

Direct and indirect effects of wheat cultivars with different levels of resistance on parasitoids and entomopathogenic fungi of cereal aphids¹

Eduardo FUENTES-CONTRERAS², Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca, Casilla 747, Talca, Chile.

Hermann M. NIEMEYER, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

Abstract: Laboratory experiments were performed to evaluate at the population level the influence of host-plant resistance on interspecific interactions of the cereal aphid *Sitobion avenae* with two phylogenetically distant species of natural enemies, the parasitoid *Aphidius rhopalosiphi* and the entomopathogenic fungus *Pandora neoaphidis*. Indirect effects of wheat resistance on interspecific interactions between parasitoid and fungus were caused by interaction modifications, *i.e.*, changes in per capita interspecific effects, as evidenced by significant statistical interactions between the effects of the fungus and wheat resistance on per capita population growth rate (PCPGR) of the parasitoid. Reduction by the fungus of parasitoid PCPGR was lower on the susceptible wheat than on the resistant wheat; this was interpreted as the population level expression of the asymmetric competitive effect of the fungus on parasitoid development. As expected from the asymmetric interaction between parasitoid and fungus, no indirect effect of the parasitoid on the fungus was found, as evidenced by no significant statistical interactions between wheat cultivar and parasitoid. However, increased fungus PCPGR was found in the presence of the parasitoids regardless of wheat cultivar; this may also be explained by increased fungal infection during parasitoid foraging. Path analysis, a statistical technique to discern the relative contributions of direct and indirect effects, also detected indirect effects between fungus and parasitoid.

Keywords: tritrophic effects, indirect interactions, path analysis, interaction modification, interaction chain, *Aphidius rhopalosiphi*, *Pandora neoaphidis*, *Sitobion avenae*.

Résumé : Par le biais d'expériences en laboratoire, nous avons évalué l'influence de la résistance d'une plante-hôte sur les interactions interspécifiques du puceron des céréales *Sitobion avenae* et de deux de ses ennemis naturels distants d'un point de vue phylogénétique, le parasitoïde *Aphidius rhopalosiphi* et le champignon entomopathogène *Pandora neoaphidis*. Les effets indirects de la résistance du blé sur les interactions entre le parasitoïde et le champignon se traduisent par des modifications statistiquement significatives des interactions entre les effets du champignon et de la résistance du blé sur le taux de croissance per capita de la population (TCPCP) du parasitoïde. Le champignon a entraîné une plus faible diminution du TCPCP du parasitoïde sur le blé sensible que sur le blé résistant, ce qui peut s'expliquer par l'expression, au niveau de la population, de l'effet compétitif asymétrique du champignon sur le développement du parasite. La présence d'une interaction asymétrique entre le parasitoïde et le champignon suggérait qu'aucun effet indirect du parasitoïde sur le champignon ne pouvait exister. Nous avons confirmé cette hypothèse en ne décelant aucune interaction significative d'un point de vue statistique entre le cultivar de blé et le parasitoïde. Néanmoins, un TCPCP plus élevé a été trouvé chez le champignon en présence de parasitoïdes (peu importe le cultivar), ce qui pourrait s'expliquer par une infection fongique plus sévère lorsque le parasitoïde se nourrit. Grâce à une analyse de direction, une technique statistique permettant de déterminer les contributions relatives des effets directs et indirects, nous avons également pu détecter des effets indirects entre le champignon et le parasitoïde.

Mots-clés : effets trophiques à 3 niveaux, interactions indirectes, analyse de direction, modification d'interaction, chaîne d'interactions, *Aphidius rhopalosiphi*, *Pandora neoaphidis*, *Sitobion avenae*.

Introduction

Indirect interactions arise when additional species affect direct interspecific interactions between two focal species (Wootton, 1994a). Such indirect interactions may constitute interaction chains, *i.e.*, changes in density of the additional species that 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, 1994a). Thus, while interaction chains are epiphenomena arising from the addition of direct effects between pairs of species, interaction modifications show the relevance of non-additive components and

preclude the extrapolation of interactions in multi-species systems from knowledge of interactions between pairs of species (Billick & Case, 1994).

Multitrophic interactions between insect herbivores and their natural enemies frequently show indirect interactions (Evans & England, 1996; Müller & Godfray, 1997; Brodeur & Rosenheim, 2000; Wajnberg & Scott, 2000). Tritrophic effects, through which plant traits may indirectly affect natural enemies of herbivorous insects, are a well-documented example of these indirect interactions (reviewed by Hare, 1992). Several mechanisms for tritrophic effects have been described, but host-plant resistance against herbivores mediated by secondary metabolites is of major relevance for aphids, as well as for their natural enemies (van Emden,

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²Author for correspondence.

1995; Dixon, 1998). Wheat chemical resistance against cereal aphids is based mainly on hydroxamic acids (Hx), a family of secondary metabolites characteristic of Gramineae (Poaceae) (Niemeyer & Pérez, 1995). Tritrophic effects associated with this mechanism of wheat resistance have been found for natural enemies of cereal aphids at the individual level (Martos, Givovich & Niemeyer, 1992; Fuentes-Contreras & Niemeyer, 1998) and may even affect interspecific interactions between phylogenetically distant groups of them, such as parasitoids and entomopathogenic fungi (Fuentes-Contreras, Pell & Niemeyer, 1998).

At the population level, Fuentes-Contreras and Niemeyer (2000) found that the joint action of the parasitoid *Aphidius rhopalosiphi* De Stephani-Pérez (Hymenoptera: Braconidae) and the entomopathogenic fungus *Pandora neoaphidis* Remaudière et Hennebert (Zygomycetes: Entomophthorales) was complementary to the effect of wheat resistance in reducing the density and population growth rate of the cereal aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). However, previously undetected interactions apparently associated with density-dependent interactions between the entomopathogenic fungus and the aphid, which were not observed at the individual level (Fuentes-Contreras, Pell & Niemeyer, 1998), were found statistically.

Population dynamics may provide further evidence of the presence of indirect interactions, mediated by wheat resistance (cultivars) through the shared aphid host, on the natural enemies as focal species. Therefore, the work of Fuentes-Contreras and Niemeyer (2000) on the population dynamics of natural enemies is subjected to further analysis in the present article in order to address statistically the presence at the population level of indirect interactions (interaction chains or interaction modifications) between the parasitoid *A. rhopalosiphi* and the entomopathogenic fungus *P. neoaphidis* of the cereal aphid *S. avenae*.

The parasitoid-fungus interaction has been described as strongly asymmetrical, *i.e.*, the competitive outcome normally favors the fungus due to its lower development time (Powell *et al.*, 1986). This asymmetry is further enhanced by wheat resistance because development time of the parasitoid is increased (Fuentes-Contreras, Pell & Niemeyer, 1998). Hence, we predicted that there would be significant statistical interactions between fungus and wheat cultivar treatments on the density and/or per capita population growth rates (PCPGR) of the parasitoid, but not between parasitoid and wheat treatments on the fungus. On the basis of these statistical interactions, we expected that the fungus would reduce the density and/or PCPGR of the parasitoid by a lesser amount on the susceptible wheat than it would on the resistant wheat. Furthermore, the relative contributions of direct and indirect effects were assessed using path analysis, with the expectation that we would observe correlations associated mainly with indirect effects of the fungus on the parasitoid and direct effects of the aphids on the parasitoid.

Methods

PLANT MATERIAL AND INSECT CULTURES

The parasitoid *Aphidius rhopalosiphi* and the cereal aphid *Sitobion avenae* were maintained on oats (*Avena sati-*

va L., cv. Nehuén), in order to obtain standardized insects not exposed to wheat resistance prior to the beginning of the experiments. *In vivo* cultures of *Pandora neoaphidis* on *S. avenae* feeding on oat were also maintained. Two spring wheat (*Triticum aestivum* L.) cultivars were used in the experiments: Huenufén (susceptible to aphids; Hx concentration on primary leaf of 6-day-old seedlings: $\bar{x} = 1.72 \pm 0.12$ mmoles kg⁻¹ fresh weight, $N = 6$) and Naofén (partially resistant toward aphids; Hx concentration on primary leaf of 6-day-old seedling: $\bar{x} = 3.02 \pm 0.17$ mmoles kg⁻¹ fresh weight, $N = 6$). All insect cultures were maintained at 23 °C ± 2 with a 16:8 (light:dark) photoperiod.

POPULATION EXPERIMENT

Plexiglass cages 40 cm × 40 cm × 40 cm were used to start 24 experimental units. Twelve 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 (fixed with water-soluble gum arabic on the tip of the seedlings), and 12 cages (six for each wheat cultivar), including six cages (three for each cultivar) that had previously received the aphid cadavers, received four previously mated parasitoid females each. This left six cages (three for each wheat cultivar) without infestation by either fungi or parasitoids. Plastic film was placed over all experimental cages to reduce ventilation and to promote fungal growth.

Pots containing new seedlings were introduced into the cages once a week, without removing the pots already present in the cage. Only when plants had died naturally from aphid attack were the pots removed; however, dead aphids with unemerged parasitoids or unsporulated fungi were left in the cage. The whole experiment lasted 56 days and was performed under the same temperature and lighting conditions as those described in the insect culture section.

Every four to five days, ten tillers were removed from each experimental unit, and live aphids as well as dead aphids with unemerged mummies or unsporulated fungi were counted. Mummies produced by the parasitoid and aphid cadavers produced by the fungus can be readily distinguished by their rounded or spindle body shape, respectively. In doubtful cases, the mummies were placed in a humid Petri dish to evaluate production of spores. Mean values of these ten samples were used to estimate the aphid, parasitoid, and fungus population densities in each experimental unit, *i.e.*, number of aphids, parasitoid mummies, and aphid cadavers produced by the fungus per tiller (N_t). Densities of the natural enemies were expressed as absolute per tiller values and relative values expressed in relation to aphid densities. This second, relative measurement has the advantage that it represents the natural enemy-to-aphid ratio, which measures the potential capacity of the natural enemy to control the aphid population. Population dynamics were monitored to the completion of nearly four parasitoid generations and nearly 12 fungus generations (*i.e.*, *ca* 14 days for each parasitoid generation and *ca* 5 days for each fungus generation).

ANALYSIS OF DATA

Absolute (per tiller) and relative (per aphid) densities of parasitoids and fungi were $\log(N_t + 10)$ transformed, while daily PCPGR of parasitoids and fungi were calculated as $1/\Delta t \log((N_{t+1} - N_t)N_t^{-1}) \text{ day}^{-1}$. Given that fungi are modular organisms (Harper, Rosen & White, 1986), the per capita concept does not have a direct application as with individual organisms 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).

Indirect interactions may be detected when density, population growth rate, or any variable associated with performance of the focal species shows significant statistical interactions between treatments with and without the additional species within ANOVA designs (Case & Bender, 1981; Wilbur & Fauth, 1990). Interaction modifications can be distinguished from interaction chains when per capita population growth rates of the focal species are included in the ANOVA as the dependent variable (Billick & Case, 1994). However, an ANOVA-based manipulative approach is logistically difficult while maintaining an adequate level of replication in multispecies systems; hence, the combined utilization of manipulative experiments and path analysis has been recommended (Wootton, 1994b). Path analysis is a statistical technique that breaks down the overall correlation between predictor and response variables into direct effects, indirect effects mediated by other variables, and spurious effects due to common causes, allowing estimation of their relative magnitude (Sokal & Rohlf, 1995).

ANALYSIS OF VARIANCE

To evaluate the effects of wheat resistance (cultivar) and fungi on parasitoids, as well as the effects of wheat resistance and parasitoids on fungi, an ANOVA design for measures repeated over time was performed. When density or PCPGR of the parasitoid was the dependent variable, wheat cultivar (Huenufén and Naofén), fungus (presence and absence), and time (7 sampling dates as within-subject repeated measures, started when parasitoids or fungi were detected in the sampling) were the independent variables. Reciprocally, when density or PCPGR of the fungus was the dependent variable, wheat cultivar (Huenufén and Naofén), parasitoid (present and absent), and time (7 sampling dates as within-subject repeated measures, started when parasitoids or fungi were detected in the sampling) were the independent variables. All statistical interactions of first and second order were analyzed. The Tukey test was used for multiple comparisons after significant effects were detected by ANOVA. For all variables, the data supported the assumptions of homoscedasticity (Barlett test, $P > 0.05$) and sphericity (Mauchley test, $P > 0.05$).

PATH ANALYSIS

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

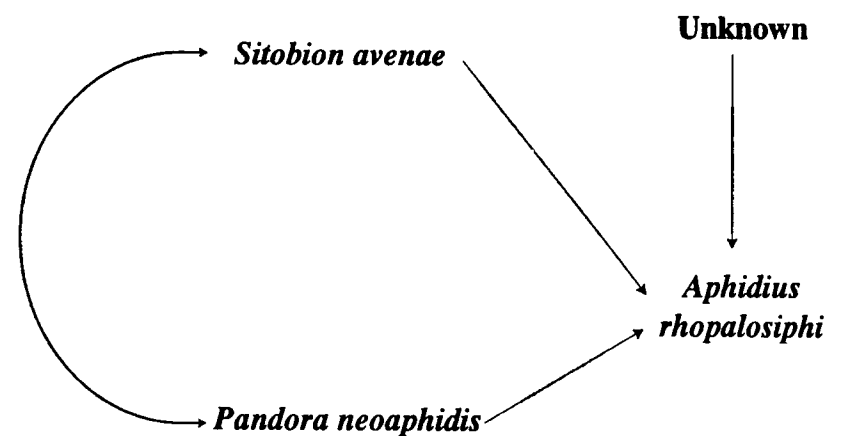


FIGURE 1. Path diagram used to evaluate direct and indirect effects on the parasitoid *Aphidius rhopalosiphi*. Straight arrows represent direct effects and curved arrow represents correlation between variables.

of the path diagram is the asymmetric effect of the fungus on the parasitoid, which is justified by the asymmetric interspecific effect detected in experiments at the individual scale, *i.e.*, there was a priority effect of the fungus that resulted in successful parasitoid emergence when oviposition took place several days before fungal infection (Powell *et al.*, 1986; Fuentes-Contreras, Pell & Niemeyer, 1998).

Data from the population dynamics of aphids, parasitoids, and fungi in treatment combinations that included all species were used for path analysis. A comparative study of path analyses for each wheat cultivar was not conducted due to the low sample size ($N = 3$) available. Instead, a single path analysis was performed, pooling data of both wheat treatments. Pooling of the data included the effect of wheat resistance through changes in aphid density or PCPGR into the analysis. The pooling of wheat resistance treatments increased the variance of the data, improving the performance of the path analysis in disentangling direct and indirect effects (Smith, Brown & Valone, 1997).

Density and PCPGR data were averaged through time, using the same method as Smith, Brown and Valone (1997), in order to avoid pseudoreplication. Absolute densities and PCPGR were analyzed with standard multiple regression, obtaining the correlation and standardized regression coefficients necessary to calculate path coefficients. Assumptions of uncorrelated residuals and linear additive relationships among variables were examined.

Results

EVALUATION OF INDIRECT EFFECTS WITH ANOVA

The ANOVA results showed that absolute density (per tiller) of parasitoids was significantly affected by fungus, time, and the statistical interaction between these factors (Table I), but not by wheat cultivar or the statistical interactions between wheat cultivar and fungus, wheat cultivar and time, and all three factors (cultivar, fungus, and time; Table I). Regardless of the wheat cultivar, higher parasitoid absolute density was found in the absence of the fungus (Figure 2a). Similarly, the parasitoid relative density (per aphid), or parasitoid-aphid ratio, showed a significant effect of the fungus, time, and the interaction between these factors (Table I). The higher parasitoid-aphid ratios were found when the fungus was absent from both wheat cultivars (Table I, Figure 2b). There was no significant statistical

TABLE I. Significance of the ANOVA addressing the effects of wheat cultivar (C) and fungus (F) through time (T) on density and PCPGR of the parasitoid, as well as of the effects of wheat cultivar (C) and parasitoid (P) through time (T) on density and PCPGR of the fungus. The ANOVA were performed with absolute (per tiller) and relative (per aphid) values of natural enemy population variables.

Effects	Absolute (per tiller)		Relative (per aphid)	
	Density	PCPGR	Density	PCPGR
PARASITOID				
Between subjects ^a				
Cultivar (C)	0.09	0.006	0.88	0.004
Fungi (F)	< 0.001	0.005	0.002	0.001
C × F	0.19	0.009	0.96	0.56
Within subjects ^b				
Time (T)	< 0.001	< 0.001	< 0.001	< 0.001
T × C	0.85	0.93	0.65	0.30
T × F	< 0.001	< 0.001	< 0.001	0.019
T × C × F	0.09	0.94	0.31	0.89
FUNGUS				
Between subjects ^a				
Cultivar (C)	0.75	0.90	0.05	0.19
Parasitoid (P)	0.80	0.59	0.005	0.02
C × P	0.46	0.87	0.48	0.99
Within subjects ^b				
Time (T)	< 0.001	< 0.001	< 0.001	< 0.001
T × C	0.87	0.89	0.09	0.83
T × P	0.22	0.65	0.02	0.35
T × C × P	0.98	0.99	0.72	0.98

^a between subjects error term

interaction between cultivar and fungus, cultivar and time, or the three factors (Table I).

The ANOVA for PCPGR of the parasitoid absolute density and relative density (parasitoid-aphid ratio) detected significant effects of wheat cultivar and fungus (Table I). A significant interaction between wheat cultivar and fungus (C × F) was found for PCPGR evaluated as absolute density, which was caused by the highest PCPGR observed on the susceptible wheat cultivar without the fungus, but there were no significant differences between the other treatment combinations (Figure 2c). By contrast, this wheat cultivar-fungus interaction (C × F) was not significant when parasitoid-aphid ratio was used to estimate PCPGR (Table I, Figure 2d). In this case, the highest PCPGR was observed on the susceptible wheat without fungus, while the lowest PCPGR was found on the resistant wheat with fungus presence (Figure 2d). There were also significant statistical interactions between fungus and time, but not between time and wheat cultivar or the three factors (Table I).

Absolute density of the fungus was not significantly affected either by wheat cultivar or parasitoid presence (Table I, Figure 3a). There were significant differences through time, but no significant statistical interactions of first order or between the three independent variables were found (Table I). By contrast, relative density of the fungus (fungus-aphid ratio) was significantly affected by parasitoid presence and marginally as well by the wheat cultivar (Table I), although no interaction between parasitoid pres-

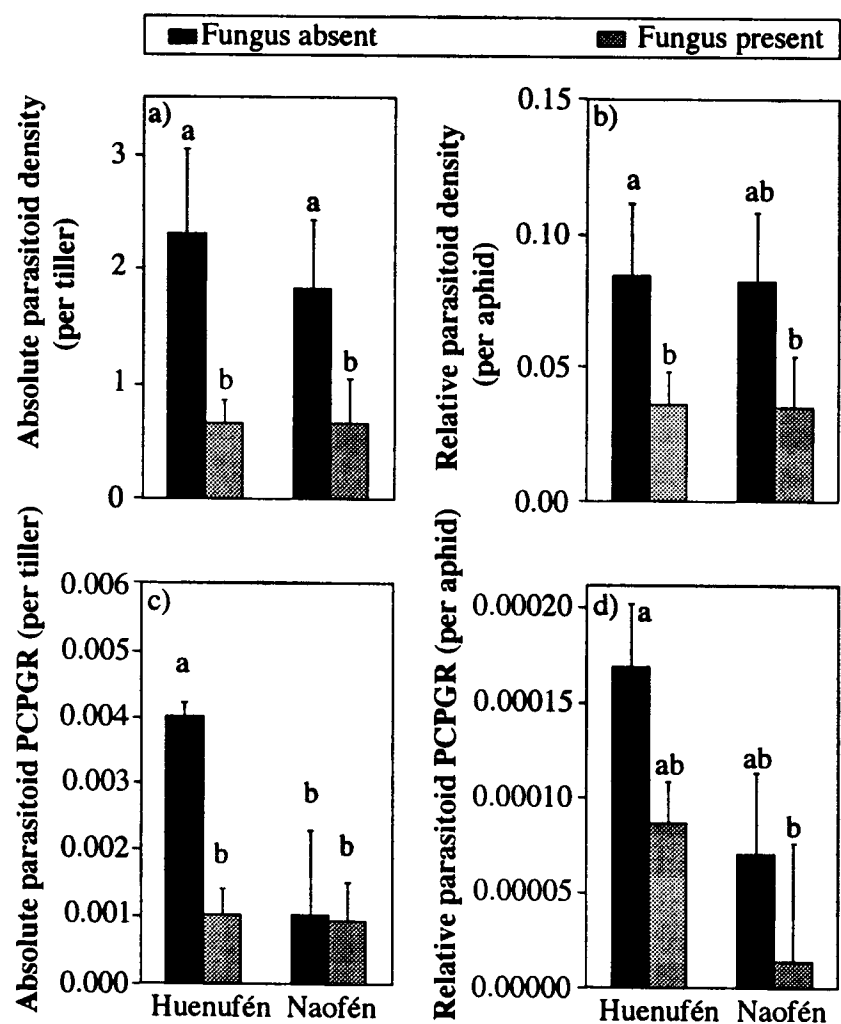


FIGURE 2. Effects of wheat cultivar and presence of the fungus on absolute, *i.e.*, per tiller (a), and relative, *i.e.*, per aphid (b), mean density of the parasitoid, and effects of wheat cultivar and presence of the fungus on absolute, *i.e.*, per tiller (c), and relative, *i.e.*, per aphid (d), PCPGR of the parasitoid. $N = 3$.

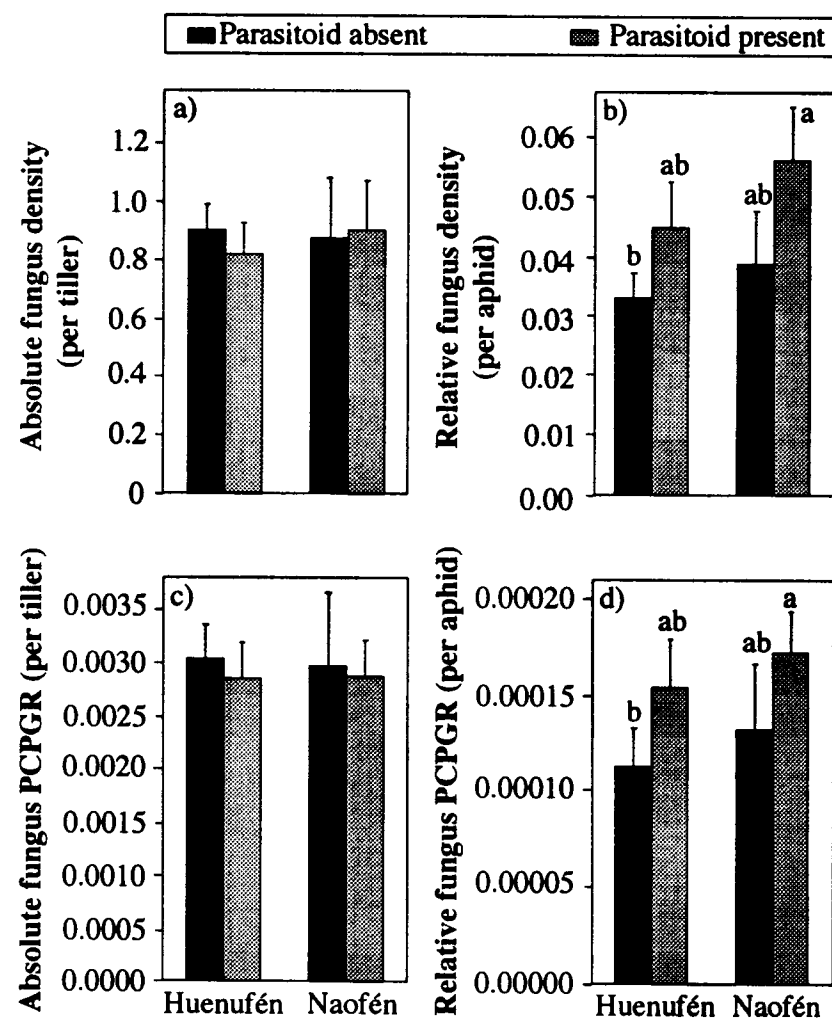


FIGURE 3. Effects of wheat cultivar and presence of the parasitoid on absolute, *i.e.*, per tiller (a), and relative, *i.e.*, per aphid (b), mean density of the fungus, and effects of wheat cultivar and presence of the parasitoid on absolute, *i.e.*, per tiller (c), and relative, *i.e.*, per aphid (d), PCPGR of the fungus. $N = 3$.

ence and wheat cultivar was found (Table I). The fungus-aphid ratio in the resistant wheat with parasitoids was higher than in the susceptible wheat without parasitoids (Figure 3b). There were also significant differences through time, and the first order interaction between time and parasitoid was significant as well (Table I).

The ANOVA for PCPGR of the fungus in absolute density (per tiller) only showed differences through time, with non-significant effects of wheat cultivar, parasitoid presence, or any statistical interaction (Table I, Figure 3c). Relative PCPGR of the fungus (fungus-aphid ratio) was significantly affected by parasitoid presence, with the highest PCPGR of the fungus-aphid ratio when the parasitoid was present in the resistant wheat (Figure 3d).

EVALUATION OF INDIRECT EFFECTS WITH PATH ANALYSIS

Multiple and partial regressions of the effects of aphid and fungus densities on parasitoid density were not significant ($P > 0.05$). In the same vein, the partial regression coefficients of both aphid and fungus density on parasitoid density were non-significant ($P > 0.05$).

Since calculation of direct and indirect effects does not require significant partial regression coefficients, the relative magnitude of path coefficients representing direct and indirect effects according to the path diagram shown in Figure 1 was evaluated. The correlation between aphid and parasitoid densities was explained mainly by the direct effect of the aphid on the parasitoid, while the indirect effect through the fungus was smaller (Table II). On the other hand, the correlation between fungus and parasitoid densities was explained more by indirect effects of the fungus through aphid density than by direct effect of the fungus on the parasitoid (Table II). However, a positive overall correlation between parasitoid and fungus density was observed, *i.e.*, high parasitoid density associated with high fungus density, which is inconsistent with the significant reduction of parasitoid density (Figure 2a) detected by the ANOVA (Table I) in treatments where the fungus was present.

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 (Table II). Furthermore, correlation between fungus and parasitoid PCPGR was very low and was influenced by direct negative effects counteracted by positive indirect effects through aphid PCPGR (Table II).

TABLE II. 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 species studied. Direct and indirect effects were calculated based on partial regression coefficients.

Interaction variable	Response effect	Direct effect	Indirect correlation	Total
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 ANOVA detected indirect effects of wheat resistance and fungus presence on the parasitoid at the population level, as revealed by the significant statistical interaction between wheat cultivar and fungus treatments ($C \times F$) on parasitoid PCPGR expressed in absolute (per tiller) terms. This indirect effect was the result of an interaction modification, *i.e.*, changes in the per capita interspecific effects of the species studied, but not an interaction chain, *i.e.*, changes mediated by the densities of the involved species. The $C \times F$ statistical interaction is explained in biological terms by the higher parasitoid PCPGR on the susceptible wheat without fungus in relation to all other treatments (Figure 2c). Since aphid density was significantly higher on the susceptible wheat without fungus (Fuentes-Contreras & Niemeyer, 2000), the differences between the susceptible wheat without fungus and other treatments were reduced when the parasitoid PCPGR was expressed relative to aphid density (parasitoid/aphid ratio) (Figure 2d), and the $C \times F$ interaction became non-significant (Table I). The loss of the significance of the $C \times F$ interaction when parasitoid density was expressed relative to aphid density (parasitoid/aphid ratio) (Table I) further reinforced our interpretation that indirect effects of fungus and wheat cultivar on the parasitoid were mediated by the aphids.

Furthermore, as expected, no indirect interactions of wheat resistance and parasitoid ($C \times P$) on fungus density or PCPGR were detected with the dependant variables expressed either in absolute (per tiller) or relative (per aphid) terms. However, when fungus density or PCPGR was expressed in relative terms, *i.e.*, fungus-aphid ratio, lower values were observed when parasitoids were absent, in particular for the susceptible wheat cultivar.

The significant interaction modifications statistically detected at the population level agreed with previous analyses of antagonistic interactions between parasitoid and fungus at the individual level, which are normally asymmetric in favor of the fungus (Powell *et al.*, 1986; Askary & Brodeur, 1999). 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, Pell & Niemeyer, 1998), further favoring the competitive dominance of the fungus. As expected, a stronger reduction by the fungus of PCPGR of the parasitoid was detected on the resistant wheat, which suggests that the actual mechanism involved in the indirect effect detected at the population level could be related to the above-described parasitoid-fungus interaction at the individual level (Fuentes-Contreras, Pell & Niemeyer, 1998).

Although no interaction between parasitoid presence and wheat cultivar was found (Table I), the presence of parasitoids significantly increased the density and PCPGR of the fungus when expressed in relative terms (fungus-aphid ratio), particularly in the resistant wheat (Figure 3b, 3d). Direct interactions are known to be absent during oviposition or infection processes since the fungus does not infect the adult parasitoid (Powell *et al.*, 1986; Brobyn, Clark & Wilding, 1988) and the parasitoid does not serve as a vector

of infective spores of the fungus (Fuentes-Contreras, Pell & Niemeyer, 1998). However, 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 encountering infective conidia (Fuentes-Contreras, Pell & Niemeyer, 1998).

Path analysis yielded conclusions similar to those of the ANOVA about the relative importance of direct and indirect effects evaluated through changes in density and PCPGR of the species studied. While direct and indirect effects were of comparable importance in explaining correlations between fungi and parasitoids, direct effects were more important in explaining the correlation between aphids and parasitoids. However, the sign of the latter correlation was inconsistent with the ANOVA results, a situation previously reported by Smith, Brown and Valone (1997) when using path analysis to detect indirect interactions previously established with ANOVA and manipulative experiments.

The magnitude of indirect effects was more important in explaining 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 *P. neoaphidis* cannot infect the immature stages of the parasitoid *A. rhopalosiphi* (Powell *et al.*, 1986); therefore, they interact indirectly through exploitative competition (Wootton, 1994a), rather than by intraguild predation (Brodeur & Rosenheim, 2000). The relevance of indirect interactions might differ in parasitoid-fungus interactions in which the host range of the fungus includes the parasitoid. In this case, the direct effect of the fungus on the parasitoid might be more important (Lacey *et al.*, 1997; Askary & Brodeur, 1999; Mesquita & Lacey, 2001).

The results of this work show the importance of indirect interactions even in simple experimental laboratory systems. In our experiment, interactions at the population level can be explained using detailed knowledge of interactions at the individual level. Indirect effects seem to be of great relevance for the understanding of aphid population dynamics in relation to biological control (Mesquita, Lacey & Leclant, 1997; Fuentes-Contreras & Niemeyer, 2000; Wajnberg & Scott, 2000). Based on the higher complexity of agroecosystems, such indirect effects (Evans & England, 1996; Müller & Godfray, 1997; Morris, Müller & Godfray, 2001) are expected to be of equal or even greater importance than the traditionally studied direct interactions between species.

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