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## Influence of plant resistance at the third trophic level: interactions between parasitoids and entomopathogenic fungi of cereal aphids

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**Abstract** Host-plant resistance can affect herbivorous insects and their natural enemies such as parasitoids and entomopathogenic fungi. This tritrophic effect acts on interspecific interactions between the two groups of natural enemies distantly related in phylogenetic terms. The intra- and extra-host aspects of the interaction between the cereal aphid parasitoid *Aphidius rhopalosiphii* and the entomopathogenic fungus *Erynia neoaphidis* developing on the grain aphid, *Sitobion avenae*, on resistant and susceptible wheat (*Triticum aestivum*) cultivars, were studied. The competitive outcome of the intra-host interaction depended on the timing of parasitoid oviposition and fungal infection and was affected by wheat resistance. In particular, survival of the parasitoid was lower on the resistant wheat cultivar than the susceptible wheat cultivar, when the competitive outcome of the interaction was favourable for either parasitoid or fungal development. Before and after this period the influence of plant resistance was not significant. Furthermore, the extra-host interaction was not affected by the wheat cultivar, although an increase in fungal infection of *S. avenae* was observed when parasitoids foraged in the experimental arena with sporulating aphid cadavers compared with foraging in the absence of sporulating cadavers. Our results showed that the host plant may affect interspecific interactions between parasitoids and fungi and that these interactions depended on the timing of parasitoid oviposition and fungal infection.

**Key words** Tritrophic effects · Host-plant resistance · Parasitoid · Entomopathogenic fungi · Competition

### Introduction

Host plant attributes, such as spatial and temporal distribution, architecture, and resistance against herbivorous insects, can affect organisms at the third trophic level (Price et al. 1980). Host-plant resistance, based on secondary metabolites, constitutes a widely studied mechanism of such “tritrophic” interactions (Hare 1992 and references therein). Research efforts in this field have focused on the evaluation of the effect of plant resistance on single species of natural enemies (e.g. Barbosa et al. 1991; English-Loeb et al. 1993; Osier et al. 1996). However, no attention has been devoted to the study of the potential influence of host plant resistance on interspecific interactions between species at the third trophic level.

Natural enemies include predators, parasitoids, and pathogens such as viruses, bacteria and fungi. Entomopathogenic fungi are a common cause of mortality in herbivorous insects (Hajek and St. Leger 1994) and, although phylogenetically very distant from insects, they are often regarded as functional members of the parasitoid guild (Eggleton and Gaston 1990). Furthermore, they have been mentioned as likely to establish “interkingdom” competition with parasitoids, i.e. an interaction where species belonging to different kingdoms contend for the same resource (Hochberg and Lawton 1990). Entomopathogenic fungi and parasitoids, with vastly different life cycles, may be affected in different ways or to different extents by host-plant resistance, and hence interspecific interactions between them may also be influenced (Dickson and Whitham 1996).

Hochberg (1991a,b) studied the competitive interaction between parasitoids and pathogens, specifically viruses, emphasising the mechanisms of the interaction. He defined two potential interactions: (1) intra-

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host interactions, during ontogenetic development of the natural enemies within a shared host; and (2) extra-host interactions, which take place outside the host and involve eventual transmission of the pathogen by the parasitoids. Intra-host interactions between parasitoids and fungi are asymmetrical and determined by the timing of parasitoid oviposition and fungal infection (Powell et al. 1986; Fransen and van Lenteren 1993). The fungus usually outcompetes the parasitoid unless the development of the latter begins several days before fungal infection. The effect of the relative timing of the attacks of natural enemies is known as the priority effect (Powell et al. 1986; Fransen and van Lenteren 1993). In relation to extra-host interactions, entomopathogenic fungi are dependent upon abiotic (e.g. wind and rain) and biotic (e.g. co-occurring insects) agents for their dispersal (Wilding 1970; Hajek and St. Leger 1994; Steinkraus et al. 1996; Pell et al. 1997). There are examples in the literature where parasitoids acted as passive vectors of entomopathogenic fungi to host populations during foraging (Poprawski et al. 1992), but there are also examples where this did not occur (Akalach et al. 1992; Furlong and Pell 1996). Parasitoids and fungi may exert negative effects on each other at the intra-host scale, but at the extra-host scale, parasitoids may aid dispersal of fungi to new host populations. An examination of both aspects is needed, therefore, to appreciate fully the net effect of the interaction between these species (Hochberg 1991a,b).

The commonest species of parasitoid and entomopathogenic fungus in cereal fields are respectively *Aphidius rhopalosiphi* De Stephani-Perez (Hymenoptera: Braconidae) and *Erynia neoaphidis* Remaudière et Hennebert (Zygomycetes: Entomophthorales). Both *A. rhopalosiphi* and *E. neoaphidis* are important components of the natural enemy complex of cereal aphids on cereal crops, coexisting spatially and temporally during nearly half of the growing season (Powell et al. 1986; Wratten and Powell 1991). In the field, negative correlations between parasitoid and fungal abundance suggest potential antagonistic or competitive interactions between them during the growing season (Powell et al. 1986). In laboratory studies it was shown that the parasitoid only reached the adult stage if oviposition had occurred at least 4 days before fungal infection (Powell et al. 1986), and that parasitoids continued to oviposit in infected aphids until one day prior to fungus-induced death (Brobyn et al. 1988). Although competition of this type has never been tested experimentally in the field, cereal aphid populations fluctuate from low levels to pest status within and between growing seasons (Carter 1994) and, under circumstances of low aphid density and high parasitoid and/or fungus densities, aphids are likely to become a limiting resource such that competition between *A. rhopalosiphi* and *E. neoaphidis* could become important. For instance, from June to July in the United Kingdom, aphid populations decrease in abundance in early sown cereals (Vorley and Wratten 1985) at a time

when both parasitoids and fungi co-exist in large populations in the same fields (Powell et al. 1986).

Wheat resistance is mainly based on hydroxamic acids (Hx), a family of secondary metabolites with antibiotic and deterrent activity against cereal aphids (Niemeyer and Pérez 1995). Resistant cultivars, although able to reduce population growth rates of aphids compared to susceptible wheat cultivars, still show considerable aphid density under field conditions (Leszczynski et al. 1989; Gianoli et al. 1996). Hence, both resistant and susceptible wheat cultivars harbour cereal aphid populations which differ in density, and eventually in their quality as hosts for parasitoids and fungi. In experiments where aphids were grown on artificial diets containing Hx not only was there reduced relative growth rates of aphids but also an increased developmental time of parasitoids (Fuentes-Contreras and Niemeyer 1998) and predators (Martos et al. 1992). The effect of Hx against several phytopathogenic fungi is variable but seems to be species-specific for different fungi on wheat (Weibull and Niemeyer 1995).

The aim of this study was to evaluate the influence of plant resistance on interspecific interactions between phylogenetically distant natural enemies, namely the parasitoid *A. rhopalosiphi* and the entomopathogenic fungus *E. neoaphidis*. We hypothesise that these species, belonging to different kingdoms, will be affected to different extents by plant resistance, which in turn, may influence the interspecific interactions between them. Intra- and extra-host aspects of the interspecific interaction between *A. rhopalosiphi* and *E. neoaphidis* were studied, specifically: (1) the influence of plant resistance on the performance of the fungus, (2) the influence of plant resistance on the priority required by the parasitoid to complete its development in hosts prior to infection by fungi, and (3) the influence of plant resistance on the role of the parasitoid as a fungal vector.

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## Materials and methods

### Natural history of the species studied

*A. rhopalosiphi* is a koinobiont, solitary parasitoid of cereal aphids. A single egg is oviposited within the aphid host. At 20°C the egg hatches after 4 days and after a further 3 days the larva has consumed all internal tissues and pupates within the cuticle of the aphid forming a characteristic "mummy". Adult eclosion occurs 7 days after pupation. *E. neoaphidis* is an entomophthoralean fungus which infects aphids from numerous different genera (Wilding and Brady 1984). Infective conidia (spores) adhere to the aphid and penetrate the cuticle directly without needing to be ingested. Once inside the host the fungus develops in the haemolymph, then rapidly invades all the tissues, killing the host within 4–6 days at 20°C (Butt et al. 1990). After host death, the fungus emerges and sporulates, actively discharging more infective conidia.

### Insect, plant and fungus cultures

A polyclonal culture of the cereal aphid *Sitobion avenae* (Fabricius), collected in wheat fields near Santiago (Chile) was maintained

on oat (*Avena sativa* L., cv. Nehuén) at  $22 \pm 5^\circ\text{C}$  in a 16:8 h light-dark photoperiod. Stock cultures of the parasitoid *A. rhopalosiphi*, also collected in wheat fields near Santiago, were maintained on *S. avenae* reared on the same oat cultivar under the same environmental conditions. Two spring wheat (*T. aestivum* L.) cultivars, Huenufén (susceptible) with a low Hx level ( $\bar{x} = 1.72 \pm 0.12 \text{ mmol kg}^{-1}$  fresh weight in primary leaves of 6-day-old seedlings,  $n = 6$ ) and Naofén (partially resistant) with a high Hx level ( $\bar{x} = 3.02 \pm 0.17 \text{ mmol kg}^{-1}$  fresh weight in primary leaves of 6-day-old seedlings,  $n = 6$ ), were used in all experiments. The fungus, *E. neoaphidis*, was collected from *Acyrtosiphon pisum* Harris aphids on broad beans near Chillán (Chile) and passaged through five generations of *S. avenae* in the laboratory before in vitro isolation onto SEMA (Sabouraud dextrose agar supplemented with egg yolk and milk, Wilding and Brobyn 1980) nutrient agar in 9-cm sterile Petri dishes. At  $20^\circ\text{C}$  fungal growth had filled the plates within 25 days, after which it was grown on in YEMG (yeast extract, semi-skimmed milk and glucose, Pell et al. 1993) liquid media in shake flasks for use in experiments. Mycelium grown in liquid culture was rinsed in water to remove nutrients and harvested by suction filtration to produce uniform mats. Mats were held at  $5^\circ\text{C}$  and 100% relative humidity (RH) overnight, then transferred to  $20^\circ\text{C}$  to encourage the production of conidia. Conidia produced in this way could be used to inoculate aphids for experiments.

#### Virulence bioassay and evaluation of lethal concentrations of the fungus

Lethal concentrations (LC) were estimated using a modified version of the Wilding (1976) spore shower bioassay. Second instar nymphs were placed onto oat leaf sections embedded in 2% water agar in Petri dishes (40 mm diameter). There were ten aphids in each dish. After the aphids had settled each Petri dish was gently placed under an inverted mat of sporulating fungus and inoculated with conidia for 5, 20, 80 or 320 min. There were five independent replicates for each time. All dishes were maintained at 100% RH during inoculation to ensure sporulation proceeded. The concentration of conidia that the aphids received in each dish was evaluated by counting the number of conidia  $\text{mm}^{-2}$  on coverslips (20 mm diameter) placed alongside the oat leaves during inoculation, as described by Furlong and Pell (1996).

After inoculation, aphids were transferred to oat plants maintained inside air-tight cages covered by plastic film to maintain a high humidity. After 24 h the plastic film was replaced with muslin to allow ventilation. Inoculated aphids were incubated in a growth chamber at  $20 \pm 10^\circ\text{C}$  and 16:8 h light-dark photoperiod, and mortality due to fungus (evaluated as aphid cadavers able to produce a detectable number of conidia) recorded daily for 6 days. Three additional dishes were treated in the same way but were not exposed to conidia and served as controls. Aphid mortality caused by the fungus and conidia concentration were subjected to logit analysis to estimate  $\text{LC}_{50}$  and  $\text{LC}_{90}$ .

#### Effect of wheat resistance on performance of the fungi

Wheat cultivar (two resistance levels) was the independent variable, while fungus performance (survival, developmental time) were the dependent variables in this experiment. Second instar aphids were maintained on oat to obtain standardised individuals not previously exposed to wheat resistance. Groups of five aphids each were infected using the spore-shower bioassay already described for a time calculated to provide an  $\text{LC}_{90}$  deposition of conidia. Each fungal mat was used only once to achieve fully independent replicates. Conidia were counted as previously described and those replications that showed values outside the 95% confidence interval for  $\text{LC}_{90}$  were discarded and the process repeated until there were ten replicates per treatment. Inoculated aphids were maintained in a growth chamber at  $25 \pm 1^\circ\text{C}$ , 100% RH in a 16:8 h light-dark photoperiod on oat for 24 h and then transferred to the two wheat treatments. Control aphids were subjected to the same manipulations except that the spore-shower was omitted.

Performance of fungi was evaluated by fungus induced mortality in inoculated aphids (i.e. the proportion of aphids dying during the assay from which the fungus successfully sporulated), and by the average developmental time of individuals that successfully reached the adult stage in each experimental group. Such a group average was regarded as the experimental unit for developmental time. Fungus success was measured as the proportion sporulating cadavers and arcsin-square root transformed to satisfy the assumptions of normality and homogeneity of variances. These results were analysed by one-way ANOVA of wheat cultivar on survival and developmental time of the fungus.

#### Effect of wheat resistance on intra-host interactions

Wheat cultivar (two resistance levels) and the different periods between parasitoid oviposition and fungal infection (five priority effects) were independent variables, while parasitoid and fungus performance (survival, developmental time) were the dependent variables in this experiment. The priority effect was evaluated when groups of aphids were infected on: (1) the same day, (2) 3, (3) 4, (4) 5, and (5) 6 days after parasitoid oviposition.

The experimental unit comprised a group of five second-instar aphids. Five experimental units were used per treatment combination and controls. Aphids were individually parasitised as described by Fuentes-Contreras and Niemeyer (1998). Parasitisation was performed on the same day, each parasitoid female being allowed to oviposit just one egg in each of the five aphids of a given experimental unit. A set of six groups of five experimental units was assigned to each wheat cultivar. Five of these groups (treatments) were inoculated with an  $\text{LC}_{90}$  dose of conidia from freshly produced mycelial mats of the fungus either on the same day of parasitism or 3, 4, 5 or 6 days thereafter. The remaining group (parasitised control) was not inoculated with fungus. An additional group of five experimental unit was assigned to each wheat cultivar, and was inoculated with fungi without previous parasitisation (fungus control).

Performance of parasitoids and fungi was evaluated by survival (the proportion of aphids at the end of the experiment from which either a parasitoid emerged, the fungus sporulated or both successfully completed their development) and by averaged developmental time of parasitoids or fungi that successfully completed their development in each experimental unit. Survival data were arcsin-square root transformed to satisfy the assumptions of normality and homogeneity of variances. Results were analysed by two-way ANOVA (wheat cultivar and priority effect) for each dependent variable (survival, developmental time of parasitoid and fungus), and Tukey tests were performed when post hoc multiple comparisons were required.

#### Effect of wheat resistance on extra-host interactions

Wheat cultivar (resistance level) and parasitoid foraging were independent variables, while fungal infection was the dependent variable in this experiment. Aphid colonies were maintained in the two wheat cultivars for at least five generations before the beginning of the experiments. Second instar aphids were allowed to settle in 60 Petri dishes (20 aphids per dish) containing wheat leaves of their respective cultivar embedded in 2% water agar. Aphid cadavers killed by *E. neoaphidis*, which were sporulating profusely, were placed individually in the centre of each of these Petri dishes. Single parasitoids were introduced in 30 of the dishes, while the remaining 30 dishes were maintained as controls without parasitoids. All dishes were placed at  $20^\circ\text{C}$  for 6 h, after which aphids from both treatments were transferred to clean dishes containing the appropriate wheat cultivars but no sporulating cadaver (15 dishes that had been foraged on by a parasitoid in the presence of a sporulating cadaver and 15 dishes that had been in the presence of a sporulating cadaver but had not been foraged on by a parasitoid).

for each wheat cultivar). The parasitoids were anaesthetised and transferred to another set of 30 dishes, each containing a further 20 second-instar nymphs on leaves of their respective wheat cultivar (15 dishes of each wheat cultivar). After 6 h of parasitoid foraging in the second set of Petri dishes, all aphids in these groups were transferred again to clean dishes of the appropriate wheat treatments. Aphid mortality due either to successful parasitoid or fungal development was subsequently recorded in all treatments and arcsin-square root transformed to satisfy the assumptions of normality and homogeneity of variances. Fungal infection was analysed by two-way ANOVA (wheat cultivar and parasitoid foraging), and Tukey tests were performed when post hoc multiple comparisons were required.

## Results

### Virulence bioassay and evaluation of lethal concentrations of the fungus

The results for the  $LC_{50}$  and  $LC_{90}$  values with estimated 95% confidence intervals were  $LC_{50} = 2.4116 \pm 0.29 \log \text{ conidia mm}^{-1}$  and  $LC_{90} = 3.489 \pm 0.5 \log \text{ conidia mm}^{-1}$ . The lethal concentrations approximately corresponded to  $LC_{50} = 47 \text{ min}$  and  $LC_{90} = 387 \text{ min}$  of exposure to the bioassay. No mortality due to fungus was observed in control replicates.

### Effect of wheat resistance on performance of the fungus

Fungal survival (Table 1) was higher at  $LC_{90}$  doses than at  $LC_{50}$  doses and the uninfected control (mean square lethal concentration = 1.283,  $F_{1,36} = 23.89$ ,  $P < 0.001$ ), but no effect of wheat cultivar (MS cultivar = 0.034,  $F_{1,36} = 0.64$ ,  $P = 0.43$ ) or significant interaction with lethal concentration was observed (MS interaction = 0.013,  $F_{1,36} = 0.25$ ,  $P = 0.62$ ) (Table 1). Developmental time (Table 1) was not affected either by lethal concentration (MS lethal concentration = 0.090,  $F_{1,36} = 1.41$ ,  $P = 0.24$ ), wheat cultivar (MS cultivar = 0.012,  $F_{1,36} = 0.19$ ,  $P = 0.67$ ) or the interaction between them (MS interaction = 0.002,  $F_{1,36} = 0.035$ ,  $P = 0.85$ ). No aphid mortality due to fungus was observed in the control treatments.

**Table 1** Effects of wheat cultivar and concentration of conidia on survival and developmental time of the fungus *Erynia neoaphidis*. Controls showed no fungus-induced mortality. Means with different letters represent significant differences following ANOVA. Survival was arcsin-square root transformed before one-way ANOVA ( $n = 10$ )

Wheat cultivar	Lethal Concentration	Survival (proportion)		Developmental time (days)	
		Mean	SE	Mean	SE
Huenufén (susceptible)	$LC_{50}$	0.46 a	0.06	3.41 a	0.09
	$LC_{90}$	0.74 b	0.04	3.52 a	0.07
Naofén (resistant)	$LC_{50}$	0.48 a	0.05	3.46 a	0.08
	$LC_{90}$	0.78 b	0.06	3.54 a	0.07

### Effect of wheat resistance on intra-host interactions

Parasitoid survival (Table 2) was affected significantly by the priority effect (MS priority = 3.891,  $F_{5,48} = 147.10$ ,  $P < 0.001$ ), but not by the wheat cultivars (MS cultivar = 0.037,  $F_{1,48} = 1.40$ ,  $P = 0.24$ ). However, a significant interaction between these variables was observed (MS interaction = 0.075,  $F_{5,48} = 2.82$ ,  $P = 0.03$ ). No parasitoid survival was observed when infection was applied the same day or 3 days after parasitoid oviposition. Parasitoid survival was significantly higher in the susceptible wheat cultivar Huenufén when infection was applied 4 days after parasitoid oviposition (Tukey test,  $P = 0.037$ ), but not when infection occurred 5 or 6 days after parasitoid oviposition (Tukey test  $P = 0.95$  for 5 days,  $P = 0.66$  for 6 days). Developmental time of the parasitoid was significantly higher in the resistant wheat cultivar Naofén (MS cultivar = 13.456,  $F_{1,32} = 89.93$ ,  $P < 0.001$ ). Priority effect (MS priority = 0.018,  $F_{3,32} = 0.12$ ,  $P = 0.95$ ) and its interaction with wheat cultivars (MS interaction = 0.065,  $F_{3,32} = 0.73$ ,  $P = 0.73$ ) did not significantly affect the developmental time of the parasitoid (Table 2).

Fungal survival (Table 3) was not affected by wheat cultivar (MS cultivar = 0.086,  $F_{1,48} = 1.44$ ,  $P = 0.24$ ), but was significantly reduced by the priority effect of parasitoid oviposition (MS priority = 2.083,  $F_{5,48} = 34.75$ ,  $P < 0.001$ ), irrespective of wheat cultivar as shown by the non-significant interaction between these variables (MS interaction = 0.066,  $F_{5,48} = 1.10$ ,  $P = 0.37$ ). Developmental time of the fungus (Table 3)

**Table 2** Effects of wheat cultivar and priority effect of parasitoid oviposition on survival and developmental time of the parasitoid *Aphidius rhopalosiphi* in aphid hosts also infected by the fungus *Erynia neoaphidis*. Survival was arcsin-square root transformed before a two-way ANOVA. Different letters in the same column represent significant differences ( $P < 0.05$ , Tukey test following significant effects in the ANOVA,  $n = 5$ )

Wheat cultivar	Priority effect	Survival (proportion)		Developmental time <sup>a</sup> (days)	
		Mean	SE	Mean	SE
Huenufén (susceptible)	0	0 a	0	–	–
	3	0 a	0	–	–
	4	0.52 c	0.04	9.48 a	0.14
	5	0.86 d	0.05	9.38 a	0.21
	6	0.84 d	0.05	9.30 a	0.13
	Control	0.92 d	0.04	9.38 a	0.22
Naofén (resistant)	0	0 a	0	–	–
	3	0 a	0	–	–
	4	0.24 b	0.08	10.40 b	0.19
	5	0.78 d	0.04	10.60 b	0.17
	6	0.94 d	0.04	10.54 b	0.16
	Control	0.88 d	0.06	10.64 b	0.18

<sup>a</sup> No parasitoids completed their development in the treatments involving 0 and 3 days of priority effect of parasitoid oviposition. Hence, these treatments were not included in the analysis of the effect of cultivar and priority on developmental time of the parasitoid

**Table 3** Effects of wheat cultivar and priority effect of parasitoid oviposition on survival and developmental time of the fungus *E. neoaphidis* in aphid hosts also parasitised by *A. rhopalosiphii*. Survival was arcsin-square root transformed before two-way ANOVA. Different letters in the same column represent significant differences ( $P < 0.05$ , Tukey test following significant effects in the ANOVA,  $n = 5$ )

Wheat cultivar	Priority effect	Survival (proportion)		Developmental time <sup>a</sup> (days)	
		Mean	SE	Mean	SE
Huenufén (susceptible)	0	0.80 c	0.06	3.44 a	0.12
	3	0.72 bc	0.10	3.60 a	0.06
	4	0.48 bc	0.05	3.56 a	0.07
	5	0.22 ab	0.06	3.40	0.17
	6	0.02 a	0.02	–	–
	Control	0.84 c	0.07	3.62 a	0.06
Naofén (resistant)	0	0.72 bc	0.10	3.50 a	0.13
	3	0.74 c	0.09	3.48 a	0.13
	4	0.54 bc	0.05	3.60 a	0.06
	5	0.04 a	0.02	–	–
	6	0.04 a	0.02	–	–
	Control	0.75 c	0.19	3.42 a	0.15

<sup>a</sup> Few fungi completed development in the treatments involving 5 and 6 days of priority effect of parasitoid oviposition. Hence, these treatments were not included in the analysis of the effect of cultivar and priority on developmental time of the fungus

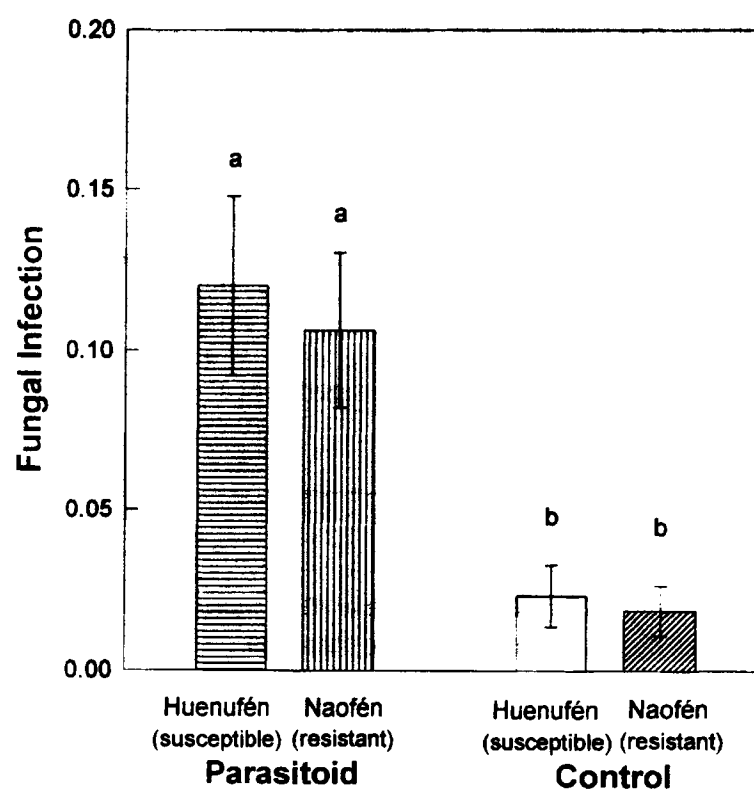
was not affected by wheat cultivar (MS cultivar = 0.016,  $F_{1,32} = 0.32$ ,  $P = 0.58$ ), priority effect (MS priority = 0.22,  $F_{3,32} = 0.43$ ,  $P = 0.73$ ), or the interaction between these variables (MS interaction = 0.27,  $F_{3,32} = 0.54$ ,  $P = 0.66$ ). Finally, when parasitoid oviposition was performed 4, 5 and 6 days before fungal infection, successful and simultaneous parasitoid and fungal development occurred on a low percentage of aphids (overall 4%).

#### Effect of wheat resistance on extra-host interactions

There were no significant differences in fungal infection between wheat treatments (MS cultivar = 0.0014,  $F_{1,58} = 0.05$ ,  $P = 0.82$ ) regardless of the presence of parasitoids in the experimental set-up (MS interaction = 0.0001,  $F_{1,58} = 0.01$ ,  $P = 0.96$ ). However, parasitoids were able to significantly increase fungal infection of aphids while foraging in Petri dishes containing a sporulating aphid cadaver (MS parasitoid = 0.6688,  $F_{1,58} = 23.97$ ,  $P < 0.001$ ) for both cultivars (Fig. 1). No fungal infection at all was obtained when the parasitoids were subsequently transferred to a second Petri dish without sporulating aphid cadavers.

#### Discussion

The results of our experiments showed that, depending on the timing of attack on the shared host, the host plant



**Fig. 1** Proportion of fungal infection in the presence of parasitoids and in the control without parasitoids. Data are means of the proportion of fungal infection and error bars represent  $\pm 1$  SE. Statistical differences were evaluated by two-way ANOVA of arcsin-square root transformed data. Significant differences are shown by different letters (Tukey test  $P < 0.05$ , following significant effects in the ANOVA)

can influence the outcome of intra-host interactions between parasitoids and fungi. Parasitoid survival was progressively reduced as the priority effect was lost, while the opposite was observed for the fungus (Table 2). When the interaction was not clearly defined in terms of which species would complete its development successfully, i.e. when fungal infection was 4 days after parasitoid oviposition, the survival of the parasitoid was significantly reduced in the resistant wheat cultivar (Table 2). In comparison, when the priority effect was favourable to the successful development of either the fungi or the parasitoid, i.e. less than 4 days of parasitoid advantage for fungal success or more than 5 days of parasitoid advantage for parasitoid success, this effect of the host-plant on the intra-host interaction between parasitoid and fungi was not significant (Table 2).

It has previously been shown that partially resistant wheat reduced aphid growth rate and increased parasitoid developmental time (Fuentes-Contreras et al. 1996, Fuentes-Contreras and Niemeyer 1998). The same effect was observed in this study in the control treatment and in parasitoids developing in shared hosts with the fungus (Table 2). The increase in developmental time of the parasitoid may increase the advantage necessary to reach the adult stage successfully (priority effect). Hence, this increase in developmental time of the parasitoid could be responsible for the observed reduction of parasitoid survival in hosts also infected by the fungi. In comparison, the fungus infecting alone or in shared hosts with the parasitoid was not affected by wheat

resistance (Table 3). Thus, differential susceptibility to wheat resistance based on Hx between parasitoids and fungi may account for the observed effect of wheat resistance on intra-host interactions between them.

Dispersal or transmission of the fungi by the parasitoid into a new experimental environment free of infective conidia did not occur. Since the parasitoid does not attempt to oviposit in sporulating aphids (Brobyn et al. 1988), it is unlikely that an infective conidium would reach the parasitoid, to be dispersed and subsequently infect other aphids. In addition, grooming behaviour is characteristic of many parasitoids and may further reduce their potential as effective fungal vectors. Only when foraging parasitoids were introduced into a conidia-containing environment was there a significant increase in fungal infection observed in aphids. In none of these experiments did wheat resistance have any significant influence. As previously described by Furlong and Pell (1996), the parasitoid would have increased aphid mobility and hence also increased the probability of conidia acquisition and therefore infection by the fungus. This increase in mobility may be caused by the release of alarm pheromone by aphids under attack by the parasitoid, which induces surrounding settled aphids to walk or to drop in order to leave the plant (Wientjens et al. 1973; Dill et al. 1990).

Theoretical analyses of host-parasitoid-pathogen dynamics have concluded that within-host competition may determine the outcome of competitive interactions between these natural enemies (Hochberg et al. 1990). Hence, the inclusion of further details of the parasitoid-fungus interaction in theoretical models, such as the influence of plant resistance on competitive hierarchies during intra-host interactions, may provide insights on how competitive mechanisms at the individual level could affect interactions at the population level (Hochberg et al. 1990).

Wheat cultivars used in this study were partially resistant and susceptible toward cereal aphids (Fuentes-Contreras and Niemeyer 1998), and are representative of the resistance levels commonly found in wheat sown in temperate areas. Inverse correlations between Hx levels (higher resistance) and population growth rates of aphids have been reported under field situations (Leszczynski et al. 1989; Gianoli et al. 1996). However, when weather conditions are favourable or pesticides are poorly applied, cereal aphids develop populations reaching pest status on susceptible as well as on partially resistant wheat cultivars (Carter 1994). Since aphids are present both in partially resistant and susceptible wheat fields and parasitoids and fungi are also commonly found co-existing spatially and temporally on wheat crops, we suggest that our laboratory results reflect interspecific interactions between parasitoids and fungi that are likely to occur in the field.

In a similar system, Dickson and Whitham (1996) reported that cottonwood (*Populus* spp.) resistance has significant influences on phylogenetically distant species of natural enemies (birds, insect predators and fungi) of

the leaf-galling aphid *Pemphigus betae* Doane under field conditions. In their experiment, the effect of cottonwood resistance on natural enemies occurred through an "interaction chain" (*sensu* Wootton 1994), i.e. plant resistance affected aphid abundance which in turn influenced natural enemies (Dickson and Whitham 1996). Our laboratory results showed that "interaction modifications" (*sensu* Wootton 1994), i.e. plant resistance affecting the interactions between parasitoids and fungus at the intra-host individual level, may also be potentially important for the aphid-parasitoid-fungus interaction.

In conclusion, at the intra-host scale *E. neoaphidis* has a competitive advantage based on its shorter developmental time, further enhanced by the reduction in *A. rhopalosiphi* survival on the resistant wheat when the outcome of the interaction is not yet defined. However, at the extra-host scale *E. neoaphidis* dispersal is not affected by the parasitoid or the resistance of the wheat cultivar. Such findings suggest that tritrophic interactions may not only affect single species of natural enemies, but also potentially their interspecific interactions.

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