Spectrum-Specific UV Egg Damage and Dispersal Responses in the Phytoseiid Predatory Mite Neoseiulus californicus (Acari: Phytoseiidae)

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ABSTRACT Solar ultraviolet-B (UVB) radiation is deleterious to plant-dwelling mites. Neoseiulus californicus (McGregor) is a predominant predator of agriculturally important pest species of spider mite. However, phytoseiid mites are more vulnerable to UVB radiation than spider mites. Thus, the UVB radiation may influence decision making in foraging phytoseiid mites whether disperse or not. We tested the difference in impact and behavioral response among wavelengths of monochromatic UV radiation using a spectroscopic light source in N. californicus in the laboratory. We also examined whether the behavioral responses of N. californicus females to UV radiation varied based on the presence of prey (Tetranychus urticae Koch) eggs and residues (webs and excreta of T. urticae foraging cue). The impact of UV radiation on the N. californicus egg hatchability varied drastically between wavelengths of ≥300 nm (0%) and ≥310 nm (100%). The N. californicus females escaped from UV radiation more quickly when they were irradiated with UV at shorter wavelength. Presence of T. urticae eggs had no effects arresting the escape of phytoseiid mites. In contrast, prey residues (including eggs) markedly detained N. californicus females from escaping under UV irradiation at ≥310 nm. However, N. californicus females quickly escaped when irradiated with UV at harmful 300 nm wavelength, regardless of prey cues. This indicates that the eyeless phytoseiid mite is capable of perceiving UV radiation, and whether escape or not is determined on the basis of harmful/harmless UV wavelength and presence/absence of foraging cues.

KEY WORDS UV avoidance, UV-B, foraging behavior, Phytoseiidae, Acari

Many plant-dwelling mites preferentially inhabit lower rather than upper leaf surfaces of their host plants (Sudo and Osakabe 2011). Solar ultraviolet-B (UVB; 280–315 nm wavelengths) radiation may be a major factor affecting the within-leaf (upper/lower) distribution of mites (Ohtsuka and Osakabe 2009, Suzuki et al. 2009, Sakai and Osakabe 2010).

The citrus red mite, Panonychus citri (McGregor) (Acari: Tetranychidae), one of a few spider mites that use upper leaf surfaces, exhibits higher tolerance to UVB radiation (Fukaya et al. 2013) and escapes from solar UV radiation more slowly than the two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae), which resides on the lower leaf surfaces (Tachi and Osakabe 2012). In a prey–predator system composed of herbivorous spider mites and predacious phytoseiid mites, the predators are more vulnerable to ambient UVB radiation than the prey, resulting in rapid emigration from places irradiated with solar UV radiation (Tachi and Osakabe 2012). Phytoseiid mites escape from not only solar UV radiation but also visible light radiation (Weintraub et al. 2007, Tachi and Osakabe 2012). Such various behavioral responses to ambient radiation potentially affect their distribution in canopy and, thus, the numerical responses of phytoseiid mites as biological control agents of spider mites.

The impact of UV radiation on the hatchability of T. urticae egg varies drastically between wavelengths of ≥300 and ≥320 nm; the hatchability is nearly 100% with UV irradiation at between 320 and 360 nm (ultraviolet-A [UVA]; 315–400 nm wavelengths), which drops to 0% with UV irradiation at 280 and 300 nm (UVB) (Sakai and Osakabe 2010). Nevertheless, T. urticae adult females do not escape from harmful UVB radiation at 280 and 300 nm; instead, they avoid harmless UVA at 320 and 340 nm (Sakai and Osakabe 2010). Previous studies have also demonstrated positive phototaxis to UVA radiation at 375 nm (Naegle et al. 1966) and the absence of a positive response against UV at ≥360 nm in T. urticae (McEnroe and Dronka 1966). T. urticae might be incapable of recognizing UVB and may thus exploit UVA at relatively short wavelengths (tentatively 320–340 nm) as a source of information to avoid ambient UVB radiation (Sakai and Osakabe 2010; cf. Suzuki et al. 2013).

However, detailed studies on the toxicity of UV wavelengths to phytoseiid mites and their behavioral responses have not been performed so far. Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) is a commercially important natural enemy of spider mites.

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in agricultural fields worldwide. Although N. californicus is a type II selective predator of Tetranychus mites (McMurtry and Croft 1997), it preys on Panonychus mites in the field (Katayama et al. 2006, Kishimoto et al. 2007, Kawashima and Jung 2010, Fadamiro et al. 2013) and also feeds on a wide range of diets such as tarsenemid mite, small herbivorous insects, and pollen (Croft et al. 1998, Castagnoli and Simoni 1999, Castagnoli et al. 2001, Easterbrook et al. 2001). N. californicus may forage not only on lower surfaces but on upper leaf surfaces as well.

However, type II as well as type I (specialized predators of Tetranychus species) phytoseiid mites exploit webs of silk threads produced by spider mites as a cue of prey existence; the webs retain phytoseiid mites and elicit prey-searching behavior (Hislop and Prokopy 1981, Hoy and Smilanick 1981, Sabelis et al. 1984, Shinmen et al. 2010). Therefore, existence of the prey cue possibly affects behavioral response of N. californicus against UV radiation.

In this study, we investigated the biological impact of monochromatic UV radiation ranging from UVB to UVA on N. californicus. We then tested UV wavelength spectrum-specific avoidance of this phytoseiid mite and the effects of prey eggs and residues (webs and excreta of spider mites) on the behavioral response.

**Materials and Methods**

**Predatory Mite and Its Prey.** The N. californicus strain had been originally collected from Japanese pear trees in Matsukawa, Nagano Prefecture, Japan (35° 36’ N, 137° 55’ E) in September 2000 and was provided by the National Institute of Agrobiological Sciences, Japan. Before the experiments, N. californicus was reared for more than two generations on a population of T. urticae (yellow-green type) that had been cultured on potted kidney bean plants in a laboratory at Kyoto University. All mites were reared in the laboratory at 25°C at a photoperiod of 16:8 (L:D) h.

**Irradiation With Monochromatic UV.** Monochromatic UV radiation at 280, 300, 310, 320, 340, 360, or 380 nm was achieved in a dark box using a 300-W xenon light source (MAX-302, Asahi Spectra Co., Tokyo, Japan) with band-pass filters and a collimator lens (RLQ-2). The wavelength spectra (indexed by relative intensity [counts]) and intensities were measured using a spectrometer (UFV-VIS F, Spectra Co-op Co., Tokyo, Japan) and a hand-held optometer (UV-3701–4, UV-3702–4, Gigahertz-Optik GmbH, Turkenfeld, Germany), respectively. The UV intensities used for experiments for both damage (egg hatchability) and behavioral response (avoidance of adult females) were 1.26 W m⁻² at 280 nm wavelength and 1.47 W m⁻² at 300, 310, 320, 340, 360, and 380 nm wavelengths (Fig. 1a).

**Damage to Egg Hatchability.** For the egg hatchability experiment, one petri dish (9 cm in diameter) was prepared for each of the seven UV wavelengths tested and for the dark control. Four kidney bean leaf discs (2 by 2 cm) were placed on water-soaked cotton in each petri dish. To prepare prey eggs for N. californicus, 10 T. urticae adult females were transferred from cultures to each leaf disk and allowed to oviposit freely for 24 h. Most adult females were likely to be mated because adult females of spider mites living in a breeding patch usually mate with guarded males immediately after the last molt. Even if some of them were virgin, T. urticae has arrhenotokous parthenogenesis and virgin females oviposit as well as mated females. The next day, after the T. urticae females were removed, five adult females of N. californicus were introduced to each leaf disk. The introduction of phyto-
seid mite females was performed petri dish by petri dish at around 90-min intervals, and the females were allowed to lay eggs for 24 h. After removing the females (5 min before exposure of eggs to UV radiation), we counted the number of phytoseid mite eggs on the leaf disks.

The eggs in a petri dish were then exposed to one of the monochromatic UV radiation levels for 60 min or assigned to the unexposed control (one petri dish). After exposure to UV (Day 0), we counted hatched and unhatched eggs on the leaf disks every day for 7 d (Day 7).

Murata and Osakabe (2013) demonstrated that the Bunsen–Roscoe reciprocity law is applicable to UVB damage in *T. urticae*. The LD$_{50}$ values (50% lethal cumulative UV dose) of *T. urticae* eggs and adult females were 0.58 and 26.12 kJ m$^{-2}$, respectively, in a laboratory whose interior was illuminated with fluorescent lamps (Murata and Osakabe 2013). Tachi and Osakabe (2012) reported that eggs of *N. californicus* were more vulnerable than those of *T. urticae*; the LD$_{50}$ value was <0.3 kJ m$^{-2}$ (eggs were exposed to UVB without visible light and moved to a laboratory condition illuminated with fluorescent lamps immediately). In the present experiment, the cumulative UV doses for 60 min were 4.54 kJ m$^{-2}$ (1.26 W m$^{-2}$) and 5.29 kJ m$^{-2}$ (1.47 W m$^{-2}$). Therefore, the cumulative doses were high enough to evaluate the biological impact on eggs but were not excessive for adult *T. urticae* females. Moreover, from the data set monitored by the Solar Radiation and Weather Monitoring Project at Kyoto Women’s University (34°59’ N, 135°47’ E; http://www.cs.kyoto-wu.ac.jp/~konami/climate/index.shtml), these UV doses were equivalent to a daily cumulative UV dose in winter (5 kJ m$^{-2}$) and the intensities were lower than the maximum intensity in summer (1.8 W m$^{-2}$) in the experimental site, Kyoto, Japan (Kyoto University 35°1’ N, 135°47’ E; Sakai et al. 2012).

We had earlier confirmed that intact residues of *T. urticae* adult females, including webs, eggs, and excreta, had no positive effects on egg hatchability after irradiation with UVB. Hatchability of eggs with and without residues was 15.9 (n = 63) and 17.7% (n = 62), respectively, after the exposure to UVB radiation at 0.31 W m$^{-2}$ (UVA + UVB: 0.45 W m$^{-2}$; UV+) for 30 min.

**Behavioral Response to UV Radiation.** A strip of kidney bean leaf (1 by 6 cm) was placed on water-soaked cotton in a rectangular plastic tray (7.5 by 22 cm). We placed a strip of wet paper (1 by 3 cm; Kimwipes, Nippon Paper Crecia Co., Tokyo, Japan) across the center of the leaf strip perpendicular to the longitudinal axis to prevent the movement of mites between the two sides (Fig. 1b). We prepared three different treatments: (1) with no prey eggs or residues (webs and excreta), (2) with prey eggs without residues, and (3) with prey eggs and residues, on one side of the leaf strip (treated side), where *N. californicus* adult females would be introduced and exposed to UV radiation under UV-transparent film in later processes. To prepare the treatment (2) with prey eggs without residues, we transferred 10–20 *T. urticae* (prey) eggs that were within 24 h after oviposition to the side of leaf strip using a fine brush. For the preparation of treatment (3) with prey eggs and residues, five *T. urticae* adult females were introduced to one side of the leaf strip, allowed to oviposit freely for 24 h, and then the *T. urticae* females were removed. As a result, the side of leaf strip harbored 10–20 prey eggs and intact prey residues.

We then introduced 10 *N. californicus* adult females to the treated side of the leaf strip, which was still divided by the wet paper. The plastic tray was kept in the laboratory for at least 10 min to allow the mites to settle to the treated side of the leaf strip. After the wet paper strip was removed, half of the leaf strip where the mites had settled (treated side) was covered with UV-transparent film (UV+; polyethylene film, 30 µm thick, Dainichi Sangyo Co., Osaka, Japan), and the other half was covered with UV-opaque film (UV--; HB3 polyester film, 25 µm thick, Teijin DuPont Films, Tokyo, Japan) stretched on a plastic frame (8.0 by 22.5 cm; Fig. 1c).

The UV-opaque film filtered out >90% of UV at wavelengths below 380 nm and >99% at wavelengths below 363 nm, while allowing the transmission of 87% of the wavelengths between 388 and 800 nm (Fig. 1d). The UV-transparent film allowed the transmission of 84% of 250–800 nm on average (Fig. 1d).

The leaf strip in the plastic tray was exposed to monochromatic UV radiation [irradiation area: 8 by 8 cm (square area)] at an assigned wavelength for 120 min or kept in a dark box without UV irradiation (dark control). We used UV at 300–380 nm wavelengths in this experiment because the irradiation with UV at 280 nm wavelength frequently made *N. californicus* incapable of walking normally, being impossible to escape as well as *T. urticae* (Sakai and Osakabe 2010). The number of individuals on UV+ (settled) and UV− (sheltered) sides of a leaf strip was counted every 10 min. To count the individuals, the plastic tray was taken out from dark box briefly (shorter than 5 s). We repeated the experiments for five times (five replications) on the different days.

**Statistical Analyses.** Effects of UV wavelength on egg hatchability were evaluated using a log-rank test.

We used “survdiff” modules in the “survival” package (Therneau and Grambsch 2000), using R software version 2.15.2 (R Core Team 2012).

We performed multiple comparisons of generalized linear mixed model (GLMM; family = binomial, logit link) among wavelengths and a dark control in each treatment. Wavelengths and a dark control were applied as categorical explanatory variables, and the numbers of individuals on the UV+ and UV− sides at each observation time were response variables. We used “glmer” modules in the “lme4” package (Bates et al. 2012) to construct GLMMs for repeated measures and “glht” in the “multcomp” package (Hothorn et al. 2008) for multiple comparisons of the GLMMs with Tukey contrasts (α = 0.05), using R software.
Results

Damage to Egg Hatchability. Eggs exposed to 280 nm \((n = 59)\) and 300–380 nm \((n = 58)\) radiation did not hatch until Day 7 (Fig. 2). In contrast, all eggs irradiated with UV at wavelengths of 310 \((n = 60), 320 (n = 64), 340 (n = 55), 360 (n = 64),\) and 380 nm \((n = 69)\) and the unexposed controls \((n = 43)\) hatched by Day 3. At these wavelengths, egg hatchability was 7.0–15.9% on Day 1 (the first day after UV exposure) and increased to 92.4% on Day 2, as did the unexposed controls. The embryonic period was not likely increased at wavelengths of 310–380 nm.

A log-rank test revealed the significant effects of UV wavelengths including unexposed control on the egg hatchability \((df = 7, \chi^2 = 407, P < 0.0001)\). In contrast, no significant effects of UV wavelengths were detected among data sets from 310 to 380 nm and the unexposed control \((df = 5, \chi^2 = 2.4, P = 0.789)\). This indicates that irradiation with UVB at wavelengths of 280 and 300 nm was harmful to \textit{N. californicus} eggs, whereas irradiation with UV at wavelengths of 310, 320, 340, 360, and 380 nm had no influence over the egg hatching.

Behavioral Response to UV radiation. With No Prey Eggs or Residues. Under dark conditions, most adult females remained on the half of the leaf strip on which they had initially settled (Fig. 3). In contrast, the phytoseiid mites irradiated with UV significantly escaped from the UV+ side to the UV− side of a leaf strip at 300, 310, 320, and 360 nm in comparison with the dark control (multiple comparisons of GLMMs, \(Pr(z) < 0.001\) at 300, 310, and 320 nm and \(Pr(z) = 0.0239\) at 360 nm). In contrast, no significant differences from the dark control were detected at 340 and 380 nm \((Pr(z) = 0.4617\) and 0.3970, respectively), suggesting slower avoidance behavior in comparison with 300 nm \((Pr(z) = 0.0421\) and 0.0551 at 340 and 380 nm, respectively). In 360 nm, although around 30% of individuals escaped from UV+ within the first 20 min, then the proportion of escaping individuals leveled off. These results indicate that \textit{N. californicus} are capable of recognizing UVB and differences in wavelengths, and they escape from UVB radiation more quickly than from UVA.

With Prey Eggs Without Residues. Females quickly escaped from UV+ at 300, 310, and 320 nm rather than...
the longer wavelengths such as 360 and 380 nm and also dark control (Fig. 4; multiple comparisons of GLMMs, \( Pr(> z ) < 0.001 \), except between 300 and 360 nm \[ Pr(> z ) = 0.00633 \] and between 310 and 360 nm \[ Pr(> z ) = 0.01573 \]). More than a half of females at 300, 310, and 320 nm moved from UV-irradiated area to the area covered with UV-opaque film within 20 min. In contrast, about a half of females at 340 nm remained on the irradiated half until 80 min, and more than a half individuals at 360 and 380 nm remained until 120 min later.

The avoidance behavior in presence of prey eggs under irradiation with the shorter wavelengths was likely quicker than that when prey eggs and residues were absent (Fig. 3), although the reason was not clear. Avoiding behavior was slower with longer the wavelength; the pattern at 340 nm was not significantly different from 360 nm \( Pr(> z ) = 0.74980 \), 360 nm was not different from 380 nm \( Pr(> z ) = 0.40571 \), and eventually 380 nm was not different from the dark control \( Pr(> z ) = 0.44887 \). Overall, the effects of prey eggs arresting the escaping of \( N. \) californicus females were small.

With Prey Eggs and Residues. Intact prey residues with eggs functioned to keep phytoseiid mites at UV + under irradiation with UV at ≥310 nm (Fig. 5). The
escaping patterns at ≥310 nm, except 320 nm, were not significantly different from that in the dark control (multiple comparisons of GLMMs, Pr(>z) = 0.1930, 0.3019, 0.2212, and 0.9257 at 310, 340, 360, and 380 nm, respectively). The pattern at 320 nm was not significantly different from that at 310, 340, and 360 nm (Pr(>z) = 0.8552, 0.8744, and 0.8255, respectively). However, the P values were 0.0412 and 0.0252 against 380 nm and the dark control, respectively. This probably reflects that a substantial number of individuals moved to the UV− side later than 60 min. Therefore, the remarkable arresting effects may be also true at 320 nm in comparison with the results in treatments (1) and (2) (Figs. 3 and 4). As prey eggs had only small effects on *N. californicus* females to stay on the leaf area irradiated with UV (Fig. 4), the factors arresting the escaping of the females were due to the function of prey residues (webs including excreta).

In contrast, the retaining effects of intact prey residues on *N. californicus* females disappeared at 300 nm (Fig. 5; i.e., females quickly escaped from UV+; Pr(>z) = 0.0343 between 300 and 320 nm and Pr(>z) < 0.001 between 300 nm and all other wavelengths). As a result, the escaping pattern was unique to 300 nm among wavelengths, suggesting that irradiation with UV at 300 nm wavelength harmful to egg hatchability (Fig. 2) helped overcome the arresting effects of prey residues on *N. californicus* females.

**Discussion**

Solar UV radiation has substantial effects on the survival and behavior of herbivorous spider mites (*Ohitsuka* and *Osakabe* 2009, *Sakai* and *Osakabe* 2010, *Sakai* et al. 2012) and predacious phytoseiid mites (*Onzo* et al. 2010, *Tachi* and *Osakabe* 2012). UVB fractions of solar radiation around 300 nm have a biological impact, and the UV damage can generally extend to longer wavelengths and frequently occurs at UVA wavelengths also in bacteria (*Coohill* and *Sagripanti* 2009) and zooplankton (*Copepoda*; *Kouwenberg* et al. 1999b). However, the biological impact on *N. californicus* eggs changed drastically at wavelengths between 300 and 310 nm, corresponding to the hatchability of *T. urticae* eggs (*Sakai* and *Osakabe* 2010). Similar active spectra have also been reported in a marine fish, the Atlantic cod *Gadus morhua* L.; UV irradiation at <305 nm strongly reduced egg hatchability, whereas irradiation at ≥312 nm caused weaker or no detrimental effects (*Kouwenberg* et al. 1999a).

As for other phytoseiid mites, *Onzo* et al. (2010) reported that not only eggs but also adult females died after irradiation emitted by a UVB lamp peaked at 312 nm. These authors irradiated eggs and mites with UVB lamps at a high intensity, 6.8 W m−2, for 30–300 min (*Onzo* et al. 2010), resulting in much higher cumulative doses of UVB (12.2–122.4 kJ m−2) than the LD50 values in phytoseiid mite eggs (<0.3 kJ m−2; *Tachi* and *Osakabe* 2012). If the UVB lamp emitted spectra of relatively broad wavelengths, the tails of spectra below 300 nm would have had deleterious effects on the survival of phytoseiid mites, which is frequently true in such equipment (*Tachi* and *Osakabe* 2012).

Many animals are unable to distinguish solar UVB radiation from other wavelengths (*Smith* and *Macagno* 1990, *Tovée* 1995, *Bancroft* et al. 2008), and some exploit the UVA fraction to avoid UVB radiation (*Bothwell* et al. 1994, *Kelly* and *Bothwell* 2002) as well as *T. urticae* (*Sakai* and *Osakabe* 2010). In contrast, the flower thrip *Frankliniella occidentalis* (*Pergande*), a herbivorous insect, possesses spectral sensitivities to UVB in its eyes (*Mazza* et al. 2010). The observed differences in UV avoidance response rates among monochromatic radiation at different wavelengths indicate the capacity of *N. californicus* females to discern variation in wavelengths of UV radiation, such as UVB from UVA. *Tachi* and *Osakabe* (2012) reported that *N. californicus* escaped from solar UV radiation more quickly than visible radiation. However, UVB recognition by *N. californicus* has never been reported so far.

Wells and excretion of spider mites and odor produced by host plants on account of the damage by spider mites serve as important prey cues for phytoseiid mites to remain in the habitat (*Hislop* and *Prokopy* 1981, *Hoy* and *Smilanick* 1981, *Sabelis* et al. 1984). *Furiuchi* et al. (2005) demonstrated that prey preference of a phytoseiid mite *Neoseiulus crosnieri* *Schicha* (*Acari: Phytoseiidae*) was determined by prey wells but not by prey eggs. In this study, the detaining effects of intact prey residues on *N. californicus* overcame escaping from UV radiation at safe wavelengths (≥310 nm). The difference in the effects detaining *N. californicus* between eggs and intact residues (including eggs) may be caused by the presence/absence of prey webs (including excreta). Because the wells remaining on the leaf strip was not likely to protect the phytoseiid mites (eggs) from UVB irradiation in our preliminary experiment, the detaining effects were probably caused by the function as prey cue, not as shelter. In contrast, *N. californicus* escaped with no regard to prey cues when mites were irradiated at harmful UVB wavelengths of 300 nm. Solar UVB intensity changes seasonally and also diurnally. Such an environmental variation and the shift in behavioral response depending on the presence/absence of prey cue would affect determination of their spatial distribution through the dynamics such as movement between interior and exterior canopy (*Villanueva* and *Childers* 2005). Study on the long-term reactions is also required to elucidate the spatiotemporal distribution of plant-dwelling mites in the field.

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