

1 Tomimori et al.: Effects of perilla and
2 UV-B pretreatment on *T. urticae*
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4 Environmental Entomology
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22 **Effects of growth phase and ultraviolet-B pretreatment in perilla leaves on the two-**
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Abstract

Perilla, *Perilla frutescens* var. *crispa*, is traditionally cultivated as an edible/medicinal crop in East Asia. Its essential oil contains many bioactive compounds that are expected to have high pharmacological functionality, as well as antimicrobial and insecticidal activity. Spider mites are a major pest group for perilla cultivation. The two-spotted spider mite, *Tetranychus urticae*, possesses divergent detoxification enzymes and has developed resistance against most acaricides. The essential oil content of perilla halves from the pre-flowering phase to the flowering phase, and ultraviolet (UV)-B radiation generally increases defense compounds. To clarify the effects of this change in essential oil content and the effects of UV-B pretreatment, we investigated the developmental success and egg production of *T. urticae* on leaves from the pre-flowering and flowering phases cultivated with and without nighttime UV-B irradiation. Both the parameters significantly increased on leaves from the flowering phase in comparison with that from the pre-flowering phase, suggesting that constitutively produced essential oil provided protection against mite pests in a growth phase-specific manner. The defense system also extended the developmental period of mites on red perilla leaves, but not on green perilla leaves, in pre-flowering phase. Although egg production was lower on red perilla leaves pretreated with UV-B, no negative effects were caused on the developmental success and duration on red and green perilla and the egg production on green perilla by UV-B pretreatment. Our findings reveal a significant impact of investment allocation of perilla plants and a small contribution of UV-B irradiation to the plant defense system.

Keywords: Plant-herbivore interactions, Medicinal crop, Essential oil, *Tetranychus urticae*

Perilla, *Perilla frutescens* (L.) Britton var. *crispa* (Thunb.) H. Deane (Lamiales: Lamiaceae), is a short-day annual herb traditionally cultivated as an edible and medicinal crop for Chinese herbal medicines, mainly in East Asia (China, Japan, and Korea). In outdoor cultivation in Japan, perilla is seeded in spring and leaves are harvested from June until the flowering stage in October or later. Conversely, year-round cultivation of perilla is possible in greenhouses; in 2014, 63% of perilla in Japan (9,592 t) was produced in greenhouses (www.maff.go.jp/j/tokei/kouhyou/tokusan_yasai). Perilla includes varieties of different colors, red perilla (f. *purpurea*) and green perilla (f. *viridis*). Purple leaves of red perilla contain high concentrations of anthocyanins (Ota et al. 2017; Fujiwara et al. 2018) and are traditionally used for natural coloring of Japanese apricot, “umeboshi.” Green perilla leaves are used as a raw food and as a garnish for processed foods made of fish, and so forth. Perilla plants also abundantly produce essential oil containing various secondary metabolites acting a biological protection mechanism.

The essential oil is secreted in glandular fistulas of perilla leaves, and is a mixture of components including perillaldehyde and limonene (Yoshida et al. 1968, 1969; Fujiwara et al. 2018; Ahmed and Tavaszi-Sarosi 2019). The essential oil also includes various other bioactive compounds, such as phenolic acids, flavonoids, and triterpenes, and therefore is expected to have pharmacological functionality (e.g., anti-allergic, antidepressant, anti-inflammatory, and anticancer effects) (Banno et al. 2004; Akihisa et al. 2006; Ito et al. 2011; Yu et al. 2017; Ahmad 2019; Kagawa et al. 2019). The antimicrobial activities of perilla plant essential oils on bacteria and fungi have also attracted attention from many researchers (Yu et al. 2017). The insecticidal effects of perilla’s essential oils have also been reported on a broad range of insect taxonomic

groups (Zhao et al. 2012; Tabanca et al. 2015; Park et al. 2016; Dong et al. 2019). However, spider mites such as the two-spotted spider mite *Tetranychus urticae* Koch and the Kanzawa spider mite *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) frequently attack perilla plants, particularly in greenhouses (Tanaka et al. 1993; Yanagida et al. 1996; Itoh et al. 2003). Because of perilla's usage as a medical material or as fresh food, it is desirable to reduce pesticide application and establish integrated pest management (IPM) strategies in perilla production. However, basic information about the biology and ecology of spider mites on perilla plants is limited.

As a new alternative control method for spider mites in strawberry greenhouses, physical control using a combination of ultraviolet-B (UV-B) lamps with light reflection sheets has been developed in Japan (UV method; Tanaka et al. 2016). The mortality of mites under UVB irradiation increases linearly with increasing cumulative UVB irradiation based on probit analyses (Sakai et al. 2012; Murata and Osakabe 2013). In the UV method, UV-B irradiation is performed at night based on the biological impact of direct UV-B irradiation on spider mites (Ohtsuka and Osakabe 2009; Sakai and Osakabe 2010) and the invalidation of photoreactivation of spider mites by maintaining darkness after UV-B irradiation (Murata and Osakabe 2014, 2017a, 2017b; Yoshioka et al. 2018). However, excessive UV-B irradiation damages to the quality of perilla leaves; in a previous study, the cumulative UV-B irradiance equivalent to that used in strawberry (Tanaka et al. 2016) caused some symptoms on perilla plants as described in the next paragraph (Ota et al. 2017).

UV-B irradiation generally increases secondary metabolites in plants, including medicinal substances (Zhang and Björn 2009; Zhang et al. 2017) and defense compounds against arthropod herbivores (Ballaré et al. 2011; Zavala et al. 2015;

Escobar-Bravo et al. 2017). The concentrations of perillaldehyde and limonene increased by 19% in UV-B-irradiated red perilla plants (51 kJ d^{-1}) in comparison with unirradiated plants, although no statistically significant difference was detected, in Nishimura et al. (2008). Simultaneously, they stated the reduction of anthocyanin concentration in UV-B irradiated plants (Nishimura et al. 2008). Marginal decrease by 7 and 9% in anthocyanin concentration was also observed in red perilla plants irradiated with UV-B at 1.3 kJ d^{-1} nighttime in Ota et al. (2017). However, the UV-B irradiation caused no destruction in the ultra-structure of adaxial epidermal cells (Ota et al. 2017), and peroxidase activity increased in the UV-B-irradiated perilla leaves (Hosokawa et al. 2016). Therefore, the lowering of anthocyanin concentration might be a result of its degradation catalyzed by vacuolar peroxidase scavenging H_2O_2 produced by UV-B irradiation (Ota et al. 2017). Perilla plants possess abundant bioactive secondary compounds, which may be appropriate materials for investigating the indirect effects of UV-B radiation on spider mites.

Plant growth phase-dependent changes in essential oil content in perilla plants have been previously reported; the content is twice as high in the pre-flowering stage than in the flowering stage (Yoshida et al. 1968, 1969; Ghimire et al. 2017). In green perilla and another Lamiaceae species, spearmint *Mentha spicata* L., it has been suggested that detoxification enzymes in *T. kanzawai* (Acari: Tetranychidae), play a role in counteracting the host defense response (Ozawa et al. 2017). However, it is unknown how the growth phase of perilla plants affects spider mite biology and how the growth phase effects interact with UV-B irradiation.

In this study, the effects of changes in leaf quality such as essential oil contents between plant growth phases and the indirect effects of UV-B irradiation on spider mites

were tested in perilla plants. For this purpose, we comparatively evaluated the developmental success and egg production (hereafter, “performance”) of *T. urticae*, on red and green perilla leaves grown and harvested in greenhouses with and without nighttime UV-B irradiation. Because spider mites can adapt or acclimate to host plants (Fry 1989; Agrawal et al. 2002), we also tested for the effects of maternal host plants on the development of their progeny after a short-term acclimation on perilla leaves.

Materials and methods

Spider Mite

The *T. urticae* (green form) population used in this study was a laboratory population originally collected from multiple areas in Japan and cultivated by mixture on potted kidney bean plants at 25–28°C for more than 9 years. Prior to experiments, 5–10 adult females were moved from the culture and introduced to a detached kidney bean leaf (5 cm in diameter) placed on water-soaked cotton in a Petri dish. Then, subcultures were established by rearing them for one or two generations in a laboratory at 25°C with a 16 h light/8 h dark photoperiod. Individuals used for experiments were picked up from the subcultures. All laboratory experiments were performed in the same laboratory.

Perilla Plants and Cultivation

Seeds of a red perilla (‘Houkouakashiso’) and a green perilla (‘Aoshiso’) were purchased from Nakahara Seed Product Co.,Ltd. (Fukuoka, Japan) and Takii Seed Co., Ltd. (Kyoto, Japan), respectively.

In 2016, the red perilla were sown on cultivating soil filled plastic trays in a greenhouse on June 19. After true leaves opened (July 13), the seedlings were

individually transplanted into pots (24 cm in diameter). Fourteen pots were placed in each of the four experimental areas in the greenhouse separated by UV-opaque polyvinyl chloride film (0.1 mm thick, Cutaceclean®Kirinain; MKV Platech, Tokyo, Japan) that completely blocked UV wavelengths shorter than 380 nm (Ohtsuka and Osakabe 2009). Two of the four areas were equipped with bulb-shaped UV-B fluorescent lamps (UV-B lamp; SPWFD24UB1PB; Panasonic Lighting Devices Co., Ltd., Takatsuki, Japan), whose output peaked at a wavelength of 310 nm (Murata and Osakabe 2013). The perilla plants were illuminated with UV-B during nighttime (00:00–03:00) from September 20, when the plants grew to more than four sections with eight leaves, until the experiments were completed. The UV-B irradiance on the perilla plants was 0.06 W m^{-2} ($0.65 \text{ kJ m}^{-2} \text{ day}^{-1}$). The temperature in the greenhouse was maintained at 15°C or higher throughout cultivation using electric heaters from October onward. All perilla plants flowered by November 17.

We tentatively measured plant height and number of leaves at sampling time in 2016; 33–47 cm high with 14–18 leaves and 25–46 cm high with 12–18 leaves in UV-B irradiated and unirradiated plants on 4–8 October; 55–67 cm high with 18–24 leaves and 43–62 cm high with 16–24 leaves in UV-B irradiated and unirradiated plants on 17 November. Leaf sizes used for experiments were 72–112 cm length $\times$ 46–85 cm width and 65–127 cm length $\times$ 42–74 cm width in UV-B irradiated and unirradiated plants on 4–8 October; 98–123 cm length $\times$ 56–80 cm width and 84–131 cm length $\times$ 49–75 cm width in UV-B irradiated and unirradiated plants on 17 November.

In 2017, the red and green perilla were sown on May 15 and transplanted into pots on June 19. Daily UV-B irradiation commenced on October 8. The red and green perilla plants flowered by December 12 and by December 1, respectively.

We sampled leaves at the second or third section from the top of the stem for use in laboratory experiments. To evaluate the performance of *T. urticae*, sampling of red perilla was performed on October 4 (used for experiments of both development and egg production; before flowering: BF), November 17 (development) and December 4 (egg production; flowering: AF), 2016, and October 30 (development) and November 3 (egg production; BF) and December 12 (development and egg production; AF), 2017. For green perilla, sampling was performed on October 30 (development) and November 3 (egg production; BF) and December 1 (development) and December 3 (egg production; AF), 2017. In addition, to evaluate maternal effects, red perilla leaves were sampled on October 5 and November 21 (BF), 2017.

Development

We prepared 20 perilla leaf discs (15 × 15 mm) on water-soaked cotton in Petri dishes (9 cm in diameter; 6–7 leaf discs per dish) for each of five plants chosen from the UV-B irradiated area (UV+) and non-irradiated control area (UV–; 100 leaf discs in total per treatment). *T. urticae* larvae were individually introduced to the leaf discs within 2 h after hatching. To synchronize egg hatching, eggs laid on kidney bean leaves were maintained at high humidity by closing the lids of Petri dishes. It is known that egg hatching is suspended under the high humidity in spider mites including *T. urticae* (Ubara and Osakabe 2015). The lids were opened the day exceeded the egg periods. Using this method, most eggs hatched within 1 h after the lid was opened. The Petri dishes were kept in the laboratory and developmental status was recorded every 24 h.

Individuals that escaped from the leaf discs were recorded as dead individuals to calculate developmental success, because the escape behavior may have been promoted

by leaf disc characteristics, such as the emission of volatiles. Prior to the experiments, we tentatively tested the leaf disc condition (15×15 mm) using kidney bean leaves as a preferred host plant for *T. urticae* and confirmed the rate of developmental success, mortality, escaping rate, and developmental duration. However, we did not combined the results on kidney bean leaves with that on perilla leaves in data analysis, because information about sex of several individuals were not recorded. Developmental duration was calculated as days from the introduction of larvae (day 0) to the day when the adult emerged.

The effects of plant growth phase and UV-B irradiation on the developmental success of *T. urticae* were evaluated by two-way analysis of variance (ANOVA) using the “aov” module in R software (R Core Team, 2014). Developmental ratios calculated for 20 leaf discs from the same plants were used as replicates. Prior to the two-way ANOVA, we applied the arcsine square root transformation for the dataset and confirmed the homogeneity of variances through Bartlett’s test using the “bartlett.test” module in R software.

The effects of plant growth phase, sex of developed mite, and UV-B irradiation on developmental duration were evaluated using generalized linear regression (GLM) analyses assuming a Gaussian distribution employing the “glm” module in R software. In the GLM analyses, we excluded interactions among explanatory variables based on the Akaike's information criterion (AIC). Developmental days of individuals that successfully developed were used as replicates.

Egg Production

One hundred perilla leaf discs (20 per plant) were prepared for each of the UV+ and

UV– treatments as well as for the development experiments described above. One hundred unmated adult *T. urticae* females were individually introduced to the leaf discs. The last molt from teleiochrysalis to adulthood was suspended using the high humidity in Petri dishes covered with lids (Ikegami et al. 2000), then the lids were opened to allow females to molt simultaneously.

Egg production per female was observed every 24 h and calculated as the number of eggs produced over 5 days from the first oviposition day. Eggs produced on leaf discs were removed each day after counting. Individuals that died from introduction until day 8 were excluded from subsequent data analyses. Individuals that produced no eggs before day 8 were recorded as having produced no eggs.

The effects of plant growth phase and UV-B irradiation on egg production were evaluated using GLM analyses assuming a gamma distribution with the “glm” module in R software. The numbers of eggs produced by individual adult females were used as replicates.

Effects of Maternal Host Plants on Development of Offspring

One kidney bean leaf disc (20 × 20 mm) and four red perilla leaf discs (20 × 20 mm) were prepared in Petri dishes. Fifty teleiochrysalis females that developed on kidney bean were introduced to each leaf disc. The Petri dishes were covered with lids to synchronize the last molt. After 48 h, the lids were opened for adult emergence, and resulting unmated adult females were allowed to oviposit for 2 days on kidney bean leaf discs and 4 days on red perilla leaf discs. After adult females were removed from the leaf discs, hatch timing was synchronized by applying high humidity. Eighty new leaf discs (15 × 15 mm) were prepared for kidney bean and red perilla, respectively. Larvae

that hatched within 2 h on kidney bean leaf discs were individually introduced to 40 new kidney bean leaf discs and 40 new red perilla leaf discs. The leaf discs were prepared from six plants (6 or 7 leaf discs per plant) in each plant species. Similarly, larvae that hatched within 2 h on red perilla leaf discs were individually introduced to 40 new kidney bean leaf discs and 40 new red perilla leaf discs.

The effects of several factors (the date of leaf picking, maternal host plants, and progeny host plants) on the developmental success of progeny were evaluated by three-way ANOVA using the “aov” module in R software. Developmental ratios calculated for leaf discs from the same plants were used as replications. Prior to the three-way ANOVA, we applied the arcsine square root transformation for the dataset and confirmed the homogeneity of variances by Bartlett’s test using the “bartlett.test” module in R software. The effects of the factors on progeny developmental duration were evaluated using GLM analyses assuming a Gaussian distribution with the “glm” module in R software. Individuals that successfully developed were considered replicates in the calculation of developmental duration.

Results

Effects of Plant Growth Phase and UV-B Irradiation on Development

The rate of developmental success, mortality, escaping rate, and developmental duration of *T. urticae* ($n = 30$) were 93%, 0%, 7%, and 5.30 ± 0.09 days (mean $\pm$ SE; including both females and males) on kidney bean leaf discs.

Most larvae did not develop into adults on BF perilla leaves, whereas approximately half or more larvae developed on AF leaves in all experiments (Fig. 1). Two-way ANOVA indicated statistically significant effects of plant growth phase on

developmental success ($F_{[1,16]} = 95.428$, $P = 3.8 \times 10^{-8}$, on red perilla in 2016; $F_{[1,16]} = 240.126$, $P = 4.69 \times 10^{-11}$ on red perilla in 2017; $F_{[1,16]} = 43.916$, $P = 5.82 \times 10^{-6}$ on green perilla in 2017). UV-B irradiation increased developmental success on red perilla in 2016 ($F_{[1,16]} = 4.619$, $P = 0.0473$) and green perilla in 2017 ($F_{[1,16]} = 4.753$, $P = 0.0445$), but had marginal effects on red perilla in 2017 ($F_{[1,16]} = 2.560$, $P = 0.129$). The difference between UV+ and UV- was minor compared to the difference between AF and BF. The interaction between plant growth phase and UV-B irradiation was not significant ($F_{[1,16]} = 0.109$, $P = 0.746$ on red perilla in 2016; $F_{[1,16]} = 0.285$, $P = 0.6$ on red perilla in 2017; $F_{[1,16]} = 0.06$, $P = 0.809$ on green perilla in 2017).

In both red perilla and green perilla, developmental duration was significantly extended on BF leaves compared to AF leaves (Table 1 and 2). Conversely, UV-B pretreatment had no effect on developmental duration on red perilla in 2017 (Table 2) and slightly shortened developmental duration on green perilla (Table 2). The degree of shortening was 0.1 day in females and males in BF and AF, respectively, and 0.5 day in females in AF, whereas no difference was observed in males in BF (Table 1).

Most individuals that died did so at the larval stage, and survival rates were therefore calculated at the completion of the first molt (protonymph emergence) in the 2016 experiments with red perilla. All individuals that died (UV+: 70.5%, UV-: 67.6%) or escaped (UV+: 24.8%, UV-: 32.4%) did so at the larval or protochrysalis stages in the BF phase. Similarly, most individuals that did not develop to adulthood (UV+: 18.5% died and 24.2% escaped, UV-: 31.7% died and 22.8% escaped) died by protonymph emergence (UV+: 16.7% died and 24.2% escaped, UV-: 30.8% died and 20.0% escaped) in the AF phase. Moreover, the majority of the extension in developmental duration in the BF phase occurred during the larval stage; the difference

between the duration in BF and AF was 1.5 and 1.7 days until protonymph emergence and until adult emergence, respectively. However, escaping rates until adult emergence were not different between BF and AF in the experiments with red perilla 2016 (Table S1 and S2).

In contrast, in the experiments 2017, escaping rates of individuals in red perilla (36.6 and 33.9% in UV+ and UV-, respectively) and green perilla (50 and 60.5% in UV+ and UV-, respectively) in BF were significantly larger than that in AF (3.9 and 6.1% in UV+ and UV-, respectively, in red perilla; 19.8 and 22.8% in UV+ and UV-, respectively, in green perilla) (Tables S1 and S2). Whereas, UV-B pretreatments had no effects on the escaping rates in all experiments (Table S2).

Effects of Plant Growth Phase and UV-B Irradiation on Egg Production

Mortality of adult females within eight days were largest in UV- of red perilla 2017 (21.4%) and tended larger in BF than AF (Tables S3 and S4). Pretreatment of UV-B irradiation had no effects on the mortalities of adult females.

Egg production was significantly lower on BF leaves than AF leaves in all experiments (Fig. 2, Table 3). UV-B treatment decreased egg production on red perilla, whereas it increased egg production on green perilla (Fig. 2, Table 3). A significant interaction between plant growth phase and UV-B treatment was detected on red perilla in 2016 (Table 3), and the difference between UV+ and UV- was greater in AF than BF. No interaction was detected in red perilla in 2017, and marginal effects were detected in green perilla (Table 3).

Effects of Maternal Host Plants on Development of Offspring

There were no effects of maternal host plant (three-way ANOVA, $F_{[1, 40]} = 1.003$, $P = 0.3227$) or leaf picking date (first or second experiments: October 5 or November 21; $F_{[1, 40]} = 2.236$, $P = 0.1427$) on developmental success (Fig. 3). In contrast, the effects of progeny host plants ($F_{[1, 40]} = 20.446$, $P = 5.36 \times 10^{-5}$) and their interaction with leaf picking date ($F_{[1, 40]} = 4.237$, $P = 0.0461$) were statistically significant. The developmental rate was lower on red perilla than on kidney bean, and it tended to be lower in the first experiment than the second experiment on red perilla, particularly if the maternal host plant was kidney bean and the progeny host plant was red perilla. There were no significant interactions between leaf picking date and maternal host plant ($F_{[1, 40]} = 2.733$, $P = 0.1061$), between maternal host plant and progeny host plant ($F_{[1, 40]} = 0.105$, $P = 0.7477$), or among all three factors ($F_{[1, 40]} = 0.807$, $P = 0.3743$).

Progeny host plants affected developmental duration. Progeny on red perilla required longer periods to develop than those on kidney bean, whereas leaf picking date and maternal host plants had no effect (Table 4 and 5). The interaction between progeny host plants and leaf picking date was also statistically significant (Table 5).

Developmental duration tended to be slightly longer in the first experiment than the second experiment in red perilla, but no change was observed for kidney bean. There was also a statistically significant interaction between maternal host plant and progeny host plant (Table 5): developmental duration of progeny on red perilla tended to be slightly shorter if their mothers' host plants were also red perilla. There was no statistically significant interaction between maternal host plant and leaf picking date (Table 5). The interaction among the three factors was excluded from GLM analyses based on Akaike's information criterion (AIC).

Discussion

Spider mites are an important pest group in perilla cultivation. Our laboratory experiments using harvested perilla leaves revealed large effects of plant growth phase on the development and egg production of *T. urticae*: perilla leaves from the BF phase were well-protected, inhibiting juvenile development and reducing egg production compared to leaves from the AF phase. This tendency was more pronounced in red perilla than green perilla, particularly with respect to developmental success and duration. Higher escaping rate during juvenile development and mortality of adult females during egg production in BF than AF in 2017 also supported better protection in BF. In contrast, escaping rate in AF did not decreased on red perilla leaves in 2016. The red perilla leaves used for development experiments were collected 17 November and 12 December in 2016 and 2017, respectively. Because essential oil content of perilla leaves is higher at the beginning than later of AF (Yoshida et al. 1968), the earlier collection date might cause higher escaping rate in 2016 than that in 2017.

These results may be related to the fact that few data points were obtained from green perilla (Tanaka et al. 1993; Yanagida et al. 1996; Itoh et al. 2003). According to Yanagida et al. (1996), leaf damage caused by spider mites infesting green perilla planted in March was most frequent in October, which was consistent with our findings on the effects of plant growth phase. Anthocyanin pattern and essential oil type (main oil) are genetically independent, although Fujiwara et al. (2018) reported that five of eight strains of red perilla contained perillaldehyde-type essential oil. The essential oil types of the remaining red perilla were perillaketone-type for two strains and (dillapiole+elemicin+myristicin)-type for one strain, while those of green perilla varies, including elsholtziaketone-, perillaketone-, perillene-, piperitenone-, myristicin-, and

(elemicin+myristicin)-types (Fujiwara et al. 2018).

Components of perilla essential oil have been demonstrated to have insecticidal effects on various herbivorous insects. Perillaldehyde exhibits strong inhibition effects against acetylcholine esterase in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), resulting in high mortality at a dose of 1 mg L⁻¹ (Park et al. 2016). Methyl perillate, a component of green perilla oil, causes high mortality in larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) (LC₅₀ = 16 mg L⁻¹) (Tabanca et al. 2015). Crude extracts of perilla essential oil have also shown strong insecticidal activity in some studies (Zhao et al. 2012; Tabanca et al. 2015). The egg production of *T. kanzawai* is suppressed on leaves of crispate green perilla (Ozawa et al. 2017), and similar effects were observed for *T. urticae* in the current study. One explanation for the change in the efficacy of the defense response in perilla plants may be a shift of investment from protection of the plant body to reproduction as an adaptive evolutionally mechanism for increasing the performance of annual plants. Indeed, the essential oil content of perilla plants is higher in the BF phase than the AF phase (Yoshida et al. 1968, 1969), although the tendency varies among plant strains (Ghimire et al. 2017). Because perilla plants possess many variable bioactive ingredients, further research on the single or combined effects of these ingredients is necessary to understand the underlying mechanisms for the performance variation between the growth phases of perilla plants.

From an adaptive perspective, selection and phenotypic plasticity may affect mite performance on unfavorable host plants. The fact that host preference is subject to additive genetic variance has long been known in spider mites, e.g., *T. urticae* against tomato and broccoli (Fry 1989; Kant et al. 2008) and *T. kanzawai* against tea and hydrangea (Gomi and Gotoh 1997). The suppression of egg production on perilla leaves

is mitigated in strains with higher expression levels of detoxification enzymes, which affect acaricide susceptibility (Ozawa et al. 2017). *Tetranychus urticae* is a notable crop pest that has developed resistance against acaricides, and elevated activities of detoxification enzymes are frequently reported in the population worldwide. Moreover, *T. urticae* genes involved in the detoxification and transport of xenobiotics enzymes are often expanded compared to insects, and respond to induction/selection by unfavorable host plants (Grbić et al. 2011; Dermauw et al. 2013; Snoeck et al. 2018). Based on the literature and current findings, investigating the mechanism of *T. urticae*'s adaptation to perilla plants may help elucidate the interactions between host plant adaptation and pesticide resistance.

Acclimation improves acceptance and performance on unfavorable host plants, even in non-adapted strains (Agrawal et al. 2002; Tajima et al. 2007). Regarding to the acclimation of *T. urticae* to red perilla, we detected maternal effects (interactions between host plants of mothers and progeny) upon developmental duration, but not developmental success. Progeny on red perilla exhibited reduced developmental success and elongated developmental duration, particularly at the larval stage. Therefore, maternal host plants did not have a significant beneficial effects on the performance of progeny, at least after a short acclimation period. On the other hand, although the red perilla leaves were picked in the BF period, developmental success and developmental duration were increased and reduced, respectively, in the second experiment vs. the first experiment evaluating maternal effects. Perilla essential oil contents, particularly components with anti-*T. urticae* bioactivity, may have begun to decrease from end of the BF period to flower bud formation in early/mid-December in our cultivation system.

Many studies have reported UV-B-mediated changes in the architecture and

physiology of plant resistance to herbivory by arthropods (Ballaré 2014; Escobar-Bravo et al. 2017). Similarly, UV-B pretreatment made *T. urticae* egg production on red perilla leaves to decrease in the current study. However, developmental success on red and green perilla leaves and developmental duration on red perilla leaves were unaffected by UV-B pretreatment of leaves. Moreover, oviposition and developmental duration were enhanced and slightly shortened, respectively, on green perilla leaves by UV-B irradiation. Similar discrepancies in indirect UV-B effects have been observed in the generalist caterpillar, *Trichoplusia ni* (Hbn.) (Lepidoptera: Noctuidae), on UV-B treated weedy forb leaves. In *Plantago lanceolate* L. (Plantaginaceae), larval development was accelerated on UV-B treated leaves, although survivorship was unaffected (McCloud and Berenbaum 1999). In contrast, the growth of *T. ni* was significantly inhibited on weedy forb plants exposed to UV-B radiation (direct and indirect effects), although no change was observed in a specialist insect, *Precis coenia* Hbn. (Lepidoptera: Nymphalidae) (McCloud and Berenbaum, 1999). Although it is not clear whether a co-evolutionary arms race between host plants and arthropod herbivores has occurred with respect to adaptations to ambient UV-B radiation, the direct biological impacts of UV-B radiation on UV-B vulnerable arthropods appear to be more significant than indirect effects through the change in plant chemicals. This seems to be the case for *T. urticae* because mortality is determined by the cumulative UV-B dose under various environmental conditions, including nighttime irradiation in greenhouses and solar radiation (Sakai et al. 2012; Murata and Osakabe 2013; Tanaka et al. 2016).

Nighttime UV-B irradiation prevents photoreactivation (Murata and Osakabe 2014, 2017a) and therefore has the potential to control spider mites in greenhouse strawberry cultivation (Tanaka et al. 2016). However, Ota et al. (2017) observed reduced leaf size

and degradation of anthocyanin in red perilla plants irradiated with UV-B at $0.54 \text{ kJ m}^{-2} \text{ day}^{-1}$ in the nighttime, which was lower than the UV-B dose practically applied in greenhouse strawberry cultivation ($1.7\text{--}2.0 \text{ kJ m}^{-2} \text{ day}^{-1}$). Simultaneous visible light irradiation prevented the degradation of anthocyanin and largely mitigated morphological changes in leaves, and daytime irradiation had no effect on anthocyanin content or leaf morphology (Ota et al. 2017), suggesting that photoreactivation contributed to the adaptation of red perilla plants to ambient UV-B irradiation. In the current study, perilla plants were irradiated with UV-B at $0.65 \text{ kJ m}^{-2} \text{ day}^{-1}$, but only a slight color change was observed in old red perilla leaves near the bottom of the stem. Although it is not known how this compares to the degree of UV-B damage reported by Ota et al. (2017), the dose used in the current study was likely a critical dose of nighttime UV-B irradiation without photoreactivation. The practical dose for spider mite control in strawberry greenhouses was determined based on the mortality obtained after a single acute dose of UV-B irradiation in a study by Murata and Osakabe (2013); the LD_{50} value for *T. urticae* eggs was 0.58 kJ m^{-2} (Tanaka et al. 2016). However, Nakai et al. (2018) found higher mortality under daily irradiation conditions than under a single acute dose of irradiation, indicating that it may be possible to design a dose regime that effectively controls spider mites without causing UV-B damage to perilla plants.

In conclusion, the defense mechanism of perilla plants is more effective against *T. urticae* in the BF period than the AF period, indicating a significant impact of investment allocation. The contribution of UV-B irradiation to the perilla plant defense system through indirect effects is surprisingly limited or absent. It is therefore appropriate to expect only direct effects of UV-B irradiation in controlling *T. urticae* on perilla plants, and this treatment is likely to be more effective for the AF period than the

470 BF period.

471

Acknowledgments

This study was supported by JSPS KAKENHI (a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan) grant number 26292029 for MO. DT, MO and MH planed this project, DT mainly performed experiments, SA supported UV-B irradiation condition design and adjustment, and MO and DT wrote the manuscript.

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Figure legends

Fig. 1 Effects of plant growth phase and UV-B irradiation on juvenile development of *T. urticae*. (a) and (b): on red perilla in 2016 and 2017, respectively; (c): on green perilla in 2017. Twenty mites per plants were tested for five plants (replicate) per treatment in each experiment. BF: before flower bud formation; AF: after flower bud formation (flowering stage); UV+: UV-B-irradiated plants; UV–: UV-B-non-irradiated plants. Vertical lines on bars show the standard errors among five plants in each treatment.

Fig. 2 Effects of plant growth phase and UV-B irradiation on egg production of *T. urticae*. (a) and (b): on red perilla in 2016 and 2017, respectively; (c): on green perilla in 2017. Number of females per plant in UV+ and UV– used for analysis were (a) 13–20 and 15–19 in BF and 15–19 and 11–20 in AF, (b) 15–19 and 12–17 in BF and 17–20 and 17–20 in AF, and (c) 15–18 and 15–20 in BF and 17–20 and 17–20 in AF, respectively. Eggs: number of eggs produced per female for the 5 initial days of its oviposition period; BF: before flower bud formation; AF: after flower bud formation (flowering stage); UV+: UV-B-irradiated plants; UV–: UV-B-non-irradiated plants. Vertical lines on bars show the standard errors among mite individuals.

Fig. 3 Effects of maternal host plants on the developmental success of progeny. K: kidney bean; R: red perilla. Leaves were collected on Oct. 5 and Nov. 21 (BF) in the first experiment and second experiment, respectively. Forty larvae per

664 treatment were used for the growth test. Vertical lines on bars show 95%
665 confidence intervals.

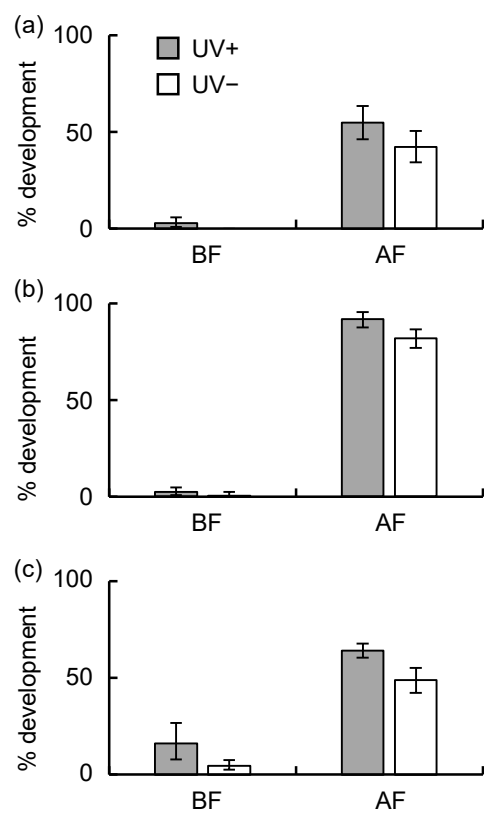


Fig. 1

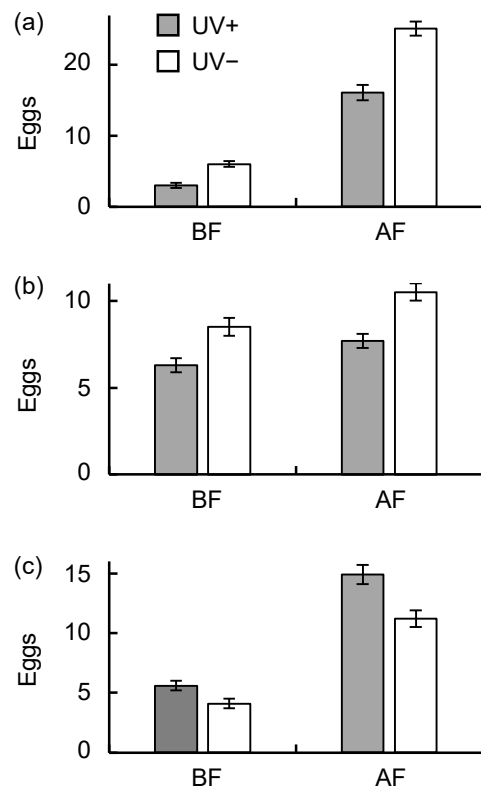


Fig. 2

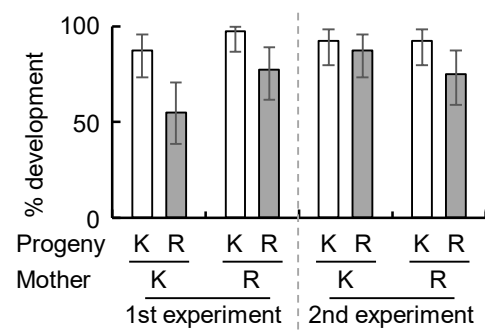


Fig. 3

Table 1 Development duration from larvae to adult emergence

		BF (Days $\pm$ SE) ^a		AF (Days $\pm$ SE) ^a	
		UV+	UV-	UV+	UV-
Red perilla					
2016	Female	8.7 $\pm$ 0.3 (3)	NA	6.9 $\pm$ 0.2 (42)	7.2 $\pm$ 0.3 (30)
	Male	8 $\pm$ 0 (2)	NA	6.4 $\pm$ 0.3 (13)	6.1 $\pm$ 0.2 (13)
2017	Female	8.3 $\pm$ 0.3 (3)	8.5 $\pm$ 0.5 (2)	6.5 $\pm$ 0.1 (56)	6.8 $\pm$ 0.1 (43)
	Male	8 (1)	8 (1)	6 $\pm$ 0.1 (34)	6 $\pm$ 0.1 (38)
Green perilla					
2017	Female	7.7 $\pm$ 0.1 (14)	7.8 $\pm$ 0.4 (5)	6.9 $\pm$ 0.1 (37)	7.4 $\pm$ 0.2 (31)
	Male	7 $\pm$ 0 (7)	7 (1)	6.8 $\pm$ 0.2 (27)	6.9 $\pm$ 0.1 (18)

^a BF: before flower bud formation, AF: flowering stage; UV+: host plants irradiated with UV-B, UV-: host plants unirradiated with UV-B; NA: not applicable; numerals in parentheses show the number of developed individuals.

Table 2 Analyses of the effects of plant growth phase and UV-B irradiation on developmental days in red perilla 2016 (a) and 2017 (b), and green perilla 2017 (c) by generalized linear model (GLM, family = Gaussian)

Factor ^a	Estimate	SE	<i>t</i> -value	<i>P</i> -value
(a) [only the data from UV+ were used for analyses]				
(Intercept)	6.8621	0.1440	47.660	$< 2 \times 10^{-16}$
PGP (BF)	1.7353	0.4439	3.909	2.49×10^{-4}
Sex (male)	-0.4935	0.2834	-1.741	0.0870
(b)				
(Intercept)	6.7230	0.1025	65.585	$< 2 \times 10^{-16}$
UV (+)	-0.1761	0.1177	-1.496	0.136
PGP (BF)	1.8411	0.3015	6.106	6.47×10^{-9}
Sex (male)	-0.6218	0.1194	-5.207	5.36×10^{-7}
(c)				
(Intercept)	7.3331	0.1134	64.650	$< 2 \times 10^{-16}$
UV (+)	-0.3157	0.1326	-2.381	0.0186
PGP (BF)	0.5326	0.1644	3.239	1.51×10^{-3}
Sex (male)	-0.3431	0.1320	-2.599	0.0104

^a UV: UV-B irradiation treatment on plants, PGP: plant growth phase

Table 3 Analyses of the effects of plant growth phase and UV-B irradiation on egg production in red perilla 2016 (a) and 2017 (b), and green perilla 2017 (c) by generalized linear model (GLM, family = gamma)

Factor ^a	Estimate	SE	<i>t</i> -value	<i>P</i> -value
(a)				
(Intercept)	0.038278	0.002595	14.750	$< 2 \times 10^{-16}$
UV (+)	0.020314	0.004574	4.441	1.22×10^{-5}
PGP (BF)	0.104112	0.009614	10.829	$< 2 \times 10^{-16}$
UV $\times$ PGP	0.082010	0.019455	4.215	3.21×10^{-5}
(b)				
(Intercept)	0.086754	0.004321	20.076	$< 2 \times 10^{-16}$
UV (+)	0.018857	0.006744	2.796	0.005458
PGP (BF)	0.028121	0.007597	3.702	2.49×10^{-4}
UV $\times$ PGP	0.002344	0.011584	0.202	0.839745
(c)				
(Intercept)	0.081857	0.004750	17.235	$< 2 \times 10^{-16}$
UV (+)	-0.018906	0.006003	-3.149	0.00177
PGP (BF)	0.115512	0.012801	9.023	$< 2 \times 10^{-16}$
UV $\times$ PGP	-0.027554	0.016339	-1.686	0.09258

^a UV: UV-B irradiation treatment on plants, PGP: plant growth phase

Table 4 Maternal effects on development duration from larvae to adult emergence of offspring

Host plants ^a		Developmental days $\pm$ SE ^b	
Mother	Progeny	1st	2nd
K	K	5.2 $\pm$ 0.1 (35)	5.3 $\pm$ 0.1 (37)
R	K	5.2 $\pm$ 0.1 (39)	5.3 $\pm$ 0.1 (37)
K	R	7.1 $\pm$ 0.3 (55)	6.6 $\pm$ 0.2 (35)
R	R	6.5 $\pm$ 0.2 (31)	6.1 $\pm$ 0.2 (30)

^a R: red perilla, K: kidney bean

^b Data are including females and males. 1st: 1st experimental periods in that test leaves were collected on 5 Oct. 2017, 2nd: 2nd experimental periods in that test leaves were collected on 21 Nov. 2017 (BF). Numerals in parentheses show the number of developed individuals.

Table 5 Analyses of the maternal effects on development duration from larvae to adult emergence of offspring by generalized linear model (GLM, family = Gaussian)

Factor ^a	Estimate	SE	<i>t</i> -value	<i>P</i> -value
(Intercept)	5.277235	0.298837	17.659	$< 2 \times 10^{-16}$
LPD	0.004089	0.180749	0.023	0.9820
MHP (perilla)	-0.222964	0.351146	-0.635	0.5260
OHP (perilla)	2.287372	0.370703	6.170	2.69×10^{-9}
LPD $\times$ MHP	0.109853	0.211203	0.520	0.6034
MHP $\times$ OHP	-0.495390	0.211702	-2.340	0.0201
LPD $\times$ OHP	-0.494397	0.212013	-2.332	0.0205

^a LPD: leaf picking date (Oct. 5 = 1 and Nov. 21 = 2), MHP: mothers' host plant, OHP: offspring's host plant

Supplementary Tables

“Effects of growth phase and ultraviolet-B pretreatment in perilla leaves on the two-spotted spider mite”. Tomimori et al.

Table S1. Escaping rate of developing *T. urticae* juveniles from red and green perilla leaves

		BF				AF			
		UV+		UV–		UV+		UV–	
	<i>n</i>	Escaping rate		<i>n</i>	Escaping rate	<i>n</i>	Escaping rate	<i>n</i>	Escaping rate
Red perilla									
2016	100	0.248		98	0.324	100	0.242	100	0.228
		(0.194–0.306)			(0.241–0.413)		(0.149–0.35)		(0.126–0.35)
2017	100	0.366		100	0.339	100	0.039	100	0.061
		(0.261–0.477)			(0.292–0.388)		(0.009–0.09)		(0.011–0.147)
Green perilla									
2017	99	0.5		100	0.605	100	0.198	100	0.228
		(0.251–0.75)			(0.463–0.738)		(0.156–0.244)		(0.114–0.366)

Figures in parentheses indicate CI. Figures in the column *n* indicate the total numbers of individuals tested over five plants (replications).

Table S2. ANOVA table for escaping rate of developing *T. urticae* juveniles from red and green perilla leaves

Factor	<i>DF</i>	Sum <i>Sq</i>	Mean <i>Sq</i>	<i>F</i> value	<i>Pr</i> ($>F$)
Red perilla 2016					
UV (+/–)	1	0.00571	0.005705	0.389	0.542
Season (BF/AF)	1	0.01625	0.016250	1.107	0.308
UV $\times$ Season	1	0.01293	0.012926	0.880	0.362
Residuals	16	0.23489	0.014681		
Red perilla 2017					
UV (+/–)	1	0.0006	0.0006	0.038	0.848
Season (BF/AF)	1	0.8456	0.8456	55.482	1.38×10^{-6}
UV $\times$ Season	1	0.0075	0.0075	0.495	0.492
Residuals	16	0.2438	0.0152		
Green perilla 2017					
UV (+/–)	1	0.0249	0.0249	0.668	0.426
Season (BF/AF)	1	0.6449	0.6449	17.289	7.41×10^{-4}
UV $\times$ Season	1	0.0060	0.0060	0.160	0.695
Residuals	16	0.5968	0.0373		

Prior to the two-way ANOVA, we applied the arcsine square root transformation for the dataset and confirmed the homogeneity of variances through Bartlett’s test using the “bartlett.test” module in R software

Table S3. Mortality of *T. urticae* females within eight days in oviposition experiments

		BF				AF			
		UV+		UV–		UV+		UV–	
	<i>n</i>	Mortality	<i>n</i>	Mortality	<i>n</i>	Mortality	<i>n</i>	Mortality	
Red perilla									
2016	100	0.138 (0.023–0.329)	97	0.101 (0.07–0.137)	99	0.096 (0.049–0.155)	85	0.027 (0.001–0.131)	
2017	100	0.132 (0.069–0.211)	100	0.214 (0.136–0.305)	100	0.015 (0.001–0.078)	100	0.054 (0.011–0.128)	
Green perilla									
2017	100	0.167 (0.12–0.22)	100	0.061 (0.002–0.195)	100	0.024 (0.001–0.078)	100	0.012 (0.001–0.058)	

Figures in parentheses indicate CI. Figures in the column *n* indicate the total numbers of females used over five plants (replications).

Table S4. ANOVA table for mortality of *T. urticae* females within eight days in oviposition experiments.

Factor	<i>DF</i>	Sum <i>Sq</i>	Mean <i>Sq</i>	<i>F</i> value	<i>Pr</i> ($>F$)
Red perilla 2016					
UV (+/–)	1	0.0529	0.05292	1.546	0.232
Season (BF/AF)	1	0.0627	0.06272	1.833	0.195
UV × Season	1	0.0102	0.01016	0.297	0.593
Residuals	16	0.5476	0.03422		
Red perilla 2017					
UV (+/–)	1	0.0603	0.06033	2.885	0.109
Season (BF/AF)	1	0.3050	0.30504	14.586	1.51×10^{-3}
UV × Season	1	0.0000	0.00000	0.000	0.995
Residuals	16	0.3346	0.02091		
Green perilla 2017					
UV (+/–)	1	0.0591	0.05906	2.196	0.158
Season (BF/AF)	1	0.2055	0.20552	7.641	1.38×10^{-2}
UV × Season	1	0.0202	0.02021	0.752	0.399
Residuals	16	0.4304	0.02690		

Prior to the two-way ANOVA, we applied the arcsine square root transformation for the dataset and confirmed the homogeneity of variances through Bartlett's test using the "bartlett.test" module in R software