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Effects of growth phase and ultraviolet-B pretreatment in perilla leaves on the two-spotted spider mite

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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

86 groups (Zhao et al. 2012; Tabanca et al. 2015; Park et al. 2016; Dong et al. 2019).
87 However, spider mites such as the two-spotted spider mite *Tetranychus urticae* Koch
88 and the Kanzawa spider mite *Tetranychus kanzawai* Kishida (Acari: Tetranychidae)
89 frequently attack perilla plants, particularly in greenhouses (Tanaka et al. 1993;
90 Yanagida et al. 1996; Itoh et al. 2003). Because of perilla's usage as a medical material
91 or as fresh food, it is desirable to reduce pesticide application and establish integrated
92 pest management (IPM) strategies in perilla production. However, basic information
93 about the biology and ecology of spider mites on perilla plants is limited.
94 As a new alternative control method for spider mites in strawberry greenhouses,
95 physical control using a combination of ultraviolet-B (UV-B) lamps with light reflection
96 sheets has been developed in Japan (UV method; Tanaka et al. 2016). The mortality of
97 mites under UVB irradiation increases linearly with increasing cumulative UVB
98 irradiation based on probit analyses (Sakai et al. 2012; Murata and Osakabe 2013). In
99 the UV method, UV-B irradiation is performed at night based on the biological impact
100 of direct UV-B irradiation on spider mites (Ohtsuka and Osakabe 2009; Sakai and
101 Osakabe 2010) and the invalidation of photoreactivation of spider mites by maintaining
102 darkness after UV-B irradiation (Murata and Osakabe 2014, 2017a, 2017b; Yoshioka et
103 al. 2018). However, excessive UV-B irradiation damages to the quality of perilla leaves;
104 in a previous study, the cumulative UV-B irradiance equivalent to that used in
105 strawberry (Tanaka et al. 2016) caused some symptoms on perilla plants as described in
106 the next paragraph (Ota et al. 2017).
107 UV-B irradiation generally increases secondary metabolites in plants, including
108 medicinal substances (Zhang and Björn 2009; Zhang et al. 2017) and defense
109 compounds against arthropod herbivores (Ballaré et al. 2011; Zavala et al. 2015;

110 Escobar-Bravo et al. 2017). The concentrations of perillaldehyde and limonene
111 increased by 19% in UV-B-irradiated red perilla plants (51 kJ d^{-1}) in comparison with
112 unirradiated plants, although no statistically significant difference was detected, in
113 Nishimura et al. (2008). Simultaneously, they stated the reduction of anthocyanin
114 concentration in UV-B irradiated plants (Nishimura et al. 2008). Marginal decrease by 7
115 and 9% in anthocyanin concentration was also observed in red perilla plants irradiated
116 with UV-B at 1.3 kJ d^{-1} nighttime in Ota et al. (2017). However, the UV-B irradiation
117 caused no destruction in the ultra-structure of adaxial epidermal cells (Ota et al. 2017),
118 and peroxidase activity increased in the UV-B-irradiated perilla leaves (Hosokawa et al.
119 2016). Therefore, the lowering of anthocyanin concentration might be a result of its
120 degradation catalyzed by vacuolar peroxidase scavenging H_2O_2 produced by UV-B
121 irradiation (Ota et al. 2017). Perilla plants possess abundant bioactive secondary
122 compounds, which may be appropriate materials for investigating the indirect effects of
123 UV-B radiation on spider mites.
124 Plant growth phase-dependent changes in essential oil content in perilla plants have
125 been previously reported; the content is twice as high in the pre-flowering stage than in
126 the flowering stage (Yoshida et al. 1968, 1969; Ghimire et al. 2017). In green perilla and
127 another Laminaceae species, spearmint *Mentha spicata* L., it has been suggested that
128 detoxification enzymes in *T. kanzawai* ~~(Acari: Tetranychidae)~~, play a role in
129 counteracting the host defense response (Ozawa et al. 2017). However, it is unknown
130 how the growth phase of perilla plants affects spider mite biology and how the growth
131 phase effects interact with UV-B irradiation.
132 In this study, the effects of changes in leaf quality such as essential oil contents
133 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

158 individually transplanted into pots (24 cm in diameter). Fourteen pots were placed in
159 each of the four experimental areas in the greenhouse separated by UV-opaque
160 polyvinyl chloride film (0.1 mm thick, Cutaceclean®Kirinain; MKV Platech, Tokyo,
161 Japan) that completely blocked UV wavelengths shorter than 380 nm (Ohtsuka and
162 Osakabe 2009). Two of the four areas were equipped with bulb-shaped UV-B
163 fluorescent lamps (UV-B lamp; SPWFD24UB1PB; Panasonic Lighting Devices Co.,
164 Ltd., Takatsuki, Japan), whose output peaked at a wavelength of 310 nm (Murata and
165 Osakabe 2013). The perilla plants were illuminated with UV-B during nighttime
166 (00:00–03:00) from September 20, when the plants grew to more than four sections
167 with eight leaves, until the experiments were completed. The UV-B irradiance on the
168 perilla plants was 0.06 W m^{-2} (0.65 kJ m^{-2} day^{-1}). The temperature in the greenhouse
169 was maintained at 15°C or higher throughout cultivation using electric heaters from
170 October onward. All perilla plants flowered by November 17.
171 We tentatively measured plant height and number of leaves at sampling time in
172 2016; 33–47 cm high with 14–18 leaves and 25–46 cm high with 12–18 leaves in UV-B
173 irradiated and unirradiated plants on 4–8 October; 55–67 cm high with 18–24 leaves
174 and 43–62 cm high with 16–24 leaves in UV-B irradiated and unirradiated plants on 17
175 November. Leaf sizes used for experiments were 72–112 cm length × 46–85 cm width
176 and 65–127 cm length × 42–74 cm width in UV-B irradiated and unirradiated plants on
177 4–8 October; 98–123 cm length × 56–80 cm width and 84–131 cm length × 49–75 cm
178 width in UV-B irradiated and unirradiated plants on 17 November.
179 In 2017, the red and green perilla were sown on May 15 and transplanted into pots
180 on June 19. Daily UV-B irradiation commenced on October 8. The red and green perilla
181 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

254 that hatched within 2 h on kidney bean leaf discs were individually introduced to 40
255 new kidney bean leaf discs and 40 new red perilla leaf discs. The leaf discs were
256 prepared from six plants (6 or 7 leaf discs per plant) in each plant species. Similarly,
257 larvae that hatched within 2 h on red perilla leaf discs were individually introduced to
258 40 new kidney bean leaf discs and 40 new red perilla leaf discs.
259 The effects of several factors (the date of leaf picking, maternal host plants, and
260 progeny host plants) on the developmental success of progeny were evaluated by three-
261 way ANOVA using the "aov" module in R software. Developmental ratios calculated
262 for leaf discs from the same plants were used as replications. Prior to the three-way
263 ANOVA, we applied the arcsine square root transformation for the dataset and
264 confirmed the homogeneity of variances by Bartlett's test using the "bartlett.test"
265 module in R software. The effects of the factors on progeny developmental duration
266 were evaluated using GLM analyses assuming a Gaussian distribution with the "glm"
267 module in R software. Individuals that successfully developed were considered
268 replicates in the calculation of developmental duration.
269
270 **Results**
271 Effects of Plant Growth Phase and UV-B Irradiation on Development
272 The rate of developmental success, mortality, escaping rate, and developmental duration
273 of *T. urticae* (n = 30) were 93%, 0%, 7%, and 5.30 ± 0.09 days (mean ± SE; including
274 both females and males) on kidney bean leaf discs.
275 Most larvae did not develop into adults on BF perilla leaves, whereas
276 approximately half or more larvae developed on AF leaves in all experiments (Fig. 1).
277 Two-way ANOVA indicated statistically significant effects of plant growth phase on

278 developmental success ($F_{[1,16]} = 95.428$, $P = 3.8 \times 10^{-8}$, on red perilla in 2016; $F_{[1,16]} =$
279 240.126, $P = 4.69 \times 10^{-11}$ on red perilla in 2017; $F_{[1,16]} = 43.916$, $P = 5.82 \times 10^{-6}$ on
280 green perilla in 2017). UV-B irradiation increased developmental success on red perilla
281 in 2016 ($F_{[1,16]} = 4.619$, $P = 0.0473$) and green perilla in 2017 ($F_{[1,16]} = 4.753$, $P =$
282 0.0445), but had marginal effects on red perilla in 2017 ($F_{[1,16]} = 2.560$, $P = 0.129$). The
283 difference between UV+ and UV− was minor compared to the difference between AF
284 and BF. The interaction between plant growth phase and UV-B irradiation was not
285 significant ($F_{[1,16]} = 0.109$, $P = 0.746$ on red perilla in 2016; $F_{[1,16]} = 0.285$, $P = 0.6$ on
286 red perilla in 2017; $F_{[1,16]} = 0.06$, $P = 0.809$ on green perilla in 2017).
287 In both red perilla and green perilla, developmental duration was significantly
288 extended on BF leaves compared to AF leaves (Table 1 and 2). Conversely, UV-B
289 pretreatment had no effect on developmental duration on red perilla in 2017 (Table 2)
290 and slightly shortened developmental duration on green perilla (Table 2). The degree of
291 shortening was 0.1 day in females and males in BF and AF, respectively, and 0.5 day in
292 females in AF, whereas no difference was observed in males in BF (Table 1).
293 Most individuals that died did so at the larval stage, and survival rates were
294 therefore calculated at the completion of the first molt (protonymph emergence) in the
295 2016 experiments with red perilla. All individuals that died (UV+: 70.5%, UV−: 67.6%)
296 or escaped (UV+: 24.8%, UV−: 32.4%) did so at the larval or protochrysalis stages in
297 the BF phase. Similarly, most individuals that did not develop to adulthood (UV+:
298 18.5% died and 24.2% escaped, UV−: 31.7% died and 22.8% escaped) died by
299 protonymph emergence (UV+: 16.7% died and 24.2% escaped, UV−: 30.8% died and
300 20.0% escaped) in the AF phase. Moreover, the majority of the extension in
301 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

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

375 Components of perilla essential oil have been demonstrated to have insecticidal
376 effects on various herbivorous insects. Perillaldehyde exhibits strong inhibition effects
377 against acetylcholine esterase in *Drosophila suzukii* (Matsumura) (Diptera:
378 Drosophilidae), resulting in high mortality at a dose of 1 mg L^{-1} (Park et al. 2016).
379 Methyl perillate, a component of green perilla oil, causes high mortality in larvae of
380 *Aedes aegypt* (L.) (Diptera: Culicidae) (LC_{50} = 16 mg L^{-1}) (Tabanca et al. 2015). Crude
381 extracts of perilla essential oil have also shown strong insecticidal activity in some
382 studies (Zhao et al. 2012; Tabanca et al. 2015). The egg production of *T. kanzawai* is
383 suppressed on leaves of crispate green perilla (Ozawa et al. 2017), and similar effects
384 were observed for *T. urticae* in the current study. One explanation for the change in the
385 efficacy of the defense response in perilla plants may be a shift of investment from
386 protection of the plant body to reproduction as an adaptive evolutionally mechanism for
387 increasing the performance of annual plants. Indeed, the essential oil content of perilla
388 plants is higher in the BF phase than the AF phase (Yoshida et al. 1968, 1969), although
389 the tendency varies among plant strains (Ghimire et al. 2017). Because perilla plants
390 possess many variable bioactive ingredients, further research on the single or combined
391 effects of these ingredients is necessary to understand the underlying mechanisms for
392 the performance variation between the growth phases of perilla plants.

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

398 is mitigated in strains with higher expression levels of detoxification enzymes, which
399 affect acaricide susceptibility (Ozawa et al. 2017). *Tetranychus urticae* is a notable crop
400 pest that has developed resistance against acaricides, and elevated activities of
401 detoxification enzymes are frequently reported in the population worldwide. Moreover,
402 *T. urticae* genes involved in the detoxification and transport of xenobiotics enzymes are
403 often expanded compared to insects, and respond to induction/selection by unfavorable
404 host plants (Grbić et al. 2011; Dermauw et al. 2013; Snoeck et al. 2018). Based on the
405 literature and current findings, investigating the mechanism of *T. urticae*‘s adaptation to
406 perilla plants may help elucidate the interactions between host plant adaptation and
407 pesticide resistance.
408 Acclimation improves acceptance and performance on unfavorable host plants,
409 even in non-adapted strains (Agrawal et al. 2002; Tajima et al. 2007). Regarding to the
410 acclimation of *T. urticae* to red perilla, we detected maternal effects (interactions
411 between host plants of mothers and progeny) upon developmental duration, but not
412 developmental success. Progeny on red perilla exhibited reduced developmental success
413 and elongated developmental duration, particularly at the larval stage. Therefore,
414 maternal host plants did not have a significant beneficial effects on the performance of
415 progeny, at least after a short acclimation period. On the other hand, although the red
416 perilla leaves were picked in the BF period, developmental success and developmental
417 duration were increased and reduced, respectively, in the second experiment vs. the first
418 experiment evaluating maternal effects. Perilla essential oil contents, particularly
419 components with anti-*T. urticae* bioactivity, may have begun to decrease from end of
420 the BF period to flower bud formation in early/mid-December in our cultivation system.
421 Many studies have reported UV-B-mediated changes in the architecture and

422 physiology of plant resistance to herbivory by arthropods (Ballaré 2014; Escobar-Bravo
423 et al. 2017). Similarly, UV-B pretreatment made *T. urticae* egg production on red perilla
424 leaves to decrease in the current study. However, developmental success on red and
425 green perilla leaves and developmental duration on red perilla leaves were unaffected by
426 UV-B pretreatment of leaves. Moreover, oviposition and developmental duration were
427 enhanced and slightly shortened, respectively, on green perilla leaves by UV-B
428 irradiation. Similar discrepancies in indirect UV-B effects have been observed in the
429 generalist caterpillar, *Trichoplusia ni* (Hbn.) (Lepidoptera: Noctuidae), on UV-B treated
430 weedy forb leaves. In *Plantago lanceolate* L. (Plantaginaceae), larval development was
431 accelerated on UV-B treated leaves, although survivorship was unaffected (McCloud
432 and Berenbaum 1999). In contrast, the growth of *T. ni* was significantly inhibited on
433 weedy forb plants exposed to UV-B radiation (direct and indirect effects), although no
434 change was observed in a specialist insect, *Precis coenia* Hbn. (Lepidoptera:
435 Nymphalidae) (McCloud and Berenbaum, 1999). Although it is not clear whether a co-
436 evolutionary arms race between host plants and arthropod herbivores has occurred with
437 respect to adaptations to ambient UV-B radiation, the direct biological impacts of UV-B
438 radiation on UV-B vulnerable arthropods appear to be more significant than indirect
439 effects through the change in plant chemicals. This seems to be the case for *T. urticae*
440 because mortality is determined by the cumulative UV-B dose under various
441 environmental conditions, including nighttime irradiation in greenhouses and solar
442 radiation (Sakai et al. 2012; Murata and Osakabe 2013; Tanaka et al. 2016).
443 Nighttime UV-B irradiation prevents photoreactivation (Murata and Osakabe 2014,
444 2017a) and therefore has the potential to control spider mites in greenhouse strawberry
445 cultivation (Tanaka et al. 2016). However, Ota et al. (2017) observed reduced leaf size

446 and degradation of anthocyanin in red perilla plants irradiated with UV-B at 0.54 kJ m^{-2}
447 day^{-1} in the nighttime, which was lower than the UV-B dose practically applied in
448 greenhouse strawberry cultivation (1.7–2.0 kJ m^{-2} day^{-1}). Simultaneous visible light
449 irradiation prevented the degradation of anthocyanin and largely mitigated
450 morphological changes in leaves, and daytime irradiation had no effect on anthocyanin
451 content or leaf morphology (Ota et al. 2017), suggesting that photoreactivation
452 contributed to the adaptation of red perilla plants to ambient UV-B irradiation. In the
453 current study, perilla plants were irradiated with UV-B at 0.65 kJ m^{-2} day^{-1}, but only a
454 slight color change was observed in old red perilla leaves near the bottom of the stem.
455 Although it is not known how this compares to the degree of UV-B damage reported by
456 Ota et al. (2017), the dose used in the current study was likely a critical dose of
457 nighttime UV-B irradiation without photoreactivation. The practical dose for spider mite
458 control in strawberry greenhouses was determined based on the mortality obtained after
459 a single acute dose of UV-B irradiation in a study by Murata and Osakabe (2013); the
460 LD_{50} value for *T. urticae* eggs was 0.58 kJ m^{-2} (Tanaka et al. 2016). However, Nakai et
461 al. (2018) found higher mortality under daily irradiation conditions than under a single
462 acute dose of irradiation, indicating that it may be possibly to design a dose regime that
463 effectively controls spider mites without causing UV-B damage to perilla plants.
464 In conclusion, the defense mechanism of perilla plants is more effective against *T.*
465 *urticae* in the BF period than the AF period, indicating a significant impact of
466 investment allocation. The contribution of UV-B irradiation to the perilla plant defense
467 system through indirect effects is surprisingly limited or absent. It is therefore
468 appropriate to expect only direct effects of UV-B irradiation in controlling *T. urticae* on
469 perilla plants, and this treatment is likely to be more effective for the AF period than the

BF period.

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References Cited

Agrawal, A. A., F. Vala, and M. W. Sabelis. 2002. Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: Adaptive plasticity? Am. Nat. 159: 553–565.

Ahmad, H. M. 2019. Ethnomedicinal, phytochemical and pharmacological investigations of *Perilla frutescens* (L.). Britt. Molecules 24: 102.

Ahmed, H. M., and S. Tavaszi-Sarosi. 2019. Identification and quantification of essential oil content and composition, total polyphenols and antioxidant capacity of *Perilla frutescens* (L.) Britt. Food Chem. 275: 730–738.

Akihisa, T., S. Kamo, T. Uchiyama, H. Akazawa, N. Banno, Y. Taguchi, K. Yasukawa. 2006. Cytotoxic activity of *Perilla frutescense* var. *japonica* leaf extract is due to high concentrations of oleanolic and ursolic acids. J. Nat. Med. 60: 331–333.

Ballaré, C. L. 2014. Light regulation of plant defense. Annu. Rev. Plant Biol. 65: 335–363.

Ballaré, C. L., M. M. Caldwell, S. D. Flint, S. A. Robinson, and J. F. Bornman. 2011. Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. Photochem. Photobiol. Sci. 10: 226–241.

Banno, N., T. Akihisa, H. Tokuda, K. Yasukawa, H. Higashihara, M. Ukiya, K. Watanabe, Y. Kimura, J. Hasegawa, and H. Nishino. 2004. Triterpene acids from the leaves of *Perilla frutescens* and their anti-inflammatory and antitumor-promoting effects. Biosci. Biotechnol. Biochem. 68: 85–90.

Dermauw, W., N. Wybouw, S. Rombauts, B. Menten, J. Vontas, M. Grbić, R. M. Clark, R. Feyereisen, and T. Van Leeuwen. 2013. A link between host plant adaptation

503 and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proc.
504 Natl. Acad. Sci. USA 110: E113–E122.
505 Dong, Z., Y. Wang, Q. Liu, B. Tian, Z. Liu. 2019. Laboratory screening of 26 essential
506 oils against *Cacopsylla chinensis* (Hemiptera: Psyllidae) and field confirmation of
507 the top performer, *Perilla frutescens* (Lamiales: Lamiaceae). J. Econ. Entomol.
508 112: 1299–1305.
509 Escobar-Bravo, R., P. G. L. Klinkhamer, K. A. Leiss. 2017. Interactive effects of UV-B
510 light with abiotic factors on plant growth and chemistry, and their consequences for
511 defense against arthropod herbivores. Front. Plant Sci. 8: 278.
512 Fry, J. D. 1989. Evolutionary adaptation to host plants in a laboratory population of the
513 phytophagous mite *Tetranychus urticae* Koch. Oecologia 81: 559–565.
514 Fujiwara, Y., M. Kono, A. Ito, M. Ito. 2018. Anthocyanins in perilla plants and dried
515 leaves. Phytochemistry 147: 158–166.
516 Ghimire, B. K., J. H. Yoo, C. Y. Yu, and I. M. Chung. 2017. GC-MS analysis of volatile
517 compounds of *Perilla frutescens* Britton var. Japonica accessions: morphological
518 and seasonal variability. Asian Pac. J. Trop. Med. 10: 643–651.
519 Gomi, K., and T. Gotoh. 1997. Genetic basis for host range in *Tetranychus kanzawai*
520 Kishida (Acari: Tetranychidae). Appl. Entomol. Zool. 32: 638–641.
521 Grbić, M., T. Van Leeuwen, R. M. Clark, S. Rombauts, P. Rouzé, V. Grbić, E. J.
522 Osborne, W. Dermauw, P. C. T. Ngoc, F. Ortego, P. Hernández-Crespo, I. Diaz, M.
523 Martinez, M. Navajas, E. Sucena, S. Magalhães, L. Nagy, R. M. Pace, S.
524 Djuranović, G. Smagghe, M. Iga, O. Christiaens, J. A. Veenstra, J. Ewer, R. M.
525 Villalobos, J. L. Hutter, S. D. Hudson, M. Velez, S. V. Yi, J. Zeng, A. Pires-daSilva,
526 F. Roch, M. Cazaux, M. Navarro, V. Zhurov, G. Acevedo, A. Bjelica, J. A. Fawcett,

527 E. Bonnet, C. Martens, G. Baele, L. Wissler, A. Sanchez-Rodriguez, L. Tirry, C.
528 Blais, K. Demeestere, S. R. Henz, T. R. Gregory, J. Mathieu, L. Verdon, L.
529 Farinelli, J. Schmutz, E. Lindquist, R. Feyereisen, and Y. Van de Peer. 2011. The
530 genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature 479:
531 487–492.
532 Hosokawa, M., N. Ota, A. Deguchi, S. Ohno, S. Aoki, and M. Osakabe. 2016. Increase
533 of peroxidase activity in perilla leaves by UV-B irradiation. Hort. Res. (Japan) 15
534 (Suppl. 1): 179.
535 Ikegami, Y., S. Yano, J. Takabayashi, and A. Takafuji. 2000. Function of quiescence of
536 *Tetranychus kanzawai* (Acari: Tetranychidae), as a defense mechanism against
537 rain. Appl. Entomol. Zool. 35: 339–343.
538 Ito, N., T. Nagai, T. Oikawa, H. Yamada, and T. Hanawa. 2011. Antidepressant-like
539 effect of l-perillaldehyde in stress-induced depression-like model mice through
540 regulation of the olfactory nervous system. Evid. Based Complement Alternat.
541 Med. 2011: 512697.
542 Itoh, K., A. Oguri, and A. Suzuki. 2003. Occurrence and control of insect pests on
543 perilla in Aichi Prefecture. Proc. Kansai Pl. Prot. 45: 71–72.
544 Kagawa, N., H. Iguchi, M. Henzan, and M. Hanaoka. 2019. Drying the leaves of *Perilla*
545 *frutescens* increases their content of anticancer nutraceuticals. Food Sci. Nutr. 7:
546 1494–1501.
547 Kant, M. R., M. W. Sabelis, M. A. Haring, and R. C. Schuurink. 2008. Intraspecific
548 variation in a generalist herbivore accounts for differential induction and impact of
549 host plant defences. Proc. R. Soc. B 275: 443–452.
550 McCloud, E. S., and M. Berenbaum. 1999. Effects of enhanced UV-B radiation on a

551 weedy forb (*Plantago lanceolate*) and its interactions with a generalist and
552 specialist herbivore. Entomol. Exp. Appl. 93: 233–247.
553 Murata, Y., and M. Osakabe. 2013. The Bunsen-Roscoe reciprocity law in ultraviolet-B-
554 induced mortality of the two-spotted spider mite *Tetranychus urticae*. J. Insect
555 Physiol. 59: 241–247.
556 Murata, Y., and M. Osakabe. 2014. Factors affecting photoreactivation in UVB-
557 irradiated herbivorous spider mite (*Tetranychus urticae*). Exp. Appl. Acarol. 63:
558 253–265.
559 Murata, Y., and M. Osakabe. 2017a. Photo-enzymatic repair of UVB-induced DNA
560 damage in the two-spotted spider mite. Exp. Appl. Acarol. 71: 15–34.
561 Murata, Y., and M. Osakabe. 2017b. Developmental phase-specific mortality after
562 ultraviolet-B radiation exposure in the two-spotted spider mite. Environ. Entomol.
563 46: 1448–1455.
564 Nakai, K., Y. Murata, and M. Osakabe. 2018. Effects of low temperature on spider mite
565 control by intermittent ultraviolet-B irradiation for practical use in greenhouse
566 strawberries. Environ. Entomol. 47: 140–147.
567 Nishimura, T., K. Ohyama, E. Goto, N. Inagaki, and T. Morota. 2008. Ultraviolet-B
568 radiation suppressed the growth and anthocyanin production of *Perilla* plants
569 grown under controlled environments with artificial light. Acta Hortic. 797: 425–
570 429.
571 Ohtsuka, K., and M. Osakabe. 2009. Deleterious effects of UV-B radiation on
572 herbivorous spider mites: they can avoid it by remaining on lower leaf surfaces.
573 Environ Entomol 38:920–929.
574 Ota, N., T. Nabeshima, M. Osakabe, S. Aoki, T. Awano, and M. Hosokawa. 2017.

575 Difference between nighttime and daytime UV-B irradiation with respect to the
576 extent of damage to perilla leaves. Hort. J. 86: 349–356.
577 Ozawa, R., H. Endo, M. Iijima, K. Sugimoto, J. Takabayashi, T. Gotoh, and G. Arimura.
578 2017. Intraspecific variation among tetranychid mites for ability to detoxify and to
579 induce plant defenses. Sci. Rep. 7: 43200.
580 Park, C. G., M. Jang, K. A. Yoon, and J. Kim. 2016. Insecticidal and
581 acetylcholinesterase inhibitory activities ofLamiaceae plant essential oils and their
582 major components against *Drosophila suzukii* (Diptera: Drosophilidae). Ind. Crop
583 Prod. 89: 507–513.
584 R Core Team. 2014. R: A Language and Environment for Statistical Computing. R
585 Foundation for Statistical Computing, Vienna. http://www.R-project.org/.
586 Sakai, Y., and M. Osakabe. 2010. Spectrum-specific damage and solar ultraviolet
587 radiation avoidance in the two-spotted spider mite. Photochem. Photobiol. 86:
588 925–932.
589 Sakai, Y., M. Sudo, and M. Osakabe. 2012. Seasonal changes in the deleterious effects
590 of solar ultraviolet-B radiation on eggs of the twospotted spider mite, *Tetranychus*
591 *urticae* (Acari: Tetranychidae). Appl. Entomol. Zool. 47: 67–73.
592 Snoeck, S., N. Wybouw, T. Van Leeuwen, and W. Dermauw. 2018. Transcriptomic
593 plasticity in the arthropod generalist *Tetranychus urticae* upon long-term
594 acclimation to different host plants. G3 8: 3865–3879.
595 Tabanca, N., B. Demirci, A. Ali, Z. Ali, E. K. Blythe, and I. A. Khan. 2015. Essential
596 oils of green and red *Perilla frutescens* as potential sources of compounds for
597 mosquito management. Ind. Crop Prod. 65: 36–44.
598 Tajima, R., K. Ohashi, M. Osakabe, and A. Takafuji. 2007. Host plents utilized during

599 the immature development of *Tetranychus kanzawai* (Acari: Tetranychidae)
600 determine the preference of the adult females for the plants. J. Acarol. Soc. Jpn. 16:
601 121–127.
602 Tanaka, H., M. Ueda, N. Mizobuchi, and M. Shibao. 1993. Control of *Tetranychus*
603 *kanzawai* Kishida on *Perilla frutescens* (B.) in a greenhouse by *Phytoseiulus*
604 *persimilis* Athias-Henriot. Proc. Kansai Pl. Prot. 35: 63–64.
605 Tanaka, M., J. Yase, S. Aoki, T. Sakurai, T. Kanto, and M. Osakabe. 2016. Physical
606 control of spider mites using ultraviolet-B with light reflection sheets in
607 greenhouse strawberries. J. Econ. Entomol. 109: 1758–1765.
608 Ubara, M., and M. Osakabe. 2015. Suspension of egg hatching caused by high humidity
609 and submergence in spider mites. Environ. Entomol. 44: 1210–1219.
610 Yanagida, K., H. Kamiwada, and K. Kusigemati. 1996. Biological studies of insects
611 feeding on the perilla, *Perilla frutescens* Britt., in Kagoshima Prefecture. Bull. Fac.
612 Agric. Kagoshima Univ. 46: 15–30.
613 Yang, L., H. Huang, and J. Wang. 2010. Antioxidant responses of citrus red mite,
614 *Panonychus citri* (McGregor) (Acari: Tetranychidae), exposed to thermal stress. J.
615 Insect Physiol. 56: 1871–1875.
616 Yoshida, T., F. Higashi, and S. Ikawa. 1968. On the oil containing tissue, the essential
617 oil contents and the chemical composition of essential oil in *Perilla* species. Jpn. J.
618 Crop Sci. 37: 118–122.
619 Yoshida, T., S. Morisada, and K. Kameoka. 1969. On the distribution of oil gland and
620 the accumulation of essential oil in *Perilla* species. Jpn. J. Crop Sci. 38: 333–337.
621 Yoshioka, Y., T. Gotoh, and T. Suzuki. 2018. UV-B susceptibility and photoreactivation
622 in embryonic development of the two-spotted spider mite, *Tetranychus urticae*.

623 Exp. Appl. Acarol. 75: 155–166.
624 Yu, H., J.-F. Qiu, L.-J. Ma, Y.-J. Hu, P. Li, and J.-B. Wan. 2017. Phytochemical and
625 phytopharmacological review of *Perilla frutescens* L. (Labiatae), a traditional
626 edible-medicinal herb in China. Food Chem. Toxicol. 108: 375–391.
627 Zavala, J. A., C. A. Mazza, F. M. Dillon, H. D. Chludil, and C. L. Ballaré. 2015.
628 Soybean resistance to stink bugs (*Nezara viridula* and *Piezodorus guildinii*)
629 increases with exposure to solar UV-B radiation and correlates with isoflavonoid
630 content in pods under field conditions. Plant Cell Environ. 38: 920–928.
631 Zhang, W. J., and L. O. Björn. 2009. The effect of ultraviolet radiation on the
632 accumulation of medicinal compounds in plants. Fitoterapia 80: 207–218.
633 Zhang, X. R., Y. H. Chen, Q. S. Guo, W. M. Wang, L. Liu, J. Fan, L. P. Cao, and C. Li.
634 2017. Short-term UV-B radiation effects on morphology, physiological traits and
635 accumulation of bioactive compounds in *Prunella vulgaris* L. J. Plant Interact. 12:
636 348–354.
637 Zhao, N. N., L. Zhou, Z. L. Liu, S. S. Du, and Z. W. Deng. 2012. Evaluation of the
638 toxicity of the essential oils of some common Chinese spices against *Liposcelis*
639 *bostrychophila*. Food Control 26: 486–490.

640 Figure legends

641

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

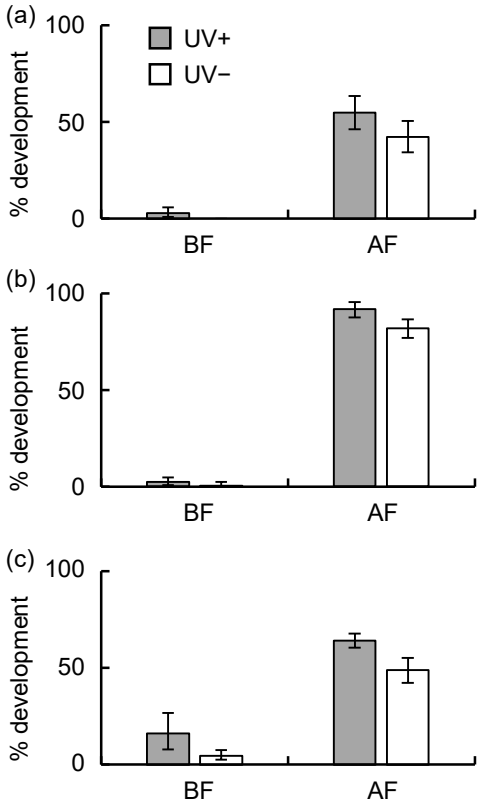
649

650 **Fig. 2** Effects of plant growth phase and UV-B irradiation on egg production of *T.*
651 *urticae*. (a) and (b): on red perilla in 2016 and 2017, respectively; (c): on green
652 perilla in 2017. Number of females per plant in UV+ and UV− used for
653 analysis were (a) 13–20 and 15–19 in BF and 15–19 and 11–20 in AF, (b) 15–
654 19 and 12–17 in BF and 17–20 and 17–20 in AF, and (c) 15–18 and 15–20 in
655 BF and 17–20 and 17–20 in AF, respectively. Eggs: number of eggs produced
656 per female for the 5 initial days of its oviposition period; BF: before flower bud
657 formation; AF: after flower bud formation (flowering stage); UV+: UV-B-
658 irradiated plants; UV−: UV-B-non-irradiated plants. Vertical lines on bars show
659 the standard errors among mite individuals.

660

661 **Fig. 3** Effects of maternal host plants on the developmental success of progeny. K:
662 kidney bean; R: red perilla. Leaves were collected on Oct. 5 and Nov. 21 (BF)
663 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.


(a)
100
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% development
UV+
UV−
BF
AF
(b)
100
50
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% development
BF
AF
(c)
100
50
0
% development
BF
AF

Fig. 1

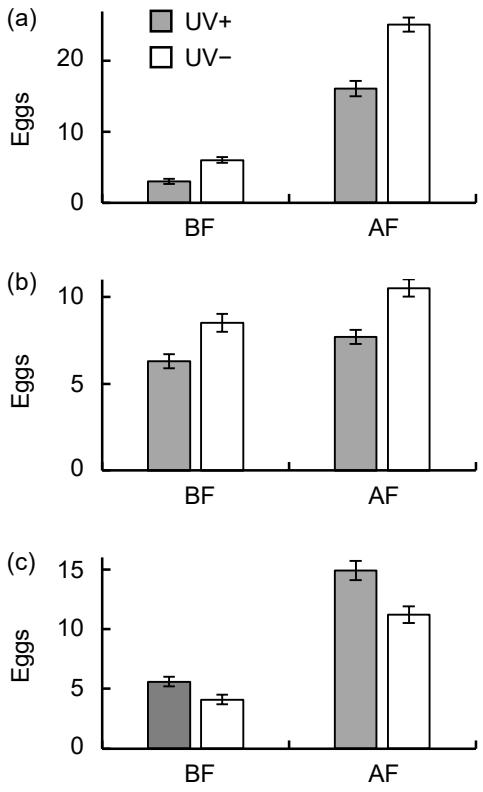

Fig. 2

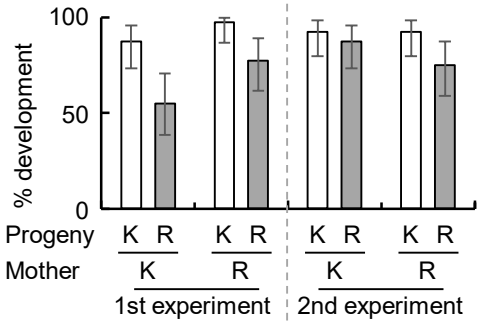
100
50
0
% development
Progeny
K R K R K R K R
Mother
K R K R
1st experiment
2nd experiment

Fig. 3

Table 1 Development duration from larvae to adult emergence

		BF (Days ± SE) [a]		AF (Days ± SE) [a]	
		UV+	UV-	UV+	UV-
Red perilla					
2016	Female	8.7 ± 0.3 (3)	NA	6.9 ± 0.2 (42)	7.2 ± 0.3 (30)
	Male	8 ± 0 (2)	NA	6.4 ± 0.3 (13)	6.1 ± 0.2 (13)
2017	Female	8.3 ± 0.3 (3)	8.5 ± 0.5 (2)	6.5 ± 0.1 (56)	6.8 ± 0.1 (43)
	Male	8 (1)	8 (1)	6 ± 0.1 (34)	6 ± 0.1 (38)
Green perilla					
2017	Female	7.7 ± 0.1 (14)	7.8 ± 0.4 (5)	6.9 ± 0.1 (37)	7.4 ± 0.2 (31)
	Male	7 ± 0 (7)	7 (1)	6.8 ± 0.2 (27)	6.9 ± 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	*t*-value	*P*-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	*t*-value	*P*-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 × 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 × 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 × 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 ± SE [b]	
Mother	Progeny	1st	2nd
K	K	5.2 ± 0.1 (35)	5.3 ± 0.1 (37)
R	K	5.2 ± 0.1 (39)	5.3 ± 0.1 (37)
K	R	7.1 ± 0.3 (55)	6.6 ± 0.2 (35)
R	R	6.5 ± 0.2 (31)	6.1 ± 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	*t*-value	*P*-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 × MHP	0.109853	0.211203	0.520	0.6034
MHP × OHP	−0.495390	0.211702	−2.340	0.0201
LPD × 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−	
	n	Escaping rate	*n*	Escaping rate	*n*	Escaping rate	*n*	Escaping rate
Red perilla								
2016	100	0.248 (0.194–0.306)	98	0.324 (0.241–0.413)	100	0.242 (0.149–0.35)	100	0.228 (0.126–0.35)
2017	100	0.366 (0.261–0.477)	100	0.339 (0.292–0.388)	100	0.039 (0.009–0.09)	100	0.061 (0.011–0.147)
Green perilla								
2017	99	0.5 (0.251–0.75)	100	0.605 (0.463–0.738)	100	0.198 (0.156–0.244)	100	0.228 (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	*DF*	Sum *Sq*	Mean *Sq*	*F* value	*Pr* (>*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 × 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 × 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 × 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−	
	n	Mortality	*n*	Mortality	*n*	Mortality	*n*	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	*DF*	Sum *Sq*	Mean *Sq*	*F* value	*Pr* (>*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