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A Preliminary Investigation of Pre-Dispersal Seed Predation by *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) in *Mimosa bimucronata* (DC.) Kuntze Trees

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Investigação Preliminar da Predação de Sementes na Fase de Pré-Dispersão por *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) em plantas de *Mimosa bimucronata* (DC.) Kuntze

RESUMO - Este é o primeiro registro de *Acanthoscelides schrankiae* Horn, predando sementes de *Mimosa bimucronata* (DC.) Kuntze. Investigou-se o padrão de oviposição e uso das sementes por *A. schrankiae* e a distribuição de frutos maduros e de predação na inflorescência. Também se comparou a matéria seca total e das sementes não predadas, a proporção de sementes predadas, e as taxas de aborto e de insetos não-emergentes, entre os diferentes quadrantes da copa de *M. bimucronata*. Para determinar as espécies ocorrentes, observou-se a emergência de bruquídeos e parasitóides, sendo que todos pertenciam às espécies *A. schrankiae* e *Horismenus* sp. (Hymenoptera: Eulophidae), respectivamente. O número médio de frutos produzidos na região mediana da inflorescência foi significativamente maior do que nas regiões inferior e superior, e as frequências (observadas e esperadas) das sementes predadas e não predadas diferiram estatisticamente entre as diferentes regiões da inflorescência. As fêmeas de *A. schrankiae* depositavam seus ovos sobre os frutos e as larvas, ao eclodirem, perfuravam o exocarpo, alcançando as sementes. A maioria dos frutos possuía de um a três ovos, sendo que somente uma larva de bruquídeo se desenvolve em cada semente. O maior valor da razão “número de ovos/fruto” e a maior porcentagem de sementes predadas foram registrados em abril. A matéria seca total e das sementes não predadas, a proporção de sementes predadas, e as taxas de aborto e de predadores não-emergentes não diferiram significativamente entre os diferentes quadrantes das copas das árvores.

PALAVRAS-CHAVE: Fabaceae, Mimosoideae, herbivoria, interação inseto-plantas

ABSTRACT - This is the first record of *Acanthoscelides schrankiae* Horn, feeding in seeds of *Mimosa bimucronata* (DC.) Kuntze. We investigated the pattern of oviposition and seed exploitation by *A. schrankiae*, and the distribution of mature fruits and seed predation in the inflorescences. We also compared the percentage of predated seeds, the total dry weight of fruits and non-predated seeds, the percentage of aborted seeds, and the percentage of non-emergent insects, among different quadrants of the *M. bimucronata* canopy. To determine the occurring species, the emergence of bruchids and parasitoids was observed in the laboratory, resulting altogether, only in individuals of *A. schrankiae* and *Horismenus* sp. (Hymenoptera: Eulophidae) species, respectively. Mean number of fruits produced in the median region of inflorescence was significantly higher than in the inferior and superior regions, and the frequencies (observed and expected) of predated and non-predated seeds differed among the different regions of inflorescence. Females of *A. schrankiae* laid their eggs on fruits, and larvae, after emergence, perforated the exocarpo to reach the seeds. Most fruits presented one to three eggs and only one bruchid larva was observed in each seed. The highest value of the rate “number of eggs/fruit” and the highest percentage of predated seeds were recorded in April. Dry weight of fruits (total) and seeds (non-predated), proportions of predated seeds, seed abortions, and non-emergent seed predators, were evenly distributed in the canopy.

KEY WORDS: Fabaceae, Mimosoideae, herbivory, insect-plant interaction

Seed predation can be an important interspecific process for regulating plant community structure and composition (Janzen 1971) since it acts as a selective force affecting abundance, distribution and evolution of plants (Harper *et*

al. 1970). Among the most important seed predators are the insects, mainly Coleoptera, Diptera, Lepidoptera, Hymenoptera, Thysanoptera and Hemiptera orders (Janzen 1971, Zhang *et al.* 1997). Bruchid beetles (Coleoptera) (62 genera worldwide) are

seed-eating insects (at larval stage) that feed in seeds of about 34 plant families; and many species represent considerable economic importance, since they damage cultivated plants (Southgate 1979, Cardona 1989, Baier & Webster 1992). Bruchidae species feed exclusively in seeds of Angiosperms, especially legumes, even though these beetles are not restricted to this plant group (Janzen 1975, Southgate 1979, Ernst et al. 1990). According to Janzen (1969) more than 10 bruchid species can be found exploring a single legume species.

Bruchid females lay their eggs on fruits or seeds and after emergence larvae perforate the exocarp, reaching the seeds. Upon reaching the seeds the larvae feed in one or more seeds. Pupation occurs inside or outside the seeds and adults emerge from circular holes (Janzen 1969). Center & Johnson (1974) reviewed the biology of 38 bruchid species and 44 host plant species, and demonstrated that: a) oviposition occurs more frequently on fruits than on seeds; b) although two thirds of the bruchid species feed in a single seed and pupate inside seeds, one third consume numerous seeds, with pupation occurring outside seeds; and c) 75% of the host plants present indehiscent or delayed dehiscent fruits, and 25% have dehiscent fruits.

Although seed predation by bruchids can be extremely important for limiting the size of plant populations, high mortality rates imposed by natural enemies of bruchids such as predators and parasitoids may reduce infestation and consequent damage to plants (Van Klinken 2005, Schmale et al. 2006). Therefore, the knowledge of potential predators and parasitoid species and the mortality rates they inflict on bruchids are of major importance. However, only a few studies have investigated such questions. Regarding to Bruchidae parasitism, for example, Schmale et al. (2002) found only the parasitoid *Horismenus ashmeadii* Dalla Torre (Hymenoptera: Eulophidae) parasitizing the beetle *Acanthoscelides obtectus* Say in bean seeds, representing 18% of parasitization. *Horismenus* sp. parasitoids have also been found parasitizing the bruchids *Amblycerus submaculatus* Pic. and *Sennius bondari* Pic. in *Senna alata* (L.) Roxb. (Fabaceae) (Ribeiro-Costa 1998). Schmale et al. (2003) also investigated the effect of resistant plants combined with the utilization of the parasitoid *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) for reduction of infestation levels of *A. obtectus* in stored beans. Schmale et al. (2003) observed that damage was significantly reduced in such circumstances and the parasitoids were very efficient, reducing the population density of *A. obtectus*.

Mimosa bimucronata (DC.) Kuntze (Fabaceae: Mimosoideae) is a perennial tree endemic to Brazil, Paraguay, Argentina, and Uruguay (Burkart 1959), reaching height of 10 m with intense ramification of branches. This species grows along wetlands and riverbanks, and on lakeshores, where even few individuals can form dense aggregations (Lorenzi 2000). *M. bimucronata* is an important agent for restoration of degraded areas, and produces wood with good quality for charcoal utilization (Reitz et al. 1983, Marchiori 1993). However, *M. bimucronata* is also an important weed when dense populations grow in pasture areas (Lorenzi 2000) because their large branches with thorns can avoid cattle access to water and fresh grass. Moreover, only a few grasses can grow below the canopy of these plants due to shading that causes loss of areas for grass management. Despite the

economic importance of *M. bimucronata*, little is known about its biology, ecology, and associated organisms.

Acanthoscelides schrankiae Horn is known to feed in seeds of 11 plant species (nine *Mimosa*, one *Acacia*, and one *Schrankia*) (Nápoles 2002), and is distributed to the Bahamas, Ecuador, United States, Mexico, Dominican Republic, Venezuela, and Brazil (Jesus Romero Nápoles, personal communication). Little is known about this bruchid beetle since information in the literature is rather scant (Johnson 1983, 1990). Here we present the first record of *A. schrankiae* feeding in seeds of *M. bimucronata*. The specific objectives of this study were: (1) to investigate the distribution of mature fruits and predation in inflorescences, and (2) to describe the patterns of oviposition and seed exploitation by *A. schrankiae*. In order to ascertain the parasitoid species of *A. schrankiae* and whether other bruchid species occur in *M. bimucronata*, we observed the emergence of bruchids and parasitoids in the laboratory. According to New (1979), some species of Coleoptera while attacking *Acacia* plants, may concentrate their damage at the superior parts of the canopy, creating an unequal pattern of predation in the tree. Therefore, we also tested the hypothesis that the spatial distribution of predation in the *M. bimucronata* canopy is equally distributed. In this case we extended our analysis by comparing the proportion of predated seeds, the total dry weight of fruits and non-predated seeds, and the percentage of aborted seeds and non-emergent insects, among the different regions of the canopy.

Material and Methods

Study site and distribution of mature fruits and predation in the inflorescence. This research was carried out from April 2002 to September 2004 in the city of Botucatu (22°52'22" S; 48°26'37" W), State of São Paulo, Brazil. The climate is typically dry mesothermal winter (Koeppen – Cwb) (Carvalho et al. 1983) and all plants were located in two areas, characterizing two distinct natural populations. Areas 1 (6,700 m²) and 2 (10,000 m²) had approximately 20 plants each, and mainly grasses surrounding plants in both populations.

To evaluate the distribution of mature fruits from different parts of the inflorescence, we collected from three to eight inflorescences per plant from a total of 11 plants randomly chosen in area 1. As plants did not produce mature fruits in synchrony, fruits were collected in 22/03/2002 and 14/04/2002 from five and six plants, respectively. Every inflorescence was divided into three equal parts: Part 1) Superior Region; Part 2) Median Region; and Part 3) Inferior Region. The superior and inferior regions consisted of fruits located at the tip and bottom (interior part) of each inflorescence, respectively. The number of mature fruits was recorded for each region of the inflorescence. After recording the number of mature fruits, 50 fruits were randomly collected from each part of the inflorescence, and the total number and percentage of predated and non-predated seeds were recorded. The Tukey test and the Chi-square test (χ^2) (Zar 1999) were used to compare fruit production and the frequencies (observed and expected) of predated and non-predated seeds among the respective inflorescence regions.

Emergence of bruchids and parasitoids, patterns of oviposition and seed exploitation, and spatial distribution of predation in plant canopy.

From January to May 2003 we collected five mature and five immature fruits per plant every fifteen days. Collections were made in five plants located in area 1 (n = 500 fruits). In the laboratory (25°C under 12h light), fruits were placed individually in plastic containers covered with *voil*, and the emergence of bruchids and parasitoids was recorded. All insects that emerged from fruits were fixed in alcohol (70%) and then sent to specialists for identification. From January to May 2004 we repeated the methodology described above. However, in this case we collected five mature and five immature fruits from eight plants (n = 800 fruits). As described above, the emergence of bruchids and parasitoids was recorded. While sampling fruits for recording the emergence of bruchids and parasitoids, we also collected another 10 fruits from each plant (n = 960 fruits). In the laboratory, these fruits were carefully dissected under a stereomicroscope, and the respective patterns of oviposition and seed exploitation by females and larvae of bruchids were recorded. In this case, samples were taken until August 2004. The number of predated and non-predated seeds was also recorded for each fruit and the percentage of predated seeds was estimated for each day of collection. The following variables were used to describe the ovipositional pattern of bruchid females: 1) the number of eggs per fruit; 2) the relationship between the fruit size (824 fruits measured with a caliper) and number of eggs laid on the fruit, and 3) the number of eggs laid per fruit through time. For the seed exploitation pattern, we described behavioral characteristics of the initial development of larvae (from eclosion to boring into the seed), and the number of larvae that developed in each seed was also recorded.

In order to investigate the distribution of fruit infestation in different locations in the *M. bimucronata* canopy, two collections of fruits were carried out (May 2003 and May 2004). We collected 100 fruits per plant in ten randomly chosen plants in area 2. The canopy of each plant was arbitrarily divided into four quadrants: Superior West, Superior East, Inferior West and Inferior East. Twenty-five fruits were collected per quadrant. In the laboratory (25°C and 12h light) fruits were placed individually in plastic containers covered with *voil* and the emergence of bruchids and parasitoids was recorded. After the emergence of all insects we calculated, for every quadrant, the proportion of predated seeds, the total dry weight of fruits and non-predated seeds, and the percentage of aborted seeds and non-emergent insects (trapped in the seeds). To compare means, Analysis of Variance (ANOVA) (Zar 1999) was computed to May 2003 and May 2004 data.

Results and Discussion

The distribution of fruits was significantly different among regions of the inflorescences, and the highest production of fruits occurred at the median region (Table 1). However, the production of fruits at the superior region did not differ statistically from production at the inferior region (Table 1). The number of seeds produced per fruit ranged from four to nine. The frequencies (observed and expected) of predated and non-predated seeds differed among the different regions

Table 1. Mean number of *M. bimucronata* fruits per region of inflorescence (n = 61 inflorescences collected).

| Region of inflorescence | Mean number of fruits (\pm SE) |
|-------------------------|-----------------------------------|
| Superior region | 34.82 (\pm 4.89) a |
| Median region | 57.44 (\pm 4.85) b |
| Inferior region | 28.31 (\pm 3.42) a |

Means followed by different letters differed statistically by the Tukey test at $P < 0.05$.

of inflorescences ($\chi^2 = 17.51$); and those fruits located in the median region presented the lowest levels of seed predation (Table 2). However, lower levels of predation concentrated in the median regions of inflorescences may be explained by the higher concentration of fruits in this region (Table 1).

Bruchid and parasitoid species that emerged from fruits of *M. bimucronata* were only *A. schrankiae* and *Horismenus* sp., respectively. *A. schrankiae* females laid their eggs on the surface of *M. bimucronata* fruits. After eclosion, larvae perforate the exocarp and reach the seeds. According to Johnson's (1981) classification for oviposition guilds, *A. schrankiae* belongs to Guild A, since oviposition occurs on fruits while on the plants. According to Johnson (1981) there are two other oviposition guilds for bruchids: Guild B – species that only oviposit on seeds exposed in fruits while still on the plant, and Guild C – species that only oviposit on seeds exposed on the substrate. Johnson & Romero (2004) observed that 77% of all bruchid species studied oviposit on fruits, 10% oviposit on seeds inside the fruits and 13% oviposit on seeds on the substrate, so the authors agree with the hypothesis that the first evolutive oviposition behavior of bruchids was probably onto fruits where they burrowed the fruit wall and fed on seeds (Guild A). Then, as evolution of fruits developed for dispersal of seeds and possible escape from bruchid predation, bruchids developed to feed in seeds in other ways (Guilds B and C) (Johnson & Romero 2004). This hypothesis can justify the higher concentration of species ovipositing on fruits.

During all observations of the pattern of seed exploitation, only one larva of *A. schrankiae* developed inside each seed until adult emergence. Redmon *et al.* (2000) studied

Table 2. Total number and percentage of predated and non-predated seeds observed in fifty fruits from each region of the inflorescences of *M. bimucronata*. Frequency of predated and non-predated seeds was compared by the Chi-square test (χ^2).

| Region of inflorescence | Predated seeds | | Non-predated seeds | |
|-------------------------|----------------|------|--------------------|------|
| | N° | % | N° | % |
| Superior region | 136 | 42.8 | 182 | 57.2 |
| Median region | 99 | 31.5 | 215 | 68.5 |
| Inferior region | 115 | 48.5 | 122 | 51.5 |

Observed frequencies differed statistically from the expected frequencies ($\chi^2 = 17.51$) at $P < 0.05$.

the biology of *Bruchidius villosus* Fabricius (Coleoptera: Bruchidae) that infests the legume *Cytisus scoparius* (L.) Link. (Fabaceae) and concluded that, apparently, the first larva to reach the seed prevents other larvae from reaching the cotyledons. Females would detect oviposition from other females and the number of eggs deposited would be adjusted to minimize offspring loss due to competition inside the fruits (Wilson 1988, Redmon et al. 2000). *A. schrankiae* females also seem to perceive previous oviposition, because in 76.5% of seeds with eggs (n = 217) only one egg per seed was found. Also, most of fruits presented one to three eggs on their surfaces (Fig. 1), and larger fruits had more eggs (Fig. 2). Redmon et al. (2000) observed a positive relationship between the number of bruchid eggs deposited and the size of fruits, corroborating the hypothesis of previous oviposition perception by bruchid females.

In the laboratory, emergence of adults of *A. schrankiae* occurred only around April. Traveset (1991) pointed out that due to the high abundance of the parasitoid *Urosigalphus* sp. (Hymenoptera: Braconidae) in immature pods of *Acacia farnesiana* (L.) Willd. (Fabaceae), females of *Mimosestes* sp. (Coleoptera: Bruchidae) would be favored if they deposit their eggs at late stage of pod maturation. It is possible that this process also occurs in the *A. schrankiae*-*Horismenus* sp. interaction, as the emergence of *A. schrankiae* started only from fruits collected near April, indicating a possible escape of bruchids during the period of highest levels of parasitoid infestation. Corroborating this hypothesis, an increase was observed in the rate “number of eggs/fruit” in fruits collected from January to the beginning of April, and the highest value was observed in April, followed by decreasing from the end of April to August (Fig. 3). Thus, the highest percentage of predated seeds was found near April (Fig. 3). At the end of March, the number of bruchid larvae inside seeds presented accentuated decreasing.

The hypothesis of damage concentration at superior parts of canopy (New 1979) was not corroborated by our data, as the proportion of predated seeds did not differ statistically

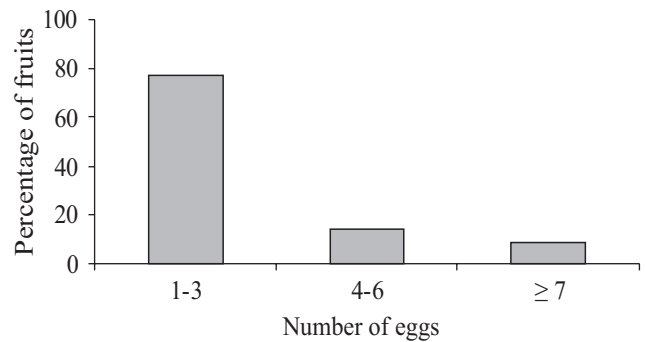


Fig. 1. Percentage of *M. bimucronata* fruits with the respective number of *A. schrankiae* eggs.

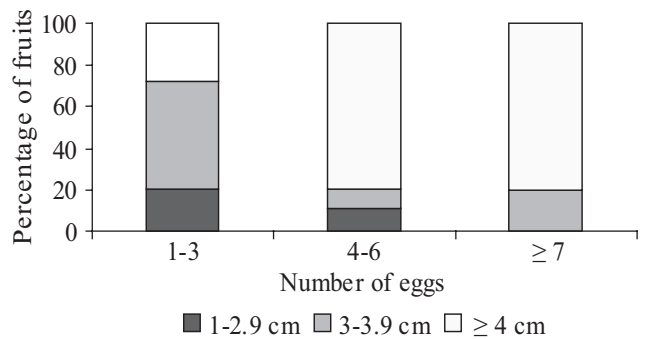


Fig. 2. Relationship between the size of *M. bimucronata* fruits (cm) and the number of *A. schrankiae* eggs.

among the different quadrants (Tables 3 and 4). Dry weight of fruits (total) and seeds (non-predated), seed abortions, and non-emergent predators, were also evenly distributed in the canopy (Tables 3 and 4).

Mean proportion of bruchids trapped in the seeds was higher in 2003 (27.80 ± 22.53) than in 2004 (1.33 ± 2.38). According to Ernst (1992) the last activity of a bruchid before emergence

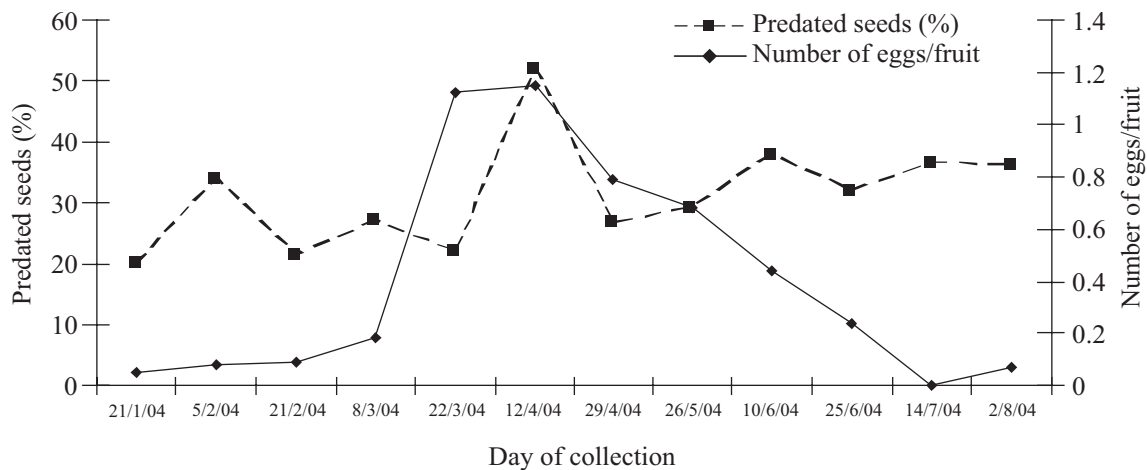


Fig. 3. Percentage of predated seeds and number of bruchid eggs per fruit recorded from January to August 2004.

Table 3. Comparison of mean values of the percentage of predated seeds, total dry weight of fruits and non-predated seeds, percentage of aborted seeds, and percentage of non-emergent insects, among different canopy quadrants [Superior West, Superior East, Inferior West and Inferior East] for fruits collected in May 2003.

| Source of variation | DF | MS | F | P |
|------------------------------------|----|----------|--------|----------|
| Total dry weight of fruits | 3 | 0.0098 | 0.0458 | 0.9868 * |
| Dry weight of non-predated seeds | 3 | 0.0848 | 0.9602 | 0.4220 * |
| Percentage of aborted seeds | 3 | 8.0841 | 0.0924 | 0.9637 * |
| Percentage of predated seed | 3 | 251.5732 | 0.5405 | 0.6576 * |
| Percentage of non-emergent insects | 3 | 22.6546 | 0.1263 | 0.9439 * |

* = non-significant at $P < 0.05$.

Table 4. Comparison of mean values of the percentage of predated seeds, total dry weight of fruits and non-predated seeds, percentage of aborted seeds, and percentage of non-emergent insects, among different canopy quadrants [Superior West, Superior East, Inferior West and Inferior East] for fruits collected in May 2004.

| Source of variation | DF | MS | F | P |
|------------------------------------|----|----------|--------|----------|
| Total dry weight of fruits | 3 | 0.0525 | 0.3987 | 0.7548 * |
| Dry weight of non-predated seeds | 3 | 0.0151 | 1.5121 | 0.2279 * |
| Percentage of aborted seeds | 3 | 56.5321 | 0.3135 | 0.8155 * |
| Percentage of predated seed | 3 | 135.3216 | 0.5793 | 0.6324 * |
| Percentage of non-emergent insects | 3 | 1.2936 | 0.2142 | 0.8859 * |

* = non-significant at $P < 0.05$.

is the boring of a hole in the seed coat. Insufficient diameter of a hole or the lack of energy to push its covering lid, may explain the difficulty presented by 6% to 17% of *Bruchidius sahlbergi* Schilsky (Coleoptera: Bruchidae) to emerge from *Acacia erioloba* E. Mey. (Fabaceae) seeds (Ernst 1992). These factors may also explain the great proportion of bruchids that did not emerge from *M. bimucronata* seeds in 2003.

In short, the results reported here suggest that only *A. schrankiae* occurs in *M. bimucronata* seeds and that this bruchid belongs to Guild A (Johnson 1981). The results also suggest that the first larva to reach the seed prevents other larvae from reaching the cotyledons because only one larva of *A. schrankiae* developed inside each seed until adult emergence. We observed that differences in the number of *M. bimucronata* fruits per region of inflorescence affected the levels of seed predation. Therefore, at this spatial scale, the pattern of seed predation was not homogeneous. However, after increasing the spatial scale to the canopy level, the damage caused by *A. schrankiae* was evenly distributed.

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