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Influence of wheat and oat cultivars on the development of the cereal aphid parasitoid *Aphidius rhopalosiphi* and the generalist aphid parasitoid *Ephedrus plagiator*

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Summary

The effects of three wheat cultivars and two oat cultivars on the development of the cereal aphid parasitoid *Aphidius rhopalosiphi* De Steph. and the generalist aphid parasitoid *Ephedrus plagiator* (Nees) (Hymenoptera: Braconidae) were evaluated in the laboratory. The level of hydroxamic acids, a family of secondary metabolites that can affect the mean relative growth rate of cereal aphids in cereals, were measured in the different cultivars. The parasitoids were reared in *Sitobion avenae* (F.) (Homoptera: Aphididae), using plants grown under greenhouse conditions. *A. rhopalosiphi* showed a longer developmental time on wheat relative to oat cultivars. This effect was accounted for by a significant increase in the time from oviposition to pupation (mummy formation), while the duration of the pupal stage remained constant between treatments. No further effects were observed in other variables evaluating *A. rhopalosiphi* performance, such as adult longevity, adult body weight and secondary sex ratio. The generalist *E. plagiator* did not show significant differences in any of the variables analysed, both between cultivars and cereal species. Hydroxamic acids levels correlated negatively with mean relative growth rates of *S. avenae*, but positively with the observed developmental time of *A. rhopalosiphi*. The results are discussed in terms of tritrophic effects and the development of breeding programmes trying to improve plant resistance to aphids.

Key words: Tritrophic interactions, biocontrol, *Aphidius, Sitobion*, plant resistance, wheat, hydroxamic acids

Introduction

Host-plant resistance can affect the natural enemies of pest aphids in several ways. In some situations resistant or partially resistant cultivars reduce the performance of aphid natural enemies (Kuo, 1986; Gowling, 1989), but in other cases they can act complementarily (Wyatt, 1970; Starks, Muniappan & Eikenbary, 1972; van Emden & Wratten, 1990). Growing evidence suggests that plant volatiles are involved in compatible or even synergistic effects between natural enemies and plant resistance (van Emden, 1984; Wickremansinghe & van

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Emden, 1992). In cereal systems, the effect on natural enemies of non-volatile allelochemicals which exhibit antibiotic and deterrent properties against cereal aphids is not yet clear. For instance, hydroxamic acids (Hx) in wheat and other Gramineae (= Poaceae) have been identified as resistance factors against cereal aphids (Niemeyer, 1988; Niemeyer & Pérez, 1995), and provide an interesting target for breeding programmes aimed at aphid resistance (Escobar & Niemeyer, 1993). Nevertheless, within integrated pest management programmes it is important to determine if increases in the plant Hx levels could also affect the action of the natural enemies of cereal aphids.

Moreover, it is likely that not all parasitoid species are affected in the same way and extent by plant allelochemicals, as has been recently proposed in relation to tritrophic interactions involving plant volatiles (Vet & Dicke, 1992).

The aim of the present study was to evaluate the influence of different wheat and oat cultivars, known to differ in their Hx levels, on the performance of two cereal aphid parasitoids (Hymenoptera: Braconidae) with different host ranges. *Aphidius rhopalosiphi* De Steph. is a specialist attacking aphids in cereal and grass systems, while *Ephedrus plagiator* (Nees) is a generalist with a wide aphid host range on a variety of host plant species (Starý, 1976).

Materials and Methods

Plant material and hydroxamic acids analysis

The experiments were performed using three wheat cultivars (Antilhue, Kona and T-1500) and two oat cultivars (Nehuen and Melys). Hx are absent in oat and are present at different levels in different wheat cultivars (Niemeyer, 1988). All the experiments were performed with eight-day old seedlings (GS 11) (Zadoks, Chang & Konzak, 1974), grown in a greenhouse at 28 ± 10°C, natural late-winter early-spring photoperiod (approximately L10:D14) and without supplementary lighting. Hx were analysed with reverse-phase high-performance liquid chromatography, as previously described by Weibull & Niemeyer (1995).

Stock cultures

Cultures of the cereal aphid *Sitobion avenae* (F.) and the parasitoids *A. rhopalosiphi* and *E. plagiator* were maintained on oat (cultivar: Melys), in order to avoid any exposure to Hx prior to the beginning of the trials. Both parasitoids were reared on *S. avenae*.

Mean Relative Growth Rate (MRGR)

In order to evaluate the effect of Hx on aphid performance, synchronised second instar nymphs (brown morph) from the stock culture were placed in clip-cages attached to the middle third of the primary leaf of the cereal seedlings (Martos, Givovich & Niemeyer, 1992). Since the Hx concentration in cereal seedlings changes significantly (Niemeyer, 1988) during the approximately 7 days of parasitoid larval development (Shirota, Carter, Rabbinge & Ankersmit, 1983), all seedlings were replaced every two days in both the MRGR and parasitoid performance experiments. MRGR values were determined as the difference between natural logarithms of final and initial weights divided by four (number of days for this experiment).

Table 1. *Mean relative growth rates (MRGR) and respective standard errors of* Sitobion avenae *reared in different wheat and oat cultivars. Values followed by different letters indicate significant differences according to nested ANOVA ($P < 0.05$). For all the cultivars* $n = 9$

	WHEAT			OAT	
	T-1500	ANTILHUE	KONA	MELYS	NEHUEN
MRGR	0.316	0.331	0.337	0.360	0.394
Standard Error	0.0089	0.0075	0.0082	0.0098	0.0155
	a	a	a	b	b

Parasitoid performance

The parasitoids were collected as mummies from oat plants in the stock culture and placed singly in small glass vials. Newly emerged parasitoids were sexed and mated overnight with access to diluted honey as a food. The mated females were experienced by allowing them to forage on aphid infested plants for one hour before being used in the trials. Synchronised aphids, as described for the MRGR evaluations, were parasitised by two or three-days-old females in gelatin capsules, each female being used only once. Potentially parasitised aphids were randomly assigned to the five cultivar treatments, using clip-cages similar to those used in the evaluation of MRGR. In order to maintain constant conditions, the experiments were transferred to a controlled environment chamber at 23 ± 1°C, 70 ± 10% r.h. and L16:D8 photoperiod. At least half of the aphids in each treatment were dissected on day four to estimate the actual oviposition and superparasitism percentages (Shirota *et al.*, 1983). The remaining aphids were reared until adult emergence, evaluating developmental time, pupal survival, longevity, adult dry mass and secondary sex ratio.

Results

Aphid performance

MRGR values for *S. avenae* (Table 1) were significantly affected by the plant species (ANOVA, $F = 26.309$, $P < 0.0004$). However, there were no significant differences in the aphid MRGR between cultivars nested within species (ANOVA, $F = 2.516$, $P = 0.072$, n.s.).

Parasitoid performance

The variables analysed for the generalist parasitoid *E. plagiator* were not significantly affected by the cereal species or cultivars (Table 2). In the case of the more specialist species *A. rhopalosiphi* (Table 3), there were statistically significant differences among cereal species in the developmental time (ANOVA, $F = 14.783$, $P < 0.0004$), but no significant differences were detected between cultivars nested within species (ANOVA, $F = 1.692$, $P = 0.177$). These differences in overall developmental time were mainly accounted for by significant effects of cereal species (ANOVA, $F = 5.2$, $P = 0.026$) and marginally non-significant effects of cultivars nested within species (ANOVA, $F = 2.542$, $P = 0.064$ n.s.) on the parasitoid larval developmental time, whilst the parasitoid pupal developmental time remained constant between species (ANOVA, $F = 1.164$, $P = 0.285$, n.s.) and cultivars (ANOVA, $F = 0.323$, $P = 0.808$, n.s.). The total parasitoid developmental time was negatively correlated with the

Table 2. *Influence of different wheat and oat cultivars on the performance of the generalist parasitoid* Ephedrus plagiator, *parasitising the cereal aphid* Sitobion avenae. *Different letters indicate statistically significant differences according to nested ANOVA ($P < 0.05$). The percentages and ratios were analysed with x^2 test ($P < 0.05$). For cultivars T-1500 and Melys n = 15, for cultivars Antilhue and Kona n = 13*

CULTIVAR	Hx levels (μg/g fw)	Total Developmental time (days)	Egg-larval Developmental times (days)	Pupal Developmental time (days)	Pupal Survival (%)	Longevity (days)	Adult Dry Mass (mg)	Secondary Sex Ratio (F/M)	Parasitism (%)	Superparasitism (%)
WHEAT										
T-1500	0.49 a	15.29 a	7.71 a	7.43 a	100 a	9.21 a	0.0519 a	0.28 a	100 a	0.0 a
ANTILHUE	0.32 b	14.64 a	7.5 a	7.14 a	93 a	10.64 a	0.0486 a	0.0 a	100 a	0.0 a
KONA	0.26 b	15.31 a	8.0 a	7.38 a	76.5 a	6.61 a	0.0439 a	0.15 a	100 a	0.0 a
OAT										
MELYS	0 c	14.85 a	7.54 a	7.31 a	86.7 a	9.07 a	0.0511 a	0.0 a	100 a	0.0 a

Table 3. *Influence of different wheat and oat cultivars on the performance of the generalist parasitoid* Aphidius rhopalosiphi, *parasitising the cereal aphid* Sitobion avenae. *Different letters indicate statistically significant differences according to nested ANOVA ($P < 0.05$). The percentages and ratios were analysed with x^2 test ($P < 0.05$). For cultivars n = 14*

CULTIVAR	Hx levels (μg/g fw)	Total Developmental time (days)	Egg-larval Developmental times (days)	Pupal Developmental time (days)	Pupal Survival (%)	Longevity (days)	Adult Dry Mass (mg)	Secondary Sex Ratio (F/M)	Parasitism (%)	Superparasitism (%)
WHEAT										
T-1500	0.49 a	12.92 a	8.0 a	4.93 a	93 a	5.36 a	0.0553 a	0.14 a	93 a	7.0 a
ANTILHUE	0.32 b	12.86 a	7.86 a	5.07 a	100	5.13 a	0.0596 a	0.07 a	79 a	7.0 a
KONA	0.26 b	12.50 a	7.50 a	5.00 a	93 a	5.23 a	0.0543 a	0.14 a	86 a	7.0 a
OAT										
MELYS	0 c	12.29 b	7.64 b	4.78 a	93 a	6.23 a	0.0660 a	0.14 a	93 a	7.0 a
NEHUEN	0 c	12.21 b	7.21 b	4.92 a	86 a	4.64 a	0.0561 a	0.07 a	86 a	7.0 a

aphid MRGR ($r = -0.89$, $P < 0.05$). No further effects were observed in other variables contributing to the performance of *A. rhopalosiphi.*

Hydroxamic acids analysis

Under greenhouse conditions the Hx levels in the three wheat cultivars were: Antilhue 0.32 ± 0.08 $\mu g\ g^{-1}$ fresh weight, T1500 0.49 ± 0.04 and Kona 0.26 ± 0.03 (x ± SE). The cultivar T-1500 showed an Hx concentration significantly higher than Kona and Antilhue (Kruskal-Wallis, $\chi^2 = 9.21$, $P < 0.01$).

The *S. avenae* MRGR values were significantly negatively correlated with the observed Hx levels in the plants utilised for the experiments ($r = -0.91$, $P < 0.05$), whilst the *A. rhopalosiphi* developmental time is positively correlated with the Hx levels in the plant ($r = 0.95$, $P < 0.05$).

Discussion

The detected increase in the developmental time of *A. rhopalosiphi* reared in aphids feeding on wheat relative to oat and the respective negative and positive correlations of *A. rhopalosiphi* developmental time with aphid MRGR and plant Hx level, suggest that a small tritrophic effect (about 5%) is operating on this specialist parasitoid species. This effect is probably of minor significance, because it only delays the parasitoid developmental time by less than one day and it does not affect parasitoid survival, longevity, adult dry mass or secondary sex ratio. Gowling (1989) working with the cereal aphid *Metopolophium dirhodum* (Walker) and *A. rhopalosiphi*, found that on the more resistant cultivar NG Avalon, the number of mature eggs at adult emergence and the weight of the adult female parasitoid were significantly lower than on the more susceptible cultivar Armada. They also detected a slight increase in the parasitoid developmental time on the partially resistant cultivar NG Avalon. However, in the study of Gowling (1989) the aphids were reared continuously on the different wheat cultivars. This fact, probably contributed to the greater tritrophic effects detected on parasitoids, because the aphids probably differed in size already at the beginning of the parasitoid development. In our experiments the parasitoids were exposed to the tritrophic effect in "standardised" aphids, which had not been exposed to wheat, and consequently to Hx.

Recently Åhman & Johanson (1994) suggested that Hx levels are strongly affected by light intensity, showing lower levels under greenhouse conditions than in laboratory experiments. In fact, the values detected in this study were significantly lower than previously observed levels for the same wheat cultivars when grown in growth chambers (J E Fuentes-Contreras, unpublished data). Under summer field conditions, Hx levels in seedlings and flag leaves are usually at the range detected in our experiments (Leszczynski, Lawrence & Bakowski, 1989; Åhman & Johanson, 1994), and in this situation these metabolites could be partially responsible for the observed reduction in the aphid MRGR as suggested by the correlation found in our experiments and those previously reported by Leszczynski *et al.* (1989) under field conditions.

At least under the conditions of this study direct tritrophic effects of Hx on parasitoids are unimportant, but a consistent reduction in the aphid MRGR is maintained, which gives support to propositions of non-antagonisms between plant resistance and biocontrol in integrated pest management systems. Moreover, Wellings & Ward (1994) have drawn attention to the benefits of partial rather than complete plant resistance in integrated pest management systems, proposing that the latter scenario could impose a strong selective pressure on pests comparable to that caused by synthetic insecticides.

Previous work with Hx tritrophic interactions has been reported by Martos *et al.* (1992), who found a biphasic relationship between the performance of a coccinellid predator and Hx levels in different wheat and oat cultivars used to feed the aphid prey. Those aphids fed on wheat cultivars with low or high-Hx levels led to a significantly shorter developmental time of the predator, as compared with intermediate Hx cultivars, suggesting that higher Hx levels in cultivars could potentiate biological control through minimisation of the antibiotic effect on the predator (Martos *et al.*, 1992; Escobar & Niemeyer, 1993). However, in contrast to predators, parasitoids are absolutely dependent on the fate of just one host individual, thus the continuous antifeeding effect in the high-Hx cultivars may eventually cause reduced aphid growth or starvation, affecting their quality as hosts for parasitoid development. Such a deleterious tritrophic effect has been detected by Campos *et al.* (1990), who reported that increasing Hx concentration in artificial diets for the European corn borer, reduced the adult weight of the parasitoid *Diadegma terebrans* (Gravenhorst) and increased its developmental time. Unfortunately, in the present work Hx levels in the greenhouse grown seedlings did not show an intermediate level and hence an eventual biphasic or linear relationship between Hx levels and parasitoid performance could not be demonstrated.

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