

**EVALUATION OF A PLANT-HERBIVORE SYSTEM IN
DETERMINING POTENTIAL EFFICACY OF A
CANDIDATE BIOLOGICAL CONTROL AGENT,
CORNOPS AQUATICUM FOR WATER HYACINTH,
*EICHHORNIA CRASSIPES***

A thesis submitted in fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

ANGELA BOWNES

December 2008

Abstract

Water hyacinth, *Eichhornia crassipes* Mart. Solms-Laubach (Pontederiaceae), a free-floating aquatic macrophyte of Neotropical origin, was introduced into South Africa as an ornamental aquarium plant in the early 1900's. By the 1970's it had reached pest proportions in dams and rivers around the country. Due to the sustainability, cost efficiency and low environmental risk associated with biological control, this has been a widely used method in an attempt to reduce infestations to below the threshold where they cause economic and ecological damage. To date, five arthropod and one pathogen biocontrol agents have been introduced for the control of water hyacinth but their impact has been variable. It is believed that their efficacy is hampered by the presence of highly eutrophic systems in South Africa in which plant growth is prolific and the negative effects of herbivory are therefore mitigated. It is for these reasons that new, potentially more damaging biocontrol agents are being considered for release.

The water hyacinth grasshopper, *Cornops aquaticum* Brünner (Orthoptera: Acrididae), which is native to South America and Mexico, was brought into quarantine in Pretoria, South Africa in 1995. Although the grasshopper was identified as one of the most damaging insects associated with water hyacinth in its native range, it has not been considered as a biocontrol agent for water hyacinth anywhere else in the world. After extensive host-range testing which revealed it to be safe for release, a release permit for this candidate agent was issued in 2007. However, host specificity testing is no longer considered to be the only important component of pre-release screening of candidate biocontrol agents. Investigating biological and ecological aspects of the plant-herbivore system that will assist in determination of potential establishment, efficacy and the ability to build up good populations in the recipient environment are some of the important factors. This thesis is a pre-release evaluation of *C. aquaticum* to determine whether it is sufficiently damaging to water hyacinth to warrant its release. It investigated interactions between the grasshopper and water hyacinth under a range of nutrient conditions found in South African water bodies as well as the impact of the grasshopper on the competitive performance of water hyacinth.

Both plant growth rates and the response of water hyacinth to herbivory by the grasshopper were influenced by nutrient availability to the plants. The ability of water hyacinth to compensate for loss of tissue through herbivory was greater under eutrophic nutrient conditions. However, a negative linear relationship was found between grasshopper biomass and water hyacinth performance parameters such as biomass accumulation and leaf production, even under eutrophic conditions. Water hyacinth's compensatory ability in terms of its potential to mitigate detrimental effects of insect feeding was dependent on the amount of damage caused by herbivory by the grasshopper. Plant biomass and the competitive ability of water hyacinth in relation to another free-floating aquatic weed species were reduced by *C. aquaticum* under eutrophic nutrient conditions, in a short space of time. It was also found that grasshopper feeding and characteristics related to their population dynamics such as fecundity and survival were significantly influenced by water nutrient availability and that environmental nutrient availability will influence the control potential of this species should it be released in South Africa. *Cornops aquaticum* shows promise as a biocontrol agent for water hyacinth but additional factors that were not investigated in this study such as compatibility with the South African climate and the current water hyacinth biocontrol agents need to be combined with these data to make a decision on its release. Possible management options for this species if it is to be introduced into South Africa are discussed.

Table of contents

Title page.....	i
Abstract.....	ii
Table of Contents.....	iv
List of Figures.....	vii
List of Tables.....	xiv
Acknowledgements.....	xv

Chapter 1: Introduction

1.1 General Introduction.....	2
1.2 The plant-herbivore system.....	7
1.2.1 Water hyacinth, <i>Eichhornia crassipes</i>	7
1.2.2 The water hyacinth grasshopper, <i>Cornops aquaticum</i>	8
1.3 The South African water hyacinth biological control programme.....	10
1.4 Recent developments in biological control.....	13
1.4.1 Pre-release efficacy testing.....	13
1.4.2 Ecology of the plant-herbivore system.....	15
1.5 Thesis outline.....	16

Chapter 2: Influence of nitrate and phosphate levels occurring in South African water bodies on the potential of *Cornops aquaticum* to growth and productivity of water hyacinth

2.1 Introduction.....	19
2.2 Materials and Methods.....	24
2.2.1 Nutrients.....	24
2.2.2 Experimental design.....	26
2.2.3 Statistical analysis.....	27
2.3 Results.....	28
2.3.1 Nitrogen and phosphorus levels of water hyacinth leaves 3, 4 and 5 from chemical analysis and evaluation of the relationship between leaf nitrogen content (%) and water nitrate levels (mgL^{-1}).....	28
2.3.2 Effect of water nutrient treatment on the wet weight of water hyacinth leaves of plants grown at high, medium and low nutrient levels.....	29
2.3.3 Effect of nutrients and herbivory on plant biomass.....	30
2.3.4 Effect of nutrients and herbivory on plant growth parameters.....	32

2.3.5 Effect of nutrients and herbivory on leaf, ramet and flower production	36
2.3.6 Effect of nutrient treatment on feeding damage and leaf preference.....	40
2.4. Discussion.....	41
 Chapter 3: Performance of <i>Cornops aquaticum</i> in response to variation in water hyacinth plant quality as influenced by environmental nitrogen (N) availability	
3.1 Introduction.....	52
3.2 Materials and Methods.....	57
3.2.1 Effect of plant nutrient levels on <i>Cornops aquaticum</i> survival, fecundity and body size.....	57
3.2.1.1 Trial 1 – Experimental design.....	57
3.2.1.2 Trial 2 – Experimental design.....	58
3.2.1.3 Statistical analysis.....	58
3.2.2 Effect of plant nutrient levels on survival and feeding and development rates of <i>Cornops aquaticum</i> nymphs.....	59
3.2.2.1 Experimental design.....	59
3.2.2.2 Statistical analysis.....	60
3.3 Results.....	61
3.3.1 Effect of nutrient levels on survival, fecundity and body size.....	61
3.3.2 Effect of nutrient treatment on survival and development rates of <i>C. aquaticum</i>	66
3.3.3 Effect of nutrient treatment on feeding rates of <i>Cornops</i> <i>aquaticum</i> nymphs.....	69
3.4 Discussion.....	72
 Chapter 4 Investigating the response of water hyacinth to herbivory by different densities of male and female <i>Cornops aquaticum</i> under eutrophic water nutrient conditions	
4.1 Introduction.....	85
4.2 Materials and Methods.....	91
4.2.1 Experimental design.....	91
4.2.2 Statistical analysis.....	92
4.3 Results.....	93
4.3.1 Effect of insect treatment on plant biomass.....	93
4.3.2 Effect of insect treatment on leaf parameters.....	96
4.3.3 Effect of insect treatment on plant growth and productivity parameters.....	99
4.3.4 Effect of insect treatment on defoliation.....	105
4.3.5 Effect of insect treatment on leaf area damage.....	106
4.4 Discussion.....	107

Chapter 5: Inter- and intraspecific competitive interactions between water hyacinth and water lettuce as influenced by <i>Cornops aquaticum</i> herbivory	
5.1 Introduction.....	121
5.2 Materials and Methods.....	126
5.2.1 <i>Experimental design</i>	126
5.2.2. <i>Statistical analysis</i>	127
5.3 Results.....	128
5.3.1 <i>Impact of herbivory on competition</i>	128
Water hyacinth.....	128
Water lettuce.....	132
5.3.2 <i>Impact of herbivory on plant biomass (mean end-weights)</i>	134
5.3.3 <i>Impact of herbivory and competition on water hyacinth biomass (total end-weights)</i>	135
5.4 Discussion.....	137
 Chapter 6: General discussion	 144
 References	 157

List of figures

Figure 2.1 Relationship between water nitrate levels (mgL^{-1}) and mean nitrogen content of leaves 3, 4 and 5 of water hyacinth plants grown at high, medium and low nutrient levels ($n = 15/\text{leaf position/nutrient treatment}$).....	29
Figure 2.2 Mean wet weight (g) of the fourth leaf of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample size, $P < 0.05$). Error bars represent the standard error of the mean.	30
Figure 2.3 Mean start and end weights (kg) of water hyacinth plants grown at high (H), medium (M) and low (L) nutrient levels in the herbivory treatment (E) and the control (C). Means compared by one-way ANOVA; those followed with the same letter are not significantly different. Error bars represent the standard error of the mean.....	31
Figure 2.4 Mean change in wet weight (kg) of water hyacinth plants in the herbivory treatment and the control in response to herbivory by <i>Cornops aquaticum</i> . Plants grown at high, medium and low nutrient levels for ten weeks. Means compared by two-way ANOVA, those with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.	32
Figure 2.5 Impact of herbivory by <i>Cornops aquaticum</i> on the number of leaves of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD for unequal sample size, $P < 0.05$). Error bars represent the standard error of the mean.....	33
Figure 2.6 Impact of herbivory by <i>Cornops aquaticum</i> on the mean length of the longest petiole (cm) of water hyacinth plants grown at high, medium or low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.....	34
Figure 2.7 Temporal development of the longest petiole (cm) in response to nutrient treatment and herbivory by <i>Cornops aquaticum</i> . Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.....	34

Figure 2.8 Impact of herbivory by <i>Cornops aquaticum</i> on the mean length of the leaf 2 petiole (cm) of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.....	35
Figure 2.9 Temporal development of the leaf 2 petiole (cm) in response to nutrient treatment and herbivory by <i>Cornops aquaticum</i> . Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.....	36
Figure 2.10 Leaf production in response to herbivory by <i>Cornops aquaticum</i> of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.....	37
Figure 2.11 Ramet production in response to herbivory by <i>Cornops aquaticum</i> of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.....	38
Figure 2.12 Temporal development of ramet production in response to nutrient treatment and herbivory by <i>Cornops aquaticum</i> . Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.....	38
Figure 2.13 Flower production in response to herbivory by <i>Cornops aquaticum</i> of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.....	39
Figure 2.14 Temporal development of flower production in response to nutrient treatment and herbivory by <i>Cornops aquaticum</i> . Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.....	40

Figure 2.15 Average feeding damage score (A) and mean position of the most damaged leaf (B) in <i>Cornops aquaticum</i> herbivory treatments. Water hyacinth plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.	41
Figure 3.1 Mean body weight of male and female grasshoppers reared on plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes; $P < 0.05$). Error bars represent the standard error of the mean.....	62
Figure 3.2 Percentage of first generation nymphs to survive from 1 st instar to adulthood and proportion of males and females among survivors reared on plants grown at high, medium and low nutrient levels (Trial 1; n = 61).....	63
Figure 3.3 Mean number of egg packets oviposited by female grasshoppers. Trial 1 females reared on plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same levels. Trial 2 females reared on plants grown at high, medium and low nutrient levels and transferred to high nutrient plants at adulthood. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.....	64
Figure 3.4 Mean number of nymphs per egg packet of female grasshoppers reared on water hyacinth plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same nutrient levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.	65
Figure 3.5 Mean weight (g) of newly emerged nymphs of female grasshoppers reared on plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.	65
Figure 3.6 Relationship between the no. of egg packets (fecundity) and body weight (g) of female grasshoppers on plants grown at high, medium and low nutrient levels.....	66

Figure 3.7 Percentage of nymphs to survive from 1 st instar to adulthood and proportions of males and females among survivors reared on cut leaves of plants grown at high, medium and low nutrient levels (n= 21).....	67
Figure 3.8 Mean number of days for development of nymphs from newly hatched 1 st instar to adulthood reared on cut leaves of plants grown at high, medium and low nutrient levels. Medians compared by Kruskal-Wallis ANOVA. No significant differences.....	68
Figure 3.9 Mean number of days for instars 1-5 of nymphs reared on cut leaves of plants grown at high, medium and low nutrient levels. Medians compared by Kruskal-Wallis ANOVA; those with the same letter are not significant different.....	69
Figure 3.10 Mean weight of faeces (g) of male and female nymphs from 1 st instar to adulthood as a measure of consumption rates. Medians compared by Kruskal-Wallis ANOVA and Mann-Whitney U tests. Those followed with the same letter are not significantly different.....	70
Figure 3.11 Mean weight of faeces (g) of male and female nymphs from 1 st instar to adulthood reared on plants grown at high, medium and low nutrient levels. Error bars represent the standard error of the mean. (■ Low female; ● Medium female; ● Low male; ▲ Medium male; ■ High female).....	71
Figure 4.1 Schematic representation of a damage curve for a candidate weed biocontrol agent adapted by McClay & Balciunas (2005) from Peterson and Higley (2001). Biocontrol agent load on the x-axis represents the number of biocontrol agents per unit of plant biomass. The y-axis is a relevant measure of plant performance or fitness such as growth rate or final biomass. The solid curve shows a damage curve for a potentially effective agent and the dotted line shows a candidate agent with little effect on plant performance even at high herbivore loads.....	88
Figure 4.2 Mean wet weight (kg) of water hyacinth plants at the start and end of the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Error bars represent the standard error of the mean.....	94

Figure 4.3 Mean weight change (kg) from the start to the end of the eight week study period of water hyacinth plants in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> . Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.	95
Figure 4.4 Regression of <i>Cornops aquaticum</i> biomass (g) and final weight (kg) of water hyacinth plants at the end of the eight week study period. Insect biomass represented by the mean weight of male or female Grasshoppers multiplied by the respective density.....	96
Figure 4.5 Mean number of leaves of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	98
Figure 4.6 Regression of <i>Cornops aquaticum</i> biomass (g) and the total number of leaves of water hyacinth plants at the end of the eight week study period. Insect biomass represented by the mean weight of male or female grasshoppers multiplied by the respective densities.....	98
Figure 4.7 Changes in the number of leaves of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> . Error bars represent the standard error of the mean.....	99
Figure 4.8 Mean length of the longest petiole (cm) of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	100
Figure 4.9 Changes in the mean length of the longest petiole (cm) of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> . Error bars represent the standard error of the mean.....	101

Figure 4.10 Mean length of the leaf 2 petiole (cm) of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	102
Figure 4.11 Changes in the mean length of the leaf 2 petiole (cm) of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> . Error bars represent the standard error of the mean.....	103
Figure 4.12 Mean number of ramets produced by water hyacinth plants in the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	104
Figure 4.13 Ramet production of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> . Error bars represent the standard error of the mean.....	104
Figure 4.14 Mean number of flowers produced by water hyacinth plants in the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	105
Figure 4.15 Mean number of petioles of water hyacinth plants defoliated at the End of the eight week study period in the herbivory treatments. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....	106

- Figure 4.16** Damage scores for the percentage of leaf area damaged on leaf 4 (A) and the average percentage of damage to all leaves (B) of water hyacinth plants at the end of the eight week study period in the herbivory treatments. Herbivory treatments included 2, 3 and 4 male or female *Cornops aquaticum*. Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).....107
- Figure 5.1** Multiple regression planes indicating the effects of *C. aquaticum* herbivory and plant densities on the inverse of the mean wet weight (kg) per water hyacinth plant. A and B compare relative competitive abilities of water hyacinth in the presence and absence of *C. aquaticum* herbivory respectively. Points indicate observations ($n = 18$) and the vertical lines between the data points indicate the residuals. Values on the X and Y axes are the original planting densities of water hyacinth and water lettuce.....131
- Figure 5.2** Multiple regression planes indicating the effects of *C. aquaticum* herbivory and plant densities on the inverse of the mean wet weight (kg) per water lettuce plant. A and B compare relative competitive abilities of water lettuce in the presence and absence of *C. aquaticum* herbivory respectively. Points indicate observations ($n = 18$) and the vertical lines between the data points indicate the residuals. Values on the X and Y axes are the original planting densities of water hyacinth and water lettuce.....133
- Figure 5.3** Mean end weights (kg) of individual water hyacinth (A) and water lettuce (B) plants in the herbivory treatments and the controls at planting densities of 3 and 9. Means followed by the same letter are not significantly different. Error bars represent the standard error of the mean.134
- Figure 5.4** Mean total water hyacinth biomass at low planting density in combination with no, or high or low levels of competition from water lettuce, either exposed to herbivory by *C. aquaticum*, or not (control). Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.....136
- Figure 5.5** Mean total water hyacinth biomass at high planting density in combination with no, high or low levels of competition from water lettuce, either exposed to herbivory by *C. aquaticum* or not (control). Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.137

List of tables

Table 1.1 Feeding guilds, distribution and release dates of biological control agents established on water hyacinth in South Africa.....	11
Table 2.1 Classification of water bodies according to nitrate levels in mgL^{-1} from the South African Water Quality Guidelines (Holmes, 1996).....	25
Table 2.2 Concentrations of nitrates and phosphates in mgL^{-1} used to represent nutrient levels found in South African river systems and impoundments ranging from highly eutrophic to oligotrophic.....	25
Table 2.3 Nitrogen and phosphorus content (% dry weight) of water hyacinth leaves from positions 3, 4 and 5. Plants grown at high, medium and low nutrient levels in the absence of herbivory by <i>Cornops aquaticum</i> (n = 15/leaf position/nutrient treatment).....	29
Table 2.4 Concentrations of nitrogen (N) and phosphorus (P) used in the present and two other studies to evaluate the impact of herbivory by biocontrol agents on water hyacinth under different nutrient regimes.....	45
Table 3.1 Mean assimilation efficiencies (AD) of 1 st to 5 th instar nymphs reared on cut water hyacinth leaves with high, medium and low nitrogen content (% dry weight).....	72
Table 4.1 Leaf production and mortality rates (leaves day^{-1}) and number of days to produce one new leaf (days leaf^{-1}) of water hyacinth plants during the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female <i>Cornops aquaticum</i> per plant.....	97
Table 5.1 Multiple regression analysis of the effects of herbivory by <i>Cornops aquaticum</i> and plant density on the reciprocal of water hyacinth yield and water lettuce yield (wet weight (kg)).....	130

Acknowledgements

I would like to thank my supervisors, Professor Martin Hill and Professor Marcus Byrne, for their guidance and constructive and positive comments on the thesis. The Working for Water (WfW) Programme are thanked for funding the research and the Agricultural Research Council-Plant Protection Research Institute for the use of facilities. A very special thanks to my parents, Sean and Lesley Bownes and my siblings Sarah, Richard and Gwynneth Bownes who are always there for support, encouragement and good advice. I'm very grateful to my mom for help with proof reading and providing delicious meals and lots of encouragement during the last days of writing. I would also like to thank the following people who have helped me in various ways:

- Ryan Brudvig for very kindly providing me with his data on water nutrient levels found in S.A. water bodies with water hyacinth
- Lawrence Mophosa for assistance with plant culture maintenance and heavy duty lifting of equipment
- Fritz Heystek and Yogi Kistensamy for help with sampling one christmas holiday
- Dr. Stephan Naser for advice on plants and insects
- Danica Lukac for helpful discussions and all the staff at Rietondale for their encouragement and friendship during my years at the Rietondale campus
- Cathy Stevens for advice on stats
- Dr. Julie Coetzee for assistance with the nutrient trial
- Ayanda Nongogo for help with processing leaf samples
- Marianne Olivier for keeping me sane and always being there for some fun and Kirsten Reid for entertaining phone calls over the last four years
- Other friends who have given me lots of encouragement: Tarryn Hunter, Laurie Whitacre and Nicky Wells

And lastly, an extra special thank you to Michael Braack for love, support, advice, help with proof reading and cups of tea and Georgie and Oliver for always keeping me company during long hours behind the computer.

Chapter 1

Introduction

1.1 General introduction

Man has facilitated the dispersion of non-indigenous species for millennia (Everett, 2000) but the negative ecological impact of these biological invasions in areas of introduction has only been recognized in the last few decades. The impact of invasive species is the second highest cause of loss of biodiversity throughout the world, after human population growth and associated activities (Vitousek et al., 1997; Pimentel, 2002). Characteristics of invasive species such as a high reproductive rate and the ability to tolerate a wide range of climatic and environmental conditions, as well as the absence of natural predators or parasites in their introduced range, are generally assumed to be responsible for their rapid establishment, spread and persistence in new environments (Keane & Crawley, 2002; Shea & Chesson, 2002). Invasive weeds are particularly successful biological invaders that threaten natural and agricultural environments throughout the world, and they are also the most significant economic pests (McFadyen, 1998). They reduce biodiversity, displace indigenous flora and fauna, disrupt farming and forestry production (Pimentel, 2002) and invade aquatic ecosystems precluding the use of river systems and impoundments.

Water hyacinth, *Eichhornia crassipes* Mart. Solms-Laubach (Pontederiaceae) is South Africa's most problematic invasive aquatic weed (Cilliers, 1991). It is also considered one of the world's worst aquatic weeds (Holm et al., 1977; Wright & Purcell, 1995) and economic losses worldwide are in the region of millions of U.S. dollars each year. The weed's invasive potential in its introduced range stems from its rapid growth rate, vegetative reproduction, its ability to re-infest via seed or flood-borne plants, the absence of natural enemies (Harley et al., 1996) and particularly in South Africa, the presence of highly eutrophic systems in which the weed proliferates (Hill & Cilliers, 1999; Hill & Olckers, 2001; Winterton & Heard, 1996; Coetzee et al., 2007; De Villiers & Thiart, 2007). Problems associated with water hyacinth infestations are a reduction in the quality of drinking water due to bad odour and colour (Hill, 1999); reductions in biodiversity as a result of limited light and oxygen in the water (Ultsch, 1973; Midgley et al., 2006) and water hyacinth having a competitive advantage over native plant species; loss of water through evapotranspiration (Timmer & Weldon, 1966; Holm et al., 1977; Lallana et al.,

1987; Wright & Purcell, 1995); damage to bridges, dam walls and canals during flooding and disruption of local recreational activities such as fishing and boating. South Africa relies heavily on water resources for the livelihood of rural communities and as an extractable resource for mining, industrial and urban uses (Jones, 2001), therefore in many cases it is imperative to manage water hyacinth infestations.

Of the methods that are available for controlling water hyacinth, biological control is the only sustainable and economically cost-effective means (van Wilgen et al., 2001) of managing infestations (Cilliers, 1991). Although water hyacinth is effectively controlled biologically in many parts of the world (Harley, 1990; Center, 1994; Julien & Orapa, 1999; Julien et al., 1996; Ogwang & Molo, 1999), South Africa in general has not achieved an adequate level of control. Five constraining factors have been identified and discussed by Hill & Cilliers (1999) and Hill & Olckers (2001) as contributing to the limited success of the five arthropod agents that have been released thus far: (1) many water bodies infested with water hyacinth are highly eutrophic, enriched with nitrates and phosphates from industrial and sewerage effluents and run-off that becomes polluted with these nutrients through agricultural activities. Water hyacinth grows prolifically under high nutrient conditions (Reddy et al., 1989; 1990) and agent populations are apparently unable to suppress plant populations with these enhanced growth rates (Coetzee et al., 2007). High levels of plant nitrogen can have a positive effect on water hyacinth biocontrol agent fecundity, growth and development (Heard & Winterton, 2000) however high water nutrient conditions predominantly have adverse effects on control by significantly reducing their efficacy in suppressing weed growth (Heard & Winterton, 2000; Coetzee et al., 2007). Tall, vigorous plants and dense water hyacinth infestations are associated with nutrient-enriched systems. Eutrophication is therefore considered to be one of the major factors hampering successful biological control in South Africa; (2) Water hyacinth proliferates in a wide range of climatic conditions in South Africa including (i) temperate summer rainfall regions at high altitudes (above 1500m) where frosting occurs frequently during the colder months (May to August); (ii) coastal, Mediterranean winter rainfall areas where frost is absent; and (iii) coastal subtropical summer rainfall areas. The agents, native to tropical and subtropical South America are

intolerant of winter temperatures in certain climatic regions. Although the agents have established throughout this climatic range, almost certainly a high mortality rate and low reproductive output during the cooler months result in a sharp decline in population densities. The plants die back in winter as a result of frost but their populations recover quickly at the onset of spring and agent populations are apparently unable to match the plants rapid increase. The result is that in temperate regions they rarely reach population densities needed to severely stress the plants. Successful biological control of water hyacinth has been limited to subtropical and tropical areas around the world (Hill & Cilliers, 1999; Julien, 2001), suggesting that climate has a significant influence on the efficacy of the agents. The effect of temperature on the development rate of insects is well known (Clarke, 1996) and low temperatures are often responsible for failure of agents to establish or have a significant impact on the target weed (McClay & Hughes, 1995; McClay, 1996; Byrne et al., 2002); (3) Plant quality also affects agent establishment and success as some agents have very specific requirements and have therefore not established at sites where water hyacinth plants are not suitable for maintaining populations. For example, the pyralid moth, *Niphograpta albigutallis* Warren (Lepidoptera: Pyralidae) has a strong preference for young, actively growing tissue on water hyacinth plants with bulbous petioles. The moth is most effective on new infestations and this preference has resulted in spatial and temporal variation in their population densities; (4) Water hyacinth infested rivers experience periodic flooding which flushes the system of the plants as well as the agents that are established. Re-infestation of water hyacinth is rapid and plants quickly reach pest proportions when released from herbivore pressure (Center et al., 1999a); (5) Interference with biological control by herbicide applications is another major factor preventing agent establishment and efficacy at many sites around South Africa. Temporary destruction of a weed population using herbicides results in mortality and dispersal of the biocontrol agents. The resultant situation is similar to that caused by flooding in that re-infestation occurs through seed germination or from plants left untreated and plant populations grow prolifically in the absence of natural enemies (Center et al., 1999a). Biological and herbicidal control can work synergistically (Jadhav et al., 2008) but requires the use of selective pesticides in a carefully managed control programme (Jones, 2001; Ueckermann

& Hill, 2001). Successful biological control of water hyacinth is often negated through injudicious herbicide applications.

Hill & Olckers (2001) also identified two other possible factors that have influenced the efficacy of introduced natural enemies in South Africa. They are: (1) techniques for establishing agents. Agents have failed to establish due to poor release effort and insufficient numbers being released for successful establishment. Establishment of agent populations is the first and most important step and is a prerequisite to control (Julien, 2001); and (2) hydrological features which have been underestimated as an important factor influencing biological control. Many of our water hyacinth sites are small impoundments that are not subject to wind and wave action, which evidently helps to break up mats that have already suffered significant damage by natural enemies. The spectacular success of biological control in Papua New Guinea and on Lake Victoria and Lake Kyoga has partly been attributed to the action of wind and waves that assisted the rate of damage and sinking of the mats (Julien & Orapa, 1999; Ogwang & Molo, 1999; Cock et al., 2000).

The limited success of agents established on water hyacinth in South Africa, identification of factors that potentially limit biocontrol and continuation of water hyacinth to invade new systems (e.g. The Little Toti River on the KwaZulu-Natal coast; Hughes, pers. comm.) and persist in those already invaded (e.g. The Vaal River) prompted consideration of additional agents that were identified as promising during surveys in South America (Cordo, 1999). This led to the introduction of the water hyacinth grasshopper, *Cornops aquaticum* Brünner (Orthoptera: Acrididae) into quarantine in South Africa for host range testing. Testing was completed in 2001, which revealed it to be oligophagous, using species in the family Pontederiaceae (Oberholzer & Hill, 2001). Release of the grasshopper was delayed, despite obtaining a release permit in 2007, for the purposes of investigating the potential of *C. aquaticum* to be a valuable introduction in to the South African biocontrol programme. The impact of the grasshopper and consideration of factors that might influence establishment and efficacy

were investigated so that a decision on its release could be based on biological data of the plant-herbivore system.

In the history of weed biocontrol, many agents have established and become abundant without bringing about effective control of the target plant (Myers, 2000; Denoth et al., 2002; McClay & Balciunas, 2005). For example, the cinnabar moth *Tyria jacobaea* L. (Lepidoptera: Arctiidae), a biocontrol agent for tansy ragwort, *Senecio jacobaea* L. (Asteraceae) became abundant at some release sites but failed to reduce plant biomass (McEvoy et al., 1993). It is ineffective but abundant agents that are likely to be associated with non-target and indirect ecological effects (Holt & Hochberg, 2001; Pearson & Callaway, 2003; 2005). For example, superabundance of agents that fail to reduce populations of their target plant can become a food source that is exploited by local consumers, leading to unnatural increases in their densities (Pearson & Callaway, 2003; 2005). Biocontrol practitioners advocated that agents should be selected not only on the basis of their host-specificity but also on their potential efficacy (Sheppard, 2003; Balciunas, 2004; McClay & Balciunas, 2005) and that studies on a candidate's potential impact should be performed prior to release (Cullen, 1992; McEvoy & Coombs, 2000; Sheppard, 2003). This allows for a more parsimonious approach and an overall reduction in the environmental and economic risks associated with each additional biocontrol agent introduction (McEvoy & Coombs, 1999). Furthermore, biological control has been criticized for not using an ecological approach and that critical factors that will influence efficacy should be well understood before an agent is released. Much of this comes with understanding plant-herbivore interactions. Both plant and insect populations are influenced by multi-trophic interactions and that an insect herbivore feeds and develops on its host plant does not guarantee success in regulating populations of their host. Furthermore, some authors have recommended proceeding with caution in multiple agent introductions in biological control (Denoth et al., 2002; Pearson & Callaway, 2005). In 54% of successful weed biocontrol projects involving multiple agents being introduced against the target weed, only one agent was responsible for bringing about effective control (Denoth et al., 2002). In some cases, the cumulative effect of more than one agent can contribute to effective control (Denoth et al. 2002) but in others, multiple agents may

act antagonistically rather than synergistically. Competitive interactions between insects that have been serially introduced to control invasive alien plants can lead to interference and displacement of one or more species (Huffaker & Kennett, 1969). Denno et al. (1995) also point out that interspecific competition is more intense amongst host-specific herbivores because they lack the option of switching host plants to avoid superior competitors. Agents that have substantial niche overlap may compete for resources such as food, space and oviposition sites, and these competitive effects may reduce control levels on the target weed. Factors important in influencing community structure, plant performance and distribution and insect and plant population dynamics needed to be investigated to make an informed decision on the release of *C. aquaticum* and on its potential role in the biological control of water hyacinth in South Africa.

1.2 The plant-herbivore system.

1.2.1 Water hyacinth, *Eichhornia crassipes*

Water hyacinth is native to the New World Tropics (Center, 1994) but was introduced into other countries as an ornamental aquatic plant for its aesthetic appeal and it is now distributed throughout the subtropical and tropical parts of the world (Pieterse, 1978; Center, 1994). It was first recorded in South Africa in the early 1900's (Gopal, 1987; Cilliers, 1991) and was soon spread to localities throughout the country by gardeners, aquarium owners and recreational boaters (Jacot Guillarmod, 1979). Flooding, currents and water birds also facilitated its spread (Edwards & Musil, 1975) with the result that it is now distributed throughout South Africa, having reached pest proportions in many systems. Despite active measures to manage the plant and maintain it at acceptable levels where its adverse effects on biodiversity and water usage are mitigated, it continues to invade new river systems and impoundments and to persist and increase in abundance in areas already invaded.

Water hyacinth is an erect, free-floating perennial herb (Center, 1994; Wright & Purcell, 1995) although roots may anchor in the substrate in shallow waters or on muddy shores (Pieterse, 1978). The plants have attractive lavender flowers and glossy dark green leaves. In free-floating plants the bulbous petioles provide buoyancy and roots are fibrous

and feather-like and vary in length according to nutrient supply in the water (Center 1994; Wright & Purcell, 1995). Water hyacinth reproduces sexually by the production of seeds which can remain dormant and viable for up to 20 years (Gopal, 1987) and asexually by the production of ramets (daughter plants). By means of vegetative reproduction, plants can double their biomass in two weeks, usually in response to high levels of nitrogen. Water hyacinth responds positively to increasing nitrogen, with increases in growth and reproductive rates proportional to nitrogen availability up to approximately 5.5 mgL⁻¹ (Reddy et al., 1989). Water hyacinth is highly competitive (Agami & Reddy, 1990; Center et al., 2005; Coetzee et al., 2005), and is usually the dominant species in aquatic communities, displacing both indigenous and other invasive species (Wright & Purcell, 1995).

1.2.2 The water hyacinth grasshopper, *Cornops aquaticum*

The grasshopper, *C. aquaticum* is a semi-aquatic species of Neotropical origin, inhabiting lowlands from southern Mexico to central Argentina and Uruguay (Adis et al., 2007). Both adults and nymphs are agile and good swimmers and the adults are strong fliers. They are diurnal, bivoltine, overwinter as adults and exhibit a sex ratio of 1:1 in the region of origin. In dense field populations in the native range, they occur at a density of one grasshopper per plant. They are susceptible to generalist predators such as spiders, frogs and fish and have a specialist egg predator, the weevil, *Ludovix fasciatus* Gyllenhal (Coleoptera: Curculionidae) (Silveira-Guido & Perkins, 1975). Female weevils deposit an egg in *C. aquaticum* egg packets and the developing larva feeds on the eggs until pupation (Hill, pers. comm.). High populations of a predaceous katydid, *Phlugis coriacea* Redtenbacher (Orthoptera: Tettigoniidae) are also associated with populations of *C. aquaticum* in Uruguay and Argentina (Silveira-Guido & Perkins, 1975).

Cornops aquaticum egg cases, containing 30 – 70 eggs, are constructed of a hard, foamy substance and are inserted into the youngest petiole by the ovipositing females, usually just above the crown of the plant (Hill & Cilliers, 1999; Oberholzer & Hill, 2001). The endophytic position of the egg case ensures that enough moisture to prevent dessication is provided during incubation and the aerenchyma tissue of the petiole prevents excessive

water uptake and drowning of the eggs (Silveira-Guido & Perkins, 1975). The eggs hatch after 25 to 30 days and the nymphs begin to feed on water hyacinth leaves immediately after hatching. The nymphs develop for 40 to 55 days (Oberholzer & Hill, 2001; Adis & Junk, 2003) passing through five to seven instars (Oberholzer & Hill, 2001; Adis & Junk, 2003; Adis et al., 2004). This variation in the number of instars has been attributed to the effect of climate conditions such as photoperiod and temperature in different geographical regions (Adis et al., 2004; Brede et al., 2007).

Perkins (1974) identified *C. aquaticum* as one of the most damaging insects associated with water hyacinth in its native range. The grasshopper was first imported into South Africa in 1995, the specimens collected from water hyacinth mats in Manaus, Brazil. Subsequent collections were made in Trinidad and Venezuela in 1996 and in Mexico in 1997. Specimens collected from all three localities were mixed together to form the laboratory culture on which all testing was done. The grasshoppers are defoliators and both nymphs and adults are extremely damaging to water hyacinth. The host-range of *C. aquaticum* was investigated by Oberholzer & Hill (2001) in a quarantine facility in Pretoria, South Africa and was determined by adult choice (oviposition) and adult and nymphal no-choice (feeding and development) trials on 64 plants in 32 families, selected on their taxonomic relationship to water hyacinth, similarity in habitat and economic importance. Oberholzer & Hill (2001) found development on two indigenous species, *Monochoria africana* Solms-Laubach (Pontederiaceae) and *Heteranthera callifolia* Kunth. (Pontederiaceae) but they were inferior hosts compared to water hyacinth and their sporadic occurrence in South Africa would prevent the grasshoppers from establishing permanent populations on either species, as was argued in the case for release of *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae), the most recent introduction into the South African water hyacinth biocontrol programme. It has been more than 10 years since this biocontrol agent was released and damage to non-target plants in the field in South Africa has not been recorded. Furthermore, water hyacinth poses a far greater risk to species that are growing sympatrically with water hyacinth through exploitative competition compared to the grasshopper. Its very specific oviposition requirements such as the aerenchyma tissue of water hyacinth petioles are

important in its specificity and the indigenous plant species would not be able to sustain populations of the grasshopper.

1.3 The South African water hyacinth biological control programme

In South Africa, the biological control programme against water hyacinth was initiated in 1974 when the water hyacinth weevil, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) was released onto infestations on rivers and dams around the country (Cilliers, 1991). The programme was terminated in 1977 when the water hyacinth biocontrol researcher was transferred to another project but was restarted in 1985 when re-releases of the weevil were made (Cilliers, 1991). *Neochetina eichhorniae* established at most water hyacinth sites around South Africa but its impact was variable. The weevil controlled the weed at New Years Dam and was effective in 'reserve' sites on other systems however it became apparent that this agent would not be successful in all areas of South Africa because of poor adaptation to the more temperate climates. Subsequently, two other insect species were released. To date, five arthropod natural enemies have been released in South Africa in an attempt to control water hyacinth. Information on the insect biocontrol agents already established in South Africa, such as release dates, distribution and feeding guilds are tabulated in Table 1.1. *Neochetina eichhorniae*'s congener, *N. bruchi* Hustache (Coleoptera: Curculionidae) was released in 1990 at five localities around the country (Cilliers, 1991) and although it established at several sites, numbers remained low (Hill & Cilliers, 1999). However, this species is of value in terms of its ability to damage water hyacinth populations in eutrophic conditions (Winterton & Heard, 1996) and it is cold-tolerant (De Loach & Cordo, 1983). *Neochetina bruchi* could therefore, theoretically, be a valuable agent at water hyacinth sites at high altitudes that are rich in nutrients and experience cold winters (Hill & Cilliers, 1999). High numbers of this agent have been reported at Roodeplaat Dam, a highly eutrophic impoundment (Cilliers, pers. comm.) but in spite of this the water hyacinth infestation remains to be one of South Africa's worst. The moth, *N. albiguttalis* was released in South Africa in 1990 (Cilliers, 1991) and became established at water hyacinth sites throughout the country (Hill & Cilliers, 1999). The moth has a broad climatic range (Cilliers, 1991) and a distinct preference for young plants and actively growing tissue. As a result this species is

particularly effective where mature water hyacinth plants have been thinned by herbicides and mechanical clearing and is also effective in restricting the spread of water hyacinth by attacking plants along the fringes of an infestation (Hill & Cilliers, 1999). The phytophagous mite, *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) was discovered on a water hyacinth infestation in South Africa in 1989, its origin or method of introduction not known (Cilliers, 1991). The mite established in the warmer areas of South Africa where very high population densities have been recorded at the end of summer (Hill & Cilliers, 1999). This agent can reach very high population densities and can be very damaging on water hyacinth suggesting it makes a significant contribution to the control of water hyacinth in South Africa. *Eccritotarsus catarinensis*, a sap-sucking mirid, was released in 1996 and is still the most recent agent to be released against water hyacinth. The mirid is established at at least 19 water hyacinth sites around the country, particularly in the warmer areas of South Africa (Coetzee, pers. comm.). It has had a substantial impact on plants growing in nutrient-rich waters such as at Hammarsdale Dam and Clairwood Quarry in KwaZulu-Natal and although the species seemed to have boom and bust cycles (King, 2008) it is becoming more abundant, widespread and consistent in its impact on water hyacinth (Hill, pers. comm.).

Table 1.1 Feeding guilds, distribution and release dates of biological control agents established on water hyacinth in South Africa.

Biocontrol agent	Year of release	Feeding guild	Main habitat
<i>Neochetina eichhorniae</i>	1974	Petiole miner	Widely distributed
<i>Neochetina bruchi</i>	1990	Petiole miner	Widely distributed
<i>Niphograpta albiguttalis</i>	1990	Petiole miner	Sporadic and localized
<i>Orthogalumna terebrantis</i>	1989	Leaf miner	Widely distributed
<i>Eccritotarsus catarinensis</i>	1996	Sap-sucker	Distributed in warmer areas

All of the agents released against water hyacinth can be very damaging and have the potential to be effective biocontrol agents but in many cases have failed to achieve an

acceptable level of control at many water hyacinth sites around the country. Hill & Olckers (2001) outlined possibilities for the variation in control levels but in general the factors responsible for success or failure are poorly understood due to insufficient post-release monitoring and little understanding of the abiotic and biotic factors that are prevalent in each system. A project funded by the Water Research Commission (WRC) in South Africa involving a collaborative team of researchers and students from University of the Witwatersrand, Rhodes University, ARC-PPRI, and University of Pretoria as well as many other supporting organizations was initiated in 2004 to evaluate the factors hampering or conducive to successful control of water hyacinth. The project addresses three of the factors considered by Hill & Olckers (2001) to contribute to the apparent limited success of the current water hyacinth biological control agents. The effects of climate and water nutrient conditions on plant and insect populations were investigated over a two-year period. Climate and water nutrient data were collected on a monthly basis at 15 water hyacinth sites around the country that were chosen to represent the range of nutrient and climatic conditions prevalent in South African river systems and impoundments with water hyacinth infestations. This aspect of the project aims to quantify the effects of climate and nutrient availability on the control potential of the current biocontrol agents and provide conclusive evidence on whether nutrient-enrichment and low winter temperatures are hampering successful control of water hyacinth. Another aspect of the project investigated the effect of sub-lethal dosages of glyphosate herbicides on both water hyacinth and the biocontrol agents to improve and largely facilitate integration of biological and chemical control of water hyacinth in South Africa. A recent survey of the biocontrol agents currently established in South Africa in water bodies in all provinces with water hyacinth, undertaken by Rhodes University, was aimed at determining whether establishment and distribution of the current biological control agents was limited by release effort. The survey revealed that control levels are better than expected but not yet at an acceptable, manageable level. Poor release effort or purely lack of releases, are considered to be major factors in the limited success of water hyacinth biocontrol in South Africa. It has been suggested that practitioners involved in water hyacinth biocontrol re-look at mass-rearing and re-distribution of the current agents and comprehensive post-release monitoring (Hill, pers. comm.).

1.4 Recent developments in biological control

1.4.1 Pre-release efficacy testing

Biological control of weeds is a widely used method for mitigating the negative effects of invasive alien plants (McFadyen, 1998) but as a result of the disastrous impacts of non-native species evident throughout the world, justification of the number and nature of biocontrol agent releases will increasingly be required (Sheppard, 2003). There is therefore pressure on practitioners to achieve both successful control and to avoid adverse indirect effects and damage to non-target plants. Furthermore, there is escalating pressure to improve post-release monitoring to quantify reasons for success or failure of biocontrol programmes (McEvoy & Coombs, 1999). It is the procedures and strategies used to select biocontrol agents that play a critical role in achieving both safety and success (McClay & Balciunas, 2005).

In the early days of weed biocontrol, efficacy was the primary focus and numerical scoring systems that were heavily weighted towards impact (Balciunas, 2004; McClay & Balciunas, 2005) were developed by Harris (1973) & Goeden (1983) to prioritize agents. Later on, extensive risk assessment became both an ethical and regulatory requirement and host-range testing of candidate agents prior to release became a prerequisite in biocontrol programmes (McClay & Balciunas, 2005). The introduction of mandatory testing meant that limited resources were invested in ensuring release of safe agents that would not damage native non-target vegetation and economically important plants and little attention was paid to efficacy. Ease of handling, transport and rearing of agents were also considerations in the selection of candidate biocontrol agents (Crawley, 1989a). McClay & Balciunas (2005) also suggest that practitioners believe that efficacy is influenced by a multitude of complex, interacting and unforeseeable factors, that accurate predictions are impossible, with selection of the best agent for a programme being referred to as the “holy grail” of weed biocontrol (McFadyen, 1998).

Harris (1979) claimed that at least four agents would be needed in weed biological control programmes to achieve successful control, implying that the cumulative effect of a suite of different insect species contribute to stress on the target plant, the outcome of

which is eventually effective control. Myers (1985) challenged the cumulative stress model, highlighting that Julien's (1982) review of 26 weed biocontrol programmes showed that in 81% of them, success was attributed to a single species, despite multiple species introductions. She tested the conventional belief by observing seed reduction on diffuse, *Centaurea diffusa* Lam. (Asteraceae) and spotted knapweed, *C. maculosa* Lam. by several introduced biological control agents. She concluded that these weed species were not under control despite a number of established biocontrol agents and it should therefore not be assumed that all established species will contribute to control of the target weed. She introduced the idea of a "lottery model" which likened success to odds in a lottery, relying on the chance introduction of an effective species.

This hit-or-miss strategy and the inefficiency of many biocontrol programmes attracted a considerable amount of criticism (McEvoy & Coombs, 1999; 2000). The lack of foresight and the idea that success is based on the chance introduction of the right species in an appropriate location was considered poor scientific practice. Mounting criticism of the science and increasing pressure to reduce the number of agents used in biocontrol programmes (Simberloff & Stiling, 1996; McEvoy & Coombs, 1999) prompted renewed interest in efficacy testing and it is now generally argued that the prudent approach is to evaluate biocontrol agents for potential efficacy in pre-release evaluations (Sheppard, 2003; Balciunas, 2004; McClay & Balciunas, 2005). Better agent screening (Myers, 2000) should improve chances of selecting the best agent, thereby increasing success rates of biocontrol programmes (McEvoy & Coombs, 1999). Pearson & Callaway (2003; 2005) also bring to attention the importance of releasing efficacious agents in avoiding non-target effects that might occur through plant compensation for herbivory by the biocontrol agent, or indirectly through food-web interactions where abundant but ineffective agents become a food source for local consumers. The occurrence of ineffective but abundant agents combined with concerns over reducing the number of biocontrol agent introductions and the need to improve predictive capabilities to alleviate criticisms of the science, has lead to the premise of pre-release efficacy testing in weed biocontrol programmes.

1.4.2 Ecology of the plant-herbivore system

Nearly a decade ago, De Clerck-Floate & Bouchier (2000) stated that classical biological control of weeds was undergoing a critical period of self-examination that was being driven by an ecological perspective. They emphasized that linking ecological theory and methods to biological data would improve establishment rates, impact and predictability in biocontrol programmes and that ecological data should be incorporated into decision-making frameworks for advancement of the science. These proposed developments for biocontrol research were prompted by the many unsuccessful biocontrol introductions and realization that an understanding of how an insect herbivore impacts plant populations is inherently difficult due to the complexity of plant-insect interactions.

Plant population dynamics are influenced by an interaction of biotic and abiotic factors such as herbivory, competition and heterogeneous environmental conditions, therefore the effect of a candidate biological control agent on individual plant fitness will not necessarily translate into effects at the population level (Crawley, 1989b; Halpern & Underwood, 2006). A plant's particular response to insect herbivory is highly variable because its potential to compensate for damage is dependent on factors such as resource availability, herbivore loads and habitat conditions that are prevalent. Furthermore, bottom-up control of insect populations through resource quality is a dominant factor limiting establishment and population growth of many biocontrol agents. Most insect herbivores are limited by the quality of their host plant and not their abundance and distribution (Price, 2000). Improving predictability of a new biocontrol agent's success in its recipient environment requires an understanding of the factors that will potentially influence populations of species at both trophic levels. Knowledge of insect fecundity, voltinism, dispersal capabilities and host plant suitability provides information for predicting the potential range and abundance of biocontrol agents. Only natural enemies with proven capabilities for significantly reducing their host's performance at some realistic density are promising biocontrol agents, justified in release (McClay & Balciunas, 2005). Structured community assemblages accounting for all herbivore species in the system, either candidate or established, must be considered so that new agents are released because they are necessary, having the potential to be superior

biocontrol agents or complement the existing biocontrol agents (McEvoy & Coombs, 1999; Pearson & Callaway, 2005).

Examining subsets and components of the plant-herbivore system and factors that will drive and modify their reciprocal responses will contribute to an understanding of their interaction strength, which are the population level effects of one species on another (Schooler & McEvoy, 2006). A better understanding of the components of the plant-herbivore system can improve establishment and success rates and largely facilitate avoidance of indirect non-target effects (De Clercke-Floate & Bouchier, 2000; Pearson & Callaway, 2003; 2005). Ecological data from the pre-release component of a programme are required to make a qualified decision on release and predictions on likely or potential impacts of the candidate biological control agent following its introduction.

1.5 Thesis outline

The principle aim of this thesis is to investigate *C. aquaticum* - water hyacinth interactions and to provide interpretations of biological and ecological data that will contribute to a holistic assessment in determining whether the grasshopper's introduction into the South African biological control programme is justifiable. The effects of nutrient availability on both trophic levels are investigated to gain insight into herbivore and host population dynamics and their physiological modifications in response to variable water nutrient conditions. The major factors influencing plant population dynamics are considered as well as the functional response of the grasshopper to the quality of its host plant. Overall, this study makes predictions on the potential of *C. aquaticum* to suppress water hyacinth growth and productivity, should it be released in South Africa.

Chapter 2 investigates the effect of water nutrient conditions found in South African water bodies with water hyacinth infestations on plant growth and productivity and therefore the influence of bottom-up control on water hyacinth populations. Nutrient-dependent compensation in response to herbivory by *C. aquaticum* is evaluated to make predictions about its capacity for controlling water hyacinth in systems with different

nutrient regimes, particularly in the type of nutrient conditions where efficacious biocontrol agents are needed.

Variability in plant quality as a result of nutrient pulses or differences in nitrogen availability could have an important influence on population fluctuations of *C. aquaticum* and therefore their potential to regulate populations of their host. The effect of various nutrient regimes on plant quality and the subsequent effect on grasshopper fecundity, feeding and development rates and survival are investigated in Chapter 3. This provides insight into potential population dynamics of the grasshopper, dependent on water nutrient conditions. Predictions on survival, dispersal, population growth rates and damage to plants are made.

With the importance of pre-release efficacy, an agent considered justified for release must be able to significantly reduce productivity, biomass and growth rates of their host plant at some realistic density. The relationship between *C. aquaticum* density and damage to water hyacinth plants is explored in Chapter 4 to determine feeding intensities needed to significantly reduce populations of water hyacinth.

In Chapter 5, the combination of competition from a similar free-floating aquatic species and herbivory by the grasshopper are evaluated in a competition study to determine how both factors might interact to influence community structure and water hyacinth population dynamics. The importance of the findings for management of water hyacinth in South Africa are discussed.

Chapter 6 is a general and complete discussion of the results, the implications of the findings for the South African biological control programme and recommendations for this agent, should it be released in South Africa.

Chapter 2

Influence of nitrate and phosphate levels occurring in South African water bodies on the potential of *Cornops aquaticum* to reduce growth and productivity of water hyacinth

2.1 Introduction

Plants are vulnerable to herbivory because they are sessile and they therefore have mechanisms to defend themselves or cope with the damage caused by feeding. Plants deter herbivory through the production of toxic secondary metabolites or morphological adaptations such as hairs and spines or they tolerate or compensate for herbivory to reduce the negative effects on their fitness levels. However, contrary to the intuitive expectation that removal of tissue would have detrimental effects for plants that don't manage to avoid being eaten, the effects on their fitness may be positive (Maschinski & Whitham, 1989), negative (Nötzold et al., 1998; Agrawal, 2004) or of no consequence (Lee & Bazazz, 1980; Van den Berg & Soehard, 2000). The ability of plants to tolerate or mitigate the negative effects of feeding is known as compensation. Plants display plasticity in their compensatory responses for herbivory which have been linked to environmental conditions such as resource availability (Trumble et al., 1993).

This plasticity of plants in their response to herbivory lead to the development of two hypotheses to explain the role that resources play in compensatory ability. The compensatory continuum hypothesis (CCH) (Hawkes & Sullivan, 2001) had its origins in Maschinski & Whitham's (1989) proposal that plants are less able to compensate for herbivory under conditions of stress such as low resource and high competition. They proposed that a continuum of responses is possible that range from negative to positive, depending on environmental conditions. This was based on their findings that the effects of herbivory on *Ipomopsis arizonica* Greene (Polemoniaceae) ranged from detrimental to beneficial according to nutrient availability, competition and timing of attack, with conditions of high fertility and low competition resulting in the highest level of compensation. Plants growing under conditions of high nutrient availability tend to have high rates of photosynthesis, biomass accumulation, growth and reproduction (Maschinski & Whitham, 1989; Meyer & Root, 1993; Fraser & Grime, 1999; Hartley & Amos, 1999; Mutikainen et al., 2000; Throop, 2005; Ripley et al., 2006; Zehnder & Hunter, 2008), and plants with high growth rates in response to high resource availability can replace lost tissue more efficiently than those with slow turnover (Coley et al., 1985). Furthermore, a certain level of herbivory in terms of leaf material removed represents a

smaller fraction of net production of a plant with high growth rates (Coley et al., 1985) therefore the high resource-high tolerance hypothesis seems the logical one. There are many examples in the literature in support of the CCH (Polley & Detling, 1989; Steinger & Müller-Schärer, 1992; Jeunger & Bergelson, 1997; Briggs, 1991). For example, *Brassica nigra* (L.) W.D.J. Koch (Brassicaceae) plants damaged by larvae of *Pieris rapae* L. (Lepidoptera: Pieridae) maintained high leaf growth rates under conditions of high soil fertility compared to those limited by nutrient availability and were able to recover more quickly when feeding ceased (Meyer, 2000). The alternate school of thought is the growth rate model (GRM) (Hawkes & Sullivan, 2001) which was based on the grazing optimization hypothesis (Hilbert et al., 1981) which postulates, based on a mathematical model, that plants with slower growth rates will have greater potential to increase growth rates in response to herbivory because they are growing at a rate below their optimal level. Plants with high growth rates have little potential to increase growth in response to herbivory because they are already growing at their maximum level (Hilbert et al., 1981). An alternative explanation is that plants typically have reduced root:shoot ratio under high resource conditions (Chapin, 1980) which has been associated with reduced tolerance (Strauss & Agrawal, 1999), presumably because nutrient uptake following herbivory for recovery is more limited. As with the CCH, there are many studies in support of the growth rate model where plants were less tolerant of herbivory under conditions of high nutrient availability (Mihaliak & Lincoln, 1989; Meyer & Root, 1993; Hartvigsen et al., 1995; Fraser & Grime, 1999; Hochwender et al., 2000).

Both the CCH and the GRM are consistent in the notion that resource availability plays a major role in determining the particular type of response of plants to herbivory (Hawkes & Sullivan, 2001). However, the effect of nutrient availability in a plant-herbivore system extends further than plant growth rates and potential for compensation. The type of herbivory can influence a plant's compensatory ability under the same nutrient conditions (Meyer & Root, 1993; Throop, 2005). For example, compensatory ability of *C. maculosa* was greatly affected by nitrogen availability but also by the type of insect, regardless of environmental nutrient conditions. Plants suffered reductions in shoot and plant biomass as a result of root herbivory by the weevil, *Cyphocelonus achates* Fahr (Coleoptera:

Curculionidae) whereas they compensated fully for root herbivory by the moth, *Agapeta zoegana* L. (Lepidoptera: Cossidae) at similar insect densities (Steinger & Müller-Schärer, 1992). Furthermore, nutrient supply influences the type and amount of chemical defense. High nitrogen availability can increase allocation to nitrogen-based chemicals such as alkaloids (Gerson & Kelsey, 1999; Dyer et al., 2004), whereas carbon-based chemicals are associated with reduced nitrogen availability (Bryant et al., 1983; 1987; Mihaliak & Lincoln, 1989), which can influence insect feeding patterns. Slow-growing species in low-resource environments typically invest more in defensive compounds compared to species in resource-rich environments (Coley et al., 1985) however Mihaliak & Lincoln (1989) caution that within-species differences in chemical defense according to nutrient availability is not comparable with inherently slow-growing species that characteristically invest more in chemical defense than do plants characteristic of resource rich environments. Resource availability can also influence susceptibility of plants to insects with different feeding patterns. Dyer et al. (2004) showed that higher levels of amide secondary metabolites produced by *Piper cenocladum* C. DC. (Piperaceae) in response to increased resource availability deterred generalist herbivores but not specialists. Foliar nitrogen content of plants increases with nutrient availability in the environment (Stiling & Moon, 2005) therefore the nutrient conditions that a plant is growing in can influence plant-herbivore interactions. Nitrogen is the essential element for insects and high levels can elicit increased development rates, fecundity and survival and influence feeding patterns (Mattson, 1980; Wheeler, 2001; Awmack & Leather, 2002; Hogendorp et al, 2006). Therefore host plant quality for insects can influence their population dynamics (Denno et al., 2003), which in turn could influence the amount of damage to plants, dependent on nutrient availability in the environment. Environmental nutrient availability can also affect leaf biomass, life cycles and turnover rates of plants (Chapin, 1980; Chabot & Hicks, 1982) and therefore their photosynthetic rates and potential for compensation.

The response of a plant targeted for biological control to herbivory by their host-specific herbivore/s would have important consequences for the success of biocontrol programmes in light of the evidence that the environmental conditions that a weed is

growing in can have an influence on its ability to compensate for damage, its population dynamics and defense mechanisms as well as performance of its herbivores. Nutrients have a heterogeneous distribution both spatially (Jackson & Caldwell, 1993) and temporally therefore all factors that vary in response to nutrients will be dynamic with a changing environment. Nutrient-enrichment, particularly nitrogen, is regarded as one of the major factors interfering with successful biological control of water hyacinth in South Africa (Hill & Olckers, 2001). Nitrogen is the key element influencing plant productivity and growth rates and a linear relationship exists between plant relative growth rates and nitrogen concentrations (Verkroost & Wassen, 2005). Several studies have quantified the response of water hyacinth to increasing levels of water nutrients and shown that the plant has increased rates of growth and productivity in response to high levels of nutrients, particularly nitrogen (Reddy et al., 1989, 1990; Heard & Winterton, 2000; Xie et al., 2004; Ripley et al. 2006, Coetzee et al., 2007). While phosphates can be limiting to plants in aquatic systems, water hyacinth is generally not phosphate-limited. The plant only requires a small amount of this nutrient for uptake of nitrogen and levels above that threshold are not of any significance. Water bodies in South Africa typically have phosphate levels higher than the minimum requirement of the plant to take up other nutrients. If water hyacinth's tolerance of herbivory is higher under conditions of high nutrient availability, this would be in agreement with the CCH.

Laboratory studies evaluating the biocontrol agents already released in South Africa have showed that under higher levels of growth and biomass accumulation (in response to high nutrient availability), the negative effects of herbivory were often mitigated and biocontrol agents had less of an impact on plant performance (Heard & Winterton, 2000; Wilson et al., 2006; Coetzee et al., 2007). However, plants are not equally tolerant of different types of herbivory (Meyer, 1993) and water hyacinth may therefore respond differently to herbivory by its biological control agents. Furthermore, a biocontrol agent's response to the quality of their host may differ between species, which can affect their population impact on the target plant. Room & Thomas (1990) showed that higher plant nitrogen levels improved biocontrol success of *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae) on *Salvinia molesta* Mitchell (Salviniaceae), by increasing

weevil population growth rates. The moth, *Samea multiplicalis* Guenée (Lepidoptera: Pyralidae) also responds positively to increasing nitrogen content of salvinia plants (Taylor, 1984) but the adults moths discriminate between plants as oviposition sites based on foliar nitrogen content (*in* Room, 1990). Increased nitrogen availability had the opposite effect on a water hyacinth biocontrol agent. Coetzee et al. (2007) found that the mirid, *E. catarinensis* had lower numbers and lower feeding rates on water hyacinth plants grown in hypertrophic nutrient conditions compared to those grown in eutrophic conditions in which nitrogen levels were ten-fold lower. Ripley et al. (2006) suggested that particular ranges of nutrient levels may be optimal for biocontrol agent establishment and efficacy, and could be decreased at nutrient availability higher or lower than the optimal levels.

With the number of water bodies in South Africa exhibiting nitrate and phosphate levels (Brudvig, pers. comm.; de Villiers & Thiart, 2007) that enhance growth rates of problematic invasive plants such as water hyacinth and in light of the evidence for water quality to reduce the efficacy of the biocontrol agents already established in South Africa, it was important to consider these factors in the evaluation of *C. aquaticum* to determine its suitability as a biocontrol agent for water hyacinth. The aim of this chapter was to (1) investigate the impact of herbivory by *C. aquaticum* on growth rates and productivity of water hyacinth plants grown at the same nutrient concentrations found in South African impoundments where water hyacinth is currently problematic; (2) investigate water hyacinth's response to herbivory by *C. aquaticum* and determine whether these responses are nutrient dependent; and (3) make predictions based on evidence from this pre-release study, on what kind of impact *C. aquaticum* will have on water hyacinth growing in different nutrient environments and by evaluating the plant's response to herbivory, determine what densities would be needed for effective control of the weed under differing nutrient regimes. For the purposes of the research presented here, Maschinski & Whitham's (1989) definitions for the types of compensation for herbivory that are commonly observed are used: (1) Overcompensation for herbivory is beneficial and occurs where production is greater compared to ungrazed plants; (2) equal compensation occurs when productivity by grazed and ungrazed plants is the same and grazed plants are

therefore not negatively affected by herbivory; (3) undercompensation occurs where herbivory is detrimental and productivity of grazed plants is less than ungrazed plants growing in the same conditions.

2.2 Materials and Methods

2.2.1 Nutrients

Three different nitrate and phosphate levels were used in this experiment and are classified as eutrophic, eutrophic/mesotrophic and oligotrophic according to the South African Water Quality Guidelines (Holmes, 1996) (Table 2.1). These classifications are based on nitrate levels since this is the most important element influencing water hyacinth growth (Reddy et al., 1989) and insect performance (Mattson, 1980). The nutrient levels were selected to represent the range of nutrient conditions found in South African systems where water hyacinth is a problem. Water samples from 15 water hyacinth sites around the country were collected on a monthly basis and analyzed for nitrates and phosphates (Brudvig, unpublished data). Averages of a year's worth of the data to represent high, medium and low levels of those nutrients were selected from the range. The eutrophic nutrient condition, here referred to as the high nutrient treatment, is representative of Mbozambo Swamp in the province of KwaZulu-Natal, a highly eutrophic impoundment situated next to a paper mill and which had the highest levels of both nitrates and phosphates out of all 15 sites. The medium nutrient treatment was on the border of eutrophic and mesotrophic. The nitrate and phosphate levels were selected from two different sites because the phosphate level from the site selected as the medium nitrate level was too low to be used in the medium nutrient treatment. The nitrate level which was the second highest after Mbozambo Swamp was representative of Hammarsdale Dam in KwaZulu-Natal and the phosphate level was representative of Warrenton Weir in the Northern Cape Province. The oligotrophic nutrient condition, here referred to as the low nutrient treatment represents average nitrate and phosphate levels of New Year's Dam in the Eastern Cape Province, an impoundment which had the lowest levels of growth enhancing nutrients out of all 15 sites. Nitrates and phosphates were added as potassium nitrate and potassium dihydrogen orthophosphate respectively. The concentrations of these nutrients for each treatment are tabulated in Table 2.2 and Table

2.1 shows the classification of levels of nitrates and phosphates in mgL^{-1} according to the South African Water Quality Guidelines (Holmes, 1996).

Table 2.1 Classification of water bodies according to nitrate levels in mgL^{-1} from the South African Water Quality Guidelines (Holmes, 1996).

Classification	Nitrate levels (mgL^{-1})
Hypertrophic	>10
Eutrophic	2.5 – 10
Mesotrophic	0.5 - 2.5
Oligotrophic	< 0.5

Table 2.2 Concentrations of nitrates and phosphates in mgL^{-1} used to represent nutrient levels found in South African river systems and impoundments ranging from highly eutrophic to oligotrophic.

	High (eutrophic)	Medium (eutrophic/mesotrophic)	Low (oligotrophic)
Nitrates (mgL^{-1})	7.6	2.52	0.034
Phosphates (mgL^{-1})	1.37	0.316	0.024

Fifteen leaves from the 3rd, 4th and 5th position ($n = 15$ per leaf position) of water hyacinth plants grown at the high, medium and low nutrient levels were analyzed using standard methods to determine concentrations of foliar nitrogen and phosphorus in response to variations in water nutrient conditions. Water hyacinth leaves are typically numbered according to the position of the petiole on the crown of the plant. Petioles are spirally arranged around the crown, with the youngest, unfurled leaf at position 0. As leaves age and new leaves are produced, they move down the crown of the plant so that each petiole position determines the age of a particular leaf (Center, 1980). Leaves 3, 4 and 5 were selected as they are the leaves that are predominantly fed upon by adult *C. aquaticum*. Table 2.2 should be referred to for the remaining chapters of the thesis as the same high, medium and low nutrient levels were used for all other trials. In experiments not

requiring manipulation of water levels, the high nutrient treatment was used as the nutrient medium so that plants were not nutrient-limited and had high rates of growth and productivity.

2.2.2 Experimental design

The trial was conducted in a quarantine glasshouse at the Plant Protection Research Institute in Pretoria, South Africa in summer from February to April 2006. Glasshouse temperatures were maintained at a maximum of 30°C and a minimum of 18°C, with a day-length of 14 hours. Water hyacinth plants obtained from stock cultures were grown in plastic tubs at a density of two plants per tub for a period of four weeks. The dimensions of the tubs were 43 x 31 x 19 cm and contained 15L of water. Nitrogen and phosphorus were added as potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) respectively. Chemicals were added at the required rate for correct concentrations and the nutrient media were replaced weekly to maintain the required nutrient supply level to the plants. Commercial chelated iron (Mircel FE 130) was also added at a rate of 1.3g/15L (Coetzee et al., 2007) and each tub was covered with a netted sleeve. Nitrates and phosphates, which are plant macronutrients were the important elements to manipulate, but micronutrients such as potassium and iron were added to the nutrient medium so that plants did not suffer from micro-nutrient deficiencies for the duration of the trial. After the four-week growth period, all daughter plants, dead leaves and stems were removed and the plants were tagged and then weighed to determine wet weight. Adult *C. aquaticum* were introduced into experimental tubs at a density of one grasshopper per plant and one male/female pair per tub. This density was selected as it was recorded by Silveiro-Guido & Perkins (1975) to be the highest density at which the grasshoppers occur in the native range. Hatching nymphs were removed from the tubs so that the trial was run at a constant insect density. Dead adult grasshoppers were removed and replaced with an adult of the same sex and as far as possible, the same age. The plants were sampled at weekly intervals and the following plant parameters were measured: number of leaves, number of ramets (daughter plants), number of flowers, leaf 2 petiole length (cm), longest petiole length (cm), leaf 4 area damage and position of the leaf with the most feeding damage. Area damage to the fourth

leaf of each plant in the herbivory treatments was assessed by visual observation and scored from 1 to 5 where 1 = 0%; 2 = >5%; 3 = 5-25%; 4 = 25-50% and 5 = 50-100% leaf material damaged or removed. Leaf 4 was selected as it was previously noted based on observation that adult *C. aquaticum* tend to feed on mature water hyacinth leaves, predominantly from the 3rd to 6th position. At each sampling interval, each new leaf, daughter plant and flower was tagged to measure productivity. Each treatment had an experimental tub and a control tub and was replicated six times resulting in a total of 36 tubs. The trial was run for a period of ten weeks after which all plants were weighed (including daughter plants and dead leaves and stems) to determine end wet weight. When the data were analyzed at the end of the trial it was found that there were differences in the amount of leaf area damage across the nutrient treatments, but since leaf size had not been controlled for in assessing leaf area damage, it was necessary to investigate whether differences in area damage could be due to differences in leaf size as a result of nutrient supply. Leaf 4 of water hyacinth plants cultured at the high, medium and low nutrient levels, but not exposed to herbivory by *C. aquaticum* were weighed to determine wet weight. One of the medium control tubs and one plant in the high control were lost due to algal contamination before the end of the trial resulting in an unequal number of replicates.

2.2.3 Statistical analysis

Chemical analysis of water hyacinth leaves (n=15 per leaf position) provided a mean level of nitrogen and phosphorus for each nutrient treatment and each leaf position. These data were used to assess the relationship between water nitrate levels (mgL^{-1}) and nitrogen content of leaves (%) at positions 3, 4 and 5 using a product-moment (Pearson's) correlation. Data on the dry weight of water hyacinth leaves and the effect of nutrients and herbivory on water hyacinth performance parameters were normally distributed, therefore parametric tests were used for the analyses. A one-way ANOVA tested for differences between the mean dry weight of the fourth leaf of water hyacinth plants grown at high, medium and low nutrient levels. The effect of nutrient treatment, herbivory by *C. aquaticum* and their combined effect on the difference in wet weight from the start to the end of the study period were analyzed using a two-way ANOVA.

Biomass data were also calculated to represent the reduction in growth increment (%) as a result of herbivory which is a powerful indication of impact of herbivory on the target plant (Wilson et al., 2005). Growth increment is the amount of biomass accumulated or lost by plants during the study period. The loss of growth increment (expressed as a percentage) is the difference between the growth increment of control plants and the growth increment of plants in the herbivory treatments. The effect of nutrient treatment, herbivory and their interactions on the growth and reproductive parameters were also analyzed by two-way ANOVA. The temporal development of significant growth and reproductive parameters are presented graphically to determine trends in their development in response to nutrient availability and herbivory. All data analyses were done in Statistica 6.0.

2.3 Results

2.3.1 Nitrogen and phosphorus levels of water hyacinth leaves 3, 4 and 5 from chemical analysis and evaluation of the relationship between leaf nitrogen content (%) and water nitrate levels (mgL^{-1})

The mean nitrogen and phosphorus (% dry weight) content of water hyacinth leaves 3, 4 and 5 from plants grown at high, medium and low nutrient levels for a period of three months are tabulated in Table 2.3. Concentrations of these nutrients in foliage increased with an increase in nutrient supply to water hyacinth. Leaves 3, 4 and 5 from each treatment were similar in their nitrogen and phosphorus levels. There was a strong positive correlation ($r = 0.953$; $P < 0.0001$) between water nitrate levels (mgL^{-1}) and the mean nitrogen content (%) of leaves of plants grown at high, medium and low nutrient levels (Fig. 2.1).

Table 2.3 Nitrogen and phosphorus content (% dry weight) of water hyacinth leaves from positions 3, 4 and 5. Plants grown at high, medium and low nutrient levels in the absence of herbivory by *Cornops aquaticum* (n = 15/leaf position/nutrient treatment).

	Leaf 3			Leaf 4			Leaf 5		
Chemical element (% g dry weight)	High	Med	Low	High	Med	Low	High	Med	Low
N	3.45	2.00	1.75	3.40	2.15	1.80	3.38	1.85	1.75
P	0.57	0.44	0.40	0.60	0.47	0.39	0.53	0.38	0.40

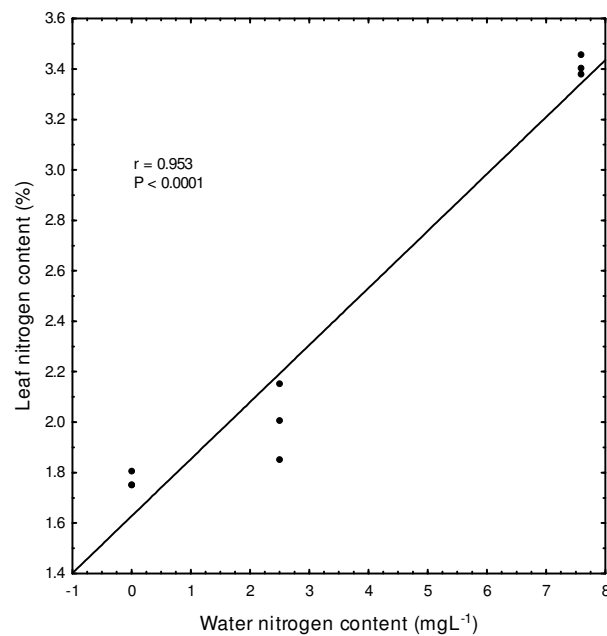


Figure 2.1 Relationship between water nitrate levels (mgL⁻¹) and mean nitrogen content of leaves 3, 4 and 5 of water hyacinth plants grown at high, medium and low nutrient levels (n = 15/leaf position/nutrient treatment).

2.3.2 Effect of water nutrient treatment on the wet weight of water hyacinth leaves of plants grown at high, medium and low nutrient levels

Water nutrient treatment had a significant effect ($F_{2;510} = 147.87$; $P < 0.0001$) (Fig. 2.2) on the mean wet weight of water hyacinth leaves. Leaf biomass was significantly different across all the nutrient treatments however there was a substantial difference in biomass between the high nutrient treatment and the medium and low nutrient treatments.

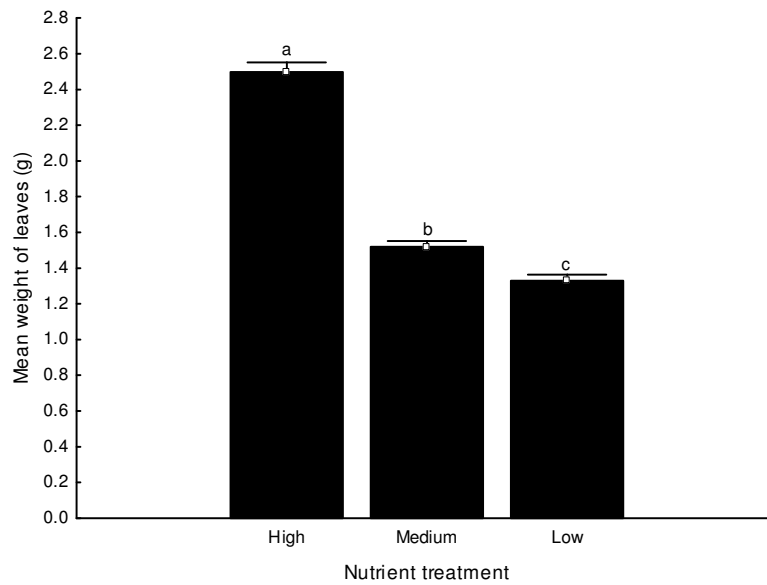


Figure 2.2 Mean wet weight (g) of the fourth leaf of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample size, $P < 0.05$). Error bars represent the standard error of the mean.

2.3.3 Effect of nutrients and herbivory on plant biomass

Despite the differences in nutrient availability there were no significant differences between the start weights of water hyacinth plants after four weeks of growth at the relevant nutrient concentrations. The accumulation of biomass of water hyacinth plants in the 10 week study period was significant in the herbivory treatment at the high nutrient level ($F_{1;10} = 16.92$; $P = 0.002$) and in control plants at both the high ($F_{1;10} = 201.36$; $P < 0.0001$) and medium ($F_{1;8} = 69.16$; $P < 0.0001$) nutrient levels (Fig. 2.3). Herbivory by *C. aquaticum* prevented biomass accumulation of water hyacinth plants grown at the medium nutrient level and plants stayed almost exactly the same weight from the start to the end of the study period. Control plants grown at the low nutrient treatment increased in wet weight from the start to the end but this was not statistically significant. The wet weight of water hyacinth plants from the start to the end of the study period was significantly reduced ($F_{1;10} = 8.80$; $P = 0.014$) by *C. aquaticum* herbivory in the low nutrient treatment. Herbivory by *C. aquaticum* caused a 67% reduction in growth increment in the high nutrient treatment, a 100% reduction in the medium nutrient

treatment and a 400% reduction in growth in the low nutrient treatment compared to control plants without herbivory.

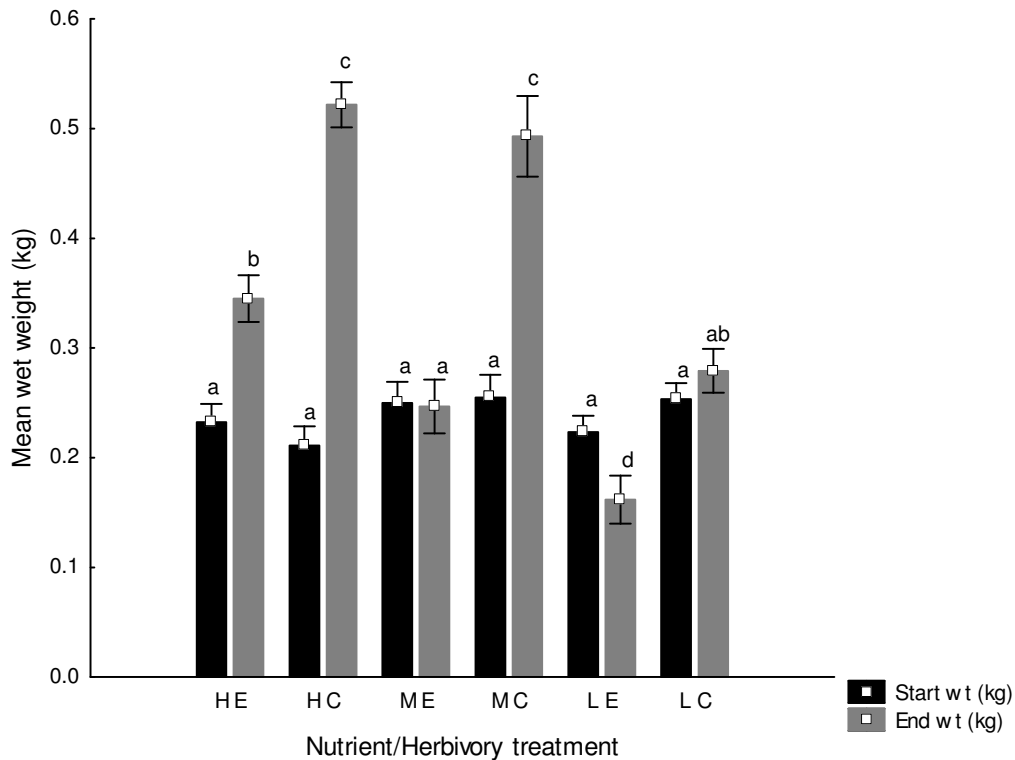


Figure 2.3 Mean start and end weights (kg) of water hyacinth plants grown at high (H), medium (M) and low (L) nutrient levels in the herbivory treatment (E) and the control (C). Means compared by one-way ANOVA; those followed with the same letter are not significantly different. Error bars represent the standard error of the mean.

The difference in wet weight of water hyacinth plants from the start to the end of the study period was significantly different between nutrient treatments ($F_{2;29} = 48.53$; $P < 0.0001$) and between the herbivory treatments and the controls ($F_{1;29} = 81.80$; $P < 0.0001$)(Fig. 2.4). The interaction of nutrient supply and herbivory was also significant ($F_{2;29} = 5.56$; $P = 0.009$). Herbivory by *C. aquaticum* caused the greatest reduction in biomass in the low nutrient treatment, and plant tolerance increased with an increase in nutrient supply to the plants.

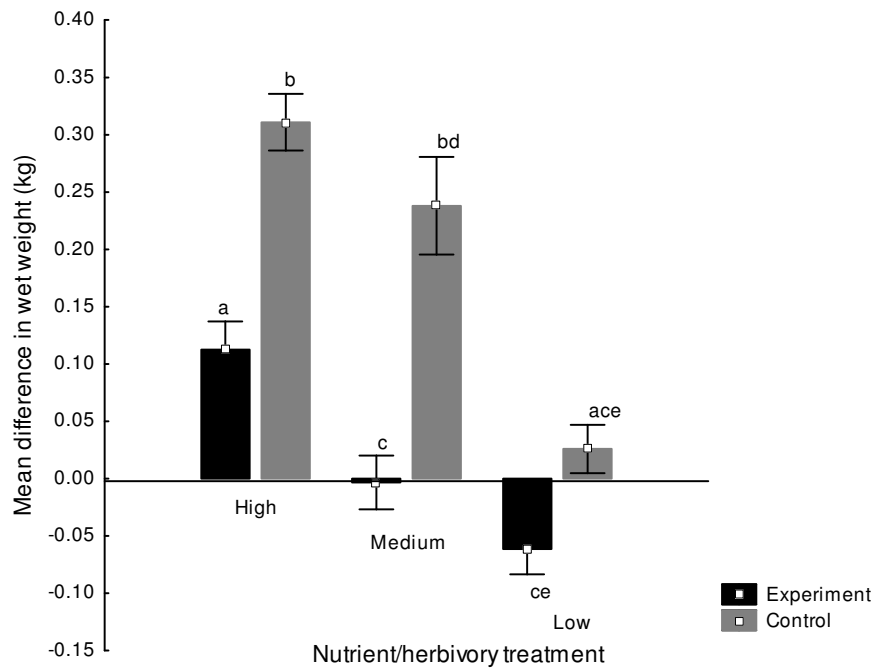


Figure 2.4 Mean change in wet weight (kg) of water hyacinth plants in the herbivory treatment and the control in response to herbivory by *Cornops aquaticum*. Plants grown at high, medium and low nutrient levels for ten weeks. Means compared by two-way ANOVA, those with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

2.3.4 Effect of nutrients and herbivory on plant growth parameters

Nutrient availability was limiting to plants in the low nutrient treatment, indicated by significant reductions in the number of leaves compared to the other two treatments ($F_{2;29} = 24.07$; $P < 0.0001$). Although herbivory by the grasshopper reduced the number of leaves in the low nutrient treatment, this was not statistically significant and plants in the high and medium nutrient treatments maintained on average, a similar number of leaves as control plants (Fig. 2.5). In fact at both nutrient levels plants had slightly higher numbers of leaves in the herbivory treatments compared to controls. The interaction of nutrients and herbivory was significant ($F_{2;29} = 3.44$; $P = 0.038$) indicating that the combination of nutrients and grasshopper herbivory had an impact on plants.

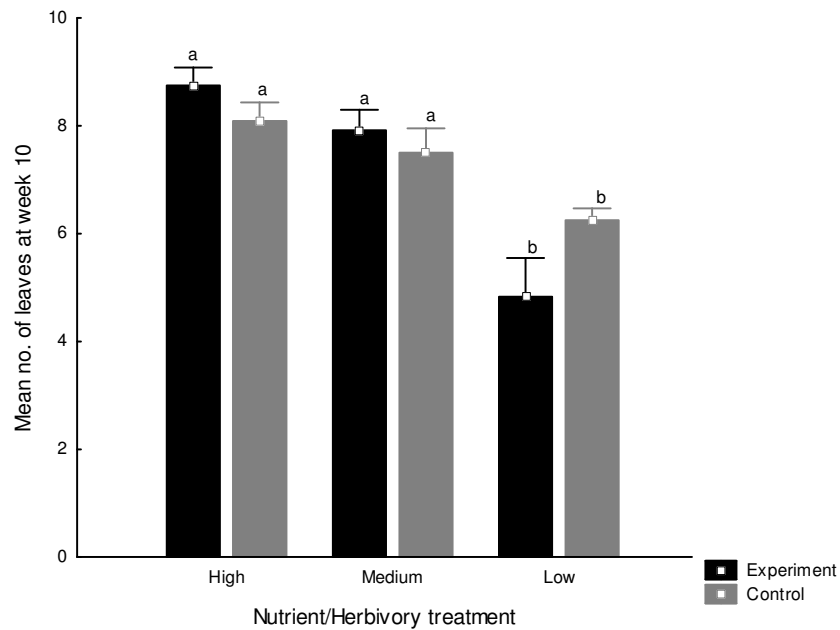


Figure 2.5 Impact of herbivory by *Cornops aquaticum* on the number of leaves of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD for unequal sample size, $P < 0.05$). Error bars represent the standard error of the mean.

Nutrient treatment had a significant effect on the length of the longest petiole (cm) of water hyacinth plants ($F_{2,29} = 89.05$; $P < 0.0001$) (Fig. 2.6). Plants were taller with elongated petioles as nutrient availability increased. Herbivory by the grasshopper caused reductions in plant height at all nutrient levels, compared to control plants, but this was only statistically significant in the high nutrient treatment ($F_{1,29} = 22.59$; $P < 0.0001$). The interaction of nutrient supply and herbivory was not significant. Nutrient treatment and herbivory had a significant effect on the length of the longest petiole (cm) over time. The mean length was decreased in all treatments except the high control plants which maintained a constant height over the ten week study period (Fig. 2.7).

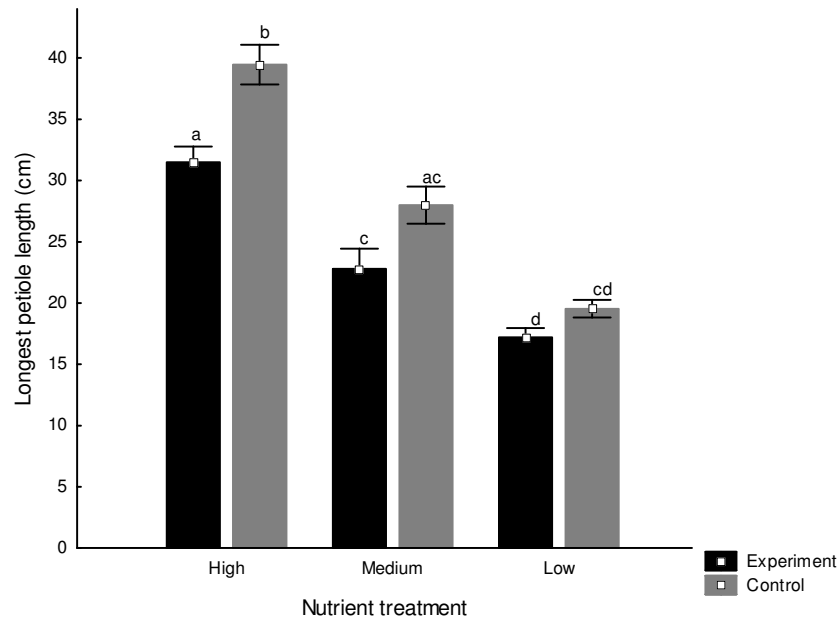


Figure 2.6 Impact of herbivory by *Cornops aquaticum* on the mean length of the longest petiole (cm) of water hyacinth plants grown at high, medium or low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

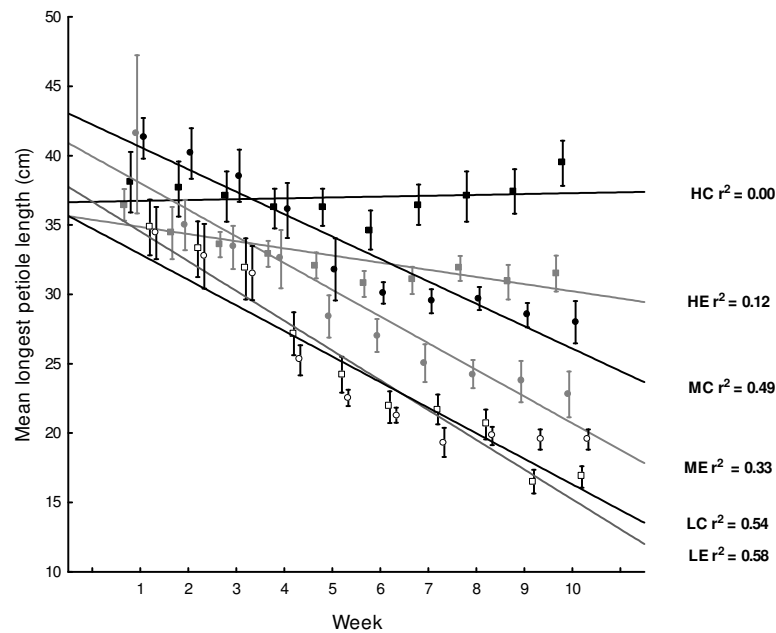


Figure 2.7 Temporal development of the longest petiole (cm) in response to nutrient treatment and herbivory by *Cornops aquaticum*. Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.

The length of the leaf 2 petiole (cm) of water hyacinth plants was significantly affected by nutrient treatment ($F_{2,29} = 108.46$; $P < 0.0001$), and by herbivory by *C. aquaticum* ($F_{1,29} = 13.13$; $P = 0.0006$)(Fig. 2.8) but the interaction of nutrient supply and herbivory was not significant. Length of the leaf 2 petiole increased as nutrient supply to the plants increased, indicating higher growth rates with higher availability of nitrogen and phosphorus. Herbivory caused reductions at all nutrient levels compared to control plants but these differences were not statistically significant.

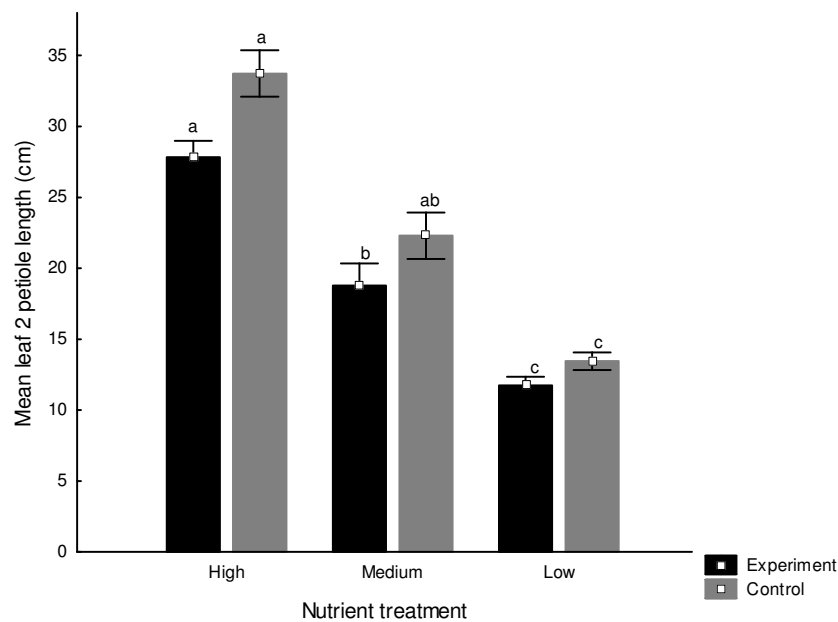


Figure 2.8 Impact of herbivory by *Cornops aquaticum* on the mean length of the leaf 2 petiole (cm) of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

There was also a decrease in the length of the leaf 2 petiole (cm) over time in the medium and low nutrient treatments in both the experimental and the control plants. There was an increase over time in the length of the leaf 2 petiole (cm) in the high control plants and the high experimental plants maintained the same mean length over the 10-week study period (Fig. 2.9).

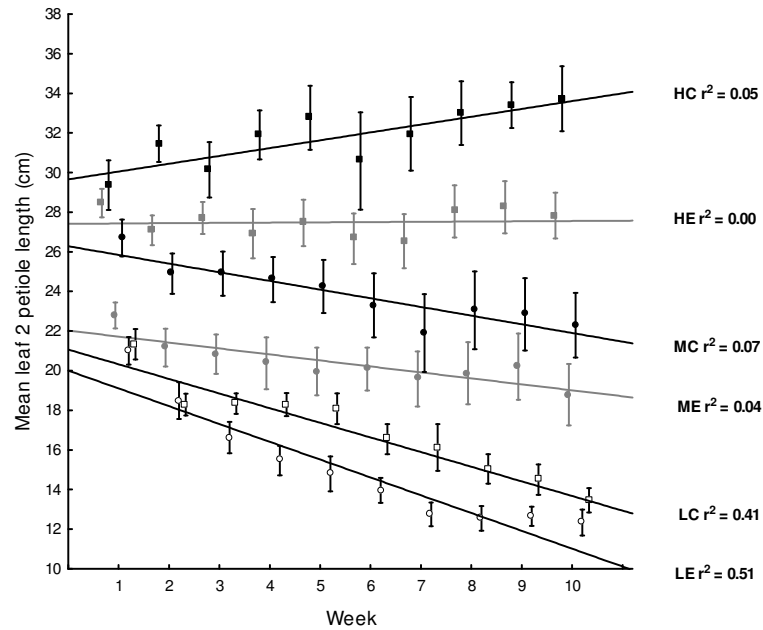


Figure 2.9 Temporal development of the leaf 2 petiole (cm) in response to nutrient treatment and herbivory by *Cornops aquaticum*. Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.

2.3.5 Effect of nutrients and herbivory on leaf, ramet and flower production

Leaf production by water hyacinth plants during the 10-week study period was reduced by nutrient availability in the low nutrient treatment compared to the high nutrient treatment ($F_{2,29} = 35.67$; $P < 0.0001$) (Fig. 2.10). Herbivory by *C. aquaticum* also only significantly reduced leaf production compared to control plants in the low nutrient treatment ($F_{1,29} = 4.79$; $P = 0.003$). Plants in the high and medium nutrient treatments maintained leaf production rates similar to control plants, being slightly higher in the medium nutrient treatment compared to control plants. The interaction between nutrient treatment and herbivory by the grasshopper was also significant ($F_{2,29} = 6.42$; $P = 0.003$).

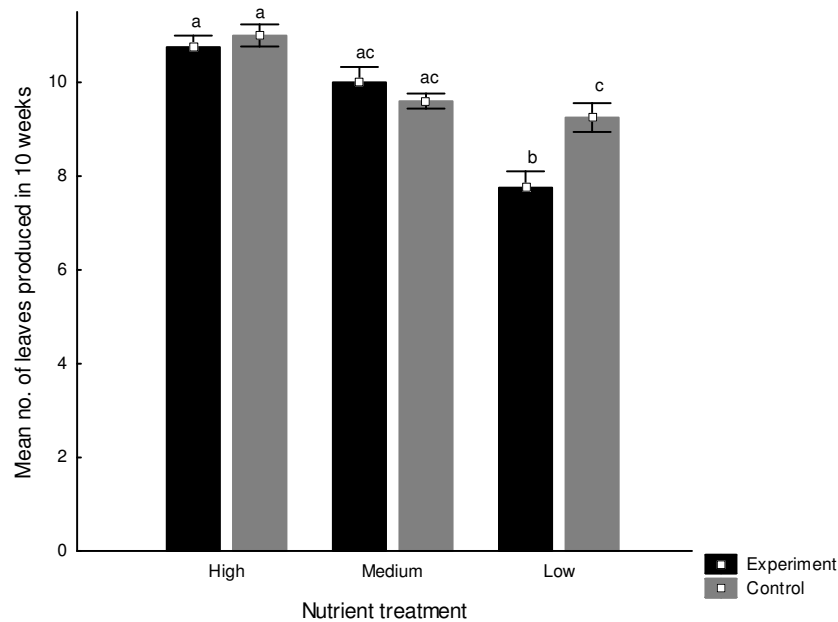


Figure 2.10 Leaf production in response to herbivory by *Cornops aquaticum* of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

Ramet production was significantly affected by nutrient supply ($F_{2,29}=12.86$; $P < 0.0001$) and by *C. aquaticum* herbivory ($F_{1,29} = 21.10$; $P < 0.0001$) (Fig. 2.11). The interaction of nutrients and herbivory was not significant. Plants produced more ramets as nutrient supply increased although productivity was similar between the high and medium nutrient treatments. Ramet production was suppressed by grasshopper herbivory in all the nutrient treatments, but this was only significant when nutrient availability was low. Ramet production increased over the ten week study period in the control plants at the high and medium nutrient treatment and only very slightly in the low nutrient treatment. Herbivory reduced the rate of increase in the number of ramets over time in the high and medium nutrient treatment and prevented an increase in the number of ramets in the low nutrient treatment (Fig. 2.12).

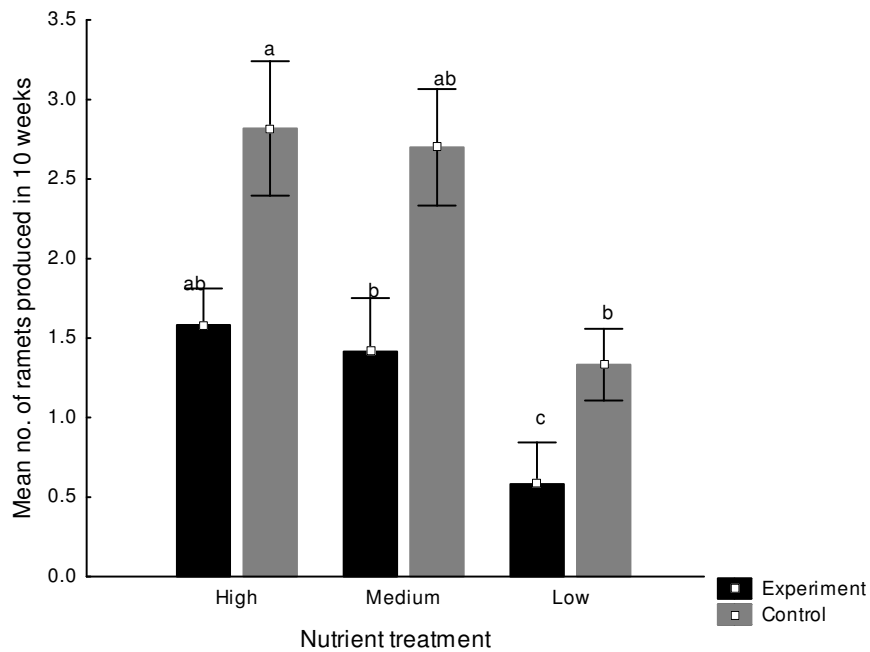


Figure 2.11 Ramet production in response to herbivory by *Cornops aquaticum* of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

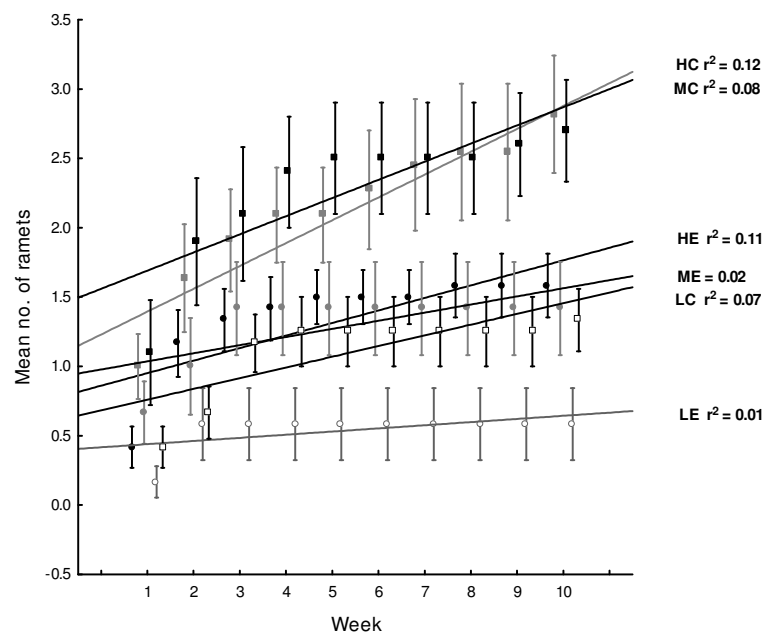


Figure 2.12 Temporal development of ramet production in response to nutrient treatment and herbivory by *Cornops aquaticum*. Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.

Nutrient supply had no effect on flower production with all plants producing between 3 and 4 flowers during the 10-week study period. However, grasshopper herbivory caused significant reductions in flower production ($F_{1,29} = 83.82$; $P < 0.0001$) (Fig 2.13) at all three nutrient levels. Investment in floral biomass was suppressed with plants producing less than 1 flower in 10 weeks in response to herbivory.

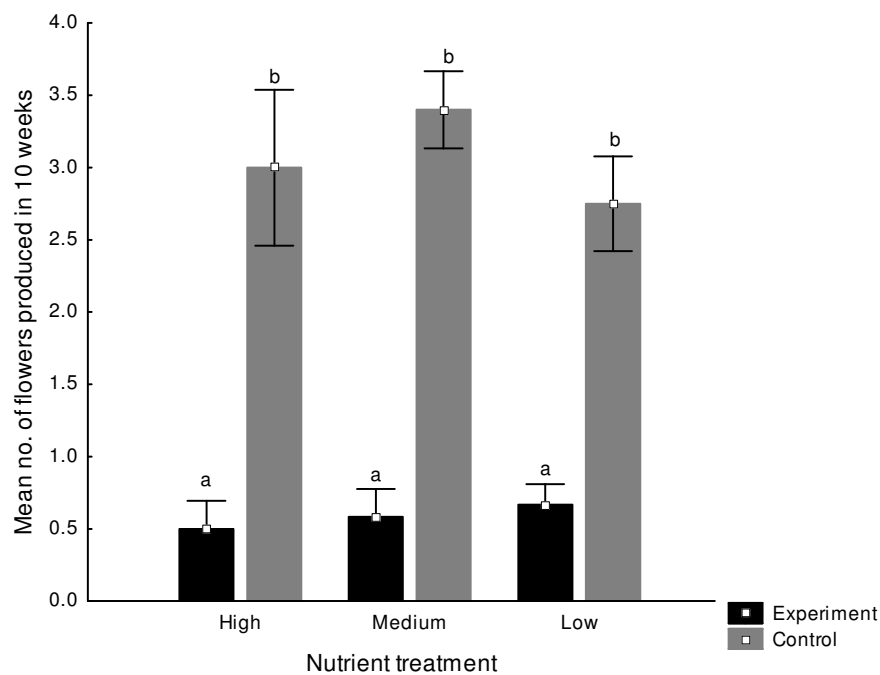


Figure 2.13 Flower production in response to herbivory by *Cornops aquaticum* of water hyacinth plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

There was a sharp increase in flower production over time in the control plants at all three nutrient treatments. Herbivory caused a significant reduction in the rate of increase in the number of flowers over the 10-weeks study period (Fig. 2.14).

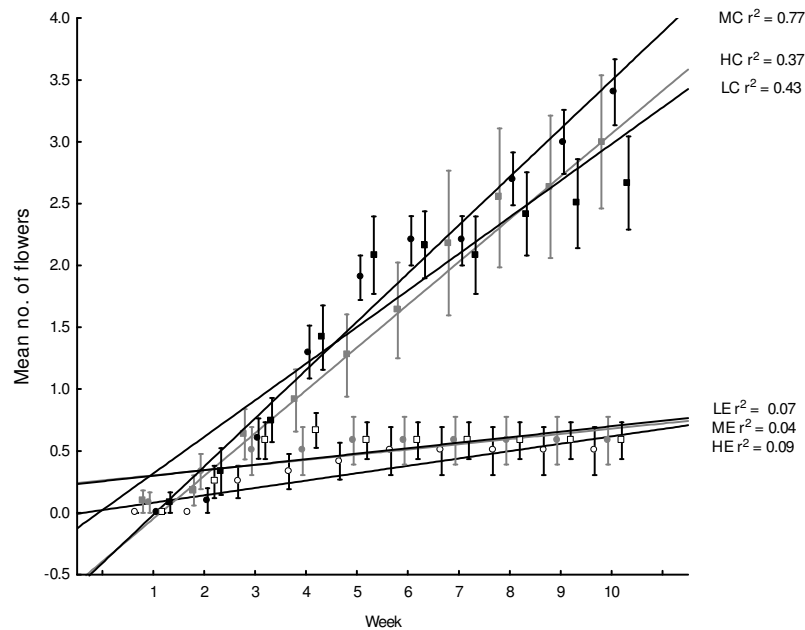


Figure 2.14 Temporal development of flower production in response to nutrient treatment and herbivory by *Cornops aquaticum*. Plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.

2.3.6 Effect of nutrient treatment on feeding damage and leaf preference

The feeding damage scores for leaf 4 at week 10 differed between the nutrient treatments. Average leaf area damage caused by *C. aquaticum* herbivory was between 5 and 25 % in the high nutrient treatment; between 25 and 50% in the medium nutrient treatment whereas between 50 and 100% of the leaf on average was damaged by grasshoppers in the low nutrient treatment. There were also differences in the position of the most damaged leaf across the nutrient treatments. Grasshoppers in the low nutrient treatment fed on average, more on the third leaf of water hyacinth plants whereas the fourth leaf had the most feeding damage in the medium nutrient treatment. Grasshoppers in the high nutrient treatment tended to have a preference for leaves 4 and 5 therefore leaf preference seemed to change depending on nutrient treatment (Fig. 2.15).

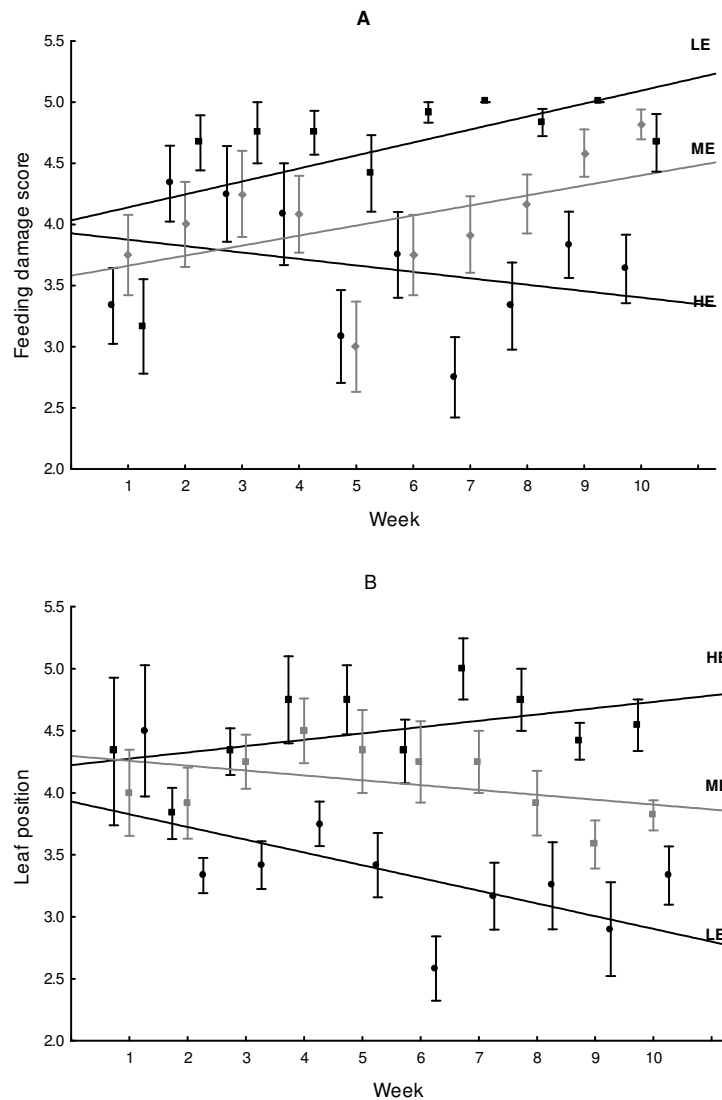


Figure 2.15 Average feeding damage score (A) and mean position of the most damaged leaf (B) in *Cornops aquaticum* herbivory treatments. Water hyacinth plants grown at high (H), medium (M) and low (L) nutrient levels. E = herbivory treatment; C = Control. Error bars represent the standard error of the mean.

2.4 Discussion

The results reported here are consistent with other studies where manipulating water nitrate and phosphate levels had significant effects on water hyacinth growth and reproduction (Reddy et al., 1989, 1990; Heard & Winterton, 2000; Xie et al., 2004; Ripley et al., 2006; Coetzee et al., 2007). The plants responded positively to increasing nitrogen and phosphorus, attaining higher biomass, longer petioles, and higher numbers

of daughter plants and leaves. The only plant parameter measured in this study that was not affected by nutrient supply was flower production. A surprising result was that plants had not responded to the different levels of nutrients in the water after four weeks of growth. Coetzee (2003) tested the effect of nitrate and phosphate levels of even greater variation compared to the nutrient levels tested here and also found no differences in water hyacinth biomass after a four-week growth period. The temporal development of the growth parameters show a gradual decline in growth in the medium and low nutrient treatments, even in the absence of herbivory and by the end of the study period which was 14 weeks since plants were cultured for the trial it was evident that water hyacinth was sensitive to nutrient availability. Nutrient availability in the low nutrient treatment was so limiting to water hyacinth that plant biomass was virtually unchanged in the herbivore-free treatment from the start to the end of the study period. The plants remained small with bulbous petioles and mimicked those in the juvenile stage, as was found by Coetzee et al. (2007) in their low nutrient treatment plants. Richards (1982) also showed that plants grown in ½ strength Hoagland's solution containing macronutrients such as N and P produced long leaves with elongated petioles while plants grown in distilled water produced small leaves with inflated petioles. It is expected that water hyacinth plants respond in the same way in the field, reinforcing the fact that nutrient loading in water bodies with water hyacinth will increase plant proliferation in those systems. Foliar nitrogen and phosphorus increased with an increase in nutrient supply to plants and the relationship between the two found here is concurrent with Gossett & Norris (1971) that leaf nitrogen content and water nitrate levels water are highly correlated. Leaf biomass also increased with nutrient supply therefore plants in the high nutrient treatment had a greater capacity for carbon gain with greater overall surface area for photosynthesis and higher photosynthetic rates that correspond with high levels of nitrogen availability (Ripley et al., 2006).

The compensatory ability of water hyacinth plants subjected to *C. aquaticum* herbivory was directly related to nutrient supply. Their response is consistent with the compensatory continuum hypothesis (CCH) (Hawkes & Sullivan, 2001) where the negative effects of herbivory were greatest under low nutrient conditions and plants had

much higher tolerance at the same herbivore density under conditions of high nutrient availability. Plants in the low nutrient treatment had less investment in leaf biomass and much lower levels of foliar nitrogen therefore carbon acquisition for those plants was limited compared to the other nutrient treatments which had a negative effect on both growth and compensatory re-growth. The positive effects of increased nitrogen and phosphorus in the medium nutrient treatment compared to the low nutrient treatment were mediated by herbivory by *C. aquaticum* so that biomass accumulation was prevented and plants stayed the same weight from the start to the end of the study period. Further confirmation that the resource/grasshopper response of water hyacinth is adequately described by the CCH and that higher growth rates characteristic of plants in high nutrient environments (Meyer & Root, 1993; Fraser & Grime, 1999; Hartley & Amos, 1999; Mutikainen et al., 2000; Throop, 2005; Zehnder & Hunter, 2008) facilitate recovery from herbivory (Coley et al., 1985) is indicated by the fact that biomass accumulation of plants without herbivory in the high and medium nutrient treatments was similar (although parameters differed between treatments) however plants only increased in wet weight in the high nutrient treatment in the presence of herbivory by *C. aquaticum*. This suggests that water hyacinth can use excess nutrients to compensate for herbivory by the grasshopper and that lost tissue can be replaced by water hyacinth plants with high growth rates in response to high nutrient availability. Regardless of greater compensatory ability of plants in the high nutrient treatment, they did not have the capacity for over- or equal-compensation in terms of biomass and herbivory by the grasshopper still significantly reduced water hyacinth's growth rates when compensatory ability was likely to be at a maximum.

There are three possibilities for the increased compensatory ability of water hyacinth under high nutrient conditions: (1) plants had higher growth and leaf turnover rates for maximum carbon acquisition and greater photosynthetic capacity therefore leaf biomass lost to herbivory could be replaced; (2) grasshopper feeding rates were reduced due to high levels of N-based compounds correlated with high levels of foliar nitrogen. Increases in N-based secondary metabolites of plants are often associated with increases in environmental nitrogen availability (Gerson & Kelsy, 1999; Dyer et al., 2004) and this

may have deterred high rates of consumption by *C. aquaticum*; and (3) grasshoppers had lower rates of consumption due to higher resource quality and lower amounts of leaf material needed to be consumed to meet their nutritional requirements. Meyer (2000) found that defoliation of *B. nigra* by *P. rapae* was strongly influenced by soil nutrient availability. Percent defoliation was 21% in high fertility compared to 48% in low soil fertility although high fertility plants lost a greater amount of leaf area to herbivory. She concluded that interactions between herbivory and nutrients are complex and can depend on factors such as total leaf area and consumption. The apparent lower damage levels to leaves in the high nutrient treatment may have been due to leaf size i.e. a leaf with a larger surface area would have a lower area damage score with the same amount of feeding. The effect that nutrient level has on feeding rates is investigated in chapter 3 and elucidates whether differential feeding patterns or damage levels were due to plant nitrogen content in terms of insect feeding or due to differences in leaf size. These three possibilities are not mutually exclusive and a combination of all three factors may have contributed to the differences in impact of the grasshopper on water hyacinth according to nutrient conditions.

Heard and Winterton (2000) and Coetzee et al. (2007) manipulated water nitrate and phosphate levels to evaluate the impact of the *Neochetina* weevils and the mirid, *E. catarinensis* on water hyacinth. Their nitrate and phosphate levels are tabulated in Table 2.4. The insect densities are not comparable (15 *E. catarinensis* per plant; 0.5 *Neochetina* per plant) but they were selected based on field densities and one grasshopper per plant is not unrealistic for the field in South Africa since it is their density in the native range. The nitrogen concentration used by Coetzee et al. (2007) in the medium nutrient treatment (eutrophic) is the threshold concentration for optimal levels of growth and reproduction in water hyacinth (Reddy et al., 1989) and is comparable with the high nutrient treatment used in the present study. Heard & Winterton's (2000) high nitrogen level is mesotrophic and is half-way between the oligotrophic and mesotrophic/eutrophic levels tested here, therefore plant productivity would have been much lower in their experiments.

Table 2.4 Concentrations of nitrogen (N) and phosphorus (P) used in the present and two other studies to evaluate the impact of herbivory by biocontrol agents on water hyacinth under different nutrient regimes.

Nutrient Treatment						
	High		Medium		Low	
	N(mgL ⁻¹)	P(mgL ⁻¹)	N (mgL ⁻¹)	P(mgL ⁻¹)	N(mgL ⁻¹)	P(mgL ⁻¹)
Heard& Winterton (2000)	1.6	1.0	0.4	0.025	No low treatment	
Coetzee et al. (2007)	50.5	2.56	5.5	2.56	0.5	0
Present study	7.6	1.37	2.52	0.316	0.034	0.024

Both Heard & Winterton (2000) and Coetzee et al. (2007) found no significant reductions in per plant water hyacinth biomass in response to herbivory at any of the nutrient levels tested whereas *C. aquaticum* caused significant reductions in biomass at all levels, including the eutrophied environment (although the *Neochetina* weevils significantly reduced total tank biomass of water hyacinth). *Cornops aquaticum* only significantly reduced ramet production in the low nutrient treatment whereas both *E. catarinensis* and the *Neochetina* weevils significantly suppressed vegetative reproduction at all the nutrient levels tested. In terms of biological control, agent efficacy should be measured by its impact on plant reproduction, which is often prolific in invasive plants. Vegetative reproduction by water hyacinth is responsible for the spread and establishment of new infestations (Edwards & Musil, 1975) and it is responsible for reinfestation of water hyacinth sites that have been cleared by chemical and biological control. The laboratory studies suggest that *E. catarinensis* has greater potential than *C. aquaticum* to reduce ramet production whereas the *Neochetina* weevils suppressed ramet production under conditions similar to the low nutrient treatment when the response of plants to *C. aquaticum* was the same in terms of clonal growth.

Flower production was not influenced by nutrient availability but was significantly reduced by feeding by *C. aquaticum* at all nutrient levels. Throop (2005) found a similar response where reproductive allocation of *Ambrosia artemisiifolia* L. (Asteraceae) was

not affected by nutrient availability but was significantly reduced by herbivory by a leaf beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). Watson & Brochier (1988) found that low nutrient conditions induced flowering in water hyacinth and suggested further studies should be conducted to investigate the effect of nitrogen concentration on flower production. The results reported here do not support the hypothesis that flower production is influenced by nitrogen availability. Flowering appeared to be stimulated by nutrient-stress in Coetzee et al.'s (2007) study but was not significantly reduced by herbivory at any of the nutrient levels tested. Although ramet production is believed to be more important than seed germination in terms of increase and spread of water hyacinth in South Africa (Hill, pers. comm.), a reduction in the seed bank would be beneficial as seed germination is also responsible for reinfestation of systems or introduction into new areas as a result of seed washing downstream.

The leaf life cycle and turnover rates that result in the maximum provision of carbon to the plant in a particular environment should be adaptively favoured (Chabot & Hicks, 1982). The number of leaves of water hyacinth plants at week 10 and leaf production were only affected by herbivory in the low nutrient treatment, where they were also affected by nutrient supply alone. Center & Spencer (1981) note the consistency in the number of water hyacinth leaves and report that “a range of 6-8 functional leaves is the normal complement for a mature water hyacinth plant and leaf production and senescence reach an equilibrium resulting in the retention of a nearly constant leaf complement”. The leaves are responsible for supplying the plants with buoyancy and an upset in the balance between leaf production and mortality rates will compromise the ability of plants to stay afloat and therefore their survival (Center & Van. 1989). The most severe impacts on water hyacinth plants can occur when leaf dynamics are disrupted and production rates are outbalanced by mortality rates as Center & Van (1989) found with high densities of the *Neochetina* weevils. Maintaining leaf biomass also maintains the capacity for carbon acquisition which is needed for growth and survival. Therefore an adaptive and compensatory response for survival would be to maintain allocation to leaf biomass, at the expense of investment in other vegetative tissue if under conditions of stress such as herbivory. Alteration in patterns of biomass allocation is a common response to herbivory

and is usually related to maintaining the highest rates of resource or carbon acquisition (Mihaliak & Lincoln, 1989; Steinger & Müller-Schärer, 1992). Heard & Winterton (2000) and Coetzee et al. (2007) observed a similar response in plants where other parameters were affected by herbivory but leaf production remained the same or increased in presence of herbivore feeding. This response of water hyacinth to herbivory in terms of leaf dynamics indicates an equal compensatory response, although, where the grasshopper is concerned, it is nutrient-dependent. Where nutrient availability is reduced, undercompensation is evident, mortality rates exceed production rates and plant survival is compromised.

The grasshoppers had a preference for younger leaves in the low nutrient treatment and preferred older, more mature leaves as nutrient supply to the plants increased. Plant nitrogen levels can influence insect behaviour and cause a change in feeding site (Mattson, 1980). However, chemical analysis of leaves 3, 4 and 5 showed that nitrogen levels were similar which is consistent with the findings of Center & Wright (1991) therefore leaf preference was not due to nitrogen requirements, and grasshoppers did not change feeding site to increase nitrogen intake. Other aspects of leaf chemistry such as defensive metabolites may have been responsible for the different feeding patterns across the nutrient levels or grasshoppers were limited by the availability of a fewer number of leaves on rosettes in the low nutrient treatment.

Despite the differences in leaf selection between the nutrient levels, adult *C. aquaticum* consistently selected mature leaves for feeding and very seldom was feeding observed on immature leaves, if they had a choice. Leaf selection by phytophagous insects is usually attributed to their specificity (Cates, 1980) and highly specialized herbivores are expected to feed on younger leaves and generalist herbivores are expected feed on older, less defended leaves. Center & Wright (1991) present a case on water hyacinth in support the generalist/specialist theory. Larvae of a generalist, the yellow woollybear, *Spilosoma virginica* F. (Lepidoptera: Arctiidae), had a preference for mature water hyacinth leaves and suffered reduced fitness when forced to consume younger leaves whereas the weevil, *N. eichhorniae* a highly specialized herbivore, consistently prefer immature leaves which

have the highest levels of toxic compounds. *Cornops aquaticum* being oligophagous (feeding on a few, closely related plants) (Oberholzer & Hill, 2001) is not as specialized on water hyacinth as the weevils and may not be adapted to cope with the higher levels of compounds that occur in young water hyacinth leaves. An alternative possibility is that mature water hyacinth leaves are a more suitable niche for adult grasshoppers and they have evolved with their host to specialize on those leaves. Leaf selection patterns by *C. aquaticum* could have significance for the biocontrol programme in terms of its compatibility with the existing biocontrol agents, and is discussed in the final chapter.

These results show that water hyacinth can take advantage of conditions of high nutrient availability but the plant is highly intolerant of stressful environmental conditions. The most severe impact on water hyacinth in this study, where plant survival was compromised, was from an interaction between grasshopper herbivory and nutrient stress. The results show that the growth and reproductive capacity of water hyacinth plants and their response to herbivory by *C. aquaticum* is largely nutrient dependent. Therefore the impact of the grasshopper on water hyacinth populations will be strongly influenced by the nutrient conditions that the plants are growing in. The results suggest that if *C. aquaticum* is released as a biocontrol agent in South Africa, it can be expected to reduce the biomass, density and spread of water hyacinth infestations. The following predictions can be made: (1) the grasshopper will provide an excellent level of control in oligotrophic nutrient conditions, when plants are nutrient stressed. At sites with nitrate and phosphate levels similar to those found in New Year's Dam in the Eastern Cape Province, the plants should suffer great reductions or loss in performance due to their slow, stunted growth rates as a result of nutrient deficiency. An inability to compensate for reductions in all growth and reproductive parameters will significantly reduce the potential for increase and spread, and disruption of leaf dynamics due to the inability of plants to equally or overcompensate for leaf loss will result in death of water hyacinth plants and reductions in water hyacinth mats; (2) At slightly eutrophic levels bordering on mesotrophic such as those found in Hammarsdale Dam, the grasshoppers will provide an adequate level of control, keeping plants in check and preventing further spread. At a density of one grasshopper per plant, herbivory will prevent increases in density, biomass and spread of

water hyacinth infestations but higher grasshopper densities will be needed to disrupt leaf dynamics, and reduce infestations by causing mortality of plants; and (3) in highly eutrophic systems, they will provide some degree of control in terms of slowing growth, productivity and biomass accumulation. However, in polluted impoundments such as Mbozambo Swamp, higher grasshopper densities will be needed to disrupt water hyacinth leaf dynamics, one of the crucial factors affecting plant survival and compensatory capacity in order to exert a more acceptable level of control. Bearing in mind that 14 out of the 15 water bodies in South Africa with water hyacinth exhibited nutrient levels below the medium levels of nitrates and phosphates used for the trial (Brudvig, pers. comm.), *C. aquaticum* would provide effective control in most of those systems. The insect density used in the present study is the same density at which they occur in the native range (Silveira-Guido & Perkins, 1975) so it would likely be a conservative density for field populations in South Africa. In the absence of its highly specialized predator, *L. fasciatus* that regulates populations of *C. aquaticum* in South America (Silveira-Guido & Perkins, 1975) we could expect to get higher population densities in South Africa.

In conclusion, nutrient availability plays a major role in water hyacinth's compensatory responses to herbivory by *C. aquaticum* therefore the nutrient status of water bodies in South Africa will have a significant influence in determining the impact of this species on water hyacinth population dynamics. This study provides further evidence that a reduction in nutrient-input into water bodies with water hyacinth will greatly reduce water hyacinth's growth potential and will reduce its ability to tolerate damage caused by herbivory. However, the response of a plant to herbivory by its host-specific herbivores is not the only factor that will influence the potential of a biocontrol agent to reduce populations of their host. There are several interacting factors responsible for influencing efficacy, one of which is the response of insects to the quality of their host. Quality, not quantity is often a limiting factor for insect populations and if phytophagous insects only have access to food of poor quality which negatively affects their performance, it is unlikely that they will become sufficiently abundant to have a significant effect on plant performance (Price, 1975; 2000). A key to success in weed biocontrol programmes is

high rates of population increase of biocontrol agents so that they reach high enough population densities to reduce weed growth. Since water nutrients and plant nutrients are highly correlated, it is likely that the nutrient conditions prevalent in South African water bodies with water hyacinth will influence not only plant response in terms of productivity and herbivory, but also insect response which will have consequences for their population dynamics. The effect of water hyacinth plant quality, as influenced by water nitrate and phosphate levels, on *C. aquaticum*'s life history characteristics is therefore investigated in chapter 3. Compensatory responses of *C. aquaticum* to varying levels of nutrients in their diet are also investigated.

Chapter 3

**Performance of *Cornops aquaticum* in response to variation
in water hyacinth plant quality as influenced by
environmental nitrogen (N) availability**

3.1 Introduction

Phytophagous insects are faced with high variability in the quality of their host plant/s, which is linked to the environmental conditions that a plant is growing in. The term host plant quality refers to the components of a host on which a phytophagous insect feeds and develops which may negatively or positively influence their performance (Awmack & Leather, 2002). Both the nutritional value and other constituents such as secondary defensive metabolites of plants can have an effect on many aspects of insect behaviour and life history characteristics (Myers & Post, 1981; Bryant et al., 1983; 1987; Taylor, 1984; 1989; Ohmart et al., 1985; Joern & Behmer, 1998; De Bruyn et al., 2002; Hogendorp et al., 2006). Nitrogen in particular is the critical nutritional element for herbivorous insects and it has been suggested that they are limited by the availability of nitrogen in their diet (White, 1976; Mattson, 1980). However, an optimal level of nitrogen in plant tissue exists for insects (Mattson, 1980) and levels above those can have detrimental effect on their physiology. Indirect effects of nitrogen availability to plants on insects include increases in plant biomass and changes in defensive chemicals (Bryant et al., 1983; 1987; Gerson & Kelsey, 1999; Throop & Lerdau, 2004). The effects can vary between generalists and specialists (Dyer et al., 2004), but environmental nitrogen availability can have a substantial impact on individual insect performance and insect population dynamics (Kytö et al., 1996; Denno et al., 2003; Xhong-xian, 2007) as a result of changes in their behaviour and life history characteristics. Host plant quality can therefore have a significant influence on the impact of insects on plant communities and populations.

Increased nitrogen in plant tissue associated with nitrogen fertilization has many positive effects on insects such as increased survival, body size, growth rates, and fecundity. Shorter development time for immature stages of insects and higher survivorship and biomass is often associated with increases in dietary nitrogen. For example, development time of the citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae) was negatively correlated with leaf N (%) concentration and body size was significantly higher with high levels of fertilizer applications (Hogendorp et al., 2006). Larval survival, body size and development of a leaf-mining fly, *Agromyza nigripes* Meigen (Diptera:

Agromyzidae) was significantly influenced by quality of their host, *Holcus lanatus* L. (Poaceae) in response to increases in the nitrogen content of Hoagland's solutions used to fertilize the grass (De Bruyn et al., 2002). Variation in larval survival rates according to plant tissue nitrogen levels in host plants have been recorded in other insect species (Myers & Post, 1981; Fox et al., 1990).

Host plant quality can have a wide range of effects on fecundity and reproductive strategies of insects. Within-species fecundity of herbivorous insects is highly variable and is linked to genetic factors, conditions during immature development as well as prevailing environmental conditions during egg development and oviposition (Honěk, 1993). Potential and realized (achieved) fecundity in insects can be influenced by a wide range of factors and sometimes the difference between the two can be substantial (Awmack & Leather, 2002). In some species, immature development determines adult body size which influences potential fecundity but food quality of females during reproduction affects achieved fecundity (Honěk, 1993). Fecundity of a leaf-feeding beetle, *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) was significantly affected by nitrogen levels in their host, *Eucalyptus blakeyli* Maiden (Myrtaceae). Females feeding on foliage with high levels of nitrogen laid significantly more eggs than females with a low-nitrogen diet (Ohmart et al., 1985). In other species, food quality during immature development affects body size, which is the major determinant of adult fecundity (Honěk, 1993). Blackenhorn (1994) found that fecundity was highly correlated with body size of the water strider, *Aquarius remigis* Say (Heteroptera: Gerridae). Food quality of females during egg development can also have an impact on fitness of their offspring. Environmentally-based maternal effects in terms of food quality of females influenced body size, growth rates and fecundity of offspring in the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) in response to the quality of a host, the red oak tree, *Quercus rubra* L. (Fagaceae) (Rossiter, 1991). Plant biomass can also influence fecundity of herbivorous insects. Female acridid grasshoppers of *Melanoplus sanguinipes* Fabricius (Orthoptera: Acrididae) responded to increased resource availability by increasing reproductive allocation, compared to sites where food was limited (Branson, 2004).

Insect feeding rates are highly variable according to both the nutritional and non-nutritional (plant secondary metabolites) profile of host plants. This variability often requires insects to compensate to meet their nutritional demands (Bernays, 1998). Compensatory consumption in response to the quality of their host plant is a common response of insects during development (Taylor & Bardner, 1968; Slansky & Feeny, 1977; Taylor, 1989; Wheeler, 2003). The two basic mechanisms used by insects to cope with low quality hosts or low nitrogen in their diets are to increase total consumption and/or to prolong periods of feeding, digestion and development (Slansky, 1981). Assimilation efficiency or approximate digestibility (AD) of ingested food usually increases with an increase in plant nitrogen content and low assimilation rates are associated with increased total consumption (Mattson, 1980). According to Mattson (1980), organisms adapted to feed on nitrogen-poor foliage and other substrates have low ADs of between 3 and 20% and those that feed on nitrogen-rich foods typically have the highest ADs of between 60 and 90%. Changes in feeding rates in response to plant quality can lead to differential impacts of herbivores on plants according to environmental conditions that plants are growing in.

The plasticity of phytophagous insects in their physiological responses to changes in the quality of their diet, as well as the variability in plant defensive metabolites with environmental nutrient availability can potentially have a profound influence on population dynamics and damage levels of insect biological control agents. This would ultimately influence their ability to control populations of the target plant. Price (2000) notes that the quality of host plants in the field is one of the major factors that limits successful implementation of biological control. Host plants are often not of suitable quality for high rates of establishment and population growth, therefore he stresses that the optimal host plant quality for a particular insect biocontrol agent should be determined for successful introductions. There have been cases where biocontrol practitioners have identified the importance of host plant quality in establishment and success of biocontrol agents. Wheeler (2001) investigated the effect of host plant quality on survival, development rates and adult biomass of the biocontrol agent, *Oxyops vitosa* Pascoe (Coleoptera: Curculionidae) for *Melaleuca quinquenervia* (Cav.) Blake

(Myrtaceae). Their findings facilitated establishment of *O. vitosa* through the development of mass-production nursery sites and selection of suitable release sites. Further work with this agent indicated that fertilization of *M. quinquenervia* to manipulate nitrogen levels had no effects on fecundity or feeding rates of *O. vitosa* but larval survival was significantly higher when reared on high-quality leaves compared to low quality leaves. The author suggested that high-fertilizer sites with *O. vitosa* will have higher rates of establishment and population build-up due to increased larval survival in response to leaf quality of their host (Wheeler, 2003). High nitrogen levels in tansy ragwort, *Senecio jacobaea* L. (Asteraceae) increased larval survival and adult fecundity of the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), a biocontrol agent, which allowed the moth populations to periodically overexploit their food supply, accentuating instability and fluctuations of moth populations (Myers & Post, 1981). Larval development and fecundity of *S. multiplicalis* were highly correlated with nitrogen levels in its host plant, *S. molesta* (Taylor, 1984). Nitrogen fertilization of a lake in Papua New Guinea after failure of establishment of a biocontrol agent for *S. molesta*, the weevil *C. salviniae*, resulted in rapid population growth of the beetles and increased damage to plants (Room & Thomas, 1985). A strong positive correlation between pupal mass and fecundity of *Gratiana spadicea* Klug (Coleoptera: Chrysomelidae) for *Solanum sisymbriifolium* Lamarck (Solanaceae) indicated suitability of this species to its host plant as they provided the necessary nutrients for high fitness levels (Cyzpionka & Hill, 2007). Stanley et al. (2007) found that high quality water hyacinth plants increased larval development rates and body weight at pupation of *Xubida infusella* Walker (Lepidoptera: Pyralidae) leading the authors to suggest that faster developing insects on high nitrogen plants would have higher rates of population increase which would lead to greater impacts on the target plant, if the agent were to be released. In general the effects of high plant quality are expected to have a positive effect on the performance of biological control agents.

Water nutrient availability and water hyacinth quality in terms of foliar nitrogen levels are correlated (chapter 2) therefore the nutrient status of water bodies could have a significant influence on the water hyacinth biocontrol agents. Efficacy of the *Neochetina*

weevils and plant quality are notably related and weevil population growth rates in the United States and Australia have been observed to be fastest on healthy, luxuriant plants (in Center & Wright, 1991; unpublished data). Heard & Winterton (2000) found in laboratory studies that development and reproductive performance of the *Neochetina* weevils were significantly affected by differences in plant quality related to water nutrient levels and higher damage levels to water hyacinth plants by *N. bruchi* at high nutrient concentrations were found due to higher numbers of offspring produced at those levels. Differences in performance of the mirid, *E. catarinensis* on water hyacinth of different qualities were found in laboratory experiments (Coetzee et al., 2005; Ripley et al., 2006), with plants growing under eutrophic conditions supporting the largest mirid populations. Despite these findings and observations, the relationship between plant quality and agent efficacy has not been evaluated in the field in South Africa to provide possible explanations for varying levels of control of water hyacinth by its biocontrol agents seen in river systems and impoundments. For example, knowledge of the range of conditions that are favourable for high rates of increase for specific agents can provide an indication of expected performance levels in particular systems if the nutrient concentrations are known and monitored. Understanding the relationship between the physiology of a biocontrol agent and target plant quality will enable predictions to be made and expectations for biocontrol in certain systems. This could contribute to management and decision-making in biocontrol programmes for water hyacinth.

The link between insect physiology and nutritional ecology or food requirements means that host plant quality may influence the biocontrol potential of *C. aquaticum* by influencing feeding rates, survival, dispersal and population fluctuations. Evaluation of the impact of *C. aquaticum* on plants grown in different nutrient regimes found in South African systems revealed that water hyacinth's response to herbivory by the grasshopper was nutrient dependent and plants were better able to compensate for loss of tissue through defoliation as nutrient supply to the plants increased (chapter 2). The results suggested that higher densities of *C. aquaticum* would be needed in eutrophic systems to reduce the density and spread of water hyacinth infestations. However, there is a need for clarity on whether these differences in damage levels were purely a result of plant

productivity or whether increased levels of damage to plants at low nutrient levels were due to the grasshoppers increasing their consumption to meet nutrient demands. Furthermore, evaluation of changes in *C. aquaticum* life history characteristics, such as fecundity and development rates, in response to plant quality would provide insight into the significance of host plant quality in the field in success of this candidate biocontrol agent. If these relationships are defined, leaf quality can be used as a predictor of success or failure of a programme in a certain system with *C. aquaticum*. Therefore the aim of this chapter was to: (1) investigate the effects of water hyacinth plant quality, as influenced by varying water nitrate and phosphate levels, on *C. aquaticum* survival, body size, fecundity and feeding and development rates; and (2) to evaluate nutrient dependent responses of the grasshopper in order to make predictions on establishment, survival, population dynamics and success of this species in systems with water hyacinth and changing nutrient regimes.

3.2 Materials and Methods

3.2.1 Effect of plant nutrient levels on Cornops aquaticum survival, fecundity and body size

3.2.1.1 Trial 1 experimental design - investigating the effect of plant nutrients on fecundity, nymphal survival and body size of adults and their offspring

This trial was conducted in a quarantine glasshouse at the Plant Protection Research Institute in Pretoria from October to December 2007. Refer to chapter 2 materials and methods for glasshouse conditions. Twenty-eight grasshoppers per nutrient treatment were reared from newly hatched first instar nymphs to adulthood on plants grown at the high, medium and low levels of nitrates and phosphates (see chapter 2 for concentrations in mgL^{-1}). These first generation nymphs were offspring of females from the *C. aquaticum* culture in quarantine at PPRI in Pretoria, maintained on water hyacinth plants fertilized with a patterned release fertilizer (Osmocote). The nymphs were reared on whole plants in rearing boxes in a quarantine glasshouse and were separated into males and females before the final moult to prevent copulation before pairing. After the final nymphal moult, grasshoppers were sexed, weighed, paired and introduced onto plants

grown at the same nutrient levels on which they were reared. There were eight pairs of adults in the high and medium nutrient treatments but only 7 in the low nutrient treatment due to a shortage of females reared through to adulthood. The trial was conducted for a period of six weeks and the number of egg packets per female, and the number of eggs per egg packet and body weight (g) of their offspring (newly hatched second generation nymphs) were recorded for each pair at each nutrient treatment.

3.2.1.2 Trial 2 experimental design – investigating immature or maternal effects of plant quality on fecundity

Significant differences in fecundity of females (first generation) and body size of the second generation nymphs were found between the nutrient levels in trial 1 so a second trial was conducted to determine whether these differences were due to plant quality during nymphal development or due to plant quality during egg development by females. This trial was conducted under the same quarantine glasshouse conditions as trial 1 from March to May 2008. The methodology for the second trial followed the same design as the first with the exception that all pairs of adults from all three treatments were introduced onto plants grown at the high nutrient level after pairing. Nymphs reared on high nutrient plants served as a control and the experimental design eliminated the possibility that a lack of oviposition sites in the low treatment in trial 1 (plants were shown to have fewer and shorter petioles in chapter 2) was the cause of fewer egg packets per female. The number of egg packets per female were recorded but the number of nymphs per egg packet and the body weight of nymphs were not recorded during this trial.

3.2.1.3 Statistical analysis

Data for body weights of male and female grasshoppers from trials 1 and 2 were not significantly different ($F_{1,92} = 1.6967$; $P = 0.2002$) therefore they were combined and compared by two-way ANOVA to test for differences between males and females and for the effect of nutrient treatment on body size of the two sexes. Survivorship of first generation nymphs from both trials (1st instar to adult) were plotted as a percentage for each nutrient treatment (survivors/total no. reared). The proportions of males and females

amongst the total number of first generation survivors from trials 1 and 2 within each nutrient treatment were also plotted as a percentage (male or female/total survivors) to show the effect of plant quality on survival. The number of egg packets per female from trials 1 and 2 were compared by one-way ANOVA to test for the effect of nutrient treatment on fecundity. The number of second generation nymphs per egg packet and their body weights (g) measured in trial 1 were also compared by one-way ANOVA. A mean body weight (g) of 1st instar nymphs that hatched from each egg packet for which data was collected was used in the statistical analysis. Tukey's HSD test for unequal sample sizes was used as a multiple range test for all ANOVAs. Nitrogen levels (%) of leaves 3, 4 and 5 (see chapter 2 for methodology) were averaged for each nutrient treatment and used to assess with a product-moment (Pearson's) correlation the relationship between plant foliar nitrogen content that females were exposed to during egg development and the number of egg packets oviposited by females at each nutrient treatment. A product-moment (Pearson's) correlation was also used for assessment of the relationship between body size (g) of females and fecundity measured by the number of egg packets. Scatterplots were used for graphical representations of these relationships.

*3.2.2 Effect of plant nutrient levels on survival and feeding and development rates of *Cornops aquaticum* nymphs*

3.2.2.1 Experimental design

To investigate the effect of plant nutrient levels on feeding and development rates of *C. aquaticum*, 16 newly emerged first instar nymphs were reared to adulthood on cut leaves of water hyacinth plants grown at high, medium and low nutrient levels (see chapter 2 for concentrations of N and P in mgL^{-1}). Nymphs were the offspring of females from the *C. aquaticum* culture (see above). Plants to be used in the experiment were grown at the three nutrient concentrations for a period of three months before the trial commenced to ensure they were fully adapted to the nutrient environment with differences in tissue nitrogen content. Leaf 4 of water hyacinth plants at all three nutrient levels were fed to nymphs for the duration of the trial to maintain consistency in foliar nitrogen levels that nymphs were exposed to during development. Nymphs were fed one new leaf every two

days. To maintain turgidity and leaf freshness, petioles of cut leaves were sealed with soft wax and the rearing containers (transparent plastic tubs) were lined with moist filter paper. The trial was conducted in a controlled environment (CE) room with a maximum temperature of 28°C, a minimum of 22°C and a photoperiod of 14:10 hrs day:night. The rearing conditions were selected as they are favourable for insect growth and development and correspond with temperatures and day length in the summer months in South Africa. Leaves were weighed before and after feeding and faecal pellets (frass) were collected as a measure of the amount of food consumed. Leaves and faeces were oven dried at 70°C to constant weight and then weighed. The number of days between each nymphal moult, number of instars, total development time and sex and weight of adult grasshoppers were recorded. Mortality rates were extremely high, possibly due to stress of handling but were particularly so in the high nutrient treatment. Up to 30 nymphs per treatment were reared in an attempt to increase the final number of replicates, but in spite of that, only low numbers of nymphs could be reared all the way through to adulthood. The possibility that nymphs were diseased was not investigated but it is highly unlikely that this was the cause of the high mortality rate as the *C. aquaticum* culture has been free of diseases since its introduction into quarantine in 1995. No male nymphs in the high nutrient treatment survived to the final moult so differences between males could only be compared between the medium and low treatments.

3.2.2.2 Statistical analysis

The number of nymphs surviving to adulthood and the proportions of males and females of the total number of survivors in each treatment were plotted as a percentage as for the fecundity trial to show the effect of plant quality on survivorship. Survival figures were calculated on the original 16 replicates for each treatment. The development data were not normally distributed so a Kruskal-Wallis ANOVA was used for comparison of the total development time for nymphs in each treatment and the number of days for each instar. The relationship between nitrogen content of leaf 4 (%) and development time were investigated using Pearson's rank order correlations. The weight of frass (g) of nymphs and the change in leaf weight (g) before and after feeding at the high, medium and low nutrient treatments were investigated using a product-moment (Pearson's)

correlation. Assimilation efficiencies/approximate digestibility (AD) of the nymphal instars across the nutrient treatments were calculated where $AD = (\text{food ingested} - \text{frass})/\text{food ingested}$. The data are expressed as a percentage. The data for the total weight of frass of nymphs during their development were not normally distributed so non-parametric statistical tests were used for analysis of the feeding data. A Mann-Whitney U test was used for comparison of two independent sample data (comparing males reared through to adulthood at the medium and low nutrient treatments). A Kruskal-Wallis ANOVA was used to compare female grasshopper feeding rates.

3.3 Results

3.3.1 Effect of nutrient levels on survival, fecundity and body size

Nutrient treatment had a significant effect ($F_{2,92} = 10.78$; $P < 0.0001$) (Fig. 3.1) on the body weight of female grasshoppers, with females reared on plants grown at the high and medium nutrient treatments weighing significantly more than females reared on low nutrient plants. Females from the medium nutrient treatment weighed more than females from the high nutrient treatment, but this was not statistically significant. Males were not affected by plant quality in terms of their biomass with nutrient treatment having no significant effect on their weight at adulthood. Body weights of female grasshoppers were significantly higher than males ($F_{1,92} = 539.89$; $P = 0.0001$) (Fig. 3.1) and the interaction between nutrient treatment and grasshopper sex was highly significant ($F_{2,92} = 9.90$; $P = 0.0001$).

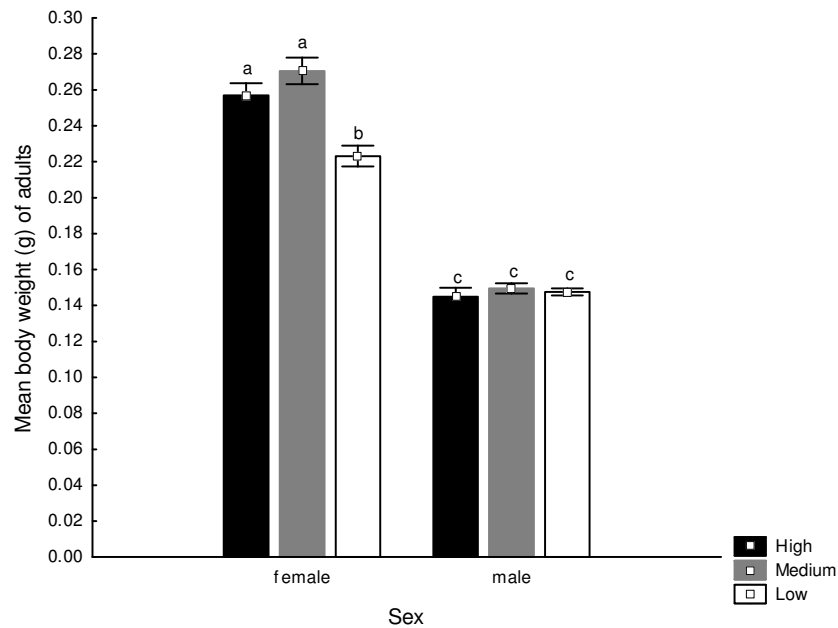


Figure 3.1 Mean body weight of male and female grasshoppers reared on plants grown at high, medium and low nutrient levels. Means compared by two-way ANOVA; those with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes; $P < 0.05$). Error bars represent the standard error of the mean.

Survivorship of nymphs to adulthood was affected by nutrient treatment which also had an effect on the proportions of males and females to be reared through to adulthood (Fig. 3.2). Total survival increased with an increase in nutrient supply to plants when nymphs were reared on whole plants indicating that higher plant quality elicits higher rates of survival in *C. aquaticum*. Higher numbers of females survived to adulthood in the high nutrient treatment, whereas the ratio was close to 50:50 in the medium nutrient treatment, and in the low nutrient treatment, higher numbers of males than females were reared all the way through to adulthood. Therefore, high quality plants favoured females, and low quality plants favoured survival of males.

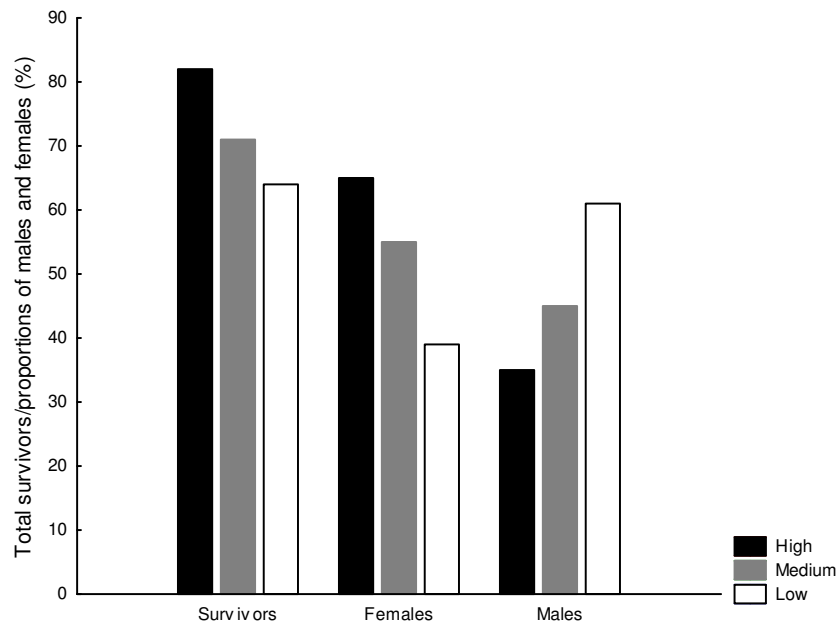


Figure 3.2 Percentage of first generation nymphs to survive from 1st instar to adulthood and proportion of males and females among survivors reared on plants grown at high, medium and low nutrient levels (Trial 1; n = 61).

Nutrient treatment had a significant effect ($F_{2,20} = 26.058$; $P < 0.0001$) on fecundity of female grasshoppers that were reared and maintained after pairing with males at adulthood on plants grown at high, medium and low nutrient levels (trial 1; Fig. 3.3). Fecundity measured by the number of egg packets per female increased with an increase in nutrient supply to plants and therefore host plant quality. When females reared on plants grown at medium and low nutrient levels were switched to high nutrient plants at adulthood and therefore a higher quality diet, fecundity measured by the number of egg packets increased at both nutrient levels (trial 2; Fig. 3.3). However, these increases within each nutrient treatment were not statistically significant and the number of egg packets was still lower than the number of egg packets of females that were reared and maintained as adults on high nutrient plants. The number of egg packets in the medium nutrient treatment was not significantly different from females in the high nutrient treatment when switching to a high quality diet. The lack of significant differences in the number of egg packets of females in the high nutrient treatment between trials 1 and 2 indicated consistent results and therefore the absence of variability in other factors that might have influenced the changes in fecundity measured as the number of egg packets.

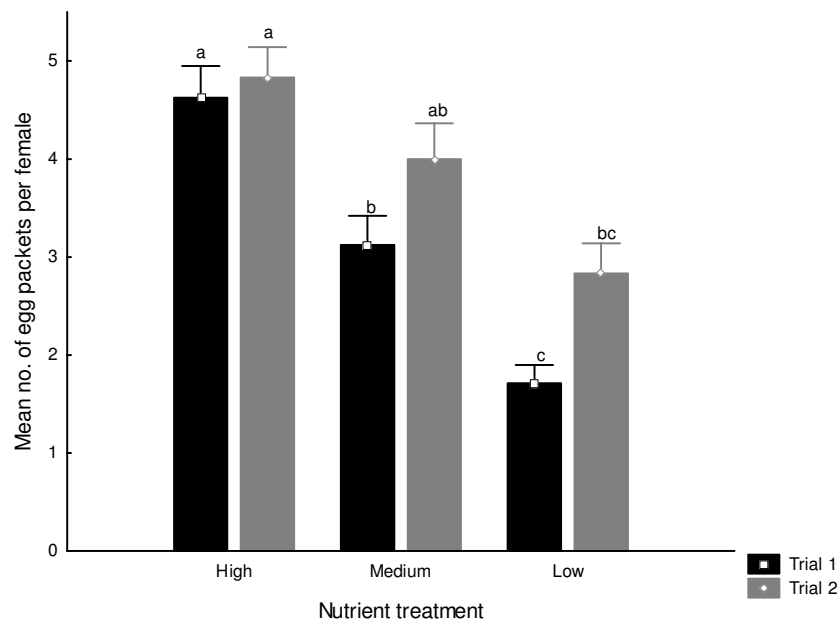


Figure 3.3 Mean number of egg packets oviposited by female grasshoppers. Trial 1 females reared on plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same levels. Trial 2 females reared on plants grown at high, medium and low nutrient levels and transferred to high nutrient plants at adulthood. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.

Nutrient treatment had a significant effect ($F_{2;18} = 7.578$; $P = 0.0041$) on the number of nymphs to hatch from egg packets of females that were reared and maintained as adults on the same quality plant (Fig. 3.4). Nymphs per egg packet increased with an increase in nutrient supply to plants, but only the high and low nutrient treatments were significantly different from one another. The same trend was evident for body weights of newly emerged 1st instar nymphs which increased with an increase in nutrient supply, ($F_{2;16} = 4.678$; $P = 0.025$) although only the high and low nutrient treatments were significantly different (Fig. 3.5).

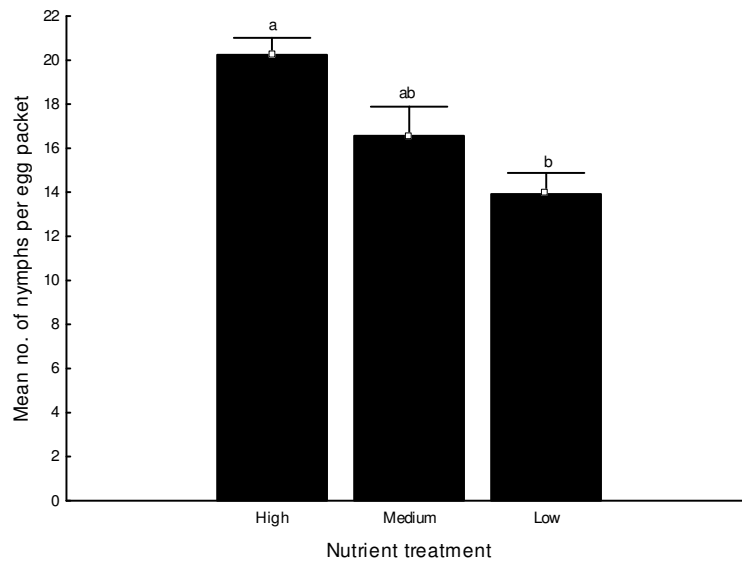


Figure 3.4 Mean number of nymphs per egg packet of female grasshoppers reared on water hyacinth plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same nutrient levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.

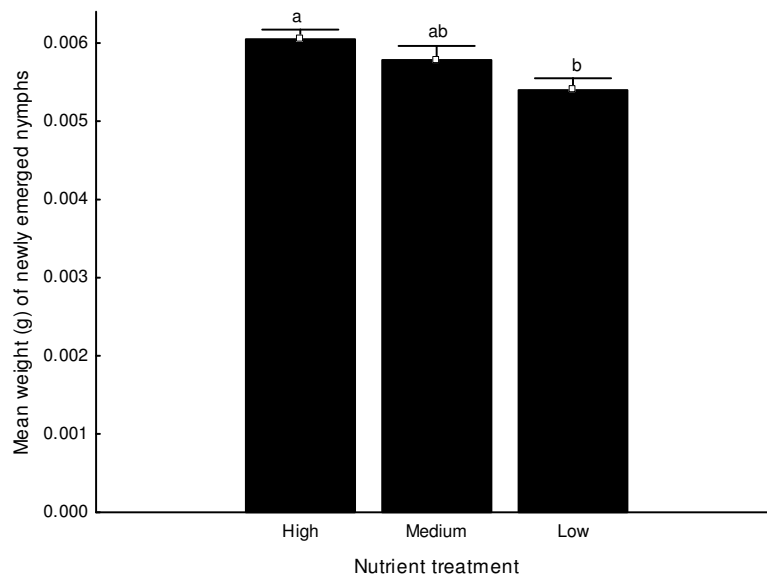


Figure 3.5 Mean weight (g) of newly emerged nymphs of female grasshoppers reared on plants grown at high, medium and low nutrient levels and transferred at adulthood to plants grown at the same levels. Means compared by one-way ANOVA, those with the same letter are not significantly different (Tukey's HSD for unequal sample sizes, $P < 0.05$). Error bars represent the standard error of the mean.

There was a weak but significant positive correlation ($r = 0.48$; $P = 0.002$) between the number of egg packets and body weight of females, suggesting that body size has an influence on female fecundity.

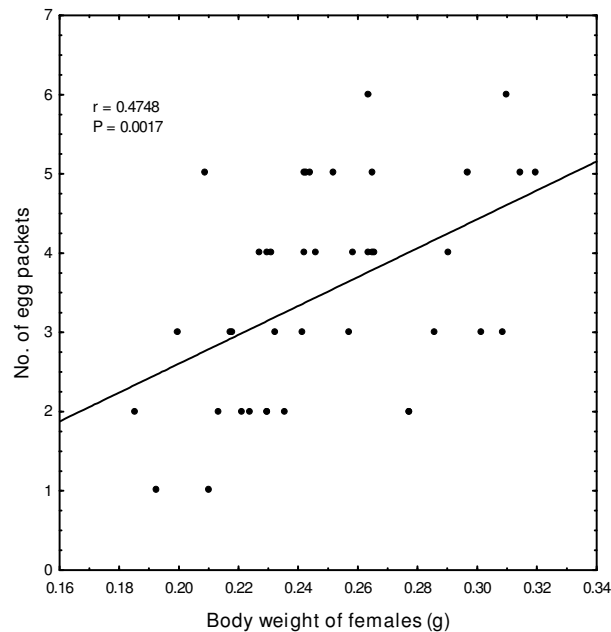


Figure 3.6 Relationship between the no. of egg packets (fecundity) and body weight (g) of female grasshoppers on plants grown at high, medium and low nutrient levels.

Numbers of egg packets per female was also significantly positively correlated ($r = 0.71$; $P < 0.0001$) with the mean nitrogen content of leaves that they predominantly fed on during the trials, therefore females feeding on higher quality leaves had greater fecundity. However, the regression only explained 50% of the variance.

3.3.2 Effects of nutrient treatment on survival and development rates of *Cornops aquaticum*

Nymphal survival (1st instar to adulthood) differed between nutrient treatments, although the trend was different to that found in the fecundity trial where nymphs were reared on whole plants as opposed to cut leaves (Fig. 3.7). In the high nutrient treatment, no males could be reared through to adulthood, despite doubling the number of replicates and only a total of four females could be reared to adulthood from over 30 nymphs. Therefore

females made up 100% of the survivors in the high nutrient treatment and overall survival was lower than the other two treatments. Survival was highest in the medium nutrient treatment and the proportions of survivors, although much lower than the fecundity trial, were almost identical to the proportions reared for the fecundity trial. In the low nutrient treatment, the total number of survivors was lower than the medium nutrient treatment but higher than the high nutrient treatment. A similar trend of highest survival of females in the high nutrient treatment, fairly equal survival rates at the medium nutrient treatment and highest survival of males in the low nutrient treatment was evident in both the fecundity and development trials where survivorship was recorded.

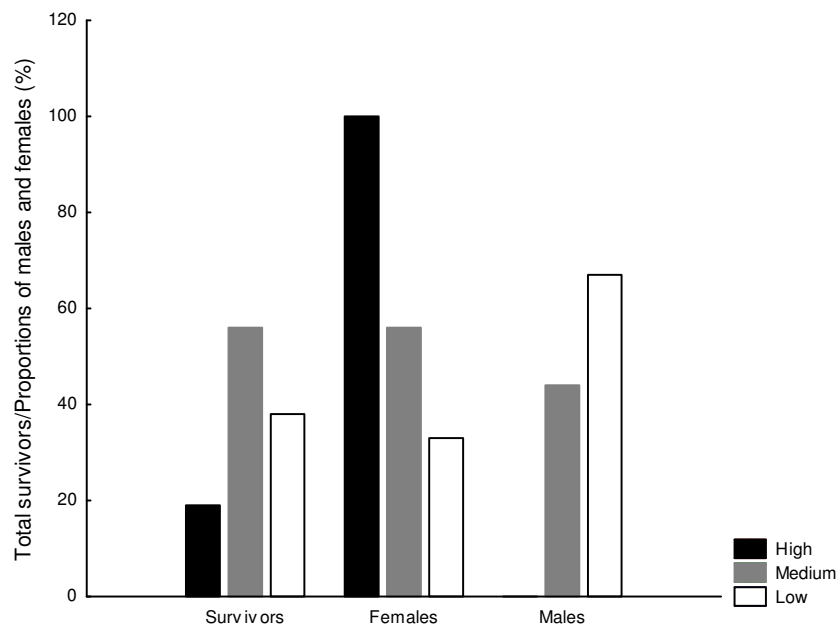


Figure 3.7 Percentage of nymphs to survive from 1st instar to adulthood and proportions of males and females among survivors reared on cut leaves of plants grown at high, medium and low nutrient levels (n= 21).

Development time of nymphs from 1st instar to adulthood was not significantly affected by leaf quality although there was a general trend for development time to decrease with an increase in nitrogen content of leaves (Fig. 3.8). The mean number of days for nymphs to complete development were 39 days (± 2.45 SD) at the high nutrient concentration, 40.5 days (± 2.24 SD) at the medium nutrient concentration and 41.9 days (± 1.89 SD)

days at the low nutrient concentration. There were no significant differences for development time between male and female grasshoppers ($F_{1;18} = 0.169$; $P = 0.686$). Pearson's rank order correlation showed a weak but significant negative correlation ($r = -0.438$; $P < 0.05$) between leaf nitrogen content and total number of days for development, but the regression only explained 19% of the variance. The mean number of days per instar did not differ significantly between treatments with the exception of the first instar where development of nymphs was slightly faster in the high nutrient treatment ($F_{2;17} = 4.33$; $P = 0.030$) compared to the medium and low nutrient treatments (Fig. 3.9). The first four development stages averaged around seven days whereas the final instar took an average of 12 days across all the nutrient treatments.

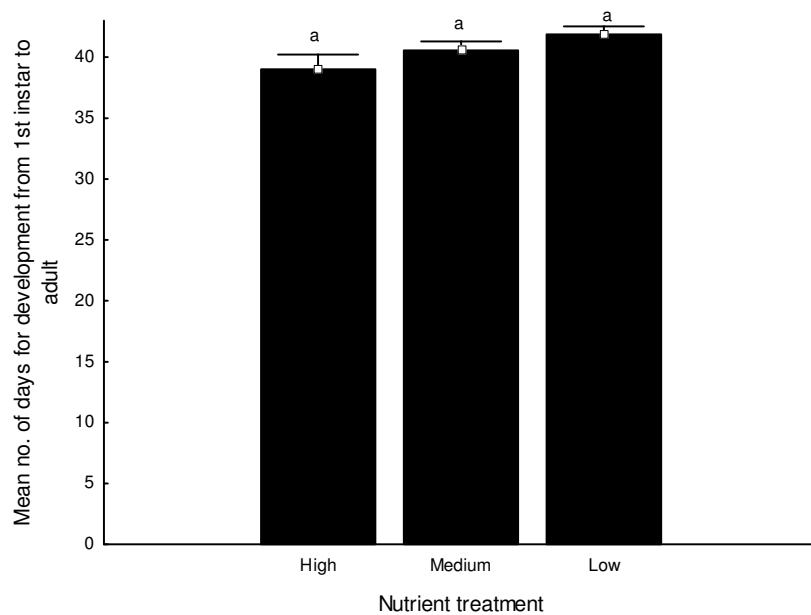


Figure 3.8 Mean number of days for development of nymphs from newly hatched 1st instar to adulthood reared on cut leaves of plants grown at high, medium and low nutrient levels. Medians compared by Kruskal-Wallis ANOVA. No significant differences.

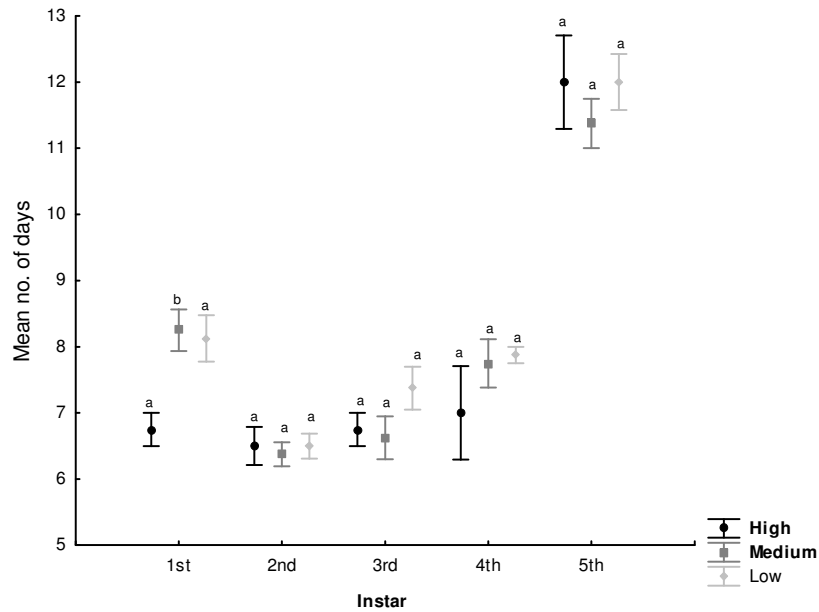


Figure 3.9 Mean number of days for instars 1-5 of nymphs reared on cut leaves of plants grown at high, medium and low nutrient levels. Medians compared by Kruskal-Wallis ANOVA; those with the same letter are not significantly different.

3.3.3 Effect of nutrient treatment on feeding rates of *Cornops aquaticum* nymphs

No correlation was found between the change in weight of water hyacinth leaves before and after feeding by the nymphs and the weight of frass ($r = -0.0016$; $P = 0.9728$). The mean total weight of frass (g) and the mean total amount of leaf material removed showed the opposite trend. The mean weight (g) of frass of male and female nymphs from 1st instar to the final moult increased with a decrease in plant quality, indicating compensatory consumption. Total consumption by females in the low treatment was significantly higher than in the high nutrient treatment ($H_{2;10} = 6.75$; $P = 0.034$) and the medium treatment was not different from either the high or the low. Weight of frass of male nymphs was significantly higher in the low treatment compared to the medium nutrient treatment ($U_{1;7} = 2.12$; $P = 0.034$). Females in the high treatment consumed significantly less than males in the low nutrient treatment ($U_{1;6} = 0.00$; $P = 0.049$) but the high female and medium male treatment were not different from one another ($U_{1;7} = 4.00$; $P = 0.480$). Female nymphs reared on leaves with medium levels of nitrogen consumed more than males reared on leaves of low ($U_{1;7} = 0.00$; $P = 0.034$) and medium ($U_{1;8} = 0.00$; $P = 0.021$) nitrogen content. Weight of frass of females in the low nutrient

treatment were significantly higher than the male medium ($U_{1;7} = 0.00$; $P = 0.034$) and the low male treatment ($U_{1;6} = 0.049$) (Fig. 3.10).

The increase in weight of frass of male and female nymphs over time from 1st instar to adulthood indicated that females in the low nutrient treatment had the highest feeding rates, followed by females in the medium nutrient treatment (Fig. 3.11). Feeding rates of males were very similar between the low and medium nutrient treatments suggesting that compensatory feeding was not as important for males as it was for females. Despite the size difference between males and females, consumption of males and females feeding on high quality plants was similar suggesting that plant nutrient levels have a substantial effect on feeding rates.

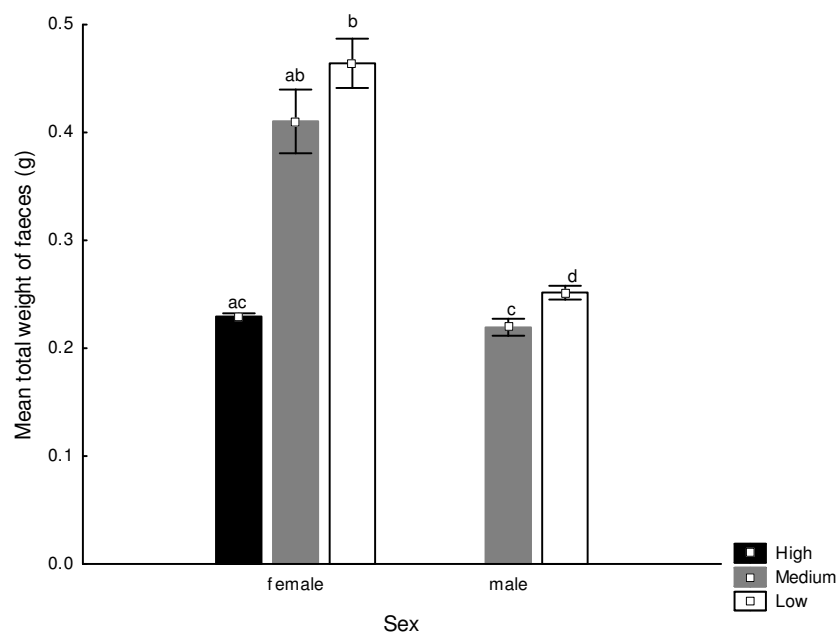


Figure 3.10 Mean weight of faeces (g) of male and female nymphs from 1st instar to adulthood as a measure of consumption rates. Medians compared by Kruskal-Wallis ANOVA and Mann-Whitney U tests. Those followed with the same letter are not significantly different.

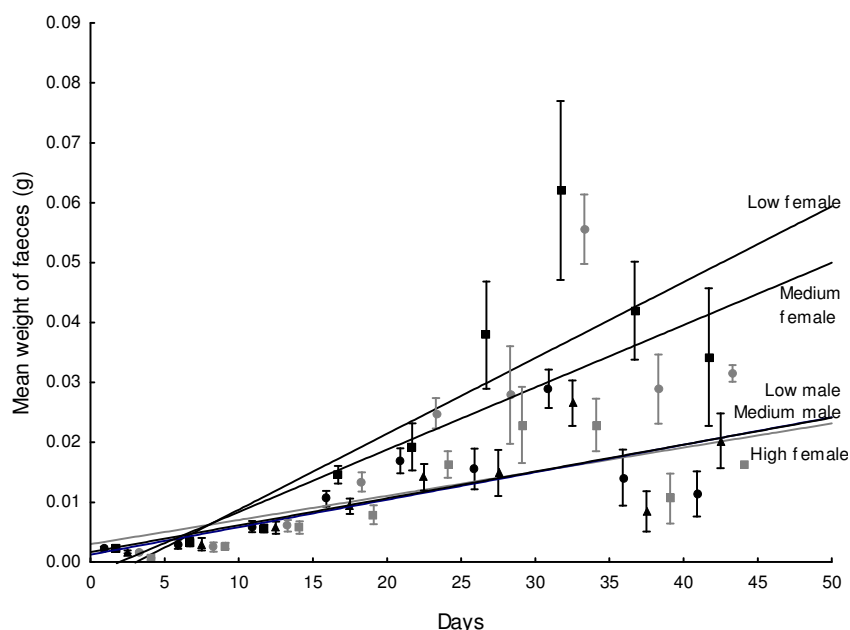


Figure 3.11 Mean weight of faeces (g) of male and female nymphs from 1st instar to adulthood reared on plants grown at high, medium and low nutrient levels. Error bars represent the standard error of the mean. (■ Low female; ● Medium female; ● Low male; ▲ Medium male; ■ High female).

The assimilation efficiencies (AD) of the nymphs feeding on leaves with high, medium and low levels of nitrogen are tabulated in Table 3.1. The ADs are exaggerated which is most likely due to the high water content of water hyacinth leaves which can be up to 90%. The ADs did show the expected trend where assimilation efficiencies of nymphs were higher when feeding on leaves of high nitrogen content. However, these data are considered to be inaccurate because the measure of consumption of the grasshoppers is skewed by the high water content of water hyacinth leaves and therefore it does not give an accurate measure of the amount of leaf material actually removed by feeding. The change in weight of leaves before and after feeding was influenced by a high percentage of water loss which was probably also highly variable between leaves. In order to use these data with confidence, a weight differencing method should have been applied whereby a mean proportion of weight loss of water hyacinth leaves due to drying should have been estimated on control leaves, so that the amount of tissue removed by the nymphs could have been calculated on dry weight by first converting the leaves to dry weight before feeding. However this was not done therefore it is believed that the change in weight is not a good measure of consumption in this experiment and is responsible for

the extreme ADs. It was clear based on observation during the trial that nymphs consumed much more leaf material and produced more frass in the low nutrient treatment, compared to nymphs in the high nutrient treatment, therefore the data on the weight of frass is used as a measure of consumption for *C. aquaticum* in this experiment as was done in a study by Burn (1981). Weight of frass was also found to be a better measure of consumption for *C. aquaticum* in a study investigating the effect of temperature on feeding rates (Bownes, unpublished data).

Table 3.1 Mean assimilation efficiencies (AD) of 1st to 5th instar nymphs reared on cut water leaves with high, medium and low nitrogen content (% dry weight).

Assimilation Efficiency % (AD) (\pm SD)			
	High	Medium	Low
1 st instar	99.75 (0.18)	99.86 (0.11)	99.93 (0.08)
2 nd instar	99.57 (0.27)	99.71 (0.15)	99.81 (0.08)
3 rd instar	99.25 (0.54)	99.14 (0.37)	99.66 (0.21)
4 th instar	97.69 (1.57)	98.39 (1.23)	99.27 (0.41)
5 th instar	97.48 (1.76)	97.32 (1.8)	99.12 (0.66)

3.4 Discussion

These results indicate that variation in the nutritional composition of water hyacinth plants, as influenced by water nutrient concentrations has a significant influence on *C. aquaticum* physiology and life history characteristics. Increasing levels of nitrogen and possibly other plant nutrients elicited increased nymphal survival (when reared on whole plants), higher body weights of adults and offspring and increased realized fecundity measured as the number of egg packets and number of eggs per egg packet of females. Development time of nymphs from first instar to the final moult was on average approximately three days longer in the low nutrient treatment compared to the high, although this was not statistically significant and is unlikely to have a significant effect on their population dynamics in the field. Feeding rates of nymphs decreased with

increasing levels of plant nitrogen and feeding rates between males and females differed significantly as a result of differences in body size and plant nutrient levels.

Cornops aquaticum is typically a species that displays considerable phenotypic plasticity in its life history characteristics. For example, the number of nymphal instars is largely determined by environmental conditions such as temperature, photoperiod and food quality (Adis et al., 2004; Capello et al., 2007) and can vary between five and seven instars. Adis & Junk (2003) recorded five or six instars whereas six or seven instars have been reported by Oberholzer & Hill (2001) in the same *C. aquaticum* culture that was tested here. Female development time is often extended by an instar, although this is also largely dependent on conditions during development (Adis et al., 2004). Other grasshopper species have sex-related variation in the number of instars (Amorim & Adis, 1995) as well as other insect groups which is considered an adaptive function for achieving higher body mass (Esperk et al., 2007) which is important in female fecundity. Only five instars were recorded in the present study and both males and females had the same number and similar development times in all the replicates. This demonstrates that the life history characteristics recorded here are only an indication of the effect that plant quality might have on their performance while other environmental conditions in the field are likely to also have a considerable effect on their life history. Nymphal development time is another trait of its biology that is highly variable with environmental conditions. Adis & Junk (2003) recorded 41 to 50 days from 1st instar to adult and approximately 50 days were recorded for development to adulthood by Oberholzer & Hill (2001). Development times reported here were considerably shorter, with an average of 41.9 days recorded in the low nutrient treatment which was the longest development time out of all three treatments. Vieira & dos Santos (2003) recorded an average of 156.2 days for development from egg to adult with an incubation period of 34.1 days, therefore nymphal development took approximately 122 days, more than double that recorded in most other studies. The nymphs in the present study were reared at favourable temperatures for development rates for insects which may explain the shorter development time compared to other studies. Variation in rearing conditions between the studies is likely to have played a role in the observed differences in development rates, therefore while plant

nutrients had no significant effects on development time, it's likely that there will be variability in the field according to temperature.

The number of nymphs per egg packet recorded in this trial was substantially lower than the number of eggs per egg packet recorded by Oberholzer & Hill (2001). They found between 30 and 70 eggs per egg packet whereas the number of nymphs to hatch per egg packet in the high nutrient treatment (which had the highest mean out of all treatments) only averaged approximately 21 nymphs. This apparent reduction in fecundity of the *C. aquaticum* culture could possibly be attributed to reduced fitness as a result of inbreeding. Oberholzer & Hill (2001) had completed biology studies on *C. aquaticum* by 2001 and new material has not been added to the current culture to increase genetic diversity since the last collections of *C. aquaticum* were made in South America in 1997. Fecundity seems to have declined in the last seven years however a new *C. aquaticum* culture will be collected from Argentina in the summer of 2008/2009 and comparative studies on the new and present cultures will be conducted.

Cornops aquaticum nymphal survival under laboratory conditions has been reported to be highly variable with temperature and photoperiod. The highest survival rate of nymphs recorded by Capello et al. (2007) was only 69% at a constant temperature of 27°C and an extreme photoperiod of a 24 hour day, which are unnatural conditions. In the present study, nymphal survival was influenced by plant nutrient levels and interestingly, survival patterns showed the opposite trend when reared on whole plants where feeding site could be selected compared to being force-fed leaves of a particular age. Survivorship was greatest at the high nutrient treatment when reared on whole plants but lowest when reared on cut leaves of position 4 from plants grown at the same nutrient concentrations. These differential survival patterns could possibly have been influenced by secondary plant chemistry which was not investigated in this study. Center & Wright (1991) suggested that the low incidence of herbivores found on water hyacinth in areas where it has been introduced, despite its abundance and often luxuriant quality, may be due to unpalatability or high levels of defensive chemicals. They investigated water hyacinth leaf chemistry and leaf preferences of a highly specialized herbivore on water

hyacinth, *N. eichhorniae* and a generalist, the yellow woollybear, *S. virginica*. They found that levels of folin-reactive compounds (total phenolics) which are digestibility-reducing substances were highest in leaf buds, lowest in the first, second and third leaves, with concentrations increasing with leaf age from the second to eighth-position leaves. *Neochetina eichhorniae* had a strong preference for young leaves, whereas the woollybear larvae preferred mature leaves which are the same leaves that are consistently selected by adult grasshoppers (chapter 2). Woollybear larvae fed fifth position leaves of water hyacinth plants weighed 72% more than larvae fed third position leaves despite the higher concentrations of phenolics in leaf 3. This suggests that other aspects of plant chemistry that differed between leaves 3 and 5 had a negative effect on woollybear growth and could possibly be the same constituents that had a negative effect on nymphal survival in the high nutrient treatment in the present study. Although levels of phenolics increase with leaf age (Center & Wright, 1991), phenolic compounds are carbon-based chemicals and are usually associated with low nitrogen conditions (Bryant et al., 1987) therefore it is probably unlikely that they were higher in the high nutrient plants, compared to the low and medium nutrient treatment where nymphal survival was higher. Fischer & Fiedler (2000) reported high larval and pupal mortality rates of the copper butterfly *Lycaena tityrus* Poda (Lepidoptera: Lycaenidae) reared on cuttings of a host plant, *Rumex acetosa* L. (Polygonaceae) of high nitrogen content compared to low nitrogen content. The authors also suggested that the different survival rates according to plant nitrogen could have been due to other changes in plant chemistry that were not measured in their study.

The increased nitrogen content of plants in the high nutrient treatments may have resulted in higher levels of nitrogen-based chemicals (Gerson & Kelsey, 1999), which possibly negatively influenced survivorship of nymphs fed the fourth leaf of plants grown in high nutrient conditions. Leaf selection by adult grasshoppers was fairly consistent in that they, given a choice, always fed on mature leaves however, nutrient demands and tolerance of different concentrations of plant nutrients and allelochemicals may be very different between immature and adult stages of *C. aquaticum*. The nutritional demands of insects are not consistent throughout their life but change with the requirements of

growth, development and reproduction (Simpson & Simpson, 1990). Leaf preferences for nymphal stages was not quantified but personal observation of their feeding patterns on whole plants in rearing boxes showed a tendency to feed on all leaves rather than only mature leaves which was very obvious with adults. Therefore, although increases in foliar nitrogen increases survival rates of *C. aquaticum*, it may be important for nymphs to select different tissues to balance their requirements and nutrient demands, as well as to avoid the potential negative effects of plant defensive compounds. Specialist insects don't have the option of changing foods but they frequently change their feeding strategies (Bernays, 1998). Bernays (1998) gives an example of lepidopteran larvae that feed on tissue rich in proteins during their early stages of development, and then move to a different plant part, with tissues rich in carbohydrates.

Although it is unlikely that phenolic compounds were responsible for the nutrient-dependent survival rates in the development trials, levels of phenolics in water hyacinth leaves may explain lower overall survivorship of nymphs in the low nutrient treatment compared to the high and medium nutrient treatments when reared on whole plants. Bryant et al. (1987) found that an increase in nitrogen fertilization lead to a reduction in foliar phenolics of young quaking aspen, *Populus tremuloides* Michx. (Salicaceae) which improved food quality and performance of *Christoneura conflictana* Walker (Lepidoptera: Tortricidae). If levels of phenolics in water hyacinth are related to nitrogen availability in the environment, this could well have been the factor responsible for reducing survival rates of nymphs on plants growing under low nutrient conditions. However, lower overall foliar nitrogen levels may have limited nymphal development. Increasing nitrogen is often correlated with higher rates of survival of immature insects developing on higher quality diets (Myers & Post, 1981; Fox et al., 1990; De Bruyn, 2002; Wheeler, 2003). Survivorship rates of nymphs reared on whole plants are the relevant data for extrapolating to the field, as the force-feeding was unnatural and under field conditions, they will be able to select feeding sites based on their nutritional requirements. Therefore, it can be concluded, that where *C. aquaticum* is concerned a high quality diet supports higher survival rates of nymphs than a low quality diet.

Cornops aquaticum fecundity was also positively associated with increasing levels of nitrogen in plant tissue. Honěk (1993) reviewed intraspecific variation in body size and fecundity in holometabolous insects. In some groups, resource quality during immature development determines adult body size and fecundity and in others, resource quality affects adult body size but fecundity is largely determined by the quality of resources available to adult females. In the latter group, adult body size will determine potential egg production, which will ultimately depend on environmental conditions. There are many examples where both increased body size within a species and increased foliar nitrogen concentrations increased insect fecundity (Ohmart et al., 1985; Blackenhorn, 1994; Hogendorp et al., 2006; Stanley et al., 2007) including orthopterans (Joern & Behmer, 2002; Branson, 2006). *Cornops aquaticum* fecundity was influenced by three interacting factors - body size (which was influenced by plant quality), food quality during nymphal development and food quality during the pre-oviposition period. Therefore, host plant quality had a significant influence on the difference between potential and realized fecundity of *C. aquaticum*. Fecundity and body size were positively, albeit weakly correlated and body size was significantly influenced by nutrient treatment. There was therefore an interaction between these factors in their effect on fecundity in that female body size increased with an increase in host plant quality during immature development and females that were reared on high quality plants were heavier and had higher numbers of egg packets. Therefore potential fecundity was likely to be influenced by body size. However, this was not the only factor that influenced fecundity of *C. aquaticum* and this is indicated by the significant differences in the number of egg packets between females in the high and medium nutrient treatments, even though females from those treatments were of similar body weight. Nitrogen levels in their food during their development as nymphs and during egg development played a role. This is evident in the fact that the switch from a low quality diet during nymphal development to a high quality diet during egg development increased fecundity but the number of egg packets for those females were not as high as females that had a high quality diet during both immature and egg development stages. Plant nitrogen content during development of immature stages can be important in determining the number of mature oöcytes (Taylor, 1984) and therefore also has an effect on potential fecundity whereas plant quality during egg development

affects realized fecundity. An exciting finding is the change in fecundity and rapid response of females to changes in food quality from their immature to post-mating development stages, suggesting that populations of *C. aquaticum* might respond quickly to a flush of nutrients into a system and the increased nitrogen content of plants should result in offspring of the next generation being more numerous, fitter and having higher survival rates. Jamil & Jyothi (1988) found a similar response in the *Neochetina* weevils where adults reared on plants growing in water with medium nutrient concentrations and then transferred to a high nitrogen environment responded to the greater availability of nutrients with increased fecundity. While nutrient levels during immature stages of development will affect potential fecundity, most likely through body size and development of oöcytes, nutrient levels in an adult female's diet will influence achieved fecundity which can increase with an increase in plant quality. Another factor that was influenced by food quality for gravid female grasshoppers was fitness of their offspring. Maternal effects according to food quality can have an effect on offspring survival, growth rates and fecundity (Rossister, 1991). The interrelatedness of the various components that influence fecundity and the offspring of *C. aquaticum* will have consequences for biocontrol through influencing their reproductive potential and fitness of their offspring.

A very surprising result was the influence of plant quality on survival of the sexes and therefore on the resultant sex ratios. Environmental sex determination has been recorded for insects where food quality influences sex ratios, usually being female-biased when food quality is high (Fox et al., 1990; Craig et al., 1999). In the present study, the differences in the sex ratios across the nutrient treatments were likely to be due to differential survival rates of the sexes, since all the nymphs were offspring of females from the *C. aquaticum* culture, which had the same quality diet. Variation in sex ratios according to different survival rates of black pineleaf scale insects, *Nuculaspis californica* Coleman (Hemiptera: Coccidae) were attributed to adaptation of males to an individual host, *Pinus ponderosa* Lawson (Pinaceae). Pines show great variability in their phytochemical defenses and local populations of the scale insects that were well adapted to an individual pine had higher proportions of males than local populations that were

poorly adapted to their individual pine (Alstad & Edmunds, 1983). This demonstrates that plant chemistry can result in differential survival rates of male and female insects of the same species, which can lead to changes in sex ratios. The patterns of survival of male and female *C. aquaticum* could possibly be attributed to plant chemistry. If female grasshoppers actively seek out plants with high tissue nitrogen, they would be better adapted to other chemical constituents associated with higher levels of plant nitrogen. A positive relationship between female body size and nitrogen levels in the host plant was shown by Stanley et al. (2007) who found that under high nutrient conditions, female pupae of *X. infusella* were heavier than males and only females increased with nutrition compared to males. *Cornops aquaticum* responded similarly where body size of females was significantly influenced by plant nutrient levels, whereas the same nutrients levels had no effect at all on adult male body size. Therefore, it is plausible that female grasshoppers are more tolerant and responsive to high nitrogen levels in water hyacinth than males. If females are better adapted to a diet with higher levels of nitrogen they would also be better adapted to cope with secondary metabolites associated with the amount of nitrogen available to a plant. Adaptations of females to cope with a diet of high nitrogen content, which would be favourable for reproductive fitness, may explain the different survival rates according to plant quality, however this remains speculative.

Mopper & Whitham (1992) suggest that sex ratios are seldom used to explain insect performance in natural systems and that it is another aspect of plant-insect interactions that is influenced by plant quality and could therefore potentially have an important influence on plant and insect population dynamics. The influence of nutrients on differential survival rates of male and female *C. aquaticum* suggest that the sex ratio may be shifted according to plant quality which would likely have a significant influence on the potential rate of increase from generation to generation. This could have a significant impact on the reproductive potential of *C. aquaticum* under different conditions. Furthermore, due to sexual size dimorphism (SSD) in this species, with females being considerably larger than males and hence their differential consumption rates, the level of control exerted by populations under particular nutrient conditions may change with a shift in the sex ratio. Under natural field conditions the grasshopper exhibits a sex ratio of

1:1 (Silveira-Guido & Perkins, 1975), as was found here in the medium nutrient treatment, however it is possible that extreme variations in nutrient availability that are perhaps not prevalent in systems in the region of origin, will indeed lead to changes in the ratios of male and female *C. aquaticum* surviving in populations with high or low levels of nitrogen in their food.

The effect of nutritional host plant quality on nymphal development was not as pronounced as other life history characteristics. Nymphs were able to maintain fairly constant development rates despite significant differences in nitrogen levels of leaves. *Cornops aquaticum* increased feeding rates and only slightly prolonged time for feeding, which are the two responses that are commonly observed to compensate for low quality diet (Slansky, 1981). Slansky & Feeny (1977) found evidence of compensatory feeding in *P. rapae*. They reported that larvae managed to stabilize their nitrogen accumulation rates through compensatory changes in feeding which permitted a relatively constant growth rate. Compensatory feeding allowed the salvinia moth, *S. multiplicalis* to ingest similar amounts of nitrogen, and maintain fairly constant growth rates although body size at adulthood was lower when fed leaves with low nitrogen content (Taylor, 1989). It is likely that the nymphs maintained fairly constant development rates through increasing their total consumption of leaf material during development and compensatory feeding. The negative correlation between body weight and development time indicated that nymphs with a longer development time weighed less than nymphs that developed more quickly. Nymphs in the low nutrient treatment were likely to have been limited in their accumulation of biomass due to a poor quality diet. Nymphs reared on high nitrogen plants had greater body weight and shorter development times again indicating the positive effects of nitrogen on *C. aquaticum* fitness levels. A negative correlation between body size and development time has been reported and is thought to be associated with organisms exposed to a range of environments varying in suitability such as in the quality of their food (Klingenberg & Spence, 1997). For example, the southern green stinkbug, *Nezara viridula* L. (Heteroptera: Pentatomidae) had a longer development time but weighed 65% less when reared on an inferior host plant (Panizzi & Saraiva, 1993).

The feeding patterns of nymphs according to the quality of their food provides evidence that the greater impact that *C. aquaticum* had on plants growing under low nutrient conditions in chapter 2 was likely to have been influenced by feeding rates of the grasshoppers. While nutrient deficiency severely stunted growth and limited water hyacinth's potential for compensatory regrowth in response to herbivory, the grasshoppers in the previous experiment possibly exhibited substantially higher feeding rates in the low nutrient treatment compared to the high, and therefore had a more devastating impact on those plants. Although consumption rates of adults were not measured and their nutritional requirements may differ from nymphs, it's plausible that both factors were responsible for the severe impact that the grasshoppers had on water hyacinth plants growing in nutrient-deficient environments. Therefore, in terms of individual performance in the field, the per capita severity of damage caused by grasshoppers in low nutrient systems will be higher than that in eutrophic systems where leaf nitrogen content is higher. However, as is indicated by the life history characteristics also investigated here, *C. aquaticum* population dynamics will be significantly affected by plant quality, therefore, their impact on water hyacinth in low nutrient systems may not necessarily be higher than their impact on plants growing under eutrophic nutrient conditions. In high nutrient systems, establishment, survival, and higher population growth rates could lead to higher population densities on water hyacinth infestations but their lower overall feeding rates might negate the benefits from higher insect densities. Likewise, lower insect densities being supported by food of lower quality might be balanced by higher per capita feeding rates of grasshoppers. Therefore feeding rates and insect densities will differ between systems, but could possibly lead to the same impact on water hyacinth.

Heard & Winterton (2000) investigated the effects of plant quality on life history characteristics of the *Neochetina* weevils and made predictions on efficacy. Studies of their life history parameters showed that high nutrient plants were superior hosts to *N. bruchi* compared to those grown at a lower nutrient concentration. Mean generation time was shorter, and net productive rate and intrinsic rate of increase were greater for weevils reared on water hyacinth grown at high nutrient levels. This comparison between the two

species suggested that *N. bruchi* would be a superior biocontrol agent under high nutrient conditions and Heard & Winterton (2000) predicted that at high nutrient concentrations, a slightly faster development time, high survival, higher fecundity and greater longevity would increase plant damage. Both Ripley et al. (2006) and Coetzee et al. (2007) found in laboratory studies that plants growing under eutrophic nutrient conditions supported higher numbers of the mirid, *E. catarinensis* compared to conditions of low nutrient availability. In spite of the positive response of insects to more nutritious plants by exhibiting greater fecundity, survival and increased development rates, water hyacinth remains most problematic in systems with high levels of nitrogen availability. Despite low feeding rates in response to the high nutrient levels in the plants from the high treatment, *C. aquaticum*, at a fairly low density, still caused significant reductions in water hyacinth growth parameters and potential for biomass accumulation.

McClay & Balciunas (2005) note that the range and abundance of introduced biocontrol agents are primarily functions of their life history characteristics and their response to the environment. These results have indicated that the nutritional quality of *C. aquaticum*'s diet has important consequences for its survival, fitness and performance and these factors are likely to have an important influence on the control potential of this species in the field. *Cornops aquaticum* developing on high-nutrient plants should have good establishment and survival and greater population increase due to greater fecundity and a female-biased sex ratio. This should result in higher exponential growth rates and higher damage levels due to higher numbers of females. These obvious advantages may be negated to some extent due to lower overall feeding rates and greater compensatory ability of water hyacinth under those conditions (chapter 2), but with high population growth rates, time taken to reach population densities that are sufficiently damaging will be significantly reduced. A low quality diet will promote increased feeding rates but a male-biased sex ratio, and reduced survival and fecundity will result in lower exponential growth rates with substantially smaller increases from generation to generation. Dispersal of this species may be influenced by a low quality diet, in that grasshoppers may actively disperse in search of a higher quality food source.

In conclusion, this species displays considerable phenotypic plasticity in response to nutrient conditions in its environment. The nutrient levels used in this trial which are typical of South African systems with water hyacinth infestations will influence *C. aquaticum* physiology and performance as a biocontrol agent. An interaction of plant quality and these factors will influence *C. aquaticum*'s population dynamics which will in turn influence water hyacinth population dynamics. Chapters 2 and 3 established that water and plant nutrient levels will influence the efficacy of *C. aquaticum* as a biocontrol agent for water hyacinth and that higher densities will be needed and attained in eutrophic systems. However, density-damage relationships between biocontrol agent and host have not been established. In order to be justified in release, *C. aquaticum* must be able to reduce water hyacinth growth and productivity at a realistic insect density in nutrient-enriched systems where more effective control of water hyacinth is needed. The relationship between *C. aquaticum* density and water hyacinth fitness parameters are investigated in chapter 4.

Chapter 4

**Investigating the response of water hyacinth to herbivory
by different densities of male and female *Cornops*
aquaticum under eutrophic water nutrient conditions**

4.1 Introduction

The dynamics of natural communities and populations are influenced by top-down (e.g. predators) and bottom-up (e.g. resources) forces that work simultaneously (Hunter & Price, 1992; Power, 1992; Walker & Jones, 2001) to determine interactions between different trophic levels. Environmental heterogeneity within species and populations will influence the relative importance of each force in structuring a particular community or population system (Hunter & Price, 1992; Turchin, 1999). The generally accepted principle amongst ecologists is that abiotic factors set the limit for potential population growth whereas biotic factors influence the resultant growth and community dynamics (Gutierrez et al., 1994; Turchin, 1999). Population densities of the middle trophic levels in particular, for example a plant-herbivore system, will largely be determined by the relative strengths of both top-down and bottom-up forces, specific to species or biological systems.

Densities of insect herbivore populations depend on predation pressure (top-down) (Hairston et al., 1960; Risch & Carroll, 1982; Matsumoto et al., 2003; Gruner, 2004; Stiling & Moon, 2005; Elderd, 2006) and the quality and defense mechanisms of their hosts (Price, 1975; Mattson, 1980; Coley et al., 1985; Hunter & McNeill, 1997; Dyer et al., 2004; Gruner, 2004; Throop & Lerdau, 2004; Stiling & Moon, 2005; Miller, 2008) as well as their density, abundance and spatial distribution (bottom-up) (Root, 1973; Crawley, 1983; Bach, 1988; Andow, 1990). Through negative feedback, insect populations respond in a density-dependent manner to populations of their host, following their cyclical patterns of abundance, although possibly with a lag period (Price, 1975; Murdoch, 1994) (but see Crawley (1983) – he claims there is no link in fluctuations in numbers between those two trophic levels and they don't always follow typical predator-prey models). Plant population dynamics are dependent on resources (bottom-up) and competition (Julien & Bourne, 1986; Mihaliak & Lincoln, 1989) as well as population densities and impact of their herbivores (top-down) (Rauscher & Feeny, 1980; Berryman et al., 1985; Julien & Bourne, 1986; Müller-Schärer & Brown, 1997; Meyer, 1993; Keane & Crawley, 2002; Matsumoto et al., 2003; Briese et al., 2004; Stiling & Moon, 2005; Elderd, 2006), although not all herbivores are equally damaging or

significantly influence the population dynamics of their host plants. Most insect herbivores affect an aspect of individual plant fitness, such as flowering, fruit or seed production or relative growth rates (Crawley, 1989b; Oosterheld, 1992) but the degree to which they regulate populations of their host will depend on many factors, one of which is the host's ability to tolerate or compensate for damage inflicted by insect feeding (Crawley, 1983).

Most plants have mechanisms for mitigating the detrimental effects of herbivory on their fitness which are reviewed by Trumble et al. (1993), Stowe et al. (2000) and Tiffin (2000). Plants that are defoliated by herbivores lose photosynthetic tissue and in order to tolerate or compensate for that kind of damage, they need to restore their capacity for carbon acquisition. Typically observed mechanisms for maintaining photosynthetic ability in response to defoliation are delayed leaf senescence (Meyer, 1998) or enhancement of photosynthetic rates of remaining leaf tissue (Nowak & Caldwell, 1984; Meyer, 1998; Gassman, 2004; Stevens et al., 2008) (compensatory photosynthesis) to maintain productivity of remaining leaves. Alteration in biomass allocation, for example through increasing investment in shoot biomass relative to root biomass, to maintain a balance in carbon and nutrient income similar to undamaged plants is also a commonly observed response (Mihaliak & Lincoln, 1989). Other re-allocation patterns include greater allocation of biomass to leaves either through increasing leaf production or increasing the size of newly produced leaves (Wallace & O'Hop, 1985; Meyer, 1998; Ding et al., 2006; Stevens et al., 2008). The efficacy of these responses in maintaining the same levels of fitness as plants not damaged by herbivory will depend on the plant, the herbivore and the intensity of damage.

Tiffin (2000) notes that tolerance to herbivore damage can play an important role in the ecological dynamics between plants and herbivores. A complete understanding of the dynamics of populations of a plant species and one of its monophagous herbivores requires an understanding of the per capita impact of herbivore feeding on plant performance. The extent to which plants will compensate for herbivory is typically investigated by plotting a damage function which is a graph of an aspect of plant

performance as a function of increasing insect density. 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, 1983b; 1989). Most plant-herbivore relationships are described by linear damage functions (Morrill et al., 1984; Pantoja et al., 1986; Crawley, 1989b; Meyer, 1998; Schooler & McEvoy, 2006; Stanley et al., 2007) where plant fitness or performance decreases linearly with an increase in insect density. When insect densities approach the carrying capacity, the linear relationship changes to a non-linear, asymptotic relationship (Schooler & McEvoy, 2006). This curvilinear relationship indicates compensation for low levels of herbivory but which decreases rapidly with increasing amounts of tissue removed (Pitan et al., 2007). A humped damage function would indicate overcompensation for herbivory (Crawley, 1989b) where fitness is increased in response to insect feeding. In some cases, exponential increases in growth rates in response to increasing levels of herbivory are evident so that plants maintain the same growth rates irrespective of the degree of herbivory (Oosterheld, 1992).

The effect that insects have on yield or performance of their hosts is well studied in agroecosystems and a damage function known as the 'damage curve' was developed for identifying how the stress produced by injury through feeding relates to a measurable reduction in plant growth, development or reproduction and hence damage (Peterson & Higley, 2001). McClay & Balciunas (2005) present an adaptation of the damage curve (Fig. 4.1) 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 load. The basic purpose of the damage curve would be to identify agents that do not have enough impact on their host to justify release, as indicated by the broken line in their schematic diagram (Fig. 4.1). A damaging and promising agent would have a damage curve that indicates that plant fitness is reduced to low levels at a high, but realistic density of the candidate agent, as indicated by the solid line in their diagram. A linear or curvilinear relationship between biocontrol agent density and plant performance would suggest that reductions in plant fitness are associated with increasing levels of herbivory by the biocontrol agent. McClay &

Balciunas (2005) therefore recommend testing different densities of agents in pre-release assessments, and that the densities used should be high enough to represent a best-case scenario. The aim is to mimic a population outbreak where the density of the agent is only limited by the availability of food. They note that testing only low densities can miss the effects of unexpected types of injury that are only evident at high biocontrol agent densities. Such studies should be performed prior to release to determine that aspect of the plant-herbivore system which will give clues as to how herbivory by a candidate agent might influence population dynamics of the target plant.

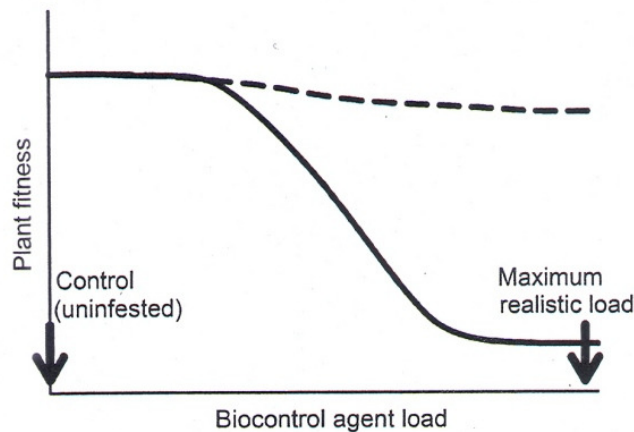


Figure 4.1 Schematic representation of a damage curve for a candidate weed biocontrol agent adapted by McClay & Balciunas (2005) from Peterson & Higley (2001). Biocontrol agent load on the x-axis represents the number of biocontrol agents per unit of plant biomass. The y-axis is a relevant measure of plant performance or fitness, such as growth rate or final biomass. The solid curve shows a damage curve for a potentially effective agent and the dotted curve shows a candidate agent with little effect on plant performance even at high herbivore loads.

There are generally two approaches to assessing per capita damage effects of candidate biological control agents in pre-release efficacy studies. The first evaluates the agent in field studies in the region of origin, by manipulating densities of the candidate (McClay & Balciunas, 2005) or excluding it from populations of the target plant and monitoring productivity in the absence and presence of herbivory. This has been the most widely used method to date, and is possibly the better method since the candidate agent is evaluated under natural conditions. For example, Balciunas and Burrows (1993) using

insecticidal exclusion experiments showed that native insects in Australia cause significant reductions in growth of *Melaleuca quinquenervia* Cav. (Myrtaceae) indicating promise for a biocontrol programme in North America. Briese (1996) conducted impact studies with the stem-boring weevil *Lixus cardui* Olivier (Coleoptera: Curculionidae) in field cages in its native France to evaluate its potential as a biocontrol agent for *Onopordum* spp. thistles in Australia. Later, two other agents were evaluated through impact studies in the native range to determine their potential as biocontrol agents for *Onopordum* thistles (Briese et al., 2002; 2003). The impact of the leaf beetle *Galerucella birmanica* Jacoby (Coleoptera: Chrysomelidae) on growth and reproduction of water chestnut, *Trapa natans* L. (Trapaceae) was evaluated in a series of pre-release assessments in the native range in China to determine its potential as a biocontrol agent for North America (Ding et al., 2006). The second approach involves studies under quarantine conditions in the recipient country, which have proved to be valuable in assessing potential impacts and assisting in decision-making on release (McClay & Balciunas, 2005). Wu et al. (1999) showed in greenhouse experiments that high densities of *Prokelisia* planthoppers caused mortality of more than 90% of *Spartina anglica* C.E. Hubbard (Poaceae) plants, and concluded that biological control using *Prokelisia* spp. could contribute to management of cordgrass. Klöppel et al. (2003) measured the impact of a gall wasp, *Aulacidea subterminalis* Nisbett (Hymenoptera: Cynipidae) on growth of *Hieracium pilosella* L. (Asteraceae) under shade- and green-house conditions and suggested that the wasp has potential to be a successful biocontrol agent. Balciunas (2004) gives an example of pre-release efficacy testing under quarantine conditions where he assessed the probable impact of a gall-forming fly, *Parafreutreta regalis* Munro (Diptera: Tephritidae) on cape ivy, *Delairea odorata* Lem. (Asteraceae) which indicated the candidate agent had good damage potential.

Most pre-release studies have evaluated different densities of the candidate agent (Wu et al., 1999; Briese et al., 2002; Balciunas, 2004; Ding et al., 2006) to determine potential efficacy and many post-release evaluations manipulate populations of biocontrol agents to determine impact at different densities (Center & Van, 1989; Center et al., 1999b; Grevstad, 1999; Briese et al., 2004; Wilson et al., 2006; Bebawi et al., 2007). They allow

for a more detailed evaluation of the target plant's response to herbivory by that particular species of insect and can elucidate whether any compensatory effects are apparent at some levels of herbivory. An alternative advantage of conducting insect density testing with biocontrol agents is to establish the functional form of density-damage relationship in order to estimate insect population densities from feeding damage to the target plant (Schooler & McEvoy, 2006).

A critical goal in pre-release studies is to attempt to understand how a particular insect herbivore might influence the abundance and distribution of its host and to gain information that can be used in species management and in predicting population dynamics and potential success. Chapter 2 investigated nutrient-dependent compensation for herbivory by the grasshoppers and revealed that higher densities than at which they occur in the native range would be needed to cause reductions in water hyacinth infestations in eutrophic environments. Therefore the next step was to determine what kind of densities would be needed in eutrophic systems where more effective control in South Africa is desirable, as well as the response of water hyacinth plants to a range of feeding intensities by the grasshopper. Investigating the relationship between insect density and damage to plants, measured by a reduction in several measures of plant fitness, would reveal whether *C. aquaticum* has the potential to reduce water hyacinth to levels where its negative effects are mitigated, and therefore justify release of the grasshopper based on potential efficacy. This study would also allow for a better understanding of how *C. aquaticum* might influence water hyacinth population dynamics at different grasshopper densities. Furthermore, there is a dearth of knowledge on whether pre-release impact studies provide the information needed to make accurate predictions on efficacy of candidate biological control agents. Comparing the data from laboratory impact studies with data from a comprehensive post-release evaluation, should the grasshopper be released in South Africa will give an indication of the value and merit in performing such studies. Therefore the aim of the chapter was to (1) investigate in a quarantine glasshouse experiment, the relationship between herbivore density and plant performance; (2) investigate the plant's ability to, under high nutrient conditions, tolerate or compensate for tissue loss at varying herbivore feeding intensities; (3) identify mechanisms by which the plant reduces the negative effects of defoliation and determine

at what density these mechanisms are no longer adequate to sustain plant production, growth and survival and (4) investigate the impact of different densities of male and female grasshoppers on water hyacinth. Sexes were separated for this trial due to consideration of a trial release consisting of males only. Due to their large size, strong size sexual dimorphism (SSD) (chapter 3) and clear differences in the reproductive segments, it would be easy to separate the sexes for a one-sex only release. At one stage of the release application process, the regulatory authorities responsible for granting release permits expressed concern over the introduction of an exotic grasshopper species and their possible indirect non-target effects. A one-sex only release with rigorous monitoring 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 for releases to have a significant impact on water hyacinth infestations.

4.2 Materials and Methods

4.2.1 Experimental design

The density trial was conducted in a quarantine glasshouse at the Plant Protection Research Institute in Pretoria from December to April 2007. See chapter 2 materials and methods for glasshouse conditions. Water hyacinth plants obtained from stock cultures were grown in plastic tubs containing 15 liters of water. The dimensions of the tubs were 43 x 31 x 19 cm. Each tub contained two water hyacinth plants and was covered with a netted sleeve. 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 high nutrient treatment, simulating nitrate and phosphate levels from a eutrophic impoundment in South Africa called Mbozambo Swamp, was used to assess the impact of different densities of the grasshoppers on plants with high rates of growth and reproduction (chapter 2). Refer to chapter 2 Materials and Methods for details on the nitrate and phosphate levels. A commercial iron chelate (Mircel FE 130) was also added at a rate of 1.3g/15L.

The tubs were arranged in a randomized block design in the glasshouse. Plants were grown for two weeks prior to the start of the study, after which all daughter plants, 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). Two tubs per replicate were left as controls. Adults were separated into males and females at each density level so that each tub had only male or only female grasshoppers. There was oviposition in the female treatments but all nymphs that hatched were removed to maintain the original herbivore densities and any dead adult grasshoppers were replaced with grasshoppers of the same sex and as far as possible, of similar age.

Plants were sampled weekly and the following parameters were measured: number of leaves, number of dead leaves, number of petioles completely defoliated, number of ramets, number of flowers, leaf 2 petiole length (cm), longest petiole length (cm), leaf 4 area damage and average leaf area damage for whole plants. Area damage to the 4th leaf of each plant and average leaf area damage per plant was scored from 1 to 5 where 1 = 0%; 2 = >5%; 3 = 5-25%; 4 = 35-50% and 5 = 50-100% of the leaf area damaged. New leaves, daughter plants and flowers were tagged at each sampling interval to measure turnover and production. Each treatment was replicated 6 times and the duration of the trial was 8 weeks. Due to a shortage of quarantine glasshouse space, the trial was staggered with the trial experiment initially consisting of four replicates followed by another two on completion of the first four. 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 mortality. Male and female grasshoppers were weighed (males n = 47; females n = 50) to obtain a mean wet weight (g) for each sex to be used for evaluating the relationship between grasshopper biomass and water hyacinth growth parameters.

4.2.2 Statistical analysis

The data from the first set of replicates did not differ significantly from the second set therefore they were combined for statistical analysis. 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

ANOVA. Two-way ANOVA's were used to analyse the effect of insect density and sex and their interaction on the growth and reproductive parameters. Tukey's HSD test was used for *post hoc* comparison of the means for all ANOVA's. Biomass data were also calculated to represent the reduction in growth increment as a result of grasshopper herbivory compared to control plants (refer to chapter 2). Data were subjected to regression analyses to determine the relationship between insect biomass (as the dependent variable) and the different measures of plant performance (as the independent variables). Insect biomass was used as a replacement for insect density, since densities of males and females were the same. Insect biomass per treatment was calculated as the mean weight of either male or female grasshoppers multiplied by the number of individuals per plant. For linear functions, a product-moment correlation was used to determine the relationship between insect density and plant damage. Only those parameters with regression coefficients that explained more than 50% of the variance are plotted with plant performance as a function of increasing insect biomass. The temporal development of the growth and reproductive parameters were also plotted to show differences between the herbivory treatments and the control over the eight week study period. All data were analysed using Statistica 6.0.

4.3 Results

4.3.1 Effect of insect treatment on plant biomass

Herbivory by female grasshoppers significantly reduced water hyacinth plants in weight from the start to the end of the study period in the 3 ($F_{1;10} = 23.31$, $P < 0.0001$) and 4 ($F_{1;10} = 81.18$; $P < 0.0001$) female treatments (Fig. 4.2). Female grasshoppers at a density of two per plant reduced water hyacinth in wet weight from the start to the end of the study period, but this was not significant. In the male treatments, there were no significant differences between the start and end weights of water hyacinth at any of the densities. The plants increased in weight at a density of 2 and 3 male grasshoppers and a density of 4 male grasshoppers per plant prevented biomass accumulation with plants remaining approximately the same weight from the start to the end of the study period. Wet weight increase of control plants was highly significant ($F_{1;26} = 119.45$; $P < 0.0001$).

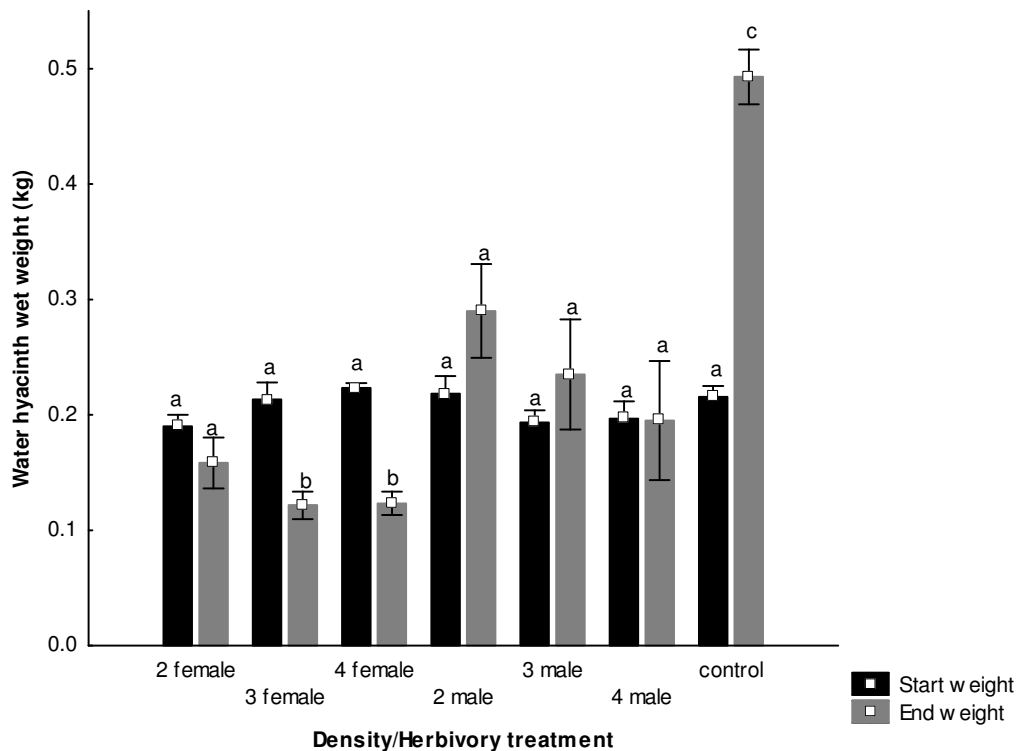


Figure 4.2 Mean wet weight (kg) of water hyacinth plants at the start and end of the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Error bars represent the standard error of the mean.

The change in wet weight of water hyacinth plants from week one to week eight was significantly different between all six herbivory treatments and the control ($F_{6,43} = 19.05$; $P < 0.0001$) (Fig. 4.3). The only significant differences in the change in plant biomass between the herbivory treatments from the start to the end of the trial were at densities of 4 females and 2 males per water hyacinth plants. A significant difference in the change in wet weight was found between the male and female treatments ($F_{1,30} = 14.13$; $P = 0.0007$) but grasshopper density had no significant effect on changes in plant biomass. The interaction between insect density and sex was not significant. The changes in plant biomass as a result of defoliation at a density of 2 females per water hyacinth plant equates to a 112% reduction in growth increment compared to undamaged plants and densities of 3 and 4 females per plant caused a loss of growth increment of 133% and 137% respectively. Feeding by male grasshoppers at a density of 2 equates to 74%

reduction in growth increment and herbivory by 3 and 4 males per plant caused 81% and 100% reductions respectively compared to undamaged plants.

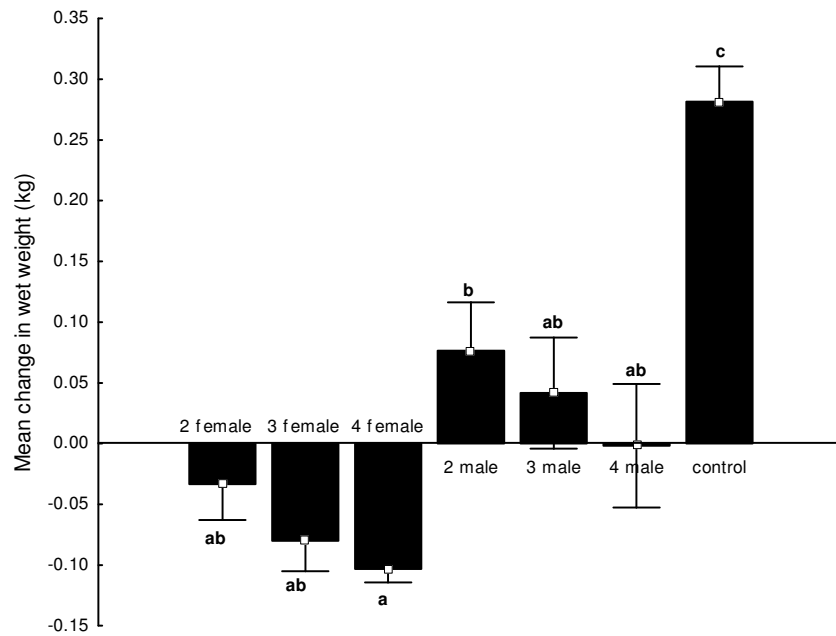


Figure 4.3 Mean weight change (kg) from the start to the end of the eight week study period of water hyacinth plants in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum*. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

The relationship between final biomass of water hyacinth plants at week eight (or at mortality of plants in the female treatments) as a function of increasing levels of herbivory was curvilinear (Fig. 4.4). The graph shows a reduction in plant performance with increasing herbivore pressure indicating some compensatory response at low feeding intensities. Exponential regression best described the relationship between total yield and insect biomass and the regression was highly significant ($F = 73.20$; $P < 0.0001$), accounting for 75% of the variance ($r^2 = 74.7$).

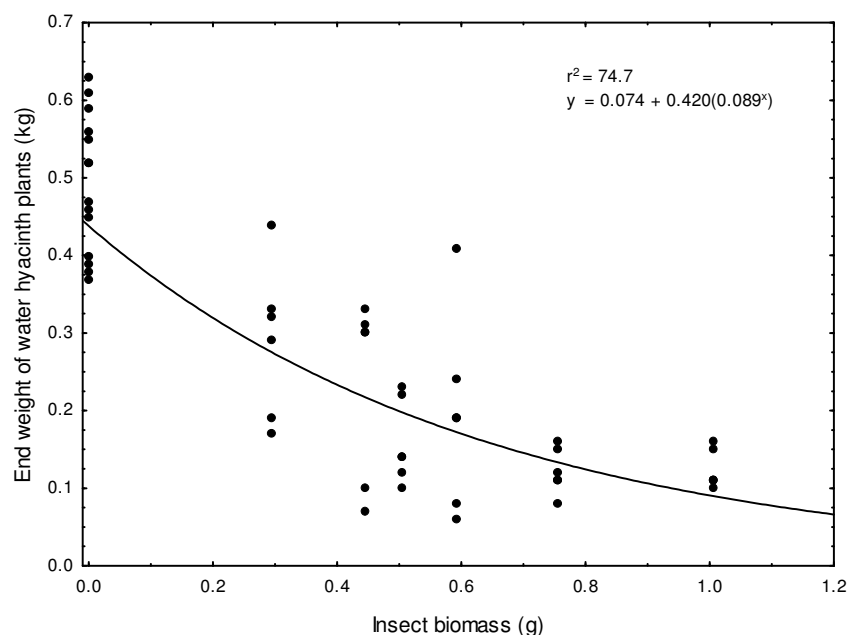


Figure 4.4 Regression of *Cornops aquaticum* biomass (g) and final weight (kg) of water hyacinth plants at the end of the eight week study period. Insect biomass represented by the mean weight of male or female grasshoppers multiplied by the respective density.

4.3.2 Effect of insect treatment on leaf parameters

Production and mortality rates of water hyacinth leaves during the study period are tabulated in Table 4.2. Leaf production rates (no. leaves day⁻¹) were significantly reduced ($F_{6;43} = 17.324$; $P < 0.0001$) in all the herbivory treatments compared to control, with the exception of the 2 and 4 male treatments. Production rates of plants with 3 and 4 females were less than half production rates of control plants. This equated to approximately 14 days to produce one new leaf in the 3 and 4 female treatments compared to control plants where it took an average of 5.8 days to produce one leaf. It took longer to produce new leaves in all the male treatments compared to the control but production rates were only significantly different from control plants in the 3 male treatment. Leaf mortality rates were not significantly different between any of the treatments. However, the number of days for leaves to senesce was longer in the 2 and 3 male treatments and in the 3 and 4 female treatments compared to control plants suggesting there might have been delayed leaf senescence in some of the treatments in response to herbivory. Leaf production rates of plants were much more severely disrupted in the female treatments compared to the

males, which was the ultimate cause of mortality. Control plants maintained a balance between leaf production and mortality rates whereas all the herbivory treatments caused leaf mortality rates to exceed leaf production rates and therefore a disruption to their leaf dynamics.

Table 4.1 Leaf production and mortality rates (leaves day⁻¹) and number of days to produce one new leaf (days leaf⁻¹) of water hyacinth plants during the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant.

	2 female (± SD)	3female (± SD)	4female (± SD)	2 male (± SD)	3 male (± SD)	4 male (± SD)	Control (± SD)
Production (leaves day ⁻¹)	0.109 (±0.03)	0.068 (±0.05)	0.067 (±0.04)	0.158 (±0.03)	0.125 (±0.02)	0.134 (±0.03)	0.174 (±0.02)
Production (days leaf ⁻¹)	10.3 (±3.77)	14.7 (±5.09)	14.09 ±2.68	6.5 (±2.44)	8.3 (±1.68)	7.8 (±1.97)	5.8 (±0.8)
Mortality (leaves day ⁻¹)	0.157 (±0.08)	0.125 (±0.08)	0.135 (±0.104)	0.132 (±0.03)	0.132 (±0.02)	0.170 ±0.05	0.159 ±0.02
Mortality (days leaf ⁻¹)	7.9 (±4.07)	7.6 (±5.8)	7.4 (±5.09)	8.17 (±2.66)	7.9 (±1.46)	6.2 (±1.63)	6.3 (±0.9)

A difference in the mean number of leaves of water hyacinth plants sampled at the end of the eight week study period was found between the male and female herbivory treatments and between the plants in female treatments and the control ($H_{6,50} = 39.67$; $P < 0.0001$). The number of leaves of plants in all three male herbivory treatments did not differ from control plants (Fig. 4.5). Grasshopper density had a significant effect on the number of leaves ($F_{2,30} = 9.56$; $P < 0.0001$) and there were highly significant differences between the male and female treatments ($F_{1,30} = 151.65$; $P < 0.0001$). The interaction of density and sex was not significant. Plants in the 3 and 4 female treatments had less than one whole leaf by the time they succumbed to the damage caused by the grasshoppers. The number of leaves of water hyacinth plants at the end of the trial was linearly related to insect biomass and the regression was highly significant ($F = 107.22$; $P < 0.0001$) (Fig. 4.6). A product moment correlation showed a significant negative correlation ($r = -0.831$; $P < 0.05$) indicating that the capacity of plants to maintain a full complement of productive leaves decreased linearly with increasing herbivore pressure.

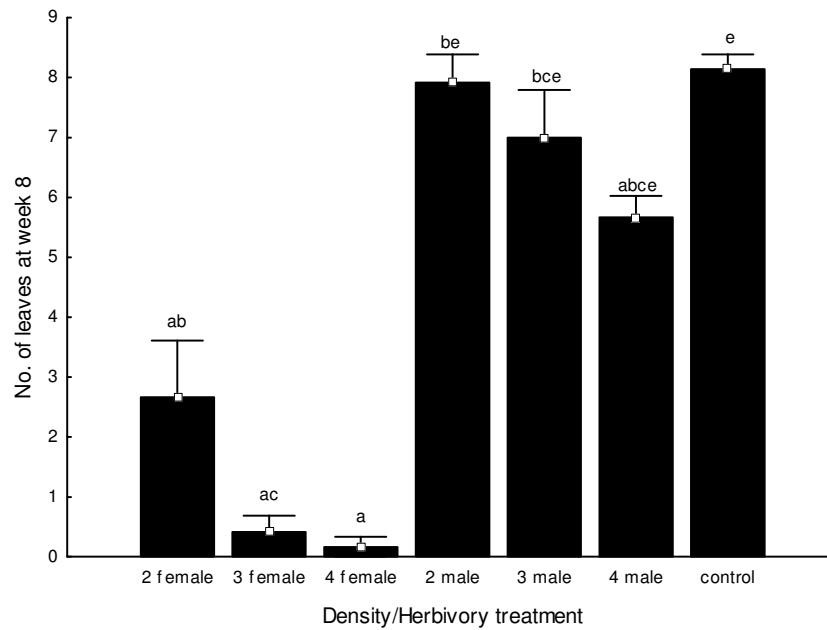


Figure 4.5 Mean number of leaves of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

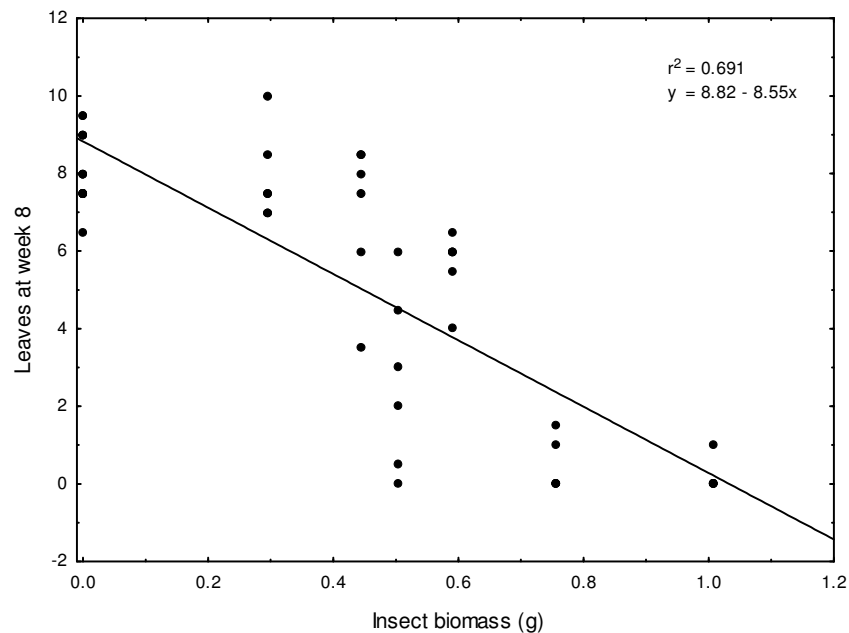


Figure 4.6 Regression of *Cornops aquaticum* biomass (g) and the total number of leaves of water hyacinth plants at the end of the eight week study period. Insect biomass represented by the mean weight of male or female grasshoppers multiplied by the respective densities.

There was a sharp decrease in leaf production from week 1 to 8 in all 3 female treatments. Leaf production decreased steadily over the study period in the 3 and 4 male treatments but it remained stable in the control and 2 male treatment (Fig. 4.7).

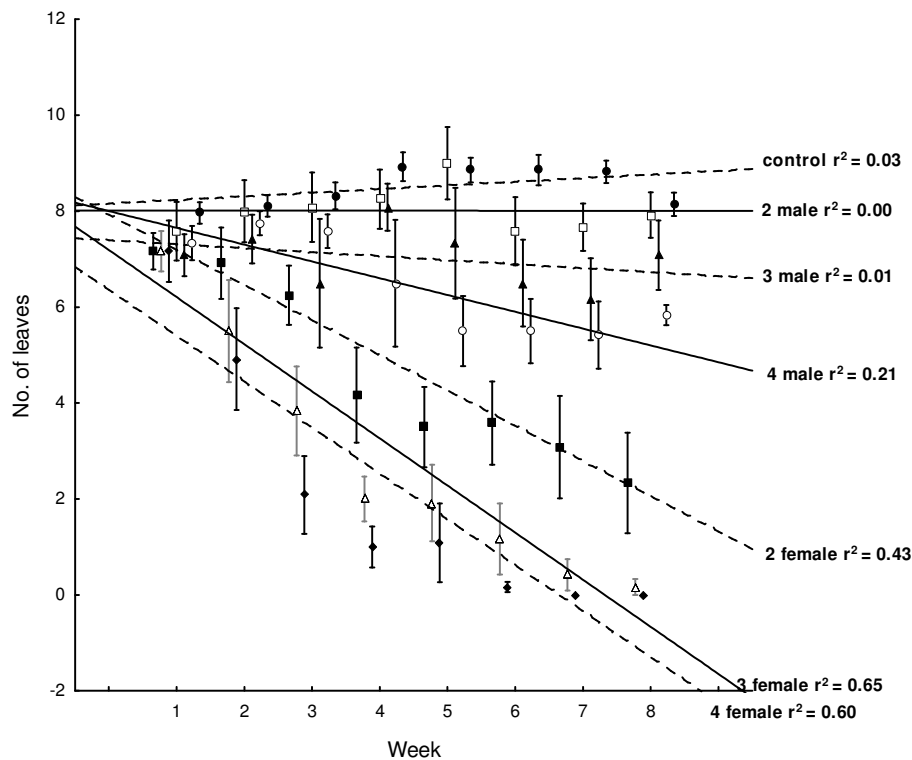


Figure 4.7 Changes in the number of leaves of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum*. Error bars represent the standard error of the mean.

4.3.3 Effect of insect treatment on plant growth and productivity parameters

Control plants were significantly taller than plants in the male and female treatments at all densities of *C. aquaticum* ($F_{6,43} = 14.05$; $P < 0.0001$) (Fig. 4.8). Leaf turnover in the 3 and 4 female treatments was so slow and the plants were killed so quickly that the petioles that were measured at the last sampling interval before they died from grasshopper herbivory had been on the plant since the start of the trial. This is indicated by the longer length of petioles of plants in the 2 female treatment compared to the 3 and 4 female treatment. The interaction between density and sex had a significant effect on

the length of the longest petiole suggesting an interaction of both factors to significantly reduce growth rates of water hyacinth plants in those treatments ($F_{2;30} = 3.48$; $P = 0.0044$) but density or sex alone had no significant effect.

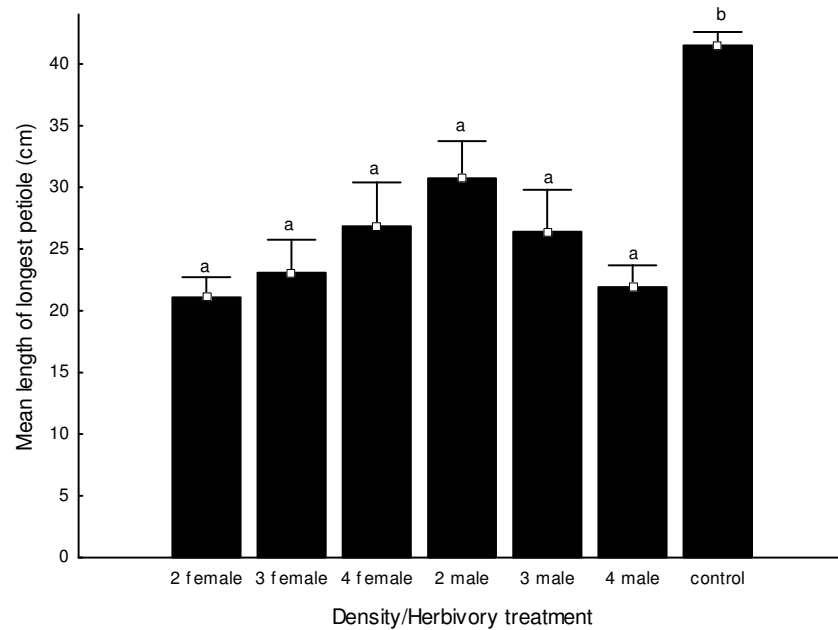


Figure 4.8 Mean length of the longest petiole (cm) of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

The mean length of the longest petiole decreased significantly over time in all the herbivory treatments, indicated by the divergence of all development lines from the control plants. There was a rapid decline in the height of water hyacinth plants in the female herbivory treatments, which indicates the substantial impact that the females had on growth rates of water hyacinth. The mean length of the longest petiole of the control plants did not increase or decrease over the eight week study period (Fig. 4.9). A significant negative correlation ($r = -0.657$; $P < 0.0001$) was found between the length of the longest petiole of plants at the end of the trial and insect biomass but the regression, although linear and significant, only explained 43% of the variance ($r^2 = 0.43$; $P < 0.0001$).

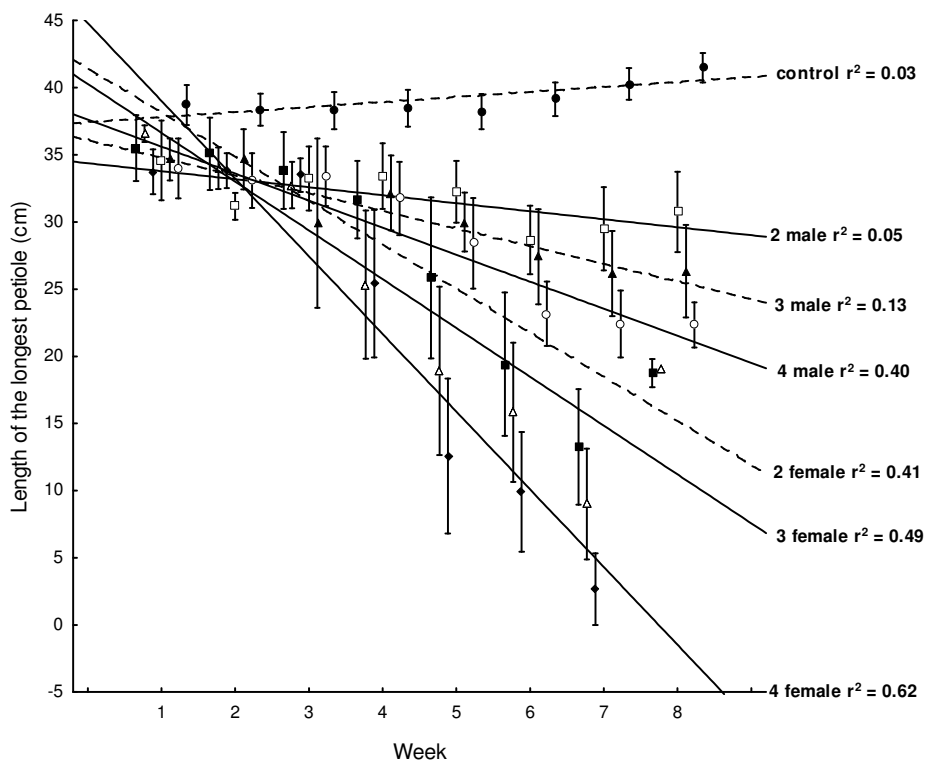


Figure 4.9 Changes in the mean length of the longest petiole (cm) of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum*. Error bars represent the standard error of the mean.

The mean length of the leaf 2 petiole was significantly greater in the control plants compared to the herbivory treatments ($F_{6,43} = 11.63$; $P < 0.0001$) (Fig. 4.10). This indicates that growth rates of plants were significantly higher in control plants compared to plants subjected to herbivory by the grasshoppers at all densities. Only the 2 male treatment was not different from the control and there were no significant differences between any of the herbivory treatments (Fig. 4.10). Grasshopper sex ($F_{1,30} = 5.43$; $P = 0.0267$) and the interaction of density and sex ($F_{2,30} = 4.39$; $P = 0.0214$) had a significant effect on leaf 2 petiole length but density did not. Insect biomass and the length of the leaf 2 petiole of plants at the end of the trial were linearly related, but the regression only explained 45% of the variation ($r^2 = 0.45$; $P < 0.0001$). There was a significant negative correlation between plant growth, measured by length of the leaf 2 petiole and insect biomass ($r = -0.6727$; $P = 0.001$).

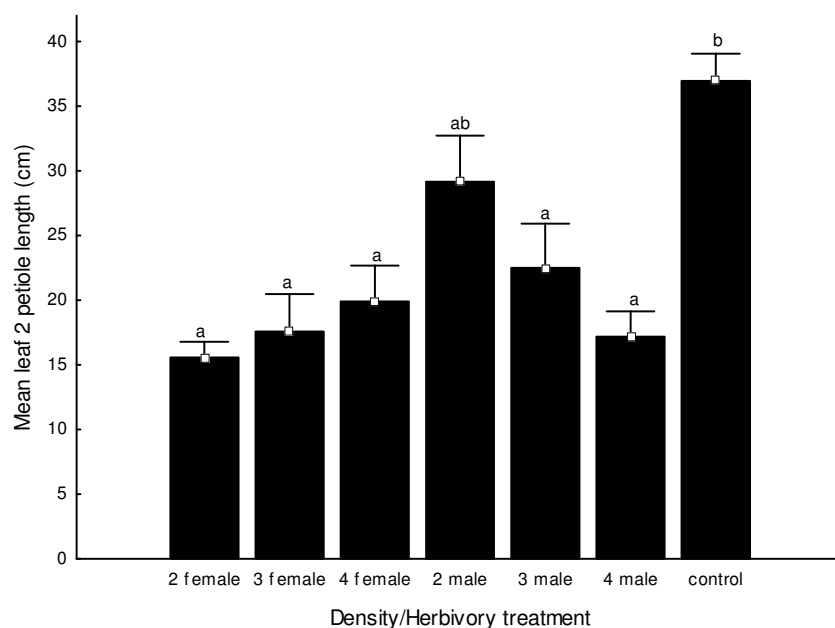


Figure 4.10 Mean length of the leaf 2 petiole (cm) of water hyacinth plants at the end of eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

The mean length of the leaf 2 petiole decreased from week one to eight in all the herbivory treatments with the exception of the 2 male treatment. The decrease was much sharper in all three female treatments compared to the 3 male and 4 male treatments. The mean length of the leaf 2 petiole increased in the control plants from the start to the end of the study period (Fig. 4.11), indicating an increase in growth rates for plants not defoliated by the grasshopper.

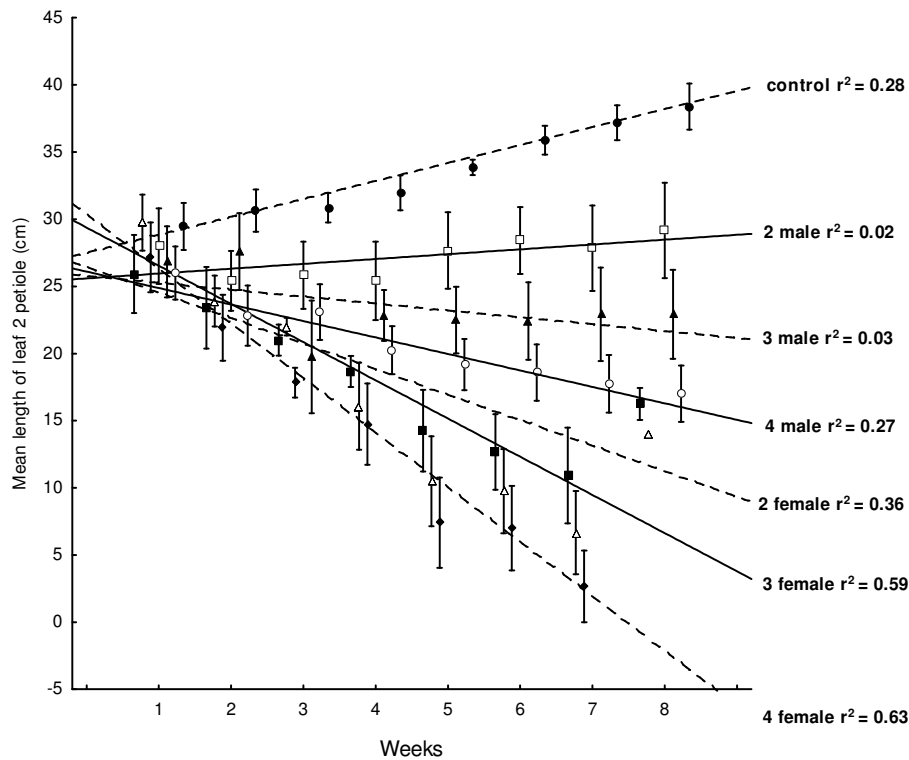


Figure 4.11 Changes in the mean length of the leaf 2 petiole (cm) of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum*. Error bars represent the standard error of the mean.

All densities of both male and female grasshoppers reduced total daughter plant production in eight weeks, although only significantly in the 4 male and 4 female treatments ($F_{6;43} = 3.89$; $P = 0.0034$) (Fig. 4.12). Despite the lack of significant differences, the temporal development of ramet production indicates that all herbivory treatments had a significant impact on vegetative reproduction of water hyacinth plants (Fig. 4.13). Control plants continued to produce daughter plants throughout the study period whereas all ramets produced by plants in the herbivory treatments were in the first four weeks of the trial, after which vegetative reproduction was suppressed by herbivory. A product-moment correlation showed a weak but significant negative correlation between insect biomass and ramet production ($r = -0.55$; $P < 0.0001$). The relationship was linear but the regression only explained 30% of the variance.

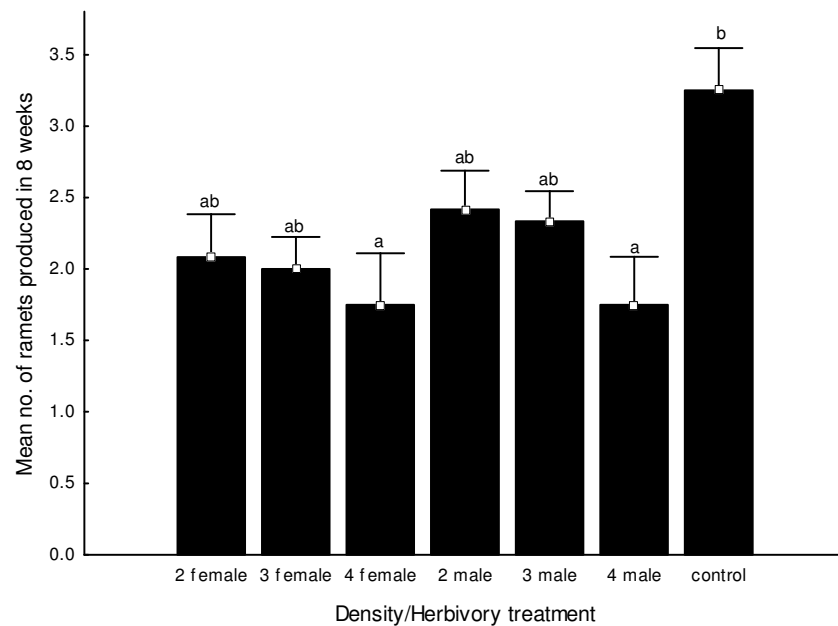


Figure 4.12 Mean number of ramets produced by water hyacinth plants in the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

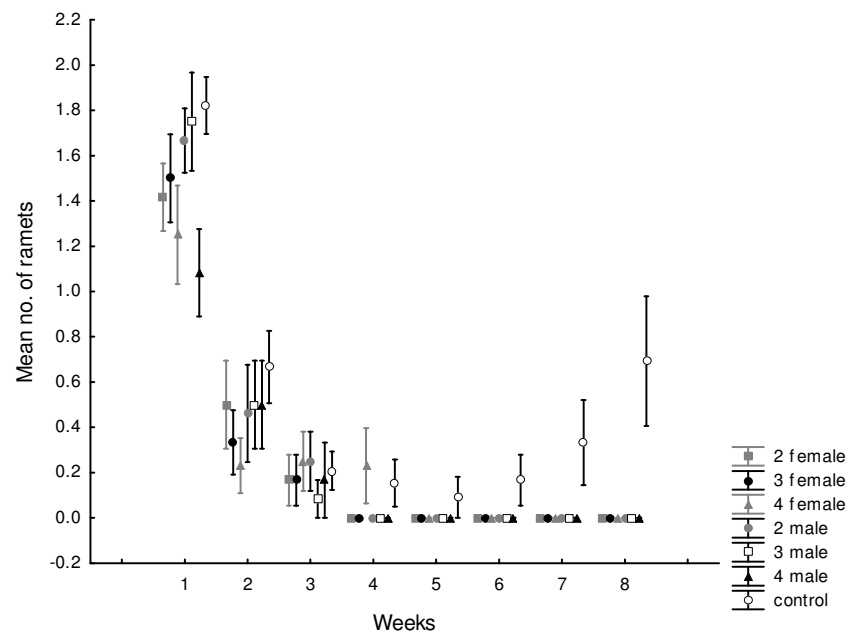


Figure 4.13 Ramet production of water hyacinth plants from week one to week eight in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum*. Error bars represent the standard error of the mean.

Flower production by plants over the study period was highly variable, and although the total number produced by plants was lower in the herbivory treatments compared to the control, none of these differences were significant (Fig. 4.14). A two-way ANOVA of the effect of density and sex on the number of flowers produced by water hyacinth plants in eight weeks showed no significant differences. The relationship between flower production and grasshopper biomass could not be clearly defined and there was no significant correlation.

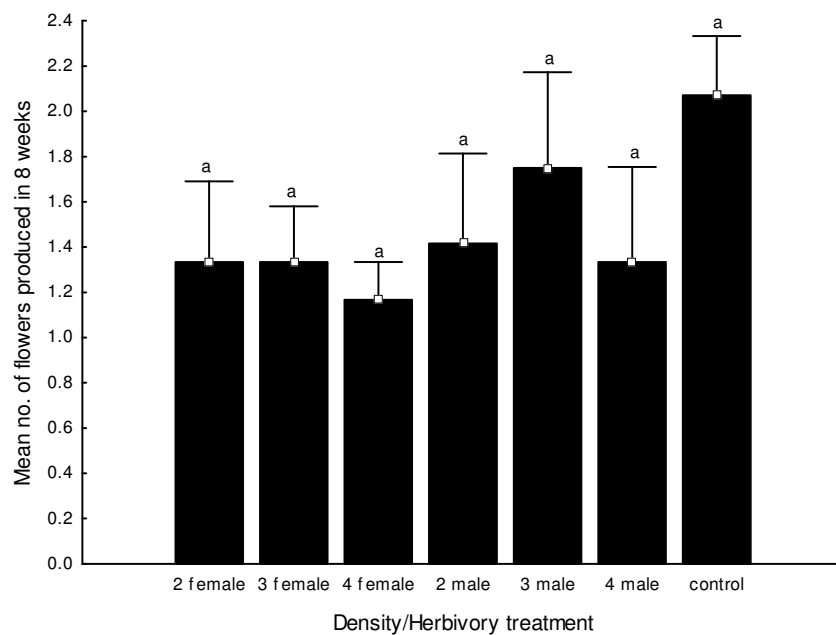


Figure 4.14 Mean number of flowers produced by water hyacinth plants in the eight week study period in the herbivory treatments and the control. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

4.3.4 Effect of insect treatment on defoliation

A two-way ANOVA of the mean number of petioles completely defoliated (entire leaf consumed) by the grasshoppers showed highly significant differences between males and females ($F_{1,30} = 192.90$; $P < 0.0001$) and between the different densities ($F_{2,30} = 10.90$; $P = 0.0003$) but the interaction of density and sex had no significant effects (Fig. 4.15). The total number of defoliated petioles increased proportionately with the amount of damage caused by herbivory by different densities of male and female grasshoppers. Less than

one petiole per plant was defoliated by male grasshoppers at all densities at the end of the trial whereas females had removed nearly all leaf material of plants by the last sampling event before they died.

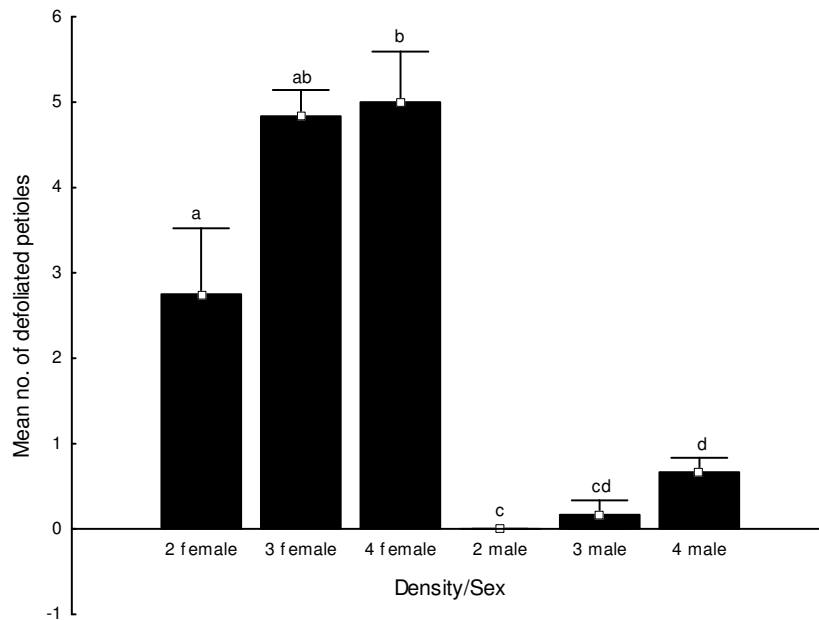


Figure 4.15 Mean number of petioles of water hyacinth plants defoliated at the end of the eight week study period in the herbivory treatments. Herbivory treatments included densities of 2, 3 and 4 male or female *Cornops aquaticum* per plant. Means compared by one-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

4.3.5 Effect of insect treatment on leaf area damage

Leaf 4 area damage and average leaf area damage was consistently between 50 and 100% in the female treatments at all three densities. Leaf area damage increased with an increase in density in the male treatments. The 2 male treatment had fairly low damage scores of between 5 and 25% for both leaf 4 and the average leaf for each plant (Fig. 4.16). This increased to between 25 and 50% in the 3 male treatment and damage to leaves in the 4 male treatment was the same as females, but this was due to the scale used and not the actual amount of damage since females removed much more leaf material. The scale used was not fine enough to separate damage levels between the female treatments and the 4 male treatment.

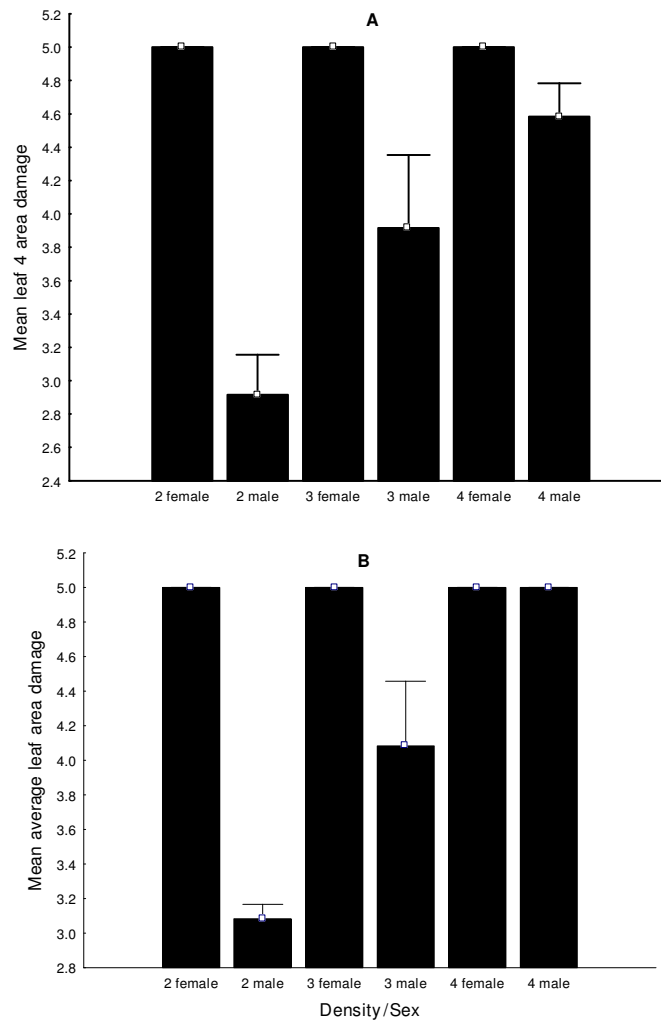


Figure 4.16 Damage scores for the percentage of leaf area damaged on leaf 4 (A) and the average percentage of damage to all leaves (B) of water hyacinth plants at the end of the eight week study period in the herbivory treatments. Herbivory treatments included 2, 3 and 4 male or female *Cornops aquaticum*. Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD $P < 0.05$).

4.4 Discussion

The results reported in this chapter suggest that water hyacinth is susceptible to medium to high levels of herbivory by *C. aquaticum*. There was a corresponding increase in damage to plants with an increase in insect biomass and the decline in water hyacinth's ability to compensate for herbivory was most often a linear function of insect biomass which is a relationship commonly observed between plants and phytophagous insects (Morrill et al., 1984; Pantoja et al., 1986; Crawley, 1989b; Meyer, 1998; Schooler &

McEvoy, 2006; Stanley et al., 2007). Even the lowest levels of herbivory by the grasshopper that were tested reduced water hyacinth growth and biomass accumulation compared to control plants. These findings under eutrophic conditions, when an abundant supply of nutrients enhances water hyacinth's ability to replace lost tissue (chapter 2), indicate potential of *C. aquaticum* to contribute to significantly reducing weed growth in eutrophic systems. The response of water hyacinth to herbivory by different grasshopper densities in terms of biomass allocation revealed some interesting strategies for compensation, survival and overall species fitness, which are discussed later in the chapter.

Although not always indicated by significant differences between the sexes, female grasshoppers with their higher feeding rates (chapter 3) were much more damaging than males. The plants were unable to compensate for leaf loss, resulting in leaf production rates too low to survive. With the exception of three plants in the 2 female treatment, all plants in the female herbivory treatments were completely stripped of all photosynthetic tissue, including the epidermis of petioles which resulted in mortality of most of the plants before the end of the trial. In the male herbivory treatments, the severity of damage was not enough to cause sufficient disruption to leaf production and mortality rates to compromise plant survival. However, an overall reduction in plant performance parameters and potential for biomass accumulation indicates that resource acquisition and assimilation were limited in all the male treatments compared to control plants. The carrying capacity of plants at the densities tested was reached well before the maximum herbivory levels, suggesting that herbivore loads in the range of two females and four male grasshoppers per plant would be sufficient to cause significant reductions in water hyacinth infestations and interfere with survivorship of established plants. The severity of damage caused by females was exacerbated by the high density of egg packets and probing holes - they use their ovipositor to pierce holes in petioles in search of suitable oviposition sites. Egg packets are likely to interfere with translocation of nutrients through the petioles into leaves and based on observation, at very high densities of females, probing holes cause water logging and weakness of petioles which then become susceptible to breaking off from the crown of the plant. If *C. aquaticum* were to be

released, higher densities of female and greater numbers of egg packets per female (chapter 3) under eutrophic conditions would, outside of feeding damage, further reduce uptake of essential nutrients to productive tissues like leaves.

Observation of water hyacinth's leaf production and mortality rates provide interesting insight into the mechanisms that plants use to reduce the negative effects caused by insect herbivory. Water hyacinth plants appear to be adapted to tolerate or compensate for injury caused by insect feeding by having rapid leaf production rates to effectively replace damaged leaves (Center, 1985), thereby compensating for lost photosynthetic tissue. For example, Center & Van (1989) found an increase in leaf production rates from 0.134 to 0.170 leaves day⁻¹ at high *Neochetina* weevil densities, most likely in an attempt to balance mortality rates, which were increased as a result of weevil herbivory. The grasshoppers did not increase leaf mortality rates but rather reduced leaf production rates. In fact, there were slight delays in leaf senescence (indicated by reduced mortality rates) in most of the herbivory treatments compared to the controls. For example, in the 2 and 3 male treatments, leaf mortality rates were delayed by 17% i.e. leaves were photosynthetically active for 17% longer than leaves of control plants. Other plants use adaptive mechanisms such as delayed leaf senescence to restore their capacity for carbon gain. Leaf senescence rates of *Solidago altissima* L. (Asteraceae) were a linear function of defoliation intensity by the leaf-feeding beetle, *Trirhabda* sp. (Coleoptera: Chrysomelidae) where the number of leaves to senesce per day decreased with an increase in the intensity of defoliation (Meyer, 1998). Delaying senescence thereby maximizing photosynthetic capacity of remaining leaves would only be of value to grazed plants. Young leaves are generally more productive than mature leaves as they have higher levels of nitrogen, and a longer life span to contribute to carbon production for the plant (Center, 1985). If the production of new leaves by plants is constrained by herbivory, maintaining the productive capacity of existing leaves by delaying leaf senescence would be an adaptation for compensatory photosynthesis. If leaf production is limited, the value of holding on to existing leaves that can still contribute to carbon gain becomes more important.

Increasing or maintaining biomass allocation to leaves at the expense of biomass allocation to other plant parts is another adaptive response commonly seen in plants to compensate for a reduced capacity for carbon acquisition as a result of insect herbivory (Wallace & O'Hop, 1985; Trumble et al. 1993; Meyer, 1998; Stowe et al., 2000; Tiffin, 2000; Ding et al. 2006; Stevens et al., 2008). Despite water hyacinth's phenotypic plasticity in their response to stress and environmental conditions, their response in maintaining the full complement of leaves typical of water hyacinth rosettes (Center & Spencer, 1981) is consistent (as much as herbivory levels allow) and is an adaptive mechanism to maintain photosynthetic capability and buoyancy (Center & Van, 1989). The leaf parameters in the male grasshopper treatments that were measured in this study were similar to control plants, although plant growth and the accumulation of biomass were significantly reduced by herbivory. The response of water hyacinth plants to *C. aquaticum* herbivory in the high nutrient treatment in chapter 2 was similar where leaf production was the same as control plants, but this was at the expense of plant growth and reproduction. Water hyacinth plants responded in the same way in studies conducted by Heard & Winterton (2000) and Coetzee et al. (2007). They found that ramet production and the accumulation of biomass were reduced by herbivory by *E. catarinensis* and the *Neochetina* weevils but the number of leaves of plants were the same or higher than control plants.

Other aquatic plants have similar mechanisms to cope with loss of photosynthetic tissue. Water chestnut, *Trapa natans* L. (Trapaceae) increased leaf production at low levels of herbivory by the biocontrol agent, *Galerucella birmanica* Jacoby (Coleoptera: Chrysomelidae, but this was at the expense of reproduction and biomass accumulation (Ding et al., 2006). However at higher insect densities, the plants were unable to compensate resulting in complete defoliation and ultimately death of plants. The water lily *Nuphar luteum* L. (Nymphaeaceae) compensated for herbivory by *Pyrraltea* (*Galerucella*) *nymphaeae* Linne (Coleoptera: Chrysomelidae) by increasing leaf production rates. Rapid turnover rates were needed to support herbivory and leaf longevity was 17 days in the presence of herbivory compared to a nearby site without herbivory where leaf longevity was 45 days (Wallace & O'Hop, 1985). The mechanisms

for compensation and biomass allocation patterns are likely to be species specific but leaf production and allocation to leaf biomass seem to be a specific adaptive response of aquatic plants for survival. Therefore, a promising biocontrol agent for water hyacinth should have the ability to disrupt leaf dynamics as was suggested by Stanley et al. (2007) for *X. infusella*. The moth reduced leaf production rates of water hyacinth under high nutrient conditions and in light of the importance of leaf production in survival of water hyacinth plants the authors suggested this indicated promise for *X. infusella* as a biocontrol agent (although not for the U.S.A. as the impact of the moth on leaf production was greater on an indigenous species, pickerel weed, *Pontederia cordata* L. (Pontederiaceae)).

The factors that govern water hyacinth population dynamics were variable in their response to herbivory. The patterns of ramet production that were evident were largely in response to the removal of daughter plants at the start of the trial as well as herbivory by the grasshopper. Removal of water hyacinth's ramets stimulated clonal growth with all plants producing a similar number of daughter plants within the first four weeks of the trial, which is why there were no significant differences in the number of ramets between the herbivory treatments and the control (with the exception of the 4 female and 2 male treatment). However, sustained herbivory at all densities of male and female grasshoppers suppressed further daughter plant production whereas control plants continued to reproduce vegetatively throughout the study period. Asexual reproduction by water hyacinth is important in the density and spread of water hyacinth populations, therefore a reduction in productivity would reduce expansion of water hyacinth mats and reduce its invasive potential. Under simulated field conditions, Center et al. (1999b) found that high densities of the *Neochetina* weevils reduced water hyacinth colony development by suppressing the expansion of mats. *Cornops aquaticum* might have some superior ability in reducing clonal growth in that even the lowest levels of herbivore pressure tested in this study, suppressed ramet production. *Salvinia molesta* plants damaged by *C. salviniae* produced the same number of ramets compared to salvinia plants without weevil herbivory however the biomass of each ramet was reduced. This was regarded by Julien & Bourne (1986) as an adaptation of a clonal plant whose

population dynamics rely solely on the production of meristematic tissue. This was not the case for water hyacinth, also a clonal plant, where investment in leaf biomass appeared to take preference over investment in ramets in response to herbivory. The investment of water hyacinth plants in flowers revealed a different pattern in their allocation to vegetative reproduction in response to grasshopper herbivory.

Flower production did not follow a trend according to the amount of herbivore pressure and was highly variable within the herbivory treatments. These results are very different from the results for flower production in chapter 2 where feeding by a density of one grasshopper per plant significantly reduced flowering compared to control plants at all nutrient levels. Both trials were conducted in summer and under controlled glasshouse conditions, therefore the differences in flowering can not be attributed to the time of year or climatic conditions. The factors that stimulate flower production in water hyacinth are poorly understood. Watson & Brochier (1998) found that nutrient-stress stimulated flowering in water hyacinth and conditions of low nutrient availability seemed to induce flowering in a study by Coetzee et al. (2007) where flower production by water hyacinth was significantly higher in the low nutrient treatment compared to the high nutrient treatment. The nutrient study in chapter 2 showed no effects of nutrient availability on flower production. These variable responses demonstrate the phenotypic plasticity of water hyacinth in its patterns of biomass allocation which seem to be dependent on the type and severity of stress. The higher feeding intensities tested compared to those in chapter 2 at the same nutrient levels may have stimulated plants to compensate for herbivore damage by increasing flower production. Compensatory responses to herbivory can depend on the type and level of stress (Oosterheld & McNaughton, 1991) and herbivory has often been believed to benefit plants by stimulating them to increase flower and seed production (Maschinski & Whitham, 1989; Pilson & Decker, 2002). Reproduction is a measure of performance at the individual level (Stowe et al., 2000) therefore if survival of a plant is compromised due to high levels of stress caused by herbivory, greater allocation to flower and therefore seed production, would favour maintenance of their genetic material in the gene pool. Water hyacinth's variability in its flowering patterns conform to the theory of Maschinski & Whitham (1989) of a

continuum of compensatory responses in plants as was evident in chapter 2, which are dependent on environmental conditions such as nutrient availability and the intensity of herbivore damage.

Intraspecific interference in feeding rates of individuals was apparent, as the damage caused by double the insect biomass did not equate to double reductions in yield. Food was limiting in the female treatments but not in the male treatments. High mortality rates of male acridid grasshoppers compared to females have been recorded, apparently due to increased interference between males to gain access to females, which allowed less time for feeding (Belovsky & Slade, 1995). Although there were no females for males to compete for in the present study, the observed feeding patterns in the male treatments may indicate density-dependent competition within the species. Density-dependent effects such as mortality and reduced reproductive allocation (Wall & Begon, 1986; Joern & Klucas, 1993; Belovsky & Slade, 1995; Branson, 2006) have been recorded for conspecific orthopterans, and were always the result of limited food availability. Extrapolating to the field from this study is constrained by the fact that the density-dependent effects were evident in a closed system, where the grasshoppers could not control their own density. In open, natural systems, they can disperse or emigrate if an increase in density-dependent effects become apparent. Both adults and nymphs are highly mobile but the nymphs are likely to suffer more from density-dependent competition for food since their dispersal capabilities are more limited, although Belovsky & Slade (1995) found both nymphs and adults of *Melanoplus* sp. (Orthoptera: Acrididae) to suffer similarly as a result of density-dependent effects. Indeed density-dependent effects can occur under natural field conditions due to resource availability, but if food was limiting to *C. aquaticum*, water hyacinth would be under excellent control. Grasshopper populations may crash or there may be increased emigration but negative feedback mechanisms should regulate populations through bottom-up control which takes effect to reduce populations in response to the density of their host (Hixon et al., 2002). Herbivore-host systems with a strong interaction should respond with cyclical fluctuations, following patterns of their herbivore/host by responding to each other's densities (Pearson & Callaway, 2003; 2005).

There is no doubt that at relatively high population densities of *C. aquaticum*, water hyacinth would be under control. However, predicting whether these population densities will be realized in the field is more difficult. Because of the stochastic nature of environmental influences, most populations are subject to random fluctuations and are inherently non-linear (Turchin, 1999; Hixon et al., 2002). From the results of chapters 2 and 3, we can predict that water hyacinth population fluctuations will largely be determined by bottom-up forces through nutrient availability (chapter 2) and grasshopper populations will largely be determined by bottom-up forces through plant quality (chapter 3) and abundance. Therefore environmental heterogeneity in nutrient availability will be important in determining population dynamics at both trophic levels. These predictions are independent of climatic influences which can be important in the abundance of both plants and insects. Based on the evidence, populations of *C. aquaticum* will fluctuate similarly to plants with fluctuating water nutrient levels, in response to quality and quantity of their hosts. In other words, plant populations will increase in response to increased resource availability (chapter 2), and grasshopper populations will increase in response to an increase in resource availability, as a result of increased fecundity and survival in response to increased tissue nitrogen levels (chapter 3) and an increase in plant biomass. Therefore, bottom-up forces might have primacy in influencing *C. aquaticum* population dynamics and this is generally believed to be the prevalent factor determining phytophagous insect community structure (Denno et al., 2003). Bottom-up effects similar to those we might expect for *C. aquaticum* were found in a sap-feeder community, whereby their density increased when the nitrogen content of their host was elevated (Denno et al., 2003).

Top-down forces are believed to be more important for specialist herbivores because their populations aren't limited by plant chemical defenses as they are for generalists (Dyer et al., 2004) but quality of their host plant might then be more important. Belovsky & Slade (1993) after a six-year experimental study found that the effect of predators on acridid grasshopper populations was minimal and Elder (2006) found that predation by spiders in particular had very little effect on acridids in their experimental herbivore community.

Cornops aquaticum is likely to be susceptible to generalist predators as they are in the region of origin (Silveira-Guido & Perkins, 1975), however, in the absence of their specialist predator, *L. fasciatus* their populations are probably more likely to be regulated by resource quality and quantity as well as climatic conditions in some parts of South Africa. The relative strength of each force is likely to be case-specific but it seems from the evidence from chapters 2 and 3 and the absence of their specialist predator that bottom-up forces might be dominant in the dynamics of *C. aquaticum* populations. Water hyacinth and grasshopper populations might be highly correlated according to prevailing nutrient conditions, although additional factors such as climate and density-dependent effects are likely to play a role.

Schooler & McEvoy (2006) introduced a method for estimating population density from feeding damage for a biological control agent. They note that plant damage is cumulative and can predict impact more effectively than estimates of insect density, which is often difficult due to insect behaviour and phenology. Insects are mostly not immobile, immature stages often develop inside plant tissue and are therefore not visible and many are not active during the day when counts of insects on plants are more feasible. They concluded from a number of studies that plant damage is a function of herbivore density and can be a surrogate for insect density when measuring population growth and spread. For example, Rhainds & English-Loeb (2003) found that the proportion of damaged fruit of strawberry plants was positively correlated with nymphal densities of the tarnished plant-bug. Schooler & McEvoy (2006) set out to establish the density-damage relationship between purple loosestrife, *Lythrum salicaria* L. (Lythraceae) and the golden loosestrife beetle, *Galerucella pusilla* Duftschmid (Coleoptera: Chrysomelidae) in order to provide an alternative to direct counts of insects to estimate herbivore density indirectly from feeding damage. Their results indicated that visual estimates of leaf area damage could be used to estimate densities of field populations of the biocontrol agent.

Increases in the amount of leaf tissue removed by *C. aquaticum* were consistent with increases in densities of male grasshoppers therefore the proportion of leaf material removed is directly associated with herbivore load. The scale used to measure damage to

leaves was not fine enough to separate damage levels between the different densities of females, with the amount of damage consistently between 50 and 100%. The results for leaf 4 area damage and average leaf area damage could be used for estimating field densities of grasshoppers and their population impact. For example, feeding damage to leaves at an average of 50 to 100% would indicate sufficient population densities and reductions in water hyacinth infestations could be expected. Feeding damage to leaves at an average of 5 to 25% would indicate population levels too low to severely reduce infestations but reductions in biomass and clonal development could be expected. Actual density estimates could be taken by measuring plant density and insect biomass as a function of the amount of observable damage to leaves (although these relationships would first have to be defined for a natural population).

In the past, it was assumed that if a biocontrol agent establishes and becomes sufficiently abundant, it would automatically contribute to control of the target plant (Myers, 1985). McClay & Balciunas (2005) listed a number of examples taken from the literature, where agents, despite becoming relatively abundant, had no significant impact on their target weeds. They suggest possible causes of ineffectiveness of abundant agents to include: (1) the use of seed-feeders against a target whose populations are not seed limited; (2) agents that feed on non-essential tissue and therefore do not significantly stress the plants; (3) the ability of target weeds to tolerate or compensate for defoliation or other kinds of damage; (4) and agents that trigger a strong induced defensive response in the target weed protecting it against further damage; and (5) damage that comes too late in the phenology of the weed to affect its reproduction or growth. Indeed, studies have shown that herbivores can have no effect on the plants they consume (Karban & Courtney 1987; Van den Berg & Soehard, 2000) and the ability of a biocontrol agent to feed and develop on a target plant does not guarantee reductions in plant vigour (Hufbauer & Roderick, 2005). This again reinforces the importance of testing candidate agents to assess whether the damage they cause has a negative affect on plant performance. Center & Van (1989) have discussed and demonstrated the importance of leaf productivity in survival of water hyacinth plants. As a defoliator, the grasshoppers feed on essential tissue directly related to plant survival, and other researchers working on water hyacinth have suggested that

water hyacinth biocontrol agents should be measured by their ability to affect leaf production (Stanley et al., 2007) suggesting that *C. aquaticum* should be a valuable biocontrol agent. The results reported here show that water hyacinth plants are sensitive to defoliation by the grasshoppers and the type of damage that they cause has a significant impact on plant performance and survival (at high densities). At low herbivore densities, although leaf production was maintained to ensure survival, other parameters that are related to the weedy characteristics of water hyacinth, such as vegetative reproduction and prolific growth rates, were reduced. This study has therefore indicated that water hyacinth plants cannot compensate effectively at any grasshopper density to maintain production and growth rates as normal without herbivory. Some compensation for leaf loss was evident at low herbivore densities but this was at the expense of other growth and reproductive parameters. Assessing damage by *C. aquaticum* against the causes for abundant but ineffective agents as suggested by McClay & Balciunas (2005): (1) is not applicable; (2) *C. aquaticum* feeds on essential tissue that severely stresses the plants. This was indicated by the corresponding reductions in plant fitness with increasing amounts of leaf tissue removed by the grasshoppers; (3) water hyacinth is not able to compensate effectively for defoliation. The relationship between grasshopper density and plant damage that was found in this study indicates that water hyacinth's compensatory ability decreases with increases in feeding intensity by the grasshoppers; and (4) although this was not measured, water hyacinth plants do not appear to trigger an induced response protecting the plants from further damage. None of the impact studies that were conducted gave any indication that grasshoppers were deterred from feeding at any stage. In some cases, herbivory continued until the plants died; (5) one of the crucial questions that remains is whether populations of *C. aquaticum* will be high enough at the critical stage of water hyacinth population development to prevent growth and spread.

A trial release consisting of only male grasshoppers is no longer a consideration since approval for the release of *C. aquaticum* was granted by the relevant authorities in 2007. While a trial release would have given a good indication of the field host-range of the grasshopper and potential non-target effects, evaluating efficacy would have been limited by: (1) the absence of natural population growth rates. While population increase could

have been simulated, this would potentially have been very inaccurate, as it is impossible to predict how *C. aquaticum* will respond to environmental variability in the field in South Africa without temperature and development data. Therefore impact of the test densities could have over- or underestimated potential impact of natural *C. aquaticum* populations which could mislead a decision on release; (2) the benefit of higher damage levels of females due to higher feeding rates and egg packets in petioles would have been missed in a male only population.

In conclusion, water hyacinth is highly susceptible to the type of damage caused by the grasshopper, even under eutrophic conditions when plant productivity is at a maximum (Reddy et al., 1989). Reductions in water hyacinth were density-dependent and the linear relationship between plant performance and insect density indicates that the ability of plants to tolerate or compensate for herbivory declines with increased herbivore pressure. Females are substantially more damaging than males and their negative effects are exacerbated through the production of egg packets and probing holes which at high densities, causes water logging of petioles. At fairly high grasshopper population densities, they will be able to reduce reproduction, growth rates, density and spread of water hyacinth. At lower herbivores densities, water hyacinth would be under good control in eutrophic conditions with reduced biomass, and reductions in density and potential for spread. With sustained herbivory at those levels, a decline in water hyacinth infestations could be expected. Densities nearing the carrying capacity of water hyacinth plants might lead to dispersal but at those kinds of grasshopper densities, water hyacinth would remain at low levels, or be severely damaged. The relationship between grasshopper density and plant performance indicates promise for *C. aquaticum* as a biological control agent for water hyacinth and based on these findings would be justified for release (McClay & Balciunas, 2005). However, top-down control is not the only dominant factor influencing plant population dynamics. Competition for resources from neighbouring plants can impact plant populations and a combination of the two factors has lead to improved levels of control of invasive weeds. Also, damage by herbivores can in some cases increase the competitive ability of plants or the production of chemicals that can have negative effects on neighbouring plants (Pearson & Callaway, 2003; 2005).

Therefore, the combination of low levels of herbivory by the grasshopper and competition from a similar free-floating aquatic species is investigated in Chapter 5.

Chapter 5

Inter- and intraspecific competitive interactions between water hyacinth and water lettuce, as influenced by *Cornops aquaticum* herbivory

5.1 Introduction

Some plant communities are botanically diverse, consisting of many coexisting species, while others can be dominated by a single species that has morphological and phenological characteristics that allow it to monopolize resources in the environment (Grime, 1979), to the detriment of other less competitive species. This asymmetry is commonly observed with invasive alien plants that grow unchecked in introduced environments, completely or relatively free from herbivore pressure, allowing them to exploit invaded habitats and outcompete indigenous flora. Two of the processes that have an important influence on the abundance and distribution of plant species in a community are competition and herbivory (Harper, 1977) and are the factors that are generally considered to be dominant in influencing plant population dynamics (Grime, 1979; Crawley, 1989b). They are therefore potentially important processes in control of invasive alien plants.

Competition (both inter- and intra-specific) with neighbouring plants is a stress factor (Grime, 1979) arising from direct interference for example through shading or indirectly as a result of exploitation of space and resources such as water and nutrients by the dominant competitor (Louda et al., 1990). The negative effects of stress on the inferior competitor can be manifested as reductions in biomass (Müller-Schärer, 1991; Steinger & Müller-Schärer, 1992; Agrawal, 2004), a change in plant architecture (Steinger & Müller-Schärer, 1992) and flowering phenology (Nötzold et al., 1998) and reductions in flower, fruit and seed production (Maschinski & Whitham, 1989; Müller-Schärer, 1991). Herbivory is a disturbance factor (Grime, 1979) that can reduce plant fitness as a result of loss of productive tissue. Reduced growth rates (Rauscher & Feeny, 1980) survival (Müller-Schärer, 1991; Sheppard et al., 2001) and competitive ability (Cottam, 1986) as well as changes in biomass allocation patterns (Mihaliak & Lincoln, 1989; Nötzold et al., 1998; Stevens et al., 2008) are some of the effects of herbivory on plants. The relative importance of each factor in structuring plant communities will depend on the species as well as the prevailing conditions such as nutrient availability (Maschinski & Whitham, 1989; Swank & Oechel, 1991), or plant biomass (Bonser & Reader, 1995; Tiffin, 2002) through influencing competitive ability or potential for compensation. For example,

supplementary nitrogen and lime increased interspecific competition by increasing biomass of competitors of creeping thistle, *Cirsium arvense* (L.) Scop. (Asteraceae), which resulted in reductions in thistle shoot density (Edwards et al., 2000). Shoot biomass of *C. maculosa* was reduced by 63% in nutrient-stressed plants infested with the weevil, *Cyphocleonus achates* Fahr. (Coleoptera: Curculionidae) compared to only 30% in plants growing at high nitrogen availability (Steinger & Müller-Schärer, 1992), which would in turn affect their competitive ability. *Ipomopsis arizonica* plants overcompensated for herbivory under high nutrient conditions without competition by increasing fruit-set, however, their potential for compensation was reduced in the presence of competing species, regardless of nutrient availability (Maschinski & Whitham, 1989). Cipollini & Bergelson (2002) suggest that resource competition can reduce the production of defensive chemicals in plants, which could lead to increased levels of herbivory. This, in turn could intensify the effects of competition on plant fitness levels. Bonser & Reader (1995), in accordance with Grime's (1979) biomass-dependent theory of plant community organization, found that the negative effects of competition and herbivory on a perennial grass *Poa compressa* L. (Poaceae) were greater at sites with high plant biomass. High levels of competition had a greater effect on fitness of *Ipomoea pupurea* Roth (Convolvulaceae) compared to lower levels of competition and the detrimental effects of selective herbivory on *I. pupurea* were amplified in the high competition environments (Tiffin, 2002).

When both competition and herbivory are present, the relative strength of each factor will also be dependent on the system. In some cases, competition can have a much greater impact on plant fitness (Müller-Schärer, 1991; Steinger & Müller-Schärer, 1992; McEvoy & Coombs, 1993; Friedli & Bacher, 2001) and in others, herbivory has been found to be a much stronger factor in reducing plant performance (Nötzold et al., 1998; Agrawal, 2004). Sheppard (1996) describes the outcomes of interactions between competition and herbivory as substitutive, multiplicative or synergistic. A substitutive reduction occurs where one factor fails to have an effect on the target plant, or where one of the factors is smothered by the other and the effects can not be detected (e.g. Müller-Schärer, 1991). The effects are multiplicative when both factors have an impact on the target plant

regardless of the presence of the other factor (e.g. Fowler & Rauscher, 1985; Doyle et al. 2007) and a synergistic interaction occurs when one of the factors changes the effect of the other (e.g. Lee & Bazzaz, 1980; Agrawal, 2004; Rand, 2004). A two-factor synergy is where both factors have an impact on a plant in isolation but the combined effect is greater than either factor on its own. Herbivory by *Apion onopordi* Kirby (Coleoptera: Apionidae) combined with grass competition showed a classical two-factor synergy where the combination of the two factors had a significantly greater impact on *C. arvensis* than the effect of each single factor (Friedli & Bacher, 2001). In some cases herbivory can increase a plant's competitive ability in its environment. For example, Callaway et al. (1999) found that the negative effects of *C. maculosa* on neighbouring plants increased with herbivory by the moth *Agapeta zoegana* Lin (Lepidoptera: Tortricidae), by stimulating the production of allelopathic chemicals. The effect of interspecific competition alone, or herbivory alone has also been found to increase plant growth and reproductive parameters (Nötzold et al., 1998), which is an indication of overcompensation in response to stress (Maschinski & Whitham, 1989).

Biocontrol practitioners are increasingly realizing the potential benefits of combining the two factors and that competition can increase or magnify the negative effects of herbivory by a biological control agent. Plant performance might only be reduced by herbivory in the presence of interspecific competition (Cottam et al., 1986; Rand, 2004) and herbivory can mediate exploitative competition through selective herbivory on the dominant competitor (Cottam, 1986). Furthermore, in some systems where both factors were investigated, competition has been found to be more effective than some biocontrol agents in reducing populations of the target weed (McEvoy & Coombs, 1993). Success in some biocontrol programmes has been attributed to a combination of grazing, and a change in the competitive status of the plant (Whitaker, 1979). For example, control of alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae) was brought about by herbivory by the biocontrol agent, *Agasicles hygrophila* Selmon & Vogt (Coleoptera: Chrysomelidae) working in combination with competition from other aquatic plants (Durden et al., 1975). A study by Ang et al. (1994) indicated that herbivory by the biological control agent *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae)

and competition from other plant species should be combined to improve control of Canada thistle, *Cirsium arvense* Scop. (Asteraceae) Herbivory by *Gastrophysa viridula* Degeer (Coleoptera: Chrysomelidae) is only effective in reducing growth of the agricultural weed, *Rumex obtusifolius* L. (Polygonaceae), in the presence of competing species (Cottam et al., 1986). In a greenhouse experiment, competition from a green cover plant, *Trifolium pretense* L. (Fabaceae), improved overall control levels of a weed, *Calystegia sepium* (L.) R. Br. (Convolvulaceae) in maize crops by a pathogen (Guntli et al., 1999). A combination of the two factors has been particularly well studied with root herbivores (Müller-Schärer, 1991; Steinger & Müller-Schärer, 1992; McEvoy & Coombs, 1993; Nötzold et al., 1998; Sheppard et al., 2001) for weeds such as tansy ragwort, *S. jacobaea*, purple loosestrife, *L. salicaria* and spotted knapweed, *C. maculosa*.

Evaluating the impact of a combination of herbivory and competition on a plant targeted for biological control is increasing in its application and methods have been developed to quantify the changes in the target weed's competitive ability. Spitters (1983) introduced the inverse linear model to determine best combinations for increased yield in mixed cropping systems, using results of mixed cropping of maize and groundnut as a model. The method was then adapted by Pantone et al. (1989) in order to develop an alternative means for evaluating the efficacy of biocontrol agents, as opposed to traditional impact studies, which measure changes in biomass and productivity in an experimental unit and a control. Their method considers the two important factors influencing plant population dynamics - herbivory and competition. The change in competitive interactions between the target plant and another plant species is measured in the presence and absence of selective feeding by the biological control agent. Van et al. (1998) used the method to evaluate the impact of insect herbivory on the competitive ability of *Hydrilla verticillata* L.f. Royle (Hydrocharitaceae) compared with an indigenous aquatic plant, *Vallisneria americana* Michx. (Hydrocharitaceae). Center et al. (2001) later suggested it as a potentially effective method for evaluating a water hyacinth biocontrol agent, and it has subsequently been used to evaluate three water hyacinth agents, the sap-sucking mirid, *E. catarinensis* (Coetzee et al., 2005) and the water hyacinth weevils, *N. bruchi* and *N. eichhorniae* (Center et al., 2005) as well as a combination of the mirid and *N. eichhorniae*

(Ajuonu et al., 2008). While these types of studies have been identified as being particularly useful for evaluating biocontrol agents with more subtle types of feeding damage such as sap-suckers or gall-formers (Pantone et al., 1989; Coetzee et al., 2005), where the direct effects of herbivore feeding may not be easily detected, they hold merit for evaluating more damaging agents as they give an indication of how herbivore feeding may shift the competitive balance, reducing the target plant's competitive edge. McClay & Balciunas (2005) also suggest it as a good method for evaluating efficacy of candidate biological control agents in pre-release studies. In addition to this, performing such studies prior to release will elucidate any compensatory responses in the form of increased competitive ability, thereby eliminating the chances of unwanted negative effects on indigenous flora as a result of increased plant performance in response to herbivory (Pearson & Callaway, 2005).

Chapters 2 and 4 investigated the impact of *C. aquaticum* herbivory on water hyacinth's fitness at varying insect densities and nutrient conditions in order to make predictions about the grasshopper's potential efficacy. This gave a good indication of the direct effects of feeding by *C. aquaticum* on water hyacinth. An understanding of how herbivory by the grasshopper might influence the competitive performance of water hyacinth would provide insight in to how selective feeding by the grasshopper might alter plant community dynamics in the field and would reveal any compensatory responses to a combination of interspecific competition and herbivory. Therefore, the aim of this chapter was to (1) evaluate the impact of *C. aquaticum* on the competitive performance of water hyacinth; (2) elucidate whether compensatory responses are evident when in competition with another plant species and (3) investigate whether a combination of herbivory and competition would result in improved control and greater impact than herbivory alone. Water lettuce was used as the competing species as was done in other studies evaluating water hyacinth biocontrol agents (Center et al., 2005; Coetzee et al., 2005; Ajuonu et al., 2008). The two plant species are often found growing together and water hyacinth is consistently the dominant species. Also, South Africa does not have indigenous free-floating aquatic macrophytes, which are needed for a comparative study. This study will contribute to furthering knowledge of the dynamics of the relationship

between water hyacinth and the grasshopper. An added advantage is that it allows for comparison with data from other studies that have evaluated other water hyacinth biocontrol agents already released in South Africa. One of the principle aims of these in-depth pre-release studies of the candidate agent is to ascertain its potential efficacy compared to the agents already established. The grasshopper would only be eligible for release if it has proven potential to be a superior biocontrol agent compared with the agents already released. Another advantage is evaluation of the two factors in combination that are the major factors influencing individuals and populations in plant communities.

5.2 Materials and Methods

5.2.1 Experimental design

The experimental design was based on that used by Coetzee et al. (2005) to evaluate the efficacy of *E. catarinensis*, the most recent agent introduction into the South African biocontrol programme for control of water hyacinth. Water hyacinth and water lettuce plants obtained from stock cultures were grown in plastic tubs in a poly-carbon quarantine glasshouse at the Plant Protection Research Institute in Pretoria, South Africa. The trial was run from November to December 2007. The tubs were 63 x 42 x 37 cm and were filled with 20L of water. The high nutrient treatment, simulating nutrient conditions of a eutrophic impoundment in South Africa, was used as the nutrient medium in this trial so that the combined effect of plant competition and herbivory could be evaluated when plant productivity and growth rates were at a maximum (Reddy et al, 1989). Refer to chapter 2 for levels of nitrogen and phosphorus. Commercial chelated iron was also added to the nutrient medium at a rate of 1.7 g/20 L of water (Coetzee et al., 2005). The nutrient medium and water were replaced on a weekly basis to maintain a good supply of nutrients to plants and each tub was enclosed with a netted sleeve.

The experimental design followed an additive series (Spitters, 1983) of factorial combinations of different densities of the two competing species. The planting densities of water hyacinth:water lettuce were 0:3, 0:9, 3:0, 3:3, 3:9, 9:0, 9:3 and 9:9 in each tub. The density matrices were repeated twice, once as the experiment with herbivore feeding

and once as the control and were replicated three times. The resulting 48 tubs were arranged in a randomized block design (Coetzee et al., 2005). The plants were grown for two weeks prior to the start of the experiment, after which, all daughter plants were removed to revert to the initial stocking densities. Adult *C. aquaticum* were introduced into the experimental tubs at a density of one pair per two water hyacinth plants. Because there were an uneven number of plants in every tub, the extra grasshopper used was always a female. The insect densities were maintained at the original density so any emerging nymphs were removed from the tubs as soon after hatching as possible, although this was not many due to the length of the trial. It was run for a period of 4 weeks, after which the two plant species in each tub were weighed to determine total wet weight (including daughter plants and dead plant material). These values for each plant species in each tub were then divided by the original plant stocking density to calculate mean wet weight per individual water hyacinth and water lettuce plant. Wet weight and dry weight are highly correlated (Agami & Reddy, 1990) so wet weight was used as it is the more practical measure.

5.2.2 Statistical analysis

The inverse linear model described by Spitters (1983), and Pantone et al. (1989) was used for analysis of the data. The competitive ability of each species was estimated using multiple linear regressions of the inverse of the mean weight-yield of each species as the dependent variable and the planting densities of each species as the independent variables. The regression equation is of the form:

$$\begin{aligned} 1/w_h &= a_{h0} + a_{hh}d_h + a_{h1} d_1, \\ 1/w_l &= a_{l0} + a_{ll}d_l + a_{lh} + a_{lh}d_h \end{aligned}$$

where $1/w_h$ is the inverse biomass yield of individual water hyacinth plants and $1/w_l$ is the inverse biomass yield of individual water lettuce plants. The respective planting densities are represented by d_h and d_l . Intraspecific competition is estimated by the coefficients, a_{hh} and a_{ll} , and interspecific competition is estimated by a_{h1} and a_{lh} in terms of their effects on the reciprocals of the yield of both plant species. The intercepts are represented by a_{h0}

and a_{l0} which are the reciprocal of the maximum weight of isolated plants. The ratio of the coefficients a_{hh}/a_{hl} measures the effects of intraspecific competition of water hyacinth on itself, relative to the effects of interspecific competition of water lettuce on water hyacinth. Likewise, the ratio of the coefficients a_{ll}/a_{lh} measure the effects of intraspecific competition by water lettuce on its own yield relative to the effect of interspecific competition by water hyacinth on the yield of the water lettuce (Pantone et al., 1989).

The data were normally distributed so a one-way ANOVA was used to analyze whether the mean end-weights of water hyacinth and water lettuce (total yield/original planting density) were significantly different in the presence and absence of *C. aquaticum* herbivory. The graphical representations of the data with surface response planes show the combined effect of water lettuce and water hyacinth planting densities on the reciprocal yield of both water hyacinth and water lettuce.

The total wet weights of water hyacinth at low (planting density = 3) and high densities (planting density = 9) were analyzed to evaluate the effects of competition from water lettuce on water hyacinth biomass in the presence or absence of herbivory by the grasshopper. The effect of three competition treatments on water hyacinth biomass were used in the analysis: no competition which was represented by the treatments where water lettuce was absent; low competition where water lettuce was grown at a density of three plants with water hyacinth; and high competition where water lettuce was grown at a density of 9 plants with water hyacinth. These data were analysed by two-way ANOVA to investigate the effects of competition, herbivory and their interaction on total water hyacinth biomass.

5.3 Results

5.3.1 Impact of herbivory on competition

Water hyacinth (*Eichhornia crassipes*)

Water hyacinth was the superior competitor of the two plants species in the presence and absence of herbivory by *C. aquaticum*. In the absence of herbivory, water hyacinth was

24 times as strong a competitor with itself as was water lettuce with water hyacinth, indicating that intra-specific competition has a much greater effect on water hyacinth than interspecific competition from water lettuce i.e. it took 24 water lettuce plants to have the same impact on water hyacinth biomass as one water hyacinth plant. In the herbivory treatment, the ratio of the competition coefficients (a_{hh}/a_{hl}) was only 12 which equates to a 50% reduction in water hyacinth's competitive ability due to selective feeding by the grasshopper.

Interspecific competition from water lettuce increased in the presence of *C. aquaticum* herbivory, as indicated by the coefficient a_{hl} (Table 5.1), which shows a 4-fold increase in water lettuce's competitive ability, although the total effect is still small. The graphical representation of the data provides a visual indication of this increase through the slightly steeper slope of the water lettuce regression (Fig. 5.1A) in the herbivory treatment compared to the flat slope in the control (Fig. 5.1 B). This indicates that without herbivore pressure, water lettuce had no effect on water hyacinth yield. Furthermore, the water lettuce density β 's are also an indication that water lettuce had very little effect on water hyacinth biomass with (water lettuce density $\beta = 0.080$) or without (water lettuce density $\beta = -0.040$) herbivory. Although the density β was substantially higher in the herbivory treatment, this was not significant.

Intraspecific competition had a much greater impact on water hyacinth yield. The steeper slopes of the regression planes on the water hyacinth density axes (Fig. 5.1 A and B) for both the herbivory treatment and the control are a visual indication that competition from conspecifics had a much greater impact on water hyacinth biomass than competition from water lettuce. The water hyacinth density β 's indicate that yield was significantly influenced by water hyacinth with (water hyacinth density $\beta = 0.958$) or without (water hyacinth density $\beta = 0.926$) herbivory.

Table 5.1 Multiple regression analysis of the effects of herbivory by *Cornops aquaticum* and plant density on the reciprocal of water hyacinth yield and water lettuce yield (wet weight (kg)).

Treatment	Regression coefficients			Intercept	R^2	F-value
Water hyacinth ($1/w_h$)	a_{hh}	a_{hl}	a_{hh}/a_{hl}	a_{h0}		
Herbivory	0.435	0.036	12.08	0.141	0.899	93.11 ($P = 0.0000$)
Control	0.287	-0.012	23.92	0.374	0.872	71.29 ($P = 0.0000$)
Water lettuce ($1/w_l$)	a_{ll}	a_{lh}	a_{ll}/a_{lh}	a_{l0}		
Herbivory	0.785	0.163	4.816	-0.363	0.825	49.46 ($P = 0.0000$)
Control	0.788	0.210	3.752	-0.781	0.920	120.61 ($P = 0.0000$)

$1/w_h$: The intercept a_{h0} estimates the reciprocal of the maximum weight of isolated water hyacinth plants. The regression coefficients a_{hh} and a_{hl} measure intra- and interspecific competition respectively for water hyacinth. The ratio a_{hh}/a_{hl} measures the effects of intraspecific competition by water hyacinth on its own weight relative to the effects of interspecific competition by water lettuce

$1/w_l$: The intercept a_{l0} estimates the reciprocal of the maximum weight of isolated water lettuce plants. The regression coefficients a_{ll} and a_{lh} measure intra- and interspecific competition, respectively, for water lettuce. The ratio a_{ll}/a_{lh} measures the effects on intraspecific competition by water lettuce on its own weight relative to the effects of interspecific competition by water hyacinth

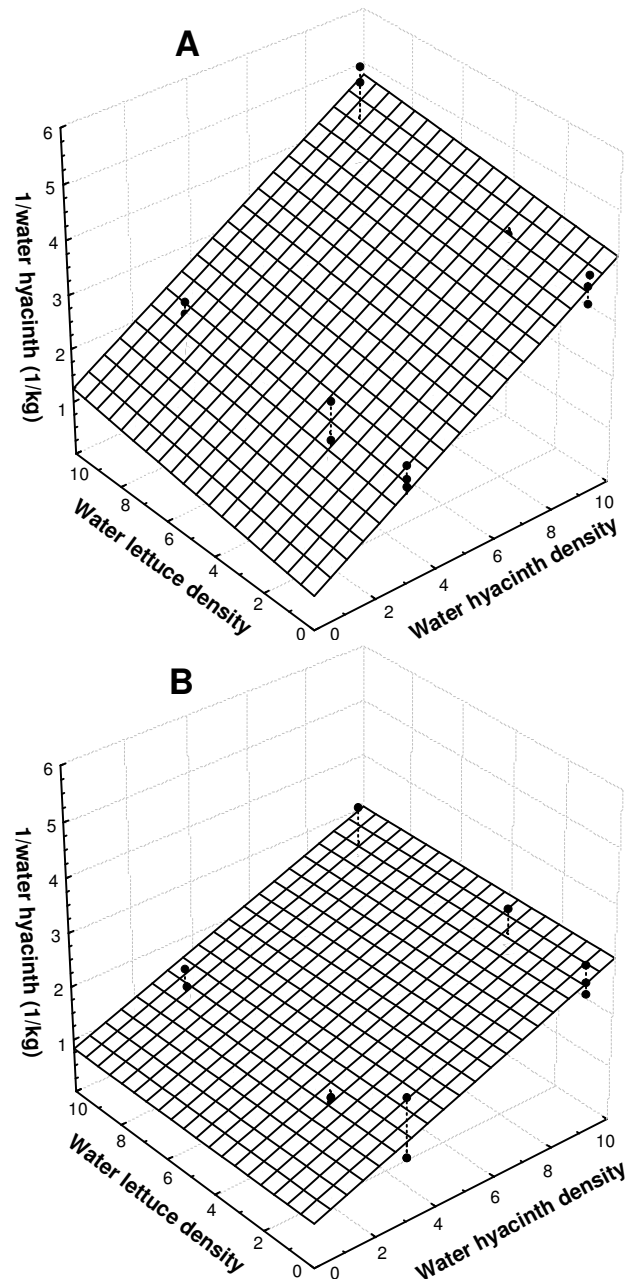


Figure 5.1 Multiple regression planes indicating the effects of *C. aquaticum* herbivory and plant densities on the inverse of the mean wet weight (kg) per water hyacinth plant. A and B compare relative competitive abilities of water hyacinth in the presence and absence of *C. aquaticum* herbivory respectively. Points indicate observations ($n = 18$) and the vertical lines between the data points indicate the residuals. Values on the X and Y axes are the original planting densities of water hyacinth and water lettuce.

Water lettuce (*Pistia stratiotes*)

Water lettuce was a weaker competitor in relation to water hyacinth. Adding one water lettuce plant had the same effect on water lettuce biomass as adding 3.8 water hyacinth plants without herbivory by *C. aquaticum*. In the presence of selective herbivory on water hyacinth, one water lettuce plant had an effect equal to 4.8 water hyacinth plants indicating a 21% increase in the competitive ability of water lettuce, when water hyacinth plants were stressed by herbivory.

The intraspecific competition coefficients (a_{ll}) are almost identical which is expected, considering that water lettuce plants were not subjected to damage from herbivory (Table 5.1). It is evident from the graphical representation of the data that the planting densities of both species had a significant impact on water lettuce yield indicated by the steep slopes in both directions. The planting density β 's show that water lettuce had a significant effect on biomass of conspecifics in both the herbivory treatment ($\beta = 0.920$) and the control ($\beta = 0.968$).

There was only a slight decrease in the interspecific competition coefficient a_{hl} (Table 5.1) in the herbivory treatment compared to the control which is visible from the surface response planes (Fig. 5.2 A and B). These show a marginally steeper slope in the control compared to the herbivory regression. The water hyacinth density β 's again show that water hyacinth density had a significant impact on water lettuce yield in the herbivory ($\beta = 0.191$) and, with a slight increase, the control ($\beta = 0.258$). These give an indication that both intra and interspecific competition were acting on water lettuce biomass.

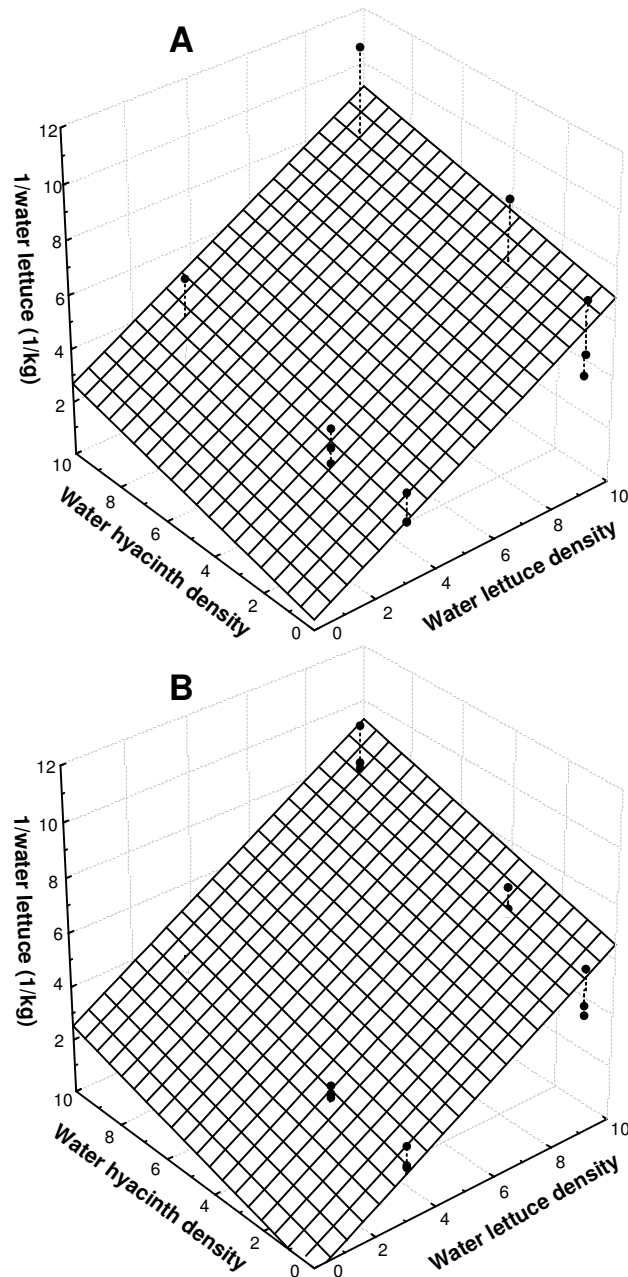


Figure 5.2 Multiple regression planes indicating the effects of *C. aquaticum* herbivory and plant densities on the inverse of the mean wet weight (kg) per water lettuce plant. A and B compare relative competitive abilities of water lettuce in the presence and absence of *C. aquaticum* herbivory respectively. Points indicate observations ($n = 18$) and the vertical lines between the data points indicate the residuals. Values on the X and Y axes are the original planting densities of water hyacinth and water lettuce.

5.3.2 Impact of herbivory on plant biomass (mean end-weights)

The mean end-weights per original water hyacinth plant were significantly lower in the herbivory treatments compared to the controls at original planting densities of both 3 and 9 water hyacinth plants (3 plants: $F_{1;16} = 7.58$, $P = 0.0140$; 9 plants: $F_{1;16} = 26.70$, $P < 0.0001$ (Fig. 5.3 A).

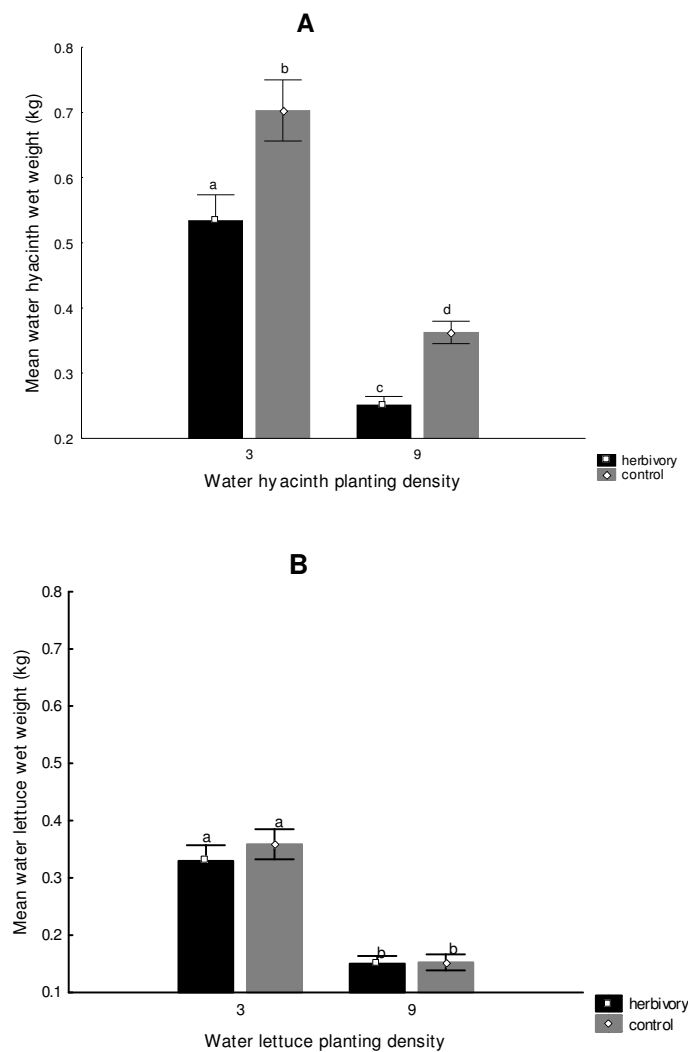


Figure 5.3 Mean end weights (kg) of individual water hyacinth (A) and water lettuce (B) plants in the herbivory treatments and the controls at planting densities of 3 and 9. Means followed by the same letter are not significantly different. Error bars represent the standard error of the mean.

There were no significant differences between the mean end-weights of water lettuce plants in the herbivory and control treatments at both planting densities. Mean wet weights were significantly lower at the original planting density of 9 compared to a planting density of 3 for both water hyacinth and water lettuce indicating that both species were affected by competition for space and possibly resources from neighbouring plants.

5.3.3 Impact of herbivory and competition on water hyacinth biomass (total end-weights)

Biomass accumulation was significantly reduced in the high competition herbivory treatment compared to the low competition control ($F_{1,12} = 6.71$; $P = 0.0236$) and these were the only significant differences that were found between any of the treatments (Fig. 5.4). Interestingly, total biomass yield for water hyacinth plants was significantly higher in the low competition treatment compared to water hyacinth grown in monoculture at the same density and therefore not subjected to interspecific competition. This may indicate a compensatory response to low levels of competition with neighbours of a competing species. The greatest reductions in biomass accumulation were from a combination of high levels of competition from water lettuce and herbivory by the grasshopper, or herbivory treatments without competition from water lettuce. Herbivory by the grasshopper had the least impact on water hyacinth biomass in combination with low levels of competition from water lettuce. The interaction between herbivory and competition was not significant.

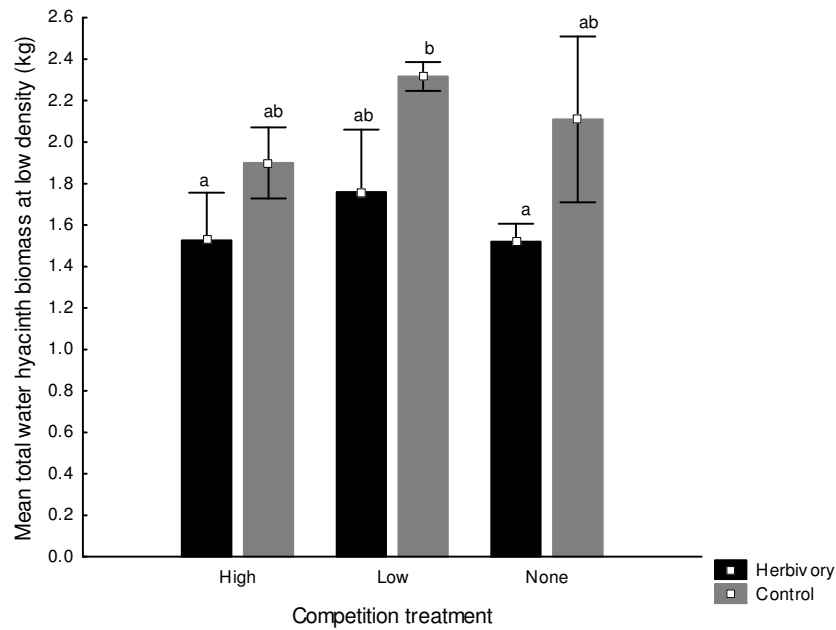


Figure 5.4 Mean total water hyacinth biomass at low planting density in combination with no, or high or low levels of competition from water lettuce, either exposed to herbivory by *C. aquaticum*, or not (control). Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

Herbivory by the grasshopper at high water hyacinth planting densities had a highly significant effect ($F_{1;12} = 25.03$; $P < 0.0001$) on total water hyacinth biomass, however both high and low levels of competition from water lettuce had no significant effects (Fig. 5.5). Although the interaction between herbivory and competition was not significant, a high level of competition from water lettuce combined with herbivory by *C. aquaticum* caused the greatest reductions in water hyacinth biomass accumulation. Competition alone had no significant effect on water hyacinth compared to the treatment where water hyacinth grew in the absence of competition. Herbivory by *C. aquaticum* alone reduced water hyacinth biomass compared to controls, therefore its negative effect on water hyacinth does not depend on the presence of other stress factors like competition.

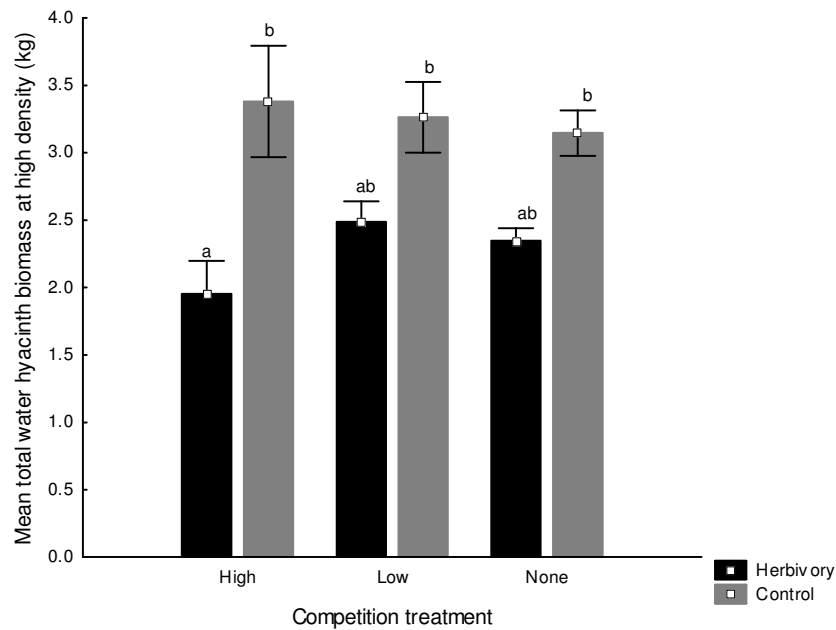


Figure 5.5 Mean total water hyacinth biomass at high planting density in combination with no, high or low levels of competition from water lettuce, either exposed to herbivory by *C. aquaticum* or not (control). Means compared by two-way ANOVA; those followed by the same letter are not significantly different (Tukey's HSD; $P < 0.05$). Error bars represent the standard error of the mean.

5.4 Discussion

Water hyacinth was clearly the dominant species compared to water lettuce and it remained the superior competitor in both the presence and absence of herbivory by the grasshopper. Agami & Reddy (1990) demonstrated using a reciprocal replacement series of water hyacinth and water lettuce planting densities, the superior competitive ability of water hyacinth compared with water lettuce and the results reported here are consistent with similar studies that evaluated the impact of water hyacinth biocontrol agents on the competitive performance of water hyacinth, when in competition with water lettuce (Center et al., 2005; Coetzee et al., 2005; Ajuonu et al., 2008). Under favourable nutrient conditions, water hyacinth's "luxuriant growth and high plasticity" (Agami & Reddy, 1990) enable it to flourish even in the presence of another weed species. Plants with the advantage of phenotypic plasticity such as water hyacinth can respond to changes in the distribution of resources and through phenotypic adjustment, for example in leaf area, extension of petioles or allocations to root biomass, can maximize capture of resources

such as light and nutrients (Grime, 1979). Also, water hyacinth's shoot:root ratio is 2:1 under high nutrient conditions, compared to only 1:1 in water lettuce, which demonstrates the superior ability of the plant for resource acquisition such as light (Agami & Reddy, 1990). These characteristics are responsible for its competitive dominance over similar species and its notoriety as a highly aggressive competitor in the aquatic environment (Wright & Purcell, 1995).

However, despite maintaining dominance in the presence of selective feeding, there was a significant decrease in water hyacinth competitive ability after only 4 weeks of herbivory by *C. aquaticum*. The combined biomass data also indicated that defoliation by the grasshopper significantly reduced the accumulation of biomass at both planting densities compared to control plants within the 4-week period. While two other water hyacinth biocontrol agents tested using the inverse linear model caused significant reductions in competitive ability, this was measured over longer periods and at densities at which they occur under field conditions in areas of introduction, as opposed to the native-range density of *C. aquaticum* tested here. Coetzee et al. (2005) found a 56% reduction in water hyacinth's competitive ability after 16 weeks of feeding by the sap-sucking mirid *E. catarinensis* at a density of 15 per plant, but no differences in plant biomass as a result of herbivory. However 40 mirids per plant reduced water hyacinth competitive ability by 101% and caused reductions in biomass after only 8 weeks (Ajuonu et al., 2008). Herbivory by the two water hyacinth weevils, *N. eichhorniae* and *N. bruchi* decreased water hyacinth's competitive performance by 98% (Center et al., 2005) after 10 weeks at a density of 4 weevils per plant. A combination of the weevil *N. eichhorniae* and *E. catarinensis* at half the density that was used in single species trials had the greatest impact on water hyacinth competitive ability, reducing it by 229% after 8 weeks of herbivory, suggesting a synergism between the two agents (Ajuonu et al., 2008). The most significant finding from the *C. aquaticum* study is a 50% reduction in water hyacinth's competitive ability and significant reductions in plant biomass after only 4 weeks of feeding and at a density of only one grasshopper per plant. This study maintained a constant insect density whereas the others allowed the test populations to increase naturally. *Neochetina bruchi* was found to be more damaging than *N.*

eichhorniae which was partly attributed to its higher fecundity and shorter development time, therefore having faster rates of population growth than its congener (Center et al., 2005). If the emerging *C. aquaticum* nymphs had been allowed to remain on plants and the study period extended, the plants would not, from personal observation and experience, have survived for much longer at those insect densities. The nymphs are extremely damaging and at the nutrient levels used in this trial, high numbers of nymphs as a result of high fecundity of females (chapter 3), would likely have contributed to rapid defoliation of water hyacinth plants within a few days of emergence of the first batch of nymphs. Therefore the grasshopper's influence on competitive ability at conservative densities, without population increase that would be found under natural conditions is critical in the comparison. Furthermore, a potential advantage that *C. aquaticum* may confer to other species in the aquatic environment compared with the *Neochetina* weevils and *E. catarinensis* is an increase in light penetration due to removal of large portions of the leaves whereas the other two species damage the surface of leaves. Defoliation might decrease the shading effect, which is partly responsible for its negative affect on other plant species (Reddy et al., 1990), whereas even high levels of sustained herbivory by the *Neochetina* weevils and *E. catarinensis* would not increase the amount of light through the canopy, unless leaf production rates were reduced. However, studies have shown that water hyacinth maintains the same number of leaves when under herbivore attack by the *Neochetina* weevils (unless at high densities) or *E. catarinensis* (Center & Van, 1989; Heard & Winterton, 2000; Coetzee et al., 2007). Based on the comparison with the current biocontrol agents that were also tested using the inverse linear model, *C. aquaticum* seems to have greater potential to reduce water hyacinth's competitive dominance.

Although water lettuce's competitive ability increased with a reduction in water hyacinth's competitive ability in the herbivory treatments, water lettuce plants did not benefit directly from herbivory by *C. aquaticum* in that their yield did not increase as water hyacinth biomass accumulation was reduced, as may have been predicted. A similar result was found by Lee & Bazzaz (1980) and Doyle et al. (2007) where undamaged plants showed no competitive release, in that they did not benefit from

selective herbivory on the competing species with an increase in their fitness. Lee & Bazzaz (1980) suggested that defoliation levels of *Abutilon theophrasti* Medic. (Malvaceae) may not have been severe enough to increase light flux in the canopy and production of new leaves by defoliated plants may not have allowed enough time for the other species to respond to additional light. Herbivory by the grasshopper at the same densities and nutrient levels used in this trial did not affect leaf production or the number of leaves of water hyacinth plants in the experiment presented in chapter 2, therefore it is unlikely that water lettuce would have benefitted through a reduction in shading after 4 weeks of herbivory. Furthermore, the duration of the trial might not have allowed enough time for water lettuce to respond to increased resource availability if water hyacinth's ability for nutrient uptake had been reduced by herbivory.

Herbivory by the grasshopper and intraspecific competition were the dominant factors influencing water hyacinth biomass. Intraspecific competition can be intense among weed species for example, high densities of knapweed reduced individual plant biomass and shoot number (Müller-Schärer, 1991). The negative effects of herbivory on water hyacinth were independent of interspecific competition however the greatest reductions in water hyacinth biomass were evident at a high water hyacinth density in combination with high competitor densities and herbivory. Nötzold et al. (1998) suggested that the types of interactions between competition and herbivory described by Sheppard (1996) can all be present in one plant-herbivore system and would likely depend on the intensity of each factor. The results reported here seem to be consistent with the idea of variable responses within the same system, depending on the intensity of the stress factor. In some cases, the effects of competition alone were negligible, whereas herbivory alone had a great impact and although the interaction between the two factors was not significant, in some cases a combination of the two had the greatest effect in reducing biomass accumulation. It appears that both substitutive and synergistic interactions were present which were dependent on the level of water hyacinth biomass and the level of competition. These results are also in accordance with the biomass-dependent theory (Grime, 1979) where increasing levels on both intra- and interspecific competition had a negative effect of water hyacinth biomass and are consistent with findings of Bonser &

Reader (1995) and Tiffin (2002) that negative competition effects are increased with increasing plant density. Resources become more limiting and plants suffer reductions in fitness associated with resource-limitation.

An interesting result was that biomass accumulation of water hyacinth plants was highest in combination with low levels of competition from water lettuce and not for plants grown in monoculture at the lowest overall plant density, as may have been expected as space and nutrients would be less limiting. Without herbivory, *L. salicaria* plants increased plant biomass, height and duration of flowering in response to competition, although the differences compared to plants free of competition were not statistically significant (Nötzold et al., 1998). The authors suggested that an increase in resource competition might have had a stimulative effect, with plants responding with increased height and biomass to become more competitive. Water hyacinth responded in a similar manner when grown in combination with *Hydrocotyle umbellata* L. (Apiaceae). Agami & Reddy (1991) found that water hyacinth biomass yield was higher when grown in a mixed culture with *H. umbellata* than when grown in monoculture at the same density. Center et al. (2005) found that water hyacinth responded to low levels of competition with water lettuce by increasing flower production. Where resources are not limiting to growth, plants seem to be adapted to compensate for low levels of competition by increasing fitness parameters that would allow them to be more competitive. Overcompensation (*sensu* Maschinski & Whitham, 1989; Chapter 2) in response to resource competition is likely to be one of water hyacinth's adaptive mechanisms as an aggressive competitor. However, at high densities of both conspecifics and competitors, they were not able to compensate, presumably space and nutrients were too limiting for that type of response.

Louda et al. (1990) suggested that herbivory will have the greatest effect on competitive interactions between plants when environmental conditions limit compensatory regrowth by the consumed competitor. Water hyacinth's potential for compensatory regrowth in response to herbivory by the grasshopper is largely nutrient-dependent (chapter 2). The nutrient medium used in this experiment was eutrophic, and nutrients were replaced

weekly, therefore plants were unlikely to have been severely nutrient-limited. Under oligotrophic nutrient conditions, such as those simulated in chapter 2, water hyacinth's competitive ability is likely to be even further reduced in combination with herbivory, due to severe reductions in plant performance as a result of nutrient-deficiency. Water hyacinth is a plant that appears to have an innate ability to capitalize on resources but plants suffer severely stunted growth and reproduction under poor nutrient conditions. Herbivory alone changes plant architecture and biomass allocation but a combination of herbivory and nutrient limitation causes drastic changes in water hyacinth's stature and potential for compensation. Under those sorts of conditions, the amount of shading and potential for resource acquisition would be significantly reduced. Changes in plant architecture can alter a plant's resource requirements and have a significant effect on its ability to acquire limited resources (Louda et al., 1990). Moreover, the higher grasshopper feeding rates under those conditions (Chapter 3) would likely exacerbate the negative effects of plant competition which would further alter competitive interactions as suggested by Cipollini & Bergelson (2002) although they suggested increased feeding rates in response to reduced levels of plant defensive chemicals.

The problem with water surfaces of many impoundments is that they are open systems much like disturbed habitats that are vulnerable to invasion by competitive species. This study shows that selective grazing of water hyacinth by *C. aquaticum* could reduce water hyacinth's competitiveness and biomass accumulation which could potentially allow other species to increase in number and density. Since South Africa does not have indigenous free-floating macrophytes, other invasive species such as water lettuce, which is widespread in the subtropical areas, would potentially invade cleared areas or take advantage of reductions in water hyacinth competitive dominance. Introducing stresses from other macrophytes that are better controlled by their biological control agents than water hyacinth is, has been proposed as a management strategy for water hyacinth in South Africa, and although this may lead to the greatest reductions in water hyacinth infestations, it should not be considered as a management tool for water hyacinth in South Africa. Purposefully trying to replace one aquatic weed with another would

complicate management strategies and according to South African legislation, cultivation of a category one plant invader is illegal.

In conclusion, herbivory by *C. aquaticum* has the potential to reduce water hyacinth vigor and biomass accumulation at conservative insect densities and in a very short space of time. By comparison with two of the most abundant and widespread biocontrol agents in South Africa, *C. aquaticum* appears to be more damaging and have greater potential to reduce the competitive performance of water hyacinth thereby reducing its invasive potential. Although combinations of competition and herbivory were investigated, the impact of the grasshopper does not depend on the presence of competing plant species, therefore *C. aquaticum* will still significantly reduce water hyacinth populations if no other plant species are growing together with water hyacinth.

Chapter 6

General discussion

Biological control of invasive alien plants as a science has undergone some positive changes in approach and practice over the last decade. The intention was to become more selective of biocontrol agents and to improve the likelihood of a successful introduction by basing a release decision on biological and ecological data in support of the candidate's potential efficacy. Biocontrol agents are non-indigenous species in their recipient environments therefore there is some degree of ecological risk associated with their introduction (McEvoy & Coombs, 1999). It is therefore an ethical requirement that future biocontrol agent releases are safe and, as far as can be predicted in pre-release screening, efficacious. Efficacy testing in the laboratory or even in the field in the native range can never guarantee success or be completely reliable predictors of the outcome of complex interactions that are likely in the new environment. However, this type of approach will ultimately allow for selection of a fewer number of agents that have the potential to control the target weed (Pearson & Callaway, 2005).

Some of the factors that require insight through testing interactions between a candidate agent and the target plant are: (1) its potential to establish and spread throughout the range of its host plant in the new environment; (2) compatibility with its host plant; (3) the potential to develop high population densities and (4) the potential to regulate populations of its host (Price, 2000; McClay & Balciunas, 2005). However, herbivory is not isolated in its effect on the target plant. Environmental heterogeneity on spatial and temporal scales will play a role in the dynamics of relationships between biocontrol agents and their host and the environmental conditions that the weed is growing in will have a significant effect on the ability of the agent to control its host plant. Therefore these factors need to be considered when designing pre-release evaluations of candidate agents.

This thesis is an example of a pre-release evaluation of a candidate biological control agent, taking factors that might influence establishment and efficacy in to account. It investigated interactions between *C. aquaticum* and water hyacinth to obtain information that will assist in determining whether the grasshopper will be a valuable introduction in to the South African biocontrol programme. Since eutrophication is considered to be one

of the major factors constraining efficacy of the current biocontrol agents, the effect of high water nutrient conditions on interactions between the two species was taken in to consideration to determine whether the grasshopper has potential to perform better under those conditions and therefore justify its release.

Despite more than 30 years of an active biological control programme for water hyacinth and South Africa having the highest numbers of agents to be released against the weed compared to other countries with water hyacinth biocontrol (Hill & Cilliers, 1999), it remains South Africa's most significant economic, social and environmentally problematic aquatic weed (van Wyk & van Wilgen, 2002). Although the current biocontrol agents have decreased the negative impact of water hyacinth (Hill & Olckers, 2001), better levels of control through biological means are desirable. It is for this reason that biocontrol practitioners are looking at introducing potentially more damaging agents for water hyacinth, with *C. aquaticum* being the forerunner under consideration for release. Host range testing of the grasshopper satisfied the requirements of a safe biological control agent without potential non-target effects on native vegetation, and permission for its release was granted in 2007. However, if a new biocontrol agent is to be considered worthy of releasing in South Africa it should: (1) have demonstrated capabilities of causing significant damage to water hyacinth, having the potential to reduce its invasiveness; (2) have the potential to contribute to control of water hyacinth under eutrophic conditions. Nutrient levels prevalent in South African water bodies have exacerbated the problems caused by water hyacinth through prolific growth, which is characteristic of plants in eutrophic environments (Reddy et al. 1989; 1990; Ripley et al., 2006; Coetzee et al., 2007, Chapter 2). This is believed to negate the impact of the current biocontrol agents in that water hyacinth's growth rates outpace the damage caused by insect feeding and lost tissue is easily replaced. Water hyacinth is considered to be well managed by the current biocontrol agents in systems where nutrients are limiting to plant growth. Therefore a condition of release for *C. aquaticum* would be its ability to significantly reduce water hyacinth's potential for growth, increase and spread under high nutrient conditions; (3) be well adapted to the quality of host plants that are typically found in South Africa so that it has the potential for high rates of establishment and

population build-up; (4) have the ability to reduce water hyacinth's competitiveness; (5) complement the existing biocontrol agents and not have the potential to displace or exclude them through interference or competition; and (6) have the potential to establish and reach damaging densities throughout the climatic range of water hyacinth in South Africa. This thesis addressed requirements one to four and five and six are discussed below.

Chapter 2 investigated the impact that herbivory by the grasshopper has on water hyacinth growing in nutrient conditions typical of South African water bodies. The results provided further evidence that water hyacinth's invasive potential is increased in eutrophic environments due to high rates of growth and reproduction and that many of the problems caused by water hyacinth would be mitigated through a reduction in nutrient input into aquatic systems. The results also provide evidence in support of the idea that water hyacinth has greater potential to compensate for the negative effects of herbivory when their growth rates are at an optimal level. Eutrophication is regarded as one of the most important factors interfering with the water hyacinth biocontrol programme (Hill & Cilliers, 1999; Hill & Olckers, 2001) and since this problem is exacerbated by population growth, urbanization and agricultural activities which are all a reality of the future, only agents that are efficacious under nutrient-enriched conditions should be considered. Although the grasshopper's impact on water hyacinth was not as severe when nutrients were in abundant supply compared to when plants were limited by nutrient availability, a conservative density of one grasshopper per plant caused significant reductions in many water hyacinth growth parameters related to its invasive potential. Therefore, the results from chapter 2 indicate promise for the grasshopper in making a contribution to biological control of water hyacinth in eutrophied environments.

Chapter 3 investigated the bottom-up effects of plant quality on *C. aquaticum* survival, feeding and life history characteristics. Since water hyacinth plant quality is highly correlated with the nutrient environment (chapter 2) and the performance of phytophagous insects can be closely linked to host plant quality, the nutrient status of water bodies with water hyacinth could potentially have a significant influence on the

population dynamics of *C. aquaticum*, as well as their damage potential. The results gave an indication that the grasshoppers are sensitive to the quality of their host plant and that nutrient availability will indeed have an effect on their population dynamics. Not only will aspects of their fitness and performance be affected, but also their feeding rates which are largely dependent on the nitrogen content of plants. This will therefore influence their per capita impact on water hyacinth in relation to environmental nitrogen availability.

Most insects used in biocontrol programmes are latent species (Price, 2000) which usually remain at stable population densities and don't have the potential to erupt and cause significant damage to host plant populations. An eruptive species has two phases, one of low density and low damage and one of high density, when they can be very damaging to host plant populations (Price et al., 1990). Price (1975; 2000) discusses how phytophagous insects with typically latent population dynamics can become eruptive in response to vigorous plants of high quality. Nitrogen fertilization in agroecosystems for improved plant growth is frequently the cause of pest problems and the status of many crop pests increase in response to nitrogen fertilization (Xhong-xian et al., 2007) by creating conditions conducive to population outbreaks of insect species with typically latent population dynamics (Price, 2000). Therefore if an invasive plant like water hyacinth is growing under conditions of high nitrogen availability, their biocontrol agents may have the potential to have eruptive population dynamics if they respond positively to vigorous plants of high quality. *Cornops aquaticum* showed a positive response to water hyacinth plants with high nitrogen content in terms of their fecundity, survival and sex ratios, therefore water hyacinth plants growing in eutrophic environments may create ideal conditions for this species to become eruptive and reach high population densities. Three of the most abundant biocontrol agents in South Africa, the *Neochetina* weevils and the mirid, *E. catarinensis*, also respond positively to increasing levels of tissue nitrogen (Heard & Winterton, 2000; Ripley et al., 2006; Coetzee et al., 2007), although population outbreaks have only been recorded for the mirid. The reasons for this remain speculative, but it is possible that their outbreaks are related to increases in plant nitrogen as a result of nutrient-enrichment. It is difficult to predict whether *C. aquaticum* will have

population outbreaks if released in South Africa, but the results reported in this thesis indicate that bottom-up control through resource availability will have an important influence on their population dynamics and therefore their ability to control water hyacinth. One of the remaining questions is whether the benefit of greater fecundity, survival and population growth rates will lead to greater impacts on water hyacinth in eutrophic conditions or whether the benefit of higher population densities will be balanced by lower feeding rates of grasshoppers and greater compensatory ability of water hyacinth under those conditions.

Chapter 4 investigated the relationship between *C. aquaticum* and water hyacinth. The results indicated that water hyacinth is sensitive to herbivory by the grasshopper and that increasing feeding intensities cause corresponding reductions in weed growth and biomass. Pearson & Callaway (2003) claim that the key to successful control of klamath weed, *Hypericum perforatum* L. (Hypericaceae) by the chrysomelid beetle, *Chrysolina quadrigemina* Suffrian (Coleoptera: Chrysomelidae) is their interaction strength (population level effects on each other). The beetle has a strong negative effect on the weed and the two species persist at densities where the negative ecological impacts of *H. perforatum* are mitigated. Results from chapter 4 (and chapter 3) suggest that the interaction strength between the grasshopper and water hyacinth is good and that there are likely to be population level effects of one species on the other (Schooler & McEvoy, 2006). If *C. aquaticum* reaches high population densities, it will reduce populations of water hyacinth, causing an associated decline in grasshopper densities. The density-damage relationship between water hyacinth and the grasshopper 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. Furthermore, biological control agents that don't suppress their hosts and don't have density-dependent feedback mechanisms can cause problems in ecosystems by remaining at high densities. Pearson & Callaway (2003; 2005) give examples of how abundant but ineffective agents can offset a cascade of negative interactions in an ecosystem. If biocontrol agents are strong enough to control their host populations their super-abundance will be short-lived and with a strong interaction, should respond in a

density-dependent manner where their numbers decline as they deplete their food source. One of the most significant findings from investigating the impact of a range of densities on *C. aquaticum* is that grasshopper biomass in the range of 2 females or 4 males per plant would be high enough to reduce populations of water hyacinth.

Lastly, chapter 5 investigated the impact of the grasshopper on the competitive performance of water hyacinth, which is a characteristic related to its invasiveness and dominance in the aquatic environment. The fact that the grasshoppers can reduce water hyacinth biomass and competitive performance after only 4 weeks of feeding is an indication of how the damage caused by defoliation limits water hyacinth's growth, even under high nutrient conditions.

Based on the results reported in this thesis, *C. aquaticum* is sufficiently damaging to water hyacinth under eutrophic conditions to be considered for release. The grasshoppers respond positively to the quality of their host plant and should therefore be able to establish and have high rates of population growth when water nutrient levels are high. The potential for strong population level effects on each other, according to environmental conditions, suggests an interaction between herbivore and host where they respond to each other's population densities. The grasshopper exerts top-down control on water hyacinth and water hyacinth exerts bottom-up control on the grasshopper.

However, the interactions investigated in this thesis are not the only factors that are due to receive consideration in the release decision for *C. aquaticum* in South Africa. There are concerns that the damage caused by the grasshopper could disrupt valuable water hyacinth biocontrol agents such as the *Neochetina* weevils, which could potentially have implications if this lead to displacement of the weevils further north in Africa (Hill, pers. comm.) where control has been highly successful (which is believed to due to a more favourable climate and lower nutrient levels as well as the type of water bodies in which water hyacinth is found) (Hill & Olckers, 2001). For this reason, interactions between the current biocontrol agents and the grasshopper will be investigated in pre-release studies. The question of interference rather than displacement is probably the critical one because

if the grasshopper reaches high enough population densities to displace or exclude the other agents, water hyacinth will be under good control. Many insect herbivores change the quality of their host plants through feeding, affecting both inter- and intraspecific interactions (Awmack & Leather, 2002). The sensitivity of the grasshopper to the quality of water hyacinth suggests that there may be antagonistic effects between the mirid and the grasshopper. The type of feeding by *E. catarinensis* could reduce *C. aquaticum* fitness through resource degradation. Resource degradation can lead to competitive displacement of species and occurs when feeding by one species reduces the quality of the resource, which can have a negative effect on the development of other species (Reitz & Trumble, 2002). The mirid can reach very high population densities in the field where they cause severe chlorosis of water hyacinth leaves. In such cases, nearly every leaf in an infestation turns brown as a result of chlorophyll extraction by the mirid. This would reduce the quality of food for the grasshopper and could potentially reduce their fecundity and overall fitness. A negative effect of the grasshopper on the mirid might be destruction of eggs and habitat since water hyacinth leaves are the habitat niche for all stages of the mirid (and other biocontrol agents like the mite, *O. terebrantis*). Competition between sap-feeding and chewing insects is thought to be infrequent (Denno et al., 1995; 2003) but interactions between the mirid and the grasshopper are worth investigating. If both species respond positively to high nitrogen levels with population outbreaks, there may be negative interactions at these high densities.

Herbivory by adult *Neochetina* weevils could have a negative effect on *C. aquaticum*. Adult weevils feed predominantly on young water hyacinth leaves and the damage they cause can change leaf chemistry (Center & Van, 1989). Since *C. aquaticum* adults have a strong preference for mature water hyacinth leaves, they will be feeding on leaves that have altered nutrient profiles in response to *Neochetina* herbivory which could affect grasshopper performance at sites where both species are present. However, the feeding patterns of *C. aquaticum* and the *Neochetina* weevils might also mean that the two species complement each other and are synergistic rather than antagonistic in their effect on water hyacinth. If the *Neochetina* weevils damage young water hyacinth leaves and the grasshoppers damage mature leaves, the effect on water hyacinth may be greater than

the effect of herbivory by either species on its own. Another factor under consideration in terms of *Neochetina* – *C. aquaticum* interactions is the effect that *Neochetina* larval mining in the petioles at high densities might have on *C. aquaticum* egg packets (Hill, pers. comm.). This could prevent the grasshoppers from establishing good populations at sites where both species are present. The interactions between *C. aquaticum* and some of the biocontrol agents already established on water hyacinth are going to be investigated in the summer of 2008/2009 under quarantine conditions in Pretoria, South Africa.

Another important factor that warrants consideration is compatibility of the grasshopper with the South African climate. Climatic conditions influence insect population dynamics and biocontrol agent establishment and success has been limited by incompatibility with climates in areas of introduction (McClay & Hughes, 1995; McClay, 1996; Byrne et al., 2002). It is also believed to be one of the major factors hampering efficacy of the current biological control agents for water hyacinth in the more temperate regions of South Africa (Hill & Cilliers, 1999; Hill & Olckers, 2001). Therefore, new agents should be fairly cold tolerant in order to try and improve control levels where other agents have failed to establish and persist through the winter months. Preliminary thermal tolerance trials with the *C. aquaticum* culture in Pretoria in quarantine suggest that this species is not very cold tolerant (Bownes, unpublished data). However, its distribution, which extends from Mexico as far south as central Argentina, around Buenos Aires (Adis et al., 2007) suggests it has a wide thermal tolerance. The current *C. aquaticum* culture is possibly a lab-adapted strain since this species has been in quarantine in Pretoria, South Africa for almost 12 years. Adaptation to the quarantine environment and loss of genetic diversity through inbreeding are some of the factors that might reduce fitness. Collection and release of a strain from its southern-most distribution, around Buenos Aires is planned which may improve chances of establishment and success in the areas of South Africa with low winter temperatures. A ‘cold-adapted’ strain of *C. aquaticum* is likely to be collected and brought in to quarantine in South Africa in the summer of 2008/2009. A cold tolerant biocontrol agent that can persist through winter and eliminate the lag phase that is evident at the beginning of the growing season when plants are quick to pick up

but insects lag behind with slower rates of growth and development, could potentially attack plants at a critical stage when they are still stressed from low temperatures.

One of the conditions for release of the grasshopper would be to demonstrate that they can be superior biocontrol agents to the agents already established on water hyacinth. Room (1990) discusses the attributes of *C. salviniae* that are likely to have contributed to its success as a biocontrol agent for salvinia. The weevil tolerates high population densities before intraspecific interference and a shortage of food trigger dispersal and reductions in feeding and development. The weevils have high per capita feeding rates and their searching efficiency is presumed to be good because they are successful in locating plants on the fringes of vegetation after salvinia mats have collapsed. As far as *C. aquaticum* is concerned, there is anecdotal evidence from the region of origin that they tend to reach high densities and are very damaging to water hyacinth. The grasshoppers seem to have high per capita feeding rates because even low densities have negative effects on plant production. Their searching efficiency is likely to be high due to high mobility of adults. They may also have good dispersal capabilities, which can be important in achieving successful control of invasive plants. Success of the biocontrol agent, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) for another aquatic weed, *Azolla filiculoides* Lamarck (Azollaceae) has partly been attributed to their dispersal ability (McConnachie et al., 2004). The mobility of the grasshoppers might be an advantage over the current biocontrol agents, which are mostly fairly immobile. Furthermore, the immature stages of agents such as the *Neochetina* weevils are susceptible to mortality when water hyacinth is sprayed with herbicide. This could be another advantage that the grasshopper may have over other species. Although some egg packets may be lost when mats sink after a herbicide application, the nymphs are highly mobile and can therefore move quickly onto unsprayed plants. Furthermore, while some egg packets may be lost, most would have hatched by the time the mats sink which can take up to a month after application. Investigating an integrated approach using both the grasshopper and herbicides for water hyacinth will be worthwhile if this species is released.

One of the most important findings from this research is that by examining subsets of the plant-herbivore system involved in a biocontrol programme, species characteristics and traits that can assist in successful implementation of the agent, can be identified. Defining relationships between plant quality and insect physiological responses can help in understanding reasons for success or failure of an agent, their population fluctuations and their ability to reduce water hyacinth infestations at a particular site. For example, in low nutrient systems, classical biological control alone may be sufficient, although it should be considered that *C. aquaticum* population growth could be limited by poor quality food. Therefore augmentative releases might need to be considered but once the grasshoppers are established and abundant, good levels of control could be expected. Alternatively, this could be achieved without augmentation but the time taken for the grasshoppers to reach damaging densities would be longer. In this study, mesotrophic nutrient conditions, bordering on eutrophic were found to be the most suitable conditions for the grasshopper to be highly effective in controlling water hyacinth. A combination of high fecundity, stable sex ratios, high feeding rates and reduced compensatory ability of water hyacinth would likely contribute to its success. Therefore, classical biological control with the grasshopper in these types of systems could be used and effective management of water hyacinth could be expected. These are examples of how water nutrient conditions and therefore plant quality can be an indicator of the likelihood of success and can be used as a decision-making tool in determining the best management strategy at a particular site.

Another example of how this information can be used in management decisions is through selection of complementary agents based on site-specific conditions that are favourable for particular species or combinations of species. Price (2000) discusses that optimal conditions should prevail or be created by local manipulation which was done and proved to be very successful for *C. salviniae* in Papua New Guinea where salvinia plants were fertilized to increase populations of the insects (Room & Thomas, 1985). In South African water bodies, which generally have sufficient nutrients for healthy plant growth, this would not be necessary. Rather than create optimal conditions, a management strategy could be selection of agents from the suite available based on their potential performance in terms of establishment, population growth and persistence

according to prevailing nutrient conditions at a particular site. For example, a combination of *C. aquaticum* and *N. bruchi* might be optimal for control of water hyacinth under eutrophic conditions. *Neochetina bruchi* is more damaging than *N. eichhorniae* on vigorous, high quality plants due to higher population growth rates (Heard & Winterton, 2000), therefore a combination of the grasshopper and *N. bruchi* might lead to good levels of control as both of these species respond positively to plants growing under eutrophic conditions. On the other hand, a species like *N. eichhorniae* that is not as responsive to different nitrogen levels (Heard & Winterton, 2000) as *C. aquaticum*, might be a better candidate for control of water hyacinth under low nutrient conditions. This is another example of how identifying prevailing conditions in systems could contribute to best management practices, decisions and expectations for biological control.

Cornops aquaticum has been well-studied in its region of origin in the last few years. These studies have shown that the life history characteristics of this species are plastic and vary according to temperature and insolation in different geographical areas (Adis et al., 2004). We can therefore expect high variability in their performance, abundance and distribution in South Africa, according to environmental conditions. High rates of establishment and population build-up can be expected in the more subtropical areas of South Africa, particularly where nutrient levels favour survival, and increased female fecundity. The grasshopper's distribution as far south as Buenos Aires (Adis et al., 2007) which is on a similar latitude to the more temperate regions of South Africa and where frosting occurs in winter, suggest that a cold-tolerant strain of the grasshopper may be able to persist through the winter months in the colder areas of South Africa. However, populations of the grasshopper may be limited by the lack of green leaf material available after a frosting event. Water hyacinth plants in the highveld region usually die back and leaves turn brown as a result of frost and extreme cold winter temperatures. The adaptability of *C. aquaticum* according to environmental conditions and its response to the quality of its host plant, suggest it may be a highly effective biological control agent for water hyacinth. Although the grasshopper was identified as extremely promising in the first surveys for water hyacinth biocontrol agents in the region of origin, it was not

considered in South Africa until more than 30 years of biocontrol and after the release of five other arthropod biocontrol agents. Concerns over specificity of a grasshopper, which are notoriously polyphagous, combined with concerns over acceptance for release of a grasshopper by government authorities are the most likely reasons for not considering this species soon after initiation of the South Africa biocontrol programme for water hyacinth.

In conclusion, while there needs to be a balance between investing copious amounts of time and resources in pre-release efficacy testing, investigations of interactions between a candidate agent and its host, will provide much of the knowledge needed to make an informed decision and improve the chances of a successful release. The research presented here has satisfied many of the conditions to justify release of the grasshopper in South Africa. It has the potential under mesotrophic/eutrophic conditions, to have high establishment and population growth rates and therefore cause high levels of damage in systems where water hyacinth is currently the most problematic. It has also shown that both nutrient supply and herbivory by the grasshopper affect plant biomass and has demonstrated the plasticity of plant-insect interactions according to prevailing environmental conditions. A continuum of plant responses to grasshopper herbivory can be expected which will be dependent on grasshopper densities and the nutrient environment. Identifying conditions at a particular site might contribute to determining best management practices should this species be introduced. Based on impact and suitability to its host plant, *C. aquaticum* should be released in South Africa as it has great potential to be a highly effective biological control agent. However, these data should be collated with data investigating other aspects that will influence the plant-herbivore system and *C. aquaticum*'s potential efficacy in order to make a holistic assessment in determining whether the grasshopper's release in South Africa is justified and necessary.

References

Adis, J. & Junk, W.J. (2003). Feeding impact and bionomics of the grasshopper *Cornops aquaticum* on water hyacinth *Eichhornia crassipes* in Central Amazonian floodplains. *Studies on Neotropical Fauna and Environment* **38**: 245-429.

Adis, J., Lhano, M., Hill, M., Junk, W., Marques, M.I. & Oberholzer, H. (2004). What determines the number of juvenile instars in the tropical grasshopper *Cornops aquaticum* (Leptysminae: Acrididae: Orthoptera)? *Studies on Neotropical Fauna and Environment* **39**: 127-132.

Adis, J., Bustorf, E., Lhano, M.G., Amedegnato, C. & Nunes, A. (2007). Distribution of *Cornops* grasshoppers (Leptysminae: Acrididae: Orthoptera) in Latin America and the Caribbean Islands. *Studies on Neotropical Fauna and Environment* **42**: 11-24.

Agami, M. & Reddy, K.R. (1990). Competition for space between *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L. cultured in nutrient-enriched water. *Aquatic Botany* **38**: 195-208.

Agami, M. & Reddy, K.R. (1991). Interrelationships between *Eichhornia crassipes* (Mart.) Solms and *Hydrocotyle umbellata* L. *Aquatic Botany* **39**: 147-157.

Agrawal, A.A. (2004). Resistance and susceptibility of milkweed: competition, root herbivory and genetic plant variation. *Ecology* **85**: 2118-2133.

Ajuonu, O., Byrne, M.J., Hill, M.P., Neuenschwander, P. & Korie, S. (2009). The effect of two biological control agents, the weevil *Neochetina eichhorniae* and the mirid *Eccritotarsus catarinensis* on water hyacinth, *Eichhornia crassipes*, grown in culture with water lettuce, *Pistia stratiotes*. *Biocontrol* **54**: 155-162.

Alstad, D.N. & Edmunds Jr., G.F. (1983). Selection, outbreeding depression, and the sex ratio of scale insects. *Science* **220**: 93-95.

Amorim, M.A. & Adis, J. (1995). Desenvolvimento ninfal do gafanhoto neotropical semi-aquático *Stenacris fissicauda fissicauda* (Brüner, 1908) (Orthoptera: Acrididae) em condições controladas. *Acta Amazonica* **25**: 73-92.

Andow, D.A. (1990). Population dynamics of an insect herbivore in simple and diverse habitats. *Ecology* **71**: 1006-1017.

Ang, B.N., Kok, L.T., Holtzman, G.I. & Wolf, D.D. (1994). Competitive growth of Canada thistle, tall fescue and crownvetch in the presence of a thistle defoliator, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae). *Biological Control* **4**: 277-284.

Awmack, C.S. & Leather, S.R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**: 817-844.

Bach, C.E. (1988). Effect of host plant patch size on herbivore density: patterns. *Ecology* **69**: 1090-1102.

Balciunas, J. (2004). Are mono-specific agents necessarily safe? The need for pre-release assessment of the probably impact of candidate biological control agents, with some examples. In: Cullen, J.M., Briese, D.T., Kriticos, D. J., Lonsdale, W.M., Morin, L., Scott, J.K. (Eds.). *Proceedings of the XI International Symposium on the Biological Control of Weeds*. CSIRO Publishing, Melbourne, Australia. pp. 252-257.

Balciunas, J.K. & Burrows, D.W. (1993). The rapid suppression of the growth of *Melaleuca quinquenervia* saplings in Australia by insects. *Journal of Aquatic Plant Management* **31**: 265-270.

Bebawi, F.F., Lockett, C.J., Davis, K.M. & Lukitsch, B.V. (2007). Damage potential of an introduced biological control agent *Agonosoma trilineatum* (F.) on bellyache bush (*Jatropha gossypifolia* L.). *Biological Control* **41**: 415-422.

Belovsky, G.E. & Slade, J.B. (1993). The role of vertebrate and invertebrate predators in a grasshopper community. *Oikos* **68**: 193-201.

Belovsky, G.E. & Slade, J.B. (1995). Dynamics of two Montana grasshopper populations: relationships among weather, food abundance and intraspecific competition. *Oecologia* **101**: 383-396.

Bernays, E.A. (1998). Evolution of feeding behaviour in insect herbivores. *Bioscience* **48**: 35-44.

Berryman, A.A., Dennis, B., Raffa, K.F. & Stenseth, N.C. (1985). Evolution of optimal group attack, with particular reference to bark beetles (Coleoptera: Scolytidae). *Ecology* **66**: 898-903.

Blackenhorn, W.U. (1994). Fitness consequences of alternative life histories in water striders *Aquarius remigis* (Heteroptera: Gerridae). *Oecologia* **97**: 354-365.

Bonser, S.P. & Reader, R.J. (1995). Plant competition and herbivory in relation to vegetation biomass. *Ecology* **76**: 2176-2183.

Branson, D.H. (2004). Relative importance of nymphal and adult resource availability for reproductive allocation in *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Journal of Orthoptera Research* **13**: 239-245.

Brede, E.G., Adis, J. & Schneider, P. (2007). What is responsible for the variance in life history traits of a South American semi-aquatic grasshopper (*Cornops aquaticum*)? A test of three possible hypotheses. *Studies on Neotropical Fauna and Environment* **42**: 225-233.

Briese, D.T. (1996). Potential impact of the stem-boring weevil *Lixus cardui* on the growth and reproductive capacity of *Onopordum* spp. thistles. *Biocontrol Science and Technology* **6**: 251-261.

Briese, D.T. Thomann, T. & Vitou, J. (2002). Impact of the rosette crown weevil *Trichosirocalus briesei* on the growth and reproduction of *Onopordum* thistles. *Journal of Applied Ecology* **39**: 688-698.

Briese, D.T., Böttcher, S., Vitou, J. & Thomann, T. (2003). Prevalence and impact of the crown fly, *Botanophila spinosa*, on its host thistle, *Onopordum acanthium*, in southern France. *Acta Oecologica* **24**: 77-86.

Briese, D.T., Pettit, W.J. & Walker, A. (2004). Evaluation of the biological control agent, *Lixus cardui*, on *Onopordum* thistles: experimental studies on agent demography and impact. *Biological Control* **31**: 165-171.

Briggs, M.A. (1991). Influence of herbivory and nutrient availability on biomass, reproduction and chemical defenses of *Lotus corniculatus*. *Functional Ecology* **5**: 780-786.

Bryant, J.P., Chapin III, F.S. & Klein, D.R. (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**: 357-368.

Bryant, J.P., Clausen, T.P., Reichardt, P.B., McCarthy, M.C. & Werner, R.A. (1987). Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortrix (*Christoneura conflictana* (Walker). *Oecologia* **73**: 513-517.

Burn, A.J. (1981). Feeding and growth in the Antarctic collembolan, *Cryptopygus antarcticus*. *Oikos* **36**: 59-64.

Byrne, M.J., Currin, S. & Hill, M.P. (2002). The influence of climate on the establishment and success of the biological control agent, *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control* **24**: 128-134.

Callaway, R.M., DeLuca, T.H. & Belliveau, W.M. (1999). Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. *Ecology* **80**: 1196-1201.

Capello, S., Adis, J. & de Wysiecki, M.L. (2007). Temperatura y fotoperiodo: qué influencia ejercen en el desarrollo ninfal de *Cornops aquaticum* (Orthoptera: Acrididae)? *Amazoniana* **XIX**: 209-216.

Cates, R.G. (1980). Feeding patterns of monophagous, oligophagous and polyphagous insect herbivores: The effect of resource abundance and plant chemistry. *Oecologia* **46**: 22-31.

Center, T.D. (1980). Biological control and its effect on production and survival of water hyacinth leaves. In: Delfosse, E.S. (Ed.). *Proceedings of the V International Symposium on Biological Control of Weeds*. CSIRO Publishing, Brisbane, Australia. pp. 393-410.

Center, T.D. (1985). Leaf life tables: A viable method for assessing sublethal effects of herbivory on water hyacinth shoots. In: Delfosse, E.S. (Ed.). *Proceedings of the VI International Symposium on Biological Control of Weeds*. Agriculture Canada, Vancouver, Canada. pp. 511-524.

Center, T.D. (1994). Biological control of water weeds: water hyacinth and water lettuce. In: Rosen, D., Bennett, F.D. & Capinera, J.L. (Eds.). *Pest Management in the Subtropics – A Florida Perspective*. Intercept Ltd., Andover. pp. 481-521.

Center, T.D. & Spencer, N.R. (1981). The phenology and growth of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in a eutrophic north-central Florida Lake. *Aquatic Botany* **10**: 1-32.

Center, T.D. & Van, T.K (1989). Alteration of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) leaf dynamics and phytochemistry by insect damage and plant density. *Aquatic Botany* **35**: 191-195.

Center, T.D. & Wright, A.D. (1991). Age and phytochemical composition of water hyacinth (Pontederiaceae) leaves determine their acceptability to *Neochetina eichhorniae* (Coleoptera: Curculionidae). *Environmental Entomology* **20**: 323-334.

Center, T.D., Dray Jr., F.A., Jubinsky, G.P. & Leslie, A.J. (1999a). Biological control of water hyacinth under conditions of maintenance management: can herbicides and insects be integrated? *Environmental Management* **23**: 241-256.

Center, T.D., Dray Jr., F.A., Jubinsky, G.P. & Leslie, A.J. (1999b). Water hyacinth weevils (*Neochetina eichhorniae* and *N. bruchi*) inhibit waterhyacinth (*Eichhornia crassipes*) colony development. *Biological Control* **15**: 39-50.

Center, T.D., Van, T.K. & Hill, M.P. (2001). Can competition experiments be used to evaluate the potential efficacy of new water hyacinth biological control agents? In: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.). *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated of Control of Water Hyacinth*. ACIAR, Australia. pp. 77-81.

Center, T.D., Van, T.K., Dray Jr., F.A., Franks, S.J., Rebelo, M.T., Pratt, P.D., & Rayamajhi, M.B. (2005). Herbivory alters competitive interactions between two invasive aquatic plants. *Biological Control* **33**: 173-185.

Chabot, B.F. & Hicks, D.J. (1982). The ecology of leaf life spans. *Annual Review of Ecology and Systematics* **13**: 229-259.

Chapin III, F.S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233-260.

Cilliers, C.J. (1991). Biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37**: 207-217.

Cippollini, D.F. & Bergelson, J. (2002). Interspecific competition affects growth and herbivore damage of *Brassica napus* in the field. *Plant Ecology* **162**: 227-231.

Clarke, A. (1996). The influence of climate change on the distribution and evolution of organisms. In: Johnston, I.A. & Bennett, A.F. (Eds.). *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge. pp. 377-408.

Cock, M., Day, R., Herren, H., Hill, M.P., Julien, M.H., Neuenschwander, P. & Ogowang, J. (2000). Harvesters get that sinking feeling. *Biocontrol News and Information* **21**: 1-8.

Coetzee, J.A. (2003). Suitability of *Eccritotarsus catarinensis* as a biological control agent of water hyacinth. PhD thesis. University of the Witwatersrand, Johannesburg.

Coetzee, J.A., Center, T.D, Byrne, M.J. & Hill, M.P. (2005). Impact of the biocontrol agent *Eccritotarsus catarinensis*, a sap-feeding mirid, on the competitive performance of waterhyacinth, *Eichhornia crassipes*. *Biological Control* **32**: 90-96.

Coetzee, J.A., Byrne, M. J. & Hill, M.P. (2007). Impact of nutrients and herbivory by *Eccritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia crassipes*. *Aquatic Botany* **86**: 179-186.

Coley, P.D., Bryant, J.P. & Chapin III, F.S. (1985). Resource availability and plant antiherbivore defense. *Science* **230**: 895-899.

Cordo, H.A. (1999). New agents for biological control of water hyacinth. *In*: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.) *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated of Control of Water Hyacinth*. ACIAR, Australia. pp. 68-74.

Cottam, D.A. (1986). The effects of slug-grazing on *Trifolium repens* and *Dactylis glomerata* in monoculture and mixed sward. *Oikos* **47**: 275-279.

Cottam, D.A., Whittaker, J.B. & Malloch, A.J.C. (1986). The effects of chrysomelid grazing and plant competition on the growth of *Rumex obtusifolius*. *Oecologia* **70**: 452-456.

Craig, T.P., Price, P.W. & Itami, K.J. (1992). Facultative sex ratio shifts by a herbivorous insect in response to variation in host plant quality. *Oecologia* **92**: 153-161.

Crawley, M.J. (1983). *Herbivory. The dynamics of animal plant interactions*. Blackwell Scientific Publications, Oxford.

Crawley, M.J. (1989a). The successes and failures of weed biocontrol using insects. *Biocontrol News and Information* **10**: 213-223.

Crawley, M.J. (1989b). Insect herbivores and plant population dynamics. *Annual Review of Entomology* **34**: 531-564.

Cullen, J.M. (1992). Predicting effectiveness: fact and Fantasy. *In*: Delfosse, E.S., Scott, R.R. (Eds.). *Proceedings of the VIII International Symposium on Biological Control of Weeds*. DSIR/CSIRO, Canterbury, New Zealand. pp. 103-109.

Cyzpionka, K. & Hill, M.P. (2007). The relationship between female pupal mass and fecundity of *Gratiana spadicea* (Klug, 1829) (Coleoptera: Chrysomelidae). *African Entomology* **15**: 380 – 382.

De Bruyn, L., Scheirs, J. & Verhagen, R. (2002). Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia* **130**: 594-599

De Clercke-Floate, R. & Bouchier, R.S. (2000). Ecological principles of biological control: from population theory to weed biocontrol practice. *In*: Spencer, N.R. (Ed.). *Proceedings of the X International Symposium on Biological Control of Weeds*. Montana State University, Bozeman, Montana. pp. 517-520.

DeLoach, C.J. & Cordo, H.A. (1983). Control of water hyacinth by *Neochetina bruchi* (Coleoptera: Curculionidae: Bagoini) in Argentina. *Environmental Entomology* **12**: 19-23.

Denno, R.F., McClure, M.S., Ott, J.R. (1995). Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annual Review of Entomology* **40**: 297-331.

Denno, R.F., Gratton, C., Döbel, H. & Finke, D.L. (2003). Predation risk affects relative strength of top-down and bottom-up impacts on insect herbivores. *Ecology* **84**: 1032-1044.

Denoth, M., Frid, L. & Myers, J.H. (2002). Multiple agents in biological control: Improving the odds? *Biological Control* **24**: 20-30.

deVilliers, S. & Thiart, C. (2007). The nutrient status of South African rivers: concentrations, trends and fluxes. *South African Journal of Science* **103**: 343-349.

- Ding, J., Blossey, B., Du, Y. & Zheng, F. (2006).** Impact of *Galerucella birmanica* (Coleoptera: Chrysomelidae) on growth and seed production of *Trapa natans*. *Biological Control* **37**: 338-345.
- Doyle, R., Grodowitz, M., Smart, M. & Owens, C. (2007).** Separate and interactive effects of competition and herbivory on the growth, expansion and tuber formation of *Hydrilla verticillata*. *Biological Control* **41**: 327-338.
- Durden, W.C., Blackburn, R.D. & Gangstad, E.O. (1975).** Control program for alligatorweed in the Southeastern States. *Hyacinth Control Journal*: 27-30.
- Dyer, L.A., Letourneau, D.K., Dodson, C.D., Tobler, M.A., Stireman, J.O. & Hsu, A. (2004).** Ecological causes and consequences of variation in defensive chemistry of a Neotropical shrub. *Ecology* **85**: 2795-2803.
- Edwards, D. & Musil, C.J. (1975).** *Eichhornia crassipes* in South Africa – A general review. *Journal of the Limnological Society of southern Africa* **7**: 23-27
- Edwards, G.R., Bourbôt, G.A. & Crawley, M.J. (2000).** Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grassland. *Journal of Applied Ecology* **37**: 321-334.
- Elder, B.D. (2006).** Disturbance-mediated trophic interactions and plant performance. *Oecologia* **147**: 261-271.
- Esperk, T., Tammaru, T., Nylin, S. & Teder, T. (2007).** Achieving high sexual size dimorphism in insects: females add instars. *Ecological Entomology* **32**: 243-256.
- Evans, J.R. (1983).** Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L). *Plant Physiology* **72**: 297-302.

Everett, R.A. (2000). Patterns and pathways of biological invasions. *Trends in Ecology and Evolution* **15**: 177-178.

Fischer, K. & Fiedler, K. (2000). Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. *Oecologia* **124**: 235-241.

Fowler, N.L. & Rauscher, M.D. (1985). Joint effects of competitors and herbivores on growth and reproduction in *Aristolochia reticulata*. *Ecology* **66**: 1580-1587.

Fox, L.R., Letourneau, Eisenbach, J. & Van Nouhuys, S. (1990). Parasitism and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia* **83**: 414-419.

Fraser, L.H. & Grime, J.P. (1999). Interacting effects of herbivory and fertility on a synthesized plant community. *Journal of Ecology* **87**: 514-525.

Friedli, J. & Bacher, S. (2001). Direct and indirect effects of a shoot-base boring weevil and plant competition on the performance of creeping thistle, *Cirsium arvense*. *Biological Control* **22**: 219-226.

Fournio, I.W. & Bourne, A.S. (1985). Feeding by *Cyrtobagous salviniae* on *Salvinia molesta* under different regimes of temperature and nitrogen content and the effects on plant growth. *Entomophaga* **30**: 279-286.

Gassman, A.J. (2004). Effect of photosynthetic efficiency and water availability on tolerance of leaf removal in *Amaranthus hybridus*. *Journal of Ecology* **92**: 882-892.

Gerson, E.A. & Kelsey, R.G. (1999). Piperidine alkaloids in nitrogen fertilized *Pinus ponderosa*. *Journal of Chemical Ecology* **25**: 2027-2039.

Goeden, R.D. (1983). Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. *Protection Ecology* **5**: 287-301.

Gopal, B. (1987). Aquatic Plant Studies 1: Water hyacinth. Amsterdam, Elsevier.

Gossett, D.R. & Norris, W.E. (1971). Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, *Eichhornia crassipes* (Mart.) Solms. *Hydrobiologia* **38**: 15-28.

Grevstad, F.S. (1999). Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. *Biological Invasions* **1**: 313-323.

Grime, J.P. (1979). Plant strategies and vegetative processes. John Wiley and Sons, Chichester.

Gruner, D.S. (2004). Attenuation of top-down and bottom-up forces in a complex terrestrial community. *Ecology* **85**: 3010-3022.

Guntli, D., Burgos, S., Kump, I., Heeb, M., Pfirter, H.A., Dèfago, G. (1999). Biological control of hedge bindweed (*Calystegia sepium*) with *Stagonospora concolvuli* LA39 in combination with competition from red clover (*Trifolium pretense*). *Biological Control* **15**: 252-258.

Gutierrez, A.P., Mills, N.J., Schreiber, S.J. & Ellis, C.K. (1994). A physiologically based tritrophic perspective on bottom-up and top-down regulation of populations. *Ecology* **75**: 2227-2242.

Haag, K.H. (1986). Effects of herbicide application on mortality and dispersive behaviour of the water hyacinth weevils, *Neochetina eichhorniae* and *Neochetina bruchi* (Coleoptera: Curculionidae). *Environmental Entomology* **15**: 1192-1198.

- Häfliger, P., Schwarzländer, M. & Blossey, B. (2006).** Impact of *Archanara geminipuncta* (Lepidoptera: Noctuidae) on aboveground biomass production of *Phragmites australis*. *Biological Control* **38**: 413-421.
- Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960).** Community structure, population control and competition. *The American Naturalist* **XCIV**: 421-425.
- Halpern, S.L. & Underwood, N. (2006).** Approaches for testing herbivore effects on plant population dynamics. *Journal of Applied Ecology* **43**: 922-929.
- Harley, K.L.S. (1990).** The role of biological control in the management of water hyacinth. *Biocontrol News and Information* **11**: 11-20.
- Harley, K.L.S., Julien, M.H. & Wright, A.D. (1996).** Water hyacinth: a Tropical worldwide problem and methods for its control. *Second International Weed Control Congress*. Department of Weed Control and Pesticide Ecology, Flakkebjerg, Denmark. pp. 639-644.
- Harper, J.L. (1977).** Population Biology of Plants. Academic Press, New York.
- Harris, P. (1973).** The selection of effective agents for the biological control of weeds. *Canadian Entomologist* **105**: 1495-1503.
- Harris, P. (1979).** Cost of biological control of weeds by insects in Canada. *Weed Science* **27**: 242-250.
- Hartley, S.E. & Amos, L. (1999).** Competitive interactions between *Nardus stricta* L. and *Calluna vulgaris* (L.) Hull: the effect of fertilizer on defoliation on above- and below-ground performance. *Journal of Ecology* **87**: 330-340.

Hartvigsen, G., Wait, D.A. & Coleman, J.S. (1995). Tri-trophic interactions influenced by resource availability: predator effects on plant performance depend on plant resources. *Oikos* **74**: 463-468.

Hawkes, C.V. & Sullivan, J.J. (2001). The impact of herbivory on plants in different resource conditions – a meta-analysis. *Ecology* **82**: 2045-2058.

Heard, T.A. & Winterton, S.L. (2000). Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology* **37**: 117-127.

Hilbert, D.W., Swift, D.M., Detling, J.K. & Dyer, M.I. (1981). Relative growth rates and the grazing optimization hypothesis. *Oecologia* **51**: 14-18.

Hill, M.P. (1999). The world's worst aquatic weed. *Pesticide Outlook* (April Ed.) pp. 58-61.

Hill, M.P. & Cilliers, C.J. (1999). A review of the arthropod natural enemies, and factors that influence their efficacy, in the biological control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), in South Africa. *African Entomology Memoir* **1**: 103-112.

Hill, M.P. & Olckers, T. (2001). Biological control initiatives against water hyacinth in South Africa: constraining factors, success and new courses of action. In: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.). *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*. ACIAR, Australia. pp. 33-38.

Hixon, M.A., Pascala, S.W. & Sandin, S.A. (2002). Population regulation: historical context and contemporary challenges of open vs. closed systems. *Ecology* **83**: 1490-1508.

Hochwender, C.G., Marquis, R.J. & Stowe, K.A. (2000). The potential for and constraints on the evolution of compensatory ability of *Asclepias syriaca*. *Oecologia* **122**: 361-370.

Hogendorp, B.K., Cloyd, R.A. & Swiader, J.M. (2006). Effect of nitrogen fertility on reproduction and development of citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), feeding on two colours of Coleus, *Solenostemon scutellarioides* L. Codd. *Environmental Entomology* **35**: 201-211.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P. (1977). The World's Worst Weeds. University Press, Honolulu, Hawaii.

Holmes, S. (1996) South African Water Quality Guidelines. World Wide Web.
www.dwaf.gov.za/IWQS/wq_guide/index.html.

Holt, R.D. & Hochberg, M.E. (2001). Indirect interactions, community modules and biological control: a theoretical perspective. *In*: Wajnberg, E., Scott, J.K. & Quimby, P.C. (Eds.). Evaluating Indirect Ecological Effects of Biological Control. CABI Publishing, Wallingford, UK. pp. 13-37.

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**: 483-492.

Hufbauer, R.A. & Roderick, G.K. (2005). Microevolution in biological control: Mechanisms, patterns and processes. *Biological Control* **35**: 227-239.

Huffaker, C.B. & Kennett, C.E. (1969). Some aspects of assessing the efficiency of natural enemies. *Canadian Entomologist* **101**: 425-447.

Hunter, M.D. & Price, P.W. (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**: 724-732.

Hunter, M.D. & McNeill, J.N. (1997). Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology* **78**: 977 – 986.

Jackson, R.B. & Cladwell, M.M. (1993). The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. *Ecology* **74**: 612-614.

Jadhav, A., Hill, M.P. & Byrne, M. (2008). Integration of a retardant does of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* **47**: 154-158.

Jamil, K. & Jyothi, K.N. (1988). Enhanced reproductive potential of *Neochetina bruchi* Hustache fed on water hyacinth plants from polluted water bodies. *Current Science* **57**: 195-197.

Jacot Guillarmod, A. (1979). Water weeds in southern Africa. *Aquatic Botany* **6**: 377-391.

Jeunger, T. & Bergelson, J. (1997). Pollen resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* **78**: 1684-1695.

Joern, A. & Klucas, G. (1993). Intra- and interspecific competition in adults of two abundant grasshoppers (Orthoptera: Acrididae) from a Sandhills grassland population. *Population Ecology* **22**: 352-361.

Joern, A. & Behmer, S.T. (2002). Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecological Entomology* **23**: 174-184.

Jones, R.W. (2001). Integrated control of water hyacinth on the Nseleni/Mposa Rivers and Lake Nsezi, KwaZulu-Natal, South Africa. *In: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.). Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth.* ACIAR, Australia. pp. 123-129.

Julien, M.H. (1982). Biological control of weeds: A world catalogue of agents and their target weeds. Commonwealth Agriculture Bureau, Surrey.

Julien, M.H. (2001). Biological control of water hyacinth with arthropods: a review to 2000. *In: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.). Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth.* ACIAR, Australia. pp. 8-20.

Julien, M.H. & Bourne, A.S. (1986). Compensatory branching and changes in nitrogen content in the aquatic weed *Salvinia molesta* in response to disbudding. *Oecologia* **70**: 250-257.

Julien, M.H. & Orapa, W. (1999). Structure and management of a successful biological control project for water hyacinth. *In: Hill, M.P., Julien, M.H. & Center, T.D. (Eds.). Proceedings of the First Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth.* Plant Protection Research Institute, Pretoria. pp. 123-134.

Julien, M.H., Harley, K.L.S., Cordo, H.A., Wright, A.D., Cilliers, C.J., Center, T.D., Confrancesco, A. & Hill, M.P. (1996). International cooperation and linkages in the management of water hyacinth with the emphasis on biological control. *In: Moran, V.C. & Hoffman, J.H. (Eds.). Proceedings of the IX International Symposium on the Biological Control of Weeds.* University of Cape Town, Cape Town. pp. 273-282.

Julien, M.H., Griffiths, M.W. & Wright, A.D. (1999). Biological Control of Water Hyacinth. ACIAR, Canberra.

Karban, R. & Courtney, S. (1987). Intraspecific host plant choice: lack of consequences for *Streptanthus tortuosus* (Cruciferae) and *Euchloe hyantis* (Lepidoptera: Pieridae). *Oikos* **48**: 243-248.

Kareiva, P. (1996). Special feature: contributions of ecology to biological control. *Ecology* **77**: 1963-1964.

Keane, R.M. & Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* **17**: 164-170.

King, A. (2008). Temperature as a catalyst for boom/bust events. *Plant Protection News* **76**: 11-12.

Klingenberg, C.P. & Spence, J.R. (1997). On the role of body size for life-history evolution. *Ecological Entomology* **22**: 55-68.

Klöppel, M., Smith, L & Syrett, P. (2003). Predicting the impact of the biocontrol agent *Aulacidea subterminalis* (Cynipidae) on growth of *Hieracium pilosella* (Asteraceae) using differing environmental conditions in New Zealand. *Biocontrol Science and Technology* **13**: 207-218.

Kytö, M., Niemelä, P. & Larsson, S. (1996). Insects on trees: population and individual response to fertilization. *Oikos* **75**: 148-159.

Lallana, V.H., Sabattini, R.A. & Del Carmen Lallana, M. (1987). Evapotranspiration from *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia herzogii* and *Azolla caroliniana* during summer in Argentina. *Aquatic Plant Management* **25**: 48-50.

Lee, T.D. & Bazzaz, F.A. (1980). Effects of defoliation and competition on growth and reproduction in the annual plant *Abutilon theophrasti*. *Journal of Ecology* **68**: 813-821.

Louda, S. M., Keeler, K.H. & Holt, R.D. (1990). Herbivore influences on plant performance and competitive interactions. *In*: Grace, J.B & Tilman, D. (Eds.). *Perspectives on Plant Competition*. Academic Press, New York. pp. 413-443.

Maschinski, J. & Whitham, T.G. (1989). The continuum of plant responses to herbivory: the influence of plant association, nutrient availability and timing. *The American Naturalist* **134**: 1-19.

Matsumoto, T. Itioka, T. & Nishida, T. (2003). Cascading effects of a specialist parasitoid on plant biomass in a citrus agroecosystem. *Ecological Research* **18**: 651-659.

Mattson, W.J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11**: 119-161.

McClay, A.S. (1996). Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents. *In*: Moran, V.C. & Hoffman, J.H. (Eds.). *Proceedings of the IX International Symposium on Biological Control of Weeds*. University of Cape Town, Cape Town. pp. 377-383.

McClay, A.S. & Hughes, R.B. (1995). Effects of temperature on developmental rate, distribution, and establishment of *Calophasia lunula* (Lepidoptera: Noctuidae), a biocontrol agent for toadflax (*Linaria* spp.). *Biological Control* **5**: 368-377.

McClay, A.S. & Balciunas, J.K. (2005). The role of pre-release efficacy assessment in selecting classical biological control agents for weeds – applying the Anna Karenina principle. *Biological Control* **35**: 197-207.

McConnachie, A.J., Hill, M.P. & Byrne, M.J. (2004). Field assessment of a frond-feeding weevil, a successful biological control agent of red water fern, *Azolla filiculoides* Lamarck (Azollaceae), in southern Africa. *Biological Control* **29**: 326-331.

McEvoy, P. & Coombs, E.M. (1993). Disturbance, competition and herbivory effects on ragwort, *Senecio jacobaea* populations. *Ecological Monographs* **63**: 55-75.

McEvoy, P. & Coombs, E.M. (1999). Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications* **9**: 387-401.

McEvoy, P. & Coombs, E.M. (2000). Why things bite back: unintended consequences of weed biological control. In: Follet, P.A., Duan, J.J. (Eds.). Nontarget Effects of Biological Control. Kluwer Academic Publishers, Boston, MA. pp. 167-194.

McFadyen, R.E. (1998). Biological control of weeds. *Annual Review of Entomology* **43**: 369-393.

Meyer, G.A. (1993). A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. *Ecology* **74**: 1101-1116.

Meyer, G.A. (1998). Mechanisms promoting recovery from defoliation in goldenrod (*Solidago altissima*). *Canadian Journal of Botany* **76**: 450-459.

Meyer, G.A. (2000). Interactive effects of soil fertility and herbivory on *Brassica nigra*. *Oikos* **88**: 433-441.

Meyer, G.A. & Root, R.B. (1993). Effects of herbivorous insects and soil fertility on reproduction of goldenrod. *Ecology* **74**: 1117-1128.

Midgley, J.M., Hill, M.P. & Villet, M.H. (2006). The effect of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), on benthic biodiversity in two impoundments on the New Year's River. *African Journal of Aquatic Science* **31**: 25-30.

Mihaliak, C.A. & Lincoln, D.E. (1989). Plant biomass partitioning and chemical defense. *Oecologia* **80**: 122-126.

Miller, T.E.X. (2008). Bottom-up, top-down and within-trophic level pressure on a cactus-feeding insect. *Ecological Entomology* **33**: 261-268.

Mopper, S. & Whitham, T.G. (1992). The plant stress paradox: effects on pinyon sawfly sex ratios and fecundity. *Ecology* **73**: 515-525.

Morrill, W.L., Ditterline, R.L. and Winstead, C. (1984). Effects of *Lygus borealis* Kelton (Hemiptera: Miridae) feeding on sanfoin seed production. *Journal of Economic Entomology* **77**: 966-968.

Müller-Schärer, H. (1991). The impact of root herbivory as a function of plant density and competition. Survival, growth and fecundity of *Centaurea maculosa* in field plots. *Journal of Applied Ecology* **28**: 759-776.

Müller-Schärer, H. & Brown, V.K. (1995). Direct and indirect effects of above and below-ground insect herbivory on plant density and performance of *Tripleurospermum perforatum* during early plant succession. *Oikos* **72**: 36-41.

Murdoch, W.W. (1994). Population regulation in theory and practice. *Ecology* **75**: 271-287.

Mutikainen, P., Walls, M., Ovaska, J., Keinänen, M., Julkunen-Tiito, R. and Vapaavuori, E. (2000). Herbivore resistance in *Betula pendula*: effect of fertilization, defoliation and plant genotype. *Ecology* **81**: 49-65.

Myers, J. H. (1985). How many insect species are necessary for successful biocontrol of weeds? In: Delfosse, E.S. (Ed.). *Proceedings of the VI International Symposium of Biological Control of Weeds*. Agric. Can, Vancouver, Canada. pp. 77-82.

Myers, J.H. (2000). What can we learn from biological control failures? In: Spencer, N.R. (Ed.). *Proceedings of the X International Symposium on Biological Control of Weeds*. USDA-ARS, Bozeman, Montana. pp. 151-154.

Myers, J.H. & Post, B.J. (1981). Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth - tansy ragwort system. *Oecologia* **48**: 151-156.

Myers, J.H., Boettner, G. & Elkinton, J. (1998). Maternal effects in gypsy moth: only sex ratio varies with population density. *Ecology* **79**: 305-314.

Nötzold, R., Blossey, B. & Newton, E. (1998). The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* **113**: 82-93.

Nowak, R.S. & Caldwell, M.M. (1984). A test of compensatory photosynthesis in the field: Implications for herbivory tolerance. *Oecologia* **61**: 311-318.

Oberholzer, I.G. & Hill, M.P. (2001). How safe is the grasshopper *Cornops aquaticum* for release on water hyacinth in South Africa. In: Julien, M.H., Hill, M.P., Center, T.D. & Jianqing, D. (Eds.). *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth..* ACIAR, Australia. pp. 82-88.

Oosterheld, M. (1992). Effect of defoliation intensity on aboveground and belowground relative growth rates. *Oecologia* **92**: 313-316.

Oosterheld, M. & McNaughton, S.J. (1991). Effect of stress and time for recovery on the amount of compensatory growth after grazing. *Oecologia* **85**: 305-313.

Ogwang, J.A. & Molo, R. (1999). Impact studies on *Neochetina bruchi* and *Neochetina eichhorniae* in Lake Kyoga, Uganda. In: Hill, M.P., Julien, M.H. & Center, T.D. (Eds.). *Proceedings of the First Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*. Plant Protection Research Institute, Pretoria. pp. 10-13.

Ohmart, C.P., Stewart, L.G. & Thomas, J.R. (1985). Effects of nitrogen concentrations of *Eucalyptus blakelyi atomaria* (Coleoptera: Chrysomelidae). *Oecologia* **68**: 41-44.

Panizzi, A.R. & Saraiva, S.I. (1993). Performance of nymphal and adult southern green stinkbug on an overwintering host plant and impact of nymph to adult food-switch. *Entomologica Experimentalis et Applicata* **68**: 109-115.

Pantoja, A., Smith, C.M. & Robinson, J.F. (1986). Effects of the fall armyworm (Lepidoptera: Noctuidae) on rice yields. *Journal of Economic Entomology* **79**: 1324-1329.

Pantone, D.J., Williams, W.A. & Maggenti, A.R. (1989). An alternative approach for evaluating the efficacy of potential biocontrol agents of weeds. 1. Inverse Linear Model. *Weed Science* **37**: 771-777.

Pearson, D.E. & Callaway, R.M. (2003). Indirect effects of host-specific biological control agents. *Trends in Ecology and Evolution* **18**: 456-461.

Pearson, D.E. & Callaway, R.M. (2005). Indirect nontarget effects of host-specific biological control agents: Implications for biological control. *Biological Control* **35**: 288-298.

Perkins, B.D. (1974). Arthropods that stress water hyacinth. *Pest Articles and News Summaries* **20**: 304-314

Peterson, R.K.D. & Higley, L.G. (2001). Illuminating the black box: the relationship between injury and yield. *In*: Peterson, R.K.D. & Higley, L.G. (Eds.). Biotic Stress and Yield Loss. CRC Press, Boca Raton. pp. 1-12.

Pieterse, A.H. (1978). Biological Control of Aquatic Weeds: Perspectives for the Tropics. *Aquatic Botany* **3**: 133-141.

Pilson, D. & Decker, K.L. (2002). Compensation for herbivory in wild sunflower: response to simulated damage by the head-clipping weevil. *Ecology* **83**: 3097-3107.

Pimentel, D. (2002). Introduction: Non-native species in the world. *In*: Biological Invasions. CRC press, Florida. pp. 3-8.

Pitan, O.O.R., Odubiyi, S.I.I. & Olatunde, G.O. (2007). Yield response of cowpea, *Vigna unguiculata* L. (Walp.), to infestation of *Aspavia armigera* F. (Hem., Pentatmidae). *Journal of Applied Entomology* **131**: 704-708.

Polley, H.W. & Detling, J.K. (1989). Defoliation, nitrogen and competition: effects on plant growth and nitrogen nutrition. *Ecology* **70**: 721-727.

Power, M.E. (1992). Top-down and bottom-up forces in food webs: do plants have primacy? *Ecology* **73**: 733-746.

Price, P.W. (1975). Insect Ecology. John Wiley & Sons, New York.

Price, P.W. (2000). Host plant resource quality, insect herbivores and biocontrol. *In*: Spencer, N.R. (Ed.). *Proceedings of the X International Symposium on Biological Control of Weeds*. Montana State University, Montana. pp 583-590.

Price, P.W., Cobb, N., Craig, T.P., Wilson Fernandes, G. Itami, J.K., Mopper, S. & Preszler, R.W. (1990). Insect herbivore population dynamics on trees and shrubs: new approaches relevant to latent and eruptive species and life table development. *In*: Bernays, E.A. (Ed.). *Insect-Plant Interactions Volume II*. CRC Press, Boca Raton. pp. 1-38.

Rand, T.A. (2004). Competition, facilitation and compensation for insect herbivory in an annual salt marsh forb. *Ecology* **85**: 2046-2052.

Rauscher, M.D. & Feeny, P. (1980). Herbivory, plant density and plant reproductive success: the effect of *Battus philenor* on *Aristolochia reticulata*. *Ecology* **61**: 905-917.

Reddy, K.R., Agami, M. & Tucker, J.C. (1989). Influence on nitrogen supply rates on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. *Aquatic Botany* **36**: 33-43.

Reddy, K.R., Agami, M. & Tucker, J.C. (1990). Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants. *Aquatic Botany* **37**: 355-365.

Reitz, S.R. & Trumble, J.T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology* **47**: 435-465.

Rhainds, M. & English-Loeb, G. (2003). Testing the resource concentration hypothesis with tarnished plant bug on strawberry: density of hosts and patch size influence the interaction between abundance of nymphs and incidence of damage. *Ecological Entomology* **28**: 348-358.

Richards, J.H. (1982). Developmental potential of axillary buds of water hyacinth, *Eichhornia crassipes* Solms. (Pontederiaceae). *American Journal of Botany* **69**: 615-622.

Ripley, B.S., Muller, E., Behenna, M., Whittington-Jones, G.M. & Hill, M.P. (2006). Biomass and photosynthetic productivity of water hyacinth (*Eichhornia crassipes*) as affected by nutrient supply and mirid (*Eccritotarsus catarinensis*) biocontrol. *Biological Control* **39**: 392-400.

Risch, S.H. & Carroll, C.R. (1982). Effect of a keystone predacious ant, *Solenopsis geminata*, on arthropods in a tropical agroecosystem. *Ecology* **63**: 1979-1983.

Room, P.M. (1990). Ecology of a simple plant-herbivore system: biological control of *Salvinia*. *Trends in Ecology and Evolution* **5**: 74-79.

Room, P.M. & Thomas, P.A. (1985). Nitrogen and establishment of a beetle for biological control of the floating weed salvinia in Papua New Guinea. *The Journal of Applied Ecology* **22**: 139-156.

Root, R.B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43**: 95-124.

Rossiter, M.C. (1991). Environmentally-based maternal effects: a hidden force in insect population dynamics. *Oecologia* **87**: 288-294.

Schooler, S.S. & McEvoy, P.B. (2006). Relationship between insect density and plant damage for the golden loosestrife beetle, *Galerucella pusilla*, on purple loosestrife (*Lythrum salicaria*). *Biological Control* **36**: 100-105.

Scriber, J.M. & Slansky, F. (1981). The nutritional ecology of immature insects. *Annual Review of Entomology* **26**: 183-211.

Shea, K. & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends in Ecology and Evolution* **17**: 170- 176.

Sheppard, A.W. (1996). The interaction between natural enemies and interspecific competition in the control of invasive pasture weeds. *In*: Moran, V.C. & Hoffman, J.H. (Eds.). *Proceedings of the IX International Symposium on Biological Control of Weeds*. University of Cape Town, Cape Town. pp. 47-53.

Sheppard, A. W. (2003). Prioritizing agents based on predicted efficacy. *In*: Jacob, H.S., Briese, D.T. (Eds.). *Improving the Selection, Testing, and Evaluation of Weed Biological Control Agents*. CRC for Australian Weed Management, Glen Osmond, Australia, pp. 11-21.

Silveira-Guido, A. & Perkins, D.B. (1975). Biology and host specificity of *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae), a potential biological control agent for water hyacinth. *Environmental Entomology* **4**: 400 – 404.

Simberloff, D. & Stiling, P. (1996). How risky is biological control? *Ecology* **77**: 1965-1974.

Simpson, S.J. & Simpson, C.L. (1990). The mechanisms of nutritional compensation by phytophagous insects. *In*: Bernays, E.A. (Ed.). *Insect-Plant Interactions Volume II*. CRC Press, Boca Raton. pp. 111-160.

Slansky, F. (1981). Insect nutrition: an adaptationist's perspective. *Florida Entomologist* **65**: 45-71.

Slansky, F. & Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological monographs* **47**: 209-228.

Spitters, C.J.T. (1983). An alternative approach to the analysis of mixed cropping experiments. 1. Estimation of competition effects. *Netherlands Journal of Agricultural Science* **31**: 1-11.

Stanley, J.N., Julien, M.H., & Center, T.D. (2007). Performance and impact of the biological control agent *Xubida infusella* (Lepidoptera: Pyralidae) on the target weed *Eichhornia crassipes* (waterhyacinth) and on a non-target plant, *Pontederia cordata* (pickerelweed) in two nutrient regimes. *Biological Control* **40**: 298-305.

Steinger, T. & Müller-Schärer, H. (1992). Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia* **91**: 141-149.

Stevens, M.T., Kruger, E.L. & Lindroth, R.L. (2008). Variation in tolerance to herbivory is mediated by differences in biomass allocation in aspen. *Functional Ecology* **22**: 40-47.

Stiling, P. & Moon, D.C. (2005). Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. *Oecologia* **142**: 413-420.

Stowe, K.A., Marquis, R.J., Hochwender, C.G. & Simms, E.L. (2000). The evolutionary ecology of tolerance to herbivore damage. *Annual Review of Ecology and Systematics* **31**: 565-595.

Strauss, S.Y. & Agrawal, A.A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* **14**: 179-185.

Swank, S.E. & Oechel, W.C. (1991). Interactions among the effects of herbivory, competition and resource limitation on chaparral herbs. *Ecology* **72**: 104-115.

Taylor, M.F.J. (1984). The dependence of development and fecundity of *Samea multiplicalis* on early larval nitrogen intake. *Journal of Insect Physiology* **30**: 779-785.

Taylor, M.F.J. (1989). Compensation for variable dietary nitrogen by larvae of the salvinia moth. *Functional Ecology* **3**: 407-416.

Throop, H.L. (2005). Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. *Oikos* **111**: 91-100.

Throop, H.L. & Lerdau, M.T. (2004). Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. *Ecosystems* **7**: 109-133.

Tiffin, P. (2000). Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology* **14**: 523-536.

Tiffin, P. (2002). Competition and timing of damage affect the pattern of selection acting on plant defenses against herbivores. *Ecology* **83**: 1981-1990.

Tilman, D. (1994). Competition and biodiversity in spatially structured habitats. *Ecology* **74**: 2-16.

Timmer, C.E. & Weldon, L.W. (1966). Evapotranspiration and pollution of water by water hyacinth. *Hyacinth Control Journal* **6**: 34-37.

Trumble, J.T., Kolodny-Hirsch, D.M. & Ting, I.P. (1993). Plant compensation for arthropod herbivory. *Annual Review of Entomology* **38**: 93-119.

Turchin, P. (1999). Population regulation: a synthetic view. *Oikos* **84**: 153-159.

Ueckermann, C. & Hill, M.P. (2001). Impact of herbicides used in water hyacinth control on natural enemies released against the weed for biological control. WRC Report No. 915/1/01. Water Research Commission, Pretoria, South Africa.

Ultsch, G.R. (1973). The effects of water hyacinths (*Eichhornia crassipes*) on the microenvironment of aquatic communities. *Archiv fuer Hydrobiologie* **72**: 460-473.

Van, T.K., Wheeler, G.S. & Center, T.D. (1998). Competitive interactions between hydrilla (*Hydrilla verticillata*) and Vallisneria (*Vallisneria americana*) as influenced by insect herbivory. *Biological Control* **11**: 185-192.

Van, T.K., Wheeler, G.S. & Center, T.D. (1999). Competition between *Hydrilla verticillata* and *Vallisneria americana* as influenced by soil fertility. *Aquatic Botany* **62**: 225-233.

Van den Berg, H. & Soehard, I. (2000). The influence of the rice bug *Leptocorisa oratorius* on rice yield. *Journal of Applied Entomology* **131**: 959-970.

Van Wilgen, B.W., Richardson, D.M., Le Maitre, D.C., Marais, C. & Magadlela, D. (2001). The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability* **3**: 145-168.

Van Wyk, E. & van Wilgen, B.W. (2002). The cost of water hyacinth control in South Africa: a case study of three options. *African Journal of Aquatic Science* **27**: 141-149.

Verkroost, A.W.M. & Wassen, M.J. (2005). A simple model for nitrogen-limited plant growth and nitrogen allocation. *Annals of Botany* **96**: 871-876.

Vieira, M. de F., Santos, A.C. dos. (2003). Life cycle of *Cornops aquaticum* (Brüner 1906) (Orthoptera: Acrididae, Leptysminae) and aspects of its food behaviour at Central Amazonia. *Acta Amazonica* **33**: 711-714.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**: 1-16.

Walker, M. & Jones, T.H. (2001). Relative roles of top-down and bottom-up forces in terrestrial tri-trophic insect-herbivore-natural enemy system. *Oikos* **93**: 177-187.

Wall, R. & Begon, M. (1986). Population density, phenotype and mortality in the grasshopper, *Chorthippus brunneus*. *Ecological Entomology* **11**: 445-456.

Wallace, J.B. & O'Hop, J. (1985). Life on a fast pad: waterlily leaf beetle impact on water lilies. *Ecology* **66**: 1534-1544.

Watson, M.A. & Brochier, J. (1988). The role of nutrient levels on flowering in water hyacinth. *Aquatic Botany* **31**: 367-372.

Wheeler, G.S. (2001). Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. *Biological Control* **22**: 256-264.

Wheeler, G.S. (2003). Minimal increase in larval performance of the biological control agent *Oxyops vitiosa* when fed *Melaleuca quinquenervia* leaves of different nitrogen levels. *Biological Control* **26**: 109-116.

White, T.C.R. (1976). Weather, food and plagues of locusts. *Oecologia* **22**: 119-134.

Wilson, J.R., Holst, N. & Rees, M. (2005). Determinants and patterns of population growth in water hyacinth. *Aquatic Botany* **81**: 51-67.

Winterton, S.L. & Heard, T.A. (1996). Eutrophication and the biological control of water hyacinth. In: Moran, V.C. & Hoffman, J.H. (Eds.). *Proceedings of the IX International Symposium on the Biological Control of Weeds*. University of Cape Town, Cape Town. pp. 481-482.

Wright, A.D. & Purcell, M.F. (1995). *Eichhornia crassipes* (Mart.) Solms-Laubach. In: Groves, R.H., Shepherd, R.C.H. & Richardson, R.G. (Eds.). *The Biology of Australian Weeds*. R.G. & F.J. Richardson, Melbourne. pp. 111-121.

Wu, M, Hacker, S., Ayres, D. & Strong, D.R. (1999). Potential of *Prokelisia* spp. as biological control agents of English cordgrass, *Spartina anglica*. *Biological Control* **16**: 267-273.

Xhong-xian, L., Xiao-ping, Y., Heong, K. & Cui, H. (2007). Effect of nitrogen fertilizer on herbivores and its stimulation to major insect pests in rice. *Rice Science* **14**: 56-66.

Xie, Y., Wen, M., Yu, D. & Li, Y. (2004). Growth and resource allocation of water hyacinth as affected by gradually increasing nutrient concentrations. *Aquatic Botany* **79**: 257-266.

Zehnder, C.B. & Hunter, M.D. (2008). Effects of nitrogen deposition on the interaction between an aphid and its host plant. *Ecological Entomology* **33**: 24-30.