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### The Oxycaryum cubense floating mat as refuge of *Cornops aquaticum* (Orthoptera: Acrididae), a new control agent of the water hyacinth

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## ORIGINAL ARTICLE

### The *Oxycaryum cubense* floating mat as refuge of *Cornops aquaticum* (Orthoptera: Acrididae), a new control agent of the water hyacinth

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*Cornops aquaticum* is a native Neotropical grasshopper frequently associated with *Eichhornia* (Pontederiaceae) spp. The aims of this research are: (1) to report the presence of this grasshopper on *Oxycaryum cubense* (Cyperaceae) in relation to climate conditions and microhabitat structure; and (2) to study and compare survivorship and occurrence of feeding and oviposition of *C. aquaticum* on *O. cubense* and the host plant (*E. azurea*). Grasshopper abundance was recorded during all seasons in both plants; field observations and no-choice tests were conducted to analyze the survivorship, feeding and oviposition of *C. aquaticum* on both plants. Results show that *C. aquaticum* is not able to feed and oviposit on *O. cubense*, but rather uses this macrophyte as refuge under specific climate conditions.

**Keywords:** *Eichhornia*; Cyperaceae; grasshopper abundance; host specificity; microhabitat selection

#### Introduction

*Cornops aquaticum* Bruner (1906) is a semi-aquatic grasshopper widely distributed in the Neotropics, from South-eastern Mexico to the South of the Buenos Aires Province, Argentina (Lhano 2006; Adis et al. 2007). The species lives in close association with Pontederiaceae floating meadows, especially the genus *Eichhornia* Kunth (1842) (Center et al. 2002). The water hyacinth is the most typical macrophyte of floating meadows in large rivers in South America (Blanco Belmonte et al. 1998) and is considered the worst pest of natural and artificial water bodies in tropical latitudes around the world (Waterhouse & Norris 1987; Julien et al. 2001; Center et al. 2002). *Cornops aquaticum* is being considered in South Africa as a biological control agent of the water hyacinth, *E. crassipes* (Bownes et al. 2010a, 2010b) because laboratory and field studies on *C. aquaticum* have demonstrated a high host plant specificity to *E. azurea* and *E. crassipes* (Zolessi 1956; Silveira Guido & Perkins 1975; Cordo 1999; Hill & Oberholzer 2000; Ferreira & Vasconcellos-Neto 2001; Oberholzer & Hill 2001; Adis & Junk 2003; Vieira & Santos 2003; Lhano et al. 2005; Franceschini et al. 2011).

Franceschini et al. (2007, 2008) showed that populations of *C. aquaticum* in North-eastern Argentina, in two wetlands with different host plants and

connectivity (the El Puente wetland, which is occasionally connected to the Paraná River during the low water phase, where *E. crassipes* is the host plant; and the Pampín wetland, an isolated lake in which *E. azurea* is found as a host plant), include adults during all months while nymphs are only absent in the coldest months. According to Franceschini (2008) the abundance of instar I nymphs, females with mature ovaries and open valves of the ovipositor indicates that reproduction of this grasshopper takes place during spring and summer (September to February). In the Pantanal of Mato Grosso, a *C. aquaticum* population living in *E. azurea* showed continual reproduction (Silva et al. 2010).

Egg-pods of *C. aquaticum* are laid inside the aerenchyma tissue of *Eichhornia* and *Pontederia* plants (Zolessi 1956; Silveira Guido & Perkins 1975), and nymphs and imagines feed on them (Carbonell 1981) suggesting a high specificity to this microhabitat. However, it has been observed, in a North-eastern Argentinean wetland, that a *C. aquaticum* population living on an *E. azurea* floating meadow also tends to be present on another macrophyte, *Oxycaryum cubense* (Poepp. & Kunth) Lye var. *paraguayense* (Maury) Pedersen (Cyperaceae).

Therefore, the aims of this paper are: (1) to report the presence of the highly host-specific *C. aquaticum*

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†In memoriam

on a non-host plant in relation to climate conditions and microhabitat structure; and (2) to study survivorship and occurrence of feeding and oviposition of *C. aquaticum* on *O. cubense* and *E. azurea* under natural conditions and in outdoor no-choice cage experiments. The hypothesis is that *C. aquaticum* is not able to feed and oviposit on *O. cubense* under natural conditions and that it only uses this macrophyte as an alternative microhabitat under specific climate conditions.

## Material and methods

### Study site

The Pampín Lake (27° 28' 54" S, 58° 44' 58" W) is located in North-eastern Argentina near the city of Corrientes, in the Riachuelo River basin (a tributary of the Paraná River). This wetland is small (78.2 ha), shallow (1–2 m deep) and surrounded by mixed patches of rainforest and native grassland. *Eichhornia azurea* was found coexisting with adjacent stands of *O. cubense* (Figure 1). *Oxycaryum cubense*

colonizes *Eichhornia* floating meadows as an epiphyte and, after becoming established, increases its density and biomass enormously, causing the death of floating plants and creating a lax soil of considerable thickness (Neiff 1982). As a result, both plants grow as adjacent vegetation stands with well-defined boundaries. Because the Pampín Lake is not connected with the Riachuelo River, the water level basically depends on the seasonal rain regime (1200–1400 mm/year), with rainy periods during the summer (December to February) and autumn (March to May). The climate of this area is classified as subtropical; summers are warm and prolonged, while winters are shorter and mild, with occasional frost days (frequency of 0.25 days/year) and temperatures not dropping lower than  $-5^{\circ}\text{C}$  (Bruniard 1981, 1996).

### Assessing the abundance of *C. aquaticum* on *O. cubense* and *E. azurea*

To assess choice of alternative microhabitat, between August 2004 and February 2006, monthly samplings



Figure 1. *Eichhornia azurea* floating meadow (in the foreground) and *Oxycaryum cubense* (in the background) floating mat at the Pampín Lake, North-eastern Argentina.

and field observations were carried out from 9:00 to 13:00 h, avoiding cloudy days. Abundance of *C. aquaticum* on *E. azurea* and *O. cubense* stands was recorded from a boat using a sweep net with a diameter of 70 cm and a depth of 1 m. Following Johnson (1989), a preset number of grasshoppers (50 individuals) was captured, registering the required time. When low abundance of grasshoppers prevented the capture of the preset number (August and September 2004), we swept the vegetation for 30 min. We used these sampling criteria, established by the "Host Insect Coevolution on Waterhyacinth Project" (Adis et al. 2004; Brede et al. 2007), to allow comparisons among *C. aquaticum* populations from other sites in South America. Abundance of nymphs and adults was expressed as the number of individuals captured per minute. Climate variables were recorded from August 2004 to February 2006 with a LiCor (LI-1200S) data logger (monthly mean of maximum temperature, monthly mean temperature, monthly mean of minimum temperature, monthly absolute maximum temperature, monthly absolute minimum temperature, difference between monthly mean of maximum and minimum temperatures and precipitation, insolation) and the weather gauge of the Colonia Benitez INTA (photoperiod, wind speed and cloudiness).

#### **Determination of vegetation parameters**

To describe the structure of the plant microhabitat, three samples of leaves were collected from inside an aluminum ring with a diameter of 30 cm in both the *O. cubense* floating mat and the *E. azurea* floating meadow. Because *C. aquaticum* is a herbivore of leaves above the waterline, submerged leaves of *E. azurea* were not included in the sampling. The leaf density and biomass and Leaf Area Index (LAI: the ratio of total upper leaf surface of vegetation divided by the surface area of the land on which the vegetation grows) were estimated following Vollenweider (1974) and Neiff et al. (2008). To calculate biomass, leaves were dried at 105°C. The above-water height of leaves for *E. azurea* and *O. cubense* was obtained by measuring 15 mature leaves per species from the waterline to the apex of the leaf. Coverage of *O. cubense* and *E. azurea* was estimated using plots of 1 m<sup>2</sup> in 4 transects (Muller-Dombois & Ellenberg 1974).

#### **Assessing survivorship, feeding and oviposition on *O. cubense* and *E. azurea***

In order to evaluate the acceptance of *O. cubense* and *E. azurea* by *C. aquaticum*, survivorship and occurrence of feeding and oviposition on both species were assessed. Experiments were performed during

summer in a screened outdoor enclosure under the influence of the local climate but without direct sun exposure following the protocol defined by "Host Insect Coevolution on Waterhyacinth Project". No-choice tests were conducted with four classes of the *C. aquaticum* population: nymphs A (instar I and II), nymphs B (instar III to VI), adult females and adult males. Cages of 50 × 50 × 50 cm containing 10 individuals from the same class were set up. Inside the cages, fresh growing plants of either *O. cubense* or *E. azurea* from the field without damage and ovipositions were maintained with water in plastic containers (43 × 30 × 11.5 cm). Leaf density within the containers was similar to the leaf density observed in the field. Damaged plants were replaced by fresh ones every three days in order to maintain food in optimal condition during the experiments. Three replications were performed for each experimental set up (12 cages and 120 grasshoppers for each plant species). The sex ratio for nymphs B was 1:1, but it was not determined for nymphs A.

The survival of individuals and the feeding on plant tissues was assessed every three days for a period of 12 days, in accordance with Lhano et al. (2005). At the end of the experiment, plants offered to adult females were carefully dissected and checked for the presence of eggs. The females were also dissected to confirm the presence of mature ovaries.

In addition, *O. cubense* and *E. azurea* plants from the lake were checked to determine whether individuals of *C. aquaticum* were capable of feeding and oviposition in these macrophytes under natural conditions. Feeding of *C. aquaticum* in the field was measured with the visual estimation method by overlapping each leaf with a clear grid and counting the number of grid cells covering the damage (Cronin et al. 1998).

#### **Statistical analysis**

Principal Component Analysis (PCA) was used to test the relationship between the abundance of *C. aquaticum* on both plant microhabitats and climate variables. Kruskal Wallis Test was used to analyze differences in climate variables between months in which *C. aquaticum* was more abundant on *E. azurea* and months in which it was more abundant on *O. cubense*. The structure of both plant microhabitats was assessed by analyzing differences in plant biomass, leaf density and above-water height of leaves with the Kruskal Wallis Test. The survivorship of *C. aquaticum* individuals feeding on *E. azurea* and *O. cubense* in no-choice experiments was analyzed with the Chi-square test. Values of  $p < 0.05$  were considered significant. Statistical analyses were performed using Infostat Software (version 1.1; Infostat 2002).

## Results

### Abundance of *C. aquaticum* on *O. cubense* and *E. azurea*

During spring and summer, the population of *C. aquaticum* was living on *E. azurea* floating meadows. As the cold months arrived, most adults left the host plant, the typical microhabitat, to live inside the adjacent *O. cubense* floating mat. The majority of nymphs continued living on *E. azurea* until their development was complete. Abundance of *C. aquaticum* was highest on *E. azurea* when temperatures were high (spring and summer: November 2004, February and March 2005, and from October to December 2005). *Cornops aquaticum* was most abundant on *O. cubense* when average temperatures were low. The highest abundances registered were 96 ind. captured/min in December 2005 on *E. azurea* and 15.7 ind. captured/min in April 2005 on *O. cubense* (Figure 2).

The PCA showed that the abundance of *C. aquaticum* individuals on *O. cubense* and *E. azurea* was related to climate variables of the study site. The first component, which explained 58% of the variation, clearly separated the months in which *C. aquaticum* was more abundant on *O. cubense* and months in which grasshoppers were more abundant on *E. azurea* (Figure 3). Higher abundance of *C. aquaticum* on *O. cubense* was related to high values of cloudiness and wind speed. On the other hand, higher abundance of grasshoppers on the *E. azurea* was related to high temperatures and high values of insolation and precipitation, and a photoperiod of 11.4–14.7 h light, typical spring and summer conditions.

Significant differences were found comparing the climate variables of months in which *C. aquaticum* was more abundant on *O. cubense* with those of

months in which it was more abundant in *E. azurea* (Kruskal Wallis Test: monthly mean of maximum temperature  $p = 0.0009$ ; monthly mean temperature  $p = 0.0013$ ; monthly mean of minimum temperature  $p = 0.0084$ ; monthly absolute maximum temperature  $p = 0.0054$ ; monthly absolute minimum temperature  $p = 0.001$ ; difference between monthly mean of maximum and minimum temperatures  $p = 0.0003$ ; insolation  $p = 0.0003$ ; photoperiod  $p = 0.0006$ ; precipitation  $p = 0.0064$ ; cloudiness  $p = 0.0021$ ; wind speed  $p = 0.0237$ ).

### Structure of the plant microhabitats

*Oxycaryum cubense* and *E. azurea* presented similar coverage during the sampling period and were the two most abundant macrophytes, occupying about 40% of the littoral zone. Because of their abundance, architecture and coverage, *E. azurea* and *O. cubense* generate different microhabitat structures. *Oxycaryum cubense* plants grow as a dense floating mat with plants reaching 25–106 cm height above the water surface ( $\bar{x} = 71.68 \pm 14.27$ ), narrow linear leaves that are densely vertically arranged. LAIs of 12–15 and densities of 1336 leaves/m<sup>2</sup> (decay period) to 2699 leaves/m<sup>2</sup> (growth period) were obtained. Leaf biomass above the water surface varied from 311 g/m<sup>2</sup> (winter) to 714 g/m<sup>2</sup> (autumn). *Eichhornia azurea* grows 15–20 cm high above the water surface. Mature leaves have round blades, sitting, in oblique or vertical direction, on long stems rooted in the banks and growing above the waterline. It had a LAI of 5 and leaf densities ranged from 141 leaves/m<sup>2</sup> (decay period) to 215 leaves/m<sup>2</sup> (growth period). Leaf biomass above the water surface varied from 18.33 g/m<sup>2</sup> (spring) to 88.3 g/m<sup>2</sup> (summer). Significant differences between both plant microhabitats were found comparing leaf

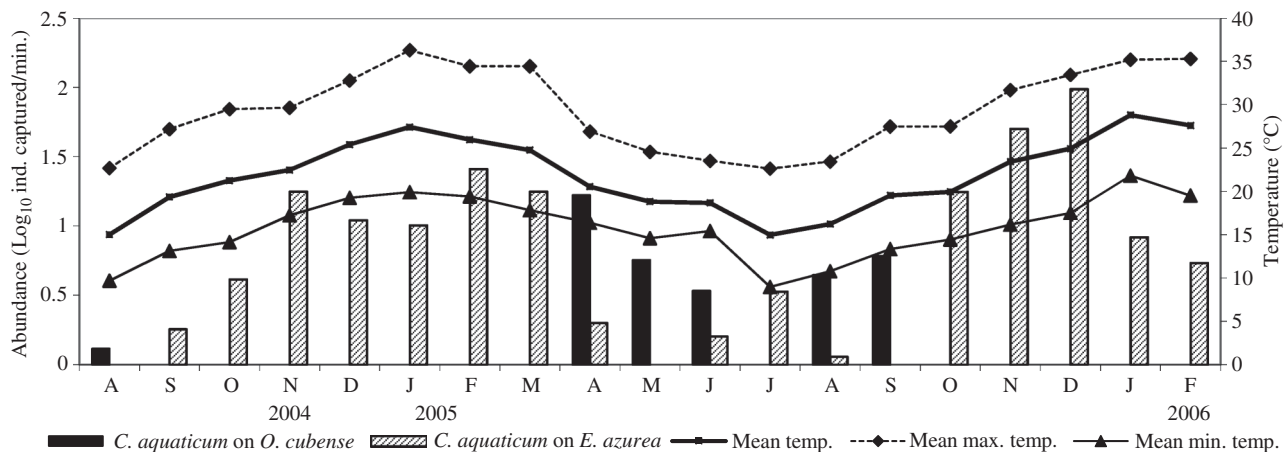


Figure 2. Monthly mean temperatures and abundance of *Cornops aquaticum* on *Oxycaryum cubense* floating mat and *Eichhornia azurea* floating meadows from August 2004 to February 2006 at Pampin wetland. Mean max. temp.: monthly mean of maximum temperature; Mean temp.: monthly mean temperature; Mean min. temp.: monthly mean of minimum temperature. Abundance is expressed as Log<sub>10</sub> of individuals captured per minute.

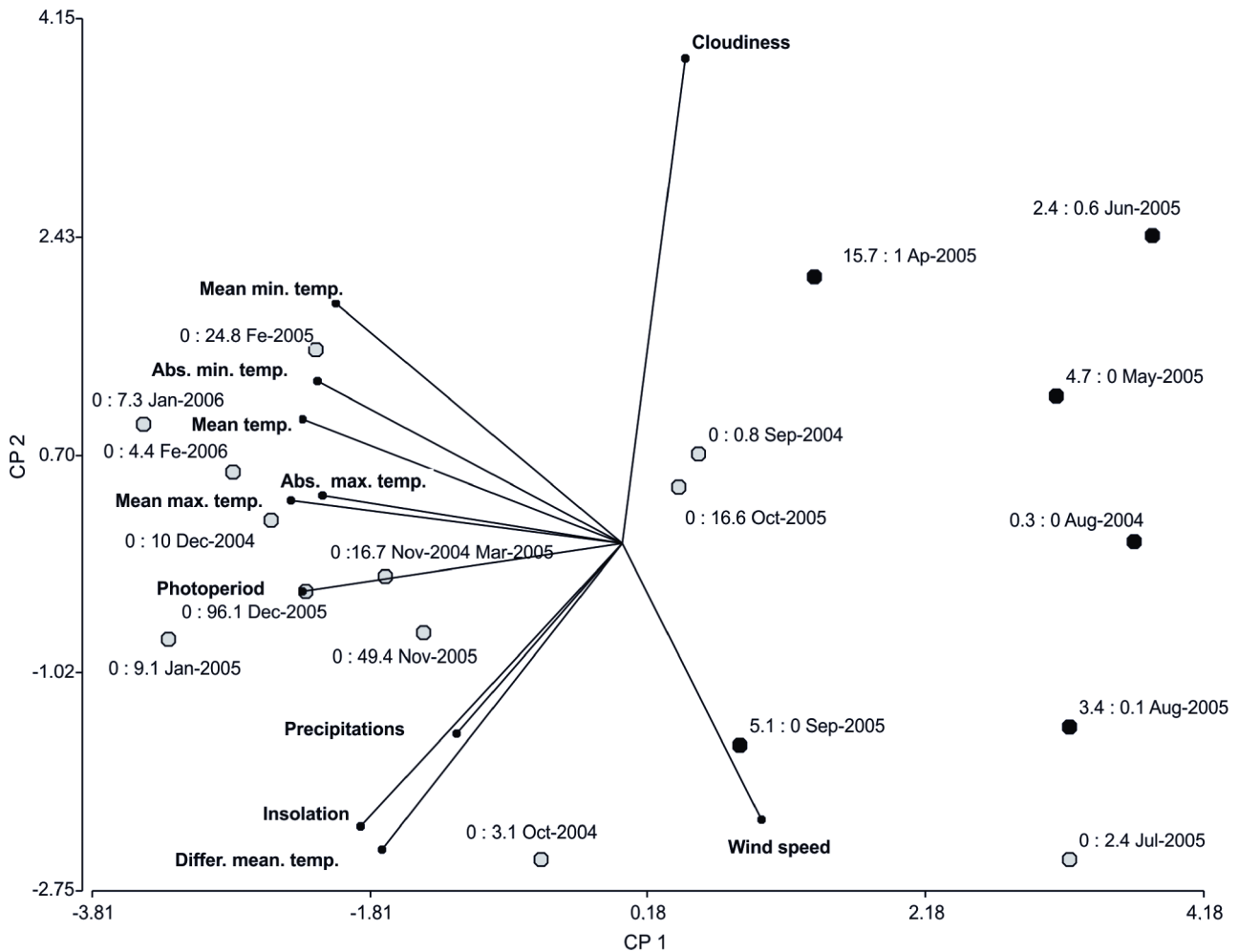


Figure 3. (Colour online) Principal Components Analysis showing months in which *Cornops aquaticum* was more abundant on *Oxycaryum cubense* (black dots) and months in which it was more abundant on *Eichhornia azurea* (gray dots). For each dot (ex. 2.4:0.6 Jun-2005), grasshopper abundance (individuals captured/min) on both plants (first: *Oxycaryum cubense*, second: *Eichhornia azurea*) and the sampling dates (from August 2004 to February 2006) are indicated. Jan: January; Fe: February; Mar: March; Ap: April; Jun: June; Jul: July; Aug: August; Sep: September; Oct: October; Nov: November; Dec: December; Mean max. temp.: monthly mean of maximum temperature; Mean temp.: monthly mean temperature; Mean min. temp.: monthly mean of minimum temperature; Abs. max. temp.: monthly absolute maximum temperature; Abs. min. temp.: monthly absolute minimum temperature; Differ. mean temp.: Difference between monthly mean of maximum and minimum temperatures.

biomass ( $p < 0.0001$ ;  $N = 24$ ), leaf density ( $p < 0.0001$ ;  $N = 24$ ) and the height of plants above the waterline ( $p < 0.0001$ ;  $N = 24$ ) with the Kruskal Wallis Test.

#### Survivorship, feeding and oviposition on *O. cubense* and *E. azurea*

Survivorship of *C. aquaticum* nymphs was higher on *E. azurea* than on *O. cubense* after 3, 6, 9 and 12 days (Figure 4). No-choice tests showed no significant differences in the final survivorship between adult females ( $X^2 = 4.3$ ,  $df = 3$ ,  $p > 0.05$ ) and adult males ( $X^2 = 0.11$ ,  $df = 3$ ,  $p > 0.05$ ). Final survivorship of nymphs on *O. cubense*

plants dropped sharply towards the end of the observation period, and was significantly different from *E. azurea* for nymphs B ( $X^2 = 6.55$ ,  $df = 3$ ,  $p < 0.05$ ) and nymphs A ( $X^2 = 27$ ,  $df = 3$ ,  $p < 0.05$ ).

Throughout the sampling periods, feeding and oviposition of *C. aquaticum* on *O. cubense* were not recorded under natural conditions. Leaf damage by nymphs and adults of *C. aquaticum* on *E. azurea* varied from 0.3% of the leaf area in winter to 16% in summer. However, feeding on *O. cubense* and *E. azurea* was recorded in the no-choice experiment for nymphs and adults of *C. aquaticum* (Figure 4), but nymphs B and adult grasshoppers caused more severe damage to *E. azurea* than to *O. cubense* plants.

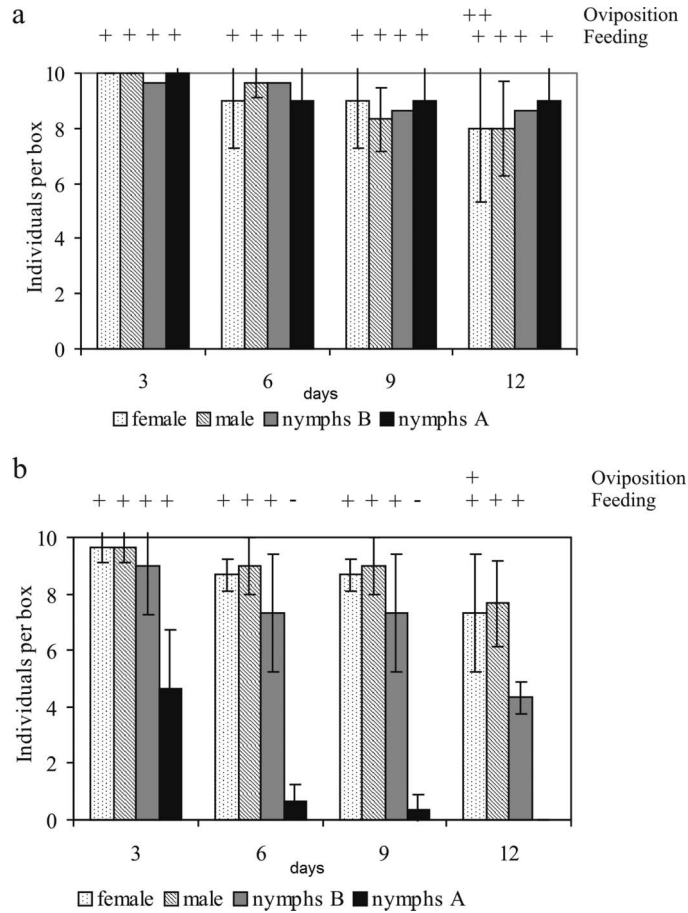


Figure 4. Occurrence of feeding and oviposition and survivorship of adults and nymphs of *Cornops aquaticum* during the no-choice cage experiments after 3, 6, 9 and 12 days on (a) *Eichhornia azurea* and (b) *Oxycaryum cubense* plants. Nymphs A: instar I and II; nymphs B: instar III to VI. Feeding: (+) occurrence of feeding (-) not feeding; oviposition: (++) endophytic (+) epiphytic.

Under natural conditions oviposition by *C. aquaticum* was recorded only inside the petioles of *E. azurea*. In the no-choice experiment oviposition took place in both plants, but only eggs laid in *E. azurea* were endophytic and viable; eggs on *O. cubense* were laid on the leaf surface and were rapidly covered by fungus. All adult females used in the experiment had mature ovaries.

**Discussion**

***Cornops aquaticum* and its alternative plant microhabitat**

Reproduction of *C. aquaticum* populations takes place during spring and summer (Franceschini et al. 2007, 2008) and high abundance of *C. aquaticum* on *E. azurea* and the absence of individuals on *O. cubense* floating mat during the reproductive period of the grasshoppers confirm the specificity of this insect

to the host plant. There is no previous information on how *C. aquaticum* behaves under adverse conditions. Our findings revealed that adults choose another plant microhabitat during the colder seasons. Differential use of microhabitats is a common strategy used by grasshoppers to avoid being exposed to adverse conditions (Willott 1997). In the locust *Locustana pardalina* (Walker 1870), adults bask on fast-drying exposed rocks and aggregate around the main stems of shrubs to increase their body temperature and avoid cool and wet conditions of the soil surface (Blanford & Thomas 2000). Grasshoppers living in temperate or arid climates prefer sheltered microhabitats over uncovered places (Chappell & Whitman 1990; Blanford & Thomas 2000). Acridids from humid tropical forest show intensive use of complementary microhabitat because the diversity of grasshoppers is high, as is the number of microhabitats that each species can use in addition to the host plants (Amédégnato 1997; 2003).

*Oxycaryum cubense* provides a more suitable refuge for grasshoppers during the cold seasons due to its architecture, specifically the vertical position, the high biomass and density of leaves with high LAI value, and its greater height above water in comparison with *E. azurea*. A similar architecture to that of *O. cubense* is found in plants of prairies. In fact, inside such vegetation, extreme temperatures and wind speed are considerably reduced, whereas levels of relative humidity are increased (Edwards & Wratten 1981). According to Chappell & Whitman (1990), heat loss in grasshoppers occurs primarily as convective exchange with the air surrounding the animal and is minimal inside dense vegetation because wind speed is low there. Wind speed would be also lower inside the *O. cubense* mats than inside the *E. azurea* meadows because leaf density is noticeably higher in the former. The relative abundance of plants can affect the selection of microhabitats by insects, and a very abundant but less acceptable plant may be chosen when the most acceptable host becomes rare (Bernays & Chapman 1994). However, because coverage of both macrophytes was similar we can discard this explanation.

#### ***Oxycaryum cubense* as refuge of *C. aquaticum***

Despite the findings that, in no-choice tests, adults survived for some weeks and even fed on *O. cubense*, the absence of endophytic oviposition indicates that the life cycle of *C. aquaticum* cannot be completed on this macrophyte. In fact, during the periods of August 2004 and April to June 2005, when *C. aquaticum* was most abundant on *O. cubense*, a high proportion of females (50% to 100%) had immature ovaries (Franceschini et al. 2007). The peak of nymphs A in October 2005 on *E. azurea* indicates that the high proportion of mature females (nearly 90%) seeking shelter in *O. cubense* during August and September 2005 had returned to the host plant to oviposit.

It is also evident from the high mortality of nymphs A and the poor survival of nymphs B that they cannot develop on *O. cubense*. Even though some feeding by the nymphs was observed they were apparently not able to successfully use this resource for their growth. Thus, *O. cubense* cannot be considered as a secondary host plant but as a temporarily used shelter during adverse weather conditions. Instead, this study confirmed the high specificity of *C. aquaticum* for feeding and reproduction on *E. azurea*.

#### **Conclusions**

Thus, given these results, there is support for the hypothesis that *C. aquaticum* is not able to feed and

oviposit on *O. cubense*, but rather uses this macrophyte as an alternative microhabitat and refuge under specific climate conditions. Further studies on the behaviour of *C. aquaticum*, specifically the use of alternative microhabitats and its feeding preferences in the field, should be carried out throughout its wide distribution range.

#### **Acknowledgments**

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