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ECOSYSTEMS

Abundance, distribution, and associated forage losses of pest grasshoppers *(Orthoptera: Acrididae)* in the Argentine Pampas

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Abstract: The goal of this study was to assess the status of *Dichroplus elongatus* and *Borellia bruneri* as actual agricultural pests in the Argentine Pampas by determining their abundance, distribution, and associated forage loss. The study was conducted in Laprida and Tandil, two counties in Buenos Aires province. In each county 20 sampling sites were established and monitored from 2012 to 2018. *B. bruneri* was more abundant and with a wider distribution in Laprida (91.4% of the sites) than in Tandil (42.1% of the sites) while *D. elongatus* abundance was significantly higher from 2012 to 2016 in Tandil than in Laprida and its distribution was wide in Laprida (75% of the sites) and very wide in Tandil (77.14%). Under field-cage conditions forage loss caused at three different densities (8, 16, and 32 ind/m^2) of *D. elongatus* and *B. bruneri* adults on a pasture of *Festuca arundinacea* was estimated. Forage loss caused by *D. elongatus* was significantly higher than that caused by *B. bruneri*. *Dichroplus elongatus* caused a significant decrease in biomass at the three densities respect to the control, while *B. bruneri* only caused a significant decrease at the highest density. Our study suggests that although the gomphocerine *B. bruneri* is an abundant and widely-distributed species capable of doing some damage in the grasslands of the southern Pampas, it is comparatively much less harmful than the melanopline *D. elongatus*.

Key words: *Borellia bruneri, Dichroplus elongatus, Dichroplus maculipennis, Festuca arundinacea,* Gomphocerinae, Melanoplinae.

INTRODUCTION

Grasshoppers constitute one of the most conspicuous groups of insects in grassland ecosystems. They play a significant ecological role as primary consumers and components of trophic chains, and in the cycling of nutrients and energy (Belovsky 2000, Guo et al. 2006, Song et al. 2018). However, some species of grasshoppers are considered harmful to agriculture and during outbreak years can destroy crops and compete with livestock for available forage (Branson et al. 2006, Mariottini et al. 2012).

Of the 204 grasshopper species known in Argentina, 19 are considered of economic importance for agriculture (Cigliano et al. 2014). Two of them are *Dichroplus elongatus* (Acrididae: Melanoplinae) and *Borellia bruneri* (Acrididae: Gomphocerinae). The melanopline *D. elongatus* is the most widely distributed species of the genus *Dichroplus* and occurs in almost all of Argentina except Tierra del Fuego, in the center and North of Chile, Uruguay, and southernmost Brazil (Carbonell et al. 2017). It is usually the dominant species in grasshopper communities of almost all grassland habitats of the Pampas region (Cigliano et al. 2000, Cigliano et al. 2014, Lange & Cigliano 2019). *Borellia bruneri* is one of the most common species of gomphocerines inhabiting the Pampas grasslands of both Argentina and Uruguay (COPR 1982, Mariottini et al. 2012, Miguel et al. 2014). It shows a wide geographic distribution, also occurring in southernmost Brazil, much of Argentina, Chile, and Uruguay (Cigliano et al. 2014, Carbonell et al. 2017). Both *D. elongatus* and *B. bruneri* undergo obligatory embryonic diapause in their life cycles and hence are univoltine (Bardi & Lange 2011, Mariottini et al. 2020). Following the categories widely accepted for defining the pest status of grasshopper species (COPR 1982), Carbonell et al. (2017) have categorized *D. elongatus* as a "Major pest of several crops" and *B. bruneri* as a "Frequent plague of importance".

In this study we report on our assessment on the status of *D. elongatus* and *B. bruneri* as actual agricultural pests in areas of the southern Argentine Pampas by determining their relative abundance, distribution, and associated forage loss.

MATERIALS AND METHODS

Estimations on abundance and distribution

The study on the abundance and distribution of *B. bruneri* and *D. elongatus* was conducted in native grasslands of Laprida and Tandil, two counties in southern Buenos Aires province (Fig. 1). In Laprida county (345.498 ha) grasslands are the dominant vegetation type, being livestock activity markedly prevalent. Approximately 45% of

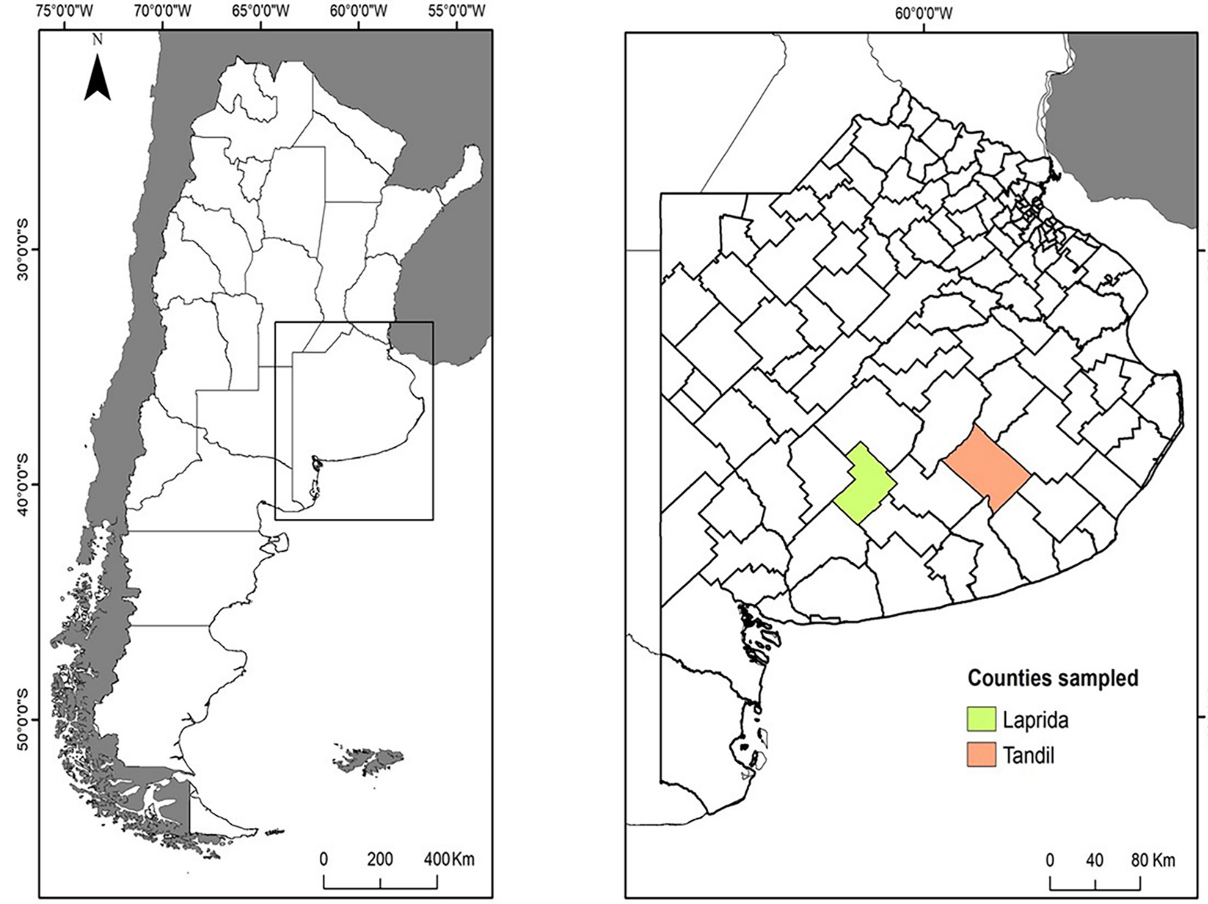

Figure 1. Study area: Laprida and Tandil counties, Buenos Aires province, Argentina.

the county's area is used for livestock production (Batista et al. 2005, Recabarren 2016). In Tandil county (493.500 ha), Sánchez et al. (1999) identified three environments: Hills, hillock plain, and distal plain. Hillock plains succeed the hilly landscape and have good aptitude for agricultural development. The distal or depressed plains succeeds altimetrically the hillock plains and it is where grasslands of *Nassella* sp. and *Piptochaetium* sp. mix with shrub and saxifolia communities (Plants associated with rocky areas) comprise the native vegetation (Vazques & Zulaica 2011).

In each county, 20 sampling sites, as evenly distributed as possible to cover as much of each county, were selected according to a visual impression of the dominant vegetation which represents the native grassland characteristics of the area (Batista et al. 1988, Torrusio et al. 2002). In both counties the sampling of the 40 sites took place between 2012 and 2018.

Like most grasshopper species in the Pampas both *D. elongatus* and *B. bruneri* are univoltine due to having obligatory embryonic diapause (COPR 1982, Bardi & Lange 2011), hatchings normally starting by mid-late spring (Mariottini et al. 2011) and populations usually peaking sometime during January, possibly the best month for collection in order to maximize chances of detection of these two species.

The abundance of *D. elongatus* and *B. bruneri* were determined from 200 sweeps of entomological nets (diameter: 40 cm, depth: 75 cm, arc of sweep: 180º) along four transects of approximately 3 m wide and 50 m long each *per* site according to Evans (1988), a method acknowledged to provide representative samples of grasshopper communities (Larson et al. 1999). Later, the individuals of *B. bruneri* and *D. elongatus* collected were counted and the relative abundance of these was estimated taking into account the abundance of these species in relation to the total number of individuals of all species collected *per* site.

In order to determine the extent of the distribution (frequency of occurrence) of *B. bruneri* and *D. elongatus*, the scale proposed by Mariottini et al. (2013) was followed. By using this scale of distribution the proportion of sites in which the given species was registered was taken into account in relation to the total number of sampled sites throughout the study period. The scale have four categories of distribution: a- (1-25%: restricted), b- (26-50%: Intermediate), c- (51-75%: Wide) and d- (>75%: Very wide), and it is important to indicate that it applies only to the study area.

Estimation of forage loss

To estimate the forage loss, the experience was carried out for a month (from January 3 to February 3, 2019) in a livestock field of *Festuca arundinacea* (a perennial forage grass that can prosper in multiple environments) (Insua et al. 2013, Bazzigalupi & Bertín 2014) located in Tandil county (37º12 '27.39''S, 59º 17' 22.53''O).

In order to estimate the forage loss (consumption + destruction rate) caused by different densities of *D. elongatus* and *B. bruneri* the methodology used by Torrusio et al. (2005) and Mariottini et al. (2018) was followed. In the study site, 21 cages of aluminum and wire mesh screen of 50 cm x 50 cm x 70 cm high (0.25 m^2) were used. In each of the cages, adult males and females of each species were placed in a 1:1 ratio (Torrusio et al. 2005). Three different densities were used according to Mariottini et al. (2018): 8 ind/m^2, 16 ind/m^2, and 32 ind/m^2. Proportionally, 2, 4, and 8 individuals were placed in each cage. Three replicates were performed *per* density tested for *D. elongatus* and *B. bruneri,* and three cages without grasshoppers (0 ind/m^2) were also established as a control. For estimating the initial plant biomass of the pasture, five samples were taken by harvesting all vegetation within an area of 0.25 m^2. After the experience, the vegetation biomass remnant in each of the cages was harvested. All harvested material was dried in an oven at 70 ° C until constant weight and weighted on a precision scale (down to 0.001 g). The forage loss was estimated considering the final plant biomass harvested in the cages with grasshoppers respect to the control cages. The experience was monitored daily to ensure the presence of all grasshopper individuals in each of the cages.

Statistical analysis

In order to compare the abundance of *B. bruneri* and *D. elongatus* over the years (2012-2018) and between the counties an ANOVA of repeated measures were used. The dependent variable was the relative abundance (estimated as percentage). Prior to performing the ANOVA, the Mauchly sphericity test was used and the adjustment of the degrees of freedom was conducted by the Greenhouse Geisser method (Scheiner & Gurevitch 2001).

In order to assess forage increase during the study the initial biomass was compared with the final biomass of control cages (without grasshoppers) through a T test with the Satterwait correction. For comparing the final biomass of *F. arundinacea* harvested in cages with different grasshopper densities and the control an analysis of variance of two-factors (ANOVA) was performed. The mean consumption *per* individual was estimated by dividing the loss of forage occurred in each of the cages by the number of individuals present *per* day. The daily consumption by *B. bruneri* and *D. elongatus* was compared through ANOVA against the consumption made by the melanopline *D. maculipennis*, the most harmful grasshopper species in the area (Mariottini et al. 2018).

In all ANOVA analysis the Fisher test (LSD) was used *a posteriori* to compare means.

RESULTS

Abundance and distribution

In Laprida, the mean species richness *per* sampling site varied between a minimum value of 4.3 ± 0.44 species recorded in 2012 and a maximum of 6.9 ± 0.34 species collected in the 2016 season. In this county, the mean total number of individuals collected *per* sampling site was lower in 2015 (77.44 ± 7.25 individuals *per* site) and higher in 2016 (201.4 ± 20.13 individuals *per* site) (Table I). In Tandil, the variation in species richness was less, the lowest value was recorded in 2015 (4.1 ± 0.31 species *per* sampling site) and the highest was in the 2017 season (4.9 ± 0.29 species/ site), while in terms of number of individuals collected *per* sampling site the lowest value was observed in 2013 (29.40 ± 18.45 individuals/site), and the highest was in 2017 (162.9 ± 29.64 individuals/site) (Table II).

Abundance of the two species considered was significantly different both between counties and years. The interaction between the factors were significant ($p<0.05$) (Table III), suggesting changes in abundance of the two species over the years. With respect to *B. bruneri* population status, the relative abundance of this species was higher in Laprida than that recorded in Tandil (Fisher LSD p: 0.00038), registering a significant difference from 2013 onwards (LSD Fisher $p<0.05$) (Fig. 2). In addition, a significant variation in abundance of this species within each county was observed. In Laprida, there was a higher abundance in 2014 (27.57 ± 3.12%) and 2016 (30.48 ± 4.24%) compared to 2012 (17.87 ± 4.35%), 2013 (21.99 ± 2.94%), and 2015 (17.38 ± 4.38%) (LSD Fisher $p <0.05$). In Tandil, the abundance of *B. bruneri* in 2012 (14.59 ± 5.78) was

Table I. Mean values (± ES) of species richness, total individuals, relative abundance and distribution of *Borellia bruneri* and *Dichroplus elongatus*, between 2012 to 2018 in Laprida county. Between brackets minimum and maximum values.

LAPRIDA	**Characteristics**		***Borellia bruneri***		***Dichroplus elongatus***	
	Species richness *per* site	**Total individuals collected *per* site**	**Relative abundance *per* site (%)**	**% of sites with presence**	**Relative abundance *per* site (%)**	**% of sites with presence**
2012	4.3 ± 0.4	105.6 ± 10.5 (25-207)	19.3 ± 4.2 (0-69.5)	95	11.1 ± 1.4 (0-66.1)	35
2013	5.5 ± 0.2	84.9 ± 6.3 (26-133)	19.7 ± 2.7 (0-47.7)	95	16.9 ± 4.6 (0-50.4)	60
2014	5.9 ± 0.6	84.1 ± 10.6 (27-203)	27.5 ± 4.2 (0-65.9)	90	17.9 ± 5.1 (0-47)	95
2015	5.6 ± 0.3	77.4 ± 7.25 (29-132)	17.7 ± 4.1 (0-55.3)	70	27.8 ± 2.9 (0-75.9)	90
2016	6.9 ± 0.3	201.4 ± 20.1 (56-384)	30.3 ± 4.3 (2-73.3)	100	15.3 ± 3.8 (0-72.8)	90
2017	5.7 ± 0.3	184.1 ± 38.5 (24-608)	22.8 ± 5.5 (0-68.4)	90	21.8 ± 5.5 (0-76.1)	85
2018	6 ± 0.4	189.3 ± 30.5 (34-405)	24.4 ± 2.5 (7.4-52)	100	15.3 ± 3.3 (0-68.1)	75

Table II. Mean values (± ES) of species richness, total individuals, relative abundance and distribution of *Borellia bruneri* and *Dichroplus elongatus*, between 2012 to 2018 in Tandil county. Between brackets minimum and maximum values.

TANDIL	**Characteristics**		***Borellia bruneri***		***Dichroplus elongatus***	
	Species richness *per* site	**Total individuals collected *per* site**	**Relative abundance *per* site (%)**	**% of sites with presence**	**Relative abundance *per* site (%)**	**% of sites with presence**
2012	4.2 ± 0.4	69.7 ± 12.3 (4-240)	14.6 ± 5.8 (0-100)	60	55.3 ± 8 (0-100)	80
2013	4.5 ± 0.3	59.4 ± 18.4 (3-285)	7.6 ± 2.7 (0-47.9)	45	49.9 ± 7.2 (0- 94.8)	85
2014	4.2 ± 0.3	91.8 ± 20.1 (7-308)	11.7 ± 5.1 (0-84)	45	49.2 ± 7.2 (0-93.2)	85
2015	4.1 ± 0.3	92.1 ± 24.8 (11-474)	6.1 ± 3.7 (0-50.1)	25	47.6 ± 6.9 (0-84.2)	85
2016	4.2 ± 0.4	146.6 ± 39.3 (6-645)	4.7 ± 2.7 (0-50.7)	25	26.4 ± 6.4 (0-90.5)	60
2017	4.9 ± 0.3	162.9 ± 29.6 (20-518)	6.2 ± 3 (0-57.3)	45	33.6 ± 6.2 (0-94.6)	80
2018	4.8 ± 0.4	136.8 ± 29.5 (34-405)	7.9 ± 3.7 (0-65.3)	50	19.1 ± 5.7 (0-68.1)	65

Table III. Results of repeated measures ANOVA for relative abundance of *Borellia bruneri* and *Dichroplus elongatus* in Laprida and Tandil counties between 2012 to 2018.

Effects	DF Effect	DF Error	F	p-value	df Effect [1]	df Error [1]	p-value [1]
Counties	1	76	1.7	0.187			
Species	1	76	29.4	0.0000			
County x species	1	76	54.6	0.0000			
Year	6	456	2.3	0.0309	4.772	362.7	0.044
Years x county	6	456	4.4	0.0002	4.772	362.7	0.0008
Years x species	6	456	3.5	0.002	4.772	362.7	0.0046
Years x species x county	6	456	1.4	0.204	4.772	362.7	0.218

[1] Adjusted probability based on the epsilon value of the Greenhouse-Geisser estimator (ε: 0.65).

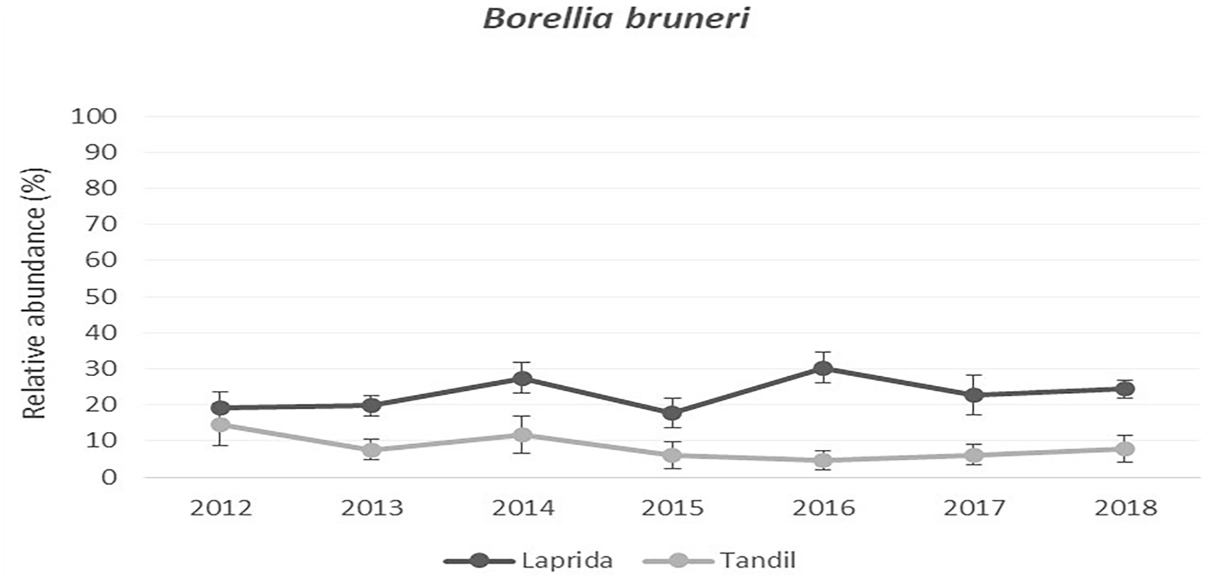

Figure 2. Relative abundance of *Borellia bruneri* in native grasslands of Laprida and Tandil counties, between 2012 to 2018.

higher (LSD Fisher p <0.05) with respect to 2015 (4.67 ± 2.69%) and 2016 (4.60 ± 2.69%).

Unlike *B.bruneri*, abundance of *D. elongatus* was significantly higher from 2012 to 2016 in Tandil relative to Laprida (LSD p <0.0001). In Tandil, the abundance of this species remained stable until 2016 when it decreased significantly. The abundance of *D. elongatus* between 2012 and 2015 was around 50% in each season (Fig. 3) and in 2016 it decreased approximately by half (26.41 ± 6.37%). Then, in 2017 (33.64 ± 6.19%) increased, and decreased again 2018 (7.19 ± 5.72%) although with no significant changes between the last two years. In Laprida, abundance of this species also remained relatively constant throughout the study, only a higher abundance was recorded in 2015 (27.8 ± 2.9%) compared to 2012 (11.07 ± 1.41%).

Regarding the distribution and considering all sites/years, *B. bruneri*, had a very wide distribution in Laprida county, having been registered in 91.4% of the sampled sites (128/140) and an intermediate distribution in Tandil of 42.14% of the total sites sampled (59/140 sites). *Dichroplus elongatus* in Laprida had a wide distribution representing 75% (105/140)

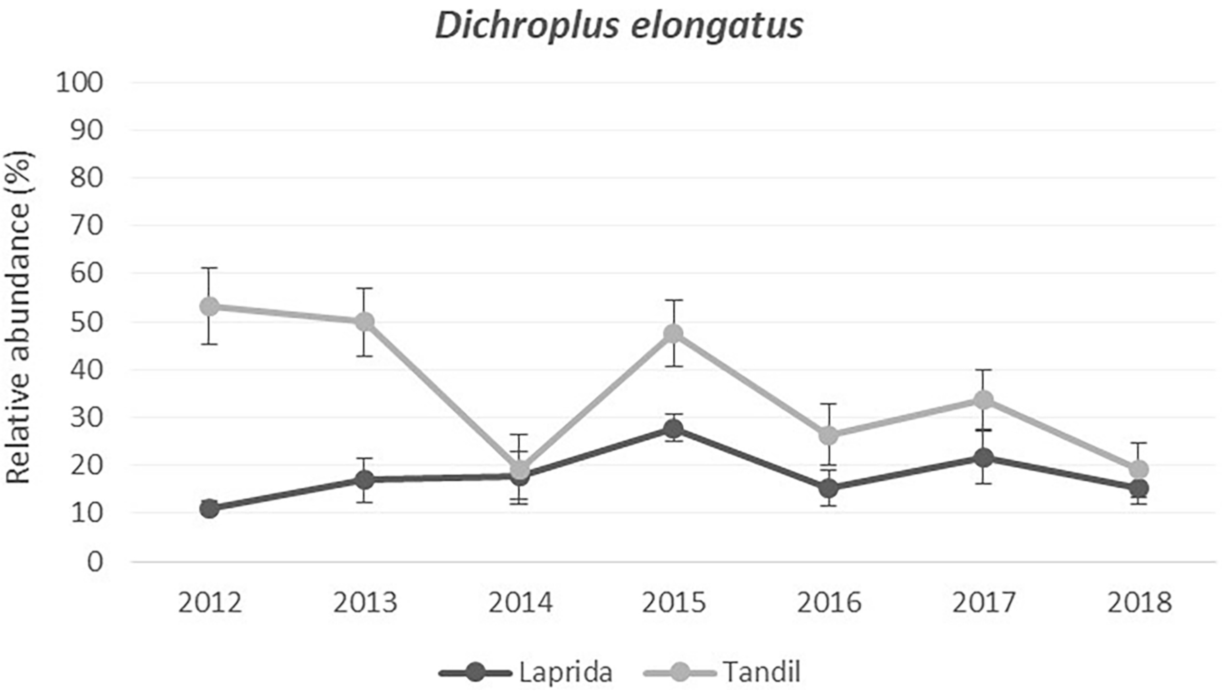

Figure 3. Relative abundance of *Dichroplus elongatus* in native grasslands of Laprida and Tandil counties, between 2012 to 2018.

of the sampled sites and in Tandil a very wide distribution of 77.14% of the sites (108/140).

Forage loss

Biomass of *F. arundinacea* estimated at the beginning and at the end (as from the cages without grasshoppers) of the experience was 182.52 ± 18.64 g/m^2 and 333.70 ± 15.22 g/m^2, respectively, and the difference between them was significant (T: 6.28 p = 0.0033). An increase in biomass of 54.7% was observed during the study.

Results of the ANOVA analysis indicated that the observed differences in forage loss were significant for both by species and by density (Table IV). The loss of forage caused by *D. elongatus* was significantly higher than that caused by *B. bruneri* (LSD Fisher p <0.005). The forage loss caused by the three tested densities of *D. elongatus* was different from the control and between them (LSD Fisher p <0.005).

The final biomass at a density of 8 ind/m^2 of *D. elongatus* was 252.19 ± 5.52 g/m^2, about 24.4% less than the final plant biomass of the control. At a density of 16 ind/m^2 the biomass was of 168.53 ± 7.59 g/m^2, approximately 49.5% less than the control, and at 32 ind/m^2 the final plant biomass was of 131.03 ± 10.26 g/m^2, 60.7% less than the control (Fig. 4).

Considering the food consumption made by *B. bruneri*, the final biomass of *F. arundinacea* at densities of 8 ind/m^2 and 16 ind/m^2 was of 293.23 ±25.18 g/m^2 and 269.46 ± 12.43 g/m^2, respectively, values that were not significantly different from the final biomass of the control (LSD Fisher p> 0.05). The decrease in final biomass caused by *B. bruneri* respect to the control at a density of

Table IV. Results of two-way ANOVA of forage loss caused by differents densities of *Borellia bruneri* and *Dichroplus elongatus* on a *Festuca arundinacea* pasture.

Factors	DF	F	p-value
Density	3	25.3	<0.0001
Species	1	12.0	0.0032
Density * species	3	1.9	0.1738

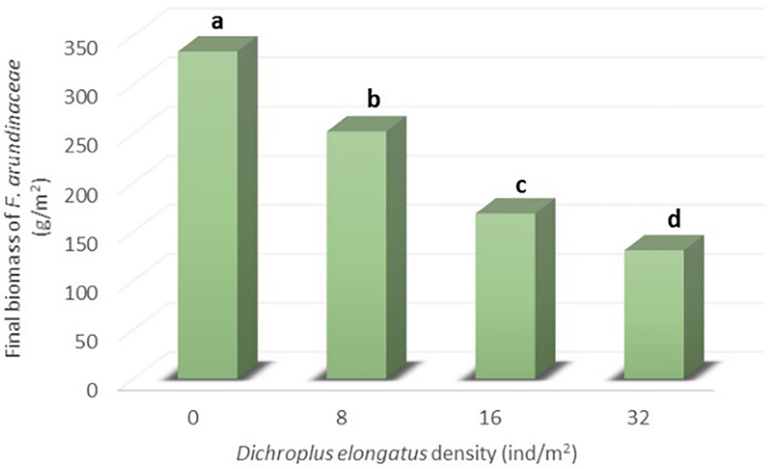

Figure 4. Mean value (± SE) of the final biomass of *Festuca arundinacea* (g/m^2) in control cages (without grasshoppers) and in the cages with the three tested densities of *Dichroplus elongatus*. Different letters indicate significant differences (LSD p <0.05).

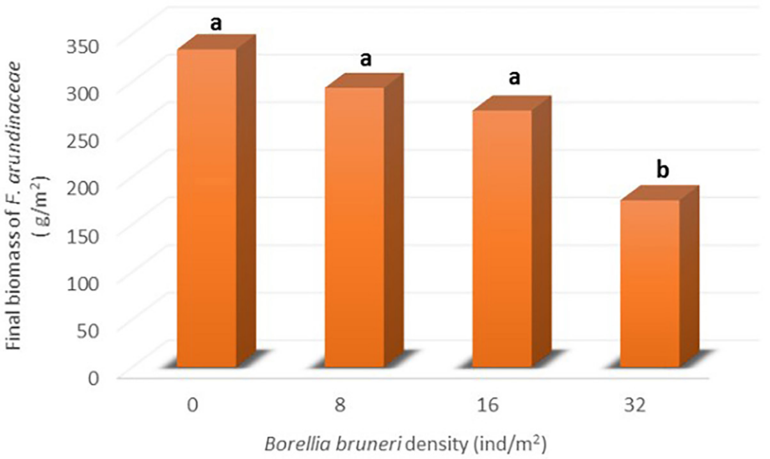

Figure 5. Mean value (± SE) of the final biomass of *Festuca arundinacea* (g/m^2) in control cages (without grasshoppers) and in the cages with the three tested densities of *Borellia bruneri*. Different letters indicate significant differences (LSD p <0.05).

8 ind/m^2 was approximately 12.13%, and at 16 ind/m^2 was of 19.25%. A significant reduction of biomass was produced with a density of 32 ind/m^2 (175.05 ± 8.23 g/m^2), decreasing plant biomass approximately in a 47.57 ± 2.47% (Fig. 5).

When compared with *D. maculipennis* (as in Mariottini et al. 2018), the daily consumption of adults of *D. elongatus* and *B. bruneri* were significantly different (ANOVA F= 7.48; p= 0.003). The consumptions of *D. elongatus* (0.30 ± 0.02 g/day) and *D. maculipennis* (0.24 ± 0.02 g/day) (Mariottini et al. 2018), which were similar to each other (LSD Fisher p>0.05), were higher (LSD Fisher: p<0.05) than the consumption made by *B. bruneri* adults (0.16 ± 0.03 g/day).

DISCUSSION

The results of the present study revealed that both *D. elongatus* and *B.bruneri* had a high relative abundance within the grasshopper communities of southern Pampas of Argentina. During the evaluated period of time (2012 to 2018) they both showed to be species with a wide distribution range and a high frequency of occurrence, having been registered year after year in the majority of the sampled sites.

Borellia bruneri was more abundant and with a higher frequency of occurrence in Laprida than in Tandil and the opposite situation occurred with *D. elongatus*, being more abundant and frequent in Tandil than in Laprida. Previous studies on different ecological aspects of grasshoppers carried out in various plant communities of the Pampas region evidenced a high association of *B. bruneri* with halophilous vegetation (Torrusio et al. 2002, De Wysiecki et al. 2004, Mariottini et al. 2012, 2013). This would explain in part the higher abundance of *B. bruneri* recorded in Laprida relative to Tandil because larger areas of the former county´s grasslands are covered with short-type of grasses than in Tandil, a habitat greatly favored by *B. bruneri* (Batista et al. 2005, Mariottini et al. 2013, Recabarren 2016).

On the other hand, *D. elongatus* is normally favored with relatively humid type of habitats in relation to more arid ones (Carbonell et al. 2017). Various studies conducted on different areas of the Pampas depicted *D. elongatus* as one of the species numerically most important and also most widely distributed (Sánchez & De Wysiecki 1993, Cigliano et al. 1995, Cigliano et al. 2000). A study made by Torrusio et al. (2002) found that *D. elongatus* was the most abundant species and was associated with implanted grasses and introduced forbs in Benito Juarez county which neighbors Laprida and Tandil. Also in Benito Juarez, Cigliano et al. (2002) recorded that *D. elongatus* was the most prevalent species during an outbreak that occurred from 2001 to 2002.

Results of the forage loss test showed that at same densities *D. elongatus* produced a greater forage loss than *B. bruneri*. At all the densities tested *D. elongatus* caused a significant decrease in the plant biomass of *F. arundinacea* respect to the control, unlike *B. bruneri* which only at a density of 32 ind/m^2 caused a significant decrease in pasture biomass. The only previous quantitative assessment on the abundance of *B. bruneri* was provided by Mariottini et al. (2012) although not as a segregated species but in combination with *D. maculipennis*, on occasion of the 2008-10 outbreak in the southern Pampas. Both species were the ones that contributed the most to the overall increase in the grasshopper communities, reaching a mean density of 40 ind/m^2 and peaks of 75 ind/m^2, and causing substantial economic losses to ranchers and farmers.

The density at which economic damage or a significant decrease in plant biomass occurs is dynamic and depends (among other variables) upon the capacity of vegetation growth, the phenological state of the vegetation, and the climatic conditions (Thompson & Garner 1996). Belovsky (2000) indicated that the water status of the plant community largely determines the magnitude of the damage caused by a certain grasshopper density. During January 2016, Mariottini et al. (2018) conducted a similar experience evaluating the loss of forage caused by *D. maculipennis,* one of the most harmful grasshopper species of Argentina (Mariottini et al. 2015, Carbonell et al. 2017). At that time, the accumulated rainfall (184.52 mml, double the average value for the month and study area) favored an increase of about five times the plant biomass in control cages (without grasshoppers). In the present study, climatic conditions were not as favorable, with lower rainfall (98mml) and an increase in plant biomass in control cages of 54.7%.

In the current work, the three densities tested for *D. elongatus* caused a significant decrease in plant biomass with respect to the control and between them. Mariottini et al. (2018) used the same densities with *D. maculipennis* observing that all three of them also caused a significant decrease in biomass in relation to the control, but there was no significant difference between the decrease caused at 8 ind/m^2 and 16 ind/m^2. This situation could have been resulted from a possible compensatory growth (Dyer et al. 1982) of *F. arundinacea* after herbivory caused by favorable precipitation conditions registered at that time.

Taking into account the results obtained here and those obtained by Mariottini et al. (2018) at equal densities, *B. bruneri* shows a lower forage consumption than *D. elongatus* and *D. maculipennis*. In addition to consumption and destruction of vegetation, another relevant factor that may determine whether a grasshopper species would constitute a pest or not is its fecundity. In this sense, both *D. elongatus* (81.09 ± 14.02 eggs/female) and *D. maculipennis* (83.3 ± 11.9 eggs/female) showed much higher fecundity than that of *B.bruneri* (37.9 ± 1.8 eggs/female) all estimated under the same laboratory conditions (De Wysiecki et al. 1997, Mariottini et al. 2011, 2020). All in all, our study suggests that although the gomphocerine *B. bruneri* is an abundant and widely-distributed species capable of doing some damage in the grasslands of the southern Pampas region, it is comparatively much less harmful than the melanoplines *D. elongatus* and *D. maculipennis*, a fact that should be taken into account when control measures are considered due to upsurges in grasshopper communities.

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