The role of the mite *Orthogalumna terebrantis* in the biological control programme for water hyacinth, *Eichhornia crassipes*, in South Africa

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Abstract

Water hyacinth (*Eichhornia crassipes*) is an aquatic macrophyte originating from the Amazon basin. Due to its beautiful appearance it has been introduced into numerous countries across the world as an ornamental pond plant. It was introduced into South Africa in the early 1900s and has since reached pest proportions in many of the country's fresh water bodies, causing significant economic and ecological losses. It is now considered to be the worst aquatic weed in South Africa. Efforts to control the spread of the weed began in the early 1970s and there have been some successes. Biological control has been used widely as an alternative to mechanical and chemical controls because it is cost-effective, self-sustaining and environmentally friendly.

To date, six biological control agents have been introduced onto water hyacinth in South Africa. However, due to factors such as cold winter temperatures and interference from chemical control, the agent populations are occasionally knocked-down and thus the impact of biological control on the weed population is variable. In addition, many South African water systems are highly eutrophic, and in these systems the plant growth may be accelerated to such an extent that the negative impact of the agents' herbivory is mitigated.

One of the agents established on the weed is the galumnid mite *Orthogalumna terebrantis*, which originates from Uruguay. In South Africa, the mite was initially discovered on two water hyacinth infestations in the Mpumalanga Province in 1989 and it is now established at 17 sites across the country. Many biological control researchers believe that the mite is a good biological control agent but, prior to this thesis, little quantitative data existed to confirm the belief. Thus, this thesis is a post-release evaluation of *O. terebrantis* in which various aspects of the mite-plant relationship were investigated to determine the efficacy of the mite and thus better understand the role of the mite in the biological control programme of water hyacinth in South Africa.

From laboratory experiments, in which mite densities were lower than densities occurring in the field, it was found that water hyacinth growth is largely unaffected by mite herbivory, except possibly at very high mite densities. When grown in high nutrient conditions the growth of the plant is so great that any affect the mite has is nullified.

Plant growth is thus more affected by nutrients than by mite herbivory. However, mite feeding was also influenced by water nutrient levels and mite herbivory was greatest on plants grown in high nutrient conditions. The presence of the mite had a positive effect on the performance of the mirid *Eccritotarsus catarinensis*, such that the interactions of the two agents together had a greater negative impact on the plant's growth than the individual agents had alone. Furthermore, water hyacinth physiological parameters, such as the plant's photosynthetic ability, were negatively impacted by the mite, even at the very low mite densities used in the study. Plant growth rate is dependent on photosynthetic ability i.e. the rate of photosynthesis, and thus a decrease in the plant's photosynthetic ability will eventually be translated into decreased plant growth rates which would ultimately result in the overall reduction of water hyacinth populations. In addition, temperature tolerance studies showed that the mite was tolerant of low temperatures. The mite already occurs at some of the coldest sites in South Africa. Therefore, the mite should be able to establish at all of the water hyacinth infestations in the country, but because it is a poor disperser it is unlikely to establish at new sites without human intervention. It is suggested that the mite be used as an additional biological control agent at sites where it does not yet occur, specifically at cold sites where some of the other, less cold-tolerant, agents have failed to establish. Finally, conditions of where, how many and how often the mite should be distributed to water hyacinth infestation in South Africa are discussed.

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CHAPTER 1

General Introduction

1. 1. INVASIVE ALIEN PLANTS

The movement of species from native to exotic environments posses a threat to biodiversity and is largely a result of the ease with which humans are now able to travel internationally, carrying with them, deliberately or otherwise, various exotic organisms. One of the main threats to biodiversity is the invasion of natural or seminatural habitats by alien organisms, particularly invasive plants, commonly referred to as weeds. Cronk and Fuller (1995) define a weed as "an alien plant spreading naturally (without the direct assistance of people) in natural and seminatural habitats, to produce a significant change in terms of composition, structure or ecosystem processes." Plant species in particular threaten the existence of endangered species and transform native ecosystems, costing national economies billions of dollars annually (Culliney, 2005). In South Africa, van Wilgen *et al*. (2001) estimated that the losses and damages due to weeds combined with the costs of control, in the fynbos area alone (which covers less than 6% of South Africa), cost the country $12 billion. Now, in 2010, the cost is likely to have risen considerably. Weeds are problematic in that they compete with useful and indigenous plants, occupy useful space, and spoil agricultural produce (Auld and Medd, 1987). An invasive plant usually gains a competitive advantage over indigenous

vegetation since it does not have natural enemies in its new habitat, thereby disturbing, and often destroying the indigenous ecology.

One method of slowing down the spread of weeds is through the use of biological control, which may be defined as "the study of relationships among weeds, their associated organisms, and the environment, followed by the manipulation of selected species of these organisms (natural enemies) to the detriment of a target weed" (Goeden and Andrés, 1999). The aim of biological control, however, is not necessarily to eliminate the invasive plant but rather to reduce the plant population to a level that is manageable and which allows other, native plants to survive (Delfosse *et al.*, 1976) and thus the principal goal of biological control of invasive plants is the protection of native biodiversity and ecosystem functioning (Van Driesche and Bellows, 1996). At the same time, sufficient pest (e.g. weed) numbers need to be maintained to enable the natural enemy (e.g. arthropod biological control agent) to survive and to be present if the pest population resurges and rises above the economic injury level.

While chemical and mechanical methods of removing invasive plants work to a certain extent and results are noticeable almost immediately, they are generally costly, time-consuming and need to be repeated (Andres and Bennett, 1975; Olckers, 1995). In addition, they have proved to be ineffective over large infestations for extended periods of time, and certain herbicides may cause adverse environmental effects (Delfosse, 1978a). In contrast, biological control is cost-effective, environmentally friendly, self-sustaining and permanent, and can be integrated with other control methods (Olckers, 1995). However, globally only 35% of agents released against invasive alien weeds have established successfully in the field (Memmott *et al.*, 1996) and many weeds are consequently not under acceptable control levels (Julien and Griffiths, 1998). Therefore, integrated control methods are often used to control weeds, with chemical and mechanical methods being used for short-term control and biological control being used as a long-term option (Ding *et al*., 1999; Kampeshi and Shantima, 1999; Paynter 2003; Buckley *et al*., 2004; Lym 2005).

Weed species found in South Africa, which include succulent cacti, herbaceous annuals and perennials, woody perennials and aquatic macrophytes, originate from South America (40%), North and Central America (28%), Australia (23%) and Europe and Asia

(9%) and their biological control agents (biocontrol agents) tend to be phytophagous insects, although mites, fish and fungal pathogens are also used against some of the weeds (Olckers *et al*., 1998).

South Africa is amongst the top five most active countries in the world conducting research in the field of biological control of weeds, together with the United States of America, Australia, Canada and New Zealand (McFadyen and Wilson, 1997). According to the Conservation of Agricultural Resources Act (Act No. 43 of 1983, amended in March 2001) there are over 100 category 1 declared weeds in South Africa; category 1 weeds are prohibited invasive alien plants which must be controlled and are forbidden (except with permission from the government) to be planted, propagated, transported or allowed to disperse as they may pose a serious health risk to humans and livestock and cause great financial losses to land users. Seventy biocontrol agent 'entities' (species or biotypes) have been introduced, released and are established on category 1 weeds in South Africa (Klein, in prep.) Eight aquatic plants fall under category 1 weeds and water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae)) is considered to be the worst aquatic weed in the country (Hill and Cilliers, 1999).

1.2. BIOLOGICAL CONTROL OF AQUATIC PLANTS

1.2.1. Globally

When exotic aquatic plants are not sufficiently controlled by natural enemies or through competition with other plant species, they become problematic because they interfere with the use of water by humans, and disrupt native fauna and aquatic flora (Forno and Julien, 2000) often resulting in the decline of native biodiversity (Van Driesche *et al.*, 2010).

The first use of invertebrates as control agents against aquatic weeds took place in the southeastern United States in 1964 when the chrysomelid beetle *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) was introduced for the control of alligatorweed *Alternanthera philoxeroides* (Martius) Grisebach (Amaranthaceae) (Andres and Bennett, 1975). Today, biocontrol agents of aquatic weeds have been released in some thirty-six countries, onto seven of the most important aquatic weeds: alligatorweed

A. philoxeroides, water lettuce *Pistia stratiotes* Linnaeus (Araceae), parrot's feather *Myriophyllum aquaticum* (Vellozo Conceição) Verdcourt (Haloragaceae), hydrilla *Hydrilla verticillata* (Linnaeus f) Royle (Hydrocharitaceae), water hyacinth *Eichhornia crassipes*, red water fern *Azolla filiculoides* Lamarck (Azollaceae) and salvinia *Salvinia molesta* D.S. Mitchell (Salviniaceae) (Julien and Griffiths, 1998; Van Driesche *et al*., 2010). Of these aquatic weeds, water hyacinth has the greatest number of agents released on it, and is under biological control in over thirty countries.

There is much variability in the degree of control of aquatic weeds between countries and regions within countries, and this is often attributed to field conditions being unsuitable for biocontrol agents in specific areas, as well as the poor management of water bodies (Julien *et al*., 1996; Stewart *et al*., 1996; Hill and Olckers, 2001). Aquatic weeds pose a particular threat to the development of African countries, where the livelihoods of many rural communities depend on the direct use of fresh water systems, which provide fish as food and a means of income (Masifwa *et al*., 2001). Nevertheless, there has been much success with biological control of aquatic weeds in Africa over the past two decades, as the local technology and knowledge has improved (Cilliers *et al*., 2003; Hill and Julien, 2004).

1.2.2. South Africa

In 1974 the weevil *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) was the first biocontrol agent to be released against an aquatic weed, water hyacinth, in South Africa (Cilliers, 1991). Some of the most important aquatic weeds and their biocontrol agents found in South Africa are listed in Table 1.1.

Although most of the aquatic weeds in South Africa are considered to be under substantial, if not complete, control, there are certain water bodies where the weeds remain a problem. For example, Hartebeespoort Dam (North-West Province) and sections of the Vaal River have particularly bad water hyacinth infestations, and as such these systems are regularly in the media since the public feels that the relevant authorities are not managing the systems correctly.

Table 1.1. The top five most important invasive aquatic weeds and their biological control agents, released in South Africa (Henderson and Cilliers, 2002).

Weed	**Biological control agent**	**Year of release**	**Degree of control of weed**
Pistia stratiotes (Araceae) Water lettuce	*Neohydronomus affinis* (Curculionidae)	1985	Complete
Azolla filiculoides (Azollaceae) Red water fern	*Stenopelmus rufinasus* (Curculionidae)	1997	Complete
Myriophyllum aquaticum (Haloragaceae) Parrot's feather	*Lysathia* sp (Chrysomelidae)	1994	Substantial
Eichhornia crassipes (Pontederiaceae) Water hyacinth	*Cercospora rodmanii* (synonymized with *C. piaropi*) (Hyphomycetes, Moniliates) *Eccrtitotarsus catarinensis* (Miridae) *Neochetina bruchi* (Curculionidae) *Neochetina eichhorniae* (Curculionidae) *Niphograpta albiguttalis* (Pyralidae) *Orthogalumna terebrantis* (Galumnidae)	1992 1996 1990/ 1996 1974/ 1985 1990 1989	Substantial
Salvinia molesta (Salviniaceae)	*Cyrtobagous salviniae* (Curculionidae)	1985	Complete

1.3. WATER HYACINTH: *EICHHORNIA CRASSIPES*

1.3.1. History and distribution

One of the most damaging and important aquatic weeds in South Africa is the water hyacinth (Cilliers, 1991; Hill and Cilliers, 1999). According to Sculthorpe (1967), it originates from tropical South America, most probably the Amazon Basin, and has an

extensive range including northern Brazil and Venezuela, parts of central South America and even the larger Caribbean islands (Edwards and Musil, 1975). The weed has spread throughout the world and is now found in south-east Asia (found there since 1902), the United States of America (since 1884), Egypt (since around 1879) and central and southern Africa (since the early 1900s) (Edwards and Musil, 1975, Cilliers, 1991).

In South Africa, *E. crassipes* was introduced as an ornamental pond plant and is now widely distributed in the eastern half of the country with greatest concentrations found along the KwaZulu-Natal coast and along the Vaal River (Edwards and Musil, 1975; Henderson and Cilliers, 2002) (Fig. 1.1). In the last 20 years it has reached pest proportions in many rivers and dams of the Eastern Cape Province, in the Hartebeespoort Dam and the Vaal River in the Gauteng Province as well as in the Crocodile River in the eastern Mpumalanga Province (Edwards and Musil, 1975).

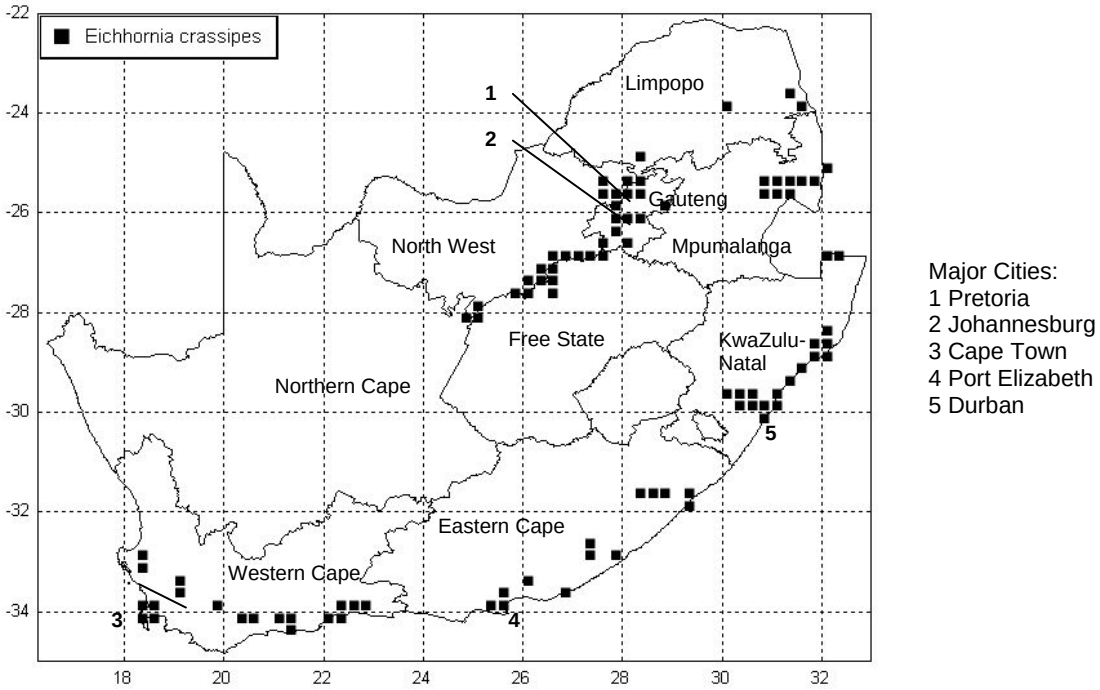

Figure 1.1. Distribution of water hyacinth (*Eichhornia crassipes*) in South Africa. (SAPIA database 2007, Agricultural Research Council – Plant Protection Research Institute).

1.3.2. Biology

The biology of *E. crassipes* is described in detail by various authors (Penfound and Earle, 1948; Edwards and Musil, 1975; Gopal, 1987). *Eichhornia crassipes* is a perennial, free-floating aquatic macrophyte with dark green leaves and pretty pale-purple flowers, and usually grows to a height of 10-20 cm. The roots are long and feathery to help balance the plant, while the petioles are swollen and filled with air to keep the plant afloat but are less swollen in dense infestations (Henderson and Cilliers, 2002). The leaves and petioles are highly plastic and may change from long and narrow petioles with elongated leaves to short and bulbous petioles with wide leaves (Penfound and Earle, 1948). Leaves are produced singly from the apical meristem and are arranged spirally around the stem, so that as the stem elongates the leaves move progressively lower on the stem and begin to senesce. Leaf production is constant and proportional to leaf mortality, so that each plant tends to maintain a constant number of leaves (Center, 1981).

In shallow water, the plant may become anchored and produce flowers and seeds, with flowering being stimulated by temperature rather than day length. In deeper water vegetative reproduction occurs through runners, called ramets or daughter plants, and these become individual “mother” plants once they have broken off at the stolon that connects them to the original mother plant. In suitable habitats (usually where the water is highly eutrophic), the population may double in number every 11 to 18 days (Edwards and Musil, 1975). The seeds are able to withstand long, dry spells and remain viable even after 15 years (Edwards and Musil, 1975), thus making chemical and mechanical control methods ineffective in the long term.

Water hyacinth can survive at a wide range of temperatures. Penfound and Earle (1948) found that the leaves were destroyed at -2.8 ºC but the plants re-sprouted. The rhizome tips froze at -5 ºC, after which the plants did not re-sprout and died. In addition, free-floating plants are less resistant to prolonged exposure to cold temperatures than rooted plants (Owens and Madsen, 1995). This implies that in South Africa only the most unusual weather conditions and the highest altitudes with low temperatures will hinder the growth and development of the plant.

The weed therefore easily becomes invasive in its introduced range because of its rapid growth rate, vegetative reproduction, the absence of natural enemies and the ability to re-infest from seeds or flood-borne plants (Harley *et al*., 1996). It is thus the plant's life cycle and survival strategies that have given water hyacinth a competitive advantage over other species (Gutiérrez *et al.*, 2001).

1.3.3. Impact on the environment

Water hyacinth does well in water where there is no other aquatic vegetation, yet it is also a fierce competitor that easily out-competes other aquatic plants (Gopal, 1987). Due to its competitive advantage, water hyacinth forms dense mats on the water surfaces of dams, rivers, lakes and canals, thus clogging waterways, preventing navigation and fishing, creating an environment suitable for the breeding of mosquitoes and bilharzias-carrying snails, and large mats of the plant may put pressure on infrastructure such as bridges and hydro-electric power stations (Pieterse, 1978; Hill, 2003).

The mats disrupt the aquatic environment by reducing water flow and decreasing light penetration, and greatly increase the loss of water through evapotranspiration (Lallana *et al*., 1987; Cilliers and Neser, 1991). Rapid rotting of large plant masses causes oxygen depletion and increases water acidity, resulting in the death of indigenous flora and fauna, thus reducing the biodiversity of entire ecosystems (Henderson and Cilliers, 2002; Midgley *et al*., 2006). McVea and Boyd (1975) showed experimentally that fish production of phytoplankton-feeding fish may decline by up to 50% where just 10% of the water surface is covered by water hyacinth, due to competition for nutrients and light between water hyacinth and the phytoplankton. In a study of Argentinean floodplain lakes it was found that the lowest mean biomass of macrobenthos was associated with lakes covered by water hyacinth (Bechara, 1996). Similarly, along the northern shores of Lake Victoria in Uganda, the abundance and diversity of aquatic macroinvertebrates decreased in water hyacinth mats moving from the boundary with open water towards the middle of the mats (Masifwa *et al.*, 2001).

1.3.4. Utilisation

About 95% of water hyacinth is made up of water (Edwards and Musil, 1975). This means that to gain a mere one tonne of dry material one would have to collect and process approximately nine tonnes of fresh plant material (Julien *et al.*, 1996). Not only would this require extensive machinery and labour, making the drying process commercially impractical, but the plants tend to occur at scattered and inaccessible locations (Julien *et al*., 1996).

Nevertheless, water hyacinth is being utilized in a hand-full of countries. For example, in the Philippines, Thailand and Indonesia the plants are use to make sandals, hats, baskets, vases, furniture and stuffing for upholstery (Lindsey and Hirt, 2000) while in India the dried fibrous stems of the plant are used to make paper and crafts (Edwards and Musil, 1975; Julien *et al.*, 1996). Some subsistence communities in China and Malaysia use water hyacinth as fodder for livestock (pigs, rabbits, chickens, fish), and in Sudan the plants are eaten by cattle during drought conditions (Lindsey and Hirt, 2000). Animals are fed this fodder even though the plant's high chlorine and potash content makes it almost unpalatable (Edwards and Musil, 1975). A Maximum Security Prison in Uganda has an ingenious use for the weed – prisoners collect the plants which are mixed with cow-dung to generate biogas. The gas is then stored and use during power outages to cook meals for the prisoners, thus saving Uganda about US $5,000 per annum (Lindsey and Hirt, 2000).

Due to the plant's high NPK content, the plant can be mixed with ash, cow dung and wood ash to produce compost (Edwards and Musil, 1975). Penfound and Earle (1948) suggested using the plant as a cheap and environmentally friendly means of decontaminating wastewater because of its high rate of nutrient absorption and fast growth rate. Rogers and Davis (1972) estimated that under favourable conditions, one hectare of water hyacinth plants could absorb the average nitrogen and phosphorus waste production of over 800 people each day. However, they also pointed out that this would only occur if the plants had a constant maximum nutrient uptake and grew continually throughout the year.

The low demand for water hyacinth products combined with the high cost of processing the raw material and the inaccessibility to most water hyacinth infestations justifies against its utilization (Julien *et al.*, 1996). Thus, considering the limited uses of water hyacinth together with the costs involved in making it usable, compared to the devastating damage the plant causes to ecosystems in South Africa and the losses involved with such damage, it becomes clear why the further spread of this plant needs to be prevented and why its existing populations must be controlled.

1.3.5. Biological control of water hyacinth in South Africa

Water hyacinth can be controlled using chemical, mechanical and biological methods, but these tend to be ineffective when used in isolation (Campbell *et al.*, 1996). Today it is generally accepted that mechanical and chemical controls have limited application due to their high cost and low sustainability (Julien, 2001). Therefore, biological control is favoured in many countries, including South Africa, where a biological control programme on water hyacinth was initiated during the early 1970s, terminated in 1977 and then restarted in 1985 (Cilliers, 1991).

More than 30 phytophagous species have been collected from water hyacinth (Andres and Bennett, 1975). Damage to water hyacinth by arthropod natural enemies occurs either by the direct removal of the plant tissue or by the decomposition of tissue that surrounds the feeding area (Perkins, 1974). Since it was unlikely that a single insect (i.e. *N. eichhorniae*) would achieve efficient control on its own (Bennett, 1977), four other arthropod natural enemy species and one pathogen have been released and established in South Africa: another leaf-feeding weevil *N. bruchi* Hustache, a petiole-mining moth *Niphograpta albiguttalis* (= *Sameodes albiguttalis* Warren) (Lepidoptera: Pyralidae), a leaf-sucking mirid *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), a leaf-mining mite *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae) and a fungal pathogen *Cercospora piaropi* Tharp (Hill and Cilliers, 1999; Hill and Olckers, 2001). Host-specificity tests have been conducted on another potential agent, the grasshopper *Cornops aquaticum* (Burner) (Orthoptera: Acrididae), and permission for the release of the agent was recently granted by the Department of Tourism and Environmental Affairs (A. Bownes and J. Coetzee pers. comm.).

The two weevils, *N. eichhorniae* and *N. bruchi*, have been more successful than the other agents (Cordo, 1999). The adults of both weevils are nocturnal and produce characteristic rectangular feeding scars, usually on the youngest leaves (Hill and Cilliers, 1999). Large numbers of feeding scars damage the plant by removing epidermal tissue, thus increasing water loss and exposing the plant to attack by pathogens (Julien, 2001). Larvae create damage by burrowing down petioles and into the crowns of the plants thus preventing flowering, and by pupating in the roots, causing damage to tissue (Hill and Cilliers, 1999; Julien, 2001). The two species co-exist, but *N. bruchi* is more cold tolerant (DeLoach and Cordo, 1983), and more effective in nutrient-enriched waters than *N. eichhorniae* (Heard and Winterton, 2000). Since the worst infestations of water hyacinth in South Africa occur in eutrophic waters at high altitudes, *N. bruchi* is an important agent at high altitude sites characterized by cold winters (Hill and Cilliers, 1999).

The adult females of the moth *N. albiguttalis* oviposit on the young leaves of water hyacinth, usually in areas where the epidermis has been damaged by weevil feeding scars (Hill and Cilliers, 1999). Larvae prefer to mine bulbous petioles of leaves, causing severe necrosis and water logging (Hill and Cilliers, 1999; Julien, 2001). The moth attacks plants along the fringes of an infestation, thus reducing net growth and the spread of the mats (Julien, 2001). Since *N. albiguttalis* prefer young, growing plants, they are most effective in areas where there is a constant supply of bulbous plants, which are usually produced after populations of mature plants have been controlled by chemical or mechanical methods (Hill and Cilliers, 1999). However, no post-release evaluations have been conducted on this agent (M. Hill pers. comm.).

The females of the mirid *E. catarinensis* insert eggs into the leaves, just below the surface of the lamina, and the nymphs (of which there are four instars) develop and feed gregariously with the adults on the leaf undersurface (Hill and Cilliers, 1999). The nymphs and adults cause chlorosis of the laminae, reducing the plant's ability to photosynthesize and therefore its ability to grow and reproduce (Julien, 2001; Ripley *et al.*, 2006). Although the mirid has established at some high altitude sites where winters are characterized by frost, it is most effective at tropical and subtropical sites (Hill and Oberholzer, 2004).

Although to date five pathogens occur on *E. crassipes* in South Africa, only *C. piaropi* has been purposefully released and is the best studied (Morris *et al.*, 1999). Morris (1990) first recorded it from a small farm dam in the Mpumalanga Province in 1986, and believes that it was introduced accidentally via a shipment of *N. eichhorniae* from Australia. The pathogen causes numerous small, dark-brown spots on the leaves and petioles of the plant which may coalesce and kill the leaves (Morris *et al*., 1999). Through translocation of naturally infected or inoculated plants, the pathogen now occurs in the Gauteng, Eastern Cape, KwaZulu-Natal, and Western Cape Provinces (Morris *et al.,* 1999).

Studies have shown that combinations of agents cause more damage to water hyacinth than agents working in isolation (Delfosse, 1978a; Moran, 2005). Thus, this thesis investigates the damage caused to water hyacinth by *O. terebrantis*, and hence its potential as an additional biocontrol agent at sites in South Africa where it does not yet occur. Based on qualitative observations, a number of specialists in the biological control of water hyacinth consider *O. terebrantis* to be a good biocontrol agent (Cordo and DeLoach, 1976; C. Cilliers and M. Hill pers. comm.). On the whole, however, the mite has been largely ignored and understudied. Therefore, the overall aim of this study is to examine the galumnid mite, *O. terebrantis*, as an additional agent in the biological control programme of water hyacinth in South Africa.

1.4. MITES (ACARI) AS AGENTS FOR PEST REDUCTION

Among the Arachnida, mites are unique in that they utilize a wide range of non-predatory life styles (Krantz and Lindquist, 1979). Gerson *et al.* (2003) define biological control of pests by mites as “acarine actions (e.g. predation, parasitism, parasitoidism, phytophagy, competition, disease transmission, other activities and any combination thereof) that reduce pest numbers and/or the extent of their damage to below the accepted economic (and medical or veterinary) injury level.” In 1868, Shimer (in Gerson *et al*., 2003) noticed that the mite *Hemisarcoptes malus* Shimer (Acari: Hemisarcoptidae), through feeding on the pest oystershell scale *Lepidosaphes ulmi* (L.) (Hemiptera: Diaspididae), greatly reduced the pest’s populations. Since then, whilst not being the first choice as candidates for biological control, mites have been recognized as natural enemies of pests, including weeds (Cromroy, 1983; Gerson *et al*., 2003).

Cromroy (1983) developed a scoring system that can be used to predict the potential effectiveness of mites in the control of weeds (Table 1.2). The scoring system is divided into 12 categories and takes into account host specificity, direct and indirect damage and compatibility with other agents.

The first weed control with acarine agents was considered in 1924 when the spider mite *Tetranychus desertorum* Banks (Acari: Tetranychidae) was found to be a significant feeder on the prickly-pear cactus *Opuntia inermis* (de Candolle) (Cactaceae) in Australia (Gerson *et al*., 2003). Up to 1998, approximately 350 organisms (invertebrates, vertebrates and pathogens) were released for the biological control of weeds world-wide, and only seven species have been mites (Julien and Griffiths, 1998). The seven mite species belong to the families Eriophyidae, Galumnidae and Tetranychidae, with the Eriophyidae having the greatest potential as candidates for weed control (Craemer, 1993). Julien and Griffiths (1998) list most of the mites being used as biocontrol agents of weeds (Appendix 1). Other acarine agents used in weed biological control include the eriophyid *Aceria liopeltus* Meyer which is used to control *Acacia nilotica* spp. *indica* (L.) Willd. ex Delile (Leguminosae) in Australia (Witt, 2004); the eriophyid *A. lantanae* is used to control *Lantana camara* L. (Verbenaceae) in South Africa (Baars and Neser, 1999) and in Australia (Walter 1999); the eriophyid *A. convolvuli* Nalepa controls *Convolvulus arvensis* L. (Convolvulaceae) (field bindweed) in Canada and the United States (Julien and Griffiths, 1998), and the eriophyid *A. cynodoniensis* (Sayed) damages *Cynodon dactylon* (L.) Pers. (Poaceae) (bermudagrass) in Florida (Cromroy, 1983). As mentioned above, although it is known that *O. terebrantis* causes damage to water hyacinth, no quantitative studies have been conducted on its damaging effects on the weed. The impact of *O. terebrantis* on water hyacinth thus forms the major component of this thesis.

Table 1.2. Cromroy's (1983) scoring system for predicting the potential effectiveness of mites in the control of weeds (score over 27 suggests a likely candidate). Using this scoring system conservatively, *Orthogalumna terebrantis* scores 23 points (scores highlighted in bold, as per present author).

1 Host specificity	
A. Broadly polyphagous (feeding on a number of unrelated genera)	0
B. Oligophagous (feeding on a limited variety of related plants)	1
C. Restricted monophagous (specialized on a biotype species)	2
D. Monophagous (specialised on a species or species group)	**3**
2 Direct damage inflicted	
A. Leaf mining or gall forming	**1**
B. Defoliating	2
3 Indirect damage inflicted	
A. None	0
B. Initial damage reduces seed production	1
C. Disease transmission or renders plant susceptible to invasion by other organisms	**3**
4 Phenology of attack	
A. Limited period of attack not increasing plant susceptibility to drought, frost, or competition from other plants	0
B. Limited period of attack but combining with another agent to cover the growing season	**2**
C. Limited period of attack increasing plant susceptibility to frost, drought, or competition from other vegetation	3
D. Prolonged attack covering the growing season	4
5 Number of generations	
A. Obligate univoltine species	1
B. Two to three generations a year, climate permitting	**2**
C. Over four generations a year, climate permitting	4
6 Average number of progeny per female per generation	
A. Under 10	0
B. 10 to 100	**1**
C. Over 100	2
7 Extrinsic mortality factors of mites	
A. Natural control largely by non-specific enemies or ecological factors	**0**
B. Subject to extensive mortality from other competitors from the host, combined with relatively common occurrence	3
C. Subject to extensive mortality from specialized enemies including diseases and relatively immune to non-specific enemies	4
8 Feeding behavior	
A. Solitary feeder (due to cannibalism, avoidance, or other intrinsic behavior)	0
B. Gregarious or colonial feeders	**2**
9 Compatibility with other control agents	
A. Compatibility poor or restricts possibilities of introductions of additional agents	0
B. Compatibility good	**2**
10 Distribution	
A. Local	**0**
B. Covers about half the range of the target weed	2
C. Covers about three-quarters of the range of the target weed	4
D. Covers full range of the target weed	6
11 Evidence of effectiveness as a control agent	
A. Failure in previous biological control attempt(s)	0
B. Controls host in native habitat or one region of introduction	**4**
C. Successful in two or more regions of the world	6
12 Dissemination	
A. Tends to form “pockets” of infestation of low density populations	1
B. Tends to form “pockets” of infestations of high density populations	**3**
C. Tends to be widespread with low density population	5
D. Tends to be widespread with high density populations	7

1.5. *ORTHOGALUMNA TEREBRANTIS*

1.5.1. Introduction

Orthogalumna terebrantis belongs to the suborder Oribatida in the family Galumnidae. Very little is known about the tiny oribatid mites, yet they are one of the most abundant and diverse groups of arthropods (Schatz and Behan-Pelletier, 2008). The suborder comprises of over 9000 named species that make up 172 families (Norton and Behan-Pelletier, 2009). Most oribatid species are non-predatory, feeding either as saprohages or microphytophages, while feeding on higher plants is usually restricted to decaying tissue (Krantz and Lindquist, 1979). The oribatid life cycle is primarily characterized by a long adult life span, slow growth and low reproductive potential (Schatz and Behan-Pelletier, 2008). In temperate zones the life cycle is often synchronized with the annual climatic cycle (Norton, 1994). Of the 1000 genera, only 6 (one of which is *O. terebrantis*) are known to contain species that feed on living plant matter (Krantz and Lindquist, 1979). Galumnidae are characterized by large and movable pteromorphs which extend interiorly and posteriorly. They have weak a smooth cuticle that has four pairs of porose areas (Gerson *et al*., 2003) (Fig. 1.2).

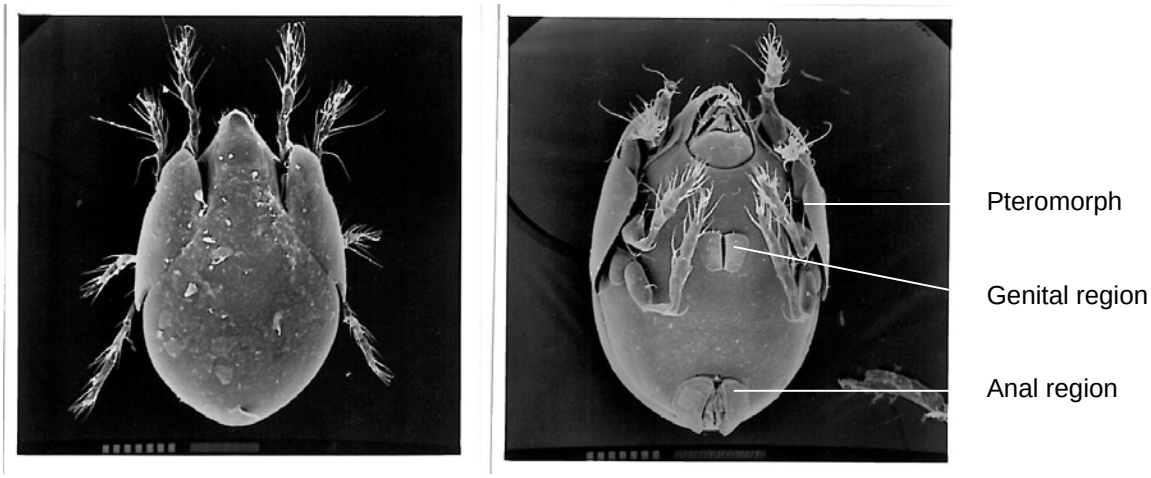

Figure 1.2. Dorsal (left photo x 150) and ventral (right photos x 200) views of *Orthogalumna terebrantis*. Photographs from C. Cilliers, courtesy of D. Kruger (Agricultural Research Council – Plant Protection Research Institute).

1.5.2. History and distribution

Orthogalumna terebrantis belongs to a small group of oribatid mites that are known mainly from Madagascar, southeastern North America and Central and South America (Bennett, 1968 in Delfosse, 1978b). The mite was first discovered in Uruguay, and is indigenous to South America (Wallwork, 1965). It is native to both North and South America (Center *et al*., 2002a). It is now present in ten countries, namely India, Zambia, Cuba, Jamaica, Malawi, Mexico, Mozambique, South Africa, Zimbabwe and the southern United States of America, with variable success as a biocontrol agent (Julien and Griffiths, 1998). The mite is highly specific to water hyacinth (Andres and Bennett, 1975), although it can feed on a relative of water hyacinth, the pickerelweed *Pontederia cordata* L. (Pontederiaceae) (Center *et al*., 2002b) and can survive on a diet of algae in situations when water hyacinth is in low supply (Sumangala and Haq, 1995). In Argentina, Cordo and DeLoach (1976) observed that the southern limit of the mite was determined by the presence of water hyacinth rather than temperature. Water hyacinth stops growing at temperatures below 10 ºC (Gopal, 1987), while adult mites can survive below 0 ºC if they are sheltered within leaf tissue (Cordo and DeLoach, 1976).

A colony of *O. terebrantis* was imported into South African quarantine in 1975, along with *N. eichhorniae*, but the colony did not survive under insectary conditions (Cilliers, 1991). Surprisingly, however, in 1989 populations of the mites were discovered on two water hyacinth infestations in the Mpumalanga Province (formally called the Eastern Transvaal Province) (Cilliers, 1991). The timing of the introduction and the method of translocation of *O. terebrantis* in South Africa remain unclear but it is likely that the mite came into South Africa from Zambia via a subsidiary of the Zambezi River.

1.5.3. Biology

Various authors have described the biology of *O. terebrantis* (Wallwork, 1965; Silveira-Guido, 1965; Perkins 1973; Cordo and DeLoach, 1976). *Orthogalumna terebrantis* has chewing mouthparts, and it is consequently a particle feeder, and this plays an important role in its biology and ecological significance as a decomposer (Norton and Behan-Pelletier, 2009). The mite is one of very few phytophagous oribatids (Cordo and DeLoach, 1975). The adults are shiny dark-brown in colour, heavily sclerotised, tear-

drop shaped, and measure about 0.3 mm in width and 0.5 mm in length. When disturbed, adults tend to cluster in protected areas such as the feeding spots of snails and weevils, broken areas of the leaf or inside abandoned tunnels created by mite nymphs (Cordo and DeLoach, 1976). Interestingly, in laboratory feeding tests Cordo and DeLoach (1975) found that mites survived longest on leaves with *Neochetina* spp. feeding scars. Silveira-Guido (1965) observed that the number of mites increased from the centre towards the border of the lamina.

Adult life duration under laboratory conditions is reported to be about 78 days at 26 ºC (Ganga-Visalakshy and Jayanth, 1991) and the egg to adult period is between 22 and 25 days at 25-26 ºC (Cordo and DeLoach, 1976; Ganga-Visalakshy and Jayanth, 1991). Adult females use their chewing mouth parts to cut small (0.075 mm) round holes in the abaxial surface of the water hyacinth lamina into which eggs are deposited, with the young central laminae being preferred for oviposition. Young leaves have a higher nitrogen content which decreases as leaves mature and then remains constant until senescence (Center and Wright, 1991), and it is possible that females are choosing leaves according to their nutrient levels. A female will typically oviposit on separate leaves, inserting an egg every fourth or so leaf (Perkins, 1973). Usually only one egg is laid per puncture (Ganga-Visalakshy and Jayanth, 1991), but Cordo and DeLoach (1976) found that up to five eggs were laid per puncture in a laboratory experiment. Reports on fecundity are highly variable: Ganga-Visalakshy and Jayanth (1991) reported an average of 58.5 eggs produced per female lifetime, as determined from laboratory observations at 26 ºC, while Delfosse (1977b) reported an average of 21.2 eggs produced per female per week, when females were held at incubator temperatures of 10 ºC during a 14 h dark phase and 30 ºC during a 10 h light phase. Eggs hatch within 7-8 days (at 26 ºC), and whitish, slow-moving larvae, with three pairs of legs and measuring less than 0.24 mm, are produced. Nymphs, of which there are three stages (proto-, deuto-, and tritonymphs), are amber coloured and have four pairs of legs. Nymphal stages are distinguished according to size (maximum lengths are 0.28 mm, 0.34 mm and 0.43 mm, respectively) (Ganga-Visalakshy and Jayanth, 1991). Roughly 15 days are needed for development through larval and nymphal stages. The nymphs continue burrowing in the galleries started by the larvae, sometimes changing direction (Silveira-Guido, 1965). Tritonymphs may leave their galleries and begin new galleries without a break in the epidermis (Cordo and DeLoach, 1976). The ratio of adult to nymph to larvae

is 1:5:10 and no sexual dimorphism exists (Wallwork, 1965; Perkins, 1973). Just before the nymphs become adults, the tritonymphal skin is shed and can often be observed lying over the body or next to the adult inside the tunnel (Wallwork, 1965).

The adult mites can survive temperatures ranging from 0 ºC to 40 ºC, although Cordo and DeLoach (1976) reported that in Argentina mite populations withstood -10 ºC and could survive for substantial periods at -5 ºC on water lettuce growing among water hyacinth. Whether the mites actually burrowed into the water lettuce or simply hid amongst the water lettuce leaves is not reported by Cordo and DeLoach (1976). The mites are able to overwinter in all developmental stages (Cordo and DeLoach, 1976). In the Buenos Aires area in Argentina, Cordo and DeLoach (1976) found that *O. terebrantis* could have up to three generations per year, but that population numbers in the same area varied noticeably from year to year. According to Perkins (1973), the number of generations per year depends on the number of days of high temperature, although the major cause of death is exposure to high temperatures and sunlight.

Field observations by Perkins (1973) indicate a possibility of two strains of the mite with obvious differences: the Florida strain can feed on pickerelweed (Gordon and Coulson, 1971 in Perkins, 1973), and tends to restrict its movement to shaded areas, while the Argentine strain feeds strictly on water hyacinth and occurs in both sunny and shaded areas. The mite has never been collected on pickerelweed in the field in South Africa, where the plant is potentially invasive (Hill *et al*., 2000).

1.5.4. Feeding damage and effect on water hyacinth

Mite feeding damage is restricted to the laminae. The greatest damage is caused by larvae and nymphs which feed on paranchymeous tissue as they develop inside the lamina. The feeding of the larval and nymphal stages produces tunnels, called galleries, which appear on the exterior of the leaf as yellowish linear streaks, giving the leaf a striped appearance. The tunnels are found between leaf veins, and extend towards the leaf apex, producing characteristic galleries of 5-10 mm in length (Cordo and DeLoach, 1976). Frass accumulates at the base of the galleries, giving the basal portion a darkened appearance. The tissue inside galleries dries up, eventually resulting in the lamina folding and breaking at the necrotic area, but without causing the lamina to fall off

completely (Silveira-Guido, 1965). Eggs tend to be laid on younger leaves, such that the amount of damage and size of galleries increases as the nymphs develop and as leaves increase in age (Cordo and DeLoach, 1976). In severe mite infestations the adjacent galleries may intersect, resulting in a complete brown appearance of the leaves. Pin holes at the distal end of the galleries on the upper surface of leaves indicate that adults have emerged from the galleries (Oberholzer, 2001; Center *et al*., 2002a). Adult mites are often found feeding in weevil feeding spots (Delfosse *et al*., 1975).

It is estimated that it would take roughly 1 month for a heavily-infested leaf to collapse (Sumangala and Haq, 1995). In addition to damaging water hyacinth by feeding on the laminae, the emergence holes of mites serve as an entry point for the pathogen *Acremonium zonatum* (Sawada) Gams (Fungi), which is disseminated by the mites and grows very well in the highly humid mite galleries, from where it causes zonate leaf spot disease (Delfosse, 1978b; Gerson *et al*., 2003). Furthermore, Delfosse (1978b) observed that the presence of mites stimulated weevils to produce significantly more eggs and to feed more, thus increasing weevil damage on the plant. The combined activities of weevils and the mite reduced water hyacinth densities by about 50% in one year (Delfosse, 1978a).

Despite the significant impact that *O. terebrantis* has on water hyacinth in Malawi (Hill, unpublished data), quantitative evaluations of the mite have generally been neglected, possibly underrating it as an important biocontrol agent (Center *et al*., 2002a). In the words of Silveira-Guido (1965), “This phytophagous mite (in spite of its size) performs such a systematic action that during the Fall and Winter field trips it is impossible to obtain healthy plants of *Eichhornia* for other works.”

1.5.5. Natural enemies of Orthogalumna terebrantis

Orthogalumna terebrantis is fairly resistant to predation and desiccation due to its sclerotised body (Haq and Sumangala, 2003). Neither Silveira-Guido (1965) nor Cordo and DeLoach (1976) found any specific parasites or predators attacking the mite in the field. However, under laboratory conditions Cordo and DeLoach (1976) showed that certain staphylinid beetles fed on adult mites at an average of 3.9mites/beetle per day,

suggesting that some mortality of mites may occur in the field due to general predators feeding on them.

1.5.6. Present status of **Orthogalumna terebrantis** *in South Africa*

Cilliers (pers. comm.) found that the mite established in subtropical and temperate climates but did not establish under Highveld conditions characterized by cold winters. High population densities, noticeable from January to March, have been recorded on the Crocodile River West in the North-West Province, Crocodile River East in the Mpumalanga Province, Bon Accord Dam in the Gauteng Province, the Letaba River in the Kruger National Park in the Northern Province, Sterkspruit below Hammarsdale Dam in KwaZulu-Natal Province, and at the Nseleni River near Richards Bay also in KwaZulu-Natal (Hill and Cilliers, 1999) and all sites from where *O. terebrantis* is recorded are indicated in Figure 1.3. However, since the mite can withstand fairly harsh temperatures (< 0ºC to > 40ºC), Hill and Cilliers (1999) suggest that further attempts should be made to establish *O. terebrantis* on the Vaal River and other colder areas in South Africa.

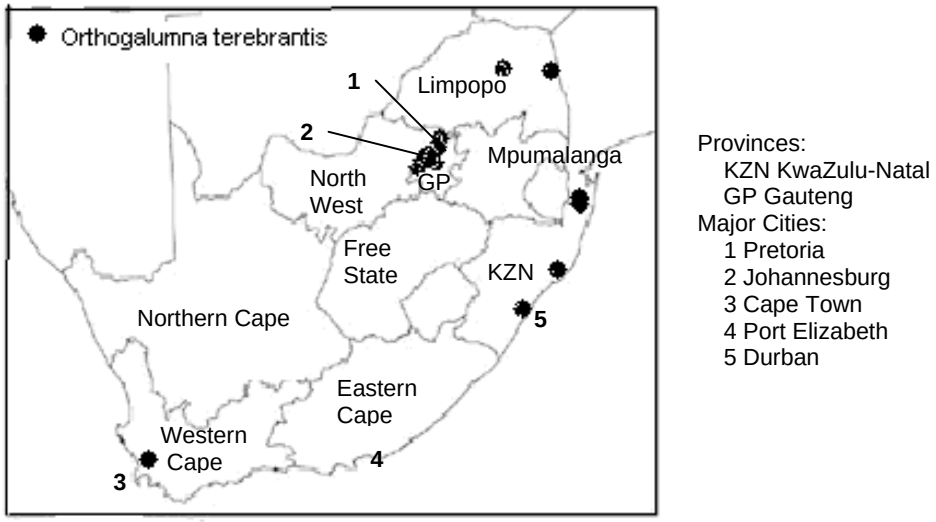

Figure 1.3. Distribution of *Orthogalumna terebrantis* in South Africa (mite establishment records obtained from the Rhodes University Aquatic Weeds Monitoring Survey database (D. Schlange) and the Integrated Management of Water Hyacinth in South Africa database (Byrne *et al.*, 2010)).

1.6. AIMS OF THESIS

Despite the fact that *O. terebrantis* affects water hyacinth directly by feeding on it and creating galleries in the laminae, thereby damaging the laminae, and indirectly by promoting the activities of weevils and by making water hyacinth more susceptible to pathogens, only a few studies have described the damage caused by *O. terebrantis* herbivory (Silveira-Guido, 1965; Perkins, 1973; Cordo and DeLoach, 1975, 1976; Ganga-Visalakshy and Jayanth, 1991) and only one study (Del Fosse, 1978a) has quantified the impact of the mite on water hyacinth growth, but this too was the combined impact caused by both the mite and *N. eichhorniae*. It is therefore possible that *O. terebrantis* has been highly underestimated as an agent for the control of water hyacinth (Hill and Cilliers, 1999; Charudattan, 1986). Thus, it is necessary that quantitative post release evaluations are conducted. Therefore, the overall aim of this thesis is to investigate the role of the mite in the biological control programme on water hyacinth in South Africa. Accordingly, the following parameters will be investigated:

1) The impact of different densities of the mite on water hyacinth growth will be tested to determine how many mites are needed for plant growth to be impacted negatively (Chapter 2, serves as a pilot study).
2) The impact of the mite on water hyacinth grown under different nutrient regimes, namely low, medium and high concentrations, will be tested. Water hyacinth grows exceptionally well under eutrophic conditions, to such an extent that damage caused by the biocontrol agents on plants growing in eutrophic waters is almost negated (Chapter 3).
3) The interaction of the mite with other biocontrol agents (*N. eichhorniae* and *E. catarinensis*) and their combined impact on water hyacinth will be investigated. The combined impact of the agents will be compared to the impact of each agent individually. Field observations show that the additional stress on water hyacinth caused by the combined action of more than one agent results in a greater reduction of plant growth parameters (Chapter 4).
4) The impact of the mite on plant physiological parameters (e.g. photosynthesis) will be tested, as well as the impact of the mite on leaf chlorophyll content. It is suspected that mite feeding lowers the leaf chlorophyll content, thereby

decreasing the plant's photosynthetic rate and thus placing stress on the plant which consequently decreases the plant's growth (Chapter 5).

5) The temperature tolerances of the mite will be determined. The lower and upper lethal temperatures (LT_{50}) and the critical minima and maxima (CT_{Min} /CT_{Max}) will be determined to investigate in which areas of South Africa the mite is likely to establish and thus serve as an additional agent (Chapter 6).

Once the above parameters have been investigated it should be possible to determine firstly, whether *O. terebrantis* should be release at sites where it dos not yet occur, as a supplementary agent to the agents already controlling water hyacinth in South Africa, and secondly, in which parts of South Africa *O. terebrantis* is likely to cause the greatest damage to water hyacinth infestations.

CHAPTER 2

The impact of herbivory by different densities of *Orthogalumna terebrantis* on water hyacinth growth parameters and leaf chlorophyll content

2.1. INTRODUCTION

Populations and communities of organisms making up ecosystems are regulated by 'top-down' and 'bottom-up' processes, where top-down processes refer to consumers suppressing the species on which they feed, and bottom-up processes refer to resources (e.g. nutrients as resources for plants, or plants as resources for herbivores) influencing the population dynamics of the organisms reliant on them (Strong, 2008). Ecologists have long debated which of these two processes is most important in structuring communities but, in general, the two processes act simultaneously between trophic levels (Hunter and Price, 1992; Power, 1992) and their importance fluctuates in response to variables such as spacial and temporal variation in the abundance of the organisms within a community (Hunter, 2001). However, as Hunter and Price (1992) point out, the removal of higher trophic levels (e.g. herbivores) leaves the lower levels (e.g. plants) present, while the reverse scenario is not true.

In a simplified food chain a plant-herbivore relationship falls into the middle trophic level, and both the plants and the herbivores are affected by top-down and bottom-up processes. The plant population is dependent on abiotic forces such as nutrient and

water supply (bottom-up) and is also affected by competition (top-down) (Mihaliak and Lincoln, 1989; Van *et al*., 1999; Niemelä *et al*., 2008) and by the population density and impact of its herbivores (top-down) (Meyer, 1993; Doyle *et al.*, 2002; Briese *et al*., 2004; Elderd, 2006). The herbivore population is in turn affected by predation (top-down) (Schoonhoven *et al*., 1998; Lang *et al.*, 1999; Elderd 2006) and competition (Karban *et al.*, 1987; Kaplan and Denno, 2007) and is dependent on plants for food and is also affected by the plant abundance and spacial distribution (bottom-up) (Lenz and Taylor, 2001; Ober and Hayes, 2008) and by the plant quality and its defence mechanisms (Dyer *et al.,* 2004; Rodriguez -Saona and Thaler, 2005).

A number of studies have examined the effects of high and low insect densities on their host plants and have found that the higher the insect density, the more adversely effected are certain plant growth parameters and the greater is the damaged to the host plant (Center and Van, 1989; Center and Jubinsky, 1996; Briese *et al*., 2002, 2004; Ding *et al*., 2006; Bebawi *et al*., 2007). Center and Van (1989) found that water hyacinth leaf mortality was 50% higher when subjected to high *N. eichhorniae* weevil populations (250 weevils per tank measuring 0.8 m by 2.2 m) than when no weevils were present. On the other hand, Center and Jubinsky (1996) showed that a mat of plants exposed to 4000 weevils grew as well as the same size mat exposed to 1000 weevils due to emigration of adult weevils, which was possibly a result of overcrowding. In addition, it has been established in weed biological control that the greater the number of individuals released the greater is the chance of establishment of the population (Memmott *et al*., 1996; Grevstad, 1999; Shea and Possingham, 2000). Furthermore, Grevstad (1999) found that release size also had a significantly positive effect on the population growth rate of the chrysomelid beetle *Galerucella calmariensis* (L.) (Coleoptera: Chrysomelidae) released on purple loosestrife, *Lythrum salicaria* L. (Lythraceae) in North America. However, insect performance may be density dependent, for example, the growth of caterpillars *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was negatively affected at high caterpillar densities due to a decrease in tomato plant quality as the caterpillar densities increased and hence as the amount of damage increased (Underwood, 2010). Studies such as these are, however, lacking for mite populations, and specifically for the mite *O. terebrantis*.

Feeding damage of *O. terebrantis* is restricted to the leaves where the greatest damage is caused by larvae and nymphs as they tunnel within the leaf tissue. Yellowish-brown streaks (galleries) occur on heavily infested leaves, and it takes

roughly one month for a leaf to collapse (Sumangala and Haq, 1995). Perkins (1973) found that heavily attacked leaves had roughly 250 mites per leaf and Cordo and DeLoach (1976) observed that high mite populations can result in up to 2500 galleries per leaf while an estimated 10 000 galleries per plant would kill the plant. Under laboratory conditions, Silveira-Guido (1965) found that an average of 180 mites per leaf with a total of over 1400 mites per plant caused observable damage, usually to the older leaves, but that this was not in proportion to the number of individuals per plant. The observations of the above authors suggest that very large numbers of mites (>200) per leaf are needed if any pressure is to be placed on water hyacinth growth. At some field sites in South Africa i.e. Yamorna Weir, Limpopo Province, as many as 500 mites per leaf are observed during summer months (own observation).

This chapter represents a methodology for determining a "lethal dose" of *O. terebrantis,* i.e. a density of mites that will have an adverse affect on water hyacinth growth parameters and leaf chlorophyll content (which would impact on the plant's photosynthetic ability), so that the correct mite density is used for future trials (Chapters 3, 4 and 5). As such, this chapter investigates the effects of four different densities of mites per plant to determine the minimum density of mites needed to cause such damage to the plant that the growth rate and photosynthetic ability of the plant is decreased.

Since research methodology focuses on "the research process and the kind of tools and procedures to be used" (Babbie and Mouton, 2001), the aims of this chapter, which serves as a pilot study for the subsequent studies, are to determine 1) what density of mites should be used to observe changes in water hyacinth growth parameters, 2) which plant growth parameters are most sensitive to mite herbivory and 3) which leaves (young or old) are the most damaged by mite herbivory. This will ensure that in future trials only mite densities are used that will have an impact on the plant and that time and effort are not wasted on measuring plant growth parameters and damage on leaves that are not affected by mite herbivory. In addition, two pieces of equipment i.e. a fluorometer and a chlorophyll meter, are compared to determine which one is best suited for measuring the chlorophyll-a content of water hyacinth leaves.

The hypothesis for this chapter is that plant growth parameters will be impacted negatively, and leaf chlorophyll content will decrease, with a greater density of the

mites on water hyacinth plants. Similarly, damage to the leaf surface area will increase with an increase in mite density.

2.2. MATERIALS AND METHODS

2.2.1. Experimental set-up

Water hyacinth plants were grown in a plastic pool (305 cm diameter and 76 cm depth) inside a glasshouse at the Plant Protection Research Institute (PPRI) in Pretoria, South Africa, during summer. Healthy plants from this stock were used for the experiment which was conducted during summer. Plastic tubs (70 cm x 40 cm and 35 cm depth) were placed in an open area outdoors and were filled with 40 L of tap water. Four free-floating plants, which had daughter plants (ramets), dead leaves and stems removed, were weighed and then placed into each tub. Initially, 1 g of Osmocote® (a slow-release fertilizer) and 3.48 g of commercial iron chelate (13% Fe) were added to the water. Iron chelate is added to prevent plant chlorosis (Newman and Haller, 1988) . Thereafter, 1.74 g of iron chelate only was added weekly. The water in each tub was replenished to 40 L once a week.

Mites collected from stock cultures kept at the PPRI, Pretoria, were placed onto the experimental plants using a fine camel-hair paintbrush, at high, medium and low densities, which were represented by 120, 80 and 40 mites per plant, respectively. No mites were added to the control treatment plants. Whilst younger mites may react differently to environmental stressed than older mites, and similarly males may react differently to stressed than females, it is not possible to determine the age or sex of *O. terebrantis* (Wallwork, 1965). However, this should not detract from experimental results.

Tubs were placed at least 0.5m apart to prevent mites from potentially moving from plants in one tub to plants in another tub. Each treatment was replicated six times. The experiment was designed as a randomised block design to account for potential environmental differences, such as shade and temperature, between treatments and replicates.

Plants were sampled weekly for six weeks and the following plant growth parameters were measured or counted on each plant: wet biomass (which included ramets),

number of leaves, number of ramets, number of flowers, length of the longest petiole, length of the second youngest petiole, root length, and the chlorophyll-a (chl-a) content of leaves 3, 4 and 5. Water hyacinth leaves are arranged in a spiral around the crown of the plant, such that the youngest, unfurled leaf is closest to the crown (Center, 1981). The leaves move down the crown of the plant as they age and new leaves are produced. The position of a leaf on the crown determines the age of that particular leaf, and for experimental purposes the leaves were numbered from the youngest (unfurled) leaf, starting at 0. The second petiole is immature and therefore still growing but is not necessarily the same length as the longest petiole due to the production of new leaves by the plant (Center, 1981). By tagging the second petiole and noting its position on the crown, it was possible to determine the number of new leaves that were produced each week. This is referred to as “leaf production” in the study. Similarly, “ramet production” refers to the number of new ramets produced each week. Flowers were not tagged and it was thus not possible to determine whether the flowers counted each week were new. Therefore, data referring to flowers simply shows the number of flowers counted per plant, and may include some new and some old flowers. Fresh wet weights (biomass) can be used instead of dry weights as water hyacinth plants exhibit little variation in their percentage of water content, which is 95% on average (Penfound and Earle, 1948).

The difference in wet weight at the beginning and the end of the experiment was calculated for plants in each density treatment and control. All growth parameters were averaged for the four plants in each tub to obtain a mean response per tub.

2.2.2. Leaf chlorophyll determination

The chlorophyll content of leaves was measured to record the effect of feeding of the mites on the leaf chlorophyll content, and thus indirectly photosynthesis, of water hyacinth. A Minolta SPAD-205 chlorophyll meter was used. The meter has two light emitting windows which emit light sequentially in red and infrared wavelengths that are passed through the leaf and converted into an electrical signal (SPAD-502 instruction manual, Minolta 1989). It is then assumed that the SPAD value thus determined by the meter indicates the amount of chlorophyll present in leaves. To obtain the overall amount of chlorophyll in each leaf, each leaf was measure at five different areas of the leaf surface, and the mean was recorded. Since the SPAD chlorophyll meter does not give an exact concentration of chlorophyll (µg cm^{-2}), the

chlorophyll content values obtained using the meter will be referred to as “relative” chlorophyll content of leaves for the purposes of this study.

To determine the actual concentration of chlorophyll-a (µg cm^{-2}) in leaves, the fluorometric method of Holm-Hansen and Riemann (1978) was modified to be applicable to plant material. A fluorometer was used instead of a spectrophotometer, which is the standard meter used for measuring plant chlorophyll content, because at the time of the experiment a spectrophotometer was not available. Leaves of varying conditions (varying damage due to mite herbivory) were selected from culture stocks where the variability in mite damage between leaves is similar to field conditions, and each leaf was punched at five different areas to obtain disks (measuring 3mm in diameter) of plant matter. These same five areas were also measured with the SPAD chlorophyll meter. Each disk was then placed in a test tube with 8 ml of 90% acetone (acetone was used to extract the chlorophyll) and stored for 24 h at -20 ºC. Thereafter the modified methods of Holm-Hansen and Riemann (1978) and Lorenzen (1967) were used. The test tubes were centrifuged at 5000 rpm for 5 minutes. Six millilitres of the acetone solution from each test tube was poured into a separate glass vial which was placed into a Turner Design 10AU Fluorometer and the initial reading (F_0) was noted. If the concentration of chl-a was above the measurable range of the fluorometer, the solution was diluted with 4ml of 90% acetone and the sample was re-measured. The vial was then removed from the fluorometer and 2 drops of HCl were added to the sample. The vial was returned into the fluorometer and the final reading (F_1) was noted. The following equation, modified for plant material, was used to obtain the chl-a concentration*:

Chl-a (µg cm^{-2}) = 0.325 ($F_0 - F_1$) (y) (8) where
F_0 = initial fluorometer reading
F_1 = final fluorometer reading after addition of HCl
y = surface area of plant disk
(8) = ml of acetone added to the sample initially. If sample was diluted as described above then multiply by 2.
*Original equation from Lorenzen (1967)

Finally, regression analysis was used to compare the relative chlorophyll content values obtained with the SPAD-205 chlorophyll meter with the actual chl-a concentration values obtain using the fluorometric method.

2.2.3. Feeding damage determination

Mite feeding damage was estimated visually and recorded on leaves 2 to 6 on all experimental plants. The leaf surface area damaged, from the abaxial surface, was scored according to a five digit scoring system, where 1 = 0% leaf surface area damaged, 2 = <5% leaf surface area damaged, 3 = 5-25% leaf surface area damaged, 4 = 26-50% leaf surface area damaged and 5 = >50% leaf surface area damaged. The data of the leaf surface area damaged by mites did not meet the normality or homogeneity requirements and were therefore square root (y + 0.5) transformed. The transformed scores were used for the analyses of the data but the actual scores were used in graphs.

In a later study (Chapter 5) an image analysis software programme called ImageJ version 1.40g (National Institute of Health, USA) was used to measure the percentage of the leaf surface area damaged by the mites (see details in Chapter 5), to give more accurate measurements. However, it was found that there was a high correlation between the ImageJ measurements and the measurements made visually ($r^2 = 0.824$, $p < 0.001$). Measuring mite damage using ImageJ is time consuming, and it was therefore decided to use visual measurements in the remaining chapters.

2.2.4. Statistical analyses

Data of all 6 weeks of sampling were combined to test whether the data of each growth parameter were normally distributed. The data were acceptably normal with homogenous treatment variances (Tables 2.1 and 2.2), and differences between treatments were therefore tested for in an analysis of variance (ANOVA). At the end of the sample period i.e. at week 6, differences in growth parameters, between the density treatments, were highlighted using a one-way ANOVA. If the F-probability from the ANOVA was significant at 5%, *post hoc* comparisons were made using Fisher's protected least significant difference (LSD) test at the 5% level of significance (Snedecor and Cochran, 1980). A factorial ANOVA was used to show whether sample time, or density treatment, or the interaction of sample time and density treatment were responsible for differences in plant growth parameters. Data for the leaf surface area damaged were tested at $p < 0.01$ due to heterogeneity of variances. A factorial ANOVA was also conducted to test for the effects of sample time and density treatment, as well as their interaction, on the leaf surface area damaged.

Regression analysis was used to highlight significant correlations between the changes in the plant growth parameters and the changes in the leaf surface area damaged, at the end of the six week sample period.

Data were analysed using the statistical programme Statistica (Version 7.0) (© StatSoft, Inc., USA).

2.3. RESULTS

The majority of data had a normal distribution ($p > 0.05$) according to both the Kolmogorov-Smirnov and Shapiro-Wilks W tests (Table 2.1). When data were not normally distributed (i.e. number leaves, number of flowers, number of ramets and the lengths of the second petioles), the variances between the different treatments were tested using Levene's test for homogeneity of variances and all the data were found to be homogenous ($p > 0.05$) (Table 2.2). Data were therefore able to be analysed using ANOVA.

Table 2.1. Distribution statistics of water hyacinth plant growth parameters when plants were grown for 6 weeks and exposed to herbivory by different densities of *Orthogalumna terebrantis*. The distribution analyses were conducted for data of all 6 weeks and all treatments combined. n = 144 and *df* = 143 for all parameters.

Plant growth parameter	Variance	Standard error of means	Kolmogorov-Smirnov *p*-value	Shapiro-Wilk W *p*-value
Weight (kg)	0.001	0.003	> 0.20	0.304
Leaf production	0.243	0.041	**< 0.01**	**< 0.001**
Ramet production	1.239	0.093	**< 0.01**	**< 0.001**
Flower production	0.424	0.054	**<0.01**	**< 0.001**
Max. petiole length (cm)	6.077	0.205	> 0.20	0.011
Leaf 2 petiole length (cm)	17.849	0.352	**< 0.01**	**< 0.001**
Root length (cm)	20.281	0.375	> 0.20	0.654
Leaf 3 chlorophyll	22.696	0.397	> 0.20	0.311
Leaf 4 chlorophyll	42.309	0.542	> 0.20	0.349
Leaf 5 chlorophyll	99.426	0.831	> 0.20	< 0.05

Chlorophyll refers to the chlorophyll content as measured with the SPAD meter. Highlighted data did not have a normal distribution (i.e. $p < 0.05$ for both normality tests, Kolmogorov-Smirnov and Shapiro-Wilk W).

Table 2.2. Statistics of Levene's tests for equality of variances for water hyacinth plant growth parameters where both the Kolmogorov-Smirnov and the Shapiro-Wilk W tests show that data are not normally distributed ($p < 0.05$). Plants were exposed to herbivory by *Orthogalumna terebrantis* at different densities (mites per plant): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites.

	Density treatments	***t*-value**	***p*-value**	***F*-ration Variance**	***p* variance**	**Levene *F***	***P* Levene**
Leaf Production	C vs H	0.060	0.952	1.088	0.805	0.929	0.338
df = 70	C vs M	0.301	0.764	1.091	0.798	0.137	0.713
Levene F (1, 70)	C vs L	0.355	0.723	1.161	0.661	0.061	0.806
	H vs M	0.236	0.814	1.003	0.993	0.299	0.586
	H vs L	0.290	0.772	1.067	0.848	0.370	0.545
	M vs L	0.058	0.954	1.064	0.855	0.009	0.923
Ramet Production	C vs H	0.223	0.825	1.017	0.960	0.026	0.871
df = 70	C vs M	0.437	0.663	1.052	0.881	0.098	0.756
Levene F (1, 70)	C vs L	0.025	0.980	1.431	0.293	1.553	0.217
	H vs M	0.230	0.827	1.070	0.842	0.223	0.638
	H vs L	-0.176	0.860	1.456	0.271	1.909	0.171
	M vs L	-0.373	0.710	1.360	0.367	0.943	0.335
Flower Production	C vs H	0.393	0.696	1.076	0.829	0.199	0.657
df = 70	C vs M	0.171	0.864	1.002	0.996	0.033	0.856
Levene F (1, 70)	C vs L	-0.046	0.963	1.370	0.356	1.119	0.294
	H vs M	-0.218	0.828	1.074	0.833	0.064	0.801
	H vs L	-0.470	0.639	1.273	0.479	0.306	0.582
	M vs L	-0.230	0.818	1.368	0.359	0.675	0.414
Leaf 2 petiole Length	C vs H	-0.131	0.896	1.110	0.759	0.157	0.693
df = 70	C vs M	-0.297	0.767	1.186	0.617	0.146	0.704
Levene F (1, 70)	C vs L	-0.611	0.991	1.011	0.975	0.076	0.783
	H vs M	-0.165	0.870	1.068	0.847	0	0.999
	H vs L	0.12	0.905	1.122	0.735	0.436	0.511
	M vs L	0.287	0.775	1.198	0.595	0.405	0.527

2.3.1. Plant growth parameters

Wet weight

There was no significant difference in plant wet weights between the density treatments at the end of six weeks ($F_{3, 20} = 1.522$; $p = 0.240$). Similarly, there was no significant difference in the change of weight of plants between weeks 1 and 6, between the treatments ($F_{3, 20} = 0.389$; $p = 0.762$) However, there were somewhat stronger differences in wet weights between weeks one and four, between the high density treatment and the low density treatment ($F_{1, 10} = 3.739$; $p = 0.082$) and the

high density treatment and the control ($F_{1, 10}$ = 4.134; p = 0.069). There was a steady increase in the wet weight of plants in all the treatments over six weeks (Fig. 2.1). Interestingly, the factorial ANOVA showed that wet weight increased significantly over the sample time ($F_{5, 120}$ = 18.11; p < 0.001) and the rate of change was significantly different between the density treatments ($F_{3, 120}$ = 18.110; p < 0.001), but the interaction of these two factors was not significant ($F_{15, 120}$ = 0.700; p = 0.780). At the end of the experiment i.e. after six weeks, a significant difference in wet weights between the control and high density treatments was revealed by Fisher's LSD test (p = 0.048).

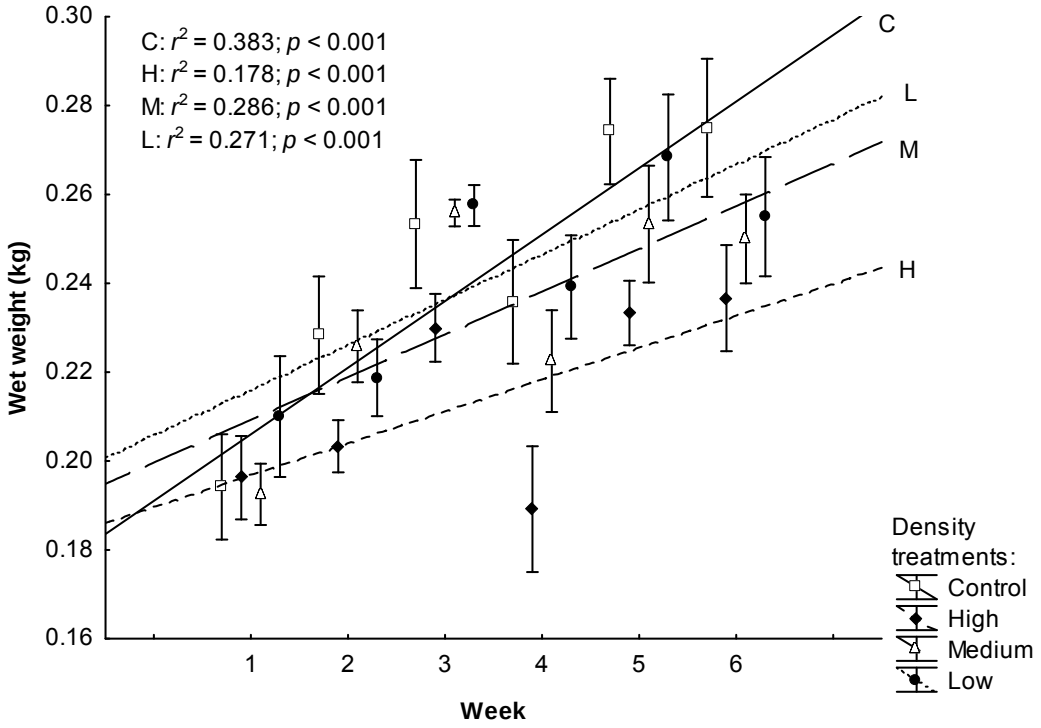

Figure 2.1. Increase in water hyacinth wet weights (including daughter plants) over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plant): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

There were no significant differences between treatments for any of the other plant parameters measured at the end of the sample period (Table 2.3).

Table 2.3. Means (± S.D.) of water hyacinth plant growth parameters and their related statistics of one-way ANOVAs, at the end of a six-week experiment when plants were exposed to herbivory by *Orthogalumna terebrantis* at different densities. Density treatments (mites per plant): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. *df* = 23 in all parameters. n = 6 for all density treatments.

	Plant growth parameters				
Density Treatment	**Weight (kg)**	**Total number of leaves produced in 6 weeks**	**Total number of ramets produced in 6 weeks**	**Average number of flowers counted per week**	**Maximum Petiole length (cm)**
C	0.28 ± 0.04	3.08 ± 0.74	5.88 ± 0.93	3.33 ± 4.07	28.15 ± 2.22
H	0.24 ± 0.03	3.21 ± 0.46	5.58 ± 0.77	3.0 ± 3.84	27.74 ± 2.11
M	0.25 ± 0.02	3.0 ± 0.39	5.38 ± 0.52	3.23 ± 3.87	28.46 ± 2.87
L	0.26 ± 0.03	3.17 ± 0.88	5.75 ± 0.65	3.38 ± 3.18	27.69 ± 2.50
SEM	0.013	0.117	0.160	0.060	0.997
F probability	0.240	0.946	0.672	0.593	0.939
LSD (5%)	n/a	n/a	n/a	n/a	n/a

	Plant growth parameters				
Density Treatment	**Leaf 2 petiole**	**Root length (cm)**	**Leaf 3 Chlorophyll**	**Leaf 4 Chlorophyll**	**Leaf 5 Chlorophyll**
	length (cm)		**Content**	**Content**	**content**
C	7.29 ± 1.42	41.96 ± 6.26	33.63 ± 3.39	28.14 ± 4.53	22.22 ± 5.17
H	7.19 ± 1.01	42.15 ± 3.44	34.58 ± 3.04	28.97 ± 4.71	20.96 ± 4.20
M	7.40 ± 0.95	41.40 ± 2.90	35.30 ± 1.22	31.02 ± 2.12	24.22 ± 5.56
L	7.94 ± 1.35	42.71 ± 4.50	35.55 ± 1.73	31.05 ± 3.43	25.25 ± 3.13
SEM	0.490	1.821	1.025	1.567	1.883
F probability	0.711	0.966	0.560	0.468	0.389
LSD (5%)	n/a	n/a	n/a	n/a	n/a

SEM is the standard error of means.
LSD is the Fisher's protected t-test least significant difference at the 5% level.

Leaf production

In contrast to the wet weights, there was a steady decrease in the number of water hyacinth leaves produced over six weeks for all treatments (Fig. 2.2). However, there were no significant differences between the numbers of leaves produced by the end of the sample period between any of the density treatments (Table 2.3). The number of leaves produced decreased significantly over the sample time ($F_{5, 120} = 94.743$; $p < 0.0001$), but the rate of change was not significantly different between the density treatments ($F_{3, 120} = 262$; $p = 0.853$), and the interaction of these two factors was also not significant ($F_{15, 120} = 0.794$; $p = 0.683$).

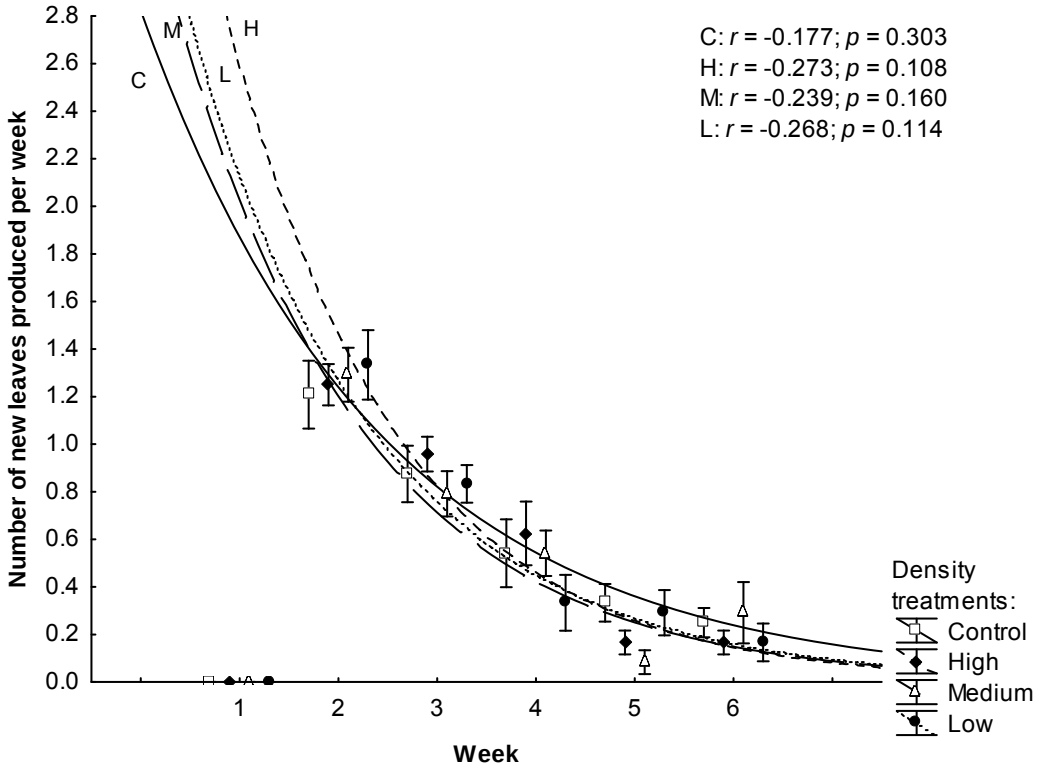

Figure 2.2. Exponential decrease in water hyacinth leaf production over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plant): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

Ramet production

Although there were no significant differences in the number of ramets produced between the treatments after six weeks (Table 2.3), the greatest mean number of ramets (6.79 ± 0.19 ramets/plant) produced by the end of the six-week sample period was observed in the control treatments. As with the production of leaves, there was a steady decrease in the production of ramets during the six week sample period for all treatments (Fig. 2.3). The number of ramets produced decreased significantly over sample time ($F_{5, 120}$ = 87.063; p < 0.001) but the rate of change was not significantly different between the density treatments ($F_{3, 120}$ = 0.304; p = 0.822). Also, the interaction between sample time and density treatments did not cause significant changes in the number of ramets produced ($F_{12, 100}$ = 0.602; p = 0.868). This suggests that time had a greater effect on ramet production than the density of mites.

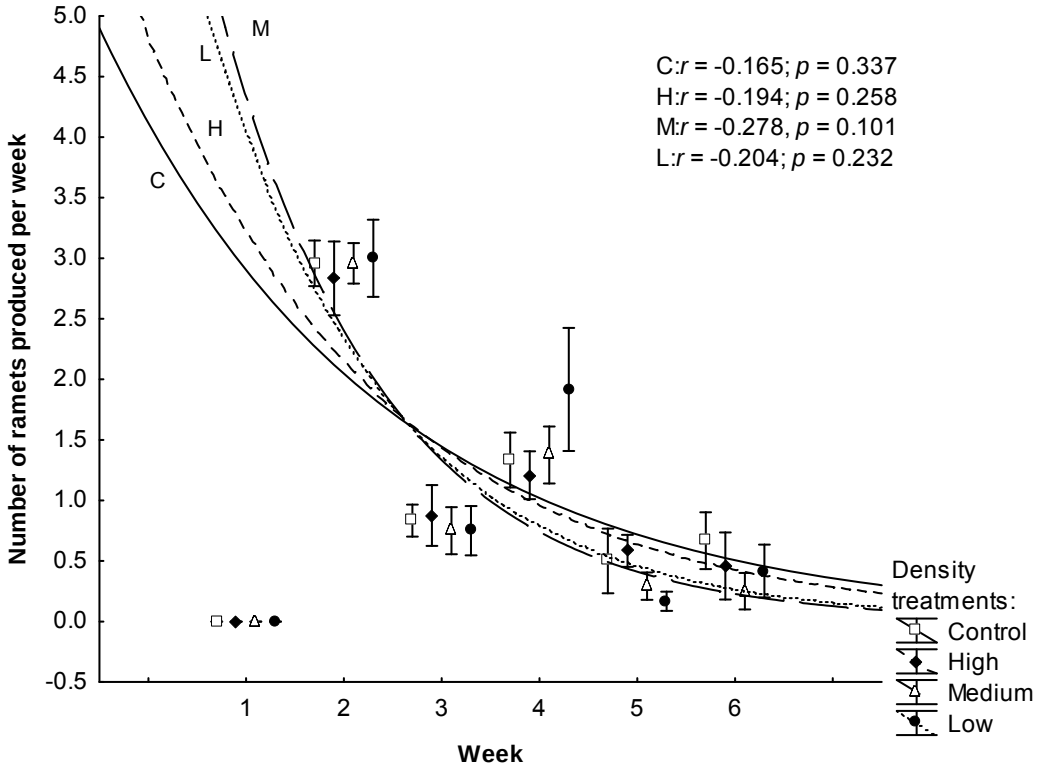

Figure 2.3. Exponential decrease in the production of water hyacinth daughter plants (ramets) over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

Flowers counted

As with the production of leaves and ramets, there was no significant difference in the number of flowers counted per plant between the treatments per week (Table 2.3). The number of flowers counted per plant increased each week throughout the study (Fig. 2.4) but, as explained in the methods, flowers were not tagged and it was therefore not possible to distinguish between new flowers produced and old flowers counted from a previous week. The number of flowers counted per plant increased significantly over sample time ($F_{5, 120} = 78.480$; $p < 0.001$), but the rate of change was not significantly different between the density treatments ($F_{3, 120} = 0.311$; $p = 0.819$), and the interaction between these two factors was also not significant ($F_{15, 120} = 0.630$; $p = 0.845$). In general, the significant differences observed in the number of flowers counted each week were due to differences that occurred between week 1 and week 6.

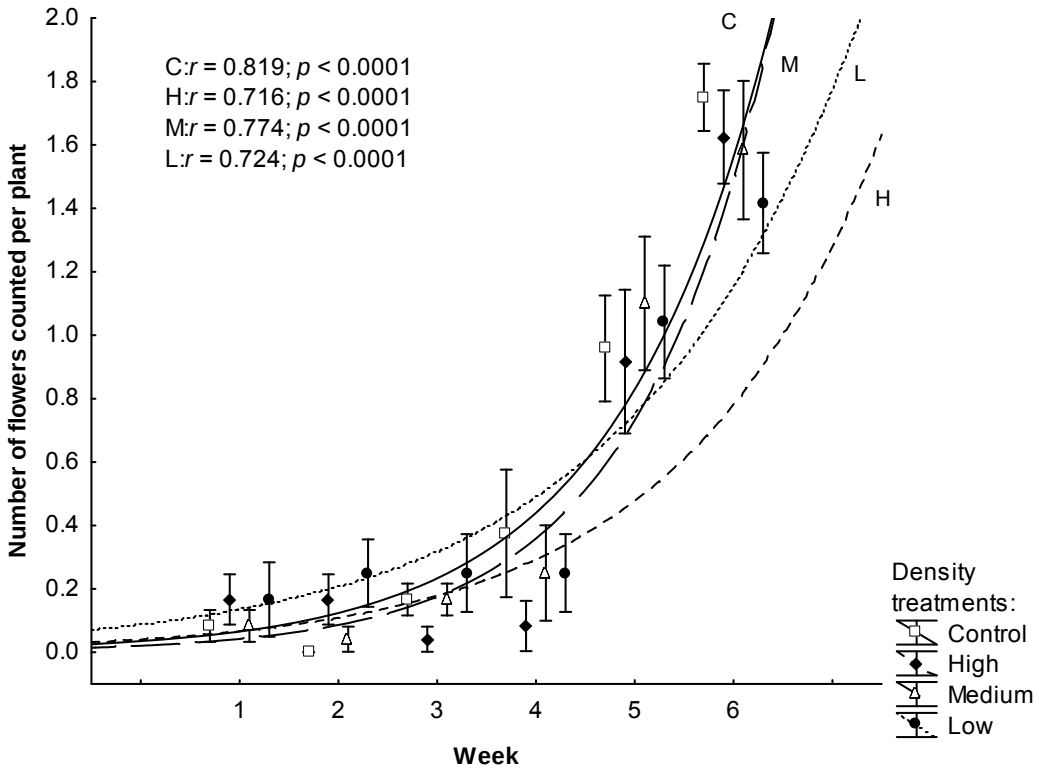

Figure 2.4. Exponential increase in the number of water hyacinth flowers counted on the plants for six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plant): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

Maximum petiole length

Maximum petiole lengths between the treatments were not significantly different after six weeks (Table 2.3). Although the lengths of the longest petioles decreased slightly in the high and low density treatments, and increased slightly in the control over six weeks (Fig. 2.5), there was no significant change in maximum petiole lengths over the sample period ($F_{5, 20} = 0.634$; $p = 0.674$) or between the rate of change between treatments ($F_{3, 120} = 0.317$; $p = 0.813$), and neither was there a significant interaction between these factors ($F_{15, 120} = 0.178$; $p = 1.0$).

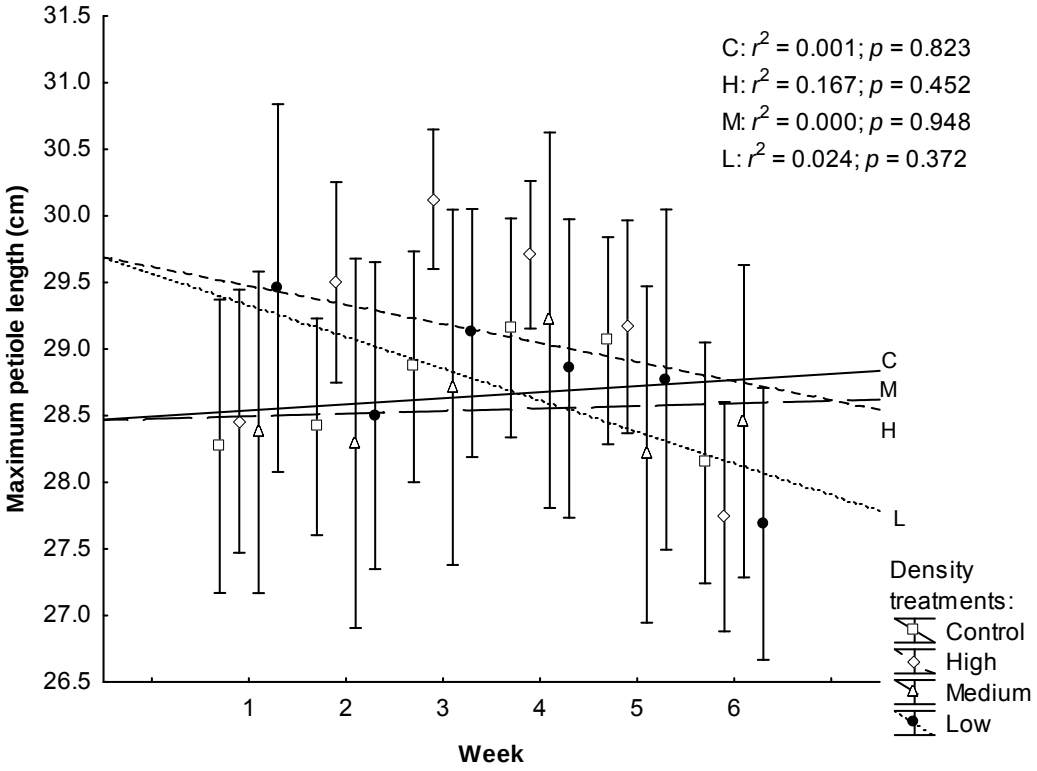

Figure 2.5. Changes in the length of the longest petiole of water hyacinth plants over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

Lengths of the second petioles

Overall, the lengths of the second petioles decreased exponentially in all treatments over the six week sample period and there was a sharp decrease in the lengths of the second petioles, in all the treatments, between week 1 and week 2, after which the lengths remained relatively stable (Fig. 2.6). At week 1, the mean lengths of the second petioles at the high, medium, low density and the control treatments were 18.71 ± 1.35 cm, 18.96 ± 3.39 cm, 16.96 ± 5.15 cm and 17.67 ± 3.29 cm, respectively, while at week 2 they were 10.65 ± 0.80 cm, 10.75 ± 0.82 cm, 10.71 ± 1.08 cm and 10.65 ± 0.50 cm, respectively. In addition, even when the data from week 1 were removed from the analysis, there was still a significant change in the lengths of the second petioles over the six week period ($F_{4, 100}$ = 53.570; p < 0.001). There was no significant difference in the rate of change in the lengths of the second petioles between the density treatments ($F_{3, 100}$ = 0.280; p = 0.843), and neither was there a significant interaction between the sample period and treatments ($F_{12, 100}$ = 0.490; p = 0.913).

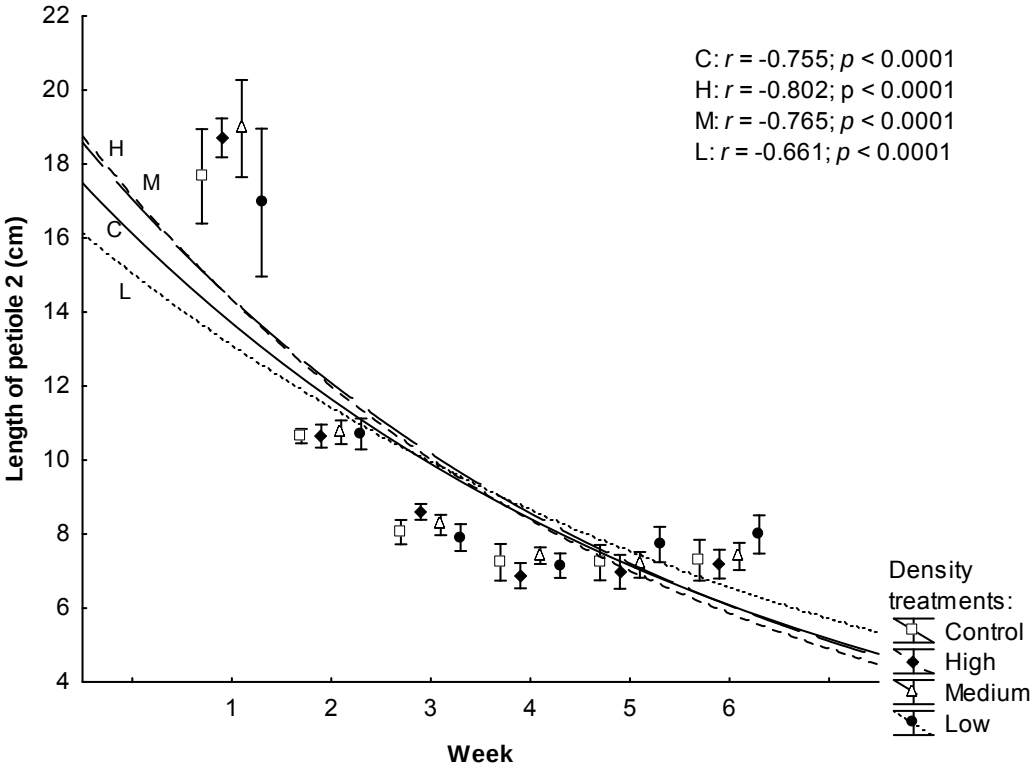

Figure 2.6. Changes in the length of leaf 2 petioles of water hyacinth plants over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

Root lengths

Root lengths were not significantly different between the treatments after six weeks (Table 2.3). In general, there was no significant change in the length of roots of water hyacinth plants over the six week sample period ($F_{5, 120}$ = 0.961; p = 0.445), although there was a slight higher increase in root length over time at the low density treatment than in the other treatments (Fig. 2.7). There was no significant difference in the rate of change in root lengths between the treatments ($F_{3, 120}$ = 0.023; p = 0.995), nor was there a significant interaction between the weeks and treatments ($F_{15, 120}$ = 0.321; p = 0.992), suggesting that the length of water hyacinth roots is not affected by time or mites, regardless of the mite densities.

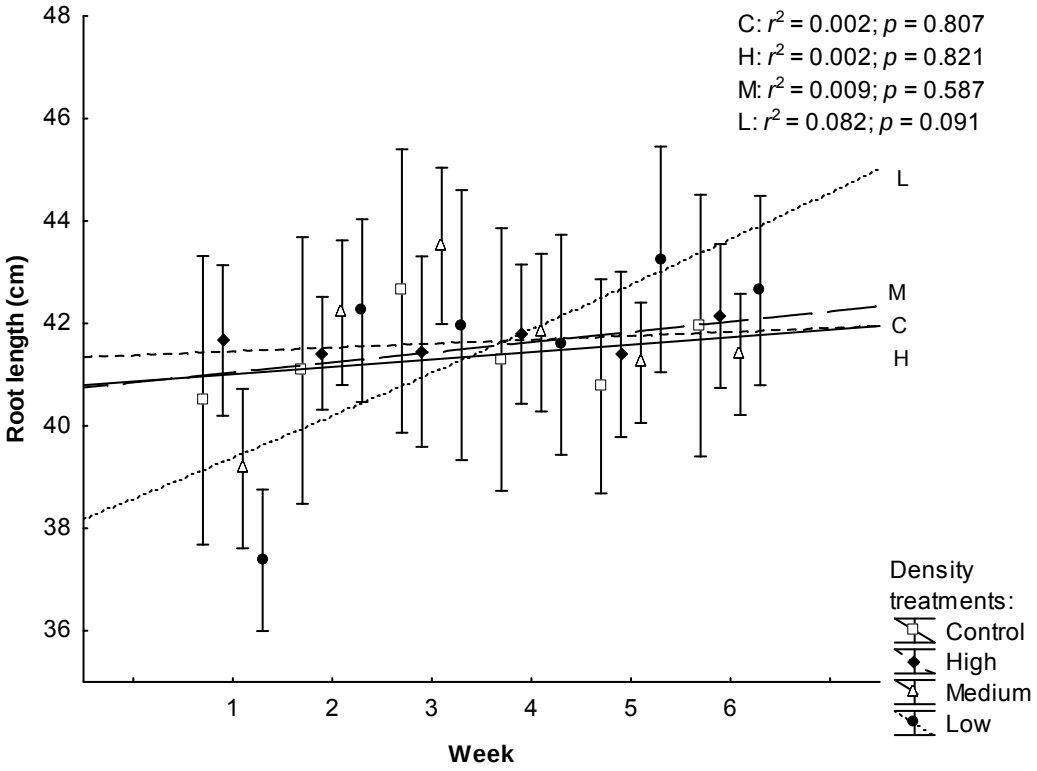

Figure 2.7. Changes in water hyacinth root lengths over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. n = 6 for all density treatments.

2.3.2 Leaf chlorophyll content

Chlorophyll content obtained using the SPAD chlorophyll meter

No significant differences were observed in the relative chlorophyll contents of leaves between the treatments, in all of the leaves measured i.e. leaves 3, 4 and 5, after six weeks (Table 2.3). Figure 2.8 shows a decrease in the leaf chlorophyll content of leaves 3, 4 and 5 over six weeks. All the leaves measured showed a significant change in chlorophyll content over the sample period, but only leaves 3 and 4 showed a significant difference in the rate of change in chlorophyll content between treatments (Table 2.4). Fisher's LSD tests revealed that, in general, the significant changes in the chlorophyll content of leaves, in all of the leaves measured, were due to significant differences between the first three weeks (weeks 1 to 3) and the last two weeks (weeks 5 and 6) of the sample period. There were no significant interactions between the weeks and treatments in all of the leaves measured (Table 2.4). There was, however, a significant difference in the chlorophyll content between

the leaves measured, for the entire sample period ($F_{2, 429}$ = 30.086; p < 0.001), as well as at the end of the sample period ($F_{2, 69}$ = 58.127; p < 0.001), with leaf 3 having the highest chlorophyll content, followed by leaf 4 and then leaf 5.

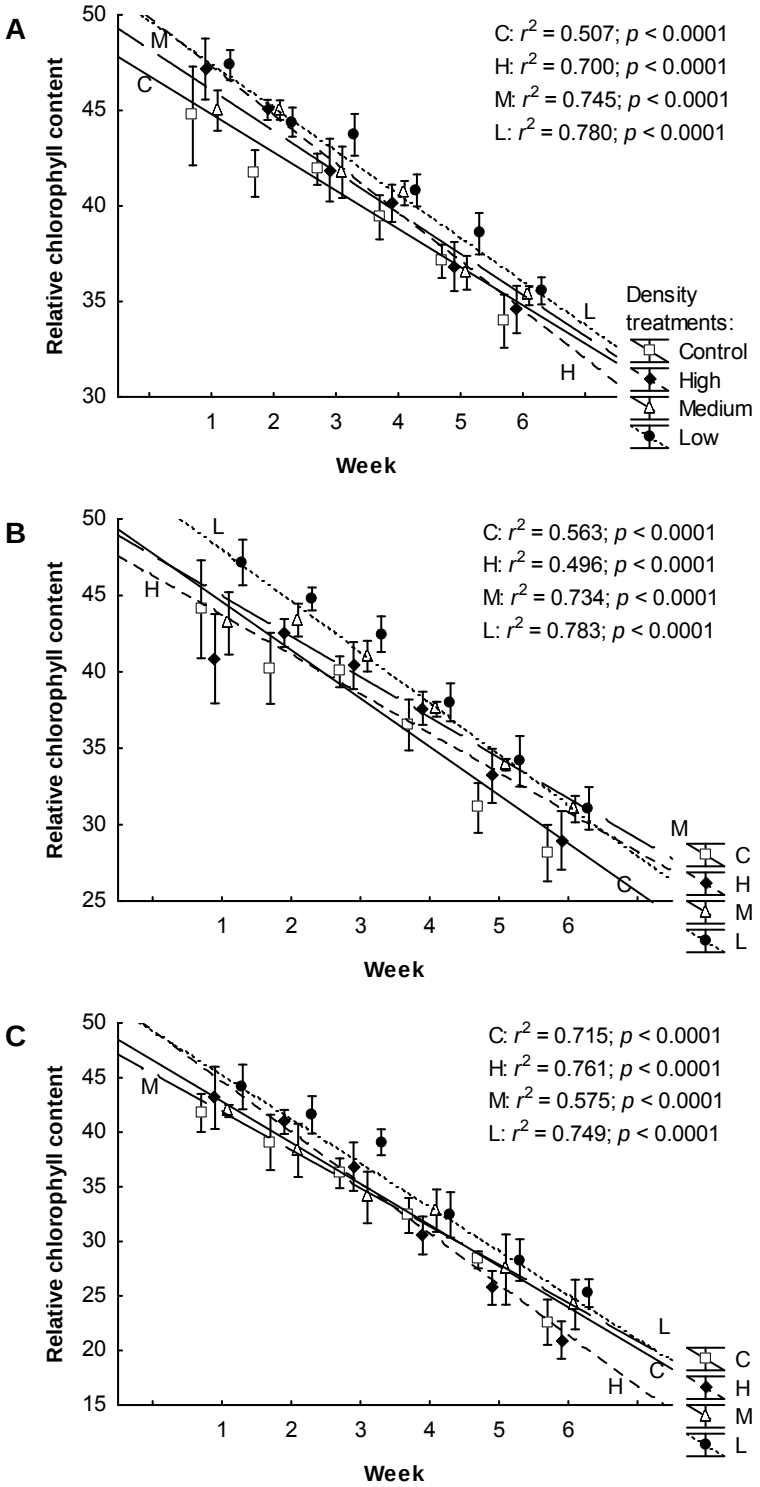

Figure 2.8. Changes in the chlorophyll content of leaves 3 (A), 4 (B) and 5 (C) of water hyacinth plants over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Chlorophyll content was measured with a SPAD chlorophyll meter. Error bars represent the standard error of the means. n = 6 for all density treatments.

Table 2.4. Statistics of factorial ANOVAs revealing significant changes in the chlorophyll content (measured with a SPAD chlorophyll meter) of leaves 3, 4 and 5 of water hyacinth plants exposed to herbivory by different densities (treatments) of *Orthogalumna terebrantis* over a six week sample period. Numbers in brackets are the degrees of freedom for each source of variation.

Factors and Interactions	**Leaf 3**		**Leaf 4**		**Leaf 5**	
	F	***p***	**F**	***P***	**F**	***p***
Week (5, 120)	53.96	< 0.0001	48.38	< 0.0001	62.49	< 0.0001
Treatment (3, 120)	3.01	0.033	3.47	0.018	1.71	0.169
Week*Treatment (15, 120)	0.50	0.938	0.33	0.992	0.44	0.962

At the end of the sample period leaf 3 had the highest relative chlorophyll content in all the treatments (Fig. 2.9). This is interesting because, as will be discussed below, leaf 3 had the greatest surface area damaged by mites.

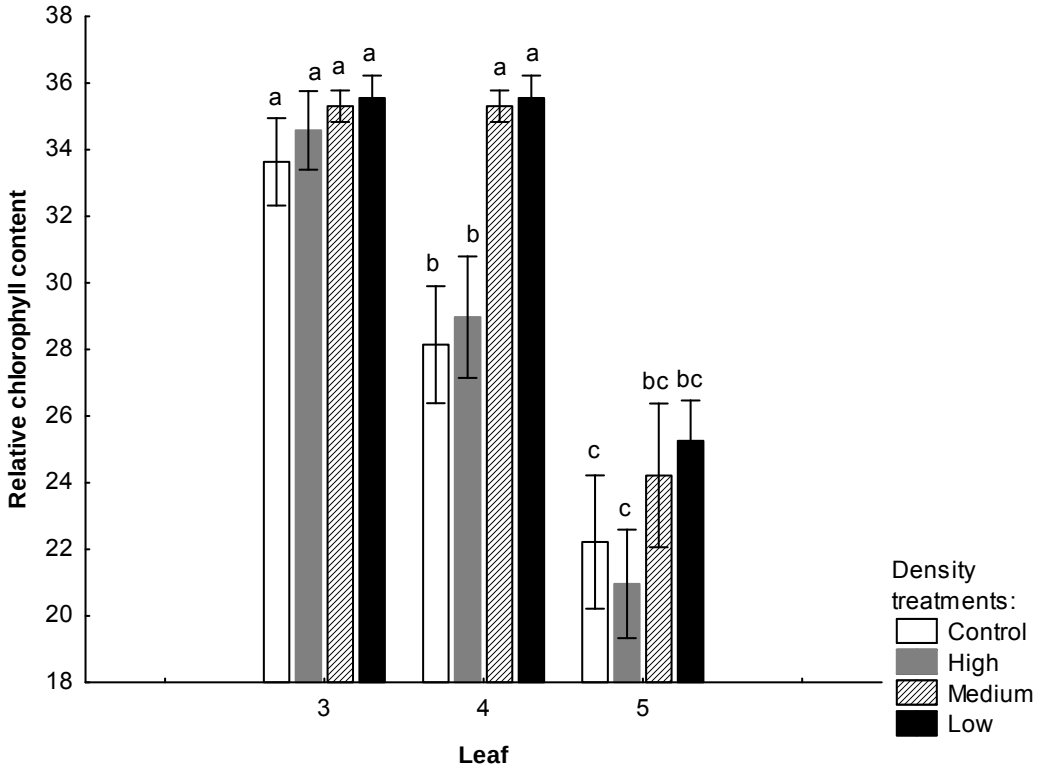

Figure 2.9. Relative chlorophyll content (measured with a SPAD chlorophyll meter) of water hyacinth leaves 3, 4 and 5 at the end of a six week sample period when plants were exposed to herbivory by *Orthogalumna terebrantis* at different densities. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 6 for all density treatments.

Chlorophyll-a content obtained using a fluorometer

Values obtained using the fluorometer demonstrated that green leaves had significantly higher chl-a concentrations ($F_{1, 208}$ = 11.322; p < 0.001) than brown or damaged leaves (Fig. 2.10). Regression analysis showed a very weak correlation between the relative chlorophyll content as obtained with the chlorophyll meter and the actual chl-a concentration as obtained with the fluorometer (r^2 = 0.01; $F_{1, 33}$ = 0.338; p = 0.565). Consequently, analyses run on the relative chlorophyll content data may not be reliable, but this is discussed further below.

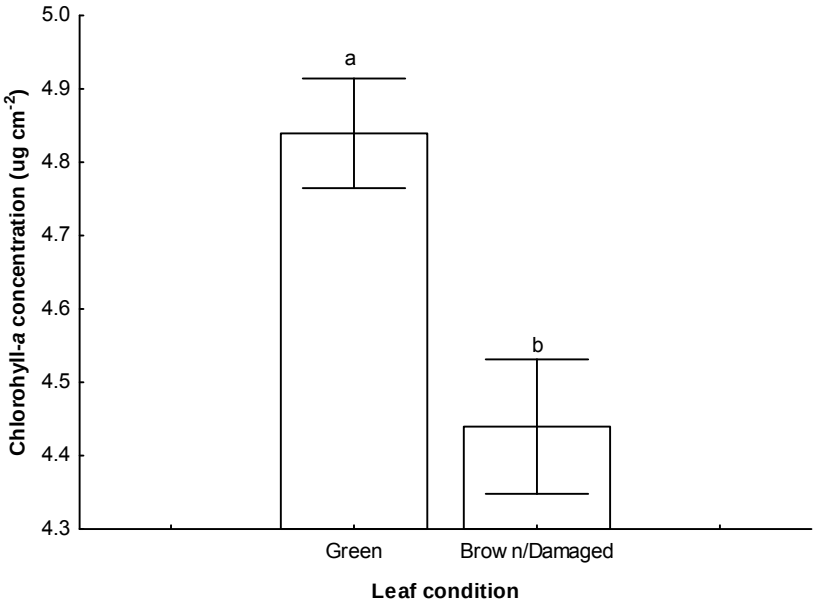

Figure 2.10. Chlorophyll-a concentrations (µg cm^{-2}) of water hyacinth leaves that had very low levels of damage (green) or very high levels of damage (brown/damaged) due to herbivory by *Orthogalumna terebrantis*. Chlorophyll content was measured using a fluorometer. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, p < 0.05). n = 19 for green leaves and 15 for brown leaves.

2.3.3. Mite damage to the leaf surface area

Difference in damage between leaves

There was a significant difference in the leaf surface area damaged between the leaves scored i.e. leaves 2 to 6, after six weeks, in all of the density treatments (high density $F_{4, 25}$ = 16.206; p < 0.001; medium density $F_{4, 25}$ = 20.848; p < 0.001 and low density $F_{4, 25}$ = 31.793; p < 0.001). In all of the density treatments leaf 3 had the

highest leaf surface area damaged (Figs. 2.11). *Post-hoc* tests revealed that the surface area damaged on leaf 3 differed significantly from all the other leaves in the medium and low treatments ($p < 0.05$), but did not differ significantly from leaf 4 in the high density treatment ($p > 0.05$). This suggests that at high densities mites are likely to damage leaves 3 and 4 to a similar extent.

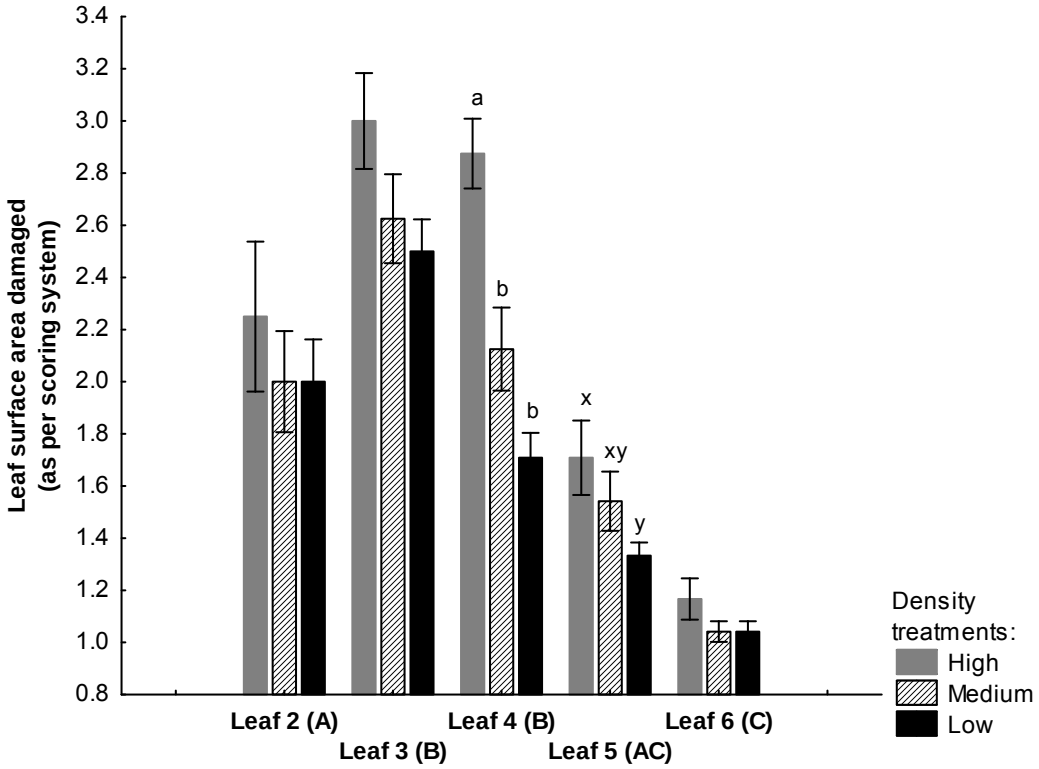

Figure 2.11. The leaf surface area damaged on different water hyacinth leaves at the end of a six-week sample period when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. The leaf surface area damaged by mites was scored according to a five digit scoring system: 1 = 0% damaged, 2 = <5% damaged, 3 = 5-25% damaged, 4 = 26-50% damaged and 5 = >50% damaged. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, and low (L) = 40 mites. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same small letter indicate no significant difference (Fisher's LSD test, $p > 0.05$) between mite densities, for each leaf analyzed separately. Error bars not followed by letters indicate no significant difference in surface area damaged between the density treatments for that leaf. Leaf labels followed by the same capital letter indicate no significant difference ($p > 0.05$) in damage between leaves. n = 6 for all density treatments.

Difference in damage between treatments

At the end of the six-week sample period, leaves 2, 3 and 4 showed significant differences in the leaf surface area damaged between the density treatments, while leaves 5 and 6 did not (Table 2.5).

Table 2.5. Damage scores of leaf surface area damaged on water hyacinth leaves through feeding by *Orthogalumna terebrantis* at different densities at the end of a six-week sample period. Density treatments (mites per plants): high (H) = 180 mites, medium (M) = 80 mites, low (L) = 40 mites and control (C) = 0 mites. Damage scores: 1 = 0% damage, 2 = <5% damage, 3 = 5-25% damage, 4 = 26-50% damage and 5 = >50% damage. Values have been square root (y + 0.5) transformed and values followed by the same letter are not significantly different (Fisher's LSD test, $p > 0.01$). Means compared by one-way ANOVA. Values in brackets are actual mean percentages (±SD) of the leaf surface area damaged. *df* = 23 for each leaf and n = 6 in all treatments.

Density treatment	Leaf 2	Leaf 3	Leaf 4
C	0.71 (0.0 ± 0.0) b	0.71 (0.0 ± 0.0) b	0.71 (0.0 ± 0.0) b
H	2.34 (5.75 ± 4.15) a	4.37 (19.88 ± 11.29) a	3.39 (12.08 ± 8.45) c
M	2.04 (4.13 ± 2.77) a	3.00 (9.38 ± 6.4) a	1.86 (3.29 ± 2.27) a
L	1.65 (2.42 ± 1.74) ab	3.10 (9.86 ± 5.72) a	1.58 (2.17 ± 1.65) ab
SEM	0.267	0.379	0.285
F probability	0.002	< 0.001	< 0.001
LSD (1%)	1.074	1.525	1.145

Density treatment	Leaf 5	Leaf 6
C	0.71 (0.0 ± 0.0) a	0.71 (0.0 ± 0.0)a
H	1.35 (1.63 ± 2.08) a	0.83 (0.17 ± 0.20) a
M	1.11 (0.79 ± 0.66) a	0.76 (0.04 ± 0.10) a
L	0.96 (0.46 ± 0.40) a	0.73 (0.04 ± 0.10) a
SEM	0.139	0.041
F probability	0.027	0.192
LSD (1%)	n/a	n/a

SEM is the standard error of means.
LSD is the Fisher's protected t-test least significant difference at the 1% level.

For the entire study period all of the leaves scored showed significant changes in the leaf surface area damaged between the treatments, and between the weeks, and the interaction between these two factors was also significant (Table 2.6). In general, Fisher's LSD tests revealed that the changes in leaf surface area damaged in leaf 2 were due to differences between the first three weeks and the last three weeks, and due to the significant difference between the control and the other treatments. In leaf 3 the changes were due to significant differences between first two weeks and the last four weeks, and, as in leaf 2, due to differences between the control and the other treatments. In leaf 4, as in leaf 2, significant changes were due to differences between the first three weeks and the last three weeks, but all of the treatments differed significantly from each other. In leaf 5, as in leaves 2 and 4, significant changes were a result of the differences between the first three weeks and the last three weeks, in addition, however, week four was significantly different from all the other weeks, while the medium and low density treatments did not differ significantly

from each other. Significant changes in leaf 6 occurred due to the difference between the first three weeks and week five, and due to the high density treatment being significantly different to the other three treatments which were not significantly different from each other.

Table 2.6. *F*-statistics of factorial ANOVAs revealing significant differences in the leaf surface areas damaged on different water hyacinth leaves by herbivory of *Orthogalumna terebrantis* between different density treatments (Dt) over a six-week sample period (W). Numbers in brackets are degrees of freedom for each source of variation.

Leaf	Source of variation		
	Density treatment (3, 120)	**Week (5, 120)**	**Dt x W (15, 120)**
2	28.068*	43.700*	5.547*
3	94.049*	94.040*	12.177*
4	83.760*	78.112*	12.740*
5	29.405*	29.480*	5.875*
6	7.966*	5.308*	1.996*

* $p < 0.05$

The surface area damaged increased over time in all of the leaves scored, with the high density treatment displaying the fastest rate of increase, followed by the medium and then the low density treatments (Figs. 2.12). It can also be seen that there was little change in the leaf surface area damaged on leaves 5 and 6 (Figs. 2.12).

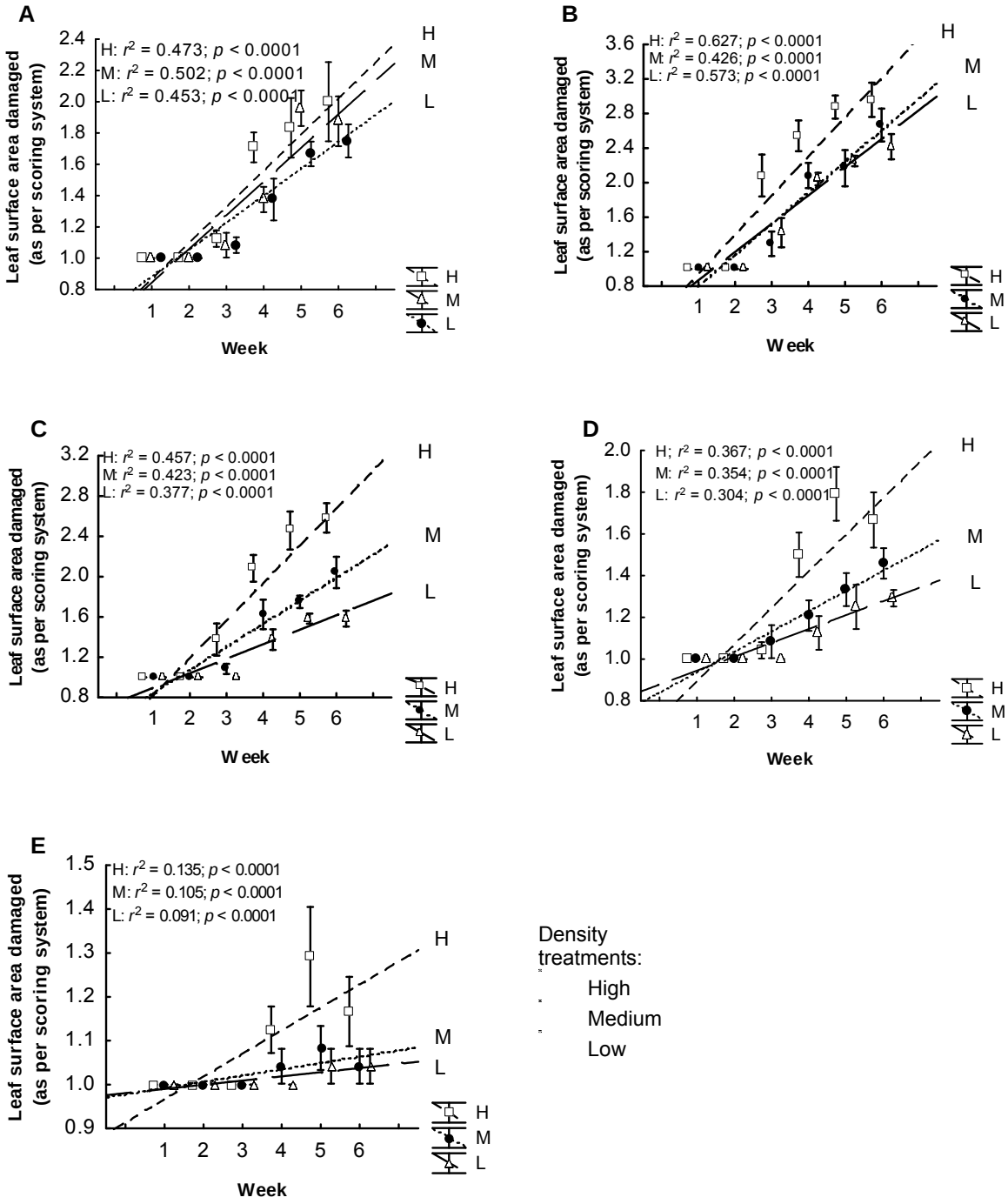

Figure 2.12. The leaf surface area damaged on leaves 2 (A), 3 (B), 4 (C), 5 (D) and 6 (E) of water hyacinth plants over six weeks, when plants were exposed to herbivory by different densities of *Orthogalumna terebrantis*. The leaf surface area damaged by mites was scored according to a five digit scoring system: 1 = 0% damaged, 2 = <5% damaged, 3 = 5-25% damaged, 4 = 26-50% damaged and 5 = >50% damaged. Densities (mites per plants): high (H) = 180 mites, medium (M) = 80 mites and low (L) = 40 mites. Error bars represent the standard error of means. n = 6 for all density treatments.

2.3.4. Effect of mite herbivory on the plant growth parameters

Since there were no significant differences in most of the plant growth parameters between the treatments (Table 2.3), but the high density treatment caused the greatest damage to leaves (Table 2.4) and the third leaf was the most damaged in all of the density treatments (Figs. 2.11), the regression analysis presented here was conducted only between the plant growth parameters of plants exposed to high mite densities and the leaf surface area damaged on the third leaf. The regression analysis was conducted for data of the final week of the experiment (week 6).

Strong correlations were found between the percentage of the leaf surface area damaged by mite herbivory and plant wet weights (Fig. 2.13) and also the relative chlorophyll content (as measured with the SPAD meter) (Fig. 2.14). On the other hand, poor correlations existed between the leaf surface area damaged and the remaining plant growth parameters (Table 2.7).

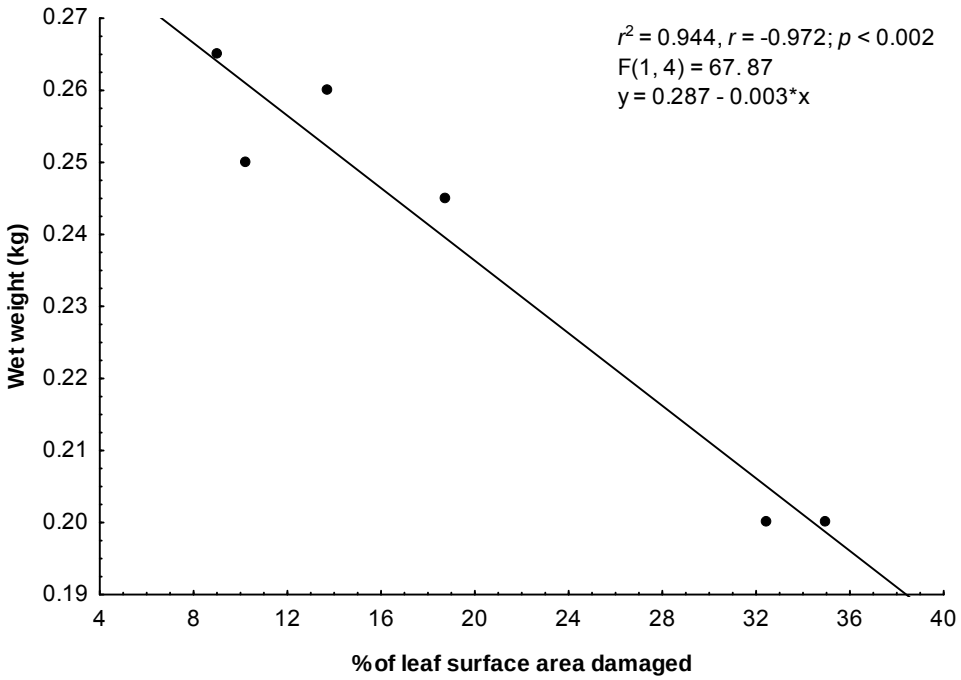

Figure 2.13. The relationship between the wet weights (ramets included) of water hyacinth plants and the leaf surface area damaged by herbivory of *Orthogalumna terebrantis* on the third leaf, at the end of six weeks when plants were exposed to a high densities of mites (180 mites per plant). n = 6.

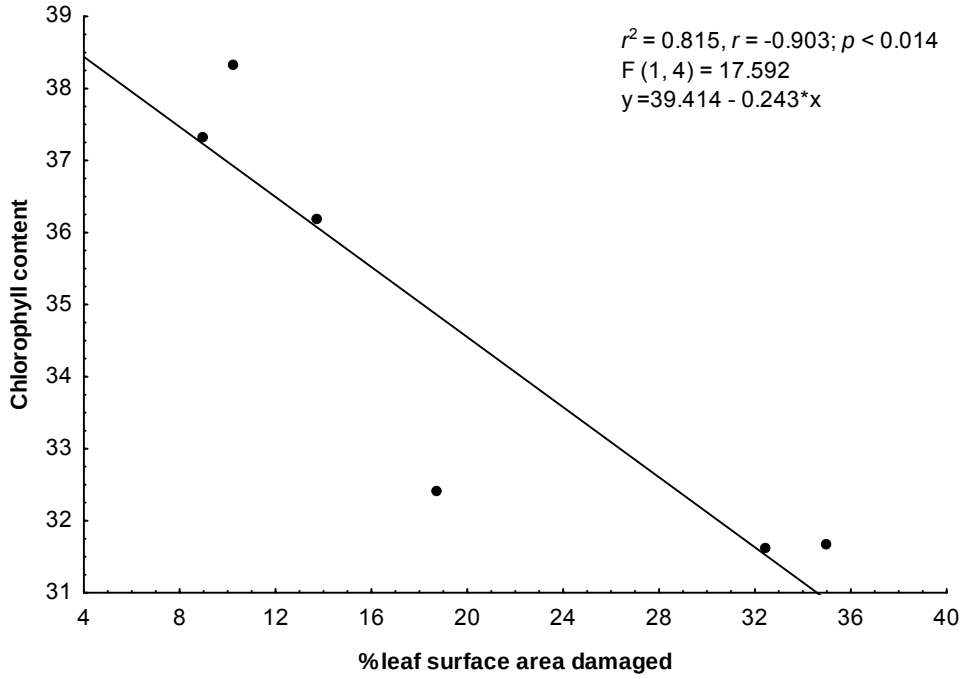

Figure 2.14. The relationship between the chlorophyll content (measured with a SPAD chlorophyll meter) of the third leaf of water hyacinth plants and the leaf surface area damaged by herbivory of *Orthogalumna terebrantis* on the third leaf, at the end of six weeks when plants were exposed to a high densities of mites (180 mites per plant). n = 6.

Table 2.7. Statistics of regression analyses showing the relationship between water hyacinth plant growth parameters and the leaf surface area damaged by herbivory of *Orthogalumna terebrantis* on the third leaf, at the end of six weeks when plants were exposed to a high densities of mites (180 mites per plant). n = 6 for all plant growth parameters.

Plant growth parameter	*F*- value (1, 4)	r^2	*p*-value
Number of leaves produced in 6 weeks	0.208	0.05	0.672
Number of ramets produced in 6 weeks	0.704	0.149	0.449
Number of flowers counted during 6 weeks	0.786	0.164	0.425
Length of the second petiole	0.032	0.008	0.867
Maximum petiole length	0.008	0.002	0.935
Root length	2.618	0.395	0.181

2.3.5. Summary of results

After a short period of six weeks there were no differences in plant growth parameters between the control and the density treatments used in this study. However, plant growth parameters showed significant changes over time, and for

certain growth parameters e.g. wet weight, slight differences did occur between the control and the high density treatment after six weeks, suggesting that given more time, high mite densities would indeed have a negative impact on water hyacinth growth parameters. On the other hand, different densities of mites produced different levels of damage to the leaf surface area in leaves 4 and 5. In general, however, the high density treatment at the beginning of the sample period i.e. week 2, caused the same amount of damaged to the leaf surface area as did, for example, the low density treatment at the end of the sample period i.e. weeks 5 and 6. This implies that over time the mite population would increase and cause incremental damage to the leaf surface area as a result. Also, plant wet weights and chlorophyll content of leaf 3 were strongly negatively related to the leaf surface area damaged, suggesting that, at high enough densities, mite herbivory would have a negative impact on certain water hyacinth growth parameters.

2.4. DISCUSSION

2.4.1. Effect of mite herbivory on the plant growth parameters

The different densities of mites used in this study did not have significantly different effects on the plant growth parameters, but the general trends showed that the high and medium density treatments had greater effects on plant growth parameters than the low density and control treatments. Briese et al. (2002) found similar results with the rosette crown weevil *Trichosirocalus briesei* Alonso-Zarazaga & Sánches-Ruiz (Coleoptera: Chrysomelidae); the stem height and plant biomass of *Onopordum* thistles decreased with an increase in the weevil densities. It may be that all of the initial mite densities chosen for this study were too low and that the range of densities used was not wide enough. To illustrate, Center and Jubinsky (1996) used increments of 1000 *N. eichhorniae* weevils to observe differences in water hyacinth mat expansion rates (initial mat size $10m^2$) when exposed to increasing weevil populations. Furthermore, Grevstad's (1999) three-year long study on a chrysomelid beetle, *G. calmariensis*, confirmed that the larger the initial release size the more likely a population is to establish. It is highly probable that in the present study the magnitude of the impact was underestimated due to the short duration of the experiment and it is therefore possible that over time, the high density treatment in this study had a better chance of increasing its population, thereby increasing its impact on the plant.

Over the six week sample period, the rates of change in the wet weights and in the chlorophyll contents of the third and fourth leaves were significantly different between the density treatments. Over time, the wet weights and the chlorophyll content of the fourth leaf changed the least at the high and medium mite densities, while the chlorophyll content of the third leaf changed the most over time. This suggests that mite densities affect the rate of growth of certain plant growth parameters, and also the rate at which the chlorophyll content of leaves changes, and that the high and medium mite densities tend to create slower changes than low mite densities for certain plant growth parameters. It is likely that, with time, the different mite densities would show a different impact on water hyacinth growth parameters.

Delfosse (1978a) found that *O. terebrantis* field populations peaked after 16 weeks, at 840 mites per plant. However, the present study was limited by time-constraints and was only able to be conducted over a short period of six weeks, using small mite densities because placing mites onto water hyacinth plants is highly time consuming. Furthermore, in a small mite such as *O. terebrantis* the egg to adult development takes about three weeks (Norton, 1994) and adults live for up to 85 days (Cordo and DeLoach, 1976). As such, the six week sample period of this study was not long enough to allow the mite population to build up to such numbers as to cause adverse effects to water hyacinth growth, regardless of the initial numbers of mites used. However, because the egg to adult period is between 22 and 25 days at 25-26 ºC (Cordo and DeLoach, 1976; Ganga-Visalakshy and Jayanth, 1991) it is possible that during the last fortnight of this experiment an undetermined number of mites may have been added those initially introduced and contributed to the damage inflicted on the plant. In addition, the effect of time was significant on all the plant growth parameters except for the root lengths and the maximum petiole lengths, so it is possible that differences in certain plant growth parameters would occur between densities given enough time. Also, mite numbers decrease in cooler weather (Perkins, 1974). This study was run towards the end of summer when ambient temperatures were decreasing, which may have lessened the mites' impact on the plants due to the decrease in population growth. Similarly, Forno (1981) found that the amount of damage to water hyacinth by *N. eichhorniae* varied with season and the duration of the insect attack.

The wet weights of the plants increased during the sample period. Since all the ramets and dead matter were removed from the plants before they were weighed at the beginning and at end of the experiment, and the production of leaves and lengths

of petioles decreased, the increase in wet weight may be explained by the increase in the production of flowers. Water hyacinth produce flowers when under stress i.e. when grown at low nutrients (Luu and Getsinger, 1990; Coetzee *et al*., 2007a). It is possible that the amounts of iron chelate and Osmocote® used in this study were not optimal for plant growth, and may have been low enough to stress the plants. In future trails, potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) will be used as the nitrogen and potassium bases, so that exact concentrations of N and P can be measured out as needed. Additionally, the tubs in which plants were kept during the study were placed outside on a cement floor and were continually in bright sunlight, causing the water to become very warm during the day. Ajuonu *et al*. (2009) mention in their methods that small water containers (similar to those used in this study) accumulate heat quickly and this has a negative impact on plant growth. It is possible that the conditions created by the experimental set-up in this study were not ideal, and placed the plants under stess, thereby causing them to flower.

Center (1981) observed that water hyacinth leaf production rates decrease towards winter and pick up again towards summer. Whilst ambient temperatures were not measured during this study, the study was conducted towards the end of summer when temperatures tend to decrease, and it is therefore possible that the observed decrease in leaf production over the six-week sample period was a reflection of seasonal changes in ambient temperatures. According to Center (1981) leaves are the ideal units to measure for water hyacinth population studies as it is easy to determine when a new leaf emerges and when it dies. A new water hyacinth leaf has a life-span of roughly two months (Center, 1981) while this study was only 6 weeks long, and therefore the leaf turn-over rates were not analysed here. However, it is noteworthy to mention that during this entire study six plants had a complete leaf turn-over i.e. all of the leaves that were on the plant at the start of the experiment died and were systematically replaced by new leaves, and of these six plants three were from low density treatment, two from the control and only one from the high density treatment. This suggests that high mite densities slow down the production of new leaves, and factors that slow down leaf production may affect stability, buoyancy and nutrition of the plant (Center and Van, 1989). This shows that *O. terebrantis* is able to negatively impact water hyacinth leaf production, which suggests that the mite is effective as a biocontrol agent.

Initially, there was an increase in the number of new ramets produced in each treatment (between week 1 and week 2), but from week 2 the ramet production gradually decreased and eventually stayed constant. It is possible that the removal of ramets from the experimental plants at the start of the experiment stimulates ramet production, thus the observed initial increase in ramet numbers, after which production slows down. Coetzee (2003) conducted a similar study to the present one, and looked at the impact of the mirid *E. catarinensis* on water hyacinth growth, and also observed an initial increase in ramet production after removing ramets from experimental plants at the start of the experiment.

A very unusual result was the exponential decrease in the lengths of the second petioles between week 1 and week 3. Water hyacinth leaf morphology is highly plastic, such that a single plant can change from having long leaves with narrow petioles to having short leaves with swollen petioles, depending on where the plant is situated in a water hyacinth mat; plants at the centre of a mat produce long leaves with narrow petioles while plants at the edge of a mat produce small, wide leaves with inflated petioles (Penfound and Earle, 1948). It is likely that in this study, as the plants were moved from stock culture pools where their densities were high and their petioles long, to the tubs where their densities were low (four plants per tub), the plants in tubs did not have the support of other plants around them to keep them upright, and so the petioles shortened and became bulbous to keep the plants afloat. In addition, water hyacinth leaf form is also influenced by factors such as nutrients (Coetzee *et al.*, 2007a) and light (Richards and Lee, 1986). It is therefore possible that the change in petiole lengths occurred because the nutrient concentration and light levels between the stock pools and the experimental tubs differed. Changes in the petiole length are thus not a good measure of the effect of herbivory on the plant.

The fact that root lengths did not change over time is not unexpected since water hyacinth root growth responds to water chemistry (Zaranyika and Ndapwadza, 1995; Xie *et al*., 2004), which was the same in all treatments in this study. Similarly, given that all treatments in this study were kept at the same water nutrient conditions, it is not unexpected that the lengths of the longest petioles remained relatively unchanged since the lengths of petioles are known to change with changing water nutrients (Coetzee *et al*., 2007a). The large variations observed in the root lengths and the maximum petiole lengths could be due to the relatively small sample size.

2.4.2. Effect of mite herbivory on the leaf chlorophyll content

The relative chlorophyll content (as measured with the chlorophyll SPAD meter) decreased significantly over time in the third, fourth and fifth leaves, which is not unusual since the majority of plants exhibit a decrease in leaf chlorophyll content as the leaves age and undergo senescence (Šesták and Čatský, 1962; Šesták, 1963; Lu *et al*., 2001; Yoo *et al*., 2003; Lefsrud *et al*., 2007). The third and fourth leaves showed a difference in the rate of change of chlorophyll content between the densities and, whilst not significant, at the end of six weeks the chlorophyll content of the leaves was lowest in plants that had been exposed to the high mite density. The chlorophyll content of the control treatment in leaf 3 and leaf 4 was lower than in the mite treatments, and in leaf 5 the chlorophyll content of the control treatment was lower compared to the medium and low mite density treatments, but higher compared to the high mite density treatment, suggesting that chlorophyll content is not influenced by the mites, particularly at the densities used in this study. This is in agreement with the results of Chapter 6, where no correlation existed between the amount of leaf tissue that was removed by mites (i.e. damage to leaves caused by mite feeding) and the chlorophyll content of leaves.

The second, third and fourth leaves were the most damaged by mite herbivory, which shows that the mites laid eggs on younger leaves and, at least at duration of six weeks, mite densities had a greater impact on younger leaves than on older leaves. However, as the plant produces new leaves over time, and therefore the position of the leaves changes, it is expect that the oldest leaves would be the most affected by mite herbivory. This in agreement Cordo and DeLoach (1976) who found that mites laid eggs on younger leaves and that the amount of damage and size of galleries increased as the nymphs developed and as leaves increased in age (Cordo and DeLoach, 1976). Young leaves may be preferred for oviposition because they tend to be less tough than older leaves. Another alternative for selection of leaves for oviposition is given by Center (1987b) who observed that the eggs of *N. eichhorniae* were most commonly found on mature leaves, with leaf age accounting for 83% of the variation in the average number of eggs per plant, and he suggested that leaf selection for oviposition may be based on the distribution of nutrients and secondary metabolites in different-aged leaves of the same plant. A number of studies on various plants have shown that different-aged leaves contain different concentrations of proteins, sugars, amino acids and chlorophyll (Rockwood, 1974; Yoshida, 2003; Lefsrud *et al*., 2007; Li *et al.*, 2010), and in general, younger leaves are more

nutritious but more toxic than mature leaves (Cates, 1980; Lambdon *et al.*, 2003). The nitrogen content of young water hyacinth leaves is greater then that of older leaves (Center and Wright, 1991), so it is plausible that female *O. terebrantis* select leaves for oviposition based on their nutritional value.

The relative chlorophyll contents of leaves, as measured with the SPAD meter, gave surprising results; the third and fourth leaves that had been damaged by mites had higher relative chlorophyll contents than the control leaves and it was expected that mite feeding would decrease the chlorophyll content. This may be explained by the fact that the chlorometer reads the amount of light passing through the plant, hence it does not give a chl-a concentration value directly, and brown leaves or leaves with many mite galleries would allow more light to pass through them than green and undamaged leaves, resulting in a higher chlorometer reading. This would also explain why after six weeks the control treatment had a lower relative chlorophyll content than the density treatments. Hence, the assumption that the SPAD-205 chlorophyll meter measures the relative chlorophyll content in leaves is incorrect, and in future chlorophyll content will be measured using a spectrophotometer after acetone extraction of the chlorophyll, as is common practice for measuring plant chlorophyll contents (Harborne, 1984; Dere *et al*., 1998).

For the measurement of chlorophyll content using a fluorometer, plants were selected from culture stocks so as to have leaves with greatly variable mite damage, in order to imitate field conditions as closely as possible. This was done because mite densities in the field are usually greater then the densities examined in this study, and it was therefore expected that there would be greater differences in chlorophyll content between highly-damaged and little-damaged leaves, as was in fact shown in the results.

2.4.3. Effect of mite herbivory on the leaf surface area damaged

High mite densities produced the greatest amount of damage to the leaf surface area, followed by the medium and low mite densities. Similar trends have been shown in other studies, for example, Schooler and McEvoy (2006) manipulated the density of adult and larval loosestrife beetles, *Galerucella pusilla* Duftschmid (Coleoptera: Chrysomelidae), and found a linear relationship between beetle density and plant damage. Schooler and McEvoy (2006) concluded that damage is an increasing function of insect density, and therefore herbivore density can be indirectly

estimated from feeding damage. The results from the present study can therefore be used to estimate adult mite densities in the field, by comparing leaf surface area damaged in the field to the damage on leaves of this study, where known densities of mites were used.

The results also showed that the leaf surface area damaged by low mite densities at six weeks was comparable to the leaf surface area damaged by high mite densities at three weeks. Similar observations were made by Center and Van (1989) who found that within 70 days the number of weevil feeding scars counted on leaves of plants subjected to low numbers of weevils (25 individuals) was the same as that on leaves subjected to high numbers of weevils (250 individuals). This implies that, in time and in good conditions, a small population of mites would increase and cause as much damage to water hyacinth leaves as an initial high mite population.

The significantly strong negative relationships between the leaf surface area damaged and the wet weights (r^2 = 0.944, p < 0.002) as well as the relative chlorophyll content of leaf 3 (r^2 = 0.815, p < 0.014) are good indications that damage to leaf surface area, due to herbivory by high mite densities, has adverse impacts on those plant parameters. On the other hand, at the end of six weeks the results show poor correlations between the damaged leaf surface area and the production of leaves and ramets, the number of flowers counted per plant, the petiole lengths and the root lengths, suggesting that these growth parameters also are largely unaffected by mite herbivory.

2.5. CONCLUSION

The densities of mites used in the present study did not have an impact on water hyacinth growth parameters and therefore the hypothesis that plant growth parameters are impacted negatively with increased mite densities must be rejected in this case. However, the hypothesis that damage to the leaf surface area increases with an increase in mite density is confirmed. This study showed that when high densities of mites are used, the changes in plant growth parameters are observed faster, but these changes will be significant only if either a) extremely high mite densities are used, which should be comparable to field densities in late summer, or b) if the study is run for a longer period of time to allow the mite population to increase. In order to be statistically sound, an experiment where plants need to be inoculated with arthropods must be set up within one week. Since placing mites onto

plants is very time consuming, future trials will be run for no less than 10 weeks to allow mite numbers to increase naturally. In addition, for future trials (Chapters 3 and 4 specifically) the following methodology will be adhered to:

1. The high mite density causes the greatest amount of damage to the leaves, and plants should therefore be inoculated with as many mites as is possible to do within a week. Only one week can be used for inoculation if the different treatments are to be statistically comparable.
2. Mite damage will be assessed on both “young” and “old” leaves i.e. leaves 3 and 4 are young leaves and leaves 5 and 6 are old leaves. This will be done because leaf 3 was found to be the most damaged in this study, and the damage on leaf 5 is said to increases with leaf age (Cordo and DeLoach, 1976). Hence, measuring young and old leaves gives a better indication of the overall damage to the plant.
3. The lengths of the longest petioles will be measured but not those of the second petioles because the second petioles change morphology from tall to short as the plants are moved from culture stocks to experimental tubs i.e. the changes in the second petiole lengths are not due to the effect of herbivory, which is the effect under investigation.
4. Since flower production and root lengths did not show any significant changes, they will not be measured unless the response of water hyacinth to different nutrient concentrations is being investigated (as in Chapter 3).
5. Experimental tubs must be kept either on grass or in a ventilated glass house so that the water inside the tubs does not get excessively warm and thus potentially impact plant growth.
6. Potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) will be used as nutrient sources so that the exact known amount of nitrogen and potassium can be used.
7. The SPAD chlorophyll meter did not give reliable chlorophyll readings, possibly due to the fact that mite feeding removes whole pieces of plant tissue and does not simply suck out chlorophyll from the plant cells, thereby creating holes in the lamina and not necessarily changing leaf colour (as happens with feeding by the mirid *E. catarinensis* which causes leaf chlorosis (Julien, 2001)). Therefore, chlorophyll will be acetone extracted and measured with a spectrophotometer.

CHAPTER 3

The impact of nutrients and herbivory by *Orthogalumna terebrantis* on water hyacinth growth parameters and leaf chlorophyll content

3.1. INTRODUCTION

Globally there is a general trend towards an increase in the nutrient status of water bodies as nutrients enter aquatic systems through surface runoff, discharge of organic industrial wastes and sewage from human settlements, and runoff from the manufacturing of fertilizers and their excessive use in agriculture (Khan and Ansari, 2005). This causes eutrophication of water bodies – a process that under natural conditions occurs slowly and enhances plant growth, but that is significantly accelerated through an increased rate of nutrient input into water bodies from anthropogenic sources (Khan and Ansari, 2005). The resultant unwanted fast growth and subsequent death of aquatic plants reduces light penetration and depletes oxygen, often leading to changes in biological diversity (Pieterse, 1978; Tiwari, 1998).

Worldwide, moderately eutrophic water is defined as having a phosphorus (P) content of 10-30 µg L^{-1} and a nitrogen (N) content of 500-1100 µg L^{-1} while oligotrophic water has a P content of 5-10 µg L^{-1} and an N content of 250-600 µg L^{-1} (Khan and Ansari, 2005).

According to the South African Water Quality Guidelines, aquatic systems in South Africa are similarly divided into trophic groups, depending on their phosphorus and nitrogen contents (Table 3.1).

Table 3.1. Average summer inorganic phosphorus and nitrogen concentrations of South African aquatic systems, modified from SA Water Quality Guidelines (Holmes, 1996).

Condition of water	Phosphorus content ($\mu g\ L^{-1}$)	Nitrogen content ($\mu g\ l^{-1}$)	Effects on ecosystem
Oligotrophic	< 5	< 500	Moderate level of species diversity; minimal growth of aquatic plants.
Mesotrophic	5 – 25	500 – 2 500	High level of species diversity; fair growth of aquatic plants.
Eutrophic	25 – 250	2 500 – 10 000	Low level of species diversity; growth of aquatic plants becomes problematic.
Hypertrophic	> 250	> 10 000	Very low level of species diversity; growth of aquatic plants very problematic.

Water hyacinth is one of several plants known world-wide as a good indicator of the level of eutrophication (Khan and Ansari, 2005), since its impressive growth rate is regularly attributed to the eutrophication of fresh water systems (Musil, 1977; Hill and Cilliers, 1999; Kampeshi and Shantima, 1999). Water hyacinth can survive in low nutrient water, such as that of the Paraná floodplains of Argentina (Carignan and Neiff, 1994) and New Years Dam in South Africa (Hill and Olckers, 2001), but it is not problematic under the low nutrient conditions. In South Africa, the weed is most abundant in highly-eutrophic waters typically found near densely populated settlements, for instance at Hartebeespoort Dam, Hammarsdale Dam and the Vaal River, where it has become a major concern for agricultural, and urban and recreational land-users (Cilliers, 1991; Hill, 2003; Jadhav *et al*., 2004; Cilliers *et al*., 2008).

Numerous studies have demonstrated that the growth and biomass of water hyacinth increases with an increase in water nutrient conditions (Gopal, 1987; Carignan and Neiff, 1994; Xie *et al*., 2004; Heard and Winterton, 2000; Ripley *et al*., 2006; Coetzee *et al.*, 2007a; Stanley *et al*., 2007) and excess nutrients may be stored in petioles (Gossett and Norris, 1971). Using a mathematical model, Wilson *et al*. (2005) predicted that under eutrophic conditions water hyacinth would grow to a density of 10kg m^{-2} in 50 days at

30ºC, but would take 120 days to reach the same density under low nutrient conditions, at any temperature.

The growth and nutrient storage of water hyacinth is most affected by nitrogen and phosphorus supply (Reddy *et al.*, 1989, 1990). Reddy *et al*. (1989, 1990) investigated the effects of nitrogen and phosphorus levels on net productivity and nutrient storage by the plant and found that maximum net productivity occurred when the plants were grown at 5.5 mg N L^{-1} and at 1.06 mg P L^{-1}. The nitrogen and phosphorus storage in plant tissue increased with both N and P supply rates, and N and P storage in plant tissue was affected by plant density in the low N (0.5 mg N L^{-1}) and low P (0.06 mg P L^{-1}) concentrations, respectively (Reddy *et al*., 1989, 1990). While many nutrients are important to plant growth and the structuring of plant communities, the N:P ratio is of particular significance (Koerselman and Meuleman, 1996). Nitrogen becomes limiting to water hyacinth growth only when the N concentration is 7 times less than that of the P concentration, and similarly the concentration of P inside water hyacinth leaves is seven times less than the N concentration (Gopal, 1987).

In addition to nutrients, plant growth is also influenced by herbivory. Plants respond to insect herbivory in numerous ways and many studies have looked at plant-insect interactions (Agrawal, 1998, 2005; Pieterse *et al*., 2001; Kessler and Baldwin, 2002; Taylor *et al*., 2003; Heil *et al*., 2004; Wise and Cummins, 2006). Plants defend themselves either by tolerating or resisting insect herbivory, which is limited by plant chemicals (Schultz, 2002), while the amount and type of plant defense is determined by resources in the environment (Coley *et al*., 1985). Plants may also compensate for insect herbivory by either completely or partially replacing yield losses caused by the herbivory (McNaughton, 1983; Trumble *et al*., 1993) and plant compensatory responses are subsequently considered to hamper the efficacy of biocontrol agents (Myers *et al*., 1990; Wirf, 2006; Watt *et al*., 2007). Herbivores, on the other hand, are not only reliant on plant availability, but also on plant quality. Insect performance and preference is highly dependent on plant quality and nutrient content (Agrawal, 1998; Karley *et al*., 2008) and global changes in CO_2 and tropospheric ozone levels are altering nutrient availability to plants, which in turn influences insect life histories and population dynamics (Fagan *et al.*, 2002; Woods *et al.*, 2003). In a study that considered metal accumulation in plants, Behmer *et al.* (2005) found that the desert locust, *Schistocerca*

gregaria (Forskål) (Orthoptera: Acrididae), lost mass as the Zinc concentration in its plant food increased. Karley *et al.* (2008) reported that the aphid *Aphis fabae* Scopoli (Homoptera: Aphididae) produced fewer nymphs on plants that had a low tissue C:N ratio. In a laboratory study, Coetzee (2003) reported an increase in the body size of the mirid *E. catarinensis* as nutrient levels in the water in which water hyacinth plants were grown, were increased. In two separate field studies, Heard and Winterton (2000) and Spencer and Ksander (2004) found that the weevil *N. bruchi* preferred to feed on plant tissue that was higher in nitrogen. For water hyacinth, younger leaves have a greater N tissue content than older leaves (Center and Wright, 1991), and leaf laminae have the greatest tissue N content, followed by stem bases and leaf petioles (Spencer and Ksander, 2004).

The impact of biocontrol agents on water hyacinth is dependant on plant nutrients and biomass, which are related to water nutrient concentrations (Moran, 2006). In highly eutrophic systems, water hyacinth growth and reproductive rates are thought to be higher than can be controlled by invertebrate enemies (Heard and Winterton, 2000; Hill and Cilliers, 1999). For example, *E. catarinensis* had a lesser impact on plant growth when plants were grown at high water nutrient concentrations than at low water nutrient concentrations (Coetzee *et al*., 2007a.) Similarly, Ripley *et al*. (2006) found that mirid herbivory decreased plant chlorophyll fluorescence and biomass accumulation when plants were grown at low nutrients, but had a minimal impact when plants were grown at high nutrients. However, at the end of the experiments conducted in the above studies, the number of adult mirids was much lower on plants grown at low nutrient levels compared to plants grown at high nutrient levels. In a study on the performance and impact of the moth *Xubida infusella* (Walker) (Lepidoptera: Pyralidae) on water hyacinth and pickerelweed, Stanley *et al.* (2007) showed that for water hyacinth the dry weight of shoots increased, and the dry weight of roots decreased, at the higher nutrient treatment compared to the lower nutrient treatment, while pupae numbers were greater in the higher nutrient treatment. Similarly, Room (1990) noted an increase in the number of *Cyrtobagous salviniae* Hustache (Coleoptera: Curculionidae) on *Salvinia molesta* D.S. Mitchell (Salviniaceae) as a response to higher quality plants - populations of *C. salviniae* declined in Papua New Guinea during the early 1980s until the nitrogen levels in *S. molesta* were increased through localized addition of fertilizer, and only once a

significant weevil population density was reached did the weevil damage raise plant N concentration sufficiently to allow the weevil population to continue increasing unaided.

Since both nutrient availability and herbivory play an important role in plant growth, and plant nutrient levels in turn influence the development and success of herbivores, the aim of this chapter was, firstly, to determine what impact different nutrient concentrations and mite herbivory had on water hyacinth growth and leaf chlorophyll content, and secondly, to determine whether plants grown in the different nutrient concentrations influenced mite herbivory and reproductive output.

3.2. MATERIALS AND METHODS

3.2.1. Experimental set-up

Water hyacinth plants were grown in a plastic pool (305 cm diameter and 76 cm depth) inside a glasshouse at the Plant Protection Research Institute (PPRI) in Pretoria, South Africa, during summer. Healthy plants from this stock were then used for the experiment. Forty-eight plastic tubs (42 cm x 30 cm and 20 cm depth) were placed in a glasshouse at the PPRI and were filled with 14 L of tap water. Two free-floating plants, with daughter plants, dead leaves and stems removed, were placed into each tub. Tubs were then divided into three groups so that plants could be grown at three different nutrient concentrations (Table 3.2).

Table 3.2. Concentrations of potassium nitrate (KNO_3) (used as the nitrogen base) and potassium dihydrogen orthophosphate (KH_2PO_4) (used as the phosphorus base) added to water used for growing water hyacinth plants at three different nutrient treatments.

Nutrient treatment	KNO_3 (mg/L)	KH_2PO_4 (mg/L)
High (eutrophic) (n = 18)	3.0	0.43
Medium (mesotrophic) (n = 14)	1.5	0.22
Low (oligotrophic) (n = 16)	0.5	0.08

* n represents the number of replicates for each treatment.

Potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) were added as the nitrogen and phosphorus bases, respectively. The nutrient levels chosen were similar to the nutrient concentrations found at three different field sites in South

Africa, namely Mbozambo Swamp, Hammarsdale Dam and Farm Dam, which were considered to be eutrophic (high nutrient treatment), mesotrophic (medium nutrient treatment) and oligotrophic (low nutrient treatment), respectively (Brudvig, *et al*., 2005). The nutrient levels chosen had an N:P ration of 7:1 which is optimal for water hyacinth growth (based on laboratory trials done by Brudvig). The high, medium and low treatments were replicated 18, 14 and 16 times, respectively. Commercial iron chelate (13% Fe) was also added to each tub at 1.3 g/ 14 L water. The plants were grown at these nutrient levels for four weeks, and the nutrients and water in each tub were replaced completely every week.

After four weeks, ramets, dead leaves and stems were again removed and the plants were weighed. 100 mites per plant were placed onto the plants in one half of the tubs (i.e. 24 tubs with mites and 24 without mites). The tubs were placed at least 0.5 m apart to prevent the mites from potentially moving between tubs. Since it is not realistic to use more mites in an experimental set-up because placing mites onto the plants is highly time-consuming (refer to Chapter 2), the duration of this study was extended to 12 weeks to allow the mite population to increase on its own. Plants with mites acted as the experimental herbivory treatment, while plants without mites acted as the control treatment. This resulted in 24 experimental and 24 control tubs, arranged in a complete randomized block design. The average day and night temperatures in the glasshouse were 28.61 ºC ± 5.47 ºC and 16 ºC ± 1.66 ºC, respectively.

Plants were sampled fortnightly for 12 weeks and the following parameters were measured on each plant: number of leaves, number of ramets, number of flowers, length of the longest petiole, root length and wet biomass (which included ramets). The numbers of flowers and root lengths were measured in this chapter because they are known to be affected by nutrients (Coetzee 2003; Luu and Getsinger, 1990). The petiole of leaf 2 was tagged in the first week and its position was observed over time. If a tagged leaf 2 died before the end of the experiment, a new leaf 2 was tagged, and again observed. The growth parameters were averaged for the two plants in each tub to obtain a mean response per tub. Water in the tubs and the nutrients were replaced weekly.

3.2.2. Feeding damage determination

Damage to leaves by mite herbivory was also noted fortnightly on the experimental plants by recording the percentage of the leaf surface area damaged on the adaxial surface of leaves 4 and 6. Leaves 4 and 6 were chosen because mite damage becomes more distinct on older leaves; females oviposit on younger leaves and as the mite nymphs develop inside the galleries, as the leaves age, the galleries to become longer over time, and hence more visible (Cordo and DeLoach, 1976). In addition, the actual number of galleries was counted in five 1cm x 1cm blocks (Fig. 3.1) on leaves 4 and 6 to determine whether mites preferred to oviposit on a specific area of the leaf. The lengths of the longest and shortest gallery in the same five blocks were measured to obtain an idea of mite development over time, since gallery lengths correspond to mite development stages (Delfosse, 1978a).

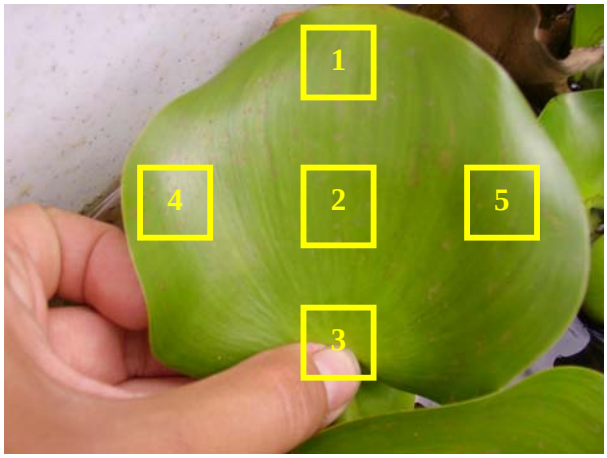

Figure 3.1. A water hyacinth leaf showing the position of the five demarcated 1 cm x 1 cm blocks in which the number of *Orthogalumna terebrantis* galleries were counted, and gallery lengths were measured. Size of blocks is not to scale.

3.2.3. Leaf chlorophyll content

The chlorophyll content (mg chl-a + chl-b L^{-1}) of leaf 3 in one of the plants in each tub was measured after 8 weeks using a UNICO® 1100 spectrophotometer, according to a modified method of Dere *et al*. (1998). Leaf 3 was used because previous work (Chapter 2) showed that this leaf had the highest chlorophyll content of all the leaves. In addition, the chlorophyll content was compared in leaves randomly selected from stocks of water hyacinth plants grown at the PPRI, half of which were kept agent free and half

that had been exposed to mites for about 10 years (C. Cilliers pers. comm.) such that the mite population was well established (> 200 mites per plant). The water hyacinth plants that had been exposed to the long-established and large mite population had leaves with a much greater surface area damaged by mites than the experimental plant leaves, which were only exposed to mites for 12 weeks, and had a low initial mite population (i.e. 100 mites per plant).

3.2.4. Statistical analyses

At the end of the sample period, differences in growth parameters, between the nutrient treatments, were highlighted using a one-way ANOVA. Similarly, a one-way ANOVA was used to determine the differences in growth parameters between herbivory treatments, at each nutrient treatment separately. The non-parametric Kruskal-Wallis test was used if data failed to meet the requirements for parametric analysis. Where the F-probability from the ANOVA was significant at 5%, *post hoc* comparisons were made using Fisher's protected least significant difference (LSD) test at the 5% level of significance (Snedecor and Cochran, 1980). If there were significant differences between herbivory treatments, data were plotted over time to see at which point these differences occurred. The position of leaf 2 was also plotted over time to show how long it took for the leaf to die.

No statistical analysis was performed on the position of leaf 2 because there was great variability within the nutrient treatments themselves i.e. sometimes only a single plant showed a change in the position of leaf 2 over a week. Since all the leaves from the original set of leaves would have died before a new leaf 2 was tagged, the graphs plotted also show the number of times a completely new set of leaves was produced in twelve weeks.

The wet weights of all plants were measured at the end of the sample period, and the difference in wet biomass at the beginning and the end of the experiment was calculated for each nutrient treatment. A one-way ANOVA was used to determine whether there were significant differences in the change of plant wet biomass between the nutrient treatments, as well as between plants that were or were not damaged by mites.

One-way ANOVAs were used to determine the differences in chlorophyll content of leaf 3 between the nutrient treatments, and between plants that had and had not been fed upon by mites. Similarly, a one-way ANOVA was used to compare leaves of agent free plants, and leaves that had been exposed to the long-established and large mite population.

A Kruskal-Wallis ANOVA was used to determine whether there were differences in the leaf surface area damaged by mites between the nutrient treatments, at the end of the sample period, for leaves 4 and 6 separately. In addition, mite damage between leaves 4 and 6 was compared at each nutrient treatment. The numbers of galleries on leaves 4 and 6 were combined for each of the 5 demarcated blocks (Figure 3.1), and a Kruskal-Wallis ANOVA was used to compare the number of galleries in each block at week 12, at each nutrient treatment separately.

The lengths of galleries measured in the five demarcated blocks were combined to obtain a mean gallery length for leaves 4 and 6. One-way ANOVA were used to determine whether there were differences in gallery lengths, and therefore mite development, between the three nutrient treatments.

Data were analysed using the statistical programme STATISTICA Version 7.0 (© StatSoft, Inc., USA).

3.3. RESULTS

3.3.1. Effect of nutrients on the plant growth parameters

Leaf and ramet production

Nutrients had a great impact on the majority of the growth parameters measured. The numbers of leaves and ramets produced after twelve weeks were significantly higher at the high nutrient treatment (leaves $F_{2, 46} = 7.276$, $p = 0.002$; ramets $F_{2, 38} = 21.776$, $p < 0.001$), compared to the medium and low nutrient treatments, which were not significantly different from each other (Figs. 3.2 and 3.3).

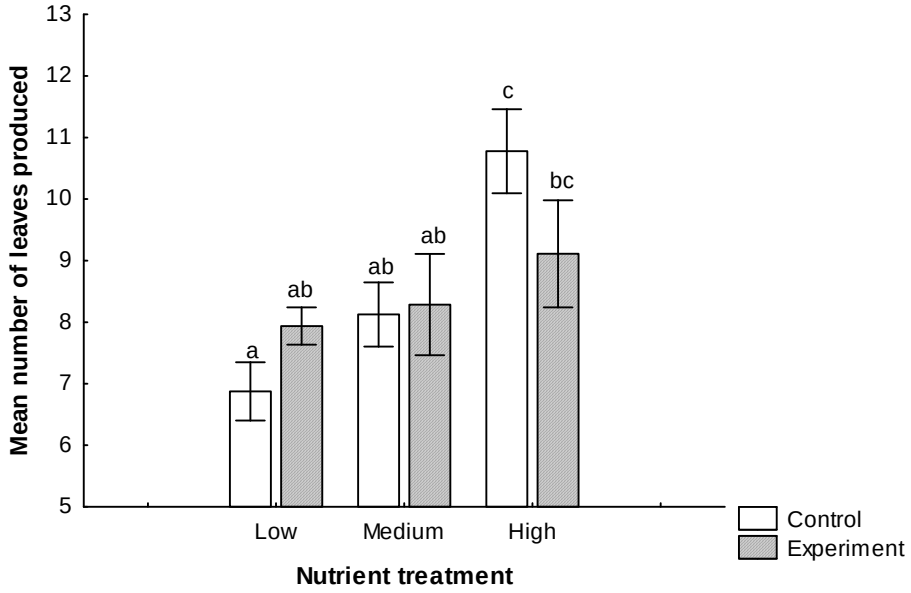

Figure 3.2. The mean number of water hyacinth leaves produced at the end of a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

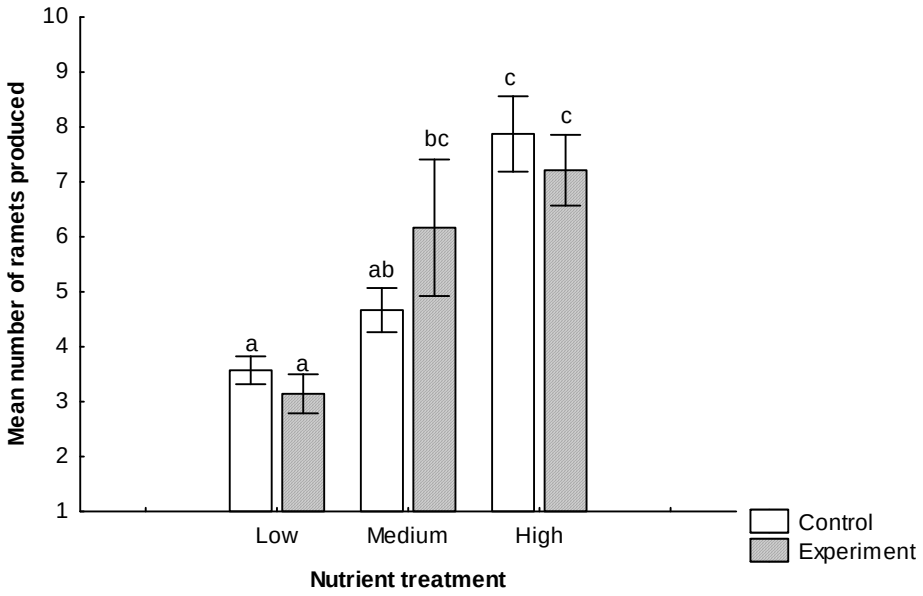

Figure 3.3. The mean number of water hyacinth ramets produced at the end of a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Petiole lengths

The lengths of the longest petioles were not significantly different between treatments at the start of the experiment ($p > 0.05$) and therefore their lengths at the end of the experiment could be compared directly. The lengths of the longest petioles were significantly longer at the high nutrient treatment compared to the medium and low nutrient treatments ($F_{2, 38} = 22.563$, $p < 0.001$), except that the experimental plants of the medium nutrient treatment had similar petiole lengths to the high nutrient plants, such that in the medium nutrient treatment the petiole lengths of the control plants were significantly shorter than the experimental plants (Fig. 3.4).

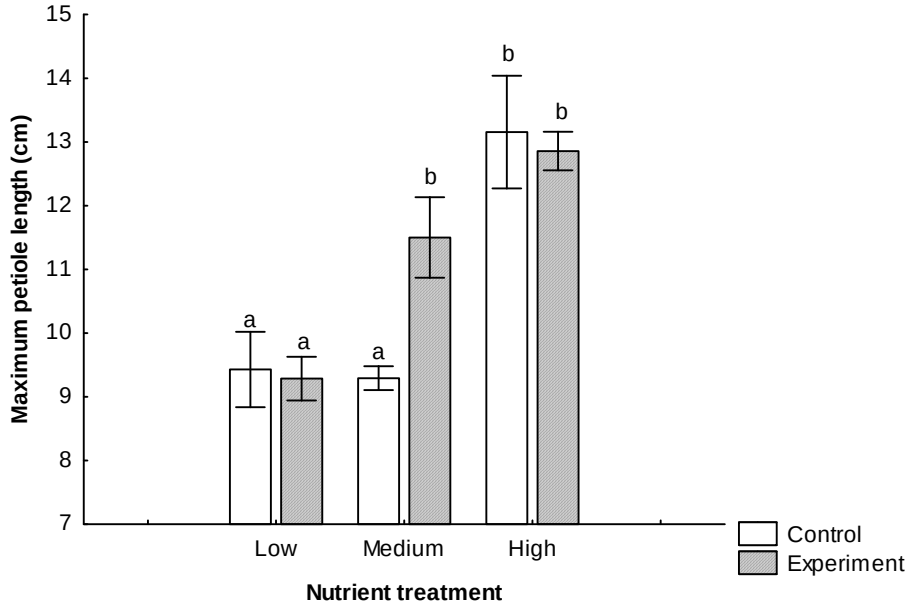

Figure 3.4. Mean lengths of the longest water hyacinth petioles at the end of a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Root length

After twelve weeks the roots of plants grown in the medium nutrient treatment were significantly longer than the roots of plants grown in the high nutrient treatment, but were not significantly different to the root lengths of plants grown in the low nutrient treatment ($F_{2, 38} = 4.314$, $p = 0.021$; Fig 3.5).

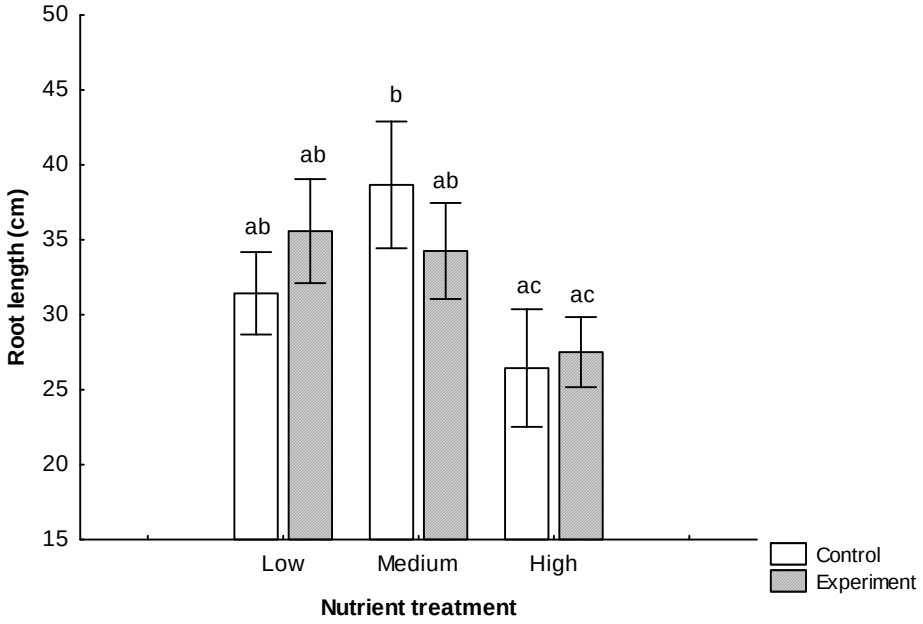

Figure 3.5. Mean root lengths of water hyacinth plants grown in low, medium or high nutrient treatments, at the end of a 12-week experiment, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Flower production

The number of flowers produced during the experiment did not differ between the nutrient treatments ($F_{2, 38} = 2.212$, $p = 0.124$; Fig. 3.6).

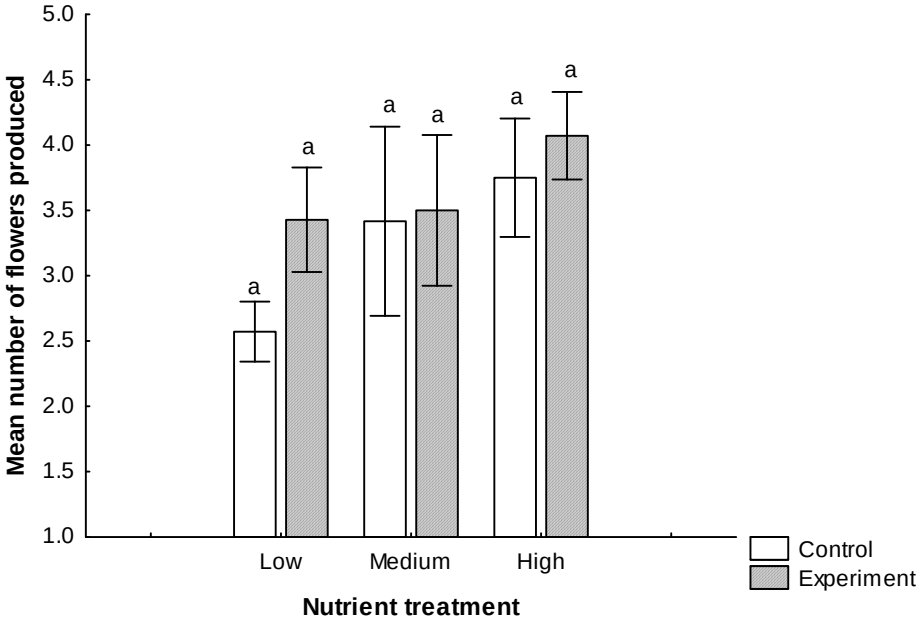

Figure 3.6. The mean number of water hyacinth flowers produced during a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Wet biomass

Similarly, there was no difference in the change of plant wet biomass accumulation, after 12 weeks, across the nutrient treatments ($F_{2,\ 38} = 1.106$, $p = 0.341$; Fig. 3.7). Interestingly, most plants lost biomass during the course of the experiment (Fig. 3.7).

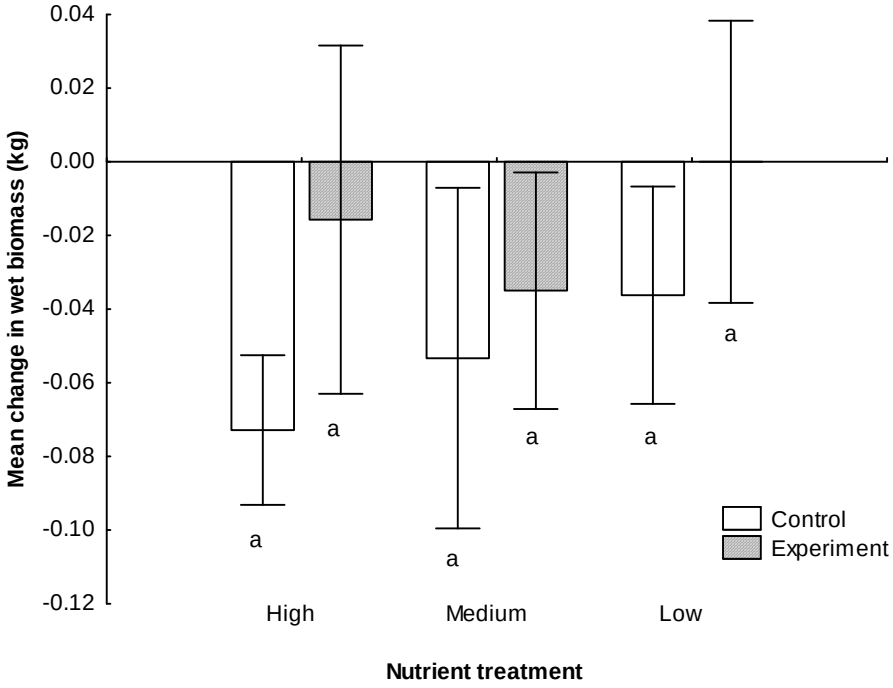

Figure 3.7. The change of water hyacinth wet biomass after a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Leaf turn-over rate

In the low and medium nutrient treatments, the majority of the plants had a complete new set of leaves by week 8, while the remaining plants had another complete new set of leaves two weeks later, at week 10 (Fig 3.8A-C). In the high nutrient treatment, one plant had a complete new set of leaves as early as week 6 (Fig. 3.8C), indicating that plants grown at a high nutrient treatment produce new leaves faster than plants grown at low and medium nutrient treatments. In addition, one plant in both the medium and the high nutrient treatment, had another complete new set of leaves i.e. third new set of leaves, at week 12 (Fig. 3.8B and 3.8C). In the low nutrient treatment, there was no significant difference in leaf production between control plants and plants that had been fed on by the mite, while there were significant differences in leaf production between control plants and plants fed on by the mites in the medium and high nutrient treatments.

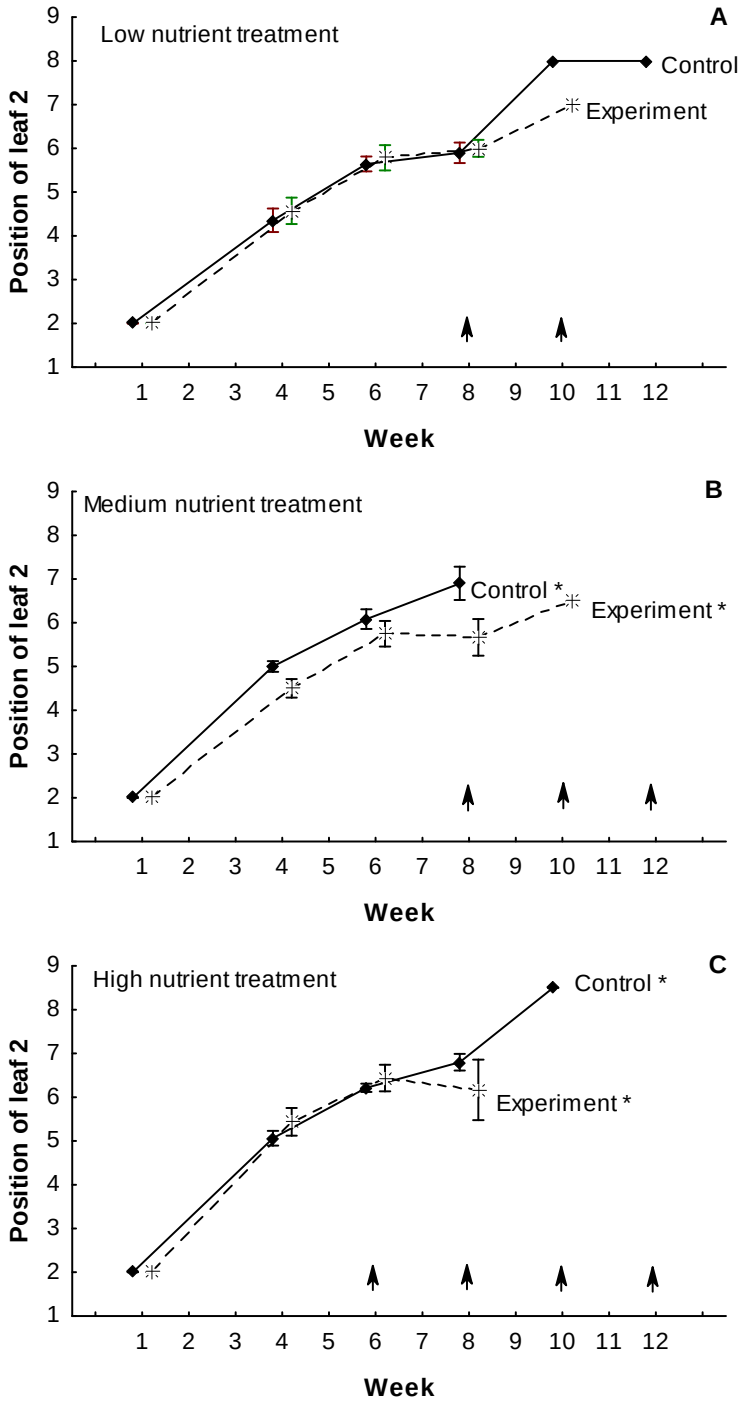

Figure 3.8. The change in the position of the second youngest leaves of water hyacinth plants over 12 weeks, when plants were grown in low (A), medium (B) or high (C) nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of the means. The curves represent the first complete set of leaves. Arrows indicate where a new set of leaves started i.e. where a new leaf 2 was tagged. Asterisks (*) behind labels indicate significant differences (Fisher's LSD test, $p < 0.05$) between herbivory treatments. n = 16 (low), 14 (medium) and 18 (high).

3.3.2. Effect of mite herbivory on the plant growth parameters

Unlike nutrients, mite herbivory had little impact on the majority of the plant growth parameters. At the end of the experiment, there were no significant differences in the number of leaves (Fig. 3.2), or ramets (Fig. 3.3), or flowers (Fig. 3.6) produced, or in root lengths (Fig. 3.6), or in the change in wet biomass (Fig. 3.7) between the herbivory treatments, at any of the nutrient concentrations (Table 3.2). However, plants that had been fed upon by mites had significantly longer maximum petioles in the medium nutrient treatment, than plants that had not been fed upon by mites (Fig. 3.4; Table 3.3). In addition, the changes in the position of leaves 2 were slightly slower in plants that had been fed upon by mites, especially in the medium nutrient treatment (Fig. 3.8).

There were no significant interactions between the nutrient and herbivory treatments, for any of the parameters measured (Table 3.3).

Table 3.3. F statistics and *p* values of one-way ANOVAs for nutrient treatment analysis and herbivory treatment analysis, and for the factorial ANOVA for a combination of nutrient x herbivory treatment analysis, showing significant differences in nutrient and herbivory treatments after 12 weeks during which water hyacinth plants were grown in different nutrient concentrations (high, medium and low) and were either subjected (experiment) or not subjected (control) to herbivory by *Orthogalumna terebrantis.* n = 18 (high), 14 (medium) and 16 (low).

Plant growth parameter	**Source of variation**				
	Nutrient treatment	Herbivory treatment (control vs experiment)			Nutrient treatment x
	(2,35)	Low (1, 12)	Medium (1, 10)	High (1, 13)	herbivory treatment (2, 35)
Leaf production	7.2; ***p* = 0.002**	0.0; *p* = 0.945	0.0; *p* = 1.0	1.8; *p* = 0.202	0.8; *p* = 0.454
Ramet production	21.8; ***p* < 0.005**	1.0; *p* = 0.348	1.3; *p* = 0.277	0.5; *p* = 0.499	1.5; *p* = 0.231
Maximum petiole length	22.6; ***p* < 0.005**	0.0; *p* = 0.838	11.2; ***p* = 0.007**	0.1; *p* = 0.767	2.7; *p* = 0.078
Root length	3.3; ***p* = 0.020**	0.89; *p* = 0.368	0.7; *p* = 0.425	0.1; *p* = 0.826	0.8; *p* = 0.475
Flower production	2.2; *p* = 0.378	3.5; *p* = 0.090	0.0; *p* = 0.930	0.8; ***p* = 0.037**	0.4; *p* = 0.669
Change in biomass	1.1; *p* = 0.341	2.2; *p* = 0.158	0.4; *p* = 0.539	0.2; *p* = 0.642	0.5; *p* = 0.629
Chlorophyll content	0.5; *p* = 0.621	2.4; *p* = 0.145	1.2; *p* = 0.30	0.0; *p* = 0.931	1.2; *p* = 0.310

Numbers in brackets are the degrees of freedom.
Significant differences have been highlighted in bold.

3.3.3. Effect of nutrients and herbivory on the leaf chlorophyll content

After 8 weeks, there were no significant differences in the chlorophyll content of leaf 3 between the nutrient treatments, or between the herbivory treatments (Table 3.4; Fig. 3.9). However, there was a significant difference in leaves that had been subject to herbivory by a long-established and large mite population, and leaves that were not exposed to mite herbivory at all ($F_{1, 20}$ = 17.808, p < 0.001; Fig.3.10).

Table 3.4. F statistics and p values of the one-way ANOVAs conducted for leaf chlorophyll contents of leaf 3 of water hyacinth plants when plants were grown in different nutrient concentrations (low, medium and high) and were either subjected (experiment) or not subjected (control) to herbivory by *Orthogalumna terebrantis*, for 8 weeks.

Source of variation for leaf chlorophyll content measure			
Nutrient treatment	**Herbivory treatment (control vs. experiment)**		
(2, 38)	Low (1, 13)	Medium (1, 9)	High (1, 13)
0.4; p = 0.621	2.4; p = 0.145	1.2; p = 0.301	0.0; p = 0.931

Numbers in brackets are the degrees of freedom.

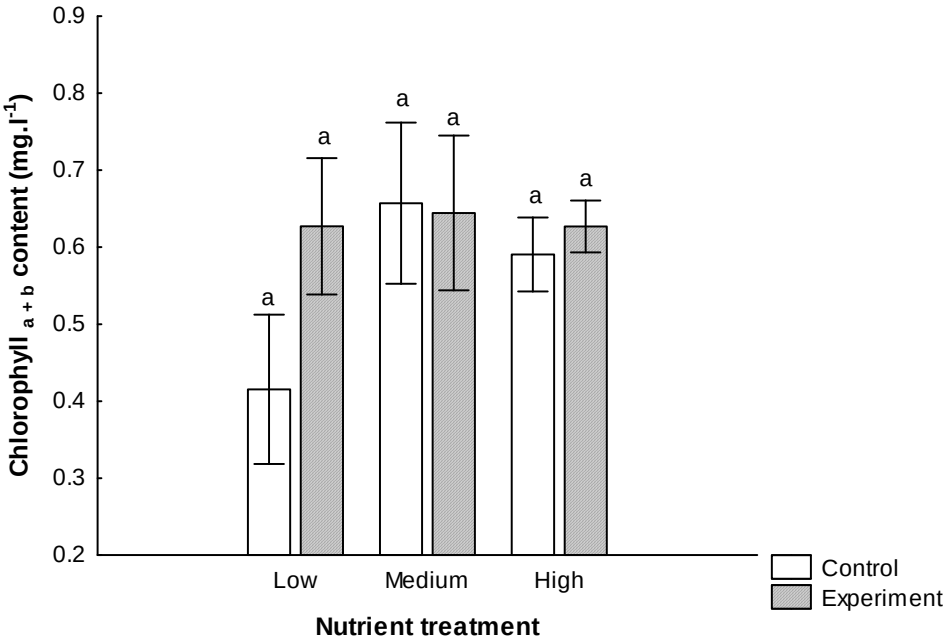

Figure 3.9. Chlorophyll content of leaf 3 of water hyacinth plants after the plants were grown for eight weeks in low, medium or high nutrient treatments, either exposed (experiment) or not exposed (control) to herbivory by *Orthogalumna terebrantis*. Error bars represent the standard error of means. Error bars followed by the same letter are not significantly different (p > 0.05). n = 16 (low), 14 (medium) and 18 (high).

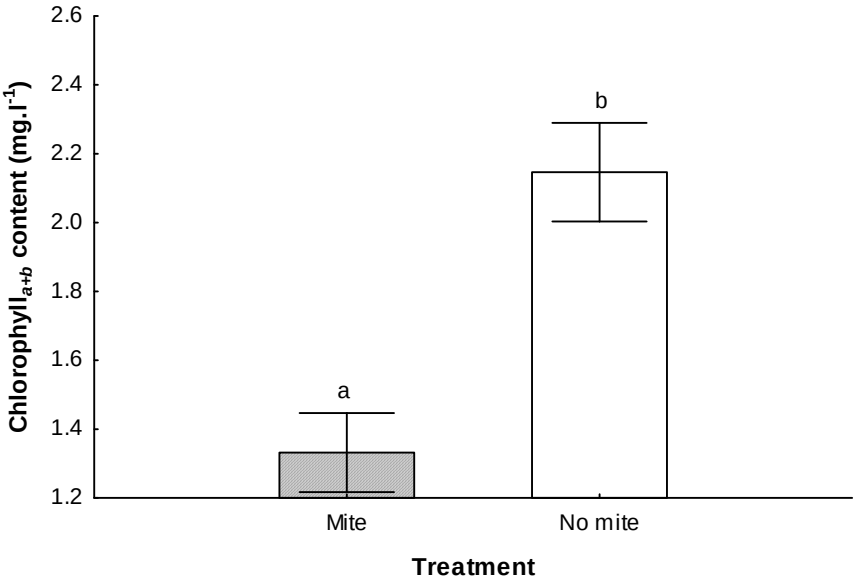

Figure 3.10. Chlorophyll content of water hyacinth leaves either exposed to herbivory by a long-established, large mite population (mite) or not at all exposed to mite herbivory (no mite). Error bars represent the standard error of means. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 6 for each treatment.

3.3.4. Effect of nutrients on mite herbivory

At the end of the 12-weeks experiment, there was a significant difference in mite herbivory between the nutrient treatments on leaf 4 ($H_{2, 22} = 7.442$, $p = 0.024$), but not on leaf 6 ($H_{2, 16} = 1.4$, $p = 0.497$; Fig 3.11). On leaf 4, mite herbivory was greatest at the high nutrient treatment (Fig. 3.11). There were no significant differences in mite herbivory between leaf 4 and leaf 6, at any of the nutrient treatments (low $H_{1, 12} = 0.534$, $p = 0.465$; medium $H_{1, 11} = 3.213$, $p = 0.073$; high $H_{1, 12} = 0.086$; $p = 0.769$). Since there were no significant differences in herbivory between leaves 4 and 6 at any of the nutrient treatments, the data were combined and then plotted over time (Fig. 3.12). Mite herbivory decreased slightly over the twelve weeks (Fig. 3.12). However, since a new leaf was produced after about 8 weeks (above, Fig. 3.8A-C), it is likely that the original leaves that were measured for mite damage had changed position, and hence different leaves were being measured i.e. younger leaves that would have been exposed to mite herbivory for a shorter time, explaining the smaller percentage of damage observed on them.

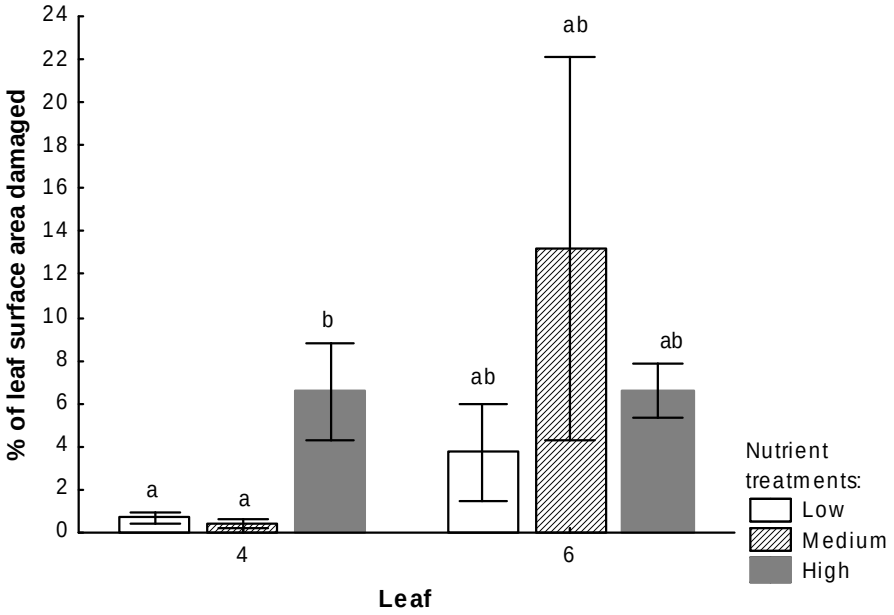

Figure 3.11. Water hyacinth leaf surface area damaged after 12 weeks of herbivory by *Orthogalumna terebrantis* on leaves 4 and 6, when plants were grown in low, medium or high nutrient treatments. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

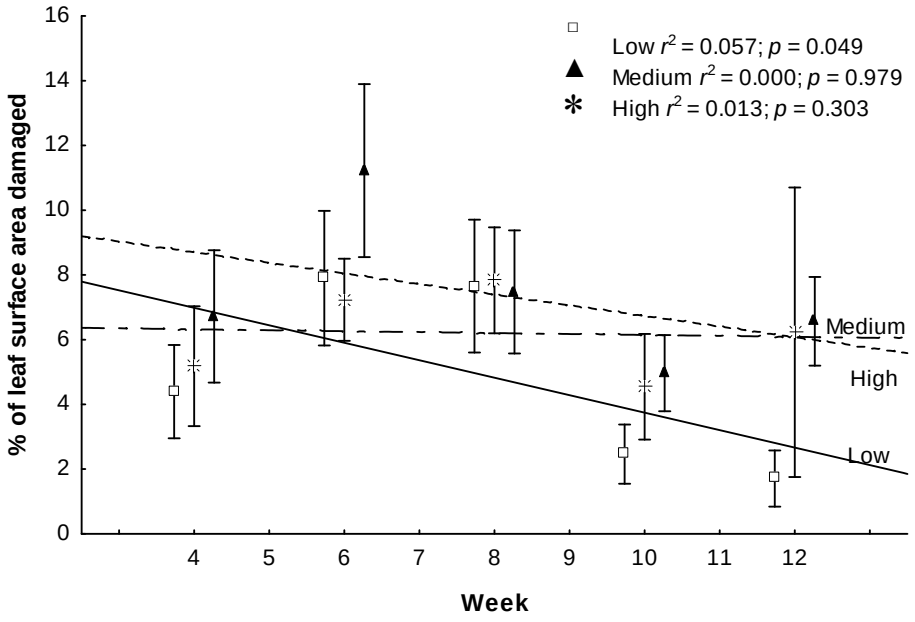

Figure 3.12. Water hyacinth leaf surface area damaged by *Orthogalumna terebrantis* over 12 weeks, when plants were grown in low, medium or high nutrient treatments. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 16 (low), 14 (medium) and 18 (high).

Since there were no differences in leaf surface area damaged between leaf 4 and leaf 6, the number of galleries in the five demarcated blocks on leaf 4 and leaf 6 were combined and analysed thereafter. After twelve weeks, there were no significant differences in the number of galleries between the five demarcated blocks in the low and medium nutrient treatments (low $H_{4, 54}$ = 2.686, p = 0.611; medium $H_{4, 66}$ = 3.415, p = 0.490), but there were significant differences in the number of galleries between the demarcated blocks in the high nutrient treatment ($H_{4, 75}$ = 14.891, p = 0.005; Fig.3.13). The positions of the blocks on the leaf were: block 1 – top centre of leaf; block 2 – centre of leaf; block 3 – bottom centre; block 4 – left edge of leaf; block 5 – right edge of leaf (Figure 3.1). In general, blocks 1 and 3 had the fewest galleries, while block 5 had the most galleries.

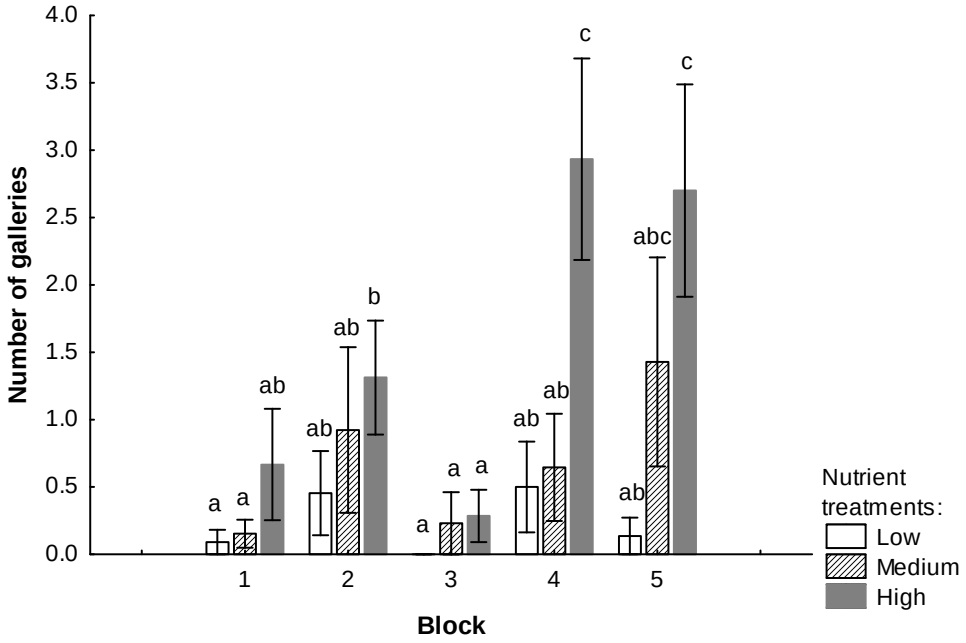

Figure 3.13. The number of *Orthogalumna terebrantis* galleries counted in five demarcated blocks (1 cm x 1 cm) on selected water hyacinth leaves, after twelve weeks of plants growing in low, medium or high nutrient treatments. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, p < 0.05). n = 16 (low), 14 (medium) and 18 (high). The positions of the blocks on the leaf were: block 1 – top centre of leaf; block 2 – centre of leaf; block 3 – bottom centre of leaf; block 4 – left edge of leaf; block 5 – right edge of leaf.

3.3.5. Effect of nutrients and time on mite development, based on gallery lengths

Gallery lengths measured on leaves 4 and 6 were combined and analysed thereafter, and were only analysed and plotted against time until week 8, which is roughly two weeks after the first generation of adult mites would have emerged (according to Delfosse (1978a)) (Fig. 3.14). The average length of galleries was 2.37 ± 0.7 mm, and the longest gallery measured during the experiment was 6.5 mm long. Gallery lengths were significantly affected by time ($F_{2, 121}$ = 18.104; p < 0.001), and increased significantly between week 4 and week 6 in the medium and high nutrient treatments, and between week 4 and week 8 in the low nutrient treatment (Fig. 3.14). Based on gallery lengths, mite development was similar in the three nutrient treatments ($F_{2, 121}$ = 1.107, p = 0.334), although mites seemed to develop slightly faster in the high nutrient treatment, where the first generation of adults emerging roughly six weeks after mites were introduced onto the plants, while in the medium and low nutrient treatments development was slower and the first generation of adult mites emerged a week later than in the high nutrient treatment, roughly seven weeks after mites were introduced onto the plants (Fig. 3.14).

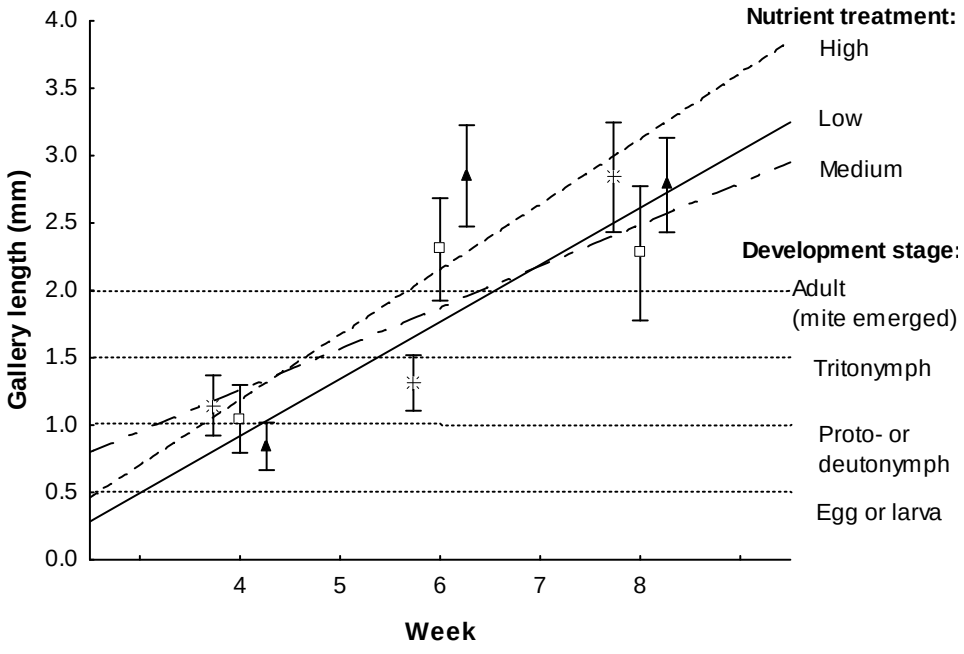

Figure 3.14. Egg to adult development of *Orthogalumna terebrantis*, based on the lengths of galleries (Delfosse, 1978a) measured on water hyacinth leaves grown in low, medium or high nutrient treatments. Error bars represent the standard error of the means. n = 16 (low), 14 (medium) and 18 (high).

3.4. DISCUSSION

The results of this study clearly demonstrate that water hyacinth growth is greatly influenced by water nutrient levels, and this is in agreement with other studies (Gossett and Norris, 1971; Watson and Brochier, 1988; Reddy *et al*., 1989, 1990; Coetzee *et al*., 2007a). Plants grown in water with a high N and P content were healthy with tall petioles, and produced more leaves and daughter plants than plants grown in water with lower N and P contents. Plants grown at low nutrients had short, bulbous petioles, and did not survive the winter season following the experiment.

The same numbers of flowers were produced in all of the nutrient treatments during this study. While a number of studies have found the production of flowers to be induced by nutrient deficiency (Richards, 1982; Watson and Brochier, 1988; Luu and Getsinger, 1990). Watson and Brochier (1988) observed that an increase as well as a decrease in nutrients could stimulate flowering. This could explain why there were no differences in flower production between the treatments. Water hyacinth root growth is, however, nutrient dependent (Zaranyika and Ndapwadza, 1995; Xie *et al*., 2004), and plants growing in low nutrient water need longer roots to increase the surface area through which nutrients can be absorbed, as was observed in the present study where plants that were grown in the low nutrient concentration had the longest roots.

An unusual result was that plants lost weight between the beginning and end of the experiment, in all three nutrient treatments. The weight loss can be explained by the shortening of petioles during the experiment. Plants used in this study had tall petioles initially since they were taken from pools with a dense water hyacinth population, where the plants could hold each other up. Once the plants were moved into plastic tubs where they had more space (two plants per tub), they no longer had the physical support of other plants to keep them up, so their petioles shortened and became more swollen to allow the plants to float on their own. In addition, plant density is inversely related to plant size and this is related to the degree of intraspecific competition for light and space (Center and Spencer, 1981). In this study, once the plants were placed into tubs where they had more room to grow, they no longer needed to compete for light and space, which may explain why the petioles shortened over time. A similar shortening of petioles was observed in Chapter 2.

The production of leaves slowed down from the beginning towards the end of the experiment. Water hyacinth leaf production rates decrease towards winter and pick up again towards summer (Center, 1981), so the decrease in leaf production during this study, which was conducted from January to April, may be explained by the gradual decrease in ambient temperatures. In general, most of the plants in this study had a complete leaf turn-over in eight weeks, which is in agreement with Center (1981) who observed that field populations of water hyacinth had a life-span of roughly two months.

Herbivory by *O. terebrantis* had little effect on water hyacinth growth. These results differ from those of Heard and Winterton (2000) and Coetzee *et al.* (2007a) who found that herbivory by *N. bruchi* and *N. eichhornia*, and by *E. catarinensis*, respectively, resulted in shorter petioles and fewer daughter plants being produced. However, plants are known to compensate for damage resulting from insect herbivores. For example, Briese *et al.* (2002) noted that slight compensation by a plant may take place where low densities of agents occur on a host plant. Similarly, in a simulated defoliation experiment, Soti and Volin (2010) showed that water hyacinth was able to compensate for a low level of continuous defoliation (10%) but not for a higher level of defoliation (80%), regardless of the nutrient conditions. The densities of mites used in this study were lower than what is usually found in field situations, and it is therefore likely that plants damaged by mites initially compensate for mite damage by increasing their flower and ramet production, and increasing their petiole lengths. However, after prolonged damage from the mites the plant growth parameters are likely to be negatively impacted, as is the case when plants are fed on by the weevils and the mirids. Other studies have found similar plant-compensatory effects, for example, Lv *et al*. (2008) reported that rice plants with stems injured by the sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) produced more tillers than uninjured plants, and that tillers with leaf injury produced larger pinnacles than uninjured tillers. Similarly, when the buds of *S. molesta* are attacked by weevils, new and previously dormant buds are activated to grow, thus negating the damage to buds (Room, 1990). It is possible that the water hyacinth in this study was compensating for mite damage by boosting some growth parameters.

In contrast, herbivory by a long-established and large mite population greatly decreased the chlorophyll content of leaves. This suggests that a mite population that is left to multiply undisturbed for a number of years can reach high enough numbers to significantly impact water hyacinth leaf chlorophyll content. When the chlorophyll content

of a plant decreases, the plant's ability to photosynthesize is compromised, and growth may be stunted. For example, Conrad and Dhileepan (2007) reported that the leaf-sucking bug *Carvalhotingis visenda* (Drake and Hambleton) (Hemiptera: Tingidae) significantly reduced the leaf chlorophyll content of cat's claw creeper, which resulted in reduced plant height and leaf biomass. In this study the number of mites introduced onto plants was very low (i.e. 100 mites per plant), when compared to mite numbers often found in the field. In South Africa as many as 200 mites per leaf are found during summer months at sites such as Mbozambo Swamp (pers. obs.), and in Argentina Delfosse (1978a) found that *O. terebrantis* populations peaked after 16 weeks, at 840 mites per plant.

Mite herbivory may cause other, more subtle effects on water hyacinth fitness which were not accounted for in this study. For example, the openings at the distal end of galleries from where adult mites emerge provide an ideal entry area for pathogens such as *Acremonium zonatum* (Sawada) Gams (Fungi) which causes zonate leaf spot disease in water hyacinth (Delfosse, 1978a; Gerson *et al*., 2003). Moran (2005) noticed that necrosis development by the fungus *C. piaropi* may be up to 10 times greater on water hyacinth plants exposed to weevils than on plants without weevils. Also, the presence of mites stimulates weevil egg production and feeding, thus increasing weevil damage to the plants (Delfosse, 1977a).

The health, and therefore nutrient status of plants, has a direct impact on insect development and survival (Strong, 1984; Room, 1990; Schoonhoven *et al.*, 1998). The numbers of mites on experimental plants was never recorded at the end of this study because of the ease with which mites are knocked off plants as one handles them. However, the greatest number of mites was always observed on plants grown in the high nutrient treatment, which also had the greatest feeding damage. This result is comparable to Heard and Winterton (2000) who found that *N. bruchi* produced more offspring on plants grown at a high nutrient concentration. Similarly, Ripley *et al*. (2006) recorded a significant rise in *E. catarinensis* adult and nymph numbers with an increasing water nutrient supply. In this study, the first generation of adults emerged a week earlier on plants in the high nutrient treatment (5.5 weeks after mites were introduced onto plants) than on plants in the medium and low nutrient treatments (6.5 weeks after mites were introduced onto plants), suggesting that the mites develop faster on plants grown at high nutrients. Stanley *et al*. (2007) found a similar result with the moth *X. infusella* whose

larval development rate and pupal weight increased on both water hyacinth and pickerelweed grown in a high nutrient treatment compared to a low nutrient treatment.

Mite development was somewhat slower in the present study than observed by Delfosse (1978a); according to Delfosse adult mites emerge roughly 6 weeks after oviposition, while in the present study mites emerged after 7 weeks. Arthropod development is influenced by temperature (Bursell, 1964; Clarke, 1967; Cloudsley-Thompson, 1970; Chown and Terblanche, 2007) and thus the decreased developmental rate in the present study was most likely due to the decreasing ambient temperatures experienced during the experimental period.

Mites preferred to oviposit along the edges of leaves rather than the centre of leaves, and this is in agreement with observation made by Silveria-Guido (1965). Although the mites can easily break through the pseudolaminae of leaves to lay eggs, they usually look for wounds created by other herbivores, or damaged areas on the leaf, from where to feed (Delfosse *et al*., 1976). It is possible that the softer edge of water hyacinth leaves, compared to the tougher centre of the leaves, makes it easier for females to oviposit there.

3.5. CONCLUSION

The results of this study demonstrate that water hyacinth grows faster and bigger in water with a high nutrient concentration, and that herbivory by mites has a small impact on water hyacinth growth, especially at high nutrient concentrations. To demonstrate, *N. bruchi* and *E. catarinensis* occur in very high numbers at Hammarsdale Dam, which in this study is used as a reference for mesotrophic nutrient conditions (1.48 mg N L^{-1} and 0.08mg P L^{-1}), but they have had very little impact on the water hyacinth population under those, relatively low, nutrient conditions (Hill and Olckers, 2001). Although the better quality of plants that grow under high nutrient conditions may be advantageous to biocontrol agents, which perform better on higher quality plants (Room, 1990) the impact of the agents becomes negligible when the plant population explodes as a result of eutrophication. The growth of water hyacinth is therefore more affected by the water nutrient status than by arthropod herbivory. This has great implications for the authorities and managers of water bodies who will need to monitor and control the amount of

nutrients entering aquatic systems if the growth and expansion of water hyacinth is to be reduced.

CHAPTER 4

Interactions of three biological control agents of water hyacinth and their impact on the plant

4.1. INTRODUCTION

During the latter part of the last century many ecologists alleged that communities, whether of insects, or birds, or marine invertebrates, were not structured so much by competition as by other process such as predation and stochastic events (Connor and Simberloff, 1984; Price, 1984; Strong, 1984; Underwood and Denley, 1984). However, recent developments in studies of indirect interactions i.e. plant-mediated competition, have challenged historical paradigms and the importance of interspecific interactions (such as competition) is now a highly debated subject. Considerable attention has been given to interspecific interactions between phytophagous insects (Denno *et al*., 1995; Denno *et al.*, 2000; Kaplan and Denno, 2007) and it has been found that interspecific interactions, specifically competition, greatly influence the performance and fitness of the insects (Kaplan and Denno, 2007), which in turn structures populations and community ecology (Denno *et al.*, 2000). Furthermore, competition theory predicts that where a mutual struggle for resources occurs between two organisms, the interactions intensify with an increase in density, ecological similarity (e.g. feeding guild) and spatiotemporal co-occurrence (Kaplan and Denno, 2007). Much research still needs to be undertaken for these contrasting views to be reconciled and May (1984) cautioned against both views when he stated

"too narrow a concern for competitive interactions may lead to important predatory species being neglected in what purports to be a community study…and, conversely, too much emphasis on prey-predator relations can cause competing species to be overlooked…"

A key manner in which phytophagous insects interact is through indirect effects involving plants (Kaplan and Denno, 2007). Herbivore damage to plants induces plant resistance mechanisms which can generally reduce herbivore populations as well as preference for the plant (Karban and Baldwin, 1997; Rodriguez-Saona and Thaler, 2005). Insect and mite induced responses of plants have been studied in various systems (Karban and Carey, 1984; Room, 1990; Agrawal, 1998; Bounfour and Tanigoshi, 2001). In a review of interspecific competition between phytophagous insects, Denno *et al*. (1995) found that host plant-mediated interspecific competition occurred in 76% of the studies reviewed, and that physical factors, natural enemies and intraspecific competition were less important in mediating interspecific competition than host-plant factors. In addition, delayed interspecific competition occurs when previous feeding by one species diminishes the performance of another species that feeds on the same plant later in the season, and this is often underestimated in instances when the interactions of two or more species are studied simultaneously. For example, in a competition study between two planthoppers, Denno *et al.* (2000) found that neither *Prokelisia dolus* Wilson nor *P. marginata* (Van Duzee) (Hemiptera: Delphacidae) suffered significant fitness reduction when they fed on cordgrass simultaneously, but when plants were previously fed on by *P. dolus* the development of *P. marginata* was protracted and its body size reduced, while plants previously fed on by *P. marginata* resulted in the prolonged development of *P. dolus.* The above results also showed an asymmetry between the two *Prokelisia* species in terms of plant-mediated competition i.e. prior feeding by *P. dolus* had an effect on the development time and body size of *P. marginata* while prior feeding by *P. marginata* only had an effect on the development time of *P. dolus*. Denno *et al.* (2000) attributed this asymmetry to the ability of *P. dolus* to better tolerate plants with depleted nitrogen levels, due to prior damage through herbivory, via compensatory feeding.

The use of multiple agents to control an invasive plant species has been both supported (Delfosse, 1978a; Charudattan, 1986; Hoffmann and Moran, 1998; Caesar, 2003, Jiménez and Balandra, 2007) and criticised (Myers, 1985; Harris, 1990; Denoth *et al*., 2002; Crowe and Bourchier, 2006). In a review on the use of multiple agents in biological control, Denoth *et al*. (2002) found that in the majority of

projects for the biological control of weeds, the likelihood of control increased as more species of agents were released. However, these authors also point out that in many instances multiple agents are used in the hope that the correct species will at some stage be released, and not necessarily that a cumulative control effect is created (Denoth *et al*., 2002). This approach has been termed the “lottery model” (Myers, 1985).

In biological control programmes, competition is quite common between agents used against insect pests, where intraguild predation may occur e.g. intraguild predation from *Orius albidipennis* (Reuter) (Heteroptera: Anthocoridae) on *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), both of which are used for the biological control of thrips on various crops (Rosenheim *et al*., 1995; Madadi *et al*., 2009). However, competition is less common between biocontrol agents used against weeds as the agents do not usually kill the weed, and at the same time the weed provides a variety of host niches ensuring that competition for space and food is potentially less limiting (Denoth *et al*., 2002). In addition, when herbivores differ in their feeding styles (sap-feeders vs. chewers), fine-scale resource partitioning occurs on individual plants (Daugherty, 2009). Differences in herbivory feeding styles also have different effects on the plant concerned. For example, the xylem sap-feeding spittlebug, *Philaenus spumatius* L. (Homoptera: Cercopidae), reduced the growth and photosynthetic rates of goldenrod more so than the leaf-chewing beetle, *Trirhabda* sp. (Coleoptera: Chrysomelidae) (Meyer, 1993; Meyer and Whitlow, 1992).

To date, five arthropod agents and one fungal pathogen have been released on water hyacinth in South Africa (Hill and Cilliers, 1999; Hill and Olckers, 2001). Although two or more agents are present at the majority of water hyacinth infestations in the country, there are still many sites without agents (M. Hill pers. comm.). Before time, effort and money is spent on introducing agents to sites that are missing particular agents, or importing new agents into quarantine facilities for further study, it is important to understand which agents are best suited to an area and, most importantly, how they will interact with each other, in order to obtain the best and most expedient weed control. Previous studies which have investigated the interactions of various biocontrol agents of water hyacinth showed that, in general, the use of multiple agents places increasing stress on the plant and reduce its growth, more so than when a single agent is used (Delfosse, 1978a; Caunter and Mohamed, 1990; Moran, 2005; Ajuonu *et al*., 2009).

Of the five arthropods that attack water hyacinth in South Africa, *N. eichhorniae* has become the most successful agent by establishing at most of the weed infestations in the country, where it has a significant impact on the plant (Hill and Cilliers, 1999; Hill and Olckers, 2001; Hill and Oberholzer, 2004). *Orthogalumna terebrantis* is found at 17 water hyacinth infestations around the country (Chapter 6), but its impact on the plant has not been evaluated (M. Hill, pers. comm.). *Eccritotarsus catarinensis* was released in South Africa in 1996 and by 2007 it was established at 20 sites, mainly along the KwaZulu-Natal coast (Coetzee *et al*., 2007b).

The present chapter investigates the impact of *O. terebrantis*, *N. eichhorniae* and *E. catarinensis* on water hyacinth growth parameters, when the agents are used alone or in combination. Adults of all three species feed on water hyacinth leaves, and both mirid and mite females oviposit on the leaf blades (Hill *et al*., 1999; Perkins, 1973) where the eggs can potentially be removed by the feeding of adult weevils, increasing the possibility of agent interaction.

The key differences between the species are that the weevil is nocturnal, mostly feeding at night, and females oviposit in the leaf petioles of young leaves (Warner, 1970; DeLoach and Cordo, 1976), while the mirid and the mite are mostly active during the day and females oviposit on the leaf blades (Hill *et al*., 1999; Cordo and DeLoach, 1975). The life cycle of the weevil ranges between 90 – 120 days and longevity is about 250 days (DeLoach and Cordo, 1976; Julien, 2001). In comparison, the mirid and the mite have a much shorter life cycle, of around three weeks and four weeks, respectively, and mirid adult longevity is about 50 days (Hill *et al.*, 1999; Stanley and Julien, 1999), while that of the mite is about 115 days (Silveira-Guido, 1965). In addition, different feeding guilds and life history stages cause damage to plants that differ in severity (Meyer, 1993; Schooler and McEvoy, 2006). Thus, in terms of having an impact on the plant, the most important aspect of the three agents' biologies is the type of damage they cause to the plant through their different feeding styles - adult weevils are "leaf-chewers" that produce rectangular feeding scars in the leaf cuticle, usually on the youngest leaves (Center, 1987a; Hill and Cilliers, 1999) and the weevil larvae are "petiole-miners" that create most damage by burrowing down the petioles and into the crowns of the plants (Hill and Cilliers, 1999; Julien, 2001; Wilson, 2002). Mirid nymphs and adults are "sap-suckers" that feed gregariously on the leaf under-surface (Hill and Cilliers, 1999), causing chlorosis of the laminae (Julien, 2001). The mite adults and nymphs are "leaf-chewers" with the adults feeding on leaf laminae, but most of the mite damage

to water hyacinth is caused by the larvae and nymphs as they feed inside the leaf immediately underneath the cuticle, thereby removing leaf tissue (Cordo and DeLoach, 1976).

The interactions between *E. catarinensis* and both *N. eichhorniae* and *N. bruchi*, and between *O. terebrantis* and *N. eichhorniae*, have been examined previously by Ajuonu *et al*. (2007) and Delfosse (1978a, 1978b), respectively. In a laboratory experiment, Ajuonu *et al*. (2007) found high mortality of the mirids on plants that had a large number of old feeding scars from adult weevils but the mirids survived well on plants that had recent feeding scars. Ajuonu *et al.* (2007) concluded that the weevils and the mirids were compatible, but the effects of the agents on water hyacinth were not measured in that study. Delfosse (1977a) showed that the weevils laid more eggs and fed more in the presence of the mites. In a later study, Delfosse (1978a) conducted a well-sampled field experiment and found that the combined effects of feeding and development of weevils and mites brought about a significant reduction in plant size and density. In addition, Delfosse (1978a) observed that the mites congregated around the feeding spots of the weevils, and this behaviour has been observed by the present author.

Synergistic effects, where multiple agents were used, have been found in other biological control programmes. For example, in South Africa the density of the invasive tree *Sesbania punicea* (Cav.) Benth. (Fabaceae) has declined significantly in areas where two or more beetles, namely *Trichapion lativentre* (Bèguin-Billecocq) (Coleoptera: Apionidae), *Rhyssomatus marginatus* Fåhraeus (Coleoptera: Curculionidae) and *Neodiplogrammus quadrivittatus* (Olivier) (Coleoptera: Curculionidae), are established (Hoffmann and Moran, 1998). Alternatively, synergistic effects have not been observed between three seed-feeding bruchid beetles, namely *Algarobius prosopis* (LeConte), *A. bottimeri* Kingsolver and *Neltumius arizonensis* Schaeffer (Coleoptera: Chrysomelidae) used for the biological control of the shrubs of *Prosopis* species (Fabaceae) in South Africa (Impson *et al*., 1999).

To date, no previous studies have looked at the interactions between *O. terebrantis* and *E. catarinensis.* Therefore, the overall aim of this study was to examine the interactive effects of *O. terebrantis*, *N. eichhorniae* and *E. catarinensis* on water hyacinth growth parameters to determine whether more time and effort should be dedicated to *O. terebrantis* as part of an augmentative biological control programme

against water hyacinth in South Africa. In addition, the effect of the interactions between the agents on each agent's performance (population density) is also investigated.

4.2. MATERIALS AND METHODS

4.2.1. Pilot studies

Pilot study 1: Testing whether mites will oviposit on plants damaged by other agents

To determine whether mites would oviposit on plants already damaged by the other water hyacinth biocontrol agents, five weevil damaged plants and five mirid damaged plants were removed from different stock cultures kept at the PPRI and used in the experiment. Five plants with no insect damage served as the control plants. The adult weevils were removed by hand, and the mirids were removed by spraying the plants with water at high pressure. Ramets and dead leaves and stems were removed from the plants before the plants were weighed and their root lengths were measured. At the beginning of the experiment, the percentage of weevil and mirid damage was recorded on leaf 2 and leaf 4 of the weevil damaged and mirid damaged plants, respectively. Leaf 2 was observed since weevils prefer to feed on younger leaves (Hill and Cilliers, 1999), and leaf 4 was observed because mirids prefer older leaves (own observation). Each plant was then placed singly into a plastic tub (70 cm x 40 cm and 35 cm depth) filled with 16 L of water. A wire ring, attached by hooks to the sides of each tub, was used to keep the plants upright inside the tubs (Fig. 4.1). Using a fine camel-hair paint brush, 100 mites were placed onto each plant.

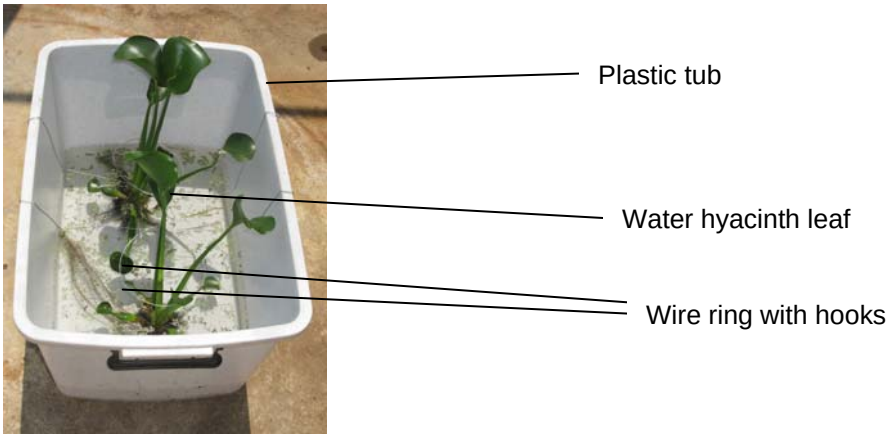

Figure 4.1. Water hyacinth plants in a plastic tub as used in the experiment, kept in position by a wire ring attached to the sides of the tub.

The tubs were placed inside a glasshouse in a randomised block design. Potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) were added to each tub as the nitrogen (2.5 mg L^{-1}) and phosphorous bases (0.4 mg L^{-1}), respectively. These specific concentrations of KNO_3 and KH_2PO_4 were used because they are representative of the high nutrient concentrations that are common in many South African fresh water systems (Holmes, 1996), and they are therefore the concentrations experienced at the majority of the field sites in which water hyacinth occurs. Commercial iron chelate (13% Fe) was also added to each tub at 1.4 g/ 16 L water. The water and nutrients in each tub were replaced weekly. The average day and night temperatures in the glasshouse were 32.7 ºC ± 5.97 ºC and 23.75 ºC ± 2.78 ºC, respectively.

Plants were sampled weekly for 8 weeks for the following plant parameters: number of leaves, number of ramets and the length of the longest petiole. To determine changes in plant biomass the plants (including their ramets) were weighed at the beginning and at the end of the experiment. Since the differently damaged plants i.e. damaged by weevils, or mirids, or not at all, were taken from different stock cultures, it was not possible to use plants of the same size. Hence, the change in the petiole lengths between the first and last week of the experiment was analysed, rather than the actual petiole lengths at the end of the experiment. Damage to leaves by mite herbivory was also noted weekly by recording the percentage of the leaf surface area damaged on the adaxial surface of leaf 5. In addition, the length of the longest gallery on leaf 5 was also recorded to determine the mite development over time.

Pilot study 2: Testing different weevil and mirid densities to determine which densities should be used in the interaction study

Before starting the interaction study where mites, weevils and mirids would be used in combination, it was important to determine what densities of weevils and mirids would cause noticeable damage to water hyacinth, to determine which densities should be used in the interaction study. Coetzee *et al*. (2005) investigated the impact of *E. catarinensis* as a single agent, and found that 15 adult mirids per plant did not cause severe damage, so the starting point of this study was to use more than 15 adult mirids per plant. Caunter and Mohamed (1990) found that 4 weevil pairs per plant, reduced leaf production, while Ajuonu *et al*. (2009) found that 2 pairs of weevils per plant significantly reduced petiole and leaf lengths, as well as the production of

flowers, but this occurred when water hyacinth was grown in competition with water lettuce (*Pistia stratiotes* L.).

Clean (insect, mite and pathogen free) and similar sized plants were taken from stock cultures kept at the PPRI in Pretoria and used in the experiment. Plants were placed singly into plastic tubs (as above) filled with 16 L of water and kept in place by a wire ring (Fig. 4.1). Onto each plant either 2, 4, 6 or 8 adult weevils, or 20, 30 or 40 adult mirids were placed. No insects were added to the control plants. Each of these treatments was replicated five times. A net (mesh size 0.5 mm x 0.5 mm) was secured over the top of each tub to prevent the agents from escaping and to create the same experimental light conditions.

The tubs were placed inside a glasshouse in a randomised block design and nutrients were added as described above. The water and nutrients in each tub were replaced weekly. The average day and night temperatures in the glasshouse were 33.29 ºC ± 6.97 ºC and 21.15 ºC ± 1.44 ºC, respectively.

The same plant parameters as in the previous pilot study were measured weekly for 8 weeks. Damage caused by weevil and mirid herbivory was measured weekly on leaves 2 and 4 respectively, by recording the percentage of the surface area damaged on the abaxial surface of the leaves.

Statistical analyses

For both of the pilot studies, one-way analysis of variance (ANOVA) was conducted at the end of the sample period to test for differences in plant growth parameters (number of leaves, number of ramets, length of the longest petiole and change in wet biomass) and in the leaf surface area damaged by herbivory, between the treatments. The ANOVA was run at a critical *p* level of 0.05 and if the F-probability from the ANOVA was significant at 5% then *post hoc* comparisons were made using Fisher's protected least significant difference (LSD) test (Snedecor and Cochran, 1980). Where data were not normally distributed and Levene's tests showed no homogeneity of variances, the Kruskal-Wallis ANOVA was used to perform the analyses.

4.2.2. Interaction study

Testing for interactive effects of herbivory by mites, weevils and mirids on water hyacinth growth and on each other's performances

Experimental set-up

Healthy, agent-free and undamaged water hyacinth plants of similar size were selected from stock cultures of the PPRI, Pretoria, South Africa, and used in the experiment. The trial was conducted during summer inside a glasshouse at the PPRI. The average day and night temperatures in the glasshouse were 30.72 ºC ± 6.74 ºC and 20.65 ºC ± 2.66 ºC respectively.

Some plants had leaves removed so that all plants had between 4 to 6 leaves at the beginning of the experiments. All ramets and dead matter were removed from the plants, before they were weighed and placed in pairs into plastic tubs. The plants were held in place by wire rings (as above). The tubs were filled with 16L of water and nutrients were added as descried above. The water and nutrients in each tub were replaced weekly.

The plants were inoculated with three agents, namely the mite *O. terebrantis*, the weevil *N. eichhorniae*, and the mirid *E. catarinensis*, in the following combinations: 1) only mites, 2) only weevils, 3) only mirids, 4) mites and weevils, 5) mites and mirids, and 6) weevils and mirids. No agents were added to the control plants. Each treatment was replicated seven times. Prior to inoculation, the weevils and mirids were sexed under a microscope to ensure a 1:1 sex ratio. Male and female *O. terebrantis* are morphologically indistinguishable (Perkins, 1973) and were therefore not sexed. In natural field populations, mites occur in approximately equal proportions of adult females and males (Walter, 2009), so it was presumed that roughly similar numbers of male and female mites were used. Mites were added at 150 mites/plant, weevils were added at 2 adult pairs/plant and mirids were added at 15 adult pairs/plant. In the single-agent treatments, the number of individuals added was doubled e.g. in the "only mites" treatment 300 mites were placed onto a plant. A net (mesh size 0.5 mm x 0.5 mm) was secured over the top of each tub to prevent the agents from escaping, and to create the same experimental light conditions. The tubs were arranged in a randomized block design.

The experiment was conducted over an 11 week period and plant growth parameters (number of leaves, number of ramets, number of flowers, length of the longest petiole and leaf turn-over) were measured once every 2 weeks. Leaf turn-over was obtained by tagging the youngest leaf at the beginning of the experiment, and noting the position of the leaf over time. A new youngest leaf was tagged if a plant had a complete leaf turn-over before the end of the experiment i.e. the tagged leaf became the oldest leaf and eventually died. The change in plant wet biomass was obtained by weighing the plants before and after the experiment, where the end wet biomass included ramets.

The damage caused by the agents was measured once every 2 weeks by recording the percentage of the abaxial leaf surface area damaged on leaves 2, 4 and 5, by each agent separately. The growth parameters and leaf damage were averaged for the two plants in each tub to obtain a mean response per tub.

Since damage to leaves by agent herbivory reflects the presence of the agent, and increases as the number of individuals increases (Center and Jubinsky, 1996; Briese *et al*., 2004; Ding *et al*., 2006; Bebawi *et al*., 2007), the percentage of leaf surface area damaged by each agent separately was used to indicate the presence and abundance of each of the agent species, in the final week of the experiment. The abundance of an agent, as determined from the leaf surface area damaged, is also used as a measure of the agent's performance (i.e. is the agent reproducing?) for the purposes of this study. To determine under which conditions (either alone or in combination with another agent) the agents performed their best i.e. under which conditions did most agents survive and/or new agents were produced, the damage caused by each agent separately was compared between treatments where the agent was used singly, or in combination with one of the other agents. Additionally, because mirids are easily seen without handling or destroying the plants, the number of mirids (adults and nymphs) was counted at the end of the experiment, for each tub where mirids were present, and compared between treatments. Mites and weevils were not counted directly since the physical disturbance of handling the plants dislodges the agents and thus hinders counting. Wright and Center (1984) found a close relationship between the number of adult weevils on a plant and the number of feeding scars on a leaf and therefore the damage to the leaf surface area caused by weevil feeding was used as a reflection of their abundance. Similarly, the percentage of the leaf surface area damaged by mite herbivory was taken to be a reflection of mite abundance.

Statistical analyses

One-way ANOVA was used to test for differences in plant growth parameters (number of leaves, number of ramets, number of flowers, length of the longest petiole and leaf turn-over rate) between the treatments, at the end of the sample period (at week 11). At the start of the experiment all the growth parameters, except for the number of flowers, were similar between treatments (no significant difference in growth parameters between the treatments, $p > 0.05$), and therefore the end point values of the parameters, measured in the final week of the experiment, were analysed directly. The numbers of flowers were highly variable at the beginning and throughout the experiment, between and within the treatments. Therefore, the flowers that were produced throughout the duration of the experiment were summed for each treatment, and the differences in the total number of flowers produced between the treatments, were analysed at the end of the experiment.

To test whether there were differences in the leaf surface area damaged between the different leaves measured (leaves 2, 4 and 5), the damage of the three agents was initially combined per leaf, and then a one-way ANOVA was used to test for differences in damage between the leaves, at the end of the sample period. Thereafter, the different leaves (2, 4, and 5) were analysed separately, using a one-way ANOVA, to test for differences in the leaf surface area damaged by agent herbivory between the treatments. To show the total damage to leaf surface area caused by herbivory, the damage caused by two agents in the case of multiple-agent treatments was combined. In addition, the damage on leaf 5 was separated out into damage caused by the individual agents, for the multiple-agent treatments only, to show which agent was responsible for the majority of the damage, at the end of the sample period. One-way ANOVA was used to test for differences between the damage caused by the individual agents.

Regression analysis was performed on the actual number of mirids counted per plant and the percentage of the surface area damaged by mirids. The results were used to test the strength of the relationship between the two variables, to ensure that the data of the percentage surface area damaged could in fact be used as a measure of insect performance. Thereafter, to determine whether agents performed better when on their own or in combination with another agent, one-way ANOVA was used to test for differences in the percentage leaf surface area damaged on leaf 5 by each agent separately, in the different treatments, at the end of the experiment. In addition, the

actual number of mirids (adults and nymphs) counted per plant where mirids were present, was compared between treatments using one-way ANOVA. The mirid numbers needed to be log transformed for the analysis to obtain a normal distribution, but the actual numbers counted are presented in the figure. In the single-agent treatments, the percentage surface area damaged, or the actual mirid numbers, was divided by 2 since in those treatments double the inoculum of agents was used at the start of the experiment.

Fisher's protected least significant difference (LSD) test was used to separate the treatment means if the differences were significant at the 5% level (Snedecor and Cochran, 1980). Where data were not normally distributed and Levene's tests showed no homogeneity of variances, the Kruskal-Wallis ANOVA was used to perform the analyses.

Data were analysed using the statistical programme STATISTICA Version 7.0 (© StatSoft, Inc., USA), in both of the pilot studies and the interaction study.

4.3. RESULTS

4.3.1. Pilot studies

Pilot study 1: Testing whether mites will oviposit on plants damaged by other agents

Plant growth parameters

At the end of the 8-week experiment, significant differences ($p < 0.05$) between the treatments were observed in the number of leaves, the number of ramets and the change in wet biomass.

The number of leaves produced by plants that were initially damaged by mirids, and then inoculated with mites, was significantly less than the leaves produced by control plants (which were not damaged initially), and plants that were initially damaged by weevils and then inoculated with mites ($F_{2, 15} = 7.4$, $p = 0.024$; Fig. 4.2). Similarly, plants that were initially damaged by mirids and weevils produced significantly fewer ramets than the control plants ($F_{2, 12} = 19.159$, $p = 0.005$; Fig. 4.3).

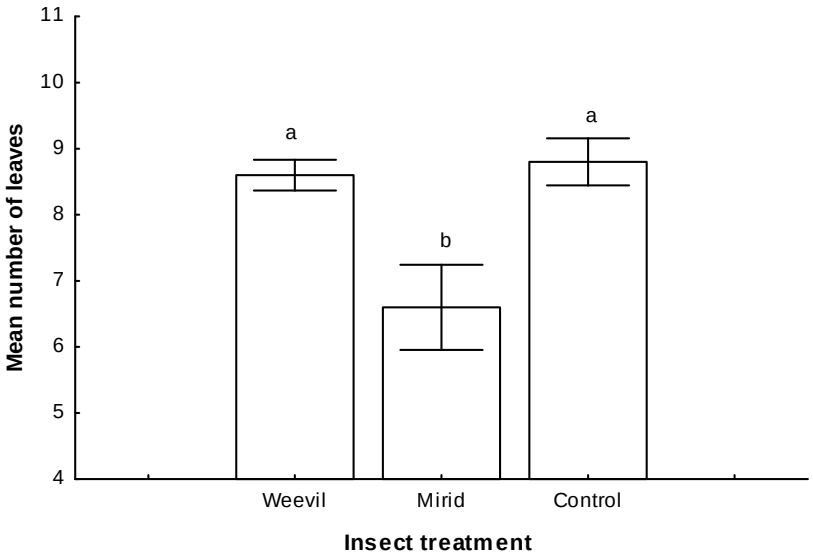

Figure 4.2. Differences in the number of leaves produced by water hyacinth plants at the end of an 8-week experiment. Plants were initially damaged by weevils or mirids, or were not damaged (control), and thereafter inoculated with 100 mites/plant. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

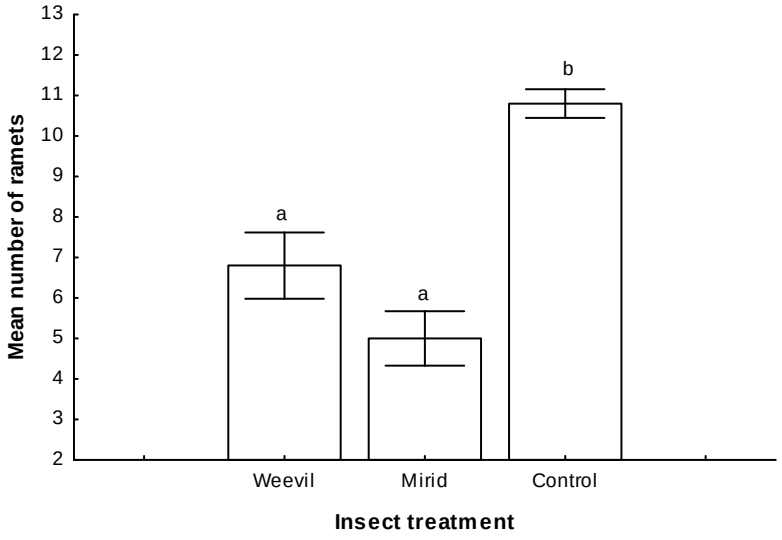

Figure 4.3. Differences in the number of ramets produced by water hyacinth plants at the end of an 8-week experiment. Plants were initially damaged by weevils or mirids, or were not damaged (control) and thereafter inoculated with 100 mites/plant. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

The control plants had the greatest increase in wet biomass after 8 weeks, and this was significantly greater than the change in wet biomass of the mirid-damaged and weevils-damaged plants ($F_{2, 12}$ = 8.603, p = 0.005). The control plants weighed the most at the end of the sample period, while the mirid-damaged plants weighed the least (Fig. 4.4).

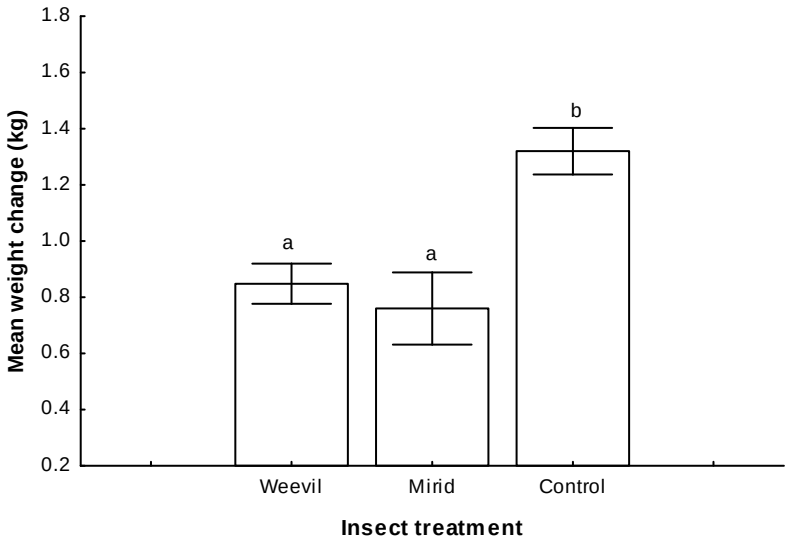

Figure 4.4. Differences in the change in wet biomass of water hyacinth plants at the end of an 8-week experiment. Plants were initially damaged by weevils or mirids, or not damaged (control) and thereafter inoculated with 100 mites/plant. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

There were no significant differences between the treatments in the change in length of the longest petioles after 8 weeks ($F_{2, 12}$ = 2.046, p = 0.172). The petiole lengths decreased significantly over time in the mirid-damaged plants (p = 0.001), but not in the weevil-damaged plants (p = 0.904) and the control plants (p = 0.216) (Fig. 4.5).

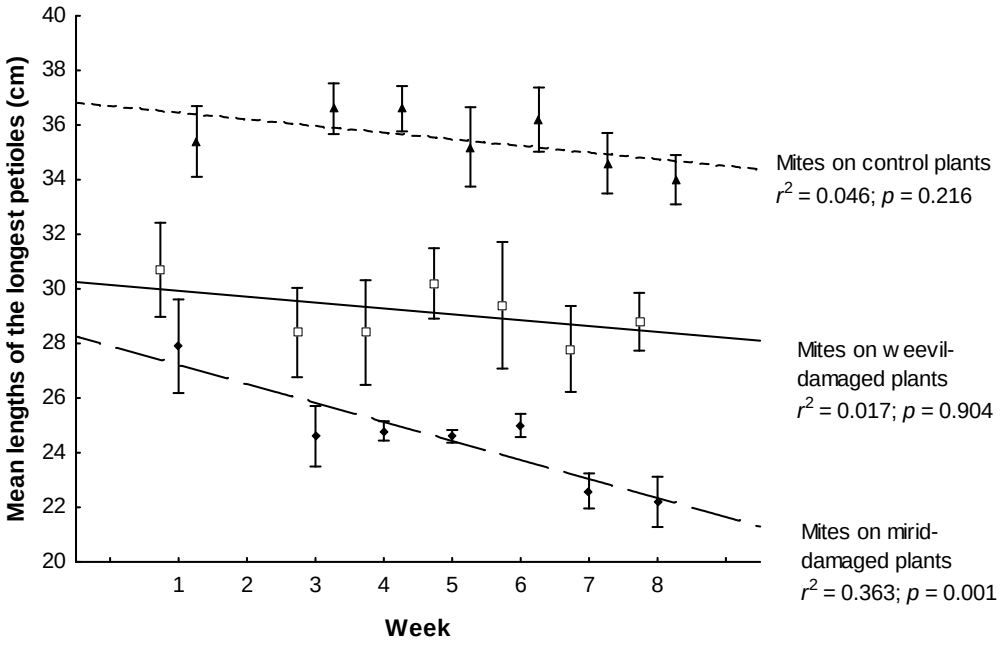

Figure 4.5. Change in the lengths of the longest water hyacinth petioles over an 8-week experiment when 100 mites/plant were placed onto plants that were initially undamaged (control), damaged by mirids or damaged by weevils. Error bars represent the standard error of the means. n = 5 for all treatments.

Damage to the leaf surface area by mite herbivory

After 8 weeks, plants that were initially damaged by mirids had significantly less surface area damaged (on leaf 5) by mite herbivory than the control plants and plants that were initially damaged by weevils ($H_{2,\ 15}$ = 8.857, p = 0.012). Over time, the leaves of the mirid-damaged plants never had more than 20% mite damage recorded, while the leaves of the weevil-damaged plants and the control plants had more than 30% and 40% mite damage recorded, respectively (Fig. 4.6).

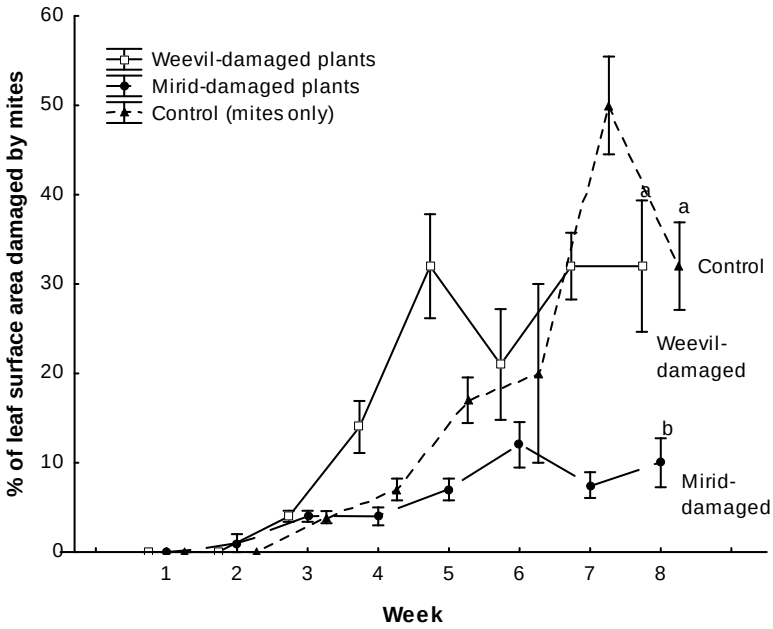

Figure 4.6. The percentage of the leaf surface area damaged by mite herbivory on leaves 5 of water hyacinth plants, during an 8-week experiment. Plants were initially damaged by weevils or mirids, or were not damaged (control) and thereafter inoculated with 100 mites/ plant. Error bars represent the standard error of the means. Means (at week 8) compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

Lengths of galleries to show mite development

During the 8-week experiment, mite galleries increased in length in all treatments, showing that mites developed on control (damage-free) plants as well as the weevil-damaged and the mirid-damaged plants. In all three treatments, adults emerged after 21 days, when the galleries were at least 2 mm long (Fig. 4.7). Initially, mites on the control plants developed faster, but by the end of the experiment there were no significant differences in the lengths of galleries ($H_{2, 15} = 2.345$, $p = 308$).

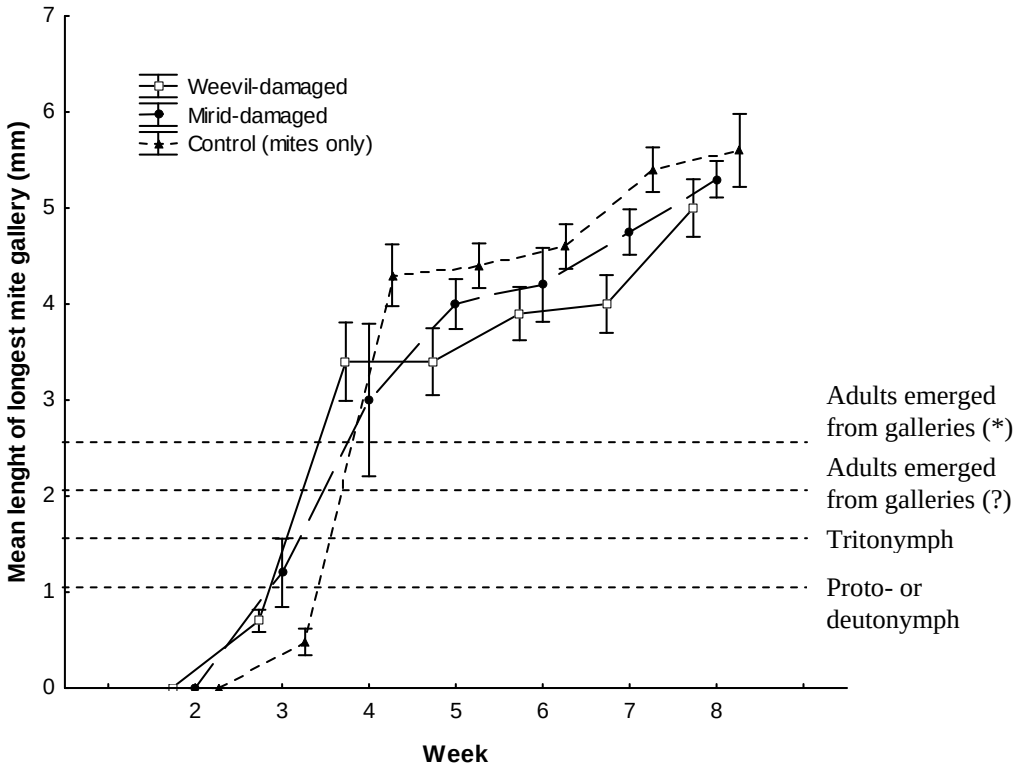

Figure 4.7. Mite development, as shown by the increasing lengths of galleries (Delfosse, 1978a) during an 8-week experiment, on plants that were initially damaged by weevils or mirids, or not damaged, and thereafter inoculated with 100 mites/plant. Error bars represent the standard error of the means. n = 5 for each treatment. (?) indicates adult emergence as observed by Delfosses (1978a) and (*) indicates adult emergence as observed in the present study.

Pilot study 2: Testing different weevil and mirid densities to determine which densities should be used in the interaction study

Plant growth parameters

At the end of the 8-week experiment, significant differences ($p < 0.05$) between the treatments (plants inoculated with either 2, 4, 6 or 8 adult weevils, or 20, 30 or 40 adult mirids, or control plants with no agents) were observed only for the change in the wet biomass of plants ($F_{7, 32} = 5.525$, $p = 0.001$). Plants that had 2 or 4 weevils on them accumulated the least amount of biomass, while plants that had 8 weevils accumulated the most biomass (Fig. 4.8). Of the mirid densities tested, plants that had 30 mirid adults on them had a significantly smaller biomass increase compared to plants that had 40 mirid adults, but this was not significantly different from the biomass change of plants that had 20 mirid adults on them (Fig. 4.8).

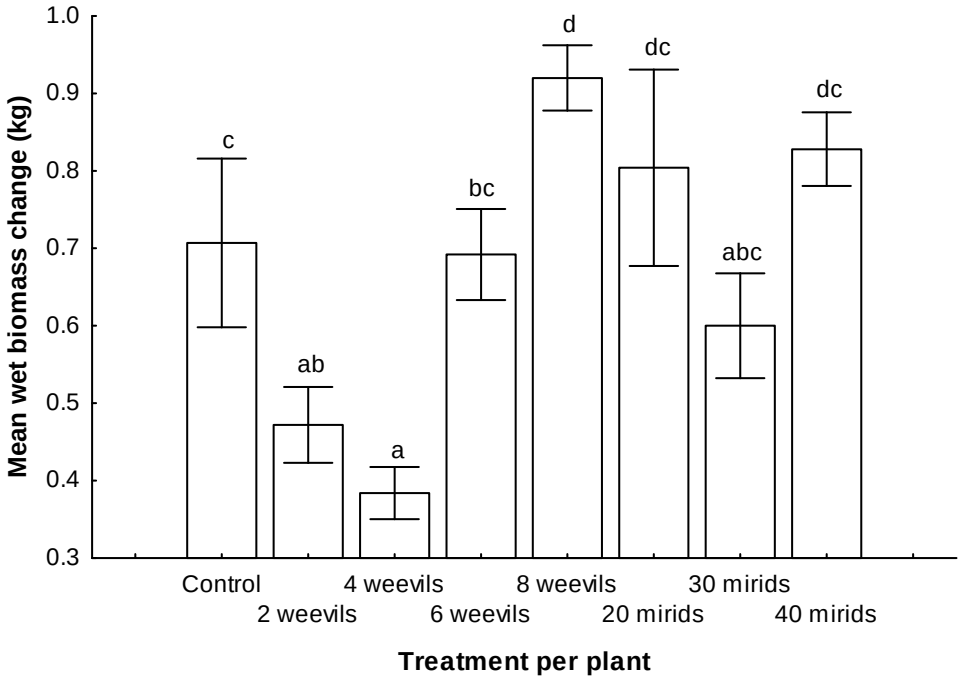

Figure 4.8. The change in wet biomass of water hyacinth plants after an 8-week experiment when exposed to herbivory by different densities of weevils and mirids. Control plants were not inoculated with agents. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

Kruskal-Wallis ANOVA revealed no significant differences ($H_{7, 40} = 13.685$, $p = 0.057$) in the lengths of the longest petioles when all the treatments were compared simultaneously (control, weevil treatments and mirid treatments) (Fig. 4.9). When only the weevil treatments were compared, petiole lengths differed significantly, with the shortest petiole length being recorded for weevil densities of 3 pairs per plant ($H_{4, 25} = 10.535$, $p = 0.032$). Similarly, there were significant differences in the number of ramets produced when only the weevils treatments were compared ($H_{4, 25} = 10.019$, $p = 0.040$) but there were no significant differences in ramet numbers when only the mirid treatments were compared to each other ($H_{3, 20} = 11.255$, $p = 0.104$), and there were also no significant differences in ramet numbers between the treatments when compared simultaneously ($H_{7, 40} = 13.431$, $p = 0.062$; Fig. 4.10).

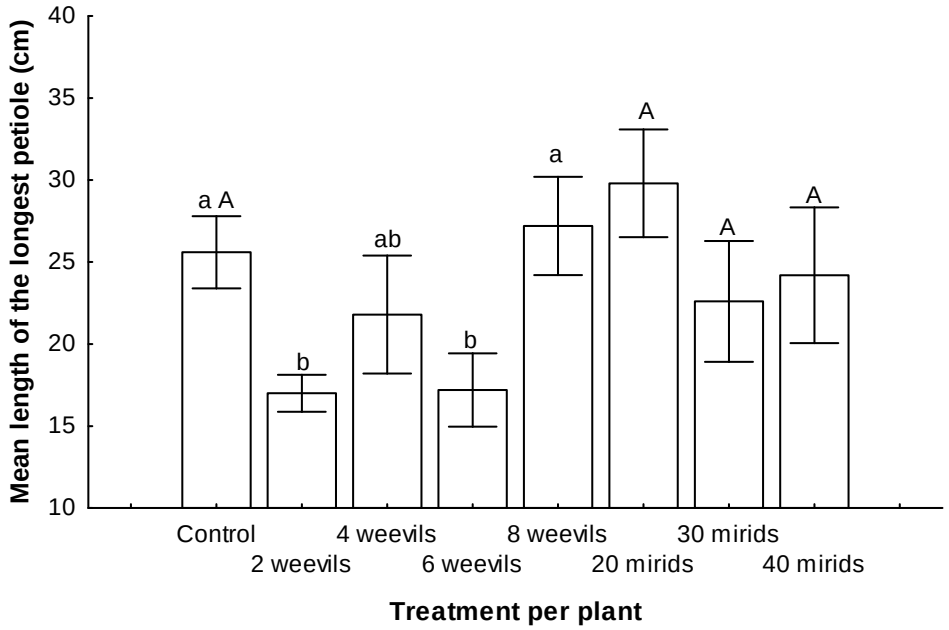

Figure 4.9. Differences in the lengths of the longest petioles of water hyacinth plants at the end of an 8-week experiment, when exposed to herbivory by different densities of weevils and mirids. For Fisher's LSD tests the weevil treatments (small letters) were compared separately to the mirids treatments (capital letters). Differences between means were compared for by one-way ANOVA. Error bars represent the standard error of the means. Error bars followed by the same letters in the same case (small or capital) are not significantly different ($p > 0.05$). n = 5 for each treatment.

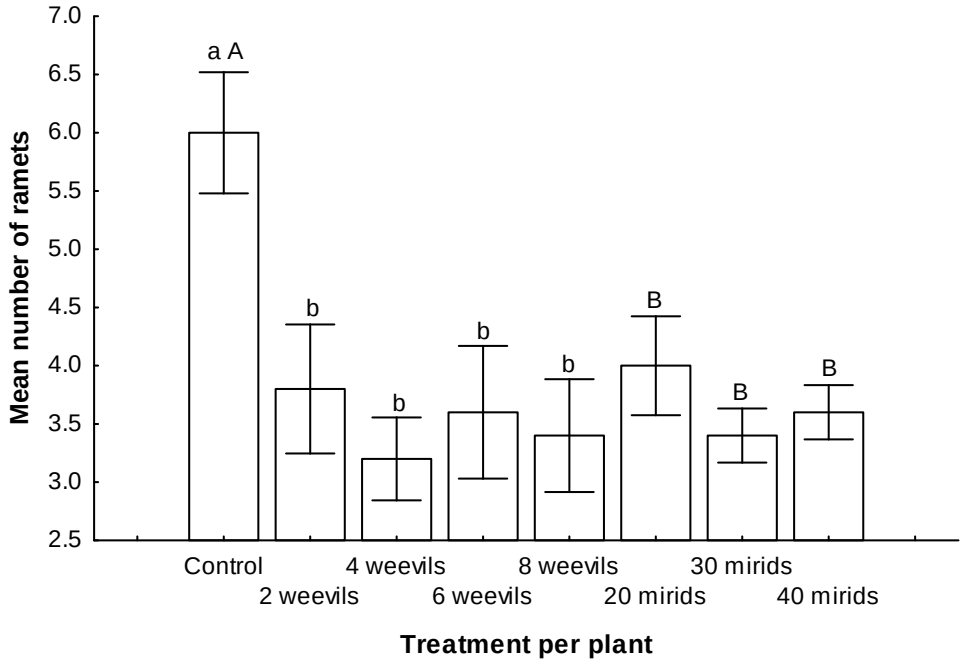

Figure 4.10. Differences in the number of ramets produced by water hyacinth plants at the end of an 8-week experiment, when exposed to herbivory by different densities of weevils and mirids. For Fisher's LSD tests weevil treatments (small letters) were compared separately to mirids treatments (capital letters). Differences between means were compared for by one-way ANOVAs. Error bars represent the standard error of the means. Error bars followed by the same letters in the same case (small or capital) are not significantly different ($p > 0.05$). n = 5 for each treatment.

Damage to the leaf surface area

After 8 weeks of the plants being exposed to different densities of weevils and mirids (plants inoculated with either 2, 4, 6 or 8 adult weevils, or 20, 30 or 40 adult mirids), the percentage of the surface area damaged on leaf 2 by weevil herbivory was significantly different between the weevil treatments ($H_{3, 20} = 9.325$, $p = 0.025$). Oddly, leaf 2 of plants that had 1 pair of weevils on them were significantly more damaged than those of plants that had 3 weevil pairs on them (Fig. 4.11). There were no significant differences in the surface area damaged on leaf 4 by mirid herbivory between the mirid treatments ($H_{2, 15} = 0.249$, $p = 0.883$).

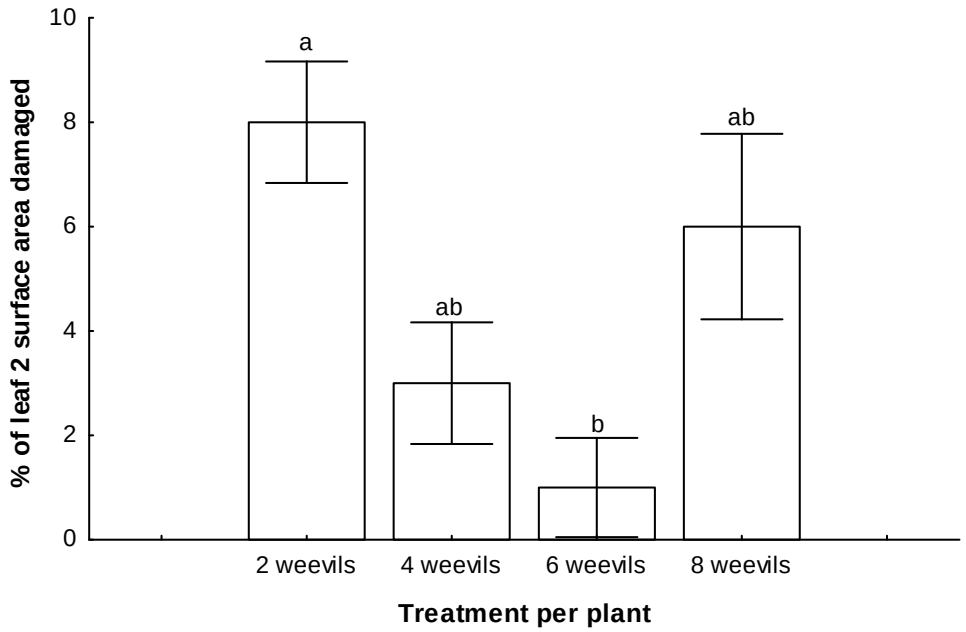

Figure 4.11. The percentage of the leaf surface area damaged by weevil herbivory on leaf 2 of water hyacinth plants when exposed to different densities of weevils for 8 weeks. Error bars represent the standard error of means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 5 for each treatment.

4.3.2. Interaction study

Effects of herbivory by various combinations of weevils, mirids and mites on plant growth parameters

Significant differences occurred between most treatments (single-agent treatments i.e. mites, weevils or mirids alone, and multiple-agent treatments i.e. combinations of mites+weevils, mites+mirids or weevils+mirids) for all of the plant parameters measured except the number of leaves (Table 4.1).

Table 4.1. *F* statistics and *p* values of one-way ANOVAs run for water hyacinth plant growth parameters, highlighting significant differences between herbivory treatments, at the end of an 11-week experiment where plants were exposed to various combinations (herbivory treatments) of three agents (*Orthogalumna terebrantis*, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*). *df* = 41 and n = 7 for all treatments.

Plant growth parameter	*F* value	*p* value
Number of leaves	2.025	0.084
Number of ramets	3.347	**0.001**
Length of longest petiole	4.573	**0.001**
Number of flowers	2.329	**0.031**
Change in wet biomass	2.412	**0.043**

Significant differences are highlighted in bold.

The most noticeable significant differences between the treatments were observed in the number of ramets produced ($F_{6, 41}$ = 3.347, p < 0.001), and the lengths of the longest petioles ($F_{6, 41}$ = 4.573, p = 0.001), at the end of the 11-week experiment. When compared to the control, significantly fewer ramets were produced by plants with mirid-feeding alone, followed closely by plants fed on by weevils only (Fig. 4.12). Similarly, the lengths of the longest petioles were significantly shorter on plants that had been fed on by only weevils, and a combination of weevils and mites, compared to the other treatments, while petioles of plants that had been fed on by only mites and a combination of mites and mirids were slightly longer than the control plants' petioles (Fig. 4.13).

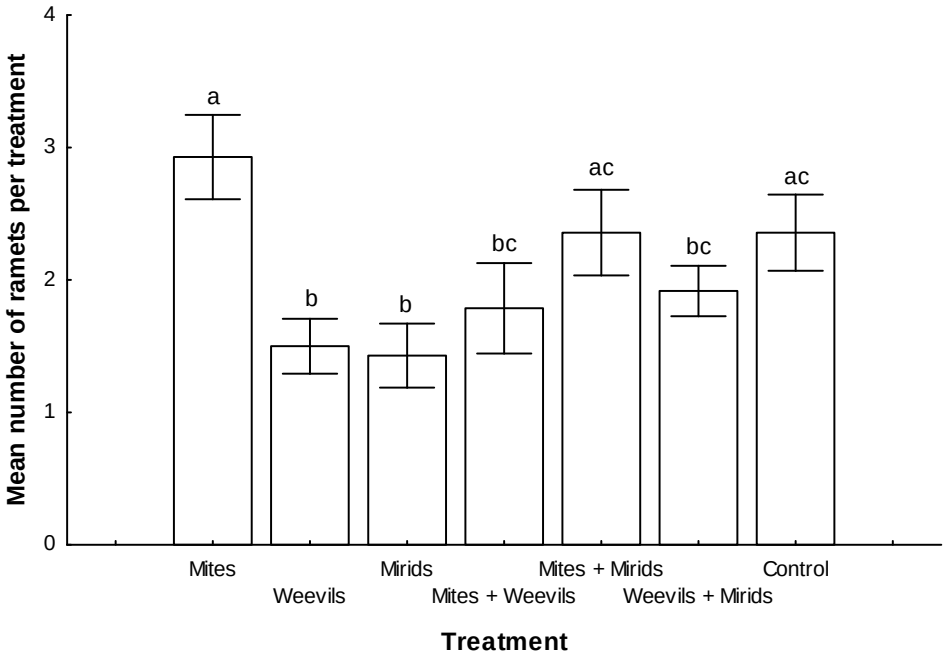

Figure 4.12. The effect of herbivory by various combinations of three agents on the total number of ramets produced by water hyacinth plants during an 11-week experiment. Numbers of individuals used in single-agent treatments: 300 mites/plant; 4 adult weevil pairs/plant; 30 adult mirid pairs/plant. For the multiple-agent treatments the numbers of individuals were halved. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

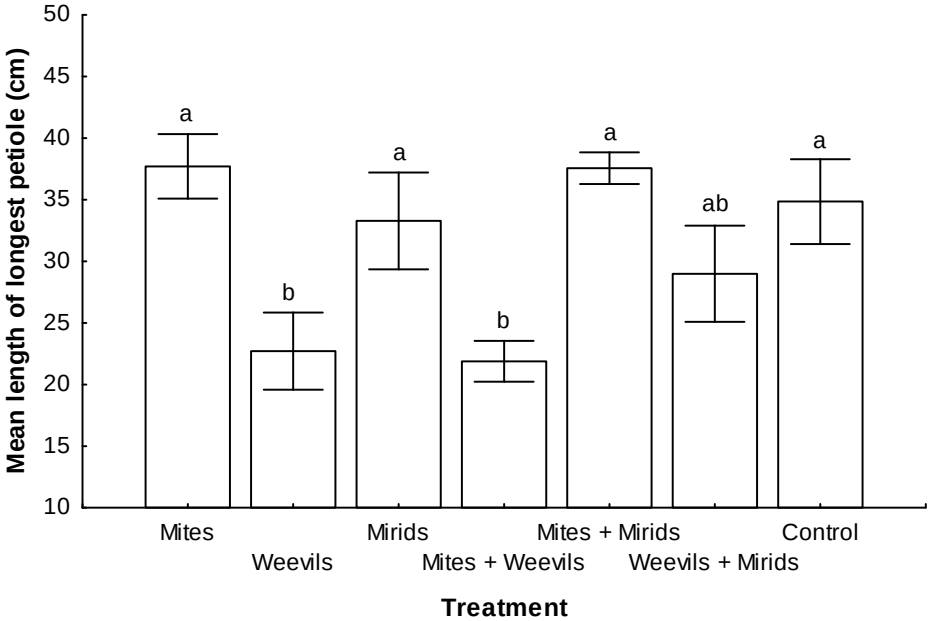

Figure 4.13. The effect of herbivory by various combinations of three agents on the lengths of the longest petioles of water hyacinth plants at the end an 11-week experiment. Numbers of individuals used in single-agent treatments: 300 mites/plant; 4 adult weevil pairs/plant; 30 adult mirid pairs/plant. For the multiple-agent treatments the numbers of individuals were halved. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

A summary of the plant growth parameters and the leaf surface area damaged by herbivory in the various treatments is given table 4.2.

Table 4.2. Means (± S.D.) of water hyacinth plant growth parameters and the mean (± S.D.) percentage of leaf surface area damaged by herbivory, at the end of an 11-week experiment, when plants were exposed to herbivory by various combinations (treatments) of three agents (*Orthogalumna terebrantis*, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*). *df* = 41 and n = 7 for all treatments. Numbers of individuals used in single-agent treatments: 300 mites/plant; 4 adult weevil pairs/plant; 30 adult mirid pairs/plant. For the multiple-agent treatments the numbers of individuals were halved.

Treatment	Total number of leaves produced in 11 weeks	Total number of ramets produced in 11 weeks	Total number of flowers produced in 11 weeks	Maximum petiole length (cm)	Change in wet biomass (kg)	% of leaf surface area damaged
Mites only	9.29 ± 1.55	2.93 ± 0.89	2.42 ± 2.72	37.71 ± 7.28	0.82 ± 0.19	30.79 ± 6.59
Weevils only	8.07 ± 1.67	1.5 ± 0.58	1.25 ± 1.51	22.71 ± 8.72	0.55 ± 0.12	45.83 ± 19.08
Mirids only	7.29 ± 0.91	1.43 ± 0.67	3.17 ± 2.82	33.29 ± 10.94	0.60 ± 0.16	62.14 ± 22.70
Mites + Weevils	7.86 ± 1.25	1.79 ± 0.95	1.75 ± 2.09	21.89 ± 4.61	0.60 ± 0.27	30.29 ± 16 76
Mites + Mirids	8.36 ± 1.57	2.36 ± 0.90	2.67 ± 2.36	37.57 ± 3.59	0.76 ± 0.21	78.29 ± 25.41
Weevils + Mirids	8.75 ± 1.41	1.92 ± 0.49	2.0 ± 2.30	29.0 ± 10.05	0.52 ± 0.17	54. 17 ± 27.46
Control	7.14 ± 1.41	2.36 ± 0.81	2.0 ± 1.76	34.86 ± 9.58	0.81 ± 0.28	n/a

Leaf turn-over rate

There were no significant differences in the number of leaves produced per week between the treatments ($F_{6, 28}$ = 0.196, p = 0.975), however, the control treatment had the lowest leaf turn-over rate while the mites only treatment had the highest leaf turn-over rate (Table 4.2). The actual rates of leaves produced per week in the different treatments are given in table 4.3.

Table 4.3. Leaf turn-over rates (new leaves produced/week) of water hyacinth plants exposed to herbivory by various combinations of three agents (*Orthogalumna terebrantis*, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*) for 11 weeks. *df* = 41 and n = 7 for all treatments. Numbers of individuals used in single-agent treatments: 300 mites/plant; 4 adult weevil pairs/plant; 30 adult mirid pairs/plant. For the multiple-agent treatments the numbers of individuals were halved.

Treatment	Mean leaf turn-over / week (±SE)
Mites only	2.02 ± 0.44
Weevils only	1.73 ± 0.46
Mirids only	1.64 ± 0.39
Mites + Weevils	1.70 ± 0.39
Mites + Mirids	1.93 ± 0.56
Weevils + Mirids	1.93 ± 0.44
Control	1.44 ± 0.48

Damage to the leaf surface area by agent herbivory

The percentage of the surface area damaged on the different leaves of a plant was significantly different between all of the three leaves measured (leaves 2, 4 and 5) ($F_{2, 113}$ = 17.415, $p < 0.001$). For leaf 2, there were no significant differences in the leaf surface area damaged by agent herbivory between any of the treatments ($F_{5, 32}$ = 1.393, $p = 0.253$). However, there were significant differences in the leaf surface area damaged by agent herbivory between some of the treatments on leaves 4 and 5, respectively (leaf 4 $F_{5, 33}$ = 3.122, $p = 0.020$; Leaf 5 $F_{5, 33}$ = 5.617, $p < 0.001$). On leaf 4, the mites+weevils treatment caused the least amount of damage to the leaf surface area, and this was significantly less ($p < 0.05$) than the damage caused by the mirids only treatment and the mites+mirids treatment (Fig. 4.14). Similarly on leaf 5, the mites+weevils treatment caused the least amount of damage to the leaf surface area, and this was significantly less ($p < 0.05$) than the damage caused by the mites only treatment, the mirids only treatment and the mites+mirids treatment (Fig. 4.14). The mites+mirids treatment caused the most amount of damage to the leaf surface area on both leaf 4 and leaf 5 (Figs. 4.14 and 4.15).

When the damage to the leaf surface area on leaf 5 was separated into damage caused by the individual agents, it was found that mirids were responsible for the majority of the damage in both the weevils+mirids and the mites+mirids treatments (mirids were responsible for 66 % and 83% of total damage in the weevils+mirids and mites+mirids treatments, respectively). The difference in damage caused by mirids in the mites+mirids treatment was significantly greater ($p < 0.05$) than the damage caused by the mites (Fig. 4.15).

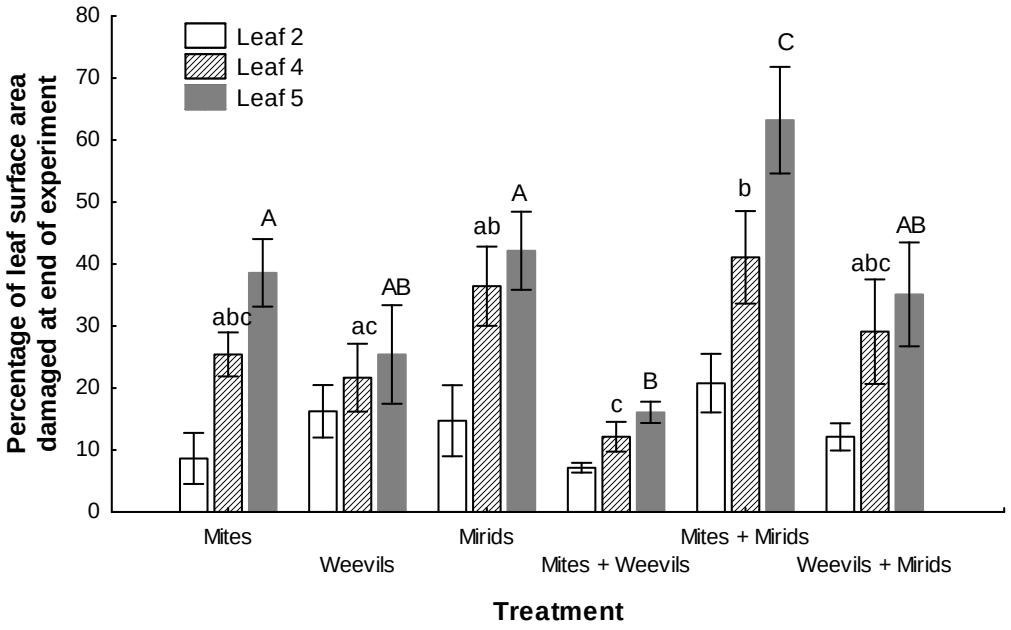

Figure 4.14. Water hyacinth leaf surface area damaged on leaves 2, 4 and 5, at the end of an 11-weeks experiment when plants were exposed to herbivory by various combinations of three agents. Numbers of individuals used in single-agent treatments: 300 mites/plant; 4 adult weevil pairs/plant; 30 adult mirid pairs/plant. For the multiple-agent treatments the numbers of individuals were halved. Differences between treatments were compared for leaf 2, leaf 4 and leaf 5 individually, using one-way ANOVAs. Error bars followed by the same lower case letters for leaf 4, and the same upper case letters for leaf 5, are not significantly different (Fisher's LSD test, $p > 0.05$). Error bars represent the standard error of means. There was no significant difference in damage found on leaf 2. n = 7 for all treatments.

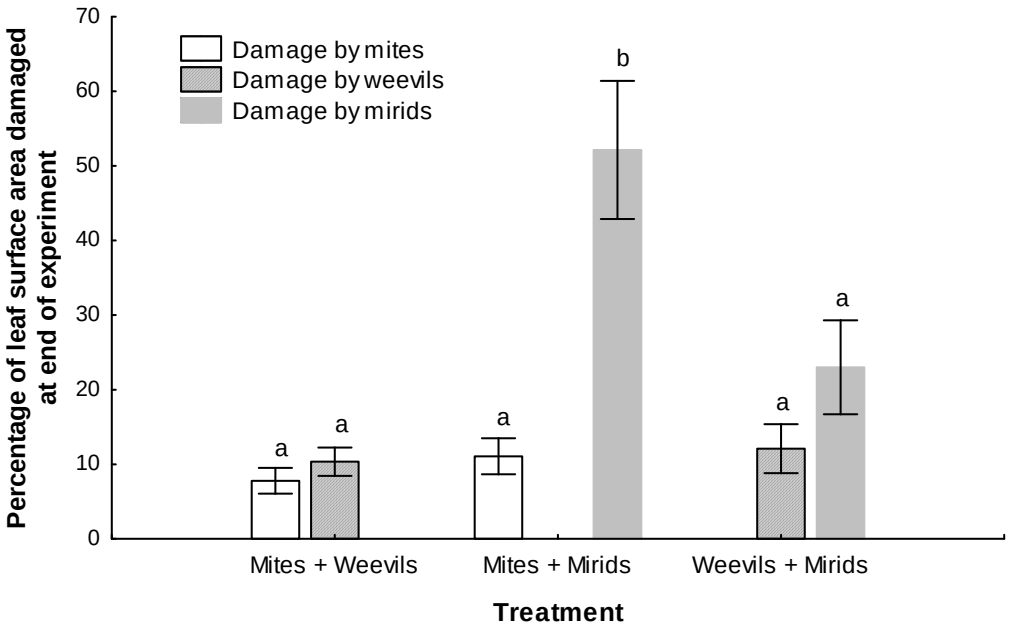

Figure 4.15. Leaf surface area damaged on leaf 5 of water hyacinth plants exposed to herbivory by different combinations of three agents, at the end of an 11-week experiment. Numbers of individuals used per treatment per plant: 150 mite, 2 adult weevil pairs and 15 adult mirid pairs. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

Effect of interactions on agent performance

There was a modest correlation between the actual number of mirids counted per plant and the percentage of the leaf surface area damaged by mirid herbivory (r^2 = 0.573, p = 0.011). Therefore, the percentage of the leaf area damaged by the agents could be taken to represent the numbers of the agents, and thus their performance.

In the mites only treatment the percentage of surface area damaged by mite herbivory on leaf 5 was significantly greater than in the mites+weevils treatment and the mites+mirids treatment ($F_{2, 18}$ = 8.552, p = 0.002). Most of the mite damage, indicating the greatest number of mites, was found in the mites only treatment, as can be seen by the greater damage to the leaf surface observed in that treatment (Fig. 4.16).

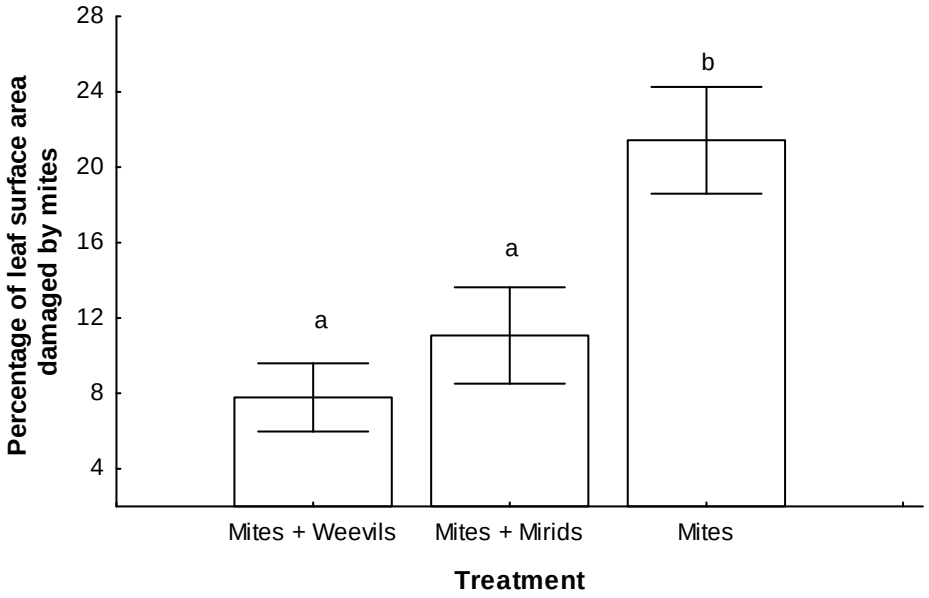

Figure 4.16. Leaf surface area damaged by mite herbivory on leaf 5 of water hyacinth plants at the end of an 11-week experiment when plants were exposed to either mites only, or a combination of mites and weevils, or mites and mirids. Numbers of individuals used per treatment per plant in the combination treatments: 150 mites, 2 adult weevil pairs and 15 adult mirid pairs. 300 mites/plant were used in the mites only treatment. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

The percentage of surface area damaged on leaf 5 by weevil herbivory in the treatments that contained weevils was greatest in the weevils only treatment, but this was not significant compared to the weevils+mites treatment or the weevils+mirids

treatment ($F_{2, 15}$ = 0.155, p = 0.858). The weevil damage, indicating the number of weevils, was similar in all three weevil treatments (Fig. 4.17).

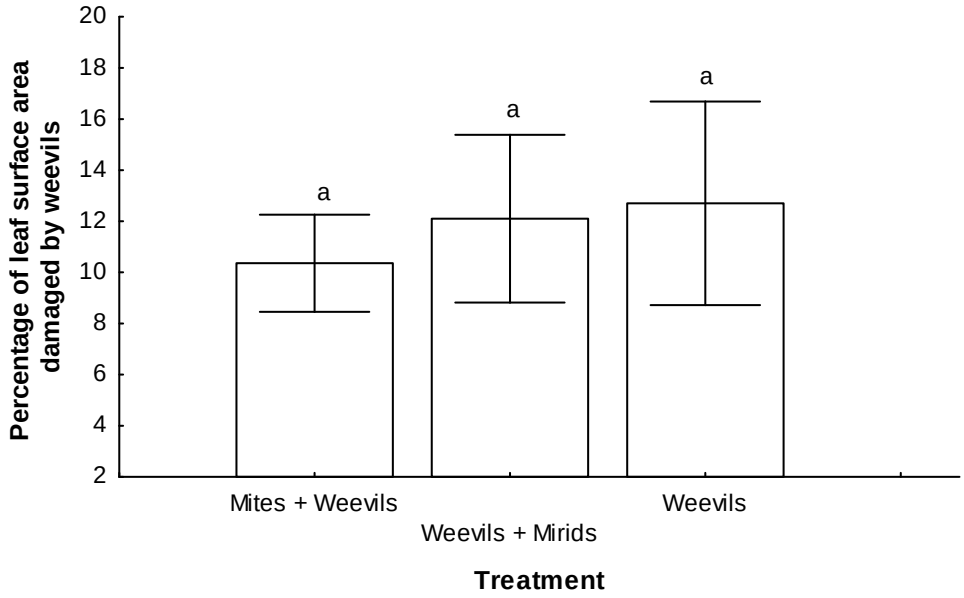

Figure 4.17. Leaf surface area damaged by weevil herbivory on leaf 5 of water hyacinth plants at the end of an 11-week experiment when plants were exposed to either weevils only, or a combination of mites and weevils, or weevils and mirids. Numbers of individuals used per treatment per plant in the combinations treatments: 150 mites, 2 adult weevil pairs and 15 adult mirid pairs. 4 adult weevil pairs/plant were used in the weevils only treatment. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

The percentage of surface area damaged on leaf 5 by mirid herbivory was significantly greater in the mirids+mites treatment compared to the mirids only treatment and the mirids+weevils treatment ($F_{2, 16}$ = 6.092, p = 0.011) (Fig. 4.18). This is comparable to the actual number of mirids counted per plant, where the most mirids per plant were counted in the mirids+mites treatment (Fig. 4.19). However, there was no significant difference in the number of mirids counted between the treatments ($H_{2, 16}$ = 1.428, p = 0.49) (Fig. 4.19).

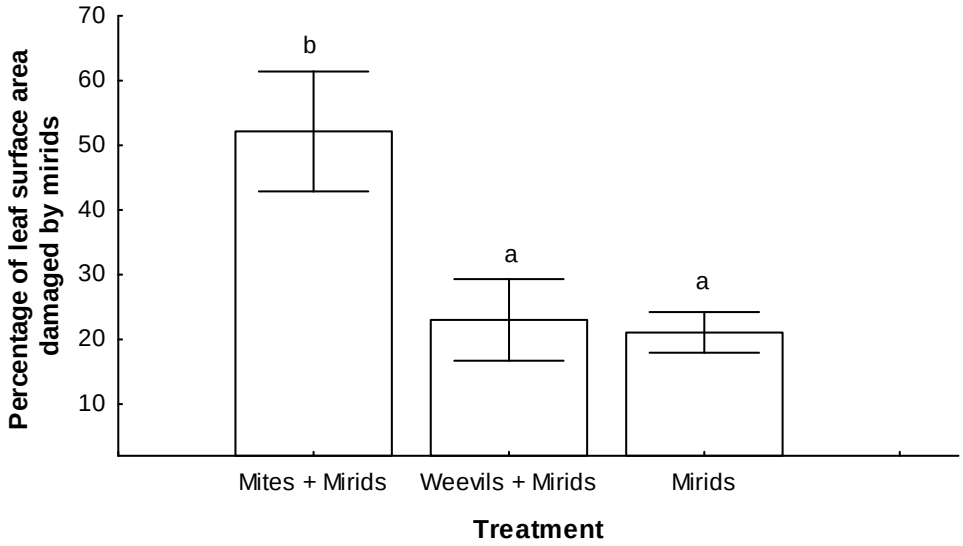

Figure 4.18. Leaf surface area damaged by mirid herbivory on leaf 5 of water hyacinth plants at the end of an 11-week experiment when plants were exposed to either mirids only, or a combination of mites and mirids, or weevils and mirids. Numbers of individuals used per treatment per plant in the combination treatments: 150 mites, 2 adult weevil pairs and 15 adult mirid pairs. 30 adult mirid pairs/plant were used in the mirids only treatment. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

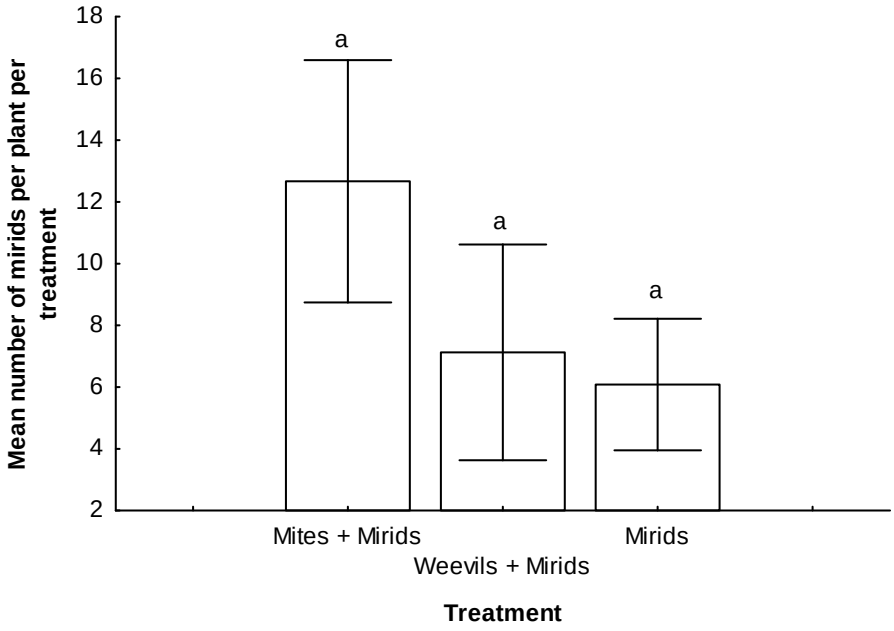

Figure 4.19. The number of mirids counted on water hyacinth plants after an 11-week experiment when the plants were exposed to either mirids only, or a combination of mirids and mites, or mirids and weevils. Numbers of individuals used per treatment per plan in the combination treatments: 150 mites, 2 adult weevil pairs and 15 adult mirid pairs. 30 adult mirid pairs/plant were used in the mirids only treatment. Error bars represent the standard error of the means. Means compared by one-way ANOVA. Error bars followed by the same letter are not significantly different (Fisher's LSD test, $p < 0.05$). n = 7 for all treatments.

4.4. DISCUSSION

In the pilot study, plants that were initially damaged by weevils and mirids grew slower over time than plants with no prior insect damage. This is evident in the shorter petioles, the reduction in ramet and leaf production and the lower biomass accumulation during the study. This is in agreement with Coetzee *et al.* (2007a) who found that herbivory by *E. catarinensis* reduced ramet production and the length of the second petioles. Similarly, Forno (1981) found that herbivory by *N. eichhorniae* reduced the numbers of leaves per plant and decreased the lengths of petioles, and Center and Durden (1986) and Grodowitz *et al*. (1991) found that extended periods of weevil feeding reduced overall plant size. In this study the petioles shortened over time, as their morphology changed from long and narrow petioles to short and bulbous petioles. The reason behind the shortening of the petioles was similarly observed in Chapters 2 and 3, and occurs as a result of moving the plants from stock cultures in pools where their densities are high and petioles long, to the experimental tubs where their densities are low and consequently they no longer have the support of other plants to keep them upright, and the plants therefore change their petiole morphology to the short bulbous form to keep afloat on their own.

Although mites were able to develop fully on both the weevil-damaged and mirid-damaged plants, the least amount of mite damage was found on the mirid-damaged plants, indicating that mites prefer to oviposit on plants not damaged by mirids as mirids feed across the entire leaf surface leaving very little healthy leaf tissue needed for mite-feeding and oviposition. Although not as damaging, scars created by weevil-feeding also limit mite feeding and oviposition. In addition to the physical damage caused by insect herbivory, changes in plant chemistry induced by insect herbivory can persist and negatively affect the fitness of later competitors for generations and seasons to come (Denno *et al*., 1995). The results suggest that adult mites preferred to oviposit on healthy, undamaged plants rather than unhealthy mirid-damaged plants, even weeks after the damage occurred. Center and Van (1989) found that adult weevil feeding reduced plant tissue nutrients, and it is likely that the sap-sucking mirid feeding does the same, making the plants less favourable to the mites. Similar effects have been shown between two biocontrol agents on *Lantana camara* L. (Verbenaceae), where the leaf-mining fly *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) avoided leaves infested by the leaf-mining beetle *Uroplata girardi* Pic (Coleoptera: Chrysomelidae), while *U. girardi* did not discriminate between leaves infested by *O. camarae* and uninfested leaves (April *et al.*, in review). Additionally,

host plant-mediated competition is not limited to insects. For example, Bounfour and Tanogoshi (2001) studied the interactions between the mites *Tetranychus urticae* Koch and *Eotetranychus carpini borealis* (Ewig) (Acari: Tetranychidae) and found that when *T. urticae* was placed onto raspberry leaves 3 days before *E. carpini borealis*, the population of *E. carpini borealis* went extinct, but when *E. carpini borealis* was placed onto raspberry leaves first, the population of *T. urticae* was affected negatively but not to the point of extinction.

Plants use various defences when under herbivore attack. For example, when a plant is damaged through herbivory, it releases anti-digestive proteins that inhibit insect digestive enzymes, thus influencing herbivore performance and/or preference (Agrawal, 1998; Kessler and Baldwin, 2002; Schultz, 2002). In a study on plant induced responses, Inbar *et al.* (1999) showed that tomato plants produce defensive proteins when attacked by the silver-leaf whitefly *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) and the vegetable leaf-miner *Liriomyza trifolii* (Burgess) (Diptera: Adromyzidae), but the degree to which the proteins are produced differed, to the point that oviposition by *L. trifolii* on *B. argentifolii* pre-infested plants was almost 31% less than on control plants, while pre-infestations by *L. trifolii* did not affect oviposition of *B. argentifolii*. It is possible that water hyacinth responded to the initial mirid herbivory by inducing a defence that made the plant less favourable to the mites, but this did not occur when the plants were initially attacked by weevils, since plants use differential defences against different types of insects (e.g. sap-suckers vs. leaf-chewers) and differential defences are also used in response to the plant part that the insects damage (Schultz, 2002; Wise and Cummins, 2006). In addition, plants respond to arthropod feeding by emitting various volatile substances which may repel other herbivores (Vet and Dicke, 1992; Turlings and Ton, 2006). Feeding by the mirids, and to a lesser extent by the weevils, may have induced water hyacinth to emit a volatile that repelled the mites, which could explain why the plants that were initially damaged by the mirids and weevils were less damaged by the mites than plants that had not previously been exposed to insect herbivory.

Adult mites are very mobile within a water hyacinth mat and in the field they are able to seek out undamaged/little-damaged plants. In an experimental set-up the agents are confined to a small number of plants and therefore oviposit on both damaged and undamaged plants. In this study less mite damaged was found on plants damaged by mirids than on plants damaged by weevils or on plants that were not damaged at all, suggesting that mites perform poorly in the presence of mirids. However, the agents

co-exist in the field. For example, at certain water hyacinth infestations in South Africa, such as sections of the Crocodile River, the two *Neochetina* weevils, the mite and the mirid occur together in high numbers during summer (own observation). Similarly, in Southern Benin the weevils and the moth *N. albiguttalis* were released together to control water hyacinth, although thus far only the weevils have established (De Groote *et al.*, 2003), and in Malawi the weevils, the mirid, the moth and the mite are being used together for the control of water hyacinth along the Shire River (Phiri *et al.*, 2001).

In the present study (Pilot study 1), adult mites emerged from galleries a few days later than what has been previously observed by Delfosse (1978a). This slight difference in emergence is possibly related to temperatures differences between the two studies, however, Delfosse does not include temperature measurements in his work and therefore direct comparisons cannot be made. Also, the galleries continue to elongate even after the adults have emerged, possibly because the adults continue to feed inside the galleries after they have emerged – adults are often seen inside “old” galleries i.e. galleries from which a mite has already emerged (own observation).

Plants that were inoculated with 2, 4, or 6 weevils accumulated less wet biomass than plants that were not damaged by insects, showing that weevils had a negative impact on plant wet biomass gain during this study. However, plants that were inoculated with 8 weevils, which was the highest weevil density tested in this study, gained significantly more biomass than the control plants which had no insects on them. The higher the number of weevils on a plant, the greater is the chance of encountering a mate, mating and hence oviposition, and this increases the number of larvae present in a plant, but this was not quantified during the study. Weevil larvae tunnel inside the petioles and the crown, causing water-logging of the plants (Cilliers, 1991). Water-logging was initially thought to explain the large biomass gain of the plants that had the most weevils on them. However, when this was tested in a separate experiment (results not presented here), there were no significant differences in the wet or dry biomass of water hyacinth plants that were exposed to 2, 4, 6 or 8 weevils after seven weeks. Therefore, in the present study (Pilot study 2) it seems more likely that the gain in biomass of plants that were exposed to the highest density of weevils was as a result of interference competition – at higher densities the weevils competed for food and thus fed less, as was seen by the smaller percentage of leaf surface area damaged by weevil feeding on plants that had higher numbers of

weevils on them i.e. plants that had 4, 6 or 8 weevils on them had less damage on leaf 2 than plants that only had 2 weevils on them.

Plants with the higher weevil densities had less leaf surface area damaged by feeding on leaf 2 than the plants that only had 2 weevils on them. Damage on leaf 2 was measured as this is the leaf preferred for feeding by weevils (Hill and Cilliers, 1999). As mentioned above, this is likely the result of interference competition. However, leaf 2 was not the only leaf fed on by weevils during the study, and older leaves were heavily attacked on plants that had higher numbers of weevils. This is supported by the fact that higher densities of insects cause greater overall damage to plants (Center and Van, 1989; Center and Jubinsky, 1996; Briese *et al*., 2004; Ding *et al*., 2006; Bebawi *et al*., 2007). In addition, the weevils move on to feed on the older leaves as the feeding area on leaf 2 becomes depleted.

In general, weevils decreased the petiole lengths and the production of ramets, and at the end of the study plants that were fed upon by weevils had shorter petioles and produced significantly fewer ramets than plants with no insects on them. These results are in agreement with those of Heard and Winterton (2000) who found that herbivory by the weevils *N. bruchi* and *N. eichhorniae* resulted in shorter petioles and fewer ramets being produced. However, the differences in the petiole lengths and the number of ramets produced were not significant between plants inoculated with different weevil densities, and it was therefore decided that it was not necessary to use more than 2 adult weevil pairs per plant in the interaction experiment as the lower weevil densities were unlikely to lessen the effect of the weevils on plant growth. Similarly in the mirid-inoculated plants, the number of ramets produced during the pilot study was significantly lower on plants inoculated with mirids compared to plants with no insects on them, but there were no significant differences in the petiole lengths and in ramet production between plants inoculated with the different mirid densities tested. Therefore, only 15 adult mirid pairs per plant were use in the interaction experiment as higher mirid densities were unlikely to cause much more damage to the plant.

The foundation of biological control of weeds is that the control agents must cause damage to the weed, which is manifested in either decreasing the growth of the weed and/or retarding its rate of invasion (DeBach, 1964). Sometimes, however, the effects of the agents on the plants are negligible or, initially, they may even appear to stimulate plant growth. For example, at the end of the interaction study, plants that

were fed on by only mites had significantly more leaves than the control plants, while the other treatments did not. Similarly, plants that were fed on by only mites also produced the most ramets during the study, but not significantly more than the control plants. These findings are similar to those observed in Chapter 3. In a study on the growth and reproduction of *Onopordum* thistles (Asteraceae) Briese *et al.* (2002) found that where low densities of the biocontrol agent, weevil *Trichosirocalus briesei* Alonso-Zarazaga & Sánchez-Ruiz (Coleoptera: Curculionidae) occurred on the host plant, slight overcompensation by the plant was observed. In this study the densities of mites were lower than what is usually found in field situations, and it is therefore likely that plants damaged by mites initially compensate for mite damage by increasing their leaf and ramet production (Chapter 3). In contrast, plants that were fed on by only mirids or only weevils, or a combination of mites and weevils, produced significantly fewer ramets than did the control plants, showing that mirids and weevils and a combination of mites and weevils have a negative impact on plant ramet production. The reduction of ramets in water hyacinth plants that are fed on by weevils and mirids has been observed in previous studies (Heard and Winterton, 2000; Coetzee *et al*., 2007a), and Delfosse (1978a) found that in the field combinations of mites and weevils significantly reduced the size and density of water hyacinth compared to reductions by either of the agents acting alone. The results of the present study differ somewhat from those of Delfosse (1978a) in that in the present study plants that were inoculated with a combination of mites and weevils produced fewer ramets than plants that were inoculated with only mites, but produced more ramets than plants inoculated with only weevils, suggesting that weevils alone are more damaging than weevils and mites together. Since water hyacinth mats increase in size through the vegetative production of ramets (Edwards and Musil, 1975), any reduction in the production of ramets would greatly diminish the spread of the plant.

Herbivory by the mites, mirids, or weevils, or the combinations of the three agents, had no affect on leaf turn-over rates. Caunter and Mohamed (1990) found that when water hyacinth was inoculated with five weevil pairs per plant, the plants produced more leaves than untreated controls, but when more weevils were used i.e. 8 or 10 pairs, the damage increased and plants produced fewer leaves than the untreated controls. In this study 2 or 4 weevil pairs were used per plant for the combined and single treatments, respectively, and the damage caused by their feeding was not enough to lower the leaf turn-over rate. Similarly, the densities of mites (150 or 300 adults per plant) and mirids (15 or 30 pairs per plant) used in this study were not

large enough to have an impact on the leaf turn-over rates. It thus follows that certain water hyacinth growth parameters will only be negatively impacted when fairly high agent densities are used, and as such augmentative releases of agents are necessary if the spread of the plant is to be slowed down. In addition, in South Africa the numbers of weevils, mites and mirids are sporadic in the field as winter minimum temperature frequently falls below the agents' development thresholds, and also cause high mortality to the agents (Byrne *at el*., 2010). This is discussed in detail in Chapter 6. Furthermore, the weevils have established at all of the water hyacinth infestations where they have been released in South Africa, but the mirids and mites have not been released at as many sites as the weevils (J. Coetzee pers. comm.), and thus augmentative releases, and new release at sites where agents do not yet occur, are necessary.

By the end of the study, the longest petioles were significantly shorter on plants that were fed on by only weevils, and a combination of mites and weevils, compared to plants fed on by either only mirids or only mites or no agents at all. Plants that were fed on by a combination of mites and weevils had the greatest negative impact on the length of the longest petioles. This is in agreement with Delfosse (1978a) who compared 2 field sites in Argentina, separated by 0.5km, one of which had water hyacinth with weevils and mites feeding on it, and the other site had water hyacinth with only mites feeding on it, and found that the size of the plants was significantly reduced in areas where the weevils and the mites co-exist. Mirids alone had no impact on the lengths of the longest petioles in the present study, and this is consistent with the experimental findings of Ajuonu *et al.* (2009). Similarly, mites had no impact on petiole lengths in the present study, and this is consistent with previous work (Chapters 2 and 3). A decrease in petiole lengths, as caused by agent herbivory, results in the plants becoming smaller over time, and this has been noted at various sites in South Africa (M. Hill pers. comm.).

At the end of the study, leaf 5 was generally more damaged compared to leaves 2 and 4. Weevils prefer to feed on younger leaves (Hill and Cilliers, 1999) and female mites tend to oviposit on younger leaves (Cordo and DeLoach, 1975), while mirids are gregarious feeders with no leaf preference (Hill *et al*., 1999), but the damage becomes more noticeable as the leaves get older (Cordo and DeLoach, 1976), as shown in this study. This occurs because the weevils feed on older leaves too, and the weevil scars accumulate on a leaf over time as the leaf moves along the apex from the youngest to the oldest position, with the older leaves being exposed to the

weevil herbivory for longer periods, and therefore older leaves are the most damaged. Mite damage, in the form of galleries, increases on older leaves because after the eggs have been laid on the younger leaves the mite nymphs develop inside the galleries and the galleries get longer while the leaf changes position along the apex, thus the longer, older and hence more damaging galleries are found on the older leaves.

The leaf surface was most damaged when the plants were exposed to herbivory by a combination of mites and mirids, while it was the least damaged when exposed to a combination of mites and weevils. It would seem, therefore, that the mites and mirids cause the most superficial damage to the leaves, but this is not translated into damage that affects plant growth. When the damage of the individual agents was examined, it was found that the mirids caused the greatest damage to the leaf surface, and this is reflected in the growth of the plants where mirids had the greatest negative impact on many of the plant growth parameters (e.g. Fig. 4.12). However, much of the weevil damage is caused by the larvae burrowing inside the petioles, and not necessarily by the adults feeding on the leaves (Hill and Cilliers, 1999; Julien, 2001). Measuring feeding damage to leaves does not in itself indicate that agents are having an impact on plant growth, but rather simply indicates the presence of the agents. In addition, insect herbivory does not simply remove leaf area, but also causes physiological changes in the plant. For example, Ripley *et al.* (2008) showed that feeding by *N. eichhorniae* reduced water hyacinth photosynthetic rates, but this reduction was not related to leaf area removal. In the field the damage to the leaf surface area caused by the agents' herbivory is much greater than was observed in this study (own observations), and undoubtedly has a greater impact on water hyacinth growth.

Effect of interactions on agent performance

Negative interactions between arthropods, such as the displacement of one arthropod by another, occurs across a wide range of taxa (Reitz and Trumble, 2002; Kaplan and Denno, 2007), and biocontrol agents are no exception (Woodburn, 1996; Louda *et al*., 2003). However, the general findings of this study show that *O. terebrantis*, *N. eichhorniae* and *E. catarinensis* can co-exist with little negative interaction.

Since the presence and abundance of agents is reflected in the damage caused to plants by agent herbivory (Wright and Center, 1984; Center and Jubinsky, 1996; Briese *et al*., 2004; Schooler and McEvoy, 2006), the percentage of the leaf surface area damaged by the agents was used as a representation of the abundance of agents in this study, and the performance of the agents (i.e. the agent's ability to reproduce) was taken to be represented by their abundance. As such, both the mirids and the weevils performed better when in combination with the mites, with the number of mirids being significantly higher on plants that had mites on them compared to plants that only had mirids on them.

All three agents utilise at least one life stage in the leaf of the plant, but because of their different life-cycles they spend different amounts of time on the leaves. The life-span of a water hyacinth leaf is roughly 8 weeks (Center, 1981), and as the leaves move down the crown of the plant as they age, the agents utilise the leaves for variable durations. *Orthogalumna terebrantis* spend their entire life-cycle (roughly 4 weeks from egg to adult stage) and adult life on the leaves, and since female mites prefer to oviposit on younger leaves the adults emerge when the water hyacinth leaves are about mid-way through their life-span. Female *E. catarinensis* also oviposit on leaves but they have no preference for younger or older leaves, and the eggs only take 15 days to develop such that the mirid nymphs and adults feed on the water hyacinth leaves throughout the leaves' life-spans, thus interactions between the mirids and the mites occur throughout each of the agents' lives. In contrast, female *N. eichhorniae* oviposit in the younger leaves and the eggs hatch within 9 to 16 days after which the hatched larvae tunnel down into the petioles, so in effect the water hyacinth leaves are initially utilised by the weevil for about 2 weeks at the beginning of the weevil's life-cycle, and only after roughly another 60 days, once the adult eclose, do new adult weevils feed on the leaves. In addition, it is possible that during feeding the mirids and the weevil adults inadvertently feed on mite eggs and/or nymphs.

Orthogalumna terebrantis performed better in the absence of *N. eichhorniae* or *E. catarinensis*. This could be attributed to a number of reasons. For example, mite eggs are so small (0.1. x 0.14 mm, Cordo and DeLoach, 1976) that adult weevils and mirids could accidentally damage them during feeding, although no evidence exists to indicate whether the weevils and mirids would purposefully seek out mite eggs (Delfosse, 1978b). Also, the feeding of adult weevils could remove entire mite galleries, which are much smaller than weevil feeding scars. Another possible reason

why mites performed less well in the presence of weevils is that plants fed on by weevils have reduced tissue nutrients (Center and Van, 1989). Although no study to date has looked at phytochemical changes in plants attacked by *E. catarinensis* it is likely that water hyacinth plants attacked by the mirids have reduced tissue nutrients much the same as when attacked by weevils, making attacked plants less favoured by other agents. Induced preference and performance has been observed in other studies on mites, for example in the two-spotted spider mite *T. urticae* which prefers cucumber plants over tomato plants, which differ in their chemical compositions (Magowski *et al*., 2003). Magowski *et al*. (2003) suggested that induced performance may be explained by induced resistance of mites to toxic secondary plant chemicals. Evidence from a number of studies suggests that rates of herbivory are lower on plants that were previously fed on by herbivores, due to induced changes in plant chemistry (Baltensweiler *et al*., 1977; Carroll and Hoffman, 1980; Denno *et al*., 2000) or the emission of herbivore-induced volatiles which may repel other herbivores (Vet and Dicke, 1992; Turlings and Ton, 2006). For example, Karban and Carey (1984) found that populations of *T. urticae* and *T. turkestani* (Ugarov and Nikolskii) (Acari: Tetranychidae) grew slower on cotton seedlings that had been exposed to the mites previously, than on seedlings that had never been exposed to the mites. Thus, behavioural responses (such as avoidance or preference) to chemical cues (such as plant volatiles as induced by herbivory), influence populations and community dynamics (Takken and Dicke, 2006). In the present study, it seems likely that herbivory by the agents changes water hyacinth chemistry and/or induces the plant to emit volatiles, which in turn either attracts or repels the other agents, as can be seen in their differential numbers on the various treatments (single agent treatments and multiple-agent treatments).

The performance of *N. eichhorniae* was similar in all of the weevil-inclusive treatments in this study, as can be seen by the similar amounts of damage caused to leaves by weevil herbivory in those treatments. Mites had neither a positive nor a negative impact on weevil performance, possibly due to their small size and the small amount of damage they caused to water hyacinth during this study. Also, mites and weevils differ in their diel activity i.e. mites are active during the day (Hill *et al*., 1999; Cordo and DeLoach, 1975) while weevils are active at night (Warner, 1970; DeLoach and Cordo, 1976), thus adults can avoid each other. In addition, the life cycle of a mite is only 4 weeks (Silveira-Guido, 1965) while that of the weevil is 13-17 weeks (DeLoach and Cordo, 1976), which means that during this 11-week study 2 new generations of mites would have been produced before even one new generation of

weevils was produced, thus reducing the chances of further interactions since no new weevils were added (through reproduction) to the plants.

In 1977(a), Delfosse conducted an experiment in which *N. eichhorniae* laid more eggs in the presence of *O. terebrantis*, and he hypothesised that this was due to the release of a greater amount of a kairomone, by the mite-damaged leaves, which supposedly acts as an oviposition stimulant. In a later study Delfosse and Perkins (1977) tested this hypothesis in part, and found that weevils were attracted to, fed more and laid more eggs on leaves that were damaged (crushed) as opposed to leaves that were in tact. Delfosse and Perkins thus concluded that the release of a kairomone from damaged water hyacinth leaves acts as an oviposition stimulant and/or phagostimulant for *N. eichhorniae* and possibly *O. terebrantis*. However, their study did not test whether leaves damaged by mite or weevil herbivory had the same effect on oviposition and feeding as crushed leaves, and therefore it cannot be concluded that mite damage stimulates weevil feeding and oviposition, and *vice versa*. In the present study, weevil abundance (as reflected in the leaf surface area damaged by weevils) was similar on plants that were inoculated with mites and weevils, and with only weevils, and thus the results of the present study do not support the suggestion of Delfosse and Perkins (1977) that mite feeding induces water hyacinth to release a kairomone which stimulates weevil feeding.

The results of the present study suggest that *E. catarinensis* had minimal impact on *N. eichhorniae*, probably because the weevils oviposit in leaf petioles (Warner, 1970; DeLoach and Cordo, 1976) while the mirids oviposit on leaf blades (Hill *et al*., 1999; Cordo and DeLoach, 1975), thus minimizing competitive interactions for available oviposition space. In addition, the diel activity patterns of weevils and mirids are different, as are their life-cycles i.e. the weevil life-cycle takes around 13-17 weeks while that of the mirid takes 3 weeks (Hill *et al*., 1999). Similarly, the feeding style of adult weevils differs to that of the mites and the mirids – the weevils remove the epidermis of leaves while both the mites and mirids extract chlorophyll from the endodermis of leaves. The different feeding styles of the agents used in this study thus ensure that the agents feed on different parts of the plant and they can consequently avoid interactions with each other.

Eccritotarsus catarinensis performed best in the presence of mites, as can be seen by the higher numbers of mirids counted on plants that had a combination of mites and mirids, compared to plants that only had mirids or a combination of mirids and

weevils on them. This is also reflected in the greater percentage of the leaf surface area damaged by mirids in the mites+mirids treatment. As discussed above, Delfosse and Perkins (1977) are of the opinion that damage to water hyacinth leaves caused by mite and weevil feeding induces the plants to release a kairomone which is thought to be an oviposition stimulant and/or a phagostimulant. However, the present study did not confirm this because, whilst the present study did not measure mirid oviposition directly, the results suggest that the effect of mite feeding on the plants did indeed stimulate mirid oviposition and feeding, but the same kairomone is thought to be released during weevil feeding (Delfosse and Perkins, 1977), and in the present study weevil feeding did not have the same effect on mirid performance as did mite feeding. On the other hand, Ajuonu *et al.* (2007) found that old weevil feeding scars had a negative effect on adult mirid survival, possibly due to the reduction of tissue nutrients caused by weevil feeding (Center and Van, 1989). The duration of this study was long enough for plants to have many old weevil scars on them, which probably reduced plant nutrient levels and made the plants less favourable to the mirids. Coetzee *et al*. (2007a) showed that poor quality plants have a negative effect on mirid performance. However, the mirids performed equally well during this study whether in combination with the weevils or alone, suggesting that there is little competition between these two agents.

4.5. CONCLUSION

Of the three agents examined in this study, *O. terebrantis* had the least impact on water hyacinth growth parameters, while *N. eichhorniae* and *E. catarinensis* both had negative impacts on the plant, but usually on different growth parameters. In combination with weevils and mirids the mites generally had no additional effect on plant growth, and were marginally detrimental (e.g. on ramet production when combined with mirids). Therefore, the cumulative effect theory does not operate in the case of the mite in combination with the weevil of the mirid. Whilst the mites performed poorly in the presence of the weevils and the mirids, they improved the performance of the mirids, and have been thought to increase weevil numbers and feeding in other studies. As such, this study is in agreement with authors who believe that interspecific competition is relatively rare and therefore is not an important factor structuring ecological communities (Price, 1984; Strong, 1984; Strong, 2008). *Orthogalumna terebrantis*, *N. eichhorniae* and *E. catarinensis* co-exist in the field in many countries, with variable success (Julien and Griffiths, 1998) and some researchers (Ajuonu *et al.*, 2009) have shown that they are compatible. In fact, this

study shows that co-existing species may help each other rather than hinder each other. It is recommended, however, that *O. terebrantis* not be developed at the expense of the other agents, although it may turn out to be better suited to some climatic regions of the country.

CHAPTER 5

Photosynthetic performance of water hyacinth as affected by *Orthogalumna terebrantis* herbivory

5.1. INTRODUCTION

The adverse effects of herbivory on plant physiological parameters such as photosynthetic efficiency have been reported for insects (Cockfield *et al.*, 1987; Buntin *et al*., 1993; Haile *et al*., 1999; Macedo *et al*., 2005; Flynn *et al*., 2006) and for mites (Sances *et al*., 1979; Sances *et al*., 1981; Haile and Higley, 2003). For example, Buntin *et al*. (1993) found a decrease in net photosynthetic rates of tomato leaves with an increase in feeding by the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), and feeding by the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), caused a 34% reduction in the photosynthetic rate of soybean (Haile and Higley, 2003). Alternatively, some studies have shown that plants are able to compensate for short-term herbivory by improving their photosynthetic efficiency (Thomson *et al*., 2003; Hayashi *et al*., 2007).

Herbivory of *O. terebrantis* did not have a great impact on water hyacinth growth parameters (Chapters 2-4), almost certainly because of the relatively small mite densities used in the experiments. It is difficult to repeat large “field-like” mite populations in the laboratory, and the amount of plant material that is removed by the mites is difficult

to measure, yet mite herbivory causes visible damage to the leaves and must consequently have some affect on the plant. Therefore, in this chapter the plant physiological parameters i.e. photosynthetic performance, are measured to determine whether mite herbivory had more subtle affects on water hyacinth than could be observed in the measurements of plant growth parameters.

When arthropods remove leaf tissue by chewing, sucking or mining the tissue, the primary effect is the reduction of photosynthetic leaf area which may affect leaf water status and thus affect stomatal responses (Aldea *et al.*, 2005). Arthropods that feed on water hyacinth either remove plant tissue directly, as is the case with *O. terebrantis* which eats away the inner leaf tissue of the laminae (Cordo and DeLoach, 1976), or they suck out the contents of cells causing chlorosis, as is the case with the mirid *E. catarinensis* (Hill and Cilliers, 1999; Julien, 2001). In both cases, the photosynthetic efficiency of water hyacinth is affected since herbivory by the mites or mirids removes and/or damages the chloroplasts found inside plant cells.

Studies of herbivory have traditionally focused on the measuring of the direct removal of plant biomass and have largely ignored the effect of herbivory on the remaining plant tissue. Recent studies show that insect herbivory triggers multifaceted physiological responses in the remaining plant tissue (tissue not directly damaged by herbivore feeding) that may reduce the photosynthetic ability of a plant more so than simply removing photosynthetic surface area (Aldea *et al.*, 2005; Nabity *et al.*, 2009). For example, the removal of leaf tissue from wild parsnip by the caterpillar *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) resulted in a reduction in photosynthesis in the remaining leaf tissue which accounted for a three-fold greater reduction in overall photosynthesis than direct leaf tissue removal i.e. pieces of the leaves cut off manually (Zangerl *et al.*, 2002), and similarly, removal of leaf tissue from water hyacinth by the weevil *N. eichhorniae* resulted in a greater decline in CO_2 assimilation rates than could be explained by the direct removal of the leaf area (Ripley *et al*., 2008). Nabity *et al.* (2009) identified four physiological mechanisms of plants by which insect herbivory indirectly suppresses photosynthesis on remaining leaf tissue: defence-induced autotoxicity, defence-induced down-regulation of photosynthesis, damaged vasculature and altered sink demands (altered energy requirements e.g. for growth of various plant organs). Different feeding guilds affect different components of the photosynthesis

process such that plants respond in a specific manner depending on the type of damaged caused (Kessler and Baldwin, 2002; Tang *et al.*, 2006; Nabity *et al.*, 2009). For example, Aldea *et al.* (2005) showed that when the corn earworm caterpillar *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) fed on soybean the effect on net photosynthesis was marginal, while chewing damage by the Mexican bean beetle *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) resulted in a considerable decrease in photosynthesis in the remaining soybean leaf tissue (Peterson *et al.*, 1998). Plant responses to injury through defoliation have been extensively studied (Peterson *et al.*, 2004), yet plant responses to injuries from other feeding guilds, such as leaf miners like *O. terebrantis*, are not well studied.

Since the early 1980s, the development of commercially available, portable and user-friendly open gas exchange systems (e.g. infra-red gas analyzers such as the LI-6400, Li-Cor Inc., Lincoln, Nebraska, USA) which are able to control CO_2 concentration, humidity, light and temperature, have enabled plant physiologists to make real-time measurements of numerous plant physiological parameters such as transpiration, uptake of CO_2, intercellular CO_2 concentration and leaf conductance (Long and Bernacchi, 2003). In addition, with the help of portable chlorophyll fluorometers plant ecophysiologists now also have the option of analyzing chlorophyll-*a* (chl-a) fluorescence, which is an indicator of primary productivity (Maxwell and Johnson, 2000). Measurements of plant gas exchange parameters, often combined with chl-a fluorescence analysis, are now employed extensively to determine photosynthetic performance, specifically in plants that are exposed to various stress conditions such as drought, high temperatures, nutrient deficiency and herbivory (Thomas and Turner, 2001; Ripley *et al*., 2004; Souza *et al*., 2004; Macedo *et al*., 2005; Jiang *et al*., 2006; Ripley *et al*., 2006; Baker *et al*., 2007). In the present study gas exchange parameters and chl-a fluorescence of water hyacinth, damaged by *O. terebrantis*, will be measured to determine the effect of the mite on the fundamental physiology of the plant.

Measurements of chl-a fluorescence are non-invasive and non-destructive, and are used to evaluate the integrity of Photosystem II (PSII), including linear electron flux and CO_2 assimilation *in vivo* (Baker, 2008). Changes on PSII are assessed by means of the fast kinetics of chl-a fluorescence that is emitted by dark-adapted leaves when they are illuminated by saturated light (Strasser *et al*., 2004). The principles behind chl-a

fluorescence are explained by Maxwell and Johnson (2000), and they are summarized in the paragraphs below.

All higher plants (plants that produce oxygen) have two types of reaction centres (RCs) located in the thylakoid membranes of chloroplasts, namely Photosystem I (PSI) and Photosystem II (PSII). Pigments inside the Photosystems absorb light which causes electrons inside the systems to reach a higher energy level, at which point the electrons are referred to as being “excited”. In order to drop the electron into a stable lower energy state, the excess energy is passed on to another pigment molecule and the electrons in that molecule now become excited, until eventually the energy is passed on to the RC where it is used in a series of enzyme reactions which produce chemical energy that ultimately drives photosynthesis. While both PSI and PSII go through similar processes, only the fluorescence signal of PSII varies with plant stress, which is why PSII is of primary interest when studying the reactions of plants to stresses (Salisbury and Ross, 1992).

When light energy enters a leaf it is trapped by chlorophyll antennae, which are light-harvesting complexes made up of protein and chlorophyll molecules (e.g. carotenoids, chlorophyll-a and chlorophyll-b) that are embedded in the thylakoid membranes. The composition and amount of the chlorophyll-protein complex is variable so that the antennae have variable sizes, depending on the incident irradiance during plant growth (Masuda *et al.*, 2003). The function of the antennae is to transfer light (in the form of photons) to the RCs where it is used in one of three ways, (a) to drive photosynthesis (photochemistry), (b) excess energy is dissipated as heat or (c) it is re-emitted as red and far-red light (fluorescence). These three processes compete so that when the efficiency of one increases it causes a decrease in the yield of the other two. Therefore, by measuring chl-a fluorescence, it is possible to obtain information about changes in photochemistry and heat dissipation of the plant being measured. Chlorophyll-a fluorescence uses only 1-2% of the total light absorbed. The spectra of fluorescent and absorbed light are different in that the peak of a fluorescence emission occurs at longer wavelengths than that of an absorption emission. Fluorescence yield is therefore easily measured by exposing a leaf to light of a known wavelength and then measuring the wavelength of the light re-emitted at longer wavelengths. This measurement is relative to the initial wavelength of the incident light since some incident light is lost while making

the measurement. This loss is accounted for by normalizing data (the fluorescence transients are normalised by dividing the F_v-values by the F_m-value) and by calculating a wide range of fluorescence parameters (see below) (Maxwell and Johnson, 2000).

Chlorophyll-a fluorescence were first observed in 1960 by Klautsky and co-workers (Klautsky *et al*, 1960 (in German), from Maxwell and Johnson, 2000). They noticed that when plants were transferred from the dark into the light, an initial increase in fluorescence occurred in a time of around 1 second. This rise occurs because electron acceptors, specifically plastoquinone (Q_A), are reduced i.e. accept an electron, downstream of PSII. When PSII absorbs light and Q_A accepts an electron, it cannot accept another electron until it has passed the first electron onto another electron acceptor (Q_B). At this time the reaction centre is said to be 'closed', causing a reduction in the efficiency of photochemistry with a corresponding increase in fluorescence yield. After a few more minutes of light exposure, the fluorescence level stars to fall again, and this is called fluorescence quenching. Thereafter, electrons are transported away from PSII at an increased rate due to the activation of enzymes involved in carbon metabolism and the opening of stomata, and this is called photochemical quenching. At the same time, energy is converted into heat at a faster rate – this is called non-photochemical quenching (*NPQ*). The amount of a fluorescence signal re-emitted by PSII can be estimated in the presence of one contributor alone, when the other contributor, usually photochemistry, is "switched off".

When plants have been in the dark (dark-adapted), the reaction centres in the photo-synthetic tissue are said to be 'open', as Q^{-}_{A} is reoxidized. At this time the majority of excitation energy is used in photochemistry and no fluorescence signal is emitted. The fluorescence intensity at this point is referred to as F_0. To "switch off" photochemical quenching, a flash of saturating light is shone onto a dark-adapted leaf, thereby closing the reaction centres, and as a result no energy is used in photochemistry. At this point the level of fluorescence is at its maximum and is referred to as maximum fluorescence (F_m). Fluorescence yield begins to fall after the first second of saturation, and this is called fluorescence quenching. Comparing F_0 with F_m gives information about the efficiency of photochemical quenching, and thus the performance of PSII. The difference between F_0 and F_m is called variable fluorescence (F_v) and represents the potential amount of energy that could be used by photochemistry. The ratio F_v/F_m (the maximum

quantum yield of primary photochemistry) measures the efficiency of PSII i.e. the quantum efficiency if all PSII centres were open, and indicates how efficient the trapping of photos is. A change in F_v/F_m occurs when there is a change in efficiency of *NPQ*. Healthy dark-adapted leaves usually have an F_v/F_m value of about 0.83 (Maxwell and Johnson, 2000). Values below this indicate that a plant has been exposed to stress. A decrease in the F_v/F_m value only occurs when there is a direct stress on PSII.

The sequence of phases from initial (F_0) to maximal (F_m) fluorescence form a curve, called the fluorescence induction transient, with several intermediate steps which have been labeled O, J, I and P (Fig. 5.1) (Strasser and Govindjee, 1992). The O-J-I-P transient changes shape when plants are exposed to stress caused by changing environmental conditions e.g. temperature, drought, chemical influences, because there is a relationship between the slope (M_0) of the curve and the amount of photons absorbed, and the amount of photos absorbed is influenced by stresses on PSII. The phases of the O-J-I-P trasnsient have been interpreted as follows: the O-J phase corresponds to a complete reduction of Q_A of PSII, the J-I phase takes place during fluorescence quenching and is controlled by the PSII water splitting activity (called the donor side of PSII), and the I-P phase corresponds to the release of fluorescence quenching by oxidized Q_A (Neubauer and Schreiber, 1987 in Strasser *et al*., 2004). The method of analysis of the transient has been called the JIP-test which is a tool that can be used to analyze environmental effects on photosynthetic organisms (Strasser *et al*, 2004). The JIP-test is used to calculate relative proportions of energy that are dissipated by different processes during the light reaction, and are referred to as energy fluxes (Strasser and Strasser, 1995). Some of the energy fluxes that the JIP-test calculates are: ABS/RC, TR_0/RC and ET_0/RC, where ABS stands for absorption, TR_0 stands for trapping and ET_0 stands for electron transport. The energy fluxes are expressed as specific energy fluxes per reaction centre or as proportions of one another, hence called flux ratios or yields. Based on a model, the JIP-test examines how light (photon flux) that is absorbed by photosynthetic antenna pigments is dissipated, mainly as heat and less as fluorescence, or is transferred as trapping flux (TR) to the reaction centres. The absorption of photons by antennae pigments is referred to as absorption flux (ABS). Inside the reaction centres the light is converted to redox energy through the reduction of Q_A to Q^-_A. Electron transport (ET) is created when Q^-_A is re-oxidized, leading to CO_2 fixation (van Heerden *et al*., 2004). The energy fluxes are used to calculate a

performance index (PI_{ABS}) which determines the potential photosynthetic activity by combining three properties of PSII: a) the density of reaction centres, b) the efficiency of the light reaction and c) a component related to forward electron transport. If a stress has an effect on these properties then the effect will be revealed in the PI_{ABS}.

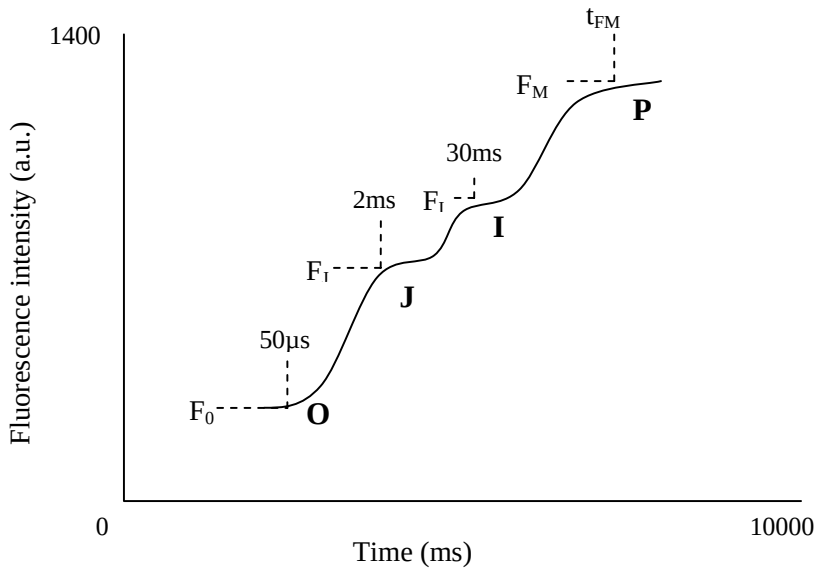

Figure 5.1. A typical chlorophyll-a fluorescence induction transient exhibited by higher plants when illuminated by saturating light, displaying a polyphasic rise with the O-J-I-P steps. The transient is plotted on a logarithmic time scale from 50µs to 1 s. The O-J-I-P markers refer to selected fluorescence data used by the JIP-test to calculate structural and functional parameters which quantify the behaviour of PSII. (Modified from Strasser *et al.*, 2004).

Female *O. terebrantis* oviposit on the youngest water hyacinth laminae and the eggs are deposited underneath the cuticle in small perforations made with their mandibles, in the middle layer of palisade cells that form the parenchyma (Silveira-Guido, 1965). As the larvae and hatched nymphs develop and feed inside the lamina, they remove plant tissue and create distinctive yellowish linear markings, called galleries, which reach a length of ± 4 mm before adults emerge (Cordo and DeLoach, 1976). Under laboratory conditions, feeding by *O. terebrantis* causes visible damage to water hyacinth laminae but has little effect on the plant's overall growth parameters even after 12 weeks (Chapters 3 and 4). However, high densities of mites that occasionally occur in the field during summer have been found to decrease leaf chlorophyll content (Chapter 2). The main hypothesis of this chapter is that mite herbivory decreases water hyacinth

photosynthetic potential (the ability of the plant to photosynthesize), which can be seen in changes to gas exchange and the functioning of PSII. Therefore, the aim of this chapter was to determine to what degree water hyacinth photosynthetic performance was affected by varying levels of mite damage, through measurements of plant gas exchange parameters and the use of the JIP-test.

5.2. MATERIALS AND METHODS

5.2.1. Experimental set-up

Water hyacinth plants were collected from stock cultures of the Plant Protection Research Institute (PPRI), Pretoria and also from Rhodes University, Grahamstown, which had varying levels of mite damage. Mites have been established on the plants at PPRI for more than 10 years, such that during summer the plants are heavily damaged by the mites. Rhodes University has been attempting to establish a mite population for the past two years, and the mite population is growing gradually but is still not large. For the purposes of this study, mites were posted on a weekly basis, from November 2009 to February 2010, from PPRI to Rhodes University, where the experiments were to be conducted. Experimental plants were continually inoculated with the PPRI mites, such that ~ 23 700 mites were introduced onto an initial 32 plants over 4 months. The plants produced ramets during that time so that by the time the experiment was conducted (in March 2010) there were > 50 plants. The experimental plants thus simulated a natural water hyacinth population where plants of different ages occur.

Stock cultures of the plants, at PPRI and Rhodes University, were kept in large plastic tubs inside polythene tunnels. The tunnels provided some protection from other water hyacinth biocontrol agents. However, it was not possible to keep all the experimental plants completely uncontaminated by other agents, so all the plants had some weevil and/or mirid damage on them, but this was relatively little compared to the mite damage, and is in fact more comparable to a field situation where usually more than one of the agents is present at any one time. Osmocote® and iron chelate were used as nutrient supplies. Plants were well-fertilized and produced ramets vegetatively. At Rhodes University, one half of the plants were inoculated with mites, while the remaining half acted as the control. The control and treated plants were kept in separate tubs inside the

same polythene tunnel. The mites are poor dispersers in terms of moving from one water hyacinth mat (one tub full of plants in the case of the experiment) to another mat, and therefore they could not get onto the control plants since the tubs were placed apart from each other.

5.2.2. Feeding intensity measurements

Prior to measuring the gas exchange and photosynthetic rates, digital photographs were taken of 10 leaves (one leaf taken from each of 10 individual plants damaged by mites) so that their surface area as well as the area damaged by mites could be measured using the image analysis software ImageJ 1.40g (National Institute of Health, USA). The fourth or fifth leaf, counting the youngest unfurled leaf closest to the apex as leaf 1, was photographed and used in the experiment. The leaves remained attached to the plant throughout the measurements. ImageJ was calibrated by including a centimeter ruler in the photographs as a reference of scale, and thereafter the percentage of the leaf area damaged by mite herbivory was determined by the software programme. This ensured that any differences in gas exchange and photosynthetic rates between leaves could be explained by differences in mite herbivory and not differences in the leaf area. The plants tended to get physically damaged during transportation from the tunnels to the laboratory, so measurements of gas exchange and photosynthetic rates were made on the leaves of the least damaged plants, either the fourth or the fifth leaf.

5.2.3. Photosynthetic gas exchange

The gas exchange parameters of replicate treated (n = 10) and control (n = 10) plants were measured using a LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, Nebraska). The gas exchange parameters measured included leaf CO_2 uptake i.e. net photosynthetic rate (*A*), leaf conductance (g_l), transpiration rate (*E*) and intercellular CO_2 concentration (C_i). In addition, *A* was plotted against C_i to show the limitations of stomata on CO_2 assimilation (Long and Bernacchi, 2003). These measurements were made on the fourth or fifth leaf, as explained above, and the leaves remained attached to the plants while the measurements were in progress. The air entering the conifer leaf chamber (7.5 cm diameter) into which the leaves were placed had a sample CO_2 concentration of 380 µmol mol^{-1}, the block temperature was set to 30ºC and the light

intensity at saturating photosynthetic photon flux (PPFD) of 1800 µmol m^{-2} s^{-1} was provided by an external halogen lamp. To ensure that leaf-to-air vapor pressure deficits would not exceed 1 kPa the sample relative humidity levels were kept at 75-80% by manually adjusting the CO_2 scrubber ($Mg(ClO_4)_2$) and a desiccant (soda lime) which are stored inside bottles attached to the console of the LI-6400 system. The gas exchange parameters are calculated automatically by the LI-6400 software, which is based on the equations of Farquhar and von Caemmerer (1982).

5.2.4. Chlorophyll-a fluorescence

The photochemical efficiency of PSII was determined on plants with different levels of mite damage, and fluorescence measurements were made on the same leaves as used for gas exchange measurements, using a Plant Efficiency Analyzer (PEA, Hansatech, UK). Five sections of each leaf were dark-adapted using leaf clips (5 mm diameter each), and the clips were kept on the leaves for a minimum of 1 hour. This ensured that all the photosystems were in 'open' states just before the measurements were made. The chl-a fluorescence parameters measured, as well as their biophysical or biochemical meanings, are given in Table 5.1. The values from the five dark-adapted sections on each leaf were combined and a mean value per leaf was calculated for each of the chl-a fluorescence parameters measured. The data were analysed using Biolyzer (Version 3, Laboratory of Bioenergetics, University of Geneva, Switzerland), which calculates JIP-test parameters.

5.2.4.1. The JIP-test

The JIP-test was performed on the original fluorescence transients that were measured (Table 5.1 (A)). Table 5.1 (B) lists the JIP-test parameters that were measured, explains their biophysical and biochemical importance, and shows how they were calculated from original fluorescence data i.e. F_0, F_m and F_v/F_m. In addition, the performance index (PI_{ABS}) was also calculated, as per the equations of Strasser *et al*. (2004). Fluorescence readings are expressed in arbitrary units and the JIP-test parameters are expressed as relative values.

Table 5.1. Selected chlorophyll-a fluorescence parameters (A) and JIP-test parameters (B) used in this study to determine the efficiency of Photosystem II (PSII) of water hyacinth exposed to varying levels of *O. terebrantis* herbivory. Biophysical or biochemical meanings are given, as well as equations showing how the selected energy fluxes of the JIP-test are calculated from the original fluorescence data (Strasser *et al*., 2004; Baker *et al*., 2007).

(A) Fluorescence parameters	**Meaning**	**Physiological relevance**
F_0	Minimal fluorescence from dark-adapted leaf, fluorescence intensity at 50 µs	Fluorescence level at which PSII primary quinine electron acceptors, plastoquinones (Q_A), are maximally oxidized (PSII reaction centres, (RCs), are 'open')
F_m	Maximal fluorescence from dark-adapted leaf	Fluorescence level at which Q_A is maximally reduced. Excitation energy is great enough to close all RCs (PSII RCs are 'closed')
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by PSII is converted to chemical energy (Q_A reduction)

'F' refers to the fluorescence emission from a dark-adapted leaf when the leaf is illuminated with saturating light. 'Fv' refers to the variable fluorescence given off by a leaf.

(B) JIP-test parameters	**Meaning**	**Calculation**
Energy fluxes expresses per Q^-_A reducing PSII reaction centres (RC)		
ABS/RC	Rate of photon absorption	$(TR_0/RC) / [(F_m – F_o)/F_m]$
TR_0/RC	Maximum rate of Q_A reduction	$(M_0/V_J) = M_0/(F_J – F_o)/(F_m – F_o)$
ET_0/RC	Rate of electron transport beyond Q_A reduction	$(TR_0/RC)(1 – V_J)$
RC/CS	Density of RCs in PSII per cross section (CS)	
Performance index on absorption basis		
PI_{ABS}	Compound function of light energy absorption, efficiency of Q_A reduction and conversion of excitation energy to electron transport	$[RC/ABS] [(TR_0/ABS)/(F_m – F_o)] [(ET_0/TR_0)/V_J)]$

The subscript '0' refers to the quantification of PSII behaviour at the onset of fluorescence induction. Abbreviations: ABS = absorption flux (occurs when photons are absorbed by the photosynthetic antenna pigments); TR = trapping flux (occurs when energy is transferred to the reaction centres); ET = electron transport flux (occurs when Q_A is reduced to Q^-_A which is then reoxidized to Q_A thereby maintaining the metabolic reactions of PSII).

5.2.5. Leaf chlorophyll content and biomass

After the completion of the above measurements, the experimental leaves were cut off from the plants and thereafter cut vertically in half, from the base to the tip of the lamina (Fig. 5.2.) and each half was weighed. Chlorophyll was extracted from one half of the

fresh leaf, using 6 ml of 100% acetone per 1 g of fresh weight. Half of a leaf was used to ensure that at least half of the area damaged by the mites was included in the extraction, since mite damage tends to be spread equally on both halves of a leaf. The plant material was ground up using an electronic Ultra-turrax grinder (Janke & Kunkel, Ike-werk), and removed by centrifuging at 6000 rpm for 7 minutes. The supernatant was poured off and the remaining pellet was re-suspended in 6 ml 100% acetone and re-centrifuged as before. The supernatants were pooled and their absorbencies were read at 663 nm and 645 nm on a PU8670 Vis/NIR spectrophotometer (Philips, UK). Pure acetone was used as the blank. The supernatants were diluted where necessary i.e. if the absorbencies were > 1. The equations of Lichtenthaler (1987) were used to determine chlorophyll-a, chlorophyll-b and chlorophyll (a + b). Chlorophyll content was expresses per gram of fresh weight. The remaining half of each leaf was weighed to obtain fresh biomass data and thereafter it was dried to constant weight for calculations of dry biomass.

Lichtenthaler's equations:

Chlorophyll-a^a = (11.24 x λ_{662}) – (2.04 x λ_{645})

Chlorophyll-b^b = (20.13 x λ_{645}) – (4.19 x λ_{662})

Total chlorophyll $(a + b)^c$ = (7.05 x λ_{662}) + (18.09 x λ_{645})

For µg chlorophyll per g fresh weight = (chlorophyll $^{(a, b \text{ or } c)}$ x solvent) / g fresh weight

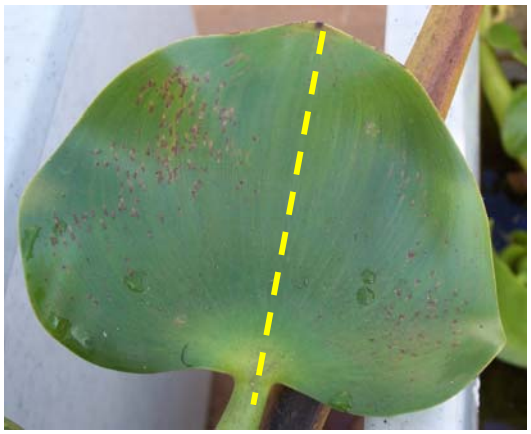

Figure 5.2. A water hyacinth leaf damaged by *Orthogalumna terebrantis*. The dashed line represents where the leaf was cut in half such that one half of the fresh leaf was used in chlorophyll extraction and the other half was weighed and then dried to obtain fresh and dry biomass.

5.2.6. Statistical analyses

Linear regressions were fitted to a) the measured gas exchange parameters, b) the chl-a fluorescence parameters, c) the JIP-test parameters, d) the leaf chlorophyll contents and e) the leaf biomass, and plotted against mite damage per leaf i.e. amount of leaf area damaged by the mites as determined by measuring the area of the mite galleries. For gas exchange parameters, where the sample CO_2 was outside of 380 µmol mol^{-1} (i.e. >390 µmol mol^{-1} or < 370 µmol mol^{-1}), the data were removed before analysis. Since only one half of a leaf was weighed to obtain biomass, the values were multiplied by 2 to obtain the biomass of a whole leaf. Data were analysed using the statistical programme STATISTICA Version 7.0 (© StatSoft, Inc., USA).

5.3. RESULTS

5.3.1. Photosynthetic gas exchange

Plants that had been exposed to mite herbivory had lowered photosynthetic rates (*A*) (Fig. 5.3). There was a strong positive correlation between the amount of leaf surface area that was damaged by the mites and the leaf conductance (g_l) (Fig. 5.4), the rate of transpiration (*E*) (Fig. 5.5) and the intercellular CO_2 concentration (C_i) (Fig. 5.6), indicating that mite damage significantly increases g_l, *E* and C_i. There was a modest decrease in *A* with an increase in C_i, (Fig. 5.7).

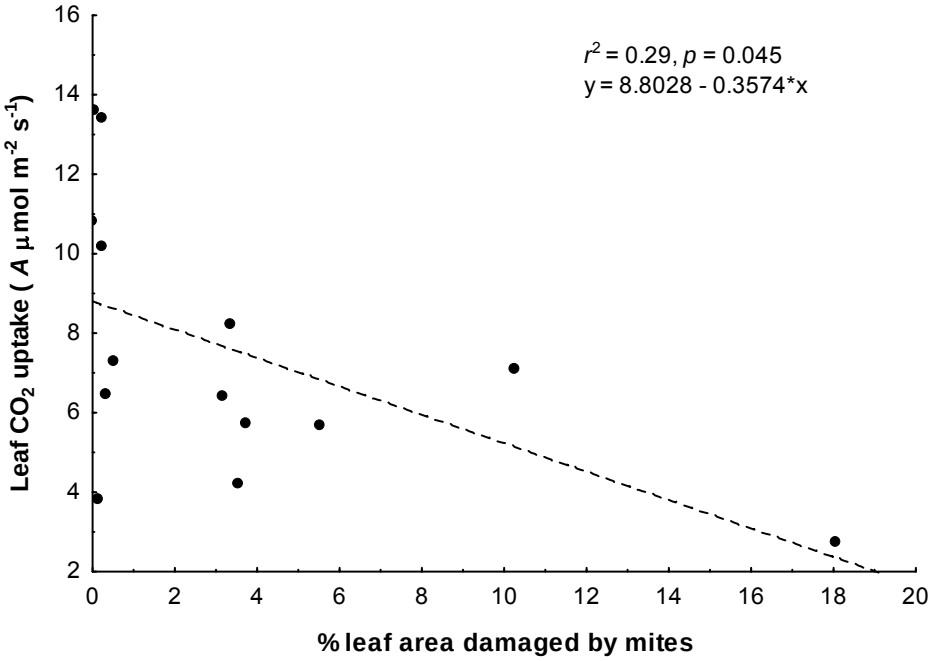

Figure 5.3. Photosynthetic rates (*A*) of water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

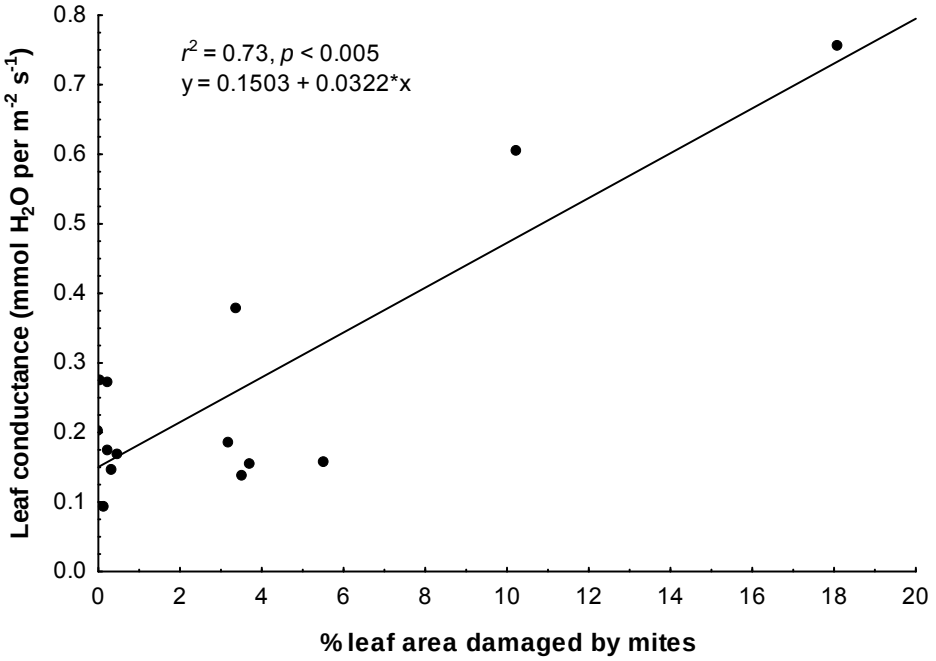

Figure 5.4. Leaf conductance rates (g_l) of water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

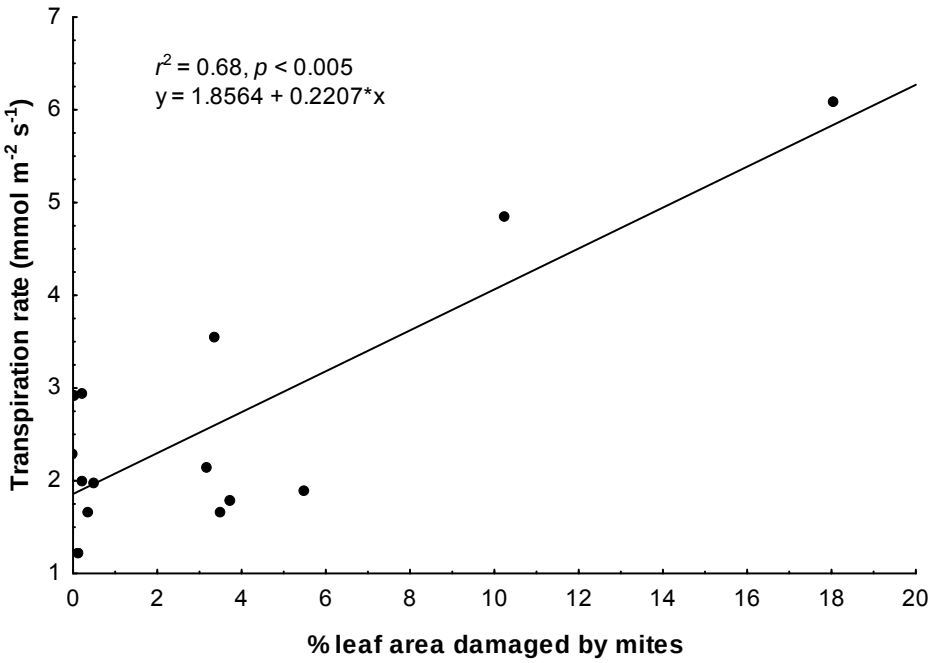

Figure 5.5. Transpiration rates (*E*) of water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

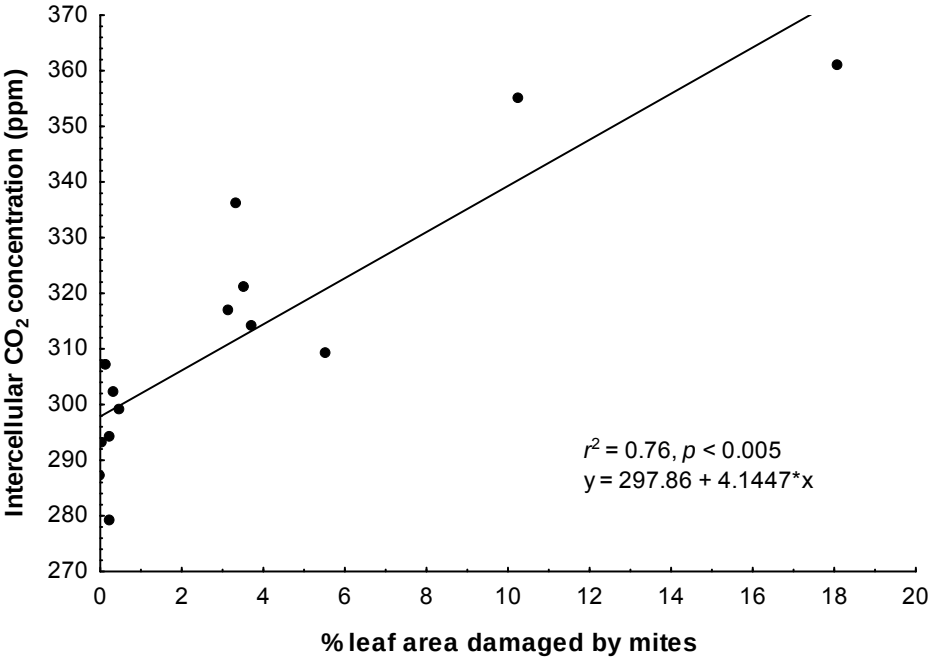

Figure 5.6. Intercellular CO_2 concentration (C_i) of water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

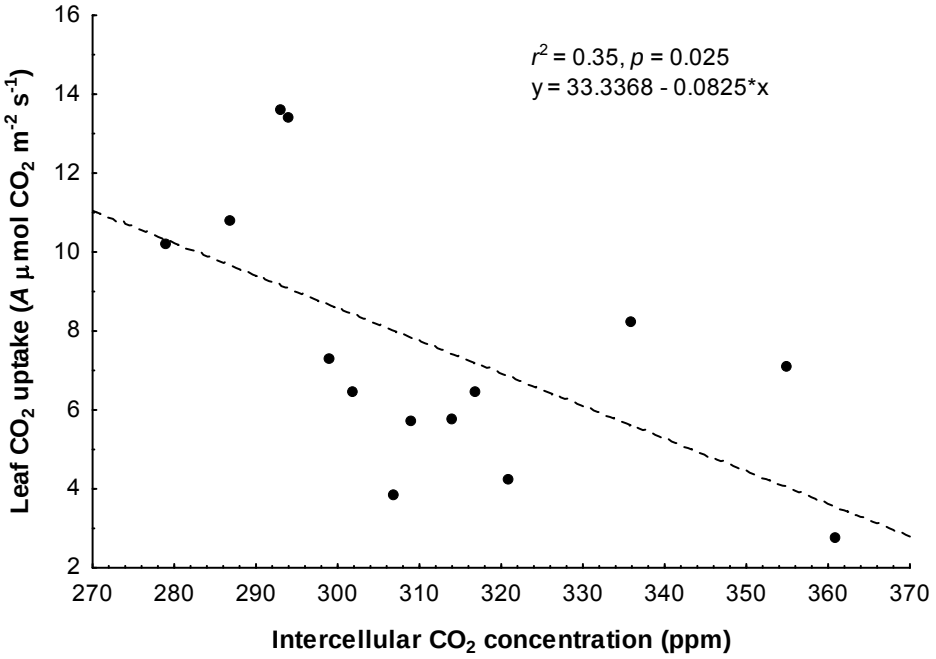

Figure 5.7. Relationship between the uptake of CO_2 (*A*) and the intercellular CO_2 concentration (C_i) of water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

5.3.2. Chlorophyll-a fluorescence

There was no correlation between F_0 and mite herbivory (r^2 = 0.001, p = 0.897). However, there was a significant decrease in F_m with an increase in mite herbivory (Fig. 5.8), and this was translated into a moderate decrease in maximum efficiency of PSII photochemistry (F_v/F_m) as the amount of leaf surface area damaged by the mites increased (Fig. 5.9), indicating that mite herbivory had a negative impact on the efficiency with which light absorbed by PSII is converted to chemical energy i.e. the efficiency at which Q_A is reduced.

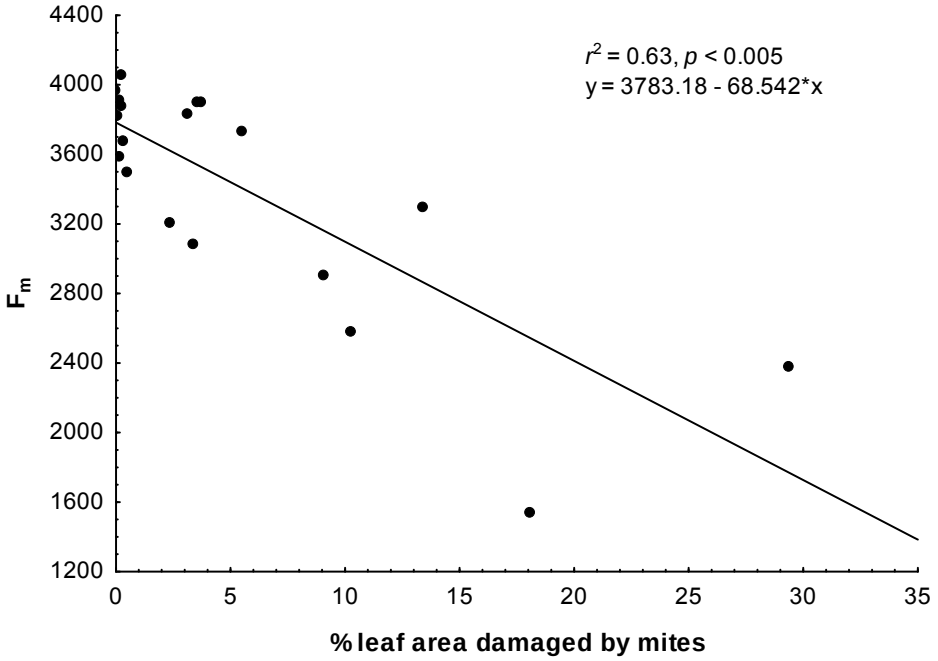

Figure 5.8. Maximal fluorescence (F_m) of dark-adapted water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

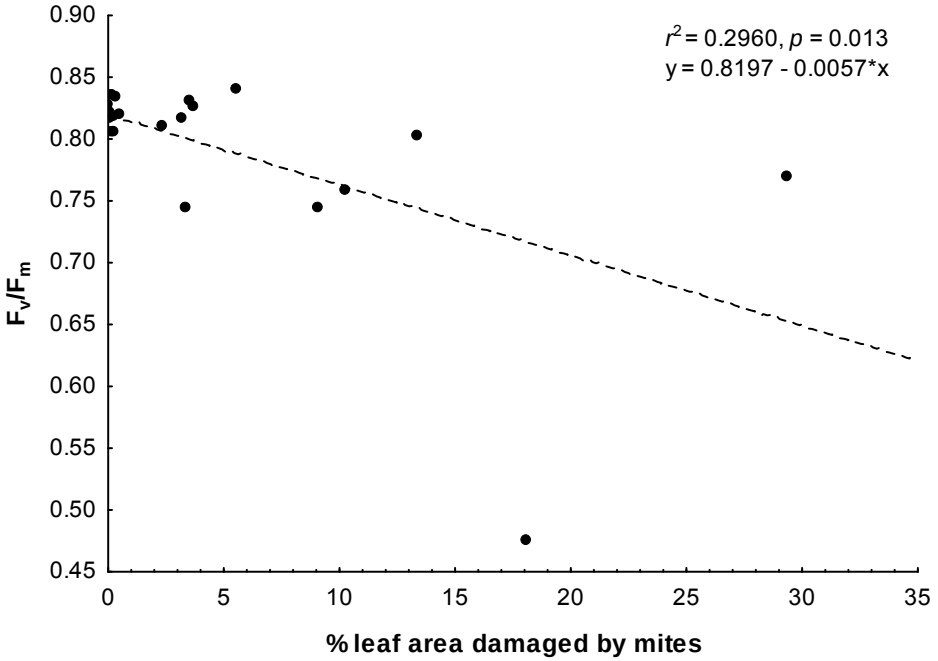

Figure 5.9. The efficiency of PSII photochemistry (F_v/F_m) of dark-adapted water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

The performance index (PI_{ABS}), which incorporates light energy absorption, the efficiency of Q_A reduction and the conversion of excitation energy to electron transport, decreased as mite herbivory increased (Fig. 5.10). The decrease in PI_{ABS} is attributed to the significantly lowered density of functional reaction centres in PSII per cross section of leaf area (Fig. 5.11). However, the decrease in PI_{ABS} occurred even though the energy fluxes (ABS/RC, TR_0/RC and ET_0/RC) per PSII reaction centre increased as mite herbivory increased (Fig. 5.12 A-C). This indicates that mite herbivory increased the rates of photon absorption (ABS/RC), the maximum rates at which excited electrons are trapped by the reaction centres resulting in Q_A reduction (TR_0/RC), and the rates of electron transport (ET_0/RC), respectively.

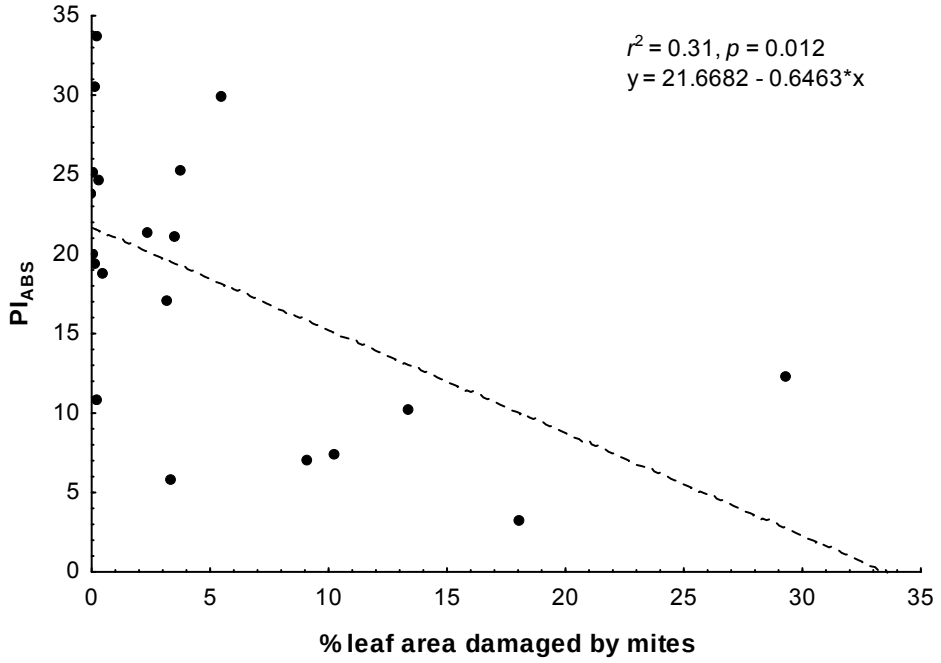

Figure 5.10. The performance index (PI_{ABS}) of dark-adapted water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

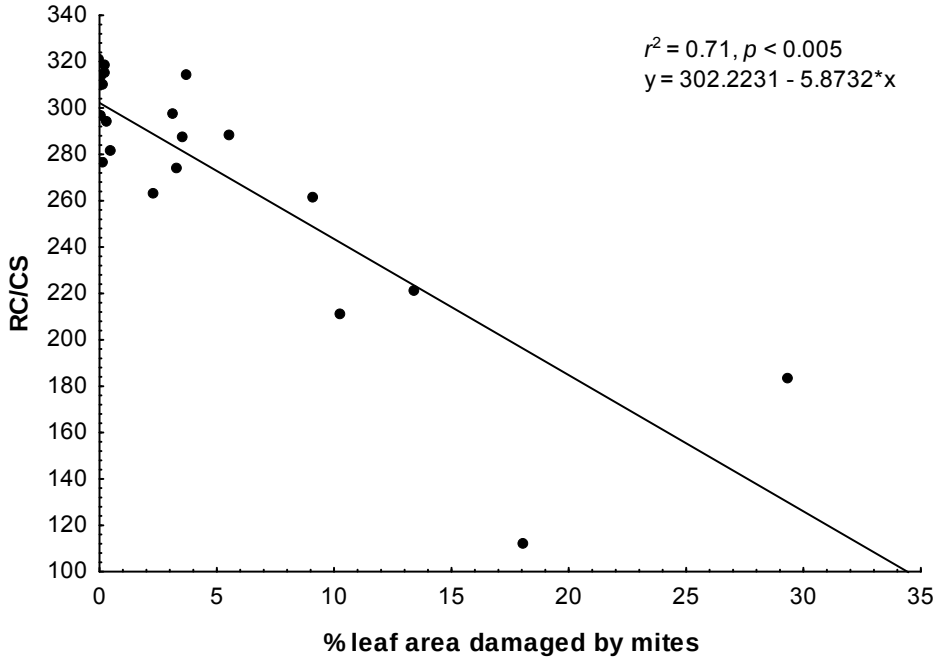

Figure 5.11. The density of functional reaction centres in PSII found per cross section of leaf area of dark-adapted water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

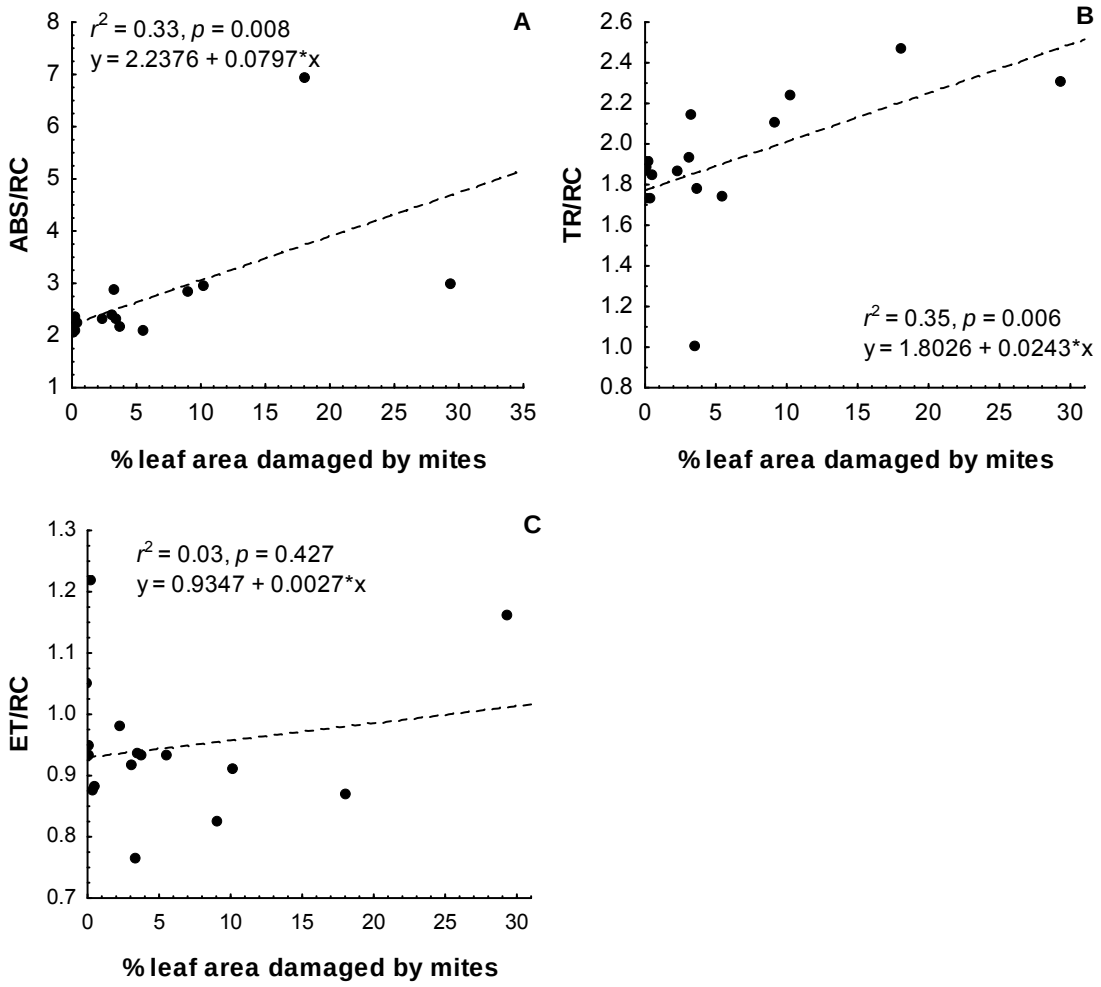

Figure 5.12. Fluorescence parameters of dark-adapted water hyacinth leaves (n = 14) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding: (A) Rate at which photos are absorbed by the photosynthetic antennae pigments in Photosystem II (ABS/RC); (B) Maximum rate at which plastoquinone (Q_A) is reduced (TR_0/RC); (C) Rate of electron transport (ET_0/RC). These fluorescence parameters are used to calculate the performance index (PI_{ABS}), which reflects the physiological state of a plant.

5.3.3. Leaf chlorophyll content and biomass

There was no correlation between the percentage of leaf area damaged by mite feeding and the leaf chlorophyll content (r^2 = 0.01, p = 0.661). This was surprising because leaf biomass decreased as mite damaged to leaves increased (Fig. 5.13).

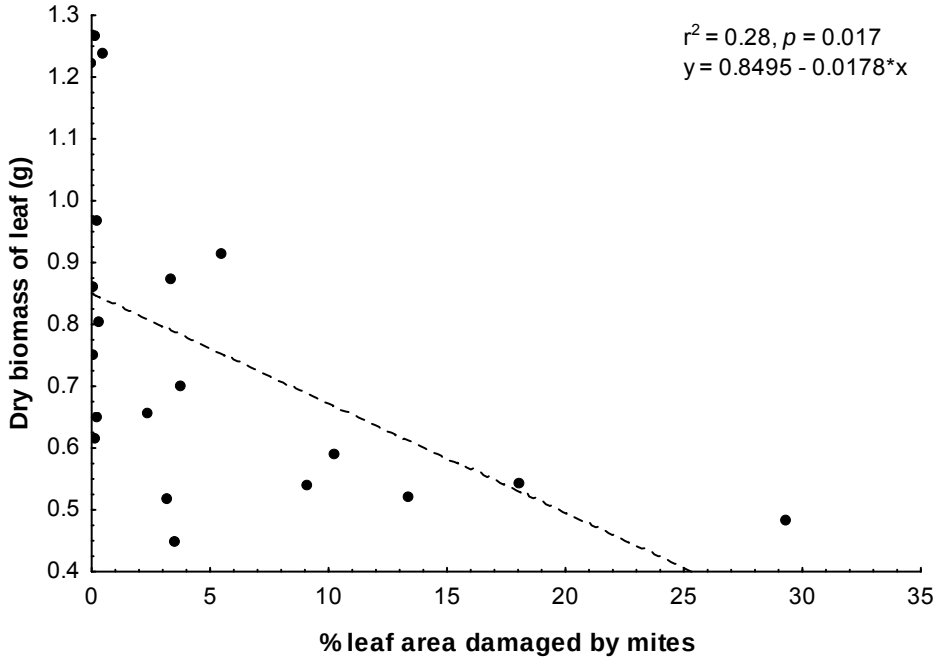

Figure 5.13. Dry biomass of water hyacinth leaves (n = 20) when the plants were exposed to varying levels of *Orthogalumna terebrantis* feeding.

5.4. DISCUSSION

Photosystem II performance decreased in plants with higher levels of damage caused by mite herbivory, as was indicated by the lowered CO_2 assimilation rate and altered fluorescence emission. Mite herbivory had a direct negative impact on PSII efficiency. This could be seen by the reduction in F_v/F_m, which is a useful relative measure of the maximum efficiency of PSII primary photochemistry: the F_v/F_m values of plants damaged by mites were generally below 0.83, a value below which plants are considered to be "unhealthy", indicating a negative effect on photochemistry (Baker, 2008), and is usually observed in plants exposed to stress, in this case stress due to mite herbivory. Since the original fluorescence data are used to work out the JIP-test parameters, the reduction in F_v/F_m was reflected in the reduction of the performance index (PI_{ABS}), which is a sensitive test of the physiological state of the plant (Strasser *et al.*, 2004). The reduction in PI_{ABS} was as a result of the reduced density of functional PSII reaction centres (RCs). A decrease in F_v/F_m is often explained by the inactivation of the RCs in PSII, since changes in F_v/F_m exhibit the same trends as changes in the density of RCs (Lu and Vonshak, 1999). These results are in agreement with those of Ripley *et al*. (2006) who noted a

similar decrease in the density of RCs of PSII on water hyacinth exposed to the mirid *E. catarinensis* relative to insect-free plants. In outdoor cultures of the filamentous cyanobacterium *Spirulina platensis* (Nordst.) Geitl. (Cyanophyceae), Lu and Vonshak (1999) observed a decrease in F_v/F_m as daily irradiance increased, and they deduced that this photoinhibition resulted in the inactivation of PSII reaction centres. While irradiance was kept constant during this study, it is possible that the damaged caused by the mites allowed more light to penetrate the laminae, leading to the photoinhibition of PSII, which inactivated the RCs.

The amount of energy absorbed per functional reaction centre (ABS/RC) increased with damage caused by mite herbivory. The expression ABS/RC can be interpreted as the average amount of chlorophyll which channels excitation energy into an RC, and can therefore be used as a measure for an average antenna size (Srivastava *et al*., 1998). Since the density of functional RCs was lower in plants with greater levels of damage, it was necessary for the RCs to compensate for the decrease in their numbers by increasing their antenna size and absorption capacity. Similarly, the observed increases in total trapping of light per functional RC (TR_0/RC), and in electron transport rates (ET_0/RC), found in this study, may also be explained as a result of the RCs compensating for their decreased numbers. The expression of TR_0/RC determines the rate by which an excited electron, called an exciton, that is trapped by an open RC causes Q_A to be reduced to Q^-_A. Therefore, TR_0/RC refers only to *active* (Q_A to be reduced to Q^-_A) centres. Since TR_0/RC is used to derive ABS/RC and ET_0/RC, these fluxes also only refer to active centres (Strasser *et al*., 2004). It must be stressed that an increase in ABS/RC means an increase in the apparent antenna size, not a structural increase of the antenna size of the biochemical complex i.e. the number of protein and chlorophyll molecules making up the antenna increases (Strasser *et al*., 2004). While working on the benthic cyanobacterium *Fischerella muscicola* (Thuret) (Cyanophyceae) Srivastava *et al*. (1998) found that when the RCs of PSII were inactivated due to the production of a secondary metabolite, named fischerellin A, the architecture of the PSII antenna changed and this had an effect on the movement of energy within the chloroplast. In this study, the energy fluxes increased as the level of mite herbivory on water hyacinth increased, and these changes may be as a result of the changing architecture of PSII antenna. The increases in the above flux ratios indicate that as the damage to plants increases through mite herbivory, so too does the light energy

absorbed by the plants. However, since both the photosynthetic rate and plant performance decreased, it would seem that either the absorbed energy is not being efficiently used by the functional RCs and is dissipated as heat instead, or the functional RCs are simply not able to compensate for the RCs that have been directly damaged by mite herbivory.

The effect of mite herbivory on chl-a fluorescence parameters was reflected in the altered gas exchange parameters. The negative effect of arthropod herbivory on water hyacinth photosynthetic rate (*A*) has previously been observed on plants damaged by the weevil *N. eichhorniae* (Ripley *et al*., 2008), and by the mirid *E. catarinensis* (Ripley *et al.*, 2006). Similarly, this study shows that water hyacinth plants that have been damaged through herbivory by *O. terebrantis* exhibit a decreased photosynthetic rate, and this rate decreases as the mite damage on the laminae increases. However, the correlation between photosynthetic rate and the leaf surface area damaged by the mite was not strong (r^2 = 0.29) due to the great variability in the photosynthetic rates of the control leaves. This may be because the leaves were taken from plants from different populations, or simply because not enough replicates were measured. Reductions in net photosynthesis are generally attributed to stomatal limitations i.e. when stomata impose resistance to the diffusion and the uptake of CO_2, specifically when plants are water-stressed (Thomas and Turner, 2001; Souza *et al*., 2004), saline-stressed (Jiang *et al*., 2006) or temperature-stressed (Fan *et al*., 2010). This is in contrast to the present study where *A* decreased despite a great increase in leaf stomatal conductance (g_l) as mite herbivory increased. This is not surprising because of the way in which the mites damage the laminae – the oviposition holes created by the females, and specifically the emergence holes created by the mites as they exit the galleries once they reach the adult stage, create large openings in the cuticle, which explains the increase in g_l, which in turn explains the observed increase in transpiration (*E*) in this study. In addition, as the mite nymphs eat away at the inner leaf tissue during their development, they create openings in the tissue and make the leaf more porous, thus making the movement of CO_2 inside the laminae less restricted. This explains the observed increase in intercellular CO_2 concentration (C_i) in the leaves damaged by mites during this study. Farquhar and Sharkey (1982) found that stomatal functioning changes the rate of CO_2 assimilation and the rate of transpiration, but stomatal closure does not necessarily reduce the availability of internal CO_2 or restrict water loss through transpiration. For

example, Tomczyk and Kropczyńska (1984) observed a decrease in transpiration (*E*) of chrysanthemum plants infested with the spider mite *T. urticae* although they observed no changes in stomatal functioning. On the other hand, a very strong correlation between *E* and g_l was observed in this study ($r^2 = 0.99$, $p < 0.001$). However, as described above, the increase in g_l is attributed to mite feeding, and it is not possible to differentiate between the effect of stomata and the effect of holes in the epidermis on leaf conductance.

Evapotranspiration, which is the loss of water from a water body through transpiration by plants, can be up to 3.2 times greater from water hyacinth than through simple transpiration by open water (Timmer and Weldon, 1966). In the present study there was a significant increase in transpiration (*E*) ($p < 0.05$; Fig. 5.5) as damage caused by mite feeding increased, even though the level of mite feeding never caused >30% damage to the laminae. In the field where mite feeding may cause up to 80% damage to the laminae during summer, the leaves eventually dry up and die (own observation). However, it would seem that at lower levels of damage, such as were examined in this study and which are sometimes seen in the field, particularly at the beginning of summer, *E* is greatly increased and is likely to cause additional water to be lost through evapotranspiration.

The net rate of CO_2 assimilation (*A*) is determined by the biophysical processes of CO_2 transport through the leaf and stomata, and also by the biochemical processes taking place inside the chloroplast, all of which are affected by environmental variables such as temperature and light (Sharkey *et al*., 2007). The response of *A* to the intercellular CO_2 concentration (C_i) is often used to quantify how the assimilation rate of CO_2 is limited by stomatal functioning (Long and Bernacchi, 2003), and usually an increase in C_i causes an increase in *A* (Sharkey *et al*., 2007). In this study, however, *A* decreased as C_i increased. This suggests that photosynthesis of water hyacinth damaged by mites is not indirectly limited by stomatal limitations imposed on CO_2 supply because the increase in C_i implies that the stomata and/or holes to the epidermis caused by mite feeding are allowing more CO_2 to be taken up by the plant. Rather, it seems that photosynthesis is affected directly by the destruction of photosynthetic leaf area caused by mite feeding.

The chlorophyll content of leaves did not change with the varying levels of mite herbivory examined in this study, and this is not surprising considering that the average amount of leaf area that was damaged by the mites was only about 15% of the entire leaf area. These results are consistent with those of Chapter 2, where the level of mite herbivory was similar to that of this study. At these relatively low levels of mite herbivory, the chlorophyll content of water hyacinth leaves is not affected, but damage caused by mite feeding does have an effect on the light reaction and photosynthetic rate. Water hyacinth normally exhibits no significant correlation between photosynthetic rate and chlorophyll content (Patterson and Duke, 1979), and this was also observed in the present study i.e. there was no correlation between F_0 and chlorophyll content (r^2 = 0.004, p = 0.79). These results are similar to those of Macedo *et al*. (2003) who found that the aphid, *Aphis glycines* Mutsamura (Hemiptera: Aphididae), at densities of > 20 individuals per leaflet had an effect on the photosynthetic rate of soybean but not on the chlorophyll content. Similarly, Tomczyk and Kropczyńska (1984) found that changes in the chlorophyll content of chrysanthemum plants damaged by the mite *T. urticae* were not proportional to changes in photosynthesis, in fact, they observed both a decrease in photosynthesis with an increase in chlorophyll content and vice versa. Tomczyk and Kropczyńska (1984) therefore concluded that a decrease in photosynthesis in mite-infested plants was not caused by a decrease in the chlorophyll content of leaves. A reduction in chlorophyll content due to herbivory has been related to reduced photosynthetic rates in other studies (Cockfield *et al*., 1987; Buntin *et al*., 1993; Haile and Higley, 2003; Ripley *et al*., 2006) but the damage caused by the insects or mites in these previous studies was usually greater than that caused by *O. terebrantis* in the present study. For example, in the study of Haile and Higley (2003) spider mite injury caused ±18% leaf chlorosis. While herbivory by *O. terebrantis* did not have an effect on chlorophyll content, the level of herbivory significantly affected leaf biomass accumulation in this study, as could be seen by the significant decrease in dry leaf biomass with an increase in mite herbivory. A similar significant decrease in leaf biomass due to *O. terebrantis* herbivory has been reported by Haq and Sumangala (2003). The leaves analysed during this study were of similar age (leaves 4 or 5 were used) and hence the decrease in biomass is unlikely to be an artifact of leaf age. A decrease in biomass due to herbivory in conjunction with a reduction in photosynthetic potential has been observed in other studies (Doyle *et al*., 2002; Ripley *et al*., 2006). In this study the removal of leaf tissue through mite feeding reduced leaf biomass directly, which offers

an explanation for the reduced light reaction performance and photosynthetic rate, since the leaf area where the light reaction and photosynthesis take place is being removed by the mites. The fact that here was no correlation between herbivory and F_0 or herbivory and chlorophyll content, suggests that the plant is compensating for mite feeding by increasing the chlorophyll content of the leaf area not damaged by mites.

5.5. CONCLUSION

This study shows that damage caused to water hyacinth laminae through *O. terebrantis* feeding decreases the plant's photosynthetic rate and light reaction performance, and this is better represented in the measurements of chl-a fluorescence than the measurements of gas exchange. It is encouraging that water hyacinth photosynthetic rates are reduced even at the low levels of damage examined in this study. Consequently, the negative impact of *O. terebrantis* herbivory on water hyacinth photosynthetic efficiency impacts plant health and, at high levels of mite herbivory, is likely to impact plant growth. Interestingly, the reduction in plant performance appears to be independent of leaf chlorophyll content, implying that the reduction of the photosynthetic rate and the light reaction performance is not simply due to the reduced leaf area i.e. reduced chlorophyll content. This study therefore adds to recent literature which supports the hypothesis that herbivory decreases a plant's photosynthetic ability more so than can be explained by the direct removal of photosynthetic surface area (Zangerl *et al.*, 2002; Aldea *et al.*, 2005; Ripley *et al*., 2008; Nabity *et al.*, 2009).

CHAPTER 6

Thermal tolerance of *Orthogalumna terebrantis* and the effect of temperature on its establishment in South Africa

6.1. INTRODUCTION

Climatic incompatibility of biocontrol agents to their areas of introduction is thought to be responsible for about forty per cent of establishment failures (Crawley, 1986). This is because the physiological tolerance of a species to environmental stresses, such as to temperature and water stress, is a major contributing factor to successful establishment after introduction to a new environment (Chown *et al.*, 2007). If a biocontrol agent has a restricted distribution in its native range then it is likely to have a narrow climatic tolerance which limits the ability of the agent to control an invasive plant over its whole distribution, especially if the weed itself has a wide climatic tolerance (Dhileepan *et al*., 2005; McClay and Hughes, 2007). Therefore, it is preferable that biocontrol agents can survive at a correspondingly broad range of climatic conditions and are able to reproduce and develop in synchrony with the target pest throughout the year (Caltagirone, 1981). Knowing the temperature range within which an organism is able to survive and reproduce therefore allows predictions to be made about where that organism will thrive.

Considerable time and money would be saved if there was some indication of an agent's thermal tolerance prior to releasing it into a new location (Byrne *et al.*, 2004;

Hughes *et al.*, 2009). Therefore, information about an organism's thermal physiology is important so that effort is not wasted on introducing agents into areas where they will not survive, or where they will perform poorly. In post-release studies, knowing the temperature tolerance of an agent may give some indication as to why the agent has established at some sites but not at others.

In order to establish successfully in a new habitat, a biocontrol agent may not only have to survive harsh conditions, such as freezing temperatures during winter, but there must also be an adequate thermal budget i.e. number of degree-days above a developmental threshold, for development and reproduction to take place (Hart *et al.*, 2002). In cold climates, therefore, cold temperatures limit the agents by not only causing mortality directly, but also by slowing down development and hence population growth. Therefore, combining climatic data with studies on the effect of temperature on arthropod reproductive and development rates is vital because this determines the number of generations that can be completed at a specific site (Stamou, 1989; McClay, 1996; Byrne *et al*., 2004; Hatherly *et al.*, 2004; Coetzee *et al*., 2007b; Pappas *et al*., 2008; Robertson *et al*., 2008), which in turn affects the density of a species in a particular population (Bursell, 1964).

Physiological responses to environmental changes take place over a range of time scales, from rapid phenotypic modifications to long-term evolutionary changes (Brakefield *et al.*, 2003; Chown and Nicolson, 2004). Phenotypic plasticity refers to the ability of an organism to change its form, movement, state or rate of activity in reaction to an environmental stimulus (West-Eberhard, 2003). The effect of temperature on the physiological responses of arthropods, such as survival, development, and feeding rates has been extensively reviewed (Bursell, 1964; Clarke, 1967; Cloudsley-Thompson, 1970; Chown and Terblanche, 2007; Bowler and Terblanche, 2008). Phenotypic responses (plasticity) to temperature can be determined by means of thermal tolerance measurements such as a) rapid heat/cold hardening, b) acclimation c) heat/cold shock or d) supercooling (Chown and Terblanche, 2007) and they are obtained either by exposing an organism to variable temperatures for a short time (a few minutes), or by exposing an organism to a set temperature for variable periods of time (a few hours). For example, hardening occurs within a few hours (usually 1-3 h) after exposure to extreme temperatures, while acclimation takes longer (a few days to weeks), generally at exposure to slightly lower-than-rearing temperatures (Sinclair and Roberts, 2005).

Much debate has arisen about whether the terms ‘acclimation’, ‘hardening’ and ‘shock' can be separated from each other as discrete categories or whether they act in continuum (Bowler, 2005; Loeschcke and Sørensen, 2005; Sinclair and Roberts, 2005). This is because the words used to describe both the responses and the treatments are very similar (Chown and Terblanche, 2007). The “Glossary of Terms for Thermal Physiology” (*Journal of Thermal Biology* 23: 75-106, 2003) define acclimation and heat shock as follows: acclimation refers to the physiological and behavioural changes occurring within an organism which reduce the strain or enhance endurance of strain caused by experimentally inducing stressful changes in particular climatic factors, and heat shock refers to a rapid, short acting molecular process associated with the synthesis of several families of heat shock proteins (Hsps) produced as a result of acute short sub-lethal heat injury (Bowler, 2005). The term ‘hardening’ is not defined in the journal but hardening usually occurs after short-term treatments (minutes to hours) at severe stress conditions. The term was first used in relation to plants and referred to “a quick, transitory ‘adaptation’ to an extreme temperature (hot or cold) that followed brief exposure at a sub-lethal temperature” (Bowler, 2005).

Acclimation (the adjustment of reversible physiological traits in response to changes in a single environmental variable in the laboratory) and acclimatization (physiological responses to environmental variables in the field) (Wilson and Franklin, 2002) refer to phenotypic changes in an individual in response to, or in advance of, a change in the environment (Huey *et al.*, 1999). The process of acclimatization is especially important during seasonal changes in climate because it allows organism to persist in fluctuating temperatures, sometimes under conditions that would otherwise prove fatal (Hoffmann, 1995). Many physiologists support the beneficial acclimation hypothesis (BAH) which states that acclimation is beneficial because an organism that has had the opportunity to acclimate to a certain environment has a performance advantage in that environment over another organism that has not had the opportunity to acclimate (Leroi *et al.*, 1994). For example, fruit flies, *Drosophila buzzatii* (Patterson and Wheeler) (Diptera: Drosophilidae), that had been acclimated to high temperature (by exposure to 38 ºC for 75 min) showed a greater resistance to heat shock (exposure to 41.9 ºC for 90 min) than individuals that had not been acclimated (Krebs and Loeschcke, 1996). On the other hand, the BAH has recently been critically scrutinized (Hoffmann, 1995; Huey *et al*., 1999; Wilson and Franklin, 2002) as a number of studies have found that acclimation is not necessarily beneficial (Zamudio *et al*., 1995; Sibly *et al*., 1997; Gibert *et al*., 2001; Stillwell and

Fox, 2005). For example, Bennett and Lenski (1997) measured the relative fitness of the bacterium *Escherichia coli* Migula (Enterobacteriaceae) acclimated to various thermal environments and found that acclimation was beneficial in only 7 out of 12 comparisons.

The physiological responses to rapid heat and cold hardening are not well understood and they differ mostly with respect to the time it takes for the response to occur, and the duration of the response (Chown and Terblanche, 2007). Briefly, rapid cold hardening only occurs in species from temperate and alpine areas (Chown and Terblanche, 2007). Some species e.g. the flesh fly *Sarcophaga bullata* Parker (Diptera: Sarcophagidae) produce glycerol during cold hardening (Yoder *et al*., 2006), while others e.g. the fruit fly *D. melanogaster* (Meigen) (Diptera: Drosophilidae) do not (Kelty and Lee, 1999). Rapid cold hardening, which can be induced either by a brief exposure (minutes to hours) to low temperatures or by the gradual cooling of an experimental organism over a range of temperatures, results in an increase in survival after cold shock exposure (Chown and Nicolson, 2004). On the other hand, heat-shock proteins (Hsps) are produced in response to both high and low temperatures experienced during heat/cold hardening, respectively, but the type of the stress (heat or cold stress) determines which specific proteins are induced (Joplin *et al*., 1990). In addition, the synthesis of Hsps in response to high temperatures ceased immediately after removal from high temperatures (Yocum and Denlinger, 1992) while Hsps synthesis may continue for days after exposure to cold temperatures (Joplin *et al.*, 1990). As with cold hardening, heat hardening at non-lethal high temperatures enables survival at even higher temperatures (Dahlgaard *et al*., 1998).

Terrestrial arthropods have evolved two general strategies to survive low temperatures, namely freeze tolerance and freeze intolerance or avoidance (Salt, 1961). Freezing intolerant species are now more commonly referred to as freeze avoiding species, since they have to avoid freezing in order to survive (Coulson and Bale, 1996). Most insect and mite species are freezing intolerant (Broufas and Koveos, 2001; Chown and Nicolson, 2004) such that the formation of ice within their tissue is fatal but is avoided through supercooling (Salt, 1961). Supercooling refers to the ability of an arthropod to depress the freezing point of its body fluids so as to keep them in a liquid state and thus avoid freezing at sub zero temperatures, and is therefore a direct measure of cold hardiness (Bale, 1987). However, many species are susceptible to chilling injury or death even when ice formation does not take

place (Kelty and Lee, 1999). A supercooling point (SCP) is reached at a temperature when the arthropod eventually freezes and dies (Sinclair *et al*., 2006). Besides supercooling to avoid freezing, freeze intolerant/avoiding species seek out overwintering sites where the microclimate temperature is distinctly different to the air temperature (Bale, 1987). For example, the beech leaf-mining weevil, *Rhynchaenus fagi* L. (Coleoptera: Curculionidae), overwinters in leaf litter on the forest floor or in the aerial canopy of conifers, where the temperature is usually > 3 ºC warmer than the temperature at ground level (Bale, 1987). Freeze intolerant species usually occur in areas where there is a low number of freezing days (Chown and Terblanche, 2007). In contrast, freeze tolerant species usually occur in areas with extremely low temperatures (Chown and Terblanche, 2007).

Temperature influences the metabolic rate, fecundity, growth rate and longevity of all organisms (Clarke, 1996). *Eichhornia crassipes* and the invertebrates associated with it originate in tropical South America (Edwards and Musil, 1975), and the weed as well as some of its natural enemies (e.g. *Cornops aquaticum* (Burner) (Orthoptera: Acrididae)) occurs as far south as Patagonia (Adis *et al*., 2007) where the temperature drops to below 0 ºC during winter (Paruelo *et al*., 1998). Water hyacinth is highly cold tolerant and only dies after 24 hrs exposure at -16 ºC (Owens and Madsen, 1995). Byrne *et al*. (2010) recorded a cessation of plant growth at water temperatures between 9.5 ºC and 10.7 ºC. Whilst water hyacinth is tolerant of cold temperatures the invertebrates that feed on it are generally less cold tolerant. For example, *E. catarinensis* has a critical thermal minimum of 1.2 ºC (Coetzee, *et al*. 2007b) and *N. eichhorniae* of 4.3 ºC (Coetzee, unpub.). In South Africa, the weed grows at sites that differ widely in their temperature, rainfall and frost conditions, but none of the sites experience temperatures low enough to kill the plant (Byrne *et al.*, 2010). On the other hand, winter minimum temperature in most regions of South Africa frequently fall below the developmental threshold temperatures of the *Neochetina* weevils and the mirid, while frosting events, which are prevalent throughout much of the interior regions may cause up to 70% mortality of the adult weevils (Byrne *et al*., 2010). As such, the South African climate is never cold enough to cause mortality to water hyacinth but may cause mortality to the biocontrol agents. Additionally, heavy frosting causes the aerial parts of water hyacinth to turn brown and die back (Owens and Madsen, 1995), which leads to a loss of habitat and food supply to the agents that overwinter on aerial parts of the plant i.e. nymphs of *O. terebrantis* (own observation), and thus results in increased mortality of the agents. Byrne *et al*. (2010) predict that *Neochetina* eggs laid prior to a frosting event as well

as the 1st and 2nd instars which occupy the upper portions of laminae are likely to be killed in a frosting event. Therefore, Byrne *et al*. (2010) predict that only the third instar larvae, which occupy the crown of the plant, overwinter successfully and can contribute to the weevil population after the winter. Heavy frosting has been known to cause local extinctions of other insect species, for example, Ehrlich *et al*. (1972) showed how the loss of a host plant due to unusual freezing temperatures in the Rocky Mountains resulted in the local extinction of the butterfly *Glaucopsyche lygdamus* Doubleday (Lepidoptera: Lycaenidae).

Some of the worst water hyacinth infestations in South Africa are found at sites where frost during the winter months is not unusual and causes many of the plants to die (Hill and Olckers, 2001). However, Byrne *et al.* (2010) conducted an 18-week long study on the growth of water hyacinth, from June to December, at one of the coldest sites in South Africa (Delta Park, Johannesburg, Gauteng Province), and found that, unless the water temperature fell below 7.5 ºC, the weed produced leaves even during winter months with an average of 0.48 leaves produced per week, for the duration of the study. More importantly, the study also showed that the plant was able to reproduce asexually during winter, with an average of 1.5 ramets produced during the study. In contrast, the *Neochetina* weevils stop laying eggs between 12-15 ºC, adults go into a complete reproductive diapause and their feeding rates decrease in colder temperature (Byrne *et al*., 2010), such that the plant's growth during winter is almost completely unhindered by biological control. Cold winters therefore seriously hamper the efficacy of biological control because low temperatures slow down the development of the agents, and frost damages the leaves of the plant to the extent that they fall off, which means that the site for feeding and oviposition of the agents is removed (Byrne *et al*., 2010).

Hill and Olckers (2001) and Hill and Cilliers (1999) have for many years thought that the asynchrony in population growth between water hyacinth and its natural enemies is a limiting factor in the control of the plant in South Africa. Recently, Byrne *et al*. (2010) used a degree-day model which showed that the speculations of the above authors were correct. Briefly, a degree-day model estimates the amount of heat that accumulates above a specific temperature threshold, so that for every degree the mean daily temperature is above a specific lower developmental threshold, one degree-day accumulates (Herms, 2004). Byrne *et al*. (2010) used the degree-day model (where they use a water temperature of 10 ºC to calculate leaf production rates, and a developmental threshold of 7.6 ºC and 10.8 ºC for pupal and larval

development, respectively, for the *Neochetina* weevils) and worked out that with the onset of spring, water hyacinth would have added 7 new leaves before the weevils even pupated, and the new generation of weevils would emerge only 3 months after the plants started to grow. Thus, the model predicts a 42 day lag period between the onset of water hyacinth growth and the time that adult weevils emerge from overwintering larvae and subject the plant to adult herbivory. At this stage the feeding damage is restricted to the leaves and has a negligible affect on the plant because of the initial small weevil population surviving winter. Since it is the larvae that are the most damaging to water hyacinth (DeLoach and Cordo, 1976), the weevils only begin to have a significant impact on the weed once the first instar F_1 generation hatches roughly 62 days later. Thus, while water hyacinth starts to re-grow and quickly increases its population through the production of ramets as the temperatures rise with the onset of spring, the resurging control agents have to build up their populations from substantially low numbers and as a consequence, the agents only reach damaging levels at the end of summer (Byrne *et al.*, 2010) resulting in a lag period during which time the weed grows virtually free from biological control. The variable success that biological control of water hyacinth has had at high altitude sites in South Africa may thus be explained by the unsuitability of control agents to their new climate, although other factors, such as high water nutrient levels and mismanagement of water bodies, also play a role.

Insect and plant distribution limits can be determined by examining their differential survival ability at temperature extremes, which can be estimated from laboratory studies (Chown and Terblanche, 2007). Insect thermal limits can be determined by two widely used methods, namely, critical thermal temperatures (CT_{Min} and CT_{Max}) and lethal temperatures (upper and lower LT_{50}). Determining critical thermal temperatures involves cooling or heating an insect from a selected starting temperature until physiological failure is reached (Terblanche *et al*., 2007). Critical thermal temperatures are reached at a point short of death when the insect loses locomotory function, and so becomes vulnerable to predation and environmental conditions which may lead to death, but from which recovery is still possible (Mitchell *et al*., 1993). The onset of chill coma takes place at the critical thermal minimum, at which time the metabolic rate drops (Sinclair and Roberts, 2005). Chill coma thus coincides with the temperature at which nerve and muscle function is lost (Goller and Esch, 1990). Lethal temperatures are physiological tolerance limits for survival when an insect is exposed to high or low temperature for a prolonged period, after which recovery is impossible (Cloudsley-Thompson, 1970). The upper and lower lethal

temperatures for many arthropods are reported in Cloudsley-Thompson (1970), and are highly variable between species, stages of development, relative humidity and acclimation (Fields, 1992). Thermal limits are influenced by factors such as age, feeding status and gender (Chown and Nicolson, 2004; Bowler and Terblanche, 2008). For example, in the Mediterranean and Natal fruit flies *Ceratitis capitata* (Wiedemann) and *C. rosa* Karsch (Diptera: Tephritidae), the CT_{Max} of the adult life-stage increased significantly with age up to 14 days, and feeding improved both CT_{Max} and CT_{Min} significantly, but thermal tolerance was similar for males and females (Nyamukondiwa and Terblanche, 2009). In addition, recent work has shown that basic experimental design has a noticeable effect on the outcome of thermal measurements (Chown *et al*., 2009; Terblanche *et al*., 2007). For example, Terblanche *et al*. (2007) found that faster rates of temperature change resulted in greater thermal tolerances in the tsetse fly *Glossina pallidipes* Austen (Diptera: Glossinidae); between heating/cooling rates of 0.06 ºC min^{-1} and 0.25 ºC min^{-1} there was a 5 ºC variation for CT_{Max} and a 10 ºC variation for CT_{Min}, and when start temperatures were high the CT_{Max} values were highest while when they were low the CT_{Min} values were lowest. It is therefore important to verify and compare the basic experimental designs of thermal tolerance studies before comparisons of critical temperatures and lethal limits are made, since the various experimental approaches used substantially affect the conclusions drawn from specific studies. While there are advantages to using standardized methods for comparing species, Sinclair (2001) strongly recommends that investigations of thermal limits be conducted using environmentally relevant conditions.

Working with mites is difficult because of their size, and consequently mites have been largely understudied in terms of their physiological responses to environmental conditions. In general, mite longevity is influenced by temperature, humidity and food availability (Walter, 2009) and mites experience shorter life spans under favourable conditions, for example, when food is scarce the females of the predatory soil Mesostigmata can fast for months with little mortality but when food is plentiful the adult lifespan is only a week long (Walter and Proctor, 1999).

Very few studies have examined the physiological responses of *O. terebrantis* to variable temperatures. Cordo and DeLoach (1976) examined the lethal limits of adults by exposing them to various temperatures between 43 ºC and -10 ºC for a duration of either 1, 4 or 8 hours. They found that the lethal high temperatures for the adult mites were between 39 ºC and 43 ºC, with survival decreasing as temperatures

and exposure time increased, and 100% mortality was observed at exposure to 43 ºC for 1 h. Lethal low temperatures were between 3 ºC and -10 ºC; only 10% of the mites died at 0 ºC even after 8 h exposure, at -5 ºC roughly 50% of the mites died and 96% mortality occurred at -10 ºC after exposure of 1 h. Delfosse (1977b) observed the oviposition, development, mortality and feeding of *O. terebrantis* inside incubators set at four temperature regimes (5-25º, 10-30º, 15-35º and 20-40 ºC) where the lower of the two temperatures at each regime (e.g. 5, 10, 15 and 20º C) corresponded to 14 h dark phase, and the higher of the two temperatures corresponded to a 10 h light phase. The mites were placed onto fresh water hyacinth laminae that were singly inserted into a 7ml test tube containing tap water. The test tubes containing the laminae with the mites were then placed into pans that were covered with glass plates to prevent mites escaping and also to keep relative humidity over 90%, and data were collected weekly for 10 weeks. The study showed that the two extreme regimes (5-25 ºC and 20-40 ºC) were the most unfavourable to mite development with the lowest numbers of emerged adults, and 100% mortality of adults occurred in the 20-40 ºC regime, at the end of the experiment. Most eggs were laid at 20-40 ºC while the highest number of larvae and nymphs developed to adults at 10-30 ºC. The two extreme regimes were also the most unsuitable for feeding. The mite mortality data of Delfosse's study (1977b) compared well to that of Cordo and DeLoach (1976). However, some aspects of the methods in the Delfosse study are unclear: (a) were the laminae replaced during the study? It is unlikely that a lamina removed from a plant would be in a good condition for the entire duration of a 10 week study as even whole water hyacinth plants kept in growth chambers look withered after two weeks (own observation); (b) how was oviposition measured? The oviposition holes of the females are only 0.1 mm in diameter (Cordo and DeLoach, 1976), and so a microscope would have had to be use to count them, implying that the laminae would have had to be removed from the incubators while the measurements were made, which would likely have disrupted the temperature regimes that the laminae were being kept at, and (c) how was development measured? The development of larvae and nymphs inside galleries can be measured by measuring the length of a gallery since galleries get longer as immatures develop (Delfosse, 1978a). In his 1977(b) study, Delfosse does not explain at what point (i.e. length of gallery) he measured "development". Since observations were made weekly, were galleries marked once they had been counted so that they would not be recounted the following week? Similarly, were the emerged adults removed each week so that they would not be recounted the following week? If not, then the calculated % mortality would be higher than actual mortality. Nevertheless, the above

studies provide a basis for a number of physiological responses (e.g. development, survival) of *O. terebrantis* to variable temperatures. From these studies it would seem that *O. terebrantis* is tolerant of a wide range of temperatures, and it is therefore unlikely to experience temperatures that would limit its survival in any region of South Africa.

Previous studies that have examined some aspects of *O. terebrantis* physiology have provided limited results, and the methods used in some are vague. This chapter therefore determines the critical thermal limits and lethal temperatures of *O. terebrantis* using standard, well-defined methods (Mitchell *et al.* 1993; Chown *et al*., 2009) and consequently adds to the results of previous studies. The first population of *O. terebrantis* was recorded in South Africa in the late 1980s and mite populations are now recorded at a number of the water hyacinth infestations around the country. The mites have thus been acclimatized to the weather conditions experienced South Africa. Therefore, the results of this study are related to ambient temperatures experienced in South Africa to verify whether temperature has influenced the establishment of the mite in the country.

6.2. MATERIALS AND METHODS

6.2.1. Critical thermal limits

The methods of Mitchell *et al.* (1993) and Chown *et al*., (2009) were modified to determine the upper and lower critical thermal limits (CT_{Max} / CT_{Min}). Adult mites were collected from the stock cultures at the PPRI, Pretoria, Gauteng Province, during the summer and were used in the experiment immediately after collection. The mites were therefore acclimatized to Pretoria summer temperatures (Dec 2008 - Feb 2009 mean daily maximum = 28.76 ± 1.57 ºC; mean daily minimum = 19.93 ± 0.67 ºC; South African Weather Bureau).

Ten adult *O. terebrantis* were placed individually into 5 ml air-filled glass vials that were closed with a tight-fitting plastic lid. For the CT_{Max} measurements a small (0.5 x 0.5 mm) piece of water hyacinth leaf was also placed into the vial to increase humidity as *O. terebrantis* mortality increases in the absence of moisture (Perkins, 1973). The vials were submerged in a Julabo F32 programmable water bath (0.1 ºC accuracy) (Julabo Co., Seelbach, Germany). The vials were fitted into a polystyrene rack in such a way that the lids were above water while the vials were in the water,

and the entire polystyrene rack kept the vials afloat. The vials were small enough to ensure that the temperature inside the vials was equivalent to that of the water bath, and this was checked by means of a thermocouple inserted into a separate vial. The programmable water bath initially heated the vials containing the mites from room temperature to 35 ºC over 5 minutes. Thereafter, the temperature was increased progressively from 35 ºC to 52 ºC at 1 ºC min^{-1}. Because of their small body size (<0.5 mm in length), the body temperature of the mites was considered to be the same as that of the chamber (Terblanche *et al*., 2007). The vials were removed briefly at each temperature stage to check visually for impaired functioning in locomotion, indicated by the mites falling to the bottom of the vial i.e. no longer walking on the sides or top of the vial. The temperature at which locomotory function was lost by each individual mite was recorded. The experiment was repeated four times using different individuals, and the combined data (n = 40) was used to calculate the CT_{Max} and its standard deviation. For measurements of the CT_{Min} the water inside the water bath was replaced with 50% antifreeze liquid. The temperature of the vials containing the mites was initially decreased from room temperature to 15 ºC over 5 minutes. Thereafter, the temperature was lowered progressively from 15 ºC to -3 ºC at 1 ºC min^{-1}. The temperature at which each individual mite fell to the bottom of the vial was recorded and the experiment was repeated six times, using different individuals (n = 60). The CT_{Min} was calculated in the same way as the CT_{Max}.

The starting temperatures were kept at 35 ºC and 15 ºC for the CT_{Max} and CT_{Min} measurements, respectively, for each repeat, to ensure that variations in the starting temperature would not influence the outcome of the experiment (Terblanche *et al*., 2007). The rate of temperature change was kept constant during the experiment since variable rates of temperature change affect the results (Chown *et al*., 2009). The rate of 1 ºC min^{-1} used in this study is the standard rate used for measurements of CT_{Min}/CT_{Max} and thus allows for comparable results. The rate of 1 ºC min^{-1} is now considered to be a low rate (Chown *et al*., 2009; Nyamukondiwa and Terblanche, 2009), but it allows for comparisons with other studies.

6.2.2. Lower and upper lethal temperatures

Lethal temperature studies were also conducted in the summer and adult mites collected from stock cultures at the PPRI, Pretoria, were used in the experiment immediately after collection. The methods of Mitchell *et al*. (1993) and Cordo and DeLoach (1976) were modified to conduct the experiment.

The lower lethal temperature (LT_{50}) was determined by placing 20 adult mites into the sealed air-filled glass vials as above, and placing them into the water bath, filled with 50% antifreeze liquid, at experimental temperatures ranging from 0 ºC to -6 ºC. The temperature of the mites inside the vials was initially lowered from room temperature to 0 ºC, in the water bath, over 20 minutes. Thereafter, the mites were exposed to each experimental temperature for two hours. Different adults were used for each experimental temperature trial (n = 20 for each experimental temperature used). After two hours at each experimental temperature the vials were removed from the water bath and the mites were gently taken out of the vials, using a fine camel-hair brush, and placed onto a piece of water hyacinth leaf (4 x 4 cm). The piece of leaf was placed inside a petri dish, closed with a lid, and left for one hour at room temperature. Insects are usually given one hour to "self right" i.e. turning back onto their feet after being turned onto their dorsal surfaces. Even when working very carefully, turning mites onto their backs is difficult, and often the mites get injured in the process. Therefore, after an hour at room temperature, the mites were observed under a microscope and were counted as "viable" either when they were walking around or when they were lying on their backs with their legs visibly moving. The number of individuals that were "viable" was recorded.

The upper LT_{50} was similarly determined. The vials containing the mites were initially heated up inside the water bath from room temperature to 35 ºC over 20 minutes. Thereafter the mites were subjected to a range of experimental temperatures, ranging from 35 ºC to 47 ºC. After exposure to each temperature for 2 hours, the mites were removed from the water bath and after an hour at room temperature the number of individuals that were "viable" was recorded, as described above. Probit analysis (Mitchell *et al.*, 1993) was used to calculate the lower and upper lethal temperatures.

6.2.3. Establishment of the mite in South Africa

Temperature data collected over two years from 15 water hyacinth sites (Byrne *et al*., 2010) was used to determine both the mean daily minimum temperatures for July, being the coldest month of the year in South Africa, and the mean daily maximum temperatures for February, being the hottest month of the year in South Africa. The temperature was recorded hourly within three microsites at each field location using Thermochron iButtons (DS1921G; Maxim Dallas Semiconductor Corporation). Using these data, it was determined that at sites where mites were present the average

minimum canopy and ambient air temperature during July was 2.36 ºC and 2.45 ºC, respectively, and the average maximum canopy and ambient air temperature during February was 37.8 ºC and 40.5 ºC, respectively. Using ARCGIS v. 9.3 a map was produced indicating areas where the mean daily minimum temperatures for July were 3 ºC or below. The temperature of 3 ºC was chosen because the CT_{Min} of *O. terebrantis* calculated in the critical thermal limits experiment was 3.1 6 ºC ± 0.52 ºC (see results). Since the locomotion of an organism is impaired at critical thermal limits, it is the temperature at which the mites would be most susceptible to external danger e.g. inability to seek out shelter from the cold and/or predation because their locomotion is affected and possibly falling off the plants into water as their grip on the surface of the plant may also be affected. The CT_{Max} values were above the mean daily maximum temperature and were therefore unlikely to be limiting.

On the map, all known sites where water hyacinth has been recorded as naturalized, according to the information in the South African Plant Invaders Atlas (SAPIA) database (Henderson, 1998), were shown. Similarly, the latest data from the Rhodes University Aquatic Weeds Monitoring Survey (D. Schlange pers. comm.) and data from the Integrated Management of Water Hyacinth in South Africa database (Byrne *et al*., 2010) were combined to show the sites where *O. terebrantis* has been recorded on water hyacinth infestations in South Africa.

6.3. RESULTS

6*.3.1. Critical thermal limits*

The temperature at which adult *O. terebrantis* lost locomotory function varied between 37 ºC and 52 ºC for the maximum critical temperatures, and between 0 ºC and -9 ºC for the minimum critical temperatures. Adults can therefore potentially remain active over a 62 ºC temperature range. The adults thus have a mean CT_{Max} of 44.52 ºC ± 0.47 ºC (n = 40) and a mean CT_{Min} of 3.1 6 ºC ± 0.52 ºC (n = 40).

6.3.2. Lower and upper lethal temperatures

The range of temperatures at which 100% mortality of *O. terebrantis* occurred were below -6 ºC and above 46 ºC. After two hours of exposure to the experimental temperatures, all adults remained alive between 0 ºC and 40 ºC. The lower and

upper LT_{50s} were calculated to be -4.28 ºC (Fig. 6.1) and 41.19 ºC (Fig. 6.2), respectively.

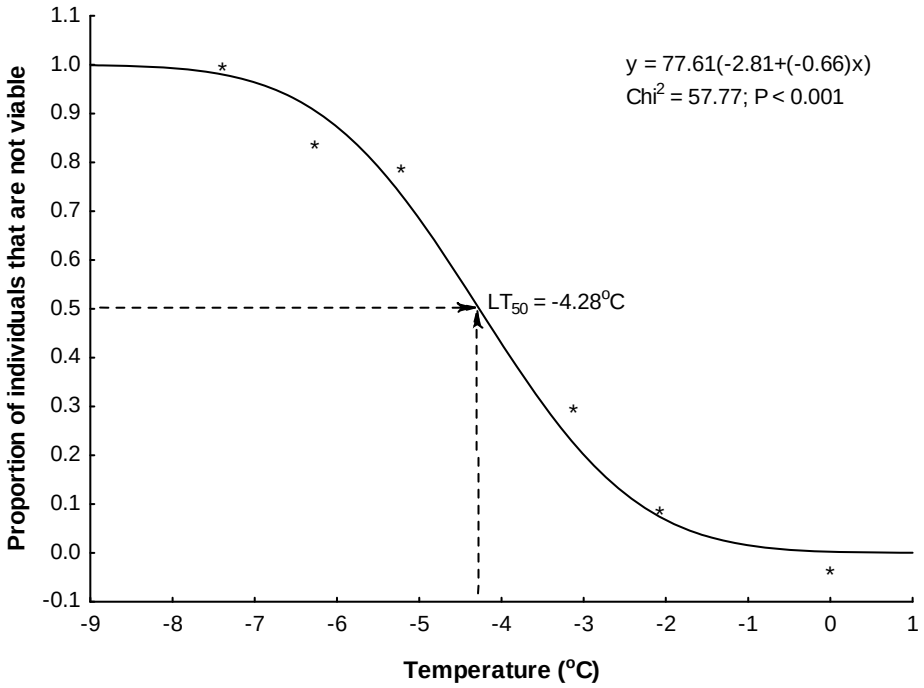

Figure 6.1. The lower lethal temperature of *Orthogalumna terebrantis* as determined by exposure to experimental temperatures for 2 hours. Probit analysis was used to calculate the LT_{50}. The * symbol indicates the actual data points used to calculate the LT_{50}.

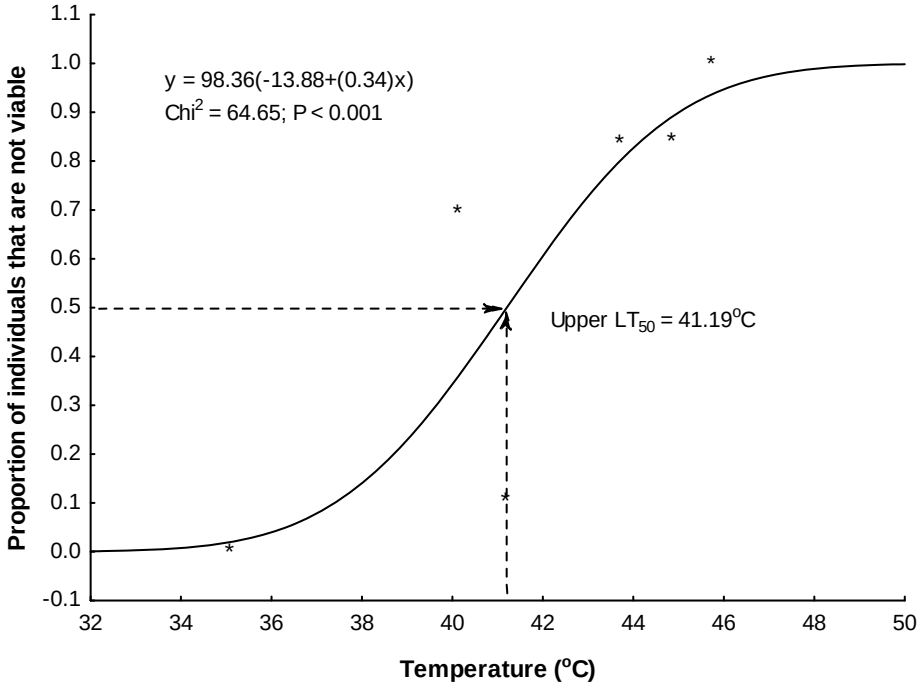

Figure 6.2. The upper lethal temperature of *Orthogalumna terebrantis* as determined by exposure to experimental temperatures for 2 hours. Probit analysis was used to calculate the LT_{50}. The * symbol indicates the actual data points used to calculate the LT_{50}.

6.3.4. Establishment of the mite in South Africa

The areas where the mean daily minimum temperatures during July are 3 ºC or below are generally found inland (white section in Figure 6.3). 43 out of 66 of the recorded water hyacinth infestations occur in the warmer coastal regions. *Orthogalumna terebrantis* is established at 17 of the recorded water hyacinth sites in South Africa, the majority of which are found in the Mpumalanga Province and the North West Province. There are some sites inland where temperatures may reach below 3 ºC at which mites have been recorded, for example, at the Roodeplaat Dam in Pretoria, Gauteng Province, and the Rooikoppies Dam which is ± 60 km north-west of Pretoria.

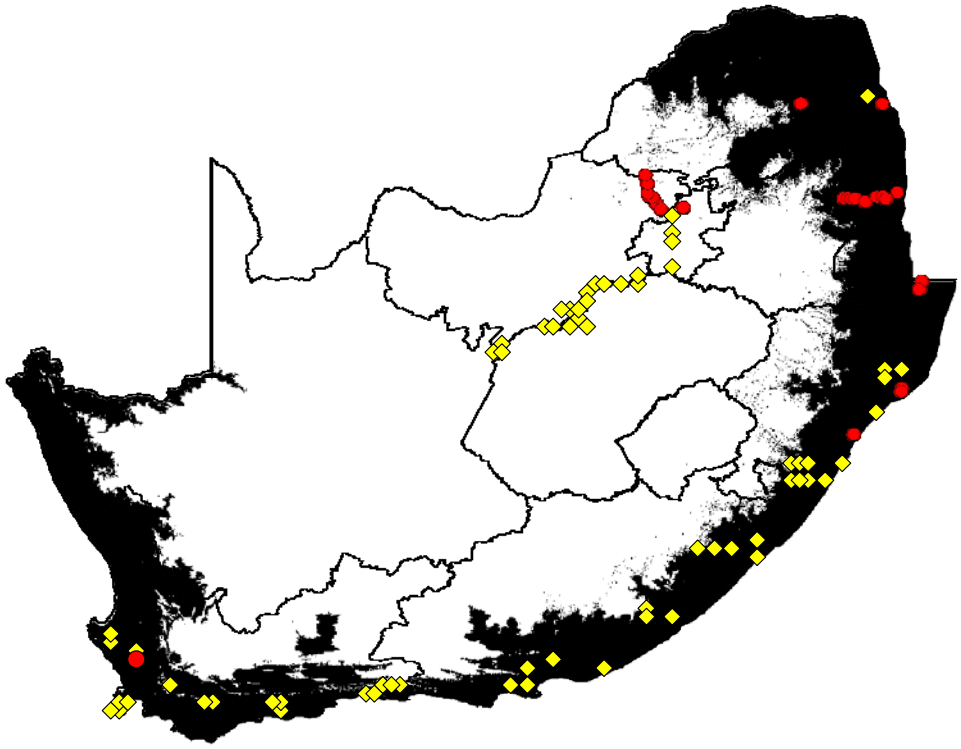

Figure 6.3. Map of South Africa showing areas where the mean daily minimum temperature during July is above (black) and below (white) 3 ºC. The diamond shapes indicate recorded sites of water hyacinth infestations (as obtained from the Southern African Plant Invaders Atlas (SAPIA) database, (Henderson, 1998)), and the circles indicate sites at which *Orthogalumna terebrantis* is recorded to be established (as obtained from the Rhodes University Aquatic Weeds Monitoring Survey database (D. Schlange) and the Integrated Management of Water Hyacinth in South Africa database (Byrne *et al*., 2010)).

6.4. DISCUSSION

The lower lethal temperature (-4.28 ºC) of adult *O. terebrantis* was much lower than their CT_{Min} (3.16 ºC ± 0.52 ºC), while the CT_{Max} (44.52 ºC ± 0.47 ºC) was higher than the upper lethal temperature (41.19 ºC). Bertram (1935) examined temperature tolerances for some 49 insects and determined a 4.1 ºC difference between temperatures where locomotion was impaired (i.e. $CT_{Min/Max}$) and temperatures where locomotion ceased (i.e. LT_{50}). This difference between the two temperature measurements is explained by the exposure time at the different temperatures i.e. in this study, for measurements of lethal temperatures the mites were exposed to a specific experimental temperature for 2 hours while for measurements of critical temperatures the mites were exposed to a specific experimental temperature for 1 minute. A number of recent studies show similar differences between critical and lethal temperatures: for the desert ant *Myrmecocystus depilis* Forel (Hymenoptera: Formicidae) the lower lethal temperature was -5 ºC after 2 hour exposure and the mean CT_{Min} was 2.5 ºC ± 0.5 ºC and was dependent on the size of the ant (Kay and Whitford, 1978); for the major workers of the harvest mite *Hodotermes mossambicus* (Hagen) (Isoptera: Hodotermitidae) the lower lethal temperature was 2.84 ºC ± 0.88 ºC after 2 hour exposure and the CT_{Min} was 7.13 ºC ± 0.35 ºC while the upper lethal temperature was 42.96 ºC ± 0.59 ºC after 2 hour exposure and the CT_{Max} was 47.27 ºC ± 0.79 ºC (Mitchell *et al*., 1993); and for the sub-Antarctic caterpillar *Pringleophaga marioni* Viette (Lepidoptera: Tineidae) there was 60% mortality at -7.5 ºC after 18 hour exposure and the CT_{Min} was -0.6 ºC ± 0.18 ºC (Klok and Chown, 1997). The differences between critical and lethal temperatures observed in the present study are therefore in agreement with those observed in other studies.

The lethal temperatures of *O. terebrantis* determined in this study are similar to those reported by Cordo and DeLoach (1976) who found lethal high and low temperatures for adults to be between 39 ºC and 43 ºC, and between 0 ºC and -10 ºC, respectively, with mortality increasing as exposure time increased. However, for the upper lethal temperature, Cordo and DeLoach observed 100% mortality at 43 ºC, whereas in the present study 100% mortality was only observed above 46 ºC, and probit analysis calculated that 50% of the population would die at 41.19 ºC. Similarly, for the lower lethal temperatures, Cordo and DeLoach observed 100% mortality only after a 4 h exposure to -10 ºC, whereas in the present study 100% was observed at -6 ºC after 2 h exposure. These differences are likely to have occurred because Cordo and DeLoach (1976) collected the mites and conducted their experiment during winter

while in the present study the mites were collected and the experiment was conducted during summer. The increased survival at colder temperatures of the mites used by Cordo and DeLoach compared to the survival of the mites used in the present study could be attributed to the fact that the mites use in the earlier study had an opportunity to acclimate to colder temperatures, thus increasing their tolerance to the colder experimental temperatures. Similarly and *vice versa*, the mites used in the present study were acclimated to warmer temperatures compared to the mites used by Cordo and DeLoach, and hence the mites in the present study were more tolerant of the higher experimental temperatures, as can be seen by their higher survival at the high experimental temperatures compared to the mites used in the earlier study. These results therefore support the beneficial acclimation hypothesis.

Bowler (2005), Loeschcke and Sørensen (2005), and Sinclair and Roberts (2005), suggest that cold hardening may be a form of acclimation, which has been indicated in *O. terebrantis* in this study. Cold hardening at non-lethal temperatures, which can be induced either by brief exposure (minutes to hours) to low temperatures (e.g. the critical thermal tolerance experiment conducted in the present study) or by gradual cooling over a range of temperatures (e.g. the lower lethal temperature experiment conducted in the present study) (Chown and Nicolson, 2004) enables survival at even lower temperatures (Dahlgaard *et al.*, 1998). The fact that the mites that had been acclimated to cold temperatures, as in the Cordo and DeLoach study (1976), had a greater tolerance to even colder temperatures compared to the mites in the present study, suggests that *O. terebrantis* can undergo cold hardening. Similarly, comparing the results of this study to the earlier study, it is likely that *O. terebrantis* also undergoes heat hardening. The occurrence of cold hardening in *O. terebrantis* may be further supported by the fact that cold hardening occurs in species from alpine and temperate regions (Chown and Terblanche, 2007) and *O. terebrantis* occurs in both tropical and temperate regions in its native and introduced range (Julien and Griffiths, 1998).

The temperature range within which *O. terebrantis* remains active is fairly wide (from 3.16 ºC ± 0.52 ºC to 44.52 ºC ± 0.47 ºC) and the thermal tolerance of the mite falls within the tolerances of other water hyacinth biocontrol agents (Table 6.2). In South Africa, the daily maximum temperatures are never higher than the CT_{Max} of the mite, and are therefore unlikely to limit its distribution. On the other hand, the daily minimum temperatures inland can get much lower than 3 ºC during winter, which is lower than the CT_{Min} of the mite. This is one possible reason why *O. terebrantis* has

not been found at the majority of the water hyacinth sites that are outside of the warmer coastal climate. However, mite populations have been recorded at sites which experience winter temperatures that are well below 3 ºC; for example, the mites have been found on water hyacinth plants at Crocodile River in Brits, Mpumalanga Province, which experience 68 days of heavy frosting between June and August (Byrne *et al.*, 2010). During winter a small number of adult mites can be found seeking refuge from the cold in the crown of the plant, as close to the water as possible (own observation, Pretoria, Gauteng Province). When examined under the microscope the mites initially look dead but after a while about a half of the mites start walking (own observation). This suggests that adults can survive cold temperatures, but that mortality is high. At the Crocodile River, the mean daily minimum water temperature in winter is 9 ºC ± 1 ºC while the canopy and air temperatures are 6 ºC ± 2 ºC and 5 ºC ± 2 ºC, respectively (Byrne *et al.*, 2010). The difference in temperature between the air, canopy and water creates microclimates within a water hyacinth infestation, such that the mites are able to overwinter even at sites that experience ambient temperatures that are potentially harmful, if not lethal. By seeking refuge near the water, the mites therefore avoid the cold air, and this explains their presence at some of the very cold inland sites. It is well documented that adult oribatid mites, the order of mites that *O. terebrantis* falls under, can withstand freezing temperatures by supercooling (Cannon and Block, 1988). Therefore, in addition to its freeze avoidance behaviour, *O. terebrantis* may be able to withstand freezing by supercooling, thus making it possible for the mites to survive at some of the coldest areas in South Africa.

Table 6.1. Thermal tolerance limits for three biological control agents released against water hyacinth in South Africa: *Orthogalumna terebrantis* (this study), *Eccritotarsus catarinensis* (Coetzee *et al.,* 2007b) and *Neochetina eichhorniae* (Coetzee, unpub.)

	Orthogalumna terebrantis	*Eccritotarsus catarinensis*	*Neochetina Eichhorniae*
Lower LT_{50}	-4.28 ºC	-3.5 ºC	-7.4 ºC
Upper LT_{50}	41.19 ºC	37.0 ºC	41.7 ºC
CT_{Min}	3.1 6 ºC ± 0.52 ºC	1.2 ºC ± 1.17 ºC	4.3 ºC
CT_{Max}	44.52 ºC ± 0.47 ºC	49.6 ºC ± 3.37 ºC	51.0 ºC

In Argentina *O. terebrantis* is able to overwinter in all stages of development and the mite populations have been known to withstand temperatures of -10 ºC and could survive for prolonged periods at -5 ºC (Cordo and DeLoach, 1976). Consequently,

Cordo and DeLoach (1976) believe that the southern range of the mite in Argentina is determined by the southern-most limit of water hyacinth and not by the direct effect of temperature on the mite. In Pretoria very few mite galleries can be found on the water hyacinth laminae during winter, mostly because the laminae are dead due to frosting, but galleries that have been found towards the end of winter i.e. end of July, were occupied by live nymphs when examined under the microscope (own observations). Immatures of oribatid mites are known to stay dormant during winter (Stamou, 1989), most probably because of the inactivity brought on by chill coma (Norton, 1994). The adults and immatures of *O. terebrantis* are thus able to survive freezing temperatures, and yet the mites have failed to establish at Delta Park, Johannesburg, Gauteng Province, despite being released there on two occasions (M. Byrne, pers. comm.). However, another possible reason explaining why the mites have not established at Delta Park is because the plants at the park are short with bulbous petioles and wide leaves while mites prefer to oviposit on plants with elongated petioles and narrower leaves (M. Hill, pers. comm., own observations). The mean daily temperatures of the water, canopy and air at Delta Park during winter are 5.5 ºC, -4.3 ºC and -3.0 ºC, respectively (Byrne *et al.*, 2010), and should therefore not be limiting to the survival of the mite. However, Delta Park experiences 101 days of frosting, which is 33 days more than is experienced at the Crocodile River where the mites are established (Byrne *et al.*, 2010), and this suggests that it is the number of frost days, and not necessarily daily temperatures, that has a negative effect on the mite population during winter. Therefore, it seems that at sites where heavy frosting occurs for the majority of the winter months, the mortality of *O. terebrantis* is so great that the population is not able to overwinter.

Water hyacinth plants continue to produce ramets during winter but as soon as temperatures rise with the onset of spring ramet production increases and new leaves are also produced (Byrne *et al.*, 2010). However, very few mite galleries can be found in winter, suggesting that *O. terebrantis* has a low winter reproductive rate, and in addition to the high winter mortality rate experienced at sites with heavy frosting, the mites build their population from substantially low numbers at the beginning of summer. Therefore, a delay in the agent's population increase is likely to limit the intensity of damage to the plants, and hence limit the level of control well into the summer months. In Argentina, two to three generations of the mites occur per year, with adults emerging in late December, March and May (Cordo and DeLoach, 1976). The time between oviposition and adult emergence takes 18-29 days 25-30 ºC (own observation), so that in South Africa, as in Argentina, two to

three generations are expected to occur during summer. However, water hyacinth may double its population every 11 to 18 days (Edwards and Musil, 1975) and thus this vast difference in population growth between the mites and the plants greatly diminishes the efficacy of the mites as biocontrol agents. Similar observations have been made in New Zealand, where low spring- and summer-temperatures inhibit population increase of the alligator weed flea-beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) and low winter temperatures cause severe over-wintering mortality, so that very little damage to the weed caused by the flee-beetle has been observed across New Zealand (Stewart *et al*., 1996).

Degree-day models are commonly used to predict whether an agent is climatically compatible to its area of introduction (Byrne *et al.*, 2004; Coetzee *et al.*, 2007b). The model uses temperature data and time to predict the number of generations an organism can complete at a specific location (McClay and Hughes, 1995). However, one shortcoming of the method is that organisms need to be reared at various fixed temperatures over long periods (Byrne *et al*., 2004), and in this study the water hyacinth plants on which the mites were being reared started browning when they were kept inside growth chambers for more than a week, often dying before the mites emerged. Therefore, there was not enough data available to meet the standards of the degree-day model, and it was thus not possible to make substantiated predictions about the number of generations that could be expected at specific sites in South Africa.

Temperature incompatibility of biocontrol agents to areas of introduction has often been used as an explanation for establishment failure (McClay and Hughes, 1995; Milan *et al*., 2006). However, the results of the present study indicate that *O. terebrantis* has a number of behavioural and physiological adaptations (i.e. freeze avoidance, acclimation, cold hardening and supercooling) which allow it to withstand even the coldest of temperatures experienced in South Africa, so that temperature incompatibility to new areas does not explain why the mite is not found at more of the water hyacinth infestations in the country. A more plausible explanation is that, since *O. terebrantis* is not a good self-disperser, the only way that it could spread to other water hyacinth infestations is by being released at the sites through human effort. Thus far, little effort has been dedicated to releasing the mites at new water hyacinth sites (M. Hill pers. comm.), mostly because it is considered to be less effective than the other water hyacinth biocontrol agents.

Oribatid mites suffer mortality when relative humidity falls below a critical level, and this level depends on the species, life stage and temperature conditions (Stamou, 1989). *Orthogalumna terebrantis* is sensitive to low humidity and adults will not survive more than a day when exposed to high temperatures and sunlight in the absence of moisture (Perkins, 1973; own observations). Whether humidity inside the galleries is different to the outside air humidity is not known, but the adult mites are often observed hiding in the galleries during the heat of the day in summer (own observation), possibly because the galleries are slightly cooler and/or more humid. Low humidity may explain the lack of establishment of the mites in the Highveld and inland regions of South Africa which are characterised by dry and sunny days for most part of the year. However, humidity close to the water i.e. inside a water hyacinth canopy, is expected to be high, and thus humidity is unlikely to affect the mites in the field. Humidity has been found to influence the establishment of other biocontrol agents. For example, the tortoise beetle *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae), which was released in South Africa as a biocontrol agent of the South American weed *Solanum sisymbriifolium* Lamarck (Solanaceae), failed to establish at some high altitude sites due to low humidity, and not necessarily extreme temperatures (Byrne *et al.*, 2002).

6.5. CONCLUSION

The lower lethal temperature of *O. terebrantis* is lower than the temperatures generally experienced within a water hyacinth canopy, even at some of the coldest sites in South Africa. This suggests that the mites should be established at almost all water hyacinth infestations in South Africa, but data indicating the actual presence of the mites in the country is to the contrary. Little effort has been expended on releasing the mites at different sites around the country, and this could explain why the mites are only established at a few sites. It seems that the effect of temperature on survival and reproduction of biocontrol agents does not, on its own, determine whether establishment will occur. The results of this study show that *O. terebrantis* is well adapted to surviving at some of the harshest temperatures experienced in South Africa. Therefore, it would be beneficial to release the mites at more water hyacinth infestations, as an additional biocontrol agent.

CHAPTER 7

General Discussion

7.1. Introduction

The South African biological control programme of water hyacinth has been active for over 35 years. Although the success of the programme has been variable, the six introduced biocontrol agents (five arthropods and one pathogen) have substantially decreased the negative impact of the weed (Hill and Olckers, 2001), such that without the agents the spread of the weed and the problems associated with weed infestations would be far greater than experienced currently. The general lack of control of the weed at certain sites in South Africa is attributed to high nutrient concentrations in fresh water systems, variable climatic conditions over the weed's range which make certain agents unsuitable to areas of introduction, and insufficient release efforts. Additionally, stochastic events such as flooding and herbicidal applications can destroy entire agent populations (Cilliers, 1991; Byrne *et al.*, 2010), further aggravating control efforts.

7.2. Establishment of *Orthogalumna terebrantis* in southern Africa

Pre-release evaluation studies, which include the testing the efficacy of potential biocontrol agents, form an integral part of biological control programmes (Balciunas,

2004). As *O. terebrantis* was found to be present at two water hyacinth infestation in South Africa in 1989 (Cilliers, 1991), without being officially released, no pre-release studies were conducted. The mite was, however, released in Zambia in 1971, and is believed to have spread from there to Mozambique (first recorded there in 1977), Malawi (recorded there in 1991), and Zimbabwe (recorded there in 1996) (Julien and Griffiths, 1998). It is therefore likely that the mite came into South Africa from Zambia, via a tributary of the Zambezi River in Zimbabwe.

It was generally believed that the mite was not cold tolerant (C. Cilliers pers. comm.) and that it would be unable to establish at cold sites, and it was therefore given little attention. In addition, sufficient control of water hyacinth at many infestations is provided by the other agents, such as the *Neochetina* weevils, and thus it was thought unnecessary to distribute the mite across South Africa. However, since the establishment of the mite was discovered, field observations made during water hyacinth monitoring surveys have found that the mite has spread to a number of new sites (Chapter 6) and that during summer the mite numbers in the field are exceptionally high. For example, in South Africa six out of the 14 water hyacinth sites that have been under long-term monitoring (> 3 years) have an established mite population, and of the six sites, three sites (Mbozambo Swamp and Enseleni River, KwaZulu-Natal Province, and Yamorna Weir, Limpopo Province) have mite herbivory levels that damage between 25% and 50% of the leaf surface area, and another two sites (Mkadhzi Spruit, Limpopo Province and sections of the Crocodile River, North-West Province) have mite herbivory levels that damage > 50% of the leaf surface area in summer (Integrated Management of Water Hyacinth in South Africa database, Byrne *et al*., 2010). However, whether the damage caused to water hyacinth by mite herbivory has contributed in any way to the purported decrease in water hyacinth infestations in South Africa is not certain.

In Malawi the mite has a negative impact on water hyacinth in certain areas (e.g. Shire River) and mite-infested leaves are being redistributed onto newly-discovered infestations of the weed (Phiri *et al*., 2001). In Zambia, on the other hand, the mite does not provide substantial control on its own, while in the remaining African countries where the mite is established (Mozambique, South Africa, Zimbabwe) its impact is largely unknown (Julien and Griffiths, 1998). Therefore, the lack of data regarding the impact of *O. terebrantis* on water hyacinth prompted further studies and so this thesis set out to

examine the efficacy of the mite. In addition, this work examines the association between a plant and an arthropod, and thus serves as an example of a plant-arthropod system.

7.3. The value of laboratory impact studies

Ideally the effect of *O. terebrantis* on the weed should have been studied entirely in the field. However, it is notoriously difficult to assess biological control programmes in the field due to the lack of controls and the great variability in biotic and abiotic factors between sites. However, since the mite is already established on water hyacinth in South Africa, the laboratory studies conducted in this thesis could be used to directly compare laboratory results with existing field records, and thus determine whether extrapolations from lab studies hold true to observations in the field.

To demonstrate the efficacy of potential biocontrol agents, laboratory studies are often carried out in situations where field studies cannot be conducted in the native range (e.g. Blossey *et al*., 1994; Smith 2005; Bownes, 2008) and pre-release host-specificity studies in particular have to be conducted in quarantine. Whilst laboratory experiments are confounded by laboratory artifacts, they do provide an opportunity to measure the effect of candidate agents on plant growth, and to test the influence of different abiotic factors, plant competition and interactions of multiple agents on the efficacy of the candidate agent (e.g. Heard and Winterton, 2000; Coetzee *et al*., 2005; Ajuonu *et al*., 2007).

An important aspect of the success of biological control is that an agent should be able to establish on its target weed (Julien and White, 1997) and it should have a negative impact on the weed so that an acceptable level of control is reached (McClay and Balciunas, 2005). *Orthogalumna terebrantis* established easily in the laboratory, even from a small population of 40 mites per plant (Chapter 2), but at the population levels obtained in the laboratory the mite did not have a significant negative impact on plant growth (Chapters 2 - 4). It is difficult to extrapolate laboratory results to field conditions as these experiments are not a true representation of a field situation, where conditions of the abiotic environment can substantially influence the agent-weed interaction (Chase, 1996; Kestenholz *et al.*, 2007). In addition, there may be large difference in the effects of introduced biocontrol agents on their target weeds in the new introduced environment compared to the native ecosystems (Keane and Crawley, 2002) and many hypotheses

exist to explain why plant invasions are so successful (Catford *et al*., 2009). An example where a biocontrol agent's efficacy was underestimated from studies in the native range and from laboratory experiments is that of the mealybug *Hypogeoccoccus festerianus* (Lizer y Trelles) (Hemiptera: Pseudococcidae) which, within three years after being released to control harrisia cactus (*Eriocereus martini* Lab.), dramatically reduced the percentage cover of the cactus, this despite its poor performance in the laboratory evaluation and high levels of parasitism and predation in the native range (McFadyen and Tomley, 1981). In contrast, the majority of lab-based host-specificity tests of weed and arthropod biocontrol agents are highly accurate (e.g. Hoelmer and Kirk, 2005) with very few reports of non-target impacts resulting from the biological control of weeds (McFadyen, 1998; Culliney, 2005).

Laboratory impact studies are therefore essential and valuable when conducting host-specificity tests, but when they are used to test agent efficacy they are limited because they cannot accurately predict the efficacy of potential biocontrol agents in the field. The best way to get around this would be to conduct field experiments in the native range, where sites with and without the natural enemy could be compared (Williams *et al.*, 2010). However, even such studies are constrained because in the native range a potential biocontrol agent and its respective weed have natural enemies which they may not have in the introduced range (Mitchell and Power, 2003), thus making comparisons of a field situation between the native country and the country of introduction difficult. Furthermore, field experiments conducted in the native country are not always possible for a number of reasons; financial constraints, difficulty with obtaining visas, policy makers not having a full understanding of biological control principles, and general bureaucracy.

In the case of *O. terebrantis*, which reaches large population numbers throughout South Africa in summer, causing leaves to brown and dry out (own observations), it is likely that the stress exerted on water hyacinth growth by mite herbivory was underestimated by the laboratory experiments conducted in this thesis, due to the substantially lower mite numbers used. Sometimes biocontrol agents are initially thought to be ineffective, until they are given time to increase their populations in the field. For example, an undescribed species of gall wasp, *Trichilogaster* sp. (Hymenoptera: Pteromalidae), released for the biological control of *Acacia pycnantha* (Fabaceae) in South Africa in

1987, was initially thought to have failed because no signs of establishment were obvious for many years after the release. Only in 1995 did the galls become abundant and currently, almost two decades later, *A. pycnantha* is being brought under successful biological control (Hoffmann *et al*., 2002). It is possible that *O. terebrantis* population numbers are still increasing and that its effect on water hyacinth has not yet been realised.

7.3.1. The validity of thermal tolerance studies

Knowing the thermal physiology of a biocontrol agent provides useful information for determining the number of generations that the agent can have at a specific site (McClay, 1996; Coetzee *et al*., 2007b; Pappas *et al*., 2008) and therefore helps to predict whether an agent will establish and become efficacious at that site. However, the temperature experienced by arthropods in the field is not necessarily the same as the standard meteorological data normally used to predict their establishment or generational turn-over at different sites (McClay and Hughes, 1995). Microclimates within sites better represent the actual temperature experience by arthropods (Byrne *et al*., 2010). For example, using a degree-day model Coetzee *et al.* (2007b) predicted that *E. catarinensis* could produce more than one generation per year at all South African water hyacinth sites, but field records show that the mirid failed to establish at some sites despite repeated releases (Byrne *et al*., 2010). Furthermore, field observations have shown that the mirid has established at sites that experience heavy frosting in winter, which were originally thought to be unsuitable for mirid establishment. Thermal tolerance studies therefore provide basic information about an organism's thermal physiology but they cannot accurately predict the survival of an organism at a given site because they do not take into account microclimate conditions nor the behavioural adaptations of the organisms (i.e. seeking shelter). Studies which measure the temperature inside an arthropod's hiding-place, which usually differs to the ambient temperature (Chapter 6), take microclimates into consideration and therefore provide more relevant data about the temperature that an arthropod is likely to experience in the field. In general, however, the use of thermal tolerance studies to predict agent establishment has very limited application in biological control programmes and should be used with caution. For example, *O. terebrantis* is established at sites where the ambient temperature falls below 3 ºC, which is below the mite's critical thermal minimum (Chapter 6), suggesting that the

temperature actually experienced by the mite is not the same as the ambient temperature.

7.4. How do we measure success in weed biological control programmes?

The crucial measure of the impact a biocontrol agent has on its host is at a landscape level, and changes at this level are usually equated with "success". A good example of this is the case of *Salvinia molesta* which invaded some 187 840 ha of water along the Senegal River and the Senegal River Delta in the year 2002, but one year after the introduction of the weevil *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) onto the weed, the weed infestation was reduced from 100% to less than 5% cover (Diop and Hill, 2009). Similarly, the success of biological control of water hyacinth has been astounding in many areas. For example, prior to the initiation of the biological control programme in Lake Victoria (divided between Kenya, Tanzania and Uganda) some 20 000 ha of the lake were covered by the weed but after the introduction of the two weevil species only 2 000 ha currently remained infested (Wilson *et al.*, 2007). Biological control ultimately aims to bring about changes at a landscape level - to transform invaded areas from land that is heavily infested with weeds to a stable ecosystem where weed populations are diminished and indigenous species can grow, thus protecting indigenous biodiversity (e.g. Van Driesche *et al*., 2010).

The effect of biocontrol agents on water hyacinth is usually studied by measuring factors such as changes in infestation size (percentage coverage at a landscape level), changes in the plant population level (e.g. seedling production) and changes at a plant growth level, all of which are site-specific (Gutiérrez *et al.*, 2001). However, landscape changes are often not obvious in biological control programmes (Hoffmann and Moran, 2008), and to understand why they do or do not occur it is important to examine population level changes. Population level changes are observed if, for example, the production of flowers or seedlings, and ramets in the case of water hyacinth, is reduced, which results in a decrease in population numbers. Plant population dynamics, which are assessed by parameters such as biomass density (e.g. wet weight per m^2) and leaf longevity (Wilson *et al*., 2001; Center, 1987a) are less frequently used to measure the effect of biological control on water hyacinth. This is possibly because changes in biomass density and percentage coverage take a longer time to observe than changes at a plant growth level,

and they are also difficult to measure due to the large scale at which the measurements need to be made.

Percentage coverage can be measured through satellite remote sensing, and biannual monitoring of water hyacinth infestations via satellite images can be more cost effective than field sampling (Byrne *et al*., 2010) as certain satellite images i.e. from Landsat 5 TM (Thermal Mapper) and SPOT IV, are freely available for research purposes from the Satellite Application Centre of the Council for Scientific and Industrial Research (CSIR) in South Africa. Furthermore, the images can provide information for very remote sites that may be inaccessible. However, remote sensing has limitations. For example, where the percentage coverage of a specific plant is being calculated, if that plant is obscured by other vegetation (e.g. riparian trees cover water hyacinth on the edges of water bodies) then the percentage coverage is underestimated. Where low to medium resolutions are used, ground-truthing is required which increases travelling costs. Also, whilst the health of plants can be measured from satellite images by using certain analyses (i.e. the Normalized Difference Vegetation Index (NDVI) (e.g. Gamon *et al*., 1995); Byrne *et al*., (2010) found a negative correlation between weevil feeding scar density and NDVI), it cannot ascertain whether the health status of the plants is being affected by herbivory or environmental conditions, or, if in fact it is herbivory that is having an impact on plant health, it cannot ascertain which herbivore is responsible.

Population level changes can be explained by changes at the plant growth level. Measurements of changes at the plant growth level are the most common method by which success of the biological control of water hyacinth is evaluated. Plant growth measures include changes over time of parameters such as petiole length, and ramet, flower and leaf production. Plant growth rates are most commonly used to examine the impact of herbivory and variable environmental conditions on the weed because (1) they are easy to measure, (2) the changes in these parameters can be examined in laboratory trials and are observed within a short time period (for example, changes in petiole length in response to different water nutrient concentrations are observed within 8 weeks (Coetzee *et al*., 2007a; Bownes, 2008; Chapter 3) and (3) they have been measured in many previous studies (Coetzee *et al*., 2007a; Ajuonu *et al*., 2009; Bownes, 2008; Byrne *et al*., 2010) and thus the results of the studies are comparable. In addition,

trends in seasonality and herbivory can be identified by long-term monitoring of plant growth parameters at a specific site.

Certain plant level changes are not necessary to measure when testing agent efficacy. For example, root length is a plant parameter often measured when examining the effect of nutrients on water hyacinth plant growth, because there is strong negative relationship between root length and water nutrient concentration (Zaranyika and Ndapwadza, 1995; Xie *et al*., 2004) thus comparing root lengths of plants from different water hyacinth infestations is indicative of the nutrient status of that water system. However, water hyacinth root length is not affected by the herbivory of *E. catarinensis* (Coetzee *et al*., 2007a) or *O. terebrantis* (Chapter 3). The larvae of the *Neochetina* weevils pupate in the roots where they cause some damage to the tissue (Julien, 2001) but whether this has an impact on root length is not known but is very unlikely. Furthermore, seed production is usually not included in plant growth parameter measures because the most widespread mode of reproduction of water hyacinth is through vegetative propagation, since the weed has low levels of sexual reproduction (only about 50% of flowers produce seeds), and germination only takes place under suitable ecological conditions, i.e. in soil of very shallow water (Barrett 1980a, b). Water hyacinth petiole and leaf morphologies are highly plastic and are largely affected by the density of the plants within a water hyacinth mat (Penfound and Earle, 1948), rather than by herbivory (Chapters 2 and 3). Therefore, the best measure of the effect of agents on water hyacinth plant growth is the change in the production of ramets, and the leaf turn-over rate, as these tend to undergo the most changes when the plant is herbivore-stressed.

The results of chapters 2, 3 and 4 show that *O. terebrantis* has little effect on water hyacinth at a plant growth level (e.g. petiole lengths, ramet production, leaf production). However, plant growth level changes are usually only negatively impacted under very severe herbivory stress, low temperatures (Byrne *et al*., 2010) or low water nutrient conditions (Coetzee *et al*., 2007a; Chapter 3). The subtle effects of herbivory may not be seen at the 'whole-plant' level, but can be detected at the cellular level by examining the plant's physiology. It is therefore important to quantify the plant's physiological status to better understand the mechanism behind plant responses to agent herbivory. It then becomes possible to correlate changes in the physiology of the plant, as caused by herbivory, to plant level changes. Water hyacinth physiological parameters (i.e. net

photosynthetic rate and functioning of Photosystem II) were negatively affected by mite herbivory, even at the very low mite densities used in the lab experiments (Chapter 5). For example, the photosynthetic rate decreased as mite herbivory increased, when only about 10% of the leaf surface area was damaged by mites. At that level of damage the calculated photosynthetic rate is about 6 µmol (CO_2) m^{-2} s^{-1} (when using the equation y = 8.8028 – 0.3574(x), Chapter 5, Fig. 5.3). Coetzee (2003) measured a similar photosynthetic rate (about 7 µmol (CO_2) m^{-2} s^{-1}) on water hyacinth leaves that had been exposed to *E. catarinensis* for two weeks, at 30 adults per leaf. If the above equation is extrapolated to a field site such as Yamorna Weir, where mite herbivory can cause up to 50% damage to the leaf surface area, then the photosynthetic rate falls below 0. The correlation between photosynthetic rate and % leaf surface area damaged, on which the equation is based, was admittedly poor (r^2 = 0.29), which to some extent explains the 0 value. Also, the equation assumes a linear relationship between the photosynthetic rate and leaf surface area damaged which may not be a true representation. A 0 value is of course only likely if the plants are kept in the dark, but it does give an indication of how low the photosynthetic rate of mite-damaged plants could be. At Yamorna Weir where both the mite and the *Neochetina* weevils, and occasionally *E. catarinensis*, cause extensive damage to the weed, the average number of ramets per plant is 0.8. If the biocontrol agents were not established at that site the ramet numbers would be far greater, possibly > 2 ramets per plant; water hyacinth plants with leaf surface area damage between 50% and 75% caused by *E. catarinensis* have a significantly reduced ramet production (Coetzee *et al.*, 2007a). Since plant growth is dependant on photosynthetic rate (Ripley *et al.*, 2006), plants whose photosynthetic rate is below optimum and decreases over time are expected to have decreased plant growth parameters, and could potentially stop growing. In a field environment, the photosynthetic rate of water hyacinth is certain to be far lower than that obtained in the lab studies in this thesis (Chapter 5). A low photosynthetic rate would eventually be translated into decreased plant growth rates, which would, over time, become visible in the population dynamics and, ultimately, the percentage coverage of water hyacinth would be expected to decrease.

Results from chapter 5 show that herbivory by the mite has a negative impact on plant physiology (at the cellular level), however, the negative effect of mite herbivory on water hyacinth at a cellular level is not seen being translated into a negative effect at a plant

growth level. This may be because (1) the time frame and the scale of the experiments conducted in this thesis needed to be limited for experimental purposes and thus differences at a plant level were not able to be observed, or (2) the plants were compensating for the low level of damage caused by mite herbivory. Slight compensation by a plant may take place where low densities of agents occur on a host plant (Briese *et al*., 2002; Soti and Volin, 2010), and it is possible that this occurred in the present studies (Chapters 2 - 4).

To measure the success of *O. terebrantis* as a biocontrol agent, this thesis examined the effect of the mite on water hyacinth at a plant growth level (Chapters 2 - 4) and a plant physiology level (Chapter 5), because the effects of herbivory at these levels are easy to quantify in laboratory experiments. At the low mite densities used in these studies, the mite is not efficacious at the plant growth level, but it is efficacious at the plant physiology level. This suggests that the mite is not a successful agent, however, success should not be measured by what has been achieved, but rather by what the situation would be like without the biocontrol agent (Hoffmann and Moran, 2008). Damage caused to water hyacinth by mite feeding makes the plant more susceptible to pathogen attack (Delfosse, 1978b; Gerson *et al*., 2003) and is thought to stimulate weevil oviposition and feeding (Delfosse, 1978b). This implies that without the mite the negative effects of the other agents would be reduced, and plant growth would thus be greater than it would be without the mite. The mite should therefore not be dismissed as “unsuccessful”.

7.5. The use of plant physiology studies in the biological control of weeds

Measurements of plant physiological parameters such as gas exchange and chlorophyll-a (chl-a) fluorescence measures are non-intrusive and widely used by plant physiologists and botanists as a standard to measure plant health in order to gain insight into plant functioning under various conditions (Long and Bernacchi, 2003). However, it appears that such measurements are scarcely used in biological control programmes; a literature search of the 10th, 11th and 12th Proceedings of the International Symposia on the Biological Control of Weeds revealed only one example (as an abstract, not a full paper) where gas exchange measurements were used to examine the effects of a biocontrol agent on an invasive plant – herbivory by the leaf-feeding weevil *Galerucella calmariensis* (L.) (Coleoptera: Chrysomelidae) increased the photosynthetic rate,

stomatal conductance and internal CO_2 concentration of purple loosestrife (*Lythrum salicaria* L. (Lythraceae)) but carbon fixation was decreased suggesting that the weed attempted to compensate for the increased loss of leaf area by increasing the carbon fixation rate (Hunt and Blossey, 2000). Hunt and Blossey (2000) state that "measurements of gas exchange provide a more complete picture of plant response to herbivory and may enhance our ability to better predict success or failure of weed control programs." The present author is aware of only three other studies where gas exchange measurements were used to examine the effects of biocontrol agents on invasive plants, those of Doyle *et al.* (2002), Coetzee (2003) and Ripley *et al.* (2006). In addition, only Ripley *et al.* (2006) and the present study (Chapter 5) made use of chl-a fluorescence measurements. Measurements of chl-a fluorescence may be used to explain changes in gas exchange, and used in combination with gas exchange measurements they provide a holistic view of what and why changes are occurring at the cellular level of a plant. A possible reason why chl-a fluorescence measurements are so rarely used by biological control researchers is because the measurements are specific to the field of botany and require expensive equipment which is not commonly available to all researchers. The measurements of plant physiology as affected by herbivory (Chapter 6) improve our understanding of plant-herbivory systems, and basic plant physiology studies should therefore become a standard procedure in determining the effect of biocontrol agents on weeds, particularly when it initially appears that the agent has no effect on plant growth despite clearly visible damage caused by agent herbivory. Since plant growth is dependent on photosynthetic rate (Salisbury and Ross, 1992; Ripley *et al.*, 2006) any physiological change in the plant that decreases the rate of photosynthesis will result in reduced growth. Setting up collaborations between botanists and biological control researchers, within or between research institutes and/or universities, would allow the sharing of equipment, facilities and knowledge, and would thereby improve our understanding of plant-arthropod relationships.

7.6. The role of *Orthogalumna terebrantis* in the biological control programme of water hyacinth

Herbivore feeding can induce changes in plant chemistry which may reduce the quality of the plant and thus have a negative effect on the fitness of other species feeding on that plant (Denno *et al*., 1995; Inbar *et al*., 1999; Reitz and Trumble, 2002). In addition to

lowering the photosynthetic rate of water hyacinth, *O. terebrantis* does not interfere significantly with the other agents tested (*E. catarinensis* and *N. eichhorniae*) but instead enhances the efficacy of *N. eichhorniae* (Chapter 4). Mite damage was lower on plants that had been exposed to the mirid, suggesting that there is potential for competitive displacement of the mite by the mirid. Mirid feeding causes chlorosis of water hyacinth leaves (Hill, 1999) resulting in reduced food quality for the mite, which could have a negative impact on mite fitness. Furthermore, feeding by the weevil and the mirid may accidentally damage or remove mite eggs and nymphs but the potential displacement of the mite by the other agents would not have a serious impact on the overall control of water hyacinth because the other agents, specifically *N. eichhorniae*, have a greater impact on the weed's growth compared to the mite (Chapter 4). The two *Neochetina* weevils control water hyacinth by themselves at many infestations in Sudan, India, Papua New Guinea, Thailand, Uganda, USA, Zimbabwe and Nigeria (Julien and Griffiths, 1998) and on Lake Victoria (Wilson *et al*., 2007), and it would therefore be unnecessary to introduce *O. terebrantis* onto water hyacinth infestations that are already under sufficient control. In South Africa, small infestations at sites such as at New Years Dam, Eastern Cape, do not require additional agents and introducing the mite there would waste valuable resources. Another interaction to consider is that between the mite and the grasshopper *C. aquaticum* which will be released at a limited number of sites in South Africa in the year 2011 (A. Bownes, pers. comm.). Feeding by the grasshopper removes large areas of the leaf tissue, potentially limiting the mite's food source and most likely physically removing the mites themselves. However, the grasshopper is one of the most damaging insects on water hyacinth in its native range (Perkins, 1974), and it has a greater impact on water hyacinth than *N. eichhorniae* and *E. catarinensis* (Bownes *et al.* (2010)), so any negative effect the grasshopper could potentially have on *O. terebrantis* would not hinder the biological control of water hyacinth on the whole.

There is much debate among biologists as to whether successful weed biological control is achieved by a single agent (Myers, 1985; Harris, 1990; Crowe and Bouchier, 2006) or the cumulative control effect of multiple agents (Charudattan, 1986; Hoffmann and Moran, 1998; Caesar, 2003). The release of multiple agents may result in interference between the agents which might reduce the overall possibility of success (Denno *et al*., 1995; Impson *et al*., 2008), and with every additional control agent released there is a potential risk of undesirable ecological consequences, i.e. direct and indirect non-target

impacts (McEvoy and Coombs, 2000; Louda *et al.*, 2003). A direct impact results from an agent feeding on a non-target plant, although this can usually be predicted from host-specificity tests, and in fact direct impacts on non-target weeds that have occurred in the past were predictable (Pemberton, 2000). An indirect impact can occur when the weed becomes a major food source for a native species (e.g. Balciunas, 2004), in which case the benefits resulting from the biological control of the weed would have to be weighed against the possible extinction of the native species feeding on that weed. In addition, Pearson and Callaway (2005) reason that the nature and strength of the interaction between a biocontrol agent and a weed influence potential, indirect, non-target effects. Therefore biocontrol agents must not only be host-specific but they must also greatly reduce their target species, and through density-dependent feedbacks, they should simultaneously reduce their own populations which would minimize risks to non-targets. The population densities of *O. terebrantis* in South Africa are not so large that density-dependent feedbacks can be predicted with certainty. However, in South Africa the mite feeds strictly on water hyacinth (M. Hill pers. comm.) and thus does not have direct non-target effects. Field observation of the mite in Florida and Argentina suggest that there may be two strains of the mite – the Florida strain feeds on water hyacinth and pickerelweed (Gordon and Coulson, 1971 in Perkins, 1973) and prefers shady areas, while the Argentine strain only feeds on water hyacinth and occurs in both sunny and shaded areas (Perkins 1974). Whilst the mite may possibly be preyed upon by other species (Cordo and DeLoach; 1976) and thus affect certain food-webs, if it is taken into account that the mite does not interact significantly with the other water hyacinth biocontrol agents (Chapter 4), the mite is unlikely to have any indirect non-target effects. In support of multiple-agent releases, reviews by Denoth *et al*. (2002) and by Stiling and Cornelissen, (2005) concluded that the likelihood of control of a weed increases when more species of agents are released, because of the expected additive effect of the agents increasing the stress on the plant. This ‘cumulative stress hypothesis’ is supported by numerous studies (Hoffmann and Moran, 1998; Caesar 2003; Seastedt *et al*., 2007; Chapter 4). If the feeding preferences and life-cycles of agents differ, niche partitioning occurs and various parts of the weed (i.e. different-aged leaves, roots, flowers) will be damaged, thus more of the plant will be impacted. The duration of the damage can also be expected to increase if the agents feed on the weed at different times, both on a daily and on a seasonal basis. The cumulative stress effects of the combined biocontrol agents may thus be additive. Eventually, this should lead to a

reduction in the size of the plants, which is translated into an overall reduction of weed infestations (Delfosse, 1978a).

The establishment and success of biocontrol agents is limited by climatic incompatibility in the introduced range of the target weed (Crawley 1986; McClay and Hughes, 1995, Byrne *et al*., 2002). This is believed to be a key factor limiting the success of the current biocontrol agents on water hyacinth in the temperate regions of South Africa (Hill and Cilliers, 1999; Hill and Olckers, 2001). The CT_{Min} of *O. terebrantis* is higher than that of *N. eichhorniae* but lower than that of *E. catarinensis* (Chapter 6). The weevil is widely distributed across the range of water hyacinth in South Africa (Rhodes University Aquatic Weeds Monitoring Survey database) while the mirid has a narrower distribution and has been predicted to have a limited distribution in South Africa due to low winter temperatures (Coetzee *et al.,* 2007b). Since the cold tolerance of the mite falls somewhere in between that of the weevil and the mirid, it can be predicted that the mite should establish and persist throughout the range of water hyacinth in South Africa. However, field records indicate otherwise; the mite is not established at the majority of the weed infestations in the country (Chapter 6). Therefore, mite establishment in South Africa is unlikely to be limited by cold temperatures, and the lack of existing mite populations is rather a result of the mite's poor ability to disperse, or possibly the lack of release effort, or incorrect release strategies. The method of release of the agents and the number of individuals released play a pivotal role in the establishment and success of an agent (McEvoy and Coombs, 2000; Hill and Cilliers, 1999). In the case of the mite, large numbers of heavily mite-infested plants need to be repeatedly released at field sites during the summer months to guarantee establishment and continual survival of the mite population, because during winter the water hyacinth biocontrol agents, including the mite, suffer high mortality rates so that they have to build up their populations from very low numbers each summer (Byrne *et al*., 2010; own observations). Augmentative releases should therefore be implemented over a number of years during summer which will allow the mite population to build up to a level where, despite winter mortalities, the mite efficacy during summer will not be compromised. The mite could be introduced at cold sites where some of the other biocontrol agents, such as *E. catarinensis*, do not survive.

According to Cromroy's (1983) scoring system, which predicts the potential effectiveness of a mite in the control of a weed (Chapter 1), *O. terebrantis* is not a likely candidate as a biocontrol agent. Some reasons for this are: (1) it only has a local distribution, (2) it has a limited period of attack, (3) it has only 2 to 3 generations per year, and (4) it is a leaf-miner as opposed to a leaf-defoliator (Cromroy, 1983). Although the results from this thesis concur that the mite is unlikely to bring water hyacinth under control on its own, it can be an effective agent when used in combination with the suite of agents already on water hyacinth. The mite should not be developed at the expense of the other agents, but where resources are available, augmentative releases of the mite could be made as the mite is likely to persist throughout the distribution range of water hyacinth in South Africa, and because of the mite's ability to cause physiological damage to the weed. Releasing the mite at new sites is uncomplicated since mite-infested plants can easily be transported to the sites, and this can be done during water hyacinth monitoring surveys conducted by universities and other interested parties such as the Working for Water Programme.

The results presented in this thesis do not provide conclusive proof that *O. terebrantis* is or is not a good biocontrol agent. Investigations of the plant-mite system in a field situation are warranted, and could be conducted in South Africa where good mite presence and absence data is already available from water hyacinth monitoring surveys such as the Rhodes University Aquatic Weeds Monitoring Survey and the Integrated Management of Water Hyacinth in South Africa Survey (Byrne *et al.*, 2010). Plans are underway to introduce the mite into the Republic of Benin (O. Ajuonu pers. comm.) and the consideration of results of this thesis, and further field studies, will be beneficial in making an informed decision as to whether and/or where in Benin the mite should be released.

7.7. Control of water hyacinth going forward

Different weed species need to be controlled in different ways, but the integration of chemical and mechanical controls with biological control is now considered to be the most effective and feasible management option for many biological control programmes (Zimmermann and Neser, 1999; Paynter, 2003; Lym, 2005; Byrne *et al*., 2010). Van Wyk and van Wilgen (2002) compared the costs associated with herbicidal control, biological

control and an integration of herbicidal and biological control of water hyacinth, and their analysis showed that integrated control is the most cost-effective control option. It is therefore important that authorities utilise integrated management programmes which are specific for each individual water hyacinth infestation, taking into consideration the site's climate and nutrient levels, so that only the agents that are likely to establish and severely damage the plant under site-specific conditions are used.

Combining the effect of a cold climate, which primarily affects the biocontrol agents (Chapter 6) and makes establishment (if any) slow and extends the amount of time it takes for an acceptable level of control to be reached, together with the eutrophication of water, which primarily affects the plant and greatly increases its growth (Chapter 3), makes the biological control of water hyacinth, especially at colder sites with high water nutrient levels, extremely difficult. Whilst biological control has helped to substantially decrease the size of water hyacinth plants and thus entire water hyacinth infestations, many studies (Kampeshi and Shantima, 1999; Coetzee *et al*., 2007a; Bownes, 2008; Byrne *et al*., 2010) including this one (Chapter 3) have shown that water hyacinth growth is more impacted by water nutrient levels than by herbivory. Therefore, to further decrease the weed infestations, in less time than it would take biological control, the nutrient levels of our fresh water systems need to be decreased. This could be achieved if the amounts of nutrients entering the water systems were reduced, but this would require strict government regulations and adherence to the law, for which there are few resources in the form of time and staff (Neysmith and Dent, 2010). Researchers therefore need to build strong relationships with government departments, such as the Department of Water Affairs and Environment, so that research outputs can be incorporated into new legislation. In addition, funding agencies need to be aware of the fact that the success of a biological control programme can often only be measured many years after the release of an agent, and therefore biological control research projects need to be allocated sufficient funding to allow for long-term monitoring.

7.8. Conclusion

Orthogalumna terebrantis reduces the photosynthetic rate of water hyacinth and is therefore potentially valuable as a biocontrol agent. However, the effects of the mite at the physiological level of the plant were not translated into plant growth level effects, and

this could be due to plant compensation or due to laboratory artifacts. The mite is compatible with the other water hyacinth biocontrol agents already released, and it is also tolerant of South African temperatures. Therefore, where resources are available, the mite should be released at more water hyacinth infestations in South Africa to provide a cumulative negative impact on the weed. However, it should not be developed at the expense of the other agents.

Appendix 1: Mites (Acari) used as biological control agents of weeds (modified from Julien and Griffiths, 1998)

Acari deliberately introduced from countries not of their origin:

Agent (Family)	Target weeds (common name(s))	Country where released	Year of release	Status	Degree of control
Aceria chondrillae (Eriophyidae)	*Chondrilla juncea* (rush skeleton, skeleton weed)	Argentina	1989	Established throughout weed's range.	Unknown.
		Australia	1971	Established readily but spread needing redistribution. Only common in drier areas.	Introduced strain specific to narrow-leaf form of the weed.
		USA	1977	Widely established in CA, ID, OR & WA. Most damaging agent in ID, OR & WA.	Decreases shoot & seed production & rosette regeneration. Effects in CA limited by indigenous mite *Typhlodromus pyri*.
Aculus hyperici (Eriophyidae)	Hypericum perforatum (St John's wart, San Juan Herb)	Australia	1991	Established at 73% of sites. Spreads slowly naturally but widely established following redistribution.	Reduces plant vigour & reproductive potential at well established sites. (Willis & Ash, 1996)
Aceria malherbae (Eriophyidae)	*Calystegia sepium* (hedge bindweed)	USA	1993	Released in Maryland.	Unconfirmed.
	Concolvulus arvensis (field bindweed)	Canada	1989	Not established in Manitoba or Saskatchewan. Establishment not confirmed in Alberta or British Columbia.	Unconfirmed.
Orthogalumna terebrantis (Galumnidae)	*Eichhornia crassipes* (water hyacinth)	India	1986	Initially released in Bangalore, then in Kerala in 1991. Established readily at all release sites.	High populations cause browning of leaves but damage confined to older leaves or shaded plants. Mite does not control weed by itself.
		Zambia	1971	Released on Kafue River.	Well established.

Abbreviations: CA = California, ID = Idaho, OR = Oregon, WA = Washington

Appendix 1 (continued)

Acari deliberately introduced from countries not of their origin (continued):

Agent (Family)	Target weeds (common name(s))	Country where released	Year of release	Status	Degree of control
Tetranychus lintearius (Tetranychidae)	*Ulex europaeus* (gorse, furze)	Hawaii	1995	Established on Hawaii & Maui. Redistribution underway.	Population increasing. Predation minimal.
		New Zealand	1989	Widespread & common in drier, cooler areas. Failed to establish in warmer climates.	Sporadic outbreaks cause severe damage. Colonies suppressed by predation from *Stethorus bifidus* (Coleoptera).
		St Helena	1995	Established & causing local damage.	Effects limited by predation from *Phytoseiulus* spp. (Acari).
		USA	1994	Released & established in CA, OR & WA. Widely distributed in CA & OR. Less widely in WA.	80% reduction in flowering in OR. Most effective in open patches in inland areas susceptible to severe winters. Has less impact in WA.

Abbreviations: CA = California, ID = Idaho, OR = Oregon, WA = Washington

Acari used in their native country:

Agent (Family)	Target weeds (common name(s))	Native country	Status	Degree of control
Aceria acroptiloni (Eriophyidae)	*Acroptilon repens* (Russian knapweed)	Ukraine	Abundance in cultivation is maintained by preserving this species on special two hectare plats among crops.	Efficiently suppressed reproduction of weed.
		Uzbekistan		Successful control of seed production observed in different crops.

Appendix 1 (continued)

Acari occurring in exotic ranges where deliberate release is not recorded:

Agent (Family)	Target weeds (common name(s))	Country where released	Year of release	Status	Degree of control
Aceria chondrillae (Eriophyidae)	*Chondrilla juncea* (rush skeleton, skeleton weed)	Canada	Pre 1993	Spread naturally following releases in USA in 1977. Established in British Columbia where 80% of seed heads is infested.	Spreading continuing.
Tetranychus opuntia (Tetranychidae)	*Opuntia stricta* (common prickly pear)	Australia	1922 or 1923	Introduced accidentally with other organisms. Spread rapidly from cages. Mite declined to scattered distributions following control of the pear by *Cactoblastis cactorum*. This species has not been deliberately released for biocontrol anywhere else.	Began providing good control together with *Daclytopius opuntiae.*
Orthogalumna terebrantis (Galumnidae)	*Eichhornia crassipes* (water hyacinth)	Cuba	1977	Accidental introduction.	Causes browning of leaves in water bodies near Havana. Impact unknown.
		Jamaica	1969	Unknown.	Impact unknown.
		Malawi	Pre 1991	Spread from Zambia following releases in 1971. Occurs extensively in Lower Shire River. Redistribution to Upper Shire River in 1996.	Appears to have depressing effect in some areas.
		Mexico	1977	Accidental introduction.	Impact unknown.
		Mozambique	1977	Accidental spread from Zambia & South Africa.	Impact unknown.

Appendix 1 (continued)

Acari occurring in exotic ranges where deliberate release is not recorded (continued):

Agent (Family)	Target weeds (common name(s))	Country where released	Year of release	Status	Degree of control
Orthogalumna terebrantis (Galumnidae)	*Eichhornia crassipes* (water hyacinth)	South Africa	1990	Found established & locally abundant in north-eastern parts of the country. Widely redistributed & now contributing to control of the weed in temperate areas but not yet in areas with coldest winters.	Impact unknown. Appears to be highly damaging in summer (own observation).
		USA	Pre 1968	Accidental introduction probably arriving at same time as the weed. Widespread & sporadic. In combination with the fungus *Acremonium zonatum* can have locally severe but temporary impact.	Provides no substantial control.
		Zimbabwe	1996	Probably spread from Zambia following release in 1971. Well established only on infestation on Lake Kariba.	Impact unknown.

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