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Do fruit traits of the *Senna occidentalis* weed influence seed predation by Bruchinae?

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ABSTRACT: (Do fruit traits of the *Senna occidentalis* weed influence seed predation by Bruchinae?). Pre-dispersal seed predation by specialist insects can affect the performance of plants, and the morphological, morphometrical and physiological traits of fruits/seeds influence the degree of predation. *Senna occidentalis* is a weed found in degraded pastures and plantations, which is poisonous to cattle and other domestic animals. This study tested the hypothesis that larger and mature *S. occidentalis* fruits have a higher probability of predation, and larger fruits have more bruchids. It also estimated the predation level and identified the bruchid species associated with this weed. Fruits were randomly selected from 42 plants from pastureland in Mato Grosso do Sul, Brazil. The probability of predation was not influenced by fruit length. Although the fruit length has been positively associated with the seed number, bruchids were not associated with fruit length. The most frequently predated fruits were those measuring between 10 to 13 cm. A possible explanation is that bruchids do not select a specific fruit length because they are not host-specific for this weed. Seed predation of about 7% was recorded for three bruchids: *Sennius bondari*, *Amblycerus submaculatus* and *Amblycerus* sp. This low level of seed predation may be insufficient to have a negative impact on the weed. Acquiring knowledge about predation rates and mechanisms that regulate a plant population can help scientists estimate the efficiency of seed-feeding insects used to biologically control weeds.

Key words: *Sennius*, *Amblycerus*, insect-plant interaction, biological control, Brazil.

RESUMO: (As características dos frutos da erva daninha *Senna occidentalis* influenciam a predação de sementes por Bruchinae?). A predação pré-dispersão de sementes causada por insetos especialistas pode afetar o desempenho das plantas, e as características morfológicas, morfométricas e fisiológicas dos frutos/sementes são atributos que influenciam o grau de predação. *Senna occidentalis* é uma erva daninha encontrada em pastos degradados e plantações, que causa envenenamento em bovinos e outros animais domésticos. Nós testamos a hipótese que frutos grandes e maduros de *S. occidentalis* teriam uma maior probabilidade de predação e que frutos maiores teriam mais bruquíneos. Nós também estimamos o nível de predação e identificamos as espécies de bruquíneos associadas com esta erva-daninha. Os frutos foram coletados de 42 plantas selecionadas aleatoriamente em pastagens no Mato Grosso do Sul, Brasil. A probabilidade de predação não foi influenciada pelo comprimento do fruto. Embora o comprimento dos frutos tenha sido positivamente relacionado com o número de sementes, os bruquíneos não foram relacionados com o comprimento do fruto. Os frutos mais frequentemente predados foram aqueles entre 10 e 13 cm. Uma possível explicação para isto é que os bruquíneos não selecionaram um comprimento de fruto específico porque eles não são hospedeiros específicos desta erva daninha. Uma predação de sementes de aproximadamente 7% foi registrada por três bruquíneos: *Sennius bondari*, *Amblycerus submaculatus* e *Amblycerus* sp. Este baixo nível de predação pode ser insuficiente para causar um impacto negativo nessa erva daninha. Entender as taxas e os mecanismos que regulam as populações de plantas é uma condição essencial para prever a eficiência dos predadores de sementes no controle biológico das ervas daninhas.

Palavras-chave: *Sennius*, *Amblycerus*, interação inseto-planta, controle biológico, Brasil.

INTRODUCTION

Seed predation is an important insect-plant interaction, which regulates the dynamics and the community structure of a plant population, and acts as a strong selective force in the evolution of traits (Harper *et al.* 1970, Janzen 1971, Rose *et al.* 2005, Kolb *et al.* 2007). Evolutionary interactions between plant and seed pre-

dators are a result of plant and insect adaptations, which include ecological, morphological, chemical, and behavioral traits (Ramírez & Traveset 2010). It has been argued that the intensity of seed predation may be affected by four main categories of attributes: vegetation traits, morphological and morphometrical traits of fruits and seeds, chemical properties and the nutritional composi-

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tion of seeds (Ramírez & Traveset 2010).

Bruchinae beetles (Coleoptera: Chrysomelidae) are important pre-dispersal seed predators in natural and agricultural habitats, and they are mainly associated with Fabaceae. Some species have a strong impact on plants, since damaged seeds cannot germinate and the viability of healthy seeds decreases when the beetles infest fruits (Tomaz *et al.* 2007). Consequently, studies have been developed to assess whether bruchids have the potential to biologically control weeds (Hoffmann & Moran 1991, Kluge & Naser 1991, Briano *et al.* 2002, Ribeiro-Costa *et al.* 2010).

Since most bruchids are host-specific, morphological, morphometrical and physiological traits of fruits and seeds are the most influential attributes regarding the degree of seed predation (Janzen 1969, Center & Johnson 1974). Some bruchid species oviposit on immature or completely-developed fruits; other species oviposit on mature fruits while yet others oviposit directly onto seeds. According to Johnson (1981), there are three oviposition guilds for bruchids and 78% of all species studied by Johnson & Romero (2004) oviposit on fruits and belong to Guild A.

Some studies show that females prefer to oviposit on large seeds, which are more nutritious for the progeny (Mitchell 1975, Cope & Fox 2003, Yang *et al.* 2006, Ostergård *et al.* 2007). It might be expected that species that oviposit on fruit walls prefer the largest fruits, which are completely developed, and that these would be the most predated. Immature fruits have a low level of predation because they do not provide enough substrate for the larva's development. The seeds inside immature fruits have different physiological conditions for the development of bruchid larvae in comparison to the mature ones.

This study assessed the seed predation of the leguminous shrub *Senna occidentalis* (L.) Link (= *Cassia occidentalis*) (Fabaceae: Caesalpinioideae). This plant is a dehiscent species with a perennial or an annual life cycle (Shaukat & Siddiqui 2007) and pantropical distribution (Lorenzi 2000). It is usually found in degraded pastures, orchards, on the edges of roads and among soybean, corn and sorghum crops (Lorenzi 2000, Saeed & Shaukat 2000) and causes serious poisoning in cattle and other domestic animals (Martins *et al.* 1986, Barth *et al.* 1994, Lorenzi 2000). Several genera and species of bruchid beetles have been identified as seed predators in *S. occidentalis*, because of the economic importance of this plant. However, little is known about morphological and morphometrical traits of fruits and seeds that influence the seed predation level.

This study tested the hypothesis that larger and mature *S. occidentalis* fruits have a higher probability of seed predation, based upon the premise that larger fruits are more attractive for oviposition. The study also assessed whether more bruchids are present on larger fruits, estimated the seed predation level and identified the bruchid species associated with the weed.

MATERIALS AND METHODS

Fruits of *Senna occidentalis* were collected during March 2008 from pastures of two annual populations in Jardim, Mato Grosso do Sul State, Brazil (Population 1: 21°28'17.50"S, 56°16'41.87"W; Population 2: 21°25'21.85"S, 56°21'58.66"W). The local climate is tropical, with mean annual temperatures ranging from 22°C to 25°C and mean annual precipitation ranging from 1400 to 1600mm (Campelo-Júnior *et al.* 1997).

The study randomly selected 21 plants from each population. From each plant, ten fruits (five mature and five immature but completely developed) were randomly collected. The fruit maturation stage was visually determined according to color (green= immature; brown= mature). A total of 420 not yet dehiscent fruits were collected from the two populations. However, three of them were not included in the study because they had partially opened.

After collection, the fruits were temporarily stored in plastic bags. Approximately seven days after collection, all of the fruits (n = 417) were measured (length, cm) and dissected to record the number of seeds per fruit. The seeds were examined to record the number of bruchid species and predated seeds. Fruits were classified as damaged or undamaged. Larvae and adults collected from dissected fruits and seeds were fixed in alcohol (70%). Some adults were pinned, labeled and identified. Bruchids were deposited in the Pe. J. S. Moure Entomology Collection of the Zoology Department at the Universidade Federal do Paraná, Curitiba, Brazil (DZUP), and in the Reference Zoological Collection of the Universidade Federal do Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

A simple logistic regression (n=261) was used to evaluate the effect of fruit size on the probability of seed predation. A two-sample Kolmogorov-Smirnov Test was used to compare the size distribution of available and damaged fruits. These analyses were carried out for all fruits and separately for mature and immature fruits to investigate whether bruchids have a preference for either of these substrates.

In order to test whether larger fruits contain more seeds, linear regression was used based only on undamaged fruits (n= 156) to exclude any effect from predators. The same analysis was used to test whether larger fruits contain more bruchids. The residuals from the linear models were graphically checked to assess both the normality and homogeneity of variances (Underwood 1997).

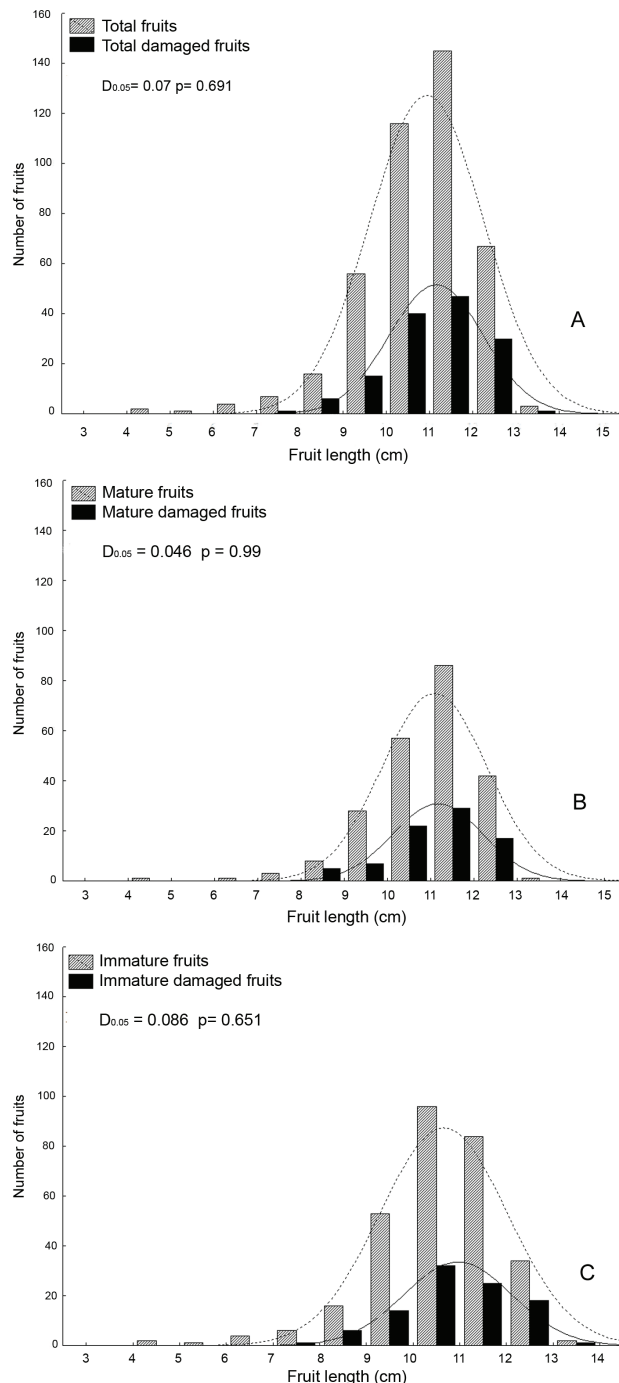
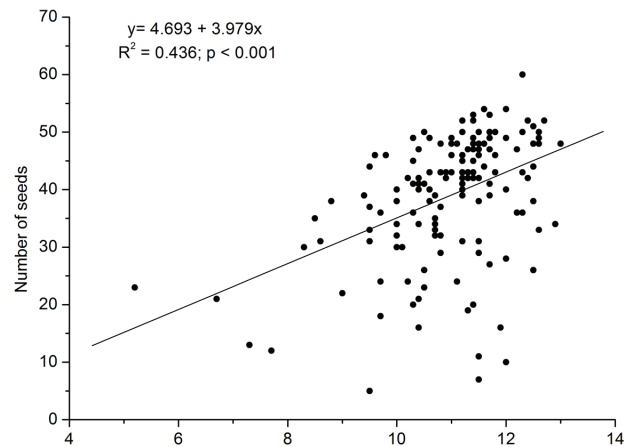
RESULTS

The logistic model showed that the probability of predation was not influenced by fruit length, regardless of the ripening stage (Table 1). The fruits size distribution curve (available and damaged by seed predators) did not differ significantly for all fruits (D=0.07, p=0.691) and followed the same pattern for mature (D=0.046,

Table 1. Logistic regression results for the effect of fruit length of *Senna occidentalis* on the probability of predation by bruchinae.

Predictor	N	β_1	SE (β_1)	G	p	Rho ²
Total fruits	417	1.228	0.087	5.991	0.014	0.011
Immature fruits	190	1.401	0.135	7.193	0.007	0.03
Mature fruits	227	1.085	0.118	0.486	0.486	0.002

Abbreviations: N, number of fruits; β_1 , parameter estimate; SE (β_1), standard error of the estimate; G, estimate of the likelihood ratio test; Rho², value of McFadden's statistic.

**Figure 1.** Length distribution of collected fruits (A), only mature (B) and only immature (C) of *S. occidentalis* showing available fruits and fruits damaged by bruchids.**Figure 2.** Regression analysis between the number of seeds and fruit length of *Senna occidentalis*.

$p=0.99$) and immature fruits ($D=0.086$, $p=0.651$). This means that bruchids mainly damaged the fruits that were the most frequent size, (from 10 to 13 cm, Fig. 1A-C). A positive significant relationship was obtained between fruit length and the number of seeds ($df=152$, $R^2=0.436$, $p<0.001$; Fig. 2), which represents a general tendency of larger fruits to have more seeds.

However, the number of bruchids was not significantly related to fruit length ($df=100$, $R^2=0.020$, $p=0.838$). Infested fruits had a mean of 2.32 ± 4.47 predated seeds per fruit. The pod length of *Senna occidentalis* varied from 4.4 to 13.4 cm, with a mean length of 10.89 ± 1.31 cm (mean $\pm$ SD). Each fruit had a mean of 35.37 ± 12.8 seeds. A total of 161 bruchid specimens (144 larvae and 17 adults) were found on infested fruits, a mean of 1.58 ± 0.8 bruchids per fruit. Bruchids caused a low level predation: about 6.6% of a total of 14,750 seeds sampled (mature and immature). The predation level for mature seeds was 7.7% of 6,728 available seeds, while for immature seeds it was 5.6% of 8,022 available seeds. This result was expected since the immature fruits were less exposed. Considering the number of predated seeds and bruchids collected, some species consumed more than one seed.

Larvae were recognized as belonging to bruchinae, but they were not identified at lower levels. The adults were identified as belonging to three species: *Sennius bondari* Pic 1929, *Amblycerus submaculatus* Pic 1927 and a new species belonging to the genus *Amblycerus*. The first belongs to Bruchini and the others to Amblycerini, both very diverse groups of the subfamily. *Amblycerus submaculatus* was the most abundant ($n=10$), followed by *Sennius bondari* ($n=5$) and *Amblycerus* sp. ($n=2$).

DISCUSSION

It has been argued that seed predation intensity may be affected by fruit and seed traits (Janzen 1969, Mitchell 1977). However, our results indicated that seed

predation of *Senna occidentalis* was not influenced by fruit length, regardless of the ripening stage. In general, there is a tendency for longer *S. occidentalis* pods to contain more seeds, but these seeds were not the most damaged by bruchids. This statement does not corroborate with our previous hypothesis that female bruchids select larger and mature fruits that provide more substrate for larvae. Ramírez & Traveset (2010) found that the incidence of pre-dispersal seed predation was independent of fruit and seed dimensions. However, the biomass and amount of starch in a seed were found to be associated with plant species predation. Furthermore, some bruchids may recognize a previous oviposition, selecting fruits with no eggs to oviposit, possibly to minimize egg loss and avoid competition among larvae within the fruit (Wilson 1988, Redmon *et al.* 2000, Silva *et al.* 2007).

Nonetheless, available and damaged fruits by seed predators had a normal size distribution curve, since damaged fruits were the most frequent in the sample. A possible explanation for this is that the bruchids do not select a specific fruit length because they are not host-specific to this weed. Morandini & Viana (2009) reported that bruchids have a preference for medium-sized fruits of *Enterolobium contortisiliquum* (Fabaceae).

The level of seed predation by bruchids in *Senna occidentalis* was lower than 7% in this study. Other studies have also found a relatively low predation rate for species of *Senna*. Sari & Ribeiro-Costa (2005) reported that 12% of *Senna multijuga* seeds were damaged in Curitiba, Brazil and Shaukat & Siddiqui (2007) found that 7.8% and 18% of *S. occidentalis* seeds were predated at two different locations in Pakistan. Although the level of seed predation was low, Shaukat & Siddiqui (2007) concluded that seed predation by bruchids was one of the factors causing seed reduction in an *S. occidentalis* seedbank, interfering with its population dynamics (Janzen 1971, Lee & Bazzaz 1982).

Johnson & Slobodchikoff (1979) stressed that most bruchids of *Senna* (= *Cassia*) are host-specific and most host plants support only a few bruchid species. Only three bruchids, *S. bondari*, *A. submaculatus* and *Amblycerus* sp., were found to be predated seeds of *S. occidentalis*, although the first two are not host-specific (Center & Johnson 1973).

Sennius bondari is found on many *Senna* species distributed in South America. In Brazil, it occurs in the States of Bahia, Rio de Janeiro, Mato Grosso do Sul, Minas Gerais, São Paulo and Paraná (Linzmeier *et al.* 2004). Larvae of *Sennius* mainly consume host plants of the subtribe Cassiinae (Caesalpinioideae: Cassieae), which include *Cassia*, *Chamaecrista* and, particularly, *Senna* (Johnson & Kingsolver 1973, Johnson 1984). The females oviposit directly onto the seed (Ribeiro-Costa 1998) when the pod valves open and the seeds are exposed (Guild B, as explained in Johnson & Romero 2004). The larva consumes only one seed during development (Ribeiro-Costa 1998). However, recent

and unpublished data on *Senna neglecta* showed that *S. bondari* is able to oviposit on pod valves of this plant (Guild A) (Ribeiro-Costa, personal communication).

Amblycerus submaculatus has already been recorded in several *Senna* species (Ribeiro-Costa 1992), as well as in *S. occidentalis*. Some species of *Amblycerus* Thunberg 1815 prey on the seeds of eleven plant families, including trees, shrubs and ornamental plants (Johnson *et al.* 2001) and they oviposit on the pod surface; they are included in Guild A (Johnson & Romero 2004) and develop at the expense of more than one seed (Ribeiro-Costa 1998).

This study showed that bruchids mainly damage fruits that are of the most frequent size, from 10 to 13 cm. However, seed predation was not influenced by fruit length and stage of ripening. Acquiring knowledge about the rates and mechanisms that regulate a plant population can be helpful in estimating how efficient a seed-feeding insect is at biologically controlling a weed. Furthermore, this study was carried out in pasture areas, subject to strong human interference. The activities that take place in these areas, such as cattle raising and burning of fields, can affect the population dynamics of *S. occidentalis* and its seed predators. Thus, caution must be taken when comparing the information found here with other studies.

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