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Article

Bruchine-Predated Seeds of *Ipomoea nil* (L.) Roth., a Cotton Crop Weed in Santiago Del Estero, Argentina

*Depredação por Bruquídeos de Sementes de **Ipomoea nil** (L.) Roth., Planta Daninha da Cultura do Algodoeiro em Santiago Del Estero, Argentina*

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ABSTRACT - The insects of the family Bruchidae (Coleoptera) are important predators; their larvae feed upon and live in the seeds of a wide variety of plants. In Argentina, there not exists background information concerning the levels of predation of ***Ipomoea nil*** (Convolvulaceae) seeds caused by these insects. Thus, the aim of this work was to record the incidence of the species of the genus *Megacerus* as predators of *I. nil* seeds, an important weed affecting cotton growing in the irrigation area of Santiago del Estero. *Megacerus maculiventris* was the only bruchine species identified as predator of this weed. The predation percentage ranged from 0.84 to 15.66% in the agricultural years 2004-2008. It was attested that the number of predated seeds per capsule is independent from the number of seeds per fruit. However, it was proved that the insect predates just one seed per capsule and the highest predation frequency occurs in fruits containing more than three seeds. The levels of predation of *I. nil* by *M. maculiventris* are low in relation to those registered in other hosts by bruchines of the genus *Megacerus*.

Keywords: Convolvulaceae, seed predators, predation rate.

*RESUMO - Os insetos da família Bruchidae (Coleoptera) são importantes depredadores; suas larvas se alimentam e vivem em sementes de uma grande variedade de plantas. Na Argentina, não há registros dos níveis de depredação de sementes de **Ipomoea nil** (Convolvulaceae) causada por esses insetos. Assim, o objetivo deste trabalho foi registrar a incidência das espécies do gênero **Megacerus** como depredadores de sementes de **I. nil**, importante planta daninha da cultura de algodoeiro em área de irrigação de Santiago del Estero. **Megacerus maculiventris** foi a única espécie de bruquídeo identificada como depredadora de sementes dessa planta daninha, tendo a porcentagem de depredação variado de 0,84% a 15,66% nos anos agrícolas de 2004 a 2008. Os resultados demonstraram que o número de sementes depredadas por cápsula é independente do número de sementes por fruto. Contudo, foi verificado que o inseto depreda uma semente por cápsula e que a maior frequência de depredação ocorre nos frutos com mais de três sementes. Os níveis de depredação de **I. nil** por **M. maculiventris** são baixos em relação aos registrados em outros hospedeiros por bruquídeos do gênero **Megacerus**.*

Palavras-chave: Convolvulaceae, predadores de sementes, taxa de depredação.

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INTRODUCTION

The cultivation of cotton, *Gossypium hirsutum* L. (Malvaceae), has a remarkable importance in the provinces of northern Argentina as a development agent of the region's economy in the primary sector (agriculture), in the industrial sector, in related services (ginners, carriage, industrial workshops, spinning mills, oil mills, etc.) and in the commercial sector of the area of influence, which together represent a large occupation of direct and indirect labor (Bonacic et al., 2006). The main provinces of Argentina producing the crop are Chaco with 69% and Santiago del Estero with 16% of planted area respectively.

Within the biotic adversities to the crop are weeds, animal pests and diseases. Weed exerts a strong competitive pressure on the crop for water, light and nutrients, causing not only yield losses, but also providing foreign matter and staining the fibers which decrease their commercial value. In addition, weed reduces the efficiency of mechanical harvesting, with losses of about 18% against the usual 4% to 5%. Weed control usually accounts for 7% to 10% of production costs, but if not controlled, the value of potential losses is much higher (Bonacic et al., 2006).

The main broadleaf weeds that make up the natural communities associated with cotton cultivation in the northwestern region of Argentina are: *Amaranthus hybridus* L. (Amaranthaceae), *Portulaca oleracea* L. (Portulacaceae), *Chenopodium album* L. (Quenopodiaceae), *Malvastrum coromandelianum* (L.) Garcke and *Sida rhombifolia* L. (Malvaceae), *Ipomoea grandifolia* (Dammer) *O'Donelle* and *Ipomoea nil* (L.) Roth. (Convolvulaceae), *Bidens subalternans* DC., *Flaveria bidentis* (L.) Kuntze, *Wedelia glauca* (Ortega) O. Hoffm. ex. Hicken and *Tagetes minuta* L. (Asteraceae), among others (Bonacic et al., 2006). *Ipomoea nil* (picotee morning glory, ivy morning glory, and Japanese morning glory) has been reported as a weed that causes problems in soybean, corn, bean and cotton crops (Marzocca et al., 1976; Moran Lemir, 1997); native to America, it is an annual, herbaceous plant with a voluble stem of 2 to 5 m in length, which presents a spring-summer cycle, blooms in summer and bears fruit in autumn (Marzocca et al., 1976; Cabrera, 1983).

In the northwestern region of Argentina, it is mainly affecting grain crops (Moran Lemir, 1997), while in Santiago del Estero, along with *I. grandifolia*, it is invasive in the cultivation of cotton. According to Franz (1985), *Ipomoea* species are moderately competitive with cotton, compared with others.

In agricultural production systems, the propagules bank in the soil is the primary resource for new weed species infestations each year. The seed bank has a significant influence on the weed populations that occur each year in a given location, which is accentuated in agro-ecosystems and therefore, a good management of this is fundamental to the success of a weed control program. The size of the bank fluctuates rapidly depending on the magnitude of weed seed introduction and losses. Many of the species are prolific in seed production, so inefficient control allows original levels to be restored from one year to the next (Acosta and Agüero, 2001).

Factors influencing seed bank entry can be classified as follows: 1) internal factors: production of weed seeds within the field; 2) external factors: agricultural implements, animals, wind, manure and other human activities (Leguizamón, 1983). Changes in bank size are determined by entry, such as seed rainfall, from local or distant sources, and exit due to germination, fungal attack, chemical control, predation, and physiological death of seeds (Cardina et al., 1996, Baskin and Baskin, 1998). Consequently, predation is one of the processes that can cause significant seed losses, both in epigeous and hypogeous banks (Nisensohn et al., 1999; Puricelli et al., 2005).

Different species of predators are able to regulate the seed density of some weed species and can modify their spatial distribution (Harper, 1977; Cavers, 1983). Seed losses through predation, even when small, influence not only their abundance and distribution in the soil, but also the subsequent stages of the weed life cycle, leading to changes in the dynamics of their populations and in the structure of the communities they comprise (Louda, 1989; Nisensohn et al., 1999). Little is known about the activity and impact of seed-feeding organisms on weed populations in agricultural ecosystems (Cardina et al., 1996).

Predators and seed dispersers include among others, insects, birds and rodents. Among the insects, several species of Coleoptera, Hymenoptera, Diptera, Lepidoptera and Thysanoptera are the main seed predators. Predation can be classified into pre and post dispersal; the first refers to the attack on the seed before it is disseminated by the mother plant, and includes several species of Diptera, Thysanoptera, Lepidoptera and some of Coleoptera. Post dispersal predation occurs when the seed is detached from the mother plant, and includes small mammals, birds and insects belonging mostly to the Coleoptera and Hymenoptera groups (Zhang et al., 1997).

Insects of the Bruchidae family (Coleoptera) are commonly known as seed weevils or beetles, as their larvae feed and live inside them. Their presence has been recorded in approximately 35 families of wild or cultivated plants (Johnson, 1981a, b, 1989; Romero et al., 1996; Johnson et al., 2003; Reyes et al., 2009). Around 85% of the registered host plants are leguminous, 4% in bindweed, 4% in arecaceae, 2% in malvaceae and the rest distributed in 29 other plant families (Johnson and Romero, 2004). Several authors mention species of bruchids associated to the seeds in wild plants of the family Convolvulaceae (Table 1).

The predation of seeds by bruchids of the genus *Megacerus Fahraeus* according to Scherer and Romanowski (2005) was observed by several authors in the genera *Ipomoea* L. and Calystegia R. Br. of the family Convolvulaceae. In other countries, it is mentioned that the seeds of *Ipomoea imperati* (Vahl) Griseb, *I. pes-caprae* (L.) R. Br., *I. leptophilla* Torr. and *I. carnea* Jacq. are preyed by species of bruchids of the genus *Megacerus* (Wilson, 1977; Frey, 1995; Castellani, 2003; Castellani and Santos, 2005). Biological aspects of *Megacerus* species have been studied by Terán and Kingsolver (1977), Johnson and Raimundez Urrutia (2008), Reyes et al. (2009), among others. In Argentina, there are no records of predation levels of *I. nil* seeds caused by bruchids, therefore the aim of this study was to determine the species of bruchids that predate seeds of this weed in the cotton agro-ecosystem and evaluate their incidence on the propagule bank.

MATERIALS AND METHODS

Identification of the species of bruchids

Individuals from *I. nil* seeds, harvested between the years of 2005 and 2008, were extracted from commercial cotton production lots located in the field of the Estación Experimental Agropecuaria La María- INTA Santiago del Estero, Argentina (28°03'S 64°15'W, elevation 182 above sea level). These were preserved in alcohol (70%) and were sent for identification to the specialist Dr. Susana Muruaga de L'Argentier (Faculty of Agricultural Sciences, National University of Jujuy, Argentina). The identified specimens are deposited in the collections of the Department of Agricultural Zoology of the Faculty of Agronomy and Agro-industries of the National University of Santiago del Estero.

Level of predation

In order to assess the level of seed predation, different tests were carried out: 1 Evaluation of differences between campaigns: from *I. nil* seeds harvested during the years of 2005 to 2008 in the cotton production lots, previously mentioned, and conserved in the laboratory. 30 seed samples of 7.32 g (approximately 300 seeds per sample) were randomly taken for each agricultural year. The seeds were placed in Petri boxes, recording the total number of seeds and the amount of predated seeds per sample and per harvest year. From the data obtained, the assumptions of normality using the modified Shapiro Wilks test and of homogeneity of variance using the Levene test were checked. Considering that these assumptions were not met, a non-parametric analysis was performed using the Kruskal Wallis test.

2- Percentage of predation per plant: the evaluation of the percentage of predation per plant and the evaluation of the number of predated seeds per fruit was carried out in a cotton plot of half a hectare, with a high infestation of *I. nil* located within a commercial cotton production lot of EEA La María – INTA Santiago del Estero, in which no agrochemical applications were made, unlike the surrounding lots, in which an average of 6 to 7 applications of the following insecticides were carried out: Chlorpyrifos, Imidacropid, Dimethoate, Cypermethrin, Lambdacialothrin and

Table 1 - Bruchidae (Coleoptera) asociated with Convolvulaceae seeds in the Americas

Species of *Megacerus*	Alternative hosts	Distribution	Reference
M. maculiventris (Fahr.)	*Convolvulus* sp. Juss. *I. aristolochiifolia* (H.B.K.) G. Don. *I. batatas* (L.) Lam. *I. cardiophylla* A. Gray *I. cholulensis* Kunth *I. dumetorum* Willd. ex Roem. & Schult. *I. grandifolia* (Dammer) O'Don. *I. hederacea* (L.) Jacq. *I. triloba* L. *I. marginisepala* ODonell, *I. nil* (L.) Roth. *I. pes-caprae* (L.) R.Br. *I. purpurea* (L.) Roth. *I. rubiflora* O'Don. *I. tricolor* Cav. *I. trifida* (Kunth) G. Don *I. violacea* L. *Merremia quinquefolia* (L.) Hallier f. *Turbina corimbosa* (L.) Raf.	Souther United States to central Argentina	Romero Gómez et al. (2009) Terán and Kingsolver (1977)
M. tricolor (Suffr.)	*I. alba* L. *I. cholulensis* Kunth, *I. crinicalyx,* S. Moore *I. heredifolia* L. *I. nil* (L.) Roth. *I parasitica* (Kunth) G. Don, *I purpurea* (L.) Roth, *I tricolor* Cav. *I. triloba* L. *M. quinquefolia* (L.) Hallier f. *Merremia aegyptia* (L.) Urban	México, Antillas, Brazil, Colombia, Costa Rica, Guatemala, Honduras, Estados Unidos	Romero Gómez et al. (2009) Reyes et al. 2009 Terán and Kingsolver (1977) Romero and Johnson (2004)
M. porosus (Sharp)	*I. crinicalyx* S. Moore *I. nil* (L.) Roth. *Merremia aegyptia,* (L.) Urban *Merremia cisoides* (Lam.) Hall. f.	From México to Brazil and Puerto Rico	Reyes et al. (2009) Terán and Kingsolver (1977)
M. cubicus (Motsch.)	*I. heredifolia* L. *I. lacunosa* L. *I. nil* (L.) Roth. *I. purpurea,* (L.) Roth *I. trifida* (Kunth) G. Don *I. triloba* L. *Merremia aegyptia* (L.) Urban *Merremia Cissoides* (Lam.) Hall. *Merremia quinquefolia* (L.) Hallier f.	Estados Unidos, México, América Central	Reyes et al. (2009) Terán and Kingsolver (1977)
M. eulophus (Er.)	*Convolvulus bonariensis* Cav. *C. laciniatus* Desr. *I. marginisepala* O'Donell *I. nil* (L.) Roth. *I. purpurea* (L.) Roth *I. rubiflora* O'Donell *I. sibirica* (L.) Pers.	Argentina, Brasil, Chile, Uruguay and Perú	Terán and Kingsolver (1977, 1992)

Triflumuron for the control of thrips (Thysanoptera), aphids and whitefly (Hemiptera), Heliothinae complex and leaf caterpillar (Lepidoptera). In this plot, 40 *I. nil* plants were marked and isolated at the beginning of the cotton crop cycle, growing without competition from other weeds. During flowering, 20 plants with vigorous growth were selected; from them, ripe fruits were harvested every 2 to 3 days from March to May 2008. The samples were taken to the laboratory and counted determining: number of capsules and number of predated seeds (n1); the latter were discarded and the rest were placed in plastic cups covered with tulle cloth and were kept for six months at room temperature until the emergence of all adult bruchids present in the seed samples (following the methodology provided by Terán and Kingsolver, 1977). After this period, the predated seeds were counted (n2). The total number of predated seeds per plant was determined as the sum of n1 and n2.

3- Assessment of the number of predated seeds per fruit: five samples of 30 capsules each were placed individually, in plastic bottles covered with tulle, in the ripening process, harvested every 10 to 15 days, from March to May 2008. The jars were kept in the laboratory at room temperature for six months, at the end of that time, the capsules were checked and the total number of seeds and predation per fruit was determined. To determine the predation preference of the number of seeds per capsule, a bivariate analysis was performed, using the CV test through contingency tables and x^2 to evaluate statistical significance.

In all the samples analyzed, seeds with an adult exit orifice were considered predated. The predation percentage was calculated from the data obtained in the trials, using the following formula:

$P = 100 \times np \div ns$ (Scherer and Romanowski, 2005)

In which *np* is the number of predated seeds and *ns* is the total number of seeds.

Statistical analyses were performed using Infostat software (Di Rienzo et al., 2013).

RESULTS AND DISCUSSION

The only bruchid species isolated from *I. nil* seed samples in the study area was *Megacerus (Serratibruchus) maculiventris* (Fahr.). The species recorded in this work is the one mentioned by Terán and Kingsolver (1977, 1992) who cited it affecting *I. nil*; however, these authors also mention the presence in this weed of *Megacerus eulophus* (Er.) in Argentina. *M. eulophus* is distributed from the south of The United States (Arizona) to central Argentina (Terán and Kingsolver, 1977; Romero Gómez et al., 2009).

The percentage of predation of *I. nil* seeds by *M. maculiventris* ranged from 0.84 to 15.66% in the years analyzed, with a peak in 2007/2008 (Table 2). The reduced predation registered during the periods of 2004/2005 and 2005/2006 could be due to the fact that in the production lots from where the seeds were harvested, insecticides were applied every 10 to 15 days, for the control of the populations of insect pests of the cotton crop and possibly, this affected the abundance of the bruchids. On the contrary, during the 2006/07 and 2007/08 periods, the significant increase in predation could be due to the fact that the area where the work was carried out was used, since September 2006, as a trap area within a cotton crop lot, with predominance of *I. nil*, without soil removal or agrochemical applications; this possibly originated an increase in the population density of *M. maculiventris*. It has been mentioned that the existence of cultivated areas without disturbance increases the abundance of weed seed predators (Menalled et al., 2000).

Table 2 - Mean percentage of *Ipomoea nil* sedes preyed by *Megacerus* (Serratibruchus) *maculiventris*. Data registered during 2005-2008 cotton crop seasons at the Estación Experimental Agropecuaria La María- INTA Santiago del Estero, Argentina

Crop year	Mean of registered seed per sample	Mean of preyed seeds per sample	P (%) (data at original scale)
2005	295.93 ± 10.21 c	2.47 ± 1.76	0.84 ± 0.60 a
2006	268.53 ± 9.71 b	5.00 ± 3.12	1.88 ± 1.21 b
2007	260.00 ± 13.40 a	13.9 ± 4.38	5.39 ±1.81 c
2008	350.00 ± 12.48 d	54.77 ± 7.07	15.66 ± 2.06 d

Different letters in the same column represent significant differences ($P \leq 0.05$) Fc = 417.47.

One *I. nil* plant, without competition, produced on average 412.4 (± 178.6) capsules and 1752.2 (± 748.4) seeds; of the total seeds produced, 338.90 (19.34%) were empty and 266.3 (15.2%) were preyed on by *M. maculiventris*. Several authors (Terán and Kingsolver, 1977; Johnson and Raimundez Urrutia, 2008; Romero Gómez et al., 2009) explained the cycle of bruchids in species of *Ipomoea*. The oviposition takes place on the mature fruit, sepals or even on already dispersed seeds of some species of this genus; they present a single period of oviposition that ceases when all the fruits have matured; the number of eggs per fruit is variable. The seeds are attacked when they are fully developed but before ripening, when the tegument is whitish and the tissues green and watery. The larva of the first stage emerges and introduces itself in the seed making a hole in the testa, to feed on the endosperm and/or cotyledons and develop completely until the adult stage. The larva before pupating marks an orifice that will be used by the adult to abandon the seed, being this a distinctive characteristic of this genus. In the present work only one larva per seed was observed and the emergence of the adult bruchids occurred during the seed dispersal period. In the laboratory, the adults gradually abandoned the seeds from the end of May to November, coinciding with what was recorded by Terán and Kingsolver (1977) and by Reyes et al. (2009).

The number of seeds per capsule varied from zero to six. Considering the amount of seeds per capsule and categorizing them into capsules with 3 seeds or less and four seeds or more, a preference of the insect in predating capsules of this last category (p=0,0174) was verified. This occurs because there is a greater supply of capsules with 4 or more seeds (Table 3).

Table 3 - Predation frequencies by *Megacerus* (Serratibruchus) *maculiventris* considering the number of *Ipomoea nil* seeds per capsules

Number of seed per capsule	Non preyed capsules		Preyed capsules		Total	
	Nr.	%	Nr.	%	Nr.	%
≤ 3	208	20.37	22	2.15	230	22.53
≥ 4	668	65.43	123	12.05	791	77.47
Total	876	85.80	145	14.20	1021	100
Chi square Pearson (p=0.0221) Significant Chi square MV-G2 (p=0.0174) Significant						

The number of predated seeds per capsule was found to be independent of the number of seeds per fruit (p=0.5966). However, it was verified that the insect predates one seed per capsule and that the highest frequency of predation occurs in fruits with more than three seeds (Table 4), nevertheless more than three predated seeds per capsule were never observed.

There are no bibliographic materials on the preference of *M. maculiventris* and other species of bruchids in the predation of one or more seeds per fruit. The percentage of predation seems to be related to the amount of fruits and seeds produced, which in turn depends on the density of plants in a given locality. The differences found in predation in different species of *Ipomoea* may be the result of differentiated reproductive strategies of host plants (Scherer and Romanowski, 2005).

Available information on the damage caused by *Megacerus* species to Convolvulaceae seeds is scarce; only a few studies mentioned predation values on *Ipomoea* species. Thus, the following values are mentioned: 8.7% in *Merremia macrocalyx* (Ruiz & Pav.) O'Donell (Convolvulaceae) by *M. flabelliger* (Fahraeus); 3.6 to 16% in *I. pes-caprae* (L.) Sweet by *M. leucospilus*; 38 to 68% in

Table 4 - Level of predation of *Ipomoea nil* seeds per capsule according to the number of seeds per fruit by the bruchid *Megacerus (Serratibruchus) maculiventris*

Number of seeds per capsule	Number of preyed seeds per sample		
	1	2	3
1	2	-	-
2	5	3	-
3	10	2	0
4	12	5	1
5	31	6	2
6	72	9	5
Total	132	25	8
Chi square Pearson (p=0.5966) Not significant Chi square MV-G2 (p=0.5582) Not significant			

Calystegia sepium (L.) R. Br. by M. *discoidus* (Say); 68% in *Ipomea imperati* (Vahl) Griseb by *M. baeri*; 74% in *I. carnea Jacq.* by *M. flabelliger* and 77% in *I. leptophylla* Torr. by *M. discoidus* (Keeler, 1980; Frey, 1995; Scherer and Romanowsky, 2005; Johnson and Raimundez Urrutia, 2008). The predation percentages of *M. maculiventris* recorded in this work on *I. nil* (0.8 to 15.65%, Table 2) are similar to those recorded for *M. flabelliger* in *M. macrocalyx* and *M. leucospilus* in *I. pes-caprae*.

The low predation percentages determined in this study, with respect to other predation values recorded in related species, may also be due to the effect of the high density in the production of fruits and seeds of this species which attenuates the record of damage (Table 4). Thus, Scherer and Romanowski (2005) mention that the predation rates of *M. baeri* and *M. reticulata* were lower in areas with higher fruit density.

To sum up, although the percentages of predation of *I. nil* seeds by *M. maculiventris* are low, the incidence of this factor added to others, such as the damage caused by other seed predatory organisms (insects, birds and rodents), added to the effects of senescence, anthropics and fungal activity, could cause a decrease in the density of seeds in the surface bank.

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