1 **Blue-green opponency and trichromatic vision in the greenhouse**

2 **whitefly (*Trialeurodes vaporariorum*)**

3 Niklas Stukenberg* and Hans-Michael Poehling

4 Leibniz-Universität Hannover, Institute of Horticultural Production Systems,
5 Section Phytomedicine, Herrenhäuser Str. 2, 30419 Hannover, Germany

6 **Corresponding author:** stukenberg@ipp.uni-hannover.de

7 **Running title:** Blue-green opponency in *T. vaporariorum*

8 **Key Words:** Wavelength-specific behaviour, visual behaviour, opponent chromatic

9 mechanism, colour vision, colour choice model, LEDs

10   **Summary statement**

11   LED based choice experiments and empirical colour choice models reveal a yet undescribed

12   blue sensitive photoreceptor and an inhibitory interaction with a green sensitive receptor.

13   **Abstract**

14   Visual orientation in the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood,

15   Hemiptera: Aleyrodidae) is the result of 'wavelength-specific behaviours'. Green-yellow elicits

16   'settling behaviour' while ultraviolet (UV) radiation initiates 'migratory behaviour'.

17   Electroretinograms of the photoreceptors' spectral efficiency showed peaks in the green and

18   the UV range and whitefly vision was said to be dichromatic.

19   In order to study the visual behaviour of *T. vaporariorum*, nineteen narrow-bandwidth LEDs

20   covering the UV-A and visible range were used in combination with light scattering acrylic glass

21   screens in a small-scale choice arena under greenhouse conditions. Multiple-choice and dual-

22   choice assays were performed, resulting in LED-based behavioural action spectra of settling

23   (green) and migratory behaviour (UV). A potential inhibitory blue-green chromatic mechanism

24   was studied by combining yellow with different blueish LEDs. Intensity dependencies were

25   illustrated by changing LED intensities.

26   Regarding the 'settling response', highest attraction was achieved by a green LED with a

27   centroid wavelength of 550 nm, while a blue LED with 469 nm proved to be most inhibitory.

28   Behaviour was distinctly intensity dependent. 'Migratory behaviour' was elicited the most by

29   the UV LED with the shortest available wavelength of 373 nm. The results clearly prove the

30   presence of a green and a yet undescribed blue sensitive photoreceptor and a blue-green

31   opponent mechanism. Furthermore, empirical colour choice models were built and receptor

32   peaks were estimated around 510 - 520 nm (green), 480 - 490 nm (blue) and 340 - 370 nm

33   (UV). Consequently, *Trialeurodes vaporariorum* possesses a trichromatic receptor setup.

34   **Introduction**

35   Visual orientation is crucial for initial host plant detection and migration in the greenhouse

36   whitefly (*Trialeurodes vaporariorum* Westwood, Hemiptera: Aleyrodidae), a worldwide

37   occurring horticultural pest in greenhouses (Byrne, 1991). Two different behavioural patterns,

38   so called 'wavelength-specific behaviours', were identified in *T. vaporariorum*. Orientation to

39   host plants is guided by a 'settling' behaviour which is elicited by green-yellow light while

40   ultraviolet (UV) radiation is responsible for a pattern which can be broadly defined as 'migratory

41   behaviour' (Coombe, 1981; 1982).

2

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

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

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

65 Many studies indicate that for herbivorous insects such as aphids, the 'settling' behaviour is
66 controlled by such an inhibitory interaction of two overlapping photoreceptors sensitive for blue
67 and green light. In this so called 'opponent mechanism' or 'blue-green opponency' the signal
68 from the blue receptor inhibits the signal from the green receptor eliciting 'settling' (Döring and
69 Chittka, 2007; Döring, 2014; Döring and Röhrig, 2016; Döring et al., 2009). This mechanism
70 facilitates to extract a constant chromatic signal that detects reflected long-wavelength light
71 (green-yellow) associated with host plants and discriminates it from short- or broad-wavelength
72 light independent from illumination intensity. It also results in a shift of the behavioural action
73 spectrum to the longer wavelength range as compared to the underlying photoreceptor
74 sensitivity and a more specific and narrow tuning in to the relevant green wavelength range.
75 An apparent shortcoming of this dichromatic mechanism is the common preference of many
76 herbivorous insects for yellow instead of green which can be explained by higher reflection in
77 the relevant green range resulting in higher relative input to the green receptor. Therefore, this

3

78   simple chromatic mechanism, which should be independent of light intensity, is influenced by

79   brightness in terms of changing blue and green photoreceptor excitation ratios. Thereby, it may

80   be that the whole mechanism lies on a mixed achromatic and chromatic axis (Döring and

81   Chittka, 2007; Kelber and Osorio, 2010; Skorupski and Chittka, 2011).

82   Similar to aphids and other herbivorous insects, *Trialeurodes vaporariorum* shows a clear

83   preference for yellow-reflecting objects. At an early stage, Moericke et al. (1966) identified a

84   'fall reflex' consistently elicited above yellow surfaces independent of the intensity of the

85   reflected colour and suggested some form of 'wavelength-specific behaviour' or colour vision.

86   This preference for yellow was later confirmed in behavioural studies with coloured surfaces,

87   and bright yellow with little to no reflectance in the violet-blue spectrum was identified as being

88   most attractive compared to darker or less saturated yellow. Violet-blue proved to be not

89   attractive and it even inhibits the attraction towards yellow. Moreover, it was shown that highly

90   reflected intensities in the green-yellow range contribute positively to their attractiveness

91   (Vaishampayan et al., 1975; Affeldt et al., 1983; Webb et al., 1985). All these results with

92   coloured surfaces have contributed to the development and use of yellow sticky traps for

93   monitoring and control of whiteflies in horticultural greenhouse crops (Böckmann et al., 2015;

94   Gillespie and Quiring, 1987).

95   In a behavioural study with monochromatic light of controlled intensities MacDowall (1972)

96   determined the spectral efficiency function for a wavelength pattern from blue to red. The

97   revealed action spectrum peaked at 550 nm and corresponded with the reflection spectrum of

98   a tobacco leaf. Coombe (1981) extensively investigated the visual behaviour using

99   monochromatic light in a 'settling' paradigm and a 'phototactic' paradigm. An action spectrum

100 for the 'settling response' was generated based on spectral sensitivity which peaked at 550

101 nm and had a second peak in the UV range at 350 nm. Based on intensity response functions

102 and different methods for the determination of 'settling' it was concluded that *T. vaporariorum*

103 exhibits 'wavelength-specific behaviour'. In the phototactic paradigm it could be shown that

104 two different antagonistic behavioural patterns are elicited by 400 nm (UV) and 550 nm (green)

105 which do not interact with each other. In a follow-up study (Coombe, 1982), it was further

106 revealed that UV elicits a variety of responses associated with migratory behaviour, such as

107 take-off behaviour and maintenance of flight. For example, increased walking activity and take-

108 off rates were observed under 400 nm light and UV was preferred over green light but only

109 during flight activity. In accordance with that, it is reported from many applied studies that

110 whiteflies show less flight activity in UV-deficient environments leading to a general avoidance

111 of such conditions (Gulidov and Poehling, 2013; Kumar and Poehling, 2006; Antignus et al.,

112 2001).

4

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.

Material and Methods

Experimental LED trap screens

149

150

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

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

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

174   For the operation and adjustment of intensities of each LED panel, a device with 16 LED drivers
175   (Mini Jolly, TCI, Saronno, Italy) was constructed. The 16 separate channels could be dimmed
176   (0-100%) by external control signals (0-10 V) which were provided by two USB analogue output
177   modules (ME RedLab 3104, Meilhaus Electronic GmbH, Alling, Germany) in combination with
178   a notebook and the software ProfiLab-Epert 4.0 (ABACOM, Ganderkesee, Germany).

179   Photon flux densities ($\mu mol\ m^{-2}\ s^{-1}$) of LEDs from the long-wave UV-A to red (UV3 - R, Table
180   1, Fig. 1) were measured and adjusted using the LI-250 A Light Meter with LI 190 Quantum
181   Sensor (LI-COR Biosciences, Lincoln, NE, USA). As the sensor is only suitable to measure
182   broadband photosynthetic active radiation (PAR, 400 – 700 nm), the sensor sensitivity data
183   provided by LI-COR (starting at 385 nm) was included in the measurement of UV and violet

184   LEDs (UV3 – V3, Table 1, Fig. 1). Extrapolation of the non-measurable parts of LED spectra

185   below 385 nm had to be conducted. For the other two UV-A LEDs (UV1, UV2, Table 1, Fig. 1),

186   the Almemo® 2390-5 datalogger (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen,

187   Germany) in combination with a UV-A sensor (Type 2.5, Indium Sensor GmbH, Neuenhagen,

188   Germany) were used. The intensities were indicated in W m^{-2} and were converted to

189   µmol m^{-2} s^{-1} using the LED spectra, Planck's constant, and Avogadro's number. The sensitivity

190   data of the sensor was included as the sensor is matched for UV-A measurement in broadband

191   sunlight. All measurements were conducted in darkness by placing the sensor directly on the

192   centre of the LED screen surface.

193   **Whiteflies**

194   Greenhouse whiteflies (*Trialeurodes vaporariorum*) were reared on tobacco plants (*Nicotiana*

195   *tabacum* L. cv. 'Xanthi') in two gauze cages (0.75 x 0.5 x 0.8 m) at the Leibniz-Universität,

196   Hannover, Institute of Horticultural Production Systems, Section Phytomedicine in Germany at

197   23 ± 3 °C. For each experimental trial, vital individuals were carefully collected with an aspirator

198   from the underside of the top leaves into a snap-on lid glass vial (h x d = 50 x 30 mm) and

199   immediately released into the experimental choice arena.

200   **LED choice arena**

201   Choice experiments were conducted close to the whitefly rearing in the same greenhouse

202   compartment. A gauze-covered flight cage (1 x 1 x 0.8 m, Fig. 2B) with a waterproof black-

203   brown plywood bottom was placed on stands at a height of one meter. The foldable front side

204   faced in northern direction and was equipped with an additional lockable circular opening (0.25

205   m diameter) enabling the releasing of whiteflies. A semicircular background made of carton

206   sprayed with matt black acrylic paint (Dupli Color, Motip Dupli GmbH, Hassmersheim,

207   Germany) was inserted into the cage at a distance of 0.7 m to the release point. The

208   background was equipped with six square holes of 0.1 x 0.1 m at a height of 0.1 m and a

209   distance of 0.05 m to each other. The LED trap screens could be optionally inserted from the

210   backside by placing them on 0.1 m high wooden blocks (Fig. 2A,B). The cage backside was

211   covered with gauze and black-silver reflective mulch film (Sunup Reflective Films, Oceanside,

212   CA, USA). The cables for each LED panel were connected from the cage backside to the LED

213   control placed under the cage.

214   The ambient solar radiation during the experiments was measured using a sensor for visible

215   light (FLA 623 PS, Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) and

216   a UV-A sensor (300 – 400 nm, Type 2.5, Indium Sensor GmbH, Neuenhagen, Germany)

217   placed next to the whitefly release point. Measurements were recorded at 20 second intervals

218   with the Almemo® 2590-4AS datalogger (Ahlborn Mess- und Regelungstechnik GmbH,

219   Holzkirchen, Germany) which was also placed under the cage. Temperature was recorded

220   with a Tinytag Plus 2 TGP-4500 datalogger (Gemini Data Loggers Ltd., Chichester, UK).

221   **Experimental overview and classification**

222   According to literature, the behavioural response to the green-yellow range corresponds with

223   'settling' while the response to the UV-violet range is presumably related to 'migratory

224   behaviour' (Coombe, 1981; 1982). The conducted experiments can be classified into

225   wavelength dependence experiments characterized by the predominant main colours (green,

226   blue, UV) and intensity experiments in the green-yellow range, resulting in four experimental

227   blocks (Table 2). Wavelength dependence experiments on the 'settling response' are referred

228   to as 'Green response experiments' (Block 1). Subsequently, 'intensity dependencies' in the

229   green-yellow range were determined (Block 2). An inhibitory blue-green chromatic mechanism

230   in the 'settling response' was studied by combining yellow LEDs of the same wavelength with

231   blueish LEDs of different wavelengths, referred to as 'Blue inhibition experiments' (Block 3).

232   Wavelength dependence experiments of the 'migratory behaviour' are referred to as 'UV

233   response experiments' (Block 4).

234   Wavelength-dependent responses were initially investigated in multiple-choice experiments

235   and subsequently relevant LEDs were selected and tested in dual-choice experiments to

236   determine standardized spectral efficiencies. All multiple-choice and dual-choice experiments

237   were performed in 2015. The experiments took place in the described choice arena and

238   replicates were conducted in consecutive trials on different daytimes and days. Trials were

239   conducted between 10:00 and 17:00 h. Experiments regarding the 'settling response' were

240   conducted from February to May. With increasing day length and brighter ambient light

241   conditions in the greenhouse, whiteflies orientated more readily to the traps, hence trial

242   durations could be reduced and number of trials per day could be increased within this time.

243   UV response experiments were conducted from September to November but suffered from

244   weaker responses and low recapture rates and trial durations were adjusted accordingly (Table

245   2).

246   In multiple-choice experiments, the LED trap screens in question (six or five) were placed in

247   the holes of the choice arena background and the order was changed randomly for each

248   replication. 150 or 200 whiteflies were released per replication and the number of trapped

249   individuals on each trap were counted after a given period (0:30 – 1:30 h). Afterwards the cage

250   was cleaned carefully from remaining whiteflies with a handheld vacuum cleaner before

251   starting the next trial. The procedure for dual-choice experiments was similar, but four holes

252   for trap screens were covered with black plastic film and only the two inner holes were

253   equipped with the two LED traps. Again, trap positions were changed randomly for each

254 replication. The measurement of ambient light conditions were averaged over each
255 experimental trial and considered in the dataset as co-variable.

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

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

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

Block 2: Intensity dependences (Exp. 6-8)

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

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

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

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

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

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

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

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

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

318　The most attractive UV LED (UV1 - 373 nm centroid wavelength) was selected as reference
319　to determine the standardized spectral efficiencies of four LED colours (test lights) from UV to
320　violet (UV3, UV4, V2, V3) in dual-choice assays (exp. 14). A standardized LED based action

10

321 spectrum of the UV response was constructed according to the procedure in the green

322 response experiments (see Block 1). The experiment was conducted with two replicates per

323 colour per day and randomized order of the colours within the day.

Colour choice models

325 An empirical colour choice model was built to describe the wavelength preference in the

326 'settling response' based on opponent chromatic interaction of a green and a yet undescribed

327 blue photoreceptor. Modelling of the UV response was performed assuming achromatic

328 processing based only on the UV receptor. As no reliable data of photoreceptor sensitivities

329 are available for whiteflies, the peak sensitivities were approximated by this method.

330 Photoreceptor sensitivity templates (Govardovskii et al., 2000) were fitted for different

331 photoreceptor peak sensitivities of a putative UV, blue, and green photoreceptor, respectively.

332 The peak sensitivities of the green and the blue receptor were altered in 5 nm steps in the

333 range of 500 - 545 nm (green) and 470 - 495 nm (blue) resulting in 60 potential combinations.

334 The peak sensitivities of the UV receptor was changed in 10 nm steps in the range of 340 -

335 370 nm.

336 The photon catch P of a photoreceptor can be calculated with the photoreceptor sensitivity

337 function $S(\lambda)$ and the spectrum of the (LED) stimulus light $I(\lambda)$ (Kelber et al., 2003):

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

339 The photon catches of each LED colour (and its combinations) were calculated for each

340 potential photoreceptor position. Photoreceptor excitations E were calculated from photon

341 catch values using a nonlinear transformation (Chittka, 1996):

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

343 This resulted in excitation values for each LED and each photoreceptor (E_{UV}, E_{B}, E_{G}) at

344 varying positions. The excitations of the colour opponent mechanism E_{opp} were calculated as

345 difference between green and blue photoreceptor excitations:

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

347 These values were connected to the LED choice datasets of the 'green response', the 'blue

348 inhibition' and the 'UV response', resulting in three separate models.

349 For the 'green response model' the mean relative choice frequencies from the 'green response

350 experiments' (exp. 1-3, 5) were combined and plotted against E_{opp} values of each receptor

351 configuration. The data from multiple-choice experiments were thereby normalized to the most

352 attractive chartreuse green LED (G4). The first dataset was built based on the outcome of exp.

353 1 and 2 which were connected via the linking green LED (G3) used in both experiments. Exp.

354   3 was taken as second dataset and the normalized spectral efficiencies from exp. 5 as third

355   dataset. A preference restriction was implemented which considers that the highest excitation

356   value should correspond with the most attractive chartreuse green LED (G4):

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

358   For the 'blue inhibition model' the data from the 'blue response experiments' with mixed yellow

359   and blue LEDs (exp. 10, 11) were plotted against E_{opp} values. Here, the indirect response was

360   the inhibition of the attraction and the highest response was referred to the most inhibiting blue

361   LED. Therefore, the mean relative choice frequencies from the multiple-choice experiment

362   (exp. 10) were inverted and normalized to the most unattractive yellow-blue combination

363   (Y2+B2). The normalized spectral efficiencies of inhibition from exp. 11 were taken as second

364   dataset. Here, the lowest excitation value should correspond with the blue LED (B2) inhibiting

365   the attraction towards yellow LEDs the most:

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

367   For the 'UV response model', achromatic processing based solely on the UV receptor was

368   assumed. Therefore, the excitation values E_{UV} were directly plotted against the normalized

369   relative response data from the multiple-choice experiments (exp. 12, 13) and the dual-choice

370   spectral efficiency experiment (exp. 14). The restriction that the highest excitation value should

371   correspond with the most attractive UV LED is described by:

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

373   All models' significant linear regressions (α = 0.05) fulfilling the preference restrictions were

374   fitted and the models were assessed based on R² values. All analyses and graphical display

375   related to the colour choice models were performed in Microsoft Excel 2016.

376   **Statistical analysis**

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

378   The multiple-choice experiments (exp. 1-4, 7-8, 9-10, 12-13) were analysed with linear models

379   using the lm() function. The response variables were the ln(x + 1) transformed numbers of

380   trapped whiteflies on each LED trap. In colour choice experiments, the explanatory variable

381   was the LED colour. The ambient light intensity (visible light or UV radiation) measured

382   throughout the experiments was included as co-variable for the experiments of the green and

383   UV response. Initial Block factors (day, daytime) of the consecutive experiments were

384   excluded after model selection using Akaike's Information Criterion (Burnham and Anderson,

385   2010). Interactions between the colour and the ambient light intensity were included in the

386   analyses of the green response experiments 2 and 3. Separate linear models were fitted to

387   analyse the total numbers of trapped whiteflies in the given time dependent on the ambient

12

388 light intensity. In the analyses of the multiple-choice experiments with different LED intensities

389 (exp. 7), LED trap intensity and its interaction with ambient light intensity were explanatory

390 variables. In the analyses of the multiple-choice experiment with equal LED intensities (exp.

391 8), the individual LED trap number and the position in the choice arena were the explanatory

392 variables. ANOVA was used to determine influences of explanatory variables and interactions

393 in the linear models. Tukey-type pairwise comparisons regarding LED colours and intensities

394 were performed at α=0.05 using the lsmeans package (Lenth, 2015).

395 The sex ratio in the multiple-choice experiment 3 was analysed with a generalized linear model

396 using the glm() function with binomial distribution and logit link. The response variable was the

397 odds ratio between males and females on each trap and the explanatory variable was the

398 colour. The dual choice experiments (exp. 5, 6, 11, 14) were analysed with generalized linear

399 models (quasibinomial, logit link). The response variable was the odds ratio between the

400 number of trapped individuals on test and reference LED traps. Explanatory variable was the

401 respective colour of the test LED. The ambient light intensity was included as co-variable for

402 the spectral efficiency experiments on green and UV response. An interaction between colour

403 and ambient light was further included in the green response analysis. Deviance analyses were

404 performed to determine influences of explanatory variables and interactions in the generalized

405 linear models. In the intensity dependence dual choice experiment (exp. 6), pairwise

406 comparisons were performed between intensity levels (α=0.05, lsmeans package). User-

407 defined interaction contrasts were created to compare intensity-dependent changes of choice

408 frequencies between colours using the package statint (Kitsche and Schaarschmidt, 2015).

409 Tukey-type comparisons on interaction contrasts were performed using the multcomp package

410 (Hothorn et al., 2008). Graphs were created using the ggplot2 and gridExtra package

411 (Wickham, 2016; Auguie, 2012).

412 **Results**

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

414 *Experiment 1*: The results showed hardly any response of whiteflies to the blue (B2 - 469 nm),

415 cyan (C - 500 nm), and blue-green (BG - 512 nm) LED, and a steep significant increase in the

416 preference among green LEDs (G1-3) with only slightly different centroid wavelengths of 524,

417 528, and 533 nm (Fig. 3A).

418 No significant influence of the ambient light or the interaction with colours were observed in

419 the fitted linear model (Fig. 4A). This indicates that whiteflies discriminated green LEDs over

420 the whole ambient light intensity range. The overall recapture rate was 69.0 ± 6.6% (Mean ±

421   s.d.) within 1:15 ± 0:10 h. A separately fitted linear model shows no significant increase of the

422   total recaptures with rising ambient light intensity.

423   *Experiment 2*: In the green range, the preference further increased revealing chartreuse green

424   (G4) with 550 nm centroid wavelength as the most attractive LED (Fig. 3B). Towards the yellow

425   spectrum with the two yellow LEDs (Y1 - 574, Y2 - 590 nm), the preference declined and only

426   a weak response to amber (A - 614 nm) and no response to the red (R - 630 nm) LED were

427   noticed.

428   In contrast to exp. 1, a significant influence of ambient light and the interaction with colour were

429   observed (both P<0.001). At darker conditions, the response to yellow was relatively stronger

430   while the corresponding response to green was weaker (Fig. 4B). With increasing ambient light

431   intensity, the response to green LEDs (G3, G4) increased while the response to yellow LEDs

432   (Y1, Y2) decreased correspondingly. This resulted in a cross-over interaction between the

433   second most attractive green (G3) and yellow (Y1) LEDs which are on average of similar

434   attractiveness but with increasing ambient light intensity G3 became more attractive. The

435   overall recapture rate was 75.6 ± 10.9% (mean ± s.d.) within 1:15 ± 0:10 h and total recaptures

436   were not influenced by ambient light.

437   *Experiment 3*: When the selected attractive green and yellow LEDs (G1-4, Y1-2) were

438   compared, the results show that the relative preferences resemble an action spectrum (Fig.

439   3C).

440   No significant influence of the ambient light but a significant interaction with the colour could

441   be determined (P=0.019). At darker conditions, the preferences were more evenly distributed

442   across all colours and with rising ambient light intensity the preference was pointed more

443   towards the most attractive chartreuse green LED (G4) while the preference towards the

444   second most attractive yellow (Y2) decreased (Fig. 4C). The overall recapture rate was 82.8 ±

445   10.0% (mean ± s.d.) within 1:15 ± 0:10 h. The totally recaptured numbers increased

446   significantly with rising ambient light (P=0.003), primarily due to the strongly increasing

447   preference for the most attractive chartreuse green (G4).

448   The ratio of females on the LED colours were 68% on G1, 72% on G2, 72% on G3, 72% on

449   G4, 81% on Y1, and 81% on Y2; the overall ratio was 74.5%. The ratio of females was slightly

450   higher on the yellow LEDs but statistically no significant effect of LED colours on the sex ratio

451   was observed (GLM, Analysis of Deviance, P=0.16).

452   *Experiment 4*: When the previously attractive range was excluded, whiteflies significantly

453   preferred the blue-green (BG - 512 nm) LED and only few landings were recorded on cyan (C

454   - 500 nm), amber, and red LED traps (Fig. 3D). The overall recapture rate was 41.9 ± 10.6%

455   (mean ± s.d.) within 1:15 ± 0:10 h.

14

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

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

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

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

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

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

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

486 *Experiment 9*: Most of the whiteflies were trapped on the LED trap with pure yellow (Y2 - 590
487 nm). Little response was obtained when yellow was additively combined with small intensities
488 of the shortest wavelength violet (V2 - 415 nm) or the longest wavelength cyan (C - 500 nm)
489 LED. Almost no trappings were recorded on the combinations with the intermediate violet (V3

15

490   - 435 nm) and blue (B1 - 447, B2 - 469 nm) LEDs. The results clearly indicate that the "settling

491   response" was inhibited by blueish light (Fig. 3E). The overall recapture rate was 92.8 ± 4.9%

492   (mean ± s.d.) within 0:30 ± 0:10 h.

493   *Experiment 10*: When the pure yellow light was excluded from the setup and the intensity of

494   blueish light was further reduced, the preferences exhibited in the previous experiment were

495   clearly emphasized. Highest trap catches were recorded on the yellow-cyan combination and

496   lowest catches on the yellow-blue combinations (B1 - 447, B2 - 469 nm). The preference

497   increased again for the adjacent violet (V3 - 435 nm) and for the shortest wavelength violet

498   (V2 - 415 nm) LED in particular. The data resemble an inverse action spectrum of inhibition of

499   the 'settling response' (Fig. 3F). The overall recapture rate was 89.7 ± 10.5% (mean ± s.d.)

500   within 0:30 ± 0:10 h.

501   *Experiment 11:* On the short wavelength side, the inhibition declined successively from UV to

502   blue (B1) and violet (V2, V3) LEDs. On the long wavelength side, the inhibition strongly

503   decreased in one big step to the cyan (C) LED. Again, the obtained action spectrum was quite

504   congruent with the one derived from the multiple-choice approach (Fig. 5). The recapture rate

505   was 75.4 ± 13.0% (mean ± s.d.) within 0:30 ± 0:10 h.

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

507   *Experiment 12*: The highest responses were recorded on the first three UV-A LEDs (UV 1-3)

508   with closely related centroid wavelengths of 373, 378, and 385 nm but these preferences did

509   not differ among each other. The preference declined over 400 nm (UV4) to the violet (V1 -

510   410, V2 - 415 nm) LEDs which showed the lowest but still detectable response (Fig. 3G).

511   A significant influence of the ambient UV radiation on the trapped numbers on the colours was

512   observed in the fitted linear model (*p*=0.003). The overall recapture rate was 46.8 ± 10.7%

513   (mean ± s.d.) within 1:30 ± 0:10 h. A separately fitted linear model showed that the totally

514   recaptured numbers decreased with rising UV radiation intensities (P=0.006).

515   *Experiment 13*: When the tested spectral range was extended to blue, the preference further

516   declined on the long wavelength violet (V3 - 435 nm) and very low responses were still

517   detected on the short wavelength blue (B1 - 447 nm) LED (Fig. 3H).

518   UV radiation had a significant influence on the trapped numbers on the colours (P=0.046). The

519   overall recapture rate was 46.8 ± 10.7% (mean ± s.d.) within 1:30 ± 0:10 h and total numbers

520   were not significantly influenced by ambient UV radiation.

521   *Experiment 14*: The response declined successively over the tested UV and violet colours but

522   was still quite prominent on the long wavelength violet (V3). The obtained half-sided action

523   spectrum was wider and not entirely congruent with the ones derived from the previous

524   multiple-choice experiments (Fig. 5). The recapture rate was 23.6 ± 10.0% (mean ± s.d.) within

525   1:30 ± 0:10 h.

Colour choice models

527   In the 'green response model' and the 'blue inhibition model', several combinations of blue and

528   green photoreceptor peak combinations led to significant linear regressions which fulfil the

529   preference restrictions (Table 3).

530   For the 'green response model', regressions with good fits ($R^2 \geq 0.8$) were found for receptor

531   peak combinations from 470 & 525 nm at widest distance to 495 & 500 nm at lowest distance

532   from each other. In the blue inhibition model, good fits ($R^2 \geq 0.9$) were found for combinations

533   from 470 & 545 nm at widest distance to 495 & 500 nm at lowest distance. Well-fitting

534   regressions which fulfil the restrictions in both models overlap at receptor combinations of 480

535   & 515 nm, 485 & 510 nm, 490 & 505 and 495 & 500 nm (Tab. 3). Selected regressions for

536   potential blue and green receptor peaks at 480 and 515 nm are shown in Fig. 7A,B. The

537   modelled potential photoreceptors based on template formulas (Govardovskii et al., 2000) and

538   the resulting theoretical relative action spectrum of the 'settling response' based on blue-green

539   opponency are shown in Fig. 5.

540   Fig. 7C shows the best fitting linear regression of the 'UV response model' with a photoreceptor

541   peak at 360 nm ($R^2 = 0.93$) and the modelled receptor is also shown in Fig. 5. The restriction

542   that the highest excitation value corresponds with the most attractive UV LED is also fulfilled

543   for adjacent receptor peaks at 340, 350, and 370 with R^2 values of 0.77, 0.90, and 0.86,

544   respectively.

Discussion

Main findings

547   This study reveals that *Trialeurodes vaporariorum* possesses a yet undescribed photoreceptor

548   sensitive towards blue light and an inhibitory blue-green chromatic mechanism which controls

549   a 'wavelength-specific behaviour' referred to as 'settling response' (Coombe, 1981). Besides

550   this chromatic processing, the behavioural control is distinctly intensity-dependent. The known

551   response to UV radiation based on a UV sensitive photoreceptor related to migratory behaviour

552   could also be confirmed in our study (Coombe, 1981; 1982). As a consequence, we could

553   conclude that *T. vaporariorum* possesses a trichromatic visual system.

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

554 The chartreuse green LED with 550 nm centroid wavelength proved to be most attractive (Fig.
555 3B,C) and consequently constitutes the peak of the LED based action spectrum of the 'settling
556 response' (Fig. 5). This meets our expectations as it is in line with earlier studies from
557 MacDowall (1972) and Coombe (1981) also showing action spectra peaking at 550 nm. As
558 only this LED was available in the region between 533 and 574 nm, it is possible that the actual
559 peak slightly differs which is also possible for both reported studies which used monochromatic
560 light in wide steps of 10 and 50 nm. When only one receptor controls the behaviour, the action
561 spectrum should roughly exhibit the shape of the underlying receptor (Skorupski and Chittka,
562 2011). But our action spectrum as well as the reported data are more narrowly tuned to the
563 green-yellow range and shifted to the longer wavelength range compared to the spectral
564 efficiency peak at 520 nm which was determined by ERG recordings by Mellor et al. (1997).
565 This discrepancy suggests the involvement of opponent processing and the extraction of
566 chromatic signals (Skorupski and Chittka, 2011). Nevertheless, from an evolutionary
567 perspective it seems natural that these action spectra peak around 550 nm which corresponds
568 quite accurately with the peak reflectance and transmittance of green leaves, corroborating the
569 fact that the visual systems of herbivores are adapted to host plant detection (MacDowall,
570 1972; Döring et al., 2009; Prokopy and Owens, 1983; Kelber and Osorio, 2010).

572 An important observation with regard to potential chromatic processing was that green LEDs
573 with similar spectra of only 4-5 nm difference could be differentiated by *T. vaporariorum* as
574 shown by the multiple-choice experiments (Fig. 3A,C). Moreover, the discrimination was
575 exhibited consistently over the whole range of ambient light intensity, whereas yellow LEDs
576 were to some extent confused with green ones at darker conditions (Fig. 4). Compared to
577 naturally reflecting objects, the constant intensity of LED light is uncoupled from illuminating
578 light intensity and should theoretically appear as brighter or darker in relation to changing
579 ambient light intensity. Colour vision is defined as the ability to detect spectral variations in the
580 light independent of the intensity (Kelber et al., 2003). Photoreceptors adapt to the intensity of
581 perceived light versus the background light by adjusting their responses through various
582 mechanisms (Laughlin and Hardie, 1978; Arshavsky, 2003; Warrant and Nilsson, 2006). This
583 avoids saturation of the photoreceptors and is a mechanism to maintain colour constancy
584 (Foster, 2011; Kemp et al., 2015). Our results therefore suggest that green LEDs are
585 discriminated based on opponent processing. In the longer wavelength range above 550 nm,
586 yellow LEDs are presumably discriminated mainly by different stimulation of the green receptor
587 with only low inhibitory input from a blue receptor. At darker conditions and relatively bright
588 LED light, the green receptor might have been saturated resulting in similar signals for different
589 wavelengths. Constant wavelength discrimination should then be possible only in the green

18

590   region with distinctly overlapping receptor sensitivities resulting in different inhibitory input from
591   a non-saturated blue receptor.

Blue-green chromatic mechanism

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

603   Descriptive evidence for the blue-green chromatic mechanism comes from the empirical colour
604   choice models built from the green response and the blue inhibition experiments (Tab. 3, Fig.
605   7A,B). Both models explain the observed colour choice behaviour and fit well into the theory
606   of opponent processing based on the difference of concurrent excitations of the green and blue
607   photoreceptors. Similar models have already been shown for aphids or the pollen beetle
608   (Döring et al., 2009; Döring et al., 2012, Döring and Röhrig). In contrast to the reported studies
609   which were based on physiological and behavioural data, reliable physiological data on
610   photoreceptor sensitivities were not available for *T. vaporariorum*. Therefore our flexible
611   approach does not enable us to estimate exact positions of the photoreceptors since linear
612   modelling based on excitation differences of several combinations of blue and green
613   photoreceptor peak sensitivities led to well-fitting linear regressions (Tab. 3). The preference
614   restriction that the highest receptor excitation should correspond with the LED of highest
615   response is thereby fulfilled either in one or the other model. The position of the green receptor
616   is limited to longer wavelengths by the preference restriction in the 'green response model'
617   while the 'blue inhibition model' sets a limit towards shorter wavelengths. Both models follow
618   a slightly different pattern with receptor peaks either far away from each other or close together
619   but have a converging area in the range where receptors are close together and the restrictions
620   are fulfilled in both models. These four combinations are 480 & 515 nm, 485 & 510 nm, 490 &
621   505 nm, and 495 & 500 nm (Tab. 3) which all lead to similarly shaped theoretical action spectra
622   peaking at 554 - 556 nm (Fig. 5). While the very close combinations appear quite unlikely with
623   regards to a reliable signal from the opponent mechanism, the more distant combinations (480
624   & 515, 485 & 510 nm) appear relatively realistic (Fig. 5). In comparison, the known
625   photoreceptor sensitivities of aphids, which are also phloem-sucking herbivores show similar

19

626 configurations. Receptor peaks for the green peach aphid *Myzus persicae* were determined

627 around 490 and 530 nm and for the pea aphid *Acyrthosiphon pisum* at 518 nm, respectively

628 (Kirchner et al., 2005; Döring et al., 2011). However, the exact positions and sensitivities of

629 photoreceptors in the greenhouse whitefly still remain uncertain from this study, but only within

630 a small range: The blue photoreceptor should be present with a peak around 480 - 490 nm,

631 while a green receptor exists between 510 - 520 nm. The presence of a green receptor around

632 520 nm is also supported by the former ERG recording by Mellor et al. (1997). Obviously, this

633 ERG investigation did not detect the blue receptor and measured a mixed peak of the green

634 and blue receptor. It is unclear why the green peak was so prominent in ERG recordings but

635 the blue photoreceptor cells may be underrepresented and contribute only a low

636 electrophysiological input which is then strongly weighted in the nervous system.

637 The possible reasons for the incongruence of both models and the inaccuracies of their

638 outcomes are diverse because they rely on simple assumptions and incalculable factors. The

639 sensitivity functions of photoreceptors based on template formulas could slightly differ from

640 real sensitivities for various reasons like self-screening properties or filter and screening

641 pigments. Moreover, the calculations from photon catches to excitation values by the nonlinear

642 transformation might not explain the reality completely. Furthermore, the relative contributions

643 of the inputs from blue and green photoreceptors most likely differ from the assumed one-to-

644 one ratio. Possible reasons for this could be different amounts of blue and green-sensitive

645 photoreceptor cells in the compound eye or different weighting of the signals in the nervous

646 system (Warrant and Nilsson, 2006; Cronin et al., 2014).

Intensity dependence in the 'settling response'

648 It could be shown that the 'settling response' exhibits a clear intensity dependence (Fig. 6)

649 which is in line with findings in whiteflies and other insects (Coombe, 1981; Scherer and Kolb,

650 1987; Booth et al., 2004). Normally, colour vision is characterized to be independent of intensity

651 and most studies implicate that behaviours are processed either purely chromatic or

652 achromatic and it often remains unclear if both aspects are involved (Kelber and Osorio, 2010).

653 But our results demonstrate that the suggested dichromatic mechanism shows both chromatic

654 and achromatic properties, hence both colour (wavelength) and intensity are crucial in the

655 'settling' behaviour. This is an aspect which has already been implied by the colour choice

656 model (see above) since excitation values as outcome of the opponent mechanism can

657 theoretically be increased at the same wavelength by increasing their intensity. Our results

658 show that within the green-yellow range of the action spectrum higher intensities can

659 compensate for not optimally attractive wavelengths, thus colour constancy is not completely

660 achieved. Furthermore, the sensitivity to relative intensity changes was higher in case of green

661 LEDs compared to yellow LEDs (Fig. 6A). This represents a further clue that an interaction

20

662 between receptors takes place, as these intensity dependencies would be parallel if they are
663 based only on one receptor, following the principal of univariance (Naka and Rushton, 1966).
664 Obviously, the intensity dependence is more distinct and stable in the green region in which
665 the action spectrum is mainly shaped by opponent processing as compared to the yellow
666 region where it should be primarily formed by the sensitivity of the green receptor.

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

675 **UV response**

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

686 A relatively ambiguous wavelength dependence was determined with no significantly
687 distinguished LEDs in the UV range below 400 nm and a high variance of the choice data (Fig.
688 3G). The attractiveness decreased at 400 nm but was still present in the blue range (Fig. 3H),
689 indicating a relatively wide sensitivity. Nevertheless, the half-sided action spectrum can most
690 likely be attributed to a uniform behaviour (Fig. 5). The observed peak of the action spectrum
691 at 373 nm allows no final conclusion about the most attractive UV LED because we could not
692 test high power LEDs with smaller wavelengths as they are not yet available. According to
693 these results, it could be assumed that the observed behaviour is based only on one receptor
694 in the UV range because no indication for a chromatic interaction with an adjacent receptor
695 could be found. This is also supported by the colour choice model which explains the data best
696 with a receptor peak sensitivity at 360 nm (Fig. 5, 7C). Therefore, it can be concluded that the
697 position and peak sensitivity of the UV receptor lies between 340 and 370 nm supporting the

698 existing study by Mellor et al. (1997) showing a UV peak around 340-350 nm in ERG
699 recordings.

700 However, the conclusion that the UV receptor does not at all interact with another receptor in
701 a behavioural context might be misleading and has to be scrutinized carefully. In a natural
702 environment, significant intensities of UV radiation are always associated with skylight which
703 contains all wavelengths while the light reflected from natural objects usually contains relatively
704 small amounts of UV. It is suggested that insects could generally use a threshold-based UV-
705 green contrast to detect skyline features and to perform landmark navigation tasks (Möller,
706 2002). This opponent interaction with inhibitory input from a distant green receptor would
707 enable insects to discriminate the sky from terrestrial objects in most cases. A UV-green
708 contrast allows a better discrimination in this context than an assumed UV-blue contrast.
709 Therefore, it might not be the total intensity but rather the UV-green ratio in the perceived light
710 which determines the classification into sky and object. Light with a UV ratio above a certain
711 threshold might be classified as sky while objects with a lower UV ratio should theoretically
712 appear as a dark silhouette.

713 We can assume that the UV radiation emitted by the traps in our setup competes with the UV
714 radiation naturally entering the cage, thus skylight and trap should appear similar in this
715 behavioural context. Theoretically, the UV traps in our setup could be perceived by the
716 whiteflies as additional entry points for skylight which elicit an 'open-space reaction' as
717 described for butterflies (Scherer and Kolb, 1987). Similarly, UV patches could be used by *T.*
718 *vaporariorum* in the natural environment to find a way out of a plant canopy in order to conduct
719 dispersal flights. But it is important to note that the solitary UV radiation emitted from LEDs in
720 our setup is highly artificial as compared to the green-yellow LEDs which basically imitate host
721 plants. Although not much UV radiation is transmitted through the greenhouse glass, the UV
722 intensity measured at the release point was frequently higher than received from the traps.
723 Only with closer distance to the traps the UV intensity became higher compared to skylight.
724 The reason why the traps with comparably low UV intensities under such daylight conditions
725 were attractive for whiteflies could be the mentioned UV-green ratio (Möller, 2002) which
726 should be high due to the lack of any green light. The possibility of a UV-green contrast
727 coincides with the antagonistic character of the behavioural pattern towards UV as compared
728 to green (Coombe, 1981; 1982). The rationale of such a UV-green contrast mechanism to
729 discriminate sky and object represents a convincing explanation but needs further
730 investigations in the future.

731 **Conclusion and Outlook**

732 Translated into the natural environment, *T. vaporariorum* uses the discussed chromatic
733 mechanism to extract a colour signal to decide if the perceived object is a host plant or not. All

22

734 objects with significant reflection in the green-yellow range (500-600 nm) and low reflection in

735 the violet-blue range (400-500 nm) are potentially seen as host plants and elicit settling. Within

736 the green-yellow range of the action spectrum the whitefly selects the brightest stimulus with

737 the highest excitation of the opponent mechanism. The intensity dependence may be used to

738 detect young leaves of brighter green or top leaves exposed to the sun and showing higher

739 reflectance or transmittance than shaded ones.

740 This study represents the first detailed LED-based investigation on whitefly visual behaviour

741 resulting in LED-based action spectra under natural sunlight conditions. This has profound

742 relevance for the basic understanding of the visual mechanism of *Trialeurodes vaporariorum*

743 and provides the basis for the improvement of visual trapping methods for monitoring and

744 control in greenhouses. A recent study already showed the effect of modifying the reflective

745 properties of yellow card traps (Sampson et al., 2018). This can be further specified according

746 to the determined wavelength and intensity dependence, the photoreceptor sensitivity

747 estimations, and the colour choice model from our study. Moreover, LEDs which enhance the

748 attractiveness of visual traps (Stukenberg et al., 2015) can be more appropriately selected

749 according to our results. The developed method generally provides great possibilities for future

750 studies on the visual ecology of insects. An important notion is that we obtained comparable

751 results regarding spectral efficiencies with multiple-choice and dual-choice experiments, thus

752 for rapid wavelength screenings time consuming dual-choice experiments could be neglected

753 in the future. The method could also be extended to more detailed LED mixing experiments

754 under controlled ambient light conditions to provide a better basis for more precise modelling

755 of photoreceptor sensitivities and interactions. Future studies should especially focus on the

756 behaviour related to UV radiation and the underlying mechanisms.

References

Affeldt, H. A., Thimijan, R. W., Smith, F. F. and Webb, R. E. (1983). Response of the greenhouse whitefly (Homoptera: Aleyrodidae) and the vegetable leafminer (Diptera: Agromyzidae) to photospectra. *J. Econ. Entomol.* **76**, 1405–1409.

Antignus, Y., Nestel, D., Cohen, S. and Lapidot, M. (2001). Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. *Environ. Entomol.* **30**, 394–399.

Arshavsky, V. Y. (2003). Protein translocation in photoreceptor light adaptation: a common theme in vertebrate and invertebrate vision. *Sci. Signal.* **2003**, pe43.

Auguie, B. (2012). *gridExtra: functions in Grid graphics*. R package version 0.9.1. URL http://CRAN.R-project.org/package=gridExtra

Böckmann, E., Hommes, M. and Meyhöfer, R. (2015). Yellow traps reloaded. What is the benefit for decision making in practice? *J. Pest. Sci.* **88**, 439–449.

Booth, D., Stewart, A. J. A. and Osorio, D. (2004). Colour vision in the glow-worm *Lampyris noctiluca* (L.) (Coleoptera: Lampyridae): evidence for a green-blue chromatic mechanism. *J. Exp. Biol.* **207**, 2373–2378.

Briscoe, A. D. and Chittka, L. (2001). The evolution of color vision in insects. *Annu. Rev. Entomol.* **46**, 471–510.

Burnham, K. P. and Anderson, D. R. (2010). *Model selection and multimodel inference. A practical information-theoretic approach*. New York, USA: Springer.

Byrne, D. N. (1991). Whitefly Biology. *Annu. Rev. Entomol.* **36**, 431–457.

Chittka, L. (1996). Optimal Sets of Color Receptors and Color Opponent Systems for Coding of Natural Objects in Insect Vision. *J. Theor. Biol.* **181**, 179–196.

Coombe, P. E. (1981). Wavelength specific behaviour of the whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *J. Comp. Physiol.* **144**, 83–90.

Coombe, P. E. (1982). Visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*. *Physiol. Entomol.* **7**, 243–251.

Cronin, T. W., Johnsen, S., Marshall, N. J. and Warrant, E. J. (2014). *Visual Ecology*. Princeton, USA: Princeton University Press.

Döring, T. F. (2014). How aphids find their host plants, and how they don't. *Ann. Appl. Biol.* **165**, 3–26.

Döring, T. F. and Chittka, L. (2007). Visual ecology of aphids—a critical review on the role of colours in host finding. *Arthropod-Plant Inte.* **1**, 3–16.

Döring, T. F. and Röhrig, K. (2016). Behavioural response of winged aphids to visual contrasts in the field. *Ann. Appl. Biol.* **168**, 421–434.

Döring, T. F., Archetti, M. and Hardie, J. (2009). Autumn leaves seen through herbivore eyes. *Proc. R. Soc. B* **276**, 121–127.

801 **Döring, T. F., Kirchner, S. M., Skorupski, P. and Hardie, J.** (2011). Spectral sensitivity of the green
802   photoreceptor of winged pea aphids. *Physiol. Entomol.* **36**, 392–396.

803 **Döring, T. F., Skellern, M., Watts, N. and Cook, S. M.** (2012). Colour choice behaviour in the pollen
804   beetle *Meligethes aeneus* (Coleoptera: Nitidulidae). *Physiol. Entomol.* **37**, 360–378.

805 **Foster, D. H.** (2011). Color constancy. *Vision research* **51**, 674–700.

806 **Gillespie, D. R. and Quiring, D.** (1987). Yellow sticky traps for detecting and monitoring greenhouse-
807   whitefly (Homoptera, Aleyrodidae) adults on greenhouse tomato crops. *J. Econ. Entomol.* **80**, 675–
808   679.

809 **Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K.** (2000). In search of
810   the visual pigment template. *Vis. Neurosci.* **17**, 509–528.

811 **Gulidov, S. and Poehling, H. M.** (2013). Control of aphids and whiteflies on Brussels sprouts by
812   means of UV-absorbing plastic films. *J. Plant. Dis. Protect.* **120**, 122–130.

813 **Hothorn, T., Bretz, F. and Westfall, P.** (2008). Simultaneous Inference in General Parametric
814   Models. *Biom. J.* **50**, 346–363.

815 **Kelber, A., Vorobyev, M. and Osorio, D.** (2003). Animal colour vision – behavioural tests and
816   physiological concepts. *Biol. Rev.* **78**, 81–118.

817 **Kelber, A. and Osorio, D.** (2010). From spectral information to animal colour vision: experiments and
818   concepts. *Proc. R. Soc. B* **277**, 1617–1625.

819 **Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T. D., Dyer, A. G.,**
820   **Hart, N. S., Marshall, J. and Whiting, M. J.** (2015). An integrative framework for the appraisal of
821   coloration in nature. *Am. Nat.* **185**, 705–724.

822 **Kirchner, S., Döring, T. and Saucke, H.** (2005). Evidence for trichromacy in the green peach aphid,
823   *Myzus persicae* (Sulz.) (Hemiptera: Aphididae). *J. Insect Physiol.* **51**, 1255–1260.

824 **Kitsche, A. and Schaarschmidt, F.** (2015). Analysis of Statistical Interactions in Factorial
825   Experiments. *J. Agro. Crop. Sci.* **201**, 69–79.

826 **Kumar, P. and Poehling, H.-M.** (2006). UV-blocking plastic films and Nets influence vectors and virus
827   transmission on greenhouse tomatoes in the humid Tropics. *Environ. Entomol.* **35**, 1069–1082.

828 **Laughlin, S. B. and Hardie, R. C.** (1978). Common strategies for light adaptation in the peripheral
829   visual systems of fly and dragonfly. *J. Comp. Physiol.* **128**, 319–340.

830 **Lenth, R. V.** (2015). *lsmeans: Least-Squares Means*. R package version 2.18. URL http://CRAN.R-
831   project.org/package=gridExtra

832 **MacDowall, F. D. H.** (1972). Phototactic action spectrum for whitefly and the question of colour vision.
833   *Can. Entomol.* **104**, 299–307.

834 **Mellor, H. E., Bellingham, J. and Anderson, M.** (1997). Spectral efficiency of the glasshouse whitefly
835   *Trialeurodes vaporariorum* and *Encarsia formosa* its hymenopteran parasitoid. *Entomol. Exp. Appl.*
836   **83**, 11–20.

837 **Moericke, V., Schneiders, H. and Vogt, B.** (1966). Flughemmung und Fallreflexhaltung als Reaktion
838   auf Gelbreiz bei *Trialeurodes vaporariorum* (Westwood). *Z. Pflanzenkr. Pflanzenschutz* **73**, 6–14.

839 **Möller, R.** (2002). Insects could exploit UV-green contrast for Landmark navigation. *J. Theor. Biol.*
840   **214**, 619–631.

841 **Naka, K. I. and Rushton, W. A. H.** (1966). An attempt to analyse colour reception by
842   electrophysiology. *J. Physiol.* **185**, 556–586.

843 **Prokopy, R. J. and Owens, E. D.** (1983). Visual detection of plants by herbivorous insects. *Annu.*
844   *Rev. Entomol.* **28**, 337–364.

845 **R Core Team** (2015). *R: A language and environment for statistical computing*. R Foundation for
846   Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

847 **Sampson, C., Covaci, A. D., Hamilton, J. G. C., Hassan, N., Al-Zaidi, S. and Kirk, W. D. J.** (2018).
848   Reduced translucency and the addition of black patterns increase the catch of the greenhouse
849   whitefly, *Trialeurodes vaporariorum*, on yellow sticky traps. *PloS one* **13**, e0193064.

850 **Scherer, C. and Kolb, G.** (1987). Behavioral experiments on the visual processing of color stimuli in
851   *Pieris brassicae* L. (Lepidoptera). *J. Comp. Physiol.* **160**, 645–656.

852 **Skorupski, P. and Chittka, L.** (2011). Is colour cognitive? *Opt. Laser Technol.* **43**, 251–260.

853 **Stukenberg, N., Gebauer, K. and Poehling, H.-M.** (2015). Light emitting diode(LED)-based trapping
854   of the greenhouse whitefly (*Trialeurodes vaporariorum*). *J. Appl. Entomol.* **139**, 268–279.

855 **Tokushima, Y., Uehara, T., Yamaguchi, T., Arikawa, K., Kainoh, Y. and Shimoda, M.** (2016).
856   Broadband Photoreceptors Are Involved in Violet Light Preference in the Parasitoid Fly *Exorista*
857   *Japonica. PloS one* **11**, e0160441.

858 **Vaishampayan, S. M., Kogan, M., Waldbauer, G. P. and Woolley, J. T.** (1975). Spectral specific
859   resonses in the visual behavior of the greenhouse whitefly, *Trialeurodes vaporariorum*
860   (Homoptera: Aleyrodidae). *Entomol. Exp. Appl.* **18**, 344–356.

861 **Warrant, E. and Nilsson, D.-E.** (2006). *Invertebrate Vision*. Cambridge, UK: Cambridge University
862   Press.

863 **Webb, R. E., Smith, F. F., Affeldt, H., Thimijan, R. W., Dudley, R. F. and Webb, H. F.** (1985).
864   Trapping greenhouse whitefly with coloured surfaces: variables affecting efficacy. *Crop Prot.* **4**,
865   381–393.

866 **Wickham, H.** (2016). *Ggplot2. Elegant graphics for data analysis*. New York, USA: Springer.

Tables

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

Table 2. Experimental overview and design

Block	No.	Design *	LEDs **	Intensity on trap screen (μmol m^{-2} s^{-1})	Replicates	Days	Trials / day	Whiteflies / trial	Trial Duration (h) ***	Dates
(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

870

871 **Table 3. R² values from linear regressions of colour choice models for different photoreceptor**
872 **combinations based on blue-green opponency.**

Green receptor peak wavelength (nm)	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	
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 R² values from significant (α=0.05) regressions are shown which fulfill the restriction $E_{\mathrm{opp}}(\mathrm{G4}) > E_{\mathrm{opp}}(\mathrm{Y1}, \mathrm{G3})$ 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.

29

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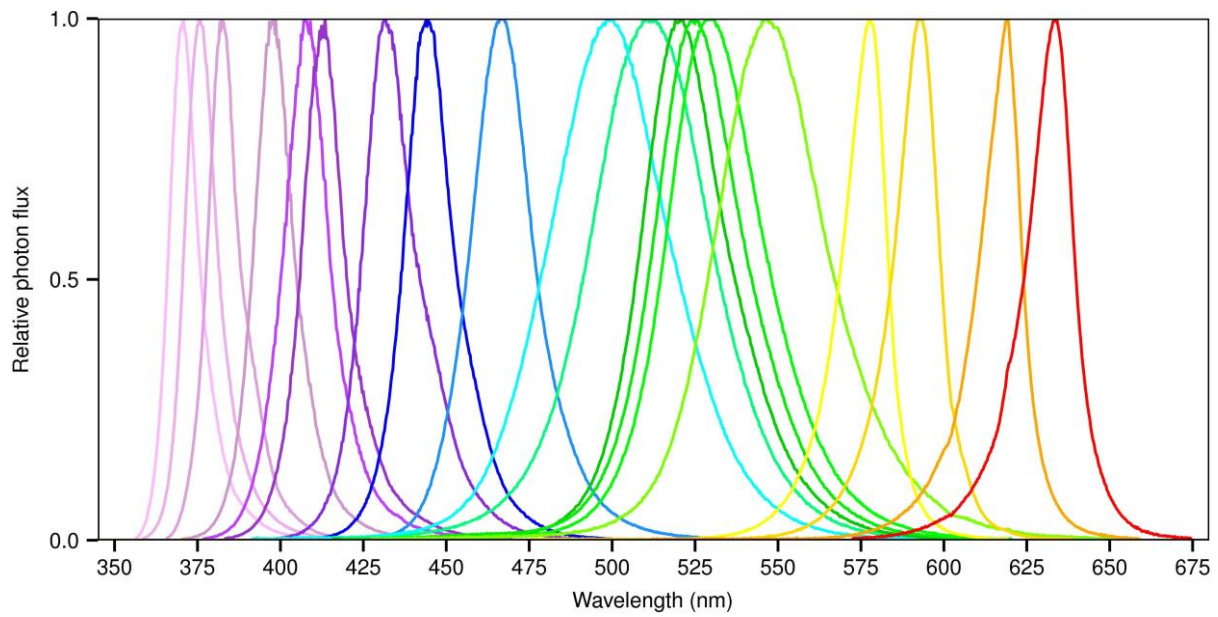
Fig. 1. Spectra of high-power LEDs used. Data refer to LED specifications given in Table 1, in spectral order.

A   **B**
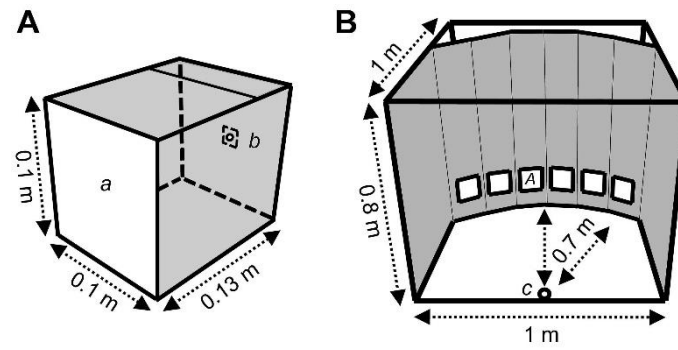
Fig. 2. Schemes of LED trap screen and choice arena. (A) LED trap screen with acrylic glass screen front side (*a*) and LED panel backside (*b*). The inner side of the box was laminated with mirror film. (B) Choice arena with whitefly release point (*c*) and position of LED traps (*A*). The background was black and the bottom was black-brown.
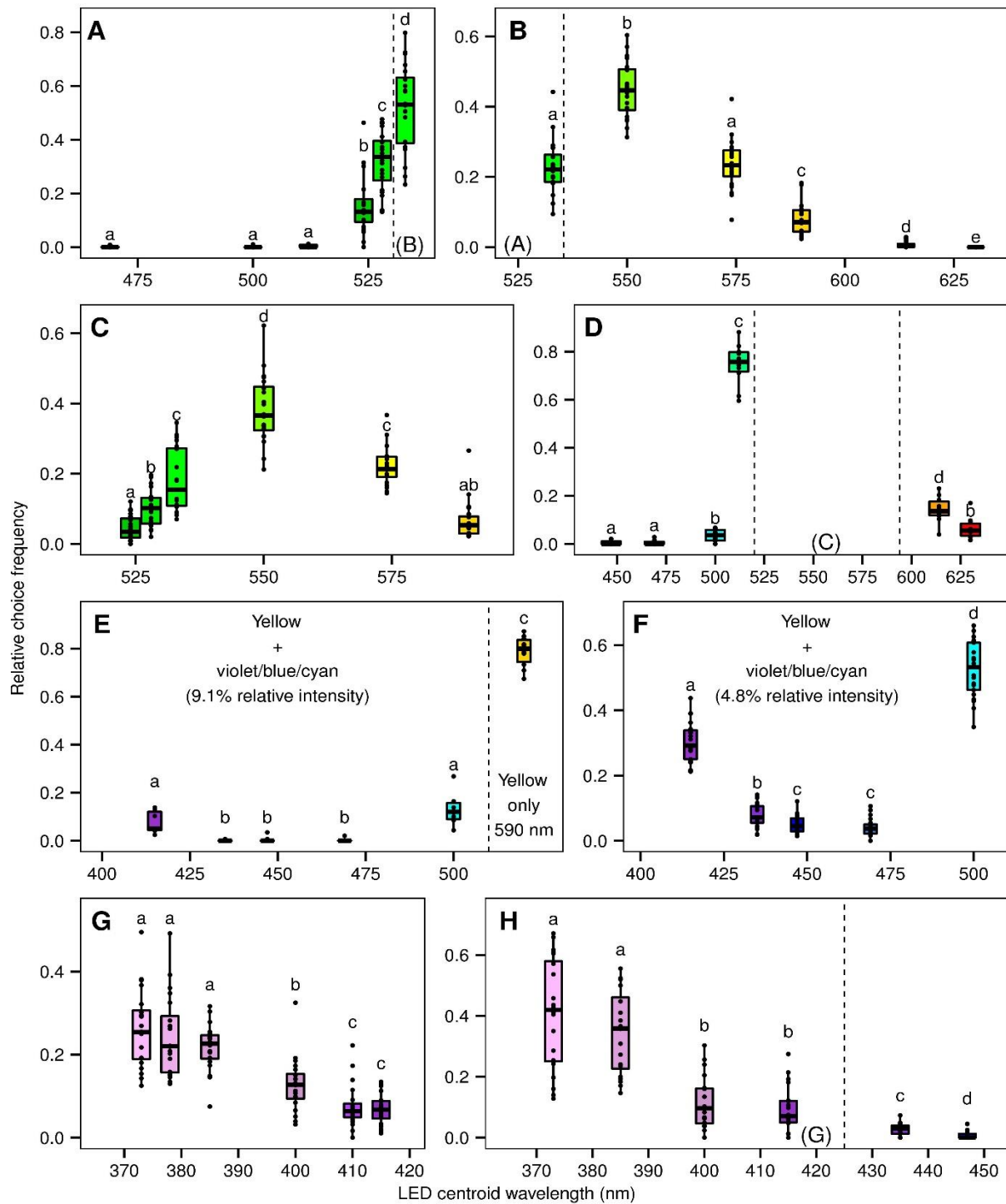
Fig. 3. Wavelength preferences of *Trialeurodes vaporariorum* in LED multiple-choice experiments. (A) blue - green (exp. 1), (B) green - red (exp. 2), (C) green - yellow (exp. 3), (D) blue & red (exp. 4), (E) yellow + violet - cyan & pure yellow (exp. 9), (F) yellow + violet - cyan (exp. 10), (G) UV - 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).
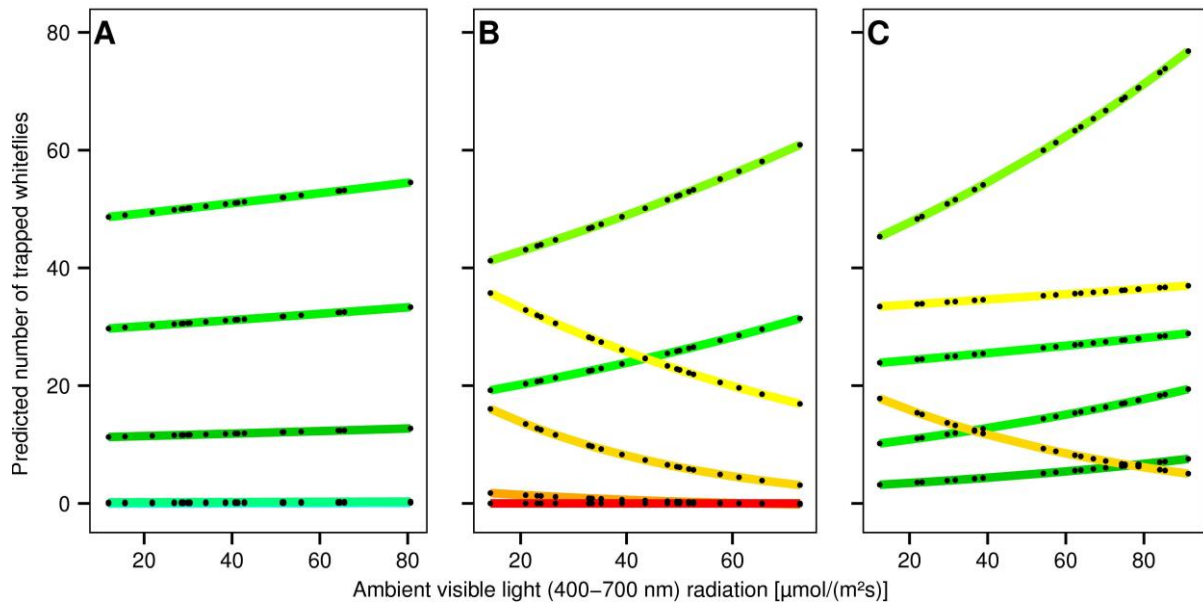
Fig. 4. Interactions of wavelength preferences in *Trialeurodes vaporariorum* with ambient light intensity in LED multiple-choice experiments based on the fitted models. Black dots connected by coloured lines show backtransformed predictions from the linear models (ln(x+1) ~ LED colour × ambient light 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).

33
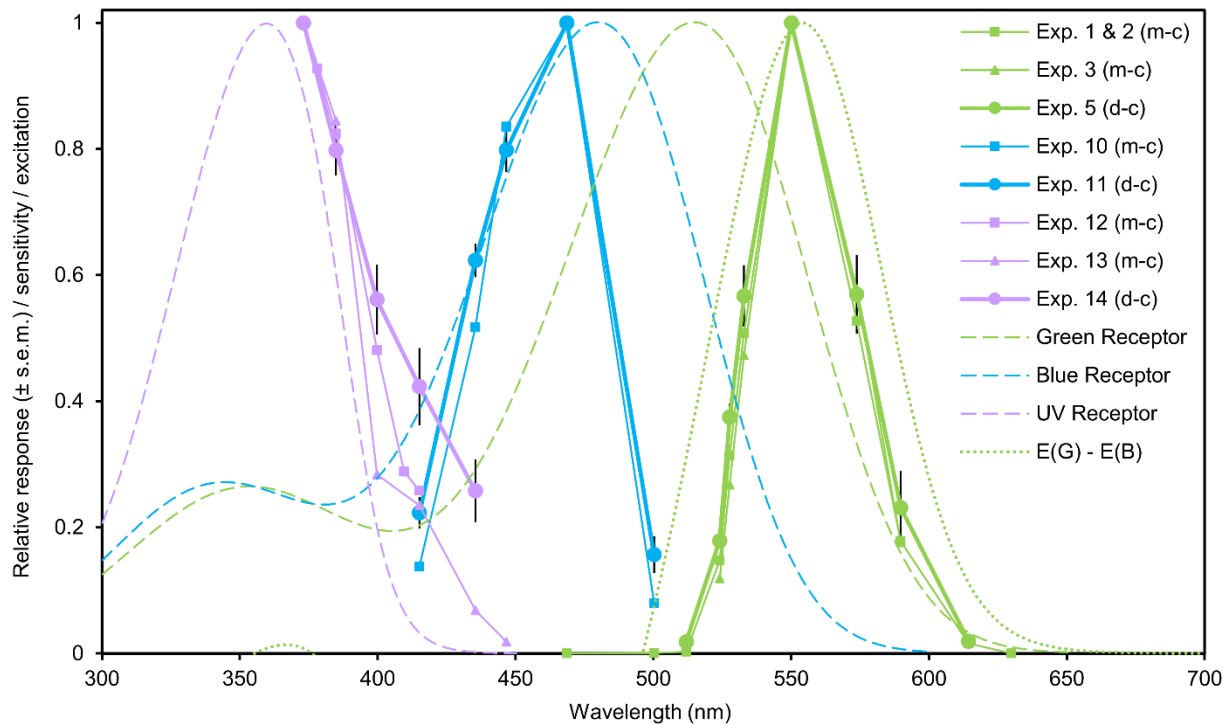
Fig. 5. Spectral efficiencies of *Trialeurodes vaporariorum* derived from LED choice experiments and modelled putative photoreceptor sensitivities with resulting theoretical action spectrum of the 'settling response' based on blue-green opponency. Coloured symbols connected by solid lines show relative 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'.

34
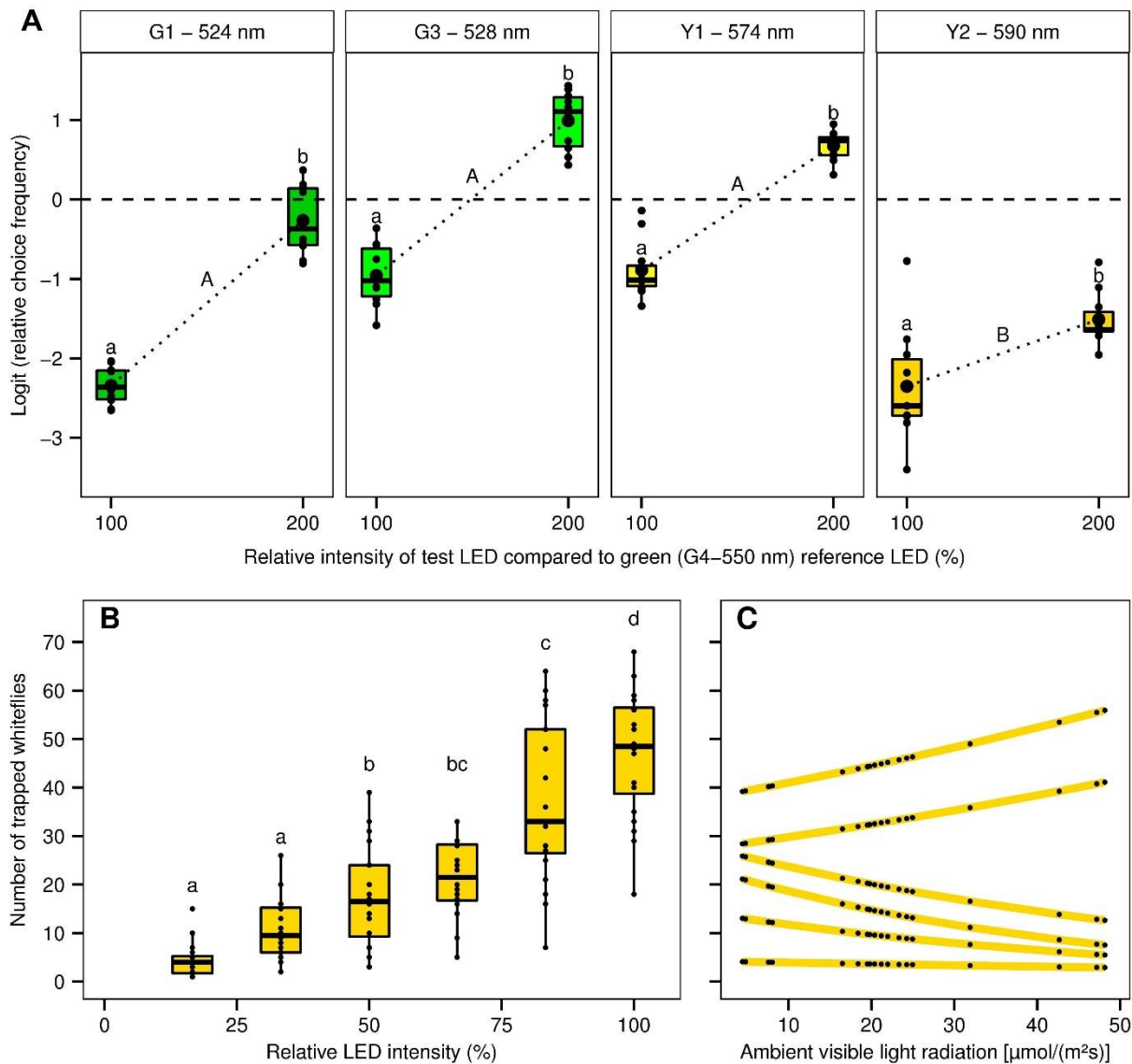
Fig. 6. Intensity dependencies in the colour choice behaviour of *Trialeurodes vaporariorum*. (A) Intensity dependences between spectral efficiencies. Panels show Logit-transformed choice frequencies in dual-choice experiments with four LED colours at equal (=100%) and double (=200%) relative intensity 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).
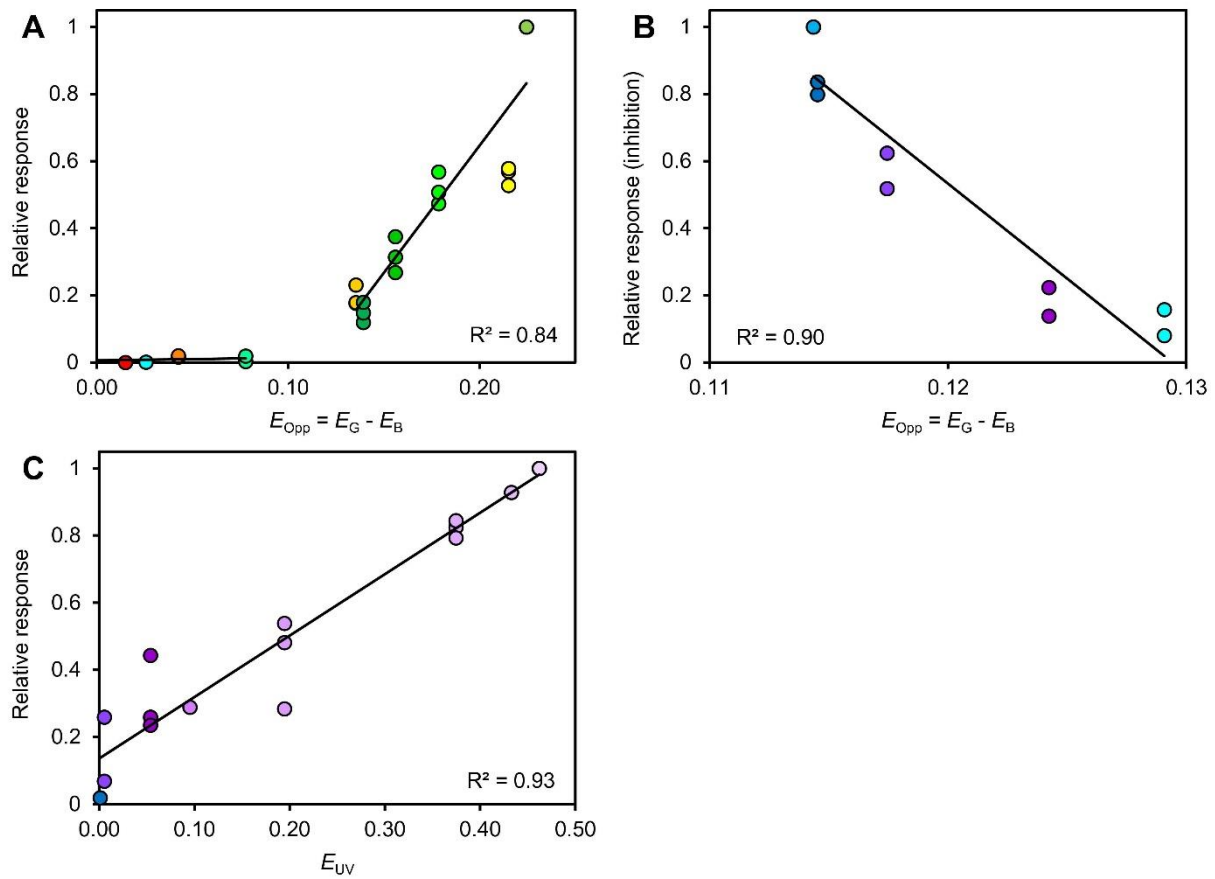
Fig. 7. Selected linear regressions of colour choice models. (A) Green response model and (B) Blue inhibition model for photoreceptor peaks at 480 nm and 515 nm based on blue-green opponency (See Table 2) (C) UV response model with photoreceptor peak at 360 nm.