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Predispersal infestation of *Vochysia haenkeana* seeds by *Lius conicus*

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ABSTRACT: The deficient development of fertile seeds of native forest plant species in Brazil limits the reproduction of these plants in various conditions. Among the limiting biotic factors in quality and quantity of the forest seeds, borer insects are quite prominent, before and after their dispersion. This study reports for the first time a host of the buprestid beetle *Lius conicus* (Gory & Laporte, 1840). The larval development of *L. conicus* takes place in the seed capsules of *Vochysia haenkeana* Mart. (Vochysiaceae), a typical tree species in the Brazilian cerrado biome. In two regions of the cerrado in Goiás State, Brazil, almost ripe fruits of *V. haenkeana* were collected directly from the plants. After natural drying, and fruit and seed processing in laboratory, damage caused by the *L. conicus* larvae was quantified and qualified. Bigger fruits were preferred as hosts. Fruits developing on the eastern side of the plant were most frequently occupied by *L. conicus*. Seed lots of bigger fruits showed damage up to 37.5% from the infestation by *L. conicus* larvae. There was only one larva per fruit, which damaged all the seeds of the capsule (three or four) and generally consumed around 26% of the seed dry mass.

Key words: Brazilian cerrado, “pau-amarelo”, seed damage, insect.

Infestação pré-dispersão de sementes de *Vochysia haenkeana* por *Lius conicus*

RESUMO: O deficiente desenvolvimento de sementes férteis de espécies florestais nativas no Brasil limita a propagação dessas plantas em diversas condições. Entre os fatores bióticos limitantes da qualidade e quantidade das sementes florestais, destacam-se os insetos broqueadores, antes e depois da sua dispersão. Este estudo relata pela primeira vez um hospedeiro do besouro buprestídeo *Lius conicus* (Gory & Laporte, 1840). O desenvolvimento larval de *L. conicus* ocorre em cápsulas com sementes de *Vochysia haenkeana* Mart. (Vochysiaceae), uma típica árvore do bioma cerrado brasileiro. Em duas regiões do cerrado, no Estado de Goiás, no Brasil, frutos quase maduros de *V. haenkeana* foram coletados diretamente das plantas. Após secagem natural e beneficiamento de frutos e sementes em laboratório, os danos causados pelas larvas de *L. conicus* foram quantificados e qualificados. Frutos maiores foram os preferidos como hospedeiros desse inseto. Os frutos produzidos pela planta, voltados para ponto o cardeal leste, foram os mais infestados por *L. conicus*. Lotes de sementes provenientes de frutos maiores apresentaram infestação por *L. conicus* de até 37,5%. Observou-se que houve apenas uma larva desse inseto por fruto, que danificou todas as sementes da cápsula (três ou quatro) e consumiu geralmente cerca de 26% da massa seca da semente.

Palavras-chave: Cerrado brasileiro, pau-amarelo, dano em semente, inseto.

INTRODUCTION

The semideciduous tree *Vochysia haenkeana* (Vochysiaceae), commonly known in the Brazilian Cerrado as “pau-amarelo”, “escorrega-macaco” or “cambarazinho” can reach a height between 8 to 20m, with a diameter of the trunk up to 60cm (NETO, 1991; RIZZI et al., 2016). Wood from *V. haenkeana* is used for

firewood, civil construction, furniture manufacturing, coffers, toys and various tool handles (VIANNA, 1980; LORENZI, 2013). LORENZI (2013) mentioned possible use of this species in silvipastoral systems, as well as an ornamental plant due to the color of its trunk and the exuberance of its yellow flowering. In addition, PASA (2011) mentioned that the bark of this tree is also used as tea for respiratory diseases.

Similar to other tree species from the cerrado, *V. haenkeana* is tolerated as a single plant or in small groups, to provide shade to the cattle, or where it is not disturbing agriculture and large monocultures. The last remaining refuges for this tree species are limited to small permanent preservation areas, close to water streams.

Seed dispersal is one of the key phases in the regeneration process of plant populations (TRAVERSE et al., 2014) and, as a component of this process, the seed predators can impose a strong selection pressure on plants (JANZEN, 1980; MARON & GARDNER, 2000). There are two basic types of seed predation: predispersal predation, which occurs while the seed is still attached to the mother plant; and post-dispersal predation, which occurs after separation from the mother plant but before the germination process begins (JANZEN, 1971). Intensity of predispersal and post-dispersal infestation by several predators contributes significantly to seed mortality, affecting the distribution of several forest species (ZHANG et al., 1997; SANTOS et al., 2001). Some studies show that the fruits and/or seeds of a great number of economically or ecologically valuable native tree species are regularly damaged by different groups of insects (HULME & BORELLI, 1999; SANTOS et al., 2001; SARI et al., 2002; MEIADO et al., 2013), many of which are strikingly prey-specific (JANZEN, 1980). Predispersal predation by insects may cause mortality in up to 80% of the seeds produced in many grassland and forest habitats (ANDERSEN, 1988).

Insect damage to seeds and embryos usually prevents them from developing and germinating (BRADFORD & SMITH, 1977; ZHANG et al., 1997; RIBEIRO et al., 2007). According to SANTOS et al. (1997) and LOUREIRO et al. (2004), the most frequent seed damaging insects of native forest species, especially *Fabaceae*, are Coleoptera belonging to *Bruchidae*, *Anthribidae*, *Curculionidae* and *Cerambycidae*, Diptera of the *Tephritidae* family and Lepidoptera of *Pyalidae* family. In addition to these, SARI et al. (2002) mentioned *Tenebrionidae* and Hymenoptera species as possible causing agents of damages in seeds of native Brazilian trees.

Buprestidae is the seventh most diverse family of the order Coleoptera, possessing around

15,000 species (BELLAMY & NELSON, 2002). In contrast with the previous groups, most buprestid species are wood-borers or leafminers (LIMA, 1953; HESPENHEIDE, 1991; CORONA & TOLEDO, 2006; HESPENHEIDE & CHABOO, 2015), with rare records as seed predators.

The aim of this study is to identify insects associated with *V. haenkeana* seeds, in the predispersal phase, as well as quantify and qualify the damages caused in the fruits and seeds by insects.

MATERIALS AND METHODS

Adult trees of *V. haenkeana* were randomly selected in two different regions of the cerrado in the Municipalities of Caldas Novas, GO, Brazil (lat. 17° 43' 34"S; long. 48° 34' 10"W; alt. 625m) and Pires do Rio, GO, Brazil (lat. 17° 03' 61"S; long. 48° 49' 46"W; alt. 755m). Both areas are small fragments of riparian forests of permanent preservation. The distance between these areas is 61km. In each one, 15 trees with diameter at breast height (DBH) bigger than 30cm and height between 8 and 15m were selected. Randomly, throughout the medium and lower canopy of the tree, 15 small branches containing about 35 still green fruits were collected. Fruits were collected from these trees at two different periods. The first removal took place in 2014 in Caldas Novas and the other in 2015 in Pires do Rio. Minimum distance between the harvested trees in each area was at least 100m, in order to guarantee the independence of the samples.

In Caldas Novas, fruits were collected once, when most of fruits presented a green color and some were already ripe before dispersing seeds. After removal from the branches, all fruits were grouped in a single sample. Fruits were dried in the natural environment in 10 ventilated (six holes, with a 2.0cm diameter) plastic containers with a 3.0L capacity. Holes in the lids were covered with a micro-perforated fabric ("tulle" type), to prevent the emerging insects from escaping. Subsequently a quantification and identification of all insects, emerging from the capsules, was conducted, as well as germination tests with the remaining seeds.

In Pires do Rio, at the selected 15 trees, unripe fruits were collected in three different states.

The first harvest was made when trees still showed abundant flowering and young fruits. The second and third harvest took place 15 and 30 days later, respectively. Procedure for collecting, drying and processing of fruits and seeds was the same as in Caldas Novas. Number of damaged fruits and seeds was quantified and capsule size (length) was measured to look for correlations between fruit size and damage. Length of fruits was measured with a digital caliper in the laboratory. Another question was if the exposure of the fruits to the sun (east or west position) would influence the quantity of damage by the insect. For this purpose, the tree was divided in half and, randomly, fruits were collected from both eastern and the western extremity side. The orientation for the collection points of fruits in the tree canopy was made with the use of a compass.

After natural drying in the laboratory, when there was no observance of emergence of adult insects in the stored seeds or capsules, the seeds were quantified and qualified. Four seed lots with 50 damaged seeds by the insect, and same amount of undamaged seeds, were weighed. For this, the seeds were placed in a forced draft oven ($105\pm 3^{\circ}\text{C}$) for 24 hours, following the recommendation by the Rules for Seed Analysis (BRASIL, 2009).

All seeds collected in Caldas Novas ($n=3120$) were classified in the following categories: undamaged, damaged seeds (by insects) and empty or deformed husks. The percentage of seeds consumed by the buprestid larvae was estimated by the difference of dry matter of undamaged and damaged by insect. For this, out of the total number of damaged and undamaged seeds ($n=50$) were randomly removed and weighed.

Germination of damaged seeds by *L. conicus* and intact seeds were compared. For this purpose, seeds were spread on germitex paper and transferred directly to germination chambers, according to the methodology in BRASIL (2009). In this treatment (germitex paper roll containing 25 seeds damaged or undamaged by the insect), with four replicates for each treatment, the seeds were allowed to germinate for 30 days. The seed was considered germinated when radicles were visible in this period.

A chi-square test (χ^2) was conducted to test the location of fruit in the tree (east or west orientation) and the intensity of the damage caused

by insects. Comparison between the fruit and the presence of *L. conicus* in it was performed using Spearman's correlation coefficient. These analyses were processed with the SISVAR 5.3 Software (FERREIRA, 2011).

RESULTS AND DISCUSSION

Regardless of the location of the fruit collection and the year of the collection, only one borer insect species in the seed of *V. haenkeana* was present. In our study, the number of emerged adult insects from the seeds was not quantified. However, confirmation of the single borer species was done with the collection of more than one hundred adult insects in the two distinct studied localities. In addition to these insects, the presence of Hymenoptera, possibly parasitoid of the young forms of the insect borer, was verified inside the plastic containers protected with "tulle". The borer insect, identified as *Lius conicus* (Gory & Laporte, 1840) (=syn. *Lius nobilis* Obenberger, 1924) belonging to the family Buprestidae, is around 5.7mm in length (Figure 1). The buprestid species was identified by the fifth author of this research. The "voucher specimen" was deposited in the entomological collection of the Universidade Estadual de Goiás, Ipameri, Goiás, Brazil.

Records of insects and their host plants are scarce for the vast majority of phytophagous insect species in Brazil, and are mainly composed of species of agricultural interest or vectors of disease (FLINT et al., 2006; SILVEIRA et al., 2008). In these records, Buprestidae preying on seeds is a rare event. Few records of *Lius* in the literature mention some species which only feed on the leaves (NAKAHARA et al., 1992; FLINT et al., 2006). However, coincidentally, CUSTÓDIO et al. (2014) reported the incidence of buprestid larvae (unidentified, but possibly the genus *Lius*) preying on *Qualea* seeds, which is also from the same plant family as the one in our study. Thus, future researches should be developed to understand the relationship between *L. conicus* and other species of Vochysiaceae in the region of our study.

The damage on the unripe fruit, caused by the insect larva, is very typical, an exudate (gum), shown in figure 1. Only one perforation with exudate

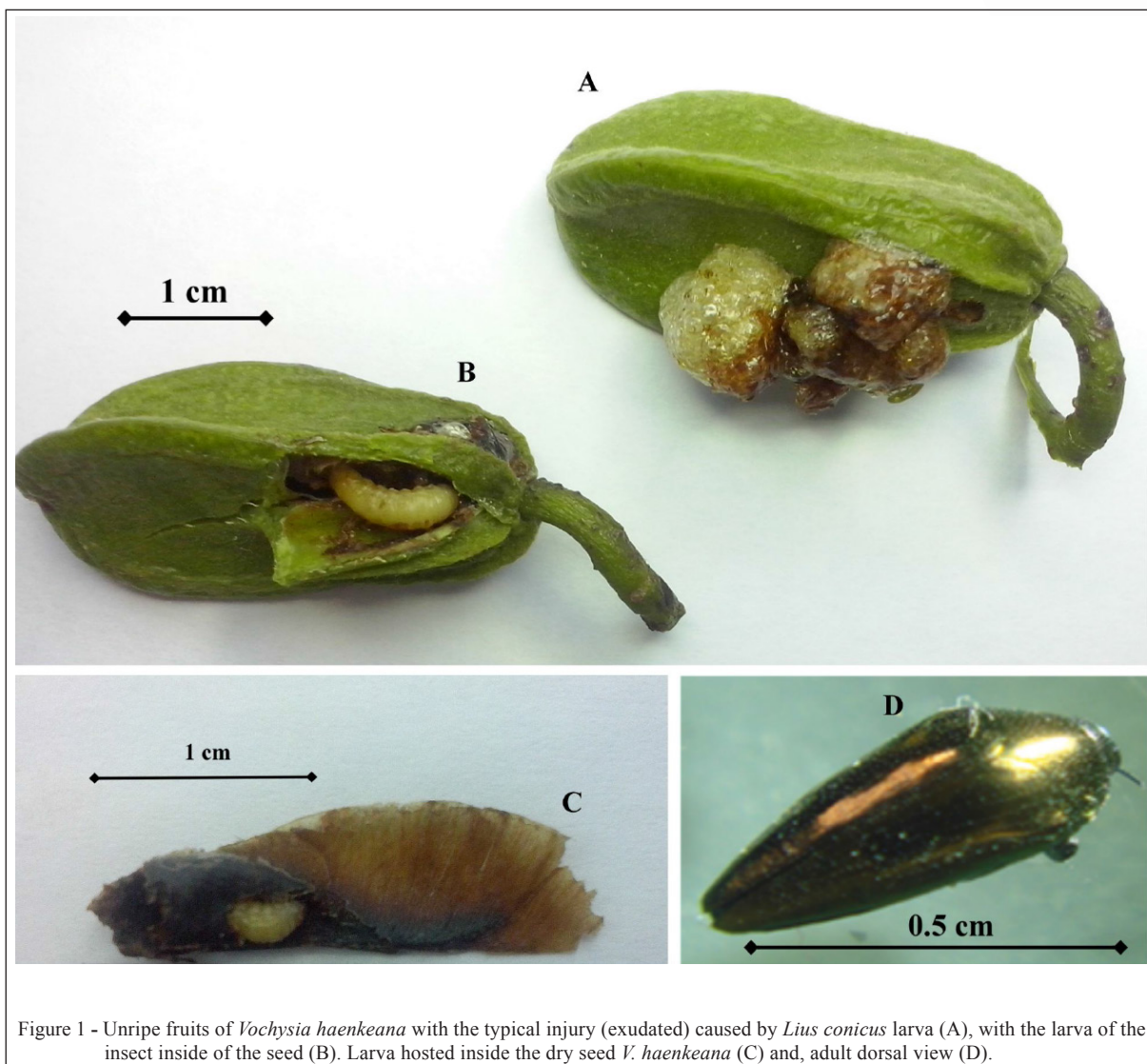


Figure 1 - Unripe fruits of *Vochysia haenkeana* with the typical injury (exudated) caused by *Lius conicus* larva (A), with the larva of the insect inside of the seed (B). Larva hosted inside the dry seed *V. haenkeana* (C) and, adult dorsal view (D).

per fruit was observed. All unripe fruit with typical signs of infestation by *L. conicus* lost all the seeds (three or four) to the buprestid larvae. Due to the fruit architecture, seeds are very close to each other and easily reachable by the larva.

The fact that no specific exit holes of the adults of *L. conicus* were observed indicates that the adult left the dehiscent fruit capsule at the time of the dispersion of the seed, similar to *Apion* spp. (Coleoptera: *Brentidae*) in seeds of *Copaifera* (*Fabaceae*) (SANTOS et al., 2015). During the natural dried seed processing, several adult insects

were removed from the capsules, which supports this hypothesis.

Of the 900 sampled fruits in Caldas Novas, after seed processing in laboratory, 62.5% contained undamaged seeds; 19.75% contained seeds destroyed by *L. conicus*, and 17.75% did not show any attack, but presented empty husks. The lower quantity of *V. haenkeana* seeds occupied by *L. conicus* when compared with intact seeds, can be a natural defense system of this plant. This strategy of defending plants in general from insects was mentioned by JANZEN (1971).

Considering the weight of five lots with 50 seeds undamaged and damaged by *L. conicus* larvae: (0.4526±0.1572g – undamaged seeds and 0.3355±0.0298g – damaged seeds), it was estimated that around 26% of seed dry matter was consumed by the insect larvae. Endosperm and embryos of seeds were preferred and almost completely consumed, whereas the wing and outer part of the seed coat were found remaining (Figure 1, *L. conicus* larva in dry seed of *V. haenkeana*).

Of the sampled fruits in Pires do Rio, (after flowering and appearance of the first fruits (n=1691), the second (n=1374), the third (n=1821) collections, 15 and 30 days after the first one) the attacks of *L. conicus* were recorded in 3.02%, 7.13% and 37.50% for each collection of fruits, respectively. A significant correlation between fruit length and its infestation by *L. conicus* (Spearman, $c=0.955$; $P<0.05$) was observed. Since during the second collection of fruits a large quantity of small and unripe fruits was observed, one can infer that the adult of this pest prefers oviposition in bigger fruits (Table 1). Female *L. conicus* prefers oviposition in fruits located towards the east part of the plant ($\chi^2=245.22$; d.f.=44; $P<0.05$) (Table 2). Maybe fruits and seeds in this orientation provide better quality, conditions or microclimate for the survival of the insect larva. This information could be useful for fruit and seed collections for the seedling production programs. For example, SANTOS et al. (2001) observed seed infestation of *Anadenanthera peregrina* L. Speg. (*Fabaceae*) by insects when found towards east and north of the tree. These authors suggest avoiding the collection

Table 1 - The length of *Vochysia haenkeana* fruits and perforated fruits by *Lius conicus*. Pires do Rio, Goiás, Brasil, 2015.

Length class (cm)	Bored fruits
<0.5	0
0.51 – 1.0	0
1.01 – 1.5	10
1.51 – 2.0	42
2.01 – 3.0	216
3.01 – 3.5	297
>3.51	265

Significant correlation between fruit length and attacks by *L. conicus* (Spearman's correlation: $r = 0.955$; $P<0.05$; $n=45$ plants).

of seeds in those areas of the plant for seedling production programs.

Undamaged seeds from Caldas Novas (67±5.1%) and from Pires do Rio (85±1.5%) germinated. None of the damaged seeds germinated. The last instar of the larva of *L. conicus* has the same length of the seed, as shown in figure 1, and it consumes nearly the whole internal content of the fruit capsule, including the seeds, or part of the seeds.

CONCLUSION

In this research, a host plant (*V. haenkeana*) of buprestid *L. conicus* is recorded for the first time. In the study area, only this insect borer was reported in the seeds. This event occurred before seed dispersal and significantly compromised seed production.

Table 2 - Injuries caused to *Vochysia haenkeana* fruits by *Lius conicus* in relation to the location in the plant. Pires do Rio, GO, Brasil, 2015.

Collection *	Perforated fruits by <i>L. conicus</i> /15 plants-----		Undamaged fruits
	East position	West position	
1 st	36	15	1640
2 nd	42	54	1278
3 rd	433	250	1138
-----Chi-square (χ^2) = 245.22; d.f. = 44; $P<0.05$ -----			

*Different occasions of fruit collection: the first, right after flowering and appearance of the first fruits, the second and third collections, 15 and 30 days after the first collection, respectively.

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