

1 **Photo-orientation regulates seasonal habitat selection in the**  
2 **two-spotted spider mite *Tetranychus urticae***

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15  
16 **SUMMARY**

17 Non-diapausing spider mites (*Tetranychus urticae*) live on the undersurface of host  
18 leaves during summer, but diapausing mites overwinter in dark hibernacula. The light  
19 environments of these habitats would differ: visible radiation (VIS) but not ultraviolet  
20 radiation (UV) would reach the undersurface, but neither would enter dark  
21 hibernacula. Thus, mites of either seasonal form could locate their preferred habitat by  
22 photo-orientation responses to UV and VIS. To investigate this possibility, we  
23 analysed the mites' locomotion behaviour on a virtual field with a programmed  
24 chequered pattern of light and dark patches in a micro-locomotion compensator. Both  
25 non-diapausing and diapausing mites moved away from UV-illuminated patches into  
26 dark patches. Non-diapausing mites moved towards VIS-illuminated patches, whereas  
27 diapausing mites did not show a preference. Our results show that non-diapausing  
28 mites avoid UV and are attracted to VIS, suggesting that this can guide them beneath  
29 a leaf. Diapausing mites simply avoid UV. The lack of preference for VIS during  
30 diapause could be due to changes in carotenoid metabolism, which also involve  
31 orange pigmentation of diapausing mites. We consider that a diapause-mediated  
32 switch of the response to VIS, together with regular avoidance of UV, plays a key role

33 in the seasonal change of habitat selection in this species. This seasonal polyphenism  
34 involves alterations not only in reproductive state and pigmentation, but also in photo-  
35 spectral responses.

36

37 **Key-words** diapause, locomotion compensator, seasonal change, ultraviolet  
38 radiation, virtual field, visible radiation

39

40 **Short title** Seasonal photo-orientation in *T. urticae*

41

## 42 INTRODUCTION

43 The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), stays  
44 predominantly beneath host leaves, where it feeds, spins webs and reproduces.  
45 Researchers have proposed that this location protects mites from fluctuations of air  
46 temperature (Gutierrez and Helle, 1985) and rain (Gutierrez and Helle, 1985; Jeppson  
47 et al., 1975) and the deleterious effects of ultraviolet radiation (UV) (Barcelo, 1981;  
48 Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010; Suzuki et al., 2009), which  
49 leaves absorb and reflect (Smith, 1986).

50 Avoidance of UV has been observed in bacteria (Bebout and Garcia-Pichel,  
51 1995; Häder, 1987; Kruschel and Castenholz, 1998), protozoa (Barcelo and Calkins,  
52 1979), nematodes (Edwards et al., 2008), echinoderms (Adams, 2001; Pennington and  
53 Emler, 1986), amphibians (Han et al., 2007; Nagl and Hofer, 1997; van de Mortel and  
54 Buttemer, 1998), fish (Fukunishi et al., 2006; Holtby and Bothwell, 2008; Kelly and  
55 Bothwell, 2002), crustacea (Barcelo and Calkins, 1979; Barcelo and Calkins, 1980;  
56 Storz and Paul, 1998), insects (Bothwell et al., 1994; Mazza et al., 1999; Mazza et al.,  
57 2002) and mites (Barcelo, 1981; Barcelo and Calkins, 1980; Onzo et al., 2010; Sakai  
58 and Osakabe, 2010; Suzuki et al., 2009). In addition, mites are attracted to visible  
59 radiation (VIS) (Dimock and David, 1985; Hussey and Parr, 1963; McEnroe and  
60 Dronka, 1966; McEnroe and Dronka, 1969; Mori, 1962; Naegele et al., 1966; Suski  
61 and Naegele, 1963a; Suski and Naegele, 1963b). Since leaves transmit some VIS  
62 (Smith, 1986), the undersurface provides an attractive environment characterised by a  
63 dominance of VIS over UV. Therefore, the localization of mites beneath leaves may  
64 be regulated by photo-orientation responses to both UV and VIS.

65            However, mites lose their attraction towards light during winter. Hussey and  
66 Parr (1963) showed that the avoidance of light was induced upon diapause in *T.*  
67 *urticae*, although they did not present light spectra. Diapause is defined as a  
68 hormonally mediated metabolic arrest with an increase in resistance to environmental  
69 stresses, a change in behaviour, and suppression of morphogenesis (Tauber and  
70 Tauber, 1981). In *T. urticae*, only adult females enter diapause to overwinter when  
71 nights lengthen and low air temperatures prevail (Veerman, 1985). Diapausing  
72 females overwinter in dark hibernacula in clods of soil, litter on the ground surface,  
73 cracks in trees, and under bark (Veerman, 1985). They remain slowly mobile, so the  
74 choice of these dark hibernacula may be explained by negative photo-orientation  
75 responses to UV, VIS or both.

76            To obtain behavioural evidence, we used a micro-locomotion compensator  
77 (Kojima et al., 2003) newly equipped with an LED lighting apparatus, which allows  
78 light-cue control in real time to simulate mosaic illumination in the environment, as  
79 well as precise acquisition of locomotion data. Here, we examined the photo-  
80 orientation behaviour in non-diapausing and diapausing females of *T. urticae* towards  
81 UV and VIS to identify possible cues responsible for their seasonal habitat selection.

82

## 83 **MATERIALS AND METHODS**

### 84 **Mites**

85 We collected a *T. urticae* population from an apple orchard in Akita (39°N), Japan,  
86 and subsequently maintained this population on kidney bean (*Phaseolus vulgaris*)  
87 leaves under a 16L:8D photoperiod at an air temperature of 25°C and a relative  
88 humidity (RH) of 50%. Adult females of random ages were introduced onto a fresh *P.*  
89 *vulgaris* leaf placed on water-soaked cotton in a Petri dish and allowed to lay eggs for  
90 24 h in the dark at 25°C. The adult females were then removed and the eggs were  
91 maintained for 5 d under the same conditions. Newly hatched larvae were reared until  
92 adulthood in photoperiod chambers (Suzuki and Takeda, 2009) under 16L:8D at 25°C  
93 or 8L:16D at 18°C, producing respectively non-diapausing and diapausing females.  
94 Adult females were considered to be in diapause if their body colour turned from  
95 yellowish-green to uniform orange, owing to the accumulation of carotenoids  
96 (Veerman, 1985).

97

98 **Instrumentation**

99 We used a locomotion compensator (LC) designed for submillimetre-sized animals,  
100 specifically, a micro-locomotion compensator (MLC; Fig. 1) that our group developed  
101 for studies of olfactory behaviour in mould mites (Kojima et al., 2003). The function  
102 of an LC, or servosphere, first developed by Kramer and Heinecke, is ‘to keep a  
103 walking animal in a small experimental field and to precisely record the intended  
104 movements’ of the animal (Kramer, 1975). The Kramer–Heinecke-type LC, with a  
105 rotating sphere, has been extensively used with various ambulatory arthropods from  
106 tiny predatory mites (*Phytoseiulus persimilis*; van Tilborg et al., 2003) to American  
107 cockroaches (*Periplaneta americana*; Bell and Kramer, 1979), mainly to analyse their  
108 olfactory (Kramer, 1975, 1976; Bell and Kramer, 1979; Thiery and Visser, 1986;  
109 Visser and Taanman, 1987; Vet and Papaj, 1992; Taneja and Guerin, 1995; McMahan  
110 and Guerin, 2000; Sakuma, 2002; van Tilborg et al., 2003) and auditory behaviours  
111 (Weber et al., 1981; Verburgt et al., 2008).

112 In addition to the automated observation of a walking animal, an LC enables  
113 the analysis of its orientation mechanisms through the closed-loop control of sensory  
114 cues with reference to the location or kinematics of the animal (Kramer, 1976; Weber  
115 et al., 1981; Sorensen and Bell, 1986; Sakuma, 2002; Kojima et al., 2003). We used  
116 an integrated system of motor and stimulator controls, which makes the feedback cue  
117 control more precise and reliable without extra position encoding for the stimulation  
118 (Sakuma, 2002). This is particularly advantageous for the cue control of a micro-  
119 scaled LC (Kojima et al., 2003). Here, we examined fine-scale localization of mites in  
120 programmed illumination fields by using the MLC driven by the control system.

121 The MLC uses a sliding glass plate, in place of a rotating sphere, to  
122 compensate for the locomotion of a test animal. This planar structure ensures very  
123 tight clearance of a test chamber above the moving floor, which helps to insulate the  
124 interior of the test chamber from the environment. The MLC comprises two major  
125 parts: a mobile stage system set on the floor, and a video system with a test chamber  
126 suspended from a hanging pillar. The mobile stage system consists of a steel stage  
127 (600 mm × 600 mm × 3 mm) fully covered with a glass plate on a black acrylic board,  
128 and a pair of orthogonal motorized slides (SPF86B10-8P, Oriental Motor Co. Ltd.,  
129 Tokyo, Japan), which move the stage to compensate for the locomotion of a test  
130 animal. The video system captures the test animal’s image with a machine vision  
131 camera (CV-M30E, JAI Ltd., Yokohama, Japan) equipped with a microscopic lens

132 (TMS-740, HOGA, Kyoto, Japan). A video tracker (G-280, OKK Inc., Tokyo, Japan)  
133 processes the image and calculates its location relative to the centre of the frame in  
134 real time to transmit the  $(x, y)$  coordinates every 1/120 s to a computer, which controls  
135 the motors by generating pulses with a motor control board (PCPG-46, Cosmotechs  
136 Co. Ltd., Kanagawa, Japan) to carry the animal back to the centre. A black acrylic test  
137 chamber (10 mm i.d.) is suspended from the hanging pillar just above the sliding glass  
138 plate, together with the LED illuminator. A clear quartz or glass cover was placed on  
139 the top of the chamber. The MLC keeps the mite inside the chamber directly beneath  
140 the camera as the mite walks about freely on the glass plate.

141 The computer logs the signed number of generated pulses every 100 ms to  
142 calculate positional shifts in later path analysis. The software also processes the shifts  
143 in real time to obtain the mite's location within a programmed chequered pattern of  
144 light and dark patches (30 mm  $\times$  30 mm) that forms a virtual field as described below.  
145 The device-control program was modified from the original version created and  
146 compiled in Turbo C++ v. 4.0 (Embarcadero Technologies Inc., Tokyo, Japan) to run  
147 under PC DOS 2000 (IBM, Tokyo, Japan) (Sakuma, 2002).

148 The illuminator consists of four each of the following LEDs, denoted by the  
149 colour and peak wavelength ( $\lambda_{\max}$ ) of their light: UV-B ( $\lambda_{\max} = 307$  nm; T9B31, Seoul  
150 Optodevice Co. Ltd., Kyunggi-do, Korea), UV-A ( $\lambda_{\max} = 370$  nm; NSHU590B,  
151 Nichia Co., Tokushima, Japan), white (NSPL500S, Nichia), blue ( $\lambda_{\max} = 466$  nm;  
152 NSPB500S, Nichia), green ( $\lambda_{\max} = 536$  nm; NSPG500S, Nichia), and red ( $\lambda_{\max} = 653$   
153 nm; GL5UR3K1, Sharp Co., Osaka, Japan). These LEDs create light patches under  
154 the control of a relay module board (RRY-32, Contec Co. Ltd., Osaka, Japan) that  
155 switches them on or off when a mite enters or leaves a predefined light patch within  
156 the virtual field. An analogue potentiometer (Model 7274, BI Technologies Co.,  
157 Fullerton, CA, USA) controls the irradiance provided by the LEDs. In addition, four  
158 infrared (IR) LEDs ( $\lambda_{\max} = 953$  nm; OSIR5113A, OptoSupply Ltd., Hong Kong,  
159 China) were installed to highlight the mite's image during video tracking, while an IR  
160 sharp cut-off filter (IR-80, Fujifilm Co., Tokyo, Japan) passes only IR to the camera.  
161 The irradiance provided by the UV LEDs was measured with a UV meter (UV-340,  
162 Custom Co., Tokyo, Japan) calibrated by a spectroradiometer (USR-45D, Ushio Inc.,  
163 Tokyo, Japan), and that provided by the VIS LEDs was measured with a  
164 spectroradiometer (MS-720, EKO Instruments Co. Ltd., Tokyo, Japan).

165

166 **Treatments**

167 Only one colour of light was supplied in each test, and only the illuminator provided  
168 light during each test. The irradiance inside the chamber under the quartz cover was  
169 set at 0.02, 0.2, or 2.0 W m<sup>-2</sup> for UV-B and UV-A, and that inside the chamber under  
170 the glass cover was set at 0.2, 2.0, or 20 W m<sup>-2</sup> for white, blue, green, and red light.  
171 As a control, a uniformly continuous dark field was maintained. All treatments were  
172 performed for 10 min in a dark room at 25°C and 50% RH.

173

174 **Data analysis**

175 The acquired positional data were analysed by using data analysis software written in  
176 Visual C++ with the MFC class library (Microsoft Co., Redmond, WA, USA)  
177 (Sakuma, 2002). To evaluate the localization of mites in light patches, we calculated  
178 an “excess proportion index” (*EPI*) (Sakuma and Fukami, 1985) as:

179 
$$EPI = (F_L - F_D) / (F_L + F_D),$$

180 where  $F_L$  and  $F_D$  are the frequencies of the stays in light and dark patches,  
181 respectively. The total number of data points ( $F_L + F_D$ ) is 6000 because the computer  
182 logs the positional data every 100 ms for 10 min. *EPI* ranges from -1 (complete  
183 localization in dark patches) to +1 (complete localization in light patches). We tested  
184 for significant differences in the mean *EPI*s between each treatment and the control by  
185 using the Mann–Whitney *U*-test.

186 The sensitivities to UV and VIS relative to those to a reference light were  
187 computed by parallel line assay (Finney, 1978) with a probit analysis program  
188 (PriProbit) designed for preference testing (Sakuma, 1998). The program iteratively  
189 maximizes the log-likelihood function of probit transformation of *EPI* on a log dose  
190 metameter, in which members are substituted with data points of light and dark  
191 patches in a graded series of irradiance, and estimates intercepts and a common slope  
192 of the log(dose)-probit equations. The relative sensitivities were calculated with the  
193 equations and plotted against the  $\lambda_{\max}$  values of the LEDs to construct an action  
194 spectrum for photo-orientation responses of mites.

195 Analyses were performed in Microsoft Excel 2010, SPSS 11.5J (SPSS Inc.,  
196 Chicago, IL, USA), SigmaPlot 12.0 (Systat Software Inc., Chicago, IL, USA), or  
197 PriProbit 1.64 (programmed by Masayuki Sakuma).

198

## 199 RESULTS

### 200 Walk paths

201 Both non-diapausing and diapausing females walked with gentle turns in the control  
202 (dark) treatment (Fig. 2A, B). However, they remained within the dark patches in the  
203 UV-B treatment (Fig. 2C, D) and the UV-A treatment (data not shown): whenever  
204 they entered a light patch, they abruptly turned back, even when the irradiance was as  
205 low as  $0.02 \text{ W m}^{-2}$ .

206 In the white light treatment, non-diapausing females exhibited the opposite  
207 responses to those in the UV-B and UV-A treatments; when they entered a dark patch,  
208 they abruptly turned back (Fig. 2E), and subsequently remained within the light patch.  
209 Similar behaviour was observed in the blue, green, and red light treatments (data not  
210 shown). This behaviour was clearly observed in the green light treatment at  $\geq 0.2 \text{ W}$   
211  $\text{m}^{-2}$  and in the other VIS treatments at  $\geq 2.0 \text{ W m}^{-2}$ . However, diapausing females  
212 failed to show any attraction towards VIS; they walked with gentle turns similar to  
213 those in the control treatment even when the white light irradiance was as high as  $20$   
214  $\text{W m}^{-2}$  (Fig. 2F). No attraction towards VIS was observed in the blue, green and red  
215 light treatments even at  $20 \text{ W m}^{-2}$  (data not shown).

216

### 217 Photo-orientation responses

218 In the UV-B and UV-A treatments, *EPI* values for the non-diapausing and diapausing  
219 females were negative and were significantly lower than those ( $\approx 0$ ) in the control  
220 treatment, except for diapausing females at a UV-A of  $0.02 \text{ W m}^{-2}$  (Fig. 3). In the VIS  
221 treatments, on the other hand, *EPIs* of non-diapausing females were positive and were  
222 significantly higher than those in the control treatment at  $\geq 0.2 \text{ W m}^{-2}$  (green) and  $\geq 2$   
223  $\text{W m}^{-2}$  (white, blue and red). In contrast, *EPIs* of diapausing females were not  
224 significantly different from the control even at  $20 \text{ W m}^{-2}$ .

225

### 226 Action spectra for photo-orientation responses

227 In non-diapausing females, the principal peak wavelength of the action spectrum was  
228 in the UV region, with a minor peak in the green region (Fig. 4). Within the VIS  
229 region, the sensitivity to light decreased in the order of white = green > blue > red  
230 light. In diapausing females, the action spectrum was fitted only in the UV region,  
231 because *EPI*  $\approx 0$  in the VIS treatments (Fig. 3). The sensitivities to UV-B and UV-A



232 were lower than those in non-diapausing females but were still higher than those to  
233 VIS in non-diapausing females.

234

## 235 **DISCUSSION**

236 Both non-diapausing and diapausing females clearly avoided UV (Figs 2C, D, 3). In  
237 contrast, only non-diapausing females were clearly attracted towards VIS (Figs 2E, 3).  
238 Our study is the first to investigate the negative and positive photo-orientation  
239 responses of terrestrial arthropods to UV and VIS in the same experimental system.  
240 Storz and Paul (1998) showed similar opposite responses to UV and VIS in the water  
241 flea *Daphnia magna* (an aquatic arthropod), and suggested that the distribution of UV  
242 and VIS in different water layers was one of the factors that controlled the vertical  
243 position of *D. magna* in water.

244 Plant leaves filter out UV from sunlight before it reaches the chloroplasts,  
245 while at the same time passing some photosynthetically active radiation (PAR; 400–  
246 700 nm), which almost overlaps the range of wavelengths in VIS (Caldwell et al.,  
247 1983). Less than 5% of UV is reflected by the upper surface of a typical leaf, whereas  
248 75% to 95% is absorbed by the epidermis, and the rest by the mesophyll, leading to  
249 little or no transmittance of UV through the leaf (Caldwell et al., 1983). Between 85%  
250 and 90% of PAR is absorbed by the leaf; the rest (particularly green light) is either  
251 reflected at the leaf's upper surface or is transmitted through the leaf (Smith, 1986).  
252 Since non-diapausing *T. urticae* females were sensitive to UV in particular and to  
253 green light (Fig. 4), they may be able to sense differences in the distributions of these  
254 wavelengths between the two sides of a leaf and use them as a cue to locate the  
255 undersurface of the leaf, like the vertical positioning of *D. magna* (Storz and Paul,  
256 1998). If non-diapausing females only avoid UV, it would be difficult for them to find  
257 the undersurface of leaves, and they might move into dark sites with increased risk of  
258 starvation. If mites were only attracted towards VIS, they would have a high risk of  
259 UV damage (Barcelo, 1981; Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010;  
260 Suzuki et al., 2009). Therefore, non-diapausing females need both UV avoidance and  
261 VIS attraction to successfully locate the undersurface of leaves.

262 Surprisingly, however, diapausing females showed no preference for VIS  
263 (Figs 2F, 3). To our knowledge, ours is the first report of a lack of response to specific  
264 wavelengths during diapause. Although Hussey and Parr (1963) showed light



265 avoidance in diapausing females of *T. urticae*, the light source (which they did not  
266 describe) would have included some UV if they used sunlight or fluorescent tubes;  
267 thus, avoidance of UV would have been induced, as we observed here (Fig. 2D). Mori  
268 (1962) showed that *T. urticae* females collected in late October (autumn) were less  
269 sensitive to light than those collected in summer in Sapporo (43°N), Japan. Although  
270 it is unclear whether the less sensitive females were true diapause forms, the result is  
271 comparable to the lack of response to VIS by our diapausing females (Fig. 2F),  
272 because Mori passed light from an incandescent bulb through a water bath and frosted  
273 glass, which would result in VIS alone. However, Mori did not take the result further  
274 to consider the winter habitat selection of *T. urticae*. Diapausing females overwinter  
275 in dark hibernacula (Veerman, 1985), which probably offer a refuge from UV as well;  
276 thus, avoidance of UV is an adaptive response also for diapausing females. Moreover,  
277 if diapausing females were attracted towards VIS, as in the case of non-diapausing  
278 females, they would risk exposure to the abundant UV in leafless trees in winter.  
279 Furthermore, a lack of preference for VIS may save energy for maintaining the  
280 photoreceptors during diapause. Therefore, the absence of attraction towards VIS is  
281 also a reasonable adaptation for diapausing females.

282 The mites showed much higher sensitivity to UV than to VIS (Fig. 4), as *D.*  
283 *magna* also showed (Storz and Paul, 1998). This difference and the lack of preference  
284 for VIS during diapause suggest that *T. urticae* has at least two types of  
285 photoreceptors. This mite has adjacent pairs of anterior and posterior eyes, on each  
286 side of the propodosoma (McEnroe, 1969; Mills, 1974), and both sets of eyes act as  
287 photoreceptors (Suski and Naegele, 1963b). McEnroe and Dronka (1969) suggested  
288 that the anterior eyes have photoreceptors for UV and VIS but the posterior eyes have  
289 photoreceptors only for UV. The hypothesis that photoreceptors for UV and VIS are  
290 independent was also proposed on the basis of electroretinogram analyses of the eyes  
291 of spiders (DeVoe, 1967) and scorpions (Machan, 1968).

292 In animals, the VIS-sensitive molecule rhodopsin and its relatives consist of an  
293 opsin and the chromophore retinal (Terakita, 2005). Beta-carotene, which is the  
294 precursor of retinal, is essential for the photoreception involved in the photoperiodic  
295 induction of diapause in the predacious mite *Amblyseius potentillae* (Van Zon et al.,  
296 1981), suggesting that opsin-based photoreceptors function also in mites. Diapausing  
297 *T. urticae* females feed only a little (Veerman, 1985), so the intake of plant  
298 carotenoids (e.g.,  $\beta$ -carotene) likely decreases. Moreover, hydroxy-keto-carotenoids,

299 which are not found in leaves but are converted from  $\beta$ -carotene, are present in greater  
300 quantities in diapausing females than in non-diapausing females, possibly because of  
301 the dissolution of these carotenoids in lipids or the lipid moiety of lipoproteins, which  
302 are present in increased amount in diapausing females, and consequently the mite's  
303 body is orange during diapause (Veerman, 1985) (see inset in Fig. 2). These facts  
304 suggest that  $\beta$ -carotene is insufficiently converted into retinal for use in opsin-based  
305 photoreceptors in diapausing females, which therefore become blind to VIS. On the  
306 other hand, sensitivity to UV was preserved even during diapause (Figs 2D, 3, 4); thus,  
307 UV photoreceptors might be a non-opsin-based type such as cryptochrome, which is  
308 sensitive to short wavelengths, possesses the flavin chromophore, and acts as a  
309 photoreceptor in the circadian clock of plants and animals (Cashmore et al., 1999).

310 Further investigations will be needed to verify the hypotheses that non-  
311 diapausing females discriminate between the two sides of a leaf by sensing spatial  
312 differences in the distribution of UV and VIS; that diapausing females select dark  
313 hibernacula by sensing spatial differences in the distribution of UV; that *T. urticae* has  
314 independent UV and VIS photoreceptors; and that the VIS photoreceptor ceases to  
315 function during diapause, probably owing to insufficient conversion of  $\beta$ -carotene to  
316 retinal. We anticipate that our findings will provide a basis for examining the role of  
317 light in the mites' habitat selection and in seasonal changes in the photoreception  
318 systems.

319 Furthermore, *T. urticae* is a hard-to-control agricultural pest because of the  
320 rapid development of pesticide resistance. Our findings may contribute to the  
321 development of a lighting method that disturbs its behaviour using artificial light  
322 sources or reflective materials. To incorporate the lighting method into integrated pest  
323 management, further investigations of the photo-orientation behaviour of natural  
324 enemies will be needed. Interestingly, the predatory mite *Typhlodromalus aripo* hides  
325 in the apex of cassava during the day and emerges at night to forage for the  
326 herbivorous mite *Mononychellus tanajoa* on young leaves (Onzo et al., 2003, 2009).  
327 Hiding in the apex during the day protects *T. aripo* against the deleterious effects of  
328 UV (Onzo et al., 2010). Therefore, providing UV cut-off materials to shelter UV-  
329 susceptible natural enemies may reinforce biological control measures.

330 This paper focuses exclusively on the seasonal change of photo-orientation  
331 responses of mites. Our results suggest that the mites compare the light intensities  
332 before and after they cross the boundary between light and dark patches, because they

333 cannot detect the direction of light in the horizontal plane with only overhead  
334 illumination. The abrupt self-steered turn triggered by the onset or cessation of light  
335 cues is likely to be the same response seen in klinokinesis and klinotaxis (Kennedy,  
336 1978). Further path analysis (to be published elsewhere) will help to reveal the photo-  
337 orientation mechanisms causing localization of the mites under illumination.

338

## 339 **LIST OF SYMBOLS AND ABBREVIATIONS**

340	<i>EPI</i>	excess proportion index
341	$F_L, F_D$	frequencies of the stays in light and dark patches, respectively
342	$\lambda_{\max}$	peak wavelength
343	LC	locomotion compensator
344	LED	light-emitting diode
345	MLC	micro-locomotion compensator
346	PAR	photosynthetically active radiation
347	UV	ultraviolet radiation
348	VIS	visible radiation

349

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- 532

533

## 534 **Figure Legends**

535 Figure 1. Schematic diagrams of (A) the micro-locomotion compensator (MLC) and  
536 (B) the bottom and (C) side views of the illuminator. Broken lines represent signal  
537 lines. The MLC keeps a walking mite centred beneath the video camera by the use of  
538 micro-stepping motors. The mite is retained within a black acrylic test chamber,  
539 which is suspended just above the sliding stage. A PC switches UV or VIS LEDs on  
540 and off according to the mite's movement on a virtual light-and-dark field.  
541 Continuous IR LEDs illuminate the mite for video tracking. The details are explained  
542 in the text.

543

544 Figure 2. Walk paths of *Tetranychus urticae* in a virtual field. (A, C, E) Non-  
545 diapausing and (B, D, F) diapausing females walked for 10 min in a chequered pattern  
546 of light and dark patches (30 mm × 30 mm) programmed on the virtual field. White  
547 and grey squares indicate light and dark patches, respectively (except in the control  
548 treatments). (A, B) Mites walked under continuous dark. (C, D) Mites avoided UV-B  
549 ( $\lambda_{\max} = 307 \text{ nm}$ ,  $2 \text{ W m}^{-2}$ ). (E, F) Only non-diapausing mites sought out white light  
550 ( $20 \text{ W m}^{-2}$ ). Colours of path portions indicate the actual illumination status. Red dots  
551 plot the mite's location every 10 s.

552

553 Figure 3. Photo-orientation responses of *Tetranychus urticae*. The effect of  
554 illumination on localization in the light or dark patches is indicated by the “excess  
555 proportion index” (*EPI*; mean ± s.e.m.): *EPI* = -1 means complete localization in dark  
556 patches, *EPI* = 0 means no bias, and *EPI* = 1 means complete localization in light  
557 patches. *EPI* was obtained from the frequencies of stays in dark and light patches for  
558 each individual, which were incremented every 100 ms for 10 min, *i.e.* 6000 points in  
559 total. Numerals in the graphs indicate the numbers of non-diapausing (open circles)  
560 and diapausing (closed triangles) individuals tested in corresponding irradiance.  
561 Values labelled with asterisks differ significantly from the control: \*\**P* < 0.01 and  
562 \*\*\**P* < 0.001 (Mann–Whitney *U*-test).

563

564 Figure 4. Action spectra for photo-orientation responses of *Tetranychus urticae*. The  
565 spectra are shown as relative sensitivities plotted against  $\lambda_{\max}$  of the LEDs. The

566 sensitivity to red light is set as zero. Sensitivities (symbols with 95% fiducial  
567 intervals) were obtained by probit analysis of localization data used in Fig. 3.  
568 Although non-diapausing females showed very high sensitivity to UV-B (Fig. 3), the  
569 value is shown in parentheses because of difficulties in calculating the fiducial  
570 interval.

Figure 1

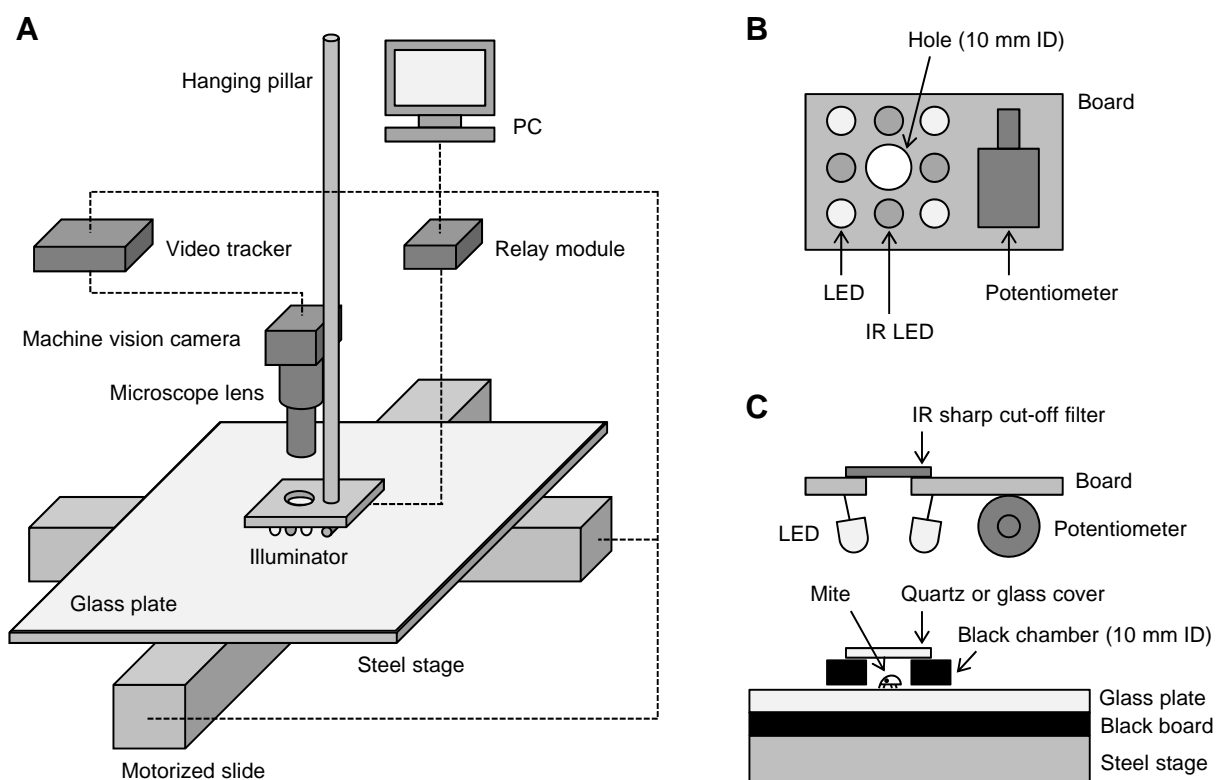


Figure 2

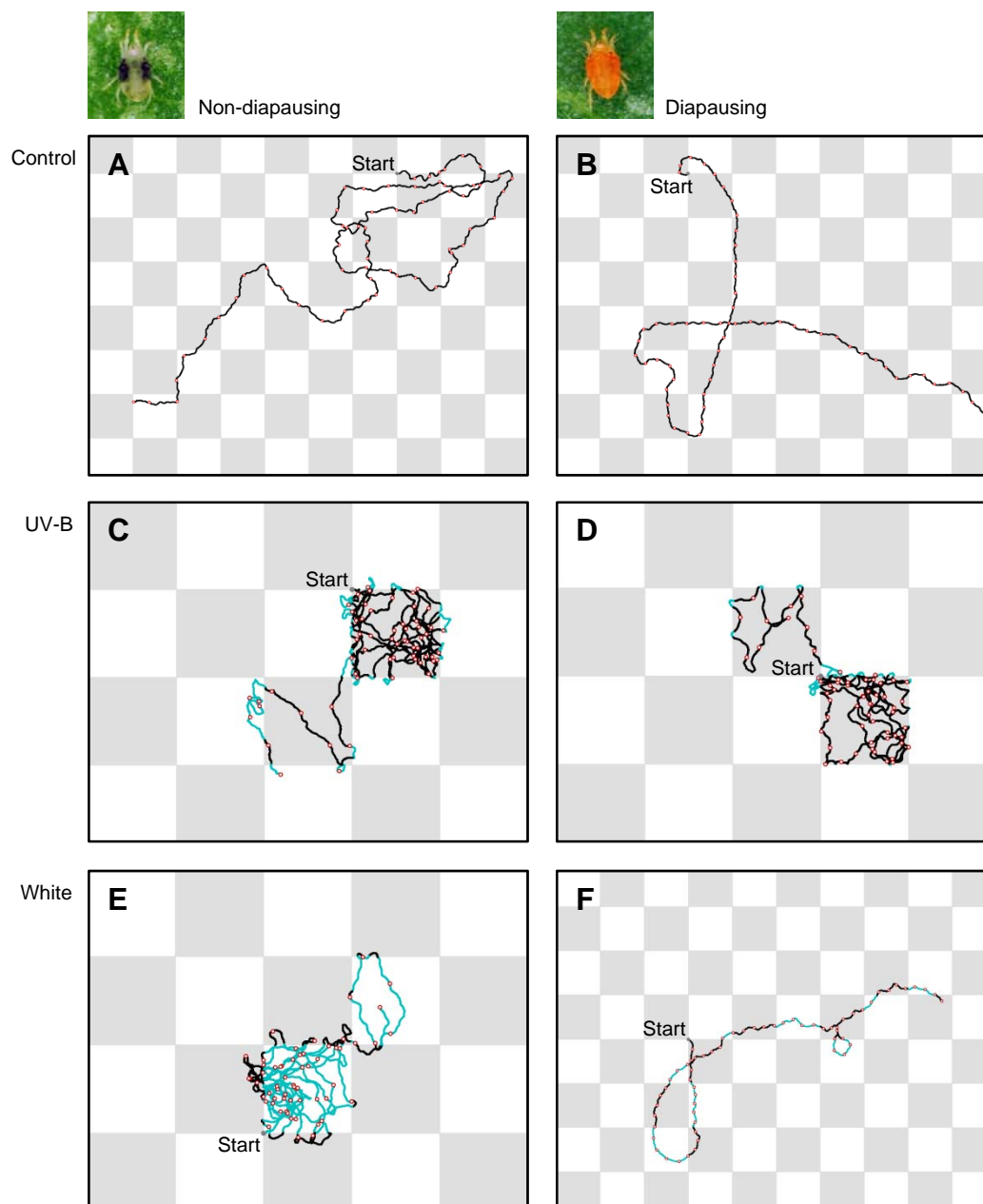


Figure 3

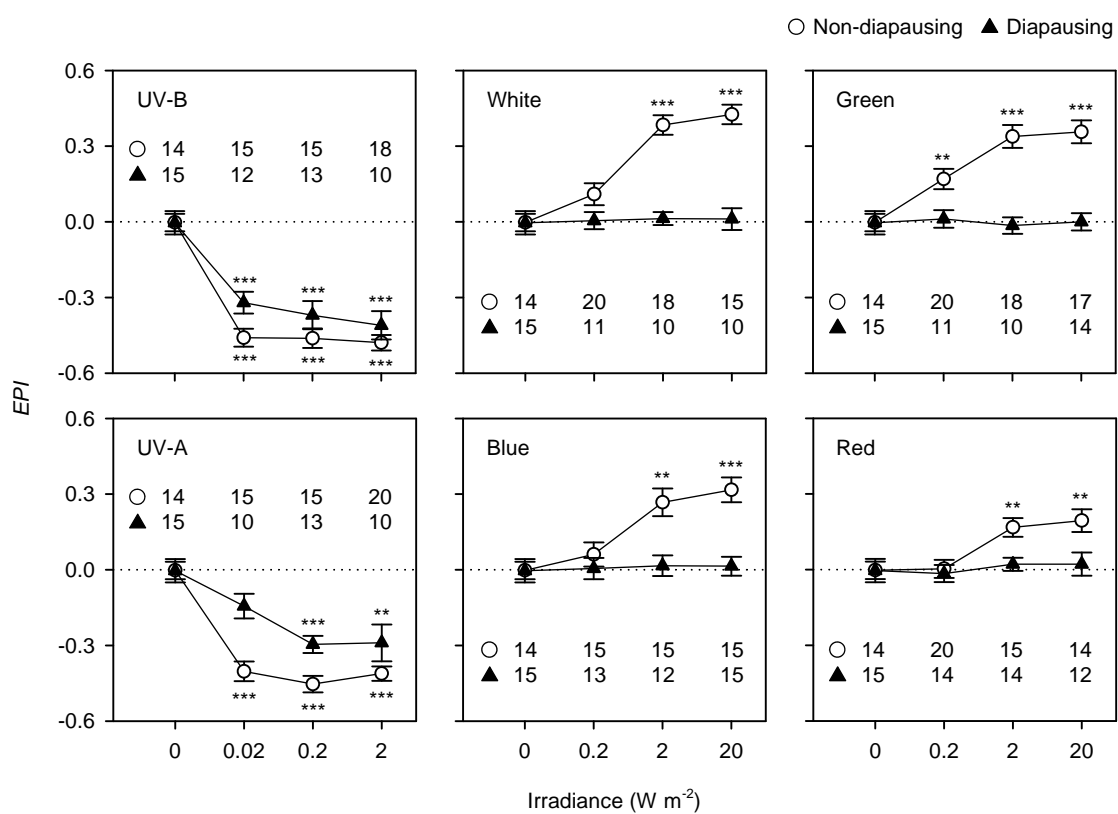


Figure 4

