FRUGIVORY OF *NEOSILBA* SPECIES (DIPTERA: LONCHAEIDAE) AND *THEPYTUS ECHELTA* (LEPIDOPTERA: LYCAENIDAE) ON *PSITTACANTHUS* (SANTALALES: LORANTHACEAE) IN ECOTONAL CERRADO-SOUTH PANTANAL, BRAZIL

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Abstract

This paper presents a survey of the insects that feed on fruits of *Psittacanthus* Martius (Santalales: Loranthaceae), a hemiparasitic mistletoe genus that infects trees in Brazil and other neotropical countries. The aim of the study was to identify candidate insects for biological control of *Psittacanthus* mistletoes. Unripe and mature fruits were collected in several localities of Cerrado, bordering South Pantanal, Southwestern Brazil, from 29 Apr 1998 to 30 Jul 2000. A total of 24,710 fruits (54 samples) of *Psittacanthus acinarius* infecting 15 species from 10 plant families were evaluated. *Psittacanthus acinarius* (Mart.) was the most abundant and frequent species of mistletoe parasitizing trees in the ecotonal Cerrado-Pantanal. From 24,710 fruits of *Psittacanthus acinarius* were obtained 1,812 insect larvae including 1,806 *Neosilba* McAlpine (Diptera: Lonchaeidae) species and 6 *Thepytus echelta* (Hewitson) (Lepidoptera: Lycaenidae). From these emerged 1,550 *Neosilba* spp. adults and 6 *T. echelta*. *Neosilba pantanense* Strikis was described from this research. Larvae of *T. echelta* occurred in fruits of *P. acinarius* parasitizing *Cecropia pachystachya* Trécul (Urticaceae) and *Anadenanthera colubrina* (Vellozo) Brenan (Fabaceae). Larvae of *Neosilba* caused no adverse effects on the germination of infected fruits of *Psittacanthus*, because they do not eat the embryo or viscin tissues. This differs from the larvae of *T. echelta* that interrupted the germination of seeds by feeding on those tissues. *Thepytus echelta* may be a promising insect for the biological control of *P. acinarius* in the ecotonal Cerrado-Pantanal, although its abundance and frequency were low throughout the sampling period.

Key Words: biological control, frugivorous insects, mistletoes, horticulture, tritrophic interactions

Resumen

Este trabajo presenta un estudio de los insectos que se alimentan de frutos de *Psittacanthus* Martius (Santalales: Loranthaceae), un género de muérdago hemiparásita que infecta a los árboles en Brasil y otros países neotropicales. El objetivo del estudio fue identificar los insectos candidatos para el control biológico de los muérdagos *Psittacanthus*. Los frutos verdes y maduros fueron recolectados en varias localidades de la sabana, en la frontera sur del Pantanal, en el suroeste de Brasil, desde el 29 abril 1998 hasta 30 julio 2000. Un total de 24.710 frutas (54 muestras) de *Psittacanthus acinarius* infectan a 15 especies de 10 familias de plantas fueron evaluadas. *P. acinarius* (Mart.) fue la especie más frecuente y abundante de muérdago que parasitan árboles en el ecotono del Cerrado-Pantanal. De 24,710 frutos de *Psittacanthus acinarius* sí obtuvieron 1,812 larvas de insectos, de los cuales 1,806 eran de especies de *Neosilba* McAlpine (Diptera: Lonchaeidae) y seis de *Thepytus echelta* (Hewitson) (Lepidoptera: Lycaenidae). A partir de estos surgieron 1,550 adultos de *Neosilb*a spp. y seis de *T. echelta*. *Neosilba pantanense* Strikis fue descrito a partir de esta investigación. Las larvas de *T. echelta* ocurrió en frutos de *P. acinarius* parasitando *Cecropia pachystachya* Trécul (Urticaceae) y *Anadenanthera colubrina* (Vellozo) Brenan (Fabaceae). Las larvas de *Neosilba* no causaron efectos adversos sobre la germinación de frutos de *Psittacanthu*s infectados porque no comen los tejidos de embriones o viscinia. Esto difiere de las larvas de *T. echelta* que interrumpe la germinación de las semillas mediante la alimentación en esos tejidos. *Thepytus echelta* puede ser un insecto prometedor para el control biológico de *P.*

acinarius en el ecotono del Cerrado-Pantanal, a pesar de su abundancia y frecuencia fueron bajos durante todo el período de muestreo.

Palabras Clave: control biológico, insectos frugívoros, meérdagos, horticultura, interacciones tiróficas

Species of Loranthaceae (Santalales) have a worldwide distribution and are composed of 75 genera and over 900 species of hemiparasitic shrubs (Nickrent 1997; Mathiasen et al. 2008). In Brazil there are reported 58 endemics species, classified in 11 genera: *Cladocolea* Tieghem, *Gaiadendron* G. Don., *Ligaria* Tieghem, *Oryctanthus* (Grisebach) Eichler, *Oryctina* Tieghem, *Passovia* H. Karstern, *Phthirusa* Martius, *Psittacanthus* Martius, *Pusillanthus* Kuijt, *Struthanthus* Martius, and *Tripodanthus* (Eichler) Tieghem (Rizzini 1995, Caires & Dettke 2011); of these, *Struthanthus* and *Psittacanthus* are the genera with the highest species richness in the family.

These mistletoes parasitize trees and bushes, even those species cultivated in rural and urban areas. In Brazil mistletoes can cause serious damage in some fruit trees (Caires et al. 2009), such as guava, *Psidium guajava* L. and jabuticaba, *Myrciaria cauliflora* (Mart.) Berg (Myrtaceae: Myrtales), and mango, *Mangifera indica* L. (Anacardiaceae: Sapindales).

Among the genera of Loranthaceae, *Psittacanthus* species (including *P. acinarius* and *P. cordatus*) are found widely distributed in Brazilian biomes. Reproduction in these mistletoes species occurs only by means of seeds, which are swallowed and spread widely in bird feces. The control of Loranthaceae is very difficult, being carried out only by cutting the infested branches, because the use of herbicides damages the host plants (Venturelli 1981).

Some *Psittacanthus* species are associated with a wide range of host plants in Brazil (Rizzini 1995; Kuijt 2009). Their major economic importance is the damage they inflict on commercial and domestic fruit and ornamental trees. The mistletoes also attack wild and ornamental trees, mainly in open environments, damaging the plant stand and decreasing their fruit production (Rizzini 1995).

Different families of Lepidoptera have been reported feeding on the Loranthaceae. Wee & Ng (2008) reported the life history of *Delias hyparete metarete* Butler (Pieridae) feeding on leafs of *Dendrophthoe pentandra* (L.) Miq. in Singapore. Kelly et al. (2008), in New Zealand, pointed out that populations of *Zelleria maculata* Philpott (Yponomeutidae) prey upon flowers of 2 species of *Peraxilla* (*P. tetrapetala* Tiegh. and *P. colensoi* Hook F.) Tieg., decreasing fruit set rates from 7.9% to 72%, depending on the altitude and fragmentation status. Uchôa & Caires (2000) found *Thepytus echelta* (Hewitson) (treated as *Thecla echelta*) feeding on fruits and seeds of *Psittacanthus* in Pantanal, Brazil. Two other species of lycaenids in the United States, *Callophrys* (formely *Mitoura*) *spinetorum* (Hewitson) and *Callophrys* (formely *Mitoura*) *johnsoni* (Skinner) are recorded as specialist herbivores on shoots of *Arceuthobium* species (Viscaceae) (Shaw et al. 2004).

Among the phytophagous insects found on mistletoes in Brazil, hairstreak butterflies (Lycaenidae, Theclinae, Eumaeini) are reported as frugivorous upon *Psittacanthus* spp. (Uchôa & Caires 2000). Lycaenidae is the second largest family of Papilionoidea (true butterflies) with about 6000 species (about 40% of all known butterflies) occurring worldwide (Fiedler 1996; Venkatesha 2005; Robbins et al. 2010). However, little is still known concerning the host plants of the Neotropical lycaenids (Silva et al. 2011).

Species of *Neosilba* McAlpine (Diptera: Lonchaeidae) are mostly Neotropical, and have been associated with fruits of several families of Angiosperms (Uchôa & Nicácio 2010; Nicácio & Uchôa 2011) including those of the mistletoe *Psittacanthus acinarius*. Currently, *Neosilba* includes 30 species of fruit flies, 25 of them being reported in Brazil (Strikis 2011; Uchôa 2012).

The aims of this paper are to survey the insects infecting fruits of 2 species of *Psittacanthus* (Loranthaceae) in the Cerrado-Pantanal ecotonal area of Southwestern Brazil, also to record which species of host trees or shrubs the mistletoe was parasitizing. An additional objective was to evaluate the effects of feeding by larvae of *Neosilba* McAlpine (Diptera: Lonchaeidae) and *T. echelta* (Lepidoptera: Lycaenidae) on the germination of *P. acinarius* (Mart.) Mart. seeds in the laboratory and in the field.

Materials and Methods

This work was part of a project entitled "Biodiversidade de Insetos Frugívoros, Plantas Hospedeiras e Inimigos Naturais no Brasil Central", whose aim was to survey the diversity of frugivorous Tephritoidea (Diptera) and their parasitoids (Hymenoptera) in the central region of Brazil from 1997 to 2007. Another part of this project, the survey of Lycaenidae larvae in *Psittacanthus* fruits, is reported here.

Thirty-three field trips were randomly carried out in the municipalities of Anastácio (S 20° 29' W 55° 49'; 170 m asl), Aquidauana (S 20° 30' W 55° 47'; 173 m asl), and Miranda (S 20° 14' W 56° 22'; 126 m asl), Mato Grosso do Sul, aiming to locate populations of *Psittacanthus* spp. in ecotonal Cerrado-South Pantanal. The sampling occurred between 29 April 1998 and 30 July 2000. Twenty-six host plants of *Psittacanthus* spp. were found and marked in the field (21 in Aquidauana, 3 in Anastácio and 2 in Miranda), and their fruiting periods monitored. Ripe fruits were collected so that the frugivorous larvae could be reared to adults in laboratory.

Two species of *Psittacanthus* were found in the study areas: *P. acinarius* (Mart.) Mart. (Caires et al. 2009 treated as *P. plagiophyllus* Eichler), and *P. cordatus* (Hoffmanns.) G. Don. (Caires et al. 2009 named as *P. calyculatus* (DC.) G. Don). However, only the first species produced a significant number of fruits with associated insects. Populations of *Psittacanthus* spp. upon their host plants have not been methodically evaluated, but 5 or more of these hemiparasitic plants may occur per host tree.

The ripe fruits of *Psittacanthus acinarius* from different host plants were collected and transported to the Laboratório de Controle Biológico de Insetos, Departamento de Biociências, Universidade Federal de Mato Grosso do Sul (UFMS), Campus Aquidauana, Mato Grosso do Sul, Brazil, where they were kept until the emergence of the associated insects, according to the methods of Uchôa & Zucchi (1999).

The fruits associated with Lepidoptera larvae were placed in cages under suitable climatic conditions for the larvae to complete their development to pupation. After emergence, adults were sacrificed with ethyl acetate, pin mounted, labeled, and finally identified by a specialist (Dr. Marcelo Duarte).

Preliminary Protocol to Evaluate Germination of *Psittacanthus acinarius* Fruits

To study germination of *P. acinarius*, 60 fruits were obtained from mistletoes parasitizing the host plant, guava, *P. guajava*. The fruits were placed in 2 transparent acrylic pots (300 mL) with their openings juxtaposed with a netted fabric (mesh size of 2 × 2 mm), forming a network in the upper pot. The samples were divided into 3 groups of 20 fruits each, which remained under observation for 15, 20 and 30 d, respectively. The observations consisted of checking the seed viability, evaluating *Psittacanthus* fruits from the field (Fig. 1).

The treatments were: 1) fruits with intact ovary (exocarp, mesocarp and endocarp) infested by

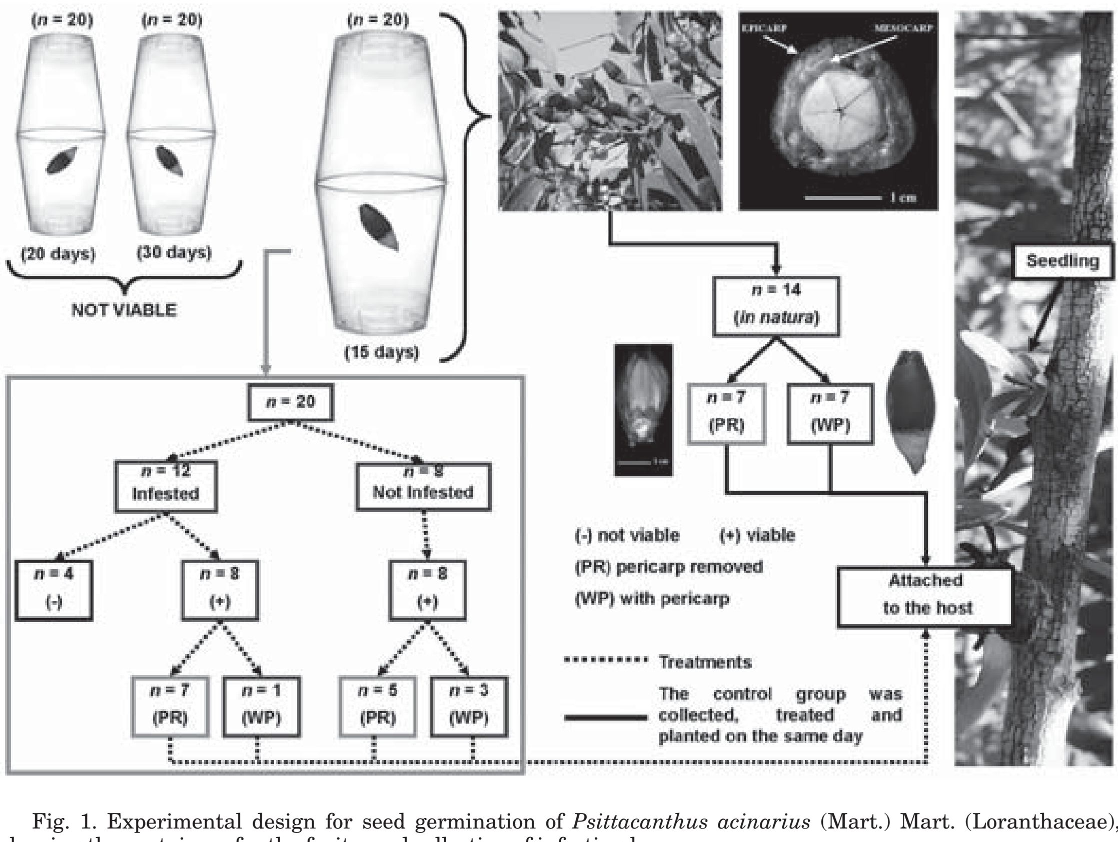

Fig. 1. Experimental design for seed germination of *Psittacanthus acinarius* (Mart.) Mart. (Loranthaceae), showing the containers for the fruits, and collection of infesting larvae.

larvae, 2) intact fruits with their ovaries not infested by larvae, 3) fruit without the integument of the ovary and infested by larvae of *Neosilba* spp. (Caires et al. 2009), and 4) fruits without the integument of ovary, but not infested by *Neosilba* larvae.

After the larvae of *Neosilba* came out of the fruits to pupate, the fruits of *P. acinarius* were submitted to 4 different experimental treatments in the field. Such fruits were tied with nylon fiber to the twigs of guava plants (*Psidium guajava*) to allow the formation of the holdfast and the emergence of the haustorium.

Germination of *Psittacanthus acinarius* in the Field

Three samples of *P. acinarius* fruits, with 20 individuals in each set (n = 60) were collected randomly in the field 15, 20 and 30 d prior to the germination treatments so as to assess the seed viability.

Considering the same sample of 20 fruits, we reserved the infested fruits and the intact ones as control group. Those fruits (n = 12) infested by larvae of *Neosilba* spp. were screened for germination screening, and 8 were viable for germination and four, were not viable. Of these viable fruits, 7 had normal cotyledons, which were placed to germinate without the seed coat, i.e., exocarp = peel and viscin, and one with the seed coat. The other 8 fruits not infested by *Neosilba* larvae were used in the germination treatment. Five of them had the seed coat removed, and 3 were kept intact. As control group we used 14 fruits collected on the same day of the germination treatment: 7 were placed without their seed coats and 7 with the seed coats (Fig. 1).

The voucher specimens of *Psittacanthus* (*P. acinarius* and *P. cordatus*) and their host plants were deposited in the Herbário Central of the Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, and in the Herbário Universidade de Brasília (UnB), Brasília, Distrito Federal, Brazil. The voucher specimens of *Thepytus echelta* (Fig. 2) are deposited in the Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (UFPR), and the specimens of *Neosilba* spp. are deposited in the Coleção Zoológica, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, São Paulo, Brazil.

Results

Two species of *Psittacanthus*, i.e., *P. cordatus* and *P. acinarius*, were found parasitizing 15 species of host trees of 10 different families, in 3 sites (counties) of the ecotonal Cerrado-South Pantanal Region (Table 1).

The insects reared from the fruits of *Psittacanthus acinarius* belonged to the orders Diptera and Lepidoptera. Six species of the genus *Neosilba* McAlpine (Lonchaeidae: Lonchaeinae) were identified as follows: *Neosilba pantanense* Strikis, *Neosilba inesperata* Strikis & Prado, *Neosilba certa* (Walker), *Neosilba pendula* (Bezzi), *Neosilba bifida* Strikis & Prado, and *Neosilba zadolicha* McAlpine & Steyskal. *Thepytus echelta* (Lycaenidae, Theclinae, Eumaeini) was the only butterfly obtained from this rearing (Table 2), and its larvae were observed feeding on the seeds of mistletoe (Fig. 3).

The most abundant and most frequent species of *Neosilba* in the fruits of *Psittacanthus* over their different host trees are the recently described species, *N. pantanense*, and *N. inesperata*, followed by *N. certa* and *N. pendula*. In contrast, *N. bifida* and *N. zadolicha* were less abundant and less frequent in the fruits of *Psittacanthus* spp. (Table 3).

The population densities of *Neosilba* species followed the peaks of fruit production of the mistletoe, *P. acinarius,* that occurred in the samples collected from Jun to Aug (Fig. 4) in both 1998 and 1999.

Discussion

In the present study, only *P. acinarius* produced a significant number of fruits suitable to evaluate insect infestations. Fruit production started in Apr and the first mature fruits appeared by May, with peaks from Jun to Aug. The fruiting season ceased in Nov in each of the 2 consecutive yr. Other species of *Psittacanthus* may have similar timing of fruiting, such as *P. calyculatus*, which produces fruits from Jul to Nov in dunes at La Mancha, Veracruz, México (Barros et al. 2001). In our study a total of 24,710 fruits were sampled, totaling 61.739 kg (Table 1).

The larvae of *T. echelta* were found feeding on fruits of *P. acinarius* parasitizing *Cecropia pachystachya* Trécul. (Rosales: Urticaceae) and *Anadenanthera colubrina* (Vellozo) Brenan (Fabales: Fabaceae) (Table 2).

Interactions between ants and the larvae of Lycaenidae have been reported so far by Atsatt (1981), Pierce et al (2002), Schmidt & Rice (2002) and Saarinen & Daniels (2006). In the present study, ant colonies were observed in the trunks of both host plants of *P. acinarius*, but no association of the ants with larvae of *T. echelta* was observed during the field sampling. We do not know if larvae of *T. echelta* and ants may have interacted during day or night. *T. echelta* is still the only hairstreak butterfly (Lycaenidae, Theclinae, Eumaeni) known to have part of its life cycle in the fruits of *P. acinarius* (Uchôa & Caires 2000).

From the guild of frugivorous insects in *P. acinarius*, larvae of the lycaenid, *T. echelta*, was the

Fig. 2. Male (above) and female (below) of *Thepytus echelta* (dorsal [right] and ventral [left] views, respectively) (Photos by Marcelo Duarte-USP, SP).

TABLE 1. INTERACTIONS BETWEEN SPECIES OF *PSITTACANTHUS* AND THEIR HOST TREES IN 3 COUNTIES FROM THE ECOTONE CERRADO-SOUTH PANTANAL, SOUTHWESTERN BRAZIL (29 APR 1998 TO 30 JUL 2000).

Host Trees of *Psittacanthus*	*P. acinarius*	*P. cordatus*	Localities
Anacardiaceae			
Astronium graveolens Jacq.	✓		Aquidauana 20° 30' S; 55° 47' W, 173 m
Myracrodruon urundeuva Allemão	✓		Aquidauana
Tapirira guianensis Aubl.	✓		Aquidauana
Boraginaceae			
Cordia sp.	✓		Aquidauana
Urticaceae			
Cecropia pachystachya Trécul	✓		Aquidauana
Combretaceae			
Terminalia catappa L.	✓		Aquidauana
Terminalia sp.	✓		Anastácio 20° 29' S; 55° 49' W, 170 m
Euphorbiaceae			
Sapium haematospermum Mül. Arg.	✓		Aquidauana
Meliaceae			
Melia azedarach L.	✓		Aquidauana
Fabaceae			
Acacia sp.	✓		Aquidauana
Anadenanthera colubrina **var**. *cebil* (Griseb.) Altschul.	✓		Aquidauana
Enterolobium contortisiliquum (Vell.) Morong	✓		Anastácio
Moraceae			
Artocarpus heterophyllus Lam.	✓		Anastácio
Myrtaceae			
Psidium guajava L.	✓	✓	Aquidauana & Miranda (20° 14' S; 56° 22' W, 126 m)
Salicaceae			
Salix babylonica L.	✓		Anastácio & Aquidauana

TABLE 2. FRUGIVORY OF *NEOSILBA* SPP. AND *THEPYTUS ECHELTA* ON RIPE FRUITS OF *PSITTACANTHUS ACINARIUS* IN ECOTONAL CERRADO-SOUTH PANTANAL, BRAZIL (APR 1998 TO JUL 2000).

Hosts of *Psittacanthus acinarius* & Number of samples (n)	Number of Fruits	Biomass of Fruits (kg)	Number of Mature Larvae	Number of Adults	Infestation of Mature Larvae by Fruit	Larval Viability (%)	Number of Adults & Frugivorous Insect Species
Anacardiaceae							
Astronium graveolens (1)	136	0.335	0	0	0.0	0.0	—
Myracrodruon urundeuva (5)	5,953	12.539	151	119	0.025	79	*Neosilba* sp. (damaged)
Tapirira guianensis (1)	140	0.342	67	67	0.471	100	5 *Neosilba inesperata* 34 *Neosilba pantanense* 28 *♀♀ *Neosilba* spp.
Boraginaceae							
Cordia sp. (1)	70	0.207	28	24	0.400	86	20 *Neosilba pantanense* 4 *♀♀ *Neosilba* spp.
Combretaceae							
Terminalia sp. (1)	260	0.669	15	15	0.058	100	2 *Neosilba pantanense* 2 *N. inesperata* 11 *♀♀ *Neosilba* spp.
Terminalia catappa (1)	352	0.804	1	1	0.003	100	*Neosilba* sp. (damaged)
Euphorbiaceae							
Sapium haematospermum (17)	11,508	31.497	1.002	805	0.087	80	298 *Neosilba pantanense* 60 *N. inesperata* 17 *N. certa* 10 *N. pendula* 2 *N. zadolicha* 418 *♀♀ *Neosilba* spp. 95 *Neosilba* sp. (damaged)
Fabaceae							
Acacia sp. (2)	1,707	3.686	60	44	0.035	73.34	4 *Neosilba pantanense* 1 *N. bifida* 2 *N. pendula* 37 *Neosilba* sp. (damaged)
Anadenanthera colubrina (4)	1,099	2.327	115	103	0.105	89.6	39 *Neosilba pantanense* 1 *N. inesperata* 3 *N. bifida* 1 *N. certa* 3 *N. pendula* 56 *♀♀*Neosilba* spp. 2 *Thepytus echelta*
Enterolobium contortisiliquum (5)	911	2.002	14	8	0.015	57	*Neosilba* spp. (damaged)

Legend: *♀♀ *Neosilba* spp. = Females of *Neosilba* that are not yet identifiable.

TABLE 2. (CONTINUED) FRUGIVORY OF *NEOSILBA* SPP. AND *THEPYTUS ECHELTA* ON RIPE FRUITS OF *PSITTACANTHUS ACINARIUS* IN ECOTONAL CERRADO-SOUTH PANTANAL, BRAZIL (APR 1998 TO JUL 2000).

Hosts of *Psittacanthus acinarius* & Number of samples (n)	Number of Fruits	Biomass of Fruits (kg)	Number of Mature Larvae	Number of Adults	Infestation of Mature Larvae by Fruit	Larval Viability (%)	Number of Adults & Frugivorous Insect Species
Meliaceae *Melia azedarach* (1)	273	0.850	19	19	0.070	100	*Neosilba* sp. (damaged)
Moraceae *Artocarpus heterophyllus* (2)	66	0.188	31	30	0.470	97	10 *Neosilba pantanense* 20 *♀♀ *Neosilba* spp.
Myrtaceae *Psidium guajava* (3)	48	0.130	11		0.2	73	4 *Neosilba pantanense* 4 *♀♀ *Neosilba* spp.
Salicaceae *Salix babylonica* (4)	1,676	4.692	143	126	0.085	88	38 *Neosilba pantanense* 9 *N. inesperata* 2 *N. bifida* 5 *N. certa* 1 *N. pendula* 2 *N. zadolicha* 69 *♀♀ *Neosilba* spp.
Urticaceae *Cecropia pachystachya* (2)	511	1.471	108	85	0.211	79	29 *Neosilba pantanense* 5 *N. inesperata* 3 *N. certa* 48 *♀♀ *Neosilba* spp. 4 *Thepytus echelta*
Total	24,710	61.739	1.765	1.454	—	82.4	—

Legend: *♀♀ *Neosilba* spp. = Females of *Neosilba* that are not yet identifiable.

Fig. 3. Larva of *Thepytus echelta* (Lycaenidae) on the peduncle of *Psittacanthus acinarius* (Loranthaceae) fruit.

only species that expressed some potential for use as a biological control agent against this mistletoe. This is because the caterpillars produced a hole in the pericarp of the fruit to reach the seeds that indeed they did eat, thus eliminating the cotyledons and the viscin. Because of this behavior *T. echelta* may completely impede seed germination. However, their population during the period of the survey was very low (Table 2).

Of all insect species reared on *P. acinarius* fruits, the most abundant (78.1%) and frequent was *N. pantanense* (Table 3). All lonchaeines were observed eating the fruit epicarp, but a bioassay conducted in the laboratory showed that no species of Lonchaeidae directly affected the seed germination of this mistletoe.

While removing the viscin over the seed and the integument we observed that the larvae of *Neosilba* fed only near the area of the mesocarp. There was no evidence indicating that the species of *Neosilba* damaged the cotyledons and viscin.

Uchôa & Caires (2000) proposed that *Thepytus echelta* (treated as *Thecla echelta*) might be considered a potential candidate for biological control of *Psittacanthus* mistletoes. Shaw et al. (2004) addressed 2 interesting issues about the herbivory of lycaenids upon species of mistletoes. First, they considered the possibility of using the larvae of *Callophrys* (formely *Mitoura*) *spinetorum* (Hewitson) and *Callophrys johnsoni* (Skinner) to control populations of dwarf mistletoe (*Arceuthobium*, Viscaceae) in the USA. Second, they argued that, with a critical reduction of the mistletoe host, these 2 species might be driven to extinction. In fact, we share the same concern regarding the use of *T. echelta* against some species of *Psittacanthus* in Brazil, because our field experience shows that the populations of *Thepytus echelta* may be few in nature.

Before recommending *T. echelta* for the biological control of the neotropical mistletoes, some questions should be addressed: Is *Thepytus echelta* a specific herbivore of *Psittacanthus acinarius*? If so, how high are its populations upon *P. acinarius* under natural conditions?

Germination of *Psittacanthus acinarius* in the Field

All fruits from 2 samples (20 and 30 d before the treatment of germination evaluation) failed to germinate. Three months after the germination treatment was started, we found that in the fruits with the intact seeds (in both the both control and fruits infested by *Neosilba* larvae) all seeds failed to germinate. On the other hand, the fruits without the exocarp (control, infested and non-infested) germinated normally. Seeds of the fruits collected 20 d before the germination treatment did not germinate although in 4 of them larvae of

Table 3. Species of *Neosilba* reared from ripe fruits of *Psittacanthus acinarius* (Mar.) mart. (Loranthaceae), sampled from fifteen host trees of the mistletoes in Ecotonal Cerrado-South Pantanal, Brazil (Apr 1998 to Jul 2000).

Species	Relative Abundance (%)	Frequency	Means	Standard Error	*CV (%)	Means comparison by Mann-Whitney Test (P < 0.05)
Neosilba pantanense	78.10	20	23.90	6.83	28.57	A
Neosilba inesperata	13.40	14	5.86	1.59	27.13	B
Neosilba certa	4.25	8	3.25	1.11	34.15	BC
Neosilba pendula	2.61	6	2.67	1.12	41.95	BC
Neosilba bifida	0.98	5	1.20	0.20	16.67	BC
Neosilba zadolicha	0.65	4	1	0	0.00	C

Legend: Kruskal-Wallis Test (P < 0.05); means followed by the same letter do not differ significantly;
*CV = Coefficient of Variation

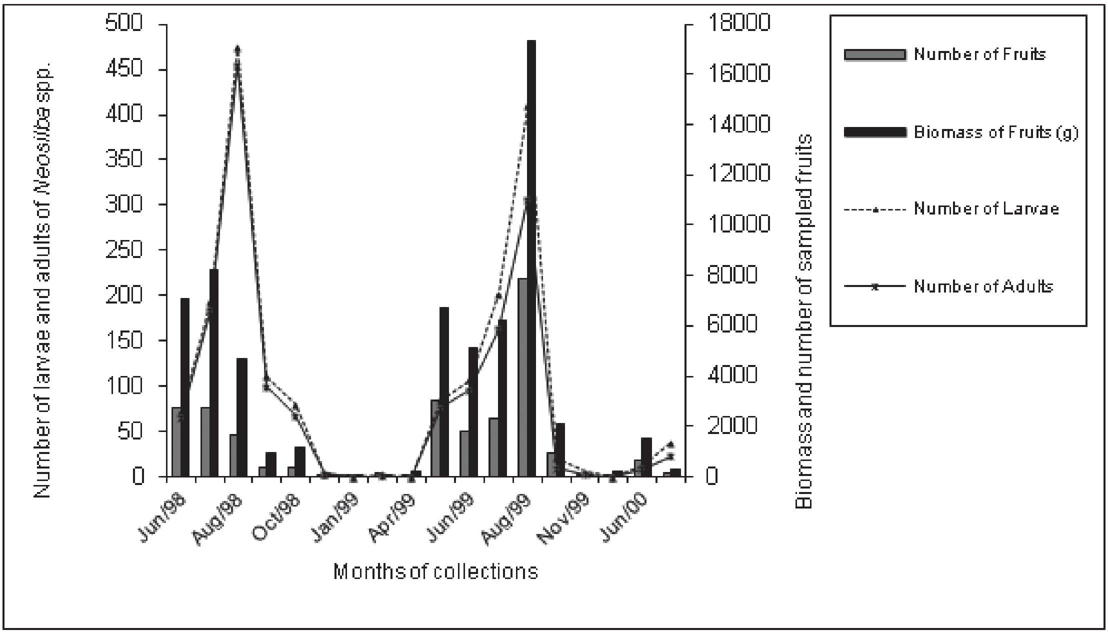

Fig. 4. Population fluctuation of larvae and adults of 6 *Neosilba* species (Diptera: Lonchaeidae) on fruits of *Psittacanthus acinarius* (Mart.) Mart. (Loranthaceae), sampled from Jun 1998 to Jul 2000 in ecotonal Cerrado-South Pantanal, Mato Grosso do Sul (Anástacio, Aquidauana, and Miranda).

Neosilba have been obtained. The fruits collected in the field 30 d before the germination assay had become useless for germination, due to the time that they had remained in the containers. Moreover, from these fruits no larva was recovered.

These findings suggest that the cotyledons of *P. acinarius* need to be exposed to the environment to continue the process of germination. Moreover, the mistletoe seedlings need to be placed on the host tree to start their parasitism. There were some cases in which the cotyledons showed darkening by the action of excreta deposited by larvae of *Neosilba* spp. or by fungal infection. In these cases, the seedlings of *P. acinarius* started the germination, but died before the hautorium could be introduced into the host. Therefore, it is possible to infer that the damage to mistletoe due to the feeding of *Neosilba* larvae was only indirect, and caused by their feces, which may promote the death of embryos due to the secondary attack of microorganisms, but this did not inhibit germination. Moreover if some birds ingest these fruits infested by species of *Neosilba*, germination probably would not be inhibited, because the larvae do not eat the cotyledons nor remove the viscin, which is responsible for attaching *P. acinarius* to the surface of the host plants.

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