Seasonal changes in the deleterious effects of solar ultraviolet-B radiation on eggs of the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

Author(s): Sakai, Yuta; Sudo, Masaaki; Osakabe, Masahiro

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Seasonal changes in the deleterious effects of solar ultraviolet-B radiation on eggs of the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

Yuta Sakai, Masaaki Sudo and Mh. Osakabe

Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Abstract

Solar ultraviolet-B (UVB) radiation has deleterious effects on plant-dwelling mites. We assessed the biological effects of UVB radiation on the eggs of the twospotted spider mite *Tetranychus urticae* Koch, under both near ambient (UV+) and UV-attenuated (UV–) conditions from spring to autumn and compared them to the effects of temperature and humidity. The ambient daily UVB irradiance increased from January to August and then decreased rapidly until December, whereas egg hatchability under UV+ was lowest in April (10.7%) and increased almost linearly until October (74.9–92.3%). In contrast, hatchability under UV– was consistently high (96.2–99.8%) through all seasons. For UV+, the stepwise multiple linear regression analysis supported the negative correlation of hatchability with cumulative UVB irradiance during egg periods (cumulative dose), but did not support that with the mean daily UVB irradiance (dose rate), suggesting that UVB-induced mortality in *T. urticae* eggs is cumulative dose dependent rather than dose rate dependent. The high mortality in April may have reflected the slower development caused by the relatively lower temperature and higher UVB radiation, increasing the cumulative dose, while the low mortality in October may have reflected the faster development caused by the relatively higher temperature and lower UVB radiation, decreasing the cumulative dose.

Keywords — Mite community, Habitat determination, UV-damage, Ambient UVB toxicity

Introduction

Solar ultraviolet radiation (UVR) has direct and indirect effects on terrestrial plant-dwelling arthropods (McCloud and Berenbaum 1999, Rousseaux et al. 2004, Ballaré et al. 2011). The small body sizes of plant-dwelling mites may be disadvantageous for protecting them against UVR damage. Such damage may alter their behavior and niche exploitation (Onzo et al. 2010, Sudo and Osakabe 2011), and in turn, their population dynamics and the composition of foliar communities.

The vulnerability of the herbivorous twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) to UVR has been documented: UVR increases egg mortality, delays juvenile development, and reduces egg production (Ohtsuka and Osakabe 2009, Sakai and Osakabe 2010). Laboratory experiments using UV lamps have suggested that the major component of solar UVR that damages the mite is UVB (280–315 nm wavelength), rather than UVA (315–400 nm wavelength; Barcelo 1981, Ohtsuka and Osakabe 2009, Suzuki et al. 2009, Sakai and Osakabe 2010). This might explain why their distribution is largely restricted to the lower leaf surfaces (Foott 1963, Osakabe et al. 2006; but see Li and Margolies 1991).

The intensity of solar UVB radiation fluctuates with season. The daily irradiance (dose rate) peaks in summer (July and August), and reaches a minimum in winter (December and January) in Japan, which is located in the mid-latitudes of northern hemisphere. This suggests that, in Japan, UVB radiation should have the greatest impact in summer (Barcelo 1981). However, UV-lamp studies of aquatic zooplankton (Copepoda; Kouwenberg et al. 1999b, Lacuna and Uye 2001), shrimp zoea (Wübben 2000), and the eggs of fish (Kouwenberg et al. 1999a) have suggested that UVR-induced mortality is not dependent on dose rate but rather depends on cumulative irradiance during developmental periods (cumulative dose), whereas the developmental rates of poikilothermic arthropods are accelerated in direct proportion to temperature increases within a comfortable range. Therefore, the cumulative dose is obviously a function of both the dose rate and temperature.
The combined actions of UVB intensity and temperature may also influence the activity of the two types of general cellular repair systems, photoenzymatic repair and nucleotide excision repair, removing the lethal lesions produced by UVB (Connelly et al. 2009, Bullock and Jeffrey 2010, Matallana-Surget et al. 2010). Although no one has attempted to measure enzymatic photoreactions, Santos (2005) reported the photoreactivation of UVB damage in *T. urticae* and the mold mite *Tyrophagus putrescentiae* (Schrank) (Acari, Acaridae). Moreover, several literatures indicated the effects of humidity and/or interactive effects of the humidity and temperature on survival of *Tetranychus* mites (Boudreaux 1958, Ferro and Chapman 1979, Perring et al. 1984). Therefore, the impact of solar UVR on mites may not simply synchronize with the seasonal fluctuation in the intensity of solar UVB radiation.

The interactions among solar UVR and other environmental factors may be essential for determining the seasonal impact of solar UVR, and may affect the evolutionary scenario of UV adaptation. However, no empirical study has examined seasonal fluctuation in the effects of solar UVR on terrestrial arthropods. *Tetranychus urticae* is a cosmopolitan and economically important herbivorous mite species found in many agricultural crops. The embryogenesis of this mite has been well established, making it a candidate as a chelicerate model organism (Grbić et al. 2007). In this study we investigated the seasonal changes in the effects of solar UVR on the eggs of *T. urticae*, under both near ambient (UV+) and UV-attenuated (UV−) conditions.

### Materials and methods

**Mites**

The *T. urticae* population used in this study was cultured in the laboratory on potted kidney bean plants at 25–28°C for at least 6 years. The plants were illuminated continuously by fluorescent lights. Several *T. urticae* populations collected from different sites have, over time, been added to this culture population.

Embryogenesis of *T. urticae* starts immediately after egg laying (Dearden et al. 2002), and larvae hatch 3–4 days and 2–3 days after oviposition at 25°C and at 27–30°C, respectively (Shih et al. 1976, Bounfour and Tanigoshi 2001).

**Experimental design**

Two shelves surrounded by a frame were set up on the roof of a four-story building at Kyoto University, Kyoto, Japan (35°02′N, 135°47′E), following Ohtsuka and Osakabe (2009). The top (roof), westward, and southward surfaces of each shelf were covered with either UV-transparent film (UV+; near ambient condition) or UV-opaque film (UV−; UV-attenuated condition). The 20-μm-thick polyethylene UV-transparent film (Kohnan Shoji, Osaka, Japan) transmitted about 80% of the ambient UVB (280–315 nm wavelength), UVA (315–400 nm wavelength), and visible light (VIS; 400–800 nm wavelength; Fig. 1). The 25-μm-thick HB3 polyester UV-opaque film (Teijin DuPont Films, Tokyo, Japan) filtered out >90% of the ambient UV at wavelengths of <380 nm (e.g., transmission was 1.6% and 0.2% at 370 nm and 360 nm, respectively), and transmitted about 80% of the VIS (Fig. 1).

Sixteen kidney bean leaf disks (2 × 2 cm) were prepared on water-soaked cotton in Petri dishes (9 cm in diameter; four leaf disks per dish). Five adult *T. urticae* females were introduced onto each leaf disk between 0900 and 1000 h. The Petri dishes were placed in a laboratory at 25°C, 16-h L: 8-h D light cycles. In the laboratory, fluorescent light were turn on at 0700 h and off at 2300 h. The next morning (0830–0900 h), the adult females were removed, and eggs that were laid on the leaf disks were counted. Eight leaf disks (two Petri dishes), including a total of 298–461 eggs (Table 1), were placed on a shelf under UV-transparent film. The remaining eight leaf disks (two Petri dishes) with similar numbers of eggs (278–446) were placed on a shelf under UV-opaque film (Table 1). Then the eggs on the leaf disks were exposed to solar radiation through the UV-transparent or UV-opaque film from 0900 to 1500 h every day, except on rainy days (or during periods of rain). The proportion of cumulative UVB radiation for the exposing time (0900–1500 h) to that for all levels of visible radiation is shown in Fig. 1.

![Fig. 1 Transmission spectrum of light passing through the UV-transparent film (broken line: UV+) and the UV opaque film (solid line: UV−).](image)

Table 1 Number of eggs set under UV-transparent (UV+) and UV-opaque (UV−) film

<table>
<thead>
<tr>
<th>Experiment date</th>
<th>UV+</th>
<th>UV−</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-28 May 2009</td>
<td>374</td>
<td>425</td>
</tr>
<tr>
<td>7-12 Aug 2009</td>
<td>298</td>
<td>278</td>
</tr>
<tr>
<td>18-23 Aug 2009</td>
<td>300</td>
<td>318</td>
</tr>
<tr>
<td>27 Oct-1 Nov 2009</td>
<td>461</td>
<td>427</td>
</tr>
<tr>
<td>14-19 Apr 2010</td>
<td>448</td>
<td>423</td>
</tr>
<tr>
<td>7-12 Oct 2010</td>
<td>413</td>
<td>395</td>
</tr>
<tr>
<td>22-27 Oct 2010</td>
<td>445</td>
<td>446</td>
</tr>
</tbody>
</table>

*a* Dates on which the eggs were exposed to solar radiation

*b* Pooled number of eggs for the eight leaf disks
day long were 77.4% (23–28 May 2009) at the minimum and 88.3% (27 Oct.–1 Nov. 2009) at the maximum (in this calculation the data of rainy days: 28 May, 9, 10 and 22 Aug. and 1 Nov. in 2009, and 16 Apr. and 9 and 25 Oct. in 2010, were excluded). The air temperature and relative humidity (RH) under each film were recorded every hour with data loggers (Hygrochron; KN Laboratories, Osaka, Japan). The data loggers were set at side of the Petri dishes with leaf disks on the shelf and shaded with corrugated paper to avoid direct irradiation by sunlight. After the exposure, the hatched and unhatched eggs were counted and the Petri dishes were returned to laboratory conditions (25°C, 16-h L: 8-h D light cycles). This process (sunlight irradiation and hatch count) was repeated every day for 6 days. In the first experiment in May 2009 we continued that process until after 8 days, but no individuals hatched on the 7th and 8th days. The experiments were begun (introduction of adult females onto the leaf disks) on 22 May, 6 and 17 August, and 26 October, 2009, and on 13 April and 6 and 21 October, 2010 (Table 1).

The ambient temperature and RH at the experimental site (Kyoto, Japan) in 2009 and 2010 were obtained from data measured by the Japan Meteorological Agency in Kyoto (35°0'1"N, 135°4'44"E; http://www.jma.go.jp/jma/index.html), and ambient daily UVB irradiance was taken from the data set monitored by the Solar Radiation and Weather Monitoring Project at Kyoto Women’s University (34°59'N, 135°4'7"E; http://www.cs.kyoto-wu.ac.jp/~konami/climate/index.shtml).

The cumulative UVB irradiance during egg periods ($I_{\text{total}}$; cumulative dose) was calculated using the formula:

$$I_{\text{total}} = I_{\text{day}} \times D_{\text{hatch(UV−)}},$$

where $I_{\text{day}}$ is the mean daily UVB irradiance (dose-rate) determined by integrating the ambient UVB irradiance for 0900–1500 h during the experimental periods, and $D_{\text{hatch(UV−)}}$ represents the mean days until hatching calculated from the number of eggs that hatched daily under UV− conditions. The daily UVB irradiance on the rainy days (zero kJ m$^{-2}$) was included with the calculation of $I_{\text{day}}$. Since it was impossible to know when unhatched eggs had died and most eggs died in the several experiments under UV+ conditions, we expediently used $I_{\text{day}}$ and $D_{\text{hatch(UV−)}}$ to estimate $I_{\text{total}}$.

The developmental rate per day ($V_{\text{day}}$) was calculated using the formula:

$$V_{\text{day}} = \frac{1}{D_{\text{hatch}}},$$

where $D_{\text{hatch}}$ represents the mean days until hatching calculated from the number of eggs that hatched daily under UV+ or UV− conditions.

Statistical analyses

Differences in the temperature and RH between the UV+ and UV− treatments were analyzed using a one-way analysis of variance (ANOVA). The mean temperature and mean RH during the periods in which the eggs were exposed to solar UVR (0900–1500 h) were calculated for the 6 days of each experiment and the data were used as variables. The effects of the UV treatments at the different dates on $V_{\text{day}}$ and on the arcsine-transformed mortality were analyzed using a two-way ANOVA. The effects of UVB ($I_{\text{total}}$ or $I_{\text{day}}$), temperature, and RH on the hatchability or $V_{\text{day}}$ under UV+ conditions, and the effects of air temperature and RH under UV− conditions were evaluated using stepwise multiple linear regression analyses. All statistical analyses were performed using JMP ver. 7.0.2 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

The mean temperature and RH on the shelves exposed to UV+ and UV− during the experimental time exposing to solar UVR (0900–1500 h) varied seasonally, with values of 21.1–37.3°C and 30–49%, respectively, but differences between UV+ and UV− were not significant (one-way ANOVA, temperature: $F_{1, 12} = 0.001$, $P = 0.9734$; RH: $F_{1, 12} = 0.0003$, $P = 0.9862$). On the shelves, the temperature was higher and the RH lower than the respective ambient mean values for the exposing times (12.2–33.2°C and 32.7–65.7%) and also for all day long (Fig. 2).

The hatchability of $T$. urticae eggs differed significantly...
between the UV+ and UV− treatments and among the experimental dates (Table 2). The ambient daily UVB irradiance increased from January to August, and then decreased rapidly until December (Fig. 2). As shown in Figure 2, egg hatchability under the UV+ condition was lowest in April (10.7%) and increased almost linearly until October (74.9–92.3%), except in early August, whereas hatchability under the UV− condition was consistently high (96.2–99.8%). $V_{day}$ also differed significantly between the UV+ ($V_{day}$: 0.191–0.219) and UV− ($V_{day}$: 0.189–0.240) treatments and experimental dates (Table 2). Moreover, significant interactions between treatments and experimental dates were also seen for both hatchability and $V_{day}$. This indicates that the hatchability and $V_{day}$ of *T. urticae* eggs are affected by the presence/absence of solar UVR, but the deleterious effects of solar UVR vary with the seasonal changes in other environmental factors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Action</th>
<th>Sequential $R^2$</th>
<th>Mallow $s^2 \times C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV+ condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatchability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{total}$</td>
<td>Add</td>
<td>0.6491</td>
<td>0.7369</td>
</tr>
<tr>
<td>RH</td>
<td>Add</td>
<td>0.7183</td>
<td>2</td>
</tr>
<tr>
<td>Temperature</td>
<td>Add</td>
<td>0.7183</td>
<td>4</td>
</tr>
<tr>
<td>Temperature</td>
<td>Remove</td>
<td>0.7183</td>
<td>2</td>
</tr>
<tr>
<td>RH</td>
<td>Remove</td>
<td>0.6491</td>
<td>0.7369</td>
</tr>
<tr>
<td>UV− condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatchability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Add</td>
<td>0.6483</td>
<td>3</td>
</tr>
<tr>
<td>RH</td>
<td>Add</td>
<td>0.6491</td>
<td>0.7369</td>
</tr>
<tr>
<td>Temperature</td>
<td>Remove</td>
<td>0.6491</td>
<td>0.7369</td>
</tr>
<tr>
<td>$V_{day}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Add</td>
<td>0.5190</td>
<td>2.4912</td>
</tr>
<tr>
<td>RH</td>
<td>Add</td>
<td>0.6496</td>
<td>3</td>
</tr>
<tr>
<td>RH</td>
<td>Remove</td>
<td>0.5190</td>
<td>2.4912</td>
</tr>
</tbody>
</table>

For the stepwise multiple linear regression analyses, $I_{total}$ or $I_{day}$ with the mean temperature and RH during the experimental periods were used as explanatory variables in UV+. The stepwise multiple linear regression analysis of egg hatchability under UV+ conditions supported a negative correlation with $I_{total}$ ($y = 1.8458298 - 0.020901x$; Table 3, Fig. 3a), but not with temperature and RH. In contrast, the analysis using $I_{day}$ instead of $I_{total}$ supported no regressions between the hatchability and explanatory variables under the UV+ condition (the results of the stepwise multiple linear regression analysis are not shown). $V_{day}$ was not affected by those factors under UV+ conditions (Fig. 3b; the results of
the stepwise multiple linear regression analysis are not shown).

Under UV− conditions, a positive correlation was supported between egg hatchability and RH \((y = 1.1427437 + 0.0071868x; \text{Table 3, Fig. 3a})\), and between \(V_{\text{day}}\) and temperature \((y = 0.1690935 + 0.0018155x; \text{Table 3, Fig. 3b})\). The effects of temperature on \(V_{\text{day}}\) under UV− conditions suggest that the range of temperature (including both daytime outside and nighttime in the laboratory) did not injure the eggs. Regarding the effects of humidity, although Ferro and Chapman (1979) reported that the hatchability of \(T. urticae\) eggs was higher under conditions of higher rather than lower humidity including both RH and vapor pressure deficit, other published studies have reported only minimal effects of such humidity on the performance of \(Tetranychus\) species, except when the humidity is low and the temperature is high.

![Fig. 3](image-url) The correlation between hatchability (a) or developmental rate \((V_{\text{day}}; \text{b})\) and the cumulative UVB irradiance during egg periods \(I_{\text{total}}\), mean temperature, or mean RH in \(T. urticae\) eggs under UV+ (solid circles) and UV− (open circles) conditions. The vertical lines in each plot indicate the standard errors. The regression lines are shown as solid lines for UV+ and broken lines for UV− only if the correlation was supported by the stepwise multiple linear regression analysis in Table 3.
(Boudreaux 1958, Perring et al. 1984). The correlation of RH with the hatchability of T. urticae eggs was also supported under UV− conditions in this study. However, the hatchability was >90% over all experimental periods under UV− conditions, and the correlation between RH and the developmental rate was not supported. Overall, we consider humidity did not affect the results in our experimental conditions that the eggs were put in the laboratory every night.

Neither hatchability nor $V_{eg}$ was correlated with temperature or RH under UV+ conditions. Instead, an obvious negative correlation of egg hatchability with $I_{rad}$ was observed, suggesting that direct adverse effects of temperature and humidity were excluded in this study. The result that the correlation of $I_{rad}$ with hatchability was not supported statistically suggests that the UVB-induced mortality is cumulative dose-dependent, rather than dose rate-dependent as in aquatic organisms (Kouwenberg et al. 1999a,b; Wübben 2000, Lacuna and Uye 2001). Although temperature did not affect the egg mortality directly, but it affected the developmental rate and thus altered egg periods that, with dose rate, determined the cumulative dose of UVB radiation. Consequently, temperature indirectly influenced the egg mortality via alteration of UVB cumulative dose. This may be a mechanism that the extent of UV damage to T. urticae eggs did not parallel the seasonal fluctuation in the intensity of ambient UVB radiation contrary to a general prospect (Barcelo 1981).

On the other hand, although little is known about UV damage-mediated general repair systems (Santos 2005), but these might contribute to the survival of eggs in later seasons. Our experiments revealed correlation of egg mortality with solar UVB radiation. However, the egg mortality under UV+ conditions was larger in April than that in August in spite of the cumulative UVB radiation was equivalent to each other. Likewise, despite the smaller cumulative UVB radiation the egg mortality in May was larger than or equivalent to that in August. Additional elaborately designed studies, such as laboratory experiments using UV lamp, are required to elucidate the effects of UV damage-mediated repair systems and may also the relationship between UV damage and temperature.

Sakai and Osakabe (2010) found that the distribution of T. urticae females was different between upper and lower leaf surfaces as a result of UV-avoidance behavior. The deleterious effects of solar UVR may be significant factors restricting plant-dwelling mites, including predacious mites, to the lower leaf surfaces or inside domatia (Ohtsuka and Osakabe 2009, Onzo et al. 2010, Sudo and Osakabe 2011). These species might occupy habitat in which they encounter predators or competitors less frequently or gain nutritional advantage through increased fecundity on the upper leaf surfaces versus the lower leaf surfaces. In fact, a part of herbivorous mites exploit not only the lower but also the upper leaf surfaces of host plants (Foott 1963, Jones and Parrella 1984, Sudo and Osakabe 2011). Of those species, the European red mite Panonychus ulmi (Koch) (Acari: Tetranychidae) moves from the lower to the upper leaf surfaces as a response to increasing density of a superior competitor T. urticae on the lower leaf surfaces (Osakabe et al. 2006). Such species may acquire any protection mechanism against solar UVR.

On the other hand, adult females of T. urticae are induced diapause with photoperiodic response in autumn and spend several months during winter after leaves fallen. Suzuki et al. (2009) revealed that the diapausing females were more tolerant to UVB radiation than non-diapausing females but avoided UVB radiation more sensitively than non-diapausing females. Body color of the diapausing females turn to orange with accumulation of carotenoids (Veerman 1974) that may function as antioxidant agents, suggesting an adaptation to solar UVB radiation in winter (Suzuki et al. 2009).

Our results show the enormous impact of solar UVR on the mites and the reduction in the deleterious effects in autumn. Simultaneously, it suggests that high dose rate (intensity) of UVB radiation with high temperature such as that in summer does not necessarily mean the greater impacts on mites. In contrast, even the intensity of UVB radiation is low the cumulative dose possibly reaches an effective range to confer lethal damage on mites after long exposure. Such seasonal, environmental condition dependent changes in the impact of solar UVR likely affect the dynamics and evolution of plant-dwelling mite communities via habitat determination and species success.

Acknowledgements

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