

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).

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

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).

113 For aphids, clear physiological evidence of a trichromatic receptor setup involving UV-sensitive
114 photoreceptors exists (Kirchner et al., 2005). In contrast, trichromacy has not been confirmed
115 in *Trialeurodes vaporariorum*. Mellor et al. (1997) investigated the physiological properties of
116 the compound eye of *T. vaporariorum* and determined its spectral efficiency using the
117 electroretinogram (ERG) technique. Efficiency peaks were identified in the green-yellow region
118 (520 nm) and in the UV region (340 nm). Furthermore, the eye is divided in a dorsal part with
119 54-55 ommatidia and a ventral part containing 29-31 ommatidia. The dorsal region was thereby
120 more sensitive to UV. Based on these results the visual system was concluded to be
121 dichromatic.

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

136 Considering the referred studies it is quite likely that *T. vaporariorum* exhibits blue-green
137 opponency and possesses a trichromatic receptor setup. Nevertheless, a clear proof and a
138 detailed characterisation of the mechanism which connects behavioural data with potential
139 photoreceptor sensitivities is still missing. LEDs are a very useful tool to study insects' visual
140 behaviour since wavelengths and intensities can be individually adjusted and combined
141 (Tokushima et al., 2016; Booth et al., 2004). In this study, we explored the visual behaviour
142 and wavelength discrimination ability of *T. vaporariorum* using a fine-tuned selection of LEDs
143 ranging from UV to red. Behavioural action spectra were generated under semi-natural
144 greenhouse conditions, thereby taking changing ambient light conditions into account. We
145 further investigated and characterized in detail the potential blue photoreceptor and the blue-
146 green chromatic mechanism by LED mixing experiments. From the data, we built simple
147 empirical colour choice models which explain the choice behaviour and enable approximate
148 estimation of the spectral location of photoreceptors.

149 **Material and Methods**

150 **Experimental LED trap screens**

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 ($\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $\mu mol\ m^{-2}\ s^{-1}$ 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 $23 \pm 3\ ^\circ C$. 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.

256 **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.

276 **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).

290 **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 $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ on the trap screen. A small
298 amount of $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (= 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 $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (= 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.

312 **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

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.

324 **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):

$$338 \quad P = \int I(\lambda)S(\lambda)d\lambda \quad (1)$$

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):

$$342 \quad E = P/(P + 1) \quad (2)$$

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:

$$346 \quad E_{opp} = E_G - E_B \quad (3)$$

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):

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

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:

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

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:

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

373 All models' significant linear regressions ($\alpha = 0.05$) fulfilling the preference restrictions were
374 fitted and the models were assessed based on R^2 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 $\text{lm}()$ 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

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 $\alpha=0.05$ 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 ($\alpha=0.05$, `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 $69.0 \pm 6.6\%$ (Mean \pm

421 s.d.) within $1:15 \pm 0:10$ 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 $75.6 \pm 10.9\%$ (mean \pm s.d.) within $1:15 \pm 0:10$ 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 $82.8 \pm$
445 10.0% (mean \pm s.d.) within $1:15 \pm 0:10$ 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 $41.9 \pm 10.6\%$
455 (mean \pm s.d.) within $1:15 \pm 0:10$ h.

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 $82.0 \pm 13.5\%$ (mean \pm s.d.) within $0:40 \pm 0:10$
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

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 $92.8 \pm 4.9\%$
492 (mean \pm s.d.) within $0:30 \pm 0:10$ 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 $89.7 \pm 10.5\%$ (mean \pm s.d.)
500 within $0:30 \pm 0:10$ 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 $75.4 \pm 13.0\%$ (mean \pm s.d.) within $0:30 \pm 0:10$ 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 $46.8 \pm 10.7\%$
513 (mean \pm s.d.) within $1:30 \pm 0:10$ 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 $46.8 \pm 10.7\%$ (mean \pm s.d.) within $1:30 \pm 0:10$ 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 $23.6 \pm 10.0\%$ (mean \pm s.d.) within
525 $1:30 \pm 0:10$ h.

526 **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.

545 **Discussion**

546 **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.

554 **Wavelength dependence of the ‘settling response’ and interaction with ambient light**

555 The chartreuse green LED with 550 nm centroid wavelength proved to be most attractive (Fig.
556 3B,C) and consequently constitutes the peak of the LED based action spectrum of the ‘settling
557 response’ (Fig. 5). This meets our expectations as it is in line with earlier studies from
558 MacDowall (1972) and Coombe (1981) also showing action spectra peaking at 550 nm. As
559 only this LED was available in the region between 533 and 574 nm, it is possible that the actual
560 peak slightly differs which is also possible for both reported studies which used monochromatic
561 light in wide steps of 10 and 50 nm. When only one receptor controls the behaviour, the action
562 spectrum should roughly exhibit the shape of the underlying receptor (Skorupski and Chittka,
563 2011). But our action spectrum as well as the reported data are more narrowly tuned to the
564 green-yellow range and shifted to the longer wavelength range compared to the spectral
565 efficiency peak at 520 nm which was determined by ERG recordings by Mellor et al. (1997).
566 This discrepancy suggests the involvement of opponent processing and the extraction of
567 chromatic signals (Skorupski and Chittka, 2011). Nevertheless, from an evolutionary
568 perspective it seems natural that these action spectra peak around 550 nm which corresponds
569 quite accurately with the peak reflectance and transmittance of green leaves, corroborating the
570 fact that the visual systems of herbivores are adapted to host plant detection (MacDowall,
571 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

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

592 **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 *Lampyrus 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

626 configurations. Receptor peaks for the green peach aphid *Myzus persicae* were determined
627 around 490 and 530 nm and for the pea aphid *Acyrtosiphon 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).

647 **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

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

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.

757 **Acknowledgements**

758 We gratefully acknowledge Prof. Dr. Thomas Döring for a fruitful discussion on the colour
759 choice model, Dr. Frank Schaarschmidt for statistical advice and Dr. Christine Dieckhoff for
760 valuable comments on language and style.

761 **Competing interests**

762 No competing interests declared.

763 **Funding**

764 This work was funded by the Federal Office for Agriculture and Food, Germany, under the
765 grant no. 2815411110. The authors take full responsibility for the content of this publication.

766 References

- 767 **Affeldt, H. A., Thimijan, R. W., Smith, F. F. and Webb, R. E.** (1983). Response of the greenhouse
768 whitefly (Homoptera: Aleyrodidae) and the vegetable leafminer (Diptera: Agromyzidae) to
769 photospectra. *J. Econ. Entomol.* **76**, 1405–1409.
- 770 **Antignus, Y., Nestel, D., Cohen, S. and Lapidot, M.** (2001). Ultraviolet-deficient greenhouse
771 environment affects whitefly attraction and flight-behavior. *Environ. Entomol.* **30**, 394–399.
- 772 **Arshavsky, V. Y.** (2003). Protein translocation in photoreceptor light adaptation: a common theme in
773 vertebrate and invertebrate vision. *Sci. Signal.* **2003**, pe43.
- 774 **Auguie, B.** (2012). *gridExtra: functions in Grid graphics*. R package version 0.9.1. URL [http://CRAN.R-](http://CRAN.R-project.org/package=gridExtra)
775 [project.org/package=gridExtra](http://CRAN.R-project.org/package=gridExtra)
- 776 **Böckmann, E., Hommes, M. and Meyhöfer, R.** (2015). Yellow traps reloaded. What is the benefit for
777 decision making in practice? *J. Pest. Sci.* **88**, 439–449.
- 778 **Booth, D., Stewart, A. J. A. and Osorio, D.** (2004). Colour vision in the glow-worm *Lampyris*
779 *noctiluca* (L.) (Coleoptera: Lampyridae): evidence for a green-blue chromatic mechanism. *J. Exp.*
780 *Biol.* **207**, 2373–2378.
- 781 **Briscoe, A. D. and Chittka, L.** (2001). The evolution of color vision in insects. *Annu. Rev. Entomol.*
782 **46**, 471–510.
- 783 **Burnham, K. P. and Anderson, D. R.** (2010). *Model selection and multimodel inference. A practical*
784 *information-theoretic approach*. New York, USA: Springer.
- 785 **Byrne, D. N.** (1991). Whitefly Biology. *Annu. Rev. Entomol.* **36**, 431–457.
- 786 **Chittka, L.** (1996). Optimal Sets of Color Receptors and Color Opponent Systems for Coding of
787 Natural Objects in Insect Vision. *J. Theor. Biol.* **181**, 179–196.
- 788 **Coombe, P. E.** (1981). Wavelength specific behaviour of the whitefly *Trialeurodes vaporariorum*
789 (Homoptera: Aleyrodidae). *J. Comp. Physiol.* **144**, 83–90.
- 790 **Coombe, P. E.** (1982). Visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*.
791 *Physiol. Entomol.* **7**, 243–251.
- 792 **Cronin, T. W., Johnsen, S., Marshall, N. J. and Warrant, E. J.** (2014). *Visual Ecology*. Princeton,
793 USA: Princeton University Press.
- 794 **Döring, T. F.** (2014). How aphids find their host plants, and how they don't. *Ann. Appl. Biol.* **165**, 3–26.
- 795 **Döring, T. F. and Chittka, L.** (2007). Visual ecology of aphids—a critical review on the role of colours
796 in host finding. *Arthropod-Plant Inte.* **1**, 3–16.
- 797 **Döring, T. F. and Röhrig, K.** (2016). Behavioural response of winged aphids to visual contrasts in the
798 field. *Ann. Appl. Biol.* **168**, 421–434.
- 799 **Döring, T. F., Archetti, M. and Hardie, J.** (2009). Autumn leaves seen through herbivore eyes. *Proc.*
800 *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). *Ismeans: Least-Squares Means*. R package version 2.18. URL [http://CRAN.R-](http://CRAN.R-project.org/package=gridExtra)
831 [project.org/package=gridExtra](http://CRAN.R-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 responses 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.

867 **Tables**

868 **Table 1. Specifications of high-power LEDs and constructed LED panels used in the**
 869 **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	Experimental		LEDs **	Intensity on trap screen ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Number of			Trial Duration (h) ***	Dates	
	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

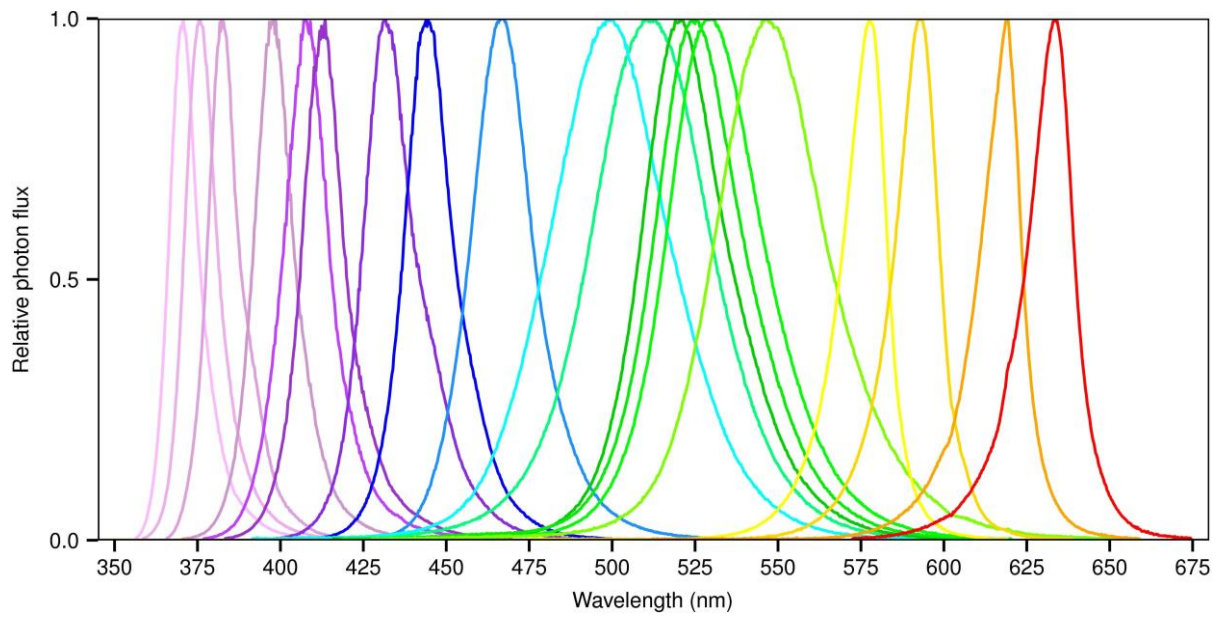
* m-c = Multiple-choice experiment, d-c = Dual-choice experiment / ** LED colour abbreviations according to Table 1 / *** Trial duration varied by $\pm 0:10$ h at most

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

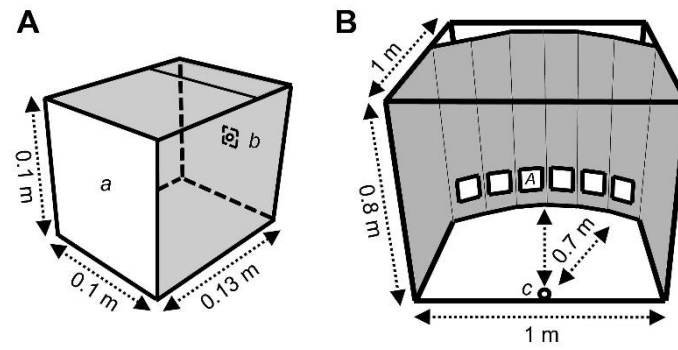
		Colour choice model											
		Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.
		Blue receptor peak wavelength (nm)											
		470	475	480	485	490	495						
Green receptor peak wavelength (nm)	500		0.32	0.48	0.68	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 R² values from significant ($\alpha=0.05$) regressions are shown which fulfill the restriction $E_{opp}(G4) > E_{opp}(Y1, G3)$ for the green response model and $E_{opp}(Y2+B2) < E_{opp}(Y2+B3)$ for the blue inhibition model. Fields framed with dotted line indicate overlapping of appropriate regressions from both models.

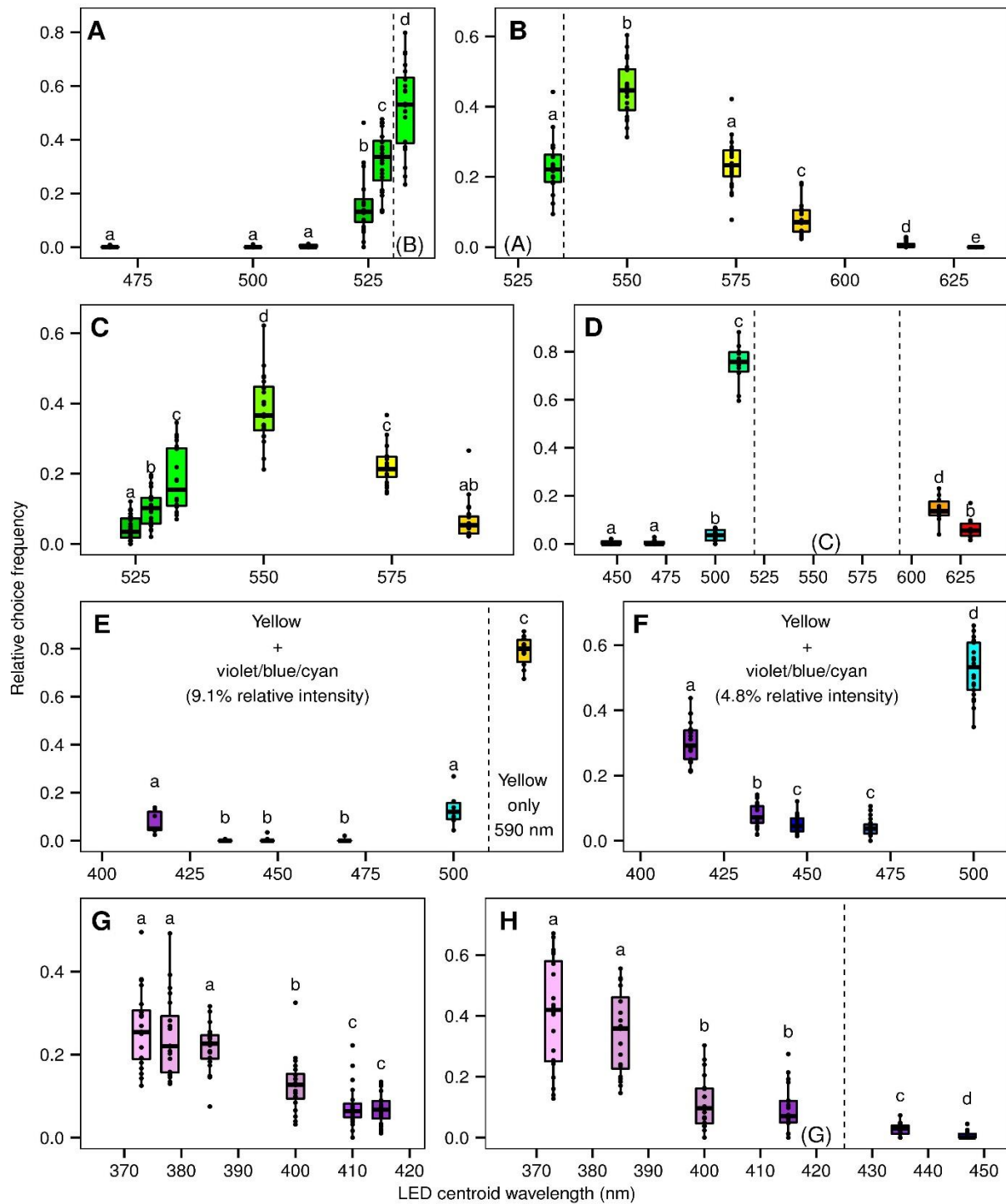
873 **Figures**



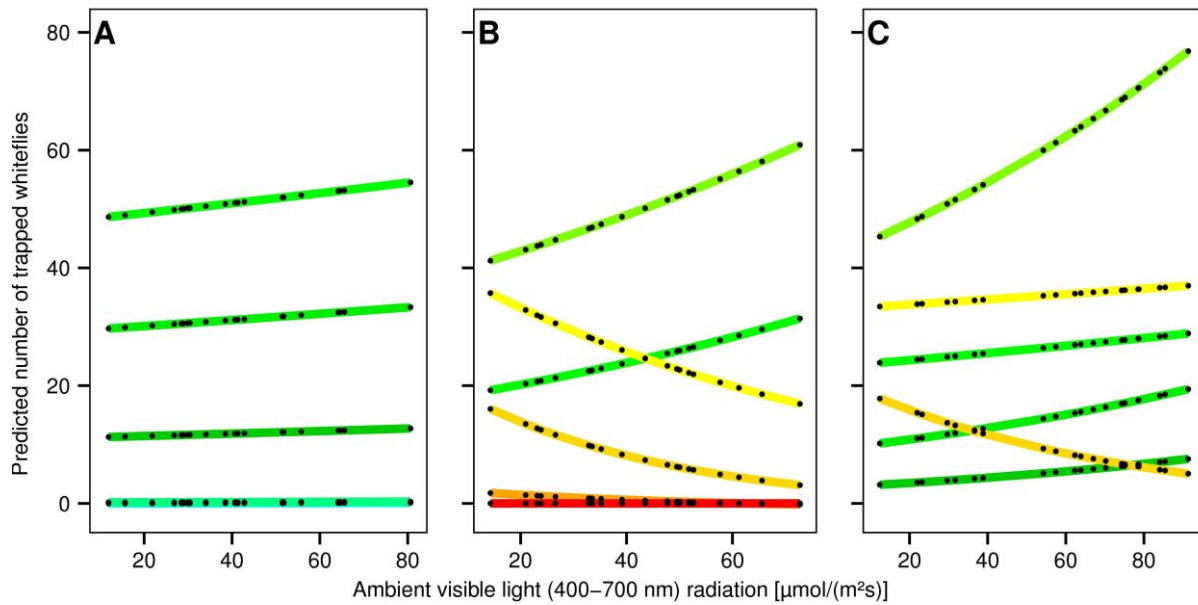
874 **Fig. 1. Spectra of high-power LEDs used.** Data refer to LED specifications given in Table 1, in spectral
875 order.



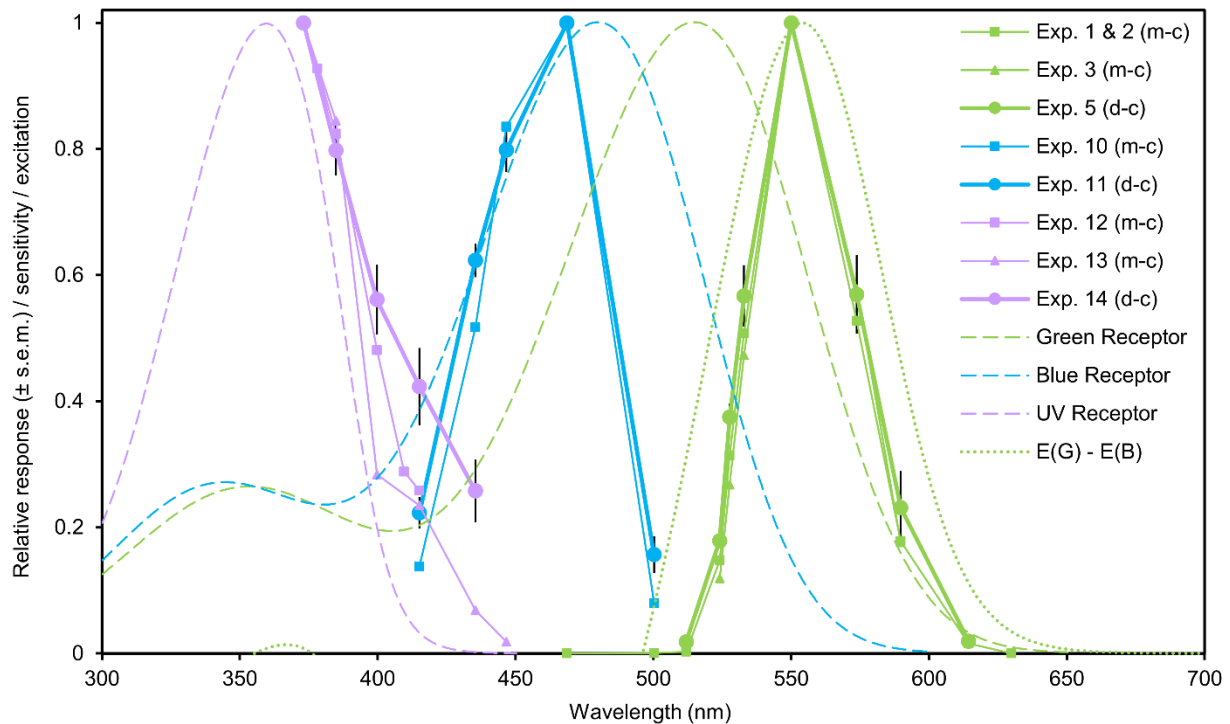
876 **Fig. 2. Schemes of LED trap screen and choice arena.** (A) LED trap screen with acrylic glass screen
877 front side (*a*) and LED panel backside (*b*). The inner side of the box was laminated with mirror film. (B)
878 Choice arena with whitefly release point (*c*) and position of LED traps (*A*).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



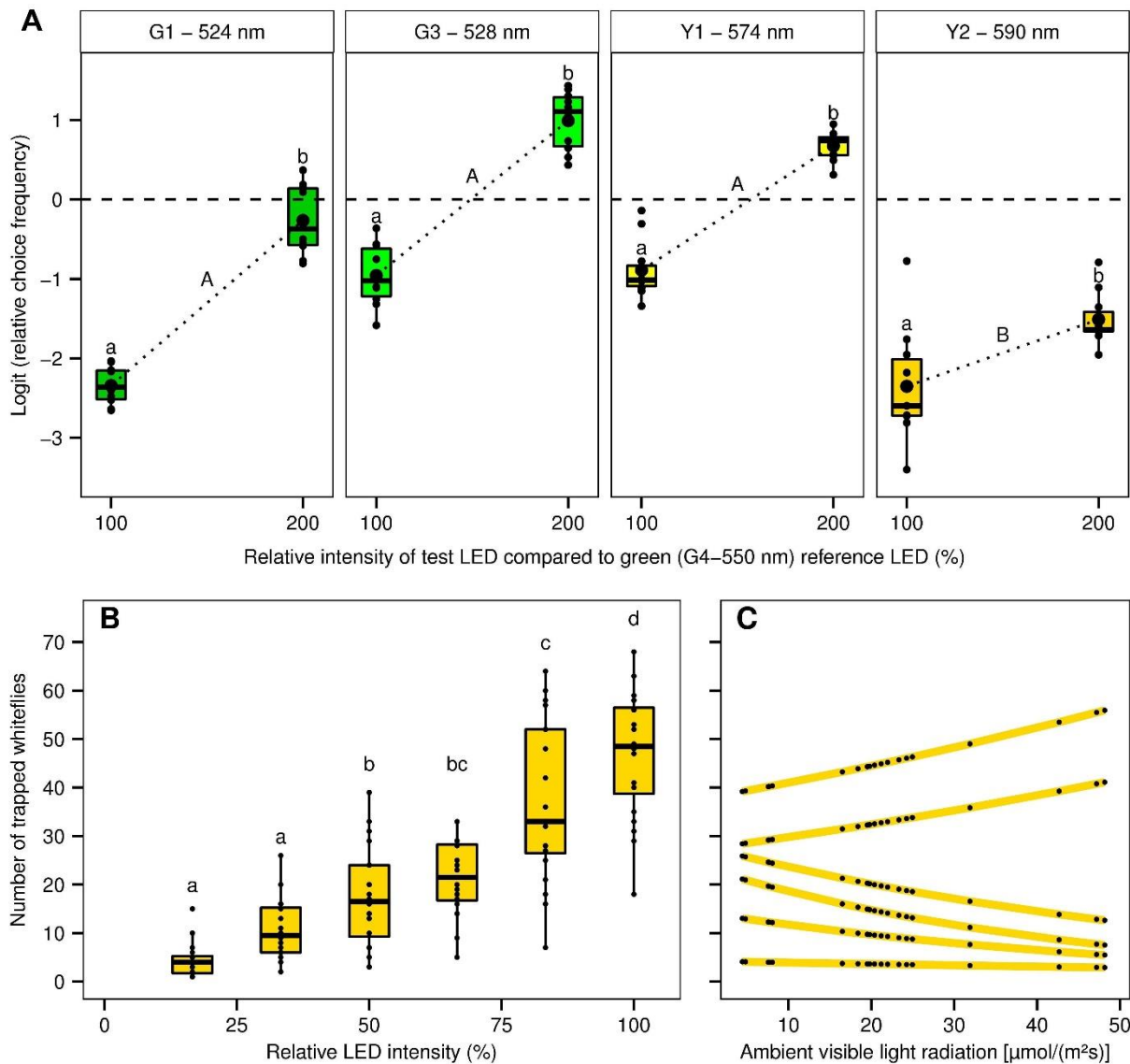
880 **Fig. 3. Wavelength preferences of *Trialeurodes vaporariorum* in LED multiple-choice**
 881 **experiments.** (A) blue - green (exp. 1), (B) green - red (exp. 2), (C) green - yellow (exp. 3), (D) blue &
 882 red (exp. 4), (E) yellow + violet - cyan & pure yellow (exp. 9), (F) yellow + violet - cyan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



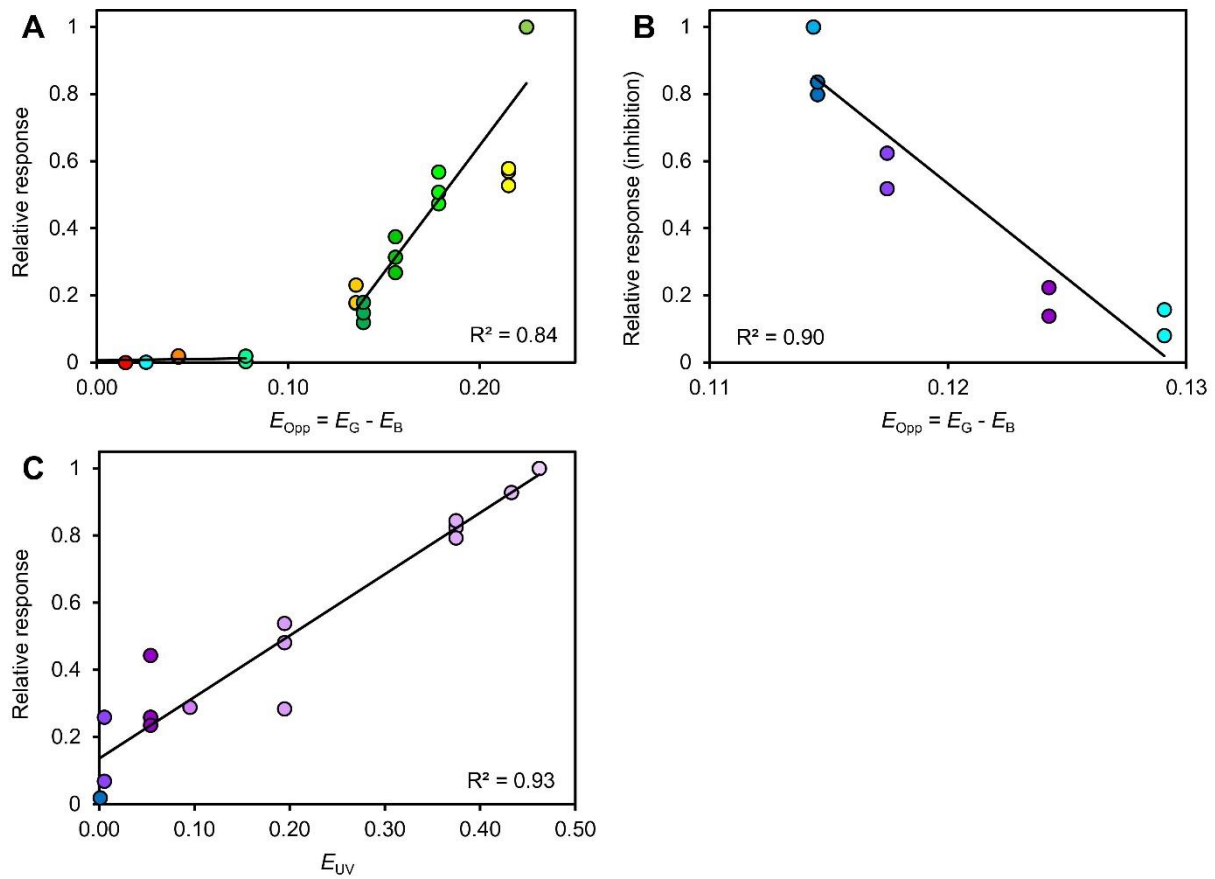
889 **Fig. 4. Interactions of wavelength preferences in *Trialeurodes vaporariorum* with ambient light**
890 **intensity in LED multiple-choice experiments based on the fitted models.** Black dots connected by
891 coloured lines show backtransformed predictions from the linear models ($\ln(x+1) \sim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



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



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



923 **Fig. 7. Selected linear regressions of colour choice models.** (A) Green response model and (B) Blue
924 inhibition model for photoreceptor peaks at 480 nm and 515 nm based on blue-green opponency (See
925 Table 2) (C) UV response model with photoreceptor peak at 360 nm.