

Capítulo 5

Biological agents that accelerate winter decay of *Eichhornia crassipes* Mart. Solms. in northeastern Argentina¹

Alicia S. Poi de Neiff² & Sylvina L. Casco³

²Profesora titular, Universidad Nacional del Nordeste (UNNE). Investigador Científico del CONICET. C. C. 291 (3400), Corrientes, Argentina. ³Becaria Interna del CONICET en el CECOAL.

Introduction

In the contemporary literature there is little doubt that macrophytes are rarely consumed alive and most of their annual production enters the detrital pathway upon senescence and death (Wetzel, c1983; Esteves, 1988). Because herbivory loss values range from about 0.5 to 8 % of the total production, many reports have suggested that herbivory is relatively unimportant in aquatic systems (Wetzel, c1983). However, there are few field studies that provide quantification of herbivore damage (Newman, 1991) and most of the recent reports have been laboratory studies of potential biological control agents.

¹ Contribution of the Projects PIP 4242/96 and PIP 0815/98 of the National Council of Scientific and Technical Research (Argentina).

High densities of invertebrates, including oligochaetes, mites, snails and insects, were reported in aquatic macrophytes in northeastern Argentina where between 3,600 and 160,000 ind per m² were present (Neiff; Poi de Neiff, 1978; Poi de Neiff; Bruquetas, 1983). Nine major groups of invertebrates were found associated with free-floating plants, encompassing over 40 families and over 78 species (Poi de Neiff; Carignan, 1997). Studies carried out to date have revealed that collector-gatherers and predators have been the most abundant feeding groups, while shredders have been fewer in number and 23 species of invertebrates (Table 1) have fed on 13 macrophyte species in northeastern Argentina.

Eichhornia crassipes has been classified in first place among the main native weeds for the investigation of biological control possibilities (DeLoach; Cordo; Crouzel, 1989). It is spreading at an alarming rate in Africa, South East Asia, Australia and North America (Julien *et al.*, 1996). Therefore, in this chapter we emphasize the most frequent natural enemies of *E. crassipes*.

On the Paraná River floodplain, many floodplain lakes are dominated by *Eichhornia crassipes*, which may cover 30-100% of the water surface. This plant shows optimum growth in warm turbid waters (~30°C) and can colonize large areas within a few months by means of vegetative propagation (Neiff; Poi de Neiff, 1978).

Eichhornia crassipes production shows a pronounced seasonality. Biomass increases from August to March and declines to about 50% of its annual maximum during the cool season (May to July). Average monthly biomass ranges from 8.6 to 24 t.ha⁻¹ dry weight, with more than 35% as root tissue (Neiff; Poi de Neiff, 1984). The winter decline could be ascribed to factors such as:

- a- Lower temperatures during winter (Lallana, 1980).
- b- Nitrogen limitation during the low water phase (Carignan; Neiff, 1992).
- c- Damage caused by insects during the growth period.

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Table 1. Herbivores and host plants found in northeastern Argentina

Herbivores	Host plant	Location	Source
<i>Tyloderma cupreum</i>	<i>Panicum elephantipes</i>	Riachuelo River Basin.	E
		Paraná River (Antequera)	A
<i>Ludovix fasciatum</i>	<i>Panicum elephantipes</i>	Paraná River (Antequera)	A
<i>Neochetina bruchi</i>	<i>Eichhornia crassipes</i>	Paraná River (Barranqueras).	B
	<i>Eichhornia azurea</i>	Paraná River (Antequera).	A
		Paraguay River (Concepción).	
<i>Neochetina eichhorniae</i>	<i>Eichhornia crassipes</i>	Paraná River (Barranqueras).	B
	<i>Eichhornia azurea</i>	Paraná River (Antequeras).	A
		Paraguay River (Concepción).	
<i>Neohydronomus pulchellus</i>	<i>Salvinia herzogii</i>	Paraná River (Antequeras).	A
	<i>Pistia stratiotes</i>	Paraná River (Barranqueras).	C
		Eastern Chaco cattail swamps.	D
<i>Argentinorhynchus breyeri</i>	<i>Pistia stratiotes</i>	Paraná River (Barranqueras)	C
<i>Ochetina bruchi</i>	<i>Salvinia herzogii</i> <i>Eichhornia azurea</i>	Riachuelo River Basin.	E
		Iberá swamps.	F
		Eastern Chaco cattail swamps.	D
<i>Ochetina fulvipes</i>	<i>Salvinia herzogii</i>	Ñeembucú swamp.	
<i>Cyrtobagous singularis</i>	<i>Salvinia herzogii</i>	Riachuelo River Basin.	E
		Eastern Chaco cattail swamps.	D
<i>Sphenophorus vilis</i>	<i>Cyperus giganteus</i>	Eastern Chaco cattail swamps.	D
	<i>Typha</i> spp.	Iberá swamps.	F
<i>Tanysphiroideus parvulus</i>	<i>Eichhornia azurea</i>	Eastern Chaco cattail swamps.	D
<i>Listronotus vulgaris</i>	<i>Salvinia herzogii</i>	Riachuelo River Basin.	E
<i>Onychylis cretata</i>	<i>Lemna gibba</i>	Eastern Chaco cattail swamps.	D
<i>Onychylis</i> sp.	<i>Ludwigia peploides</i>	Riachuelo River Basin.	E
<i>Ilodytes lembulus</i>	<i>Egeria najas</i>	Riachuelo River Basin.	G
<i>Ilodytes lintriculus</i>	<i>Egeria najas</i>	Iberá swamps.	F
	<i>Cabomba australis</i>	Iberá swamps.	F
<i>Hydrotimetes natans</i>	<i>Egeria najas</i>	Iberá swamps.	F
	<i>Cabomba australis</i>	Iberá swamps.	F
	<i>Potamogeton</i> sp.	Iberá swamps.	F
<i>Stenopelmus minutus</i>	<i>Salvinia herzogii</i>	Eastern Chaco cattail swamps.	D
	<i>Eichhornia azurea</i>	Eastern Chaco cattail swamps.	D
	<i>Lemna gibba</i>	Eastern Chaco cattail swamps.	D
<i>Stenopelmus brunneus</i>	<i>Salvinia herzogii</i>	Riachuelo River Basin.	E
<i>Lyxellus</i> sp.	<i>Hydrocotyle ranunculoides</i>	Guaycurú and Salado rivers.	H
<i>Paulinia acuminata</i>	<i>Salvinia herzogii</i>	Paraná River (Antequeras)	A
	<i>Azolla caroliniana</i>	Riachuelo River Basin	I
		Iberá swamps	F
		Eastern Chaco cattail swamps.	D
<i>Cornops aquaticum</i>	<i>Eichhornia crassipes</i>	Paraná River (Antequeras)	A
<i>Omalonyx unguis</i>	<i>Eichhornia crassipes</i> <i>Salvinia herzogii</i>	Paraná River (Barranqueras)	B
		Paraná River (Antequeras)	A
		Paraná River (Barranqueras)	B
		Eastern Chaco cattail swamps.	D
		Riachuelo River Basin	E

A- Casco and Poi de Neiff, 1998; B- Poi de Neiff, Neiff and Bonetto, 1977; C- Neiff and Poi de Neiff, 1978; D- Bruquetas de Zozaya., 1986a; E- Bruquetas de Zozaya, 1986b; F- Poi de Neiff, 1992; G- Poi de Neiff, 1979; H- Poi de Neiff, 1990; I- Bonetto *et al.*, 1978

In relation to the last hypothesis, we studied the effect caused by herbivorous insects on *E. crassipes* populations. For this purpose, the results obtained in bodies of water, where plants presented differences in vigor (proportion of dry leaves and nutritional quality) and density (number of leaves per area), were compared.

Material and methods

The studies were conducted between March 1997 and March 1998 in two natural lakes (Site A, 27°27'S, 58°55'W and Site B, 27°26'S, 58°51'W) located on the Paraná River floodplain. Samplings in an artificial reservoir (Site C, 27°28'S, 58°44'W) free of the hydrosedimentological fluctuations of the river were carried out simultaneously (Figure 1).

The studied area has hot summers and mild winters. During the sampling period, maximum air temperature reached 39°C in January 1997 and there were unusually high values in the spring of the same year (40°C in October). Lowest minimum temperatures (near 0°C) were recorded between June and August. There were thermic variations in these months, with maximum temperatures exceeding 30°C.

Sites A and B are shallow (0.40 to 2 m deep), small (200 x 2,000 m) and densely vegetated by monospecific formations of *E. crassipes*. At site A, plant coverage is close to 100% of the water surface and the root system can reach the bottom of the lake in low water periods. At site B, coverage by *E. crassipes* is higher than 70%. Connection with the river occurs various times per year, when the water level measured at the Corrientes Port exceeds 4.20 m (Site B) or 4.80 m (Site A).

Site C is an artificial environment filled with water coming from Site A. *E. crassipes* plants were placed in such a manner to cover 70 % of the water surface.

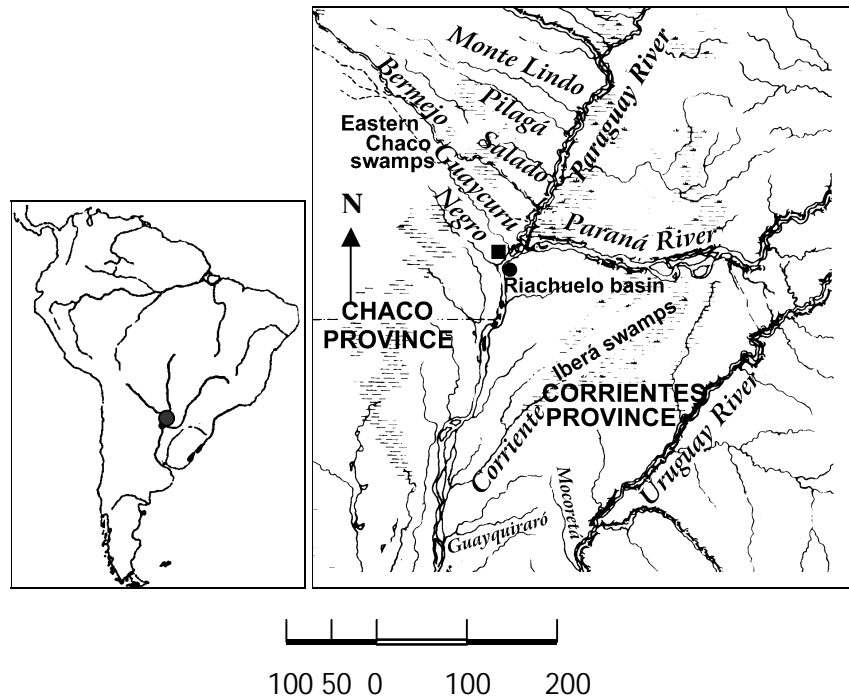


Figure 1. Location map of northeastern Argentina showing the environments mentioned in Table 1 and the sampling sites. ■ Sites A and B (right bank of the Paraná River) • Site C (left bank)

Three (3) samples were taken monthly at each site at a distance of 5 m from the edge, along a transect with a ring of 3,000 cm². Leaf density per m² and leaf length from the petiole base to the end of the leaf sheet were estimated. Density estimations referred to the number of leaves and not the number of plants, due to the high variability in the number of leaves of each plant. The vigor of *E. crassipes* populations was evaluated by counting the total number of leaves per m² and the percentage of green, standing dead, and wilted leaves.

Subsamples were taken at the end of the maximum growth period (March) to determine the nutritional quality of the leaves. They were dried

at 60°C and chemically analyzed by the Laboratory of Animal Nutrition (Catholic University of Buenos Aires) using standardized techniques.

The number of leaves damaged by both species of *Neochetina* (*bruchii* + *eichhorniae*) and by an undetermined species of *Thrypticus* was quantified.

The number of *Thrypticus* tunnels per leaf and the length of the affected area in the petiole were also measured. The damage index was obtained as:

$$DI = \frac{\text{Number of attacked leaves} \times \text{number of tunnels per leaf}}{\text{number of total leaves}}$$

The significance of the comparisons was tested using an analysis of variance (ANOVA) of all normalized data (log transformed). The data, expressed as percentages, were transformed using the arcsine square root procedure (Zar, 1984).

The correlation between the number of leaves per m² and the number of *Thrypticus* tunnels per leaf was checked using the Spearman coefficient (Steel; Torrie, 1985).

Results

The mean monthly leaf densities (green, standing dead and wilted leaves) are represented in Figure 2. The average annual leaf densities, calculated from total density per m², were 487 (Site C), 319 (Site A) and 295 (Site B). When the ANOVA was used to compare the three sites, significant differences were found in the number of standing dead leaves ($F_{2,115} = 85.3$, $P < 0.01$); however, the number of green leaves per m² was similar ($F_{2,115} = 1.79$, $P > 0.05$). The floodplain lakes were colonized by more vigorous plants than the reservoir. Due to the "El Niño" effect during the study period, there were frequent nutrient inputs into the lakes during the floods of the Paraná River, and exceptionally high temperature

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recordings in the winter and the spring. At Site C, plant growth was limited by the nutrient availability in and the size of the reservoir (Neiff *et al.*, 2001).

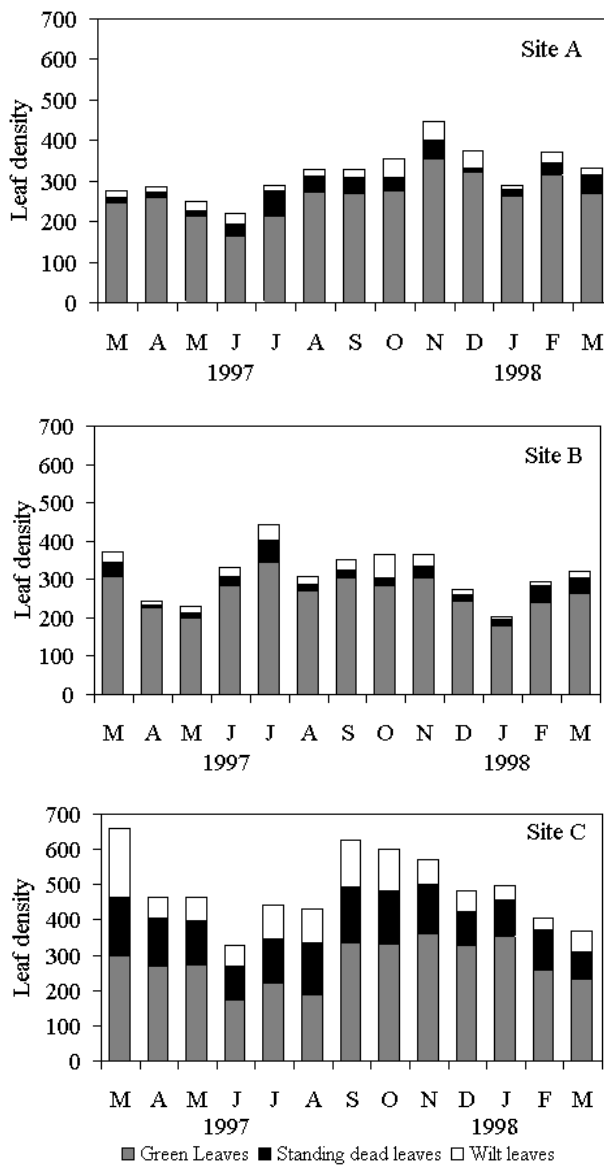


Figure 2. Variation in the number of green, standing dead and wilted leaves during sampling at the three studied sites. The sum of the three divisions corresponds to the total number of leaves per m²

The proportion of nitrogen, gross protein and lignin of the leaves was similar at the three studied sites (Table 2). At Site C, the leaves showed a lower content of total phosphorus. According to Gerloff (1970), the mean value found (0.13%, expressed as dry weight) at this site is within the quantity range of phosphorus needed to produce growth in submerged plants.

Table 2. Elemental concentrations of nitrogen, phosphorus and lignin, expressed as dry weight of *E. crassipes* green leaves in March 1998

%	Site A	Site B	Site C
Total nitrogen	1.58 ± 0.03	1.34 ± 0.01	1.44 ± 0.04
Gross protein	9.94 ± 0.2	8.40 ± 0.11	9.02 ± 0.2
Total phosphorus	0.27 ± 0.003	0.24 ± 0.02	0.13 ± 0.005
Acid detergent fiber	29.6 ± 0.35	28.8 ± 0.7	27.0 ± 0.3
Lignin	1.04 ± 0.12	0.96 ± 0.06	0.91 ± 0.14

Damage produced by Thrypticus sp. (Diptera, Dolichopodidae)

Thrypticus larvae of an undetermined species, which are only a few mm long, bore small tunnels in the base of the petioles of *Eichhornia crassipes*. The tunnels, situated within the aerenchyma tissues, are open at either end. Attacked leaves are recognized by two holes 4-10 mm apart. Each larva occupies a tunnel and moves in both directions to feed. In a cross section, the aerenchyma appears dark because of the decay of neighboring cells.

The number of *Thrypticus* tunnels per leaf was comparatively low at Site C (Figure 3), with a maximum of 8 tunnels per leaf. When all values were compared, difference between sites was significant ($F_{8,103} = 4.59$ $P < 0.05$).

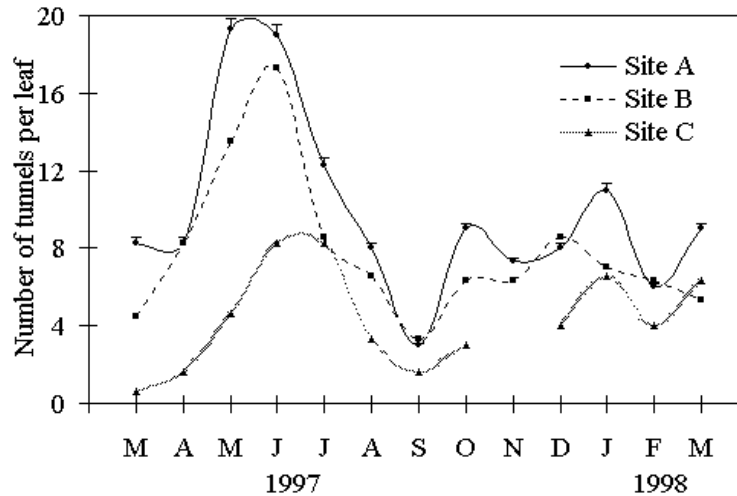


Figure 3. Variations in the number of tunnels per leaf produced by *Thrypticus* sp larvae

From the analysis of all samples in the three environments ($n= 116$), a negative correlation ($r = -0.70$) was found between the number of plants per m^2 and the number of *Thrypticus* tunnels per leaf. The degree of adjustment was not higher since the number of leaves per m^2 was low in September, and the green leaves that were beginning a new seasonal period had a scarce quantity of tunnels.

Proportionally, the largest quantity of attacked leaves (Table 3) was recorded in January (Sites A and B) and March (Site C) 1998. There was significant difference in the percentages of attacked leaves at the three sites ($F_{2,114} = 11.85$, $P < 0.01$).

If we consider the damage index (Table 3), the relationships between sites are maintained, although most attacked leaves correspond to May (Site A) or June (Sites B and C). This is due to the fact that only the presence and absence of attack is considered in the percentages; in contrast, the damage index shows its magnitude when including the number of tunnels per leaf.

Table 3. Mean percentage and damage index (DI) of petioles attacked by *Thrypticus* sp. in the studied environments

	Site A		Site B		Site C	
	Percentage	DI	Percentage	DI	Percentage	DI
March-97	42.4	3.5	25.1	1.1	1.8	0.01
April-97	44.2	3.6	41.8	3.4	10.2	0.17
May-97	48.0	9.2	34.5	4.6	21.3	0.99
June-97	32.5	6.1	33.8	5.8	28.8	2.4
July-97	19.3	2.3	11.3	0.9	23.3	1.9
August-97	19.3	1.7	16.4	1.1	9.03	0.30
September-97	17.9	0.5	16.2	0.5	4.3	0.07
October-97	42.0	3.7	35.2	2.2	9.6	0.29
November-97	29.9	2.2	37.9	2.4	-	-
December-97	54.9	4.3	50.8	4.4	21.5	0.86
January-98	58.7	6.4	53.0	3.7	35.1	2.3
February-98	43.4	2.6	47.7	3.0	21.3	0.85
March-98	49.7	4.4	46.6	2.5	40.0	2.5

Knowing the number of tunnels per leaf and the number of leaves attacked by *Thrypticus* per m², we were able to estimate the number of tunnels per m². As is shown in Figure 4, the highest mean value was recorded in the lakes (Sites A and B), where there were up to 2,200 tunnels per m², while at Site C there were no more than 1,200 tunnels per m².

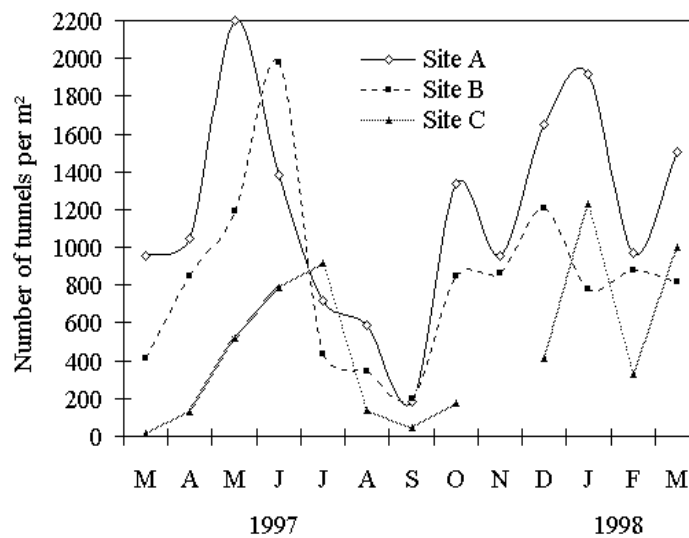


Figure 4. Comparison of number of tunnels per m² at the three studied sites

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The mean height of the attacked leaves during an annual cycle (March 1997-March 1998) was always higher than the mean height of all leaves (Figure 5). The selection of microhabitats was determined correlating the ratio of the height of the attacked leaves to the height of all leaves with the mean height of the leaves at each site. For this purpose, the mean heights of the three replicates ($n= 200$) were considered (March 1997-March 1998), which implied the analysis of 2,600 petioles per site.

The ratio had a higher fit at sites A and B ($R^2= 0.7643$ and 0.8724 , respectively) than at Site C, where a larger dispersion of the mean data was observed. The absence of selection towards the highest leaves would result in a horizontal line at $y = 1.0$ (Figure 5).

The area affected by the larvae of *Thrypticus* extends from the base of the petiole (close to the water surface) to a mean height that varies according to the sampling site and the time of year (Table 4). Attack height at sites A and B varied between 4 and 12 cm and was lower at Site C. These values represent up to a maximum of 14% of the total length of the attacked leaves.

Table 4. Mean height (cm) of the area affected by *Thrypticus* sp. in the petiole of *Eichhornia crassipes*

	Site A	Site B	Site C
March-97	7.0 ± 4	2.6 ± 1.9	-
April-97	11.7 ± 4.4	10.7 ± 4.1	6.9 ± 2.3
May-97	10.0 ± 4.4	10.4 ± 5	5.6 ± 3.9
June-97	10.6 ± 4.5	12.2 ± 5	7.0 ± 4.4
July-97	10.4 ± 5.7	8.6 ± 4.9	9.3 ± 8.3
August-97	8.2 ± 5.1	6.9 ± 5.2	6.2 ± 4.5
September-97	4.0 ± 3.8	4.2 ± 3.3	1.6 ± 1.8
October-97	7.5 ± 4.5	6.0 ± 3.2	3.6 ± 3.2

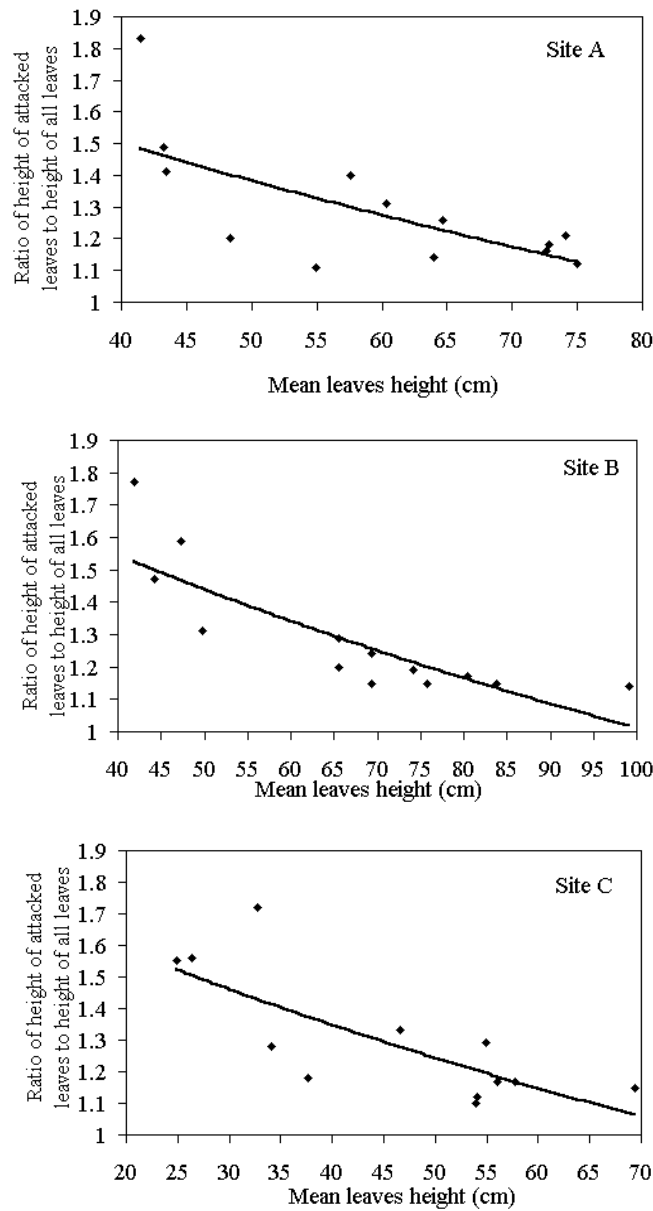


Figure 5. Interrelationship between the height ratio of attacked leaves to the height of all leaves and mean height of leaves. Exponential curve fitting: $R^2 = 0.5064$ (Site A), $R^2 = 0.7201$ (Site B), $R^2 = 0.5995$ (Site C)

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At the three sites, a lower extension of the affected area was observed in August and September (Table 4). In the latter month, the percentage, in relation to the height of the attacked leaves, varied between 4 (Site C) and 6% (Sites A and B).

Damage produced by Neochetina bruchi and N. eichhorniae (Coleoptera, Curculionidae)

In the floodplain lakes, the adult populations of *N. bruchi* and *N. eichhorniae* together reached a peak of 120 individuals per m² (Casco; Poi de Neiff, 1998) in the fall. They cause feeding spots on leaves and petioles by removing the epidermal layer. Spots made by *N. bruchi* or *N. eichhorniae* are indistinguishable from each other. Both species produced damage throughout the year, affecting 81 to 98 % of the total leaf number (Table 5). The number of leaves attacked by *Neochetina* spp. was significantly higher in the floodplain lakes (Sites A and B) than in the reservoir (Site C) ($F_{2,112} = 4.04$, $P < 0.01$). At the latter site, the mean percentage of damaged leaves varied between 63 and 96 % (Table 5), and the number of adults per m² was comparatively low, with a maximum value of 47 individuals per m² in March 1997 (Figure 6).

Table 5. Mean percentage of leaves attacked by *Neochetina* spp. at the studied sites

	Site A	Site B	Site C
March-97	91.6 ± 5.91	97.95 ± 1.26	91.74 ± 0.23
April-97	92.99 ± 1.83	92.99 ± 1.83	95.98 ± 1.27
May-97	90.05 ± 2.3	91.19 ± 0.05	88.91 ± 1.01
June-97	86.89 ± 6.14	86.44 ± 2.41	93 ± 3.12
July-97	84.78 ± 5.16	81.22 ± 4.49	85.19 ± 3.14
August-97	93.93 ± 1.61	86.41 ± 1.48	63.44 ± 5.5
September-97	90.51 ± 2.07	81.47 ± 3.33	83.28 ± 1.94
October-97	89.25 ± 0.95	89.25 ± 0.95	78.95 ± 3.98
November-97	87.71 ± 2.48	89.3 ± 1.12	88.45 ± 4.68
December-97	89.73 ± 3.31	87.54 ± 4.25	86.79 ± 4.83
January-98	89.29 ± 0.85	93.77 ± 1.95	88.15 ± 2.87
February-98	86.04 ± 0.73	86.21 ± 1.95	86.21 ± 1.95
March-98	91.34 ± 2.73	92.1 ± 2.32	86.51 ± 2.9

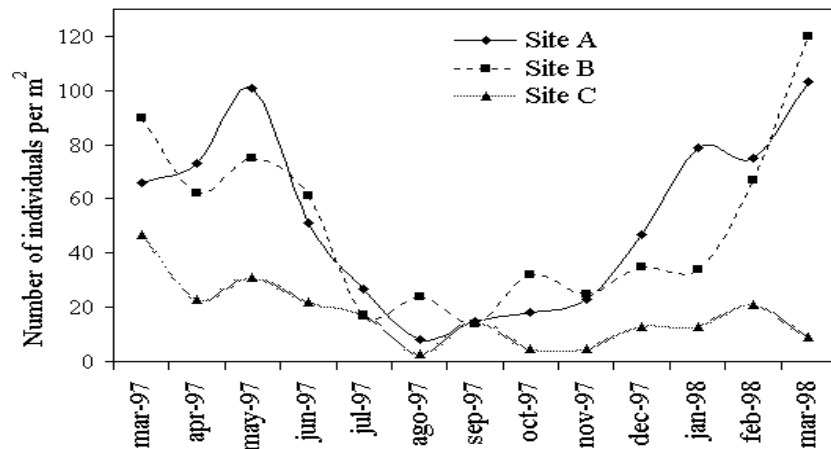


Figure 6. Variations in the density of *Neochetina* spp. at the three studied sites

Discussion

As a result of this study, it can be stated that the combined effects of two species of *Neochetina* and an undetermined species of *Thrypticus* cause extensive damage in *Eichhornia crassipes* populations in natural floodplain lakes during the high water phase of the Paraná River. However, no significant reduction in water hyacinth lake surface coverage was observed, and the proportion of wilted leaves was low.

The *Neochetina* species produce damage throughout the year, although in the reservoir the percentage of attacked leaves decreased in August. From the comparison of sites, a higher density of *Neochetina* spp. (than in the reservoir) was observed in the natural lakes, linked to the hydrodynamics of the Paraná River. In cases where there was a high population level (up to 80 ind.m⁻²), an average of 300 feeding spots were found on each leaf (Casco; Poi de Neiff, 1998). The populations of *E. crassipes* in the lagoons showed a higher vigor, verified by the quantity of

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green leaves and their nutrient content. Several authors (Room; Julien; Forno, 1989; Julien *et al.*, 1996; Center; Dray, 1992) found that diverse phytophagores prefer high vigor plants. The nutrient content of plants has been pointed out as one of the main plant characteristics that are vitally important to herbivores (Mattson, 1980). In this study, the three sites showed similar nitrogen and lignin concentrations in the leaves of *E. crassipes*, but the total phosphorus content of the green leaves in the artificial reservoir showed very low values, which would indicate the scarcity of this nutrient in the water. Regrettably, the major effect of the floods on the nitrogen content of the green leaves was detected too late (TN=2.5% in August 1998), when the extended flood regimes ended (Neiff; Poi de Neiff; Casco, 2001).

The damage index of petioles attacked by *Thrypticus* sp. was low in September, when the annual outbreak of the host population began. Independent of sampling site and the degree of attack, there was a microhabitat selection by *Thrypticus* sp. This insect prefers large-sized leaves (more mature) and the petiole base, up to a height lower than 14% of the total height of the leaves. There is a density dependent effect on the leaves per area in the selection of sites, since an inverse relationship with the tunnel abundance was found.

During the studied period, we found that the water hyacinth population did not have grasshopper damage. However, field observation indicated that *Cornops aquaticum* (Acrididae) is present at the study sites during low water (Poi de Neiff; Bruquetas, 1983). Approximately 12 individuals per m² were found in the densest field population (Poi de Neiff; Neiff; Bonetto, 1977) in Barranqueras Lake (Chaco Province). Details of the biology of this species have been given by Zolessi (1956) and Silveira Guido and Perkins (1975). *Sameodes albigutallis* (Lepidoptera, Pyralidae) was occasionally recorded in the studied floodplain lakes, where the elongated growth form of *E. crassipes* is dominant. According to

Julien *et al.* (1996), the larvae prefer the smaller bulbous leaf form, which is characteristic of water hyacinths invading open water.

The highest population density of *Neochetina* spp., the highest number of *Thrypticus* tunnels per leaf and the highest damage index were recorded in the fall. According to Neiff and Poi de Neiff (1984), during this period the decay in the annual biomass curve of *E. crassipes* is pronounced. *E. crassipes* detritus accumulation averaged 5.1 g dry wt m⁻²day⁻¹ (Poi de Neiff; Solís De Chiozza, 1994), with the maximum dry weight of organic input in March and the minimum in August. Therefore, invertebrate damage appears to account for the seasonal decay of the plants at the study sites and herbivores would have an even greater effect on detrital inputs than the consumption estimates suggest. Unfortunately, production and detritus accumulation experiments without herbivores cannot easily be reproduced at our study sites, where natural enemies laid eggs within plants that propagate by vegetative reproduction. In similar experiments in Florida (Moorhead; Reddy; Graetz, 1988), where *E. crassipes* became a serious problem due to its great reproductive rate and lack of effective natural enemies, detritus accumulation occurred at a relatively constant rate throughout the year. These results corroborate ours and indicate the great potential in using these natural enemies in *E. crassipes* biological control programs.

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