CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Volume 75 N° 4 OCTOBER ▪ DECEMBER 2015

ISSN 0718-5820 Printed version
ISSN 0718-5839 Online version

CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Volume 75 Nº 4 | OCTOBER ▪ DECEMBER 2015

RESEARCH ARTICLES

SCIENTIFIC NOTES

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CHILEANJAR

RESEARCH

Resistance in alfalfa to *Aphis craccivora* Koch

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The cowpea aphid, *Aphis craccivora* Koch (Aphididae), is considered to be one of the major pests in the semiarid Pampas of Argentina and in other alfalfa (*Medicago sativa* L.) growing areas of the world. In the present study the antibiotic, antixenotic, and tolerance resistance of eight alfalfa cultivars to *A. craccivora*, were investigated under laboratory conditions at 24 ± 1 °C, 65 ± 10% relative humidity, and 14:10 h photoperiod. Antibiosis experiments showed significant differences in the developmental time and adult longevity of the cowpea aphid among the alfalfa cultivars. Intrinsic rate of natural increase (r_m) for apterous aphids varied significantly with alfalfa cultivars on which aphids were reared. This value ranged from 0.04 to 0.16 females female^{-1} d^{-1}, which was the lowest on 'Medina'. Additionally, the estimated net reproductive rate (R_0) and finite rate of increase (λ) for apterous aphids were the lowest on 'Medina'. For the antixenosis experiment significant difference was found in aphid's preference to the alfalfa cultivars. 'Carmina' and 'Victoria' were the least preferred by the apterous aphids. For the tolerance experiment 'Carmina', 'Monarca', 'SPS6550' and 'Victoria' were more tolerant than the other cultivars to *A. craccivora*. Therefore, our results demonstrated that among the investigated cultivars 'Medina' displayed antibiosis to *A. craccivora*, 'Carmina' and 'Victoria' expressed antixenosis and tolerance, and 'Monarca' and 'SPS6550' were tolerant to this aphid pest.

Key words: Antibiosis, antixenosis, cowpea aphid, host plant resistance tolerance.

INTRODUCTION

Medicago sativa L. (Fabaceae) is one of major forage crops through the world and in Argentina cover an area of 6.9 million ha (Yuegao and Cash, 2009). It is a perennial crop that is harvested several times each year. It produces a high forage yield, composed of stems and leaves, with a high protein concentration but a moderate energy value (Julier et al., 2008).

Alfalfa is a versatile crop that can be used for pasture, hay, silage, or green chop. As a result of its versatility, yield potential, and quality, alfalfa can be used successfully in many types of livestock feeding programs. It can also play an important role in crop rotations since it supplies substantial amounts of organic N to subsequent crops and has numerous other positive effects on soil fertility, soil structure, and soil health (Shebl et al., 2008; Li and Brummer, 2012).

Alfalfa's dense canopy and crown structure affords a wide variety of habitats and niches for exploitation by a diverse array of organisms (Shebl et al., 2008; Pons et al., 2013). While most of alfalfa's inhabitants have little or no impact on it as a crop, a few are capable of causing extensive damage.

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Received: 28 November 2014.
Accepted: 8 July 2015.
doi:10.4067/S0718-58392015000500010

The cowpea aphid, *Aphis craccivora* Koch, is considered to be one of the major pests in the semiarid Pampas of Argentina and in other alfalfa growing areas of the world (Ortego et al., 2004; Ferreira da Silva and Bleicher, 2010). This species can remove considerable amounts of liquid and nutrients from phloem, and plant heavily infested are stunted in growth and display shortened internodes and chlorosis of the foliage (Hill, 2008). Indeed, *A. craccivora* causes major yield losses due to the transmission of two viruses, *Alfalfa enation virus* (AEV) and *Alfalfa mosaic virus* (AMV) for which it is the most important vector (Das et al., 2008; Bejerman et al., 2011; Wintermantel and Natwick, 2012; Manfrino et al., 2014).

Control of aphids using insecticide has proven difficult because this pest often develop insecticide resistance (Mokbel and Mohamed, 2009). Thus, host plant resistance aphids in agricultural crops offers a sustainable means of combating this pest. Resistance to aphids involves a variety of mechanisms (Smith, 2005), including maintenance of plant growth and seed production despite aphid infestation (tolerance), reduction in aphid preference (antixenosis) or repression of aphid growth and development (antibiosis) (Kamphuis et al., 2013). Accordingly, we have focused on evaluation of antixenosis, antibiosis, and tolerance of *M. sativa* cultivars against *A. craccivora*.

MATERIALS AND METHODS

Plant material and insects

Eight cultivars of alfalfa grown in the semiarid Pampas of Argentina were selected for this study: 'Garufa', 'SPS 6550', 'Victoria' with fall dormancy 6 and 'Carmina', 'Esperanza', 'Monarca' with fall dormancy 8 and 'Medina' with fall dormancy 9 (Spada, 2006; 2007). The alfalfa seeds were obtained from the Instituto Nacional de Tecnología Agropecuaria (INTA) in Bordenave, Argentina, and were planted individually in 10-cm diameter clay pots filled with Entic Haplustoll soil (Soil Survey Staff, 1999) fertilized at commercial rates NPK 00-46-00. The plant grew at 24 ± 1 °C, 65 ± 10% RH, and a photoperiod of 14:10 h.

The insects of *Aphis craccivora* Koch were obtained from colonies established in alfalfa crop field collections and maintained on alfalfa 'Garufa', 'SPS 6550', 'Victoria', 'Carmina', 'Esperanza', 'Medina', and 'Monarca' in wood-framed cages (35 × 35 × 70 cm) in the laboratory under the aforementioned conditions. The aphid population was reared for several generations before experiments were conducted. The host plant resistance to *A. craccivora* was studied in the laboratory at 24 ± 1 °C, 65 ± 10% RH, and a photoperiod of 14:10 h.

Evaluation of resistance by antibiosis (development, fecundity, and life table parameters)

To evaluate developmental time and survival of immature stages, fecundity and longevity of adults, approximately 50 adult apterous aphids were randomly chosen from the rearing colonies and placed on the leaf surface, each confined inside a clip cage (1 cm diameter × 1 cm height) to prevent escape and parasitism. They were permitted to produce nymphs for 24 h and then the adult aphids were eliminated from the leaf clip cage. Each plant received one aphid nymph that was confined to the first true leaf. These nymphs were monitored daily to assess the aphid's performance on alfalfa. After maturity and the beginning of reproduction, adult mortality and fecundity were recorded daily, and the offspring were removed from each leaf cage until each adult aphid died. We estimated the fecundity of 20 adult aphids for each cultivar in this study. Differences in fecundity, longevity, and development time were analyzed using ANOVA and test of least significant difference (LSD, $p < 0.05$).

Life tables were constructed based on Birch (1948) and Southwood and Henderson (2000). The survival rate for adults from birth to age x (l_x), fecundity (m_x, total number of offspring produced at age x), and m_x (female offspring produced at age x) were measured according to Birch (1948). From these data, the intrinsic rate of increase (r_m, females female^{-1} d^{-1}), net reproductive rate (R_0, females female^{-1} generation^{-1}), finite rate of increase (λ, individuals female^{-1} d^{-1}), mean generation time (T), and doubling time (DT, d) were estimated with software written for this purpose (La Rossa and Kahn, 2003).

Differences in r_m and other life table parameters were tested for significance and the variance was estimated by the Jackknife method (Maia De et al., 2000). Jackknife pseudo-values were calculated with a computer program (La Rossa and Kahn, 2003) and the mean Jackknife pseudovalue for each treatment was subjected to ANOVA. Least significant difference (LSD) was employed to compare r_m and other life table parameters on different cultivars of alfalfa.

Evaluation of antixenotic resistance (non-preference)

The eight alfalfa cultivars were randomized and planted in a circular pattern 3 cm from the edge of a 32.5 cm-diameter pot. When plants were 5 to 8 cm tall, seven aphid adult per plant were released on the soil in the center of the pot. Plants and aphids were covered with plastic cages (30 cm in diameter by 50 cm high) with a cloth top and cloth-covered ventilation holes on the sides. The aphids were allowed 48 h to select the plant of their choice, at which time the numbers on each plant were recorded. The test included 10 replicates (pots). The results were analyzed using ANOVA and test of least significant difference (LSD, $p < 0.05$).

Evaluation of tolerance

Tolerance was evaluated by comparing dry weight of infested plants with that of healthy plants. The eight cultivars were grown in 10-cm diameter clay pots filled with Entic Haplustoll soil with 1 seed sown per pot. Twenty seeds in total for each cultivar were planted, 10 for infestation and 10 for use as negative controls (not infested). The plants were arranged in the greenhouse in a randomized design. At the three leaf stage, five apterous *A. craccivora* females were placed on each of the plants to be infested. The plants were then individually covered with tulle netting, to prevent external contamination. The plants were checked every 2 d. If necessary, individual aphids were moved from one plant to another, to ensure that the same level of infestation was maintained in all infested plants, throughout the experiment. After 1-mo, plants were cut at the level of the collar and placed individually in paper bags, transferred to the laboratory and placed in an oven at 75 °C for 72 h to determine dry weight. The results were analyzed using *t*-Student test ($p < 0.05$).

The decrease in dry weight due to infestation was determined as follows:

Decrease in dry weight (%) = $[(W_1 - W_2) \times 100]/W1$,

where W_1 is the dry weight of non-infested plants (g) and W_2 is the dry weight of infested plants (g) (Laamari et al., 2008).

RESULTS AND DISCUSSION

The percentage of nymphal mortality was different for each cultivar of alfalfa. The highest percentage of nymphal mortality was found on 'Medina' (90%) (Table 1, Figure 1). The development time of aphids was significantly different for cultivar tested ($p < 0.05$). Nymphs reared

Table 1. Nymphal mortality, developmental time, adult longevity, and fecundity of *Aphis craccivora* reared on eight alfalfa cultivars.

	Developmental data (mean ± SD)[1,2]				
Cultivar	Nymphal mortality (%)	Developmental time	Adult longevity	Total number of offspring/female	Number of offspring/ reproduction day
	%	d	d		
ACA 605	30	7.90 ± 4.24b	16.10 ± 9.94b	8.71 ± 3.99a	1.09 ± 0.58a
Carmina	30	9.15 ± 3.91b	16.4 ± 8.86b	11.54 ± 6.09a	1.51 ± 0.60a
Esperanza	30	7.15 ± 3.76ab	16.2 ± 10.02b	13.64 ± 7.92a	1.51 ± 0.51a
Garufa	25	7.90 ± 3.86b	18.15 ± 9.30b	10.13 ± 6.35a	1.54 ± 1.08a
Medina	90	4.70 ± 4.19a	6.25 ± 7.76a	13.50 ± 0.71a	1.06 ± 0.48a
Monarca	10	8.65 ± 3.01b	17.90 ± 6.87b	11.22 ± 5.60a	1.59 ± 0.52a
SPS6550	35	8.15 ± 4.84b	15.55 ± 9.68b	10.46 ± 4.24a	1.43 ± 0.50a
Victoria	30	8.05 ± 3.83b	15.55 ± 8.51b	10.71 ± 4.87a	1.86 ± 0.72a

SD: Standard error.
[1]Means within columns followed by the same letters are not significantly different (LSD, P > 0.05).
[2]Sample size is 20 (apterous aphid tested) for each parameter.

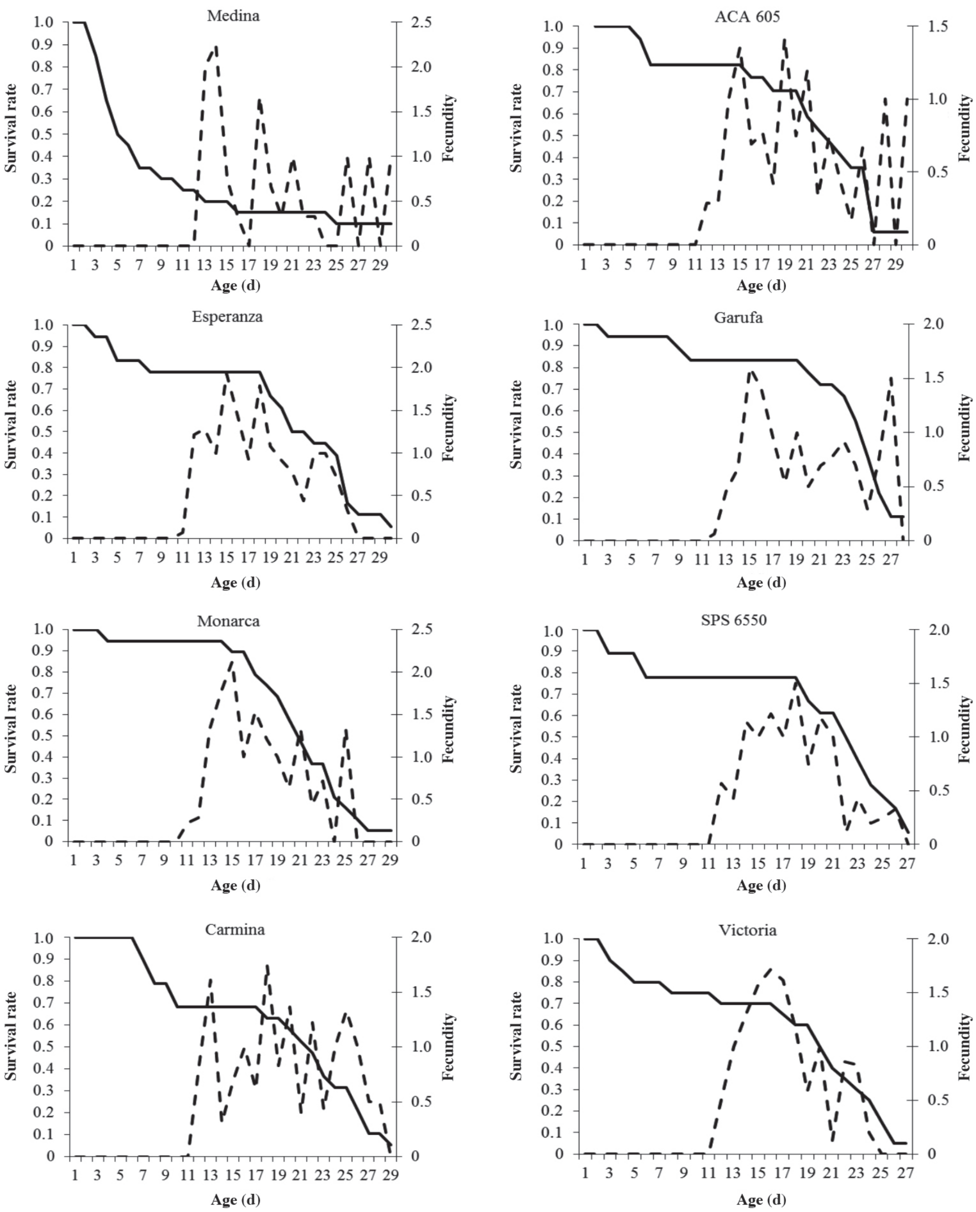

Figure 1. Daily survival rate (—) and fecundity (---) of *Aphis craccivora* on eight alfalfa cultivars under laboratory conditions.

on 'Medina' had a shorter developmental time (4.70 d) than those reared on 'ACA 605', 'Carmina', 'Garufa', 'Monarca', 'SPS 6550', and 'Victoria' (Table 1).

Life expectancy of 1-d-old nymphs on the first day was 18.65, 18.39, 16.89, 16.75, 16.61, 16.06, 16.05, and 8.3 day on 'Garufa', 'Monarca', 'Carmina', 'Esperanza', 'ACA 605', 'SPS 6550', 'Victoria' and 'Medina', respectively (Figure 2).

Alfalfa's cultivars showed no significant effects on numbers of offspring per female or offspring per day ($p > 0.05$). However significant effects were observed on aphid longevity. Aphids had a shorter average longevity in 'Medina' than on other cultivars ($p < 0.05$). The longer value of longevity was found in 'Garufa' and 'Monarca' (18.15 and 17.90 d, respectively) (Table 1). *Aphis craccivora* had the highest mean number of offspring per female on 'Esperanza' (13.64) and the lowest on 'ACA 605' (8.71) (Table 1). The means of offspring produced per day was highest on 'Victoria' (1.86) and the lowest on 'Medina' (1.06) (Table 1, Figure 1).

The value of the aphid net reproductive rate (R_0) showed difference between 'Medina' and the other cultivars tested ($p < 0.05$). Aphids fed on 'Monarca' and 'Esperanza' had the highest R_0 values (10.63 and 10.54

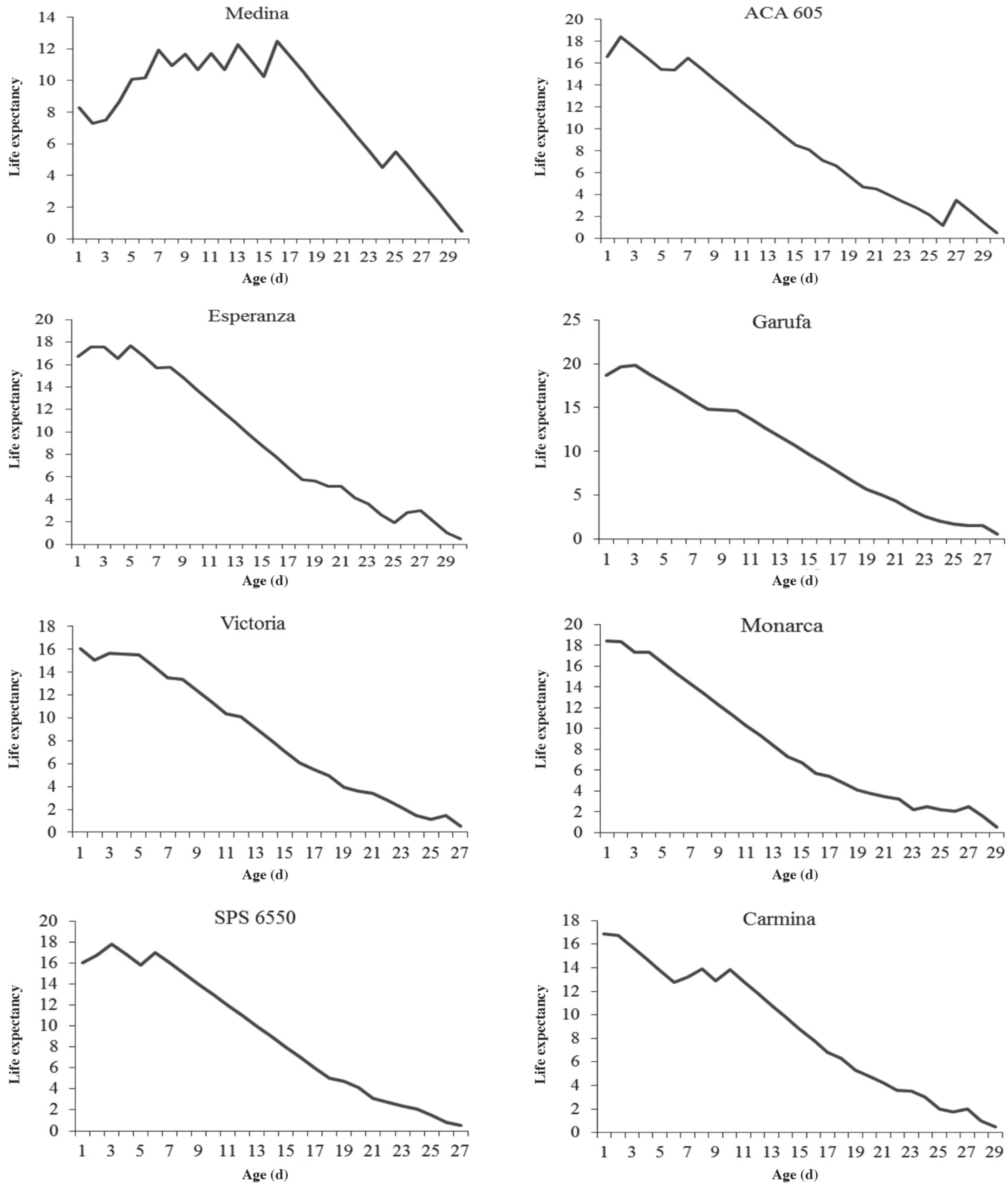

Figure 2. Life expectancy of *Aphis craccivora* on eight alfalfa cultivars under laboratory conditions.

aphids aphid^{-1}, respectively) while on 'Medina' had the lowest value (2.02 aphids aphid^{-1}) (Table 2). The mean generation time values of *A. craccivora* on 'Medina' (19.88) was longer than on the other cultivars ($p > 0.05$). The lowest values of mean generation times were found on 'Monarca', 'Victoria' and 'Esperanza' (15.25, 15.46, and 15.59 aphids, respectively) (Table 2). In addition, doubling times (DT) and the finite rate of increase (λ) of the cowpea aphid population were significant different between 'Medina' and other cultivars tested ($p < 0.05$) (Table 2). The intrinsic rate of natural increase (r_m) of viviparous apterae of *A. craccivora* was different between 'Medina' and other cultivars of alfalfa ($p < 0.05$). The r_m value was the highest on 'Monarca' (0.16 nymphs aphid^{-1} d^{-1}) compared to the aphids reared on the other cultivars. This estimated value of the eight cultivars varied from 0.04 to 0.16 females female^{-1} d^{-1}. Finally, the lowest r_m value was attained when the aphid populations were reared on 'Medina' (0.04 nymphs aphid^{-1} d^{-1}) (Table 2).

'Medina' seemed to display the highest level of antibiosis-mediated resistance to the aphids, regardless of the biological parameter considered. The significantly lower r_m, shorter duration of reproductive life and the high nymphal mortality on 'Medina' may reflect poor aphid nutrition. This may result from the difference in leaf cuticle structure, the composition of epicuticular lipids (Cameron et al., 2006), or different kinds of saponins and content (Golawska et al., 2012). Some studies have suggested that substances synthesized after aphids infestation may play a role in resistance through antibiosis (Laamari et al., 2008; Sadek et al., 2013).

The proportion of adult aphids differed sharply among alfalfa cultivars ($p < 0.05$). 'ACA605', 'Garufa', 'Medina', and 'Monarca' were the most preferred by *A. craccivora* (2.66 aphids stem^{-1}). 'Carmina' and 'Victoria' showed a high level of antixenosis (0 aphids stem^{-1}) (Table 3). This resistance may be caused by physical plant factors like color and shape of leaves (Laamari et al., 2008). Some authors have suggested that aphids tend to be more attracted to large leaves than to narrow leaves (Oyetunji et al., 2014). Searches for cultivars with particular characteristics in terms of leaf color and shape might lead to the obtaining of new cultivars displaying resistance by antixenosis.

Table 3. Preferences of *Aphis craccivora* for eight alfalfa cultivars.

Cultivar	Mean ± Standard deviation
ACA 605	2.66 ± 7b
Carmina	0.00 ± 0.00a
Esperanza	2.33 ± 1b
Garufa	2.66 ± 4.75b
Medina	2.66 ± 4.75b
Monarca	2.66 ± 4b
SPS 6550	2.00 ± 2.25b
Victoria	0.00 ± 0.00a

The different letters indicate significant differences among the cultivars of alfalfa ($p < 0.05$).

'Carmina', 'Monarca', 'SPS 6550', and 'Victoria' were more tolerant to *A. craccivora* than the other cultivars (Table 4). Infestation decreased dry weight by only 10.53% for 'SPS 6550' and 26.70% for 'Monarca' (Figure 3).

Table 4. Tolerance of eight alfalfa cultivars to *Aphis craccivora*.

	Mean of dry weight ± SD (g)		
Cultivar	Infested plants	Non-infested plants	*t*-Student*
ACA 605	0.4248 ± 0.08	0.7584 ± 0.11	2.45*
Carmina	0.3602 ± 0.02	0.7175 ± 0.10	1.78
Esperanza	0.4664 ± 0.003	0.8171 ± 0.03	3.40*
Garufa	0.4197 ± 0.01	0.6325 ± 0.01	2.95*
Medina	0.5988 ± 0.03	0.8905 ± 0.11	2.47*
Monarca	0.494 ± 0.06	0.6685 ± 0.10	1.49
SPS 6550	0.7149 ± 0.12	0.799 ± 0.09	0.54
Victoria	0.3157 ± 0.02	0.5172 ± 0.03	1.53

*Indicate significant differences among the cultivars of alfalfa ($p < 0.05$).

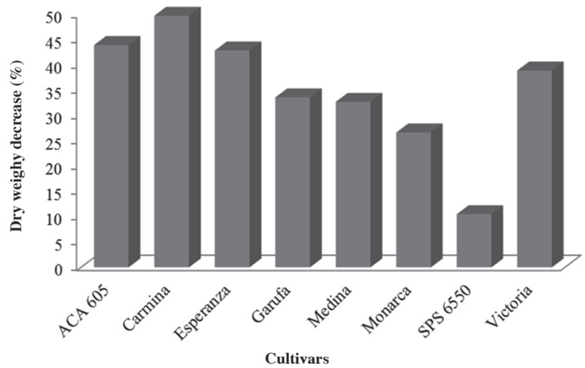

Figure 3. Decrease in dry weight of alfalfa cultivars.

Table 2. Life table parameters of *Aphis craccivora* reared on eight alfalfa cultivars.

	Parameter (mean ± SD)				
Cultivar	R_0	T (d)	DT (d)	λ	r_m
ACA 605	7.40 ± 5.32b	17.16 ± 3.66a	5.87 ± 2.40a	1.12 ± 0.05b	0.12 ± 0.05b
Carmina	7.90 ± 7.61b	16.49 ± 3.58a	5.40 ± 3.26a	1.13 ± 0.08b	0.13 ± 0.07b
Esperanza	10.57 ± 9.58b	15.59 ± 1.98a	4.50 ± 2.18a	1.16 ± 0.08b	0.15 ± 0.07b
Garufa	8.36 ± 7.33b	16.97 ± 1.94a	5.44 ± 2.53a	1.13 ± 0.06b	0.13 ± 0.06b
Medina	2.02 ± 21.18a	19.88 ± 7.45b	222.90 ± 484.35b	1.06 ± 0.23a	0.04 ± 0.06a
Monarca	10.63 ± 6.18b	15.25 ± 1.39a	4.44 ± 1.11a	1.16 ± 0.05b	0.16 ± 0.04b
SPS 6550	7.56 ± 6.34b	16.00 ± 2.06a	5.39 ± 2.63a	1.14 ± 0.07b	0.13 ± 0.18b
Victoria	7.50 ± 6.46b	15.46 ± 2.19a	5.22 ± 2.54a	1.14 ± 0.07b	0.13 ± 0.06b

Means within columns followed by the same letters are not significantly different (LSD, $p > 0.05$).
R_0: Net reproductive rate; T: mean generation time; DT: doubling time; λ: finite rate of increase; r_m: intrinsic rate of increase.

'Monarca' and 'SPS 6550' showed a high level of tolerance to infestation, with a very small decrease in dry weight in the presence of aphids. Alfalfa cultivars in which aphid infestation caused only a small decrease in DM suffered little damage due to *A. craccivora*. Loss of vegetative tissue due to infestation was much lower than in the resistant cultivars ('ACA 605' and 'Esperanza').

CONCLUSIONS

Our results demonstrated that among the investigated cultivars 'Medina' displayed antibiosis to *A. craccivora*, 'Carmina' and 'Victoria' expressed antixenosis and tolerance, and 'Monarca' and 'SPS6550' were tolerant to this aphid pest.

The deployment and proper management of alfalfa aphid-resistance cultivars has the potential to greatly reduce the frequency of aphicide applications and the ensuing economic and environmental cost in alfalfa production systems.

ACKNOWLEDGEMENTS

Financial assistance was provided by SECYT-UNS. Authors thank CONICET for a research fellowship to Jorge Bizet-Turovsky.

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