Assessing density–damage relationships between water hyacinth and its grasshopper herbivore

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Abstract

Plants are variable in their responses to insect herbivory. Experimental increases in densities of phytophagous insects can reveal the type of plant response to herbivory in terms of impact and compensatory ability. The relationship between insect density and plant damage of a grasshopper, *Cornops aquaticum* Brüner (Orthoptera: Acrididae: Tetrataeniini), a candidate biological control agent, and an invasive aquatic plant, water hyacinth, *Eichhornia crassipes* Mart. Solms-Laubach (Pontederiaceae), was investigated to assess potential damage to the weed. The impact of different densities of male and female grasshoppers on *E. crassipes* growth parameters was determined in a quarantine glasshouse experiment. Damage curves indicated that the relationship between plant biomass reduction and insect density was curvilinear whereas leaf production was linear. Female *C. aquaticum* were more damaging than males, causing high rates of plant mortality before the end of the trial at densities of three and four per plant. Feeding by *C. aquaticum* significantly reduced the total plant biomass and the number of leaves produced, and female grasshoppers caused a greater reduction in the number of leaves produced by water hyacinth plants than males. Grasshopper herbivory suppressed vegetative reproduction in *E. crassipes*, suggesting *C. aquaticum* could contribute to a reduction in the density and spread of *E. crassipes* infestations. The results showed that *E. crassipes* vigour and productivity decreases with an increase in feeding intensity by the grasshopper. *Cornops aquaticum* should therefore be considered for release in South Africa based on its host specificity and potential impact on *E. crassipes*.

Introduction

Water hyacinth, *Eichhornia crassipes* Mart. Solms-Laubach (Pontederiaceae), is a highly invasive aquatic weed in South Africa that has been targeted for classical biological control since the early 1970s. The programme was initiated with the release of the weevil *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and to date, five arthropods and one pathogen have been introduced (Hill & Cilliers, 1999). This is highest of any country in the world with an *E. crassipes* biocontrol programme, yet South Africa has had comparatively little success. Although the biocontrol programme has reduced the overall negative

impacts of *E. crassipes*, it was apparent by the end of the 1990s that certain conditions in South Africa have constrained efficacy of the biocontrol agents (Hill & Olckers, 2001). Nitrate and phosphate-enriched surface waters (deVilliers & Thiart, 2007) facilitate explosive plant growth rates (Reddy et al., 1989, 1990) and under these conditions, the negative impact of herbivory by the biocontrol agents is reduced (Heard & Winterton, 2000; Coetzee et al., 2007). Additionally, cold winter temperatures deplete agent populations and their reproductive potential, which respond slowly compared to plant populations at the onset of the growing season (Byrne et al., 2010).

Unsatisfactory levels of control of *E. crassipes* have led to consideration of potentially more damaging and climatically adapted biocontrol agents. The grasshopper *Cornops aquaticum* Brüner (Orthoptera: Acrididae: Tetrataeniini), was the most promising candidate agent based on its host

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specificity (Oberholzer & Hill, 2001), its wide natural distribution extending to climatically similar regions to South Africa (Adis et al., 2007), and reports on its damage potential from the native range (Perkins, 1974). However, evaluating a candidate biocontrol agent's potential efficacy to assist a decision on release has become an important component of pre-release studies (Sheppard, 2003; Balciunas, 2004; McClay & Balciunas, 2005). A series of pre-release impact studies were initiated in 2005 to determine potential efficacy of *C. aquaticum* and its eligibility for release in South Africa.

A critical goal of pre-release efficacy studies in biological control of weeds is to attempt to understand how a particular insect herbivore might influence the abundance of its host plant. Experimental increases in herbivore densities test a range of feeding intensities and the shape of the damage function provides information on the nature and extent of plant compensation for herbivory (Crawley, 1983, 1989). Most plant–herbivore relationships are described by linear damage functions (Meyer, 1998; Schooler & McEvoy, 2006; Stanley et al., 2007) where plant fitness or performance decreases linearly with an increase in insect density. When densities approach the carrying capacity of the herbivore, the linear relationship changes to a non-linear, asymptotic relationship (Schooler & McEvoy, 2006). McClay & Balciunas (2005) present an adaptation of a damage curve used for analysis of the effect of crop pests on yield (Peterson & Higley, 2001), to represent the impact of a biological control agent at various densities on a target weed. It relates a critical aspect of weed performance, such as seed production, growth rate, or final biomass to biocontrol agent density. The purpose of the damage curve is to identify agents that do not have sufficient impact on their host to justify release.

This paper investigates the relationship between *C. aquaticum* biomass and plant damage, and compares the impact of different densities of male and female grasshoppers on *E. crassipes* growth and reproduction. The principle aim was to determine how different grasshopper densities will affect *E. crassipes* characteristics related to its invasiveness. Sexes were separated due to consideration of a trial release of males only. Because of their large size, marked size sexual dimorphism, and clear differences in the external genitalia, it is easy to separate the sexes for a one-sex only release. At an early stage of the release application process, the regulatory authorities expressed concern over the introduction of an exotic grasshopper and possible indirect non-target effects. A one-sex only release was suggested to dispel concerns over unwanted non-target effects to native vegetation. It was, therefore, necessary to evaluate the impact of male and female grasshoppers separately in order to provide an indication of the number of males needed to have a significant impact on *E. crassipes* infestations.

Materials and methods

Experimental organisms

Eichhornia crassipes is native to the New World Tropics (Center, 1994) and its aesthetic appeal has encouraged its spread throughout the world as an ornamental aquatic plant. It is an erect, free-floating perennial herb (Center, 1994) with attractive lavender flowers and glossy dark green leaves. Bulbous petioles provide buoyancy to free-floating plants and roots are fibrous and feather-like and vary in length according to nutrient supply in the water (Wright & Purcell, 1995). *Eichhornia crassipes* reproduces sexually by the production of seeds and asexually by the production of ramets (daughter plants). The plant responds positively to nitrogen, with increases in growth and reproductive rates proportional to nitrogen availability up to approximately 5.5 mg l^{-1} (Reddy et al., 1989). *Eichhornia crassipes* is highly competitive (Coetzee et al., 2005; Bownes et al., 2010) and is usually the dominant species in aquatic communities, displacing both indigenous and other invasive species (Wright & Purcell, 1995).

Cornops aquaticum is a semi-aquatic grasshopper of Neotropical origin which inhabits lowlands from Mexico to central Argentina and Uruguay (Adis et al., 2007). The grasshopper was first collected from *E. crassipes* mats in Manaus, Brazil in 1995 during a survey conducted by ARC-PPRI, South Africa. Subsequent collections were made in Trinidad and Venezuela in 1996 and Mexico in 1997 (Hill & Cilliers, 1999). *Cornops aquaticum* is oligophagous (Oberholzer & Hill, 2001) with a strong preference for water hyacinth. Its specific oviposition requirements preclude many plants species from sustaining populations of the grasshopper.

Both nymphal and adult *C. aquaticum* are defoliators and are extremely damaging to *E. crassipes*. Nymphs and adults are agile and good swimmers and the adults are strong fliers. According to Silveira-Guido & Perkins (1975) they are diurnal, bivoltine, overwinter as adults, and exhibit a sex ratio of 1:1 in the region of origin. When abundant, field populations occur at a density of one grasshopper per plant.

The biology of *C. aquaticum* was studied by Hill & Oberholzer (2000) and Oberholzer & Hill (2001). Egg cases, containing 30–70 eggs are constructed of a hard, foamy substance and are inserted into the youngest petiole, usually just above the crown of the plant. Eggs hatch after 25–30 days and the nymphs begin to feed immediately after hatching. Nymphal development from first instar to adult takes 36–55 days (Oberholzer & Hill, 2001; Adis &

Junk, 2003; Bownes et al., 2010), passing through 5–7 instars (Oberholzer & Hill, 2001; Adis & Junk, 2003).

Experimental design

The density trial was conducted in a quarantine glasshouse in Pretoria, South Africa from 11 January 2007 to 8 March 2007. *Eichhornia crassipes* plants obtained from stock cultures were grown in plastic tubs ($43 \times 31 \times 19$ cm) containing 15 l of water. Each tub contained two *E. crassipes* plants from which the ramets had been removed and was covered with a net sleeve to confine the insects to the tubs. Nutrients and water were replaced on a weekly basis to maintain an adequate supply of nitrogen and phosphorus to the plants for the duration of the trial. The nutrient treatment simulated average nitrate and phosphate levels from a highly eutrophic impoundment in South Africa (R Brudvig, DWAF, pers. comm.) and was used to assess the impact of different densities of *C. aquaticum* on plants with high rates of growth and reproduction (Reddy et al., 1989). Nitrates and phosphates were added to the water as potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) at a rate of 7.6 mg N l^{-1} and 1.37 mg P l^{-1}, respectively. A commercial iron chelate (Mircel FE 130, Ocean Agriculture (Pty) Ltd, Muldersdrift, South Africa) was also added at a rate of 1.3 g 15 l^{-1}.

The tubs were arranged in a randomized block design in the glasshouse. Plants were grown for 2 weeks prior to the start of the study, after which all ramets, dead leaves, and stems were removed and the plants weighed to determine wet weight. Adult *C. aquaticum* were introduced into the experimental tubs at a density of two, three, and four grasshoppers per plant (= four, six, and eight grasshoppers per tub). The impact of one grasshopper per plant under eutrophic nutrient conditions was investigated by Bownes (2009) and indicated that higher densities would be needed to cause a significant reduction in growth and productivity of the plant. Two tubs per replicate were left as controls and each treatment was replicated six times. Adults were separated into males and females at each density level so that each tub had only male or only female grasshoppers. Oviposition occurred in the female treatments but all nymphs were removed immediately to maintain the original herbivore densities, and any dead adult grasshoppers were replaced with grasshoppers of the same sex and as a far as possible, of similar age.

Plants were sampled weekly to record the number of leaves and ramets on each *E. crassipes* rosette. New leaves and ramets were tagged and counted at each sampling interval to record the number of leaves and ramets produced during the study period. Each treatment was replicated six times and the duration of the trial was 8 weeks, at which time all surviving plants were weighed

to determine end wet weight. As there were two plants per tub, plant data for each tub were averaged to obtain a mean for each replicate, i.e., tub. Most of the plants in the female treatments died before the end of the trial due to herbivory, therefore the data presented for those treatments are the last measurements recorded before death. A random sample of male and female grasshoppers were weighed (males n = 47; females n = 50) to obtain a mean wet weight (g) for each sex, to evaluate the relationship between grasshopper biomass and *E. crassipes* growth parameters.

Statistical analysis

All the data were normally distributed therefore the means of the biomass data and the growth and reproductive parameters between the different herbivory treatments and the controls were compared by one-way analysis of variance (ANOVA). Two-way ANOVAs were used to analyse the effect of insect density and sex and their interaction on growth and reproductive parameters. Tukey's HSD test was used for post-hoc comparison of the means for all ANOVAs (Zar, 1998). Data were subjected to regression analyses to determine the relationship between insect biomass (as the independent variable) and the different measures of plant performance (as the dependent variables). Because densities of males and females were the same, insect biomass was used as a surrogate for insect density. Insect biomass per treatment was calculated as a mean weight of either male or female grasshoppers multiplied by the number of individuals per plant. Insect biomass per tub was therefore highest in treatments with four *C. aquaticum* females per plant and lowest with two *C. aquaticum* males per plant. For linear functions, a product–moment correlation was used to determine the relationship between insect density and plant damage. The temporal development of daughter plant (ramet) production was plotted to show differences between the herbivory treatments and the control over the 8-week study period. All data were analysed using Statistica 6.0. (Statsoft, 2001).

Results

The relationship between final biomass of *E. crassipes* plants at week 8 (or at death of plants in the female treatments) as a function of increasing insect biomass was curvilinear (Figure 1). The damage curve shows a reduction in plant biomass with increasing herbivore pressure. Exponential regression best described the relationship between total yield and insect biomass and the regression was highly significant ($F_{6,43} = 73.20$, $P<0.0001$) accounting for 75% of the variance.
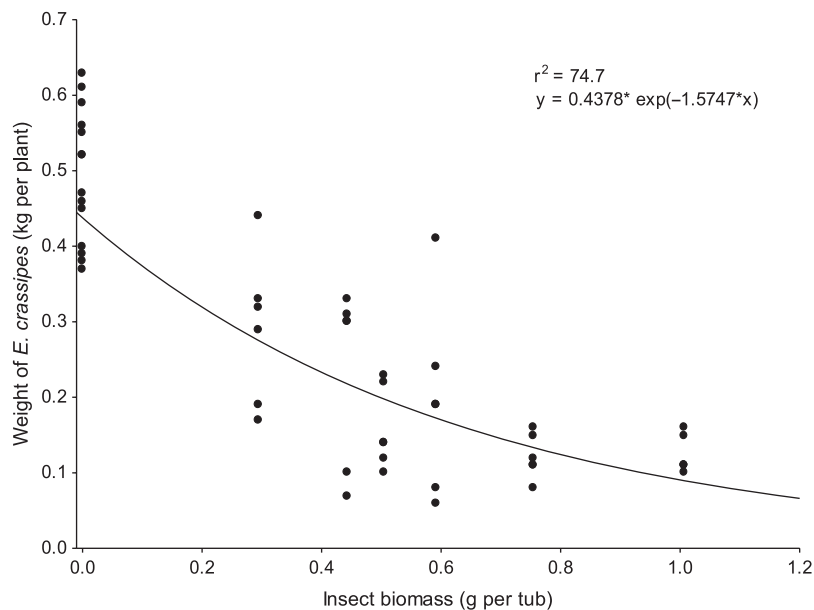
Figure 1 Regression of *Cornops aquaticum* biomass (g/tub) and final weight (kg/plant) of *Eichhornia crassipes* plants at the end of the 8-week study period. Insect biomass is represented by a mean weight of male or female grasshoppers multiplied by their respective density. Four *C. aquaticum* females/plant represent the highest insect biomass and two *C. aquaticum* males/plant represent the lowest insect biomass.

The mean number of leaves produced by *E. crassipes* plants in 8 weeks (i.e., leaf production) was linearly related to insect biomass and the regression was highly significant ($F_{6,43}$ = 108.57, P<0.0001) (Figure 2), explaining 69% of the variation. A product–moment correlation indicated that leaf production was significantly negatively correlated
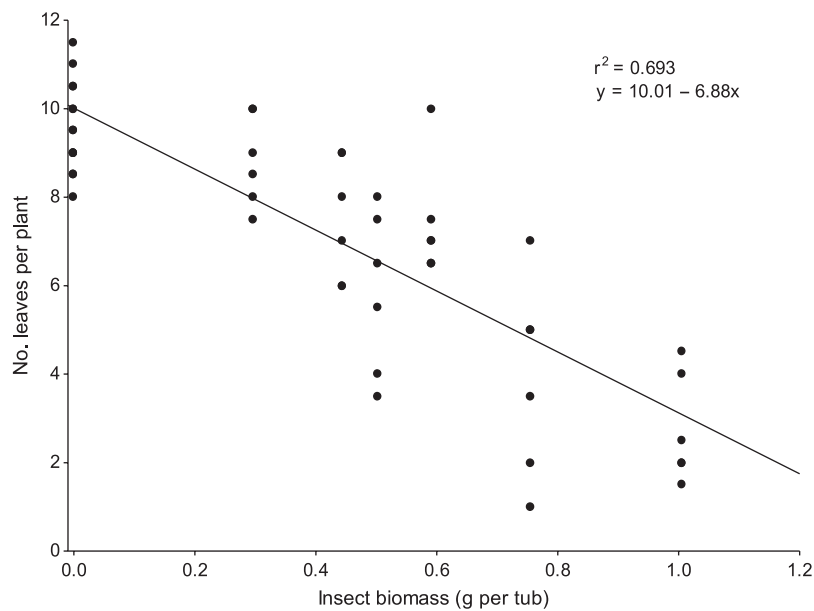

Figure 2 Regression of *Cornops aquaticum* biomass (g/tub) and the total number of leaves produced by *Eichhornia crassipes* plants during the 8-week study period. Insect biomass is represented by a mean weight of male or female grasshoppers multiplied by their respective density.

(r = −0.83, P<0.05) with insect biomass which indicates that leaf production decreased proportionately to the increase in insect biomass and sustained feeding damage.

Cornops aquaticum herbivory at densities of three and four female grasshoppers per plant caused high rates of plant mortality ($F_{6,43}$ = 19.41, P<0.0001) compared to all other herbivory treatments. Three and four females per plant caused 83 and 100% mortality, respectively, whereas mortality rates in the two-female (33%) and three- and four-male (8%) treatments were significantly lower (Figure 3).

The change in wet weight of *E. crassipes* plants from week 1 to week 8 was significantly different between the six herbivory treatments and the control ($F_{6,43}$ = 19.05, P<0.0001) (Figure 4). Insect density had no significant effects on the change in wet weight of *E. crassipes* plants, but the four-female and two-male herbivory treatments were significantly different from one another ($F_{1,30}$ = 14.13, P = 0.0007) (Figure 4). The interaction between density and sex was not significant (Figure 4).

A difference in the mean number of leaves on *E. crassipes* rosettes (i.e., total no. of leaves per rosette), sampled at the end of the 8-week study period, was found between male and female herbivory treatments and between the female grasshopper treatments and the controls ($F_{6,43}$ = 52.51, P<0.0001) (Figure 5). Grasshopper density had a significant effect on the number of leaves ($F_{2,30}$ = 9.56, P<0.0001) and there were significant differences between the male and female treatments ($F_{1,30}$ = 151.65, P<0.0001)

(Figure 5). The interaction of density and sex was not significant. The number of leaves on *E. crassipes* plants at the end of the trial was linearly related to insect biomass and the regression was significant ($F_{6,43}$ = 107.22, P<0.0001). A product–moment correlation showed a significant negative correlation (r = −0.831, P<0.05) indicating the capacity of plants to maintain the full complement of productive leaves decreased linearly with increasing herbivore pressure.

All densities of both male and female grasshoppers caused a reduction in ramet production but this was only significant in the four-male and four-female treatments ($F_{6,43}$ = 3.89, P = 0.0034). Despite the lack of significant differences, the temporal reduction in ramet production (Figure 6) indicated that all herbivory treatments had a significant impact on vegetative reproduction of *E. crassipes* plants. Control plants continued to produce ramets throughout the study period whereas all ramets produced by plants in the herbivory treatments appeared in the first 4 weeks of the trial, after which vegetative reproduction was suppressed by *C. aquaticum* herbivory.

Discussion

The results reported here indicate that *E. crassipes* is highly susceptible to feeding damage by *C. aquaticum*, where increasing feeding intensities caused corresponding reductions in weed growth and biomass. The decline in
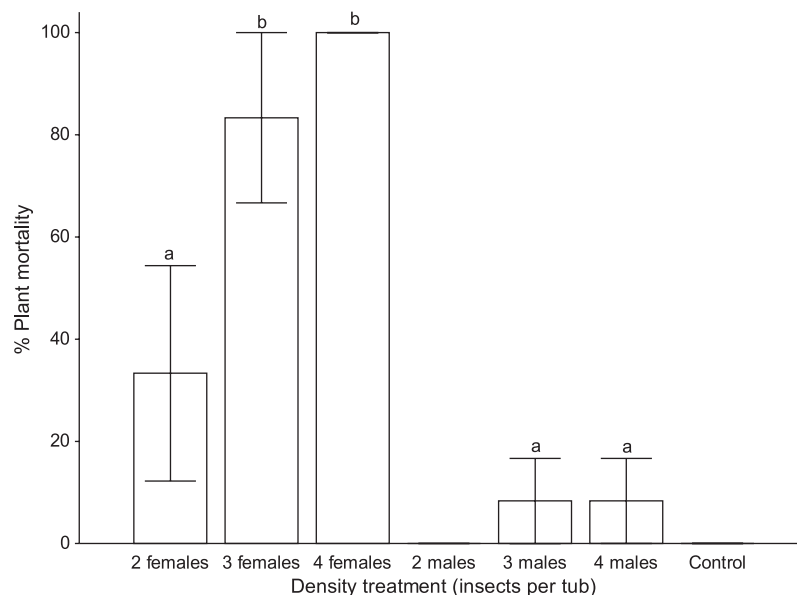

Figure 3 Mean (± SEM) % mortality of *Eichhornia crassipes* plants at the termination of the 8 weeks. Means compared by one-way ANOVA; bars capped with different letters are significantly different (Tukey's HSD: P<0.05).
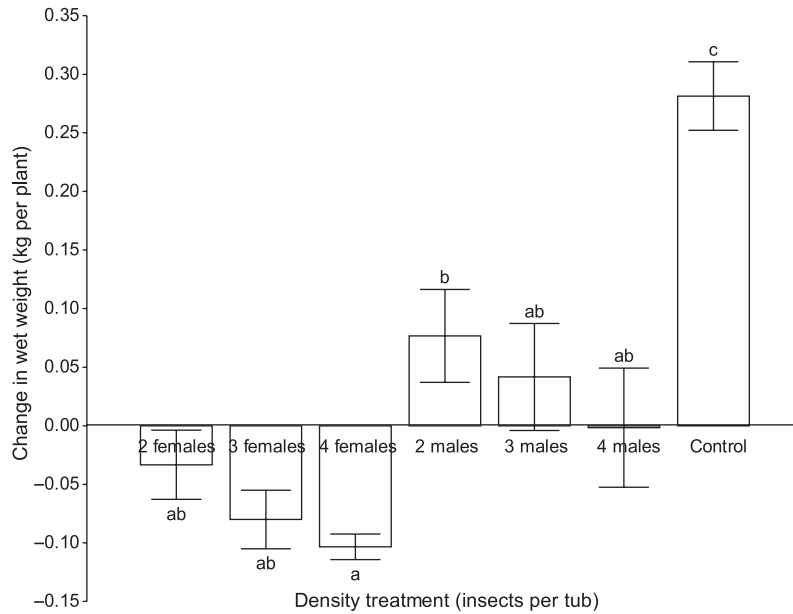
Figure 4 Mean (± SEM) weight change (kg/plant) of *Eichhornia crassipes* over 8 weeks of herbivory by *Cornops aquaticum*. Means compared by one-way ANOVA; bars capped with the same letter are not significantly different (Tukey's HSD: P>0.05).

Figure 5 Mean (± SEM) number of leaves on *Eichhornia crassipes* plants over 8 weeks of herbivory by *Cornops aquaticum*. Means compared by one-way ANOVA; bars capped with the same letter are not significantly different (Tukey's HSD: P>0.05).

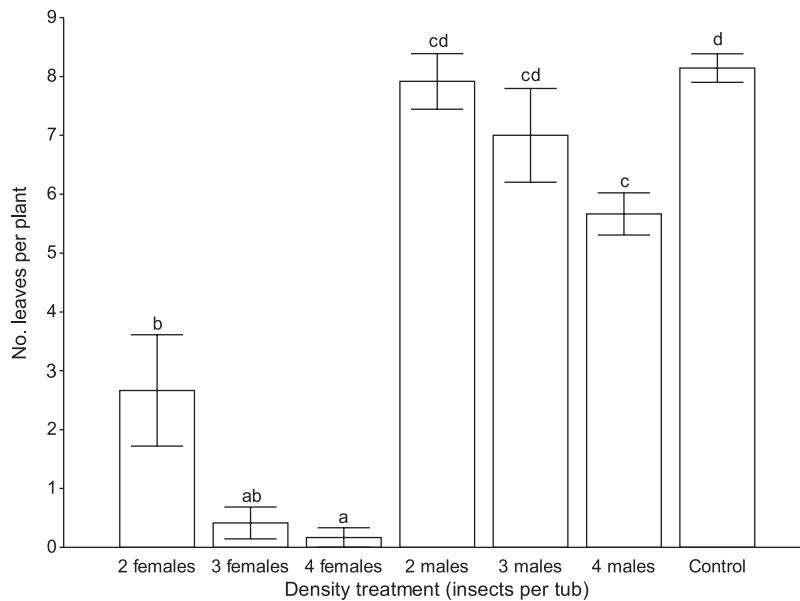*E. crassipes*' ability to compensate for herbivory was a linear or curvilinear function of insect biomass, which is a relationship commonly observed between plants and phytophagous insects (Meyer, 1998; Schooler & McEvoy, 2006; Stanley et al., 2007). Overall, the results suggest that herbivore loads greater than one grasshopper per plant are sufficient to cause significant reductions in *E. crassipes* bio-mass and vegetative reproduction under eutrophic nutrient conditions. The density–damage relationships between *E. crassipes* and *C. aquaticum* found in this study satisfies the condition of McClay & Balciunas (2005) of a promising biocontrol agent whereby a candidate is only justified in release if it has, at realistic field densities, the ability to reduce fitness of their host plant.
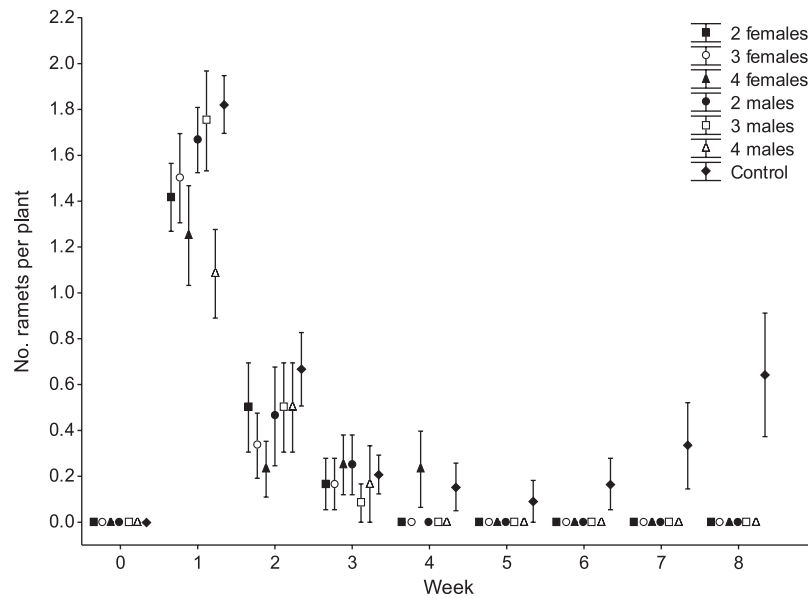
Figure 6 Mean (± SEM) ramet production of *Eichhornia crassipes* plants from week 0 to week 8 of herbivory by *Cornops aquaticum*.

Female grasshoppers were much more damaging than males, probably due to their higher feeding rates (Bownes, 2009) and oviposition behaviour. Plants were unable to compensate for leaf loss, resulting in leaf production and growth rates too low for plants to survive. The severity of damage was exacerbated by the high density of egg packets and ovipositor-probing holes. Egg packets are likely to interfere with translocation of nutrients, and probing holes cause water logging and weakening of the petioles which can then break off from the crown of the plant. In the male herbivory treatments, the severity of damage was not sufficient to compromise plant survival. However, an overall reduction in plant performance parameters and potential for growth and accumulation of biomass indicates that resource acquisition and assimilation were limited in all the male treatments compared to control plants.

Patterns of ramet production were initially a response to removal of ramets at the start of the trial, probably due to increased light penetration to the crown (Méthy & Roy, 1993), then as a response to herbivory by *C. aquaticum*. Removing the ramets stimulated clonal growth, with all plants producing a similar number within the first 4 weeks of the trial. Sustained herbivory at all densities of male and female grasshoppers suppressed further ramet production, despite having fewer leaves and ramets to block out light (Méthy et al., 1990), whereas control plants continued to reproduce vegetatively throughout the study period. Asexual reproduction drives the increase in *E. crassipes* popu-

lations (Byrne et al., 2010) therefore a reduction in ramet production should reduce the expansion rate of *E. crassipes* mats and reduce its invasive potential.

There is no doubt that at high population densities, the grasshopper would suppress density increase and spread of *E. crassipes*. However, predicting whether these grasshopper population densities will be realized in the field is difficult. *Neochetina eichhorniae* and the mirid *Eccritotarsus catarinensis* Carvalho are currently the most widespread and damaging biocontrol agents for *E. crassipes* in South Africa. Their population dynamics and impact on *E. crassipes* populations are dependent on water nutrient (Heard & Winterton, 2000; Coetzee et al., 2007; Byrne et al., 2010) and climatic (Byrne et al., 2010) conditions. *Eichhornia crassipes* leaf nitrogen content is highly correlated with water nitrate levels (Gossett & Norris, 1971; Bownes, 2009) so plant quality, for insect growth and development, is high under eutrophic nutrient conditions. Center & Dray (2010) demonstrated that bottom-up regulation of biocontrol agent populations can influence agent efficacy and expectations for biological control programmes, however, not all insects respond similarly to variation in plant quality. For example, *N. eichhorniae*'s congener, *Neochetina bruchi* Hustache responds positivity to increased leaf tissue nitrogen whereas *N. eichhorniae* has similar reproductive output and rates of population growth under a range of nutritional conditions (Heard & Winterton, 2000; Center & Dray, 2010). The complexity and interrelatedness of water and plant nutrient levels and their impact on plant

and insect growth and development makes it difficult to predict their exact effects on efficacy of candidate biocontrol agents. *Cornops aquaticum*'s per capita impact on *E. crassipes* is similarly influenced by nutrient availability and therefore plant growth rates, and the grasshopper exhibits nutrient-dependent plasticity in its feeding rates and reproduction (Bownes, 2009). We can therefore expect the nutrient status of water bodies in South Africa to influence the efficacy of *C. aquaticum*. However, by comparison of *C. aquaticum*'s impact on *E. crassipes* competitive performance with that of *E. catarinensis* and the *Neochetina* weevils, the grasshopper has a greater impact on plant performance under eutrophic nutrient conditions (Bownes et al., 2010). Furthermore, interaction studies investigating the impact of combinations of the grasshopper with *N. eichhorniae* and *E. catarinensis* on *E. crassipes* revealed that the optimal combination for the greatest impact on plant productivity was a combination of *C. aquaticum* and *N. eichhorniae* (A King & A Nongogo, unpubl.). Their effect in suppressing *E. crassipes* growth and productivity was greater than any other combination of the agents tested and any of the agents in isolation.

The findings from this and other studies (Bownes, 2009; Bownes et al., 2010) predict high levels of agent impact. However, reports from Mexico where *C. aquaticum* is adventive, indicate that this species does not contribute to control of *E. crassipes* (Martínez Jiménez & Gómez Balandra Ma, 2007). Furthermore, densities of 30 *C. aquaticum* individuals m^{-2} in the Amazonian floodplain could not prevent vegetative reproduction and therefore development of *E. crassipes* mats (Adis & Junk, 2003). It is for these reasons that all ecological and impact data as well as results from the interaction trials will be collated to take a holistic approach in deciding whether the grasshopper will be a worthwhile introduction into the South African biocontrol programme.

In conclusion, *E. crassipes* is highly susceptible to the type of damage caused by *C. aquaticum*, even under eutrophic conditions. These results indicate that *E. crassipes* cannot compensate effectively at any grasshopper density to maintain its prolific production and growth rates. Reductions in *E. crassipes* growth were insect density-dependent and the relationship between grasshopper density and plant performance indicates promise for *C. aquaticum* as a biological control agent for *E. crassipes*, and based on these findings would be justified for release.

Acknowledgements

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