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Article

Scotophase interruption with LEDs and OLEDs to inhibit photoperiodic induction of diapause in *Tetranychus urticae* and *T. kanzawai* (Acari: Tetranychidae)

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Abstract

Tetranychus urticae Koch and *T. kanzawai* Kishida enter facultative diapause in response to short-day photoperiods. To determine the effect of various colors of light-emitting diodes (LEDs) and organic light-emitting diodes (OLEDs) on diapause induction, both species were reared under different photoperiods at 18°C and 2.0 W m⁻² light intensity, in which photon flux density (PFD) was 7.9–11.0 μmol m⁻² s⁻¹ depending on light quality. Under blue, green, and white LEDs, critical photoperiods were ca. 13.5:10.5 h L: D for *T. urticae* and ca. 12.5: 11.5 h L: D for *T. kanzawai*, but no diapause was induced in either species under red LEDs. Under blue, green, orange, and white OLEDs, the critical light phases were ca. 13.3–13.5 h for *T. urticae* and ca. 12.5 h for *T. kanzawai*. The inhibitory effects of the duration, quality, and intensity of scotophase-interrupting lights on diapause induction in both species were tested under an 8:16 h L: D photoperiod. In *T. urticae* females, diapause induction was prevented by interrupting the scotophase with 1 h of light from all colors of LEDs or OLEDs except red LEDs. However, in *T. kanzawai* females, diapause was fully induced with 1-h scotophase interruption of all light colors and types, even when the PFD was as high as 20 μmol m⁻² s⁻¹. Interrupting the scotophase with 3 h of 20 μmol m⁻² s⁻¹ light from blue, green, and white LEDs, and from blue, green, orange, and white OLEDs completely inhibited diapause induction in both species. When interrupting the dark phase with 3 h of light at the lowest intensity tested (0.2 μmol m⁻² s⁻¹), blue and green inhibited diapause induction in *T. urticae*, whereas only blue light inhibited diapause induction in *T. kanzawai*. Therefore, blue LED and OLED performed best to inhibit diapause of *T. urticae* and *T. kanzawai* at 3-h scotophase interruption even at low light intensity.

Key words: Tetranychidae, light-emitting diodes, organic light-emitting diodes, scotophase interruption, diapause inhibition

Introduction

Spider mites are major agricultural pests worldwide and are difficult to control with pesticides alone because of their rapid development of resistance to both acaricides and insecticides (Van Leeuwen *et al.* 2010; Sparks & Nauen 2015). Recently, light of different colors and different intensities has been proposed to control insect and mite growth (Shimoda & Honda 2013). The two-spotted spider mite, *Tetranychus urticae* Koch, and the Kanzawa spider mite, *T. kanzawai* Kishida, are the most common and most serious pest mite species in Japan (Takafuji *et al.* 2000), and they can enter reproductive diapause in response to short photoperiods and/or low temperatures (Veerman 1985; Takafuji *et al.* 2003). Diapause induction in *T. urticae* and *T. kanzawai* is sensitive to the photoperiod

(Parr & Hussey 1966; Veerman 1977, 2001; Gotoh & Shinkaji 1981; Mochizuki & Takafuji 1996; Kroon *et al.* 1997; Morishita & Takafuji 1999; Takafuji & Gotoh 1999; Takafuji *et al.* 2003; Suzuki *et al.* 2007; Suzuki & Takeda 2009; Shah *et al.* 2011a), but only one study has examined the effects of different light colors on diapause induction (Suzuki *et al.* 2008).

Long-day arthropods (those that enter diapause when the day length falls below a threshold) are highly sensitive to interruption of the scotophase (the dark phase in the light/dark cycle), so that diapause induced by long nights could be inhibited by light pulses (Beck 1980; Saunders 2002). Adult female *T. urticae* and *T. kanzawai* enter diapause when exposed to long nights during immature stages, and diapause has been successfully inhibited by either extending the light periods (Suzuki *et al.* 2007) or applying scotophase-interrupting light (Vaz Nunes & Veerman 1984; Suzuki *et al.* 2011; Shah *et al.* 2011b). Scotophase-interrupting experiments on mites have long been conducted to investigate the photoperiodic response of diapause (Lees 1953; Hussey 1972; Shah *et al.* 2011b), but diapause inhibition under different light qualities (colors) and intensities has not been investigated. Light-emitting diodes (LEDs) and organic light-emitting diodes (OLEDs) are new light sources that are expected to replace conventional “vacuum system” light sources, e.g., incandescent lamps, fluorescent tubes, and high-intensity discharge lamps (Jou *et al.* 2015). Compared with LEDs, OLEDs can be made to be extremely thin, small, and remarkably flexible—they are composed of organic compounds that light up when fed electricity (Karzazi 2014). In particular, LED technology has been put to practical use in plant production systems because of its capacity to produce various colors of monochromatic lights, and to closely and uniformly irradiate plants. OLED technology may be used in plant cultivation systems within a few years (Jou *et al.* 2015). Different spectral combinations can also affect plant growth and development (Barreiro *et al.* 1992). Plants also showed a high degree of physiological and morphological plasticity in response to changes in light quality (Barreiro *et al.* 1992). In arthropods, development, reproduction, diapause, and behavior, are often regulated by photoperiod, temperature, light quality, and light intensity (Tauber *et al.* 1986; Gotoh & Kameyama 2014).

Therefore, our current study aimed to determine the effects of light quality on diapause induction in two spider mite species, *T. urticae* and *T. kanzawai*, using LEDs and OLEDs. We also focused on scotophase interruption to elucidate its inhibitory effect on diapause induction of these two species using different light qualities and photon flux densities (PFDs) of both LEDs and OLEDs.

Materials and methods

Rearing of spider mites

Tetranychus urticae was collected from strawberry, *Fragaria × ananassa* Duch., in Tokoro (44°6'54"N–144°2'12"E), Hokkaido, Japan on 30 May 2014, and *T. kanzawai* was collected from red clover, *Trifolium pretense* L., in Soubetsu (42°33'N–140°50'33"E), Hokkaido, Japan on 28 July 2008. Laboratory stocks were maintained on leaf discs (16 cm²) of common bean, *Phaseolus vulgaris* L., placed on water-saturated polyurethane mats in plastic dishes (9 cm diameter, 2 cm depth) and kept at 25 ± 1°C and 60–70% RH under a 16:8 h L:D photoperiod. Both spider mite species are generalist herbivores and they achieve excellent performance on common bean leaves. The leaves were replaced whenever they appeared to dry out or be overexploited by feeding mites.

Experimental devices

LED ‘photoperiodic bottles’ (300 ml; Suzuki *et al.* 2007, 2011) and OLED ‘photoperiodic boxes’ (150 × 125 × 50 mm; Fig. 1) were used to determine photoperiodic curves for diapause induction and

conducting scotophase interruption experiments under different light qualities and intensities. Each LED in a ‘photoperiodic bottle’ was connected with a pulse-width modulator, which regulates light intensity. Four colors of LED were used: blue [peak wavelength (λ_p) = 469 nm; E1L51-3B; Toyoda Gosei Co. Ltd., Aichi, Japan], green (λ_p = 529 nm; TLGE183P; Toshiba Co., Tokyo, Japan), red (λ_p = 659 nm; GL5UR3K1; Sharp Co., Osaka, Japan), and white LEDs (λ_p = 459 nm; NSPW500BS; Nichia Co., Tokushima, Japan) (Fig. 2A). Air exchange in LED bottles was provided by four 1 cm holes per bottle, and the bottles were covered with a gas-permeable membrane filter (Milliseal; pore diameter = 0.5 mm, Nihon Millipore K.K., Tokyo, Japan) (Suzuki *et al.* 2007, 2011).

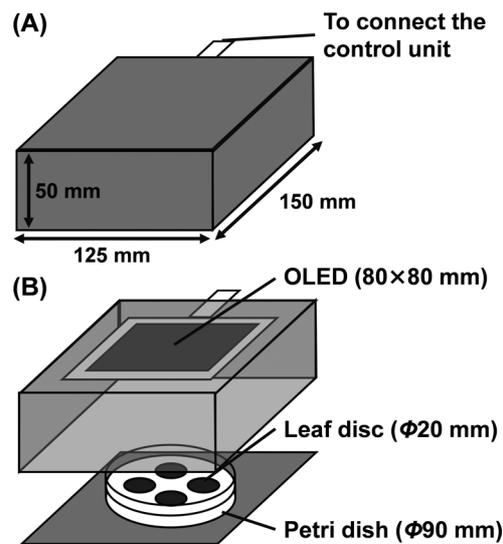


FIGURE 1. OLED photoperiodic box (150 mm × 125 mm × 50 mm) used in experiments; schematics of (A) the box and (B) components. A Petri dish (90-mm diameter) was placed in each box. Air temperature was set at 18°C both inside and outside of the box.

In each ‘photoperiodic box,’ a single color of OLED light was wired to an externally mounted data logger/controller (GK100; ESD Co. Ltd., Tokyo, Japan), which regulates photoperiod (Fig. 1). Different colors of OLED were used: blue (λ_p = 472 nm; KN-DW-B-BL, Kaneka, Osaka, Japan), green (λ_p = 533 nm; KN-DW-B-GR, Kaneka), orange (λ_p = 613 nm; KN-DW-B-RE, Kaneka), and white OLEDs (λ_p = 610 nm; KN-DW-B-30, Kaneka) in photoperiodic boxes (Fig. 2B). During the experiments, the LED bottles and OLED boxes with *T. urticae* or *T. kanzawai* were placed in an incubator.

Effect of light quality on T. urticae and T. kanzawai diapause induction

Ten *T. urticae* and ten *T. kanzawai* adult females arbitrarily collected from the rearing cultures were allowed to lay eggs separately on fresh *P. vulgaris* leaf discs (2 cm diameter) for 24 h at 25±1°C and 60–70% RH under a 16:8 h L:D photoperiod. The females were removed after 24 h, and the eggs laid were counted and used for photoperiodic induction analysis with three replications each in LED bottles and four replications each in OLED boxes. The eggs were transferred to the LED bottles or OLED boxes to determine how different light colors affected diapause induction of *T. urticae* and *T. kanzawai*. Four light colors were examined to evaluate diapause induction at 18°C and under different photoperiods: 10:14, 12:12, 12.5:11.5, 13:11, 13.5:10.5, and 14:10 h L:D for *T. urticae*; and L:D 10:14, 11:13, 12:12, 12.5:11.5, 13:11, and 14:10 h L:D for *T. kanzawai*. Light intensity was set

at 2 W m^{-2} ($7.9\text{--}11.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for LEDs and $7.9\text{--}10.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for OLEDs, depending on light quality). In each photoperiod, the number of females tested ranged from 38 to 165. When female teleiochrysales emerged under a particular photoperiod, females were introduced onto new bean leaf discs (1 cm diameter) and kept separately under the same environmental conditions, together with two adult males obtained from the stock cultures, for mating. The newly emerged females were observed at 24-h intervals for 10 d to assess their body color and whether they could lay eggs. If body color was pale or bright orange without oviposition, they were then determined to be ‘diapausing females’ (Veerman 1985; Takafuji & Gotoh 1999). Whether females without distinct pale or bright orange body color were in diapause was determined based on if they oviposited (Takafuji *et al.* 2003). The photoperiodic response curves for *T. urticae* and *T. kanzawai* were generated based on these observations, and the critical photoperiods were determined from the curves.

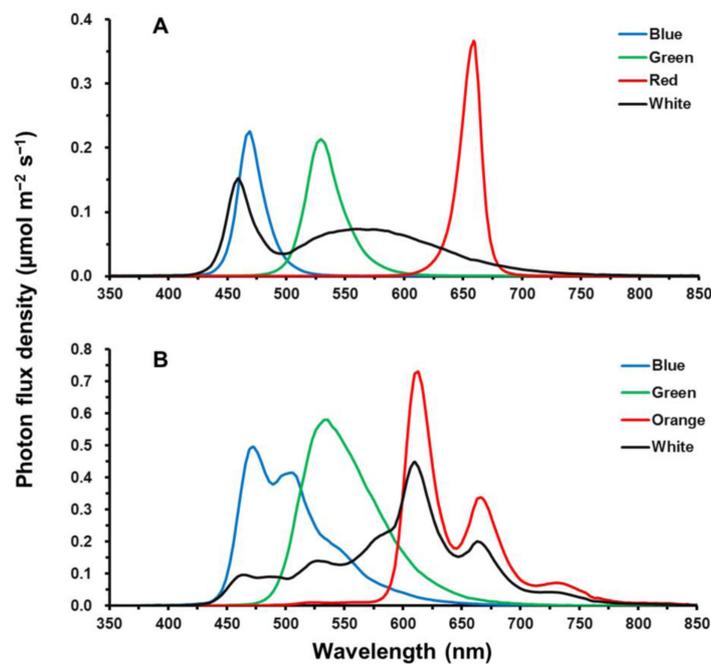


FIGURE 2. Spectral distribution of different colors of (A) LEDs and (B) OLEDs when the irradiance was set at 100 W m^{-2} .

Effect of light intensity and duration on diapause inhibition

For most insects, dark period duration in day-night cycles is a crucial factor for diapause determination. Therefore, we designed an experiment to explore whether this phenomenon exists in *T. urticae* and *T. kanzawai* under both LEDs and OLEDs. Durations of scotophase-interrupting light were set at 1- or 3-h under different combinations of PFD (0.2 , 2 , and $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and light colors (blue, green, and white colors for LEDs; blue, green, orange, and white colors for OLEDs). Red LEDs were only examined at $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for *T. urticae* and *T. kanzawai*, because neither species was sensitive to red light from LEDs; this was done to confirm if red light was ineffective. Light intensity was recorded with an irradiance meter cosine corrector (HD2302.01, Delta OHM, Padua, Italy) and the light intensity transformed to expected PFD based on the spectral data measured with a spectral radiometer (MS-720, Eko Instruments, Tokyo, Japan). Eggs of *T. urticae* and *T. kanzawai* collected using the method described above were transferred to the LED bottles or OLED

boxes to determine the effect of different types of scotophase interruption on diapause inhibition with six replications each in LEDs and seven replications each in OLEDs at 18°C. For each PFD and light color, the number of females tested ranged from 39 to 116. The diapause-inducing photoperiod of 8:16 h L: D was adopted, in which scotophase was systematically interrupted by a single 1- or 3-h light pulse halfway through scotophase. No scotophase interruption was used as the control (100% diapause). Diapause conditions of *T. urticae* and *T. kanzawai* were evaluated as previously described (Veerman 1985; Takafuji & Gotoh 1999; Takafuji *et al.* 2003).

Data analysis

An arcsine square root transformation was applied to normalize percentage of diapausing females prior to analysis. One-way analysis of variance (ANOVA) was used to infer the effect of light quality on the incidence of diapause. One-way ANOVA was also used to test for significant differences ($\alpha = 0.05$) of scotophase interruption in diapause induction of *T. urticae* and *T. kanzawai* to elucidate the effect of PFD and light quality using LEDs and OLEDs. Mean values were compared among factors using Tukey's honestly significant difference (HSD) test. All analyses were performed using IBM SPSS version 22 (IBM 2013).

Results

Effect of light quality on diapause induction in *T. urticae* and *T. kanzawai*

Different light quality (blue, green, and white LEDs; blue, green, orange, and white OLEDs) did not strongly affect diapause induction of *T. urticae* and *T. kanzawai* (Fig. 3). The critical photoperiods of *T. urticae* were ca. 13.5: 10.5 h L: D for LEDs and ca. 13.3–13.5 h light for OLEDs (Fig. 3 A, C), whereas those of *T. kanzawai* were ca. 12.5 h light for both LEDs and OLEDs (Fig. 3 B, D). No diapause induction was found under red LEDs in either species (Fig. 3 A, B). Diapause induction completely failed under 14:10 h L: D of photophase for *T. urticae* and in 13:11 h L: D for *T. kanzawai* under all colors.

Effect of intensity and duration of scotophase-interrupting light on diapause inhibition

One-hour interruption of scotophase using different colors of LEDs midway through scotophase (8:16 h L: D) and three light intensities showed variable diapause inhibition responses in *T. urticae* (Fig. 4). No diapause inhibition was observed under 1-h interruptions in *T. kanzawai*, with the exception of 98.5% (66/67) of females under blue LEDs at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. No diapause inhibition occurred in either species under red LEDs, even at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 1- and 3-h scotophase interruptions. In *T. urticae*, percentages of diapause decreased with increasing light intensity, but some females entered diapause even at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after 1-h of scotophase interruption with blue, green, and white LEDs. Under 3-h interruptions at 0.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, diapause induction of *T. urticae* was effectively inhibited with blue and green LEDs, whereas inhibition was only observed under blue LEDs for *T. kanzawai*. At 2 and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, blue, green, and white LEDs inhibited diapause induction in both *T. urticae* and *T. kanzawai*.

Variable diapause inhibition responses were observed in *T. urticae* under 1-h scotophase interruptions based on different colors of OLEDs at three light intensities (Fig. 5). No diapause inhibition was observed under 1-h interruptions in *T. kanzawai*, with the exception of 98.5% (67/68) of females under blue and white OLEDs, even at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Even 0.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 3-h scotophase-interrupting light from blue and green OLEDs were effective for inhibiting diapause induction of *T. urticae*, whereas the inhibition was only observed under blue OLEDs for *T. kanzawai*. Orange OLEDs showed higher diapause inhibition in *T. urticae* (11% diapause) than *T. kanzawai*

(91% diapause) at $2 \mu\text{mol m}^{-2} \text{s}^{-1}$. At $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, blue, green, orange, and white OLEDs completely averted diapause induction in both species.

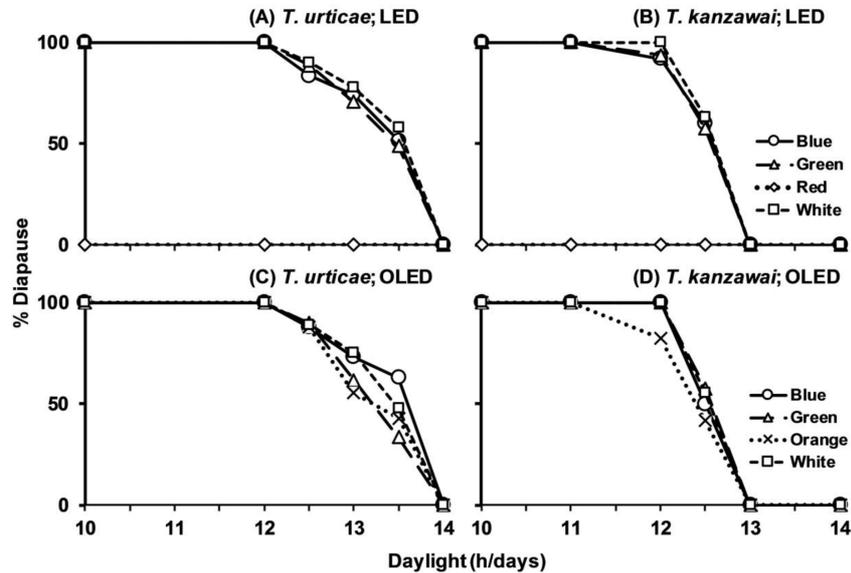


FIGURE 3. Photoperiodic response curves of diapause induction of (A, C) *T. urticae* and (B, D) *T. kanzawai* under different colors of (A, B) LEDs and (C, D) OLEDs. Air temperature, irradiance and PFD of the light period were set at 18°C , 2.0 W m^{-2} , and $7.9\text{--}11.0 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

Discussion

Our study revealed that diapause induction decreases with increasing photophase and completely failed at 14:10 h L: D and 13:11 h L: D in *T. urticae* and *T. kanzawai*, respectively, under both LEDs and OLEDs. Blue, green, and white LEDs, and blue, green, orange, and white OLEDs inhibited diapause induction under 3-h interruptions in the middle of the scotophase (8:16 h L: D) at $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *T. urticae* and *T. kanzawai*. Both species were insensitive to red LEDs.

The critical photoperiods of *T. urticae* were ca. 13.5:11.5 h L: D under LEDs and ca. 13.3–13.5 h light under OLEDs, whereas those of *T. kanzawai* were ca. 12.5:11.5 h L: D under LEDs and OLEDs. Light quality did not affect photoperiodic induction of diapause in *T. urticae* and *T. kanzawai*. Therefore, no significant effect was observed in critical photoperiods under different color LEDs and OLEDs in this study. It was reported that *T. urticae* are able to enter diapause under blue (475 nm), green (574 nm), or orange (612 nm) LEDs, but they are insensitive to red (658 nm) LEDs (Suzuki *et al.* 2008), which is consistent with our results. However, the critical photoperiod of *T. urticae* in our study was longer than those in populations collected from Sapporo (12.8 h light, Gotoh and Shinkaji 1981; 13:11 h L:D, Gotoh 1986), whereas the critical photoperiod of *T. kanzawai* was slightly shorter than that in a population collected from Soubetsu, Hokkaido (13:11 h L:D, Shah *et al.* 2011a), although the same strain of *T. kanzawai* was used in this study. This discrepancy might be due to a few-degree temperature fluctuation inside the incubators between the two experiments, or long-term rearing of females under laboratory conditions might produce a lower diapause induction rate as a result of artificial selection (Ito 2009). Both species were maintained in the laboratory for a variable period; that might affect the photoperiodic response of both species, although the critical photoperiods at least of *T. urticae* are not different from those of a previous

report (Gotoh & Shinkaji 1981). Either way, critical photoperiod variation among populations in either species is difficult to compare, because populations were collected from different geographical locations and altitudes, and their environmental conditions other than photoperiod, temperature, and humidity may also differ (Danilevskii 1965; Parr & Hussey 1966; Gotoh & Shinkaji 1981; Morishita & Takafuji 1999; Koveos *et al.* 1999; Suzuki & Takeda 2009). Although in both species the various light colors did not affect the critical photoperiods, they did have a differential inhibitory effect on diapause when used for scotophase interruption. These results may indicate that different physiological mechanisms are involved in the two processes. Different photoreceptor pigments are involved in circadian entrainment and in photoperiodism (Veerman 2001; Suzuki *et al.* 2008). Moreover, among insects and mites, a photoreceptor pigment is involved in photoperiodism, which may be coupled with a carotenoid-derived chromophore but displays different sensitivity to light quality (Veerman 2001; Suzuki *et al.* 2008).

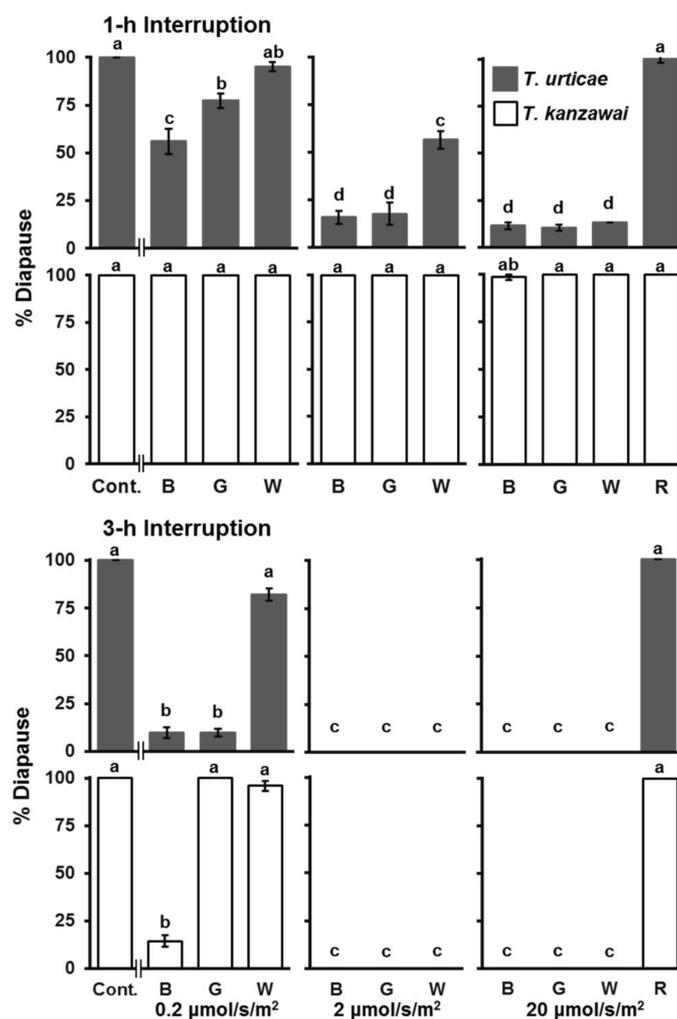


FIGURE 4. Effects of 1- or 3-h scotophase-interrupting lights applied midway through the 16 h dark period on diapause incidence in *T. urticae* and *T. kanzawai* with LEDs that were turned on at 7.5 and 6.5 h after the start of the dark period for 1- and 3-h interruptions, respectively. PFD of in blue, green, orange, and white LEDs were set at 0.2, 2, and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Red LED was only set at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Data were arcsine-transformed before analysis. Means followed by different letters are significantly different at $P < 0.05$ by Tukey's HSD test after ANOVA. Analyses were separately carried out for 1- and 3-h interruptions.

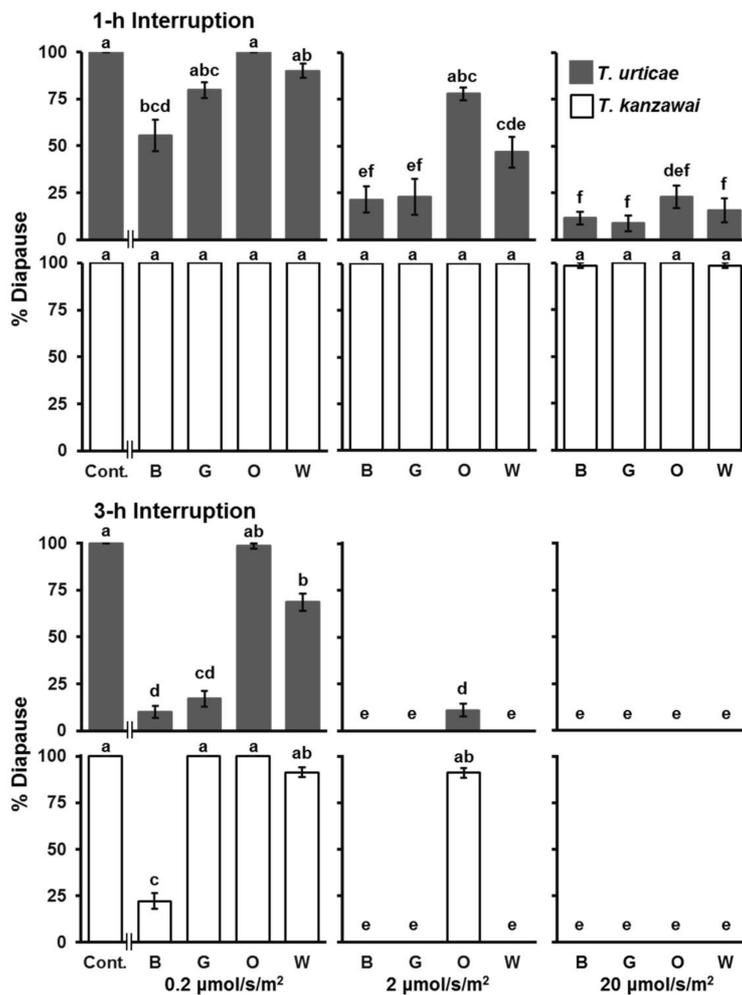


FIGURE 5. Effects of 1- or 3-h scotophase-interrupting lights applied midway through the 16 h dark period on diapause incidence of *T. urticae* and *T. kanzawai* with blue, green, orange, and white OLEDs that were turned on at 7.5 and 6.5 h after the start of the dark period for 1- and 3-h interruptions, respectively. PFD was set at 0.2, 2, and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Data were arcsine-transformed before analysis. Means followed by different letters are significantly different at $P < 0.05$ by Tukey's HSD test after ANOVA. Analyses were separately carried out for 1- and 3-h interruptions.

The effect of scotophase interruption on diapause inhibition has been investigated for several insect species (Koveos *et al.* 1993; Saunders 2002), but scotophase interruption experiments using different light colors and intensity have only been conducted in a few spider mite species. Inhibition of diapause induction in the European red mite, *Panonychus ulmi* (Koch), increased with increased duration of scotophase-interrupting light applied midway through a long dark period using fluorescent tubes (Lees 1953). Hussey (1972) reported that a 2-h scotophase-interrupting light applied midway through a long dark period was more effective (12% incidence of diapause) than a 1-h interruption (75–96% incidence of diapause) in *T. urticae*, when using fluorescent tubes. This study also showed that 3-h scotophase-interrupting light was more effective than 1-h interruption for diapause inhibition of *T. urticae* and *T. kanzawai* when using blue, green, and white LEDs, and blue, green, orange, and white OLEDs. Although 1-h interruption with both LEDs and OLEDs was

ineffective for successful inhibition of diapause in *T. kanzawai* even at $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, the same light intensity consistently reduced diapause induction of *T. urticae*. We therefore suggest that increasing the intensity of scotophase-interrupting light along with increasing interruption duration is appropriate for preventing diapause induction.

The spectral wavelength distribution of orange OLED overlaps with that of white OLED (Fig. 2), yet 3-h interruption with orange OLED had a strong inhibitory effect on *T. urticae* but not on *T. kanzawai* at $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD (Fig. 5). This might be related to the differential absorption of the orange color by the tested mite species. When the intensity of 3-h scotophase-interrupting light was as low as $0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, blue and green LEDs and OLEDs had greater diapause-averting effects in *T. urticae*, whereas only blue LEDs and OLEDs averted diapause in *T. kanzawai* (Figs. 4 and 5). High sensitivity of photoperiodic responses in several arthropods was found under blue or blue–green regions (400–550 nm) of the light spectrum (Saunders 2012), which is comparable to the results in the present study.

Plant physiological processes such as flowering, dormancy, and tuberization are controlled by photoperiod in a wide range of species (Jackson 2009). Blue LED produced higher biomass and leaf area in potato, radish (Yorio *et al.* 2001), and lettuce (Stutte *et al.* 2009). Blue LED was recommended to use as the preferred light source for higher nutritional quality to improve the growth and development of non-heading Chinese cabbage (Li *et al.* 2012). Partially supplemented blue LED promoted stem and internode elongation growth without any inhibitory effect on flower bud formation of a chrysanthemum, *Chrysanthemum morifolium* Ramat (Jeong *et al.* 2014). Furthermore, OLEDs closely mimicked natural lights with desirable colors and functions as ideal light sources for plant growth (Jou *et al.* 2015). Therefore, OLEDs might contribute to high efficiency in plant cultivation systems within a few years.

This study revealed the effects of different colors of LEDs and OLEDs on diapause induction and scotophase interruption on inhibiting diapause in *T. urticae* and *T. kanzawai*. We demonstrated that 3-h scotophase-interrupting light from blue LEDs and OLEDs have potential utility for effective pest-control strategy of spider mites while optimizing plant growth in greenhouses.

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