Performance and feeding preference of *Cornops aquaticum* (Orthoptera: Acrididae) on *Eichhornia crassipes* and crop plants in native area

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Abstract

The semiaquatic grasshopper *Cornops aquaticum* Bruner, which is highly specific of *Eichhornia crassipes* (Pontederiaceae), constitutes one of the most abundant herbivores in the Neotropical wetlands and a possible biological control agent of this weed in South Africa. The aims of this study were 1) to assess the performance of each age category of *C. aquaticum* on crop plants and the host plant *E. crassipes*; 2) to assess the feeding preference of this grasshopper on crop plants in the presence and absence of *E. crassipes*; and 3) to determine the presence of nymphs and adults of this grasshopper on crops and in the adjacent wetlands where *Eichhornia* plants are present. Crop plants included in this study were *Zea mays*, *Oryza sativa*, *Saccharum officinarum* and *Ilex paraguariensis*. The age categories of *C. aquaticum* population were nymphs A (instar I and II), nymphs B (instar III to VI) and adults (females and males). To assess grasshopper performance on *E. crassipes* and crop plants, survival and oviposition were recorded during the no-choice tests. To evaluate feeding preference, choice tests with crop plants in the presence and absence of *E. crassipes* were performed. Abundance of *C. aquaticum* in the crop patches and the wetlands vegetation was assessed using an entomological net. During the no-choice tests, high survival of most *C. aquaticum* individuals was observed on *Z. mays* and *E. crassipes*, except for nymphs A fed on *Z. mays*. Ovipositions were registered in *E. crassipes*, *Z. mays* and *S. officinarum*, but only eggs laid in *E. crassipes* were endophytic and viable. *C. aquaticum* showed high preference to *E. crassipes*, whereas *Z. mays* and *S. officinarum* were the preferred plants in the choice test without *E. crassipes*. We did not find *C. aquaticum* individuals in the crop patches of *Z. mays*, *O. sativa* and *S. officinarum*, whereas abundance of this grasshopper varied between 28 and 62 individuals captured per minute on *Eichhornia* floating meadows near these cultivated areas. Our results confirm the high specificity of *C. aquaticum* to *E. crassipes* because eggs laid on crop plants are not viable and nymphs A have no possibilities of surviving when fed on these plants. Finally, we recommend carrying out choice tests and field samplings throughout the whole native range of *C. aquaticum*.

Key words

host-specificity, Leptysminae, semi-aquatic grasshopper, water hyacinth

Introduction

The semi-aquatic grasshopper *Cornops aquaticum* Bruner (1906) is of particular interest as a herbivore in the Neotropical wetlands due to the high values of population abundance (Braga *et al.* 2012, Franceschini *et al.* 2013), together with high biomass per individual (Gallardo *et al.* in press) and high consumption rates (Adis & Junk 2003, Braga *et al.* 2014). This grasshopper is native to South America and inhabits lowlands from Southern Mexico to Central Argentina and Uruguay (Adis *et al.* 2007).

Several laboratory tests and field observations in native areas have shown the high specificity of *C. aquaticum* to Pontederiaceae, mainly *Eichhornia crassipes*. This plant provides the insect with food and an adequate substrate for the endophytic oviposition (Center *et al.* 2002, Lhano *et al.* 2005, Silveira Guido & Perkins 1975, Vieira & Santos 2003).

Eichhornia crassipes, commonly known as the water hyacinth, is an aquatic plant originating in the Amazon Basin of Brazil and its distribution native area ranges from tropical to semi-tropical regions of America (Gopal 1987). Outside its native range, it is considered one of the most important weeds of the natural and artificial aquatic systems around the world (Center *et al.* 2002, Waterhouse & Norris 1987).

According to Cordo (1999), *C. aquaticum* is considered an intermediate priority category among the candidates for a biological control agent of *E. crassipes* since the specificity is under testing. A quarantine population of this grasshopper has existed in South Africa for 15 years and it is planned to be released in order to control this weed (Bownes *et al.* 2010, Oberholzer & Hill 2001).

Although *C. aquaticum* is considered highly specific to feed on Pontederiaceae, mainly on *E. crassipes*, some studies in native areas have provided evidence that this grasshopper is oligophagous under field conditions and polyphagous under laboratory conditions (Ferreira & Vasconcellos-Neto 2001, Capello *et al.* 2011). Also, adults of *C. aquaticum* can move from *Eichhornia* plants to a non-host plant under specific climatic conditions (Ferreira & Vasconcellos-Neto 2001, Franceschini *et al.* 2011).

Performance and feeding preference of *C. aquaticum* on crop plants and vegetable species have been scarcely evaluated in native areas, especially under natural conditions (Lhano *et al.* 2005, Silveira Guido & Perkins 1975).

In non-native areas, Hill and Oberholzer (2000) assessed a quarantine population of *C. aquaticum* in the ability of first instar nymphs and adult individuals to survive and oviposit in several cultivated and wetland plants under laboratory conditions. A complete nymphal development of this grasshopper on *Canna indica*, *Musa paradisiaca* and on the South African Pontederiaceae *Heteranthera callifolia*, was recorded (Oberholzer & Hill 2001).

However, it remains unclear whether the performance of *C. aquaticum* on crop plants varies in different age categories of these

grasshopper populations and the capacity of each category to survive and eventually feed on crop plants in native areas. Considering the occurrence of cultivated areas at subsistence and agro-industrial scale near the wetlands where *C. aquaticum* and *Eichhornia* plants are present, it is necessary for a quantitative evaluation of the feeding preference and abundance of individuals in the crops to determine if this grasshopper will choose them as food items. Oberholzer and Hill (2001), in regards to the possibility of releasing *C. aquaticum* in South Africa, pointed out the necessity of carrying out research to test the feeding behaviour of this grasshopper under more natural conditions in order to clarify the host specificity, including field trials and preference tests with nymphs and adults in native areas.

Thus, the aims of this study were 1) to assess the performance of each age category of *C. aquaticum* on crop plants and the host plant *E. crassipes*; 2) to assess the feeding preference of this grasshopper on crop plants in the presence and absence of *E. crassipes*; and 3) to determine the presence of nymphs and adults of this grasshopper on crops and in the adjacent wetlands where the *Eichhornia* plants are present.

We hypothesize, first, that this grasshopper has the capacity to survive on crop plants and may choose those as food item during the feeding preference tests. Our second hypothesis is that under field condition, high population abundance and oviposition occur only in *E. crassipes*.

Material and methods

Crop plants used in this study were *Zea mays* (maize), *Oryza sativa* (rice), *Saccharum officinarum* (sugarcane) and *Ilex paraguariensis* (mate). These crops were selected due to their high economic importance in agricultural ecosystems of the Neotropic, particularly in the Northeast of Argentina. The patches of these crops and vegetables, which are cultivated at subsistence or agro-industrial scale, frequently alternate with the wetlands due to the good condition of soil nutrients and water availability of these habitats (Benzaquén *et al.* 2013, Junk 2000, Neiff 2004). The climate in the Northeast of Argentina is subtropical, with warm and long summers and mild winters with a frequency of frost days of 0.25 days/year (Bruniard 1981, 1996). Experiments and field samplings were carried out in summer because, in natural conditions, herbivorous damage on *E. crassipes* is higher in this season and all age categories of *C. aquaticum* are present in the grasshopper populations (Franceschini *et al.* 2013). During the experiments and field samplings (27th January-07th February; 27-29th March 2007), daily mean temperatures ranged from 19.0-30.1°C.

Performance and feeding preference tests were performed in a greenhouse located in the Centro de Ecología Aplicada del Litoral (27°28′14″ S - 58°50′ 24″ W) at 2 km from the wetlands and cultivated habitats, under the influence of the local climate and without direct sun exposure, following the criteria of Vieira and Santos (2003) and Martinez *et al.* (2013).

Cornops aquaticum individuals were collected using an entomological net (70 cm of diameter and 1 m of depth) in an *E. crassipes* floating meadow from a lake of the Paraná River floodplain. Before the tests, grasshoppers were placed in cages without food and remained starved for four hours to avoid effects of prior experience and learning (Bernays & Chapman 1994).

Performance tests.—In order to evaluate the performance of *C. aquaticum* on the crop plants and *E. crassipes* (host plant), survival and oviposition were recorded by means of no-choice tests, with grasshoppers confined onto one plant species at a time. No-choice tests were conducted with four categories of the *C. aquaticum* population: nymphs A (instars I and II), nymphs B (instars III to VI), adult females and adult males. Cages of 50 × 50 × 50 cm containing 10 individuals from the same age category were set up.

Inside the cages, fresh growing plants of *E. crassipes* and crops without feeding damage and ovipositions were maintained with water in plastic containers (43 × 30 × 11.5 cm). Leaf density of plants within the containers was similar to the leaf density of *E. crassipes* and crops observed in the field. Damaged plants were replaced by fresh ones every three days in order to maintain food in optimal conditions during the experiments. We performed three replications of each 20 plant-insect age category combinations (5 plant species × 4 insect age categories), with a total of 120 grasshoppers for each plant species (Total N= 600 grasshoppers). The sex ratio for nymphs B was 1:1, but it was not determined for nymphs A. The survival of individuals was assessed every three days for a period of 12 days according to Lhano *et al.* (2005). At the end of the experiment, plants offered to adult females were carefully dissected and checked to detect the presence of eggs. The females of *C. aquaticum* were also dissected to confirm the presence of mature ovaries.

Feeding preference tests.—Multiple-choice tests were performed in two distinct forms: the traditional choice test in which all crop species and the host plant were offered together to the grasshoppers and the choice-minus-target test, where all crop species were offered together without the host plant (Sheppard *et al.* 2005). The choice-minus-target tests allowed us to predict the feeding preference that *C. aquaticum* should have on crops in the hypothetical situation that the host plant, *E. crassipes*, became absent in field conditions. For practical reasons, in this paper we use the terms "choice-with-target tests" for those that include *E. crassipes* in the choice and "choice-minus-target tests" for those without *E. crassipes*.

Choice tests were conducted with nymphs B, adult females and adult males. These nymphs were selected because last instars of *C. aquaticum* have higher mobility and higher number of plants in their diet (Capello *et al.* 2011).

Cages of 50 × 50 × 50 cm containing 10 grasshoppers from the same age category were set up. Leaf rations of *E. crassipes* and crop plants were placed in a 250 cm^3 glass with water to maintain leaf turgidity and were distributed equidistant from each other. We performed five replications of each 6 choice test-insect age category combinations (2 choice tests: choice-with-target test and choice-minus-target test × 3 insect age categories: nymphs B, adult males and adult females), with a total of 150 grasshoppers for each choice test (Total N= 300 grasshoppers). The sex ratio for nymphs was 1:1. The leaf rations, instead of the growing plants, allowed us to quantify biomass consumed in each plant species, offering similar amounts of food and visual stimuli to the grasshoppers during the choice tests (Squitier & Capinera 2002). Leaf rations were previously weighed to obtain the initial food fresh weight, and then were offered to the grasshoppers for 24 hours (Squitier & Capinera 2002). We used 24 hours as the period of experiments to avoid the possibility that the grasshoppers chose one species of plant because the alternative more preferred plant was excessively damaged (Mansor & Buckingham 1989).

To calculate the dry weight (dw) of the rations offered at the beginning of the choice tests, 10 similar leaf rations were also prepared as a control; their fresh weight was recorded, and then they were dried at 60°C to obtain their constant dry weight and the conversion factor was calculated.

Table 1. Performance of *Cornops aquaticum* adults and nymphs comparing values of final survival on the host plant *Eichhornia crassipes* (reference values) with those from the crop plants with the Chi-square test (X^2). Performance is expressed as the mean number of grasshoppers surviving at 12 days and (±) indicate standard deviation. (%) indicate percentage of final survival at 12 days of the experiments. (*) in the X^2 values indicate significant differences (p<0.05, df=3).

	Eichhornia crassipes	*Oryza sativa*	*Saccharum officinarum*	*Zea mays*	*Ilex paraguariensis*
Adult females		0.33 ±0.58	3.67 ±1.53	7.33±2.08	0.00 ±0.00
	9.67±0.58	3.3%	36.7%	73.3%	0%
	96.7%	X^2=27.10*	X^2=11.54*	X^2=2.71	X^2=29*
Adult males		0.33 ±0.58	4.00 ±1.00	8.00 ±2.00	0.00 ±0.00
	9.33 ±0.58	3.3%	40%	80%	0%
	93.3%	X^2=26.11*	X^2=9.37*	X^2=1.51	X^2=28*
Nymphs B		0.00 ±0.00	2.00 ±1.00	7.00 ±1.73	0.00 ±0.00
	7.00 ±1.73	0%	20%	70%	0%
	70%	X^2=21*	X^2=11.11*	X^2=2.50	X^2=21*
Nymphs A		0.00 ±0.00	0.00 ±0.00	0.33±0.58	0.00 ±0.00
	9.33 ±1.15	0%	0%	3.3%	0%
	93.3%	X^2=28*	X^2=28*	X^2=26.13*	X^2=28*

After the feeding period, the remaining leaf rations of each plant were collected and dried at 60 °C to obtain the constant weight. For each plant species, mg of food consumed by grasshopper per hour was calculated and feeding preference was determined assuming that the most consumed plant was the most preferred.

Presence of C. aquaticum *on cultivated patches and wetlands.*— Grasshopper sampling was carried out in the Riachuelo River Basin and in the Paraná River floodplain. To assess the presence of *C. aquaticum* on crop plants, field observations were carried out from 9:00 to 13:00 hours in cultivated patches of *Z. mays* (27°26'53''S, 58°51'21''O and 27°29'20''S, 58°45'82''O), *O. sativa* (27°42'12''S, 58°46'2''O) and *S. officinarum* (27°29'18''S, 58°46'42''O) that were between 500 to 1000 m to the wetlands with *Eichhornia* plants. We swept the crop patches and the adjacent *Eichhornia* floating meadows using an entomological net. In the cultivated patches, sampling was carried out along the edge and center, walking between 30-40 minutes. In the *Eichhornia* floating meadows, we swept the net for 1 minute from a boat in three different points located at the center and edge of the vegetation. We recorded the number of *C. aquaticum* individuals captured per minute from the vegetation. We were not able to access the Riachuelo River by boat to sweep the *Eichhornia* plants associated with *O. sativa* for security reasons. In this particular case, the presence of *C. aquaticum* was evaluated indirectly by identifying the feeding damage caused by this grasshopper on the host plant leaves.

Statistical analysis.—To evaluate the performance of *C. aquaticum*, we compared the final survival of grasshoppers on *E. crassipes* and the crop plants using a Chi Square Test. Differences on performance and feeding preference between *C. aquaticum* age categories were assessed using the Generalized Linear Mixed Models (GLMM) with post hoc tests. Differences were considered to be statistically significant at p <0.05. Statistical analyses were performed using Infostat Software (Di Rienzo *et al.* 2012).

Results

Performance of C. aquaticum *on* E. crassipes *and crops plants.*— Performance of *C. aquaticum* varied between the plant species and the age categories of the grasshopper population. Survival of grasshoppers fed on the *E. crassipes* and *Z. mays* was high during all the tests, except for nymphs A fed on *Z. mays*. The lowest performance of nymphs and adults of *C. aquaticum* was obtained on *O. sativa* and *I. paraguariensis*. Significant differences in survival between age categories of *C. aquaticum* were found in *E. crassipes* and crop plants on almost every day of the experiment (Fig. 1).

Results obtained in final survival of *C. aquaticum* on different plant species show that this grasshopper had the high performance feeding on *E. crassipes* and on *Z. mays*, except for nymphs A fed on *Z. mays*. Final survival of nymphs B and adults fed on *Z. mays* was not significantly different from the final survival of these grasshopper categories fed on *E. crassipes* (X^2 Test, p>0.05 df=3), whereas final survival of nymphs A fed on *Z. mays* was significantly lower than the final survival of nymphs A fed on *E. crassipes* (X^2 Test, p<0.05 df=3). Percentage of final survival of *C. aquaticum* on *E. crassipes* varied from 70 to 96.7% and from 3 to 80% on *Z. mays* (Table 1). Final survival of *C. aquaticum* fed on *O. sativa*, *S. officinarum* and *I. paraguariensis* was significantly lower than the final survival of this grasshopper fed on *E. crassipes* (X^2 Test, p<0.05 df= 3). Percentage of final survival was intermediate in *S. officinarum* (0-40%) and notably low (0-3.3%) in *O. sativa* and *I. paraguariensis* (Table 1).

Analysis and dissection of the plant materials show that ovipositions of *C. aquaticum* took place in *E. crassipes*, *S. officinarum* and *Z. mays* plants, but only eggs laid on the host plant *E. crassipes* were endophytic and viable. Eggs of *C. aquaticum* from *E. crassipes* were elliptical (20.8± 3 mm long × 4.4± 0.74 mm lat) and had 6 to 24 (12± 5.2) eggs. These eggs were covered with a yellowish foamy substance, which acts as a cement, gluing and protecting them. In a cross section, the protein substance around the egg forms a considerably thick layer consisting of thin sheets delimiting numerous air spaces, the structure being similar to the aerenchyma of the petiole of *E. crassipes* (Fig. 2). Ovipositions on *S. officinarum* and *Z. mays* had a smaller number of eggs than those from *E. crassipes*; the protein substance covering the eggs was also scarce. Eggs were laid on leaves and on the part of the stem that is surrounded by the leaf sheath. These ovipositions were quickly covered by fungi and lost their viability. Ovipositions were not recorded on *I. paraguariensis* and *O. sativa* plants. The adult females from *E. crassipes* and crop plants cages had mature ovaries.

Feeding preference of C. aquaticum *on crop plants.*—In the choice-with-target test, *C. aquaticum* nymphs and adults showed high preference to *E. crassipes* and low preference to the crop plants. Significant differences in consumption between plant species were obtained in adult females, adult males and nymphs B (GLMM, p< 0.05, N= 25). In the choice-with-target test, mean consumption

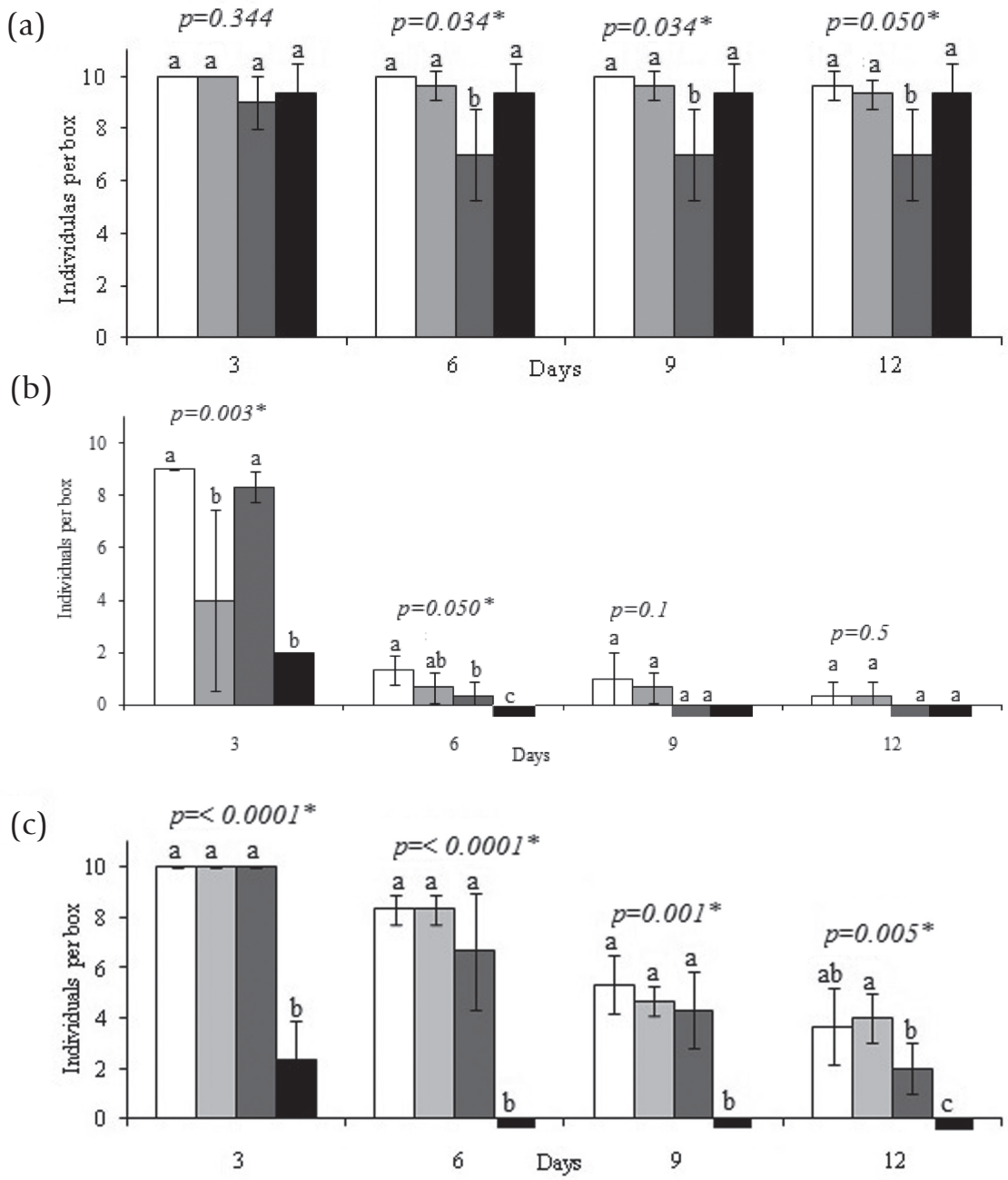

Fig. 1. Survivorship of *Cornops aquaticum* during the no-choice cage experiments after 3, 6, 9 and 12 days on the host plant *Eichhornia crassipes* (a), *Oryza sativa* (b), *Saccharum officinarum* (c), *Zea mays* (d) and *Ilex paraguariensis* (e). Nymphs A: instar I and II; nymphs B: instars III to VI. Adult females, adults male, nymphs B and nymphs A are indicated with the color white, light grey, dark grey and black of the bars, respectively. Bars below the zero value indicate no survivorship in the age category. (*) in the p values indicate significant differences on survival between age categories of *C. aquaticum* with the Generalized Linear Mixed Models (p< 0.05). Different letters indicate significant differences on survival between grasshopper age categories with the Post hoc test.

by this grasshopper reached up to 2 mg of food per individual per hour in *E. crassipes*. Among the crop plant species, *Z. mays* tended to have highest values of consumption, reaching up to 0.4 mg per individual per hour (Table 2).

In the choice-minus-target test, *C. aquaticum* presented lower values of consumption in comparison to the consumption observed in the choice-with-target test. Differences in consumption by *C. aquaticum* between crop plants were obtained only in adult females and nymphs B, which preferred *Z. mays* and *S.officinarum* (GLMM, <0.05, N= 20). Consumption by adult males tended to be higher in *Z. mays*, but differences between crop plants were not significant. Mean consumption of *C. aquaticum* reached up to 0.54 mg of food per individual per hour in *Z. mays*. Unlike the choice-with-target test, in the choice-minus-target test adult females of *C. aquaticum* consumed *I. paraguariensis* (Table 2).

C. aquaticum *on crop patches and* Eichhornia *floating meadows.—Cornops aquaticum* nymphs and adults were not recorded in *Z. mays*, *O.*

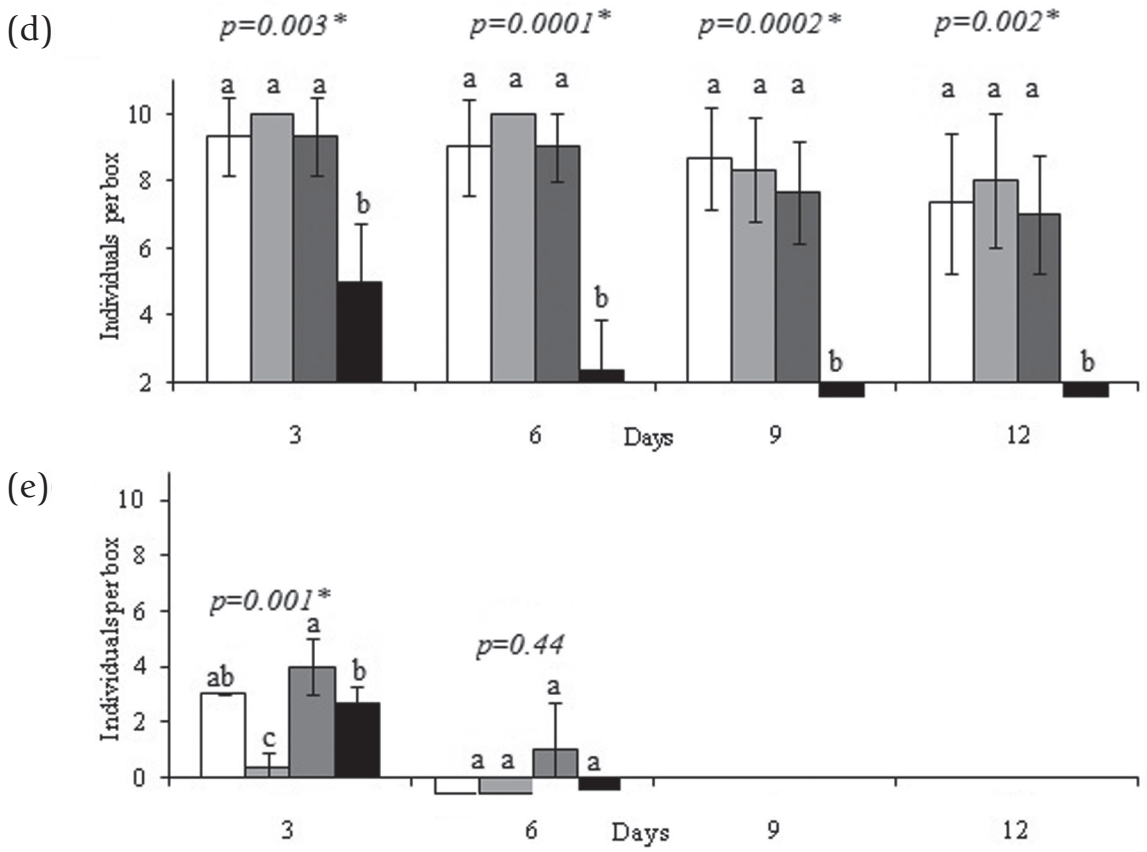

Fig. 1. Continued.

Table 2. Feeding preference of *Cornops aquaticum* adults and nymphs B on crop plants, showing the consumption of grasshoppers during the choice-with-target test and choice-minus-target tests. Consumption of grasshoppers is expressed as mg of plant consumed per individual per hour. (±) is standard deviation and (*) in the *p* values indicate significant differences on consumption between plant species with the Generalized Linear Mixed Models ($p < 0.05$). Different letters indicate significant differences on consumption between plant species with the Post hoc test.

Choice-with-target test						
	Zea mays	*Oryza sativa*	*Saccharum officinarum*	*Ilex paraguariensis*	*Eichhornia crassipes*	*p* values
Adult females	0.30^{b} ±0.16	0.05^{b} ±0.06	0.10^{b} ±0.23	0.00^{b} ±0.00	2.00^{a} ±0.81	<0.0001 *
Adult males	0.40^{b} ±0.37	0.17^{b} ±0.33	0.05^{b} ±0.09	0.06^{b} ±0.07	1.25^{a} ±0.34	<0.0001 *
Nymphs B	0.31^{b} ±0.24	0.25^{b} ±0.24	0.17^{b} ±0.26	0.15^{b} ±0.10	1.28^{a} ±0.26	<0.0001 *
Choice-minus-target test						
	Zea mays	*Oryza sativa*	*Saccharum officinarum*	*Ilex paraguariensis*	*p* values	
Adult females	0.54^{a} ±0.37	0.14^{b} ±0.16	0.38^{a} ±0.31	0.10^{b} ±0.05	0.047*	
Adult males	0.40^{a} ± 0.41	0.04^{a} ±0.07	0.18^{a} ±0.33	0.07^{a} ±0.09	0.263	
Nymphs B	0.42^{a} ±0.34	0.05^{b} ±0.06	0.45^{a} ±0.27	0.07^{b} ±0.03	0.009*	

sativa and *S. officinarum* crop patches. Some terrestrial Acridoidea and Tettigonoidea species were observed in *O. sativa* and *S. officinarum* crops, but the plants were scarcely damaged. We recorded high abundance of nymphs and adults of *C. aquaticum* on the *Eichhornia* floating meadows associated with *Z. mays* and *S. officinarum*, varying in abundance between 28 and 62 individuals captured per minute, respectively. *C. aquaticum* nymphs represented between 83.32 and 82.25% of the total abundance of grasshoppers on the host plants. In the floating meadows of the Riachuelo River, which was associated with *O. sativa* crops, leaves of *Eichhornia* plants were intensely damaged by grasshoppers. Despite the fact that we were unable to access by boat to sweep this floating meadow, the pattern of leaf

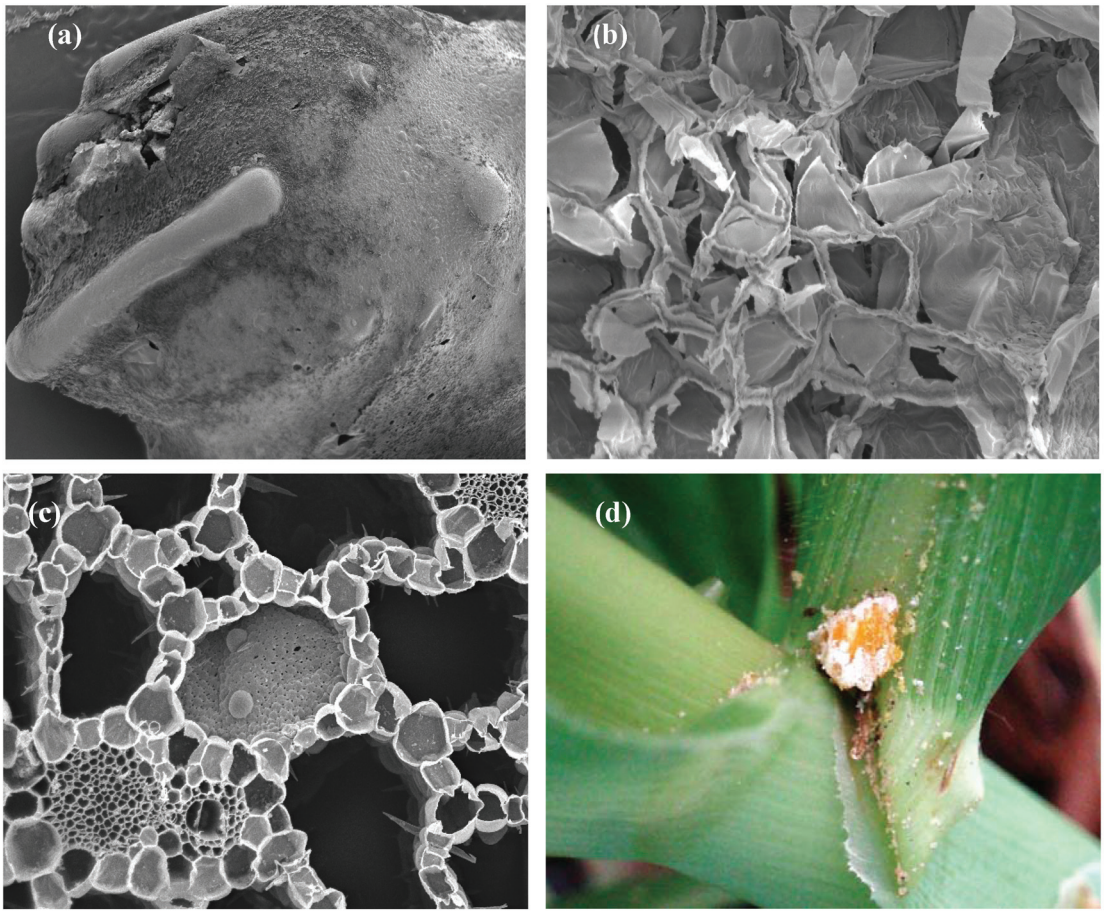

Fig. 2. Endophytic (viable) and epiphytic (unviable) oviposition of *Cornops aquaticum* females during the no-choice tests. (a) Eggs laid inside the petioles of *Eichhornia crassipes;* (b) Layer of the protein substance that recover the endophytic eggs of *C. aquaticum*; (c) aerenchyma tissue of *E. crassipes* petiole, where endophytic eggs were laid during the oviposition; (d) epiphytic unviable eggs laid on the shoot of *Zea mays*.

damage was similar to that produced by *C. aquaticum* in the *Eichhornia* floating meadows in the study area. In the irrigation channels of *O. sativa*, the most common macrophyte was *Sagittaria montevidensis*, and *Eichhornia* species were absent during sampling. In the sampled wetlands, coverage of the host plants varied between 40 to 80%.

Discussion

Survival differences of each age category of the *C. aquaticum* population obtained on *E. crassipes* and crop plants show that this aspect should be taken into account in order to evaluate the performance of this grasshopper among plant species.

In the Pantanal of Mato Grosso (Brazil), *C. aquaticum* nymphs between first and six instars had low final survival when fed on plants cultivated near the wetland habitats, like *Lactuca sativa* (lettuce) and *Brassica oleraceae* var *acephala* (kale) (Lhano *et al.* 2005).

High survival of *C. aquaticum* fed on *Z. mays* indicates that nymphs B and adults have the capacity to survive, to include this crop as a food item and to eventually feed on this crop. However, high mortality of nymphs A and no viable ovipositions indicate that this grasshopper cannot colonize maize successfully. In addition, we did not find this grasshopper in patches of this crop when *Eichhornia* plants were abundant in the adjacent wetlands. In the same study area, despite the fact that *C. aquaticum* had high performance and high abundance under field conditions on *Oxycaryum cubense* (Cyperaceae), this grasshopper did not act as a herbivore of this plant when *Eichhornia* plants were abundant in the wetlands and only used this cyperaceous as a refuge during cold seasons (Franceschini *et al.* 2011). High performance of *C. aquaticum* on the non-host plant *Pistia stratiotes* was also reported in the Central Amazonia by Vieira and Santos (2003).

In quarantine populations reared in South Africa, performance of first instar nymphs of *C. aquaticum* was assessed separately in 20 crops and other cultivated plants that grow near the wetland habitats (Oberholzer & Hill 2001). No feeding was recorded on the majority of species tested and most nymphs died during the first week of the experiment. Poor performance of the first instar nymphs on *Z. mays*, *S. officinarum* and *O. sativa* observed in quarantine populations of South Africa (Oberholzer & Hill 2001) is in agreement with our results obtained on nymphs A. Complete development of nymphs on *Musa paradisiaca* (banana) and *Canna indica* (canna) and the Pontederiaceae *Heteranthera callifolia* reported by these authors suggests that more studies of feeding preference and performance of different age categories of *C. aquaticum* are necessary in native and non-native areas.

Shoot and leaf anatomy of the crop plants are not compatible to endophytic oviposition and survival of nymphs A of *C. aquaticum* (Boeger & Adis 2007) and chemical factors also could play an important role (Bernays & Champan 1994, Hinks & Olfert 1999). No viable oviposition on the crop plant and endophytic oviposition only on *E. crassipes* indicate that *C. aquaticum* cannot colonize these crop plants and this is in agreement with results obtained in native

areas that confirm the specificity of this grasshopper to oviposit in the host plants (Ferreira & Vasconcellos-Neto 2001, Franceschini *et al.* 2011, Silveira Guido & Perkins 1975). Low performance and high mortality of nymphs A obtained from the crop plants in our study support this statement. Smaller oviposition and a low number of eggs of *C. aquaticum* found on *S. officinarum* and *Z. mays* plants agree with the findings of Awmack and Leather (2002) who pointed out that females of herbivorous insects tend to have a reduced number of eggs and poorer yolk content in plants with low nutritional quality. Eggs of *C. aquaticum* laid on *S. officinarum* and *Z. mays* could also lose their viability because the microclimate conditions are not stable outside the plant tissue for development and eggs are more susceptible to fungi or predators (Braker 1989, 1991).

Choice test with *E. crassipes* showed that *C. aquaticum* nymphs B and adults had a high feeding preference for this plant. This agrees with almost all results obtained under field and laboratory conditions (Capello *et al.* 2011, Ferreira & Vasconcellos Neto 2001, Silveira Guido & Perkins 1975). However, Ferreira and Vasconcellos Neto (2001) pointed out that adult females of *C. aquaticum* in the Mato Grosso wetlands may prefer another wetland plant during the dry season instead of *Eichhornia* plants under field condition.

Cornops aquaticum showed a low feeding preference of *O. sativa* and *S. officinarum* in the choice-with-target test and this is in agreement with the results obtained by Silveira Guido and Perkins (1975). The mixture of different plant species in the diet of polyphagous grasshoppers improves the nutrient balance of these insects (Bernays & Chapman 1994, Bernays & Minkenberg 1997). In this sense, the low amount of crop plants consumed by *C. aquaticum* in the choice test that included *E. crassipes* would provide the extra nutrients to achieve the balance necessary to the grasshopper's physiology. The fact that non-host plants are found in the feces and gut contents of *C. aquaticum* nymphs and adults collected from the field agrees with this statement (Capello *et al.* 2011, Ferreira & Vasconcellos Neto 2001).

High preference of *C. aquaticum* to *Z. mays* and *S. officinarum* in the choice-minus-target test indicates that this grasshopper is able to feed and choose these crop plants as food item when the host plant *E. crassipes* is absent. High to intermediate performance of *C. aquaticum* to survive on both crop plants support these results and show the importance to carry out performance and preference tests simultaneously when feeding behaviour is an aspect to be studied. Because *Z. mays* and *S. officinarum* are plants with C_4 photosynthetic pathway, results obtained in the non-choice test and choice-minus-target test did not agree with those found by several authors (Caswell *et al.* 1973, Hinks & Olfert 1999, Scheirs *et al.* 2001). The herbivorous insects tend to avoid C_4 plants, because Kranz leaf anatomy concentrates proteins and carbohydrates in difficult-to-digest bundle sheath cells (Caswell *et al.* 1973, Scheirs *et al.* 2001). Adis and Victoria (2001) reported C_3 photosynthetic pathway to *E. crassipes*. No *C. aquaticum* individuals were recorded in the crop patches adjacent to *E. crassipes* wetlands. This is in agreement with the observations made by Silveira Guido and Perkins (1975). Despite that, we did not find *Eichhornia* plants in the irrigation channels and cultivated areas of *O. sativa;* Maltchik *et al.* (2011) mentioned that this environment provides suitable habitats and ecological conditions for many freshwater insects and Pontederiaceae plants, including *E. crassipes*.

We did not include sampling in cultivated patches of *I. paraguariensis*, but analysis of Orthoptera material collected in an agro-industrial cultivation indicates that *C. aquaticum* individuals were not present under field conditions in this crop (Franceschini & De Wysiecki unpubl.). This would confirm the negligible performance and feeding preference obtained in the no-choice and choice tests. Considering that nymphs A cannot survive on the crop plants studied, our previous hypothesis that *C. aquaticum* has the capacity to survive on crop plants and may choose them as food items during the feeding preference tests must be partially accepted. We accept our second hypothesis that under field conditions high population abundance and eggs laid of *C. aquaticum* occur only on the host plants *E. crassipes*.

Finally, we recommend continuing with the feeding behaviour studies through the native distribution area of *C. aquaticum*, especially near the wetlands habitats where coverage of the host plants decrease by prolonged dry seasons or anthropic effect. In non-native areas where specific predators of *C. aquaticum* are absent, studies on genetics, feeding preference and prey-predator interactions would contribute substantially to predict the success of this grasshopper as a biological control agent of *E. crassipes*.

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