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Abstract

The introduction of exotic commercial plants represents a change in the food resources for the communities of herbivores. The blueberry is native to the northern hemisphere and was recently introduced in Argentina, so we expect to find polyphagous tortricids and a low complexity in the tortricid–parasitoid community. Tortricids are exophytic leaf-rollers and flower and fruit feeders, they can feed on different plant structures, and they may be present in every blueberry phenological stage. The aims of this study were (a) to estimate the relative abundance of tortricids in different plant structures and phenological stages of blueberry, (b) to evaluate the relative importance of the different parasitoid guilds, and (c) to describe the tortricid–parasitoid community in blueberry fields of Argentina. The abundance of tortricids in blueberries was low and mainly localized to flowers and fruits. Five parasitoid guilds were identified: early larval endoparasitoids (*Apanteles* sp. and *Dolichogenidea* m1 and m2), larval–prepupal endoparasitoids (*Austroearinus* sp.), larval–pupal endoparasitoids (Ichneumonidae), larval ectoparasitoids (Eulophidae), and pupal endoparasitoids (*Brachymeria* sp. and *Conura* sp.). Most parasitoids were koinobiont larval endoparasitoids. The tortricid–parasitoid food web was very simple in comparison to those of other systems, with high values of vulnerability and connectance. The results of this study suggest that the abundance of tortricids in blueberry crop in Argentina is low. From the point of view of production, the risk of economic losses and the likelihood of direct damage to the fruit would be very low.

Introduction

The introduction of exotic cultivated plants represents a change in the landscape, primarily in terms of food resources for the communities of herbivores already present in the ecosystem (Russell 1989, Jervis & Kidd 1996, Hochberg & Ives 2000). The herbivorous insects that will adapt most quickly to the use of this new resource in the region will consist of native or established polyphagous (Strong *et al* 1984) and ectophagous (Kennedy & Southwood 1984) insects. Temporal heterogeneity of the landscape and the spatial arrangement of habitat elements are essential for species diversity (Burel 1992). This has consequences for survival and population abundance of arthropods because the suitability of resources depends on the crops present (Price *et al* 2011). The blueberry, *Vaccinium corymbosum* (Ericaceae), is native to the northern hemisphere and was introduced in Argentina in the 1990s as an alternative crop. Blueberry crops are mainly located in Tucumán, Entre Ríos, and Buenos Aires provinces (Rocca & Greco 2011).

In Argentina, a large number of insects have been reported using blueberry as a resource throughout the year, being the external chewers the richest guild at the family level (Rocca & Greco 2011). Tortricidae (Lepidoptera) deserve special attention within this guild because species such as *Choristoneura rosaceana* (Harris) and *Grapholita packardi* (Zeller) are

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important pests of blueberries in regions where the crop originates (Mallampalli & Isaacs 2002, Calvo & Molina 2003). Four species of Tortricidae have been described as affecting blueberries in Argentina: *Clarkeulia bourquini* (Clarke), known from Brazil, Uruguay, and Argentina; *Clarkeulia deceptiva* (Clarke) recorded for Brazil and Argentina; *Argyrotaenia sphaleropa* (Meyrick), a common and widespread species, with records in Bolivia, Brazil, Uruguay, and Argentina; and *Platynota meridionalis* Brown, a newly described species that was found in Argentina (Brown 2013, Rocca & Brown 2013). These species feed on a variety of different plant structures (leaves, buds, flowers, and fruits) and they are considered exophytic leaf, leaf-rollers, and flower and fruit feeders (Betancourt & Scatoni 1995, Rocca & Brown 2013), so they may be present in all of blueberry's phenological stages.

Parasitoids are the main natural enemies of tortricids. Tortricids offer numerous niches for parasitoids, generating potentially complex food webs. The parasitoid complex of tortricids and their life cycles has been widely studied (Coop & Croft 1990; Mills 1992, 1993, 1994, Trematerra & Bown 2004). Mills (1992, 1993) found a great number of guilds in the tortricid parasitoids of the Holarctic region, taking into account the life histories and the ways in which hosts can be used by parasitoids. However, due to the recent introduction of blueberry crop in Argentina, we expect to find low complexity in the tortricid–parasitoid community.

The aims of this study were (a) to estimate the relative abundance of tortricids in different plant structures and phenological stages of blueberry crop of Argentina, (b) to evaluate the relative importance of the different parasitoid guilds, and (c) to describe the tortricid–parasitoid community in blueberry fields.

Material and Methods

This study was conducted from June 2006 to December 2008 in blueberry fields situated in four localities of Buenos Aires: Gobernador Castro (GC) (33° 38′ 9.7″ S, 59° 51′ 6.4″ W), San Pedro (SP) (33° 42′ 6.9″ S, 59° 51′ 8.9″ W), Chascomús (Ch) (35° 40′ 42.7″ S, 57° 56′ 55.8″ W), and Colonia Urquiza (CU) (34° 57′ 2.7″ S, 58° 04′ 55.9″ W). The blueberry fields were situated in environments surrounded by crops: in Gobernador Castro, the field was surrounded by citrus, soybean crop, and small vegetable orchards; in San Pedro, it was surrounded by soybean crop fields; in Chascomús, it was surrounded by extensive fields (soybean, corn), and in Colonia Urquiza, it was surrounded by horticultural (mainly tomato, sweet pepper, collard, lettuce) and floricultural crop fields (chrysanthemum, clove pink, freesia, lisianthus, rose, gypsophila, lily, gerbera daisy). Insecticides, mainly the contact and systemic aphicides, are applied to blueberries in Argentina when growers consider that pest density is high (Rocca *et al* 2009).

A random sampling design was used in the aerial part of the plant. Sixty sample units were taken at random monthly in each field. The sample unit consisted of four subsample units: three leaves, three buds, three flowers, and three fruits. The number of larvae and pupae of Tortricidae and parasitoid cocoons was counted and placed in Petri dishes until tortricid adult or parasitoid emergence.

Relative abundance of tortricids in different phenological stages and plant structures

The mean and standard error of the total number of tortricids per sample unit in each phenological stage (Table 1) were estimated. Generalized linear/nonlinear models (Lindsey 1997) were used to examine the relationship between abundance of tortricids, phenological stages, and localities. Phenological stages (V2: summer budding; V3: dormancy and presence of senescent leaves; V1/R1: budding starts, spring budding, and flower bud formation; V1/R2: spring budding and flowering starts; R3: open flower, fruit formation, unripened fruit; R4: ripe fruit) and localities (GC, SP, Ch, and CU) were added as categorical variables, assuming a normal distribution of residuals and the log link function. The quantile–quantile plot was inspected to test the distribution of deviance of the residuals from normality (Lindsey 1997, Fox 2008). The statistical significance of each variable was tested in the model by a forward stepwise procedure, and those that contributed to the most significant change in deviance from the null model were retained. The change in the deviance was tested using a chi-square distribution with a significance level of 0.05.

The relative abundance of tortricids in each plant structure for each phenological stage was calculated with data from all fields and sampling dates as

Number of tortricids in (i)/total number of tortricids where (i) corresponds to leaves (leaves and buds), flowers, or fruits.

The tortricid abundance in different plant structures for each phenological stage was examined by ANOVA, after applying a logarithmic transformation of data. Following a significant ANOVA, Tukey's HSD pairwise comparison was used. When the assumptions of ANOVA were not fulfilled, the Kruskal–Wallis test was used, followed by multiple comparisons of the ranks of the means.

Parasitoid guild of Tortricidae

Parasitoid guilds of Tortricidae were recognized following the classification made by Mills (1992, 1993, 1994) which are defined by the host stage attacked by the parasitoid, the host stage killed by the parasitoid, and the mode of parasitism, ectoparasitism, or endoparasitism. A series of 12 guilds were

Table 1 Phenological stages of blueberry crop in Buenos Aires province, Argentina, and resource available to tortricids.

Phenological stages		Month	Resources
V2	Summer budding	Jan–Feb–Mar	Leaves, vegetative buds, occasionally flowers
V3	Dormancy and presence of senescent leaves	Apr–May	Leaves, relict vegetative buds, occasionally flowers
V1/R1	Budding starts, spring budding, and flower bud formation	June–July	Leaves, occasionally vegetative buds, flower buds
V1/R2	Spring budding and flowering starts	Ago–Sep	Leaves, vegetative buds, flowers
R3	Open flower, fruit formation, unripened fruit	Oct	Leaves, vegetative buds, flowers, immature fruits
R4	Ripe fruit	Nov–Dec	Leaves, vegetative buds, relict flowers, fruits

defined: one using the host egg (egg endoparasitoid), three using the host larva (early larval endoparasitoid, late larval endoparasitoid, and larval ectoparasitoid), four using the host prepupa (egg–prepupal endoparasitoid, larval–prepupal endoparasitoid, larval–prepupal ectoparasitoid, and prepupal–pupal ectoparasitoid), two using the pupa (larval–pupal endoparasitoid and pupal endoparasitoid), and two using the host adult (larval–adult endoparasitoid and adult endoparasitoid) as a resource. One scheme was made to characterize the parasitoid guild of tortricids taking into account all localities and the entire study period. To estimate the percentage of parasitism of each guild, first we calculated the percentage of parasitism of each parasitoid species for each species of tortricid as follows: the number of parasitoids of the species A which emerged from tortricids of the species B/total number of individuals of species B (parasitized+not parasitized) * 100. The percentage of parasitism by guild was calculated adding the percentage of parasitism of each species belonging to the same guild, and then the percentages of parasitism by guild were compared using the Kruskal–Wallis test.

Food webs of Tortricidae and their larval parasitoids

Tortricids and parasitoids were identified to species level or to morphospecies in those cases in which the species identification was not possible. Because of their low abundance, the species of *Clarkeulia* were treated as a single taxon. The quantitative food web of the tortricid–parasitoid community was constructed from tortricid larvae found in the samples and the parasitoids that emerged from these larvae. Pupal parasitoids were not taken into account because it was not possible to identify the host species.

For the graphic representation, the total abundance, accumulated in all localities and over the 3 years of sampling, was considered, and gregarious parasitoids were treated as a single individual per host in the analysis. The size of the food web is given by the number of species involved in all trophic levels. We use vulnerability, number of real links, connectance, and the Berger–Parker index as metrics to describe the community structure. The qualitative vulnerability is the number of parasitoids per herbivore species (Dormann *et al* 2009), and it was calculated by dividing the number of parasitoid species by the

number of tortricid species. As measures of the complexity of the community, we considered the number of real links that represents the real trophic interactions (L) and the connectance (C) that is the proportion of all possible interactions that take place within a system. Connectance in food webs is normally calculated as L/S^2 (Dunne *et al* 2002), where S is the species richness and L is the number of trophic links in the web, respectively. This measure is less suitable for host–parasitoid webs as it allows the possibility of hosts feeding on hosts, which is impossible, and parasitoids feeding on parasitoids, which we did not measure. Therefore, the connectance was calculated as

$$C = \frac{L}{HP}$$

where H represents the total number of hosts and P represents parasitoids, being HP the maximum possible number of host–parasitoid associations. Thus, L/HP is a measure of the fraction of realized links (Valladares *et al* 2001).

Results

Relative abundance of tortricids in different phenological stages and plant structures

Neither the phenological stages of blueberries nor the various localities explained the variation in tortricid abundance (Table 2). The abundance of tortricids ranged between 0.02 and 0.52 individuals per sample unit, and it was numerically

Table 2 Summary of the forward stepwise procedure used to build a multiple regression model for the relative abundance of tortricids between sites and blueberry phenological stages. The model assumes a normal distribution of errors and uses the log link function.

Model	Deviance	Change in deviance	df	p
No terms added (null model)	2.17		66	
Int+phenological stages	1.69	0.47	61	0.99
Int+localities	1.95	0.22	63	0.97
Int+phenological stages+localities	1.07	1.10	43	1

higher in the phenological stages of V2 and V3; however, it was not significantly different from the other stages. The highest abundance was found in Chascomús while the lowest abundance of tortricids was found in Colonia Urquiza. On the other hand, San Pedro and Gobernador Castro had similar levels of tortricids (Fig 1).

In blueberry phenological stages of V3, V1/R1, and V1/R2, there were no significant differences among plant structures. In phenological stages of R3 and R4, the number of tortricids found in each plant structure was higher on leaves and buds than on flowers and fruits (R3: $F=8.39$, df=2, $p=0.001$; R4: $H_{(2, N=24)}=13.02$, $p=0.001$). In general, in all phenological stages, there were more tortricids on leaves and buds, except in V1/R2 where they were more abundant on flowers (Fig 2).

Parasitoid guild of Tortricidae

There were no parasitoids reared from *Clarkeulia* species. Most of the parasitoids of *A. sphaleropa* and *P. meridionalis* (62.5%) were koinobiont larval endoparasitoids. Two species were idiobiont pupal endoparasitoids and one was a larval gregarious ectoparasitoid (Fig 3). The total percentage of parasitism recorded was 18.2±8.8 (mean±SE), and all parasitoid guilds caused similar percentage of parasitism ($H_{(4, N=20)}=4.23$, $p=0.38$) (Fig 4).

Food web of Tortricidae and their larval parasitoids

The total numbers of tortricids and parasitoids sampled were 389 and 39, respectively. The food web was simple, formed by four tortricid and six parasitoid species. *Argyrotaenia sphaleropa* was the most abundant species of tortricids (Berger–Parker index=0.73), followed by *P. meridionalis*, while *C. bourquini* and *C. deceptiva* were poorly represented. *Dolichogenidea* sp. morph 1 was the most abundant species among parasitoids (Berger–Parker index=0.59), and its main host was *A. sphaleropa* (Fig 5). The values of the community metrics were as follows: vulnerability=2 (there were two species of parasitoids for each tortricid species), number of real links=7, and connectance=0.39.

Discussion

Based on literature records and specimen data from collections, it is likely that all tortricids recorded in this study are polyphagous (Silva *et al* 1968, Betancourt & Scatoni 1995, Pastrana 2004). Therefore, our results confirm the hypothesis that the tortricids colonizing blueberries in Argentina are native or established ectophagous (Kennedy & Southwood 1984, Strong *et al* 1984). Because the blueberry is a recently introduced crop, these tortricid species have moved from native hosts or other cultivated hosts onto cultivated blueberries. The most abundant species was *A. sphaleropa*, cited in Argentina as a pest of apple in the High Valley of Río Negro and associated mainly with stone fruit, pome fruit, vines, and natural vegetation (Silva *et al* 1968; Betancourt & Scatoni 1995, Pastrana 2004). Their presence in blueberry fields of San Pedro and Gobernador Castro might be related to surrounding crops, which are citrus and stone fruit. In all the blueberry fields, there were areas of natural vegetation near the crop, which may represent a source from which colonization of blueberries in Chascomús and Colonia Urquiza may occur.

In this study, tortricids were present in all phenological stages, mainly on leaves and buds, and their abundance was generally low. But their presence on fruits could be a constraint to comply with the sanitary quality demands of foreign markets. It is known that tortricids do not cause significant economic losses unless they feed during the flowering stage, preventing fruit formation, or else interrupt fruit formation by directly damaging them (Elsner & Whalon
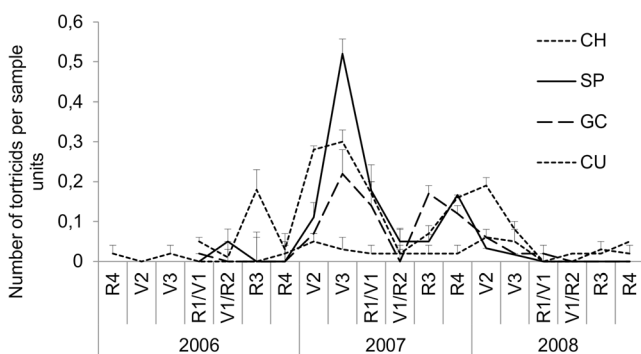

Fig 1 Abundance of tortricids in blueberry crops in Buenos Aires. *Bars* show +SE. *V2* summer budding, *V3* dormancy and presence of senescent leaves, *V1/R1* budding starts, spring budding, and flower bud formation, *V1/R2* spring budding and flowering starts, *R3* open flower, fruit formation, unripened fruit, *R4* ripe fruit, *CH* Chascomús, *SP* San Pedro, *GC* Gobernador Castro, *CU* Colonia Urquiza.
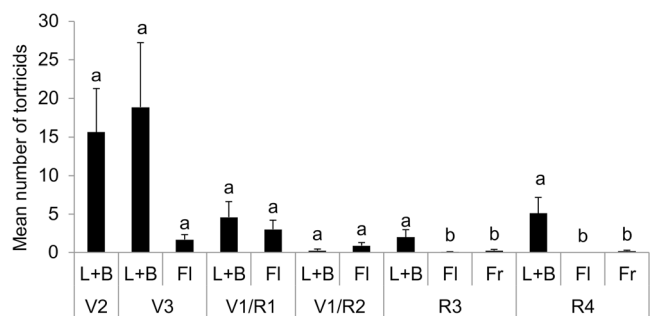

Fig 2 Mean number of tortricids (data from 3 years and four fields) in different plant structures for each blueberry phenological stage. *Bars* show +SE. *Different letters* indicate significant differences ($p<0.05$). *V2* summer budding, *V3* dormancy and presence of senescent leaves, *V1/R1* budding starts, spring budding, and flower bud formation, *V1/R2* spring budding and flowering starts, *R3* open flower, fruit formation, unripened fruit, *R4* ripe fruit, *L+B* leaves+buds, *Fl* flowers, *Fr* fruits.

Fig 3 Parasitoid guilds of Tortricidae in blueberry fields of Buenos Aires. The host life cycle is represented by a *circle* with *E* indicating egg, *EL* early larva, *ML* mid larva, *LL* late larva, *pP* prepupa, *P* pupa, and *Ad* adult stages. The *lines* begin in the host stage attacked and end up (————●) in the host stage killed by the parasitoid (*ELN* early larval endoparasitoid, *L-pPN* larval–prepupal endoparasitoid, *L-PN* larval–pupal endoparasitoid, *LLC* late larval ectoparasitoid, *PN* pupal endoparasitoid).

PN: *Brachymeria* sp. (I), *Conura* sp. (I). (Chalcididae)

ELN: *Apanteles* sp. (K), *Dolichogenidea* (K) m1 & m2. (Braconidae: Microgastrinae)

L-pPN: *Austroearinus* sp. (K). (Braconidae: Agathidinae)

LLC: Eulophidae (K or I)
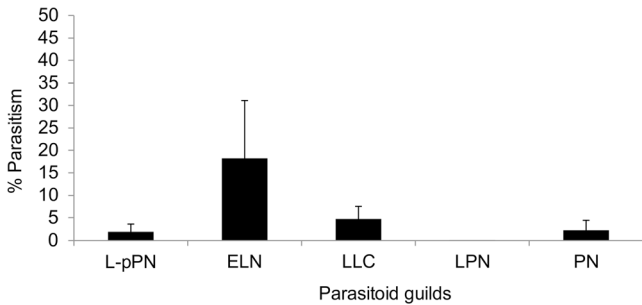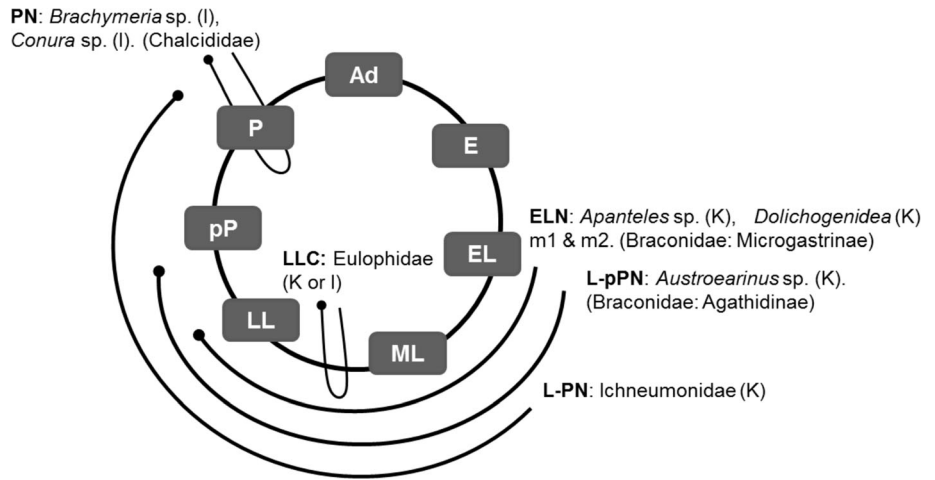
L-PN: Ichneumonidae (K)

Fig 4 Percentage of parasitism caused by parasitoid guilds to Tortricidae in blueberry fields of Buenos Aires (*L-pPN* larval–prepupal endoparasitoid, *ELN* early larval endoparasitoid, *LLC* late larval ectoparasitoid, *LPN*, larval–pupal endoparasitoid, *PN* pupal endoparasitoid).

1998). Mallampalli & Isaacs (2002) recorded similar abundances (between 0.02 and 0.05) of *G. packardi* on blueberry fruits in MI (USA) using comparable sampling design.
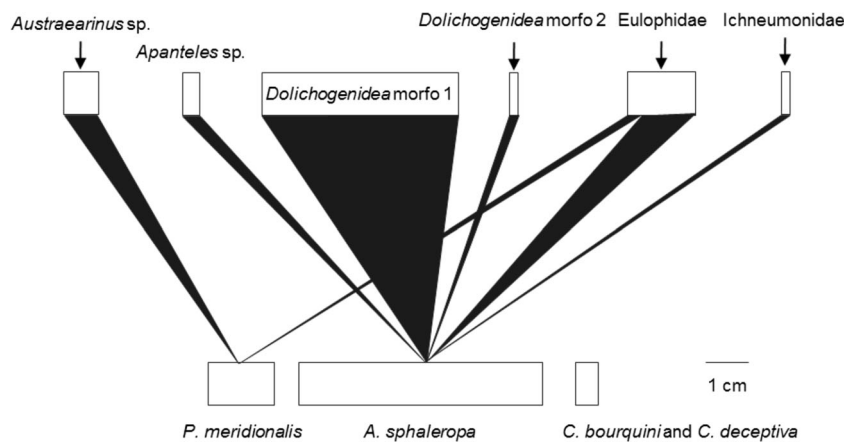
The most important causes of mortality of tortricids are their parasitoids (Mills 1992), and in this study, although the majority of species were endoparasitoids, we also recorded ectoparasitoids. The larval ectoparasitoid guild is often related to host living concealed within plant structures that offer some shelter from desiccation, abrasion, and enemies (Mills 1994).

The more important larval endoparasitoids of *A. sphaleropa* and *P. meridionalis* were *Dolichogenidea* sp. (morph 1) and *Austroearinus* sp., respectively. The genus *Dolichogenidea*, not recorded in the USA, comprised the main parasitoids of tortricids in vineyards in New Zealand (Thomas 1987). The genus *Austroearinus* is relatively new to science (Sharkey 2006). Other genera that have been mentioned as the most important tortricid larval parasitoids in South America are *Apanteles* and *Bracon* (Braconidae) in Uruguay (Betancourt & Scatoni 1995) and *Apanteles*, *Cotesia*, and *Exochus* in Perú (Núñez 2008). We recorded only one species of Eulophidae among the larval ectoparasitoids that parasitized both species of tortricids.

It was not possible for us to associate the pupal parasitoids *Brachymeria* spp. and *Conura* spp. with a particular species of tortricid because pupae collected in the field were not

Fig 5 Quantitative food web of tortricids and their parasitoids in blueberries of Buenos Aires. *Bars* show species abundance (*1 cm=* 50 tortricids and 5 parasitoids). The *width of the lines connecting the species from the two trophic levels* indicates the strength of the interaction.

Austraearinus sp.

Apanteles sp.

Dolichogenidea morfo 2   Eulophidae   Ichneumonidae

Dolichogenidea morfo 1

P. meridionalis   *A. sphaleropa*   *C. bourquini* and *C. deceptiva*

1 cm

identified at the species level. Even though parasitoid species vary in different locale or regions, we think that *A. sphaleropa* could be the main host for *Brachymeria* because it is the most common pupal parasitoid of this tortricid in Uruguay (Betancourt & Scatoni 1995).

The tortricid–parasitoid food web of blueberries in Argentina is very simple relative to those of other systems (Memmott *et al* 1994). This simplification is characteristic of agricultural systems compared to natural systems (Memmott & Godfray 1994, Valladares & Salvo 1999). Due to the short time that elapsed since the introduction of the crop in Argentina, a low number of parasitoid species per host species (low vulnerability) would be expected, because the majority of insects colonizing introduced plants are herbivores, and natural enemies follow them with some delay (Strong *et al* 1984). However, we found more than one parasitoid species per host species probably because tortricids and parasitoids belong to the regional pool of species and their interactions already exist in the landscape where blueberries were established. The number of real links was high compared with the number of all possible trophic links, so the tortricid–parasitoid food web in blueberry showed a high value of connectance. The connectance is a good indicator of the resilience of the community and generally is considered independent of the web size; also, food webs with high values of connectance are more robust to species loss (Martínez 1992, Dunne *et al* 2002).

High densities of tortricids could affect the vegetative growth of plants and therefore diminish the quality and quantity of fruits (Maust *et al* 1999). The results of this study suggest that the abundance of tortricids in blueberry crops in Argentina is low probably because tortricids that colonized blueberries in Argentina from other host plants may still require a longer period to become adapted to this new host plant. Moreover, the mortality caused by parasitoids could also be a limiting factor to allow for population growth of tortricids in blueberry fields in Argentina. From the point of view of production, the risk of economic losses and the likelihood of direct damage to the fruit would be very low.

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