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RESEARCH NOTE

Abundance and prevalence of *Aphidius avenae* (Hymenoptera: Braconidae: Aphidiinae) in Chile

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Abstract

A. Peñalver-Cruz, S. Ortiz-Martínez, C. Villegas, Ž. Tomanović, F. Zepeda-Paulo, V. Žikić, and B. Lavandero. 2017. Abundance and prevalence of *Aphidius avenae* (Hymenoptera: Braconidae: Aphidiinae) in Chile. Cien. Inv. Agr. 44(2): 207-214. During the samplings described here, *Aphidius avenae* (= *picipes*) (Haliday, 1834) was collected. This species has been reported in Chile but has never been studied in terms of its relative abundance and prevalence in central and southern Chile by using classical or molecular methods. The composition of the parasitoid assemblages on several cereal aphid hosts was examined for three seasons in agricultural landscapes. In this note, we describe the prevalence of this parasitoid species in cereal aphids and other aphid hosts and discuss the abundance of this parasitoid compared to other aphid parasitoids and the probable origins of this important aphid parasitoid species in Chile. The results indicate that *A. avenae* has a low prevalence compared to other main parasitoids such as *Aphidius ervi* (Haliday) and that it has been found parasitizing the grain aphid (*Sitobion avenae* (Fabricius)) and the bird cherry-oat aphid (*Rhopalosiphum padi* (Linnaeus)) in wheat and the foxglove aphid (*Aulacorthum solani* (Kaltenbach)) in tomato. Although the introduction of *A. avenae* in Chile as a biological agent in 1992 was not successful, we here confirm the presence of this aphid parasitoid wasp in Chile and discuss the factors that could explain the low prevalence of this parasitoid species in the country.

Key words: Aphids, braconidae, hosts, parasitoids, tomato, wheat.

Introduction

As parasitoids, aphidiines are important natural enemies of many aphids in natural and agricultural areas worldwide. Cereal aphids constitute

a large group of pest species that directly and indirectly affect the yield of cereals globally. A rich assemblage of parasitoids is co-occurring and potentially interacting in cereal systems in Chile (Zepeda-Paulo *et al.*, 2013). Of all Aphidiinae present in Chile, the vast majority were introduced for biological control programs (Starý, 1995). Only species from the genus *Pseudephedrus* that are

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associated with aphids on *Nothofagus* are considered native to the Chilean ecosystems (Starý, 1995). *Aphidius avenae* (accepted name) has a rich synonym history: *A. crithmi* (Marshall, 1896), *A. granarius* (Marshall, 1896), *A. pascuorum* (Marshall, 1896), *Lysiphlebus hungaricus* (Györfi, 1958), and *A. caraganae* (Starý, 1963) as well as the first name under which it was described, *A. picipes* (Nees, 1811) (Starý, 1974).

Despite a prevailing interest due to the pest aphids in agriculture and even forestry, previous studies have not paid attention to *A. avenae* in Chile. This species was well known from Holarctic and Oriental zones. The only record of *A. avenae* in Neotropical areas, including Chile, was reported by Starý (1995), who indicated that there was a release performed in the fifth region of central Chile during 1992 on cereals and alfalfa.

The present note aims to confirm and discuss the presence of *A. avenae* in Chile and its prevalence and origins for future reference, as this species is important to aphid control in cereal systems in Europe.

Materials and methods

Parasitoids were obtained during the collection of aphids from two different studies in Chile (Zepeda-Paulo *et al.*, 2013; Raymond *et al.*, 2015). Data were based on samples from three seasons and were reviewed to confirm species identification by taxonomical experts via classical and molecular methods as described below.

Parasitoid procurement

The first study was performed in wheat, potato and tomato fields in central (S 35°24', W 71°40') and southern (S 39°51', W 73°7') Chile from 2010 to 2011. Randomly selected plants were sampled for aphids. The grain aphid (*Sitobion avenae* (Fabricius)) and the bird cherry-oat aphid

(*Rhopalosiphum padi* (Linnaeus)) were collected in wheat, and the foxglove aphid (*Aulacorthum solani* (Kaltenbach)) was collected in tomato. Aphids were maintained in growth chambers (20 °C, 50–60 RH, D16/N8 of photoperiod) until they were mummified hosts of parasitoid larvae and adult wasp emergence was registered.

The second sampling period was performed from October to December of 2013 (on five sampling dates) in 20 wheat fields of central Chile (S 35°58', W 70°38'). In each field, for each of the sampling dates, three sub-sampling locations were separated by 50 m, and 60 wheat tillers were sampled for all apterous *S. avenae*. Aphids were then placed in 99% ethanol at 4 °C until further molecular and taxonomic analyses (Blackman and Eastop, 1984) could be completed in the laboratory.

The emerged parasitoid adults were either dry-mounted or ethanol-preserved, and a part of them was dissected and mounted on microscopic slides (Neo-Mount medium). The identification key for parasitoids used in this study was Starý (1995). Most of the material was deposited in the collection of Universidad de Talca, and voucher specimens of the subfamily Aphidiinae were sent to the Institute of Zoology at the Faculty of Belgrade (University of Belgrade, Serbia) and to the Department of Biology and Ecology at the Faculty of Sciences and Mathematics in the University of Niš, Serbia.

Assessment of parasitoid species using molecular methods

Samples of *S. avenae* stored in 99% ethanol were analyzed first by extracting DNA using the Salting Out protocol (Sunnucks and Hales, 1996) and then using a multiplex PCR test as described by Traugott *et al.* (2008). Species-specific primers developed by Traugott *et al.* (2008) for accurately identifying the immature stages (eggs, larvae and pupae) of 10 parasitoid species associated with *S. avenae* were used. A genus-specific primer was

also used to identify other *Aphidius* spp. where markers were not available. From the previous studies in which rearing aphids and mummies were involved, we detected some of the parasitoid species present in Chile, with exception of *Aphidius matricariae* (Haliday) and *Aphidius colemani* (Viereck).

Statistical analysis

All data were analyzed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The results obtained from the molecular analysis were analyzed using an ANOVA and a Post Hoc by using Tukey's HSD (honest significant difference) statistical test to execute pairwise multiple comparisons of the examined variables.

Results

A total of 954 parasitoids were obtained from reared aphids collected in the field (samples from 2010 to 2011) and 231 samples were positive for at least one parasitoid species from a total of 641 collected field aphids (samples from 2013). Of these, 63 *A. avenae* specimens were obtained from reared aphids, and 10 were detected through molecular methods. This present report confirms the presence of *A. avenae* in Chile, which was identified for the first time by molecular methods. *Aphidius avenae* was found mainly in the samples collected in the central area of Chile, with only a few individuals found in the south in the Los Rios region (Table 1). The occurrence of

this parasitoid is low compared to that of other parasitoids of the main aphids in wheat or tomato. However, *A. avenae* has been found parasitizing *S. avenae* and *R. padi* in wheat and *A. solani* in tomato. During the sampling, an *A. avenae* was encountered in an unidentified host on potato plants and one individual of *S. avenae* on an oat plant. *Sitobion avenae* is the host where this parasitoid was found more often (Table 1). However, parasitoids such as *A. ervi*, *Praon volucre* (Haliday) and *A. uzbekistanicus* parasitized more *S. avenae* compared to *A. avenae* (Figure 1). *Aulacorthum solani* was the main aphid collected on tomato plants (Table 1). *Aphidius avenae* (2.2%) and *Praon volucre* (2.2%) were the least abundant parasitoids emerging from the mummies of *A. solani*. *Aphidius avenae* emerged from mummies of *A. solani* in lower densities compared to *A. matricariae* (24%), *A. colemani* (22.9%), *Aphidius uzbekistanicus* (Luzhetskii) (15.4%) and other individuals from the super family Chalcidoidea (33.3%). Among the samples collected from *R. padi*, approximately 9% were parasitized by *A. avenae*, 37% by *Lysiphlebus testaceipes* (Cresson), 25% by *A. colemani* and 22% by *A. uzbekistanicus* (Figure 1). Additionally, 59% of the collected *A. avenae* specimens were male (Table 1).

As expected from the data obtained in previous investigation years, the parasitoid species detected through molecular methods were (in descending relative abundance): *Aphidius ervi* (Haliday), *A. uzbekistanicus*, *Aphidius rhopalosiphii* (De Stefani-Perez), *P. gallicum* (Starý) and *A. avenae* (Figure 2). *Aphidius avenae* was detected less frequently than were the other parasitoid species

Table 1. Abundance of female (F) and male (M) *Aphidius avenae* in two locations of Chile in different aphid hosts/ crop associations. Field samplings were conducted during 2010 to 2011.

| Aphid host/ Location and crop (F/M) | Central Chile (Talca) | | | South Chile (Valdivia) | |
|-------------------------------------|-----------------------|-----------------------------|--------------------------|------------------------|----------------------|
| | <i>Triticum spp.</i> | <i>Solanum lycopersicum</i> | <i>Solanum tuberosum</i> | <i>Avena sativa</i> | <i>Triticum spp.</i> |
| <i>Sitobion avenae</i> | 42 (20/22) | | | 3 (0/3) | 1 (0/1) |
| <i>Rhopalosiphum padi</i> | 6 (1/5) | | | | |
| <i>Aulacorthum solani</i> | | 6 (3/3) | | | |
| Mummy | 4 (4/0) | | 1 | | |

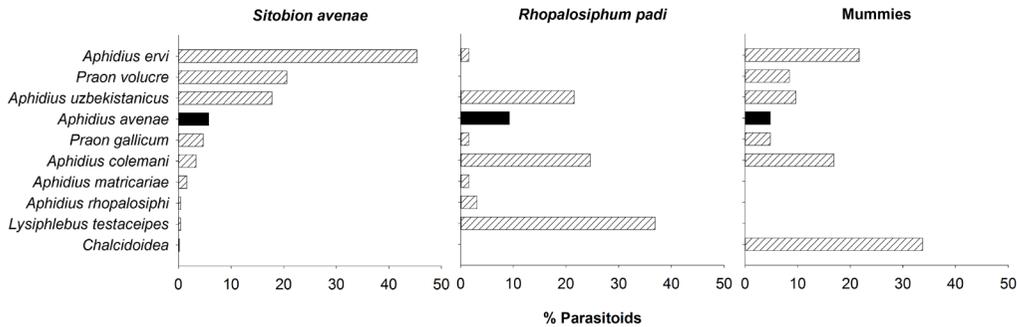


Figure 1. Results of the percentage of occurrence of different parasitoid species per each aphid host during field samplings of 2010 and 2011 in wheat.

(Figure 1 and 2). Additionally, the only secondary parasitoid detected using molecular methods was *Dendrocerus carpenteri* (Curtis), which was also found in samples with *A. avenae* (Figure 2).

Based on the results obtained from the molecular analysis, *A. avenae* was detected in the same samples in multiparasitism with other parasitoids such as *A. ervi*, *A. uzbekistanicus* and *A. rhopalosiphii*. Furthermore, *A. avenae* was detected without other parasitoids only in one sample.

Discussion

Originally, individuals from the family Aphidiinae were distributed similarly to their host groups, which are mainly in the mild to subtropical zones of the northern hemisphere, with some peculiarities in the southern hemisphere and in the tropics (Blackman and Eastop, 1984). Despite the primary parasitoid-aphid-plant associations, the faunal distribution of aphidiines has been altered by the introduction of species. Cereal aphids and aphidiines in Chile are mainly introduced (Starý, 1995). In the case of *Aphidius avenae*, although a release was performed, this parasitoid has not been as successful as it is in the northern hemisphere. Zepeda-Paulo *et al.* (2013) reported *Aphidius avenae* in Chile, and the present study confirms its presence via classical and molecular methods, adding it to the list of aphidiines that prevail in Chilean agroecosystems.

Species interactions, such as competition, can be limiting factors for the prevalence of species in an ecosystem. The presence of alternative hosts for parasitoids sustains their population emerging after winter and allows this population to increase in such a way that also increases control of crop pests (Langer *et al.*, 1997). Contrary to the behavior of polyphagous predators, parasitoids do not always alternate among hosts (Langer and Hance, 2004). This, indeed, could be one of the reasons for which *A. avenae* has a low prevalence in Chile compared to agroecosystems of the northern hemisphere. *Aphidius avenae* is a generalist parasitoid, and it competes with other aphid parasitoids of the genus *Aphidius* such as *A. ervi*, *A. uzbekistanicus* and *A. rhopalosiphii*. Based on the present results, *A. ervi* and *A. uzbekistanicus* are stronger competitors compared to *A. avenae*. These two aphid parasitoids were introduced in Chile to control certain aphid pest populations (Starý, 1995). These species may be able to switch between alternative aphid hosts, which enabled them to increase in prevalence after their release in Chile and become more successful compared to *A. avenae*. Interestingly, while *A. rhopalosiphii* was one of the predominant parasitoids detected in *S. avenae* using molecular methods, it was barely identified as an emerging adult from the rearing of field-collected aphids in central Chile, although this could also be altered by the seasonal effect. Molecular methods have the advantage of detecting several parasitoid species in one host sample, increasing knowledge of the competi-

tion between species. However, it is not possible to determine the “winning” species without the classical methods of rearing. Therefore, this may be interpreted as a case of high larval mortality due to competition with other parasitoid species. *Aphidius avenae* is known to coexist with other parasitoid species; one well-studied interaction is that of *A. rhopalosiphii* in western Europe attacking *S. avenae* (Le Lann *et al.*, 2011). Although these two species are closely related, sympatric parasitoid species, they have different thermal tolerance, body size, phenology and response to aphid defensive behaviors (Van Baaren *et al.*, 2004). For instance, these last authors explain that *S. avenae* defended itself less under the attack of *A. avenae* compared to the attack of *A. rhopalosiphii* and that *A. avenae* totally rejected aphids that were already hosting other parasitoids. The results of the present paper show that *A. avenae* most frequently (in 90% of cases) shared their host with other aphid parasitoids from the *Aphidius* genus. However, it is unknown whether *A. avenae* parasitized the host before the other parasitoids. In fact, the literature suggests that *A. avenae* prefers unparasitized hosts and rejects patches with the aphid pheromone alarm (Van Baaren *et al.* 2004). Generalist parasitoids, such as *A. avenae*, are presumed to decline in their host use efficiency compared to specialist parasitoids, which are more likely to increase the use of shared hosts, taking advantage of the trade-off of generalists (Straub *et al.*, 2011). Therefore, the possible increase of shared hosts by *A. avenae* in Chile could be related to the possible reduction of host availability for *A. avenae*, as they may specialize more than in other regions of the northern hemisphere. With such high intraspecific competition among parasitoids, the factors that influence the success of a parasitoid species may include the parasitoid species itself, being intrinsically superior to others and the age of the parasitoids, as older larvae are more likely to kill younger competitors (Brodeur and Rosenheim, 2000). However, as it is known that *A. avenae* preferred those aphids that were unparasitized, the larvae of this parasitoid may have failed against the younger larvae of its

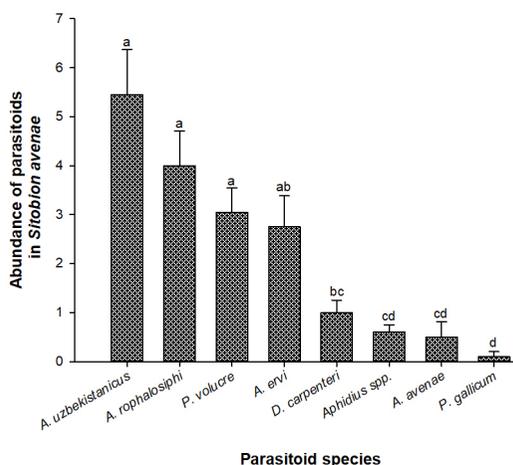


Figure 2. Results of the abundance of different parasitoid species (*Aphidius uzbekistanicus*, *Aphidius rhopalosiphii*, *Praon volucra*, *Aphidius ervi*, *Dendrocerus carpenteri*, *Aphidius spp.*, *Aphidius avenae* and *Praon gallicum*) found in the aphid *Sitobion avenae*, evaluated with a molecular technique in wheat fields.

competitors inside their host; in this way, the prevalence of *A. avenae* may be reduced with respect to other aphid parasitoids in the guild. There may be other factors that could influence the prevalence of *A. avenae*. For instance, there are differences in prevalence of *A. avenae* among western European populations, which could respond to intraregional variations (Andrade *et al.* 2015); however, the factors that explain this variation of *A. avenae* in Europe has not been addressed. Another factor, such as the effect of facultative protective endosymbionts, is not clear for this species, microbial endosymbionts in Chilean populations of *S. avenae* could select against *A. avenae*. Furthermore, there is evidence that the populations of *S. avenae* in Chile do not possess strains of endosymbionts that protect against *A. ervi* and possibly not against *A. uzbekistanicus* and *A. rhopalosiphii* (Zepeda-Paulo *et al.*, 2016).

In areas of Brittany (France), *A. avenae* has recently been detected in a non-diapausing form, largely due to the global increase of temperatures. However, originally, this parasitoid was reported to diapause outside cereal crops and return to this crop during spring (Van Baaren *et al.*, 2004; Le Lann *et al.*, 2011). Therefore, the presence and

diversity of adjacent vegetation to cereal crops could potentially alter the overwintering and, consequently, the prevalence of *A. avenae*. Central Chile has a similar climate to that of Brittany (France) during winter, with drier months after the crop season; however, the vegetation and its configuration in Chile are likely to be different due largely to the high endemism because of the Andes and the younger history of agriculture compared to Europe. Given that the vegetation present around cereal crops in Chile may differ from what *A. avenae* needs, the prevalence of this species could certainly be affected. There is also a lack of information on which other aphid species of Chilean native plants could potentially act as alternative hosts. Further research on ecological engineering of cereal crops in Chile could examine the effects of the native vegetation on ecosystem services of introduced aphid parasitoids such as *A. avenae*.

Biological control agents have been historically considered to independently and in parallel affect their prey/host. However, in field conditions, it is known that the natural enemies of herbivores interact with their own natural enemies, thus reducing the effects on the control of pest populations (Brodeur and Rosenheim, 2000). The parasitoid guild found in the present report shows evidence of a hyperparasitoid of *A. avenae*, which was detected with great abundance early in the season. This fourth trophic level interaction should also be considered a factor that could affect the abundance of *A. avenae* in the sampled areas.

Finally, based on the results presented here, *A. avenae* in Chile is an example of a poorly estab-

lished introduction of a natural enemy to control cereal aphids. The introduction of biological agents for successful biological control should consider more factors than host availability, such as feeding behavior, food accessibility, potential competitors, refuge for overwintering and regional and temporal factors that can influence the guild of natural enemies. This note reports *A. avenae* and some possible intraguild interactions; however, further studies in Chile including *A. avenae* could help better understand its role in cereal agroecosystems.

The results of field sampling in central southern Chile provide evidence of the abundance and interactions of *Aphidius avenae* with other aphid parasitoids using classical and molecular methods, demonstrating that this aphid parasitoid is present at low frequencies in the agroecosystems of Chile.

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Resumen

A. Peñalver-Cruz, S. Ortiz-Martínez, C. Villegas, Ž. Tomanović, F. Zepeda-Paulo, V. Žikić, y B. Lavandero. 2017. Abundancia y prevalencia de *Aphidius avenae* (Hymenoptera: Braconidae: Aphidiinae) en Chile. Cien. Inv. Agr. 44(2): 207-214. Durante los muestreos descritos en la presente, se colectó *Aphidius avenae* (= *picipes*) (Haliday, 1834). A pesar de que esta especie ya fue reportada anteriormente en Chile, nunca ha sido estudiada en términos de la abundancia relativa y la prevalencia en el centro y sur de Chile mediante métodos clásicos y moleculares. La composición de la colección de parasitoides de varios huéspedes de áfidos en cereales fue examinada por tres temporadas en paisajes agrícolas. En esta nota, se describe la prevalencia de esta especie de parasitoide en áfidos de cereales y en otros huéspedes, la abundancia de este parasitoide comparado a otros parasitoides de áfidos y se discute el posible origen de esta importante especie de parasitoide de áfidos en Chile. Los resultados indican que *A. avenae* tiene baja prevalencia comparado a otros parasitoides como *Aphidius ervi* (Haliday) y que ha sido encontrado parasitando al pulgón de la espiga (*Sitobion avenae* (Fabricius)) y al pulgón de la avena (*Rhopalosiphum padi* (Linnaeus)) en trigo, y al pulgón de las solanáceas *Aulacorthum solani* (Kaltenbach)) en tomate. Aunque la introducción de *A. avenae* en Chile como controlador biológico en 1992 no fue satisfactoria, en esta nota se confirma que este parasitoide de áfidos está presente en Chile y se discuten los factores que pueden explicar su baja prevalencia en el país.

Palabras clave: Áfidos, Braconidae, huéspedes, parasitoides, tomate, trigo.

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