Journal of Insect Physiology 62 (2014) 1–10

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

An LED-based UV-B irradiation system for tiny organisms: System description and demonstration experiment to determine the hatchability of eggs from four *Tetranychus* spider mite species from Okinawa

Takeshi Suzuki [a,b,c,d,e,*], Yoshio Yoshioka [a], Olga Tsarsitalidou [b], Vivi Ntalia [b], Suguru Ohno [f], Katsumi Ohyama [c], Yasuki Kitashima [a], Tetsuo Gotoh [a], Makio Takeda [d], Dimitris S. Koveos [b]

[a] *Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300-0393, Japan*
[b] *Faculty of Agriculture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*
[c] *Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Chiba 277-0882, Japan*
[d] *Graduate School of Agricultural Science, Kobe University, Nada, Kobe 657-8501, Japan*
[e] *Japan Society for the Promotion of Science, Chiyoda, Tokyo 102-0083, Japan*
[f] *Ishigaki Branch, Okinawa Prefectural Agricultural Research Center, Ishigaki, Okinawa 907-0003, Japan*

ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form 9 January 2014
Accepted 10 January 2014
Available online 21 January 2014

Keywords:
Irradiance
Photolyase
Photoreactivation
Pulse-width modulator
UV-B irradiation

ABSTRACT

We developed a computer-based system for controlling the photoperiod and irradiance of UV-B and white light from a 5 × 5 light-emitting diode (LED) matrix (100 × 100 mm). In this system, the LED matrix was installed in each of four irradiation boxes and controlled by pulse-width modulators so that each box can independently emit UV-B and white light at irradiances of up to 1.5 and 4.0 W m^{-2}, respectively, or a combination of both light types. We used this system to examine the hatchabilities of the eggs of four *Tetranychus* spider mite species (*T. urticae*, *T. kanzawai*, *T. piercei* and *T. okinawanus*) collected from Okinawa Island under UV-B irradiation alone or simultaneous irradiation with white light for 12 h d^{-1} at 25 °C. Although no eggs of any species hatched under the UV-B irradiation, even when the irradiance was as low as 0.02 W m^{-2}, the hatchabilities increased to >90% under simultaneous irradiation with 4.0 W m^{-2} white light. At 0.06 W m^{-2} UV-B, *T. okinawanus* eggs hatched (15% hatchability) under simultaneous irradiation with white light, whereas other species showed hatchabilities <1%. These results suggest that photolyases activated by white light may reduce UV-B–induced DNA damage in spider mite eggs and that the greater UV-B tolerance of *T. okinawanus* may explain its dominance on plants in seashore environments, which have a higher risk of exposure to reflected UV-B even on the undersurface of leaves. Our system will be useful for further examination of photophysiological responses of tiny organisms because of its ability to precisely control radiation conditions.

1. Introduction

Because of the deleterious effects of ultraviolet-B radiation (UV-B; 280–315 nm), its application has been considered in pest control, particularly for tiny organisms such as insects and mites (Suzuki, 2012). Spider mites (Acari: Tetranychidae) are one of the agricultural pests that are hardest to control because of their rapid development of pesticide resistance (Dermauw et al., 2013). Therefore, investigation of their susceptibility to UV-B is important to determine whether this light offers an alternative measure for controlling spider mites. Although spider mites are small, their susceptibility to UV-B has often been investigated using large light sources such as fluorescent lamps (Barcelo, 1981; Barcelo and Calkins, 1980; Fukaya et al., 2013; Mazza et al., 1999; Murata and Osakabe, 2013; Ohtsuka and Osakabe, 2009; Santos, 2005; Tachi and Osakabe, 2012), xenon lamps (Sakai and Osakabe, 2010; Suzuki et al., 2009), halogen lamps (Mazza et al., 2010) or sunlight (Fukaya et al., 2013; Mazza et al., 2002; Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010; Sakai et al., 2012; Tachi and Osakabe, 2012). However, such large UV-B irradiation systems occupy a large space and take some time for the irradiance to stabilize. In addition, spectral apparatuses such as diffraction gratings or filters specific to UV-B are needed.

Abbreviations: 6-4PP, pyrimidine (6-4) pyrimidone photoproduct; AT, air temperature; CPD, cyclobutane pyrimidine dimer; DAT, days after treatment; DR, duty ratio; λ_{max}, peak wavelength; LD_{50}, median lethal dose for 50% hatchability; LED, light-emitting diode; PC, personal computer; PIC, peripheral interface controller; PWM, pulse-width modulator; ROS, reactive oxygen species; SNSR, sensor; SWO, saw-wave oscillator; UV, ultraviolet radiation; WT, white light.

* Corresponding author at: Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300-0393, Japan. Tel./fax.: +81 29 888 8560.

E-mail address: suzukit@mx.ibaraki.ac.jp (T. Suzuki).

0022-1910/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2014.01.005

Recently developed deep-UV light-emitting diodes (LEDs), with wavelengths ranging from 210 to 365 nm, can solve the problems with conventional UV-B sources and have therefore attracted intense research interest because of their potential applications in (1) air, water and surface sterilization and decontamination; (2) UV curing of various substances (e.g., inks, glues, polymers); and (3) biomedical and analytical instrumentation (Shur and Gaska, 2010). In addition, the combination of deep-UV LEDs with other LED colors lets researchers investigate the interactions of photoeffects such as photoreactivation, which is the reversal of UV damage to a biological system using near-UV or visible radiation (Dulbecco, 1949; Kelner, 1949; Sancar, 2003). Furthermore, pulse-width modulators (PWMs), which regulate the duty ratio (DR) of electric voltage supplied to the LEDs at a constant frequency, can control the irradiance quantitatively (Suzuki et al., 2011). In addition to the abovementioned uses of deep-UV LEDs, these advantages therefore make them a powerful tool for investigating the UV-B susceptibility of tiny organisms.

In the present study, we developed a space-saving system for irradiating tiny organisms with various irradiances of UV-B, white light, or both from an LED matrix controlled by PWMs. We also conducted an egg hatchability test using four agricultural pests, spider mite species in genus *Tetranychus* (*T. urticae* Koch, *T. kanzawai* Kishida, *T. piercei* McGregor and *T. okinawanus* Ehara). These mites were collected from Okinawa Island, a subtropical area of Japan that receives relatively high doses of UV-B.

2. Materials and methods

2.1. UV-B irradiation system

The UV-B irradiation system consists of a personal computer (PC), four 16-bit peripheral interface controllers (PICs; PIC24FJ64-GA002; Microchip Technology, Chandler, AZ, USA), four saw-wave oscillators (SWOs) with single-precision timers (NE555; Texas Instruments, Dallas, TX, USA), eight PWMs (two per box) equipped with comparators (LM339; National Semiconductor Co., Santa Clara, CA, USA) and digital potentiometers (MCP42010, Microchip Technology), four aluminum boxes (200 × 150 × 125 mm), up to 64 UV-B LEDs (LED_{UV}; λ_{max} = 304 nm; UVCLEAN300TO39FW; Sensor Electronic Technology, Columbia, SC, USA), up to 36 white LEDs (LED_{WT}; NSPW515BS; Nichia, Tokushima, Japan) and an incubator (123 L in volume, 700 × 1018 × 580 mm; MIR-154; Sanyo Electric, Osaka, Japan) (Fig. 1). Details of the software required to run this system are provided in Section 2.2.

Each box (Fig. 2A and B) consists of a board with 25 sockets in a 5 × 5 grid that contains the LEDs, two sensors ($SNSR_{IN}$ and $SNSR_{OUT}$; SHT75; Sensirion, Zurich, Switzerland) for measuring air temperature (AT) and relative humidity (RH) inside and outside the box, a fan (60 × 60 × 25 mm; San Cooler 60; Sanyo Denki, Tokyo, Japan) for circulating air from outside to inside the box and a Petri dish for rearing the test mites on leaf disks, as described below. The fan's rotation is controlled by software that we created to maintain an internal AT in the boxes similar to the external temperature. Up to 16 LED_{UV} and 9 LED_{WT} were arrayed on the board by connecting them to the sockets in a specific pattern (Fig. 2C). Four boxes were placed in the incubator to control their AT (Fig. 2D). The front of each box can be covered by an aluminum plate (110 × 150 mm) to prevent leakage of harmful UV-B, and a hole (50 mm in diameter) was cut in the bottom of the box to allow measurements of spectral distribution and irradiance.

The spectral distributions of the LED_{UV} and LED_{WT} light (Fig. 3) were measured using a spectroradiometer (USR-45D; Ushio, Tokyo, Japan). The irradiances of the UV-B and white light were measured using a radiometer (HD2302.0; Delta OHM, Padova, Italy) with

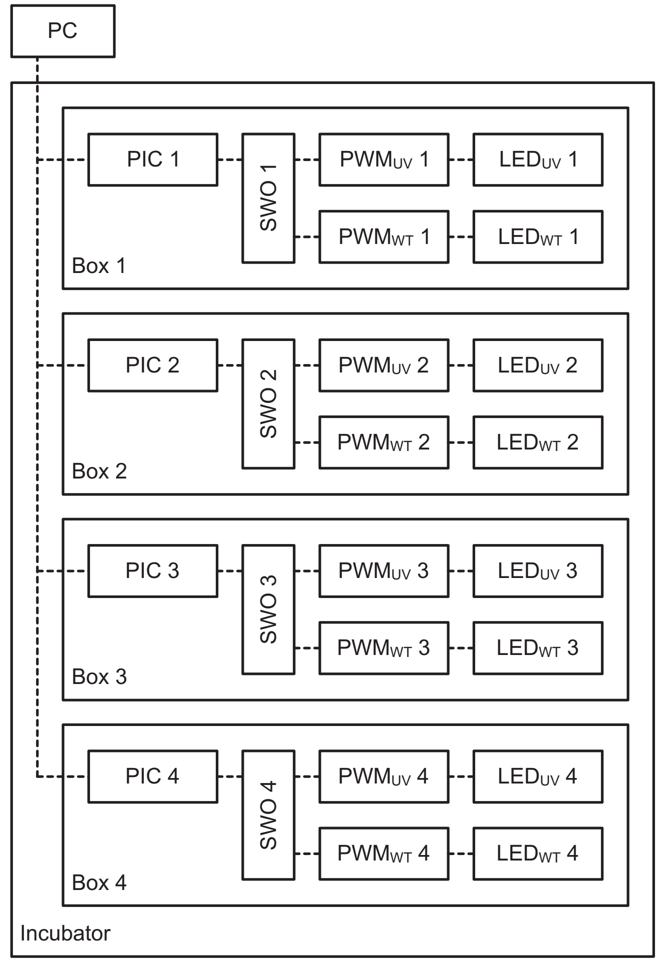

Fig. 1. A circuit block diagram of the LED-based UV-B irradiation system. LED, light-emitting diode; PC, personal computer; PIC, peripheral interface controller; PWM, pulse-width modulator; SWO, saw-wave oscillator. Subscripts are "UV" for UV-B and "WT" for white light. The system can simultaneously control the irradiances of UV-B and white light inside four different aluminum boxes (Boxes 1–4) by changing the duty ratios of the LEDs for UV-B and white light using the associated PWMs (PWM_{UV} and PWM_{WT}, respectively) at a 12 kHz frequency generated by the SWO.

probes for UV-B (LP471UVB; Delta Ohm) and visible radiation (LP471RAD; Delta Ohm), which were independently controlled in each box using the PWM_{UV} and PWM_{WT} devices. The PWMs regulated the DR of the electric voltage supplied to the LEDs at a constant frequency of 12 kHz generated by the SWOs. For detailed information on the DR control, see Suzuki et al. (2011).

2.2. System control software

In the software, the initial inputs were the DR set points DR_{UV} and DR_{WT} for LED_{UV} and LED_{WT}, respectively (Fig. 4). The software subsequently creates a timetable consisting of times (every 1 min), assigned box numbers, and DR_{UV} and DR_{WT} corresponding to the assigned box numbers. Based on the timetable, LED_{UV} and LED_{WT} in each box were driven at the programmed DR_{UV} and DR_{WT}, respectively. DR_{UV} and DR_{WT} can be set at intervals of 1%. The software was written in Visual Basic 2008 (Express Edition; Microsoft, Redmond, WA, USA) and ran under the Microsoft Windows 7 operating system on the PC.

2.3. Mites

Populations of *T. urticae* (green form), *T. kanzawai*, *T. piercei* and *T. okinawanus* were originally collected from chrysanthemums (9