Interacciones Indirectas Entre Especies del Tercer Nivel Trófico Mediadas por Metabolitos Secundarios de la Planta Hospedera

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A mi familia,

Pauli, María José y Carlita

A mis padres,

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INDICE DE MATERIAS

Niemeyer. Additive effects of wheat resistance and parasitoids on population dynamics of the cereal aphid *Sitobion avenae*.

This subchapter has been submitted to Ecological Entomology as Fuentes-Contreras E. & H.M. Niemeyer. Indirect effects of wheat resistance on interspecific interactions between a parasitoid and an entomopathogenic fungus of cereal aphids.

RESUMEN

El estudio de interacciones indirectas (i.e. efectos indirectos e interacciones de orden superior) ha sido enfocado analizando efectos entre especies dentro del mismo nivel trófico, efectos de especies de niveles tróficos superiores sobre especies de niveles tróficos inferiores y la acción conjunta de estos dos procesos. Sin embargo, aún no se ha enfatizado la eventual existencia de efectos ascendentes en términos relativos desde niveles tróficos inferiores sobre las interacciones entre especies de los niveles tróficos superiores.

En esta tesis se analizaron los efectos indirectos que podrían generar los metabolitos secundarios de las plantas, a través de los insectos herbívoros, sobre las interacciones interespecíficas que pueden establecerse entre organismos del tercer nivel trófico. Se seleccionaron especies del tercer nivel trófico alejadas filogenéticamente y que utilizan el mismo recurso en forma similar, ya que se ha postulado que debido a sus diferencias en ciclo de vida, es probable que sean afectadas en forma diferencial por los metabolitos secundarios de las plantas.

El sistema de estudio fue especialmente seleccionado para esta tesis y estuvo compuesto por cultivares de trigo (*Triticum aestivum* L.) que difieren en sus concentraciones de ácidos hidroxámicos (Hx), metabolitos secundarios que confieren resistencia a las plantas contra los áfidos de los cereales, el áfido

Sitobion avenae (F.) y como organismos del tercer nivel trófico alejados filogenéticamente el parasitoide *Aphidius rhopalosiphi* De Steph. y el hongo entomopatógeno *Erynia neoaphidis* Remaudière et Hennebert.

Los resultados obtenidos mostraron que el tiempo de desarrollo del parasitoide aumentó con el nivel de Hx en los cultivares de trigo. Por el contrario el hongo no fue afectado por la concentración de Hx en el trigo. La interacción competitiva dentro del mismo áfido entre parasitoide y hongo fue influenciada por el nivel de Hx en el cultivar de trigo dependiendo del estado de desarrollo del parasitoide. Solamente en aquel momento en que el resultado de la interacción entre parasitoide y hongo no está claramente definido, el nivel de Hx del trigo afectó negativamente la sobrevivencia del parasitoide favoreciendo el éxito competitivo del hongo. La dispersión del hongo y la probabilidad de infección del hongo en presencia del parasitoide no fueron afectadas por el nivel de Hx del cultivar de trigo.

A nivel poblacional se observaron efectos indirectos del trigo, asociados a los niveles de Hx, sobre la interacción interespecífica entre parasitoide y hongo. Estos efectos indirectos estaban compuestos por cadenas de interacción y modificaciones de interacción. Este estudio demuestra el potencial impacto de las interacciones indirectas ascendentes, al afectar diferencialmente el desempeño de las especies interactuantes en el tercer nivel trófico, e influir así indirectamente sobre el resultado de la interacción competitiva entre enemigos naturales alejados filogenéticamente.

ABSTRACT

The study of indirect interactions (i.e. indirect effects and higher order interactions) has been addressed through the analysis of effects between species within the same trophic level, effects of species of upper trophic levels on species of lower trophic levels, and the joint operation of these two processes. However, the possible existence of ascending effects in relative terms from lower trophic levels on the interactions between species at upper trophic levels has not been emphasised.

In this thesis the indirect effects of secondary metabolites from plants on the interspecific interactions between organisms of the third trophic level were studied. Phylogenetically distant species from the third trophic level using the same resources in a similar way were selected since differences in their life cycle were likely to result in different tritrophic effects from secondary plant metabolites.

The study system was particularly selected for this thesis and was composed by wheat cultivars (*Triticum aestivum* L.), which differ in their concentrations of hydroxamic acids (Hx), secondary plant metabolites known to confer resistance against cereal aphids, the cereal aphid *Sitobion avenae* (F.), and the parasitoid *Aphidius rhopalosiphi* De Steph. and the entomopathogenic fungus *Erynia neoaphidis*.

The results obtained showed that Hx in resistant wheat cultivars increased developmental time of the parasitoid. On the contrary, the fungus was not affected by wheat resistance. The competitive interaction within the same aphid host between the parasitoid and the fungus was affected by Hx levels in wheat depending on the developmental stage of the parasitoid. Only when the competitive outcome of the interaction between parasitoid and fungus was not defined, Hx levels in wheat reduced the parasitoid survival and favoured fungal successful development. Fungal dispersal and probability of fungal infection mediated by the parasitoid were not affected by wheat resistance.

Indirect effects of wheat, associated to Hx levels, on interspecific interactions between parasitoid and fungus were also observed at the population level. These indirect effects were composed by both interaction chains and interaction modifications. This thesis demonstrates the potential impact of ascending indirect interactions on the performance of species at the third trophic level, and in turn indirectly on the outcome of interspecific interactions between phylogenetically distant species of natural enemies.

1. INTRODUCTION

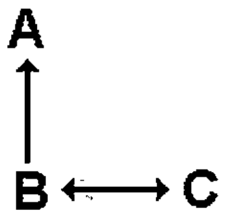
Assemblages of species have been initially studied under the assumption of additive interactions between species (May 1973), *i.e.* that the outcome of interactions in multi-species systems could be predicted from interaction between pairs of species. Such approach was based on the empirical estimation of: i) the parameters of logistic population growth, and ii) the interaction coefficients between all possible combinations of pairs of species under study. These values would produce a community matrix able to predict the dynamics and properties of multi-species systems at equilibrium (Vandermeer 1970, May 1973). However, empirical evidence supporting the existence of non-linear density-dependence (Ayala *et al.* 1973) and non-additive effects in experimental laboratory systems questioned the utility of this approach (Wilbur 1972, Neill 1974, Table 1). Although there is also empirical evidence supporting at least on qualitative basis the predictions of the community matrix approach (Vandermeer 1969, Pomerantz 1981, Gilpin *et al.* 1986), the empirical and theoretical relevance of non-additive effects and indirect interactions is widely recognised at present, hence precluding the utilisation of a reductionist approach to study interspecific interactions in multi-species systems (Wilbur & Fauth 1990, Strauss 1991, Wootton 1993, Billick & Case 1994, Wootton 1994b, Abrams 1995).

1.1 Interaction chains and interaction modifications in ecological communities

Interspecific interactions can be direct, where physical contact of any sort between interacting species is required (*e.g.* predation, interference competition, some types of mutualisms), or can also be indirect, where the interaction between two focal species occurs through changes in other species present in the system (*e.g.* exploitative competition whenever the shared resource is another species of the system, apparent competition, etc.) (Wootton 1994a). Indirect interactions have been classified into two main groups: i) **interaction chains** (I.C.), also known as indirect effects and ii) **interaction modifications** (I.M.), also known as higher order interactions (Wootton 1993).

Interaction chains group all indirect interactions mediated by changes in the density of a third species which affect the density of the two focal interacting species (Figure 1.a), while interaction modifications refer to changes caused by a third species on the *per capita* (individual) interspecific effect of the direct interaction established between focal species (Figure 1 b). These definitions clarify that interaction chains are just epiphenomena resulting from the successive addition of direct effects between species pairs. On the contrary, interaction modifications are the non-additive component precluding the extrapolation of the dynamics of interactions in multi-species systems from the knowledge of dynamics of pairs of species (Billick & Case 1994).

a) *INTERACTION CHAIN*



b) *INTERACTION MODIFICATION*

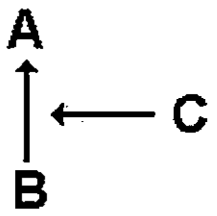


Figure 1. Scheme of species interactions representing a) interaction chain and b) interaction modification. A, B = focal species; C = third species. Figure modified from Wootton (1993).

Although I.C. are widely acknowledged as relevant interactions in several ecological systems (*e.g.* Wilbur & Fauth 1990, Strauss 1991, Wootton 1993, Billick & Case 1994, Wootton 1994b, Abrams 1995), it is empirically very difficulty to distinguish the relative importance of I.C. and I.M. This situation is particularly relevant in ecological systems where densities of focal species change during the experiment. At present there is no formal protocol to disentangle such relative contributions (Billick & Case 1994), or to evaluate their joint effect in relation to direct interactions (Abrams 1995, Smith *et al.* 1997).

Billick & Case (1994) propose a statistical test of the interaction between principal effects of an ANOVA to evidence the presence of indirect interactions. When the *per capita* population growth rate of the focal species is used as the dependent variable to test the statistical significance of the interaction term in the ANOVA, an I.C. can be postulated. However, such analysis should not be restricted to the detection of statistically significant interactions, but also to the study of mechanisms eventually responsible of the potential interaction modification (Kareiva 1994). Moreover, since detailed mechanistic studies allow the evaluation and manipulation of individual responses of the focal species in the presence and absence of a third species, they seem to be the most direct way to qualitatively distinguish the presence of I.C. or I.M. (Werner 1992, Adler & Morris 1994). Unfortunately, only few studies have been able to detect I.C. and provide experimental support of a mechanistic basis to explain their results (Wooton 1994a).

1.2 Top-down and bottom-up forces in species assemblages and the importance of indirect interactions

The relative importance of top-down and bottom-up forces on the structure and dynamics of natural populations and communities has been a long term subject for discussion in ecology (*e.g.* Hairston *et al.* 1960, Menge & Sutherland 1976). At present there is consensus about the simultaneous action of both processes in natural communities (Leibold 1989, Hunter & Price 1992, Menge 1992, Power 1992, Strong 1992), and subsequent research has been focused upon the factors affecting the expression and transmission of top-down and bottom-up influences in the trophic web (Morris 1992, Preszler & Boecklen 1996)

Connections within and between trophic levels are based on interspecific interactions. Since indirect interactions involve more than two species they constitute important contributions to the propagation of indirect influences. Studies explicitly addressing this issue have focused on predation and competition within and between trophic levels (Figure 2) and have been reviewed by Strauss (1991) and Wootton (1994b). Table 1 shows a selective review of empirical studies addressing indirect effects as a main topic. It can be seen that studies have been performed on vertebrates and invertebrates, including several aquatic and terrestrial habitats. Experiments ranging from controlled laboratory conditions to field exclusions have been used apparently depending on body size and longevity of the organisms under study. Microrganisms and insects seem to be preferred for laboratory studies, while vertebrates in aquatic systems are frequently studied in water tanks under semicontrolled conditions. Field studies are performed with intertidal organisms, insects on plants and small vertebrates.

Table 1. Selective review of empirical studies regarding indirect effects. Interactions are competition (C), predation (P), mutualism (M). Indirect effects are interaction modification (I.M.) and interaction chain (I.C.) and "none" when they were not observed. Types of studies were classified as performed under laboratory conditions (L), semicontrolled water tank or greenhouse conditions (SC) and field conditions (F).

Taxa	Interaction	Indirect Effects	Type	Reference
amphibians	C,P	I.C.	SC	Wilbur (1972)
microcrustaceans	C	I.M.	L	Neill (1974)
hydras	C	I.M.	L	Case & Bender (1981)
herbs/ simulated herbivory	C, P	none	L	Fowler & Rausher (1985)
aquatic insects, amphibians	C	I.M./I.C.	SC	Morin *et al.* (1988)
amphibians, Odonata naiads	P	none	SC	Van Buskirk (1988)
fishes, crustaceans	C, P	I.M.	SC	Martin *et al.* (1989)
fishes, amphibians, crustacenas	P	I.M.	SC	Huang & Sih (1990)
amphibians, Odonata naiads	C, P	I.M./I.C.	SC	Wilbur & Fauth (1990)
fishes, zooplancton	P	I.M./I.C.	SC	Turner & Mittelbach (1990)
Drosophila spp. (Diptera)	C	I.C.	L	Worthen & Moore (1991)
fishes, amphibians, isopods	P	I.M.	SC	Huang & Sih (1991)
aphids, flea-beetles, chrysopids	P	none	F	Morris (1992)
spider-mites	C	I.C.	F	English-Loeb & Karban (1993)
protists	C,P	I.C.	L	Lawler (1993)
amphibians, Odonata naiads	P	I.M.	SC	Wissinger & McGrady (1993)
aphids, ants, flea-beetles	M,C,P	I.C.	F	Floate & Whitham (1994)
intertidal invertebrates, birds	C,P	I.M.	F	Wooton (1993, 1994a)
mantids, mirid bugs	C,P	I.C.	F	Fagan & Hurd (1994)
parasitoids, weevils, crucifer	P	I.C.	F	Gómez & Zamora (1995)
aphids, arthropods, fungi, birds	C,P	I.C.	F	Dickson & Whitham (1996)
parasitoids, weevils, aphids	P	I.M.	F	Evans & England (1996)
parasitoids, coccinellid, aphids	P	I.M.	F	Ferguson & Stiling (1996)
amphibians, Odonata naiads	P	I.M:	SC	Peacor & Werner (1997)
fish, aquatic insects	C,P	I.M.	SC	Diehl (1997)
herbs, rodents	C,P	none	F	Smith *et al.* (1997)
parasitoids, coccinellids, aphids	P	I.C.	F	Müller & Godfray (1997)

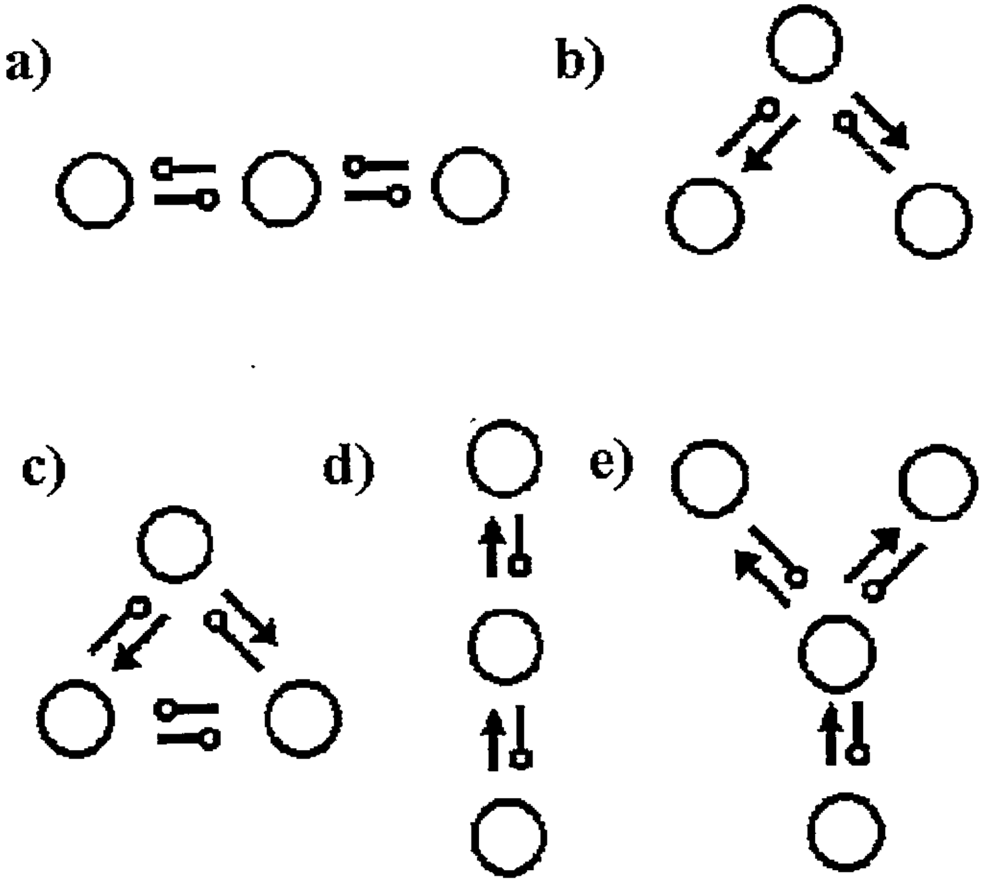


Figure 2. Interspecific interactions involving: a) competitive interactions within the same trophic level (*e.g.*, Levine 1976, Lawlor 1979), b) predation from adjacent trophic levels (*e.g.* Paine 1966, Holt 1977, c) competition and/or predation between several trophic levels (Figure 1.c) (*e.g.* Lubchenco 1978, Paine 1980, Kotler & Holt 1989), d) tritrophic interactions (*e.g.* Barbosa *et al.* 1991, Stamp *et al.* 1991) and e) tritrophic interactions affecting exploitative competition between species at the third trophic level (this thesis).

1.3 Tritrophic effects and inter-kingdom competition

An example of indirect interactions are the "tritrophic" effects (Price *et al.* 1980) widely described in insect-plant interactions (Figure 2.d.) (Miller & Kerfoot 1987), where host plants may have an impact on populations of herbivores and ultimately on their natural enemies (Hunter & Price 1992). The architecture (Kareiva & Sahakian 1990) and secondary metabolites of the host plant have been suggested as important factors behind these interactions (Price *et al.* 1980, Hare 1992). For instance, host-plant species or genotypes which differing in their content of secondary metabolites, have been demonstrated to influence the physiological state, behaviour, survival and reproduction of herbivorous insects, which in turn affect the performance of their microparasites, parasitoids and predators (Price *et al.* 1980, Barbosa & Letorneau 1988, Hare 1992). However, all these studies have considered only isolated species (*i.e.* non-interacting) of the third trophic level (*e.g.* Barbosa *et al.* 1991, Stamp *et al.* 1991, Marvier 1996), remaining unexplored the potential influence of secondary metabolites on interspecific the interactions at the third trophic level (Figure 2.e.).

Although there are only few examples in the literature about the participation of secondary metabolites as mediators of interspecific interactions (*e.g.* Faeth 1986, Karban *et al.* 1987; Krischick *et al.* 1991, Bultman *et al.* 1997), it has been mentioned that chemical signals may be of major importance for indirect interactions (Miller & Kerfoot 1987).

Hochberg & Lawton (1990) have proposed that natural enemies used in biological control of herbivores in agricultural ecosystems, including microparasites (viruses, bacteria and fungi), macroparasites (nematodes), parasitoids and predators (arthropods), represent an ecological system where "competition between phylogeneticaly distant taxa" (in particular kingdoms) seems to be as common as in benthic intertidal systems (Woodin & Jackson 1979). It is indeed with such organisms belonging to distantly related taxa where it seems more probable to detect indirect interactions mediated by secondary metabolites (Karban *et al.* 1987). In particular, parasitoids and entomopathogenic fungi exhibit vastly different life cycles exploiting the same resource in a similar way (Eggleton & Gaston 1990).

1.4 Tritrophic effects and the system under study

Tritrophic effects involving aphids and their natural enemies have been previously reviewed by van Emden (1984, 1995) and van Emden & Wratten (1990). These reviews are focused on the integration of biological control and crop resistance within strategies of integrated management of aphid pests. Among the several examples discussed, partial wheat resistance and the action of parasitoids are mentioned as examples of complementary and even synergistic reductions of aphid populations (Salto *et al.* 1983, van Emden & Wratten 1990, Gowling & van Emden, 1994).

Since the biochemical basis of wheat resistance toward aphids is reasonably well established this thesis was undertaken using spring wheat *Triticum aestivum* (Linnaeus). The English grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) was selected as herbivore; while the cereal aphid parasitoid *Aphidius rhopalosiphi* De Stephani-Perez (Hymenoptera: Braconidae) and the entomopathogenic fungus *Erynia neoaphidis* Remaudierè et Hennebert (Zygomycetes: Entomophtorales) were used as phylogenetically distant natural enemies (Figure 3).

The main secondary metabolites conferring resistance against aphids in wheat and several other Gramineae (Poaceae) are Hydroxamic acids (Hx) (Niemeyer 1988, Niemeyer & Pérez 1995, Fuentes-Contreras *et al.* in press). These compounds show antifeeding and antibiotic properties against cereal aphids and other pests, representing interesting targets for breeding programs aimed to increase wheat resistance. The main Hx in wheat is DIMBOA (2,4-dihidroxy-7-methoxy-1,4-benzoxazin-3-one), which is naturally present as glucoside. Only following plant damage the glucoside is transformed into the respective aglucone by endo-β-glucosidases (Figure 4).

This study system was built artificially under laboratory conditions in order to analyse direct and indirect effects from host plants on interactions between natural enemies. In this sense, our study was intended to represent any general insect-plant interaction more than to accurately represent the complexities found in a particular field situation.

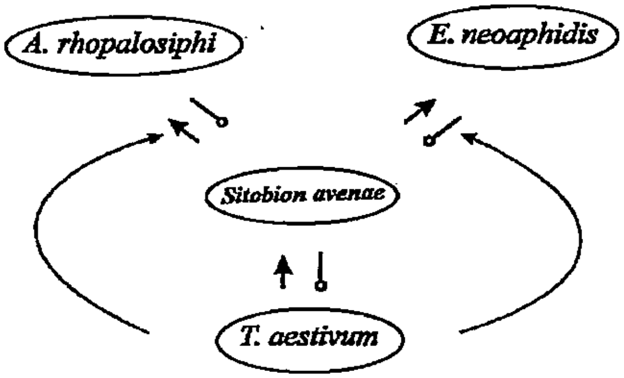
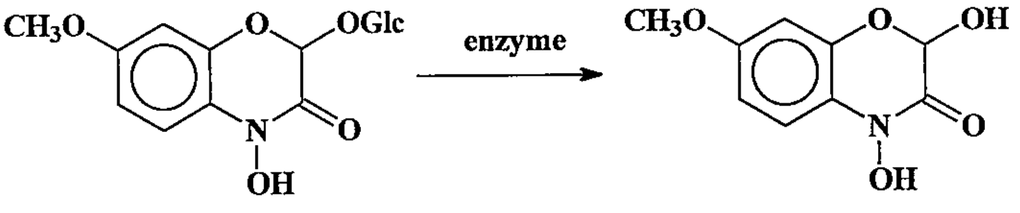


Figure 3. The system under study showing interspecific interactions. Interaction chains are the addition of sequential direct interactions, while curved arrows represent interaction modifications caused by wheat on aphid-parasitoid and aphid-fungus interactions.



DIMBOA-glucoside

DIMBOA

Figure 4. Enzymatic hydrolysis of DIMBOA-glucoside to produce the aglucone DIMBOA.

1.4.1 Parasitoid and fungus natural history

Parasitoids are insects whose larvae develop by feeding on the bodies of other arthropods, usually insects (Godfray 1994). The parasitoids of aphids are members of the families Braconidae and Aphelinidae. However, just one subfamily of Braconidae (Aphidiinae) has evolved in close association with aphids and represents their main parasitoid group (Starý 1987).

The most diverse taxon within the Aphidiinae is the genus *Aphidius*, which has several species widely used in biological control programs against aphid pests around the world. These parasitoids are koinobionts, solitary and with haplo-diploid sex determination (Hågvar & Hofsvang 1991).

The life cycle of *Aphidius rhopalosiphi* is summarised as follows: a single egg is oviposited within the aphid host. At 20 ^{0}C the egg hatches after four days, and after a further three days the larva has consumed all internal tissues and pupates within the cuticle of the aphid forming a characteristic "mummy". At 20 ^{0}C adult eclosion occurs seven days after pupation (Shirota *et al.* 1982, Sequeira & Mackauer 1992).

The entomopathogenic fungi are members of the order Entomophthorales within the Zygomicetes. They are a common source of mortality for herbivorous insects, able to lead to epizootics that may substantially reduce population levels of their hosts (Hajek & St. Leger 1994). For aphids, the most important entomopathogenic group of fungi are species of the genus *Erynia* (Latgé &

Papierok 1987). In particular, *Erynia neoaphidis* infects aphids from numerous different genera (Wilding & Brady 1985). Infective conidia (spores) adhere to the aphid and penetrate the cuticle directly without the requirement of ingestion. Once inside the host, the fungus develops in the haemolymph, then rapidly invades all the tissues killing the host within four to six days at 20 °C (Butt *et al.* 1990). After host death, the fungus emerges and sporulates, actively discharging more conidia.

At present, cereal aphids in Chile are under control by the action of several natural enemies (Zúñiga 1987); among them, parasitoids and entomopathogenic fungi can be found in cereal fields and wild grasses (Gerding *et al.* 1989, Starý *et al.* 1993, Starý 1994).

1.4.2 Potential for indirect effects and interaction modifications in the system under study

In the system under study there are direct interactions (Figure 3), such as aphid herbivory on wheat and parasitism and infection on aphids. However, parasitoids and fungi do not seem to interfere directly between them during the intra-host interaction, *i.e.* the fungus does not invade the body of the parasitoid and the parasitod does not feed from fungal tissues (Powell *et al.* 1986). This absence of intra-guild predation (Polis & Holt 1992) is not rare among parasitoids and fungi, but much more common among predators and parasitoids (Rosenheim et al. 1993, Ferguson & Stiling 1996). Furthermore, since *A. rhopalosiphi* does not attack sporulating aphids (Brobyn *et al.* 1988) its potential as fungal vector seems to be rather limited and hence this direct interaction at the extra-host level is also unlikely. Interactions between parasitoids and fungi are probably limited to competitive exploitation at the intra and extra-host level, *i.e.* when developing within shared aphid hosts and when attacking or infecting new aphid hosts, and therefore fully indirect through aphids (Mesquita *et al.* 1997). In this way wheat resistance could affect the interaction between parasitoid and fungus while affecting the aphid-parasitoid and aphid-fungus interactions.

Interaction modifications are expected at several levels of the system under study. At the intra-host level, plant antixenosis or antibiosis toward aphids increases developmental time and reduces body size and fecundity of females of parasitoids maintained on resistant cereals (Kuo 1986, Gowling 1989, Reed *et al.* 1991, Farid *et al.* 1998a,b). No such effects have been reported for entomopathogenic fungi, but resistance against cereal aphids in wheat may also negatively affect phytopathogenic fungi (Niemeyer 1988). Changes in aphid-parasitoid or aphid-fungus interaction may affect the outcome of the intra-host interaction between parasitoid and fungus. Successful development of the parasitoid in shared hosts with the fungus is determined by the timing of parasitoid oviposition and fungus infection. The parasitoid requires to oviposit several days before fungal infection in order to reach the adult stage, otherwise the fungus completes its development alone (Powell *et al.* 1986). This "priority effect" could be affected by changes in developmental times or mortality rates induced by wheat resistance on either the parasitoid or the fungus.

At the extra-host level, non-infected (healthy) aphids are able to display defensive reactions when facing a parasitoid attack. The success of these defensive behaviours, such as "bucking" or "kicking" the parasitoid (Gardner *et al.* 1984) are determined by aphid body size (Mackauer & Völkl 1993, Mackauer *et al.* 1996). Wheat resistance may affect aphid growth rate and hence adult body size, affecting concomitantly aphid defensive potential against parasitoids. In addition, *A. rhopalosiphi* avoids to oviposit in aphids sporulating or close to death from fungal infection (Brobyn *et al.* 1988). Hence, the role of the parasitoid as fungal vector seems to be of minor concern. On the other hand, parasitoid attack increases aphid mobility and consequently may increase the probability of fungal infection. Since aphids feeding on resistant wheat cultivars are restless and show a higher probability to leave the plant when facing a parasitoid attack (Gowling & van Emden 1994), an eventual increase on fungal infection associated to parasitoid activity could be higher on resistant rather than on susceptible wheat cultivars.

Interaction modifications in the system under study might be also detected at the population level. Billick & Case (1994) established that the statistical tests previously proposed by Case & Bender (1981) and by Wootton (1993) can be equivalent to the statistical interaction between principal effects of an ANOVA. They also suggest to use the *per capita* population growth rate of the focal species as the dependent variable to test the statistical significance of the interaction term in the ANOVA.

Interaction chains are also probably important in addition to interaction modifications. Changes in aphid growth, survival or fecundity are translated into changes in population density through time. In particular, Spiller & Llewellyn (1986) reported that intrinsic population growth rates (r_m) are affected by wheat species and cultivars, while Thackray *et al.* (1990) found negative correlations between Hx level in wheat cultivars and r_m of several species of cereal aphids. Such changes in r_m of the aphids is likely to cause an interaction chain on parasitoid or fungus density as predicted by mathematical models developed by Hochberg *et al.* (1990) for host-parasitoid-microparasite systems.

The present thesis aims to study indirect interactions between plants and phylogenetically distant species at the third trophic level. The study system was built in the laboratory to evaluate the presence of interaction modifications caused by wheat cultivars, with different levels of resistance against aphids, on parasitoids and fungi. The interactions between plants, aphids and natural enemies will be verified and the mechanisms responsible identified. Then, the attention will be placed on tritrophic effects acting on interspecific interactions between parasitoids and fungi, and the mechanisms potentially responsible of such tritrophic effects.

1.5 General objectives and hypotheses

1) **To evaluate mechanisms by which wheat resistance based on Hydroxamic acids (Hx) could cause interaction modifications between parasitoids and fungi of cereal aphids at the individual level.** With regard to this objective the following hypotheses will be tested:

- 1.1 If indirect effects of wheat resistance on natural enemies (parasitoids and fungi) are present in the study system, then the performance (*e.g.* developmental time and survival) of the natural enemies (parasitoids and fungi) will be reduced on the resistant wheat cultivar in relation to the susceptible wheat cultivar (Chapter 2 and 3).
- 1.2 If indirect effects of wheat resistance on the parasitoid are present in the study system, then the oviposition of the parasitoid will be reduced in the susceptible wheat cultivar in relation to the resistant wheat cultivar (Chapter 2).
- 1.3 If indirect effects of wheat resistance on the interaction between natural enemies (parasitoids and fungi) at the intra-host level are present in the study system there are two possible subhypotheses: First, if the fungus is more affected than the parasitoid, then wheat resistance at reducing fungus performance will enhance parasitoid survival in shared aphid hosts, decreasing the time advantage required by the parasitoid to complete its development. Second, if the parasitoid is more affected than the fungus, then

wheat resistance at reducing parasitoid performance will enhance fungus survival in shared aphid hosts, increasing the time advantage required by the parasitoid to complete its development (Chapter 3).

- 1.4 If indirect effects of wheat resistance on the interaction between natural enemies (parasitoids and fungi) at the extra-host level are present in the study system, then parasitoids through vectoring spores of the fungus or increasing aphid mobility will increase fungal infection of aphids on the resistant wheat cultivar in the relation to the susceptible wheat cultivar (Chapter 3).

2) **To evaluate the effect of wheat resistance based on Hydroxamic acids (Hx) on interactions at the population level between parasitoids and fungi of cereal aphids.** With regard to this objective the following hypotheses will be tested:

- 2.1 If indirect effects of wheat resistance on the interaction between natural enemies (parasitoids and fungi) at the population level are present in the study system, then wheat resistance will affect either the per capita interspecific effect of the fungus[1] on the parasitoid or the per capita interspecific effect of the parasitoid on the fungus, *i.e.* the per capita

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[1] *Per capita* effects are referred as suggested by Billick & Case (1994) in order to maintain formal terms in the hypothesis. However, the modular organisation of the fungi precludes the proper utilisation of this nomencalture.

interspecific effect of the fungus on the parasitoid or vice versa will be different when they are developing on resistant or susceptible wheat cultivars (Chapter 4).

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2.1 Influence of wheat and oat cultivars on the development of specialist and generalist parasitoids of cereal aphids

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 programs trying to improve plant resistance to aphids.

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 complementary (Wyatt 1970, Starks *et al.* 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 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 1994).

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 (Nehuén 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-days old seedlings (G.S. 11) (Zadoks *et al.* 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 *et al.* 1992). Since the Hx concentration in cereal seedlings changes significantly (Niemeyer 1988) during the approximately seven days of parasitoid larval development (Shirota *et al.* 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).

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 parasitized by two or three-days-old females in gelatine capsules, each female being used only once. Potentially parasitized 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).

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

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

	Wheat			**Oat**	
	T-1500	**Antilhue**	**Kona**	**Melys**	**Nehuén**
MRGR	0.316	0.331	0.337	0.360	0.394
SE	0.0089	0.0075	0.0082	0.0098	0.0155
	a	a	a	b	b

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) on the parasitoid larval developmental time, whilst the parasitoid pupal developmental time remained constant between species (ANOVA, F = 1.164, P = 0.285) and cultivars (ANOVA, F =0.323, P = 0.808). The total parasitoid developmental time was negatively correlated with the aphid MRGR ($r = -0.89$, $P < 0.05$). No further effects were observed in other variables contributing to the performance of *A. rhopalosiphi*.

Table 2: Influence of 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.

	Hx level (g/ g fw)	Total developmenta l time (days)	Egg-larval developmental time (days)	Pupal developmenta l 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	7.50 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	8.00 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
Nehuén	-	-	-	-	-	-	-	-	-	-

Table 3: Influence of wheat and oat cultivars on the performance of the specialist 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 ($P < 0.05$). N = 14.

	Hx level (g/ g fw)	Total developmental time (days)	Egg-larval developmental time (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 a	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
Nehuén	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

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

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 & Johansson (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 (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 *et al.* 1989, Åhman & Johansson 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.* (1991), 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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2.2 Effects of DIMBOA-glucoside on host acceptance and suitability of *Sitobion avenae* (Hemiptera: Aphididae) to the cereal aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)

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* were evaluated. Aphids showed a reduction in mean relative growth rate and in body size in the wheat cultivar with higher Hx level. The reduction in aphid size was related to a decreased success in avoiding parasitoid oviposition. A minor increase on *A. rhopalosiphi* developmental time was observed in 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. These evidences are discussed in relation to the compatible utilisation of host-plant resistance and biological control in integrated pest management.

INTRODUCTION

Several morphological and/or chemical attributes of the host-plant may confer resistance towards herbivorous insects. However, resistance to herbivorous insects can also affect significantly the performance of their natural enemies (Price *et al.* 1980, Vet & Dicke 1992, Hare 1992) producing negative, compatible 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 & 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 & Pérez 1995). These compounds could be a target for breeding programs aiming to increase resistance towards cereal aphids (Escobar & Niemeyer 1993). Nevertheless, within 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, a *ca.* 5% increase in developmental time, on the cereal aphid parasitoid *Aphidius rhopalosiphi* De Steph. in wheat (*Triticum aestivum* L.) when compared to 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 behaviour 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, Aphid and Parasitoid Stock Cultures. All the 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 ($\bar{x}$ = 1.72 ± 0.12 mmoles / Kg fr. wt.) and Naofén ($\bar{x}$ = 3.02 ± 0.17 mmoles / Kg fr. 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 reversed phase high performance liquid chromatography (HPLC), as previously described by Weibull & 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, Germany). Purity was also evaluated by HPLC and ranged between 95 and 98 %.

Influence of Wheat Cultivars on Host Acceptance by the Parasitoid and Aphid Defensive Behaviour. 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 synchronised 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 & Wright 1992, Braimah & van Emden 1994), only oat leaves were used in the experimental arena, so wheat volatiles from different cultivars that would affect parasitoid behaviour during the experiments were absent. After allowing aphids to settle for 10 minutes, a naive female of the parasitoid was introduced in the arena. All parasitoid females were mated, and 2 to 3 day-old, being used only once. The behaviour 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 five minutes. The following parasitoid and aphid behavioural 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 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. Synchronised second instar aphids were placed inside clip-cages on the wheat seedlings as described previously in Fuentes-Contreras *et al.* (1996). All the 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). Synchronised 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 & Niemeyer 1995).

RESULTS

Effect of Wheat Cultivars on Aphid-Parasitoid Interaction. Behaviour 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 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.

Table 1. Behavioural events during *S. avenae* acceptance process by *A. rhopalosiphi*. 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 (α = 0.05). N = 22.

Wheat Cultivar	***A. rhopalosiphi***			***S. avenae***		
Hx level (mean ± SE) mmol / Kg fr. wt.	**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

Table 2. Effect of wheat cultivars on the performance of *S. avenae* and *A. rhopalosiphi*. 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

Wheat cultivars Hx level (mean ± SE) mmol / Kg fr. wt.	***Sitobion avenae*** **MRGR** (μg/μg/day)	***Aphidius rhopalosiphi*** **total developmental time** (days)	***Aphidius rhopalosiphi*** **egg-larval developmental time** (days)	***Aphidius rhopalosiphi*** **pupal developmental time** (days)	***Aphidius rhopalosiphi*** **survival** (%)	***Aphidius rhopalosiphi*** **body mass** (mg)	***Aphidius rhopalosiphi*** **secondary sex ratio** (F/M)
Huenufén (1.72 ± 0.12)	0.330a	8.95 a	5.41 a	3.55 a	100 a	0.0399 a	0.48 a
Naofén (3.02 ± 0.17)	0.262 b	10.23 b	6.64 b	3.59 a	100 a	0.0363 a	0.60 a

Table 3. Effect of DIMBOA-glc in artificial diets on the performance of *S. avenae* and *A. rhopalosiphi*. 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, *A. rhopalosiphi* N = 40

[DIMBOA-glc]	***Sitobion avenae* MRGR** (µg/µg/day)	***Aphidius rhopalosiphi* total developmental time** (days)	***Aphidius rhopalosiphi* egg-larval developmental time** (days)	***Aphidius rhopalosiphi* pupal developmental time** (days)	***Aphidius rhopalosiphi* total survival** (%)	***Aphidius rhopalosiphi* egg-larval survival** (%)	***Aphidius rhopalosiphi* body mass** (mg)	***Aphidius rhopalosiphi* secondary sex ratio** (F/M)
0 mM	0.194 a	16.33 a	9.01 a	7.40 a	65 a	80 a	0.0328 a	0.58 a
2 mM	0.128 b	16.72 ab	9.79 ab	7.28 a	60 a	65 b	0.0332 a	0.5 a
4 mM	0.105 c	17.8 b	10.46 b	7.25 a	50 a	55 b	0.0316 a	0.55 a

Aphid and Parasitoid Performance in Artificial Diets. MRGR of the aphid significantly decreased as DIMBOA-glc concentration increased (Table 3). In the same way that for the experiment with plants, data from both parasitoid sexes were pooled based on non-significant 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 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, parasitoid overall survival was not significantly affected by DIMBOA-glc, but a partial reduction on the egg-larval survival was detected as DIMBOA-glc concentration increased (Table 3). No further effects were detected in other variables of parasitoid performance.

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 & 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, in the wheat cultivar with higher Hx level. Aphid body size influences the success of aphid defensive reactions against attack by natural enemies (Gerling *et al.* 1990, Kouamé & Mackauer 1991, Gross 1993). Our results showed that aphids grown in wheat cultivar Naofén (high Hx level) were found and attacked by parasitoids at frequencies similar to aphids from cultivar Huenufén (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 concentration in the phloem sap of the cultivars used herein 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 the proper test of the effect of the compound on parasitoid performance. Furthermore, higher concentrations of Hx in diets reduce aphid survival (Niemeyer & Pérez 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. Developmental time of the parasitoid was reduced in comparable magnitude by DIMBOA-glc concentration in plants and in artificial diets (*ca.* 1 day). However, total developmental time of the parasitoid was much lower in the plants than in the diets, and hence in relative terms the increase in developmental time in the plant is *ca.* 10 %, while in the diets 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 i) behaviour, as an increase in successful stabs in aphids reared on wheat cultivars that have higher levels of the compound, and to ii) life history traits, as a minor increase in developmental time. From an applied 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, Hx deleterious influence on overall survival of the parasitoid is not significant and any potential reduction in parasitoid body size seems to be compensated by increased developmental time.

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

SUMMARY

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 rhopalosiphi* 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 decreased on the resistant wheat cultivar compared with 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.

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 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 on interspecific interactions between species in 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 & St. Leger 1994) and, although phylogenetically very distant from insects, they are often regarded as functional members of the parasitoid guild (Eggleton & 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 & Lawton 1990). Entomopathogenic fungi and parasitoids, showing 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 & Whitham 1996).

Hochberg (1991a,b) studied the competitive interaction between parasitoids and pathogens, specifically virus's, emphasising the mechanisms of the interaction. He defined two potential interactions: i) intra-host interactions, during ontogenetic development of the natural enemies within a shared host; and ii) 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 & van Lenteren 1993, Furlong & Pell 1997). The fungus usually outcompetes the parasitoid unless the development of the latter begins several days before fungal infection. The influence of timing between the attacks of natural enemies is known as the priority effect (Powell *et al.* 1986, Fransen & 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 & St. Leger 1994, Steinkraus *et al.* 1996, Pell *et al.* 1997). There are examples in the literature where parasitoids have passively vectored 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 & 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 vector fungi to new host populations aiding dispersal. An examination of both aspects is needed, therefore, to appreciate fully the net effect of the interaction between these species (Hochberg 1991a,b).

The most common 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 & 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 four 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 UK, aphid populations decrease in abundance in early sown cereals (Vorley & 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 & 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 (Leszcynski *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 & 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 & 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: i) the influence of plant resistance on the performance of the fungus, ii) the influence of plant resistance on the priority required by the parasitoid to complete its development in hosts prior to infection by fungi, and iii) the influence of plant resistance on the role of the parasitoid as a fungal vector.

MATERIALS AND METHODS

Natural history of the species studied. Aphidius rhopalosiphi is a koinobiont, solitary parasitoid of cereal aphids. A single egg is oviposited within the aphid host. At 20 ^{0}C the egg hatches after four days and after a further three days the larva has consumed all internal tissues and pupates within the cuticle of the aphid forming a characteristic "mummy". Adult eclosion occurs seven days after pupation. *Erynia neoaphidis* is an entomophthoralean fungus which infects aphids from numerous different genera (Wilding & Brady 1984). Infective conidia (spores) adhere to the aphid and penetrate the cuticle directly without the requirement for ingestion. Once inside the host the fungus develops in the haemolymph, then rapidly invades all the tissues, killing the host within four-six days at 20^{0}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 ± 5 °C in a 16:8 light-dark photoperiod. Stock cultures of the parasitoid *A. rhopalosiphi*, also collected in wheat fields near Santiago (Chile), were maintained on *S. avenae* reared on the same oat cultivar under the same environmental conditions. Two spring wheat (*Triticum aestivum* L.) cultivars, Huenufén susceptible with a low Hx level ($\bar{x}$ = 1.72 ± 0.12 mmoles / Kg fr. wt. on primary leaf of 6-day old seedlings, N = 6) and Naofén partially resistant with a high Hx level ($\bar{x}$ = 3.02 ± 0.17 mmoles / Kg fr. wt. on primary leaf of 6-day old seedlings, N = 6), were used in all experiments. The fungus, *E. neoaphidis*, was collected from *Acyrthosiphon 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 nutrient agar in 9 cm. sterile Petri dishes. (SEMA = Sabouraud dextrose agar supplemented with egg yolk and milk, Wilding & Brobyn 1980). At 20 °C fungal growth had filled the plates within 25 days after which time it was grown on in YEMG liquid media in shake flasks (YEMG = Yeast extract, semi-skimmed milk and glucose, Pell et al. 1993) 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 °C and 100 % R.H. overnight then transferred to 20 °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 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 10 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 minutes. There were five independent replicates for each time. All dishes were maintained at 100 % R.H. 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 per mm^2 on coverslips (20 mm. diameter) placed alongside the oat leaves during inoculation, as described by Furlong & 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 hours the plastic film was replaced with muslin to allow ventilation. Inoculated aphids were incubated in a growth chamber at 20 ± 1°C and 16:8 light-dark photoperiod and mortality due to fungus (evaluated as aphid cadavers able to produce a detectable number of conidia) recording daily for six 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 in order to estimate LC_{50} and 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 in order 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 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 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 ± 1 °C, 100% humidity in a 16:8 light-dark photoperiod on oat for 24 hours and then transferred to the two wheat treatments. Control aphids were subjected to the same manipulations except 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. Fungus success was measured as the proportion sporulating cadavers and subjected to Arcsin√x to satisfy the assumptions of normality and homogeneity of variances. These results were analysed with 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: i) the same day, ii) three, iii) four, iv) five, and v) six days after parasitoid oviposition.

The experimental unit was composed by 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 & 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 a LC_{90} dose of conidia from freshly produced mycelial mats of the fungus either on the same day of parasitism or three, four, five or six 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 was subjected to the Arcsin√x transformation 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. *Erynia neoaphidis*-killed aphid cadavers, 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 °C for six hours, after which time aphids from both treatments were transferred to clean dishes containing the appropriate wheat cultivars but no sporulating cadaver (15 dishes of which had been foraged on by a parasitoid in the presence of a sporulating cadaver and 15 dishes which 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 twenty second instar nymphs on leaves of their respective wheat cultivar (15 dishes of each wheat cultivar). After six hours 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. 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 conidia mm^{-1} and $LC_{90} = 3.489 \pm 0.5$ log conidia mm^{-1}. The lethal concentrations approximately corresponded to LC_{50} = 47 minutes and LC_{90} = 387 minutes 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) increased at LC_{90} doses compared to LC_{50} doses and the uninfected control (MS 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√x transformed to perform the statistical analysis. Sample size = 10

Wheat cultivar	**Lethal Concentration**	**Survival (proportion)**		**Developmental time (days)**	
		Mean	SE	Mean	SE
Huenufén	**LC_{50}**	0.46 a	0.06	3.41 a	0.09
(susceptible)	**LC_{90}**	0.74 b	0.04	3.52 a	0.07
Naofén	**LC_{50}**	0.48 a	0.05	3.46 a	0.08
(resistant)	**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 three days after parasitoid oviposition. Parasitoid survival was significantly higher in the susceptible wheat cultivar Huenufén when infection was applied four days after parasitoid oviposition (Tukey test, P = 0.037), but not when infection occurred five or six days after parasitoid oviposition (Tukey test P = 0.95 for five days, P = 0.66 for six 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) were not significant on 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) 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 four, five and six days before fungal infection, a low percentage of aphids (overall 4 %) allowed successful and simultaneous parasitoid and fungal development.

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√x transformed to perform the statistical analysis. Sample size = 5. 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

Wheat cultivar	**Priority Effect**	**Survival** (proportion)		**Developmental time** (days)	
		Mean	SE	Mean	SE
Huenufén	**0**	0	0	-	-
(susceptible)	**3**	0	0	-	-
	4	0.52	0.04	9.48	0.14
	5	0.86	0.05	9.38	0.21
	6	0.84	0.05	9.30	0.13
	Control	0.92	0.04	9.38	0.22
Naofén	**0**	0	0	-	-
(resistant)	**3**	0	0	-	-
	4	0.24	0.08	10.40	0.19
	5	0.78	0.04	10.60	0.17
	6	0.94	0.04	10.54	0.16
	Control	0.88	0.06	10.64	0.18

Table 3: Effects of wheat cultivar and priority effect of parasitoid oviposition on survival and developmental time of the fungus *Erynia neoaphidis* in aphid hosts also parasitised by *Aphidius rhopalosiphi*. Survival was Arcsin√x transformed to perform the statistical analysis. Sample size = 5. Little fungus 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.

Wheat cultivar	**Priority Effect**	**Survival (proportion)**		**Developmental time (days)**	
		Mean	SE	Mean	SE
Huenufén	**0**	0.80	0.06	3.44	0.12
(susceptible)	**3**	0.72	0.10	3.60	0.06
	4	0.48	0.05	3.56	0.07
	5	0.22	0.06	3.40	0.17
	6	0.02	0.02	-	-
	Control	0.84	0.07	3.62	0.06
Naofén	**0**	0.72	0.10	3.50	0.13
(resistant)	**3**	0.74	0.09	3.48	0.13
	4	0.54	0.05	3.6	0.06
	5	0.04	0.02	-	-
	6	0.04	0.02	-	-
	Control	0.75	0.19	3.42	0.15

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 (Figure 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 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 four days after parasitoid oviposition, the survival of the parasitoid was significantly reduced in the resistant wheat cultivar (Table 2).

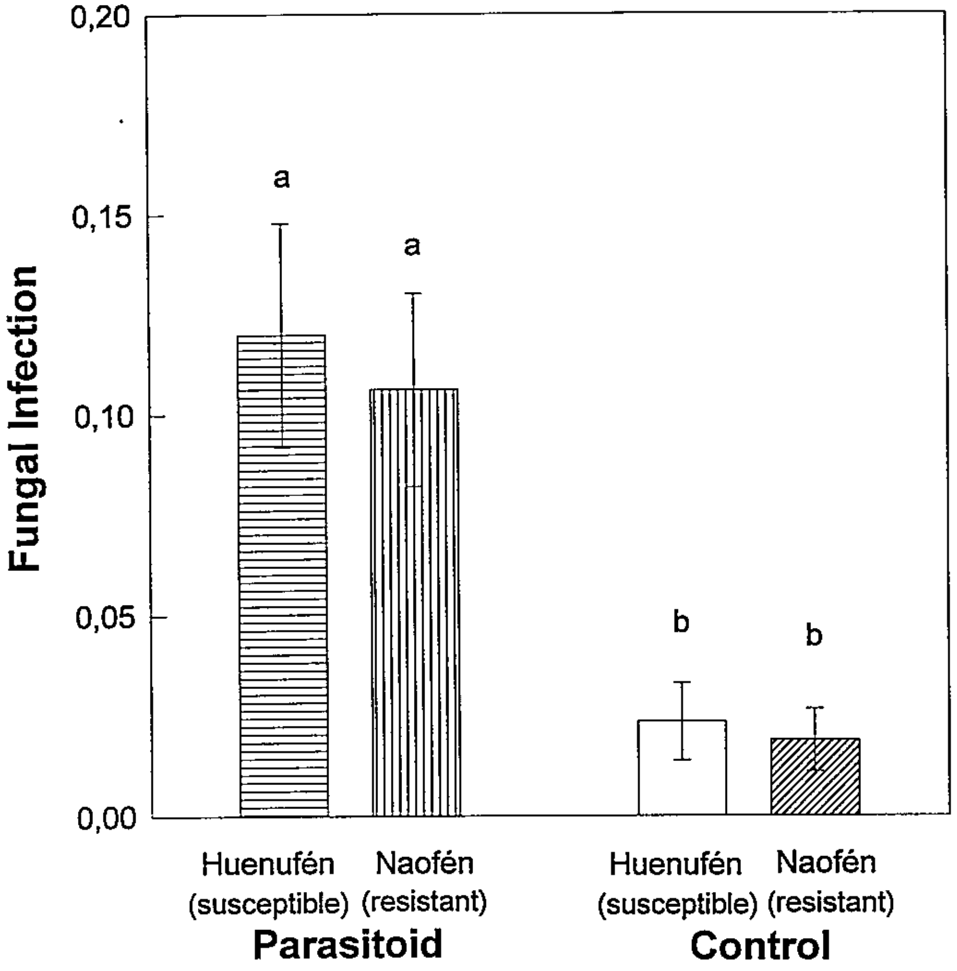

Figure 2: Proportion of fungal infection in the presence of parasitoids and in the control without parasitoids. Data are means of the proportion of fungal infection and error bars represent ± 1 standard error. Statistical differences were evaluated by two-way ANOVA of Arcsin$\sqrt{x}$ transformed data. Significant differences are shown by different letters.

In comparison, when the priority effect was favourable to the successful development of either the fungi or the parasitoid, *i.e.* less than four days of parasitoid advantage for fungal success or more than five days of parasitoid advantage for parasitoid success, this influence of the host-plant in the intra-host interaction between parasitoid and fungi was not significant (Table 2).

It was previously shown that partially resistant wheat reduced aphid growth rate and increased parasitoid developmental time (Fuentes-Contreras *et al.* 1996, Fuentes-Contreras & Niemeyer 1998). The same effect was observed in the present 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 its potential as effective fungal vectors. Only when foraging parasitoids were introduced into a conidia-containing environment there was 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 & 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 & 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 (Leszcynski *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 sytem, Dickson & 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 & 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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4.1 Additive effects of wheat resistance and parasitoids on population dynamics of the cereal aphid *Sitobion avenae*

INTRODUCTION

The influence of host-plant resistance on the action of natural enemies of herbivorous pests can be antagonistic, but also complementary (simply additive) or synergistic (Hare 1992). Such tritrophic effects have been studied measuring the impact of resistant plant species or genotypes on development, survival, fecundity, sex ratio, body size and behaviour of natural enemies. However, tritrophic effects at the individual level may not necessarily impact at the population level in the same modality or extent (Hare 1992).

van Emden & Wratten (1992) reviewed tritrophic effects in the biological control of aphids, concluding that complementary or synergistic interactions between partial resistance of the host-plant and natural enemies seem to be the general pattern. Previous studies have shown that partial resistance of wheat can produce tritrophic effects in aphid-parasitoid interactions at the individual level, such as increases in developmental time of the parasitoid, reductions in the aphid defensive success when facing a parasitoid attack (Fuentes-Contreras *et al.* 1996, Fuentes-Contreras & Niemeyer 1998), and reductions in parasitoid body size (van Emden & Wratten 1992). The present work evaluates the expression of these tritrophic effects at the population level.

Aphid populations were maintained on wheat cultivars with different levels of resistance, in the presence and absence of parasitoids. The wheat cultivars were known to differ in their levels of hydroxamic acids (Hx), a family of secondary metabolites conferring resistance toward aphids in wheat and several other cereals (Niemeyer & Pérez 1995). The effects of wheat resistance and parasitoids through time on aphid population parameters, such as density, population growth rate and per capita population growth rate were evaluated.

MATERIALS AND METHODS

Plant and insect cultures. The parasitoid *Aphidius rhopalosiphi* De Stephani-Perez and the cereal aphid *Sitobion avenae* (Fabricius) were maintained on oat (*Avena sativa* L.), a cereal lacking Hx, previously to the experiment. Two spring wheat (*Triticum aestivum* L.) cultivars: Huenufén (susceptible, Hx concentration in seedlings, $\bar{x} = 1.72 \pm 0.12$ mmoles / Kg fr. wt.) and Naofén (partially resistant, $\bar{x} = 3.02 \pm 0.17$ mmoles / Kg fr. wt.) were used in the experiments. All insect cultures were maintained at 23 ± 2 °C, L16:D8 light-dark photoperiod.

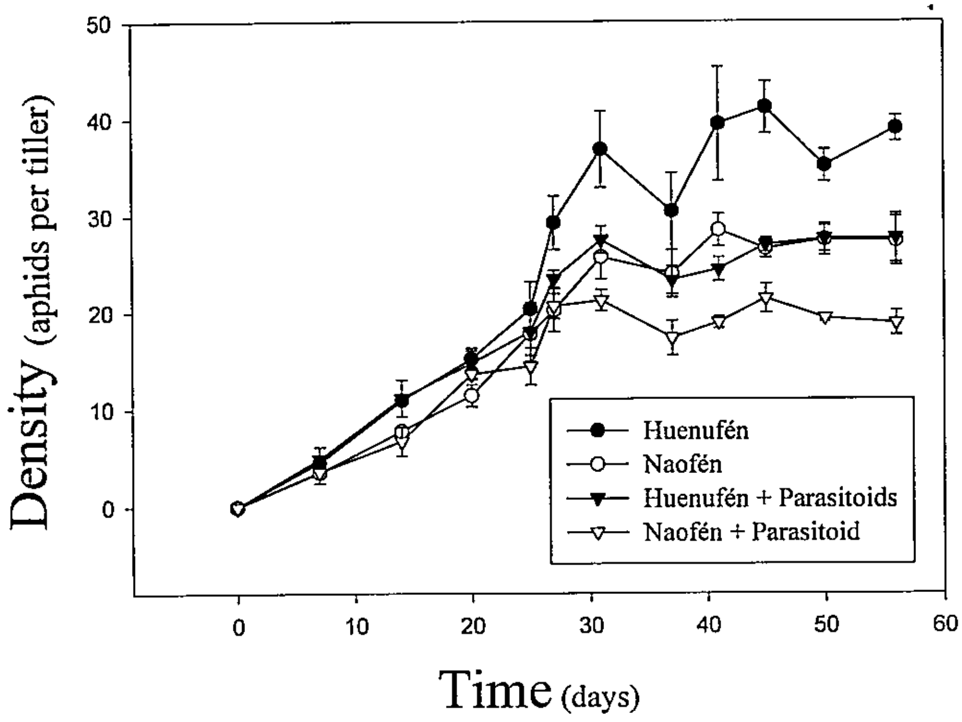
Population experiment. Plexiglass cages 40 x 40 x 40 cm were used to start twelve experimental populations. Six cages were assigned to the wheat cultivar Naofén, while the remaining six cages were assigned to the susceptible wheat Huenufén. Half of the cages were subsequently assigned to the aphid treatment and the other half to the aphid and parasitoid treatment. Hence, three replicates were used for every treatment combination. Sowing was performed at a density of 50 seeds per pot (volume 450 ml). Two weeks later, when wheat seedlings reached growth stage 14 (Zadoks *et al.* 1974), fifty adult aphids were placed on them. The parasitoid treatment also received four previously mated parasitoid females following aphid transfer. Pots containing new seedlings were introduced into the cages once a week, without removing the pots already present in the cage. Only when plants naturally died from aphid attack they were removed, but leaving all not emerged mummies in the cage. The whole experiment was performed under the same environmental conditions described in the insect culture section.

Sampling and statistical analyses. Every four to five days aphids and not emerged mummies present in ten tillers from every cage, were removed from the colony and counted. Mean values of these ten samples were used as the experimental unit to estimate aphid population density, i.e. number of aphids per tiller. Population dynamics were followed up to completion of nearly four parasitoid generations. Population growth rate of aphids (PGR) was calculated as the difference between population density in N_t and N_{t+1}, while *per capita* population growth rate of aphids (PCPGR) was calculated dividing PGR by density at N_t. The dependent variables density, PGR and PCPGR were compared using an ANOVA design for repeated measures in order to assess the effects of wheat cultivar, parasitoids, time (sampling date) and their statistical interaction.

RESULTS AND DISCUSSION

ANOVA results showed that aphid density (Figure 1) was higher on the susceptible wheat cultivar Huenufén (M.S. = 978.6, F(1,8) = 83.2 , $P < 0.001$), and also higher in the treatment without parasitoids (M.S. = 697.4, F(1,8) = 59.3, $P < 0.001$). There were significant differences in aphid density among sampling dates (M.S. = 1310.5, F(10,80) = 112.1, $P < 0.001$), and significant statistical interactions between sampling date and wheat cultivar (M.S. = 38.6, F(10,80) = 3.3, $P< 0.001$), and sampling date and parasitoid treatment (M.S. = 66.7, F(10,80) = 5.7, $P < 0.001$) were observed. However, the statistical interaction between wheat cultivar and parasitoid treatment was only nearly significant (M.S. = 55.8, F(10,80) = 4.7, $P = 0.061$). This later statistical interaction reveals that wheat cultivar and parasitoids influence aphid density in a rather independent ways, although there is an almost significant tendency to reduce aphid density in a synergistic way.

Figure 1: Density (individuals/tiller) of aphids in the different wheat cultivars and parasitoid treatments. Values are means ± one standard error.



Results from ANOVA of PGR and PCPGR showed that the aphid population grew faster on the susceptible wheat cultivar Huenufén (PGR, M.S. = 2.6, F(1,8) = 10.4, P = 0.012; PCPGR, M.S. = 0.0015, F(1,8) = 6.8, P = 0.031), PGR was higher in the treatment without parasitoids (M.S. = 1.4, F(1,8) = 5.5, P = 0.047), but no significant differences between treatments with or without parasitoids were observed for PCPGR (M.S. = 0.0006, F(1,8) = 2.8, P = 0.135).

There were also significant differences in PGR and PCPGR among sampling dates (PGR, M.S. = 10.2, F(10,80) = 6.1, P < 0.001; PCPGR, M.S. = 0.008, F(10,80) = 6.2, P < 0.001), but no statistical interactions were found among any independent variables either for PGR or PCPGR.

The results suggested that the effects of parasitoids and wheat resistance on density, PGR and PCPGR of aphids are simply additive, *i.e.* both effects just added their respective contributions toward the reduction of aphid density, PGR and PCPGR. Since the interaction term between wheat cultivar and parasitoid treatment on aphid density was close to the significance level of a synergistic effect, a cautionary conclusion must be taken with respect to this dependent variable. Density and PGR of aphids was significantly reduced by parasitoids, but PCPGR of aphids was not affected by this treatment. This result suggests that the parasitoid influence on aphid population dynamics is mediated by changes in density of the aphids, but not by changes in the interspecific effect of the average parasitoid on the average aphid, *i.e.* an individual level process such as those previously reported for this aphid-parasitoid interaction.

Previous work revealed an increase in developmental time of the parasitoid in the partially resistant wheat cultivar (Fuentes-Contreras *et al.* 1996; Fuentes-Contreras & Niemeyer 1998), but such reduction seems to be not relevant enough to affect aphid population dynamics. At the time scale required for few parasitoid generations (three or four months), which is the most common situation in parasitoids attacking aphids growing on arable and greenhouse crops (Rochat 1997), it is unlikely that any interference between wheat resistance and parasitoid control will be found. The increase in parasitoid oviposition success on aphids grown in the resistant cultivar (Fuentes-Contreras & Niemeyer 1998) may be responsible for the almost significant higher aphid control by parasitoids in this case. However, other potential mechanisms may also be responsible, such as aphid mortality associated to an increase in falling behaviour of aphids on partially resistant wheat cultivars under parasitoid presence (Gowling & van Emden 1994).

In conclusion, partial wheat resistance based on Hx is complementary with the action of parasitoids against cereal aphids, and both alternatives can be readily implemented in integrated pest management strategies.

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4.2 Wheat resistance affects interactions at the population level between parasitoids and entomopathogenic fungus of cereal aphids

ABSTRACT

1. The cereal aphid parasitoid *Aphidius rhopalosiphi* and the entomopathogenic fungus *Erynia neoaphidis* growing on the aphid *Sitobion avenae*, were used to evaluate the influence of wheat resistance on interspecific interactions between natural enemies at the population level.

2. Indirect effects of wheat resistance on interspecific interactions between parasitoid and fungus were detected by ANOVA. Such indirect effects were caused by interaction chains and interaction modifications, i.e. changes in density and *per capita* interspecific effects respectively, as evidenced by significant statistical interactions between the effects of the fungus and wheat resistance on both density and *per capita* population growth rate of the parasitoid.

3. Path analysis, a statistical technique to discern the relative contributions of direct and indirect effects, also detected indirect effects between fungus and parasitoid, although the amount of variability explained by the multiple regression was low.

INTRODUCTION

Indirect interactions arise when additional species affect direct interspecific interaction between two focal species (Strauss 1991, Wootton 1994a). These interactions are widely recognised as important elements in the structure of species assemblages and their relevance has been highlighted in several ecological systems (Wootton 1994a). Such indirect interactions may be "interaction chains", *i.e.* changes in density of the additional species which in turn affect the densities of the focal species, or "interaction modifications", *i.e.* changes caused by the additional species on the *per capita* interspecific effects between focal species (Wootton 1993, 1994a). Therefore, interaction chains are just epiphenomena arising from the addition of direct effects between pairs of species, whereas interaction modifications show the relevance of non-additive components, thus precluding the extrapolation of interactions in multi-species systems from the knowledge of interactions between pairs of species (Billick & Case 1994).

Conceptual distinction between direct and indirect interactions, as well as between interaction chains and interaction modifications, is unambiguous and heuristic. However, the empirical assessment of their presence and relative contributions is difficult (Case & Bender 1994, Wootton 1994b, Smith *et al.* 1997). Indirect interactions can be detected when density, population growth rates or any variable associated with performance of the focal species, show significant statistical interactions between treatments with and without the additional species within ANOVA designs (*e.g.* Case & Bender, 1981; Wilbur & Fauth, 1990). Case & Bender (1994) further developed this approach stating that interaction modifications could be distinguished from interaction chains, when *per capita* population growth rates of the focal species were the dependent variable showing significant statistical interactions in the ANOVA. In this last case, the study of mechanisms underlying the interaction modification also represent strong evidence to support the existence of such indirect interactions (Adler & Morris 1994, Kareiva 1994, Wooton 1994a).

In multispecies systems the manipulative approach based on ANOVA is logistically difficult while maintaining adequate replication, hence the combined utilisation of manipulative experiments and path analysis (Kingsolver & Schemske 1994) has been recomended (Wootton 1994b). Path analysis is a statistical technique to decompose the overall correlation between predictor and response variables into their direct effects, indirect effects mediated by other variables, and spurious effects due to common causes, allowing to estimate their relative magnitude (Sokal & Rohlf 1995).

Multitrophic interactions between insect herbivores and their natural enemies frequently show indirect interactions (*e.g.* Settle & Wilson 1990, Rosenheim *et al.* 1993, Ferguson & Stiling 1996, Evans & England 1996, Müller & Godfray 1997). An interesting and well documented case of these indirect interactions are "tritrophic effects", where plant traits indirectly affect natural enemies of herbivorous insects (Price *et al.* 1980, Hare & Luck 1991). Several mechanisms for "tritrophic" effects have been described, but host-plant resistance based on secondary metabolites is of major relevance in several cases (reviewed by Hare 1992).

Wheat resistance against cereal aphids is based mainly on hydroxamic acids (Hx), a family of secondary metabolites characteristic of Gramineae (Poaceae) (Niemeyer & Pérez 1995). Resistance based on these metabolites is able to affect interspecific interactions between phylogenetically distant groups of natural enemies, such as parasitoids and entomopathogenic fungi at the individual level (Fuentes-Contreras & Niemeyer 1998, Fuentes-Contreras *et al.* in press). However, it is not clear whether this influence is also significant at the population level, therefore representing an indirect interaction mediated by interaction chains (changes in population densities) or by interaction modifications (changes in *per capita* interspecific effects).

In the present study we evaluated the effect of wheat resistance on parasitoid-fungus interaction at the population level. In particular, i) we addressed the presence of indirect interactions, and ii) we evaluated whether interaction modifications and/or interaction chains are present in an experimental system under laboratory conditions. Furthermore, we discerned the relative contributions of direct and indirect effects using path analysis.

MATERIALS AND METHODS

Plant material and insect cultures. The parasitoid *Aphidius rhopalosiphi* De Stephani-Pérez and the cereal aphid *Sitobion avenae* (Fabricius) were maintained on oat (*Avena sativa* L., cv. Nehuén), a highly susceptible cereal species, previously to the experiments. *In vivo* cultures of *Erynia neoaphidis* Remaudière *et* Hennebert on *S. avenae* feeding on oat were also maintained and continuously produced fresh mummies. Two spring wheats (*Triticum aestivum* L.) cultivars were used in the experiments: Huenufén (susceptible, Hx concentration on primary leaf of 6-day old seedlings, $\bar{x}$ = 1.72 ± 0.12 mmoles Kg^{-1} fresh weight, N = 6) and Naofén (partially resistant, Hx concentration on primary leaf of 6-day old seedlings, $\bar{x}$ = 3.02 ± 0.17 mmoles Kg^{-1} fresh weight, N = 6). All insect cultures were maintained at 23 ± 2 °C, L16:D8 light-dark photoperiod.

Population experiment. Plexiglass cages 40 x 40 x 40 cm were used to start 24 experimental units. 12 cages were assigned to the partially resistant wheat cultivar Naofén, while the remaining 12 cages were assigned to the susceptible wheat Huenufén. Sowing was performed at a density of 50 seeds per pot (volume 450 ml). Two weeks later, when wheat seedlings reached growth stage 14 (Zadoks *et al.* 1974), 50 adult aphids were placed on the plants. Following aphid settlement, 12 cages (six for each wheat cultivar) received four sporulating aphid cadavers each. Subsequently, 12 cages (six for each wheat cultivar) received four previously mated parasitoid females, including six cages (three for each wheat cultivar) which previously received the fungi.

Pots containing new seedlings were introduced into the cages once a week, without removing the pots already present in the cage. Only when plants naturally died from aphid attack the pots were removed, but leaving all not emerged or sporurated mummies in the cage. The whole experiment was performed under the same environmental conditions described in the insect culture section.

Sampling. Every four to five days ten tillers from every cage, were removed from the colony and aphids and not emerged or sporulated mummies were counted. Mummies produced by the parasitoid or the fungus can be readily distinguished by their rounded or spindle body shape respectively. Only in doubtful cases the mummies were placed in a humid Petri dish to evalute production of spores. Mean values of these ten samples were used to estimate aphid, parasitoid or fungus population density at each experimental unit, i.e. number of aphids, parasitoid and fungus mummies per tiller. Population dynamics were followed up to the completion of four parasitoid generations. Density was transformed to log (N + 10) and daily *per capita* growth rate of the populations (PCPGR) was calculated as $((N_t - N_{t+1})\ N_t^{-1})\ day^{-1}$.

Given that fungi are modular organisms (Harper *et al.* 1986), the *per capita* concept does not have a direct application as with individual organism such as parasitoids. This shortcoming has been solved in several studies of population dynamics of parasitoids and insect pathogens, by regarding a host killed by the pathogen as the equivalent of a host killed by the parasitoid (Hochberg & Lawton, 1990). Such approach is particularly useful for solitary parasitoids such as the braconid species we studied.

Statistical analyses. Density and PCPGR of parasitoids and fungi were compared using a two way ANOVA for repeated measures in order to assess the effects of wheat cultivar, fungus, time (sampling date) and their statistical interactions of first and second order. Assumption of normality and homoscedasticity of variances were fulfilled after the above mentioned logarithmic transformation.

Readable introductions to path analysis are found in Sokal & Rohlf (1995) and their potential for studies in ecology and evolution has been discussed by Kingsolver & Schemske (1991) and Mitchell (1992). Based on our previous knowledge of the system a path diagram was proposed (Figure 1) and used to evaluate the relative contributions of direct and indirect effects of aphids and fungi (predictor or independent variables) on parasitoids (response or dependent variable). The main feature of the path diagram is the asymmetric effect of the fungus on the parasitoid, which is justified in the asymmetric

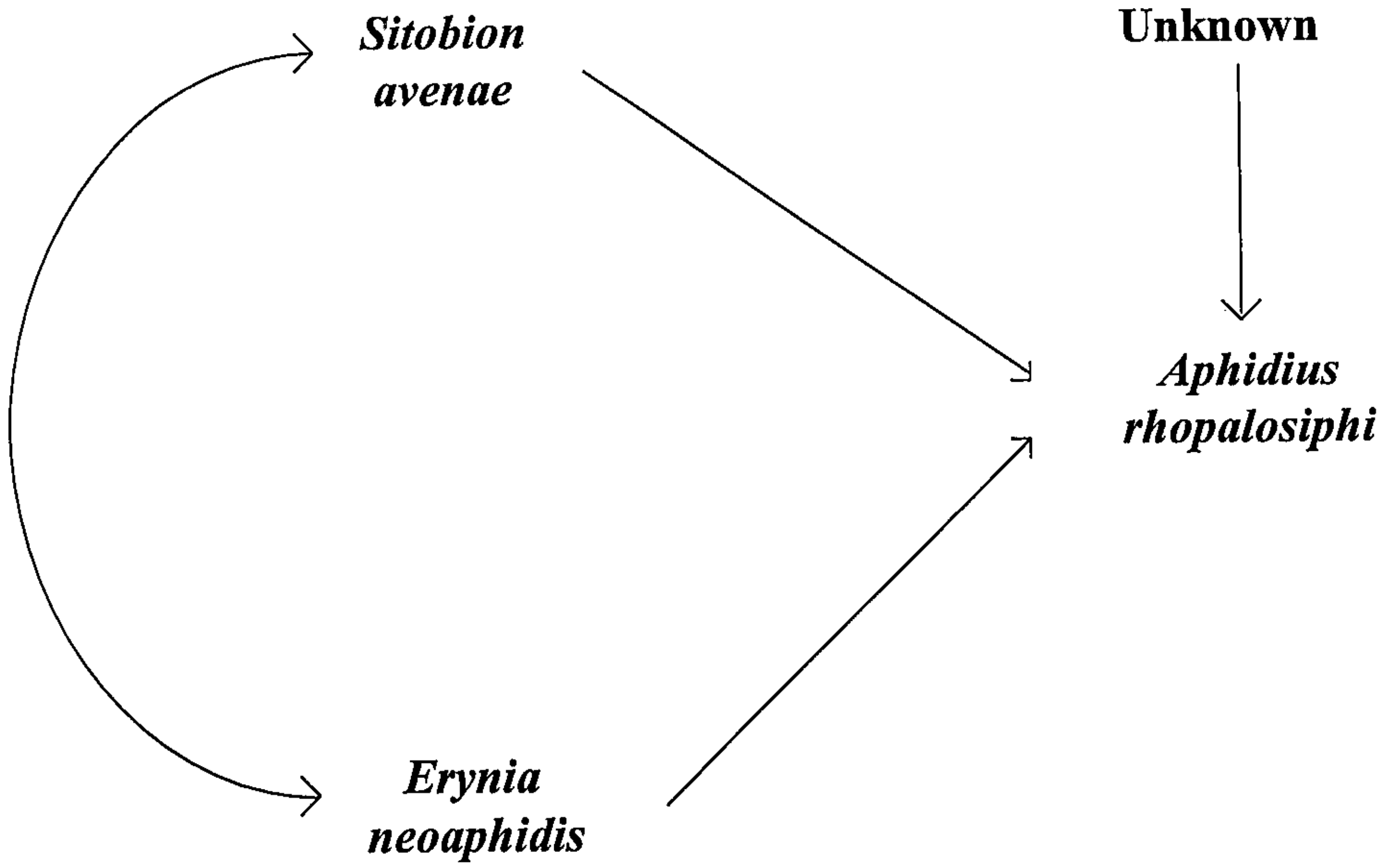

Figure 1. Path diagram showing the direct and indirect effects analysed in this study.

interspecific effect detected in experiments at the individual scale, *i.e.* there was a priority effect of the fungus that resulted in parasitoid successful emergence when oviposition took place several days in advance (Fuentes-Contreras *et al.* in press).

Data from population dynamics of aphids, parasitoids and fungi in treatment combinations that included all species were used for path analysis. Due to low sample size (N = 3), both wheat treatments were pooled. Density and PCPGR data were averaged through time to avoid pseudoreplication (see Smith *et al.* 1997). Densities and PCPGR were analysed with standard multiple regression, obtaining the correlation and the standardised regression coefficients necessary to calculate path coefficients. Assumptions of uncorrelated residuals and linear additive relationships among varaibles were examined.

RESULTS

Evaluation of indirect effects with ANOVA. Results of parasitoid and fungus population dynamics are summarised as mean values across all sampling dates in Table 1 and 2 respectively. These values are presented solely to describe broad patterns of population change. Density of parasitoids was lower in the resistant wheat cultivar (M.S. = 0.0059, F(1,8) = 8.3, P = 0.021), in presence of fungi (M.S. = 0.0153, F(1,8) = 21.5, P = 0.002) and was significantly affected by time (M.S. = 0.0215, F(6, 48) = 57.7, P < 0.001) (Table 1). There were significant statistical interactions between wheat cultivar and fungus (M.S. = 0.0055, F(1,8) = 7.7, P = 0.024), fungus and time (M.S. = 0.0043, F(6,48) = 11.6, P < 0.001) and the full interaction between the three variables was also significant (M.S. = 0.0017, F(6,48) = 4.7, P < 0.001). Finally, the statistical interaction between wheat cultivar and time was non-significant (M.S. = 0.0017, F(6,48) = 4.7, P = 0.065).

Table 1. Effects of wheat cultivar and presence of the fungus on density and PCPGR of the parasitoid. N = 3.

Wheat Cultivar	Fungus	Density mean (s.e.)	PCPGR mean (s.e.)
Huenufén	Absent	2.30 (0.38)	0.00400 (0.00015)
Huenufén	Present	0.66 (0.11)	0.00110 (0.00021)
Naofén	Absent	1.84 (0.31)	0.00117 (0.00068)
Naofén	Present	0.65 (0.20)	0.00098 (0.00034)

PCPGR of the parasitoid showed similar results (Table 1), it was lower in the resistant wheat cultivar (M.S. = 0.00002, F(1,8) = 11.9, P = 0.009), in presence of fungi (M.S. = 0.00016, F(1,8) = 87.3, P < 0.001) and was significantly affected by time (M.S. = 0.00281, F(6,48) = 70.5, P < 0.001). The statistical interaction between wheat cultivar and fungus was significant (M.S. = 0.00002, F(1,8) = 9.3, P = 0.016) as well as all remaining first order statistical interactions and the full interaction between the three variables (P < 0.05).

Table 2. Effects of wheat cultivar and presence of the parasitoid on density and PCPGR of the fungus N = 3.

Wheat Cultivar	Parasitoid	Density mean (s.e.)	PCPGR mean (s.e.)
Huenufén	Absent	0.91 (0.04)	0.00302 (0.00016)
Huenufén	Present	0.82 (0.05)	0.00285 (0.00017)
Naofén	Absent	0.87 (0.11)	0.00296 (0.00036)
Naofén	Present	0.90 (0.08)	0.00286 (0.00018)

Density of the fungi was not significantly affected either by wheat cultivar (M.S. = 0.00002, F(1,8) = 0.1, P = 0.752) or parasitoid presence (M.S. = 0.00001, F(1,8) = 0.07, P = 0.796) (Table 2). There were significant differences through time (M.S. = 0.01080, F(6,48) = 7.7, P = 0.024), but no significant statistical interactions of first order or between the three independent variables. Similar results were obtained for PCPGR of the fungi (Table 2), with time as the only significant effect (M.S. = 0.00007, F(6,48) = 6.86, P < 0.001). Finally, since an asymmetric effect of the fungus on the parasitoid was observed, the ANOVA results agreed with the path diagram initially proposed.

Evaluation of indirect effects with path analysis. Multiple regression of the effects of aphid and fungus densities on parasitoid density was not significant (M.S. = 1.37, F (2,3) = 0.47, R^2 = 0.24, P = 0.66). In the same vein, the partial regression coefficients of both aphid (b = 0.42, S.E.= 0.63, P = 0.55) and fungus density (b = 0.12, S.E. = 0.63, P = 0.86) on parasitoid density were non-significant.

Since calculation of direct and indirect effects do not require significant partial regression coefficients, we evaluated the relative magnitude of path coefficients representing direct and indirect effects according to the path diagram shown in Figure 1. The correlation between aphid and parasitoid density was explained mainly by the direct effect of the aphid on the parasitoid, while the indirect effect through the fungus is negligible (Table 3). On the other hand, the correlation between fungus and parasitoid density was explained to a higher extent by indirect effects of the fungus through aphid density than by direct effect of the fungus on the parasitoid (Table 3).

Results of PCPGR were similar to those obtained with density, i.e. a non-significant regression with non-significant partial regression coefficients (P > 0.05). Correlation between aphid and parasitoid PCPGR was explained mainly by direct effects and to a lesser extent by a negative indirect effect mediated by the fungus PCPGR (Table 3). Furthermore, correlation between fungus and parasitoid PCPGR was very low and influenced by direct negative effects counteracted by positive indirect effects through aphid PCPGR (Table 3).

Table 3. Partition of total correlation between the variables of the path diagram shown in Figure 1, evaluated through changes in density and *per capita* population growth rate (PCPGR) of the studied species. Direct and indirect effects were calculated based on partial regression coefficients.

Interaction	Response Variable	Direct Effect	Indirect Effect	Total Correlation
Aphid-Parasitoid	Density	0.41	0.07	0.48
Fungus-Parasitoid	Density	0.11	0.25	0.36
Aphid-Parasitoid	PCPGR	0.74	-0.10	0.64
Fungus-Parasitoid	PCPGR	-0.24	0.30	0.06

DISCUSSION

The experimental results analysed by ANOVA detected indirect effects of wheat resistance and fungus presence on parasitoid density. This indirect effect is result of interaction modifications and interaction chains, *i.e.* changes in both the *per capita* interspecific effects and densities of the studied species.

Significant interaction modifications now detected, agreed with previous analyses of antagonistic interactions between parasitoid and fungus at individual level. Those studies showed that resistant wheat cultivars (higher Hx levels) increased developmental time of parasitoids (Fuentes-Contreras *et al.* 1996, Fuentes-Contreras & Niemeyer 1998), which resulted in reduced parasitoid survival in aphid hosts also infected with the fungus (Fuentes-Contreras *et al.* in press).

Path analysis yielded similar conclusions about relative importance of direct and indirect effects evaluated through changes in density and PCPGR of the species studied. Direct effects were more important than indirect ones to explain the correlation between aphids and parasitoids, while the magnitude of indirect effects were of comparable importance in explaining correlations between fungi and parasitoids. Unfortunately, the statistical testing of path coefficients is far from straightforward (Smith *et al.* 1997), and since in our path diagram the number of equations to describe the model is equal to the number of parameters to be estimated, no maximum-likelihood methods based on structural equation modeling procedures can be applied (Smith *et al.* 1997).

The magnitude of indirect effects were more important to explain the correlation between fungus and parasitoid than between aphid and parasitoid. These results could be explained by the nature of the fungus-parasitoid interaction. During development within the same aphid host, the fungus can not infect the inmature stages of the parasitoid (Powell *et al.* 1986), therefore they interact indirectly through exploitative competition (Wootton 1994a) rather than by intraguild predation (Polis & Holt 1992, Rosenheim *et al.* 1993). On the other hand, during oviposition or infection processes direct interactions are absent since the fungus does not infect the adult parasitoid (Powell *et al.* 1986, Brobyn *et al.* 1988), nor the parasitoid serves as a vector of infective spores of the fungus (Fuentes-Contreras *et al.* in press). Furthermore, fungus infection of aphids is indirectly increased in the presence of parasitoids, an effect likely to be mediated by an increase in aphid activity resulting in a higher probability of encounter with infective conidia (Fuentes-Contreras *et al.* in press).

Laboratory results allowed us to recognise indirect interactions and to disentangle their mechanisms in the system under study. Under field conditions, such as cereal fields in temperate areas, the species studied represent main components of this assemblage coexisting spatially and temporally during the growing season (Wratten & Powell 1991). Depending on wether and on pesticides application, cereal aphids can develop considerable populations on both susceptible and partially resistant wheat cultivars, thus supporting large populations of parasitoids and entomopathogenic fungi. Although no experimental test of competition has been performed, correlational evidence suggests competitive interactions among parasitoids and fungi in cereal crops. We propose that such competitive interactions already observed in the laboratory, and potentially present in the field may also be affected by wheat resistance. In this scenario, further support to the relevance of indirect interactions in assemblages of insects associated to agricultural systems will be obtained (Settle & Wilson 1990, Rosenheim et al. 1993, Ferguson & Stiling 1996, Evans & England 1996, Müller & Godfray 1997).

ANOVA and path analysis seem to represent complementary statistical techniques, which can be sequentially applied in order to evaluate the presence and relative importance of indirect effects (Wootton 1994a, b). The experimental manipulation of one species and the evaluation of covariation of other species via path analysis, have allowed the identification of strongly interacting species likely to be involved in indirect interactions. Based on this information further hypothetical path diagrams can be tested with additional manipulative experiments (Wootton 1994b). Beside this approach, Smith *et al.* (1997) evaluated whether path analysis could detect direct and indirect interactions previously established by experimental manipulations and ANOVA. Such analysis concluded that the utility of path analysis is severely hampered by inappropriate specifications in the path diagram, which often resulted in varying and uninterpretable results. Finally, they suggest that application of this statistical tool may be more useful in simple and controlled laboratory systems. Our results are partially supporting this last assertion, although low sample size reduced the power of the multiple regression analysis, which resulted in non-significant models and partial regression coeficients explaining a low proportion of total observed variability.

In conclusion, indirect effects of wheat resistance on interspecific interactions between parasitoid and fungus at the population level were detected by ANOVA. Such indirect effects were caused by interaction chains and interaction modifications, as evidenced by significant statistical interactions between the effects of the fungus and wheat resistance on both density and PCPGR of the parasitoid. Path analysis also detected indirect effects of relative importance between fungus and parasitoid, although the amount of variability explained by the model was rather low.

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5. FINAL DISCUSSION AND CONCLUSIONS

Effects of wheat resistance on aphids, parasitoids and fungi. As previously reported, significant reductions of mean relative growth rate (MRGR) of the cereal aphid *Sitobion avenae* were observed in wheat cultivars with high levels of hydroxamic acids (Hx) relative to low levels of Hx. In particular, the wheat cultivar Huenufén and Naofén were regarded as susceptible and partially resistant respectively against this aphid.

To evaluate the influence of host-plant resistance on the development of parasitoids, an initial study comparing a specialist and a generalist species was conducted. The specialist parasitoid *Aphidius rhopalosiphi* increased its developmental time, with no significant changes in survival or adult body size, on wheat cultivars in relation to more susceptible oat cultivars, a cereal species lacking Hx. On the other hand, no effects of wheat resistance were observed on the generalist parasitoid *Ephedrus plagiator.* Such results suggested that the specialist parasitoid was able to modify its life history attributes to encompass the growth patterns of its cereal aphid host on plants with different levels of resistance. Therefore, further work was undertaken with this parasitoid species.

When wheat cultivars differing in their resistance level were used to evaluate their influence on *A. rhopalosiphi* development, a significant increase in developmental time with non-significant differences in adult body size or survival was observed. The increase in developmental time was mainly accounted for by an increase in egg-larval developmental time, a period during which the parasitoid larva is feeding and growing inside the aphid host. Since previous studies have shown that aphid parasitoids use resources to maximise size and then to reduce developmental time, the reduction in parasitoid performance based on the reduction of aphid MRGR might be finally compensated by an increase in developmental time in order to maintain adult body size. Since the increase in parasitoid developmental time was observed on: i) wheat cultivars in relation to oat cultivars, ii) wheat cultivars with higher as compared with lower Hx levels, and iii) artificial diets with higher Hx levels, I conclude that these effects was caused by Hx. Since wheat resistance based on Hx reduced parasitoid performance, evidenced as an increase in developmental time, I support the hypothesis of indirect effects of wheat resistance on the parasitoid *A. rhopalosiphi* (hypothesis 1.1). These results agreed with the previous reports of tritrophic effects of the host plants on natural enemies of herbivorous insects in several natural and managed insect-plant systems (Price *et al.* 1980; Hare 1992), and particularly with natural enemies of aphids on crops (van Emden 1995).

Wheat resistance also affected the oviposition success of the parasitoid through changes in the efficiency of defensive behaviour of aphids during parasitoid attacks. Parasitoid or aphid behaviour was not significantly affected, *i.e.* number of oviposition attempts or defensive reactions of aphids was similar on susceptible and resistant wheat cultivars. However, the number of successful stabs on aphids was higher in the more resistant wheat cultivar. High Hx concentration in the plant is known to reduce aphid ingestion rate, which probably caused the observed reduction in aphid MRGR. Consequently, body size of aphids of similar age is lower in the wheat cultivar with higher Hx level, which in turn decreases the probability of counteracting parasitoid stabbing attacks during oviposition. Since oviposition success of *A. rhopalosiphi* was lower in the resistant wheat cultivar in relation to the susceptible wheat cultivar, I support hypothesis of indirect effects of wheat resistance on the oviposition process of the parasitoid *A. rhopalosiphi* (hypothesis 1.2). The relationship between host body size and success of parasitoid oviposition have been found in several systems (Gross 1993), including aphids (Mackauer *et al.* 1996). However, such direct interaction between aphid and parasitoid is affected indirectly by the host-plant, a tritrophic effect previously proposed in the literature, but not previously supported empirically (van Emden & Wratten 1990).

The obtained results can be also discussed in relation to behavioural and life history characteristics of *A. rhopalosiphi.* This species present high fecundity, moderate longevity and short handling time, and hence foraging females probably are limited by time rather than by available eggs. In this scenario, parasitoid females should not invest time and energy in large aphids, because they are more successful in avoiding oviposition and hence reduce the limited time available for the parasitoid to find new hosts for its large number of eggs (Mackauer *et al.* 1996). In the same vein, life-history strategy of this parasitoid is based on a low resource investment in each egg with development highly dependent on host-derived substances. In this case, changes in aphid quality associated with the host-plant are expected to produce adaptive adjustment in life history attributes of the parasitoid, such as developmental time or size instead of attributes related with fecundity (Mackauer *et al.* 1996).

On the contrary, wheat resistance did not show any significant effect on the fungus *Erynia neoaphidis.* Fungal survival or developmental time was not influenced by wheat resistance, even though different doses (lethal concentrations) of infecting spores were used. Since wheat resistance did not affected the performance of the entomopathogenic fungus *E. neoaphidis*, I reject the hypothesis of indirect effects of wheat resistance on *E. neoaphidis.* This indirect effect was previously proposed by van Emden & Wratten (1990), but still there is no empirical support for this hypothesis.

Effects of wheat resistance on parasitoid-fungus interaction at individual level. Interspecific interactions between parasitoids and entomopathogenic fungi may include: i) intra-host interactions, during ontogenetic development of the natural enemies within a shared host; and ii) extra-host interactions, which involve eventual transmission of the pathogen by the parasitoids.

Previous studies (Powell *et al.* 1986) have shown that the result of the intra-host interaction between parasitoids and fungi is asymmetrical and determined by the timing of parasitoid oviposition and fungal infection. The fungus usually outcompetes the parasitoid, unless parasitoid oviposition has been held at least four days before fungal infection. Since the parasitoid was affected by wheat resistance increasing its developmental time while the fungus was not affected by wheat resistance at all, wheat resistance modified the result of the intra-host interaction between parasitoid and fungus through changes in the advantage required by the parasitoid to complete its development in aphid hosts also infected by the fungus. This was observed as a significant reduction of survival of the parasitoid on the resistant wheat cultivar, when the competitive outcome of the interaction was favourable for either parasitoid or fungal development (four days of parasitoid advantage). Before and after this period the influence of plant resistance was not significant. Since the parasitoid was more affected than the fungus by wheat resistance, parasitoid survival in shared aphid hosts was reduced further increasing the time advantage required to complete its development. Based on these results, I support the hypothesis of the indirect effect of wheat resistance on the interaction between the parasitoid *A. rhopalosiphi* and the fungus *E. neoaphidis* at the intra-host level (hypothesis 1.3). Tritrophic effects have been studied emphasising the effects of the host plant on single species of natural enemies (autoecological approach) (Hare 1992), while this thesis first recognise the importance of tritrophic effects of the host plant on interspecific interactions between species of natural enemies distantly related in phylogenetic terms.

On the other hand, extra-host interactions were not affected by wheat resistance, *i.e.* no fungal dispersal mediated by parasitoids was observed either on resistant or susceptible wheat cultivars. However, regardless of wheat cultivar an increase in fungal infection of the aphids was observed when parasitoids foraged in presence of sporulating aphid cadavers, apparently caused by an increase in aphid activity which enhances their probability of fungal infection. The non significant effect of wheat resistance on the interaction between parasitoid and fungus allow me to reject the hypothesis of an indirect effect of wheat resistance on the extra-host interaction between the parasitoid *A. rhopalosiphi* and the fungus *E. neoaphidis* (hypothesis 1.4). This was the first attempt to evaluate the existence of indirect effects of the host plant on the extra-host interactions between parasitoids and fungi.

The effect of wheat resistance on the intra-host interaction between parasitoid and fungus could influence the interspecific interaction at the population level, such as has been suggested by mathematical models previously developed for microparasite-parasitoid interactions (Hochberg *et al.* 1990). In this case, wheat resistance may cause "interaction chains", *i.e.* changes in density of aphids which in turn affected density of parasitoid and fungus, or "interaction modifications", *i.e.* changes caused by wheat resistance in aphid quality that affected *per capita* interspecific effects between parasitoid and fungus (Wootton 1994).

Effect of wheat resistance on parasitoid-fungus interaction at the population level. Indirect effects of wheat resistance on interspecific interactions between parasitoid and fungus at the population level were detected by ANOVA. Such indirect effects were caused by interaction chains and interaction modifications, as evidenced by significant statistical interactions between the effects of the fungus and wheat resistance on both density and *per capita* population growth rate (PCPGR) of the parasitoid. On the contrary, no significant statistical interactions between the effects of the parasitoid and wheat resistance on both density and PCPGR of the fungus were observed. These results agreed with: i) the reduction of parasitoid survival when the outcome of the interaction at the intra-host scale was not defined, hence causing an increase in the priority effect required by the parasitoid to complete its development, and ii) the non-significant influence of wheat resistance on the extra-host interaction. Finally, path analysis also detected indirect effects of relative importance between fungus and parasitoid, although the amount of variability explained by the model was rather low. The significant statistical interaction between wheat resistance and fungus effects on PCPGR of the parasitoid, and the non significant interaction between wheat resistance and parasitoid effects on PCPGR of the fungus allow me to support the hypothesis of the indirect effect of wheat resistance on the *per capita* interspecific effect of the fungus on the parasitoid, but not vice versa (hypothesis 2.1).

In summary, I have found evidence to support the presence of indirect interactions caused by wheat resistance and fungus effects on parasitoid population dynamics under controlled laboratory conditions. Such indirect interaction is caused by both changes in density and *per capita* interspecific effect of the fungus on the parasitoid, and not vice versa. A mechanism likely to explain these results is the increase in developmental time of the parasitoid on the resistant wheat cultivar in relation to the susceptible wheat cultivar. This delay in parasitoid development caused a decrease in parasitoid survival in the host shared with the fungus, when the outcome of the intra-host interaction can result in either parasitoid or fungus successful development (day four after oviposition). This thesis provides mechanistic evidences at the individual level and statistical evidences at the population level on the importance of interaction modifications caused by host-plant resistance.

As a general conclusion the results outlined in this thesis support the general hypothesis of the existence and relevance of indirect interactions of the host plant on the interspecific interaction between natural enemies at the third trophic level. Indirect interactions have been widely observed in several ecological systems (Wootton 1994), but the system under study showed a new type of indirect interaction through which host-plants can not only affect single species of natural enemies, but also the interspecific interactions at the third trophic level. Particularly important is the detection of interaction modifications at individual and population levels, which combines the statistical detection of interaction modifications with the mechanistic explanation of these results (Kareiva 1994).

Parasitoids and fungi were chosen based on their biological differences and my hypothesis may not be supported with natural enemies phylogenetically closer (Hochberg & Lawton 1990), which may be affected by the resistance of the host plant in a more similar way. Therefore, the results of this thesis may be conditioned by the biological characteristics of the natural enemies selected in this study system.

This thesis emphasise the influence of bottom-up influences and tritrophic effects on the structure of assemblages of herbivorous insects and their natural enemies. Tritrophic effects will be no longer restricted to single species of natural, enemies, whereas a more synecological approach should be also considered.

In general terms, this thesis also further emphasise the relevance of indirect interactions and interaction modifications in multi-species assemblages, *i.e.* the dynamics of even simple multi-species assemblages under laboratory conditions can not be extrapolated from the dynamics of isolated species. Such results may indicate that indirect interactions and particularly interaction modifications should be very common in complex ecological systems (Wootton 1994).

The analytical approach chosen in this thesis allowed to clarify potential mechanisms involved, integrating studies at population and individual levels. Finally, from a methodological point of view, when experimental manipulation is feasible, the ANOVA is recognised as a powerful statistical tool to detect the existence of indirect interactions and interaction modifications (Billick & Case 1994). This results agreed with other studies aimed to evaluate the benefits of ANOVA in relation to path analysis (Smith *et al.* 1997).

The approach and the experimental system of this thesis were selected to evaluate a specific hypothesis of indirect interaction. In this sense, the results should be interpreted focusing on their generality rather than on their realism. However, a general framework to evaluate the importance of tritrophic effects under field conditions may be possible under particular circumstances. For instance, in field situations with no significant levels of other groups of natural enemies like predators, and where coexistence of the studied species at important densities occurs during the growing season, the results observed in laboratory experiments may also be found. Field studies will be necessary to support this view and to evaluate whether the encouraging insights from laboratory experiments are actually effective for the compatible use of host-plant resistance and natural enemies in integrated pest management. Finally, the generality of the results herein obtained should be tested in further studies evaluating the influence of host-plant secondary metabolites on interspecific interactions between herbivorous insects and their natural enemies.

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