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Effects of Light Intensity and Wavelength on Diapause in *Plodia interpunctella*

Shigeru Kikukawa*, Shin Katou, Takamasa Matsuda, Akitoshi Miyashita, Tarou Tsuzuki

Biological Institute, Faculty of Science, University of Toyama, Toyama, Japan

Abstract

The Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), may diapause as a last (5^{th}) instar larva in response to the preceding photoperiod. At 25°C, light intensities above 200μW/cm^2 (daylight fluorescence at *c*. 400-750 nm) on the surface of the larval diet prevented diapause in a long day of LD 16:8 h (L=light and D=dark) and induced it in a short day of LD 12:12 h. Blue (400-495 nm), green (505-575 nm) or red (610-750 nm) filtered light at 200μW/cm^2 prevented and induced diapause in LD 16:8 h and LD 12:12 h, respectively. In another experiment, larvae were exposed to LD 16:8 h but for 4-h light period after light-on (dawn) or before light-off (dusk) were kept at different wavelengths (i.e., blue, green or red) maintained at 200μW/cm^2. If the larvae are insensitive to colored light, the effective photoperiod will be 12 h and diapause will be induced. Most of the larvae averted diapause, indicating that they were wavelength-sensitive in early or late scotophase. Next, the midnight of LD 12:12 h was interrupted with a 2-h light pulse of the different wavelengths at intensities of 100, 200 and 300μW/cm^2. Interrupting the scotophase by >200μW/cm^2 light prevented diapause at all wavelengths, indicating that diapause in this insect is determined by a wide range of the visible spectrum.

Keywords

Indian Meal Moth, Larval Diapause, Light Intensity, Wavelength, Spectral Sensitivity

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1. Introduction

From the effects of spectral energy on the biological time-measuring system, researchers have inferred the nature of the photo-sensitive pigments and the kinetics of photoperiod reception in many species of insects and mites [reviewed in 1-3]. Saunders [3] listed various species for which the spectral sensitivity of the photoperiodic response (including diapause determination) has been investigated. Amini *et al.* [4] recently exposed *Tetranychus urticae* and *T. kanzawai* to various wavelengths of light-emitting diodes (LEDs) and organic LEDs. The light from LEDs can also induce diapause in some species [e.g., 5 and 6]. Each of the tested species responds with characteristic features to different wavelengths of light. However, a general trend of the photoperiodic response of insects and mites at various wavelengths has yet to be determined.

As a preliminary study, the present paper investigates the range of wavelengths that control diapause in the Indian meal moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). This insect may enter diapause in its last (5^{th}) larval instar in response to photoperiod and/or thermoperiod during its larval life [7-9].

2. Materials and Methods

The *P. interpunctella* strain used in the experiments and the maintenance conditions of the stock culture are described in a

* Corresponding author
E-mail address: kikukawa@sci.u-toyama.ac.jp (S. Kikukawa)

previous report [10]. The light was sourced from two 15-W daylight fluorescent tubes (Akarinbou FL15EX-D-A, Hitachi Lighting Co. Ltd.), which emitted at wavelengths ranging from *c*. 400 to *c*. 750 nm. For wavelength analysis, blue (400-495 nm), green (505-575 nm) and red (610-750 nm) filters (DIF-50S-BLE, DIF-50S-GRE and DIF-50S-RED, respectively, Sigma Koki Co. Ltd.) were used. An additional filter (HAF-50S-30H, Sigma Koki Co. Ltd.) that absorbs infrared rays in the 800-2000 nm range was superimposed on each color filter. Light intensity was measured by a photo-sensor (MES-101, Koito Kogyou Co. Ltd.) placed on the surface of the rearing medium (food). The intensity was adjusted to the required level by changing the distance between the light source and the rearing container in the incubator (Hitachi incubator CRB-14L). Photoperiodic cycles were controlled by a 24-h time switch or by manual adjustment. The experimental protocols will be described appropriately in the following section.

3. Results

3.1. Effective Light Intensity for Diapause Determination

To determine the effective light intensity for determining larval diapause, the rearing cups were maintained under a diapause-preventing LD cycle of 16:8 h or a diapause-inducing LD 12:12 h at 25°C (Table 1). The light intensity was measured on the surface of the rearing medium (rice bran) in which the larvae were fed. The light intensity was varied as 100, 200, 300 and >500μW/cm^2. In the long day of LD 16:8 h, diapause was completely suppressed (i.e., 0% diapause) at intensities exceeding 200μW/cm^2, but in the short day of LD 12:12 h, it was strongly induced (>91%) at >200μW/cm^2. This suggests that at light intensities of 200μW/cm^2 appear to be sufficient for the photoperiodic diapause determination.

Table 1. The incidence of larval diapause of *Plodia interpunctella* under various light intensities in the photophase of 16 and 12 h light at 25°C. Light energy is measured at a surface of larval food in a plastic container. Light source is daylight fluorescent tubes.

Light intensity (μW/cm^2)	% Diapause			
	LD 16:8 h	(no.)*	LD 12:12 h	(no.)*
100	16	(86)	87	(84)
200	0	(92)	93	(90)
300	0	(96)	91	(80)
>500	0	(91)	100	(98)

*sample size.

3.2. Effective Wavelength

This experiment determined the range of effective wavelengths for the diapause determination. The larvae were exposed to blue (400-495 nm), green (505-575 nm) and red (610-750 nm) light at 25°C (Table 2). The light intensity was maintained at 200μW/cm^2 and the LD cycle was varied as 16:8 and 12:12. The incidence of diapause was significantly low (<7%) and high (>92%), respectively. This implies that the range of the visible light (from blue to red) was effective in diapause determination.

Table 2. Effects of wavelengths on the diapause determination under LD 16:8 h and LD 12:12 h at 25°C. Light intensity is maintained at 200μW/cm^2. Control = daylight fluorescent tubes.

Light	% Diapause			
	LD 16:8 h	(no.)*	LD 12:12 h	(no.)*
Control	0	(92)	93	(90)
Blue	7	(97)	92	(93)
Green	0	(82)	97	(90)
Red	1	(85)	96	(98)

*sample size.

3.3. Effective Wavelengths for Dawn and Dusk

In LD 16:8 h at 25°C, the larvae were exposed to blue, green or red light (200μW/cm^2) for 4 h after light-on or before light-off. During the remaining 12 h, they remained under the daylight fluorescent tubes (200μW/cm^2). As indicated in Table 2, LD 16:8 h induced no diapause (i.e., 0%) by using daylight fluorescent tubes at 200μW/cm^2. If the larvae were insensitive to light of specific wavelengths given 4 h after dawn or before dusk, the effective photoperiod would be LD 12:12 h rather than LD 16:8 h, and a high proportion of the larvae would enter diapause. When the post dawn 4-h illumination was blue or green, diapause was induced moderately and red light strongly prevented diapause (Table 3). In contrast, diapause was not induced by any wavelength of colored light applied at 4 h before dusk; percentage diapause was generally low (13-17%). These results indicated that light intensity of 200μW/cm^2 was sufficient for diapause prevention by 4 h dawn or dusk regardless of the wavelength of the colored light. No statistically significant difference among the percentage diapause shown in Table 3 was detected.

Table 3. Effects of wavelengths on the diapause induction under LD 16:8 h at 25°C. The insects are exposed to blue, green or red light during 4-h dawn or dusk. During 12-h photophase, they are under daylight fluorescent tubes. Light intensity is kept at 200μW/cm^2.

Light	% Diapause			
	Dawn	(no.)*	Dusk	(no.)*
Blue	39	(80)	14	(81)
Green	33	(86)	13	(89)
Red	5	(88)	17	(92)

*sample size.

3.4. Night Interruption by Various Wavelengths at Different Light Intensities

The night interruption experiment was conducted at 25°C with a background photoperiod of LD 12:12 h (a strong diapause-inducing condition). A 2-h light pulse was inserted at mid-scotophase, giving a photoperiod regime of LD 12:5:2:5 h. During the main photophase of 12 h, the insects were maintained at 200μW/cm^2 by using daylight fluorescent tubes. Table 4 showed that under daylight fluorescent tubes during a 2-h light pulse of LD 12:5:2:5 h, the percentage diapause was 49 with 100μW/cm^2 but it was 0 with 300μW/cm^2. To analyze the effects of wavelengths interrupting the scotophase, blue, green or red light pulse was inserted in the middle of 12-h scotophase at the light intensity of 100, 200 or 300μW/cm^2.

When blue, green or red light pulse of 300μW/cm^2 interrupted the scotophase, no larvae entered diapause (Table 4). Generally the incidence of diapause increased as the light intensity of the interrupting pulse decreased. Red light pulse of 100μW/cm^2 did not prevent diapause; the percentage diapause was 84%, not significantly different from that of the control (daylight) group. According to the results in Table 4, light energies above 200μW/cm^2 are required for diapause prevention by night interruption, regardless of wavelength. The critical threshold (50% diapause) was <100μW/cm^2 for blue and green light, and 100- 200μW/cm^2 for red light.

Table 4. Night interruption of LD 12:12 h by 2-h light pulse during which wavelength and intensity of light is varied at 25°C. Photo-regime is thus LD 12:5:2:5 h. The main photophase of 12 h is illuminated by daylight fluorescent tubes (200μW/cm^2). Control = daylight fluorescent tubes.

Light pulse	% Diapause					
	Light intensity (μW/cm^2) in light pulse					
	100	(no.)*	200	(no.)*	300	(no.)*
Control	49	(95)	24	(93)	0	(98)
Blue	39	(95)	6	(98)	0	(94)
Green	20	(95)	32	(96)	0	(93)
Red	84	(91)	4	(91)	0	(92)

*sample size.

4. Discussion

The Indian meal moth, *Plodia interpunctella*, is a model insect for analyzing the induction of larval diapause by the photoperiodic clock. The larvae of this species possess a timer that mainly measures the length of dark time [9, 11-14]. The present study determines the intensity and wavelength of light that effectively controls diapause. Photoperiodic responses were triggered at daylight intensities above 200μW/cm^2. Because the light intensity was measured on the surface of the larval feed, the light intensity received by the larvae should be below 200μW/cm^2. So far as studied, the wavelengths ranges 400-495 nm, 505-575 nm and 610-750 nm are effective in the photoperiodic photo-reception.

Species such as *Pectinophora gossypiella* [15], *Pieris brassicae* [16] and *Actias selene* [17] are sensitive to red light, whereas others such as *Antheraea pernyi* [18,19], *Laspeyresia pomonella* [19], *Adoxophyes orana* [20], *Aleyrodes proletella* [21] and *T. urticae* [5] are insensitive to red light (see also [3]). However, the wavelength sensitivities under different light intensity have not been appropriately determined in some cases. Diapause in *Nasonia vitripennis* [22] is most sensitive between 554 and 586 nm, and the critical thresholds are at 0.02 and 0.46μW/cm^2 in dawn and dusk, respectively. In *P. interpunctella,* diapause is suppressed by red light at 200μW/cm^2 in dawn. However, the diapause incidence does not significantly differ among red, blue and green lights, indicating that the larvae are sensitive to a broad range of wavelengths in both dawn and dusk. The critical threshold should be determined in this species.

In *Megoura viciae*, Lees [23] examined the dark responses of the night timer that determines the reproductive form. Adopting a night interruption technique, he identified four stages of the dark reactions; the blue-sensitive stage, a light insensitive stage, a blue- and red- activated stage, and a second light-insensitive stage. In the present study, the available information for analyzing the dark reaction of *P. interpunctella* was limited, because the wavelength effect on diapause inhibition was tested only in an LD 12:5:2:5 h night interruption. Red light at 100μW/cm^2 appears to be less effective than blue and green lights in the mid-night, but the difference was not statistically significant. Systematic night interruption by light pulses of various wavelengths are required to understand the dynamics of the night timer in this species.

5. Conclusions

The spectral effects on the photoreceptors of *T. urticae* [5] and *Sarcophaga similis* [6], which govern their photoperiodic responses, were analyzed in previous LED studies. This experimental treatment would clarify the clock system of *P. interpunctella.* As has been suggested by Saunders [3], such investigation might also identify the photoreceptor constituents (i.e., cryptochrome and opsins). Photoperiodic photoreception in *P. interpunctella* requires further study.

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