Effect of wheat resistance, the parasitoid *Aphidius rhopalosiphi*, and the entomopathogenic fungus *Pandora neoaphidis*, on population dynamics of the cereal aphid *Sitobion avenae*

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Abstract

The influence of wheat (*Triticum aestivum* L.) resistance, the parasitoid *Aphidius rhopalosiphi* De Stephani-Perez (Hymenoptera: Braconidae) and the entomopathogenic fungus *Pandora neoaphidis* (Remaudière et Hennebert) Humber (Zygomycetes: Entomophthorales) on the density and population growth rate of the cereal aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) was studied under laboratory conditions. Partial wheat resistance was based on hydroxamic acids, a family of secondary metabolites characteristic of several cultivated cereals. The partial resistance of wheat cultivar Naofén, the action of the parasitoid and the joint action of the parasitoid and fungus, reduced aphid density. The lowest aphid densities were obtained with the combination of the parasitoid and the fungus, but wheat resistance under these circumstances did not improve aphid control. Significant reductions of population growth rate (PGR) of aphids were obtained with the joint action of wheat resistance and natural enemies. In particular, the combined effects of parasitoids and fungi showed significantly lower PGR than the control without natural enemies in both wheat cultivars. Our results support the hypothesis that wheat resistance and the utilization of biological control agents could be complementary strategies in an integrated pest management program against cereal aphids.

Introduction

Cereal aphids are important pests in several temperate areas around the world, either by direct damage or by transmission of barley yellow dwarf virus (BYDV) (Carter et al., 1980; Mann et al., 1997). Natural enemies are present or have been introduced to control cereal aphids such as *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) in Europe, and *Diuraphis noxia* (Kurdjumov) in North America. Wheat germplasm with partial resistance against aphids is available, while resistance genes from wild relatives of commercial cereals are being incorporated into breeding programs (Escobar & Niemeyer, 1993; Wellings & Ward, 1994; Niemeyer & Pérez, 1995). Therefore, host plant resistance and the use of biological control are being implemented as complementary strategies within integrated pest management (IPM) of aphids on cereals.

Several studies have shown either no significant or only mild negative effects of partial resistance on the development of parasitoids of cereal aphids (Salto et al., 1983; Kuo, 1986; Reed et al., 1991; Fuentes-Contreras et al., 1996; Fuentes-Contreras & Niemeyer, 1998; Farid et al., 1998a). Such minor effects at the individual level seem not to be translated at the population level, where wheat resistance can reduce the population growth rate of aphids without affecting or even with enhancement of aphid control by parasitoids (Starks et al., 1972; van Emden, 1986; van Emden &
Wratten, 1990; Gowling & van Emden, 1994; Farid et al., 1998b).

Although entomopathogenic fungi also represent important mortality factors against aphids on irrigated cereals (Feng et al., 1991; Wraight et al., 1993), tritrophic interactions between cereal resistance and fungi on aphids have been less studied (Fuentes-Contreras et al., 1998). The interaction between natural enemies of aphids can also be affected by the resistance level of the host plant. In particular, the outcome of the competitive interaction between the parasitoid Aphidius rhopalosiphi and the entomopathogenic fungus Pandora neoaphidis was favored toward the fungus when the aphid S. avenae was grown on a partially resistant wheat cultivar (Fuentes-Contreras et al., 1998). In general, the results with parasitoids and entomopathogenic fungi support the hypothesis that complementary or even synergistic interactions of partial resistance of the host plant and natural enemies are a common phenomenon for aphids feeding on cereals (van Emden & Wratten, 1990; van Emden, 1995).

The joint action of different groups of natural enemies may also be detrimental, inconsequential or synergistic to control aphids on cereals. Powell et al. (1986) and Brobyn et al. (1988) showed antagonistic interactions between a parasitoid and an entomopathogenic fungus at the individual level. On the other hand, Poprawski et al. (1992) found an increase in fungal action in presence of parasitoids under laboratory conditions, whereas Mesquita et al. (1997) reported an additive effect between parasitoids and fungi when both natural enemies were used against confined populations of aphids under field conditions.

The aim of this study was to evaluate the effect of cereal resistance on the single and joint action of the parasitoid A. rhopalosiphi and the entomopathogenic fungus P. neoaphidis on parasitoids of the cereal aphid S. avenae under laboratory conditions. Aphid populations were maintained on wheat (Triticum aestivum) cultivars with different levels of resistance, in the presence of parasitoids, fungi, and both species of natural enemies. The wheat cultivars were known to differ in their levels of hydroxamic acids (Hx), a family of secondary metabolites in wheat and several other cereals conferring resistance toward aphids (Niemeyer & Pérez, 1995). The effects of wheat resistance, parasitoids and fungi through time on aphid population parameters, such as density and population growth rate were evaluated.

Materials and methods

Plant and insect cultures. Stock cultures of the parasitoid, A. rhopalosiphi, and the cereal aphid, S. avenae, were maintained on oat (Avena sativa L.), a cereal lacking Hx (Niemeyer & Pérez, 1995). The fungus P. neoaphidis was maintained as an in vivo culture on S. avenae, also on oat. All insect cultures were maintained at 23 ± 2°C, L16:D8 light-dark photoperiod.

The insects were collected from wheat fields at INIA-La Plativa in Santiago (Chile), and the fungus was isolated from Acyrthosiphon pisum (Harris) from broad bean fields at INIA-Carillanca near Chillán (Chile). The fungus was passed through S. avenae for at least five generations before beginning the experiments. Fungus infection was performed with the spore shower methodology (Fuentes-Contreras et al., 1998), but instead of fungus hyphae from in vitro cultures the whole aphid cadavers were used as sources of conidia (spores).

Two spring wheat (T. aestivum) cultivars were used: Huenufén (susceptible, Hx concentration in seedlings, x̄ = 1.72 ± 0.12 mmoles/kg fr. wt.) and Naofén (partially resistant, x̄ = 3.02 ± 0.17 mmoles/kg fr. wt.), since they were known to differ in their resistance level against cereal aphids (Fuentes-Contreras et al., 1996).

Population experiment. Plexiglass cages (40 × 40 × 40 cm) were used to start 24 experimental populations. Twelve cages were assigned to the wheat cultivar Naofén, while the remaining 12 cages were assigned to the susceptible wheat cultivar Huenufén. For each wheat cultivar three cages were randomly assigned to each of the following treatments: (i) aphid, (ii) aphid plus parasitoid, (iii) aphid plus fungus, and (iv) aphid plus parasitoid and fungus.

Sowing was performed with sterile soil at a density of 50 seeds/pot (volume 450 ml). Two weeks later, when wheat seedlings reached growth stage 14 (Zadoks et al., 1974), two pots per cage were transferred to all cages, and a total of 50 adult aphids (apterae) per cage were gently placed on the seedlings. The parasitoid treatment received four previously mated parasitoid females per cage, the fungus treatment received four sporulating aphid cadavers per cage, and the parasitoid-fungus treatment received both (four mated parasitoid females and four sporulating aphid cadavers per cage), following aphid transfer. The sporulating aphid cadavers were fixed with ara-
bic gum (water soluble) on the tip of the seedlings and plastic sheet was placed over all experimental cages to reduce ventilation and to promote high humidity and fungal infection. One pot per cage containing new seedlings, was introduced into the cages once a week, without removing the pots already present in the cage. When plants died naturally from aphid attack, these pots were removed, but leaving any aphids still present and all non-emerged parasitoid mummies or presumably non-sporulated aphid cadavers in the cage. The whole experiment lasted 56 days and was performed under the same temperature and lighting conditions described in the insect culture section.

**Sampling and statistical analyses.** Every four to five days, aphids present on ten tillers from every cage (including parasitised or infected individuals), were gently removed from the colony with a brush and preserved in vials with 70% ethanol for subsequent counting and storage. Mean values of these ten samples were used as the experimental unit to estimate aphid population density, i.e. number of aphids per tiller ($N_t$). Population dynamics were followed until completion of four parasitoid generations and nearly 12 fungus generations.

Aphid densities were $\log(x + 10)$ transformed and compared using an ANOVA design for repeated measures. Population growth rates of aphids were calculated as $1/\Delta t \log (N_t/N_{t+1})$ (days$^{-1}$) and compared using an ANOVA design for repeated measures. The data supported the assumptions of homoscedasticity (Barlett test, $P > 0.05$) and sphericity (Mauchley test, $P > 0.05$). The principal effects of the ANOVA were: (i) wheat cultivar (susceptible and partially resistant), (ii) natural enemies (parasitoids, fungi, both natural enemies, and control with no natural enemies), and (iii) time (11 sampling dates as within subject repeated measures).

**Results**

Aphid density (Figure 1A, B) was significantly affected by wheat cultivar ($M.S. = 0.128, F_{(1,16)} = 41.5, P < 0.001$). There were also significant differences among natural enemy treatments ($M.S. = 0.205, F_{(3,16)} = 66.30, P < 0.001$) and among sampling dates ($M.S. = 0.440, F_{(10,160)} = 330.63, P < 0.001$). No statistically significant interactions were observed between wheat cultivar and time ($M.S. = 0.03, F_{(10,160)} = 1.18, P = 0.31$) and between wheat cultivar, natural enemy and time effects ($M.S. = 0.001, F_{(30,160)} = 0.82, P = 0.74$).

Significant statistical interactions between wheat cultivar and natural enemy effects ($M.S. = 0.30, F_{(3,16)} = 9.83, P < 0.001$) and between natural enemy and time effects ($M.S. = 0.006, F_{(30,160)} = 4.40, P < 0.001$) were found. The interactions between wheat cultivar and natural enemy effects are shown in Figure 2. Multiple comparisons (Tukey test) revealed that the wheat cultivar Naoftén reduced the density of aphids in relation to the wheat cultivar Huenufén in the control and parasitoid treatments, whereas no significant differences between wheat cultivars were present in the fungus and parasitoid plus fungus treatments. Nevertheless, the lowest aphid densities were obtained when both parasitoid and fungus were used in combination, regardless of wheat cultivar, and with the parasitoid on the partially resistant wheat cultivar Huenufén (Figure 2).
**Table 1.** Mean population growth rate expressed in [aphid/(aphid * days^{-1})] for the cereal aphid, *S. avenae*, on susceptible (Huenufén) and partially resistant (Naofén) wheat cultivars with parasitoids, fungi, parasitoids + fungi, and no natural enemies (control). Letters show significant differences with the multiple comparisons Tukey test (*P* < 0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Huenufén mean (standard error)</th>
<th>Naofén mean (standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0147 (0.0019) a</td>
<td>0.0104 (0.0006) a</td>
</tr>
<tr>
<td>Parasitoid</td>
<td>0.0114 (0.0010) a</td>
<td>0.0100 (0.0009) a</td>
</tr>
<tr>
<td>Fungus</td>
<td>0.0104 (0.0005) a</td>
<td>0.0103 (0.0006) a</td>
</tr>
<tr>
<td>Parasitoid + Fungus</td>
<td>0.0075 (0.0002) b</td>
<td>0.0073 (0.0003) b</td>
</tr>
</tbody>
</table>

**Figure 2.** Mean density (individuals/tiller) of the cereal aphid, *S. avenae*, on the susceptible and resistant wheat cultivars under different natural enemy treatments. Values are means ± two standard errors.

Population growth rate of aphids (PGR) (Table 1) was significantly higher in the susceptible wheat cultivar Huenufén than in the partially resistant wheat cultivar Naofén (*M.S. = 0.00014, F_{1,16} = 5.57, P = 0.03*). There were also significant differences in aphid PGR between natural enemy treatments (*M.S. = 0.0003, F_{3,16} = 11.69, P < 0.001*), and between sampling dates (*M.S. = 0.00299, F_{10,160} = 17.78, P < 0.001*). No significant statistical interactions were found between any combination of principal effects, i.e. between wheat cultivars and natural enemies, wheat cultivars and time, natural enemies and time, and finally wheat cultivars, natural enemies and time. The Tukey test for multiple comparisons among natural enemy treatments revealed that the combination of parasitoids and fungi significantly reduced the aphid PGR, whilst either parasitoid or fungus treatments did not significantly reduce the aphid PGR in relation to the control for both wheat cultivars.

**Discussion**

Our experiments on cereal aphid population dynamics suggest that partial wheat resistance based on Hx, resulted in a reduction in aphid density mediated by a decrease in aphid population growth rate. Previously reported antixenotic, antifeeding or antibiotic effects of Hx on cereal aphids (Niemeyer & Pérez, 1995), can be responsible for the observed effect of wheat resistance in our aphid populations.

Wheat resistance as well as the combined introduction of parasitoids and fungi reduced density and population growth rate of aphids. Parasitoids alone reduced the density of the aphids, but no significant effect on population growth rate was observed. Previous work in this system at the individual level had revealed an increase in developmental time of the parasitoid in the partially resistant wheat cultivar (Fuentes-Contreras et al., 1996; Fuentes-Contreras & Niemeyer, 1998). However, such increase is apparently not relevant enough to affect aphid population dynamics. This situation is likely to prevail in the case of parasitoids attacking aphids growing on arable and greenhouse crops, since the duration of these crops (three to five months) allows the development of only a few generations of parasitoids (six to ten generations) (Rochat, 1997), thus precluding an accumulation of the effect of wheat resistance on developmental time.

Fungi alone reduced aphid density only in the susceptible wheat cultivar compared with the control. Fuentes-Contreras et al. (1998) previously reported no effect of wheat resistance on fungus development or dispersal at the individual level; hence, a significant impact of fungi on aphid density, regardless of wheat cultivar, should be expected. Consequently, our results suggest the occurrence of a deleterious effect of wheat resistance on the influence of fungi on aphid density.
This effect might be result of a density-dependent influence of wheat resistance on the fungi, which was not observed in individual level experiments previously performed by Fuentes-Contreras et al. (1998). Based on a comparison of the Hx levels of the wheats used in the present study and the results of Givovich et al. (1992), we may suggest that cereal aphids feeding on the partially resistant wheat cultivar Naofén would produce more honeydew with higher Hx concentration than cereal aphids feeding on the susceptible wheat cultivar Huenafén. Hence, accumulation of honeydew with higher concentration of Hx on the leaves and body surface of the aphids, may reduce the infection or dispersal of the fungal conidia (spores).

Mesquita et al. (1997) have detected a complementary action between the fungus Paecilomyces fumosoroseus (Wize) Brown et Smith, and the parasitoid Aphelinus asychis Walker on populations of D. noxia. Our study also found the lowest aphid densities when both natural enemies were combined, and therefore a complementary action was observed. The benefits of this combination of parasitoids and fungi was further evidenced by significant reductions in aphid population growth rate. Wheat resistance did not further affect aphid density when both natural enemies were used. This effect was expected, since aphid densities in the parasitoid treatment were lower on the resistant wheat, whereas in the fungus treatment wheat resistance did not have a significant effect on aphid density.

The observed complementary action of parasitoids and fungi might be related with the host-range of the fungus or the parasitoid, which may eventually allow the invasion of larval parasitoid tissues by fungal hyphae or the ingestion of fungal tissues by the parasitoid larva. As suggested by microscopic studies performed by Powell et al. (1986), this is not the case for A. rhopalispili and P. neoaphidis interacting inside cereal aphids. However, in a similar system the susceptibility of the larvae of the parasitoid Aphidius nigripes Ashmead toward fungal infection by Verticillium lecanii (Zimm.) has been detected by Askary & Brodeur (1999). In contrast with the strictly aphidophagous P. neoaphidis, the fungus V. lecanii shows a wide host-range (including insects, mites, spiders, nematodes and even phytopathogenic fungi) (Askary et al., 1999), which might suggest that for this latter species a complementary action with parasitoids would not be necessarily expected.

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. The best results were obtained with the combination of parasitoids and fungi, but wheat resistance under these circumstances did not improve aphid control. Since resistance based on Hx does not counteract the action of natural enemies, it does not represent a cost for wheat in terms of yield (Gianoli et al., 1996), and it confers resistance against a wide range of pests and pathogens (Niemyer & Pérez, 1995); therefore, we further support its utilization in breeding programs. Finally, our results were obtained in the laboratory, with aphids at high densities kept in rather small volumes, and high humidity conditions. These may not represent the natural conditions prevalent in cereal fields. Further studies should evaluate the combined effects of wheat resistance, parasitoids and fungi under field conditions.

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