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Role of cyanide-resistant respiration during light-induced attraction of predators to herbivore-infested leaves

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Abstract

The present work showed that spider mite–infested leaves placed in the light were more attractive to predatory mites than the infested leaves placed in the dark; furthermore, an increase in the light intensity enhanced this attractiveness. However, the increase of the light intensity did not change the attractiveness of the uninfested leaves to predatory mites. The capacity of cyanide-resistant respiration and the photosynthetic rates of both the infested and uninfested leaves increased with increasing light intensities, whereas the photosystem (PS) II chlorophyll (Chl) fluorescence decreased. The increase of the capacity of cyanide-resistant respiration in the infested leaves was more dramatic than that in the uninfested leaves, whereas the values of photosynthetic rates and Chl fluorescence were lower in the infested leaves than those in the uninfested leaves. Treatment of the infested and uninfested leaves with 1 mM salicylhydroxamic acid (SHAM, an inhibitor of cyanide-resistant respiration) decreased photosynthetic rates and caused further reductions in PSII fluorescence, especially under a higher light intensity. In contrast, the effects of SHAM on PSII fluorescence parameters and photosynthetic rates of the infested leaves were more dramatic than on those of the uninfested leaves. The treatment with SHAM did not significantly change the attractiveness of the infested or uninfested leaves to the predatory mites under all of the light intensities tested. These results indicated that cyanide-resistant respiration was not directly associated with the light-induced attraction of predators to plants, but it could play a role in the protection of photosynthesis. Such role might become relatively more important when photosynthesis is impaired by herbivores infestation.

Additional key words: cyanide-resistant respiration; herbivore attack; light; photosynthesis; predatory mites.

Introduction

Light drives the production of reducing equivalents from the photosynthetic electron transport chain, which can be used in reductive biosynthetic reactions within cells. However, when the production of reducing equivalents exceeds amounts required by the plant, there is an increased risk of electron leakage from the photosynthetic electron transport chain. The leaked electrons reduce molecular oxygen to reactive oxygen species, which can cause oxidative damage to the photosynthetic apparatus and thereby the loss of photosynthetic function (Dat *et al*. 2000).

Plants have several mechanisms operated in cells to avoid this risk. Some authors reported that an excess of reducing equivalents generated in illuminated chloroplasts can be transported to the mitochondria *via* the malate/oxaloacetate shuttle and can be dissipated by the mitochondrial respiratory chain (Allen 2002, Raghavendra and Padmasree 2003). The ability of plant mitochondrial respiratory chain to dissipate reducing equivalents from chloroplasts is mainly attributed to the alternative oxidase (AOX), which catalyzes the cyanide-resistant alternative pathway. Due to the presence of AOX, the electrons produced by the oxidation of reducing equivalents can flow from ubiquinone directly

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Abbreviations: AOX – alternative oxidase; D – dark; L – low-light treatment; H – high-light treatment; HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HIPVs – herbivore-induced plant volatiles; IL – infested leaves; F_0' – minimal fluorescence of the light-adapted state; F_m' – light-adapted maximum fluorescence yield; F_q'/F_m' – the PSII operating efficiency; F_q'/F_v' – PSII efficiency factor; F_v'/F_m' – PSII maximum efficiency; F_s – fluorescence yield at the steady-state photosynthesis; KCN – potassium cyanide; PSII – photosystem II; PAR – photosynthetically active radiation; P_N – photosynthetic rate; SD – standard deviation; SHAM – salicylhydroxamic acid; V_{alt} – capacity of cyanide-resistant respiration; V_t – total respiration rate; UL – uninfested leaves.
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to AOX and, thus, be uncoupled from ATP synthesis (Millenaar and Lambers 2003). As a result, the operation of cyanide-resistant respiration in plants enables to dissipate the reducing energy equivalents into heat (Ordog *et al*. 2002, Millenaar and Lambers 2003). Many studies have reported that the rate of the cyanide-resistant respiration is enhanced by light (Ribas-Carbo *et al*. 2000, Zhang *et al*. 2010), and the inhibition of the cyanide-resistant respiration can cause an increase of the proportion of reduced PSII centres and a decrease of photosynthetic rate (Yoshida *et al*. 2006, 2007; Zhang *et al*. 2011, 2012). Thus, cyanide-resistant respiration is thought to be a major extra-chloroplastic sink of reducing equivalents, preventing the accumulation of excessive reducing equivalents produced in the chloroplasts and thereby decreasing the probability of loss of photosynthetic activity under light conditions (Padmasree *et al*. 2002, Raghavendra and Padmasree 2003, Bartoli *et al*. 2005, Yoshida *et al*. 2006, 2007).

Recently, some works have suggested that light, as an external environmental factor, can induce plant defense responses against biotic stress (Bolton 2009). For example, it is well known that when attacked by herbivorous arthropods, plants can defend themselves indirectly by attracting the carnivorous natural enemies of herbivores (Sabelis *et al*. 2001). Light has been found to be responsible for driving this defense response of plants. This was demonstrated by the observation that *Tetranychus urticae*–infested bean leaves placed in the light attracted the predatory mite, *Amblyseius womersleyi*, whereas the infested leaves, which were placed in the dark, did not attract the predator (Maeda *et al*. 2000). However, it is still unknown whether light-induced plant defense response against biotic stress, such as attracting the carnivorous natural enemies of herbivores, could be associated with cyanide-resistant respiration. However, if light or photosynthetic activity is required for the indirect defense response, as mentioned above, the function of cyanide-resistant respiration in dissipating reducing equivalents from the chloroplasts could be required for plants to express their light-induced defenses responses or to be beneficial for the protection of photosynthesis.

In the present work, we used a model tritrophic system consisting of kidney bean (*Phaseolus vulgaris*) plants, two-spotted spider mites (*T. urticae*), and predatory mites (*Phytoseiulus persimilis*) in an effort to assess and discuss the role of cyanide-resistant respiration, when light is required for the herbivore-infested leaves to attract the natural enemies of herbivores.

Materials and methods

Plant material and culture condition: Kidney bean seeds (*Phaseolus vulgaris*) were sterilized with 1% NaClO for 10 min and then washed immediately with distilled water. The seeds were soaked in distilled water for 12 h at room temperature and germinated at 26°C for 24 h on damp gauze. The germinated seeds were planted in plastic pots containing a loam soil in a climate-controlled chamber. During the growth period, the air temperature, relative air humidity, light/dark period, and photosynthetic active radiation (PAR) of daytime were 25 ± 2°C, 50 ± 10%, 16 h of light/8 h of dark, and 100 μmol(photon) m^{-2} s^{-1}, respectively. The seedlings (2–3 weeks old) with fully developed primary leaves were offered to the spider mites.

Mite culture conditions: Herbivorous spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*) were obtained from a laboratory-maintained culture. *T. urticae* was reared on kidney bean plants and *P. persimilis* was reared on a plastic board placed on foam plastic in a tray filled with water. Excised bean leaves, which were highly infested with *T. urticae*, were provided as a food source every 2 or 3 d.

Treatments of the bean leaves: For *T. urticae* infestation, 30 adult female spider mites were evenly distributed on the fully expanded, primary leaves of the bean plants with a fine brush. The *T. urticae*-infested and -uninfested plants were incubated under the same environmental conditions as above for 10 d prior to the experiment. At the end of the 16-h light period on the 10^{th} d after *T. urticae* infestation, *T. urticae*-infested (IL) and -uninfested leaves (UL) were cut with the petiole and placed in a 2-L glass bottle with some water to prevent desiccation.

In order to investigate the possible role of cyanide-resistant respiration, 1 mM SHAM (from a 0.4 M stock in ethanol), which is a well-known inhibitor of AOX activity (Chivasa and Carr 1998, Amor *et al*. 2000, Bartoli *et al*. 2005, Yoshida *et al*. 2006), was used in the present work. The concentration was sufficiently low to avoid the possible side effects of this inhibitor and it has been reported to have no direct influence on CO_2-dependent O_2 evolution and Chl fluorescence parameters (Amor *et al*. 2000, Bartoli *et al*. 2005, Yoshida *et al*. 2006). After being maintained in the dark for 4 h to adapt to the condition, 1 mM SHAM was supplied through the petiole of the detached leaves for 4 h in the dark to inhibit the AOX activity (Martim *et al*. 2009). The supplements of the solvent (a ethanol solution with the concentration equivalent to that in the 1 mM SHAM solution diluted from the stock) alone to the *T. urticae* IL and UL was used as the control under the same conditions. The plants were then transferred either from the dark to one of two light intensities [85 (low-light, L) and 240 (high-light, H) μmol(photon) m^{-2} s^{-1}] for 3 h or they were kept in the dark (in the bottle, which was covered completely with black paper).

Measurements of attraction of predatory mites: The 2-L glass bottles containing ten *T. urticae* IL were used as the odor source. The odor-source bottles were connected with a Y-shaped olfactometer (10-cm arm length, 12-cm stem length, and 4-cm internal diameter). Air, which was first purified by passing through activated charcoal, was pulled through the odor-source bottles and then fed through both arms of the Y-tube olfactometer at 5×10^{-5} m^3 s^{-1}. Female predatory mites were individually introduced at a starting point (downwind end) on a Y-shaped iron wire, which was fixed in the center of the olfactometer, and their behavior was observed for 5 min. If the predator walked across the end line marked on the upwind end of the Y-shaped wire, the predator was considered making a 'final choice'. If the predator did not make a 'final choice' within 5 min, the response was classified as 'no choice'. The arm in the olfactometer was switched after every five bioassays (Maeda *et al*. 2000). In each experiment, 36 or more predatory mites were tested within a time frame of about 3 h. The results of the experiments were subjected to a χ^2 test.

Measurements of leaf respiration: Before the respiration measurement, the fully expanded, primary leaves were washed twice to remove the *T. urticae* individuals. The leaves were then weighted and cut into small pieces with a razor blade. The pieces were placed at once in an assay buffer (20 mM Hepes, 0.2 mM $CaCl_2$, pH 7.2) and incubated in the dark for 10 min to allow wound respiration to subside. The total respiration and the capacity of cyanide-resistant respiration of the leaf pieces was measured in an air-tight cuvette at 25°C using a Clark-type electrode according to Feng *et al*. (2010). The total respiration rate (V_t) was measured in the absence of any respiratory inhibitors. The capacity of cyanide-resistant respiration (V_{alt}) was obtained by measuring the leaf oxygen uptake in the presence of 1 mM KCN, which was corrected by subtraction of the residual respiration. The residual respiration was measured in the presence of 5 mM SHAM and 1 mM KCN (Bingham and Farrar, 1989). Each measurement was performed for 3 min. The results represented the average of four independent experiments.

Measurements of photosynthesis and Chl fluorescence: The net photosynthetic rate (P_N) of fully expanded, primary leaves was measured using a portable, open-flow gas exchange analyzer (*PP Systems*, Hertfordshire, UK); the flow rate of air through the leaf chamber was set to 5×10^{-6} m^3 s^{-1}.

Chl fluorescence parameters of fully expanded, primary leaves were measured using a fluorescence modulated system (*PAM 2500 fluorometer*, *Walz*, Germany). At any given irradiance, the fluorescence yield at steady-state photosynthesis (F_s) was measured. Then, the light-adapted maximum fluorescence yield (F_m') produced by a 0.5 s saturating flash was determined. Minimal fluorescence of the light-adapted state (F_0') was then measured with a far red pulse in the absence of the actinic light. The PSII operating efficiency was defined as $F_q'/F_m' = (F_m' - F_s)/F_m'$, the PSII maximum efficiency was defined as $F_v'/F_m' = (F_m' - F_0')/F_m'$, and the PSII efficiency factor was defined as $F_q'/F_v' = (F_m' - F_s)/(F_m' - F_0')$ according to Possell *et al*. (2010).

Statistical analysis: The results were expressed as the mean ± standard deviation (SD). The data were statistically evaluated using the standard deviation and *t*-test methods. A difference was considered to be statistically significant when $P<0.05$.

Results

The attraction of predators to *T. urticae*-infested leaves was light dependent: Under the dark, the *T. urticae* IL did not attract significantly the predatory mites more than UL did (Fig. 1*A*). However, IL placed in the light were significantly more attractive to the predatory mites than UL placed under the same light intensity (Fig. 1*A*).

In the present work, it was observed that the *T. urticae* IL placed under L were significantly more attractive to the predatory mites than IL placed in the dark (D) (Fig. 1*B*). IL exposed to H were significantly more attractive to the predatory mites than those exposed to L during 3 h of observation (Fig. 1*B*). This indicated that an increase in the light intensity could enhance the attractiveness of IL to predatory mites. In contrast, UL exposed to L were not significantly more attractive to predatory mites than those maintained in D. The increase of the light intensity from 85 to 240 μmol(photon) m^{-2} s^{-1} did not also significantly change the attractiveness of UL to predatory mites (Fig. 1*C*). Control experiments showed that illuminating odor-source bottles containing no plants with different light intensities had no significant effect on the olfactory responses of the predatory mites (Fig. 1*D*).

The changes in the respiration, photosynthetic rates, and Chl fluorescence parameters of plants under different light intensities: Under D, IL and UL had similar level of V_t and V_{alt} (Fig. 2*A*,*B*). After illumination, the values of V_t and V_{alt} in both IL and UL increased with the time of illumination, and the increase in the light intensity from 85 to 240 μmol(photon) m^{-2} s^{-1} further enhanced their values. The increase of V_{alt} seemed more dramatic in IL than that in UL. After exposure to H for 2 and 3 h, V_{alt} of IL became significantly higher than that of UL (Fig. 2*B*).

The values of P_N of both IL and UL increased with the increase in the light intensity (Fig. 3). However, the Chl fluorescence parameter, F_q'/F_m', decreased in both IL

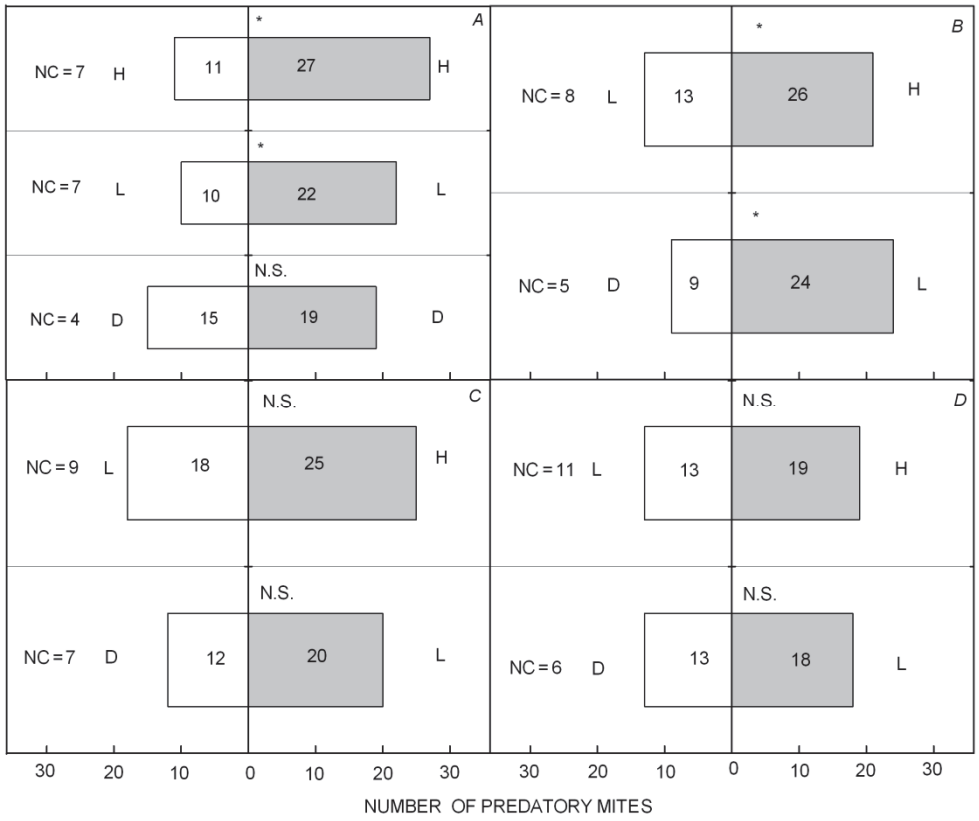

Fig. 1. Responses of predatory mites (*P. persimilis*) in a Y-tube olfactometer to the odor from the leaves subjected to different treatments. *A*: the uninfested leaves (*white*) *vs*. the *T. urticae*-infested leaves (*grey*). *B*: the infested leaves under different light intensities. *C*: the uninfested leaves under different light intensities. *D*: the odor-source bottles without leaves under different light intensities. The number of the predators that made a final choice is shown in each bar. The predators that did not reach the end of either olfactometer arm within 5 min (NC: no choice) were excluded from the statistical analyses. * – statistically significant preferences in a choice test ($\chi 2$ test: $P<0.05$). N.S. – not significant. D – dark; L – low-light treatment; H – high-light treatment.

and UL, when the light intensity increased, and this was accompanied by declines in both F_v'/F_m' and F_q'/F_v' (Fig. 4). After the exposure to H for 3 h, P_N was significantly lower in IL than that in UL (Fig. 3). The values of F_q'/F_m' and $F_q'/F_{v'}$ were significantly lower in IL than those in UL under the light conditions (Fig. 4*A*,*C*). The exposure to H for 1 and 3 h caused significantly lower F_v'/F_m' in IL compared with UL (Fig. 4*B*).

The SHAM effects on photosynthetic rate, Chl fluorescence, and the attraction of predators to plants under different light intensities: The treatment with 1 mM SHAM inhibited the cyanide-resistant oxygen uptake of both IL and UL by 55–60% (Table 1). Although IL and UL had different levels of the V_{alt} (Fig. 2*B*) under different light conditions (including different times and intensities of irradiance), there was no significant difference in the inhibition of the cyanide-resistant oxygen uptake by 1 mM SHAM among the leaves (Table 1). Therefore, 1 mM SHAM was used in the subsequent experiments.

Under the dark condition, the SHAM treatment did not significantly affect P_N or PSII Chl fluorescence of IL or UL (Table 2). However, under the illumination, the values of P_N and PSII fluorescence parameters tended to be lower in the SHAM-treated leaves than in the control (*i.e.*, the same leaves illuminated under the same intensity in the absence of SHAM), as shown in Table 2. During 3 h of the illumination at L, F_q'/F_m', F_v'/F_m', and F_q'/F_v' of IL decreased by 4.5–7.8%, 3.3–4.7%, and 1.4–3.7%, respectively, after the SHAM treatment. In UL, F_q'/F_m', $F_v'/F_{m'}$, and F_q'/F_v' decreased by 0.5–1.8%, 1.3–0.3%, and –1.01.7%, respectively, after the SHAM treatment. SHAM also caused a decrease in P_N of 12.0–15.9% in IL and of 8.3–7.8% in UL at this light intensity, respectively, but the differences in the P_N values between the SHAM-treated leaves and control were not statistically significant (Table 2). The effects of SHAM on P_N and Chl fluorescence parameters were more severe in the leaves exposed to H compared with the leaves exposed to L. At H, $F_{q'}/F_{m'}$, F_v'/F_m', and F_q'/F_v' of IL decreased by 30.4–33.8%, 22.4–23.9%, and 9.9–14.6%, respectively, after the SHAM treatment during 3 h of illumination. In UL, F_q'/F_m', F_v'/F_m', and F_q'/F_v' decreased by 17.8–19.5%, 11.9–12.2%, and 7.0–8.4%, respectively, after the SHAM treatment at H. P_N of IL and UL decreased significantly

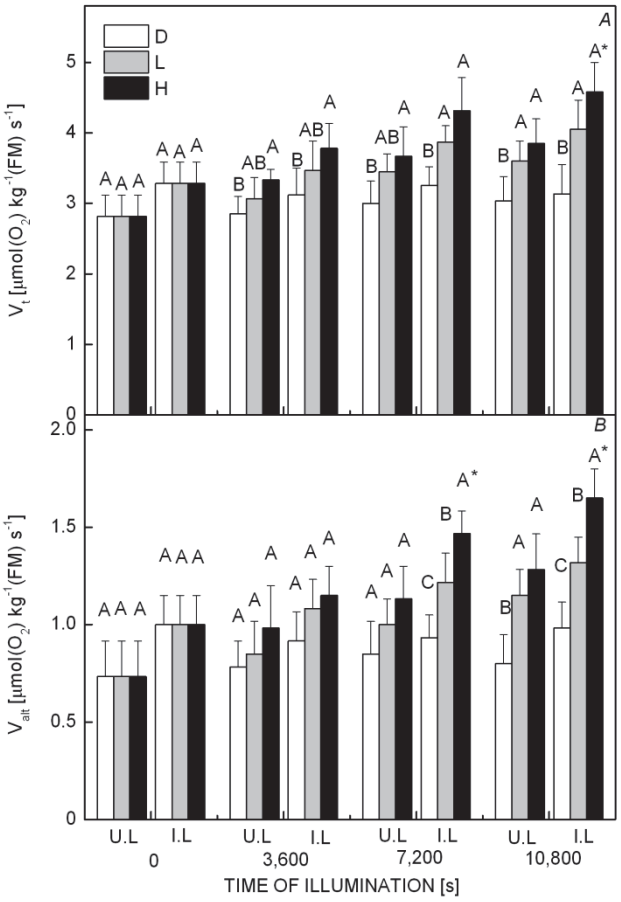

Fig. 2. The effects of illumination on the total respiration (V_t) (*A*) and cyanide-resistant respiration (V_{alt}) (*B*) in the *T. urticae*-infested and -uninfested leaves that were exposed to different light intensities respectively for 0, 1, 2, and 3 h. Each value represents the mean ± SD (vertical bars) of four independent experiments. *Different letters* denote significant differences from the treatment of the infested or uninfested leaves with different light intensities at the same time point (P<0.05). * – statistically significant differences between the infested and uninfested leaves under the same treatment (P<0.05). V_{alt} – capacity of cyanide-resistant respiration; V_t – total respiration rate; D – dark; L – low-light treatment; H – high-light treatment; IL – infested leaves; UL – uninfested leaves.

by 33.3–37.5% and 21.6–22.1%, respectively, after the SHAM treatment at H. These observations showed that the inhibition of the cyanide-resistant respiration caused the decline both in P_N and PSII activity, especially under H. The reductions were more dramatic in IL than in UL.

Under the same light intensity, the attraction of the predatory mites to UL without SHAM treatment was compared with that of UL treated with SHAM (Fig. 5*A*). The results showed that the treatment with SHAM did not significantly change the attraction of the predatory mites to UL (Fig. 5*A*). Treatment with SHAM did not also significantly affect the attractiveness of IL to the predatory mites under all of the light intensities tested (Fig. 5*B*). Furthermore, under the condition of illumination, IL treated with SHAM were still more attractive to the predatory mites than UL placed under the same light intensity (Fig. 5*C*).

Discussion

Light enhanced the attractiveness of herbivore-infested plants to predatory mites: Our present work showed that IL placed in the light attracted more predatory mite than UL (Fig. 1*A*). This observation showed that when attacked by herbivorous arthropods, plants could attract more natural enemies of herbivores. However, IL placed in D did not attract the predatory mites significantly more than UL did (Fig. 1*A*), indicating that light could be required for the herbivore-infested leaves to attract the natural herbivore enemies. In addition, the attraction of the predatory mites to IL increased with the increase of the light intensity from 0, 85 to 240 μmol(photon) m^{-2} s^{-1} (Fig. 1*B*). In contrast, illumination of UL with different light intensities had no significant effects on the attractiveness of the leaves to predatory mites (Fig. 1*C*). These observations suggest that the presence of light or the increase in the light intensity could enhance the attractiveness of plants to predatory mites, only when plants are attacked by herbivores.

Many works have revealed that the production of a specific blend of volatile organic compounds is triggered by the attack of arthropod herbivores. These volatile organic compounds, termed 'herbivore-induced plant volatiles' (HIPVs), may function as an indirect defense to attract natural enemies of the herbivores (Ozawa *et al*. 2000, Dicke *et al*. 2003, Degenhardt *et al*. 2009). In agreement with our observations above, the emission of HIPVs has been found to be very low in D, but it increased dramatically, when the plants were exposed to illumination (Loughrin *et al*. 1994, Turlings *et al*. 1995, Maeda *et al*. 2000). Considering that *T. urticae* could be more active and spent more time by feeding during the day (Maeda *et al*. 2000), it is likely that herbivores could cause more damage to the leaves in the light, thus possibly inducing more biosynthesis of HIPVs. However, this cannot explain the present observation that IL in D did not attract the predatory mites significantly more than UL did (Fig. 1*A*). Gouinguené *et al*. (2002) reported that the level of the emitted volatiles by corn leaves treated with the oral secretion of herbivores increased dramatically with the increase of the light intensities, while the untreated leaves emitted very small amount of volatiles and their emissions were not significantly different at different light intensities. Thus, these phenomena could reflect a fact that the biosynthesis or

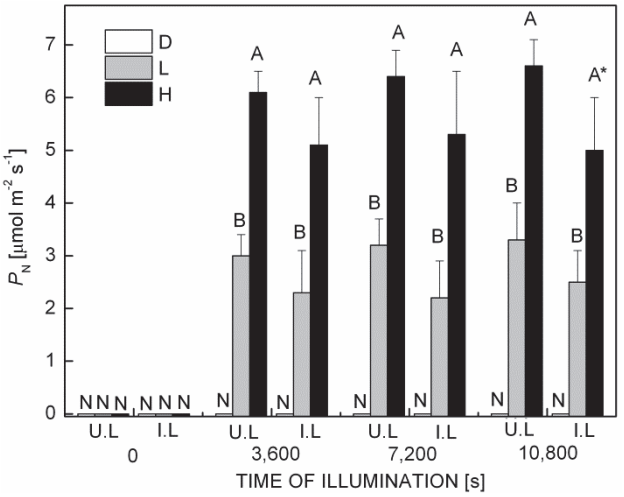

Fig. 3. The effects of illumination on the photosynthetic rates (P_N) of the *T. urticae*-infested and -uninfested leaves that were exposed to different light intensities respectively for 0, 1, 2, and 3 h. Each value represents the mean ± SD (vertical bars) of four independent experiments. *Different letters* denote significant differences from the treatment of the infested or uninfested leaves with different light intensities at the same time point (P<0.05). * – statistically significant differences between the infested and uninfested leaves under the same treatment (P<0.05). D – dark; L – low-light treatment; H – high light treatment; N – no detectable P_N; IL – infested leaves; UL – uninfested leaves.

emission of HIPVs of plants is closely correlated with light received by plants. From a biochemical perspective, isoprenoids are the most important and predominant compounds of HIPVs (Staudt and Lhoutellier 2011). It has been recognized that light upregulates the expression of many genes related to isoprenoid biosynthesis and it results in an activated synthesis of these compounds (Mandel *et al*. 1996, Carretero-Paulet *et al*. 2002).

Cyanide-resistant respiration could play a role in protection of photosynthesis: In the present work, when the increase of the light intensity enhanced the attraction of the predatory mites to the *T. urticae* IL, it was observed that the values of F_q'/F_v' and F_v'/F_m' decreased (Fig. 4*C*,*B*). The ratio of F_q'/F_v' can be used to imply qualitative changes in Q_A redox state. F_v'/F_m' estimates the maximum quantum efficiency of PSII photochemistry in the illuminated leaf, when Q_A is maximally oxidized and thus it can be used to assess the contributions of nonphotochemical quenching (NPQ) to changes in the PSII operating efficiency (Baker *et al*. 2007, Baker and Oxborough 2004). Because an increase in both the portion of reduced PSII centres and in dissipation of absorbed light energy through NPQ is a common response of leaves to a rise of light intensity, the observed declines of F_q'/F_v' and F_v'/F_m' in IL with the increase

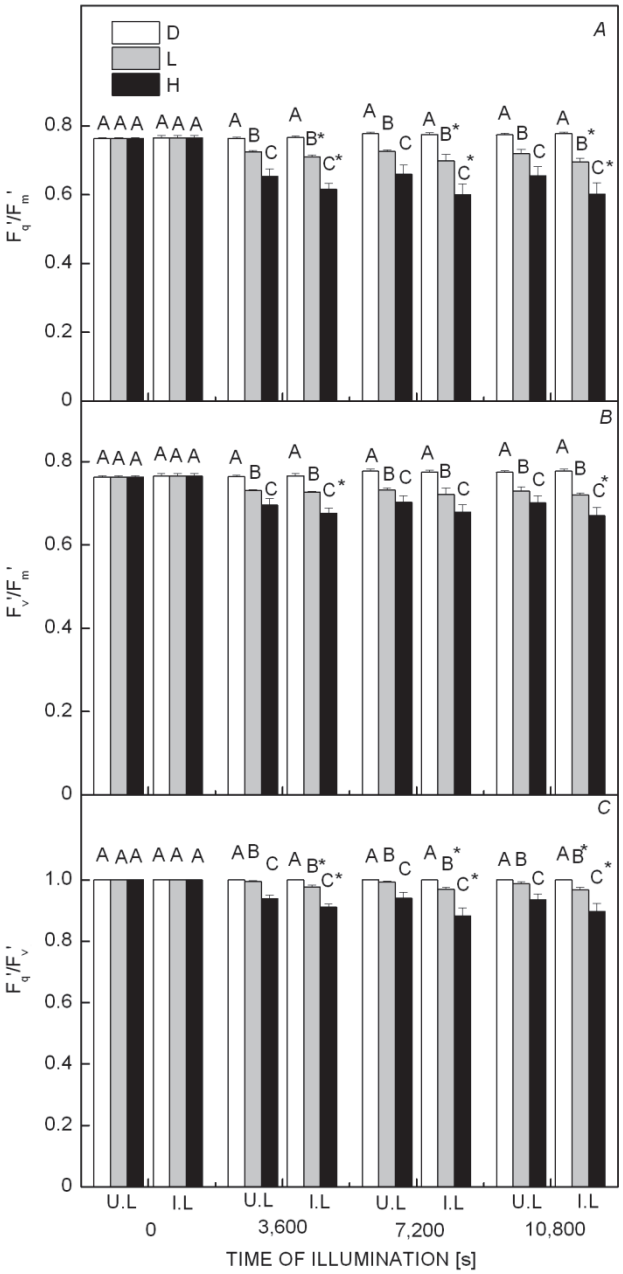

Fig. 4. The effects of illumination on the PSII operating efficiency (*A*), the PSII maximum efficiency (*B*), and the PSII efficiency factor (*C*) of the *T. urticae*-infested and -uninfested leaves that were exposed to different light intensities respectively for 0, 1, 2, and 3 h. Each value represents the mean ± SD (vertical bars) of five independent experiments. *Different letters* denote significant differences from the treatment of the infested or uninfested leaves with different light intensities at the same time point (P<0.05). * – statistically significant differences between the infested and uninfested leaves under the same treatment (P<0.05). D – dark; L – low-light treatment; H – high-light treatment; F_q'/F_m' – the PSII operating efficiency; F_q'/F_v' – the PSII efficiency factor; F_v'/F_m' – PSII maximum efficiency; IL – infested leaves; UL – uninfested leaves.

Table 1. *In vivo* inhibition of the cyanide-resistant respiration of *T. urticae*-infested and uninfested leaves by 1 mM SHAM. Oxygen uptake by the infested or uninfested leaves with 1 mM KCN in the absence of SHAM is denoted as the 100% value for maximal cyanide-resistant respiration capacity. Each value represents the mean ± SD (vertical bars) of three independent experiments.

Leaf	PAR [μmol(photon) m^{-2} s^{-1}]	Time of illumination [s]			
		0	3,600	7,200	10,800
Uninfested	0	59.8 ± 6.5	55.8 ± 9.4	57.5 ± 9.6	55.5 ± 13.3
	80	59.8. ± 6.5	60.0 ± 8.2	54.5 ± 11.0	54.0 ± 9.2
	240	59.8. ± 6.5	59.5 ± 9.0	59.8 ± 7.2	53.3 ± 11.0
Infested	0	56.0 ± 7.7	55.0 ± 5.8	57.3 ± 4.5	55.8 ± 7.7
	80	56.0 ± 7.7	56.0 ± 4.0	57.0 ± 5.7	60.0 ± 8.1
	240	56.0 ± 7.7	59.8 ± 7.2	60.5 ± 3.0	58.0 ± 4.0

Table 2. The effects of SHAM on the photosynthetic rates (P_N) and PSII chlorophyll (Chl) fluorescence of the *T. urticae*-infested and -uninfested leaves under different light conditions. The values of P_N and PSII Chl fluorescence of the infested and uninfested leaves, which were illuminated with different light intensities for 0, 1, 2, 3 h in the absence of SHAM, are denoted as the control (100%) (as presented in Figs. 3,4). Each value represents the mean ± SD of four or five independent experiments. * – statistically significant difference from the treatment of SHAM (P<0.05). - – not detectable P_N.

Leaf	PAR [μmol(photon) m^{-2} s^{-1}]	Illumination time [s]	% of control P_N	F_q'/F_m'	F_v'/F_m'	F_q'/F_v'
Uninfested	0	0	-	99.7 ± 0.3	99.7 ± 0.3	100.0 ± 0.0
		3,600	-	99.3 ± 0.6	99.3 ± 0.6	100.0 ± 0.0
		7,200	-	99.9 ± 0.8	99.9 ± 0.8	100.0 ± 0.0
		10,800	-	100.0 ± 0.8	100.0 ± 0.8	100.0 ± 0.0
	80	0	-	99.7 ± 0.3	99.7 ± 0.3	100.0 ± 0.0
		3,600	91.7 ± 11.1	98.2 ± 1.5*	99.7 ± 0.3	98.4 ± 1.3*
		7,200	92.2 ± 15.6	98.5 ± 0.6*	99.7 ± 0.5	99.0 ± 0.6*
		10,800	91.7 ± 18.9	99.5 ± 1.2	101.3 ± 0.9	98.3 ± 0.6*
	240	0	-	99.7 ± 0.3	99.7 ± 0.3	100.0 ± 0.0
		3,600	77.9 ± 12.6*	80.5 ± 4.1*	88.0 ± 2.2*	91.6 ± 2.4*
		7,200	78.5 ± 11.8*	81.6 ± 7.3*	88.1 ± 4.1*	92.4 ± 3.9*
		10,800	78.4 ± 9.9*	82.2 ± 10.1*	87.8 ± 6.9*	93.0 ± 4.6*
Infested	0	0	-	100.0 ± 0.5	100.0 ± 0.5	100.0 ± 0.0
		3,600	-	100.2 ± 0.6	100.2 ± 0.6	100.0 ± 0.0
		7,200	-	99.9 ± 0.8	99.9 ± 0.8	100.0 ± 0.0
		10,800	-	99.8 ± 0.8	99.8 ± 0.8	100.0 ± 0.0
	80	0	-	100.0 ± 0.5	100.0 ± 0.5	100.0 ± 0.0
		3,600	85.6 ± 15.6	92.2 ± 4.0*	95.6 ± 1.8*	96.3 ± 2.7*
		7,200	84.1 ± 17.2	95.5 ± 3.0*	96.7 ± 1.9*	98.6 ± 1.6
		10,800	88.0 ± 19.0	92.7 ± 6.3*	95.3 ± 3.8*	97.0 ± 3.2
	240	0	-	100.0 ± 0.5	100.0 ± 0.5	100.0 ± 0.0
		3,600	66.7 ± 9.2*	66.2 ± 9.5*	76.1 ± 3.2*	85.4 ± 8.3*
		7,200	65.6 ± 13.3*	69.5 ± 8.6*	77.6 ± 5.1*	89.3 ± 5.3*
		10,800	62.5 ± 16.9*	69.6 ± 4.0*	77.4 ± 1.6*	90.1 ± 4.0*

of the light intensity were not surprising. As the mathematical product of the F_q'/F_v' and F_v'/F_m' (Baker *et al*. 2007), the F_q'/F_m' (= $F_q'/F_v' \times F_v'/F_m$) was also found to be lowered due to the decrease of both the F_q'/F_v' and F_v'/F_m' (Fig. 4).

When the increase of the light intensity enhanced the attraction of the predatory mites to IL, the level of V_{alt} in IL increased significantly (Fig. 2*B*). The treatment with SHAM caused a decrease of both the F_q'/F_v' and P_N of IL exposed to light (Table 2). And, the declines of the F_q'/F_v' and P_N were more severe in IL exposed to the higher light intensity than those exposed to the lower light intensity (Table 2). These observations suggest that cyanide-resistant respiration could play a role in preventing the excessive accumulation of reducing equivalents in the photosynthetic electron transport chain and thereby lowering the probability of loss of photosynthetic activity during the light-induced attraction of predators to herbivore-infested leaves, especially, when higher light induced more attraction of predators.

After the SHAM treatment, the significant decrease of F_v'/F_m' in IL exposing to light (especially to the higher light intensity (Table 2) could reflect that additional NPQ was produced, when cyanide-resistant respiration was inhibited. It could, as a compensatory response, alleviate the over-reduction of PSII caused by the loss of the

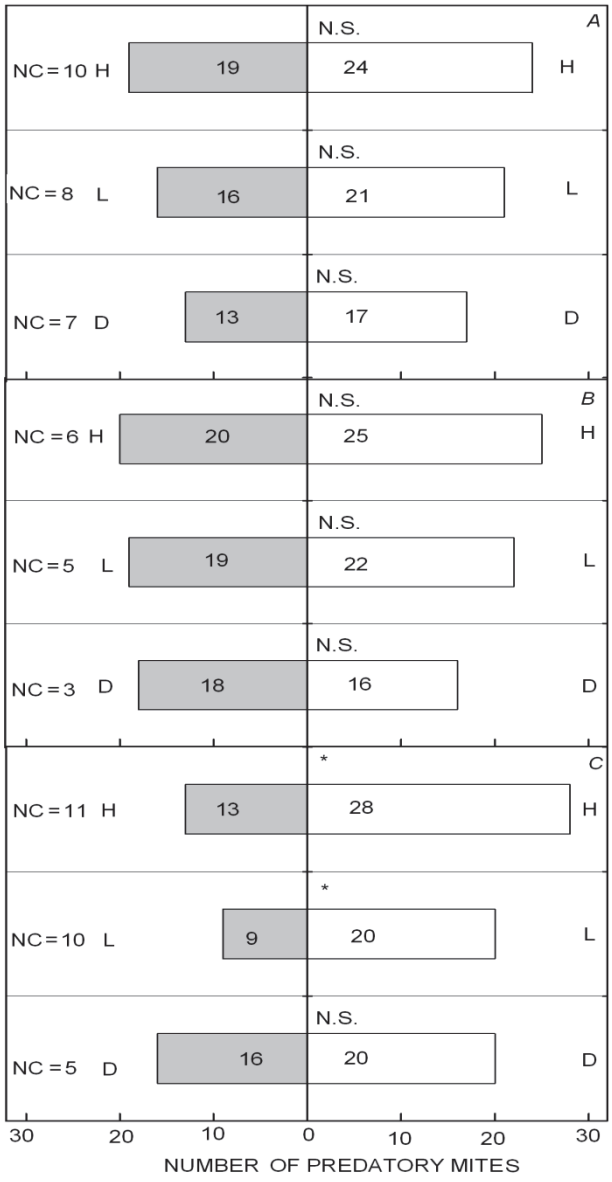

Fig. 5. Responses of predatory mites (*P. persimilis*) in a Y-tube olfactometer to the odor from the leaves subjected to different treatments. *A*: the uninfested leaves treated with SHAM (*grey*) *vs*. the uninfested leaves without SHAM treatment (*white*). *B*: the infested leaves treated with SHAM (*grey*) *vs*. the infested leaves without SHAM treatment (*white*). *C*: the uninfested leaves without SHAM treatment (*grey*) *vs*. the infested leaves treated with SHAM (*white*). N.S – no statistically significant preferences in a choice test (χ^2 test: $P<0.05$). The number of the predators that made a final choice is shown in each bar. The predators that did not reach the end of either olfactometer arm within 5 min (NC: no choice) were excluded from the statistical analyses. N.S. – no significant preferences. D – dark; L – low-light treatment; H – high-light treatment.

function of cyanide-resistant respiration (because NPQ contributes to the oxidation of PSII centres by dissipating the absorbed light energy). However, even though such a compensatory photoprotection pathway actually occurred, it did not seem to be able to replace the function of cyanide-resistant respiration, considering the observed decrease of the F_q'/F_v' and P_N in the presence of SHAM (Table 2).

Although the increase of the light intensity had no significant effects on the attractiveness of UL to predatory mites (Fig. 1*C*), the effects of the light intensity or SHAM on the PSII chlorophyll fluorescence, P_N, and V_{alt} of UL were similar to those of IL (Tables 1,2; Figs. 2*B*,4). These observations suggested that cyanide-resistant respiration in UL could also play a role in a protection of photosynthesis, as it did in IL. Thus, the function of cyanide-resistant respiration in protecting photosynthesis is not particular for the light-induced defense responses of the herbivore-infested plants.

We also noted that the values of the Chl fluorescence parameters and P_N in IL were lower than those in UL under the illumination (Figs. 3,4). There is accumulating evidence that insect herbivory reduces photosynthetic carbon fixation and photochemical efficiency of PSII (Kerchev *et al*. 2012,). In addition, the present work showed that the effects of SHAM on the PSII and P_N of IL were more dramatic than on those of UL (Table 2), indicating that the importance of cyanide-resistant respiration in the protection of photosynthesis of plants could become relatively more important, when photosynthesis was impaired by *T. urticae* infestation.

Cyanide-resistant respiration was not a component of the attractiveness of plants to predatory mites: The light-induced attractiveness of herbivore-infested plants to the natural enemies of herbivores could be explained by two mechanisms. First, as mentioned above, light can upregulate the expression of isoprenoid biosynthesis-related genes (Mandel *et al*. 1996, Carretero-Paulet *et al*. 2002). Second, the ability of light to enhance the synthesis of isoprenoids could be also dependent on photosynthesis, since the major bulk of the primary carbon substrate for their biosynthesis is thought to be provided by photosynthesis (Arimura *et al*. 2009, Staudt and Lhoutellier 2011). However, Winter (2010) revealed that ultraviolet radiation, nitrogen deficiency, and heavy metal stress, all of which can decrease photosynthetic activity of plants, did not affect the attractiveness of herbivore-infested plants to the natural enemies of herbivores, indicating that photosynthetic activity is not a determining factor for the attraction of predators to the herbivore-infested leaves. We also found that when SHAM treatment significantly decreased P_N of both IL and UL, which were exposed to H (Table 2), the attraction of the predatory mites to the leaves was not significantly affected (Fig. 5*A*,*B*). Under the condition of illumination, the *T. urticae*-infested leaves treated with SHAM were still more attractive to the predatory mites than the uninfested leaves (Fig. 5*C*). Farag and Paré (2002) found that monoterpenes and sesquiterpenes were not labeled with the $^{13}CO_2$ gas in tomato plants infested

with tobacco hornworms during the photoperiod. A possible explanation for these observations is that the carbon substrate for light-induced biosynthesis of HIPVs could be mainly attributed to stored or older carbon sources than to immediately produced photosynthates.

In conclusion, cyanide-resistant respiration could not be especially linked to the light-induced defense responses of the herbivore-infested plants, but it could serve plants to protect photosynthesis, especially, when photosynthesis was impaired by herbivores infestation.

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