Temperature and Dietary Effects on UV Damage to Spider Mites and Phytoseiid Mites

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Contents

Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch, is an economically important agricultural pest worldwide because of significant acaricide resistance development. Recently, a novel spider mite control method by nighttime UV-B irradiation in strawberry greenhouses has been developed in Japan. However, the control effects by UV-B irradiation are reduced by increased overlap of crop leaves. Previous studies suggeste that a phytoseiid mite, *Neoseiulus californicus* McGregor, well known biological control agent, hides from UV-B irradiation and entering the shade of leaves, leading to efficient predation of spider mites that survive in the shade. In this study, I aimed to evaluate concurrent use of UV-B technology and biological control with *N. californicus* toward establishment of integrated pest management (IPM).

Consequently, I obtained three important findings. First, although phytoseiids are vulnerable to a single UV-B irradiation, *N. californicus* eggs were resistant to UV-B radiation under the daily nighttime irradiation condition. I identified a range of doses that were lethal to *T. urticae* eggs but not to *N. californicus* eggs. Second, the ovicidal effect by nighttime UV-B irradiation was temperature dependent; more efficient in lower temperature against both *T. urticae* and *N. californicus* eggs. Third, strawberry pollen contained abundant spermidine derivatives with powerful antioxidant activity, and survival of *N. californicus* under UV irradiation increased via pollen diet. Other than these, I also investigated the effects of temperature and timing of UV-B irradiation on photoreactivation and embryogenesis in *T. urticae* eggs. These findings show the advantage of simultaneous use of UV-B and the phytoseiid mite for IPM systems.

Chapter 1

General introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan agricultural pest that harms numerous crops (Bolland et al. 1998). This spider mite has high reproductive capacity and short-cycle and developed resistance to most of the commercially available acaricides (Van Leeuwen et al. 2010, 2015), resulting in chemical control failure worldwide. In integrated pest management (IPM), the need for reducing the acaricide applications urges researchers to develop efficient control strategy other than chemical control such as physical and biological control methods (Dara 2019).

Based on the lethal damage inflicted on *T. urticae* by ambient and artificial ultraviolet-B (UV-B) radiation (Barcelo 1981; Ohtsuka and Osakabe 2009; Suzuki et al. 2009; Sakai and Osakabe 2010), a novel physical control method for the management of spider mites in strawberry greenhouses using UV-B lamps and light reflection sheet (UV method) has been developed by Tanaka et al. (2016). UV-B radiation induces resistance of strawberry plants against powdery mildew (Kanto et al. 2009, 2014). Thus, the UV method enables simultaneous control of herbivorous and disease pests, drastically reducing the need for pesticide applications.

The major biological effects of UV-B radiation are the direct generation of DNA lesions, such as cyclobutane pyrimidine dimers (CPDs, Murata and Osakabe 2017a), and oxidative stress resulting from the generation of reactive oxygen species that cause damage such as lipid peroxidation (Atarashi et al. 2017). As an adaptation to ambient UV-B radiation, spider mites and many other organisms undergo photoreactivation involving photoenzymatic repair (PER) of CPDs using light energy (Thoma 1999; Sinha and Häder 2002; Weber 2005; Rastogi et al. 2010; Murata and Osakabe 2014, 2017a) increasing their survival during UV-B irradiation (Santos 2005; Murata and Osakabe 2014; Koveos et al. 2017; Yoshioka et al. 2018). Conveniently, the photoreactivation of eggs of the two-spotted spider mite *T. urticae* can be blocked by keeping them in the dark (~4 h) after UV-B irradiation (Murata and Osakabe 2014; Yoshioka et al. 2018).

Therefore, in the UV method, UV-B irradiation is performed for 3 h at midnight, resulting in excellent control of *T. urticae* (Tanaka et al. 2016).

The degree of UV-B-induced damage differs according to biotic and abiotic factors (Häder et al. 2007; Alton et al. 2017). Of those factors, temperature likely affects survival under UV-B radiation because of its influence on the rate of development, physiological protection systems, behavior, and ecology. However, the thermal dependence of UV-B damage is controversial. The growth rate of the marine protozoan *Parauronema acutum* (Philasterida: Parauronematidae) is higher at 20°C than at 15 or 25°C under UV-B irradiation (Hamsher et al. 2018). The marine bacterium *Sphingopyxis alaskensis* (Sphingomonadales: Sphingomonadaceae) suffers greater DNA damage at 24°C than at 12°C (Matallana-Surget et al. 2010), but the sensitivity to UV-B of *Escherichia coli* (Enterobacterales: Enterobacteriaceae) is higher at 20°C than at 37°C (Mangoli et al. 2014). The tolerance to UV-B increases with increasing temperature in *Daphnia catawba* (Cladocera: Daphniidae) and the calanoid copepod *Leptodiaptomus minutus* (Calanoida: Diaptomidae), but it decreases in the rotifer *Asplanchna girodi* (Ploima: Asplanchnidae) (Williamson et al. 2002). Embryos and tadpoles of the frog *Limnodynastes peronii* (Anura: Limnodynastidae) suffers greater damage from UV-B radiation at 20°C than at 30°C (Van Uitregt et al. 2007).

Most plant-dwelling mites including spider mites and phytoseiid mites prefer to stay on the lower leaf surfaces of host plants as an adaption to solar UV-B radiation (Ohtsuka and Osakabe 2009; Sudo and Osakabe 2011). The control effect on spider mites is proportional to the daily cumulative UV-B irradiance on the lower leaf surfaces, where spider mites reside (Tanaka et al. 2016), suggesting importance of the magnitude of UV-B irradiation of the lower leaf surface. To ensure that UV-B irradiation of sufficient intensity reaches the lower leaf surface via light reflection sheets, Tanaka et al. (2016) used wider intervals between rows and plants than is typical for commercial greenhouses. However, as leaves grow and produce shade, the effect of UV-B irradiation may degrade due to leaf overlap. Excessive UV-B radiation causes leaf sunscald, which is more likely in winter (Tanaka et al. 2016). Therefore, optimal UV-B irradiance and a sustainable control agent are needed to manage pests in strawberry greenhouses.

Phytoseiid mites are widely used as biological control agents of herbivorous mites and insects in horticulture (Gerson 2014). *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is known as a highly effective agent of biological control of spider mites worldwide (Ahn et al. 2010; Khanamani et al. 2017; Ajila et al. 2019). Phytoseiid mites can use pollen as alternative or supplementary food resources to aid their survival and reproduction in the absence of their primary prey (McMurtry and Croft 1997). *Neoseiulus californicus* eggs are more vulnerable to a single acute dose of UV-B irradiation than are *T. urticae* eggs (Tachi and Osakabe 2012). Studies indicate that *N. californicus* tends to hide from leaf areas irradiated with UV-B (Tachi and Osakabe 2012, 2014) and move to non-irradiated areas, where *T. urticae* might also survive, suggesting greater predation efficacy under UV-B irradiation. Although hiding *T. urticae* itself on lower leaf surfaces probably dampens the control effect of artificial UV-B irradiation, similar taxis of *N. californicus* protects itself from UV-B irradiation, suggesting simultaneous applications of phytoseiid mites and UV method which promotes the adoption of IPM. Nakai et al. (2018) reported that *N. californicus* eggs are more resistant to daily nighttime UV-B radiation than *T. urticae* eggs. However, dose response and temperature dependence of the mortality between the pest mite and phytoseiid mite caused by daily nighttime UV-B irradiation should be studied in order to improve IPM program.

Photoreactivation was observed in *T. urticae* (Murata and Osakabe 2014) and *N. californicus* (Nakai et al. 2018; Sugioka et al. 2018). Koveos et al. (2017) showed that phytoseiid mite eggs had greater resistance to UV-B than did *T. urticae* eggs under continuous UV-B irradiation without photoreactivation, but the resistance reversed under simultaneous visible light (VIS) irradiation. However, the UV-B irradiation is performed in midnight without simultaneous VIS irradiation in the UV method, differing from Koveos et al. (2017).

Like the UV-B damage, the degree of photoreactivation efficiency may be affected by temperature. Connelly et al. (2009) found that photoenzymatic repair removed DNA damage more rapidly at 10°C than at 20°C in four *Daphnia* species (Cladocera: Daphniidae). In contrast, the PER repair was more efficient at high temperatures in *Daphnia pulicaria* (Cladocera: Daphniidae) (MacFadyen et al. 2004). Quek and Hu (2008) reported that higher photoreactivation levels were observed in *E. coli* when

exposed to optimum growth temperature than those with too high and too low temperatures. Elucidation of photoreactivation phenomenon and its temperature dependence will enhance our understanding of the influence of UV-B and VIS radiation on prey-predator systems in plant-dwelling mite community.

Egg is the most sensitive stage of *T. urticae* to UV-B irradiation (Murata and Osakabe 2013). The susceptibility to UV-B of *T. urticae* eggs peaked at 24–48 h after oviposition and subsequently decreased until hatching (Murata and Osakabe 2014, Yoshioka et al. 2018). The period 24–48 h after oviposition roughly corresponds to the larval body formation stage based on the germinal disk (Dearden et al. 2002). However, details about the UV-B-induced damage to embryogenesis and morphogenesis of *T. urticae* eggs has been unknown. Moreover, the first exposure of UV-B irradiation for *T. urticae* eggs is in the period 0–24 h after oviposition because the UV method is performed every night. It is necessary to study how UV-B irradiation damages embryos of newborn eggs under a low and intermittent condition since the UV-B intensity is not very high in the greenhouses for avoiding leaf sunscald.

UV-B irradiation of *N. californicus* eggs affects the development of hatched larvae (Nakai et al. 2018). Therefore, protecting phytoseiid mites from UV-B radiation is still important for the concurrent use of phytoseiid mites with UV-B lamps. Sugioka et al. (2018) reported increased UV-B resistance in *N. californicus* feeding on tea and peach pollen. Pollen grains contain bioactive substances, such as antioxidants, to protect germ cells from solar UV-B radiation (Feng et al. 2000; Žilić et al. 2014). Several antioxidants have been identified from peach and tea pollen extracts, and tea pollen contains high concentrations of very active antioxidants, such as catechins (Sugioka et al. 2018). However, it is not easy for farmers to collect enough tea or peach pollen in a short time for spreading across the greenhouses. In strawberry greenhouses, flowers bloom continuously, making strawberry pollen more available than other pollen. If *N. californicus* can use strawberry pollen as an alternative food and the pollen-diet mitigates its UV-damage, it would be advantageous for the concurrent use of *N. californicus* with the UV method in strawberry greenhouses.

With the serious development of acaricide resistance in spider mites in the greenhouses, the new effective IPM strategy based on the UV method becomes attractive. This study

aims to study about the UV-B susceptibility and photoreactivation capacity between *T. urticae* and *N. californicus*, and evaluation of pollen from host plants as a protective ingredients for phytoseiid mite from UV-B irradiation. If UV-B resistance and photoreactivation capacity is higher in *N. californicus* than *T. urticae*, it is convenient to release the phytoseiid mite in a UV-B-irradiated greenhouses for higher control effect in spider mites and reduction of acaricide is expected. If strawberry pollen diet can protect *N. californicus* against UV-B, it is expected to make the IPM system (combination of the UV method with phytoseiid mites) more sustaining in greenhouses.

In Chapter 3, I aimed to clarify the UV-B dose response of biological impact and the effects of temperature on the biological impact in pest (prey) and predatory mites. For that purpose, I simulated daily nighttime UV-B irradiation in a strawberry greenhouse using a growth chamber. First, I investigated the effect of daily UV-B irradiance on *T. urticae* and *N. californicus* eggs and analyzed the 50% lethal dose (LD_{50}) per day. Second, I evaluated the effect of temperature on the impact of UV-B irradiance at around the LD_{50} value.

In Chapter 4, I also clarified the effects of temperature on photoreactivation in pest (prey) and predatory mites after temperature effect on UV-B irradiation was clear in Chapter 3. I compared the importance of photoreactivation to survival of eggs between *T. urticae* and *N. californicus* after UV-B irradiation and evaluate the effect of temperature on the photoreactivation efficiency in these two species.

In Chapter 5, I aimed to clarify the effects of temperature on the speed of embryonic development of *T. urticae* and influence of UV-B on embryogenesis which may explain the UV-B susceptibility among different temperatures in Chapter 3. First, I investigated the effects of temperature in embryogenesis of *T. urticae* eggs by observing embryonic development during the whole egg duration. Second, I evaluated the effects of UV-B irradiated timing on embryonic termination and found out the most vulnerable stage to UV-B irradiation.

In Chapter 6, to establish spider mite management combining UV method with biological control, I evaluated the quality of strawberry pollen as an alternative food and a source of UV-B protection ingredients. I compared the juvenile development and egg production of *N. californicus* between pollen and mite diet. Then, I investigated survival

rate and egg production of *N. californicus* between strawberry pollen and mite diet after UV-B irradiation. At last, I evaluated the antioxidant activity in strawberry pollen and identified the main antioxidant compounds in strawberry pollen.

Chapter 2

Materials, equipment, and laboratory conditions

Mites

The *T. urticae* population used in this study comprised several local populations in Japan and had been reared on kidney bean (*Phaseolus vulgaris*) plants in a laboratory at 25–28°C for over a decade.

The *N. californicus* strain was purchased in January 2017 (Spical® EX, Arystra Life Science Co., Tokyo, Japan) and fed *T. urticae* on kidney bean leaf discs (5 cm in diameter) placed on water-soaked cotton in Petri dishes (9 cm in diameter) at 25°C and 80% relative humidity under a 16:8 (L:D) h photoperiod.

Pollen (Chapter 6)

Buds of strawberry *Fragaria* × *ananassa* variety "Tochiotome" were collected from a greenhouse (without UV-B lamps) at the Tochigi Prefectural Agricultural Experiment Station, Utsunomiya, Japan in April 2018 and December 2018 to March 2019. Buds of tea *Camellia sinensis* were collected in October 2017 and 2018 from plants at the Kitashirakawa Experimental Field of Kyoto University, Kyoto, Japan. Anthers were separated from the buds using tweezers. After the anthers dehisced, pollen was gathered from the anthers using a sieve. The pollen was frozen at -20 °C until used in experiments.

UV-B and fluorescent light used in investigation of UV-B-induced mortality and dysplasia embryonic (Chapter 3, 4 and 5)

The irradiation equipment was identical to that of Murata and Osakabe (2014, 2017a) and Nakai et al. (2018). Briefly, a UV-B lamp (6 W; Panasonic Co. Life Solution Company, Osaka, Japan) was affixed to a shelf 67 cm overhead in a growth chamber illuminated with fluorescent light at ~7,000 lx from 08:00 to 22:00 (14-h light/10-h darkness). The UV-B irradiation peaked at 310 nm (Fig. 2-1). UV-B irradiance and wavelength spectra was measured using an irradiance meter ($X1_1$) with a detector head for UV-B (UV-3702-4; Gigahertz-Optik, Türkenfeld, Germany) and a spectrometer

(UFV-VIS F; Spectra Co-op Co., Tokyo, Japan), respectively. The fluorescent light irradiance was measured using a Light Meter 840006 (Sper Scientific, Ltd., Scottsdale, AZ).

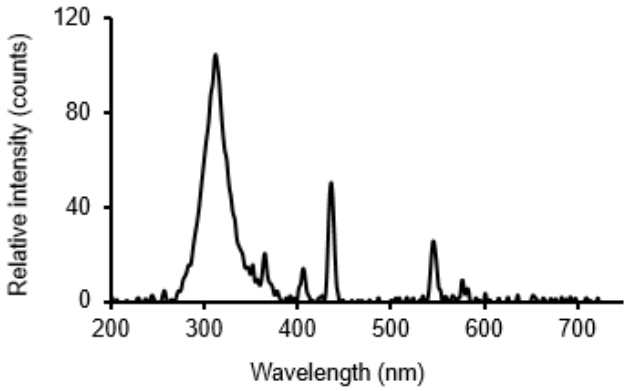

Fig. 2-1 Wavelength spectrums of UV-B lamp (0.16 W m^{-2}) in growth chamber.

In Chapter 3, daily nighttime UV-B irradiation was administered at an intensity adjusted to the experimental dose to Petri dishes on a shelf in the growth chamber from 00:00 to 03:00. The initial UV-B intensity (0.16 W m^{-2}) was adjusted to 0.005–0.04 W m^{-2} (0.054–0.432 kJ m^{-2} d^{-1}) by covering the UV-B lamp with polyethylene sheets of appropriate thickness.

Visible light irradiation (VIS) used in investigation of photoreactivation (Chapter 4)

Two halogen lamps (130 W; JDR110V-85WHM/K7-H; Ushio Lighting Co. Ltd., Tokyo, Japan; set at an interval of 22.5 cm) were affixed to a shelf 67 cm overhead in a growth chamber illuminated with fluorescent light at ~7,000 lx from 08:00 to 22:00 (14-h light/10-h darkness). The halogen lamp irradiation included a small UV-A fraction (Fig. 2-2). The fluorescent light irradiance and wavelength spectra was measured using a Light Meter 840006 (Sper Scientific, Ltd., Scottsdale, AZ) and a spectrometer (UFV-VIS F; Spectra Co-op Co., Tokyo, Japan), respectively.

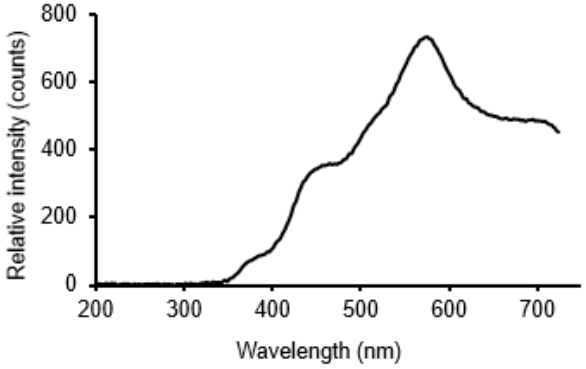

Fig. 2-2 Wavelength spectrums of halogen lamps in growth chamber.

UV-B irradiation system used in investigation of protective effect of strawberry pollen (Chapter 6)

UV-B irradiation of adult female *N. californicus* was performed in a laboratory at 25 °C. The laboratory was illuminated with fluorescent light at ~ 100 lx. Females on kidney bean leaf squares (3.5 cm square) in Petri dishes were placed 0.45 m from an overhead UV-B lamp (20 W; UV-B intensity: 1.43 W m^{-2}, peak wavelength: 312 nm; YGRFX21701GH; Panasonic, Osaka, Japan) (Fig. 2-3) in a steel rack (1.9 × 0.6 × 0.6 m; height × width × depth). The steel rack was encircled with UV-opaque film (0.1-mm thick polyvinyl chloride; Cutaceclean Kirinain; MKV Platech, Tokyo, Japan) to ensure that no UV-B irradiation leaked out. As an unirradiated control, Petri dishes with adult females were placed on another steel rack adjacent to the UV-B-irradiated rack.

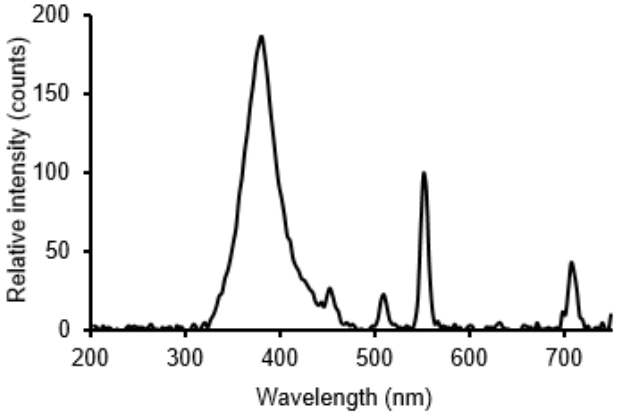

Fig. 2-3 Wavelength spectrums of UV-B lamp (1.43 W m^{-2}) fixed on experimental rack in laboratory.

Chapter 3

Dose response and temperature dependence of the mortality of *Tetranychus urticae* and *Neoseiulus californicus* eggs under daily nighttime UV-B irradiation

Ambient ultraviolet (UV)-B radiation reduces herbivory by arthropods (Rousseaux et al. 2001; Ballaré et al. 2011). Because of the development of pesticide resistance by economically important agricultural pests, the biological impact of UV-B and its utility for pest control have been investigated (Mazza et al. 2002; Kuhlmann and Müller 2009; Burdick et al. 2015; Yin et al. 2018). *Neoseiulus californicus* eggs are more vulnerable to a single acute dose of UV-B irradiation than are *T. urticae* eggs (Tachi and Osakabe 2012). However, the UV method is performed at midnight every day. More information about the susceptibility of *T. urticae* and *N. californicus* eggs under the daily nighttime UV-B is required.

The degree of UV-B-induced damage differs according to biotic and abiotic factors (Häder et al. 2007; Alton et al. 2017). Of those factors, temperature likely affects survival under UV-B radiation because of its influence on the rate of development, physiological protection systems, behavior, and ecology. However, the thermal dependence of UV-B damage is controversial. The growth rate of the marine protozoan *P. acutum* is higher at 20°C than at 15 or 25°C under UV-B irradiation (Hamsher et al. 2018). The marine bacterium *S. alaskensis* suffers greater DNA damage at 24°C than at 12°C (Matallana-Surget et al. 2010), but the sensitivity to UV-B of *E. coli* is higher at 20°C than at 37°C (Mangoli et al. 2014). The tolerance to UV-B increases with increasing temperature in *D. catawba* and the calanoid copepod *L. minutus*, but it decreases in the rotifer *A. girodi* (Williamson et al. 2002). Connelly et al. (2009) found that DNA repair occurred more rapidly, and the survival rate was higher, at 10°C than at 20°C in four *Daphnia* species. In contrast, the survival rate was lower and DNA repair was slower at low temperatures in *D. pulicaria* (MacFadyen et al. 2004). Embryos and tadpoles of the frog *L. peronii*

suffers greater damage from UV-B radiation at 20°C than at 30°C (Van Uitregt et al. 2007).

In this chapter, I aimed to clarify the UV-B dose response of biological impact and the effects of temperature on the biological impact in pest (prey) and predatory mites. For that purpose, I simulated daily nighttime UV-B irradiation in a strawberry greenhouse using a growth chamber. First, I investigated the effect of daily UV-B irradiance on *T. urticae* and *N. californicus* eggs and analyzed the 50% lethal dose (LD_{50}) per day. Second, I evaluated the effect of temperature on the impact of UV-B irradiance at around the LD_{50} value.

Section 1 UV-B dose response in *T. urticae* and *N. californicus* eggs

- Materials and methods

Four Petri dishes (9 cm diameter) were prepared with *T. urticae* and *N. californicus*. For *T. urticae*, four kidney bean leaf squares (2 cm square) were placed on water-soaked cotton in a Petri dish. Next, five adult *T. urticae* females were introduced to each kidney bean disc. For UV-B irradiation (UV+), two of the four Petri dishes were placed in a plastic basket (27 × 20 × 8.5 cm; length × width × height), the top of which was covered with UV-B-transparent polymethylpentene wrap (Japanese Consumers' Cooperative Union, Tokyo, Japan). The plastic wrap transmits >90% of UV-B and VIS radiation. For UV-B non-irradiation (UV−), the other two Petri dishes were placed in a plastic basket covered with UV-opaque film (HB3 polyester film; DuPont Teijin Films, Chester, VA, USA), which completely filters out UV at wavelengths <380 nm (Sakai and Osakabe 2010). The two plastic baskets were immediately placed on the shelf in the growth chamber with daily nighttime UV-B irradiation. The females were removed from all leaf squares after 24 h (day 0). Intact eggs laid on the leaf squares were reared in the growth chamber continuously. Hatchability was checked after 6 days (day 6). The experiment was conducted once per UV-B intensity at 25°C. The numbers of *T. urticae* eggs were 88–231 and 90–227 per irradiance under the UV− and UV+ treatments, respectively.

For *N. californicus*, a single piece of kidney bean leaf square (4 cm square) was placed

on water-soaked cotton in each Petri dish because they tend to escape from UV-B-irradiated areas (Tachi and Osakabe 2012, 2014). Prior to the introduction of *N. californicus*, 10 adult *T. urticae* females were introduced to each leaf square in the four Petri dishes. Two of the four Petri dishes were placed in a plastic basket for UV+ and UV− treatment in the growth chamber. After 24 h, 10 adult females of *N. californicus* were introduced to all leaf squares in the four Petri dishes and reared together with *T. urticae* females and eggs as prey for further 24 h. Next, all adult females of *T. urticae* and *N. californicus* were removed from the leaf squares. Intact eggs of *N. californicus* laid on the leaf were counted (day 0) and continuously reared in the growth chamber. Hatchability was checked after 3 days (day 3). Because *N. californicus* females produced fewer eggs than *T. urticae* females, the experiments were conducted twice for each UV-B intensity at 25°C. The numbers of *N. californicus* eggs were 65–128 and 68–130 per irradiance (total of two experiments) under UV− and UV+, respectively.

To determine the days on which egg hatching should be evaluated, I investigated hatching days at the test temperatures considering the thermal effects discussed below. The times to hatching at 18, 25, and 30°C were 12.3 ± 0.1, 4.5 ± 0.1, and 3.5 ± 0.1 days (± SE), respectively, for *T. urticae* (n = 38) and 5.9 ± 0.1, 2.0 ± 0, and 1.6 ± 0.1 days, respectively, for *N. californicus* (n = 39–40) (Fig. 3-1).

(a)

(b)

(c)

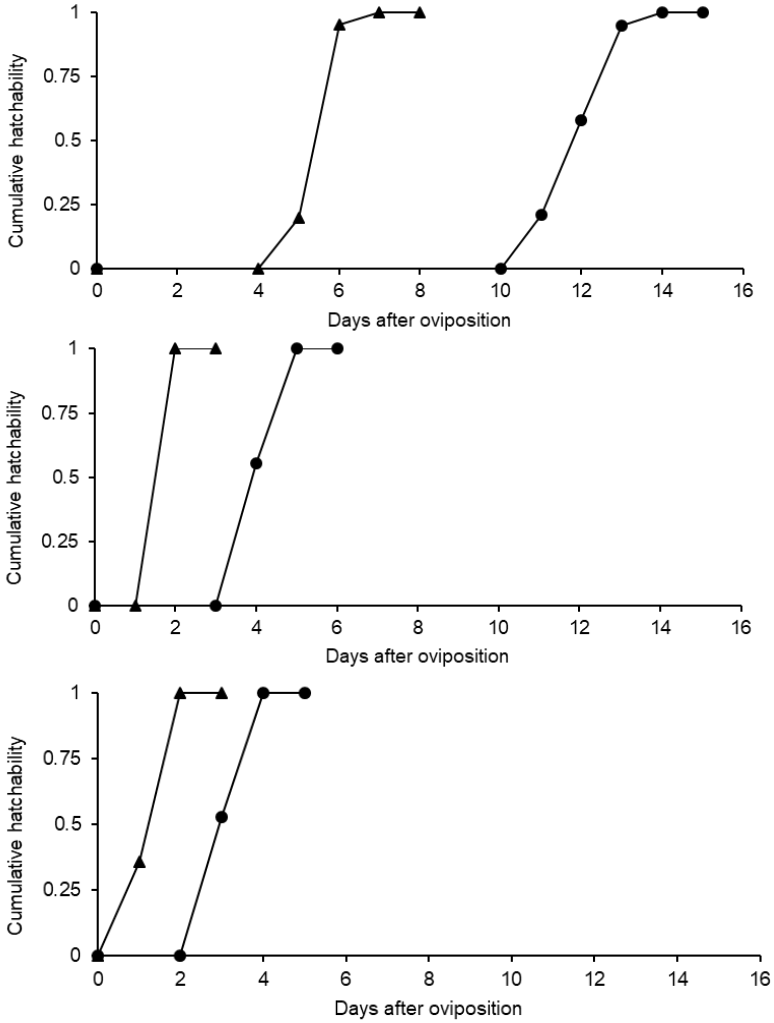

Fig. 3-1 Cumulative hatchability of *T. urticae* and *N. californicus* eggs after oviposition at 18˚C (a), 25˚C (b) and 30˚C (c). Solid circles and triangles are *T. urticae* and *N. californicus*, respectively.

- Data analysis

Mortality was corrected according to Abbott's formula (Abbott 1925):

$$M = \frac{Y - X}{Y},$$

where *M*, *X*, and *Y* represent the corrected mortality, hatchability under UV+, and hatchability under UV−, respectively. I summed the number of eggs on all leaf squares to calculate the corrected mortality.

The corrected mortalities were transformed to probits ($\Phi[0.5] = 5$). To evaluate the effect of UV-B irradiation on mortality, the data set for each mite species was subjected to analysis of covariance (ANCOVA) using R software (ver. 3.6.0; R Core Team 2017) following Aoki (2011). In ANCOVA, the base-10 logarithmic cumulative UV-B irradiances per day, mortality probits, and species were used as the independent, dependent, and group variables, respectively. After ANCOVA, a linear regression analysis was applied to the data set using the "lm" module of R software. The LD_{50} values and associated standard errors (SE) were obtained using the "dose.p" module in the MASS package of R software.

- Results

The hatchability of *T. urticae* and *N. californicus* eggs decreased with increasing daily cumulative UV-B irradiance under UV+, whereas 93.8–100% and 95.5–100% of *T. urticae* and *N. californicus* eggs hatched, respectively, under UV− (Fig. 3-2). *Neoseiulus californicus* eggs showed greater tolerance to UV-B irradiance than did *T. urticae* eggs at 25°C. The hatchability of *T. urticae* decreased from 91.1% to 2.7% when UV-B irradiance increased from 0.054 to 0.2 kJ m^{-2} d^{-1}, whereas it of *N. californicus* decreased from 92.6% to 10.1% as UV-B irradiance increased from 0.135 to 0.432 kJ m^{-2} d^{-1}.

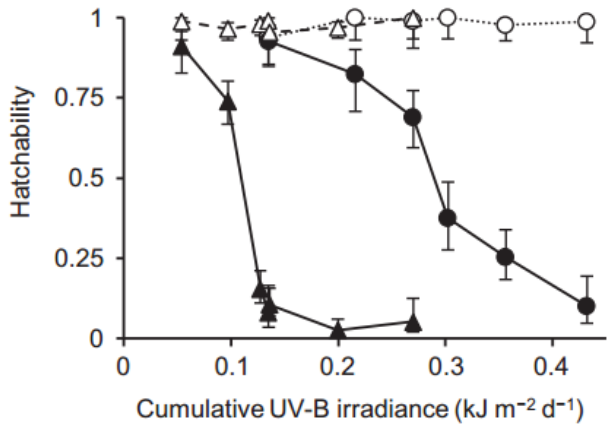

Fig. 3-2 Relationship between cumulative UV-B irradiance and hatchability of *T. urticae* and *N. californicus* eggs. Solid and open circles, *N. californicus* in UV+ and UV−, respectively; solid and open triangles, *T. urticae* under UV+ and UV−, respectively. Vertical lines, 95% CIs.

The mortality of *T. urticae* eggs peaked at 97.4% at 0.2 kJ m^{-2} d^{-1}, so I removed the 0.27 kJ m^{-2} d^{-1} data from the probit analysis. The probit mortality was linearly correlated with the logarithmic daily cumulative UV-B irradiance (Fig. 3-3). The LD_{50} values were 0.095 kJ m^{-2} d^{-1} (95% CI, 0.082–0.111 kJ m^{-2} d^{-1}) and 0.29 kJ m^{-2} d^{-1} (0.276–0.305 kJ m^{-2} d^{-1}) for *T. urticae* and *N. californicus*, respectively. ANCOVA revealed that the average value after adjustment for covariates was significantly different between *T. urticae* and *N. californicus* ($F_{[1, 10]}$ = 61.1, $P = 1.44 \times 10^{-5}$), whereas the slopes of the regression lines of the probit mortality against the logarithmic UV-B irradiation were not significantly different between the two species ($F_{[1, 9]}$ = 1.60, P = 0.237).

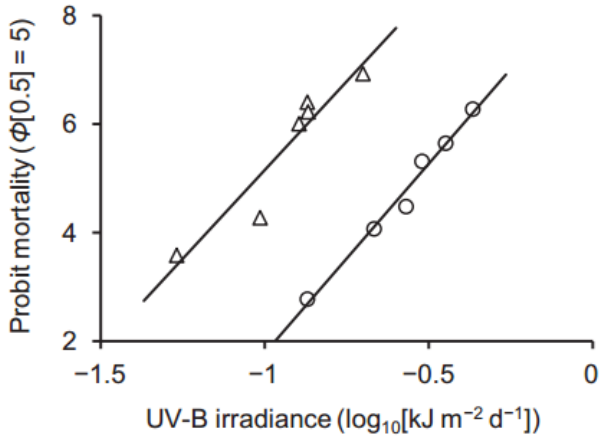

Fig. 3-3 Effect of UV-B dose on mortality. Open circles and triangles, *N. californicus* and *T. urticae*, respectively. The equation of the regression line and the R^2 and *P*-values were $y = 6.9912x + 8.7606$, 0.978, and 1.17×10^{-4}, respectively, for *N. californicus* and $y = 6.544x + 11.685$, 0.8674, and 4.38×10^{-3}, respectively, for *T. urticae*.

Of the *N. californicus* eggs, 92.6% and 83.4% hatched under UV+ at irradiances of 0.135 and 0.216 kJ m^{-2} d^{-1}, while only 8.0% of *T. urticae* eggs hatched at 0.135 kJ m^{-2} d^{-1} (UV+). This indicates that these levels of irradiance seriously damage *T. urticae* eggs but confer no or slight damage to *N. californicus* eggs.

Section 2 Effect of temperature on UV-B damage

- Materials and methods

The biological effect according to temperature was evaluated at 18, 25, and 30°C. The UV-B intensities were 0.009 W m^{-2} (0.0972 kJ m^{-2} d^{-1}) and 0.025 W m^{-2} (0.27 kJ m^{-2} d^{-1}) for *T. urticae* and *N. californicus*, respectively, with reference to the LD_{50} values obtained in the UV-B dose–response experiments. For *T. urticae*, I also tested 0.0125 W m^{-2} (0.135 kJ m^{-2} d^{-1}) at 25°C and 30°C because Nakai et al. (2018) found 70.8% hatchability for *T. urticae* at 25°C and 0% at 20°C under 0.012 W m^{-2} (0.13 kJ m^{-2} d^{-1}) irradiation.

Leaf squares bearing *T. urticae* and *N. californicus* eggs were prepared. The number of eggs per leaf square was adjusted to 20 by removing excess eggs (80 eggs per Petri dish; day 0) after removal of female *T. urticae* mites because excessive egg production was expected at 30°C. Such excessive egg production was not expected for *N. californicus*, so its eggs were used without modification. The experiments were performed in triplicate. The total number of *T. urticae* eggs per condition was 160 per replication (two Petri dishes per replication), and the numbers of *N. californicus* eggs for assays at 18, 25, and 30°C were 12–21, 25–36, and 24–52 per replication (two Petri dishes per replication), respectively, under UV− and 22–25, 33–40, and 46–64, respectively, under UV+. Hatchability at 18, 25 and 30°C was checked on day 13, 6, and 4, respectively, for *T. urticae* and on day 6, 3, and 2, respectively, for *N. californicus*. In the first replication of each temperature, I confirmed that no eggs hatched on the next day of the survey date.

- Data analysis

Corrected mortality was calculated for each replication and subjected to arcsine root transformation. The effect of temperature on mortality was assessed by one-way analysis of variance (ANOVA) using the “aov” module in R software followed by a *post hoc* test for multiple comparisons by Tukey honestly significant difference (HSD) test using the

"TukeyHSD" module in R software. For homoscedasticity, the corrected mortalities were subjected to arcsine root transformation followed by Bartlett's test for homogeneity of variance using the "Bartlett.test" module in R software.

- Results

The hatchability of *T. urticae* eggs under UV− was 93.5–97.7% and 95.5–96.1% after UV-B irradiation at 0.0972 kJ m^{-2} d^{-1} and 0.135 kJ m^{-2} d^{-1}, respectively. The corrected hatchability (= 1 – M) under UV+ was lowest at 18°C and increased as temperature increased to 30°C at 0.0972 kJ m^{-2} d^{-1} (one-way ANOVA; $F_{[2, 6]} = 145.2$, $P = 8.29 \times 10^{-6}$; Fig. 3-4a). At 0.135 kJ m^{-2} d^{-1}, more *T. urticae* eggs hatched at 30°C than at 25°C (one-way ANOVA; $F_{[1, 4]} = 46.1$, $P = 0.00245$; Fig. 3-4b).

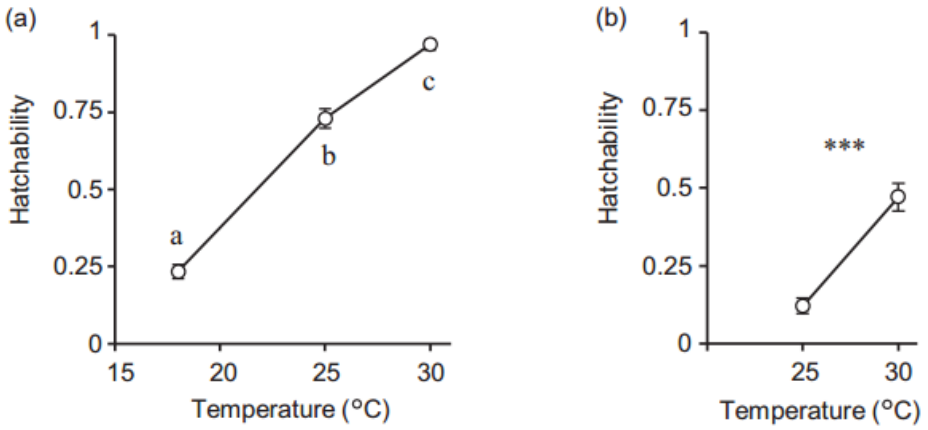

Fig. 3-4 Effect of temperature on hatchability of *T. urticae* eggs irradiated with UV-B at (a) 0.0972 kJ m^{-2} d^{-1} and (b) 0.135 kJ m^{-2} d^{-1}. Different letters in (a) indicate significant differences among temperatures (Tukey HSD test, $P < 0.05$). Asterisks in (b) indicate significant differences between temperatures (ANOVA, $P < 0.001$). Vertical lines indicate SE.

Of the *N. californicus* eggs, 98.3–99.7% hatched under UV− at 0.27 kJ m^{-2} d^{-1}. The corrected hatchability under UV+ was lowest at 18°C and increased as the temperature increased to 30°C (one-way ANOVA; $F_{[2, 6]} = 72.16$, $P = 6.36 \times 10^{-5}$; Fig. 3-5), as was true for *T. urticae*. *N. californicus* eggs were more resistant to daily nighttime UV-B irradiation than *T. urticae* eggs, irrespective of temperature.

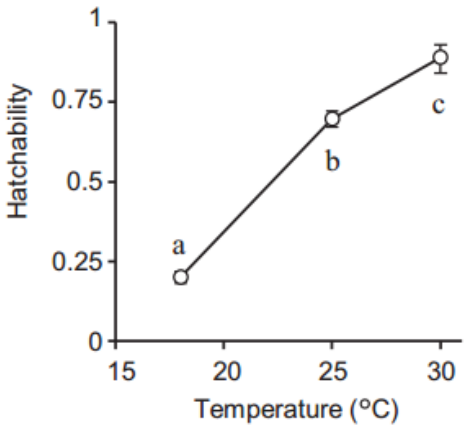

Fig. 3-5 Effect of temperature on the hatchability of *N. californicus* eggs irradiated with UV-B at 0.27 kJ m^{-2} d^{-1}. Different letters indicate significant differences among temperatures (Tukey HSD test, $P < 0.05$). Vertical lines indicate SE.

Section 3 Discussion

The LD_{50} value of daily nighttime UV-B irradiation in *T. urticae* eggs (0.095 kJ m^{-2} d^{-1}) was lower than that of a single UV-B irradiation (0.58 kJ m^{-2}; Murata and Osakabe 2013). However, in that previous study, VIS and UV-B were applied simultaneously (Murata and Osakabe 2013), so that substantial photoreactivation likely occurred (Murata and Osakabe 2014, Murata and Osakabe 2017a). On the other hand, photoreactivation of *T. urticae* eggs is largely abolished if UV-B-irradiated mites remain in darkness for 4 h (Murata and Osakabe 2014). This is why UV-B irradiation is performed in greenhouses strawberries at midnight (Tanaka et al. 2016). In this chapter, the time lag between UV-B and VIS irradiation was 5 h, so that photoreactivation was likely abolished in *T. urticae* eggs, although the effect on *N. californicus* eggs is unknown.

Murata and Osakabe (2014) showed that the mortality of *T. urticae* eggs irradiated with UV-B at 0.288 kJ m^{-2} and kept in darkness without photoreactivation at 25°C was 85%, but it decreased to 32% if the eggs were irradiated with VIS immediately after a single UV-B irradiation. The regression line in section 1 showed a 99.9% mortality at 0.288 kJ m^{-2} d^{-1}, suggesting that daily nighttime UV-B irradiation was equivalent to or more effective than a single UV-B irradiation without photoreactivation. According to Nakai et al. (2018), the mortality of *N. californicus* eggs after UV-B irradiation at 0.288 kJ m^{-2}

without photoreactivation at 25°C was 83.3%, equivalent to the rate for *T. urticae*. However, contrary to *T. urticae*, the mortality at the same daily dose in this chapter was calculated to 49.2% using the regression line for *N. californicus*, suggesting that daily nighttime UV-B irradiation conferred less damage on *N. californicus* eggs than did a single UV-B irradiation. In Nakai et al. (2018), the hatchability of *N. californicus* eggs exposed to VIS radiation after UV-B irradiation recovered to 94.2%, indicating effective photoreactivation. Therefore, daily nighttime UV-B irradiation may partially suppress photoreactivation in *N. californicus* eggs, but the underlying mechanisms are unclear.

In Section 1, range of daily UV-B irradiance (0.13–0.22 kJ m^{-2} d^{-1} at 25°C) that killed most *T. urticae* eggs, but not many *N. californicus* eggs was found. Nakai et al. (2018) also suggested that the mortality of *N. californicus* eggs, unlike that of *T. urticae*, was not increased at 20°C compared to 25°C. However, I found decreased and increased hatchability at lower and higher temperatures, respectively, in both *T. urticae* and *N. californicus* eggs. The magnitude of the response to temperature was similar, suggesting that the range of daily UV-B irradiance that damaged *T. urticae* eggs but not *N. californicus* eggs was maintained, but it shifted according to temperature. The larval fitness of the common frog, *Rana temporaria* (Anura: Ranidae), is affected by exposure to UV-B radiation at their embryo stage (Pahkala et al. 2001). The survival rate of *N. californicus* larvae hatched from UV-B-irradiated eggs was also decreased in Nakai et al. (2018). Therefore, to understand the comprehensive damage to *N. californicus* eggs by UV-B, evaluation including developmental success of larvae hatched from UV-B irradiated eggs is required.

The marked photoreactivation suggests the importance of repair of UV-B-induced DNA lesions after exposure to UV-B radiation in *T. urticae* (Murata and Osakabe 2014). *T. urticae* has four CPD-photolyase genes (Grbić et al. 2011). Murata and Osakabe (2017a) reported that 90% of CPDs produced by 0.288 kJ m^{-2} UV-B radiation in *T. urticae* larvae was repaired by the VIS irradiation for 30 min at 25°C. In contrast, the *T. urticae* genome does not harbor (6-4) photoproduct ((6-4)PP)-photolyase genes (Grbić et al. 2011); (6-4)PPs are likely repaired by nucleotide excision repair (NER) and/or other repair systems. Murata and Osakabe (2017a) reported complete disappearance of (6-4)PPs at 24 h after UV-B irradiation of *T. urticae* larvae. The susceptibility to UV-B of

T. urticae eggs peaked at 24–48 h after oviposition and subsequently decreased until hatching (Murata and Osakabe 2014, Yoshioka et al. 2018). The period 24–48 h after oviposition roughly corresponds to the larval body formation stage based on the germinal disk (Dearden et al. 2002). Most *T. urticae* larvae irradiated with UV-B immediately after hatching developed to the protochrysalis stage but died due to failed molting, morphological defects, and abnormal contraction of the integuments (Murata and Osakabe 2017b). Although the effect of temperature on UV-B damage has been discussed based on temperature-dependent changes in the activity of repair enzymes, the effect on survival varies among organisms and according to several environmental factors (Alton and Franklin 2017; Connelly et al. 2009; Alton and Franklin 2012).

Considering the above together with the neutralization of photoreactivation in *T. urticae* eggs by the time lag between UV-B and VIS irradiation, repair of DNA damage prior to the stages that require active gene expression (e.g., embryogenesis and morphogenesis) is likely essential for survival of *T. urticae* exposed to UV-B radiation. In this context, temperature may influence the magnitude of UV-B-mediated damage to *T. urticae* eggs by daily nighttime irradiation depending on the shift of egg stages in which they exposed to UV-B irradiation and change in the duration of UV-B vulnerable stages.

This idea may explain the difference between *T. urticae* and *N. californicus*, as it may be due to the more rapid development of the latter; egg duration was about 3.06 and 1.7 (female) days at 25°C in *T. urticae* and *N. californicus*, respectively (Bounfou and Tanigoshi 2001; Gotoh et al. 2004). According to Sugioka et al. (2018), the susceptibility to UV-B of *N. californicus* eggs peaked at 8–16 h after oviposition at 25°C. Then, they quickly increase resistance against UV-B irradiation after 16 h from oviposition at 25°C (Sugioka et al. 2018). Therefore, more and fewer eggs might have attained the resistant stage by the next UV-B irradiation time at 30°C and 18°C, respectively. On the other hand, Koveos et al. (2017) showed that phytoseiid mite eggs had greater resistance to UV-B than did *T. urticae* eggs under continuous UV-B irradiation without photoreactivation, but the reverse was the case under simultaneous VIS irradiation. Although *N. californicus* also shows significant photoreactivation after a single UV-B irradiation following VIS irradiation (Nakai et al. 2018; Sugioka et al. 2018), *T. urticae*

might largely depends on photoreactivation rather than phytoseiid mites as described by Koveos et al. (2017).

Reactive oxygen species production is also important in considerations of the biological impact of UV-B radiation. Although most plant-dwelling mites avoid solar UV radiation by residing on the lower leaf surface of host plants (Sudo and Osakabe 2011), the citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), remains on the upper leaf surfaces and shows greater tolerance to UV-B radiation than *T. urticae* (Fukaya et al. 2013). *Panonychus citri* constitutively produces and accumulates astaxanthin, an eminent antioxidant (Metcalf et al. 1962; Bryon et al. 2017). However, the survival rates of wild-type or albino (no astaxanthin) strains of *P. citri* under UV-B radiation were not different, meaning that astaxanthin did not affect survival, in spite of its effect that significantly reduced accumulation of lipid peroxides (Atarashi et al. 2017). In contrast, *N. californicus* eggs produced by females fed on *P. citri* showed higher hatchability after a single UV-B irradiation without photoreactivation in comparison with that produced by females fed on *T. urticae* (Nakai et al. 2018). Similarly, the UV-B resistance of *N. californicus* (females and eggs) was improved by antioxidants from pollen, an alternative food for phytoseiid mites (Koveos et al 2017), implying that the systems used to adapt to UV-B differ between *T. urticae* and *N. californicus*.

This chapter revealed difference in the response to UV-B between pest mites and their predators and showed temperature dependent changes of the effect of UV-B on mites without photoreactivation. These findings indicate that low temperatures in winter enhance the effect of UV-B radiation on *T. urticae* and decreased UV-B irradiance is profitable not only for preservation of strawberry plants from the damage by UV-B irradiation but also for that of the predators. In spring, degradation of UV-B efficacy is caused by not only shadowing due to plant growth but also temperature-dependent changes in the vulnerability of *T. urticae*. The higher tolerance of phytoseiid mites than spider mites makes possible the application of UV-B doses that are lethal for spider mites but safe for phytoseiid mites. Overall, it would be concluded that combined use of phytoseiid mites with UV-B lamps is advantageous to spider mite management in strawberry greenhouses. This strongly supports the possibility of commercially practical utilization of the UV method (Tanaka et al. 2016). To determine the physiological

mechanisms underlying the differences between *T. urticae* and *N. californicus* in the effects of temperature and UV-B and the role of irradiation conditions, further studies are required.

Chapter 4

Temperature dependence of photoreactivation of *T. urticae* and *N. californicus* eggs after a single acute UV-B irradiation

Murata and Osakabe (2014) reported that up to 57% of *T. urticae* eggs were reactivated by the photoreactivation after exposure to UV-B radiation. In Nakai et al. (2018), the hatchability of *N. californicus* eggs exposed to VIS radiation after UV-B irradiation recovered from 16.7% to 94.2%, indicating effective photoreactivation. Koveos et al. (2017) showed that phytoseiid mite eggs had greater resistance to UV-B than did *T. urticae* eggs under continuous UV-B irradiation without photoreactivation, but the reverse was the case under simultaneous VIS irradiation, suggesting *T. urticae* might largely depends on photoreactivation rather than phytoseiid mites.

In Chapter 3, the temperature dependence on UV-B-induced mortality of *T. urticae* and *N. californicus* eggs has been confirmed. However, the thermal dependence of photoreactivation in *T. urticae* and *N. californicus* is still unknown. Connelly et al. (2009) found that photoenzymatic repair removed DNA damage more rapidly at 10°C than at 20°C in four *Daphnia* species. In contrast, the PER repair was more efficient at high temperatures in *D. pulicaria* (MacFadyen et al. 2004). Quek and Hu (2008) reported that higher photoreactivation levels were observed in *E. coli* when exposed to optimum growth temperature than those with too high and too low temperatures.

In this chapter, I aimed to clarify the effect of temperature on the photoreactivation efficiency in *T. urticae* and *N. californicus* eggs. First, I compared the capacity for photoreactivation in *T. urticae* and *N. californicus* at 25°C. Then, photoreactivation efficiency was evaluated among 18°C, 25°C, and 30°C.

Section 1 Temperature dependence of photoreactivation

- Materials and methods

For *T. urticae*, four pieces of kidney bean leaf squares (2 cm square) are placed on water-soaked cotton in each of two petri dishes (9 cm diameter). Five adult *T. urticae* females were introduced to each leaf square and allowed to lay egg for 24 h in growth chamber with 14:10 (L:D) h photoperiod at 25°C. Then, the females were removed and eggs laid on the leaf squares are counted. These Petri dishes were placed in the darkened growth chamber and immediately exposed to UV-B irradiation (0.16 W m^{-2}) for 30 min (0.288 kJ m^{-2}) at 25°C. To clarify the effect of temperature on the photoreactivation efficiency, after UV-B irradiation, temperature in growth chamber was changed to 18°C, and 30°C or keep at 25°C, respectively. After 30 min, a Petri dish assigned to be the photoreactivation-free control (VIS−) was kept dark. The other petri dish were exposed to visible light for 90 min (VIS+). Then, all the Petri dishes were kept dark in the growth chamber (day 0). The numbers of *T. urticae* eggs for assays were 122–185 and 136–161 in VIS− and VIS+ treatments at 18°C, 104–191 and 136–156 at 25°C, and 138–178 and 128–165 at 30°C, respectively.

For *N. californicus*, a single piece of kidney bean leaf square (3.5 cm square) was placed on water-soaked cotton in each Petri dish. Prior to the introduction of *N. californicus*, 10 adult *T. urticae* females were introduced to each leaf square in the two Petri dishes. After 24 h, 10 adult females of *N. californicus* were introduced to all leaf squares in the two Petri dishes and reared together with *T. urticae* females and eggs as prey for further 24 h. Next, all adult females of *T. urticae* and *N. californicus* were removed from the leaf squares. Intact eggs of *N. californicus* laid on the leaf were counted. Two Petri dishes were separated into two treatments as *T. urticae* eggs. The UV-B exposure time was 30 min (0.288 kJ m^{-2}) and 15 min (0.144 kJ m^{-2}). The numbers of *N. californicus* eggs for 30 min assays were 23–36 and 28–36 in the VIS− and VIS+ at 18°C, 20–21 and 19–28 at 25°C, and 25–31 and 30–35 at 30°C. The numbers of *N. californicus* eggs for 15 min assays were 26–32 and 25–30 in the VIS− and VIS+ at 18°C, 20–32 and 22–31 at 25°C, and 25–28 and 25–30 at 30°C, respectively.

The experiments were replicated four times. Hatched and unhatched eggs at 18, 25 and 30°C was counted on day 13, day 6, and day 4, respectively, for *T. urticae* and on day 6, day 3, and day 2, respectively, for *N. californicus*.

- Data analysis

Effect of photoreactivation in *T. urticae* and *N. californicus* after 30 min UV-B (0.288 kJ m^{-2}) and 90 min VIS irradiation at 25°C were compared first. Hatchability was calculated for each replication and subjected to arcsine root transformation. The effect of species and VIS irradiation after UV-B irradiation on hatchability was assessed by two-way analysis of variance (ANOVA) using the "aov" module in R software. For homoscedasticity, the hatchability were subjected to arcsine root transformation followed by Bartlett's test for homogeneity of variance using the "Bartlett.test" module in R software.

After Photoreactivation capacity was confirmed in *T. urticae* and *N. californicus*, effect of temperature on photoreactivation efficiency was then evaluated. Photoreactivation efficiency was calculated according to Kelner's formula (Kelner, 1951):

$$S = \frac{Y-X}{1-X},$$

where *S*, *X*, and *Y* represent the photoreactivation efficiency, egg hatchability of photoreactivation-free control (VIS−), and hatchability of eggs exposed to VIS (VIS+), respectively.

The effect of temperature on photoreactivation efficiency of per species was assessed by linear regression analysis using the "lm" module in R software.

- Results

At 25°C, hatchability of photoreactivation-free control (VIS−), eggs of *T. urticae* and *N. californicus* were seriously damaged by a single acute UV-B irradiation (0.288 kJ m^{-2}). The hatchability after UV-B irradiation without photoreactivation was 19.1% and 4.0% in *T. urticae* and *N. californicus*, respectively (Fig. 4-1). Hatchability recovered to 62.7% by VIS irradiation (VIS+) in *T. urticae* and 20.7% in *N. californicus*. Two-way ANOVA

revealed that factors of VIS irradiation and mite species exerted significant effect on the egg hatchability (VIS: $P = 3.09 \times 10^{-7}$; species: $P = 5.08 \times 10^{-7}$). The interaction between VIS irradiation and species was significant ($P = 0.0189$), suggesting that photoreactivation was more effective in the eggs of *T. urticae*.

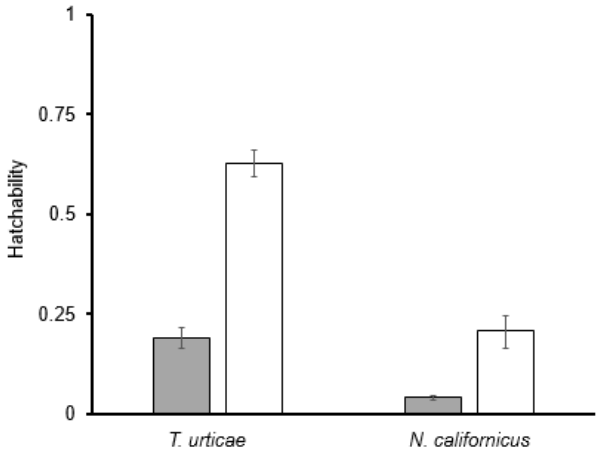

Fig. 4-1 Effect of Photoreactivation on egg hatchability of *T. urticae* and *N. californicus* after 0.288 kJ m^{-2} UV-B irradiation at 25°C. Gray and white bars represent photoreactivation-free control (VIS−) and photoreactivation treatment (VIS+), respectively. Vertical lines on bars show standard errors.

When UV-B irradiance was 0.288 kJ m^{-2}, photoreactivation efficiency of *T. urticae* eggs was 62.9%, 54.1% and 52.5% at 18°C, 25°C and 30°C, respectively, which significantly decreased with increasing temperature in *T. urticae* eggs (Fig. 4-2, linear regression analysis, $P = 0.01419$). Photoreactivation of *T. urticae* was most efficient at 18°C. For *N. californicus* eggs, photoreactivation efficiency was 6.7%, 16.6% and 14.5% at 18°C, 25°C and 30°C, respectively, which was no difference among three temperatures (Fig. 4-2, $P = 0.153$).

When UV-B irradiance was reduced to 0.144 kJ m^{-2}, photoreactivation efficiency of *N. californicus* eggs was 70.6%, 58.9% and 60.3%, respectively, showing a significant decreasing tendency with increasing temperature ($P = 0.03211$) as well as trend of that in *T. urticae* eggs after UV-B irradiance at 0.288 kJ m^{-2} (Fig. 4-2).

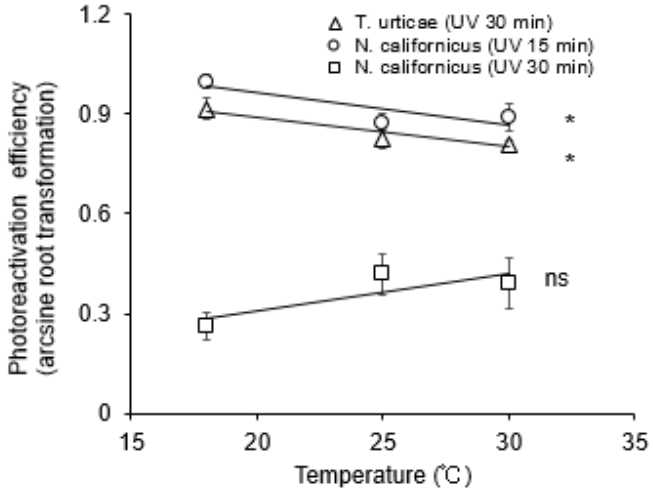

Fig. 4-2 Effects of temperature on egg photoreactivation. Open circles, square and triangles represent *N. californicus* (UV 15 min), *N. californicus* (UV 30 min) and *T. urticae* (UV 30 min), respectively. The equations of the regression lines: $y = -0.009564x + 1.153395$ for *N. californicus* (UV 15 min), $y = 0.011466x + 0.079004$ for *N. californicus* (UV 30 min) and $y = -0.009062x + 1.071369$ for *T. urticae* (UV 30 min), respectively. Asterisks show significant differences among temperatures (linear regression analysis, $P < 0.05$).

Section 2 Discussion

T. urticae eggs showed more effective photoreactivation than *N. californicus* after 0.288 kJ m^{-2} UV-B irradiation and VIS irradiation, suggesting that *T. urticae* largely depends on photoreactivation rather than phytoseiid mites.

According to Nakai et al. (2018), the hatchability of *N. californicus* eggs after UV-B irradiation at 0.288 kJ m^{-2} without photoreactivation at 25°C was 17%, marginally higher than the hatchability in this study (4.0%). Furthermore, in this chapter, the hatchability of *N. californicus* eggs exposed to VIS radiation only recovered to 20.7% after UV-B irradiation which was considerably lower than the recovered hatchability (94%) described in Nakai et al. (2018). Although the UV-B and VIS irradiance in this study was similar to Nakai et al. (2018), additional 30 min time lag (temperature adjustment) between UV-B and VIS irradiation significantly reduced the photoreactivation efficiency of *N. californicus* eggs. In Murata and Osakabe (2014), hatchability of *T. urticae* eggs was

decreased approximately 25% by a 1 h time lag comparing to no time lag treatment. The drastic reduction of hatchability caused by a short time lag in this chapter, suggesting the time sensitivity of DNA repair by photo-enzymatic in *N. californicus* eggs.

T. urticae has four CPD-photolyase genes (Grbić et al. 2011). Murata and Osakabe (2017a) reported that 90% of CPDs produced by 0.288 kJ m^{-2} UV-B radiation in *T. urticae* larvae was repaired by the VIS irradiation at 25°C. However, the information about the PER system in *N. californicus* is limited. Considering the results in this study, it is likely that DNA damage needs to be repaired more hastily in *N. californicus* than *T. urticae* after UV-B irradiation. To demonstrate this hypothesis, detailed molecular analysis is necessary in further research.

Fifty-four percent of *T. urticae* eggs were reactivated due to photoreactivation after 0.288 kJ m^{-2} UV-B irradiation at 25°C, corresponding to the finding that photoreactivation efficiency of *T. urticae* eggs levelled off at 57% after same UV-B irradiance despite of increasing cumulative VIS irradiance at 25°C (Murata and Osakabe 2014). Sixty-three percent of *T. urticae* eggs were reactivated by photoreactivation at 18°C in this chapter. Considering the 30 min time lag, photoreactivation efficiency likely has higher saturation point at 18°C, compared to 25°C.

The effects of temperature on survivorship and photoreactivation from UV-B damage are contentious. In *Daphnia* species, some species are susceptible to UVB damage at low temperature than higher temperature but the opposite is true in other species (Williamson et al. 2002; Macfadyen et al. 2004; Connelly et al. 2009). UV-B-induced mortality increased with temperature decreasing in *T. urticae* and *N. californicus* eggs (Chapter 3). However, photoreactivation efficiency decreased with temperature increasing. Reverse trend in temperature response of UV-B damage and photo-enzymatic repair in *T. urticae* and *N. californicus* induces different net UV-B damage (total UV-B damage minus repair) which is related to survival among temperatures.

Chapter 5

UV damage analysis of embryogenesis in *T. urticae*

Egg is the most sensitive stage of *T. urticae* to UV-B irradiation (Murata and Osakabe 2013). The susceptibility to UV-B of *T. urticae* eggs peaked at 24–48 h after oviposition and subsequently decreased until hatching (Murata and Osakabe 2014; Yoshioka et al. 2018). At 25°C, the period 24–48 h after oviposition roughly corresponds to the larval body formation stage based on the germinal disk (Dearden et al. 2002). To understand about the susceptibility to UV-B damage of *T. urticae* eggs in different developmental stage, it is necessary to study UV-B damage in eggs by observing embryogenesis and morphogenesis.

Previous studies on the sea urchin *Paracentrotus lividus* (Camarodonta: Parechinidae) reported higher UV-B susceptibility in 16-cell embryo than mesenchyme blastulae stage (Bonaventura et al. 2005, 2006). In *T. urticae*, the 16-cell embryo and the blastoderm appears approximately 5 h and 12 h after oviposition, respectively (Dearden et al. 2002). In the UV method, UV-B irradiation is performed at midnight for 3 h (Tanaka et al. 2016). For *T. urticae* eggs, the first exposure of UV-B irradiation in the greenhouses is 0–24 h after oviposition, which includes several early stages of embryogenesis such as cleavage and formation of the blastula (Dearden et al. 2002). To explain how the UV method works effectively in the greenhouses, details about susceptibility to UV-B in early stages of embryogenesis of *T. urticae* eggs are required.

Temperature significantly affects the egg development duration of *T. urticae* (Bayu et al. 2017). The mortality of *T. urticae* eggs was decreased with temperature increasing (Chapter 3). It is likely that temperature influences the mortality of *T. urticae* eggs by shifting egg stages in which they exposed to UV-B irradiation and change in the duration of UV-B vulnerable stages. To demonstrate this hypothesis, investigation into effect of temperature on speed of embryonic development and shift of susceptibility peak of egg stage is necessary.

In this chapter, I aimed to clarify the effects of temperature on the speed of embryonic development of *T. urticae* and influence of UV-B on embryogenesis. First, I investigated

the effects of temperature in embryogenesis of *T. urticae* eggs by observing embryonic development during the whole egg duration. Second, I evaluated the effects of UV-B irradiated timing on stage of embryonic termination and detected the most sensitive stage to UV-B irradiation.

Section 1 Effect of temperature on embryogenesis

- Materials and methods

Prior to the experiment on temperature effect, embryogenesis duration and hatchability between liquid paraffin and leaf square was compared to evaluate whether liquid paraffin had an effect on embryogenesis. A single kidney bean leaf square (3.5 cm square) was placed on water-soaked cotton in a Petri dish. Sixty *T. urticae* adult females were introduced to the leaf square and allowed to lay eggs in growth chamber with 14:10 (L:D) h photoperiod at 25°C. After 1 h, all adult females were removed. Five eggs were transferred to a single concave microscope slide (TOSHIN RIKO Co. Ltd., Tokyo, Japan) without any pre-treatment. Then, liquid paraffin was added to fill the concave and a micro cover glass (MATSUNAMI GLASS Ind. Ltd., Osaka, Japan) was used to cover the slide. The eggs were observed by using a differential interference contrast microscope (DIC; OPTIPHOT-2, Nikon, Tokyo, Japan) with a digital camera (COOLPIX P100, Nikon, Tokyo, Japan). Every day, four eggs from leaf square were transferred to the microscope slide to compare the duration with the five eggs that transferred soon after oviposition. The development stage was distinguished by referring to Dearden et al. (2002). On the other hand, sixty *T. urticae* adult females were introduced to the leaf square and allowed to lay eggs. After 3 h, all adult females were removed. Forty-six eggs were then transferred to a microscope slide. Thirty-three eggs were transferred to a new leaf square. The microscope slide and petri dish was kept in the growth chamber (day 0). Hatched eggs were counted after 6 days.

After preliminary experiments, embryogenesis duration of *T. urticae* eggs were observed at 18˚C, 25˚C and 30˚C, respectively. Sixty *T. urticae* adult females were introduced to the leaf square and allowed to lay eggs in growth chamber in each

temperature. After 1 h, all adult females were removed. Eight eggs were transferred to a single concave microscope slide and observed by the DIC. The durations that easily to be distinguished were chosen to be compared among temperatures.

- Data analysis

Statistical analyses were performed using R software. In preliminary experiments, duration between microscope slide and leaf square was assessed by two sample t-test using the "t.test" module. For homoscedasticity, the duration were subjected to Bartlett's test for homogeneity of variance using the "Bartlett.test" module in R software. Hatchability was evaluated by Fisher's exact test by "fisher.test" module.

In the experiment of temperature effect on egg duration, differences in duration from egg oviposition to hatch among temperatures were analyzed by a generalized linear model (GLM) assuming Gaussian distribution using "glm" module followed by multiple comparison by the Tukey–Kramer method using "glht" module of "multcomp" package (Hothorn et al. 2008) in R software.

- Results

Table 5-1 Embryogenesis duration and hatchability between liquid paraffin and on leaf square

	Microscope slide [c]	Leaf square [c]
Leg primordia appearance (h) [a]	26.0 ± 0.6 (5)	26.5 ± 1.0 (4)
Eye points became colored (h) [a]	67 ± 0.6 (5)	68.7 ± 0.3 (3)
Hatchability (%) [b]	26.1 (46)	97.0 (33) [*]

[a] Duration from oviposition to this stage. There is no difference between two treatments (Two sample t-test).

[b] Hatchability of eggs on microscope slide and leaf square (Fisher's excat test, $P < 0.05$).

[c] Numbers in parentheses indicate the total number of individuals for observation on microscope slide or leaf square.

Leg primordia appeared about 26.0 h and 26.5 h and eye points became colored about 67.0 h and 68.7 h after oviposition on microscope slide and leaf square, respectively. Liquid paraffin had no effect on embryonic development (two sample t-test, leg primordia: P = 0.6647; eye points: P = 0.07356, Table 5-1). However, liquid paraffin significantly reduced hatchability on microscope slide (26.1%), comparing to hatchability (97.0%) on leaf square (Fisher's exact test, $P = 5.184\times10^{-11}$, Table 5-1).

Tetranychus urticae eggs and embryos were observed with DIC microscopy (Fig. 5-1). At 25 ˚C, nucleus was not clear soon after laying (AL 0 h) and then became visible in the center of the egg about 1 h later (Fig. 5-1A and B). Nine cell divisions then occurred within the egg, approximately one per hour (Fig. 5-1C and D), producing a blastoderm with a layer of cells surrounding the inner yolk-filled center [Fig. 5-1E, Dearden et al. (2002)]. The egg remained stationary for several hours. Leg buds appeared 25 h after laying (Fig. 5-1F). Limbs and body then grew continuously (Fig. 5-1G, H and I). The eye points became colored 63 h after laying (Fig. 5-1J).

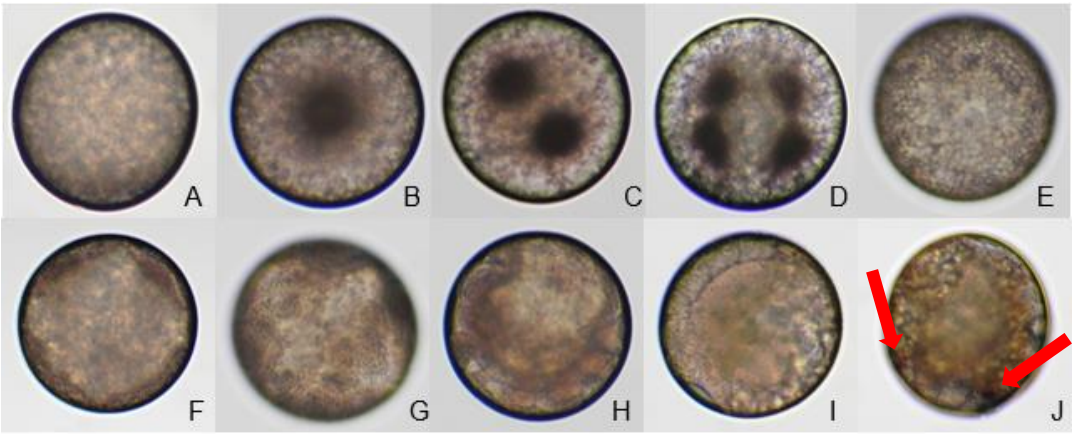

Fig. 5-1 Embryogenesis in the *Tetranychus urticae* egg at 25°C. Egg laid after (A) 0 h. Uncleaved egg. (B) 1 h. The nucleus becomes visible in the center of the egg. (C) 2 h. First division (D) 3 h. Second division (E) 12 h. Blastoderm stage. (F) 25 h. Appearance of leg primordia on both sides. (G) 29 h. Limbs growth. (ventral view) (H) 36 h. Limb growth (lateral view). (I) 57 h. Body growth (lateral view). (J) 63 h. Eye points become colored (arrowheads indicate the eyes).

About the embryogenesis of *T. urticae* eggs, temperature had a significant effect on embryogenesis speed. Duration of each stage was approximately 1.5 times faster at 30˚C

than at 25˚C. The duration of per stage was slowest at 18˚C, about 2 times slower than at 25˚C (Table 5-2).

Table 5-2 Embryogenesis in the *Tetranychus urticae* at 18°C, 25°C and 30°C.

Stage	Time after egg laying (h)		
	30˚C	25˚C	18˚C
Cleavage started	1.0 ± 0 a [a]	1.0 ± 0 a	3.0 ± 0 b
Cleavage ended	8.5 ± 0.3 a	12.0 ± 0 b	23.3 ± 0.3 c
Primordia appeared	18.3 ± 0.7 a	24.7 ± 0.3 b	50.3 ± 0.7 c
Eye points became colored	46.3 ± 0.3 a	63.3 ± 2.0 b	137 ± 0.9 c
Hatched (leaf)	66.3 ± 0.4 a	100.1 ± 0.9 b	222 ± 8 c

[a] Same letters at right in the column indicate no significant difference in per duration among different temperatures by Tukey-Kramer method ($P > 0.05$).

Section 2 Effect of UV-B irradiation on embryogenesis

- Materials and methods

Effects of different timing of single UV-B irradiation on embryogenic development

A single kidney bean leaf square (3.5 cm square) was placed on water-soaked cotton in a Petri dish. Sixty *T. urticae* adult females were introduced to the leaf square and allowed to lay eggs. After 1 h, all adult females were removed, and eggs laid on the leaf square were counted. The Petri dishes were then kept in growth chamber [25°C, 14:10 (L:D) h] until UV-B treatment. By this method, eggs that 0–1 h (AL 0 h), 6–7 h (AL 6 h), 12–13 h (AL 12 h), 24–25 h (AL 24 h), 36–37 h (AL 36 h), 48– 49 h (AL 48 h), 60–61 h (AL 60 h) and 72–73 h (AL 72 h) after oviposition were prepared, respectively. The Petri dishes were then irradiated with UV-B (0.16 W m^{-2}) for 45 min (0.432 kJ m^{-2}). A control treatment (UV−) was also prepared. Some eggs were randomly chosen to put onto microscope slide for DIC observation and others were left on the leaf square. The numbers of eggs of per treatment were shown in Table 5-3. All the eggs were subsequently kept in darkness in the growth chamber until observation was finished. The hatched eggs were

counted 6 days (AL 0 h, AL 6 h, AL 12 h, AL 24 h and control treatment) and 4 days (AL 36 h, AL 48 h, AL 60 h and AL 72 h) later.

Embryogenesis suppression effects by intermittent UV-B irradiation

Eggs within 1 h after oviposition (AL 0 h) were prepared as mentioned above. UV-B intensity was adjusted to (0.0135 W m^{-2}) by covering the UV-B lamp with polyethylene sheets of appropriate thickness as Chapter 3 to simulate the daily nighttime irradiation. The eggs were irradiated with UV-B for 3 h/day for 1 day (0.146 kJ m^{-2} d^{-1}), 2 days (0.292 kJ m^{-2} d^{-1}) and 3 days (0.437 kJ m^{-2}). Accumulative UV-B irradiation after 3 days was similar to a single acute irradiation (0.432 kJ m^{-2}) in the previous experiment (*Effects of different timing of single UV-B irradiation on embryogenic development*). The numbers of eggs of per treatment were shown in Table 5-4. Petri dishes were put into the growth chamber (day 0) were always kept dark except for UV-B irradiation. The hatched eggs were counted 6 days later.

- Data analysis

Effects of different timing of single UV-B irradiation on embryogenic development

Difference of hatchability among different irradiated timing except control treatment (UV−) on microscope slide and leaf square was evaluated by Fisher's exact test in by "fisher.test" module followed by a *post hoc* test for a multiple comparison by holm method using "fisher.multcomp" module of "RVAideMemoire" package in R software.

Embryogenesis suppression effects by intermittent UV-B irradiation

Difference of hatchability among different irradiated frequency on microscope slide and leaf square was evaluated by Fisher's exact test in by "fisher.test" module followed by a *post hoc* test for a multiple comparison by holm method using "fisher.multcomp" module of "RVAideMemoire" package in R software.

- Results

Effects of different timing of single UV-B irradiation on embryogenic development

AL 0 h and 6 h eggs were irradiated in early cleavage stage. After irradiation, cell divisions continued for at least 6 h (Fig. 5-2). One day after irradiation, however embryogenesis ceased at the late cleavage stage, and thus germ disc never appeared. At that time, flatten germ disc appeared in the eggs of control treatment. Eggs of AL 12 h, AL 24 h, AL 36 h, AL 48 h and AL 60 h were irradiated with UV-B after the cleavage finished. Their eye points were colored despite of UV-B irradiation as control treatment (Fig. 5-2). However, these eggs became orange colour gradually and could not hatch both on leaf square (Fig. 5-2, Fig. 5-3 and Table 5-3). Although AL 72 h eggs were irradiated after eye points became colored, the hatchability was still low on the leaf square, compared to the control treatment (Table 5-3).

Hatchability (except UV− treatment) was significantly different among irradiated timing treatments on microscope slide (Fisher's exact test $P = 0.02435$; Table 5-3) but no differences were found in the *post hoc* test. On leaf square, hatchability was not different among irradiated timing treatments (Fisher's exact test $P = 0.08923$; Table 5-3).

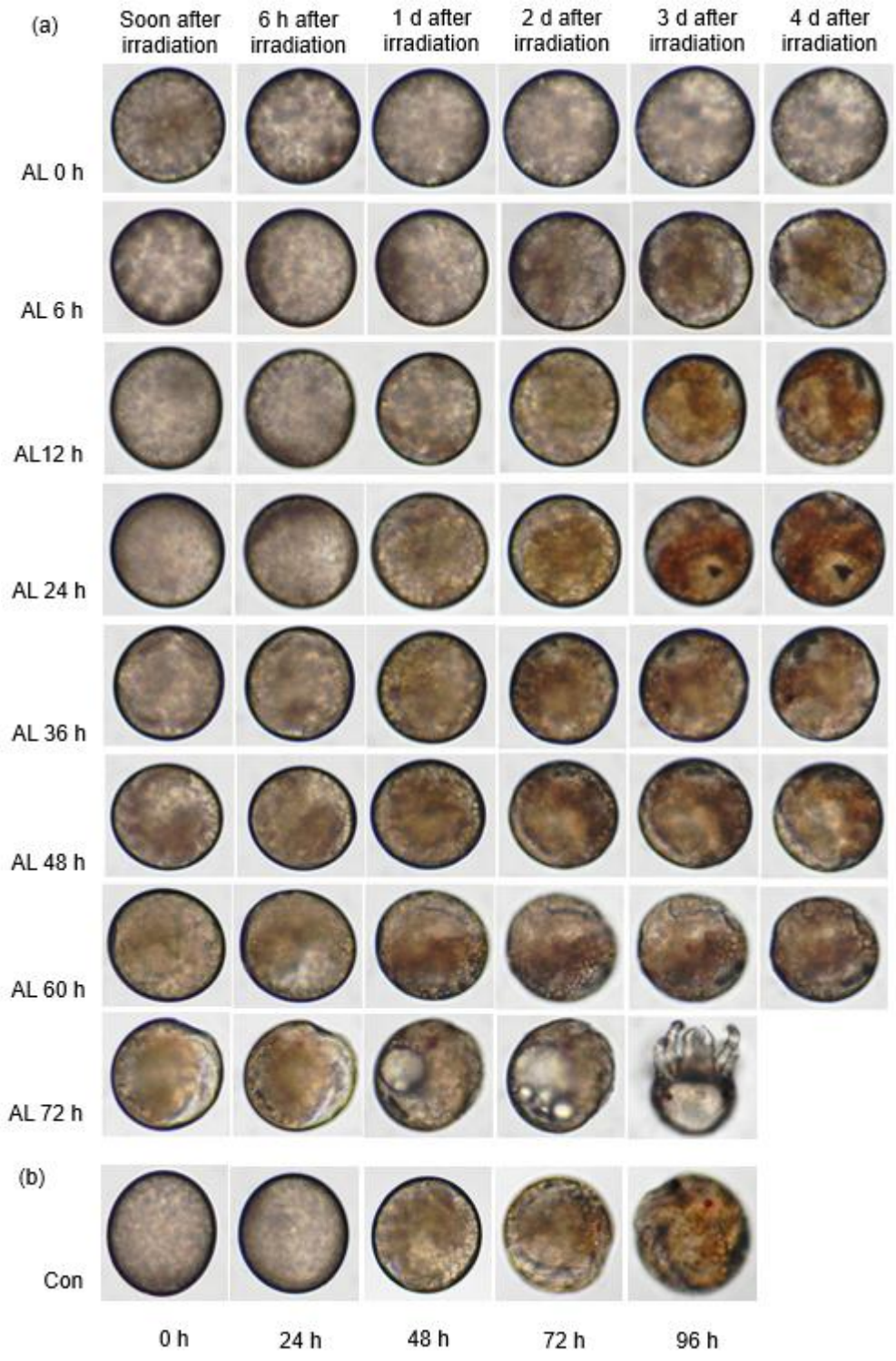

Fig. 5-2 UV-B Damage to embryogenesis in the *Tetranychus urticae* egg at 25°C. (a) Embryogenesis with different UV-B exposure (0.432 kJ m^{-2}) timing after laying. (b) Embryogenesis without UV-B irradiation.

Fig. 5-3 *Tetranychus urticae* eggs (AL 24 h) with (right) or without (left) UV-B irradiation 4 d after treatment. Their eyes were colored in both treatment.

Table 5-3 Hatchability on microscope slide and on leaf square after UV-B irradiation.

Eggs laid after (h) [a]	Hatchability on the microscope slide (%) [b]	Hatchability on leaf square (%) [c]
AL 0	16.7 (18) [d]	14.8 (27) [d]
AL 6	5.6 (18)	0 (28)
AL 12	0 (7)	0 (15)
AL 24	0 (6)	0 (13)
AL 36	0 (8)	0 (17)
AL 48	0 (6)	0 (20)
AL 60	40.0 (5)	11.7 (17)
AL 72	50.0 (8)	7.4 (27)
Control (UV-)	14.3 (7)	100 (13)

[a] Timing of exposure to UV-B irradiation after oviposition.

[b] Hatchability of eggs on microscope slide after UV-B irradiation.

[c] Hatchability of eggs on leaf square after UV-B irradiation.

[d] Numbers in parentheses indicate the total number of eggs used for observation on microscope slide or leaf square.

Embryogenesis suppression effects by intermittent UV-B irradiation

In the previous experiment (effects of different irradiated timing), development of AL 0 h eggs terminated before blastoderm and germ disc appearance by a single UV-B irradiation at 0.432 kJ m^{-2}, suggesting cleavage stage was vulnerable to UV-B irradiation.

However, when the UV-B irradiation was decreased to 0.145 kJ m^{-2}, AL 0 h eggs could complete embryogenesis (Fig. 5-4a) and 60% hatched on the leaf square (Table 5-4). UV-B damage possibly accumulated with exposure frequency increasing because the unusual orange color was getting dark in day 5 (Fig. 5-4 a, b and c). Hatchability on the microscope slide was not significantly different among three treatments (Fisher's exact test P = 0.25; Table 5-4) because liquid paraffin possibly suppressed hatch of eggs on slide (Section 1). Meanwhile, hatchability on leaf square significantly decreased with exposure frequency (accumulative irradiation) increasing (Fisher's exact test, P = 0.0003518, Table 5-4).

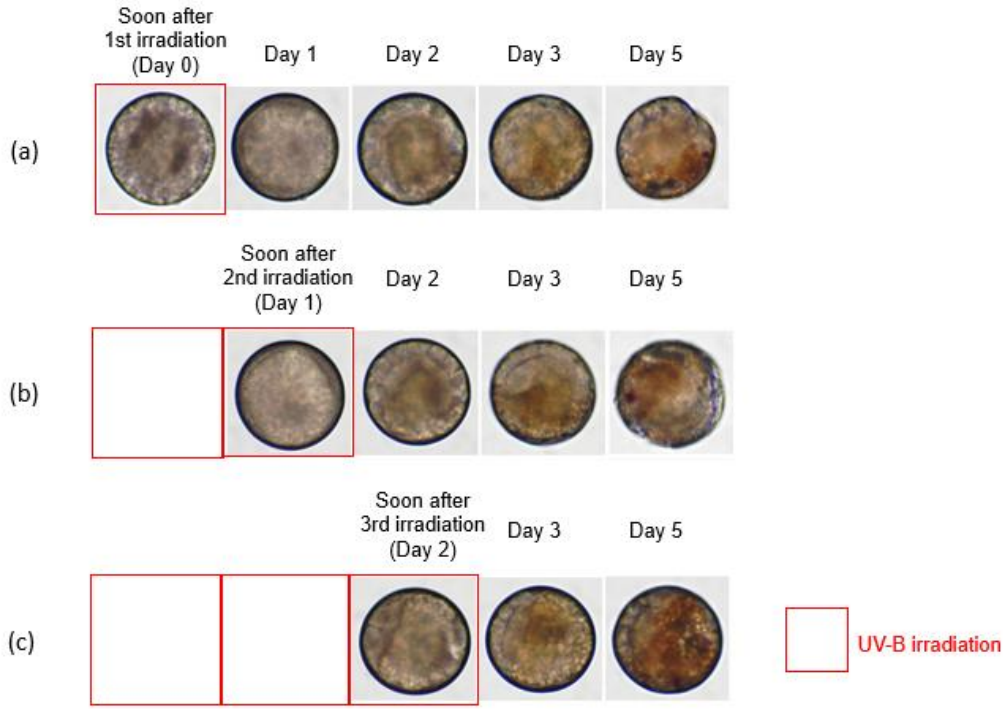

Fig. 5-4 UV-B Damage to embryogenesis in the *Tetranychus urticae* egg at 25°C. Embryogenesis irradiated with different UV-B exposure frequency (a) 1 day (UV-B: 0.146 kJ m^{-2}), (b) 2 days (0.292 kJ m^{-2}) and (c) 3 days (0.437 kJ m^{-2}). The eggs were irradiated with UV-B (first time) soon after oviposition (AL 0h).

Table 5-4 Hatchability of AL 0 h eggs on microscope slide and leaf square after intermittent UV-B irradiation

UV-B irradiation frequency [a]	Hatchability on microscope slide (%) [b d]	Hatchability on leaf square (%) [c d]
1 day (0.146 kJ m^{-2})	40.0 (5)	60.0 (10) [A]
2 days (0.292 kJ m^{-2})	20.0 (5)	10.0 (10) [AB]
3 days (0.437 kJ m^{-2})	0 (6)	0 (18) [B]

[a] Exposure to different frequency of UV-B irradiation.

[b] Hatchability on microscope slide after UV-B irradiation.

[c] Hatchability of eggs on leaf square after UV-B irradiation. Same capital letters at right in the column indicate no significant difference between hatchability of 3 treatments (Fisher's exact test, $P < 0.05$).

[d] Numbers in parentheses indicate the total number of eggs used for observation on microscope slide or leaf square.

Section 3 Discussion

As many other organisms, high temperature and low temperature, within the proper temperature range for growth, shortens and prolongs egg duration in *T. urticae*, respectively (Bayu et al. 2017). At 25˚C, the timing of leg buds appearance in Section 1 (24.7 h after oviposition) is similar to a previous report that initial limb primordia appeared 23 h after oviposition Dearden et al. (2002). Eye points became colored about 63 h after oviposition in this study, when eggs hatched approximately 39 h after oviposition in study of Dearden et al. (2002), suggesting longer time for completing the subsequent body formation in this study.

In section 2, UV-B irradiation to eggs at cleavage stage (AL 0 and 6 h) inhibited the formation of blastula. Akimoto et al. (1983) reported that UV-C irradiation of animal hemisphere and vegetal hemisphere of 8, 16 cell embryos of sea urchin *Hemicentrotus pulcherrimus* (Echinoida: Strongylocentrotidae) induced the arrest of normal development at gastrula stage and blastula stage, respectively. High dose of UV-B radiation of 16 cell embryos of another sea urchin *P. lividus* resulted in significant number

of abnormal blastula (Bonaventura et al. 2006). Moreover, several reports suggest that the blastula stage is the pivotal stage when apoptosis is activated to eliminate damaged cells (Ikegami et al. 1999; Finkielstein et al. 2001; Vega and Epel 2004). In Section 2, UV-B irradiation may influence the embryogenesis by affecting the cell division, resulting abnormal blastula which induced termination of embryogenesis.

UV-B seems to induce delay in development. Murata and Osakabe (2017b) reported *T. urticae* larvae after UV-B irradiation has a longer larval duration to protochrysalis than control treatment. Bonaventura et al. (2006) reported no differences in hsp70 (one of the heat shock protein genes) transcripts levels in early cleavage embryos of *P. lividus* from 1 to 24 h after UV-B exposure, and they suggested that only a post-transcriptional regulation is involved in the cellular response to UV-B stress involves. UV-B irradiation of mesenchyme blastula embryos of *P. lividus* induced a delay and/or inhibition of gastrulation (Bonaventura et al. 2005). However, there is no significant delay of embryo development in UV-B-irradiated eggs comparing to control treatment (Fig. 5-2, AL 12–60 h treatment, 1–3 d after irradiation). Unlike larvae, UV-B did not delay the developmental process in embryogenesis of *T. urticae* eggs.

UV-B-irradiated eggs could develop (Fig. 5-2, AL 12–60 h treatment), but could not hatch (Table 5-3). Similarly, most *T. urticae* larvae irradiated with UV-B immediately after hatching developed to the protochrysalis stage but died due to failed molting, morphological defects, and abnormal contraction of the integuments (Murata and Osakabe 2017b). UV-B irradiation possibly has a carry-over lethal effect on later life stages.

The mortality of *T. urticae* is proportional to the cumulative UV-B irradiance (Murata and Osakabe 2013). Murata and Osakabe (2014) showed that the mortality of *T. urticae* eggs irradiated with UV-B at 0.288 kJ m^{-2} and kept in darkness without photoreactivation at 25°C was 85%. The regression line in Chapter 3 showed a 99.9% mortality at 0.288 kJ m^{-2}, suggesting that daily nighttime UV-B irradiation (Chapter 3) was more effective than a single UV-B irradiation without photoreactivation. In Section 2, I confirmed that UV-B damage was proportional to cumulative UV-B irradiation no matter a single irradiation or intermittent irradiation, explaining the reason of higher mortality of daily nighttime

UV-B irradiation (the UV method) than a single UV-B irradiation because cumulative UV-B irradiation under the UV method is more than under a single irradiation .

Chapter 6

Strawberry pollen as a source of UV-B protection ingredients for the phytoseiid mite *N. californicus*

In Chapter 3, *N. californicus* showed greater resistance to UV-B than *T. urticae*, making possible the application of UV-B doses that are lethal for spider mites but safe for phytoseiid mites. UV-B radiation may adversely affect the survival of phytoseiid mites (Nakai et al. 2018). Therefore, protecting phytoseiid mites from UV-B radiation is still important for the concurrent use of phytoseiid mites with UV-B lamps. Sugioka et al. (2018) reported increased UV-B resistance in *N. californicus* feeding on tea and peach pollen. Pollen grains contain bioactive substances, such as antioxidants, to protect germ cells from solar UV-B radiation (Feng et al. 2000; Žilić et al. 2014). Several antioxidants have been identified from peach and tea pollen extracts, and tea pollen contains high concentrations of very active antioxidants, such as catechins (Sugioka et al. 2018). In strawberry greenhouses, flowers bloom continuously, making strawberry pollen more available than other pollen. If *N. californicus* can use strawberry pollen as an alternative food and the pollen-diet mitigates its UV-damage, it would be advantageous for the concurrent use of *N. californicus* with the UV method in strawberry greenhouses.

In this chapter, to establish spider mite management combining UV method with biological control, I evaluated the quality of strawberry pollen as an alternative food and a source of UV-B protection ingredients. I compared the juvenile development and egg production of *N. californicus* between pollen and mite diet. Then, I investigated survival rate and egg production of *N. californicus* between strawberry pollen and mite diet after UV-B irradiation. At last, I evaluated the antioxidant activity in strawberry pollen by Oxygen radical absorbance capacity (ORAC) measurement and identified the main antioxidant compounds in strawberry pollen.

Section 1 Evaluation of strawberry pollen as an alternative food

- Materials and methods

Juvenile development

Development success and duration from egg to adult emergence of *N. californicus* feeding on strawberry pollen (pollen diet) and *T. urticae* (mite diet, control) was tested at 25°C under a 16:8 (L:D) h photoperiod. Newly laid *N. californicus* eggs produced by 20 adult females in 24 h were transferred individually to kidney bean leaf squares (2 cm square) on water-soaked cotton in six Petri dishes (9 cm diameter, four leaf squares per dish) (day 0). Three Petri dishes each were provided with pollen (12 eggs) and mite (12 eggs) diets. For the mite diet, two *T. urtica*e females were introduced to leaf squares the day before egg introduction. For the pollen diet, a small amount of strawberry pollen was spread over the leaf squares. A piece of film (Parafilm, Bemis, Neenah, WI, USA; 0.5 cm square) was put on every leaf square to provide shelter for *N. californicus*. To determine development periods, I observed the developmental stage of each *N. californicus* individual every 24 h until adult emergence. The prey and pollen were replenished every 2 days until adult emergence. This experiment was performed three times (36 eggs total for each diet).

Statistical analyses were performed using R software. I excluded an unhatched egg in mite diet and escaped mites for the analysis of developmental time. To assess the escape rate and developmental success, data of the triplicate experiments were combined, and the effects of diet were analyzed by Fisher's exact test using "fisher.test" module. The difference in development duration from egg to adult emergence between diets was evaluated by one-way analysis of variance (ANOVA) using the "aov" module. Prior to the one-way ANOVA, homogeneity of variance between diets was confirmed with Bartlett's test using "Bartlett.test" module.

Egg production

Adult female *N. californicus* fed *T. urticae* that had molted within 4 days were individually introduced onto six kidney bean leaf squares (1.5 cm square) placed on

water-soaked cotton in six (the 1st and 2nd experiments [block]) or nine (the 3rd experiment) Petri dishes (day 0). A piece of Parafilm was put on every leaf square to provide shelter for *N. californicus*. For the first 6 days (day 0–6), one third of the females were assigned to *T. urtica*e (Tu–Tu), strawberry pollen (SP–Tu), and tea pollen (TP–Tu). All of the females were reared on *T. urticae* for days 7–18. *Neoseiulus californicus* eggs and hatched larvae on the leaf squares were counted and removed every 3 days. Prey mites or pollen were added every 2 days during the experiments. If the leaf squares became rotten, all of the females were transferred to new leaf squares.

Table 6-1 Change of *N. californicus* female state in the egg production experiments classified by diet during the first 6 days.

State	day 3	day 6	day 9	day 12	day 15	day 18
Mite-diet						
Alive	42	40	37	37	34	27
Escaped	0	0	1	1	2	7
Dead	0	2	4	4	6	8
Tea pollen-diet						
Alive	39	34	34	32	27	27
Escaped	3	8	8	9	10	10
Dead	0	0	0	1	5	5
Strawberry pollen-diet						
Alive	39	37	35	34	28	28
Escaped	3	5	5	6	9	9
Dead	0	0	2	2	5	5

P-values by 3 × 3 Fisher's exact test for State × Diet on day 6 and day 18 are 0.0056 and 0.8329, respectively.

This experiment was performed three times, so 42 females were raised on each diet. Although more *N. californicus* females escaped in pollen-diets than mite-diet (day 6; Fisher's exact test $P = 0.0056$), state of the females (alive, escaped, and dead) on day 18 was not significantly different among diets (Fisher's exact test $P = 0.8329$; Table 6-1).

Because it is well known that phytoseiid mites use threads produced by spider mites for prey search (Hoy and Smilanick 1981; Yano and Osakabe 2009; Shinmen et al. 2010), the absence of threads produced by *T. urticae* that arrest *N. californicus* (Tachi and Osakabe 2014) probably caused the escape from pollen diet. Therefore, females that escaped or died were excluded from the analyses. I also removed the data for a non-ovipositing female because copulation likely failed. Consequently, each diet had valid data from 27 females.

For the statistical analyses, +1 was added to the number of eggs produced by individual females, and the total was log-transformed. Differences in egg production among diets for the first 6 days and over 18 days were analyzed by a generalized linear model (GLM) assuming Gaussian distribution using "glm" module followed by multiple comparison by the Tukey–Kramer method using "glht" module of "multcomp" package (Hothorn et al. 2008). The change in egg production by day for each diet, assuming random effects on individuals, was analyzed with a linear mixed model (LMM) using "lme" module in "nlme" package, followed by a multiple comparison by Tukey–Kramer method using "glht" module of "multcomp" package.

- Results

Juvenile development

In the experiments, three *N. californicus* larvae escaped from leaf squares in the pollen diet group, and one unhatched egg and four escaped larvae were observed in the mite diet group. The escape rate was not significantly different between diets (Fisher's exact test, $P = 0.710$; Table 6-2). Of the hatched larvae, 100% developed to adulthood under both diets, except for the escaped larvae. The time from egg to adult emergence was significantly shorter on the pollen diet [4.42 ± 0.12 d (±SE)] than mite diet (4.87 ± 0.15 d) (one-way ANOVA, $P = 0.022$).

Table 6-2 Escape rate, developmental success, and premature duration (Mean ± SE) of *Neoseiulus californicus* from egg to adult fed on a diet of strawberry pollen and *Tetranychus urticae* at 25 °C, 80% RH.

	Pollen-diet [a]	Mite-diet [a]	*P* values
Escape rate (%) [b]	8.33 ± 0 (36)	11.62 ± 0.06 (35)	0.710
Developmental success (%) [b]	100 ± 0 (33)	100 ± 0 (31)	1
Premature duration (days) [c]	4.42 ± 0.12 (33)	4.87 ± 0.15 (31)	0.022

[a] Numbers in parentheses indicate the total number of individuals for three replications.

[b] Calculation of percentages exclude an unhatched egg and escaped individuals in "Escape rate" and "Developmental success", respectively. Statistical significance in the difference between diets was evaluated by Fisher's exact test.

[c] Durations from egg to adult emergence. Statistical significance in the difference between diets was evaluated by one-way ANOVA ($P < 0.05$).

Egg production

Egg production of *N. californicus* females fed strawberry (SP–Tu) and tea (TP–Tu) pollen during the first 6 days when they fed on pollen was similar (SP–Tu: 3.4 ± 0.3 SE eggs, TP–Tu: 4.3 ± 0.5 eggs; Tukey–Kramer method, $P = 0.379$), but was significantly lower than that for females fed *T. urtica*e (Tu–Tu: 15.3 ± 0.5 eggs; Tukey–Kramer method, $P < 0.001$; Fig. 6-1a). The egg production by Tu–Tu females peaked on days 4–6 (8.1 ± 0.3 eggs in 3 days) and dropped on days 7–9 due to depletion of sperm (Fig. 6-1b).

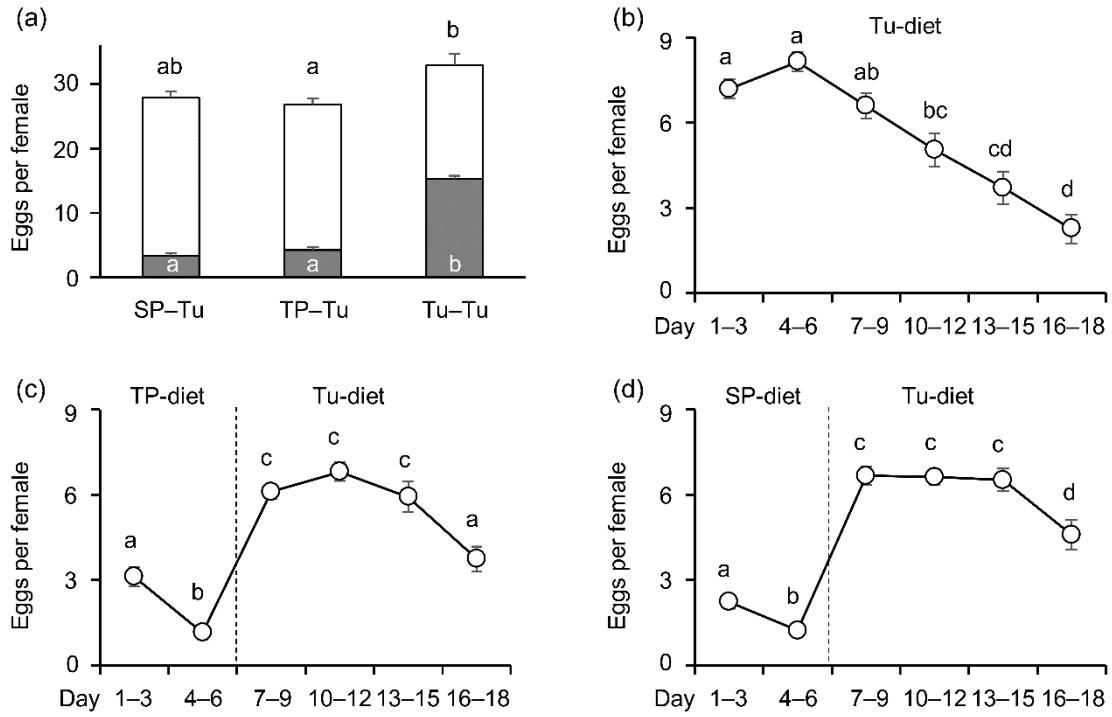

Fig. 6-1 Effects of diet on egg production by *Neoseiulus californicus* females. (a) Solid and open bars are the numbers of eggs produced on days 1–6 when females fed on different diets and for days 7–18 when females fed on *T. urticae* in all treatments. Different letters above the open bars and within black bars show significant differences in the number of eggs between diet treatments for days 1–18 and for the first 6 days, respectively, by the Tukey–Kramer method ($P < 0.05$). (b–d) Change in the number of eggs produced in 3 days in (b) Tu–Tu, (c) TP–Tu, and (d) SP–Tu treatments. Tu, TP, and SP indicate mite (*T. urticae*), tea pollen, and strawberry pollen diets, respectively. Vertical lines in each plot show the standard error (SE). Different letters above plots indicate significant differences by Tukey–Kramer method ($P < 0.05$).

Compared with Tu–Tu females, oviposition was extremely low on day 1–3 for females fed strawberry (SP–Tu females) or tea (TP–Tu females) pollen. Oviposition by SP–Tu and TP–Tu females was lowest on days 4–6 (1.2 ± 0.2 and 1.1 ± 0.2 eggs in 3 days, respectively; Fig. 6-1c, d). However, their egg production increased immediately when fed *T. urticae* on days 7–9 (6.7 ± 0.3 and 6.1 ± 0.2 eggs in SP–Tu and TP–Tu females, respectively; Tukey–Kramer method, $P < 0.001$ between days 4–6 and subsequent days for both SP–Tu and TP–Tu; Fig. 6-1c, d) and was similar to that of Tu–Tu females on days 7–9 (6.6 ± 0.5 eggs; Fig. 6-1b). Oviposition by SP–Tu and TP–Tu females remained

high for 9 days (days 7–15; Tukey-Kramer method, $P > 0.999$ and $P > 0.565$ among SP–Tu and TP–Tu, respectively) and declined on days 16–18 (Tukey–Kramer method, $P = 4.84 \times 10^{-3}$ and $P = 0.027$ for SP–Tu and TP–Tu, respectively, between days 13–15 and days 16–18; Fig. 6-1c, d).

Thus, the difference in the total number of eggs between pollen and mite-fed females for the entire experiment (18 days) was slightly mitigated. The Tu–Tu females laid significantly more eggs (32.9 ± 1.8) than the TP–Tu females (26.9 ± 0.9) (Tukey–Kramer method, $P = 0.0118$), whereas the total number of eggs laid by SP–Tu females (27.9 ± 1.0) was marginally, but not significantly, different from those laid by Tu–Tu (Tukey–Kramer method, $P = 0.0548$) and TP–Tu females (Tukey–Kramer method, $P = 0.8428$).

Section 2 Effects of the strawberry pollen diet on UV-B resistance

- Materials and methods

Effects of UV-B pretreatment on the survival of adult females

A single kidney bean leaf square (3.5 cm square) was placed on water-soaked cotton in each of eight Petri dishes. Ten adult female *T. urticae* were introduced into each of four Petri dishes (mite diet). All Petri dishes were kept in a laboratory at 25°C under a 16:8 (L:D) h photoperiod. After 24 h, a surplus amount of strawberry pollen was spread over the leaf squares in the remaining four Petri dishes where *T. urticae* was absent (pollen diet). Then, six adult female *N. californicus* reared on *T. urticae* were introduced to each of the eight leaf squares. Three days later, all females were removed. The eggs and larvae of *N. californicus* on the leaf squares were allowed to develop on *T. urticae* or strawberry pollen for a further 6 days until adult emergence.

Five newly emerged adult females on each of eight leaf squares (40 females in total) were transferred to another eight Petri dishes including a single leaf square (3.5 cm square). Four Petri dishes (two each for pollen and mite diets) were irradiated with UV-B (UV+, 1.43 W m^{-2}) for 150 min (12.87 kJ m^{-2}); the remaining four Petri dishes (two each for pollen and mite diets) were placed on a nearby rack without UV-B irradiation (UV−) (see Chapter 2). After UV-B irradiation, adult females were individually moved

to new kidney bean leaf squares (1.5 cm square) on water-soaked cotton in plastic cases (14 × 10 × 1.8 cm; length × width × height). For the mite diet, two adult *T. urticae* females were introduced to the new leaf squares the day before introduction of *N. californicus* females. For the pollen diet, a small amount of strawberry pollen was spread on the new leaf squares. A piece of Parafilm was put on every leaf square to provide shelter.

Then, the *N. californicus* females were reared on mite or pollen in the laboratory at 25°C under a 16:8 (L:D) h photoperiod (day 0). Their survival and any UV-B damage were observed daily until day 6. The UV-B damage level was determined by stimulating females with a fine small brush and categorizing them as follows: (a) walked normally; (b) walked, but sluggishly; (c) did not walk; and (d) dead. Spider mites and pollen were added every 2 days during the rearing experiments. This experiment was replicated seven times.

I preliminarily confirmed that the individuals at level (c) never recovered as in Sugioka et al (2018). Thus, individuals in (a) and (b) were grouped as survivors, and those in (c) and (d) were presumed dead. Data for the adult females that escaped from the leaf squares were excluded from the analyses because of no differences in escape rate between UV+ and UV− treatment (Fisher's exact test, pollen diet: $P = 0.6368$, mite diet: $P = 1$) . Consequently, 61 and 63 females for UV+ treatments were evaluated for the pollen and mite diets, respectively, and 58 and 62 females for UV−.

Survival curves were estimated by the Kaplan–Meier method using "survfit" function in R software. The difference in survival curve between UV+ and UV− for each diet was tested separately by the log-rank test using "survdiff" function in "survival" package.

Effects of UV-B pretreatment on egg production

Adult *N. californicus* females that developed on *T. urticae* (mite diet) and strawberry pollen (pollen diet) were irradiated with UV-B and then reared individually in the same manner as part of female survival to observe egg production. The UV-B irradiation period was shortened to 90 min (7.72 kJ m^{-2}) to reduce the damage to females and keep them alive for the experiment. The eggs and hatched larvae on each leaf square were counted and removed on day 3 and 6 after UV-B irradiation. Spider mites and pollen were added every 2 days during the experiment, which was replicated five times.

No differences were found in non-ovipositing and escaped females between UV+ and UV− treatment (Fisher's exact test, pollen diet: $P = 0.2851$, mite diet: $P = 0.4094$; Table 6-3). Therefore, I excluded the data for escaped and non-ovipositing adult females. Consequently, oviposition data for 35 and 33 females were evaluated for the pollen and mite diets for UV+ treatments, respectively, and 32 and 37 for the UV− treatment.

The effects of diet and UV-B irradiation on the total number of eggs produced over 6 days were evaluated by GLM assuming a Poisson distribution using "glm" module in R software. Then, using the combination of diets with or without UV-B irradiation as treatment, we performed multiple comparison of GLMs (Poisson distribution) in the "glm" module based on the Tukey–Kramer method using "glht" module of "multcomp" package. The difference in the number of eggs produced during day 1–3 and day 4–6 for each combination of diet and UV-B treatment was evaluated with a generalized linear mixed model (GLMM) assuming random effects on individuals using "glmmML" module of "glmmML" package.

Table 6-3 *N. californicus* female state in the egg production experiments 6 days after a single acute UV-B treatment.

State	UV+	UV−
Strawberry pollen-diet		
Ovipositing	35	32
Non-ovipositing	7	4
Escaped	8	14
Mite-diet		
Ovipositing	33	37
Non-ovipositing	4	1
Escaped	13	12

P-values by 2 × 3 Fisher's exact test for State × UV treatment of strawberry pollen and mite diet are 0.2851 and 0.4094, respectively.

- Results

Effects of UV-B pretreatment on the survival of adult females

Under UV− treatment, 98 and 92% of the females survived until day 6 on the pollen and mite diets, respectively (log-rank test, $P = 0.1$; Fig. 6-2). By contrast, with the UV+ treatment, many females walked, but were sluggish (symptom b), with both pollen and mite diets after UV-B pretreatment, and the symptoms progressed over time (Fig. S6-1). Nevertheless, survival of females (symptom a + b; Fig. 6-3) was slightly higher on the pollen diet (16 *vs*. 5%) on day 6 (Fig. 6-2). Consequently, the survival of females after UV-B irradiation was significantly longer with the pollen diet (log-rank test, $P = 0.008$; Fig. 6-2).

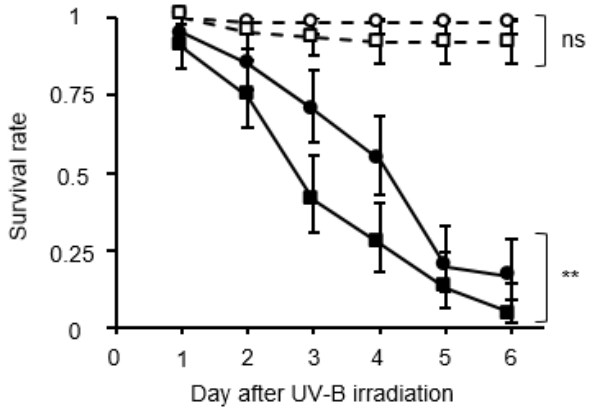

Fig. 6-2 Effects of pollen and mite diet on the survival of *N. californicus* adult females after irradiated with a single acute dose of UV-B (UV+) or not (UV−). The vertical lines in each plot are the 95% confidence interval (CI). Asterisks indicate significant differences in survival curves between diets by the log-rank test ($P < 0.01$).

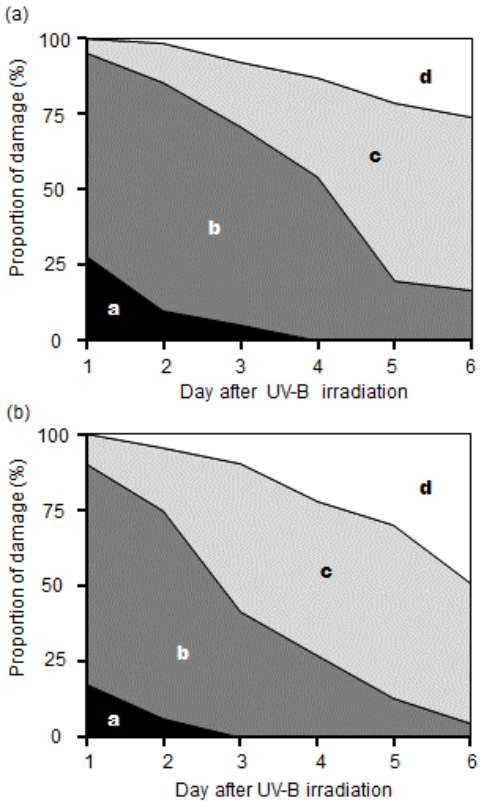

Fig. 6-3 Proportion of damage levels in *Neoseiulus californicus* adult females after UV-B irradiation (UV+). (a) pollen-diet, (b) mite-diet. a: walked normally, b: walked but sluggish, c: did not walk, d: died.

Effects of UV-B pretreatment on egg production

The number of eggs produced by *N. californicus* females on days 1–6 was not significantly different between the pollen (14.4 ± 0.4 SE) and mite (15.5 ± 0.5) diet in UV− treatment (Tukey–Kramer method, $P = 0.711$). The UV+ treatment significantly decreased egg production with both diets (Tukey–Kramer method, $P < 0.001$), but more eggs were laid with the pollen diet (7.7 ± 0.5 *vs*. 5.3 ± 0.4) in UV+ (Tukey–Kramer method, $P = 2.09 \times 10^{-3}$; Fig. 6-4). Egg production was lower on days 4–6 than on days 1–3 in UV+ (GLMM, $P = 8.27 \times 10^{-4}$ and 8.14×10^{-5} for pollen and mite diet, respectively), whereas no significant difference was detected with UV− under the pollen

diet (GLMM, P = 0.862), and a marginal increase was observed on the second 3 days with the mite diet (GLMM, P = 0.0592; Fig. 6-4).

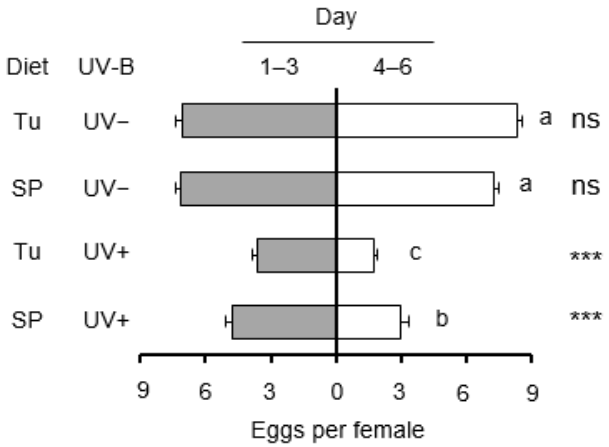

Fig. 6-4 Effects of diet on egg production by *N. californicus* adult females with (UV+) or without (UV−) single acute UV-B irradiation pretreatment. Tu and SP indicate *T. urticae* and strawberry pollen, respectively. Solid and open bars show the number of eggs produced for the first and second 3-day periods, respectively, after UV-B pretreatment. Different letters on the right-hand bars indicate significant differences in the number of eggs produced in 6 days (Tukey–Kramer method, $P < 0.05$). Asterisks show significant differences between day 1–3 and day 4–6 in a treatment (GLMM, *** $P <$ 0.001). Horizontal lines at the ends of bars show the SE.

Section 3 Antioxidant capacities of pollen compounds

- Materials and methods

Oxygen radical absorbance capacity (ORAC) measurement of intact strawberry and tea pollen extracts

I first examined the ORAC of methanol solutions of strawberry pollen and tea pollen. Strawberry pollen (155.1 mg fresh weight [FW]) collected in April 2018 was immersed in methanol (60.05 mg FW mL^{-1}) at 4°C for 101 days (SP-Extract 1), and tea pollen (255 mg, FW) collected in October 2017 was immersed in methanol (60.7 mg FW mL^{-1}) at 4°C for 49 days (TP-Extract 1). The ORAC of the supernatants of SP-Extract 1 and TP-

Extract 1 was measured. The remaining SP-Extract 1 was fractionated using high-performance liquid chromatography (HPLC) in following part (Fractioning and ORAC measurement of the strawberry pollen extract).

ORAC analysis followed Huang et al. (2002) using a microplate reader (SH-9000Lab; Corona Electric, Hitachinaka, Japan), with some modification. A fluorescein solution (94.4 nM; FL) was prepared in 75 mM phosphate buffer (pH 7.0). Methanol solutions containing 50, 25, 12.5, and 6.25 μM Trolox were used to calibrate the antioxidant activity. First, 20 μL of SP-Extract 1 and TP-Extract 1 (300 and 1,214 ng FW equivalents, respectively), blanks (methanol), and the Trolox solutions were dispensed into cells of a 96-well microplate, and then FL (200 μL) was added to each cell. After incubation for 30 min at 37 ◦C, 75 μL of 31.7 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) phosphate buffer solution was added to each cell. The decrease in fluorescence intensity (excitation at 485 nm; emission at 520 nm) was recorded for 90 min at 2-minute intervals using the microplate reader. I used four technical replicates placed on the microplate in quadruplicate with point symmetry in each analysis.

Three independent replicate assays were performed. The ORAC values were computed using SF6 software (Corona Electric). Differences in the ORAC of SP-Extract 1 and TP-Extract 1 were analyzed by one-way ANOVA using “aov” module after Bartlett’s test using “Bartlett.test” module in R software.

Fractioning and ORAC measurement of the strawberry pollen extract

SP-Extract 1 was concentrated in vacuo after filtration with a filter paper (No. 2, ADVANTEC, Tokyo, Japan) to obtain 29 mg of extract. The extract resolved with methanol (SP-Extract 2; 114 ng) was subjected to ORAC analysis as described in previous part (ORAC measurement of intact strawberry and tea pollen extracts) with the following HPLC fractions (455 ng SP-Extract 2 equivalent each).

SP-Extract 2 was subjected to preparative HPLC on L-7100 series (Hitachi, Tokyo, Japan) consisting of an L-7100 pump, L-7400 detector, and YMC-Pack ODS-AQ column (6.0-mm inner diameter × 100 mm; YMC, Kyoto, Japan). The flow rate was 1.0 mL min^{-1}, and the detection wavelength 254 nm. The extract was eluted with 50% methanol containing 0.1% acetic acid. The components eluted at retention times (t_R) 0–10.6

[fraction (Fr). 1], 10.6–15.7 (Fr. 2), 15.7–18.2 (Fr. 3), 18.2–23.2 (Fr. 4), 23.2–26.5 (Fr. 5), and 26.5–32.2 (Fr. 6) min [see Fig. 5-6 (a)] were collected. After Fr. 6, the component eluted with 100% methanol was collected as Fr. 7. Fr. 2, 4, and 6 were concentrated *in vacuo* to give compound 1 and mixtures of 2–4 and 5–7 (see Table 6-3), respectively. The contents were quantified using HPLC. The contents of 1–7 were calculated with a calibration curve between the weight and peak area using the standard *N*',*N*",*N*"'-tri-*p*-coumaroylspermidine, assuming the same molar extinction coefficient of 1–7.

Variation in the ORAC of Fr. 1–7 was evaluated by fitting to the linear mixed model, assuming random effects on replication using "lmer" module in "lme4" package, and "glht" module in "multcomp" package was used for a subsequent multiple comparison in R software..

Identification of antioxidants in strawberry pollen

Liquid chromatography mass spectrometer (LC-MS) analyses were performed on an ACQUITY UPLC system H class and a Xevo G2-S QTof MS (Waters, MA, USA) with an ACQUITY UPLC BEH C18 column (polar size 1.7 μm, 2.1 mm inner diameter × 100 mm; Waters). The analyses were conducted under the following conditions: eluent, 20–30% acetonitrile containing 0.1% formic acid for 10 min; flow rate, 0.3 mL/min; detection, UV 254 nm. The MS were operated in electron spray ionization of positive mode with the following parameters: column oven, 35°C; capillary voltage, 3.0 kV; cone voltage, 40 V; source temperature, 150°C; desolvation temperature, 500°C; cone gas, 50 L/h; desolvation gas, 800 L h^{-1}. The data processing was performed using MassLynx ver. 4.1 (Waters).

- Results

ORAC of intact pollen extracts and HPLC fractions

The ORAC of SP-Extract 1 was 1,491 ± 88 μmol Trolox equivalent (TE) (g FW) $^{-1}$, nearly triple that of TP-Extract 1 (436 ± 26 μmol TE (g FW) $^{-1}$; one-way ANOVA, $P = 2.65 \times 10^{-3}$; Fig. 6-5).

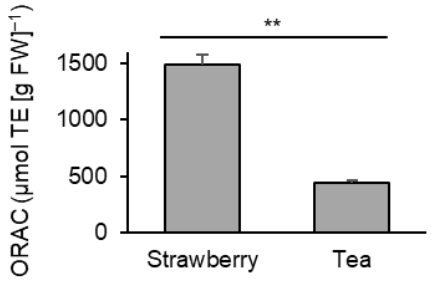

Fig. 6-5 ORAC of intact methanol extracts from strawberry (SP-Extract 1) and tea (TP-Extract 1) pollen. Vertical lines above the bars indicate the SE. Asterisks indicate statistically significant differences by one-way ANOVA ($P < 0.01$).

Table 6-4 Oxygen radical absorbance capacity (ORAC) of strawberry pollen extract (SP-Extract 2) and HPLC fractions (Fr. 1–7).

Samples	ORAC value [a] (µmol TE [g Extract 2]$^{-1}$) ± SE		Contribution rate (%) [b]
SP-Extract 2	2,657 ± 56		100
Fr. 1	707 ± 20	b	26.6
Fr. 2	377 ± 22	c	14.2
Fr. 3	49 ± 12	de	1.9
Fr. 4	989 ± 38	a	37.2
Fr. 5	44 ± 8	e	1.7
Fr. 6	130 ± 12	d	4.9
Fr. 7	131 ± 7	d	4.9
Sum of Fr. 1–7	2,428 ± 36		91.4

[a] Trolox equivalent per g extract. Same letters at right in the column indicate no significant difference between ORAC values of HPLC fractions by Tukey-Kramer method ($P > 0.05$).

[b] Percentage of ORAC value for each fraction to intact extract.

The ORAC of Fr. 1–7 was 91.4% that of SP-Extract 2 (Table 6-4). Fr. 4 had the highest ORAC (Tukey–Kramer method, $P < 0.001$ compared with all other fractions), and contributed 37.2% of the antioxidant capacity of SP-Extraction 2 (Table 6-4). Fr. 2 had the second highest antioxidant capacity (Table 6-4; Tukey–Kramer method, $P < 0.001$ relative to all other fractions). The ORAC of Fr. 6 was marginally higher than those of Fr. 3 and 5, which contained no HPLC peaks [Table 6-4, Fig. 6-6(a); Tukey–Kramer method, $P = 0.0556$ and 0.0334, respectively].

Identification of antioxidants in strawberry pollen

Table 6-5 Contents of antioxidants in strawberry pollen.

Compound	Fr. No [a]	t_R (min) [b]	*m/z* $[M+H]^+$ [c]	Content (mg [g FW]$^{-1}$) [d]
N'-caffeoyl-*N''*,*N'''*-di-*p*-coumaroylspermidine (1)	Fr. 2	13.1	600.273	4.6
E-Z isomer of 1 (2)	Fr. 4	19.2	600.273	29.3
N'-caffeoyl-*N''*-*p*-coumaroyl-*N'''*-feruloylspermidine (3)	Fr. 4	20.8	630.282	
N',*N''*-dicaffeoyl-*N'''*-*p*-coumaroylspermidine (4)	Fr. 4	20.8	616.264	
N',*N''*,*N'''*-tri-*p*-coumaroylspermidine (5)	Fr. 6	27.5	584.275	3.0
E-Z isomer of 4 (6)	Fr. 6	29.6	616.264	
N',*N''*-di-*p*-coumaroyl-*N'''*-feruloylspermidine (7)	Fr. 6	29.6	614.286	

[a] The range of collection times for each fraction is shown in Fig. 4a.

[b] Retention time in HPLC.

[c] The MS were operated in electron spray ionization of positive mode.

[d] Contents of antioxidants in pollen.

The extract yielded compounds 1–7 as major constituents in the HPLC analysis (Table 6-5). The peaks corresponding to 2–4 and 5–7 partially overlapped, so 2–4 and 5–7 were obtained as mixtures in Fr. 4 and 6, respectively, due to difficulty with the separation [Fig. 6-6 (a)]. LC-MS analysis of the extract identified 1–7 as hydroxycinnamoyl spermidine derivatives [Table 6-5, Fig. 6-6 (b)], based on the comparison of mass spectra with

reported data (Hanhineva et al. 2008). The exact position and *E*-*Z* configuration of each hydroxycinnamoyl group were not determined. A non-targeted analysis of the spatial metabolite composition of strawberry (*Fragaria* × *ananassa*) has revealed that 1–7 are predominant in the stamen and pistil (Hanhineva et al. 2008). Of the compounds analyzed here, Fr. 4 (29.3 mg [g FW] $^{-1}$) had the largest content in strawberry pollen.

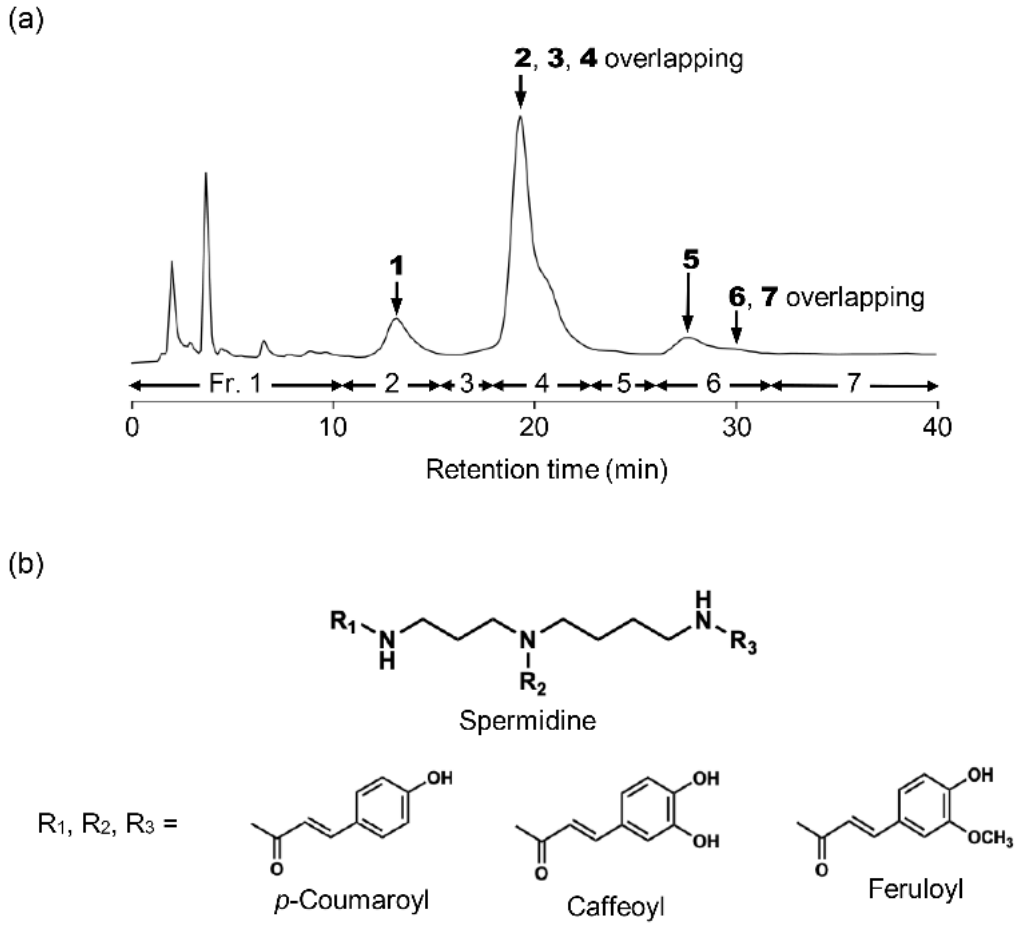

Fig. 6-5 HPLC analysis of strawberry pollen extracts (a) and major constituents in the pollen extract (b). Fr. 1–7 show the collection times of each fraction, and bold figures above peaks indicate the numbered antioxidant compound identified (see Table 6-5).

Section 4 Discussion

Gugole Ottaviano et al. (2015) reported prolongation of development and no egg production in *N. californicus* despite high developmental success (80–90%) when fed strawberry pollen. By contrast, we found complete development (100%) with a shortened juvenile stage (Section 1) in *N. californicus* fed strawberry pollen compared with individuals fed *T. urticae*. The premature durations in this study were shorter than that on *T. urticae* and another pollen diet tested in Iran (Soltaniyan et al. 2018) and on castor bean pollen in Brazil (Marafeli et al. 2014) but equivalent to that on *T. urticae* in Japan (Gotoh et al. 2004).

The egg production by *N. californicus* females fed *T. urticae* dropped significantly to 22 and 28% that of Tu–Tu when fed on strawberry (0.6 eggs day^{-1} $female^{-1}$) or tea (0.7 eggs day^{-1} $female^{-1}$) pollen (Section 1). Kishimoto et al. (2014) reported that *N. californicus* females reared on tea pollen produced 2.2 eggs $female^{-1}$ day^{-1}. The fecundity of females continuously reared on *T. urticae* (2.6 and 2.6 eggs $female^{-1}$ day^{-1} for the first 6 days; Section 1 and 2 [UV−], respectively) and strawberry pollen (2.4 eggs $female^{-1}$ day^{-1}; Section 2 [UV−]) in the current study was similar to that reported by Kishimoto et al. (2014). Therefore, the diet of juveniles or their mothers might affect the ability to use or the preference for pollen diets by *N. californicus* females via acclimation or maternal effects.

Although oviposition by SP–Tu and TP–Tu females decreased while feeding on pollen, their fecundity recovered immediately after shifting their diet to *T. urticae* (2.2 and 2.1 eggs day^{-1} $female^{-1}$ in SP–Tu and TP–Tu, respectively, for days 7–15). Egg production by *N. californicus* females was reduced by feeding on cattail (*Typha* sp.) pollen for 20 days, but recovered soon after the diet was shifted to *T. urticae* without additional copulation (Ajila et al. 2019). Interestingly, a similar recovery of suppressed fecundity after starting to feed on *T. urticae* was observed with long-term rearing of *N. californicus* on an artificial diet (Ogawa and Osakabe 2008). *N. californicus* reared on pollen can optimize its fecundity quickly after starting to feed on *T. urticae*, whereas the optimization of fecundity while feeding on pollen requires several generations (Khanamani et al. 2017; Castagnoli and Simoni 1999; Castagnoli et al. 2001). This suggests a possible

contribution of strawberry pollen to the persistence of *N. californicus* when the prey density is low in strawberry greenhouses. Ajila et al. (2019) showed that biological control of *T. urticae* by *N. californicus* in strawberries can be boosted by supplementary feeding with cattail pollen.

All compounds identified from strawberry pollen were spermidine derivatives bound to hydroxycinnamates: caffeic acid, ferulic acid, and *p*-coumaric acid. Dietary hydroxycinnamates can protect animals from reactive oxygen species (ROS) *in vivo* (Kroon and Williamson 1999). The antioxidant activities of hydroxycinnamoyl spermidine derivatives vary due to the chemical properties of bound hydroxycinnamates, such as the number of hydroxyl groups (Cao et al. 1997; Shahidi and Ambigaipalan 2015). Several literatures indicated pollen specificity of tri-substituted spermidine derivatives (Strack et al. 1990; Grienenberger et al. 2009; Vogt 2018). Of the three hydroxycinnamates in the spermidine derivatives identified from strawberry pollen, caffeic acid has the greatest radical scavenging activity, followed by ferulic acid (Castelluccio et al. 1995; Kikuzaki et al. 2002; Shahidi and Chandrasekara 2010; Nabi and Liu 2012). In fact, tri-substituted spermidine derivatives with either caffeoyl or feruloyl moiety, or both have a significant radical scavenging capacity, whereas the activity of N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine is limited (Sugioka et al. 2018; Zamble et al. 2006). Of the HPLC fractions, Fr. 4 contained the most compounds (*i.e.*, 2, 3, and 4) with one or two caffeoyl/feruloyl groups and made the greatest contribution to antioxidant capacity (37.2%). Compound 1, which has a caffeoyl group, contributed substantially to the antioxidant capacity in Fr. 2 (14.2%). The ORAC analysis indicated that 1–4 are major contributors to the high antioxidant capacity of strawberry pollen. Similarly, the presence of compounds 1 (or 2), 4, 5 and *N'*,*N''*,*N'''*-tri-caffeoylspermidine and the high antioxidant activities including ORAC were reported in rose bee pollen extracts (Yang et al. 2019).

Sugioka et al. (2018) found that tea pollen had higher antioxidant activity than peach pollen. The protective effects of the tea pollen diet on *N. californicus* against UV-B radiation were thought to be due to catechins (Sugioka et al. 2018). I found that strawberry pollen had a very high ORAC compared to tea pollen. Compounds 1–4 in strawberry

pollen, which contain one or two caffeoyl/feruloyl groups, likely have higher antioxidant activity than the catechins in tea pollen.

Sugioka et al. (2018) also reported higher survival of adult females and egg hatchability of *N. californicus* reared on tea and peach pollen than those on *T. urticae* after UV-B irradiation. In my study, the survival of *N. californicus* females reared on strawberry pollen was significantly higher than that of females reared on *T. urticae* after UV-B irradiation. Moreover, females reared on strawberry pollen laid more eggs after the UV-B irradiation than those fed *T. urticae*.

The caffeoyl groups abundant in compounds 1–4 of strawberry pollen have high scavenging activity against various reactive species, including singlet oxygen (Shahidi and Chandrasekara 2010; Foley et al. 1999), and effectively inhibit lipid peroxidation (Shahidi and Ambigaipalan 2015; Gülçin 2006). A significant reduction in lipid peroxidation after UV-B irradiation with the antioxidant astaxanthin, was demonstrated in the citrus red mite, *Panonychus citri* (McGregor) (Atarashi et al. 2017). The hatchability of eggs produced by *N. californicus* females fed *P. citri* and the development of larvae were higher than those of females fed *T. urticae* (Nakai et al. 2018). The inhibition of lipid peroxidation may contribute to UV-B resistance in *N. californicus*. The consumption of antioxidants in pollen is likely advantageous for *N. californicus*, mitigating the damage from UV-B irradiation.

Chapter 7

General discussion

Ambient UV-B radiation generates deleterious damage to *T. urticae* and forces it to stay on the lower leaf surfaces of host plants as an adaption to solar UV-B radiation (Ohtsuka and Osakabe 2009; Sakai and Osakabe 2010), because leaves contain compounds that shield against ambient UV radiation to protect their inner organs (Rousseaux et al. 2004; Izaguirre et al. 2007). However, it is difficult for artificial UV-B irradiation to reach lower side of leaves due to leaf overlap induced by elevated temperatures in April and May, possibly degrades the control effect in spider mites in strawberry greenhouses. Studies indicate that *N. californicus* escapes from leaf areas irradiated with UV-B (Tachi and Osakabe 2012, 2014) and move to non-irradiated areas, where *T. urticae* might also survive, suggesting possibly greater predation efficacy under UV-B irradiation. Although *N. californicus* eggs are more vulnerable to a single acute dose of UV-B irradiation than *T. urticae* eggs (Tachi and Osakabe 2012), in this study, I found that *N. californicus* showed greater resistance to UV-B than *T. urticae* eggs under the UV method (Chapter 3), making possible the application of UV-B doses that are lethal for spider mites but safe for phytoseiid mites.

Low temperature decreased hatchability of *T. urticae* eggs (Chapter 3), indicating that we could maintain the control effect of UV-B radiation on *T. urticae* by decreasing the UV-B irradiance in winter which is profitable for preservation of strawberry plants from sunscald risk. This strongly supports the possibility of commercially practical utilization of the UV method (Tanaka et al. 2016). On the other hand, Nakai et al. (2018) reported that UV-B damage was greater at 20°C than 25°C in *T. urticae*, but not in *N. californicus*. However, I found hatchability of *N. californicus* eggs was also decreased by low temperature, suggesting it is necessary to protect the phytoseiid mite by reducing UV-B irradiation in winter.

Effective photoreactivation is reported in both *T. urticae* and *N. californicus* eggs (Murata and Osakabe 2014; Nakai et al. 2018). Koveos et al. (2017) reported that *T. urticae* eggs were more resistant to UV-B radiation than phytoseiid mite eggs under

continuous UV-B irradiation with simultaneous VIS irradiation. Thus, this study first reported the difference in photoreactivation efficiency between *T. urticae* and *N. californicus* eggs. I also demonstrated its temperature dependence under UV-B irradiation with subsequent VIS irradiation which is more similar to the UV method condition. The photoreactivation efficiency of *T. urticae* eggs levelled off at 57% despite of increasing cumulative VIS irradiance after 0.288 kJ m^{-2} UV-B (Murata and Osakabe 2014). 54% of *T. urticae* eggs were reactivated by VIS irradiation comparing to 17% of *N. californicus* eggs after 0.288 kJ m^{-2} UV-B at 25°C, suggesting that *T. urticae* might largely depends on photoreactivation rather than phytoseiid mites with subsequent VIS irradiation (Chapter 4). The photoreactivation of *T. urticae* eggs can be blocked in the UV method which has a sufficient time lag between UV-B irradiation and sunrise (Tanaka et al. 2016).

The mortality of *T. urticae* is proportional to the cumulative UV-B irradiance (Murata and Osakabe 2013). UV-B damage was likely accumulated in mites under intermittent irradiation as a single irradiation (Chapter 5). The times to hatching at 18, 25, and 30°C were 12.3, 4.5, and 3.5 days, respectively, for *T. urticae* and 5.9, 2.0, and 1.6 days, respectively, for *N. californicus* (Chapter 3). Egg duration of *T. urticae* is longer than *N. californicus*, resulting more UV-B accumulation than *N. californicus* under daily nighttime UV-B irradiation during egg stage, which is the most sensitive stage of spider mites to UV-B. In same species, egg duration was quite different among temperatures, inducing more and less UV-B accumulation at low and high temperature, respectively. This may explain the variation of hatchability after daily nighttime UV-B irradiation among different temperatures in *T. urticae* and *N. californicus*, respectively (Chapter 3).

Cleavage stage embryos are highly vulnerable to UV-B radiation (Chapter 5). In 25 and 30°C, it is expected that more than half of *T. urticae* eggs were irradiated after cleavage finished under the UV method, because it took only 12 h and 8.5 h to finish the cleavage in 25 and 30°C, respectively. These result explain higher hatchability of *T. urticae* after UV-B irradiation in 25 and 30°C under the UV method. Moreover, embryos conducting repaid cell division during cleavage stage do not perform an apoptotic response until the mid-blastula transition, which is the pivotal stage to eliminate damaged cells (Ikegami et al. 1999; Finkielstein et al. 2001). AL 0 h eggs stopped development after 0.432 kJ m^{-2} UV-B irradiation but continued to develop until hatch after 0.145 kJ

m^{-2} UV-B irradiation. This divergence indicated that damaged cells could be eliminated by apoptosis, later in development under low UV-B dose. But the apoptotic mechanism was not sufficient to revert the defective morphologies because DNA-damaged cells was very large when high doses were used.

Low temperature increased photoreactivation efficiency of *T. urticae* eggs (Chapter 4). However, considering the high mortality of *T. urticae* eggs at low temperature (Chapter 3) and 5 h time lag under the UV method, the control effect of the UV method in spider mites would not decline in winter.

UV-B irradiation of *N. californicus* eggs affects the development of hatched larvae (Nakai et al. 2018). Therefore, protecting phytoseiid mites from UV-B radiation is still important for the concurrent use of phytoseiid mites with UV-B lamps in greenhouses. Sugioka et al. (2018) found that tea pollen and peach pollen increased UV-B resistance in *N. californicus*. Complete developmental rate in *N. californicus* fed strawberry pollen and recovery of suppressed fecundity after starting to feed on *T. urticae* (Chapter 6), suggesting a possible contribution of strawberry pollen to the persistence of *N. californicus* when the prey density is low in strawberry greenhouses. In strawberry greenhouses, flowers bloom continuously; therefore, strawberry pollen it is expected to maintain the *N. californicus* population during long cultivation period.

Strawberry pollen showed higher antioxidant activity compared to tea pollen (Chapter 6). Pollen grains contain bioactive substances, such as antioxidants, to protect germ cells from solar UV-B radiation (Feng et al. 2000; Žilić et al. 2014). *N. californicus* can be fed on different pollen (Gugole Ottaviano et al. 2015; Khanamani et al. 2017), consumption of pollen from host plants may help *N. californicus* to maintain population and protect the phytoseiid mites from UV-B radiation in released site.

Tetranychus urticae has developed high resistance to most of the commercially available acaricides (Van Leeuwen et al. 2010, 2015), resulting in chemical control failure worldwide. The UV method and phytoseiid mites become good candidates for effective IPM. This study reveals the higher susceptibility in spider mites than phytoseiid mites under the nighttime intermittent UV-B irradiation, suggesting the possibility of simultaneous application of UV-B irradiation and phytoseiid mites in controlling the spider mites. Moreover, this study reports strawberry pollen was an adequate alternative

food source and UV-B protection ingredients for *N. californicus*, which makes combination of the UV method with phytoseiid mites more sustaining in greenhouses. By simultaneous application of the UV method with phytoseiid mites, it is expected to reduce the need for pesticide applications in the IPM system.

Reference

Abbott WS, A method of computing the effectiveness of an insectide. *J Econ Entomol* **18**:265–267 (1925).

Ahn JJ, Kim KW and Lee JH, Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves. *J Appl Entomol* **134**:98–104 (2010).

Ajila HEV, Colares F, Lemos F, Marques PH, Franklin EC, do Vale WS, Oliveira EE, Venzon M and Pallini A, Supplementary food for *Neoseiulus californicus* boosts biological control of *Tetranychus urticae* on strawberry. *Pest Manag Sci* **75**:1986–1992 (2019).

Akimoto Y, Shiroya T and Egami N, Abnormal morphogenesis of sea urchin embryo induced by UV partial irradiation given at cleavage stage. *Journal of radiation research* **24**:197-202 (1983).

Alton LA and Franklin CE, Do high temperatures enhance the negative effects of ultraviolet-B radiation in embryonic and larval amphibians? *Biol Open* **1**:897–903 (2012).

Alton LA and Franklin CE, Drivers of amphibian declines: effects of ultraviolet radiation and interactions with other environmental factors. *Climate Change Responses* **4**, 6 (2017).

Aoki S, *Statistic Analysis Using R*. Ohmsha, Tokyo, Japan (2011).

Atarashi M, Manabe Y, Kishimoto H, Sugawara T and Osakabe M, Antioxidant protection by astaxanthin in the citrus red mite (Acari: Tetranychidae). *Environ Entomol* **46**:1143–1150 (2017).

Ballaré CL, Caldwell MM, Flint SD, Robinson SA and Bornman JF, Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochem Photobiol Sci* **10**:226–241 (2011).

Barcelo JA, Photoeffects of visible and ultraviolet radiation on the two-spotted spider mite, *Tetranychus urticae*. *Photochem Photobiol* **33**:703–706 (1981).

Bayu MSYI, Ullah MS, Takano Y and Gotoh T, Impact of constant versus fluctuating temperatures on the development and life history parameters of *Tetranychus urticae*, (Acari: Tetranychidae). *Exp Appl Acarol* **72**:205-227 (2017).

Bolland HR, Gutierrez J and Flechtmann CHW, World catalogue of the spider mite family (Acari: Tetranychidae), Brill, Leiden, The Netherlands (1998).

Bonaventura R, Poma V, Costa C and Matranga V, UVB radiation prevents skeleton growth and stimulates the expression of stress markers in sea urchin embryos. *Biochem Biophys Res Commun* **328**:150-157 (2005).

Bonaventura R, Poma V, Russo R, Zito F and Matranga V. Effects of UV-B radiation on development and hsp70 expression in sea urchin cleavage embryos. *Marine biology* **149**:79-86 (2006).

Bounfour M and Tanigosh LK, Effect of temperature on development and demographic parameters of *Tetranychus urticae* and *Eotetranychus carpini borealis* (Acari: Tetranychidae). *Ann Entomol Soc Am* **94**:400–404 (2001).

Bryon A, Kurlovs AH, Dermauw W, Greenhalgh R, Riga M, Grbić M, Tirry L, Osakabe M, Vontas J, Clark RM and Van Leeuwen T, Disruption of a horizontally transferred phytoene desaturase abolishes carotenoid accumulation and diapause in *Tetranychus urticae*. *Proc Natl Acad Sci USA* **114**:E5871–E5880 (2017).

Burdick S, Prischmann-Voldseth D and Harmon JP, Density and distribution of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) in response to UV radiation. *Popul Ecol* **57**:457–466 (2015).

Cao G, Sofic E and Prior RL, Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radic Biol Med* **22**:749–760 (1997).

Castagnoli M and Simoni S, Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). *Exp Appl Acarol* **23**:217–234 (1999).

Castagnoli M, Simoni S and Nachman G, Short-term changes in consumption and oviposition rates of *Neoseiulus californicus* strains (Acari: Phytoseiidae) after a diet shift. *Exp Appl Acarol* **25**:969–983 (2001).

Castelluccio C, Paganga G, Melikian N, Bolwell GP, Pridham J, Sampson J and Rice-Evans C, Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Lett* **368**:188–192 (1995).

Connelly SJ, Moeller RE, Sanchez G and Mitchell DL, Temperature effects on survival and DNA repair in four freshwater cladoceran *Daphnia* species exposed to UV radiation. *Photochem Photobiol* **85**:144–152 (2009).

Dara SK, The new integrated pest management paradigm for the modern age, *J Integr Pest Manag* **10**: 1–9 (2019).

Dearden PK, Donly C and Grbić M, Expression of pair-rule gene homologues in a chelicerate: early patterning of the two-spotted spider mite *Tetranychus urticae*. *Development* **129**:5461–5472 (2002).

Feng H, An L, Tan L, Hou Z and Wang X, Effect of enhanced ultraviolet-B radiation on pollen germination and tube growth of 19 taxa in vitro. *Environ Exp Bot* **43**:45–53 (2000).

Finkielstein CV, Lewellyn AL and Maller JL, The midblastula transition in Xenopus embryos activates multiple pathways to prevent apoptosis in response to DNA damage. *Proc Natl Acad Sci* **98**:1006-1011 (2001).

Foley S, Navaratnam S, McGarvey DJ, Land EJ, Truscott TG and Rice-Evans CA, Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. *Free Radical Bio Med* **26**:1202–1208 (1999).

Fukaya M, Uesugi R, Ohashi H, Sakai Y, Sudo M, Kasai A, Kishimoto H and Osakabe M, Tolerance to solar ultraviolet-B radiation in the citrus red mite, an upper surface user of host plant leaves. *Photochem Photobiol* **89**:424–431 (2013).

Gerson U, Pest control by mites (Acari): present and future. *Acarologia* 54:371–394 (2014).

Gotoh T, Yamaguchi K and Mori K, Effect of temperature on life history of the predatory mite *Amblyseius* (*Neoseiulus*) *californicus* (Acari: Phytoseiidae). *Exp Appl Acarol* **32**:15–30 (2004).

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Rouzé P, Grbić V, Osborne EJ, Dermauw W, Ngoc PCT, Ortego F, Hernández-Crespo P, Diaz I, Martinez M, Navajas M, Sucena É, Magalhães S, Nagy L, Pace RM, Djuranović S, Smagghe G, Iga M, Christiaens O, Veenstra JA, Ewer J, Villalobos RM, Hutter JL, Hudson SD, Velez M, Yi SV, Zeng J, Pires-daSilva A, Roch F, Cazaux M, Navarro M, Zhurov V, Acevedo G, Bjelica A, Fawcett JA, Bonnet E, Martens C, Baele G, Wissler L, Sanchez-Rodriguez A, Tirry L, Blais C, Demeestere K, Henz SR, Gregory TR, Mathieu J, Verdon L, Farinelli L, Schmutz J, Lindquist E, Feyereisen R and Van de Peer Y, The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* **479**:487–492 (2011).

Grienenberger E, Besseau S, Geoffroy P, Debayle D, Heintz D, Lapierre C, Pollet B, Heitz T and Legrand M, A BAHD acyltransferase is expressed in the tapetum of Arabidopsis anthers and is involved in the synthesis of hydroxycinnamoyl spermidines. *Plant J* **58**:246–259 (2009).

Gugole Ottaviano MF, Cédola CV, Sánchez NE and Greco NM, Conservation biological control in strawberry: effect of different pollen on development, survival, and reproduction of *Neoseiulus californicus* (Acari: Phytoseiidae). *Exp Appl Acarol* **67**:507–521 (2015).

Häder DP, Kumar HD, Smith RC and Worrest RC, Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochem Photobiol Sci* **6**:267–285 (2007).

Hamsher SE, Sung CYT and Sanders RW, Effects of temperature and photorepair radiation on a marine ciliate exposed to UVB radiation. *J Eukaryot Microbiol* **65**:458–467 (2018).

Hothorn T, Bretz F and Westfall P, Simultaneous inference in general parametric models. *Biometrical J* **50**:346–363 (2008).

Huang D, Ou B, Hampsch-Woodill M, Flanagan JA and Prior RL, High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agric Food Chem* **50**:4437–4444 (2002).

Hoy M and Smilanick JM, Non-random prey location by the phytoseiid predator *Metaseiulus occidentalis*: differential responses to several spider mite species. *Entomol Exp Appl* **29**:241–253 (1981).

Ikegami R, Hunter P and Yager TD, Developmental activation of the capability to undergo checkpoint-induced apoptosis in the early zebrafish embryo. *Dev boil* **209**:409-433 (1999).

Izaguirre MM, Mazza CA, Svatoš A, Baldwin IT and Ballaré CL, Solar ultraviolet-B radiation and insect herbivory trigger partially overlapping phenolic responses in *Nicotiana attenuata* and *Nicotiana longiflora*. *Ann Bot* **99**: 103–109 (2007).

Kanto T, Matsuura K, Yamada M, Usami T and Amemiya Y (2009) UV-B radiation for control of strawberry powdery mildew. *Acta Hortic* **842**:359–362.

Kanto T, Matsuura K, Ogawa T, Yamada M, Ishiwata M, Usami T and Amemiya Y, A new UV-B lighting system controls powdery mildew of strawberry. *Acta Hortic* **1049**:655–660 (2014).

Kelner A, Action spectra for photoreactivation of ultraviolet-irradiate. Escherichia coli and Streptomyces griseus. *J Gen Physiol* 34:835–852 (1951).

Khanamani M, Fathipour Y, Talebi AA and Mehrabadi M, Linking pollen quality and performance of *Neoseiulus californicus* (Acari: Phytoseiidae) in two-spotted spider mite management programmes. *Pest Manag Sci* **73**:452–461 (2017).

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K and Taniguchi H, Antioxidant properties of ferulic acid and its related compounds. *J Agr Food Chem* **50**:2161–2168 (2002).

Kishimoto H, Ohira Y and Adachi I, Effect of different plant pollens on the development and oviposition of seven native phytoseiid species (Acari: Phytoseiidae) in Japan. *Appl Entomol Zool* **49**:19–25 (2014).

Koveos DS, Suzuki T, Terzidou A, Kokkari A, Floros G, Damos P and Kouloussis NA, Egg hatching response to a range of ultraviolet-B (UV-B) radiation doses for four predatory mites and the herbivorous spider mite *Tetranychus urticae*. *Exp Appl Acarol* **71**:35–46 (2017).

Kroon PA and Williamson G, Hydroxycinnamates in plants and food: current and future perspectives. *J Sci Food Agr* **79**:355–361 (1999).

Kuhlmann F and Müller C, Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. *Environ Exp Bot* **66**:61–68 (2009).

MacFadyen EJ, Williamson CE, Grad G, Lowery M, Jeffrey WH and Mitchell DL, Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. *Global Change Biol* **10**:408–416 (2004).

Mangoli S, Rath D, Goswami M and Jawali N, Increased ultraviolet radiation sensitivity of *Escherichia coli* grown at low temperature. *Can J Microbiol* **60**:327–331 (2014).

Marafeli PP, Reis PR, da Silveira EC, Souza-Pimentel GC and de Toledo MA, Life history of *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) fed with

castor bean (*Ricinus communis* L.) pollen in laboratory conditions. *Braz J Biol* **74**:691–697 (2014).

Matallana-Surget S, Douki T, Meador JA, Cavicchioli R and Joux F, Influence of growth temperature and starvation state on survival and DNA damage induction in the marine bacterium *Sphingopyxis alaskensis* exposed to UV radiation. *J Photoch. Photobio B* **100**:51–56 (2010).

Mazza CA, Izaguirre MM, Zavala J, Scopel AL and Ballaré CL, Insect perception of ambient ultraviolet-B radiation. *Ecol Lett* **5**:722–726 (2002).

McMurtry JA and Croft BA, Life-styles of phytoseiid mites and their roles in biological control. *Annu Rev Entomol* **42**: 291-321 (1997).

Metcalf RL and Newell IM, Investigation of the biochronies of mites. *Ann Entomol Soc Am* **55**:350–353 (1962).

Murata Y, Osakabe M, The Bunsen–Roscoe reciprocity law in ultraviolet-B-induced mortality of the two-spotted spider mite *Tetranychus urticae*. *J Insect Physiol* **59**:241–247 (2013).

Murata Y and Osakabe M, Factors affecting photoreactivation in UVB-irradiated herbivorous spider mite (*Tetranychus urticae*). *Exp Appl Acarol* **63**:253–265 (2014).

Murata Y and Osakabe M, Photo-enzymatic repair of UVB-induced DNA damage in the two-spotted spider mite *Tetranychus urticae*. *Exp Appl Acarol* **71**, 15–34 (2017a).

Murata Y and Osakabe M, Developmental phase-specific mortality after ultraviolet-B radiation exposure in the two-spotted spider mite. *Environ Entomol* **46**:1448–1455 (2017b).

Nabi G and Liu Z, Ferulic and coumaric acids: application to release oxidative stress of DNA and methyl linoleate. *J Food Biochem* **36**:38–45 (2012).

Nakai K, Murata Y and Osakabe M, Effects of low temperature on spider mite control by intermittent ultraviolet-B irradiation for practical use in greenhouse strawberries. *Environ Entomol* **47**:140–147 (2018).

Ogawa Y and Osakabe M, Development, long-term survival, and the maintenance of fertility in *Neoseiulus californicus* (Acari: Phytoseiidae) reared on an artificial diet. *Exp Appl Acarol* **45**:123–136 (2008).

Ohtsuka K and Osakabe M, Deleterious effects of UV-B radiation on herbivorous spider mites: they can avoid it by remaining on lower leaf surfaces. *Environ Entomol* **38**:920–929 (2009).

Onzo A, Sabelis MW and Hanna R, Effects of ultraviolet radiation on predatory mites and the role of refuges in plant structures. *Environ Entomol* **39**:695–701 (2010).

Pahkala M, Laurila A and Merilä J, Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. *Proc R Soc Lond B* **268**:1699–1706 (2001).

Rastogi RP, Richa, Kumar A, Tyagi MB and Sinha RP, Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J Nucleic Acids* **2010**:592980 (2010).

Quek P and Hu J, Influence of photoreactivating light intensity and incubation temperature on photoreactivation of *Escherichia coli* following LP and MP UV disinfection. *J Appl Microbiol* 105:124-133 (2008).

R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2017).

Rousseaux MC, Scopel AL, Searles PS, Caldwell MM, Sala OE and Ballaré CL, Responses to solar ultraviolet-B radiation in an shrub-dominated natural ecosystem of Tierra del Fuego (southern Argentina). *Global Change Biol* **7**:467–478 (2001).

Rousseaux MC, Julkunen-Tiitto R, Searles PS, Scopel AL, Aphalo PJ and Ballaré CL, Solar UV-B radiation affects leaf quality and insect herbivory in the southern beech tree *Nothofagus antarctica*. *Oecologia* **138**:505–512 (2004).

Sakai Y and Osakabe M, Spectrum-specific damage and solar ultraviolet radiation avoidance in the two-spotted spider mite. *Photochem Photobiol* **86**:925–932 (2010).

Santos CD, Photoreactivation of ultraviolet-B damage in *Tyrophagus putrescentiae* (Acari: Acaridae) and *Tetranychus urticae* (Acari: Tetranychidae). *Internat J Acarol* **31**:429–431 (2005).

Shahidi F and Chandrasekara A, Hydroxycinnamates and their in vitro and in vivo antioxidant activities. *Phytochem Rev* **9**:147–170 (2010).

Shahidi F and Ambigaipalan P, Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects. A review. *J Funct Foods* **18**:820–829 (2015).

Shinmen T, Yano S and Osakabe M, The predatory mite *Neoseiulus womersleyi* (Acari: Phytoseiidae) follows extracts of trails left by the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Exp Appl Acarol* **52**:111–118 (2010).

Sinha RP and Häder DP, UV-induced DNA damage and repair: a review. *Photochem Photobiol Sci* **1**:225–236 (2002).

Soltaniyan A, Kheradmand K, Fathipour Y and Shirdel D, Suitability of pollen from different plant species as alternative food sources for *Neoseiulus californicus* (Acari: Phytoseiidae) in comparison with a natural prey. *J Econ Entomol* **111**:2046–2052 (2018).

Strack D, Eilert U, Wray V, Wolff J and Jaggy H, Tricoumaroylspermidine in flowers of Rosaceae. *Phytochemistry* **29**:2893–2896 (1990).

Sudo M and Osakabe M, Do plant mites commonly prefer the underside of leaves? *Exp Appl Acarol* **55**:25–38 (2011).

Sugioka N, Kawakami M, Hirai N and Osakabe M, A pollen diet confers ultraviolet-B resistance in phytoseiid mites by providing antioxidants. *Front Ecol Evol* **6**:133 (2018).

Suzuki T, Watanabe M and Takeda M, UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. *J Insect Physiol* **55**:649–654 (2009).

Tachi F and Osakabe M, Vulnerability and behavioral response to ultraviolet radiation in the components of a foliar mite prey-predator system. *Naturwissenschaften* **99**:1031–1038 (2012).

Tachi F and Osakabe M, Spectrum-specific UV egg damage and dispersal responses in the phytoseiid predatory mite *Neoseiulus californicus* (Acari: Phytoseiidae). *Environ Entomol* **43**:787–794 (2014).

Tanaka M, Yase J, Aoki S, Sakurai T, Kanto T and Osakabe M, Physical control of spider mites using ultraviolet-B with light reflection sheets in greenhouse strawberries. *J. Econ. Entomol.* **109**:1758–1765 (2016).

Thoma F, Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. *EMBO J* **18**:6585–6598 (1999).

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W and Tirry L, Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. *Insect Biochem Mol Biol* **40**:563–572 (2010).

Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W, The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. *Pestic Biochem Physiol* **121**:12–21 (2015).

Van Uitregt VO, Wilson RS and Franklin CE, Cooler temperatures increase sensitivity to ultraviolet B radiation in embryos and larvae of the frog *Limnodynastes peronei*. *Global Change Biol* **13**:1114–1121 (2007).

Vega RL and Epel D, Stress-induced apoptosis in sea urchin embryogenesis. *Mar Environ Res* **58**:799–802 (2004).

Vogt T, Unusual spermine-conjugated hydroxycinnamic acids on pollen: function and evolutionary advantage. *J Exp Bot* **69**:5311–5318 (2018).

Weber S, Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase. *Biochim Biophys Acta* **1707**:1–23 (2005).

Williamson CE, Grad G, De Lange HJ, Gilroy S and Karapelou DM, Temperature-dependent ultraviolet responses in zooplankton: implications of climate change. *Limnol Oceanogr* **47**:1844–1848 (2002).

Yang Y, Zhang JI, Zhou Q, Wang L, Huang W and Wang R, Effect of ultrasonic and ball-milling treatment on cell wall, nutrients, and antioxidant capacitry of rose (*Rosa rugose*) bee pollen, and identification of bioactive components. *J Sci Food Agric* **99**:5350–5357 (2019).

Yano S and Osakabe M, Do spider mite-infested plants and spider mite trails attract predatory mites? *Ecol Res* **24**:1173–1178 (2009).

Yin WD, Hoffmann AA, Gu XB and Ma CS, Behavioral thermoregulation in a small herbivore avoids direct UVB damage. *J Insect Physiol* **107**:276–283 (2018).

Yoshioka, Y., T. Gotoh and T. Suzuki, UV-B susceptibility and photoreactivation in embryonic development of the two-spotted spider mite, *Tetranychus urticae*. *Exp*

Zamble A, Sahpaz S, Hennebelle T, Carato P and Bailleul F, N^1,N^5,N^{10}-Tris (4-hydroxycinnamoyl)spermidines from *Microdesmis keayana* roots. *Chem Biodivers* **3**:982–989 (2006).

Žilić S, Vančetović J, Janković M and Maksimović V, Chemical composition, bioactive compounds, antioxidant capacity and stability of floral maize (*Zea mays* L.) pollen. *J Funct Foods* **10**:65–74 (2014).

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