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DIMBOA GLUCOSIDE, A WHEAT CHEMICAL DEFENSE, AFFECTS HOST ACCEPTANCE AND SUITABILITY OF *Sitobion avenae* TO THE CEREAL APHID PARASITOID *Aphidius rhopalosiphi*

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Abstract—The influence of hydroxamic acids (Hx), plant secondary metabolites associated with aphid resistance in wheat, on the host acceptance and suitability of the aphid *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphi* was evaluated. Aphids showed a reduction in mean relative growth rate and in body size in the wheat cultivar with higher Hx level. Reduction in aphid size was related to a decreased success in avoiding parasitoid oviposition. A minor increase in *A. rhopalosiphi* developmental time was observed in aphids feeding on the higher Hx cultivar. Experiments with different concentrations of DIMBOA glucoside, the main Hx in wheat, in artificial diets showed an increase in parasitoid developmental time at the highest concentration, with no change in other performance variables. The evidence is discussed in relation to the compatible utilization of host-plant resistance and biological control in integrated pest management.

Key Words—Tritrophic interaction, aphid, *Sitobion avenae*, parasitoid, *Aphidius rhopalosiphi*, oviposition, defensive behavior, DIMBOA.

INTRODUCTION

Several morphological and/or chemical attributes of a host plant may confer resistance towards herbivorous insects. Resistance to herbivorous insects, however, can also significantly affect the performance of their natural enemies (Price et al., 1980; Vet and Dicke, 1992; Hare, 1992) producing negative, compatible,

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or even synergistic interactions between host-plant resistance and biological control.

In particular for aphids, plant allelochemicals such as volatile, epidermal, and internal metabolites constitute potential resistance factors that can play important roles in aphid–plant interactions (Niemeyer, 1990; Pickett et al., 1992). In relation to tritrophic effects, van Emden and Wratten (1990) reviewed the influence of the plant on aphids and their natural enemies, suggesting that the emphasis on deleterious tritrophic effects might not reflect the prevalent situation.

In wheat and other Poaceae, hydroxamic acids (Hx), a family of plant secondary metabolites, have been identified as resistance factors showing deterrent and antibiotic properties against cereal aphids (Niemeyer and Pérez, 1995). These compounds could be a target for breeding programs aimed at increasing resistance towards cereal aphids (Escobar and Niemeyer, 1993). Nevertheless, in integrated pest management strategies it is necessary to evaluate the potential influence of the proposed increase in Hx levels on biological control agents.

With regard to the process of host selection in parasitoids of cereal aphids, host acceptance and suitability might be influenced by plant secondary metabolites such as Hx. Studies addressing host acceptance of cereal aphid parasitoids in relation to these metabolites are not available. However, in relation to aphid suitability Fuentes-Contreras et al. (1996) found a small tritrophic effect, ca. 5% increase in developmental time, on the cereal aphid parasitoid *Aphidius rhopalosiphi* De Steph. feeding on wheat (*Triticum aestivum* L.) when compared to those feeding on oat (*Avena sativa* L.), a cereal lacking Hx.

In order to evaluate further the potential influence of Hx on parasitoids of cereal aphids, we studied the effect of wheat cultivars with different Hx levels on the host acceptance behavior of the cereal aphid parasitoid. *A. rhopalosiphi* and the respective defensive reactions to parasitoid attack of the English grain aphid *Sitobion avenae* (F.). In addition, we evaluated the influence of 2-O-β-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-glc), the main Hx in wheat, on *S. avenae* suitability for *A. rhopalosiphi* development when aphids fed on wheat cultivars or artificial diets with different levels of Hx.

METHODS AND MATERIALS

Plant Material and Aphid and Parasitoid Stock Culture. All experiments were performed with *S. avenae* from a stock culture maintained on oat (cv. Nehuén) and *A. rhopalosiphi* from a stock culture maintained on *S. avenae* reared on the same oat cultivar. The experiments involving plants were performed using two spring wheat cultivars with different levels of DIMBOA-glc: Huenufén ($\overline{X} = 1.72 \pm 0.12$ mmol/kg fresh wt) and Naofén ($\overline{X} = 3.02 \pm 0.17$

mmol/kg fresh wt) (concentration on primary leaf of 6-day-old seedlings, N = 6).

Plant Analysis of Hydroxamic Acids and Their Isolation. Quantification of DIMBOA-glc in plants was performed with reverse-phase high-performance liquid chromatography (HPLC), as previously described by Weibull and Niemeyer (1995). DIMBOA-glc for HPLC standards and experiments with artificial diets was isolated from *Zea mays* L. (CV T55s) according to the protocol described by Hartenstein et al. (1993). The identity of the products obtained was checked by HPLC against standards provided by Dr. Dieter Sicker (Leipzig University, Leipzig, Germany). Purity was also evaluated by HPLC and ranged between 95 and 98%.

Influence of Wheat Cultivars on Host Acceptance by Parasitoid and Aphid Defensive Behavior. Before the beginning of the trials, colonies of *S. avenae* were transferred for at least four generations to the above-mentioned wheat cultivars (Huenufén and Naofén). Two synchronized third-instar nymphs, one from each wheat cultivar, were placed singly on oat leaves inside petri dishes (35 mm diameter, 10 mm height). Since volatile compounds of the plant might affect attack rate of the parasitoid (Powell and Wright, 1992; Braimah and van Emden, 1994), only oat leaves were used in the experimental arena, so wheat volatiles from different cultivars that would affect parasitoid behavior during the experiments were absent. After allowing aphids to settle for 10 min, a naive female of the parasitoid was introduced in the arena. All parasitoid females were mated, 2–3 days old, and were used only once. The behavior of the parasitoid and the aphid was observed under a Nikon stereoscopic microscope and recorded continuously with the software "The Observer." The observation period lasted for 5 min. The following parasitoid and aphid behavioral events were recorded: (1) encounter—female parasitoid approaching the aphid and tapping the aphid body with its antennae; (2) attack—female parasitoid bending the abdomen forward and reaching or not reaching the aphid body; (3) stab—female parasitoid reaching the aphid body with the ovipositor; (4) kicking—aphid kicking backwards with its hind legs after being contacted by the foraging parasitoid; and finally (5) cornicle secretion—droplets of cornicle secretion observed after a parasitoid contact.

Influence of Wheat Cultivars on Parasitoid and Aphid Performance. Mean relative growth rate (MRGR) of *S. avenae* was evaluated during a four-day period. Synchronized second-instar aphids were placed inside clip-cages on the wheat seedlings as described previously in Fuentes-Contreras et al. (1996). All experiments were performed with 6-day-old, growth stage 11 (Zadoks et al., 1974) seedlings grown under the following environmental conditions: 25 ± 1°C, 16L:8D, 3000 lux. In the same way, the effects of the wheat cultivars on *A. rhopalosiphi* development were evaluated following the protocol described in Fuentes-Contreras et al. (1996).

Artificial Diet Experiments. Artificial diets were prepared following Febvay et al. (1988). Synchronized third-instar aphids from the stock culture were parasitized as described above for plants, but the aphids were transferred to Plexiglas cages with artificial diet inside Parafilm sachets provided instead of wheat seedlings. Two DIMBOA-glc concentrations (2 and 4 mM) and a control without the compound were used in the experiments. These concentrations represent physiological concentrations found by the aphid while feeding in the cultivars used in our experiments (Givovich and Niemeyer, 1995).

RESULTS

Effect of Wheat Cultivars on Aphid–Parasitoid Interaction. Behavior of the female parasitoid in terms of frequency of encounters and attack attempts on the host was not significantly different between the two wheat cultivars. Additionally, aphid defensive reactions, such as kicking or production of cornicle secretion, were not significantly different between wheat cultivars (Table 1). However, the frequency of stabs was significantly higher on cultivar Naofén, which shows a higher level of Hx than the cultivar Huenufén (Table 1).

Effect of Wheat Cultivars in Aphid and Parasitoid Development. MRGR of the aphid was significantly lower in cultivar Naofén (Table 2). Since the sex ratio of the parasitoids was not significantly different between wheat cultivars (Table 2), data from other variables of parasitoid performance were pooled for both sexes. Total developmental time of the parasitoids significantly increased by approximately one day in cultivar Naofén (Table 2). This increase in developmental time was accounted for by a significant increase in egg–larval developmental time, whereas pupal developmental time remained not significantly different between cultivars (Table 2). No other variables, such as survival or body mass, were affected by the wheat cultivars.

Aphids and Parasitoid Performance in Artificial Diets. MRGR of the aphid significantly decreased as DIMBOA-glc concentration increased (Table 3). In the same way as for the experiment with plants, data from both parasitoid sexes were pooled, based on nonsignificant differences in sex ratio between treatments. A significant increase in total developmental time of the parasitoids was observed in DIMBOA-glc diets in relation to the control diet, although there was no significant difference between 2 and 4 mM DIMBOA-glc (Table 3). The increase in total developmental time was associated with a significant increase in the egg-larval developmental time of treatments containing DIMBOA-glc. There were no significant differences in pupal developmental time (Table 3). In addition, overall parasitoid survival was not significantly affected by DIMBOA-glc, but a partial reduction in egg–larval survival was detected as DIMBOA-glc concentration increased (Table 3). No further effects were detected in other variables of parasitoid performance.

TABLE 1. BEHAVIORAL EVENTS DURING *S. avenae* ACCEPTANCE PROCESS BY *A. rhopalosiphi*[a]

Wheat cultivar Hx level (mean ± SE, mmol/kg fresh wt)	*A. rhopalosiphi*			*S. avenae*		
	Encounter	Attack	Stab	Kick	Cornicle secretion	Body size mg
Huenufén (1.72 ± 0.12)	2.95(0, 34)a	0.91(0, 19)a	0.23(0.06)a	2.50(0.35)a	0.18(0.11)a	0.230(0.009)a
Naofén (3.02 ± 0.17)	2.45(0, 36)a	1.05(0, 14)a	(0.48(0.06)b	2.06(0.27)a	0.23(0.12)a	0.124(0.008)b

[a] Values (number of events) given are means; standard errors in parenthesis. Values in each column followed by the same letter are not significantly different according to the Wilcoxon matched-pair test ($\alpha = 0.05$). $N = 22$.

TABLE 2. EFFECT OF WHEAT CULTIVARS ON PERFORMANCE OF *S. avenae* AND *A. rhopalosiphi*[a]

Wheat cultivars Hx level (mean ± SE, mmol/kg fresh wt)	*S. avenae* MRGR (μg/μg/day)	*A. rhopalosiphi* development time (days)			*A. rhopalosiphi* survival (%)	*A. rhopalosiphi* body mass (mg)	*A. rhopalosiphi* secondary sex ratio (F/M)
		Total	Egg-larval	Pupal			
Huenufén (1.72 ± 0.12)	0.330a	8.95a	5.41a	3.55a	100a	0.0399a	0.48a
Naofén (3.02 ± 0.17)	0.262b	10.23b	6.64b	3.59a	100a	0.0363a	0.60a

[a]Values given are means. Values in the same column followed by the same letter are not significantly different according to the Kruskal-Wallis test (α = 0.05). Sample size: *S. avenae*, N = 21; *A. rhopalosiphi*, N = 25.

TABLE 3. EFFECT OF DIMBOA-glc IN ARTIFICIAL DIETS ON PERFORMANCE OF *S. avenae* AND *A. rhopalosiphi*[a]

DIMBOA-glc concentration	*S. avenae* MRGR (μg/μg/day)	*A. rhopalosiphi* development time (days)			*A. rhopalosiphi* survival (%)		*A. rhopalosiphi* body mass (mg)	*A. rhopalosiphi* secondary sex ratio (F/M)
		Total	Egg-larval	Pupal	Total	Egg-larval		
0 mM	0.194a	16.33a	9.01a	7.40a	65a	80a	0.0328a	0.58a
2 mM	0.128b	16.72ab	9.79ab	7.28a	60a	65b	0.0332a	0.5a
4 mM	0.105c	17.8b	10.46b	7.25a	50a	55b	0.0316a	0.55a

[a] Values given are means. Values in each column followed by the same letter are not significantly different according to the Kruskal-Wallis test ($P \leq 0.05$). Sample size, *S. avenae*, $N = 21$, and *A. rhopalosiphi*, $N = 40$.

DISCUSSION

Several studies have shown that different cereal species (Reed et al., 1992; Messina et al., 1995; Fuentes-Contreras et al., 1996) and cultivars (Kuo, 1984) may influence host suitability to natural enemies of aphids. Since Hx in wheat and other Poaceae exert a deleterious effect on aphid performance (Niemeyer and Pérez, 1995), they represent a potential mechanism to explain tritrophic effects involving natural enemies (e.g., Martos et al., 1992). In the present study, this negative effect on aphids was expressed in the reduction of MRGR, and consequently of body size, on the wheat cultivar with the higher Hx level. Aphid body size influences the success of aphid defensive reactions against attack by natural enemies (Gerling et al., 1990, Kouamé and Mackauer, 1991; Gross, 1993). Our results showed that aphids grown on wheat cultivar Naofén (high Hx level) were found and attacked by parasitoids at freqencies similar to aphids from the Huenufén cultivar (low Hx level). Likewise, aphids from both cultivars showed kicking and production of cornicle secretion with similar frequencies. However, the stabbing success of the parasitoids was lower in aphids from the susceptible cultivar Huenufén. This result may be tentatively explained by an increase in the success of avoidance of parasitoid stabbing in each kicking reaction of bigger aphids from the susceptible cultivar Huenufén, i.e., larger aphids would kick more effectively. Furthermore, these results are comparable to those of Campos et al. (1990), who also detected an increase in parasitization by *Diadegma terebrans* (Gravenhorst) on larvae of the European corn-borer reared in artificial diets containing Hx with respect to control diets (Campos et al., 1990).

An increase in total developmental time accounted for by an increase in egg–larval developmental time was observed in the high-Hx cultivar Naofén and in the artificial diet with highest Hx concentration. Concentrations of secondary metabolites in artificial diets should reflect physiological concentrations experienced by the aphid when feeding from sieve elements. Based on data from Givovich et al. (1994), it was possible to estimate that DIMBOA-glc concentrations in the phloem sap of the cultivars used here range from 0 to 4 mM. Since the range of DIMBOA-glc concentrations provided in the present artificial diets lies within the range mentioned above, results of the experiments with artificial diets substantiate those obtained with plants.

Aphid ingestion volumes are much lower from diets than from phloem sap (Klingauf, 1987). Thus, concentrations used in the artificial diets were probably exposing the parasitoids developing inside the aphids to rather low levels of Hx. However, higher Hx concentrations in diets produce antifeeding effects on aphids (Niemeyer et al., 1989) and hence preclude proper testing of the effect of the compound on parasitoid performance. Furthermore, higher concentrations of Hx in diets reduce aphid survival (Niemeyer and Peréz, 1995) and consequently would increase parasitoid mortality within the aphids. This effect could be responsible for the reduction in parasitoid survival during its egg–larval development. Development time of the parasitoid was reduced in comparable magnitude by DIMBOA-glc concentration in plants and in artificial diets (ca. 1 day). However, total development time of the parasitoid was much lower in the plants than in the diets, and hence in relative terms, the increase in development time in the plant is ca. 10%, while in the diets it is ca. 5%. This difference could be ascribed to the lower ingestion of the compound in diets.

In conclusion, the tritrophic effect of DIMBOA-glc on *A. rhopalosiphi* is confined to behavior, as an increase in successful stabs in aphids reared on wheat cultivars that have higher levels of the compound, and to life history traits, as a minor increase in development time. From a practical point of view, our results support the compatibility of biological control with an increase in Hx levels through breeding programs, as suggested by Campos et al. (1990) and Martos et al. (1992). Plants with higher Hx concentration would reduce aphid MRGR, thus increasing the proportion of smaller aphids in the population and facilitating parasitoid oviposition attacks. Moreover, the deleterious influence of Hx on overall survival of the parasitoid is not significant, and any potential reduction in parasitoid body size seems to be compensated by increased development time.

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