Interactions between three biological control agents of water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) in South Africa

THESIS

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Abstract

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) is a free-floating perennial weed that is regarded as the worst aquatic weed in the world because of its negative impacts on aquatic ecosystems. It is native to the Amazon Basin of South America, but since the late 1800s has spread throughout the world. The first record of the weed in South Africa was noted in 1908 on the Cape Flats and in KwaZulu-Natal, but it is now dispersed throughout the country. Mechanical and chemical control methods have been used against the weed, but biological control is considered the most cost-effective, sustainable and environmentally friendly intervention.

Currently, nine biological control agents have been released against water hyacinth in South Africa, and *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) is used most widely to control it. However, in some sites, water hyacinth mats have still not been brought under control because of eutrophic waters and cool temperatures. It was therefore necessary to release new biological control agents to complement the impact of *N. eichhorniae*.

Megamelus scutellaris Berg (Hemiptera: Delphacidae) was released in 2013, but little is known about how it interacts with other agents already present in South Africa. It is likely to compete with the established biological control agent, *Eccritotarsus eichhorniae* Henry (Heteroptera: Miridae), because they are both sap suckers. On the other hand, *N. eichhorniae* is the most widespread and thus the most important biological control agent for water hyacinth. The aim of this study, then, was to determine the interactions between the two sap-sucking agents in South Africa that presumably occupy similar niches on the plant,

and the interaction between *M. scutellerais* and *N. eichhorniae,* the most widely distributed and abundant agent in South Africa.

Three experiments were conducted at the Waainek Research Facility at Rhodes University, Grahamstown, Eastern Cape, South Africa. Plants were grown for two weeks and insect species were inoculated singly or in combination. Water hyacinth, plant growth parameters and insect parameters were measured every 14 days for a period of 12 weeks.

The results of the study showed that feeding by either *E. eichhorniae* or *M. scutellaris* had no effect on the feeding of the other agent. Both agents reduced all the measured plant growth parameters equally, either singly or in combination (*i.e. E. eichhorniae* or *M. scutellaris* alone or together). The interaction between the two agents appears neutral and agents are likely to complement each other in the field.

Prior feeding by *E. eichhorniae* or *M. scutellaris* on water hyacinth did not affect the subsequent feeding by either agent. *Megamelus scutellaris* prefers healthy fresh water hyacinth plants. In addition, planthoppers performed best in combination with the weevil, especially on plants with new weevil feeding scars.

The results of the study showed that *M. scutellaris* is compatible with other biological control agents of water hyacinth that are already established in South Africa. Therefore, the introduction of *M. scutellaris* may enhance the biological control of water hyacinth in South Africa.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 INVASIVE ALIEN PLANTS

Invasive alien plants are environmentally problematic in South Africa, threatening the functioning of indigenous ecosystems, competing with native plants for natural resources, causing a loss of native biodiversity and modifying habitats (Myers & Bazely, 2003; Brooks *et al.,* 2004; Nel *et al*., 2004; Pyšek & Richardson, 2010). Several control methods, including manual, mechanical and chemical, have been used in attempts to control alien plant infestations throughout the world and, while some have been successful, some are expensive. For example, Working for Water of the Department of Environmental Affairs of South Africa has spent R3.2 billion on alien plant control since its inception in 1995 (van Wilgen *et al.,* 2012). Mechanical and chemical control is short-lived and can even accelerate the re-establishment of the weeds (Van Driesche *et al.,* 2010). On the other hand, biological control, using host-specific natural enemies of invasive plants, is a sustainable, economical, and long-term management option that can be used effectively to control alien plant populations, thereby restoring native vegetation and ecosystem services (Zimmermann *et al.,* 2004; Van Driesche *et al.,* 2010). The focus of this thesis is to determine whether a newly released agent for South Africa's worst aquatic weed, water hyacinth (*Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), will increase the level of control of the weed in line with the statement above.

1.1.1 Biological control

Biological control of invasive alien plant species uses natural enemies (insects, mites, bacteria, or fungi) to reduce the population of the targeted invasive alien plants to below ecological or economic thresholds (De Bach & Rosen, 1991; Harley & Forno, 1992; Van Lenteren, 2000; Eilenberg *et al.,* 2001; Wilgen & De Lange, 2011). Biological control can be implemented in three ways, viz. classical, augmentative, or conservation biological control (Greathead, 1995; Eilenberg *et al.,* 2001; Culliney, 2005; Tscharntke *et al.,* 2007).

Classical biological control involves identifying the natural enemies of an invasive pest and importing, testing and establishing these natural enemies to control the pest (Harley & Forno, 1992; Culliney, 2005). Augmentative biological control involves the release of a large number of natural enemies in a specific area such as a field, garden, or greenhouse to suppress a pest population. In the augmentative approach, the natural enemies are released periodically, either inundatively or through inoculation. Inundation means releasing a high number of individuals that are expected to have an immediate impact on the pest population, whereas inoculation involves releasing smaller numbers of individuals that are expected to provide control in the future by increasing their numbers through reproduction (Eilenberg *et al.,* 2001). Lastly, conservation biological control involves protecting the natural enemies that already exist in the region by providing non-host food resources and reducing the use of insecticides (Eilenberg *et al.,* 2001; Culliney, 2005). Of these approaches, classical biological control is most strongly recommended for invasive alien plants because it is regarded as the only tool that can permanently reduce the ecological and economic impacts of these plants (Culliney, 2005; Sheppard *et al.,* 2005).

1.1.2 History of biological control of invasive alien plants in South Africa

Biological control programmes against invasive alien plants have been conducted throughout the world since the beginning of the 20th century and have used a wide range of biological control agents and invasive species (Klein, 2011). In South Africa, biological control of weeds started in 1913 with the release of the cochineal insect, *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae), to control drooping prickly pear, *Opuntia monacantha* Haworth (Cactaceae) (Moran *et al.,* 2013). Jointed cactus infested about 850 000 ha in the drier Eastern Cape, but the cochineal insect effectively reduced the area invaded by this weed to below 100 000 ha (Moran & Zimmermann, 1991; Zimmermann *et al.,* 2004; Moran *et al.,* 2013). Initially, biological control programmes expanded to other invasive species that posed threats to agriculture, but now include some of the worst environmental weeds in South Africa (Klein, 2011). Since the release of the first control agent, 146 biological control agent species (which include invertebrates and pathogens) have been released against 57 weed species in South Africa (Klein *et al.*, 2011).

1.1.3 The use of single vs multiple agents in weed biological control

The introduction of multiple agents in programmes to control weeds biologically has become a subject of debate (Jackson & Myers, 2008). Some researchers argue that releasing multiple agents results in competition between the agents and, ultimately, less effective control (Denoth *et al*, 2002; Myers & Bazely, 2003; Myers, 2008). For example, Crower and Boucheir (2006) state that when two or more agents are released on a target weed, competition occurs, as in the case of the gall fly, *Urophora affinis* Frauenfeld (Diptera: Tephritidae), and the weevil, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), which were released onto spotted knapweed, *Centaurea stoebe micranthos* L. (Asteraceae) in various combinations in southern British Columbia, Canada. The results of the study showed that the two agents competed with each other in that when the agents were released together, there was less impact on plant growth than when the agents were released separately. Conversely, Pecora and Dunn (1989) showed that the introduction of multiple agents was additive and caused greater damage to the targeted weed population. They supported their statement with an experiment conducted on leafy spurge with six *Aphthona* spp (all leaf beetles (Coleoptera: Chrysomelidae) that prefer different habitats. The six species (*Aphthona flava* Pemberton and Rees, *A. cyparissiae* Pemberton, *A. czwalinae* Pemberton, *A. nigriscutis* Foudras, *A. adbominalis* Duftschmid and *A. lacertosa* Guilebeau) were effective in controlling the weed because they all attack different parts of the plant. Some attack leaves, while others feed on shoot tips, stems, the root crown, and deep secondary roots. This combination was necessary for the successful biological control of this weed (Pecora & Dunn, 1989; Anderson *et al.*, 2000; Baker & Webber, 2008). Myers (1985) suggested two models to explain how the introduction of multiple agents can lead to successful biological control, namely the lottery model and the cumulative stress model. The lottery model refers to the simultaneous introduction of multiple agents with the expectation that only one agent will be successful (Myers, 1985), while the cumulative model means introducing multiple agents sequentially that attack different parts of the plant (Anderson *et al.,* 2000). Denoth *et al.* (2002) and Myers (2008) reviewed biological control of weed programmes where multiple agents were introduced and both studies showed that, despite multiple agents being released simultaneously, one agent was usually sufficient to control the target weed (Myers, 1985, 2008; Denoth *et al.,* 2002) (Table 1.1).

Table 1.1: Successful biological control programmes where multiple agents have been released (from Myers, 1985, 2008; Denoth *et al.,* 2002).

Weed	Country	Number of agents released	Successful agents	Lottery/Cumulative model		Ref.
				Lottery	Cumulative	
Centaurea diffusa Lamarch (Asteraceae)	Canada	Twelve agents	*Larinus minutus* Gyllenhal (Coleoptera: Curculionidae)	Yes	No	Boucher *et al*., 2002
Ageratina riparia King & Robinson (Asteraceae)	New Zealand and Hawaii	Two agents (New Zealand) Three agents (Hawaii)	*Entyloma ageratinae* Barreto &. Evans (Entylomatales: Entylonataceae)	Yes	No	Trujillo, 2005.
Mimosa pigra Linnaeus (Fabaceae).	Australia	Six agents	*Carmenta mimosa* Eichlin & Possoa (Lepidoptera: Sesiidae).	Yes	No	Paynter, 2005
Centaurea stoebe Lamarch (Asteraceae)	Canada	Twelve agents	*Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae)	Yes	No	Story *et al*., 2006
Asparagus asparagoides (Linnaues) Druce (Asparagaceae)	Australia	Three agents	*Puccinia myrsiphylli* (Thuem) Winter (Pucciniales: Pucciniaceae)	Yes	No	Morin & Edwards, 2006; Morin *et al*., 2006
Carduus nutans Linnaeus (Asteraceae)	New Zealand	Two agents	*Urophora solstitialis* Linnaeus (Diptera: Tephritidae)	Yes	No	Groenteman *et al*., 2007
Lythrum salicaria Linnaeus (Lythraceae)	Canada	Two agents	*Galerucella calmariensis* Linnaeus (Coleptera: Chrysomelidae)	No	Yes	Blossey *et al*., 2001

			and *Galerucella pusilla* Linnaeus (Coleptera: Chrysomelidae)			
Senecio jacobaea Linnaeus (Asteraceae)	Oregon	Two agents	*Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae)	Yes	No	James *et al.*, 1992
Ageratina adenophora King & Robinson (Asteraceae)	South Africa	Two agents	*Procecidochares utilis* Stone (Diptera: Trypetidae) and *Passalora ageratinae* Crous & Wood (Mycosphaerellales: Mycosphaerellaceae)	No	Yes	Heystek *et al.*, 2011; Buccellato *et al.*, 2012

Even though Table 1.1 shows that a single biological control agent species is sufficient to control the target weed (Myers, 2008), a cumulative stress model is often also required to control weeds, especially those whose environmental range might be greater than that of their introduced natural enemies (Anderson *et al.,* 2000; Myers, 2008). The introduction of multiple agents requires that they interact synergistically in order to reduce the invasiveness of the weed (McEvoy & Coombs, 1999). A good example of the cumulative stress model is the biological control of *Sesbania punicea* (Cavanilles) Bentham (Fabaceae) in South Africa instigated by Hoffmann and Moran (1998), who studied the impact of three weevil species on *S. punicea* populations. The three weevils selected were *Trichapion lativentre* Beguin-Billecocq (Coleoptera: Curculionidae), which primarily destroys the flower-buds; *Neodiplogrammus quadrivittatus* Olivier (Coleoptera: Curculionidae), a stem borer, the larvae of which bore into the trunk and stems, and *Rhyssomatus marginatus* Fahraeus

(Coleoptera: Curculionidae) that destroys the developing seeds. This study concluded that all three biological control agents were necessary to control *S. punicea* successfully, since the absence of any one or more of these species resulted in reduced control (Hoffmann & Moran, 1998). Although there are different opinions about the validity of the two models (lottery and cumulative), both play an important role in explaining the success of biological control programmes (Myers, 1985, 2008; Denoth & Myers, 2002; Denoth *et al*., 2002; Jackson & Myers, 2008). More recently, it has been suggested that a cautionary approach should be adopted and one agent species should be released first and if it does not significantly reduce the density of the weed, the introduction of other agent species is justified (Jackson & Myers, 2008). The introduction of multiple agents for the biological control of water hyacinth in South Africa (presented below) is an example of such a programme.

1.2 LITERATURE REVIEW

1.2.1 Introduction

Water hyacinth is a free-floating perennial weed that is branded the worst aquatic weed in the world because of its negative impacts on aquatic ecosystems (see Center *et al.,* 1999; Van Wyk & van Wilgen, 2002; Hill *et al*., 2012). The weed is dispersed mainly through human activities and its rapid spread in the absence of natural enemies creates extensive floating mats that are difficult to control and costs millions of dollars in countries that it invades (De Lange & Van Wilgen, 2010; Chamier *et al*., 2012). Water hyacinth is one of eight species in the genus *Eichhornia* (Cook, 1998; Strange *et al*., 2004; Coetzee *et al*., 2009) and of the eight species water hyacinth is currently the only invasive species (Coetzee *et al*., 2009).

1.2.2 Biology and ecology

A mature water hyacinth plant can grow to up to 1 metre in height. It consists of long, pendant roots, rhizomes, stolons, leaves, inflorescences and fruit clusters. Leaves are a shiny, dark green, growing in rosettes with distinctive, erect, swollen, bladder-like petioles (Center *et al*., 1999b) (Fig. 1.1). Flowers are pale violet or blue and the inflorescence bears 6–10 lily-like flowers, each 4–7 cm in diameter (Barrett, 1989). The upper petal has a prominent dark blue, yellow-centred patch. Fruit contains capsules with fine seeds that are viable for 20 years (Barrett, 1989).

Figure 1.1: *Eichhornia crassipes* (Drawn by Wilma Roux, first published in Henderson & Cilliers 2002).

The weed flowers in summer (October – January) and grows abundantly during its flowering season and reproduces both sexually and asexually (Villamagna, 2009). Sexual reproduction is through flower and seed production. Flower stalks bend back into the water after they are pollinated and release the seeds into the water body once they are mature. Seeds sink to the bottom and germinate following favourable conditions (Zhang *et al.*, 2010). Vegetative reproduction through the production of daughter plants (ramets) occurs from late spring through to autumn in sub-tropical and temperate regions (Villamagna, 2009). Daughter plant stolons break off at the water surface, forming new plants which can multiply rapidly, doubling in population size within a period of 5–10 days under favourable conditions (Dar *et al.*, 2011; Patil *et al.*, 2011). Water hyacinth grows very well in temperatures from 28 ^{0}C to 30 ^{0}C and temperatures above 33 ^{0}C obstruct further growth (Center *et al.*, 2002).

At -3 ^{0}C, water hyacinth lasts for 12 hours and the leaves are destroyed, and at -5 ^{0}C the plant dies within 48 hours (USEPA, 1988).

The growth of the weed is significantly influenced by nutrient levels in the water, mainly nitrogen, phosphorus and potassium (Reddy *et al.*, 1989, 1990). Rivers that suffer from nutrient pollution, in particular nitrogen and phosphorus, are the best places for the rapid growth of water hyacinth (USEPA, 1988). The plant tolerates drought through seeds and can survive in moist sediments up to several months (Center *et al.*, 2002). Normally, water hyacinth grows best in pH of 5.5–7.0 (Lu, 2009). However, the plant can tolerate pH values from 4-10 (El-Gendy *et al.*, 2004).

1.2.3 Origin and distribution of water hyacinth

Water hyacinth is native to the Amazon Basin of South America, but has spread throughout the world since the late 1800s (Center, 1994; Julien *et al.*, 1996; Zhang *et al.*, 2010). It was first introduced into the African continent at the end of the nineteenth century. After that it spread very quickly to tropical and sub-tropical regions of Africa, where it disturbs many wetlands, rivers and lakes (Gopal, 1987) (Table 1.2).

Table 1.2: Distribution of water hyacinth in African countries, with first recorded dates and the infested sites and regions (Jones, 2009; Akpabey, 2012).

Country	First record	Infected sites and regions	References
Angola	1972	Kwanza River and related irrigation networks.	Navarro & Phiri, 2000.
Benin	1977	So and Ouémé rivers,	Navarro & Phiri, 2000.

		Lake Nokoune.	
Burkina Faso	1989	?	Navarro & Phiri, 2000.
Burundi	1957	Kagera River.	Navarro & Phiri, 2000.
Cameroon		Country's wetlands.	Barrett, 1989.
Central African Republic	1982	Sangha River.	Desembo pers. comm., cited by Akpabey, 2012.
Congo	1950 - 51	Ntombo & Kouiloa Rivers.	Gopal 1987.
Cote d' Ivoire	1980s	Comoe, Bandama and Sassandra rivers. Tabbo, Buyo and Grah dams.	Koffi Koffi *et al.,* 1999.
Democratic Republic of Congo	1952	Congo River.	Navarro & Phiri, 2000
Egypt	1932	Nile River and related irrigation networks, and northern lakes.	Fayad, 1999; Navarro & Phiri, 2000.
Equatorial Guinea	?	?	Barrett, 1989.
Ethiopia	1956	Lake Tana. Baro, Gillo and Pibor Rivers	Navarro & Phiri, 2000.
Gabon	?	?	Barrett, 1989.
Ghana	1984	Tano Lagoon and Acca/ Tema water areas.	Navarro & Phiri, 2000.
Guinea-Bissau	?	?	Barrett, 1989.
Kenya	1982	Lakes Victoria and Naivasha.	Ochiel *et al.,* 1999.
Liberia	?	?	Barrett, 1989.
Malawi	1960's	Zambezi and Shire rivers. Lake Malawi.	Navarro & Phiri, 2000.
Mali	1987	Niger River.	Navarro & Phiri, 2000.
Mozambique	1946	Incomati River.	Navarro & Phiri, 2000.
Niger Republic	1987	?	Navarro & Phiri, 2000.
Nigeria	1984	Coastal areas of Ogun, Ondo, Edo, Delta, Bayelsa, Cross River and Akwa Ibom.	Navarro & Phiri, 2000.
Rwanda	1957	Kagera River.	Navarro & Phiri, 2000.

Senegal	2003	?	Diop pers. comm., cited by Akpabey, 2012.
Sierra-Leone	?	?	Barrett, 1989.
South Africa	1908	Water bodies of the country, mainly in Western Cape, Free State, Mpumalanga and Eastern Cape.	Stent, 1913; Cilliers, 1991.
Tanzania	1955	Lake Victoria; Pangani, Kagera and Sigi Rivers.	Navarro & Phiri, 2000.
The Gambia	?	?	Barrett, 1989.
Togo	1987	?	Navarro & Phiri, 2000.
Uganda	1988	Lakes Victoria, Kyoga and Kwania.	Ogwang & Molo, 1999.
Zambia	1960	Kafue River and Kafue Dam.	Kampeshi & Shantima, 1999.
Zimbabwe	1937	Lake Chivero and Manyame River.	Chikwenhere *et al.,* 1999; Navarro & Phiri, 2000.

1.2.4 Introduction to South Africa

According to Stent (1913), water hyacinth in South Africa was first recorded in 1908 on the Cape Flats and in KwaZulu-Natal. However, Du Toit (1938) and Edwards and Musil (1975) (in Gopal 1987), reported that the first introduction of water hyacinth into South Africa was in 1910 in KwaZulu-Natal. It is now dispersed throughout the country (Figure 1.2) and has become a serious pest plant in the country's wetlands and freshwater bodies (Henderson, 2001).

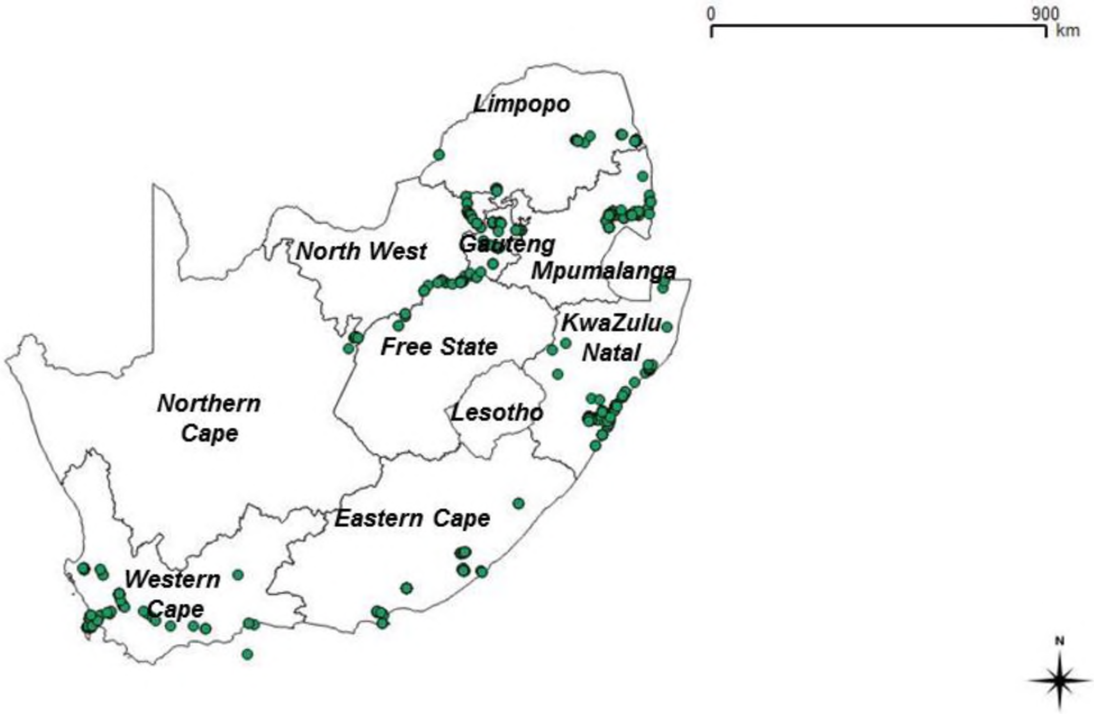


Figure 1.2: Distribution of water hyacinth in South Africa. (SAPIA Database, ARC –PPRI).

1.2.5 Negative impacts

Negative impacts of water hyacinth can be divided into its effects on the environment and on the socio-economy (Hill, 1999; Coetzee *et al.*, 2009). Large mats of water hyacinth plants prevent oxygen and light penetrating from the air to the water surface, resulting in the decrease of oxygen production by indigenous plant species. Plants die and sink to the bottom, and decomposition further depletes oxygen content in the water body (EEA, 2012). Low oxygen content reduces phytoplankton and zooplankton levels, resulting in the total disturbance of the aquatic ecosystem. Furthermore, the low concentration of dissolved oxygen speeds up phosphorus production by the decomposed biomass, which accelerates eutrophication. Algae and bacteria blooms from the decomposed biomass cause taste and odour problems in drinking water and increase the cost of purification (van Wyk & van

Wilgen, 2002). Harmful animals, such as crocodiles and snakes, use floating water hyacinth mats as hiding places from which to attack other animals and people (Ndimele *et al.*, 2011; Patel, 2012). Roots, leaves and stems of the plant provide breeding grounds for mosquitoes, which are vectors for malaria. Between 1994 and 2008, water hyacinth infestations increased the incidence of cholera infections in Nyanza Province in Kenya (Feikin *et al.*, 2010)

The dense mats of water hyacinth obstruct human activities by restricting fishing from the shore, boat navigation and tourism (Ndimele *et al.*, 2011; Patel, 2012). The Lake Victoria fish catchment rate decreased by 45 % because water hyacinth mats blocked access to fishing grounds (Kateregga & Sterner, 2009) and, at some sites in Nigeria, water hyacinth makes fishing impossible (Ndimele *et al.*, 2011). The rapid growth of water hyacinth affects many large hydropower schemes throughout the world. For example, the Owen Falls hydro power scheme at Jinja in Uganda was compromised by water hyacinth infestation, with the costs for cleaning estimated at US$1 million per annum (Mailu, 2001). In the Brahmaputra River in India, the weed blocks irrigation channels and obstructs water flow to crop fields (Patel, 2012). In Cameroon, the communities of Bwene and Bonjo, and the Wouri River Basin are the victims of floods during the rainy season that are exacerbated by water hyacinth (Mujingni, 2012).

1.3. CONTROL OPTIONS

1.3.1. Introduction

Four popular control methods are used to eradicate and control water hyacinth, namely, manual, mechanical, herbicide and biological, or a combination of these in an integrated approach (Cilliers, 1991). Each control has its benefits and drawbacks (Cilliers *et al*., 1996).

1.3.2 Manual control

Water hyacinth can be controlled by hand pulling the weed from the water surface (Patel, 2012). According to recent work done by the Environmental Planning and Climate Protection Department of eThekwini Municipality (EPCPD, 2014), the method has been used for job creation in South Africa (EPCPD, 2014) and the Working for Water Programme of the Department of Environmental Affairs in South Africa employs about 30 000 people annually for this purpose (De Lange *et al.,* 2010). The EPCPD and Parks, Leisure and Cemeteries Department (PLC) co-ordinate the clearing of water hyacinth in the uMbilo River catchment as part of the Durban Community Ecosystem-based Adaptation (CEBA) Initiative, employing members from local areas of uMbilo (EPCPD, 2014). Local businesses also participate in a variety of activities such as Invasive Alien Plant (IAP) control, tree planting, waste collection and recycling (EPCPD, 2014). Manual control was used on Lake Chivero in Zimbabwe, where 500 people were employed and 500 tonnes of water hyacinth were removed, but, typical of this intervention, the weed rapidly regenerated (Coetzee *et al*., 2009; Byrne *et al*., 2010). Manual removal of water hyacinth is appropriate only for small areas of less than 1 ha. Manual control is also very expensive in terms of labour costs and there is the added risk of workers drowning (Coetzee *et al*., 2009).

1.3.3 Mechanical control

Mechanical control is conducted using boats, bulldozers, conveyors and mechanical harvesters (Malik, 2007). The control costs are often very high (Hill, 1999), but the method has proved to be successful in localized areas where water hyacinth has caused a water body to become impenetrable (Cilliers, 1991). Mechanical control was practised on Benoni Lakes, a series of three 10–20 ha lakes in Gauteng, South Africa (Rwizi, 2014; Hoy *et al.,* 2015). The lakes were invaded by water hyacinth plants which had a negative impact on areas such as Lakeside Mall because the lake view had completely disappeared, and no water sport activities were possible (Rwizi, 2014; Hoy, 2015). The Ekurhuleni Municipality employed people and hired harvesters and crusher boats to clear up the lake and the control was successful (Hoy, 2015). Although the lakes were cleared, the cost was high, at R7.5 million per annum. Non-target species are also affected in mechanical control programmes (Cilliers, 1991; Center *et al*., 1999a). In addition, regeneration of the infestation occurs through seed germination and vegetative growth of plant material left behind which is able to regrow rapidly (Center *et al*., 1999a). Finally, this form of control interferes with the establishment of biocontrol agents.

1.3.4 Herbicidal control

Herbicidal control is often the most widely used method to control water hyacinth. Chemicals are applied by spraying water hyacinth plants with herbicides such as 2,4-D; Diquat and Glyphosate (Julien *et al*., 1999; Coetzee *et al*., 2009; EPCPD, 2014). This method is commonly used in South Africa and it offers a quick solution to the pressing problem, since it takes only six weeks for the plant to die and sink (Dagno *et al*., 2012). Herbicides were used on Hartebeespoort Dam, South Africa in the 1970s to control the weed (Ashton *et al*., 1979). The Environmental Planning and Climate Protection Department of eThekwini Municipality (EPCPD, 2014) applied herbicides in Umdloti Estuary. Unfortunately, however, the operation resulted in the subsequent decay of plant biomass, which in turn caused a sharp decline in the dissolved oxygen concentrations in the estuary, resulting in a fish kill incident (EPCPD, 2014). Regardless of the success of the method, herbicide control is not environmentally friendly, since it kills non-target phytoplankton, zooplankton and other aquatic plants, contaminates drinking water and threatens human health (Julien *et al*., 1999; Malik, 2007; Coetzee *et al*., 2009; Dagno *et al*., 2012). Glyphosate mixtures also contain toxic surfactants which are associated with the death of zooplankton (Relyea, 2005a, b, c). Furthermore, this method is expensive and requires specialised training and safety measures before application (Dagno *et al*., 2012). Finally, if plants are missed, regeneration of the infestation usually occurs soon after, through germination of the seed bank (Coetzee *et al*., 2009).

1.3.5 Biological control

Biological control of water hyacinth is considered the most environmentally friendly and sustainable control method, since it does not require long-term maintenance, is low cost and it has no negative impact on the environment (Cilliers, 1991). Biological control of water hyacinth is widely used throughout the world where the weed is a problem, with the mainstays of biological control being the weevils *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae), which were initially tested against 274 plant species in 77 families worldwide (Julien *et al*., 1999).

Water hyacinth was the first aquatic weed to be targeted for biological control in South Africa, in 1974, and the first biological control agent released against the weed was the weevil, *N. eichhorniae* (Cilliers, 1991). To date, nine biological control agents have been released against the weed in South Africa (Coetzee *et al.,* 2011). After the first biological control efforts with water hyacinth, other aquatic weeds such as *Salvinia molesta* Mitch. (Salviniaceae); *Pistia stratiotes* Linnaeus (Araceae); *Azolla filiculoides* Lamarck. (Azollaceae) and *Myriophyllum aquaticum* (Vellozo) Verdcourt (Haloragaceae) were also successfully brought under control (Coetzee *et al.*, 2011). Successful control of water hyacinth was shown at New Year's Dam, Alicedale, Eastern Cape, South Africa (Hill & Olckers, 2001), where 200 *N. eichhorniae* adults were the only agents released against the weed in January 1990, with another 1000 adults released in October that year. Within four years, the infestation was reduced to just 20% (Hill & Coetzee, 2017).

1.4 BIOLOGICAL CONTROL AGENTS RELEASED IN SOUTH AFRICA

South Africa has released more agents against water hyacinth than any other country in the world. The information on these agents is presented below.

Neochetina eichhorniae is a stem borer and leaf feeder released in 1974, and which is now widely established (Cilliers, 1991). The fungal pathogen, *Cercospora piaropi* Tharp (Mycospharellalles: Mycosphaerellaceae), was released in 1987 (Coetzee *et al.*, 2011; Patel, 2012; Ray & Hill, 2012). *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae), a leaf-mining mite, was released two years later (Hill & Cilliers, 1999; Oberholzer, 2001). *Neochetina bruchi*, a stem borer weevil, was released in 1990 and again in 1996 (Coetzee *et al.*, 2011). *Niphograpta albiguttalis* Warren (Lepidoptera: Crambidae), a petiole borer, was also released in 1990 (Julien *et al*., 2001b; Center *et al*., 2002). *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae), a leaf sucker, was released in 1996 (Julien, 2001; Hill *et al*., 1999; Coetzee *et al*., 2005, 2009), and in 2007, *Eccritotarsus eichhorniae* Henry*,* another mirid leaf sucker, was released (Paterson *et al.,* 2016). *Cornops aquaticum* Brüner (Orthoptera: Acrididae), a leaf feeder, was released in 2011, but its establishment is not confirmed (Bownes, 2010; Winston *et al.,* 2014). *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a leaf hopper, was released in 2013 (Sosa *et al*., 2004, 2005; 2007a, b; Tipping *et al*., 2008, 2011, 2014).

1.5 BARRIERS TO SUCCESSFUL BIOLOGICAL CONTROL OF*EICHHORNIACRASSIPES* IN SOUTH AFRICA

Despite the nine biological control agents released against water hyacinth in South Africa, the control status of the weed has been less successful than elsewhere in the world. Lack of success has been ascribed to eutrophic waters and climatic conditions that result in rapid growth of the plant in summer and cold winters that reduce biological control agent populations (Hill & Olckers, 2001; Julien, 2001). Extremely low winter temperatures cause high mortality of biological control agents and a low reproduction rate in those surviving. However, water hyacinth plants grow very rapidly in summer and biological control agents fail to reach the level at which they can cause significant damage until the end of summer because they are still recovering from the cold winter (Cilliers & Hill, 1996). Further, because most South Africa wetlands are small and shallow and not exposed to wind and wave action, water hyacinth plants do not sink properly after damage, and the roots merely rest on the substrate and regrow (Hill & Olckers, 2001). These conditions prompted Hill and Olckers (2001) to suggest that multiple agents were required to control water hyacinth in South Africa and subsequently three additional species were released with no consideration of how they would interact. The number of agents now released against water hyacinth make South Africa an ideal case studyl for investigating the effects of multiple agents.

1.6 INTERACTIONS BETWEEN BIOLOGICAL CONTROL AGENTS OF WATER HYACINTH

The interactions between biological control agents can have complex and unexpected consequences for weed control. Biological control agents may compete with each other for natural resources, or may complement each other to control the target weed (Ajuonu *et al.*, 2003). Because a number of control agent species have been released against water hyacinth in South Africa, there are a number of potential ecological interactions that may occur between them, thereby affecting the ultimate success of the control programme (Coetzee *et al.*, 2009). Several studies have been conducted to determine the interactions between various combinations of water hyacinth control agents. Early studies by Del Fosse (1997a, b, 1978) examined the interactions between the leaf-mining mite *O. terebrantis* and the weevil, *N. eichhorniae* and recorded a positive relationship between the two agents, *i.e.* in the presence of weevil, the mite produced more eggs and fed more extensively (Del Fosse, 1997a, b, 1978). The best example of water hyacinth agents complementing each other has been noted between the two *Neochetina* weevil species, *N. eichhorniae* and *N. bruchi*, where the control of water hyacinth is enhanced when the two agents occur together, especially under eutrophic conditions (Julien *et al.*, 1999). Another example is the research into the feeding behaviour and spatial distribution of two planthoppers, *Megamelus scutellaris* and *Taosa longula* Remes Lenicov (Hemiptera: Dictyopharidae), a study which showed that the agents complement each other and can be released together to control water hyacinth (Hernánderz *et al.*, 2011a, b). Ajuonu *et al.* (2007) and Weyl and Hill (2012) examined the interactions between *Eccritotarsus catarinensis* and *N. eichhorniae* and *N. bruchi.* While these authors found little negative interaction between these agents, Ajuonu *et al.,* (2007) discovered that the establishment of the mirid in the field was not successful where weevils had been established for some time. The performance of the mirid (adults and nymphs) significantly decreases when it is exposed to plants with a large number of old feeding scars created by weevils. However, where feeding scars are fresh, the mirid performed significantly better. The effect of mirid feeding on the weevil populations was not considered (Ajuonu *et al.*, 2007).

Additional studies investigated the interactions between the weevil (*N. eichhorniae*), the leaf-mining mite (*O. terebrantis*), and the sap-sucking mirid (*E. catarinensis*) in single and paired combinations (Marlin *et al.*, 2013a). The mirids and the weevils performed better in combination, with little negative interaction. *Orthogalumna terebrantis* performed better in the absence of *N. eichhorniae* and *E. catarinensis*, but all three of these agents can co-exist in the field (Marlin *et al.*, 2013a). In another study, both weevil species were released against water hyacinth plants grown at different nutrient levels, from low to high. *Neochetina bruchi* reduced water hyacinth growth significantly more than *N. eichhorniae* at high nutrient concentrations, and thus *N. bruchi* was the most effective agent in controlling water hyacinth when released under eutrophic conditions in the field (Heard & Winterton, 2000).

1.7 THESIS OUTLINE AND AIMS

In general, the previous studies show that multiple agents may complement rather than hinder each other (Marlin *et al.*, 2013a). Even though nine biological control agents have been released against water hyacinth in South Africa (Coetzee *et al.*, 2011), the biological control programme for water hyacinth in South Africa has still not been as successful as it could be, and since the suggestion by Hill and Olckers (2001) that introducing additional agents could have a greater impact on the control of the weed, new agents have been released with little consideration given to agent interactions.

This study investigates the impact of the two recently released sap-sucking agents, *Megamelus scutellaris* and *Eccritotarsuseichhorniae,* together with the well-known and successful weevil, *N. eichhorniae,* on water hyacinth growth parameters. The overall aim of the thesis is to determine if the addition of two new biological control agents, *M. scutellaris* and *E. eichhorniae,* in South Africa for water hyacinth control is compatible with the most widespread agent species, *N. eichhorniae*. The results of the study will improve the effective biological control programme of water hyacinth in South Africa.

CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

This chapter outlines the research design and methodology for the experiments conducted using various combinations of biological control agents to determine whether the interactions between the agents affect the level of control of water hyacinth. The methods include the experimental design, data collection and data analyses. All three experiments were conducted under control conditions in a polyurethane tunnel and in a shade house at the Waainek Research Facility (S 33^0 30$'$ 94.55$''$, E 26^0 50$'$ 06.25$''$) at Rhodes University, Grahamstown, Eastern Cape, South Africa.

2.1.1 Study species

2.1.1.1 Neochetina eichhorniae Hustache (Coleoptera: Curculionidae)

Description

Neochetina eichhorniae was the first agent released against water hyacinth in South Africa in 1974 (Julien *et al*., 2001b; King, 2011). The nocturnal adults are 4 mm long, grey in colour and lay their eggs (which are small, whitish, slender and soft) underneath the epidermis of the leaves (Center, 1994).

Life history

Eggs hatch at 20 ^{0}C ten days after they have been laid. Larvae tunnel inside the petiole and into the crown, causing water logging and, ultimately, the death of the plant (DeLoach & Cordo, 1976). Pupation occurs on the roots of the plant below the surface of the water. Larvae take 60–90 days to reach the adult stage. The adults emerge seven days after pupation (Center, 1994; Julien *et al.,* 2001a), and feed on the leaves of the water hyacinth plant (Center, 1994). Adult feeding causes distinctive feeding scars on the leaf surface which are clearly visible and easily recognised. The presence of weevil larvae is evident from the streaks of necrotic tissue just beneath the epidermis of the petiole (Center, 1994). *Neochetina eichhorniae* feeding damage kills leaves, and the larva causes water logging of the petioles; eventually the whole plant dies and the mats sink (Julien *et al.*, 1999; Heard & Winterton, 2000).

2.1.1.2 *Eccritotarsus eichhorniae* Henry (Heteroptera: Miridae)

Description

Eccritotarsus eichhorniae is a sap-feeding mirid native to South America (Paterson *et al.,* 2016). The mirid was collected in Peru and released in South Africa in 2007 (Winston *et al.*, 2014).

Life History

Eccritotarsus eichhorniae biology is very similar to that described by Hill *et al.* (1999) for *E. catarinensis* (Henry, 2017). Mating occurs on the surface of a water hyacinth leaf, and eggs are laid horizontally and separately into the leaf tissue, mainly on the abaxial surface, and hatch after nine days (Julien, 1999; Hill *et al.,* 1999; Coetzee *et al.,* 2009). Nymphs are pale or creamy white and nearly transparent, with visible red eyes. They vary in length, with the first instar being about 0.09 mm and the fourth instar about 2.83 mm (Hill *et al.,* 1999). Nymphs take 15 days to reach the adult stage, and they feed in groups on the under-surface of the leaves. Both adults and nymphs produce black frass on both sides of the leaves (Hill *et al.,* 1999; Coetzee *et al.,* 2005). Adults are slender with pale legs and reddish eyes and hyaline patches on the wings. The abdomen of males is slender with a yellow tip, while the abdomen of females is rounded and entirely black (Hill *et al.,* 1999). Adults are very active and easily disturbed and they react by hiding underneath the leaf surface or flying off (Coetzee *et al.,* 2005).

2.1.1.3 Megamelus scutellaris Berg (Hemiptera: Delphacidae)

Description

Megamelus scutellaris is a phloem-feeding bug native to Peru, Brazil, Uruguay, Argentina, and all localities where water hyacinth grows naturally in South America (Sosa *et al.,* 2004, 2005; Fitzgerald & Tipping, 2013; Heard *et al.,* 2014; Sutton *et al*., 2016). *Megamelus scutellaris* was released in South Africa in 2013 after host-specificity studies showed the same results observed in Argentina and in the United States of America in that the insect was specific to water hyacinth (Coetzee, 2013).

Life History

Megamelus scutellaris produces multiple, overlapping generations annually (Sosa *et al.,* 2004, 2005, 2007a, b; Tipping *et al.,* 2008, 2011; Hernandez *et al.,* 2011b). Adults have two wing forms: long-winged (the flying form) and short-winged (the non-flying form). When nutrient levels in the water hyacinth are very low, *M. scutellaris* develop wings so that they can disperse to alternative hosts where nutrient levels are higher (Sosa *et al.,* 2004, 2005, 2007a, b; Tipping *et al.,* 2011; Fitzgerald & Tipping, 2014).

Mating occurs at the base of the water hyacinth plant (Sosa *et al.,* 2005) and also on the upper leaves (Tipping *et al.,* 2008). Females lay pairs of eggs within the leaf tissue a few days after mating (Tipping *et al.,* 2008). The eggs are oval, with one end pointed and the other rounded (Sosa *et al.,* 2005). They are milky-white when laid, turning to yellowish-white with reddish eye spots before they hatch (Sosa *et al.,* 2005).

Nymphs hatch seven to 13 days after the eggs were laid, depending upon the temperature (Sosa *et al.,* 2005; Tipping *et al.,* 2008). They develop through five instars, feeding on both petiole surfaces and leaf stems (Sosa *et al.,* 2005; Tipping *et al.,* 2008). Nymphs take 25 days to reach the adult stage after they hatch, depending upon temperature (Sosa *et al.,* 2005). Adults are about 2.5 to 3 mm (males) and 3 to 3.7 mm (females) in body length, pale cream to dark brown (Sosa *et al.,* 2005) in colour, with a lifespan of about 80 days or more (Tipping *et al.,* 2008). *Megamelus scutellaris* feeding allows pathogen entry, which causes more damage to the plant (Sutton *et al.,* 2016). According to Sosa *et al.* (2005), *M. scutellaris* immature stages overwinter in decaying mats of water hyacinth in Argentina (Sosa *et al*., 2005).

The planthopper feeds by inserting its stylet into the water hyacinth, piercing the plant tissues and cells to reach the sap (Sosa *et al.,* 2005). During penetration, the insect secretes saliva, forming a stylet sheath that acts to hold the stylets together, and enable lubrication and movement towards food sources (Sogawa, 1982). Feeding by *M. scutellaris* damages the petiole which becomes waterlogged, thereby reducing plant toughness and causing the tissue to rot. The damage of the planthopper is evident when leaves of water hyacinth plants start to turn brown, and sooty mould develops on the leaves of the plants (Coetzee, 2013).

2.2 Interactions between *Eccritotarsus eichhorniae* and *Megamelus scutellaris* and their impact on water hyacinth growth

2.2.1 Introduction

The aim of this study was to quantify the interactions between two sap-sucking bugs, *Eccritotarsus eichhorniae* and *Megamelus scutellaris*, and to investigate the effect that these interactions would have on the control of water hyacinth.

2.2.2 Experimental set-up

The experiment was conducted in late summer of 2015, from February to May. Healthy and undamaged water hyacinth plants with a height of 20–30 cm, with four to six leaves, were selected from stock cultures grown in plastic pools at the Waainek Research Facility at Rhodes University, Grahamstown. Seventy 10 L tubs (33 cm by 27 cm, 18 cm deep) were filled with tap water, and two insect-free plants were placed in each tub. Each tub was fertilized with 1.52 g of Ludwig's Vigorosa fertilizer group 1 (N: P: K ratio 5: 1: 5) to provide 8 mg N. L^{-1} which is representative of eutrophic water in South Africa (Coetzee *et al*., 2007). The nutrient concentration was chosen because Holmes (1996) showed that, according to South African Water Quality standards, nitrogen and phosphorus concentrations of these levels are found in impoundments in South Africa, and these concentrations are similar to those used by Coetzee *et al*. (2007) in water hyacinth studies. To prevent chlorosis of the plants, 1 g of commercial iron chelate was added to each tub. Plants were grown for a period of two weeks, allowing them to acclimate to the new environment. During acclimation water levels were maintained weekly. Control treatments were gauze-covered cages, while a procedural control had no insects and no gauze.

Procedural control tubs were set up to compare the effect of reduced light as a result of the netting on the plants.

Two agents, the mirid, *E. eichhorniae,* and the plant hopper, *M. scutellaris* from the same generation were inoculated into each tub, in combinations as shown in Table 2.1. The densities were chosen based on field observations where the agents have established. No biological control agents were inoculated into the two control treatments. Each treatment was replicated 10 times.

Table 2.1: Combination of species and total number of individual insects inoculated in the treatments of the experiment (*Ee* = *E. eichhorniae* and *Ms* = *M. scutellaris*)

Treatment	**Inoculation**
One	*Ms* (20 Ms)
Two	*Ee* (20 Ee)
Three	*Ms* +*Ee* (10 Ms +10 Ee)
Four	*Ms* +*Ee* (15 Ms +5 Ee)
Five	*Ms* +*Ee* (5 Ms +15 Ee)
Six	Control (insect-free plants)
Seven	Procedural control (insect-free plants without netting)

Figure 2.1: Experimental set-up under semi-shade conditions at the Waainek Research Facility.

After two weeks of acclimation, plants in each tub were weighed and several other plant parameters (number of leaves, number of ramets (daughter plants), plant height, leaf width, leaf surface area and chlorophyll content) were measured to obtain initial plant measures. Insects were then added to the plants in the different experimental combinations shown in Table 2.1. *Megamelus scutellaris* and *E. eichhorniae* were sourced from the mass-rearing culture maintained at the Waainek Research Facility. A fine mesh net covered each tub (except the procedural control treatment tubs) to prevent the agents from escaping.

2.2.3 Data collection

Data were collected every 14 days throughout the sampling period of 12 weeks.

2.2.3.1 Plant growth parameters

The experiment was conducted over a period of 12 weeks and plant growth parameters mentioned above were measured once every two weeks. The chlorophyll content was measured from the fourth expanded leaf (leaf 4) of each plant, using an Apogee CCM-200 plus chlorophyll content meter (ADC BioScientific Ltd, Hoddeson, United Kingdom). Wet weight was measured again at the end of the experiment, using a digital bench-top kitchen scale (Clicks©, South Africa). The change in plant biomass was assessed by weighing the plants before and after the experiment; wet biomass included a number of ramets. Fresh weight was measured instead of dry weight because the two measures are highly correlated (T.D. Center, unpublished data) and fresh weight was the more convenient measure.

2.2.3.2 Insect parameters

Damage caused by both agents was measured every two weeks, usually by recording the percentage area damaged in the abaxial surface area of leaf 4. Both agents preferred to feed on younger leaves, the first and second expanded leaves (leaves 1 and 2), but damage was more evident on leaf 4. Leaf 4 was chosen in this study because a study by Marlin *et al.*, (2013) revealed that older leaves are exposed to the herbivory for a longer time and the damage caused by the agents is cumulative. The visual estimation method was used to record the total damage caused by the agents (Coetzee *et al.*, 2007). A different scale to that used by Coetzee *et al.* (2007) was chosen for this study because the feeding damage of *M. scutellaris* covers the surface area of the leaf in a short period of time and damage by both agents looks similar. The surface area damage on the leaf by the agents indicates the presence and the population increase of the agents (Weyl & Hill, 2012a; Marlin *et al.*, 2013a) and so the percentage area damaged by each agent in a single treatment was measured. At the end of the experiment, *E. eichhorniae* and *M. scutellaris* were collected from the cage and counted to measure the population size of each agent.

Eccritotarsus eichhorniae Henry (Heteroptera: Miridae)

Feeding intensity was scored by estimating damage using a scale of 1–3, where 1 is slight speckling and 3 is almost total chlorosis of the leaf, which appears yellow to white. The data were recorded by one observer throughout the experiment.

Megamelus scutellaris Berg (Hemiptera: Delphacidae)

Percentage area covered by the feeding of *M. scutellaris* was scored using a scale 1 to 3 on the adaxial surface area of leaf 4. Feeding parameters recorded included sooty mould and oviposition scars, which are the scars caused by the *M. scutellaris* female when laying eggs in the petiole of a plant. Scars were recorded by counting the number of scars on each petiole.

Each feeding parameter was recorded for the agent in single and in combination treatments.

2.2.4 Data analysis

All the data recorded during the experiment were analysed using the statistical programme, STATISTICA Version 13 (© StatSoft, Inc., USA).

2.2.4.1 Plant growth parameters

There were no significant differences between the treatments, $P < 0.05$ on plant growth parameters measured before the experiment. General Linear Model (GLM) one-way ANOVAs were conducted to test differences in plant growth parameters between the treatments, at the beginning and the end of the experiment (at week 12). A Tukey HSD post-hoc test was conducted to test for the significant differences in the homogeneous groups. A one-way ANOVA was conducted for each plant growth parameter to compare the insect treatments and the two controls in order to determine whether the netting had an effect on plant growth.

2.2.4.2 Insect parameters

The level of feeding damage by each agent in single and in combination treatments was used as an indication of the insects' performance. The difference between the performance of the insects in the treatments determined whether there was a synergistic, antagonistic, or neutral relationship between them. Two one-way ANOVAs were used, one for each of the agent species. ANOVAs were used to determine whether the agents performed better in combination with each other, or singly. The damage caused by the agent species was separated into the feeding parameters caused by each agent. Each feeding parameter was separated into damage caused by each agent separately and then compared to the damage caused by both agents in combination treatments in order to determine whether the level of feeding by each agent was affected or not by the presence of another agent. A Tukey HSD post-hoc test was used to test the significant differences to identify homogeneous groups.

2.3 Impact of prior feeding by the two agents *Eccritotarsus eichhorniae* and *Megamelus scutellaris* on subsequent feeding by the two agents

2.3.1 Introduction

The purpose of the study was to investigate whether prior feeding by either agent influenced the subsequent performance of the agents of both species in order to determine whether *E. eichhorniae* and *M. scutellaris* can be released together in the field, or whether one of the agents can be released in an area where the other had already established.

2.3.2 Experimental design

The experiment was run in early summer 2015, from August to December and consisted of five treatments, with each treatment replicated ten times (Table 2.2). Fifty tubs were filled with water, and two water hyacinth plants sourced from stock cultures were placed in each tub. Twenty adults of each species were placed in the respective treatments for four weeks to allow for the establishment of prior feeding. At the end of four weeks, plant growth parameters were taken, then another 20 adults of each species were placed in the respective treatments (Table 2.2) to determine whether prior feeding affected feeding by the newly released specimens of the agent.

Table 2.2 The combinations of species inoculated in the treatments of the experiment (*E. eichhorniae* and *M. scutellaris*)

Treatment	**Inoculation**
One	Prior *E. eichhorniae;* subsequent *M. scutellaris.*
Two	Prior *M. scutellaris;* subsequent *E. eichhorniae*
Three	Prior *E. eichhorniae*; subsequent *E. eichhorniae*
Four	Prior *M. scutellaris*; subsequent *M. scutellaris*
Five	Insect-free control

2.3.3 Data collection

Data were collected every 14 days throughout the sampling period of 12 weeks.

2.3.3.1 Plant growth parameters

Plant growth parameters (number of leaves, number of ramets (daughter plants), plant height, leaf width, leaf surface area, and chlorophyll content) were measured every 14 days for a period of 12 weeks. Wet weight and chlorophyll content were measured at the end of the experiment to avoid insect escape.

2.3.3.2 Agent performance

Insect species were inoculated in each tub at different times with the exception of the control treatment. The first group of *E. eichhorniae* and *M. scutellaris* was inoculated as the primary or prior feeding agents. The two agents were allowed to feed for a period of 14 weeks. After that, the insect parameters of both species were recorded, as in experiment one. The second group of *E. eichhorniae* and *M. scutellaris* was inoculated to feed as the subsequent feeding agents. This was done to determine whether *E. eichhorniae* and *M. scutellaris* would be able to feed after feeding damage by the other agents had already been caused. Feeding parameters were recorded every two weeks for a period of 16 weeks. At the end of the experiment, *E. eichhorniae* and *M. scutellaris* were collected in each tub and counted to measure the insect populations.

2.3.4 Data analysis

STATISTICA Version 13 (© StatSoft, Inc., USA) was used to analyse all the data collected during the experiment and at the end of the sampling period.

2.3.4.1 Plant growth parameters

All plant growth parameters recorded at the beginning of the experiment showed no significant differences between the treatments, $P < 0.05$. The differences in plant growth parameters between the treatments at the end of the sample period (at week 12) were tested by conducting GLM one-way ANOVAs. A Tukey HSD post-hoc test was used to identify homogeneous groups. The impact on plant growth parameters was noted on parameters measured at the end of the experiment.

2.3.4.2 Agent performance singly and in combination

The area damage by each insect singly and in combination was used to measure their performance and the population of both agents was used to measure the abundance. A one-way ANOVA was conducted for each agent to determine whether the prior feeding agents affected the performance of the subsequent feeding agents. A Tukey HSD post-hoc test was used to identify homogeneous groups. The damage of both agents was compared with the single and in-combination treatments, as well as the fresh and old feeding scars for both agents.

2.4 The establishment of *Megamelus scutellaris* on water hyacinth plants extensively damaged by the weevil, *Neochetina eichhorniae*

2.4.1 Introduction

The purpose of this part of the study was to examine the effects of *N. eichhorniae* feeding damage on the establishment of, and feeding by, the planthopper. *Neochetina eichhorniae* is the most widely established agent in South Africa (Coetzee *et al.*, 2011), and as such, the sites where *M. scutellaris* will be released are likely to have been damaged by weevil feeding.

2.4.2 Study site

Cultures of water hyacinth plants with extensive *N. eichhorniae* feeding scars were collected from the New Year's Dam, near Alicedale, Eastern Cape. Plants were maintained in 3000 L plastic pools in greenhouse tunnels at the Waainek Research Facility prior to the start of the study.

2.4.2.1 New Year's Dam

The New Year's Dam is a reservoir located at S 33^{0} 18$^{'}$ 6.84$^{''}$; E 26^{0} 6$^{'}$ 45.36$^{''}$ near Alicedale (EC). The dam is 80 ha in size and it is 294 metres above mean sea level (Fraser *et al.,* 2016). Water hyacinth plants were first noted in the area in 1988, and by 1990, the plants covered 80% of the dam (Hill, 2003). A biological control programme was initiated at the dam in 1990 with the release of 200 *N. eichhorniae* weevils. By 1994, feeding by the weevils had reduced water hyacinth cover to 20% (Hill, 2003). On average the leaves of these plants had between 100 to 150 adult weevil-feeding scars. Currently the dam supplies water to the local population of 7000 people for domestic and agricultural use (Doudernski, 2004; Urban - Econ, 2012).

2.4.3 Experimental set-up

Plants with old *N. eichhorniae* feeding scars and insect-free plants were selected from the water hyacinth plant pools described above. Five treatments were set up and each treatment was replicated ten times. Fifty 20 L tubs were filled with tap water and two insect-free plants were placed in the tubs of treatments 1, 2 and 3. Plants with old *N. eichhorniae* feeding scars were placed in treatment 4. *Neochetina eichhorniae* and *M. scutellaris* were inoculated singly and in combination treatments (Table 2.3). Prior to the inoculation of the agents, weevils were sexed to ensure a 1:1 sex ratio. *Megamelus scutellaris* adults were not sexed, but were randomly selected, as they have a 50:50 sex ratio. A fine mesh net covered each tub to prevent the agents from escaping.

Table 2.3 The combinations of species inoculated in the treatments of the experiment (*Megamelus scutellaris* (*Ms*) and *Neochetina eichhorniae* (*Ne*)).

Treatment	**Inoculation of insect species**
One	*Ne alone*
Two	*Ms alone*
Three	*Ne* + *Ms* on *fresh feeding*
Four	*Ms* on old feeding
Five	Control (Insect-free plants)

In a natural field population, two to seven weevils were noted to be sufficient to control one water hyacinth plant (Marlin *et al.*, 2013), whereas ten *M. scutellaris* are required to control one plant (Pers. obs). The stocking densities of both agents chosen were sufficient to cause visible damage to the plant and reduce some plant parameters (Ajuonu *et al.*, 2007; Byrne *et al.*, 2010; Coetzee *et al.*, 2010;; Weyl & Hill, 2012a; Firehun *et al.*, 2015).

2.4.4 Data collection

Data were collected every 14 days for a period of 12 weeks.

2.4.4.1 Plant growth parameters

The experiment was run for a period of 12 weeks. Plant growth parameters were measured as in experiments 1 and 2. Wet weight was used to measure a relative growth rate for each treatment.

2.4.4.2 Insect performance

Neochetina eichhorniae feeding scars were counted on both sides of the second, third and fourth leaf, whilst the number of petioles mined by the larvae was recorded at the end of the experiment. The number of feeding scars observed on the water hyacinth plant per leaf was recorded using a scale of 1–3 (1 = 0–100; 2 = 100–150; 3 = 150–220). This scale was used because Ajuonu *et al.* (2007) noted that one leaf of a water hyacinth plant could have up to 212 feeding scars. Weevil feeding scars were recorded in single and combination treatments on leaf 2, 3 and 4. At the end of the experiment numbers of petioles mined were measured. In addition, the percentage area damaged by *M. scutellaris* on leaves 2, 3 and 4 was measured. To compare the effect of weevil feeding scars on feeding by the planthopper, feeding damage of both agents on leaf 4 was measured in order to assess the cumulative feeding damage. The number of the planthoppers and the weevils in each tub were counted at the end of the experiment to determine the effect of the weevil feeding scars on the mortality of the planthopper.

2.4.5 Statistical analysis

All data recorded during the experiment were analysed using the statistical programme, STATISTICA Version 13 (© StatSoft, Inc., USA).

2.4.5.1 Plant parameters

GLM one-way ANOVAs were conducted to test for differences in plant growth parameters between the insect treatments after the experiment.

2.4.5.2 Insect performance

Feeding damage by both agents was separated into single and combination insect treatments for leaves 2, 3 and 4 in order to measure on which leaf the planthopper or the weevil performed best when in combination, or separately. Feeding scars by both agents were also separated into fresh and old feeding scars in single and in combination treatments in order to determine whether the agents can be released in areas where one has already established or whether they can both be released at new sites. The effect of old weevil scars on the establishment of *M. scutellaris* was determined by counting the number of surviving planthopper adults in each tub. A one-way ANOVA was conducted to test differences between the insect treatments in fresh and old feedings. A Tukey HSD post-hoc test was conducted to separate significant differences between the treatments and to identify homogeneous groups.

CHAPTER 3: RESULTS

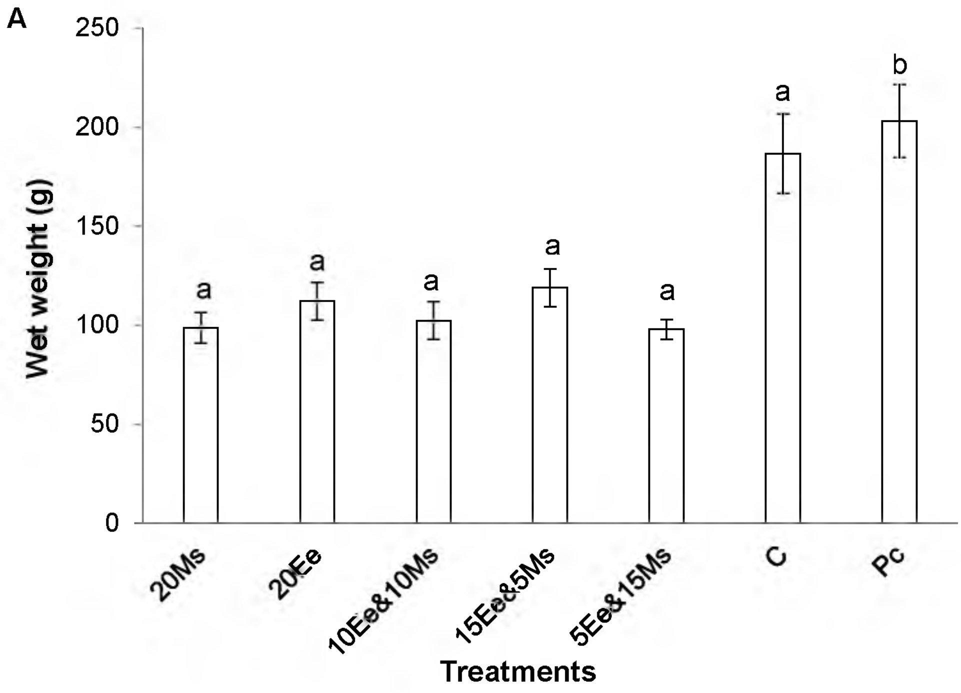
3. Introduction

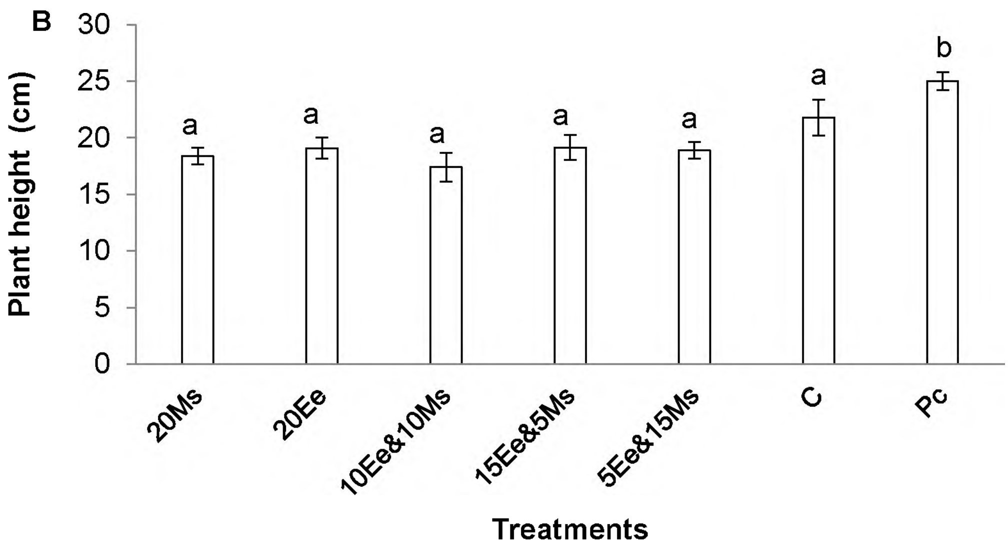
The interactions between the three biological control agents; *M. scutellaris*, *E. eichhorniae* and *N. eichhorniae* on water hyacinth were investigated by conducting three different experiments under control conditions in a polyurethane tunnel and in a shade house (see Chapter 2). Results of the three experiments were analysed separately.

3.1: Interactions between *Eccritotarsus eichhorniae* and *Megamelus scutellaris* and their impact on water hyacinth growth

3.1.1 Effect of herbivory on plant growth parameters

At the end of the 12-week sampling period, insect feeding by *M. scutellaris* and *E. eichhorniae*, both in the single and in combination treatments, significantly impacted a number of water hyacinth plant growth parameters, notably wet weight ($F_{6,63}$ = 12.57 P < 0.0001; Figure 3.1 A), plant height ($F_{6,63}$ = 6.01 P < 0.0001; Figure 3.1 B), number of ramets ($F_{6,63}$ = 21.74 P < 0.0001; Figure 3.1 C) and chlorophyll content ($F_{6,63}$ = 24.12 P < 0.0001; Figure 3.1 D). However, there were no significant differences between the insect treatment combinations, highlighting that it did not matter whether the species fed in combination, or alone (Figure 3.1 A–D). While there were no significant differences between the control and the procedural control in the wet weight and chlorophyll content, plant height and number of ramets were significantly higher in procedural controls, indicating that the netting had an effect on these parameters (Figure 3.1 A–D).


A
250
200
150
100
50
0
Wet weight (g)
a
a
a
a
a
a
b
20Ms
20Ee
10Ee&10Ms
15Ee&5Ms
5Ee&15Ms
C
Pc
Treatments


B
30
25
20
15
10
5
0
Plant height (cm)
a
a
a
a
a
a
b
20Ms
20Ee
10Ee&10Ms
15Ee&5Ms
5Ee&15Ms
C
Pc
Treatments

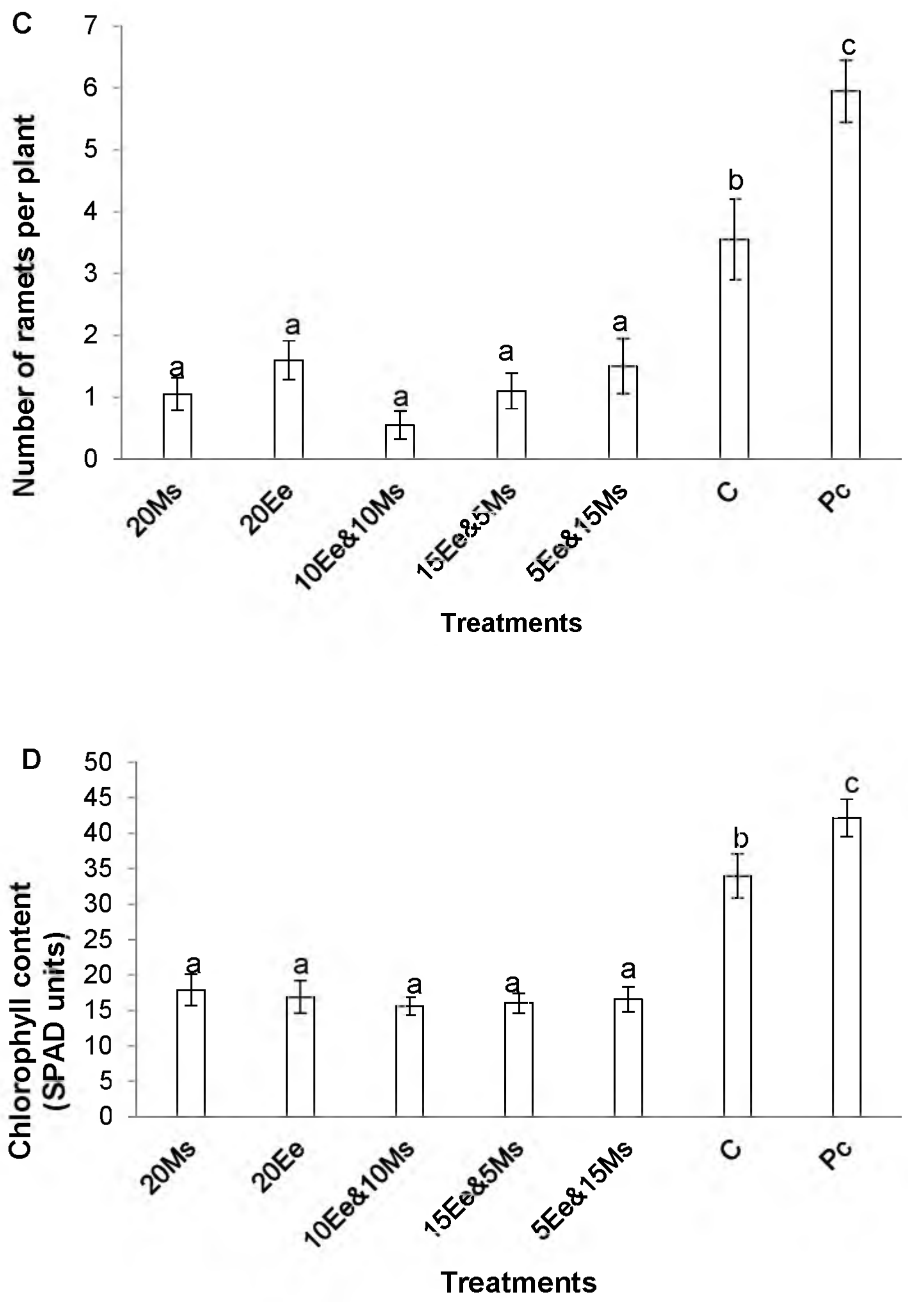


Figure 3.1. The effect of herbivory of two agents, *E. eichhorniae* (Ee) and *M. scutellaris* (Ms), in single or combination treatments on water hyacinth plant growth parameters, viz. wet weight (A), plant height (B), number of ramets (C), and chlorophyll content (D), after 12 weeks. C = control treatment, Pc = Procedural control. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

3.1.2 Insect parameters

The percentage area damaged by both agents on leaf 4 in single and combination treatments was significantly different. The combination of both agents caused the least amount of damage to the leaf, but was similar to the damage caused by *M. scutellaris* alone ($F_{3, 196}$ = 7.37 $P < 0.0011$; Figure 3.2 B), while *E. eichhorniae* caused significantly more damage when it was alone ($F_{3, 196}$ = 30.03 $P < 0.0001$; Figure 3.2 A). The feeding damage score for the control treatment was zero, as expected. After 12 weeks of the sampling period, feeding by *M. scutellaris* or *E. eichhorniae,* individually or in combination, significantly impacted water hyacinth plant growth (Figure 3.3 A–C). However, *E. eichhorniae* alone had a significantly greater impact in terms of the feeding intensity score ($F_{3, 196}$ = 9.67 $P < 0.0001$; Figure 3.3 A). Similar results were obtained for *M. scutellaris* alone with regard to sooty mould ($F_{3, 196}$ = 6.67 $P < 0.0002$; Figure 3.3 B), and oviposition scars ($F_{3, 196}$ = 5.43 $P <$ 0.0013; Figure 3.3 C). The number of insects collected at the end of the experiment differed significantly between the insect treatments (Figure 3.4 A–B). Significantly more *E. eichhorniae* were collected from the 15 Ee and 5 Ms treatment ($F_{4, 45}$ = 110.45 $P < 0.0001$), followed by *M. scutellaris* from the 10 Ms and 10 Ee treatment ($F_{4, 45}$ = 62. 59 $P < 0.0001$). Additionally, when the agents were separated into single treatments, a higher number of adults was collected for both insect species than for the combination treatments.

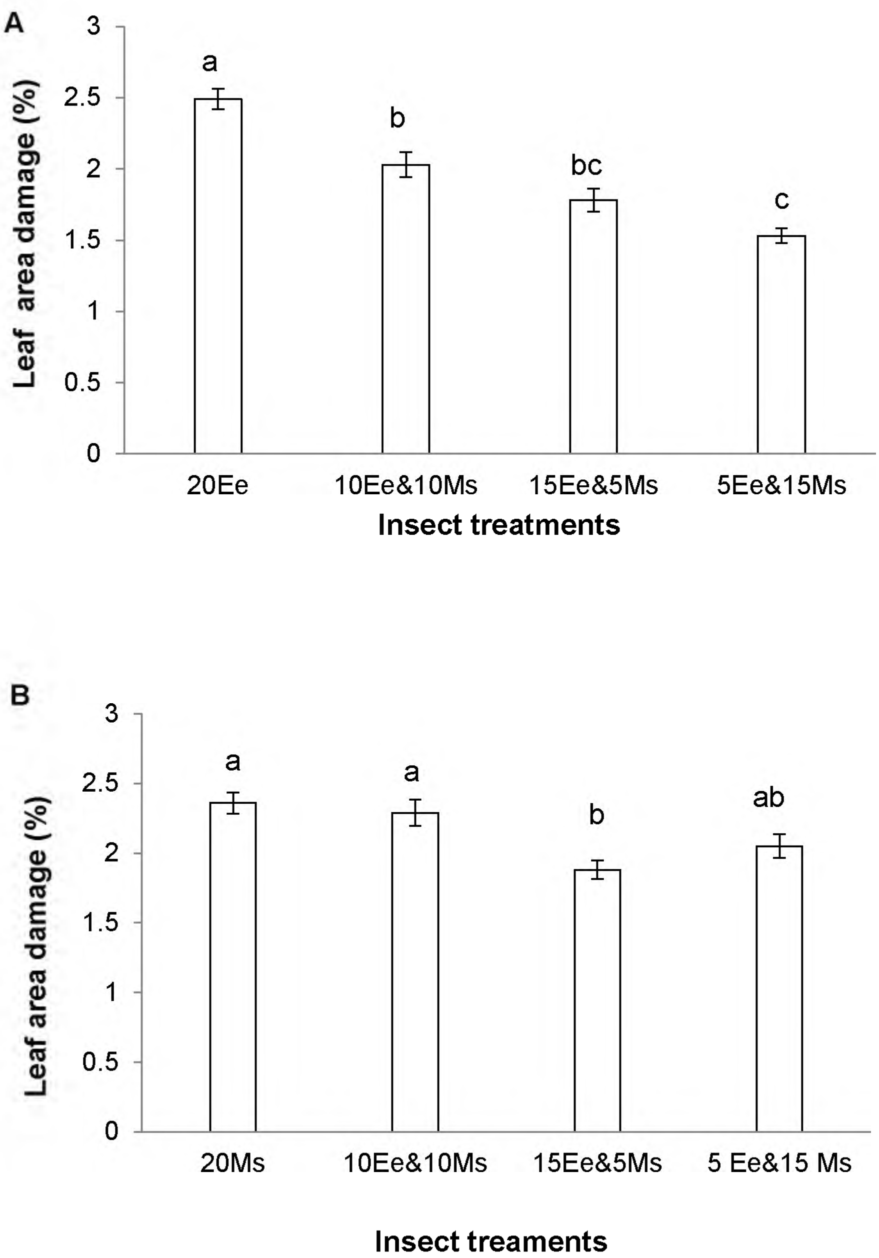


Figure 3.2: Percentage area damaged on leaf 4 of water hyacinth plants exposed to herbivory by different combinations of the two agents, *E. eichhorniae* (Ee) (A) and *M. scutellaris* (Ms) (B), after 12 weeks. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

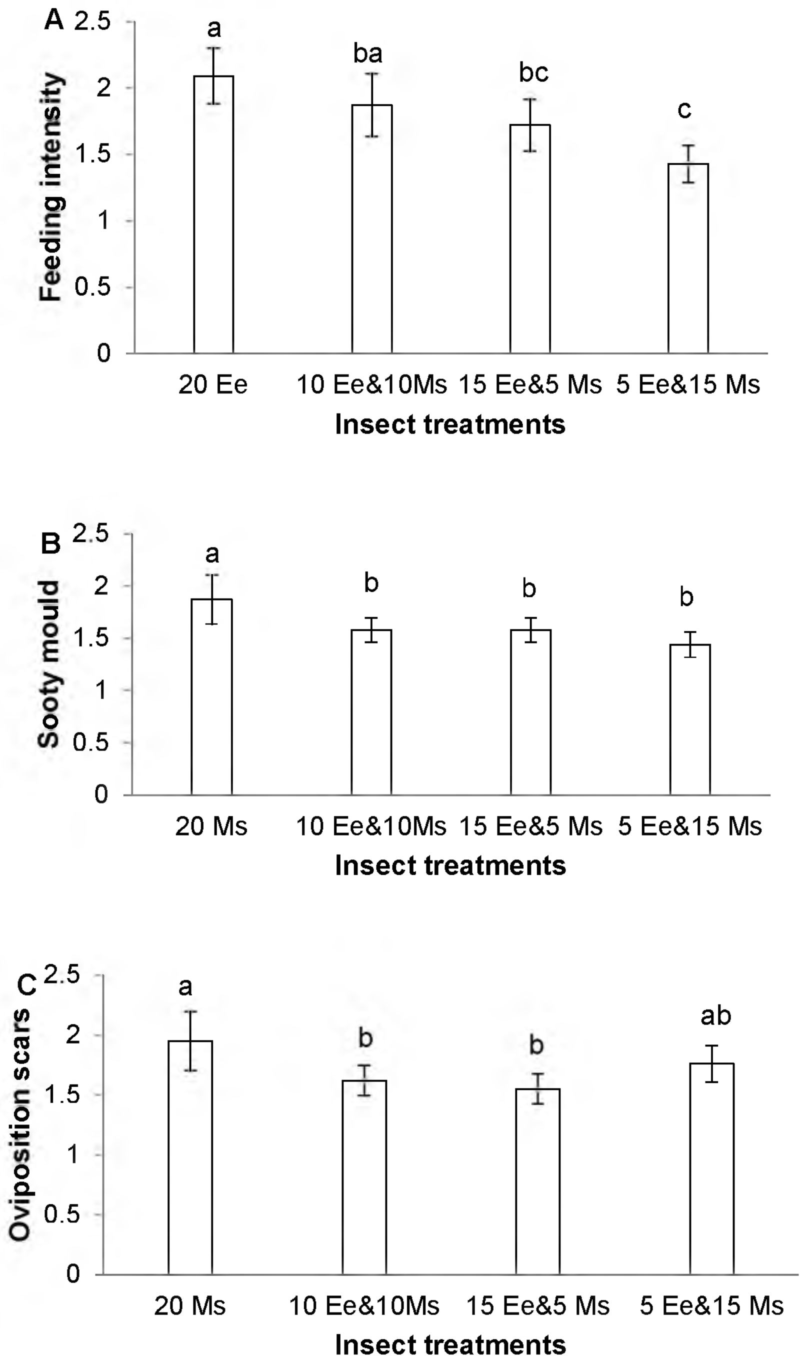
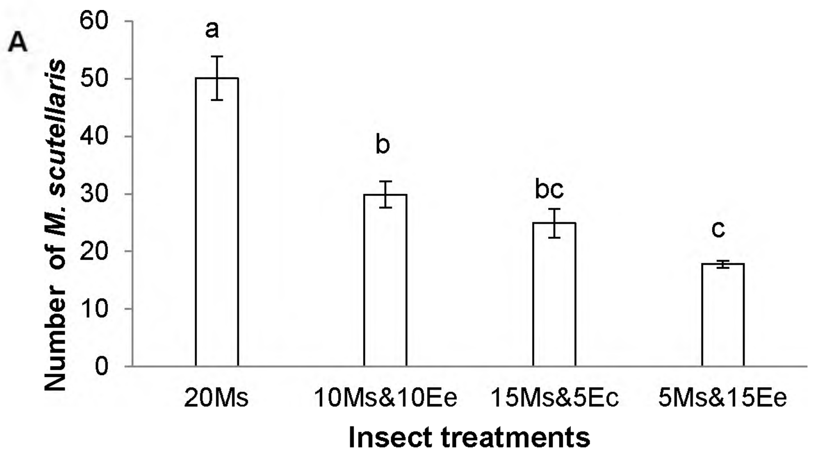


Figure 3.3: Insect feeding damage on leaf 4, after 12 weeks exposure to herbivory by various combinations of the two agents, *E. eichhorniae* (Ee) and *M. scutellaris* (Ms) and in single treatments. Feeding intensity score (A), Sooty mould score (B), and Oviposition scars (C) caused by the two agents in combination and separately. The total damage was separated into the damage caused by each agent species separately. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).



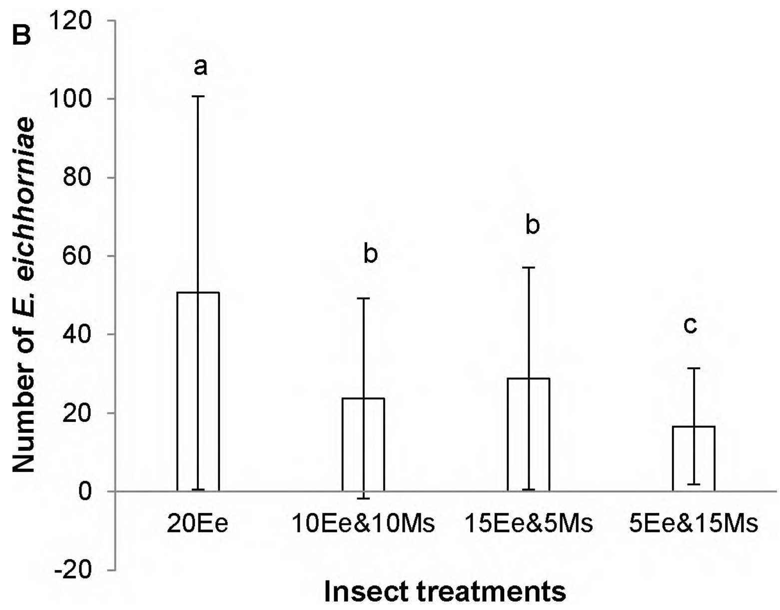
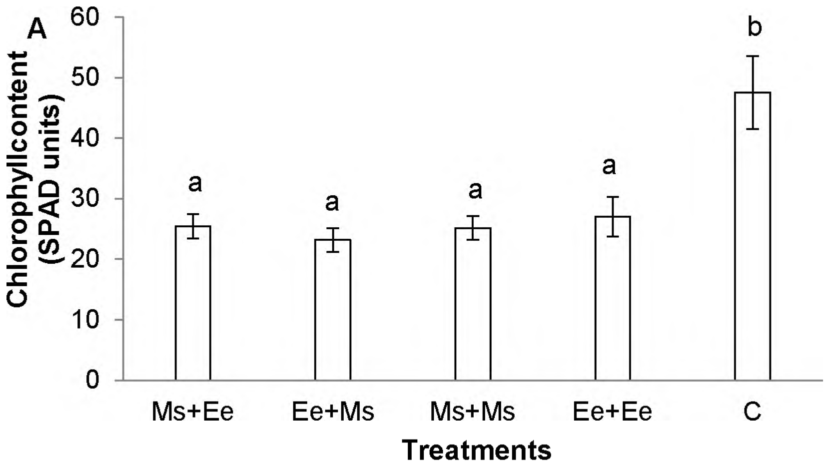


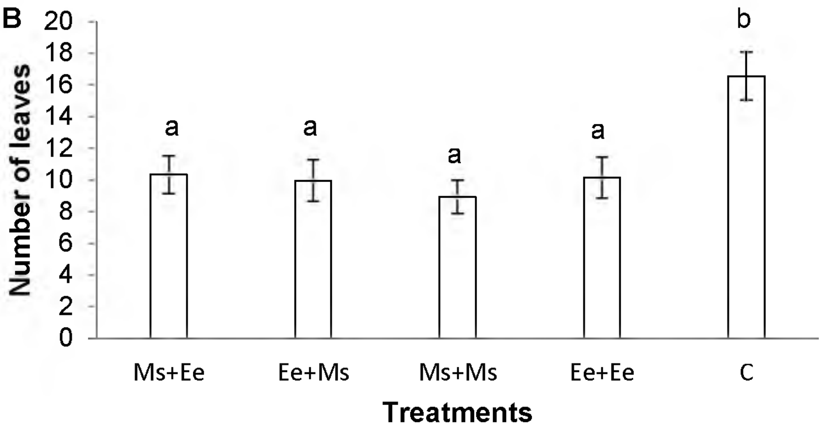
Figure 3.4: Insect population of *M.scutellaris* (Ms) (A) or *E. eichhorniae* (Ee) (B) separately and in combination, over 12 weeks. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

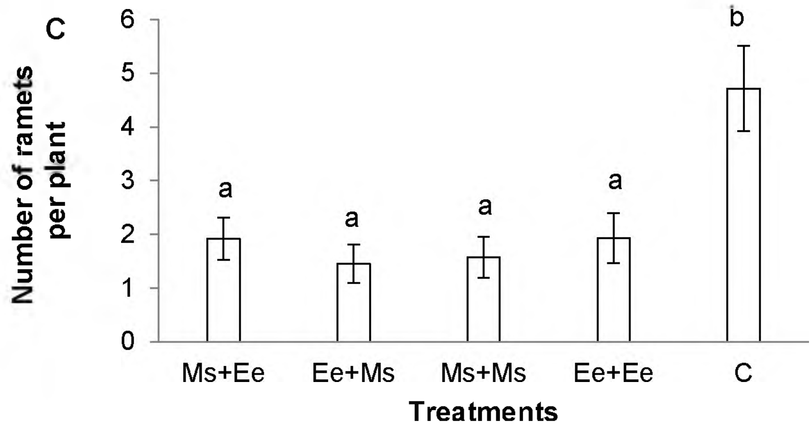
3.2 Impact of prior feeding by the two agents *Eccritotarsus eichhorniae* and *Megamelus scutellaris* on subsequent feeding by both agents

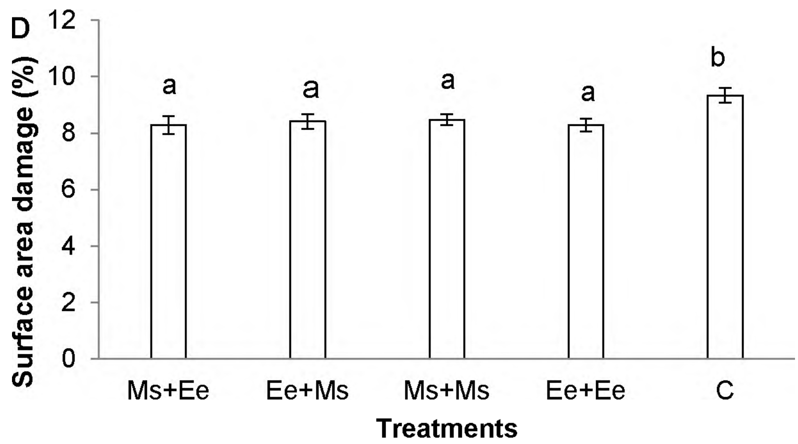
3.2.1 Plant growth parameters

After the sampling period, no significant differences were measured between the insect treatments. Significant differences were found only between the insect treatments and the control, notably in chlorophyll content ($F_{4,144} = 25.85$ $P < 0.0001$; Figure 3.5 A), number of leaves ($F_{4,144} = 17.01$ $P < 0.0001$; Figure 3.5 B), number of ramets ($F_{4,144} = 21.65$ $P < 0.0001$; Figure 3.5 C), leaf surface area ($F_{4,144} = 9.61$ $P < 0.0001$; Figure 3.5 D), leaf width ($F_{4,144} = 9.65$ $P < 0.0001$; Figure 3.5 E) and plant height ($F_{4,144} = 2.69$ $P < 0.0332$; Figure 3.5 F).




B
Number of leaves
0
2
4
6
8
10
12
14
16
18
20
a
a
a
a
b
Ms+Ee
Ee+Ms
Ms+Ms
Ee+Ee
C
Treatments


C
Number of ramets per plant
0
1
2
3
4
5
6
a
a
a
a
b
Ms+Ee
Ee+Ms
Ms+Ms
Ee+Ee
C
Treatments


D
Surface area damage (%)
0
2
4
6
8
10
12
a
a
a
a
b
Ms+Ee
Ee+Ms
Ms+Ms
Ee+Ee
C
Treatments

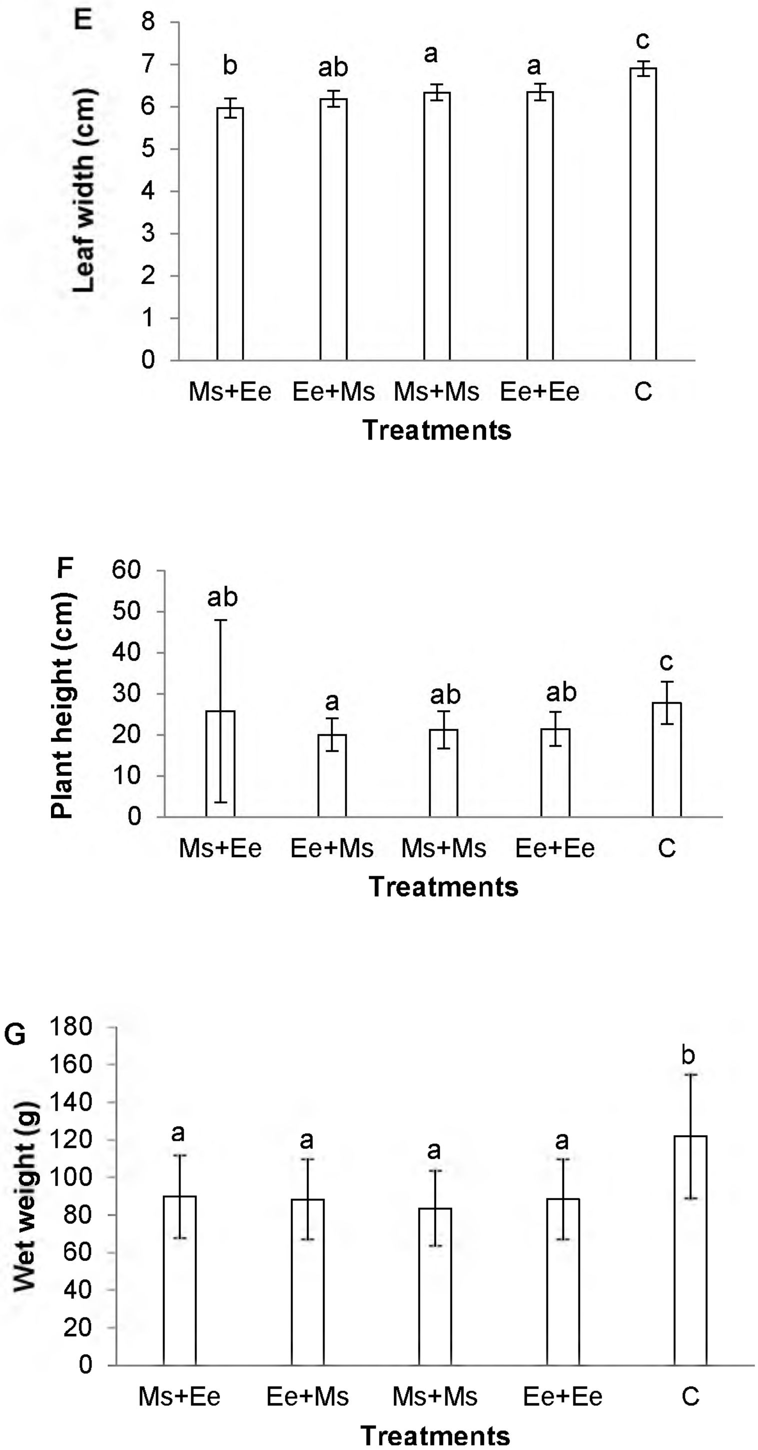


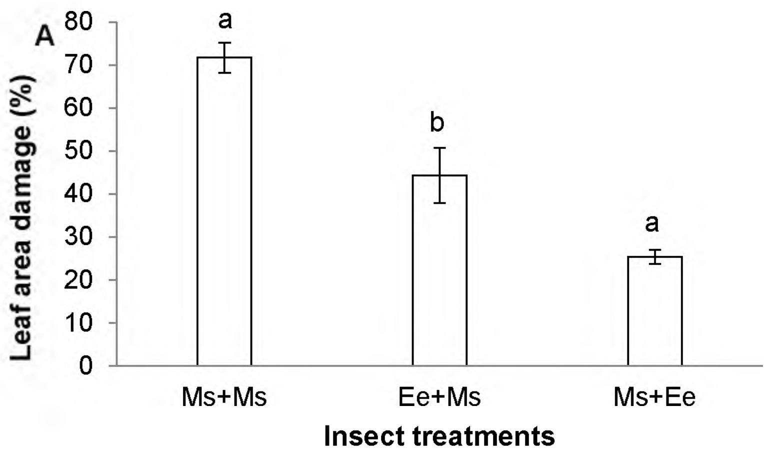
Figure 3.5: The effect of herbivory, *E. eichhorniae* and *M. scutellaris* (singly and in combination) on water hyacinth plant growth parameters after 12 weeks. Chlorophyll content (A), Number of leaves (B), Number of ramets (C), Leaf surface area (D), Leaf width (E), Longest petiole (F) and Wet weight (G). Error bars represent the standard error of the mean. Letters above the error bars shows significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

3.2.2 Insect parameters

The percentage of the area of leaf 4 damaged by prior *E. eichhorniae* and subsequent *E. eichhorniae* (Ee+Ee) or prior *M. scutellaris* and subsequent *M. scutellaris* (Ms+Ms) and combination treatments - prior *M. scutellaris* and subsequent *E. eichhorniae* (Ms+Ee) or prior *E. eichhorniae* and subsequent *M. scutellaris* (Ee+Ms) was statistically different between the treatments.

When the damage was separated into each insect treatment, *M. scutellaris* and *E. eichhorniae* dominated when they were inoculated as primary feeders: Ms+Ee treatment ($F_{2, 177}$= 12. 13 P < 0.0001; Figure 3.6 B) and (Ee+Ms) treatment ($F_{2, 177}$= 35. 49 P < 0.0001; Figure 3.6 A). No significant differences were recorded on single treatments of agents: (Ee+Ee) treatment and (Ms+Ms) treatment (Figure 3.6 A–B). No significant differences were measured between the combination insect treatments (Ms+Ee) or (Ee+Ms) treatment. The significant differences were only noted between the combination treatments and the single treatments: prior *M. scutellaris* and subsequent *M. scutellaris* (Ms+Ms), for the sooty mould score ($F_{2, 177}$= 22. 851 P < 0.0001; Figure 3.7 B) and oviposition scars ($F_{2, 177}$ = 19. 255 P < 0.0001; Figure 3.7 C). The number of *E. eichhorniae* and *M. scutellaris* collected from each tub at the end of the experiment in single treatments and in combination treatments were statistically different between the treatments (Figure 3.8 A–B). Significantly more *E. eichhorniae* were collected from the single treatment: prior *E. eichhorniae* and subsequent *E. eichhorniae* (Ee+Ee) treatment, followed by the combination treatment; prior *E. eichhorniae* and subsequent *M. scutellaris* (Ee+Ms). There was a decline in the number of *E. eichhorniae* on the prior *M. scutellaris* and subsequent *E. eichhorniae* (Ms+Ee) treatment

($F_{3.36}$ = 120.5 P < 0.0001; Figure 3.8 A). Similar results were also shown for *M. scutellaris*: more *M. scutellaris* were recorded from the single treatment; prior *M. scutellaris* and subsequent *M. scutellaris* (Ms+Ms), followed by combination treatment; prior *M. scutellaris* and subsequent *E. eichhorniae* (Ms+Ee). Prior feeding by *E. eichhorniae* had an impact on subsequent feeding on *M.scutellaris* on *E. eichhorniae* followed by *M. scutellaris* (Ee+Ms) treatment, a decline in number of *M. scutellaris* was recorded ($F_{3.36}$ = 65.03 P < 0.0001; Figure 3.8 B).



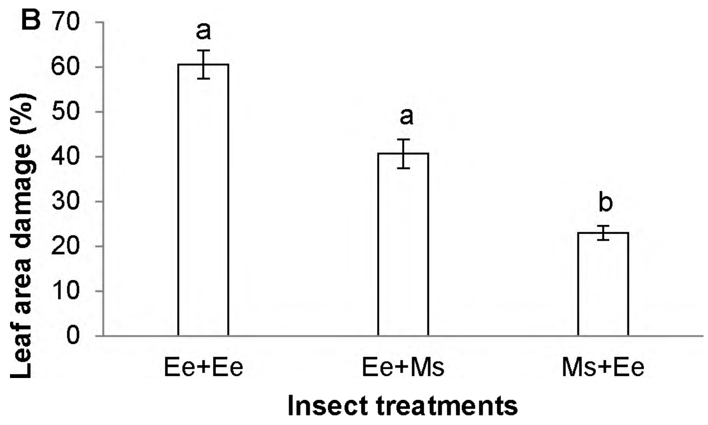
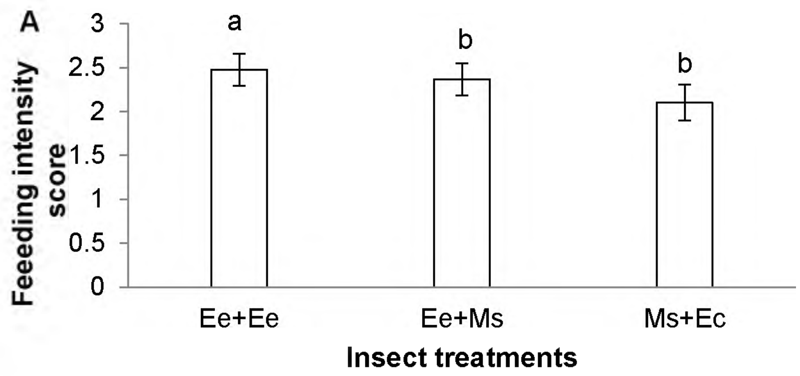
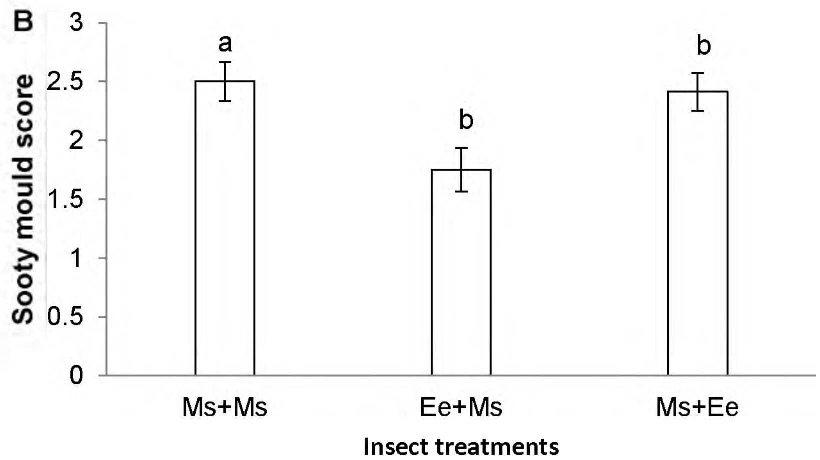


Figure 3.6: Percentage area damaged on leaf 4 of water hyacinth plants exposed to herbivory by different combinations of the two agents, *M.scutellaris* (Ms) (A) and *E. eichhorniae* (Ee) (B), after 12 weeks. The total damage was separated into the damage caused by each agent species separately. Error bars represent the standard error of the mean. Means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).





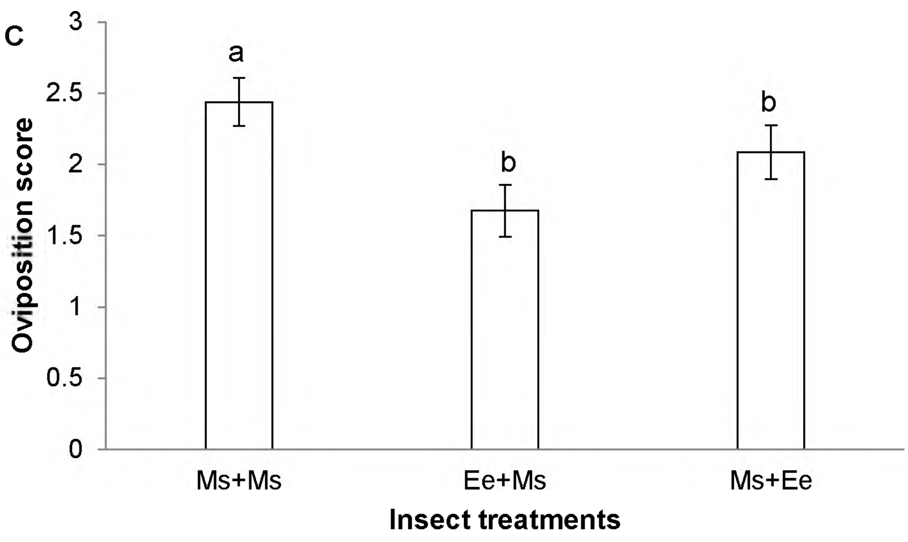
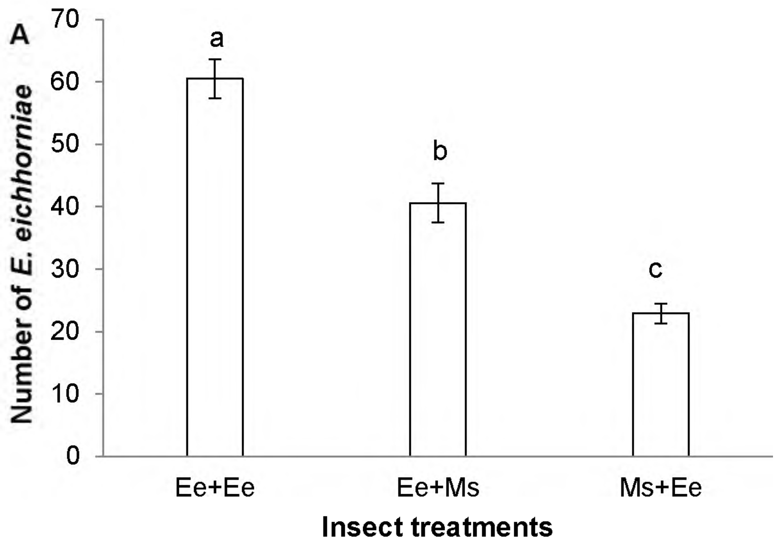


Figure 3.7: Insect feeding damage on leaf 4, after 12 weeks' exposure to herbivory by various combinations of two agents *E. eichhorniae* (Ee) and *M. scutellaris* (Ms). Feeding intensity score (A) Sooty mould score (B) and Oviposition scars (C) damaged by the two agents in combination and separately. The total damage was separated into the damage caused by each agent species separately. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).



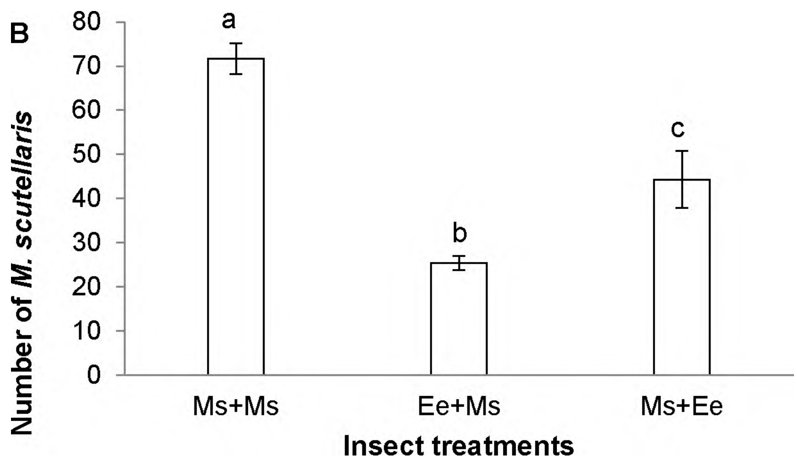


Figure 3.8: Insect population of *E. eichhorniae* (Ee) and *M. scutellaris* (Ms) in single and combination treatment. *E. eichhorniae* (A) ; *M. scutellaris* (B), over 12 weeks. Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

3.3 The establishment of *Megamelus scutellaris* on water hyacinth plants extensively damaged by the weevil, *Neochetina eichhorniae*.

3.3.1 Plant growth parameters

At the end of 12 weeks, no significant differences ($p < 0.05$) were recorded between the insect treatments for any of the plant growth parameters measured. However, significant differences were noted between the insect treatments and the control chlorophyll content ($F_{4, 45}$ = 4. 967 $P < 0.0021$; Figure 3.9 A) and control wet weight ($F_{4, 45}$ = 1. 353 $P <$ 0. 0001; Figure 3.9 B) only.

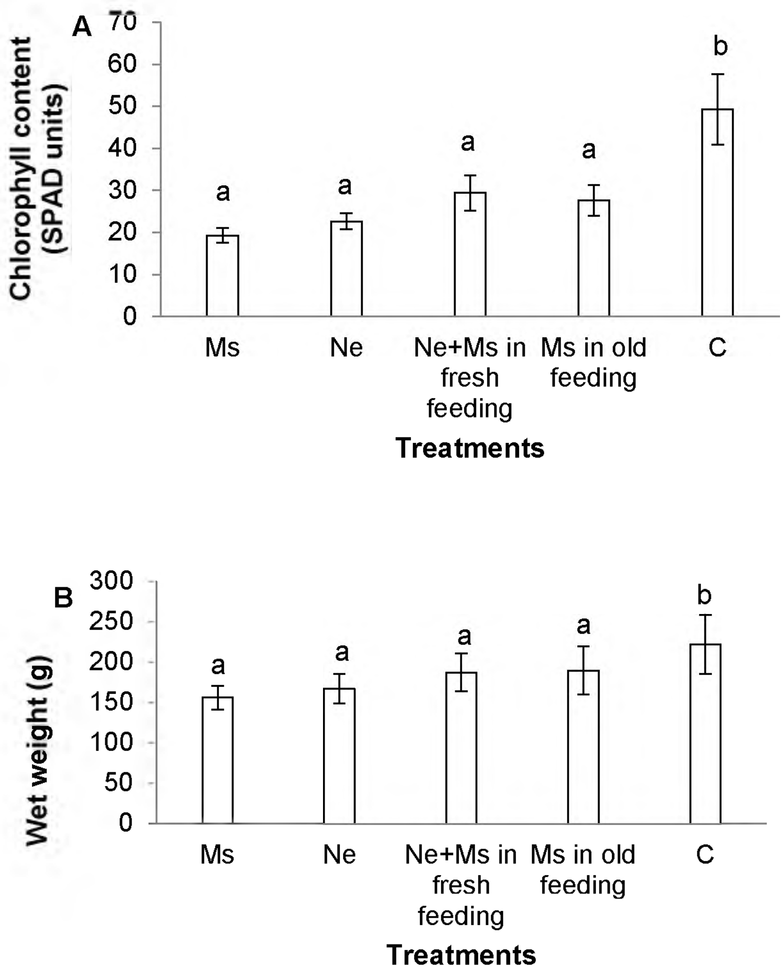


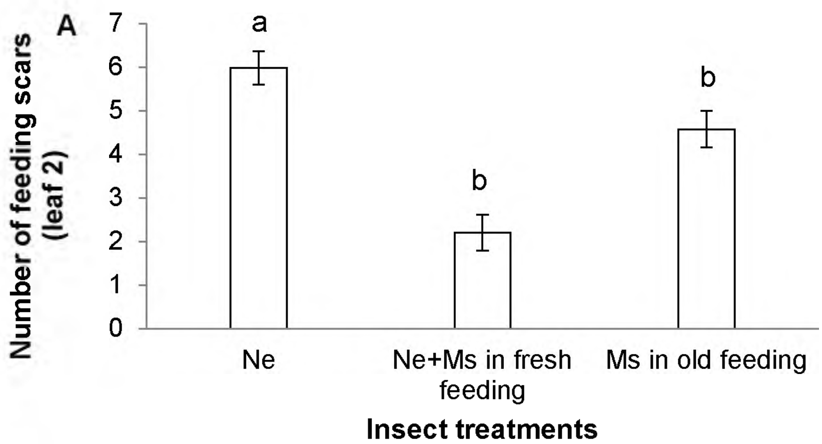
Figure 3.9: The effect of herbivory, *M. scutellaris* and *N. eichhorniae* (singly and in combination) on water hyacinth plant growth parameters: Chlorophyll content (A) and Wet weight (B). Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

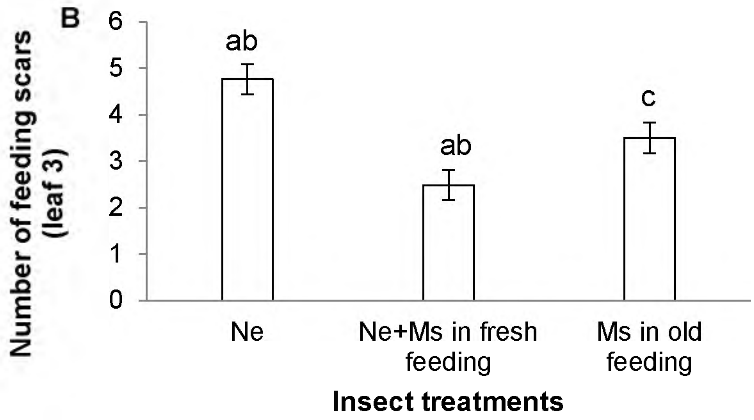
3.3.2 Insect parameters

At the end of the 12-week sample period, differences in *N. eichhorniae* feeding scars between the leaves 2, 3, and 4 were recorded (Figure 3.10 A–B), where leaf 2 had significantly more feeding scars (Figure 3.10 A). When the feeding scars were separated into each insect treatment (*N. eichhorniae* alone, or *N. eichhorniae* and *M. scutellaris* on fresh feeding, and *N. eichhorniae* and *M. scutellaris* on old feeding), *N. eichhorniae* in the single treatment produced more scars. No significant differences were recorded in the number of feeding scars in the *N. eichhorniae* and *M. scutellaris* on the old feeding treatment and *N.*

eichhorniae and *M. scutellaris* on fresh feeding ($F_{2,54}$ = 20. 806 P < 0.0001; Figure 3.10 A). There were no significant differences in the number of feeding scars on leaf 3 in any insect treatments ($F_{2, 54}$ = 0.8385 P < 0.4379; Figure 3.10 B). Leaf 4 had fewer feeding scars for all insect treatments when compared with other leaves ($F_{2, 54}$ = 6.1929 P < 0.003; Figure 3.10 C). However, there was a decline in the number of feeding scars in the *N. eichhorniae* and *M. scutellaris* on old feeding treatment, while there was a slight increase in weevil feeding scars in the *N. eichhorniae* and *M. scutellaris* on fresh feeding treatment. *Neochetina eichhorniae* alone produced the highest number of feeding scars (Figure 3.10 C).

Differences in the percentage of feeding damage by *M. scutellaris* on leaves 2, 3 and 4 were measured. However, there were no significant differences between the insect treatments on leaf 2 ($F_{2, 53}$= 2.9631 P < 0.0622; Figure 3.11 A). Significant differences between the insect treatments were measured for leaf 3 ($F_{2, 53}$= 17. 943 P < 0.0001; Figure 3.11 B), while there were no significant differences between *M. scutellaris* on single treatment, or *N. eichhorniae* and *M. scutellaris* on old feeding. However, *N. eichhorniae* and *M. scutellaris* in the fresh feeding treatment caused the greatest damage (Figure 3.11B). There was a high percentage of feeding damage on leaf 4, notably in the *M. scutellaris* single treatment ($F_{2, 53}$= 9.904 P < 0.0002; Figure 3.11 C). The number of petioles mined by *N. eichhorniae* per water hyacinth plant were significantly different in all insect treatments ($F_{2, 87}$= 92. 953 P < 0.0001; Figure 3.12). *Neochetina eichhorniae* and *M. scutellaris* on old feeding had a significantly greater number of mined petioles (ranging from 4 to 8 per plant), while the *N. eichhorniae* alone treatment had between 2 to 5 per plant. However, fewer mined petioles per plant were recorded for the *N. eichhorniae* and *M. scutellaris* on fresh feeding treatment, where petioles mined ranged between1 to 3 per plant (Figure 3.12). The number of adults for both *M.scutellaris* and *N. eichhorniae* were significantly different between the insect treatments (Figure 3.13 A–B). Significantly more *M. scutellaris* were recorded on the fresh feeding ($F_{3, 36}$ = 71. 721 P < 0. 0001; Figure 3.13 A) and *N. eichhorniae* in old feedings ($F_{3, 36}$ = 47. 649 P < 0. 0001; Figure 3.13 B).





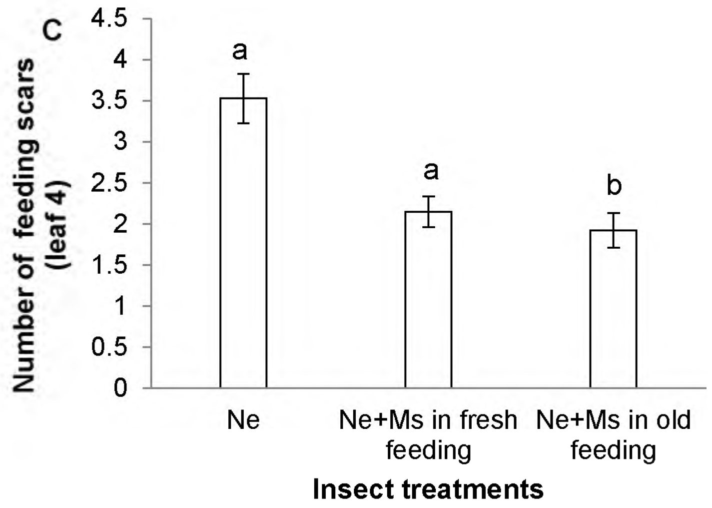
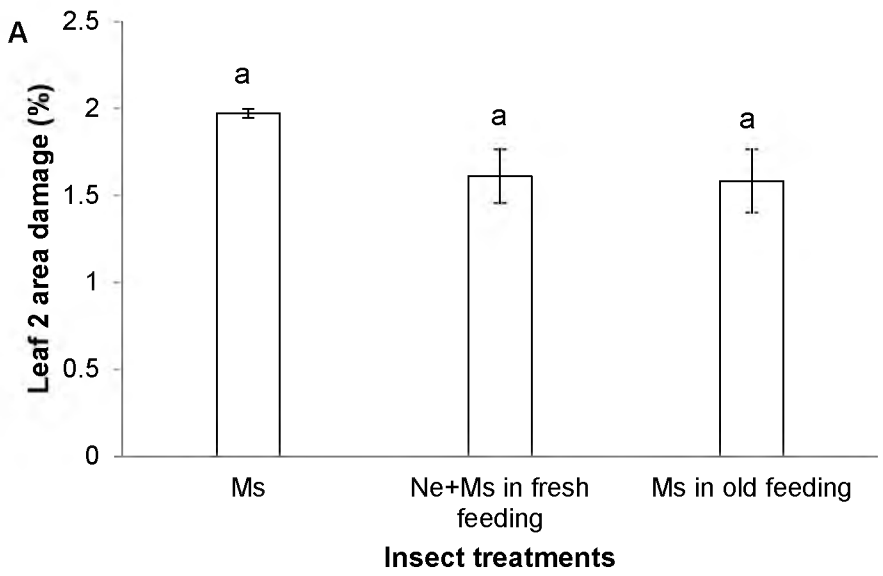
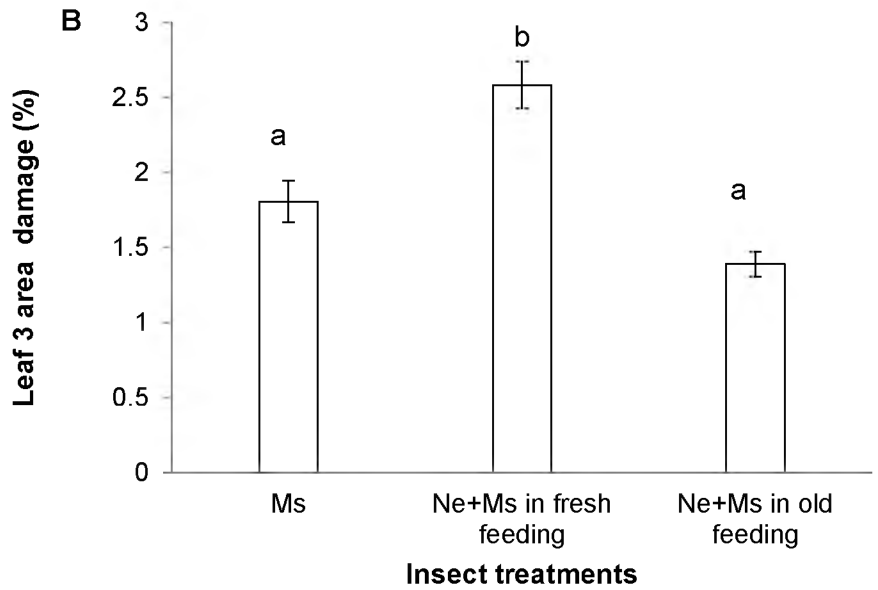


Figure 3.10*:* The number of *N. eichhorniae* feeding scars on leaf 2 (A), leaf 3 (B) and leaf 4 (C) in each insect treatment. *N. eichhorniae* (Ne); *M. scutellaris* (Ms). Error bars represent the standard error of the mean. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).


A
Leaf 2 area damage (%)
2.5
2
1.5
1
0.5
0
a
a
a
Ms
Ne+Ms in fresh feeding
Ms in old feeding
Insect treatments


B
Leaf 3 area damage (%)
3
2.5
2
1.5
1
0.5
0
a
b
a
Ms
Ne+Ms in fresh feeding
Ne+Ms in old feeding
Insect treatments

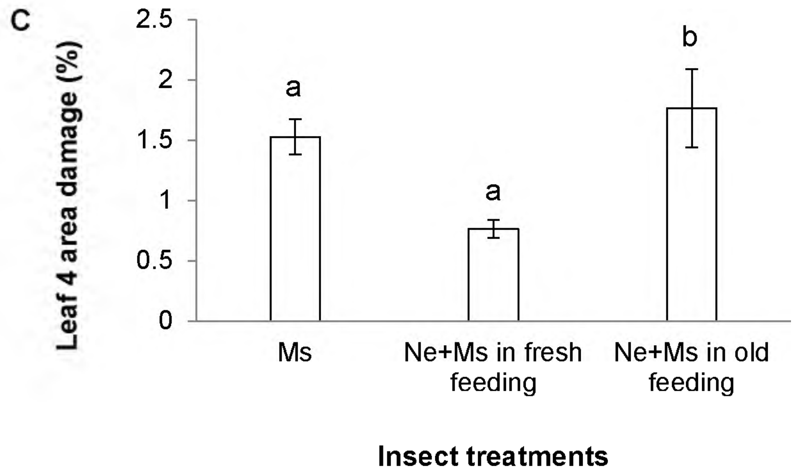


Figure 3.11: Percentage area damaged by the *M. scutellaris* on leaf 2 (A), leaf 3 (B) and leaf 4 (C) in each insect treatment. *N. eichhorniae* (Ne); *M. scutellaris* (Ms). Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

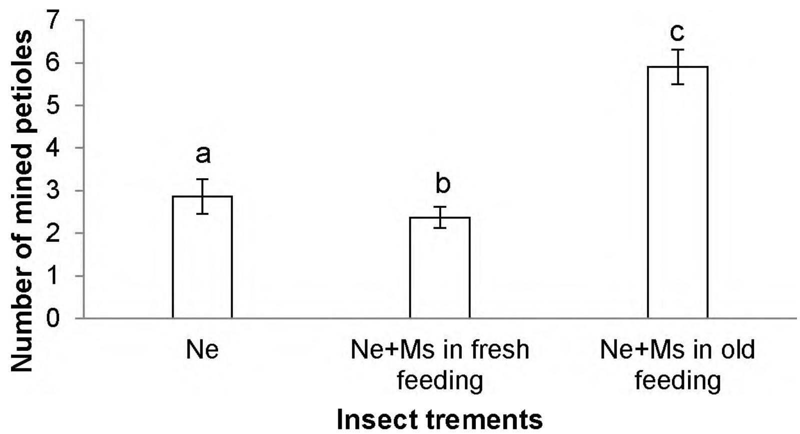
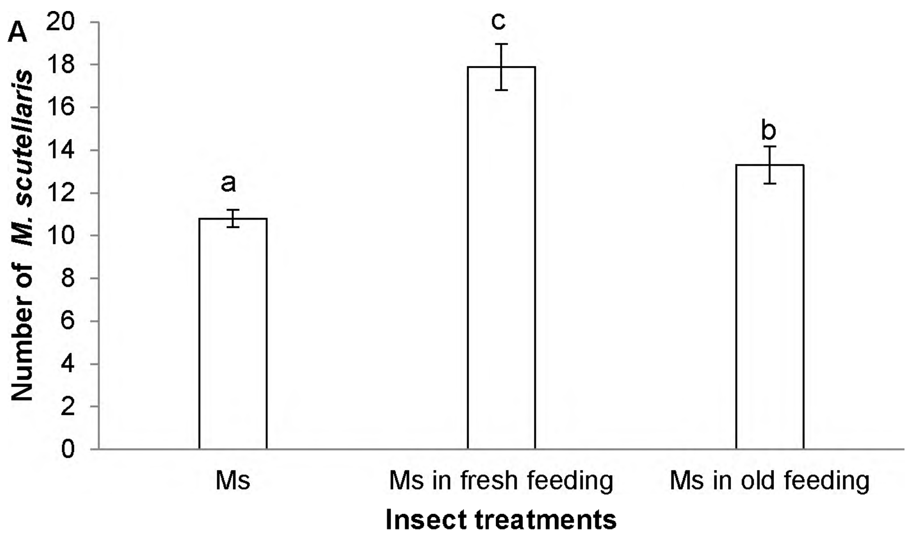


Figure 3.12: The number of water hyacinth petioles mined by weevil larvae in each insect treatment. *N. eichhorniae* (Ne); *M. scutellaris* (Ms). Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).



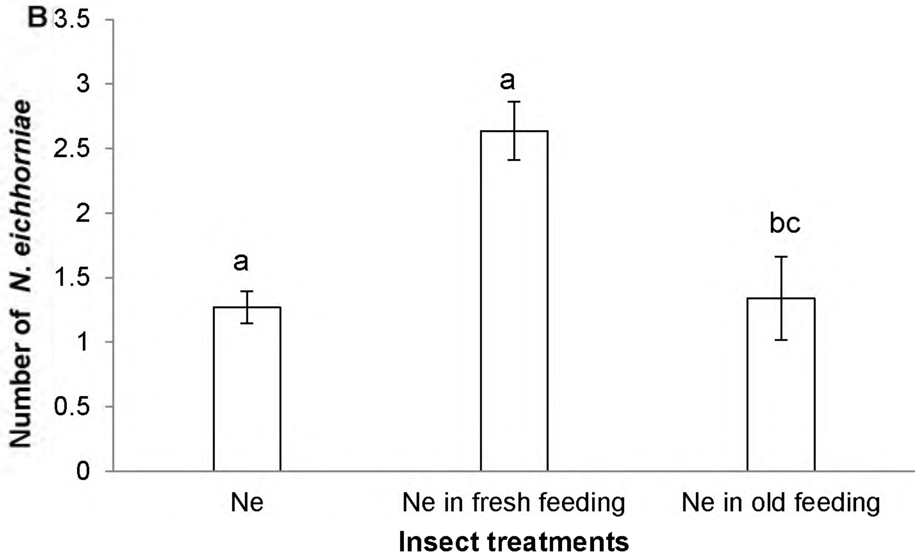


Figure 3.13: Insect population of *M. scutellaris* (Ms) (A) or *N. eichhorniae* (Ne) (B) and in combination, over 12 weeks. Letters above the error bars show significant differences; means followed by the same letter are not statistically different (Tukey HSD test, $P < 0.05$).

3.4 Summary of Results

The results of the study showed that feeding by either *E. eichhorniae* or *M. scutellaris* had no effect on the feeding of the other agent. Both agents reduced all the measured plant growth parameters equally, either singly or in combination (*i.e. E. eichhorniae* or *M. scutellaris* alone or together). The interaction between the two agents appears neutral and agents are thus likely to complement each other in the field.

Prior feeding by *E. eichhorniae* or *M. scutellaris* on water hyacinth did not affect the subsequent feeding by either agent. *Megamelus scutellaris* prefered healthy fresh water hyacinth plants, and a lower number of oviposition scars and sooty mould were noted when *M. scutellaris* was introduced as a subsequent feeding agent.

Planthoppers performed best in combination with the weevil, *N. eichhorniae*, especially on plants with new feeding scars. The interaction between the two agents appears synergistic.

CHAPTER 4: GENERAL DISCUSSION

4.1 Introduction

The biological control of water hyacinth in South Africa is variable and could be considered less successful than elsewhere in the world (Hill & Olckers, 2001; Julien, 2001). To date, nine biological control agents have been released against the weed in South Africa. However, water hyacinth is still regarded as the worst aquatic weed in the country, because of its negative impacts on aquatic ecosystems and the difficulty of controlling it (Center *et al.*, 2002; Hill *et al.*, 2001). Successful biological control of water hyacinth in South Africa was achieved at one site, the New Year's Dam (Alicedale, Eastern Cape), with the release of one agent, *N. eichhorniae* (Hill & Coetzee, 2017), but the weed still poses a problem at most sites around the country (Coetzee *et al.*, 2011). Hill and Olckers (2001) proposed that, in order to improve the biological control of water hyacinth in South Africa, there was a need to target and release new agents. Since that time, three additional agents, *Cornops aquaticum*, *Megamelus scutellaris* and *Eccritotarsus eichhorniae,* have been released.

Several studies have shown that interactions between biological control agent species do not always result in better control of the target weed (Denoth *et al.*, 2002). For example, Crowe and Bocheir (2006) examined interspecific interactions between *Urophora affinis* Frauenfeld (Diptera: Tephritidae) and *Larinus minutus* Gyllenhaal (Coleoptera: Curculionidae) when mutually released against spotted knapweed, *Centaurea stoebe micranthos*, formerly *Centaurea maculosa* Lamarck (Asteraceae) in North America. Their results revealed that increased numbers of biological control agents that use similar resources on the target plant end up competing for resources and could reduce the overall impact on the weed. Similarly, a study by Blossey *et al*. (1996) revealed that competition between biological control agents against the control of purple loosestrife, *Lythrum salicaria* Linnaeus (Lythraceae), in North America also had unintended consequences. Four biological control agents were released against purple loosestrife, *viz.* two leaf feeders, *Galerucella calmariensis* Linnaeus (Coleoptera: Chrysomelidae) and *Galerucella pusilla* Duftshmidt (Coleoptera: Chrysomelidae), a flower feeder, *Nanophyes marmoratus* Goeze (Coleoptera: Brentidae) and a seed feeder, *Nanophyes brevis* Boheman (Coleoptera: Brentidae). The two leaf-feeding agents caused heavy defoliation, resulting in the suppression of purple loosestrife flowering, and a shortage of food for the flower feeder, *N. marmoratus* (Blossey *et al.,*1994a, b; Blossey *et al.,*1996).

However, some authors subscribe to the philosophy of 'the more the merrier' (see Julien, 1985; Myers, 1985; Denoth *et al*., 2002; Myers, 2008). Julien (1982) reviewed 26 weed species and showed that, on average, four agent species were released for each weed species. Of the 26 weed species examined, 81% of the weed species were successfully controlled by a single agent, four weed species needed two insect agents, and in one study, a weed species required three agents (Julien, 1985; Myers, 1985). Denoth *et al*. (2002) and Myers (2008) found that in 55% of the studies that they reviewed, a single agent was sufficient for the successful control of the target weed (Denoth, 2008; Myers, 2008;). So, there is evidence for and against the use of multiple species as biological control agents. In South Africa, given that water hyacinth is considered to be under substantial, but not complete, control, addition of new agents must improve the level of control and not reduce control through antagonistic interactions with already established agents. Therefore, the aim of this thesis was to determine whether or not the new biological control agents of water hyacinth in South Africa, *M. scutellaris* and *E. eichhorniae,* were compatible with each other or other insect species already established.

4.2 Direct interactions

The results of the current study showed that *M. scutellaris* and *E. eichhorniae* reduced most of the plant growth parameters equally, either singly or in combination; results which support Weyl and Hill (2012a, b) who also investigated the interaction between three biological control agents of water hyacinth in South Africa, namely, *E. catarinensis*, *N. bruchi* and *N. eichhorniae*, and showed that there were no significant differences in impact when between one and three agents were released on the experimental plants. In contrast, Marlin *et al*. (2013b) examined the interactive effects of the agents *O. terebrantis* (mite), *N. eichhorniae* (weevil) and *E. catarinensis* (mirid)*,* singly or in pairwise combinations on water hyacinth growth. The results revealed that each agent and each combination of the agents impact water hyacinth plant growth parameters differently (Marlin *et al*., 2013b). The leaf surface area was most damaged by a combination of mites and mirids, while the combination of mites and weevils reduced plant height. The overall findings of the study showed that the three biological control agents could co-exist in the field with a slightly negative interaction (Marlin *et al*., 2013b).

In the current study, the feeding by *E. eichhorniae* and *M. scutellaris* did not affect the feeding of the other agent. In contrast, Turner *et al*. (2010) examined the effect of the rust fungus, *Puccinia mysiphylli* (Thuem) Winter (Pucciniales: Pucciniaceae), and an undescribed leafhopper (Tribe *Erythroneurini*, formerly referred to as *Zygina sp.*) on bridal creeper, *Asparagus asparagoides* (Linnaeus) Druce (Asparagoideae), in Australia and showed that the combination of the two agents had a greater effect in the control of the bridal creeper plant than each of them individually.

A study by Buccellato *et al.* (2012) contradicted with the results of the current study. Interactions between a stem gall fly, *Procecidochares utilis* King & Robinson (Diptera: Tephritidae), and a leaf-spot fungal pathogen, *Passalora ageratinae* Crous & Wood (Mycosphaerellales: Mycosphaerellaceae), on the vegetative growth of *Ageratina adenophora* King & Robinson (Asteraceae) in South Africa was studied. This study showed that *P. utilis* caused the greatest reduction in vegetative growth of *A. adenophora*, while the pathogen, *P. ageratinae,* was responsible for reducing the production of the side-shoots. Although the impact of the two agents was responsible for different plant variables, the impact of the two agents in combination led to an overall additive effect on the damage caused to crofton weed in keeping with the cumulative stress model (Buccellato *et al.*, 2012).

Seastedt *et al.* (2007) reported that cumulative stress model could be applied to a flower weevil, *Larinus minutus* Gyllenhaal (Coleoptera: Curculionidae), and two root feeders, *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae) and *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae), in the biological control of diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae) and spotted knapweed, *Centaurea stoebe* Lamarck (Asteraceae) in Colorado, USA. The results showed that there were no negative interactions between the biological control agents released on either weed species, as they feed on different parts of the plants. Their results demonstrated that, although some antagonistic interactions do occur between multiple agents released for the control of *Centaurea* species, these interactions were not sufficient to negate what seemed to be a strong negative effect on the target plant species by the combination of agents, which is constrast with the results of the current study (Seastedt *et al.*, 2007).

Ray and Hill (2016) examined the effect of *E. catarinensis* on the efficacy of different agents on the pathogen *Acremonium zonatum* (Sawada) Gams (Hypocreaceae) for the biological control of water hyacinth. The results of that study showed that low mirid densities enhanced the pathogen development, while high mirid densities reduced development of *the pathogen*. However, the overall results of their study showed that the combination of *E. catarinensis* and *A. zonatum* had a significant negative impact on water hyacinth growth and the agents should ideally be used in combination (Ray & Hill, 2016).

Overall it therefore seems that agent combination effects on water hyacinth management are determined by the specific agent species involved, since different species have different interactions amongst one another.

4.3 Indirect interactions

In the current study (3.2 in chapter 3), the prior feeding by *E. eichhorniae* or *M. scutellaris* on water hyacinth growth did not affect the subsequent feeding by *M. scutellaris* or *E. eichhorniae*. However, lower numbers of oviposition scars and coverage of sooty mould caused by *M. scutellaris* was recorded when *M. scutellaris* was introduced after *E. eichhorniae*, showing that *M. scutellaris* prefers to oviposit on healthy and undamaged plants. A similar study on the interactions between *O. terebrantis*, *N. eichhorniae* and *E. catarinensis* on impacting water hyacinth plant growth, showed that *O. terebrantis* also preferred to oviposit on undamaged healthy plants than on ones damaged by *E. catarinensis* (Marlin *et al.*, 2013a).

The results of the current study also revealed that planthoppers performed best in combination with the weevil, especially on plants with new weevil feeding scars. A study by Center and Van (1989) reported that feeding by the *Neochetina* weevils reduced plant nutrients, which could be the reason why the plants were less attractive to *M. scutellaris*. The results of the current study mirror those of Ajuonu *et al.* (2007) who demonstrated that the weevils did not influence the establishment of the mirid (*E. catarinensis*) in the field, but when *E. catarinensis* was introduced in large numbers onto plants with old weevil feeding scars, the performance of the mirid, both as adults and nymphs, was reduced. However, when the mirid was introduced onto plants with fresh weevil feeding scars, better performance by the mirid was recorded (Ajuonu *et al.* 2007). *Megamelus scutellaris* is a multivoltine species that completes a generation within a short period of time (Tipping *et al.*, 2011). Feeding by the planthopper damages the petiole of water hyacinth which leads to water logging, reducing plant buoyancy and causing the tissue to rot (Sosa *et al.,* 2005; Tipping *et al.*, 2008). Although *M. scutellaris* is a sap feeder and is diurnal, while *N. eichhorniae* is a chewer and nocturnal (Weyl & Hill 2012a), the results of the current study show that the planthopper performed best in combination with the weevil, except on plants with old weevil feeding scars.

4.4 The potential value of *Eccritotarsus eichhorniae* and *Megamelus scutellaris*

The establishment and non-establishment of biocontrol agents in biological control programmes of targeted weeds are often unknown (Julien *et al.,* 1999; Hill & Olckers, 2001). *Megamelus scutellaris* and *E. eichhorniae* are two sap-sucking agents of water hyacinth, and they both significantly reduce water hyacinth mats (Coetzee *et al*., 2007a, b, 2008; Sosa *et al.,* 2005, 2007a, b; Tipping *et al.,* 2014). However, it is possible that the two biological control agents may or may not interact in the field because of their different climatic preferences (Coetzee *et al*., 2007b, 2008; Sosa *et al.,* 2005, 2007; Tipping *et al.,* 2014). *Megamelus scutellaris* prefers cooler temperatures (Sosa *et al.,* 2005, 2007; Tipping *et al.,* 2014). Similar results have been recorded from the study conducted by Tipping *et al*. (2014) to determine the overwintering and establishment of *M. scutellaris* populations in Florida, USA. *Megamelus scutellaris* was released in 10 different sites (covered, shaded, and open) and the findings of the study showed that *M. scutellaris* populations survived at many sites, including the coldest ones. Overwintering of *M. scutellaris* was confirmed in some areas for three consecutive years. The establishment of *M. scutellaris* was also more abundant at sites with cover and shading that in open sites (Tipping *et al*., 2014). In contrast, studies in South Africa revealed that at cooler temperatures the developmental rate of *M. scutellaris* was low and, although at warmer temperatures the developmental rate of *M. scutellaris* was faster, development ceased at 30 ^{0}C (Coetzee, 2013).

The thermal physiology of *E. eichhorniae* has been researched, but not yet published (Coetzee, unpublished data). However, the thermal tolerance of this insect is similar to that of *E. catarinensis* (Coetzee *et al.,* 2007), which showed that the insect struggles to establish and impact water hyacinth in cooler, high-lying areas of the country. Thus, there is likely to be a spatial separation of *E. eichhorniae* and *M. scutellaris,* with the latter being more abundant and successful in cooler regions. It is thus recommended that, despite the lack of negative interaction between the two agents and *N. eichhorniae,* releases of *M. scutellaris* should be focussed in the cooler areas of the Highveld and Western Cape, while *E. eichhorniae* should be released in the warmer regions of KwaZulu-Natal, Mpumalanga, and Limpopo.

4.5 Multiple vs single releases

Neochetina eichhorniae has been successfully used to control water hyacinth mats at New Year's Dam (Alicedale, Eastern Cape) in South Africa for the past 16 years (Hill & Coetzee, 2017), but this level of control has not been observed elsewhere in South Africa (Hill & Olckers, 2001). Many successful studies of water hyacinth are noted when either or both *N. eichhorniae* and *N. bruchi* are released, as they complement each other (Julien *et al*., 1999; Julien, 2001). Successful control has been recorded in different localities around the world, mainly Australia, India, USA, Papua New Guinea, the three Lake Victoria countries (Uganda, Tanzania and Kenya), and Thailand (Julien *et al.,* 1999; Julien, 2000).

Hill and Olckers (2001) stated four factors that enhance the growth of the weed and reduce biological control success, namely temperature, herbicides, size of water body, and water nutrient status, of which the latter (in terms of eutrophication) is the most Important (Hill & Olckers, 2001; Hill & Coetzee, 2017). Hammarsdale Dam in KwaZulu-Natal typifies such a eutrophic water body, where *N. eichhorniae* and *E. catarinensis* were released. The two biological control agents established, but failed to control water hyacinth mats in the lake because of the rapid growth rate of the plant (Hill & Olckers, 2001).

Apart from climate and eutrophication, herbicides affect the success of biocontrol, since some herbicides are toxic to the agents (Coetzee *et al.,* 2012). The size of a water body can impact biological control by influencing wind action. For instance, some of the waterways infested by water hyacinth in South Africa are small, and there is no wind action to break up mats of agent-infested weed (Hill & Olckers, 2001; Hill & Coetzee, 2017).

Although the nine established biological control agents released against water hyacinth mats to date played an important role in controlling the weed in different sites around South Africa (Coetzee *et al.,* 2009), the factors discussed above have hindered biological control programmes from achieving total success and further addition of agents is required (Hill & Olckers, 2001; Hill & Coetzee, 2017).

One of the promising potential biological control agents is *Cornops aquaticum* (Orthoptera: Acrididae), an extremely damaging agent which has recently been released in South Africa to control water hyacinth infestations (Hill & Oberholzer, 2000). Nymphs and adults of the grasshopper defoliate the plant, resulting in severe damage. Adults chew large holes in the leaves while the early instar nymphs create scars by scraping tissue from the surface, causing more defoliation of the plant (Bownes *et al.,* 2010). Although the agent has been released, it has not yet established.

Another potential agent, *Xubida infusella* Walker (Lepidoptera: Pyralidae), a stalk borer, was first imported into South Africa in 1998 to complement the impact of *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae). The larvae feed on the leaf petiole and tunnel inside the petiole all the way into the rhizomes. Larval tunnelling causes the petiole to wilt and die, hence weakening the plant and destroying the meristems of the plant (Coetzee *et al.,* 2009; Julien *et al.,* 2001; Center *et al.,* 2002). Problems with rearing and its ineffectiveness in Australia resulted in this species being shelved.

Thrypticus truncatus Bickel & Herna´ndez and *T. sagittatus* Bickel & Herna´ndez (Diptera, Dolichopodidae) are stem-mining flies of water hyacinth native to South America (Hernández *et al*., 2007). Their feeding impact defoliates the plant, increasing the number of rotten leaves (Cordo *et al.,* 2000; Hernandez *et al.,* 2007). However, they are not considered damaging enough to warrant further investigation.

Taosa longula Remes Lenicov (Hemiptera: Dictyopharidae), is a water hyacinth planthopper that feeds and reproduces on water hyacinth. The feeding behaviour of the planthopper is similar to that of *M. scutellaris*, causing chlorosis, shorter leaves and resulting in a weakened, stunted plant. *Taosa longula* can tolerate extremely low, freezing winter temperatures. Host-specificity studies showed that the planthopper is highly specific and significantly damages the water hyacinth plants (Zvereva *et al.,* 2010; Hernandez *et al.,* 2011a, b; Sacco *et al.,* 2013). However, once again, rearing difficulties have necessitated further investigation.

4.6 Conclusions

Considerable effort has been focussed on the biological control of water hyacinth in South Africa (Coetzee *et al.,* 2011). Although probably unintentional, a cumulative stress model was adapted where some nine different agent species were released over a 40-year period. Despite this, complete control of the weed in most areas is yet to be achieved, although biological control has no doubt reduced its invasiveness. It is unlikely that additional agents will result in better control and a more holistic approach to the management of this weed, that includes pollution control as part of an integrated management strategy, is needed.

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