Arch. Biol. Sci., Belgrade, 65 (1), 247-254, 2013 DOI:10.2298/ABS1301247H

PROBING BEHAVIORS OF *SITOBION AVENAE* (HEMIPTERA: APHIDIDAE) ON ENHANCED UV-B IRRADIATED PLANTS

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Abstract - UV-B induced changes in plants can influence sap-feeding insects through mechanisms that have not been studied. Herein the grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), was monitored on barley plants under the treatments of control [0 kJ/ (m^2.d)], ambient UV-B [60 kJ/ (m^2.d)], and enhanced UV-B [120 kJ/ (m^2.d)] irradiation. Electrical penetration graph (EPG) techniques were used to record aphid probing behaviors. *E*nhanced UV-B irradiated plants negatively affected probing behaviors of *S. avenae compared with control plants*. In particular, phloem factors that could diminish sieve element acceptance appeared to be involved, as reflected by smaller number of phloem phase, shorter phloem ingestion, and fewer aphids reaching the sustained phloem ingestion phase (E2>10min). On the other hand, factors from leaf surface, epidermis, and mesophyll cannot be excluded, as reflected by higher number of non-probing, longer non-probing and pathway phase, and later the time to first probe.

Key words: Grain aphid, electrical penetration graph, UV-B irradiated barley

INTRODUCTION

Ultraviolet-B (UV-B) is a very narrow band at the short wavelength (280-315nm) end of the daylight spectrum. Due to the human-induced destruction of the UV-B-absorbing stratospheric layer of ozone, the amount of UV-B radiation reaching the surface of the earth has increased during the last two decades (Björn et al., 1998; Randel et al., 1999). This has led to concerns about potential biological impacts on the environment (Madronich et al., 1998; McCloud and Berenbaum, 1999). Changes in plant morphology and physiology in response to UV-B exposure are numerous. The most characteristic responses of plant morphology to UV-B are an increase in leaf thickness and an inhibition of plant growth (Jansen et al., 1998; Zavala and Ravetta, 2002; Caldwell et al., 2003). Moreover, UV-B can affect the cuticle compositions, phytohormone levels, proteinase inhibitor activities, as well as levels of many secondary plant metabolites to varying extents (Kuhlmann and Muller, 2011).

UV-B-induced changes in plant texture and metabolism can influence herbivorous insects including sap-feeding insects. The cabbage aphid, *Brevicoryne brassicae,* reproduced less on broccoli plants grown under high UV-B than on plants grown under low UV-B radiation (Kuhlman and Muller, 2010). Artificial UV-B radiation reduced survivorship and egg production of the spider mite, *Tetranychus urticae* and egg hatchability of the predaceous phytoseiid mite, *Neoseiulus womersleyi* (Ohtsuka and Osakabe, 2009). The UV-B absorbing plastic film used for greenhouse covers may have a significant influence on both the initial immigration and distribution of the greenhouse whitefly, *Trialeurodes vaporariorum*, into greenhouses (Mutwiwa et al., 2005).The UV-B

UV-absorbing plastic significantly reduced the movement of the leafhopper *Orosius orientalis* (Weintraub et al., 2008). However, these studies focused on the effect of UV-B on sap-feeding insect biology performance. The defense mechanisms of UV-B irradiated plants against sap-feeding insects have been little documented.

The electrical penetration graph (EPG) technique is frequently employed to characterize the host-plant effect on sap-feeding insects, as it allows the exploration of interactions between insects' probing behavior and plant tissues that may display some form of defense mechanism (McLean and Kinsey, 1964; Tjallingii, 1988 and 2006). Using this method, one electrode is implanted into the substrate supporting the plant and the other is positioned on the dorsal region of the insect using a drop of silver stain. The circuit is completed when the insect inserts its stylet into the plant tissue in order to probe the plant to feed. At this point, the variation in voltage can be recorded by computer software to construct a penetration graph. Each waveform generated by this system characterizes a particular type of probing activity and this, together with the location of the stylet, can be used to determine the non-probing, pathway, and phloem phases of insect probing (Tjallingii and Prado, 2001).

The grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) is one of the most abundant and economically important pests on cereal crops. They damage their host by direct probing and by transmitting diseases (Stern, 1967; Fereres et al., 1989). In our previous studies, the enhanced UV-B-irradiated plants negatively affected the development and reproduction of *S. avenae* (Hu, unpublished data). According to the effect of UV-B radiation on aphids through changing plant morphology and physiology, we hypothesized that UV-B irradiation of plants could modulate *S. avenae* probing behaviors. The electrical penetration graph (EPG) technique was used to analyze the aphid probing activities that occur before and during sap ingestion from phloem sieve elements. The objectives of this study were to elucidate the defense mechanisms of UV-B-irradiated plants to sap-feeding aphids.

MATERIALS AND METHODS

Plant material and growth conditions

Experiments were conducted using nine environmental growth chambers (conditions: 20 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 (L:D) h) located at the Bio-Test laboratory, Sagerheide, Germany. Fluorescent bulbs (115V, Philips Company, Eindhoven, Netherlands) were mounted in growth chambers to provide 250 μE/cm^2/s of light intensity at the leaf surface.

Barley, *Hordeum sativum* Jess (var. 'Lomerit', Intergrano Agrohandel Sp. z o.o. Lubuskie, Germany) was grown in 2009 in plastic pots (14 cm in height, 12 cm in diameter) with three seeds per pot using a growing medium (N: P: K = 20:20:20, Einheitserde- und Humuswerke Gebr. Patzer GmbH and Co. KG, Germany). Experimental plants were watered as needed. When the plants were at the 2-3 leaf stage, the strongest one was selected and maintained in each pot for UV-B radiation.

UV-B radiation treatments

During a pilot study in Rostock (54°09′N, 12°08′E), Germany, in May 2009, the natural daily UV-B dose of 60 kJ/(m^2.d) (UV meter, Honle UV technology, Gräfelfing, Germany) was measured under clear-sky conditions at the top of the barley. We therefore utilized the value of 60 kJ/ (m^2.d) as the current ambient UV-B level ('ambient UV-B').

The UV-B radiation treatments in this study were as follows. Treatment (i) plants were irradiated with UV-B for 8 h a day in a growth chamber equipped with one UV-B lamp (UVB-313; Q-Panel, Cleveland, OH), which was placed 22 cm above the plants [2.09W/ m^2, 60 kJ/ (m^2.d) 'ambient UV-B']. Treatment (ii) plants were irradiated with UV-B for 8 h a day in a growth chamber equipped with two UV-B lamps, which were placed 20 cm above the plants [4.20W/ m^2, 120 kJ/ (m^2.d), 'enhanced UV-

B']. Treatment (iii) was not treated (control). To get rid of UV-C (under 290 nm) radiation, all UV-B lamps were blocked with a cellulose diacetate filter (Clarifoil, Derby, UK). Fifteen plants were placed in each growth chamber (one plant per pot), with three growth chambers per treatment (a total of 45 plants per treatment). Pot placement was re-randomized in each chamber daily. Plants were used for testing the probing behaviors of aphids after 10 days UV-B irradiation.

Insect stocks

Single apterous grain aphid, *Sitobion avenae,* was originally collected from a field near collected Rostock (54°09′ N, 12°08′ E), Germany, in 2008 and transferred to barley plants (var. 'Lomerit'). The plants were maintained in insect-rearing tents (60×60×60cm, MegaView Science Co., Ltd., Taiwan) under the growth chamber conditions described above for one year. Newly cultured barley plants were exchanged weekly. The aphids were observed every 3 days, and excess aphids were removed to keep the aphid population under low-density conditions.

Aphid probing behaviors

The Giga-8 DC-EPG (W.F.Tjallingii, University of Wageningen, The Netherlands) was used to examine aphid probing behavior on the abaxial face of the third fully developed leaf from the plant top. To insert one aphid and one plant in an electrical circuit, a thin gold wire (20 μm diameter and 2 cm long) was tethered at the dorsum of the aphid by conductive silver paint (W.F.Tjallingii, University of Wageningen, The Netherlands); the other electrode was inserted in the dampened soil of the potted plant. Before an aphid was used for the EPG recording, it was allowed to acclimate to the tethering by allowing it to crawl on a solid surface without probing for 1 h. For each treatment plant, 45 replicates (one aphid per plant) were conducted and the recordings were conducted continuously for 8 h during the daytime (9:00-17:00).

Aphid probing behaviors were recorded by their EPG waveforms using PROBE 3.5 software (EPG, W.F.Tjallingii, University of Wageningen, The Netherlands). Three behavioral phases, each of which were characterized by one or more waveforms, could be distinguished: (i) non-probing phase (waveform Np) where the insect is not piercing into the plant tissues; (ii) pathway phase (waveform C), the main activity before reaching the sieve elements in the phloem, including primary penetration through plant tissues, often with cell punctures, and salivation; and (iii) phloem phase, formed by two waveforms, E1 and E2. The E1 waveform is formed by salivation into the phloem elements and the E2 waveform is formed by passive phloem sap ingestion (Tjallingii, 1988). Other waveforms were also acquired but are not presented here because they did not provide significant information on aphid probing behavior.

Experimental design and data analysis

The current study utilized a randomized complete block design. The following EPG parameters were recorded and recognized through different waveforms: number of non-probing (n_Np); sum of non-probing phase (s_Np); time to first probe (t >1Pr); sum of the pathway phase (s_C); time to first phloem phase in experiment (t >1E); time to first phloem phase in probe (t >1E/Pr); number of phloem phases (n_E); sum of phloem salivation (s_E1); sum of phloem ingestion(s_E2); time to first sustained phloem ingestion in experiment (E2 > 10 min)(t >1sE2); and time to first sustained phloem ingestion in probe (E2 > 10 min) (t >1sE2/Pr) (Table 1). Statistical analyses were performed using SPSS 17.0 software (Chicago, IL, USA). The data were transformed to log (base-10 logarithm) to fit the normal distribution. Nested ANOVA was done to determine whether the effect of UV-B treatment was significant, with UV-B irradiation treatment as a main factor and growing in independent growth chamber as a subgroup nested within the UV-B irradiation treatment. One-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$) was also performed. The percentage of aphids with a sustained phloem ingestion phase in treatment and control plants were analyzed by χ^2-test with continuity correction.

RESULTS

Probing behavior of the non-probing and pathway phase

Nested ANOVA tests revealed that there were no significant differences among the subgroups (nested within UV-B treatment) for the number of non-probing (n_Np), sum of non-probing phase (s_Np), time to first probe (t > 1Pr), sum of the pathway phase (s_C), and time to first phloem phase in experiment (t >1E), but there were significant differences among UV-B treatments for those variables (Table 1). Compared to the control and ambient UV-B plants, *S. avenae* fed on enhanced UV-B plants showed significantly longer s_Np and s_C (s_Np : 83.01±2.51 min vs 58.51±2.38 min or 70.01±2.45 min; s_C: 250.78±5.85 min vs 190.47±5.75 min or 210.37±5.91 min), significantly higher n_Np (26.09 ±1.14 vs 19.65±0.89 or 22.69±0.90), and significantly later t > 1Pr and t >1E (t > 1Pr:19.51 ±0.79 min vs 13.36±0.78 min or 15.83±0.90 min; t >1E: 329.40±6.54 min vs 264.93±6.54 min or 299.53±6.51 min) (Tukey's HSD, P <0.05; Fig. 1, A-E). Moreover, the value of s_Np, s_C and t >1Pr were significantly increased on ambient UV-B plants compared to control plants (Tukey's HSD, P <0.05; Fig. 1, B, D, E).

Probing behavior of phloem phase

Nested ANOVA on the values of number of phloem phase (n_E), sum of phloem salivation (s_E1), sum of phloem ingestion (s_E2), time to first sustained phloem ingestion in experiment (E2 > 10min) (t >1sE2), and time to first sustained phloem ingestion in probe (E2 > 10min) (t >1sE2/Pr), showed a significant difference among UV-B treatments. However, there were no significant differences among subgroups (nested within UV-B treatment) (Table 1). The n_E, s_E2 were significantly decreased for *S. avenae* fed on enhanced UV-B plants compared to those fed on the control or ambient UV-B plants (n_E: 2.644±0.16 vs 3.96±0.19 or 3.75±0.18; s_E2: 86.75±5.12 min vs 157.13±20.34 min or 108.51±5.16 min; Tukey's HSD, P <0.05; Fig. 2; A,C). Furthermore, compared to control plants, *S. avenae* fed on ambient UV-B plants showed significantly shorter s_E2 (Tukey's HSD, P <0.05; Fig. 2; C). No significant differences of s_E1 for *S. avenae* were found among control, ambient UV-B treatment, and enhanced UV-B treatment plants (21.91±0.71 min vs 21.11±0.65 min vs 21.68±0.70; Tukey's HSD, P >0.05; Fig. 2, B).

Compared to the control plants, *S. avenae* fed on enhanced UV-B and ambient UV-B plants showed significantly later t >1sE2 (297.83±4.62 min or 329.47±4.75 min vs 356.05 ± 4.90 min; Tukey's HSD, P <0.05; Fig. 2, D). No significant differences of t >1sE2/Pr for *S. avenae* were found among on control, ambient UV-B, and enhanced UV-B plants (200.45 ± 6.25 min vs 207.72 ± 6.65 min vs 209.11 ± 6.44; Tukey's HSD, P >0.05; Fig. 2, E). In the course of this experiment, after 5 h monitoring, a lower percentage of aphids showed sustained phloem ingestion phase (E2 >10 min) on the enhanced UV-B plants than aphids on the control and ambient plants (χ^2 =6.16-28.67, P = 0.000-0.013, Fig. 3).

DISCUSSION

The probing behavior performance of aphids provides invaluable clues for understanding the defense mechanism of UV-B-irradiated plants to sap-probing aphid at tissue levels. As mentioned earlier, the whole process of aphid probing behavior starts with labial contact with the plant surface, followed by the penetration of its stylets through successive tissue layers between the epidermis and phloem, eventually targeting on the sieve element of the phloem. Therefore, plant tissue factors can play various roles in the initiation, maintenance, and cessation of each subsequent event in the probing process contact by aphid (Lei et al., 1999).

On the enhanced UV-B-irradiated barley plants, the number of non-probing (n_Np), sum of non-probing phase (s_Np), and time to first probe (t > 1Pr) for *S. avenae* were significantly increased when compared with the control plants (Fig. 1). The fact that these activities occurred before the probing, strongly suggests that the defense factors are present on the surface tissue layer in enhanced UV-B-irradi-

Table 1. *F* values and significance (*P*) from nested ANOVA tests of effects of UV-B treatment and independent growth chamber site (nested within UV-B treatment) on variables of probing behavior.

Parameters	Symbol	UV-B treatment		Subgroup(Independent growth chamber site)	
		$F_{2,6}$	*P*	$F_{6,109\text{-}126}$	*P*
Number of non-probing	n_Np	26.388	0.001	0.394	0.882
Sum of non-probing	s_Np	43.945	<0.001	0.559	0.762
Time to first probe	t > 1Pr	15.771	0.004	0.963	0.453
Sum of the pathway phase	s_C	43.199	<0.001	0.631	0.705
Time to first phloem phase in experiment	t >1E	13.415	0.006	1.890	0.087
Time to first phloem phase in probe	t >1E/Pr	1.286	0.343	6.922	<0.001
Number of phloem phase	n_E	9.342	0.014	2.149	0.052
Sum of phloem salivation	s_E1	1.867	0.234	0.185	0.981
Sum of phloem ingestion	s_E2	7.636	0.022	1.100	0.366
Time to first sustained phloem ingestion in experiment(E2 > 10min)	t >1sE2	65.329	<0.001	0.591	0.737
Time to first sustained phloem ingestion in probe (E2 > 10min)	t >1sE2/Pr	0.438	0.667	1.204	0.310

ated plants. Steinmüller and Tevini (1985) reported that enhanced UV-B radiation could cause a 28% increase in leaf wax layers in different crop plant species. Next to thickness, the chemical composition of waxes has been shown to be influenced by UV-B radiation (Tevini and Steinmüller, 1987; Barnes et al., 1996). It is possible that UV-B can change the physical properties or the chemistry of the plant surface and these changes can be considered as a decreasing host plant susceptibility to the aphids.

Sitobion avenae showed longer sum of the pathway phase (s_C) and later time to first phloem phase in the experiment (t >1E) on enhanced UV-B-irradiated plants compared with control plants (Fig. 1). This result strongly indicates that the plant epidermis or mesophyll layers also might play an important role in the defense of enhanced UV-B-irradiated plants against *S. avenae*. Plants generally adapt to changes in UV-B radiation by activating the induction of protective compounds (sunscreens). These sunscreens include phenolic compounds derived from phenylalanine (flavonoids and other phenylpropanoid derivatives, such as sinapate esters) that accumulate in large quantities in the vacuoles of epidermal cells and effectively attenuate the UV-B irradiation (Caldwell et al., 2003; Jenkins and Brown, 2007). Changes in enhanced UV-B-irradiated plant sunscreens may lead to decreased host plant acceptance of the aphids.

Apart from the surface and epidermis/mesophyll layers, phloem tissue layers were often typical features associated with defenses as reported in EPG studies performed on aphid-plant combinations (Sauge et al., 1998; Kaloshian et al., 2000). From our data, the number of phloem phase (n_E) and sum of phloem ingestion (s_E2) for *S. avenae* were significantly decreased, the time to first sustained phloem ingestion in experiment (E2 >10 min) (t >1sE2) was significantly increased on enhanced UV-B-irradiated plants when compared with control plants (Fig. 2). Moreover, fewer aphids reached sustained phloem ingestion (E2 >10 min) within the 8 h experiments (Fig. 3). The previous laboratory studies have shown that UV-B radiation can alter leaf phloem-layer characteristics, such as total leaf N, available carbohydrates, fiber (Lindroth et al., 2000), and free amino acids (Salt et al., 1998). It is possible that changes in the nutrition composition in enhanced UV-B-irradi-

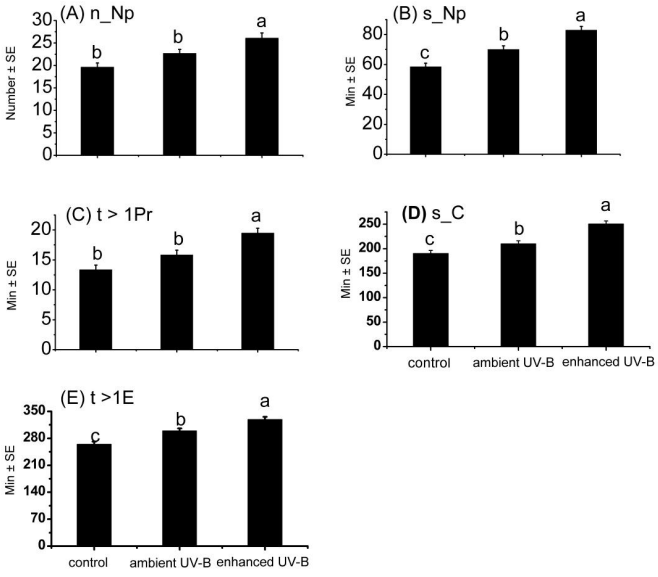

Fig. 1 Electronically monitored probing behaviors (mean ± SE, n = 45) of *S. avenae* on control, ambient UV-B and enhanced UV-B-treated plants during the 8 h access experiments. n_Np: number of non-probing, s_Np: sum of non-probing, t > 1Pr: time to first probe, s_C: sum of the pathway phase, t >1E: time to first phloem phase in experiment. Bars with different lower-case letters are significantly different from one another according to Tukey's HSD test (P <0.05).

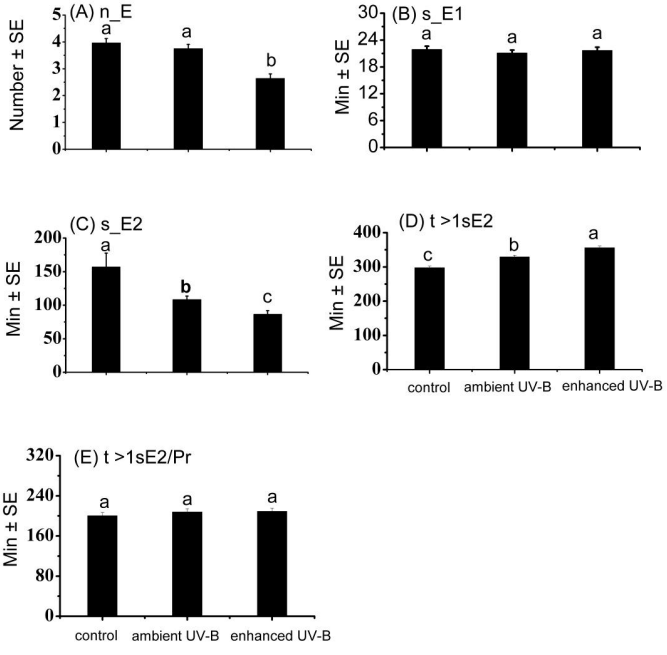

Fig. 2 Electronically monitored probing behaviors (mean ± SE, n = 36-45) of *S. avenae* on control, ambient UV-B and enhanced UV-B-treated plants during the 8h access experiments. n_E: number of phloem phase, s_E1: sum of phloem salivation, s_E2: sum of phloem ingestion, t >1sE2: time to first sustained phloem ingestion in experiment (E2 > 10 min), t >1sE2/Pr: time to first sustained phloem ingestion in probe (E2 >10 min). Bars with different lower-case letters are significantly different from one another according to Tukey's HSD test (P <0.05).

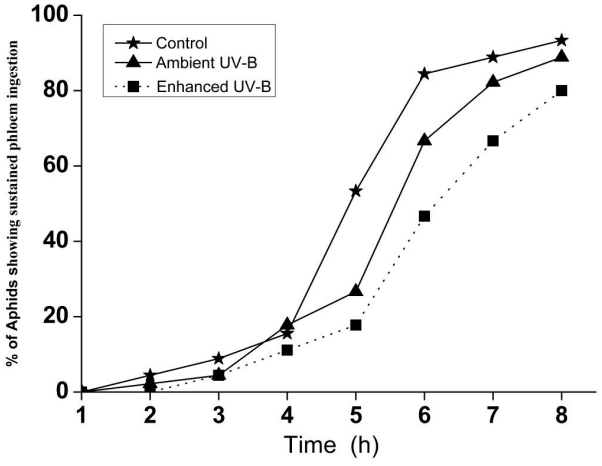

Fig. 3. Percentage of *S. avenae* reaching the sustained phloem sap ingestion phase (E2 > 10 min) on control, ambient UV-B and enhanced UV-B-treated plants during 8 h experiments.

ated plant can also be considered as decreased host plant acceptance to the aphids. Together with our results, such variations in diet may explain the negative effect on development and reproduction of *S. avenae* when fed on enhanced UV-B-irradiated plants (Hu, unpublished data).

In this study, we tested the hypothesis that enhanced UV-B-irradiated plants negatively affected the probing behaviors of *S. avenae*. In particular, phloem factors diminishing sieve element acceptance appear to be involved, as reflected by fewer aphids reaching sustained phloem ingestion within the 8 h experiment, smaller number of phloem phase (n_E), shorter sum of phloem ingestion(s_E2), and later time to first sustained phloem ingestion in experiment (t >1sE2). On the other hand, factors from the leaf surface, epidermis, and mesophyll cannot be excluded, as reflected by the higher number of non-probing (n_Np), longer sum of non-probing phase and pathway phase (s_Np, s_C), and later time to first probe (t > 1Pr) and time to first phloem phase in experiment (t >1E). Based on the results of this study, we conclude that populations of *S. avenae* will be significantly decreased under sustainable enhanced UV-B in the future. Future studies are needed to evaluate the significance of the findings of this study under field conditions.

Acknowledgments - The authors thank Dr. John Richard Schrock and Dr. De-guang Liu for their critical reviews of this manuscript. This project was funded by the 'Bundesanstalt für Landwirtschaft und Ernährung' (BLE) in the framework of bilateral collaboration (Project 3/04-05) between the PR China and Germany, and the National Natural Science Foundation of China (Grant Nos. 39970112 and 30470268).

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