The effect of shade on the biological control of *Salvinia molesta* D.S. Mitchell [Salviniaceae] by the weevil, *Cyrtobagous salviniae* Calder and Sands [Curculionidae]

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Abstract

Salvinia molesta, a floating aquatic weed, is a global menace in many water bodies and waterways. The weed disrupts the ecological balance wherever it invades and also has wide ranging economic and health impacts. Its impact has resulted in the need to control it, and while chemical and mechanical control are often ineffective, biological control by the weevil *Cyrtobagous salviniae* is largely successful. However, in many parts of the world, including South Africa, biological control of *S. molesta* has been less effective where the weed grows as an understory species. Shallow and shaded waters characteristically found at the margins of water bodies provide a refuge for *S. molesta*. Therefore, the aim of the study was to determine the effect of shade on the efficacy of biological control of *S. molesta*.

Investigations into the problems associated with control in the shade were carried out in a greenhouse and in the field. In controlled greenhouse experiments, plants were grown at high and low nutrient levels in individual mesocosms, at three varying levels of shade, where half the mesocosms were inoculated with *C. salviniae*. Plants in high nutrient conditions were significantly more productive in terms of biomass accumulation, compared to those grown at low nutrient levels, in both the absence and presence of herbivory at all levels of shade tested. Plants grown in the shade and in high nutrient conditions had significantly higher quality compared to the ones exposed to full sun. Higher plant quality in the shade consequently resulted in accelerated fecundity for *C. salviniae* resulting in significantly higher weevil populations. Furthermore, less damage was recorded on plants in the shade, possibly due to the high nitrogen concentrations which may have deterred grazing by *C. salviniae*. However, in the absence of shading, plants were of lower quality and consequently sustained more damage from herbivores despite lower weevil populations.

In contrast to the greenhouse, there were no differences in biomass recorded in the field at two nutrient levels and in the presence of herbivory for both shade and open sites. Nutrients added to high nutrient treatment quadrats diffused evenly across the water body resulting in uniform nutrient distribution, hence uniform plant biomass and carbon-nitrogen ratio. Despite the lack of statistical differences, more weevils were found in the full sun plots, while modest populations were recorded in the shade. Higher weevil populations consequently led to more damage in the sun, a situation that has been observed in most field sites in South Africa.

The results therefore suggest that nutrients were pivotal in plant growth compared to light regimes (amount of PAR). Plant quality significantly influenced weevil populations and plant damage in both studies, demonstrating that plant nitrogen plays a crucial role in the plant-herbivore system. Furthermore, in both the greenhouse and field, despite lack of statistical differences, plants exposed to full sunlight suffered more damage than shade plants, pointing towards better control in the sun compared to the shade, suggesting that there is greater preference of sun-exposed plants for grazing by the weevils. The results from this study add to the growing body of literature that plant quality is a major factor in determining the success of aquatic weed biological control programmes.

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Chapter 1: General Introduction and Literature Review

1 Introduction

Disturbances in aquatic systems of South Africa have left them vulnerable to invasion by a number of floating aquatic weeds. These disturbances are mainly anthropogenic, including agricultural activities and an increase in urbanization due to accelerated human population growth. The majority of these weeds have been targets of biological control wherever they occur. Biological control entails the use of the weed's natural enemies to keep their populations in check and at acceptable levels, and in South Africa, biological control for an array of invasive plant species, especially terrestrial ones started as early as 1913 (Moran *et al.*, 2005). Most aquatic weeds are considered to be under control following releases of host specific agents (Coetzee *et al.*, 2011). There are however, some instances where the desirable control of these weeds has not been realized and this could be attributed to some local environmental factors where these weeds at times occur. *Salvinia molesta* D.S. Mitchell (Salviniaceae) is an example of a weed where biological control is mostly excellent, but at some sites it is not as effective (van Oosterhout, 2006; Owens *et al.*, 2011; Sullivan and Postle, 2010).

Salvinia molesta is a ubiquitous aquatic invader which is named after Antonio Maria Salvini (1633-1729). The species name '*molesta*' is derived from the Latin word '*molestus*' which points out its weediness as it is a nuisance across the world, especially affecting natural water bodies as well as artificial impoundments (Room, 1990; Julien *et al.*, 2009). *Salvinia molesta* is an aquatic fern native to southeastern Brazil and is known by a variety of names, which include Kariba weed due to its successful invasion of Lake Kariba in 1959 (then the world's largest man-made water reservoir); African pyle, water fern and salvinia (Chikwenhere and Keswani, 1997; Madsen and Wersal, 2008; Osborne, 2012). It is part of a larger group of *Salvinia*, the *Salvinia auriculata* Aublet (Salviniaceae) complex, and it was at first identified as a form of *S. auriculata* until 1972 when it was reclassified as *S. molesta* based on the male sporocarps which hang from the rhizome (Room *et al.*, 1981; Room, 1990; Julien *et al.*, 2009).

Due to the problems associated with this weed, control measures were put in place, and these include chemical, mechanical and biological control, where biological control proved most effective. Although biological control by the weevil, *Cyrtobagous salviniae* Calder and Sands (Curculionidae), has proved to be successful (Doeleman, 1989; Chikwenhere and Keswani, 1997; Forno and Smith, 1999; Diop and Hill, 2009; Coetzee *et al.*, 2011), there has been less control achieved in areas where the weed grows as an understory species (van Oosterhout, 2006; Owens *et al.*, 2011). Shallow waters which occur in the shade have been observed to provide a haven for *S. molesta* to establish, and when combined with the effect of falling water levels or eutrophication, the nuisance proportions of the weed may increase (Adams *et al.*, 2002; Owens *et al.*, 2011). This study therefore focused on the effects that shade has on the plant quality and its subsequent biological control.

1.1 Description and biology of *Salvinia molesta*

Salvinia molesta bears pairs of floating leaves which have numerous, water proof trichomes on the upper surface. These trichomes have a cage like appearance closely resembling egg-beaters, commonly associated with the *S. auriculata* complex (Oliver, 1993; Julien *et al.*, 2009). Leaves have an oval shape and tend to fold upwards at tertiary stages of an infestation (Julien *et al.*, 2009). *Salvinia molesta* also possesses horizontal rhizomes that may be found at the surface or about 1-2 cm below the water surface (Room and Kerr, 1983). The plants possess aerenchyma tissue which is found in the rhizomes and it is this tissue that gives rise to buoyancy of the plants (Julien *et al.*, 2009). *Salvinia molesta* also has modified leaves below the pairs of leaves and these have a filamentous appearance and act as roots (Oliver, 1993; van Oosterhout, 2006). Room and Kerr (1983) state that the roots hang from the rhizome to depths of about 4cm in nutrient rich water and can even extend down to depths of 50 cm below water surface in oligotrophic waters. A pair of leaves and a floating root occur at a node and they thus form a ramet, and a single ramet or interconnected ramets form a plant (Julien *et al.*, 2009). A single plant referred to as a phenet, is formed by colonies of ramets of up to 100 plants growing together, thus forming floating mats on the surface of the water (van Oosterhout, 2006).

Salvinia molesta expresses three growth forms according to the varying extent of crowding: (i) the small-leafed primary growth form is associated with the invasion of open water and also characteristic of plants recovering from damage, (ii) a larger leafed secondary growth form has leaves slightly folded upwards, and (iii) a tertiary form which is characterised by mature stands of larger deeply folded and densely packed leaves (van Oosterhout, 2006). The growth forms are variable and comprise a range of forms within a growing population where they change temporally and spatially, depending on their local environment.

Salvinia molesta, a sterile pentaploid, reproduces by means of vegetative reproduction where viable fragments give rise to new plants (Julien *et al.*, 2009). The plants propagate via apical and axillary buds to produce nodes, where every node bears buds that develop into branches. When ideal conditions persist, *S. molesta* plants exhibit apical dominance, where the apical buds grow more strongly than the rest of the buds enabling the plants to spread from the terminal ends (Room, 1988). Due to the lack of sexual reproduction, population growth and fluctuations are a result of rhizome extension, branching and death as well as fragmentation at varying sections of the plants. Each node has the capability of producing three axillary buds, and up to six when under stress, leading to the formation of new plants (Room, 1988, 1990; Julien *et al.*, 2009).

Plant samples collected from many parts of the world failed to show any morphological differences when cultured under optimum conditions therefore proving that the plant has remained genetically static (Room, 1990). Over the years, herbivores have also shown indiscriminate grazing of the different plant populations, further consolidating the notion that *S. molesta* may have been the single largest genetically static species (Room, 1990).

High light intensities coupled with high water temperatures and nutrient supply promote the growth of *S. molesta* (Oliver, 1993). The optimal temperature for *S. molesta* growth is 30°C while no growth occurs below 10°C or above 40°C (Room and Thomas, 1986). On the other hand, Cary and Weerts (1983) suggest that ideal temperatures for growth are in the range of 24-28°C. The exposure of *S. molesta* to adverse conditions with temperatures below -3°C and above 43°C has been observed to kill the plant (Julien *et al.*, 2009). However, in tropical regions, the impact of high leaf temperatures above 40°C is ameliorated by the buffering effect of water where temperatures are maintained below 40°C (Storrs and Julien, 1996). Exposed plants usually succumb to frosts and only when covered by other *S. molesta* plants, as well as other vegetation, or protected by the temperature-buffering effect of water, do they stand a chance of survival. *Salvinia molesta* may then take advantage of the buffering effect of water to extend its range into temperate climates (Julien *et al.*, 2009). The weed also has the capacity to regenerate after being exposed to stresses such as frosts, droughts and extreme heat with new leaves appearing on seemingly dead, brown weed mats. Buds have been known to retain viability in dry and very hot conditions if they are sheltered within a multi-layered weed mat (van Oosterhout, 2006).

The level of nutrients within the growing medium can profoundly affect the appearance of the plants at any growth stage. Nutrients are often optimal when *S. molesta* exhibits a healthy deep hue of green with vigorous growth typically characterized by wedge shaped plants. Long, thin, yellowish plants often indicate poor nutrition and this may reduce the success of biological control as well as the performance of some herbicides (van Oosterhout, 2006). Ideal temperatures of 30°C and high nutrient levels often facilitate the doubling of the infestation size to about three days (van Oosterhout, 2006), while Parsons and Cuthbertson (2001) reported that an infestation can double in two days.

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1.2 Distribution and pest status

Salvinia molesta is widely spread across many tropical and subtropical regions of the world. Its native range is in a small area in southeastern Brazil (Forno and Harley, 1979). In its native range, *S. molesta* forms part of the floating and emergent vegetation, is home to a variety of herbivores and is kept in check through the equilibria achieved between the plant and its natural enemies (Forno and Bourne, 1984; 1985). It is mostly found occupying wetlands, natural lagoons and swamps, rivers and stream margins, as well as artificial dams and drainage channels (Forno and Harley, 1979).

Outside its native range, the weed is found in tropical and subtropical climates on all continents, as well as warm temperate regions such as parts of southern Africa, the United States and southern Australia. *Salvinia molesta* was first recorded growing in Sri Lanka in 1939, Australia in 1952 and in Lake Kariba, Zimbabwe in 1961 (Marshal and Junor, 1981; Room and Julien, 1995). The weed was first reported in the USA in 1995 and has since become established in 12 states. It is widely distributed across southern and southeastern parts of Asia, Papua New Guinea, New Caledonia, French Polynesia, Fiji and New Zealand in the south Pacific (Julien *et al.*, 2009). *Salvinia molesta* has also been found in Europe in a botanical garden at Ponta Delgarda, Azores Islands, Portugal and in Pozo del Merro, Latium in Italy. The low winter temperatures characteristic of Europe may be the only factor restricting its growth and spread, despite most of the catchment systems being eutrophic and the fact that there is high commercial trade in this plant in the aquaria and water-garden industries (Julien *et al.*, 2009).

According to the *Global Invasive Species Programme* (GISP) database, *S. molesta* is an important global invasive species which has made it into the Australian Top 20 Weeds of National Significance as it was elected to be one of the worst alien invasive species in 2013 when it replaced the rinderpest virus (van Oosterhout, 2006; Courchamp, 2013). In most countries, it has been classified as a noxious weed and its culturing and movement are prohibited (van Oosterhout, 2006). In the USA, *S. molesta* and other members of the *Salvinia* species are on the Federal Noxious Weed List as governed by the Noxious Weed Act of 1974, which restricts movement of the weed across state lines without a permit (Julien *et al.*, 2009). In South Africa, the presence of the weed dates to the early 1900s, being most troublesome in Mpumalanga, Kwa-Zulu Natal and the Western and Eastern Cape provinces. It has successfully invaded both inland areas above 500m altitude and coastal regions (Hill, 2003) and has since been listed as a Category 1b weed that has to be controlled under the National Environmental Management: Biodiversity Act (No. 10 of 2004) (NEMBA). Most African countries such as Botswana, Namibia, Zambia and Zimbabwe recognize *S. molesta* as a noxious aquatic plant but there is lack of legislation to deal with it; however, control measures against it have been put in place (Julien *et al.*, 2009).

1.3 Mode of invasion

Slow moving and still fresh water with abundant nutrients, as well as open stretches of water are open to invasion by *S. molesta*. Faster flowing water may be colonized when *S. molesta* is anchored by other vegetation and any depth of water is susceptible to colonization. Tertiary mats of *S. molesta* are able to persist for long periods of up to 12 months on mud which only caters for those plants closest to the moist ground. Shallow waters in warmer climates can reach temperatures above those desirable for *S. molesta* growth, thereby reducing the chances of survival (van Oosterhout, 2006). *Salvinia molesta* can form dense mats which kill submerged plants by light deprivation (Hennecke and Postle, 2006). Another strategy is that it lowers water flow and turbulence of water, effectively reducing the amount of oxygen that enters the water column; this in turn leads to the suffocation of submerged macrophytes and reduction in photosynthesis which is responsible for the oxygenation of the water column (Room and Thomas, 1986).

Invasion commonly occurs in disturbed areas, although undisturbed ones may also be colonized. These disturbed habitats can be flood canals, rice paddies, artificial lakes and hydroelectric facilities (Oliver, 1983). In natural settings, the dispersal of salvinia is mediated by water flow or wind and by animals which utilize natural water courses such as birds (worldwide), capybara (South America), hippopotamus (Africa) and in Australasia, water buffalo (Room and Julien, 1995; Julien *et al.*, 2009). The spread of *S. molesta* is through viable fragments (nodes or apical buds) which are responsible for giving rise to new plants. Boats and vehicles which enter infested waters also act as agents of dispersal of *S. molesta* to new areas. *Salvinia molesta* tends to be invasive in areas where man has put in place artificial modifications, including alteration of flows and high nutrient concentrations as a result of dams, diversions and excessive use of agricultural chemicals in a catchment area (Room and Thomas, 1986). The weed has also been widely spread by humans via the aquarium trade as water garden plants may be contaminated with propagules of the weed, thus enhancing its spread across the world (Julien *et al.*, 2009).

1.3.1 Impacts of *Salvinia molesta*

Salvinia molesta has wide ranging impacts both ecologically and economically, as well as health impacts for humans. In the ecological sense, *S. molesta* is known to outcompete and even displace native flora, thus upsetting ecosystem balances in its exotic range. According to Diop and Hill (2009), *S. molesta* invaded the Senegal River Delta, home to a variety of birds, some of which are endemic to that region. The aesthetic value of the Delta, which is a World Heritage Site under the Ramsar Convention, was also eroded as a result of this invasion. The prolific growth characteristic of this weed may result in the total coverage of a water body, ultimately leading to habitat degradation where mats prevent the diffusion of atmospheric oxygen into the water leading to anoxic conditions (Flores and Wendel, 2001). Decaying *S. molesta* which sinks to the bottom consumes dissolved oxygen, and the resultant reduction of oxygen in the water is also characterized by high concentrations of carbon dioxide and hydrogen sulphide in the water, leading to the suffocation of other aquatic organisms (Oliver, 1993; Doeleman, 1989). The high growth rate of *S. molesta*, coupled with the slow rate of decomposition leads to the locking up of essential nutrients within its plant tissues, and this in turn, impedes on the process of nutrient cycling. Phytoplankton and macrophytes are thus deprived of the nutrients which are essential in their role as primary producers, and other higher trophic levels are negatively affected. In this regard, *S. molesta* alters the nutrient dynamics of the water bodies it colonizes (Oliver, 1993).

Salvinia molesta also has economic impacts on human livelihoods where mats restrict utilization of both artificial and natural impoundments, as well as rivers for recreational and agricultural activities (Hill, 2003). In regions with livelihoods hugely dependent on aquaculture and water transport, severe losses are incurred due to these infestations where there is disruption of water traffic, commercial and sport fishing (Flores and Wendel, 2001). In the agro-based economies of low rainfall regions, *S. molesta* blocks irrigation pipes and fills in irrigation canals by accelerating siltation rates and losses in livestock are also incurred due to drowning in the infested waters. In Sri Lanka, rice production was severely affected where *S. molesta* actively competes with crops for space and nutrients. The Department of Agriculture in Sri Lanka, estimated losses in the affected areas to have been about 2-3% (Doeleman, 1989). Thornhill *et al.* (2007) suggested that infestation in Senegal led to a shift in the supply of rice where there was little of the product on the market. This raised prices for consumers and production costs for the farmers increased due to the factoring in of abatement costs. In this regard, the cost of living for people in the affected area was raised.

Drinking water quality is also affected, especially in terms of bad odour, colour and turbidity which tend to increase cost of water purification for the responsible authorities (Hill, 2003). Health costs are also incurred due to incidences of the weed which harbours vectors of diseases. Particular concern is raised by the resurgence of mosquito borne diseases such as malaria, filariasis, dengue fever and encephalitis and control of these diseases also raises health costs for the affected countries, such as in Sri Lanka, where about 30% of the health budget of 1987 was dedicated to controlling mosquito borne diseases (Doeleman, 1989).

1.4 Control of *Salvinia molesta*

The management of the weed requires site specific applications where priorities depend on the prevailing climate, the nature and use of the water body in question, extent of the infestation and the available resources for the undertaking. Methods of control entail biological, chemical and mechanical, as well as manual removal of the weed and the management options should be applied according to the uniqueness of the situation prevailing at a particular site (van Oosterhout, 2006).

Mechanical and chemical control provide temporary relief, however they are not environmentally friendly and financially sustainable options where the water is utilized for drinking, recreation and fishing. Biological control is thus left as the only safe option which will not hamper the utilization of water for the named uses (Hennecke and Postle, 2006).

1.4.1 Mechanical control

Mechanical control entails the physical harvesting of the weed. Booms have been employed to accumulate or localize the extent of weed mats prior to the use of machines to harvest the weed, however very little success is ever achieved, often at a huge cost, as was shown in the Hawkesbury River in Australia (van Oosterhout, 2006). Costs of the management activities far outstrip the benefits accrued from the application of this control option as the weed growth rates are higher than the removal rates. Lack of follow-up management and monitoring gives way for resurgence by the remaining plants (Room and Thomas, 1986).

There have been very few instances where complete control of the weed by this method has been possible. Such instances are mainly confined to new infestations where small amounts of *S. molesta* are detected early or small infestations on enclosed water bodies of up to 1ha in area (van Oosterhout, 2006). Follow-up measures are therefore essential to guard against re-invasion by new plants or resurgence by the missed plants (Julien *et al.*, 2009). Post-treatment monitoring is rarely done due to the costs associated with it and as such, mechanical control usually fails to provide sustainable control in the long term (Chikwenhere and Keswani, 1997).

1.4.2 Chemical control

Herbicides have been used to combat *S. molesta* in a number of countries across the world. In Australia, diquat, glyphosate, calcium dodecyl benzene sulphonate and orange oil have been used (van Oosterhout, 2006), while in South Africa diquat and 2, 4-dichlorophenoxyacetic acid were used but are no longer permitted leaving glyphosate as the only registered herbicide (Julien *et al.*, 2009). In the USA, permitted herbicides include diquat dibromide, fluridone, glyphosate and several chelated copper compounds. The application of herbicides is usually done using hand guns, boom sprayers and aircraft; however, herbicides are indiscriminate, affecting even non-target plants. There are a number of limitations to this control method, and these include reduced ease of access to some weed infestations, high costs of materials, and time consuming application of treatments, often requiring repeated applications (Julien *et al.*, 2009). In addition, the rows of trichomes on the upper surface of the *S. molesta* fronds act as a waterproof barrier to herbicides. The barrier then necessitates the use of a wetting agent before the application of a contact herbicide can be done (Oliver, 1993), and this only serves to raise the costs of control even further.

In 2009, the costs for chemicals alone were estimated to be in the range of between $210 to $900 per ha (Julien *et al.*, 2009). Some countries have imposed prohibitions or strict requirements for the use of herbicides near to or on the water itself. In the USA, post spray intervals have to be observed before cattle are allowed to drink from treated water bodies with the exception where copper compounds have been used (Julien *et al.*, 2009). Herbicides may have very rapid depleting effects against the weed biomass, but in the long run, do not provide a cost effective and environmentally friendly solution to the weed problem (Thomas and Room, 1986).

1.4.3 Biological control

Biological control is an ecologically sound method based on the use of natural enemies of the target weed to keep its populations at acceptable levels (Pieterse *et al.*, 2002). In South Africa, biological control for an array of aquatic invasive weeds was initiated in 1974 with the release of the weevil *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) on water hyacinth, *Eichhornia crassipes* (Mart) Solms-Laub. (Pontederiaceae). *Salvinia molesta* and other problematic water weeds in South Africa have been brought under control through the use of their natural enemies (Coetzee *et al.*, 2011)

A number of biological control agents were tested on *S. molesta* in Australia by CSIRO, and these comprise two weevils *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) and *C. salviniae* Calder and Sands, the moth *Samea multiplicalis* Guenee (Lepidoptera: Pyralidae) and a grasshopper *Paulinia acuminata* De Geer (Orthoptera: Pauliniidae) (Julien *et al.*, 2009). These insects were identified and isolated, having been found in association with *S. auriculata* in Guyana and Trinidad in 1960. The insects underwent host specificity testing before being released in the early 1970s in southern Africa and Fiji (Room, 1990).

Cyrtobagous singularis, S. multiplicalis and *P. acuminata* failed to control the weed after they were released against *S. molesta* in Zambia and Botswana, even though they had successfully established after they had been collected in Trinidad in 1975 (Calder and Sands, 1985). A weevil thought to be *C. singularis* was released in Australia in 1980 where it established and successfully controlled *S. molesta*. This weevil had been collected in southeastern Brazil on *S. molesta* and showed greater success in control compared to the Trinidad specimens, which was attributed to it being a different biotype to the ones collected from *S. auriculata*. Studies were carried out on the successful weevil whereupon it was reported to be a different species by Sands in 1983, and was reclassified as *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (Calder and Sands, 1985).

When *C. salviniae* initially eclose, adults are brown and gradually darken over the first five days to black synonymous with mature adults (Julien *et al.*, 2009). The external features of the weevils as described by Calder and Sands (1985) are as follows: male weevils have a length of 1.66-2.50mm with a mean of 1.76mm and a width of 0.85-1.10mm and a mean of 0.88mm. The rostrum antennae and legs are reddish brown while the head, pronotum and elytra have subcircular yellowish peltate scales. The legs and dorsal surface of the rostrum have numerous punctures. The head has frons with subcircular punctures with yellowish scales and the eyes are oval. Ventrite 1 is shallowly depressed, medially flat, bordered anteriorly by a semicircular raised flange. Females are 1.72-2.50mm in length, and have a mean of 2.20, the width is 0.96-1.2mm with a mean of 1.16mm. Ventrite 1 is broader, its posterior margin slightly convex; ventrite 5 posterior margin is more rounded.

The adults can usually be located feeding on young leaves, 'roots' and leaf buds. Because adults are sub-aquatic, they can respire under water by means of an air bubble which is held between their hind legs and lower body. This behaviour also doubles as a survival strategy when there are extreme temperatures as the weevils may utilize the temperature buffering properties of water (Julien *et al.*, 2009).

Forno *et al.* (1983) conducted studies at 25.5°C where adults mated more than once from five days after emergence and pre-oviposition behaviour was observed between 6-14 days. Oviposition sites are commonly feeding holes in young unopened leaves or developing 'roots' below the unopened leaves. Eggs measure 0.5mm x 0.24mm, and normally hatch after 10 days at temperatures greater than 19 °C (Julien *et al.*, 2009; Sullivan and Postle, 2010; Mukherjee *et al.*, 2014). The larvae are white, crescent shaped and measure 1mm in length, and upon hatching, feed on terminal buds, then petioles, rhizomes and 'roots'. Larvae go through three instars during their development which takes between 14-28 days depending on the prevailing temperature (van Oosterhout, 2006), and at third instar stage they measure about 2.6mm in length (Julien *et al.*, 2009). Larval development is optimal between 21˚C and 30˚C with nitrogen levels within host plant tissue being a major factor. It is notable that development fails at temperatures below 17˚C (Sands *et al.*, 1983), however recent studies revealed that development may occur at temperatures as low as 16.3˚C under laboratory conditions, with pupal development occurring at about 15 ˚C (Hennecke and Postle, 2006). After the third instar, larvae then spin a cocoon of root hairs which is 2-2.6mm in diameter which they attach to the 'roots' below the water surface, and pupal development takes about 9-15 days, with the generation time being between 42 and 68 days (van Oosterhout, 2006). If daily maximum temperatures remain below 20˚C for several months, weevil populations are unlikely to persist (Room *et al.*, 1989). Thus, the temperature at which the weevils can initiate reproduction and complete their developmental stages is crucial to their success as biological control agents (Sullivan and Postle, 2010).

Cyrtobagous salviniae is a very effective herbivore on *S. molesta* where both adults and larvae inflict serious damage to the plants. The feeding behaviour can stimulate the plants to produce more buds in a drive to compensate for the loss of buds, but these are also subsequently eaten (Julien *et al.*, 2009). The compensatory reaction by the weed eventually depletes the plant's resources and reserves leading to an initial slowing of growth and eventually a halt. Adults mostly feed on tender apical buds, leaves and roots, thus preventing growth, whereas larvae tunnel inside the rhizome and destroy the plant structure in the process (van Oosterhout, 2006). Tertiary stage *S. molesta* suffer damage almost solely on buds due to adult feeding, while terminal leaves of primary and secondary plants are also prone to damage (Sands and Schotz, 1985). Feeding rates increase with an increase in temperature while increases in nitrogen have no effect on feeding rates (Forno *et al.*, 1983).) High plant nitrogen serves to stimulate oviposition, and both oviposition and feeding mostly occur nocturnally (Sands *et al.*, 1986; Schotz and Sands, 1988).

First instar larvae have been observed to tunnel and feed on young buds while the second and third instar larvae tunnel and feed within rhizomes and 'roots'. In experiments conducted by Sands and Schotz (1985), it was noted that on primary and secondary stage plants, that larvae were particularly damaging to the protostele, endoderm, meristems and a greater proportion of the aerenchyma tissue, but when feeding on the tertiary stage plants, feeding was mostly restricted to the meristems, endoderm and aerenchyma. When the larvae feed on nodes, there is disintegration of internodes coupled with the separation of leaves (Sands and Schotz, 1985). According to Sands *et al.*, (1983) each larva has been known to cause damage to 1.2 to 2.4 sections of rhizome including internodes, thus effectively suppressing regrowth. Sands and Schotz (1985) noted that the behaviour of the larvae was inclined to destroying only one bud before tunneling inside the rhizome.

Feeding by both adults and larvae cumulatively results in the suppression of plant growth due to the destruction of meristematic tissue, rhizomes and ramets thus disconnecting the root-shoot link. Plants fail to compensate for the damage due to the combined effect of the two feeding strategies and eventually deteriorate. Weevil damage is evident in the form of a gradually expanding brown patch on a weed mat. The brown *S. molesta* becomes water logged after the destruction of the aerenchyma tissue and sinks to the bottom of the water body creating a patch of open water (van Oosterhout, 2006; Julien *et al.*, 2009). In studies carried out by Forno and Bourne (1985), females inflicted more damage than males in temperatures ranging from 25°C to 30°C. At low insect densities, females were observed to cause approximately 10% more damage than males and at high densities approximately 20% more. Adults caused overall damage of about 41% per bud at 25°C, and at high densities, mean damage was 45% at 20°C and 87% at 35°C.

Cyrtobagous salviniae is a relatively slow disperser (Hill, 2003); until high populations deplete food resources (young buds of *S. molesta*) and then flight dispersal occurs due to scarcity of food (Thomas and Room, 1986). Release rates of the weevil to achieve control vary where 300 adults/m^2 have been observed to successfully control the weed (Room, 1988; van Oosterhout, 2006). On the other hand, Tipping and Center (2005) found that a population of 100 adults/m^2 was effective while Schlettwein and Bethune (1992) found 50 adults/m^2 to be effective. In optimal conditions, weevils can attain populations which allow them to deplete plants much faster than the plants can compensate for damage, causing reduction in the carrying capacity of the host plants, leading to starvation of the weevils. In this regard a cycle of population build ups and crashes occurs, rarely ever achieving a total equilibrium between host and herbivore (van Oosterhout, 2006).

The control of *S. molesta* in many tropical and subtropical countries has been achieved through the release of *C. salviniae* in the affected areas (Room *et al.*, 1981; Room 1988; Thomas and Room, 1986). The control achieved after the adoption of the weevil as the biological control agent of choice facilitated its release with minimal pre-release studies in the exotic range of the weed, including in South Africa (Thomas and Room, 1986). The successes recorded in the use of biological control have proven it to be very effective against *S. molesta* and it is now the first choice strategy, at times applied in conjunction with other management options.

In Australia, *S. molesta* control was achieved after the introduction of *C. salviniae* from Brazil in 1980, and the first release was at Lake Moondarra near Mount Isa (van Oosterhout, 2006). According to Room (1990), the introduced weevil population of about a few thousand expanded, clearing about 30 000 tonnes of *S. molesta* in one year, whereas van Oosterhout (2006) wrote that an estimated 50 000 tonnes of *S. molesta* was decimated within 11 months. Subsequent releases in tropical and subtropical Australia resulted in consistent successes; however, in temperate climates, weevils were found to be effective only under suitable conditions (van Oosterhout, 2006). Biological control in temperate regions may be impeded by the cooler climate but field trials carried out at the Hawkesbury-Nepean Catchment, New South Wales, Australia showed the possibility of successful biological control of *S. molesta* in temperate regions (Room, 1990; Hennecke and Postle, 2006). More than 18 countries have benefited from releases of *C. salviniae* resulting in alleviation of some of the problems for human communities dependent on wetland ecological systems that had been most affected by the infestations (Julien *et al.*, 2009).

In Zimbabwe, a cost benefit analysis was undertaken on the attempted control options and it revealed that physical and chemical methods were expensive while biological control proved to be effective, sustainable and cost effective (Chikwenhere and Keswani, 1997). The initial high costs associated with *S. molesta* control can be significantly lowered in the long run by the application of biological control measures, carefully planned use of herbicides and consistent small-scale mechanical control. Adequate resources should be mobilized to cater for monitoring and follow up each year as an ongoing process (van Oosterhout, 2006).

1.4.4 Control in South Africa

Salvinia molesta biological control in South Africa commenced with the release of *C. salviniae* in 1985. Next to *E. crassipes*, *S. molesta* was considered the most problematic aquatic invader in South Africa (Coetzee *et al.*, 2011). The proven success of the control measures in Australia, Papua New Guinea and Namibia ensured the direct introduction of *C. salviniae* into South Africa in September 1985, from Eastern Caprivi, Namibia without going through the usual host specificity tests after Australian research had proved the weevil to be host specific (Cilliers *et al.*, 2003).

Control of the weed was achieved throughout the Limpopo, KwaZulu-Natal, Mpumalanga and the Eastern and Western Cape provinces, even when faced with concerns that there would be limited success in the temperate regions (Forno and Bourne, 1985; Coetzee *et al.*, 2011). At the end of the 1990s, biological control of *S. molesta* was viewed to have been achieved with a great measure of success and it was concluded that no further control measures were necessary, with the exception of further releases of the weevil to newly infested sites (Cilliers *et al.*, 2003; Coetzee *et al.*, 2011).

Very little has been published on the biological control of *S. molesta* in South Africa, despite the success of the programme. Nationwide, post-release evaluations from January 2008 to 2010 demonstrated that less than 10% of an estimated 300 survey sites had *S. molesta* present and the majority of these were new incidences of the weed. The evaluation showed that in spite of the weed spreading; detection had been enhanced by improved surveying methods, thus increasing the knowledge of its known distribution in South Africa (Coetzee *et al.*, 2011). A concern arose however in 2008, where only six of the 17 survey sites had *C. salviniae* present, thus highlighting the need for better application of the biological control programme. Since then, due to intensified monitoring and culturing, releases of *C. salviniae* have increased through the combined effort of the South African Sugar Research Institute (SASRI), City of Cape Town and Rhodes University which have established mass-rearing facilities. These efforts have ensured that *C. salviniae* has been distributed to all new sites, especially during periods of high growth, thus enhancing rapid control of the weed (Coetzee *et al.*, 2011).

A recent example of control success was on a farm in the Greytown area, KwaZulu-Natal. In January 2009, the dam was completely covered by the weed but releases of *C. salviniae* throughout the summer of 2009 ensured that the site was cleared with only 5% remaining by January 2010. In general, implementation of biological control has managed to bring *S. molesta* under control with most sites in South Africa being almost free of the weed (Coetzee *et al.*, 2011).

1.4.5 Influence of nitrogen and temperatures on biological control of *Salvinia molesta*

Biological control of *S. molesta* though effective, has not achieved uniform success across all areas of infestation and this can be attributed to conditions prevailing in those sites. This variation may be affected by a number of abiotic factors that have been found to influence the growth of *S. molesta* and these include conductivity, salinity, pH, temperature, light intensity and the abundance of nitrogen (Mitchell *et al.*, 1980). These factors regulate the growth and population dynamics of *S. molesta* in both laboratory and field conditions in the absence of natural enemies (Cary and Weerts, 1983); however, these factors also act to induce phenotypic differences in the plants (Room *et al.*, 1989). In the same manner, temperature and nitrogen availability of the host plants explain variation in developmental rates, survival and fecundity of the biological control agent *C. salviniae* (Tipping and Center, 2003).

Optimal conditions for both plant and control agent are closely related, with temperatures close to 30˚C being optimal for both. High water temperatures coupled with high light intensities promotes the growth of *S. molesta* in the same manner they influence insect development. Sands *et al.* (1983) found that adults exposed to constant temperatures of 23˚C, 27˚C and 31˚C lived on average 163.1, 116.9, and 101.5 days respectively. Adults have been found to lay eggs at a constant temperature of 19˚C with an average of 0.019 eggs /day being laid (Hennecke and Postle, 2006). Temperatures of about 27 ˚C allow for maximal egg laying at rates of 0.45 eggs/day, while temperatures of below 21 ˚C are characterized by negligible laying where rates of laying eggs at 21 ˚C and 19 ˚C are 0.041 and 0.019 respectively (Hennecke and Postle, 2006). Eggs are able to hatch after 10 days even where the prevailing temperatures can be as low as 19 ˚C, while larval development also occurs at temperatures of not less than 16.3 ˚C, whereas pupal development is possible at about 19˚C (Sullivan and Postle, 2010). In such situations where *C. salviniae* starts reproduction at the lower quartile of the optimum temperature range, they can possibly build up populations faster than the recovery of the weed usually after winter. The high populations of *C. salviniae* cause a lot of damage to *S. molesta* thereby suppressing growth of the plants, and effectively bring it under control (Harms *et al.*, 2009; Sullivan and Postle, 2010)

Temperature alone does not influence plants and their associated herbivores, but nitrogen as well has a significant role to play (Mitchell, 1974). Room (1990) explored the effects of two water-nitrogen concentration levels on *S. molesta* and climate at three latitudes, on plant population dynamics as well as damage inflicted by weevil feeding and found that temperature and nitrogen had an influence on the population dynamics of the host plant and the weevils. The populations of the weevils were found to increase faster on plants with high nitrogen content. The tissue nitrogen content of *S. molesta* ranges from 0.6 to 4.0% of the total dry weight (Room and Thomas, 1986a). The doubling of female fecundity results from increases in tissue nitrogen concentration (Hennecke and Postle, 2006). Larval tunneling within the plant rhizomes usually leads to the destruction of many sections of rhizome when there is low plant tissue nitrogen concentration (less than 1.5% dry weight) and fewer sections of rhizome are destroyed at higher tissue nitrogen concentrations (Room *et al.*, 1989). In areas where high temperatures occur, combined with high nitrogen in water, control of *S. molesta* is achieved in contrast to low temperature and nitrogen sites. Occurrence of high temperatures and nitrogen favours high population growth rates for *C. salviniae* (Sullivan *et al.*, 2011).

Weevil development and establishment is much faster on *S. molesta* that grows as a single layer, in open areas receiving full sun. However, for reasons yet to be understood, the weevils have been observed to fail to control *S. molesta* that grows as an understory species (van Oosterhout, 2006). Though weevils are able to effect control on a shaded infestation, small amounts of *S. molesta* always survive, taking refuge along the edges of water bodies or in shaded areas and this appears to be the case in South Africa.

1.5.1 Morphology of plants growing in shade

Research has shown that understory plant species experience opening and closure of the canopy at various times. This has resulted in shade or light tolerant plants but not necessarily giving rise to obligate shade or light plants, especially in tropical ecosystems (Lüttge, 1997). There are three strategies for plants dealing with shade conditions (i) shade avoidance (obligate sun plants); (ii) shade tolerant (facultative sun or shade plants) and, (iii) shade adapted (obligate shade plants) (Lambers *et al.*, 2008). Shade tolerant plants have been shown to have high leaf areas instead of thickness. Some of the morphological characteristics of shade plants are that they tend to be larger than those growing in the light even though they are the same species (Lüttge, 1997). In plants which tolerate moderate shade, leaves are significantly larger in terms of leaf area as compared to those growing in full light or dense shade (Marenco and Reis, 1998; Semchenko *et al.*, 2012). *Salvinia molesta* for example has been shown to have large leaves characteristic of the tertiary growth form when growing under moderate shade, however, where there is dense shade, they tend to remain in the primary stage and loosely cover the surface of the water (Owens *et al.*, 2011). The primary growth form found mostly under dense shade facilitates invasion and re-invasion by the weed as it has small leaves and long, fragile internodes which break easily, thereby ensuring the plant is easily dispersed for colonization of a water body (Mitchell and Tur, 1975).

1.5.2 Influence of shade and full sun on plant growth, quality and herbivory

Shade has a net facilitative effect on biomass of most herbaceous plants. Prolonged life spans of plants are typical of understory species (Semchenko *et al.*, 2012). Excess light, however, can inhibit photosynthesis and lead to photo-oxidative destruction of the photosynthetic apparatus, thereby decreasing the photosynthetic rates of plants growing in direct sunlight and hence a reduction in their life spans (Bote and Struik, 2011). The relative growth rates of plants grown in shade have also been reported to be higher as compared to those in full light (Marenco and Reis, 1998). *Salvinia molesta* is such a plant which suffers from light saturation leading to reduced growth (McFarland *et al.*, 2004). In a study by Owens *et al.* (2011), it was shown that *S. molesta* has high growth rates when exposed to full sunlight but even better growth was experienced by plants growing in moderate shade, while under dense shade little growth was achieved. In a study on two submerged macrophytes (*Elodea nuttallii* St. John (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae)), it was discovered that shade does not inhibit the establishment and growth of the plants; however, high growth rates were found in moderate shade compared to full light and dense shade (Zefferman, 2014).

Understory species mostly in the tropics tend to increase chlorophyll content per unit leaf area giving them a dark-green pigmentation (Lambers *et al.*, 2008). The high concentrations of chlorophyll in the leaves gives rise to a lower protein/chlorophyll ratio in shade plants owing to a reduction in the amount of RUBISCO, the single major protein and nitrogen containing compound in plant leaves (Lüttge, 1997). The abundance of chlorophyll in shade plants gives rise to less available soluble protein in leaf tissue when compared to plants in full sun, however in terms of total nitrogen, including non-protein nitrogen, shade leaves tend to have more nitrogen (Lüttge, 1997). Less available soluble protein nitrogen is probably attributable to low rates of proteolysis in shade leaves as they realize lower rates of leaf senescence which usually results in higher soluble protein availability (White, 1984).

Shade leaves have been reported to record photosynthetic yields similar to light leaves only if there has been an additional supply of nitrogen to them (Lüttge, 1997). The explanation for this could possibly be the fact that plants can then invest more nitrogen to the formation of the carbon fixing enzymes which then would increase the amount of nitrogen in the leaves (Lambers *et al.*, 2008). Evans (1989) stated that nitrogen availability in leaves is mainly dependent on the growth irradiance with much of the nitrogen being found in leaves growing at high irradiance. During the course of a plant's growth cycle, the leaves respond to varying irradiances by nitrogen allocation between proteins such that the tissue has variable nitrogen concentrations (Evans, 1996).

Dry weight of *S. molesta* plants growing in very dense shade is lower than that of those growing in full sunlight (Owens *et al.*, 2011). There is also a stark contrast in the leaf colour of plants growing under different amounts of sunlight and these express varying phenotypes with respect to pigmentation, *S. molesta* in the shade has a much deeper hue of green leaf pigmentation (Lüttge, 1997; Owens *et al.*, 2011).

Nitrogen content in plants is one plant characteristics that is essential for herbivores. Nitrogen plays a pivotal role in metabolic processes; cellular structure and genetic coding making it a critical element in the growth of all organisms (Mattson, 1980). Excessive irradiance has been identified as a stress factor which tends to stimulate nitrogen mobilisation in leaves exposed to high incident irradiance (Mattson, 1980; Roháček *et al.*, 2008). Protein synthesis in leaves tends to be inhibited by excessive radiation while accelerating the rate of senescence (White, 1984). When such stress occurs, plants react by reducing several functions, some of which include the photosynthetic performance, mobilisation and translocation of metabolites, the consequences of which is a decline in plant vitality and it is at this moment that herbivores have the greatest impact on plants when there is more available nitrogen unlocked from the plant tissues (Roháček *et al.*, 2008). Mattson (1980) reported that with the exception of young plant tissues, senescent leaves have the highest concentration of soluble protein nitrogen which means that when the tissue protein is hydrolysed, the plant tissue quality with regards to soluble protein-nitrogen is momentarily high and this is favourable to the herbivores.

Insect herbivores have a high preference for sun-exposed leaves of plants and aphids, coccids, sawflies and some Lepidoptera, have been reported to have high survival on the sun exposed leaves (Roháček *et al.*, 2008). The release of nitrogen from plant tissues makes it more available for the herbivores, hence the high preference for the sun exposed leaves (White, 1984). High defoliation rates have been recorded in sun exposed leaves where herbivores impact heavily on them leaving the shade leaves the more abundant foliage. A distinct foliage die back of sun exposed leaves is always evident after such heavy herbivory owing to the preference for the sun exposed leaves (White, 1984).

Shaded areas typically have different temperature regimes when compared with those exposed to full sunlight. Temperatures typically influence food consumption and insect growth rates (Kingsolver and Woods, 1998). Food intake by most insect herbivores has been reported to increase from between 20°C and 30°C with little consumption after 30°C and decreases being recorded above 35°C (Lemoine *et al.*, 2014). The salvinia weevil, *C. salviniae* was shown to have high feeding rates at temperatures between 25°C and 30°C with feeding completely stopping below 13°C (Forno and Bourne, 1985). Consumption rates have been known to decrease if the temperatures exceed the thermal limits of the herbivore; however, fluctuations in food consumption are species specific as not all herbivores will show a similar trend (Kingsolver and Woods, 1998). There have been predictions of exponential increase in herbivory with increase in temperature and this increase may outstrip primary productivity by plants, therefore a net decrease of plant biomass may result (Lemoine *et al.*, 2014). Increases in herbivory with a rise in temperature can be attributed to high metabolic rates which rise exponentially with the temperatures (Kingsolver and Woods, 1998).

1.5.3 Plant quality and its influence on herbivores

Insects have been shown to mostly utilize high quality plant material and this is true for specialist herbivores, especially those used in biological control of weeds (Price, 2000). With regards to oviposition, female phytophagous insects show a very strong preference for high quality food and this is closely related to the survival of the larvae as extra nitrogen stimulates health, growth, reproduction, and survival for most organisms (Price, 2000; Throop and Lerdau, 2004). In the plant-insect herbivore interaction of *S. molesta* and *C. salviniae*, it was shown that reproduction was accelerated when there was an increase in plant tissue nitrogen content when optimum temperatures were also prevailing (Room and Thomas, 1985; Sands *et al.*, 1986). Insect herbivores mostly attack new plant growth or young tissues, mainly buds, stems and leaves and it has been shown across most insect feeding guilds that they mostly utilize plants which are actively growing (Price, 2000). Insect herbivores also choose high quality food materials to offset costs of high metabolic rates which normally rise with an increase in temperature (Lemoine *et al.*, 2014).

There are instances where herbivory responds negatively to increased plant tissue nitrogen such as a decrease in the consumption rates of plants by insect herbivores. Forno *et al.* (1983) highlighted that *C. salviniae* feeding only increased with an increase in temperature and not with an increase in nitrogen. The herbivores most likely respond this way so as to maintain their nitrogen intake and keep it at a constant or optimum rate thus avoiding nitrogen toxicity which would otherwise impair their performance (Throop and Lerdau, 2004). However, in some instances, there is an increase in the consumption of foliage mostly due to the low efficiency of some herbivores in sequestering the nitrogen with some having about 50% nitrogen use efficiency. Under these circumstances, herbivores have to eat as much as four units of food to assimilate a unit of protein nitrogen (Mattson, 1980). It was reported by Kingsolver and Woods (1998) that low dietary nitrogen or protein may only affect insect growth and reproduction rates negatively while at the same time inducing increased herbivory in an effort to meet their dietary needs.

Past research has highlighted that there has been less success in controlling *S. molesta* growing in the shade in South Africa and elsewhere. This study sought to determine how shade influences the growth of the weed as well as the quality of the plants in terms of tissue nitrogen content. Reproduction and population dynamics of the associated herbivore, *C. salviniae* would most likely be influenced by the conditions determining plant growth and quality, hence the success of biological control of the weed under shade conditions.

1.6 Research Questions

1. Does *S. molesta* grown in the shade possess higher nitrogen concentrations, a determinant of plant quality, compared to *S. molesta* grown in full sunlight?

In many areas that have been invaded by *S. molesta*, temperatures remain optimum for most of the year and therefore nitrogen becomes the main limiting factor to plant growth. However, in nitrogen poor waters, the *S. molesta* plants exhibit longer ‘roots’, larger leaves and have fewer branches compared to high nitrogen conditions (Julien *et al.*, 2009). When nitrogen is abundant, it is stored and an increase in vegetative growth results through branching and earlier fragmentation of colonies of ramets (Room *et al.*, 1989).

To answer this question, elemental analysis of *S. molesta* plants investigated the nitrogen content in various tissues of the plants. Plant tissue nitrogen concentration was used as a measure of plant quality. Water samples from both shade and light treatments were collected to evaluate water nitrogen which ultimately influenced plant quality.

2. Does shade influence the biomass accumulation of *S. molesta*?

Relative growth rates of *S. molesta* in the shade when temperatures are high (especially in summer) tend to increase in comparison to growth in the full sun. Shade has been noted to be a temperature regulator for the plants and is also responsible for regulating the amount of light that is received by the plants (Usha Rani and Bhambie, 1983). Furthermore, Usha Rani and Bhambie (1983) found that *S. molesta* growth is inhibited by light saturation (about 1003 $\mu mol/m^{-2}s^{-1}$) especially under natural conditions, while McFarland *et al.* (2004) found biomass doubling rates of 9.8 days and 11.8 days in full light and shade respectively. In this regard, temperature and light levels have a profound influence on plant growth.

In order to determine the influence of shade and resulting light availability on relative growth rates, plant growth was measured by biomass accumulation rates. Plant biomasses were then compared to ascertain any differences between treatments.

3. Does shade affect the feeding behaviour of the weevil *C. salviniae,* and therefore damage to *S. molesta*?

Adult weevils primarily feed on buds which have higher nitrogen content, at temperatures ranging between 13°C and 33°C (Sullivan and Postle, 2010). Vigorous plants sustain more damage at optimal conditions and the success of biological control is more likely where there is vigorous plant growth (Room *et al.*, 1989). At temperatures of approximately 15 °C, adult weevils have been observed to spend about 10 days feeding on one bud with less than 5% damage recorded. However, at temperatures of between 30 °C and 35 °C, adults tend to abandon a bud just after three days due to extensive leaf damage (Forno and Bourne, 1985).

To investigate this question, plant damage was assessed and compared between shade and full light. Conditions in both full light and shade were recorded to ascertain the differences in temperatures between light and shade conditions. Fluctuations in the biomass measures of the plants were used to assess the feeding impacts of the weevils.

4. How do differences in plant quality between shaded and open sites influence weevil and population growth?

Plant nitrogen content influences the development of *C. salviniae*. In nitrogen poor plants, the weevils take longer to develop (Room *et al.*, 1989; Sullivan and Postle, 2010). The ideal plant nitrogen concentration for the weevil is more than 3% dry weight (Room *et al.*, 1989). An increase of just 0.5% dry weight plant nitrogen results in a decrease in the total developmental time of the larvae by about 4.1 days at most temperatures, while doubling female fecundity (Room *et al.*, 1989).

To answer the above question, weevil populations were monitored and recorded at fixed intervals, where adults were found and enumerated.

Chapter 2 : Materials and Methods

The effect of shade on the biological control of *S. molesta* was investigated in two studies, the first in a greenhouse and the other in the field. The greenhouse experiment was carried out at Waainek Research Facility, Rhodes University, GPS co-ordinates, S33° 30.9455' E26° 50.0625'. The field experiment was carried out at a quarry dam in Grahamstown GPS co-ordinates, S33° 32.0038' E26° 50.7704'.

2.1 Greenhouse study

In order to determine the effect of varying degrees of shade on *S. molesta* growth and the subsequent level of control exerted by *C. salviniae,* a greenhouse study was conducted. The study ran from May 2014 to December 2014. Three experimental treatments were set up inside the greenhouse at three levels of shade; 80% shade, 40% shade and 0% shade (full light) (Fig. 2.1). Shade cloth of the corresponding shading intensities was employed to cover tubs used in the experiment and each shade treatment had a total of 16 tubs.

For each of the shade treatments, plants were grown at two nutrient levels, high (15mg N/L^{-1}) and low (0.3mg N/L^{-1}) in 65L of tap water. The two nutrient levels were representative of a eutrophic (high nutrients) and an oligotrophic (low nutrient) system in which plants may grow under field conditions (DWAF, 1996). Half the tubs under each shade level were used for the high nutrient treatment and slow release fertilizer was used (Multicote 8 [13:6:20]) while the other half was not subjected to nutrient enrichment. The plants were obtained from an insect free culture of *S. molesta* at the Rhodes University mass rearing facility so as to eliminate the possibility of bias from an already established population of *C. salviniae* on the plants. Each of the tubs initially had five tertiary stage plants which were pre-weighed at the start of the experiment. Plants were left for 7 days to acclimate to the new conditions before the start of the experiment. Three hundred adult *C. salviniae* were obtained from the South African Sugarcane Research Institute (SASRI) (Mt. Edgecombe, KwaZulu-Natal). Half the tubs in each shade and nutrient treatment were inoculated with a pair of *C. salviniae* per plant, giving a total of five pairs of weevils per tub. Since sexing the weevils individually would have been difficult and time limited, sexing was done *in copula*. The other half of the tubs remained as insect free controls. Each of the treatments was replicated four times giving a total of 48 tubs inside the greenhouse.

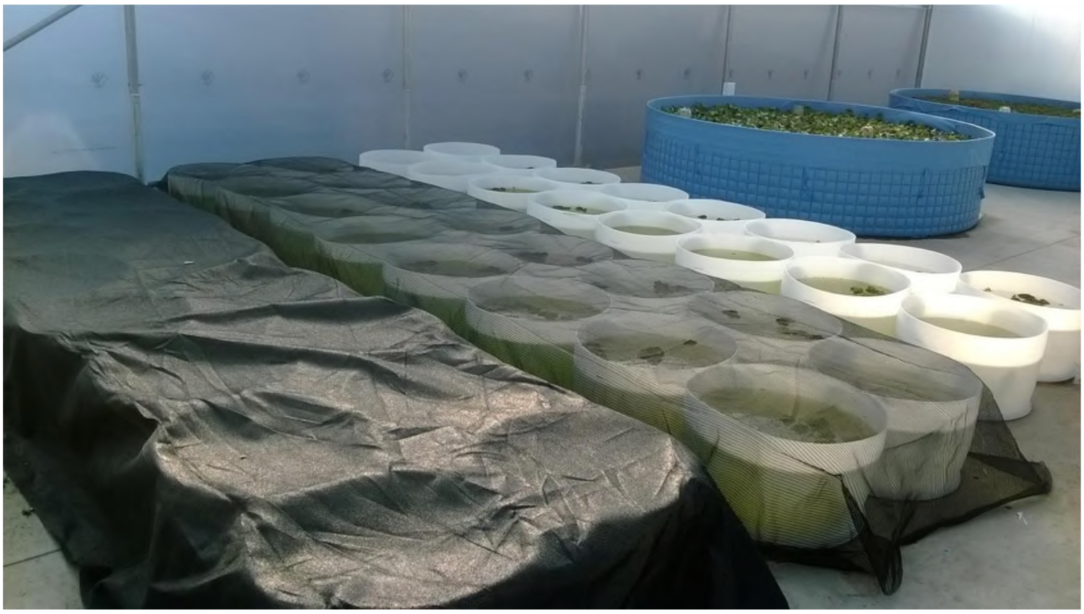


Figure 2.1: The experiment within the greenhouse had three treatments: dense shade, moderate shade and no shade and each experiment had four treatments with four replicates each.

Thirty days after inoculation with weevils, plant biomass was measured and again every 14 days thereafter. Biomass was measured for each of the five plants in each tub for all the treatments. The weed mat constantly fragmented as the plants grew, however, only five plants per tub were randomly selected and weighed to maintain consistency from the beginning of the experiment. Total plant biomass was also measured for each tub per treatment as plants were fragmenting as they were growing. If only per plant biomass was considered, it may have given a biased measure of plant growth as fragmentation gave the impression that plant growth was decreasing, hence total tub biomass was more accurate compared to per plant biomass in this situation. Leaf chlorophyll was measured to ascertain if there were differences in chlorophyll concentrations between the three levels of shade. A chlorophyll meter (Model CCM 200, Apogee instruments) was used to measure chlorophyll for five randomly chosen plants per tub in each shade level, at 14 day intervals.

Plant damage was also quantified at 14 day intervals by counting the number of leaves showing the characteristic ‘shot hole’ weevil feeding scars on the leaves. To quantify the leaf damage, 100 leaves per tub were counted and the number of damaged leaves was presented as a percentage of the total enumerated leaves. Weevil populations were counted at 40 day intervals to track population growth. The interval between sampling events was due to the relatively long developmental time of the weevils which ranged from 42-68 days. In this experiment, dead weevils were replaced with new ones at the end of the winter season due to the low populations that were recorded.

Environmental factors likely to influence plant growth were also recorded. Water and surface temperature data were collected using Thermachron iButtons (Climastats Environmental Monitoring software, Version 4). Temperatures were recorded at 1 hour intervals for the duration of the experiment. The amount of Photosynthetically Active Radiation (PAR) received by plants at water surface level was measured with an Apogee Instruments Quantum Meter (Model MQ-200), under each shade treatment at 60 minute time intervals from 08.00-16.00 hours every 14 days. Physiochemical properties of the water (pH, conductivity, total dissolved salts [TDS], salinity and dissolved oxygen) were recorded at monthly intervals using a Multi-Parameter meter (PCSTestr 35 Series) and a dissolved oxygen meter (DO Pen 850045 Sper Scientific). Ammonium and nitrate in the water were analysed using Vernier Ammonium Ion-Selective and Nitrate Ion-Selective electrodes at monthly intervals. These water parameters were measured to determine the effect of the physiochemical properties of water on plant growth with special emphasis on ammonium and nitrate which most likely influenced plant quality.

Plant carbon and nitrogen content was analysed monthly to give a measure of plant quality. Destructive sampling was employed and it did not in any way interfere with biomass measures as biomass was measured prior to dissection of plants for plant quality samples. Samples were oven dried at a constant 60°C for three days and then sealed in neutral graduated micro test tubes with caps (1.5ml Eppendorf tubes) and stored in re-sealable packets filled with Silica Gel Blue (self-indicating) to keep them dry before weighing. The dried plant parts were ground into powder using a mortar and pestle, before grinding each sample the mortar and pestle were cleaned out using methanol and dried using paper towels to avoid contamination from each preceding sample. After grinding, plant powder was weighed into tin capsules (1.8-2.2mg plant powder per tin capsule) using an Ohaus Discovery Semi-Micro Balance (Model DV214C). The equipment used for weighing was also cleaned with methanol to avoid contamination of samples. The weighed plant samples were sent to the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria for elemental analysis.

2.2 Statistical analysis

Only data obtained at the end of the trials were used in statistical analyses. Data were analysed in NCSS 10, PAST v2, Primer 6, R (v3.2.2) and in Statistica (v12.5). PCA was run in Primer 6 and PAST was used to confirm the PCA using loadings which are not provided in Primer. Comparisons for variation in data were carried out at the end of the trials. One-way ANOVA was used to check for differences in PAR as well as in checking for significance of water nutrient levels. Factorial ANOVA followed by Fisher LSD mean separation test in Statistica was used to determine differences between means for plant biomass, plant quality, weevil populations and leaf damage. G*power 3.1.9.2 was used to test statistical power of analyses which did not have significant differences. Automated model selection in R using the 'dredge' function which generates combinations (subsets) of terms in the global model, with optional rules for model inclusion was used to find the best fit model from the full complicated model of all variables. Generalized Linear Models (GLZs [Gaussian and Poisson distributions, log function]) were used to assess the effect of plant quality (plant C:N), PAR and shade on weevil populations. Effects of ammonium, nitrate and shade on plant quality were also assessed using GLZs. NCSS10 was used to generate 3D visualizations of the relationship between plant biomass, quality and plant C:N ratio. Shapiro normality test and plots of residuals against fitted values in R were used to check data for normality before analysis and dispersion was required to be close to 1 to validate the model fit.

Field study

In order to determine the effect of shade microclimate under natural conditions, a field study was conducted on a quarry dam at the Hippies Farm: 3 Chimneys extension, Grahamstown, (S33° 32.0038', E26° 50.7704'). The field site is approximately 2.3 km from the university.

2.3 Description of study site

This site was a closed lentic system with a dam that was approximately 0.457 hectares and the deepest part measured was 2.5m after it was intentionally filled in to reduce depth for recreational purposes. This dam was totally covered by *S. molesta* which was intentionally introduced to beautify and diversify the ecosystem in 2009 (Davies, pers. comms., 2014). The site also has a housing complex next to it with a number of houses situated there. Recreational activities such as swimming, braais (barbecues) and parties also take place at this site. Before *S. molesta* took over the site, fishing was a common activity which ceased due to the infestation.

A cliff overlooks the dam on the eastern side and casts a large shadow for most of the day along the margins of the water body, and this is especially prevalent during winter (Fig 2.2). At the foot of this cliff, remnants of its history as a quarry dam are visible in the form of an unstable slope of bricks lining the edge of the water body. The extent of the shade is less during summer as the normally shaded part gets some full light for a few hours during the day before the whole dam is cast into shade again in the late afternoon. The effect of the shadow is also enhanced by the overhanging riparian vegetation which is evergreen, leading to little filtered light reaching this part of the dam.

Before the start of the experiment, water samples were collected at varying depths across the water column using a Niskin bottle water sampler to measure the nutrient status. The samples were analysed for nitrogen content using Vernier Ammonium Ion-Selective and Nitrate Ion-Selective electrodes and the results showed that the water body was oligotrophic (Table 2.1). The oligotrophic status of this dam was identified in line with South Africa Water Quality Guidelines- Aquatic ecosystem which state that when inorganic nitrogen concentration (ammonium) is less than 0.5mg/L, a water body is oligotrophic (DWAF, 1996).

Table 2.1: Initial water ammonium and nitrate values sampled at varying depths down the water column.

Depth (m)	**Ammonium (mg/L)**	**Nitrate (mg/L)**
Surface	0.2	1.0
0.5	0.2	1.2
1.0	0.1	1.3
1.5	0.2	1.1
2.0	0.1	1.5
2.5	0.2	1.8

Figure 2.2: The study site at Three Chimneys farm, covered by *Salvinia molesta*.

2.3.1 Experimental procedure and data collection

Data was collected every month for 9 months, beginning April 2014 and was terminated in December 2014. Two areas that had differing exposure to full sun were identified, a zone under constant shade throughout the day, and another one which received full sunlight most of the day (Fig 2.3). A total of twelve 1m^2 quadrats were placed randomly across the dam, six in the shade and six in full sun. In each zone (shade and light), half of the quadrats were enriched with nitrogen fertilizer because of the oligotrophic status of the dam (Table 2.1), to determine the effect of high quality versus low quality plants on the level of biological control.

Nutrient enrichment was achieved using Multicote 8 (13:6:20) at a rate of 15mg/l for a volume of 1m^3 of water in each of six high nutrient quadrats (three in either zone) and for the controls no nutrient manipulations were done. In each nutrient enriched quadrat, fertilizer was put into five nutrient dispensers to try and evenly distribute the nutrients in the quadrats. In each quadrat, 100 *C. salviniae* adults were released, similar to the release rate by Tipping and Center (2005) to see the effect that herbivory would have on plants under high and low nutrients both in the shade and full sun.

Plant biomass data (wet mass) were collected every 14 days to give a measure of plant growth rates. In each quadrat, an area of $0.1m^2$ was randomly selected and sampled, and ten plants from this area were randomly selected and wet mass measures were recorded. The plants were then packed into ziplock bags and put in a cooler box full of ice to keep them fresh before being transported back to the laboratory for plant quality assessment. Leaf chlorophyll measures were recorded to determine differences in chlorophyll concentrations between the shade and full sun quadrats. A chlorophyll meter (Model CCM200, Apogee instruments) was used to measure chlorophyll for ten randomly chosen plants per quadrat in each shade level, at 14 day intervals.

To quantify feeding damage, 100 fronds were counted from the sampled plants per quadrat and the number of damaged leaves was presented as a percentage of the total number of fronds enumerated. Weevil populations were monitored every 6 weeks owing to the relatively long insect development from egg to adult (42-68 days). The number of adults found in each sample of $0.1m^2$ was recorded.

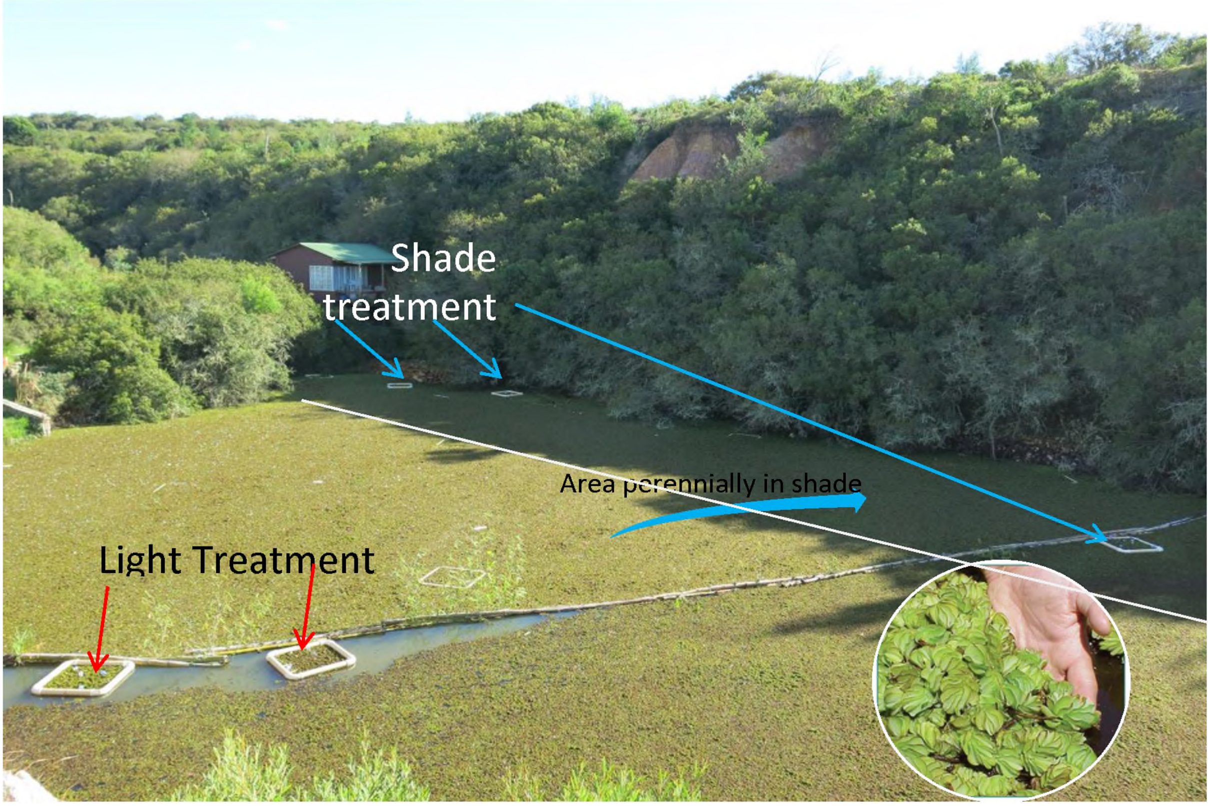


Figure 2.3: Quadrats were randomly laid out in the two zones of shade and full sun in the field site.

Photosynthetically active radiation (PAR) was measured every 14 days in both zones using a Quantum Meter (Apogee instruments: Model MQ-200). This was done to compare the amount of PAR received by plants in both the shade and full sunlight zones. The PAR recordings were made at 60 minute intervals from 08.00 hrs to 16.00 hrs. The solar data were further consolidated using ArcMap 10.1 (Advanced Student Edition) where monthly solar averages for Grahamstown were recorded using this software. Shade mapping was also done at the site to determine the extent of the shade from 08.00 hrs to 16.00 hrs at two week intervals and photographs of the study area were taken every thirty minutes during these time periods.

Conductivity, pH, total dissolved salts, salinity and dissolved oxygen were also recorded using a Multi-Parameter meter (PCSTestr 35 Series) and dissolved oxygen meter (DO Pen 850045 Sper Scientific), and all these were recorded on site. These water parameters were measured at monthly intervals. Temperatures were recorded at this site using Thermachron iButtons (Climastats Environmental Monitoring software, Version 4). The temperatures were recorded 5cm above and below the water surface to capture air and water temperatures. The data loggers were set to record the temperatures at hourly intervals every day throughout the duration of the study. Two data loggers were set up under the shade conditions and another two under full sunlight.

Water nitrogen content was measured at monthly intervals to determine the water nutrient status in the nutrient enriched and non-enriched quadrats. Water samples were taken down the water column from the surface to the floor of the dam using a Niskin bottle water sampler at an interval of 0.5 m. The water samples were put in vials which were then placed on ice before being transported to the laboratory where they were tested for ammonium and nitrate using Vernier Ammonium Ion-Selective and Nitrate Ion-Selective electrodes.

Plant nitrogen content was evaluated every 30 days by collecting different plant parts (buds, leaves, rhizomes and roots) from five plants in each quadrat for all treatments. Samples were oven dried at a constant 60°C for three days and then sealed in neutral graduated test tubes (1.5ml Eppendorf tubes) and stored in re-sealable packets filled with Silica Gel Blue (self-indicating) to keep them dry before weighing. The dried plant parts were ground to fine powder using a mortar and pestle prior to weighing. In between sample grinding, the mortar and pestle were cleaned with methanol and tissue paper to avoid contamination of samples. The samples were weighed into tin capsules at 2mg powder and samples were then sent to the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria for analysis.

2.3.2 Statistics

Data from the last sampling period of the field trials were analysed in NCSS 10, PAST v2, Primer 6, R (v3.2.2) and in Statistica (v12.5). PCA was run in Primer 6 to explore the relationship between biological and environmental data. PAST was used to validate the PCA in Primer where loadings gave the contribution of each environmental variable as these are not provided in Primer. T-tests in R were used to determine differences between means of shaded and unshaded zones for PAR, water ammonium and nitrate, plant biomass, plant quality, weevil populations and leaf damage, for the shaded and unshaded plots, as well as for the high and low nutrient treatments. A power analysis was conducted in G*power (3.1.9.2) to test the statistical power of the treatments for leaf damage and plant quality. Automated model selection in R used the 'dredge' function to generate combinations (subsets) of terms in the global model, with optional rules for model inclusion to find the best fit model from the full complicated model of all variables. The relationship between plant mass, nitrogen, nitrate, ammonium and the response of weevils populations as well as leaf damage at given temperatures was assessed using a negative binomial generalized linear model (link =”log”) in R. Generalized Linear Models (GLZs [Gaussian and Poisson distributions, log-link function]) were used to assess the effect of plant quality (plant C:N ratio), PAR and shade on weevil populations. Effects of ammonium, nitrate and shade on plant quality were also assessed using GLZs. NCSS10 was used to generate regression plots for the GLZs. Plots of residuals against fitted values in R were used to check data for normality before analysis and dispersion was required to be close to 1 to validate the model fit.

Chapter 3 : Results

The effect of shade on the biological control of *S. molesta* by the weevil, *C. salviniae*, was assessed under controlled conditions in a greenhouse, and then in the field. Results were analysed separately for each experiment.

3.1 Greenhouse study

3.1.1 Photosynthetically Active Radiation (PAR) and temperature

The aim of this thesis was to determine whether shade influences the level of biological control exerted by the weevil, *C. salviniae*, on *S. molesta* in the field. PAR is a proxy for measuring the amount of energy available to a plant for photosynthesis, which in turn affects aspects of plant growth (Gliessman, 2007). Differences in plant growth subsequently affect agent performance and consequently, the level of control. Therefore, three shade levels (dense, moderate and low) were experimentally manipulated in the greenhouse study in an attempt to extrapolate the results of herbivory on *S. molesta* to the field. PAR was significantly higher at the low shade level, followed by moderate shade, and lowest under dense shade ($F_{(2, 45)} = 610.94$, $P < 0.0001$) (Fig. 3.1), confirming that increasing shade would reduce the amount of available energy to *S. molesta*, justifying the experimental setup in the greenhouse. Mean PAR values were 72.63 μmol m^{2} s^{1}±3.10, 368.13 μmol m^{2} s^{1}± and 645.13 μmol m^{2} s^{1}±15.51 in dense, moderate and low shade, respectively.

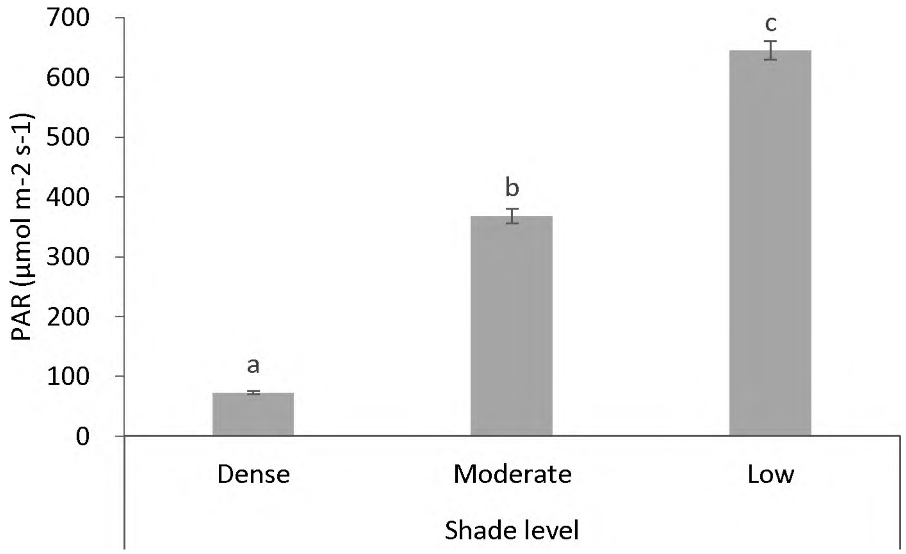


Figure 3.1: Photosynthetically Active Radiation (PAR) at three levels of shade - dense, moderate and low. Means compared using One-way ANOVA, error bars represent S.E., means followed by different letters are significantly different.

3.1.2 Leaf chlorophyll concentration

Leaf chlorophyll was measured at the three levels of shading, under two nutrient levels with half of the experiment subject to herbivory. The results revealed that leaf chlorophyll concentration was significantly higher in the dense shade compared to the low shade (D.F.=102, F=14.1575, P< 0.0001) (Fig. 3.2). However, herbivory and nutrient supply did not influence leaf chlorophyll.

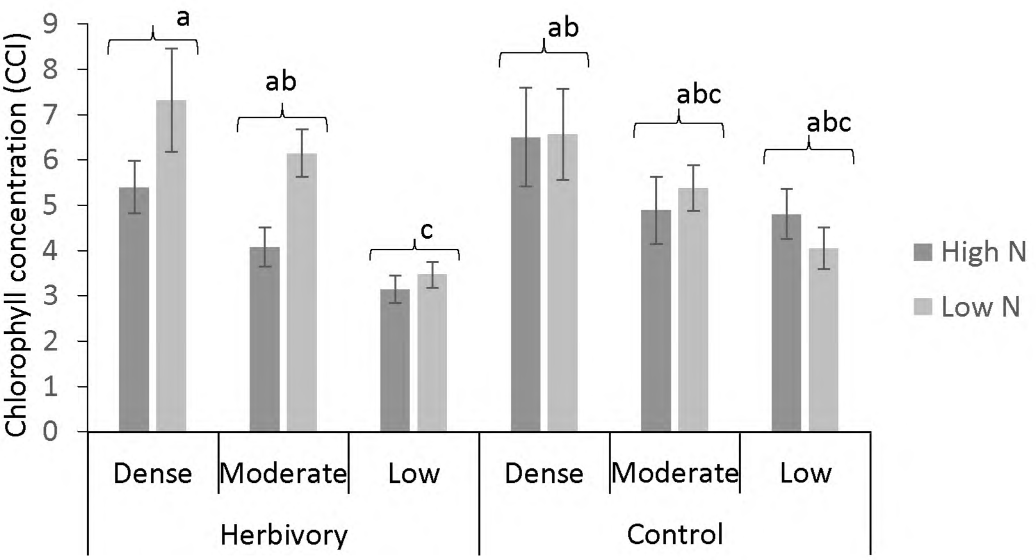


Figure 3.2: Leaf chlorophyll concentration of *Salvinia molesta* grown at three levels of shade, at two nutrient levels and herbivory levels. Means compared using factorial ANOVA, Error bars represent S.E., and means followed by different letters are significantly different.

3.1.3 Plant biomass

Biomass of *S. molesta* plants was only significantly affected by water nutrient levels; neither shade nor herbivory treatments (nor any interaction) had a significant impact on the biomass (Table 3.1). Significantly greater biomass was recorded at high nutrient levels compared to low nutrient levels (Table 3.1, Fig. 3.3).

Modelling of both the independent (nutrient level, shade level and herbivory) and continuous variables (water nitrate and ammonia concentration, PAR, temperature, plant C:N ratio) in a Generalized Linear Model (GLM) attempted to elucidate the most influential combination of factors on plant biomass. The first model was the most complicated and included all independent and continuous variables prior to model selection. The model was then checked for collinearity of variables and also for a high variance inflated factor (VIF), after which all variables found to be collinear and with inflated variance were removed. The remaining variables were again checked for collinearity and high VIF, before the actual GLM was run.

The best fit model was obtained from variables with the lowest AIC score, combined with a delta score of less than 3. The model selection process excluded shade level, treatment (herbivory /control) and temperature due to collinearity and high VIF (>5). A dispersion parameter close to 1 for the whole model validated model fit (Table 3.2). The model showed that biomass was significantly lower in the low nutrient treatment than the high nutrient one, thereby confirming the ANOVA results (Table 3.2; Fig. 3.3; Fig. 3.4). These differences are illustrated by the red (high nutrients) and blue (low nutrients) surface response planes in Fig. 3.4. The model also showed that water nitrate level and plant C:N ratio were significantly correlated with plant biomass, while water ammonium level did not have a significant influence on plant biomass. In high nutrient conditions, there was a positive relationship between plant biomass and water nitrate and plant C:N, while no relationship was observed in the low nutrient conditions (Fig. 3.4).

Table 3.1: Factorial ANOVA results illustrating the effect of shade level, nutrient levels, treatment (herbivory vs control) and their interactions on *Salvinia molesta* biomass. Values in bold indicate significant differences in means.

Effect	**SS**	**DF**	**MS**	**F**	***P***
Intercept	**7215252**	**1**	**7215252**	**59.27122**	**0.0000**
Shade level	127058	2	63529	0.52187	0.5978
Herbivory	438154	1	438154	3.59931	0.0658
Nutrients	**1272054**	**1**	**1272054**	**10.44956**	**0.0026**
Shade level*Treatment	34533	2	17267	0.14184	0.8682
Shade level*Nutrients	258738	2	129369	1.06273	0.3561
Treatment*Nutrients	210940	1	210940	1.73281	0.1963
Shade level*Treatment*Nutrients	33197	2	16599	0.13635	0.8729
Error	4382381	36	121733		

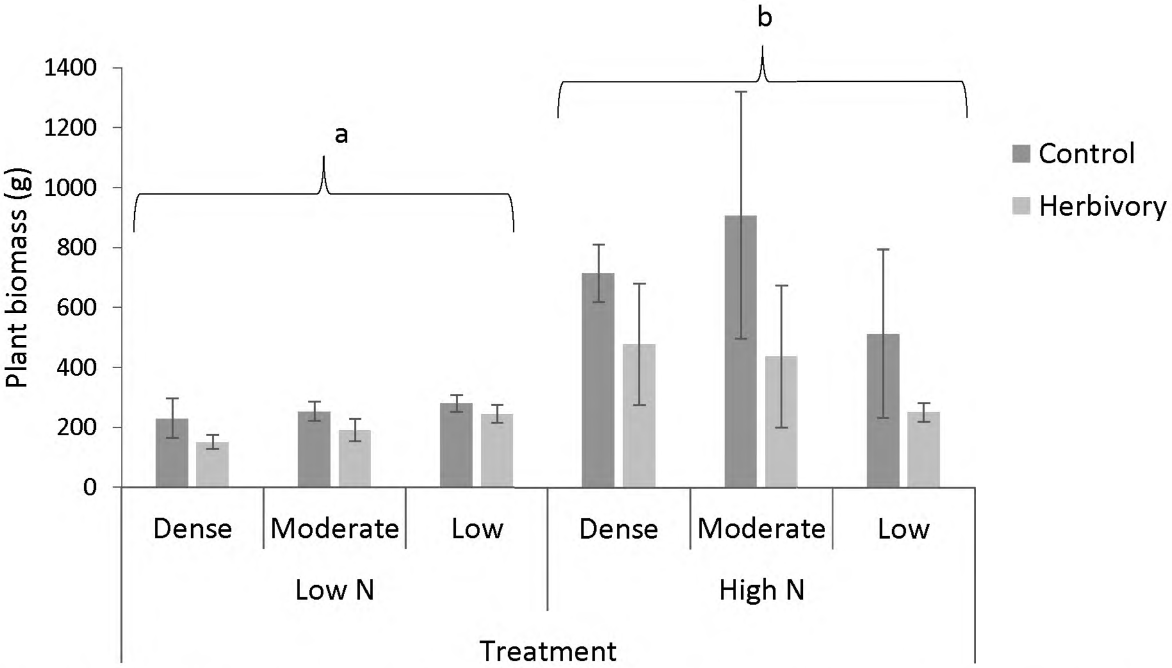


Figure 3.3: Plant biomass across all levels of testing under shade, nutrients and treatment. Means compared by factorial ANOVA, error bars represent standard error; letters indicate significant differences in biomass between nutrient levels.

Table 3.2: Summary of Generalized Linear Model results for the effect of water nutrient treatment level, water ammonium and nitrate concentrations, and plant C:N ratio on plant biomass. Values in bold depict significant variables.

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	**5.722538**	**0.354445**	**16.145**	**< 2e-16**
Nutrients	**-0.645772**	**0.244747**	**-2.639**	**0.0083**
Nitrate	**0.169340**	**0.082345**	**2.056**	**0.0397**
Ammonium	-2.339315	1.393527	-1.679	0.0932
C:N	**0.009100**	**0.004561**	**1.995**	**0.0460**
Observations		48		
Log Likelihood		-316.106		
AIC		648.213		
Dispersion		1.18537		

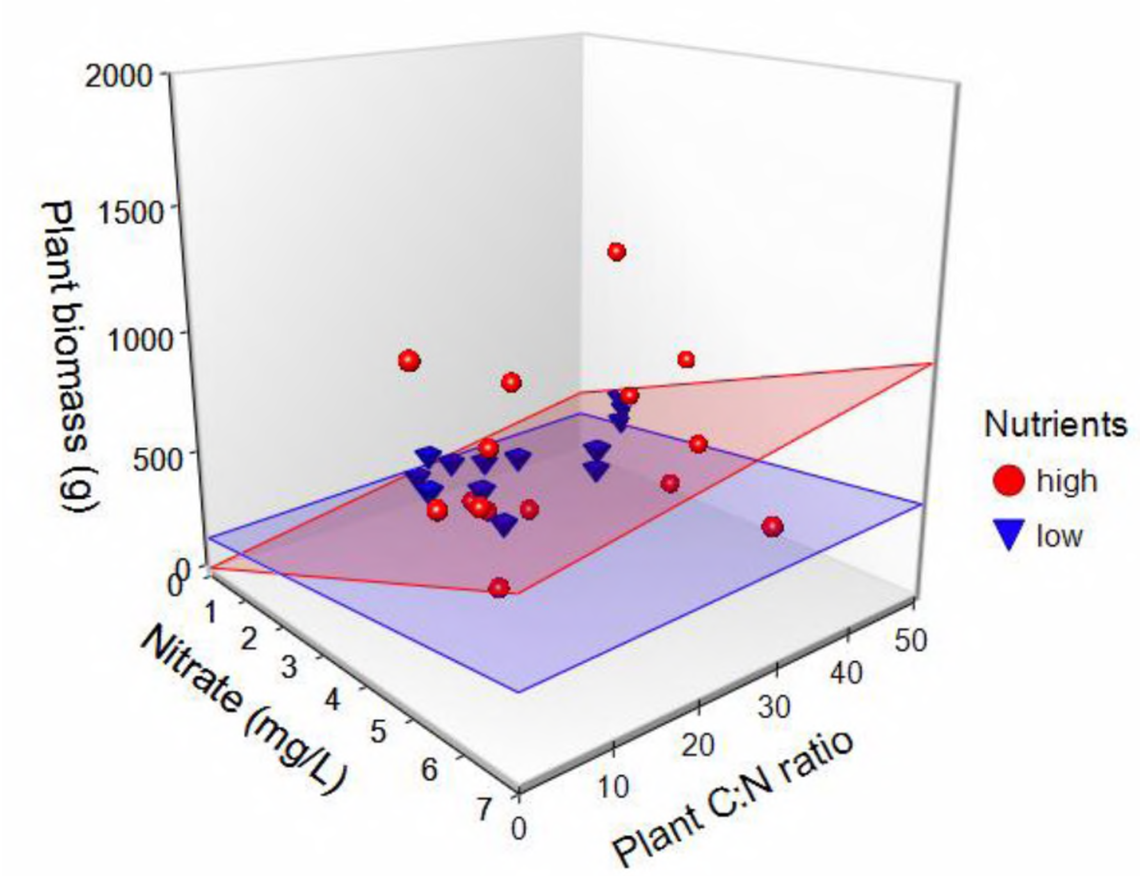


Figure 3.4: Surface response plane of the effect of water nitrate and plant C:N ratio on plant biomass. Points represent observations, n=24.

3.1.4 Plant quality

Plant carbon to nitrogen ratio (C:N) was used as a measure of plant quality, where a higher C:N represented poorer plant quality. Shade level and treatment (herbivory/ control) significantly affected plant quality, while converse to the biomass model, water nutrient level did not significantly influence plant quality (Table 3.3). There were no significant interactions between any of the independent variables (Table 3.3).

Plants grown at dense shade level had a significantly lower mean C:N (31.63±6.898.) than those grown at moderate shade levels (46.19±6.357), which were significantly lower than those grown at low shade levels (57.91±5.089) suggesting that plant quality increased with an increase in shade level (Fig. 3.5). Across the treatments, plants exposed to herbivory had a significantly lower mean C:N (36.41±7.377) than those grown in the absence of herbivory (53.91±6.163) (Fig. 3.5).

Table 3.3: Factorial ANOVA results illustrating the effect of nutrients, shade level and treatment (herbivory and control), and their interactions on *Salvinia molesta* plant quality (C:N ratio). Values in bold indicate significant differences in mean C:N ratios

Effect	**SS**	**D.F**	**MS**	**F**	***P***
Intercept	**101278.4**	**1**	**101278.4**	**464.4159**	**0.000000**
Shade level	**6435.0**	**2**	**3217.5**	**14.7540**	**0.000021**
Treatment	**4356.5**	**1**	**4356.5**	**19.9771**	**0.000075**
Nutrients	645.1	1	645.1	2.9582	0.094031
Shade level*Treatment	94.6	2	47.3	0.2169	0.806044
Shade level*Nutrients	192.9	2	96.5	0.4423	0.646010
Treatment*Nutrients	279.8	1	279.8	1.2830	0.264822
Shade*Treatment*Nutrients	474.7	2	237.3	1.0883	0.347596
Error	7850.8	36	218.1		

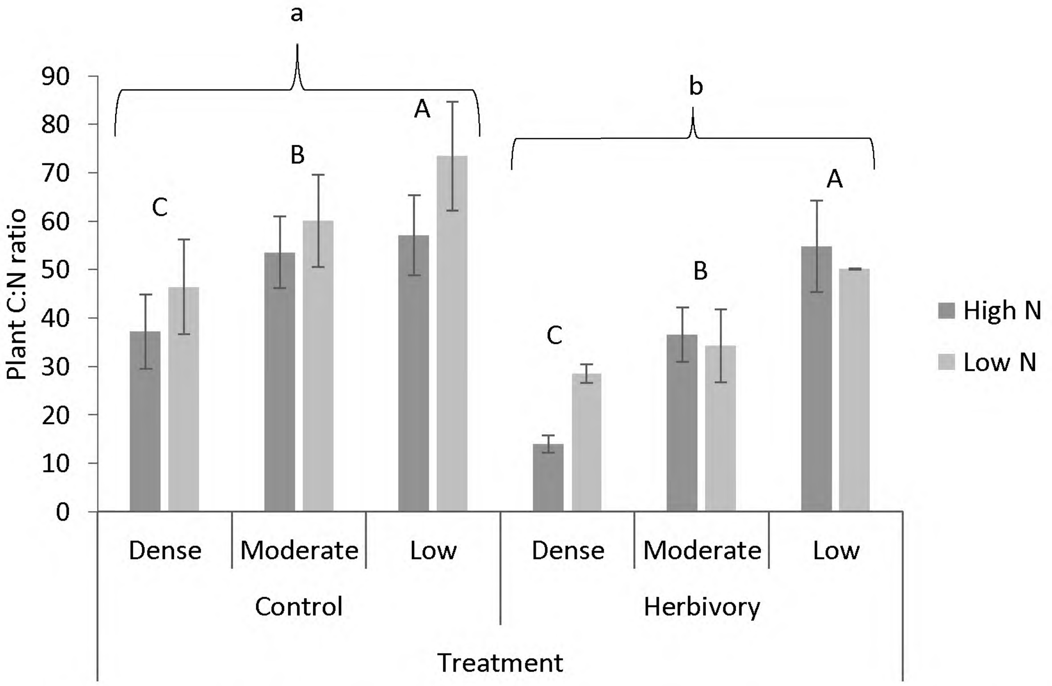


Figure 3.5: Mean plant C:N ratios of *Salvinia molesta* grown at three levels of shade at two nutrient levels, in the presence or absence of herbivory. Means compared by factorial ANOVA, error bars represent S.E., different lower case letters depict significantly different means between herbivory treatments, while upper case letters represent significant differences between shade treatments.

Once again, GLM modelling of the independent (shade level, nutrients and herbivory) and continuous (water ammonium and nitrate, temperature, weevil population, leaf damage) variables was used to explain the patterns in plant C:N. Variables with the lowest AIC and a delta score of less than 3 were used for the best fit model. Model selection excluded shade level, temperatures, weevil population and plant damage from the final model due to collinearity and high VIF. The dispersion after running the model was close to 1, validating that the model was a good fit (Table 3.4). The herbivory treatment was significantly different from the control treatments and this confirmed the ANOVA results (Tables 3.4 and 3.3; Fig. 3.5). In addition, water ammonium concentration had a significant effect on plant quality (Table 3.4). There was however, a weak, non-significant correlation between water ammonium and plant C:N, both in the absence and presence of herbivory (R^2= 0.054, P= 0.2719 and R^2= 0.093, P= 0.1463) (Fig. 3.6).

Table 3.4: Generalized Linear Model summary for plant C:N as predicted by treatment (herbivory), water ammonium and nitrate, and water nutrient levels (low).

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	**3.709**	**0.267**	**13.880**	**< 2e-16**
Treatment-h	**-0.444**	**0.128**	**-3.450**	**0.0005**
Ammonium	**2.204**	**0.870**	**2.534**	**0.0112**
Nitrate	-0.052	0.059	-0.885	0.3760
Nutrients-l	0.133	0.164	0.813	0.4161
Observations		48		
Log Likelihood		-204.578		
AIC		419.156		
Dispersion		1.2395		

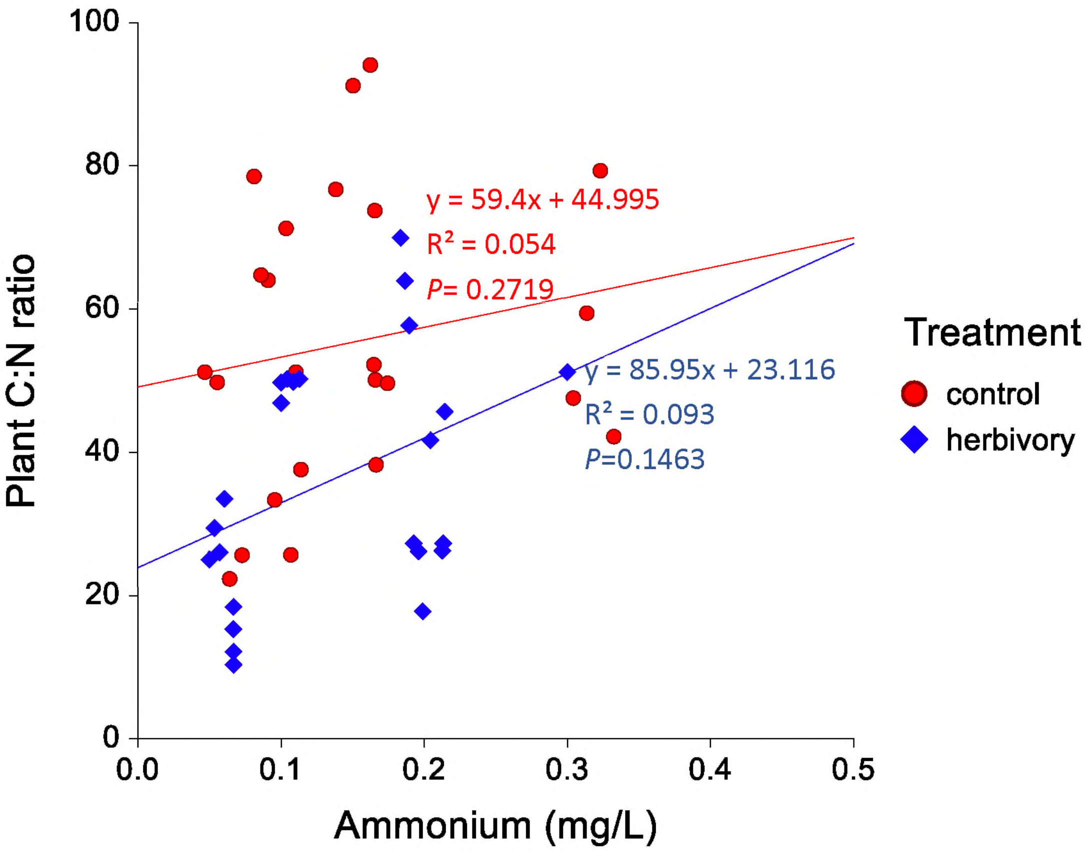


Figure 3.6: Regression analysis of plant C:N response to water ammonium concentration in the presence and absence of herbivory . Points indicate observations, n=24.

3.1.5 Weevil populations

Weevil population sizes were not significantly different between the three levels of shade, nor was there a difference between nutrient levels (Table 3.5). It is important to note that nutrients revealed a nearly significant trend in weevil populations where DF = 1, F = 3.8882 and *P* = 0.06419 (Table 3.5). There were also no significant interactions between the variables. Although not significantly different, there were more weevils under dense shade compared to the other two shade levels in the high nutrient conditions (Fig. 3.7). The lack of significance could have been the result of large variation in the data as a consequence of a small sample size (n=4). Thus a post hoc power analysis was conducted to ascertain if the null hypothesis was true. The power analysis for shade level factor was done at v_1= 2, v_2=21, *k* =3, N=24, α=0.05, effect size (f) = 0.704 and power (1-β) was set at 0.80 (where v_1 = numerator DF, v_2= denominator DF, *k* =number of groups, α = significance level), giving a power of 0.82, indicating that the test had sufficient statistical power, and the differences were indeed not significant. Power analysis for the nutrient levels was conducted at v_1= 1,

v_2=22, k= 2, N=24, α=0.05, f= 0.564 and 1-β= 0.80, resulting in a power of 0.75, leading to the conclusion that there was insufficient power due to the small sample size, and the hypothesis that nutrient levels did not influence weevil population size could be rejected.

Table 3.5: Factorial ANOVA results for differences in *Cyrtobagous salviniae* populations between shade levels and nutrient treatments. Values in bold are significant.

Effect	SS	DF	MS	F	*P*
Intercept	**1162.04**	**1**	**1162.04**	**7.16757**	**0.01538**
Shade level	643.083	2	321.542	1.9833	0.16657
Nutrients	630.375	1	630.375	3.8882	0.06419
Shade level*Nutrients	527.25	2	263.625	1.62606	0.2243
Error	2918.25	18	162.125		

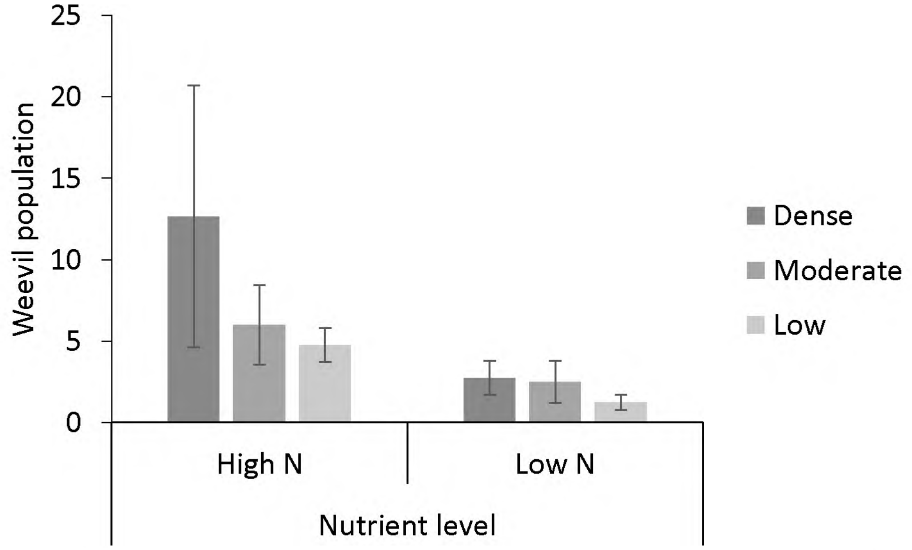


Figure 3.7: *Cyrtobagous salviniae* populations at three shade levels under high and low nutrients. Means were compared using factorial ANOVA, error bars are S.E.

The best fit model (GLM) of independent (shade level, water nutrients) and continuous variables (water ammonium, nitrate, plant C:N ratio, temperature) on weevil population size was selected from variables which had the lowest AIC and a delta score of less than 3. The model selection process excluded shade level from the final model due to collinearity. After running the model, a dispersion close to 1 validated the model as the best fit model (Table 3.6). Plant quality (C:N ratio) had a significant influence on weevil population, while none of the other variables was significantly correlated with weevil population size (Table 3.6). However, the effect of low nutrients (Nutrients-l) revealed nearly significant trends in weevil population (Table 3.6) . Weevils were negatively correlated to plant C:N, however this was weak and not significant (Fig. 3.8).

Table 3.6: Generalized Linear Modelling for weevil populations response to ammonium, nitrate, C:N ratio, temperatures and water nutrient levels. Values in bold are significant

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	6.18776	10.11992	0.611	0.5409
Temps	-0.12976	0.40940	-0.317	0.7513
Nitrate	0.24197	0.34067	0.710	0.4775
Nutrients-l	-1.30554	0.67521	-1.934	0.0532
Ammonium	-1.22220	6.26702	-0.195	0.8454
C.N	**-0.03095**	**0.01521**	**-2.035**	**0.0418**
Observations	24			
Log Likelihood	-61.112			
AIC	134.223			
Dispersion	0.8617166			

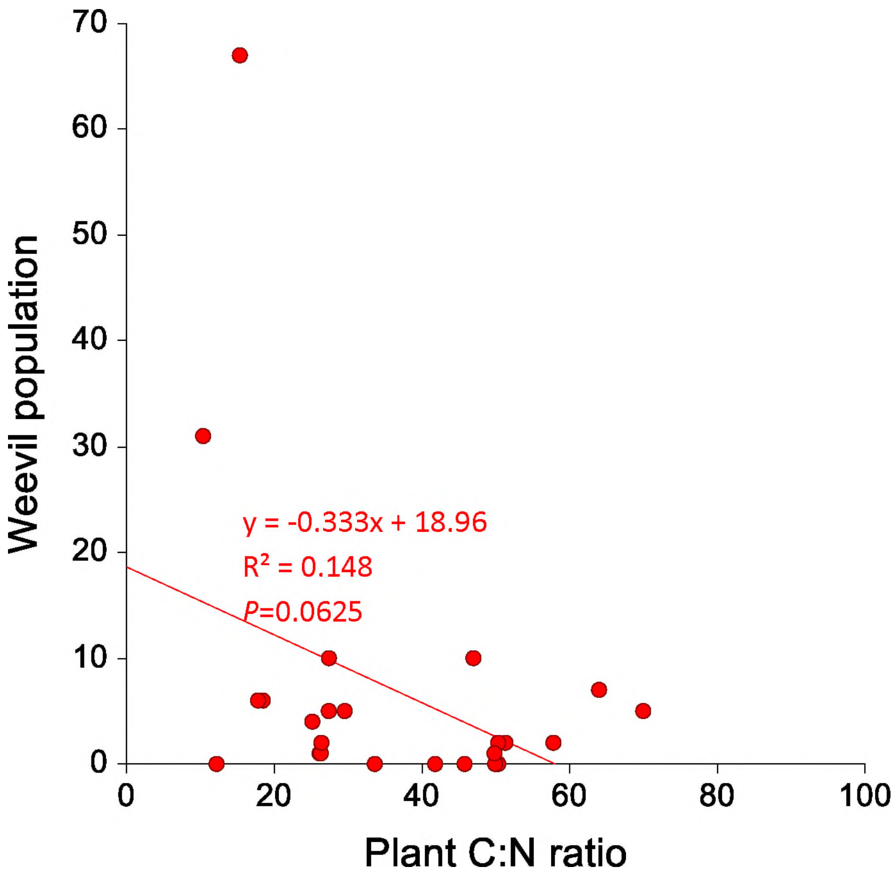


Figure 3.8: Linear regression between weevil population and plant C:N ratio. Points are observations, n=24.

3.1.6 Leaf damage

Leaf damage was significantly affected by shade levels as well as the interaction between shade and water nutrient levels (Table 3.7). Water nutrients alone did not explain any differences in leaf damage (Table 3.7). The highest leaf damage was recorded in the no shade treatment, while the shade treatments did not have any differences in leaf damage (Fig. 3.9).

Plants grown in the dense and moderate shade suffered significantly less plant damage with a mean of 54.37% ± 5.697and 53.62% ± 7.518, respectively, compared to low shade with a mean of 71.37% ± 4.004. Mean plant damage in low nutrient conditions was 55.66 % ±5.682, while in high nutrients it was 63.91% ± 4.560.

Table 3.7: Factorial ANOVA indicating differences in leaf damage between shade levels and nutrients. Values in bold are significant.

Effect	D.F	Wald	*P*
Intercept	**1**	**7597.254**	**0.000000**
Shade level	**2**	**9.641**	**0.008061**
Nutrients	1	1.812	0.178258
Shade level*Nutrients	**2**	**7.418**	**0.024498**

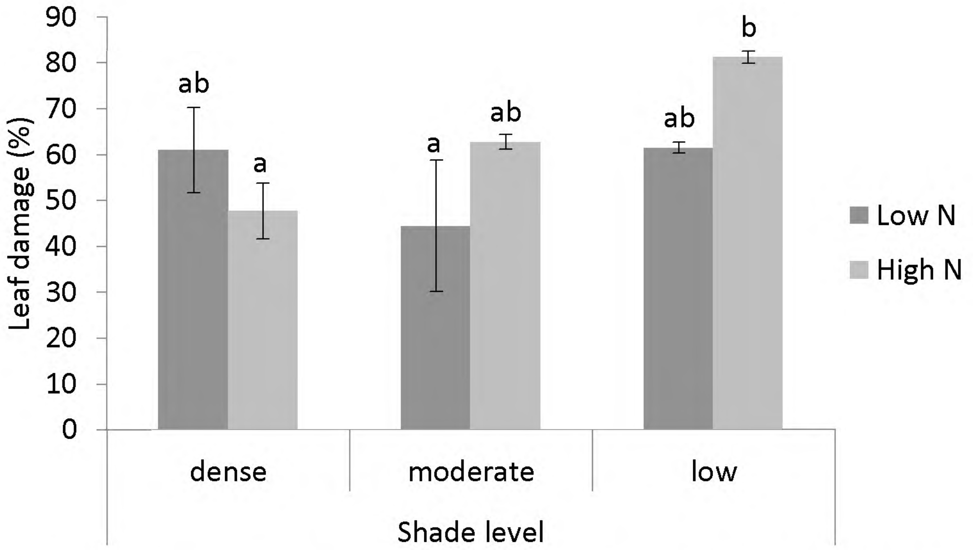


Figure 3.9: Percentage leaf damage under three shade levels (dense, moderate and low), at two nutrient levels, high and low. Means were compared using factorial ANOVA, error bars represent S.E., means with the same letter are not significantly different.

The best fit model describing leaf damage was selected from variables which had the lowest AICs and delta scores of less than 3. Model selection excluded shade levels and temperatures due to collinearity and high VIF values. A dispersion close to 1 proved the model to be a good fit (Table 3.8). Water nutrient levels and plant C:N significantly influenced leaf damage while both water ammonium and nitrate did not have a significant effect (Table 3.8). There was a significant, positive correlation between leaf damage and plant C:N ratio at high nutrient levels, but at low nutrient levels, there was a weak, non-significant correlation (Fig. 3.10).

Table 3.8: Generalized linear modelling of leaf damage as predicted by water nutrients, nitrate, ammonium, plant C:N ratio and weevil population. Values in bold are significant.

Coefficients	Estimate	Std. Error	z value	*P*
(Intercept)	4.298436	0.294037	14.619	<2e-16
C.N	**0.010109**	**0.003968**	**2.547**	**0.0109**
Nitrate	-0.140468	0.087957	-1.597	0.1103
Ammonium	-1.010245	1.135871	-0.889	0.3738
Nutrients-l	**-0.389373**	**0.172312**	**-2.260**	**0.0238**
Observations	24			
Log Likelihood	-104.252			
AIC	218.504			
Dispersion	1.18			

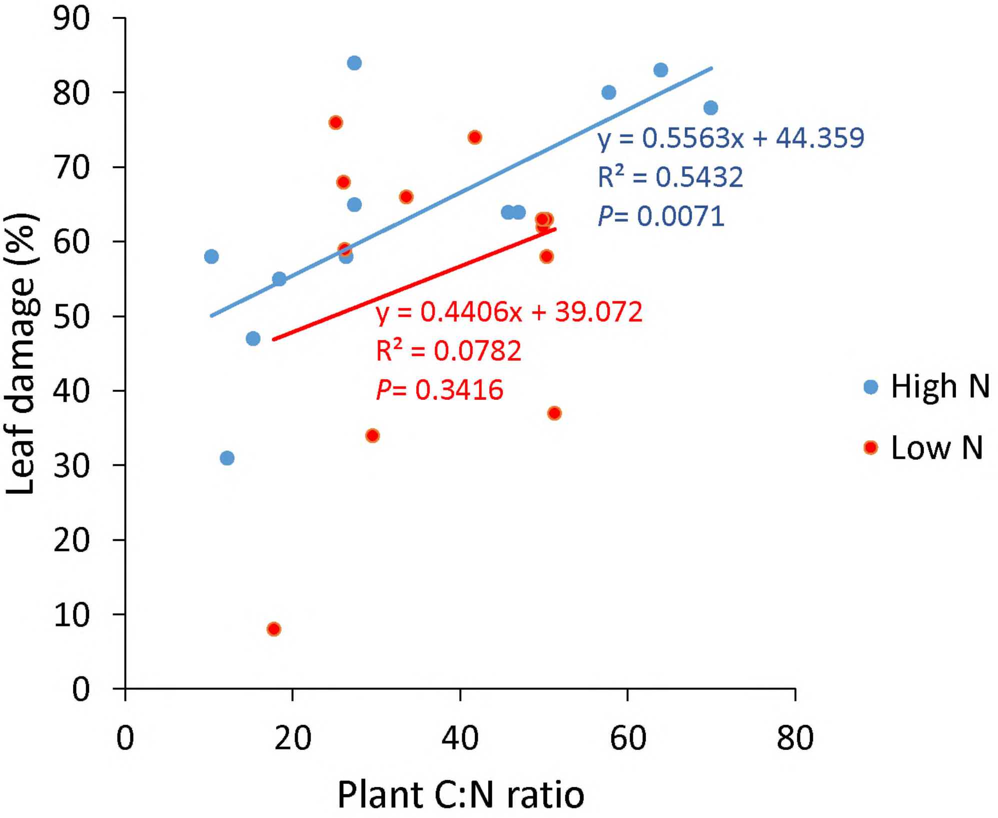


Figure 3.10: Relationship between leaf damage (%) and plant C:N in low and high nutrient conditions. Points represent observations, n=12, lines are regression trend lines.

3.1.7 Principal Component Analysis

The Principal Component Analysis which provided a spatial representation of the influence of the independent and continuous environmental variables on the dependent variables showed a clear separation between dense shade, and low and moderate shade which were not clearly separated (Fig. 3.11). In dense shade, there was also a clear separation between the nutrient treatments, where high nutrient treatments aligned with nitrate, while the low nutrient ones deviated from it (Fig 3.11). Ammonium and PAR strongly influenced the moderate and low shade treatments (Fig 3.11). Furthermore, there was lack of separation between these two treatments with nutrient treatments also being closely aligned, leading to the conclusion that these two were very similar. The PCA thus explains the lack of differences in some variables in the preceding sections where weevil populations and leaf damage were not different with nutrient treatments.

Water nitrate concentration had a significant effect on the whole experiment as the high nutrient treatments of dense shade were clustered close to nitrate which contributed 30.74% in PC2 (Fig. 3.11). Water ammonium (22.56%) and PAR (46.71%) were the major predictors in PC1 where moderate and low shade at low nutrients were grouped together. The first 2 PCs explained a cumulative total variation of 100%, indicating a good fit of the model.

The BEST function was used to show the combination of environmental variables which best explained variation, indicating that PAR, ammonium and nitrate best explained variation (r= 1, P= 0.001). A PERMANOVA test of significance revealed that shade level and nutrients, as well as the interaction of the two variables, had a significant effect on biological variables (Table 3.9). Tests of differences using ANOSIM between all shade levels was significant, however there was a weak correlation between them (r= 0.172, P= 0.002). Pairwise tests in ANOSIM showed dense and low shade to be significantly different (r= 0.39, P= 0.001). Again, ANOSIM test of significance between nutrient groups revealed a significant difference between the two nutrient groups (r = 0.244, P= 0.002).

Table 3.9: Summary PERMANOVA table of results for effect of shade and nutrients on the biological variables in the greenhouse.

Source	**DF**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Permutations**
Shade level	2	115.29	57.647	61.297	0.001	999
Nutrients	1	26.468	26.468	28.144	0.001	999
Shade*Nutrients	2	6.739	3.3695	3.5829	0.012	997
Res	42	39.499	0.94045			
Total	47	188				

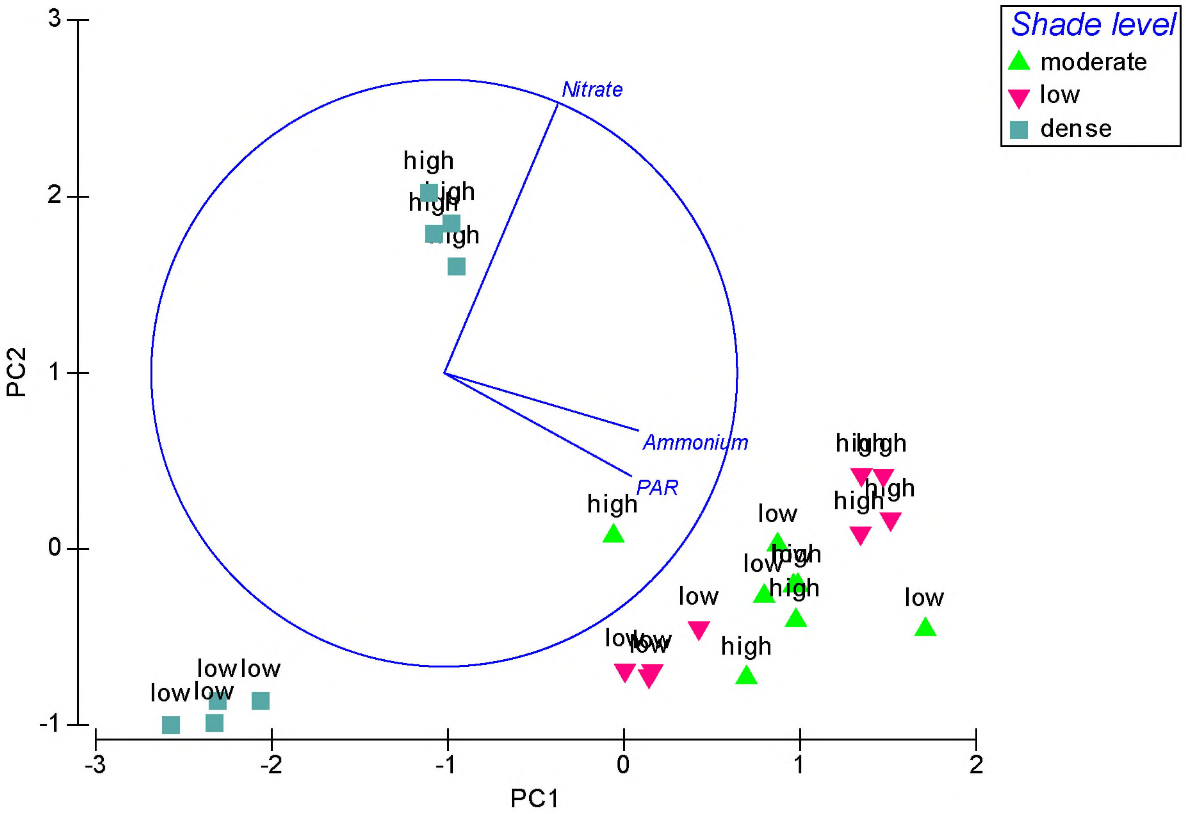


Figure 3.11: Principal Component Analysis bi-plot illustrating the relationship between three shade levels; dense, moderate and low, as well as the best combination of environmental variables at high and low nutrient levels, to explain patterns in the dependent variables.

3.2 Field Results

3.2.1 Photosynthetically Active Radiation (PAR)

Patterns for PAR in the shade and full sun in the field were similar to those in the greenhouse study, where PAR was significantly higher in full sun than shade ($t_{(9)}$ = -7.616, P = 0.00003) (Fig. 3.12). Mean PAR in the shade was 227 µmol m^2 s^1±115, while in full sun it was 1150 µmol m^2 s^1±149.32. Once again, this confirmed the hypothesis that *S. molesta* growing in the shade received less energy available for photosynthesis. Compared to the greenhouse, there was close to double the amount of PAR received by plants in the field in both the shade and full sun. This was due to the greenhouse material intercepting some of the PAR and possibly reflecting a certain percentage of it.

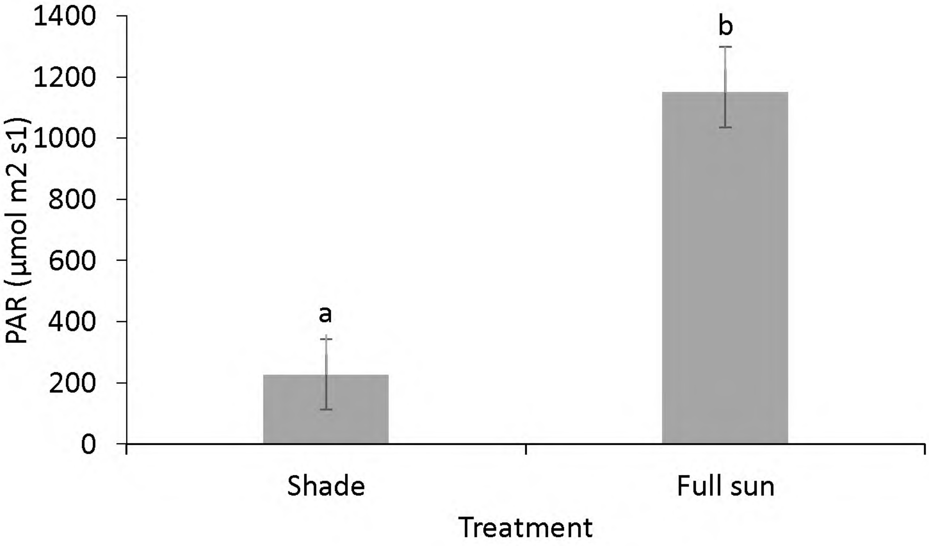


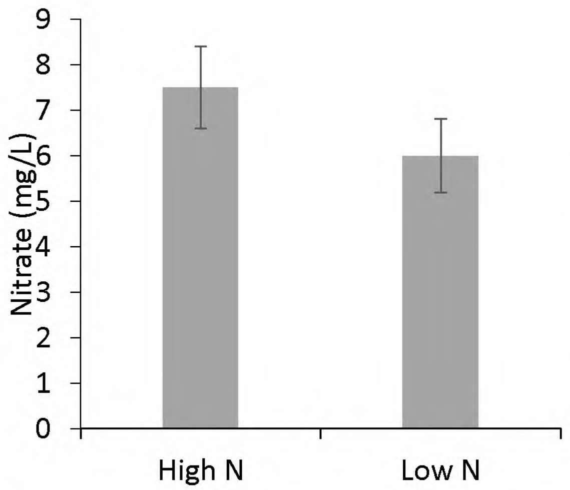
Figure 3.12: Photosynthetically Active Radiation in the shade and full sun in the field, error bars represent SE.

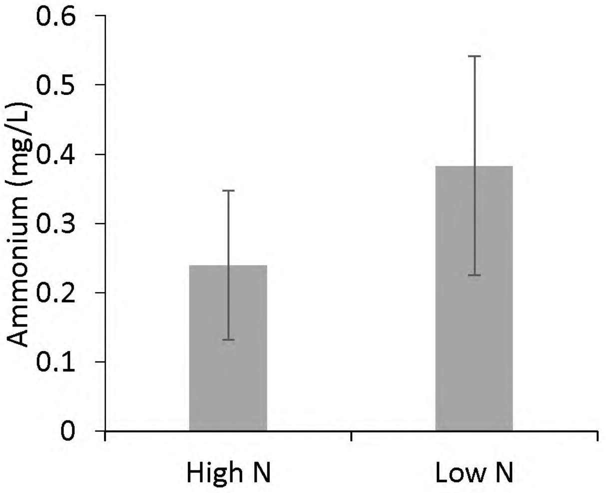
3.2.3 Nutrient enrichment

Half the quadrats in the field were directly enriched with nutrients, while the other half were not manipulated. However, there were no significant differences in water nitrate ($t_{(9)}$=1.2444, P=0.2447), nor ammonium ($t_{(9)}$= -0.7172, P= 0.4914) between the high and low nutrient treatments (Fig. 3.13a and b). Due to the lack of significant differences between nutrient treatments, this variable was left out of future analyses as it did not explain any variation in biomass. Lack of differences was most probably due to diffusion of nutrients from the enriched quadrats into the water body. There was, however, significantly more water nitrate in the full sun than in the shade ($t_{(9)}$=4.5122, *P*=0.004), but ammonium was not significantly different between the two sites ($t_{(9)}$=1.5993, *P*= 0.1442) (Fig. 3.13c and d). The differences between the full sun and shade may be the result of the differences in depth between the shade and sun sites, as the shaded sites were shallower, which may have concentrated the water nutrients. In comparison to the greenhouse, enrichment did not play a role in the field because the system is an open waterbody, compared to the greenhouse, where experiments were set up in individual mesocosms that maintained different conditions from the next mesocosm.

a

b





c

d

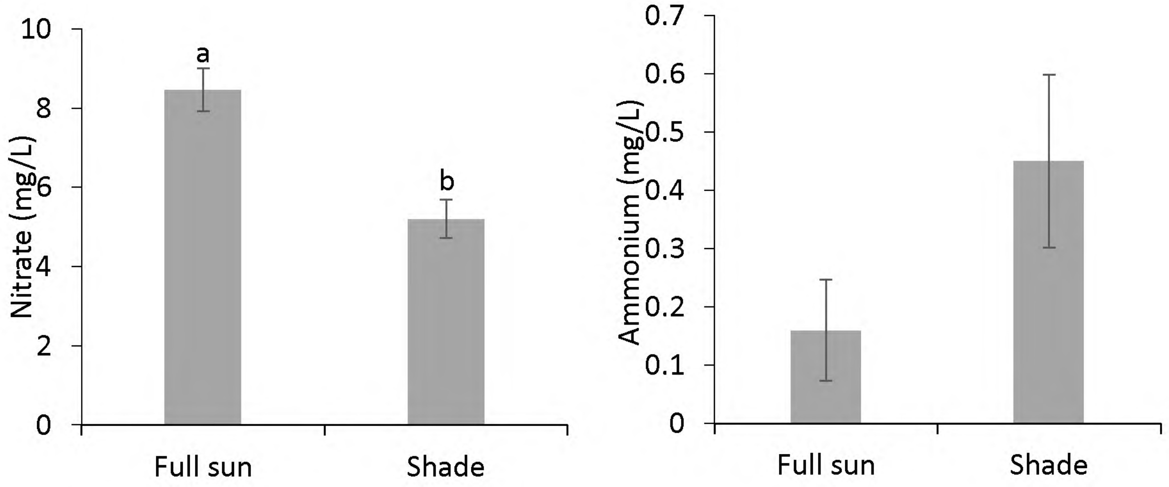


Figure 3.13: Water nutrient concentrations, nitrate (a and c) and ammonium (b and d), in both high (enriched) and low (non-enriched) nutrient plots, as well as in full sun and shade. Means were compared by *t*-tests, error bars represent SE.

3.2.3 Leaf chlorophyll concentration

Leaves of *S. molesta* growing in the shade exhibit a darker green pigmentation compared to those growing in the sun. Leaf chlorophyll was therefore measured for plants in both the shade and full sun to ascertain if any differences exist. There was significantly more chlorophyll in leaves of plants growing in the shade (D.F.= 118, *F*= 13.134, *P*< 0.0001) (Fig. 3.14). Greater chlorophyll concentrations therefore enhanced light capture in low PAR conditions in the shade.

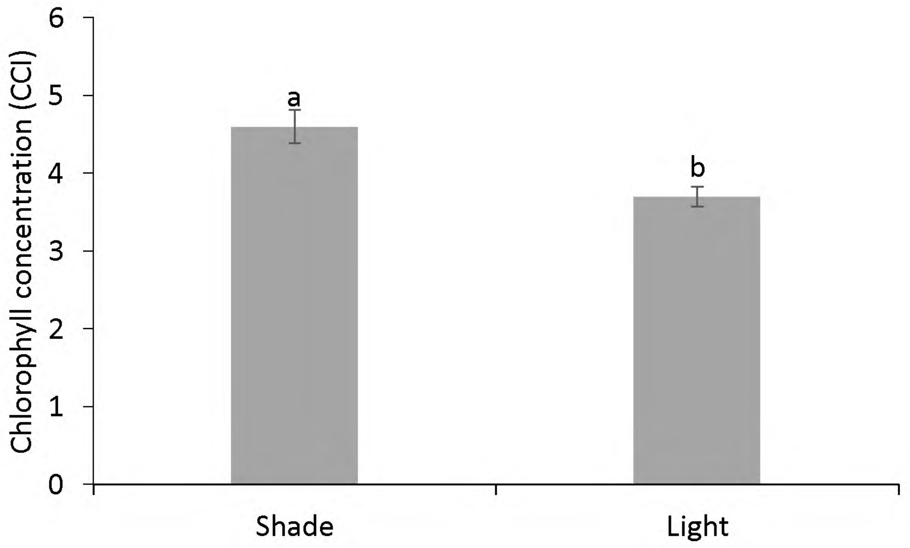


Figure 3.14: Leaf chlorophyll concentration in the shade and full sun. Means compared by One-Way ANOVA, error bars represent S.E., and means followed by different letters are significantly different.

3.2.3 Plant biomass

There were no significant differences in plant biomass between full sun and shaded quadrats, $t_{(9)}=0.7493$, $P=0.4728$ (Fig. 3.15) and a similar result was measured in the greenhouse where shade levels did not yield any differences (Table 3.2). To check if the results failed to show differences due to low statistical power, a post hoc power analysis was conducted at $v_1= 1$, $v_2= 9$, $k= 2$, $N=11$, $\alpha=0.05$, $f=1.08$ and $1-\beta= 0.80$. The results of the power analysis revealed a high statistical power of 0.8104, hence the null hypothesis was accepted. The lack of differences was most likely due to uniformity of nutrient conditions in the water body.

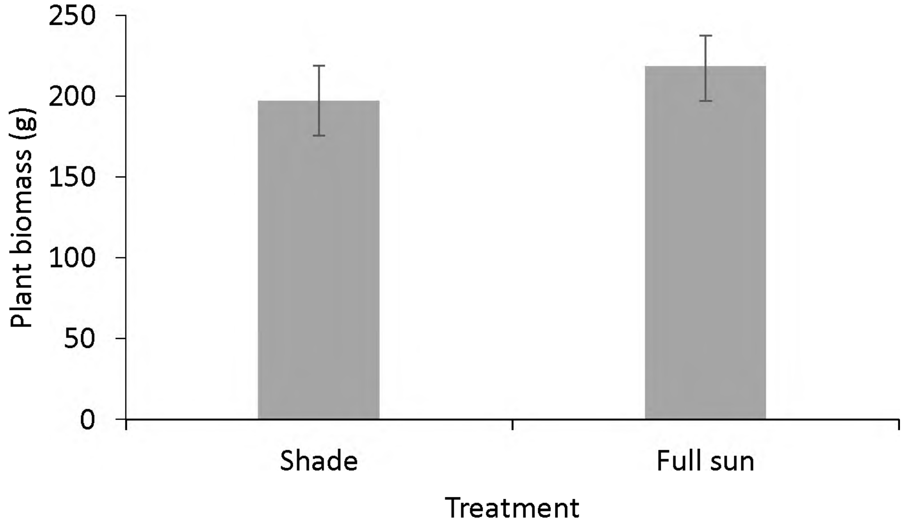


Figure 3.15: Plant biomass in full sun and shade treatments. Means compared by t test, error bars represent S.E., where mean biomass was not significantly different between treatments.

The best fit model (GLM) resulted from independent (treatment) and continuous variables (weevil population, leaf damage, water ammonium and nitrate, temperatures, plant C:N ratio) which obtained the lowest AIC scores and delta scores of less than 3. The model selection process excluded all variables except for water nitrate due to collinearity and high VIF scores. A dispersion parameter of close to 1 validated the model as a good fit (Table 3.10).

Water nitrate did not significantly influence plant biomass, and this was the converse of what was seen in the greenhouse where nitrate had a significant effect (Table 3.10; Table 3.2).

Table 3.10: Summary of generalized linear modelling for plant biomass response to water nutrient levels.

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	253.706	33.338	7.610	6.24e-05
NO_3	-5.315	4.772	-1.114	0.298
Observations	10			
Log Likelihood	-45.672			
AIC	95.343			
Dispersion	1.312935			

3.2.4 Plant quality

Shade and full sun did not have a significant effect on plant quality which was measured as C:N ($t_{(6)}$=1.6737, *P*= 0.1496) (Fig. 3.16). The result was again the converse of what was seen in the greenhouse where shade level had a significant effect on plant quality (Table 3.3). To assess if the lack of differences was due to low statistical power, a post hoc power analysis was conducted at v_1= 1, v_2= 9, *k*= 2, N=11, α=0.05, f=1.08 and 1-β= 0.80. The result of the power analysis was 0.8092, which indicated high power of the analysis, indicating that the null hypothesis was true. Therefore, the lack of significant results was not due to limited sample size.

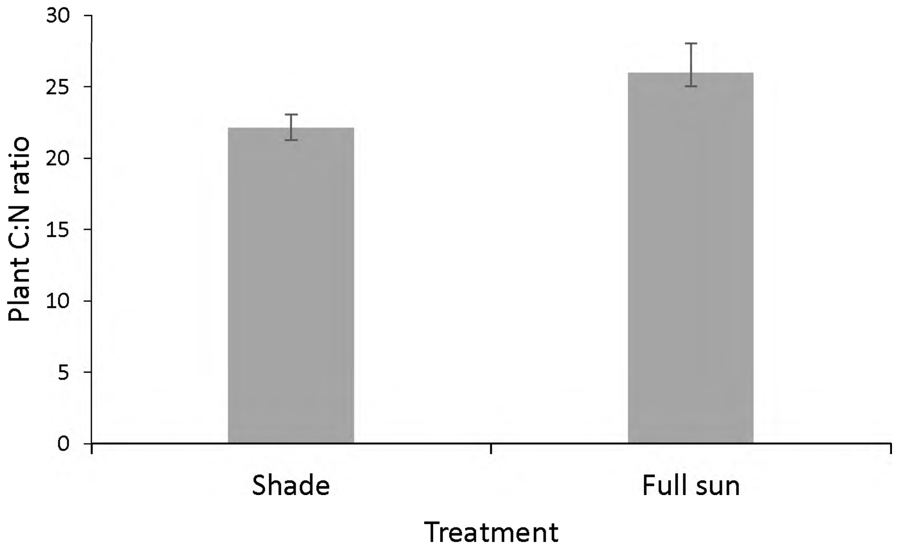


Figure 3.16: Plant quality (C:N) in full sun and shade treatments. Means compared by *t* test, error bars represent S.E., means were not significantly different.

Modelling of the independent (treatment) and continuous (weevil population, leaf damage, temperature) variables was used to explain variation in plant C:N. The best fit model for a generalized linear model was obtained from variables which had the lowest AICs and delta scores of less than 3. The model selection process excluded temperature, and treatment (shade/full sun) from the final model due to collinearity. After running the model, the dispersion parameter close to 1 validated the model to be the best fit (Table 3.11). Plant C:N ratio was significantly correlated with weevil population sizes and leaf damage (%) where the highest plant C:N ratio was observed at high weevil population and leaf damage (Table 3.11; Fig. 3.17). It can be concluded that herbivory lowered plant quality as plants had higher C:N as herbivory increased.

Table 3.11: Generalized Linear Modelling of the influence of weevil population size and leaf damage on plant quality (C:N). Values in bold are significant

Coefficients:	Estimate	Std. Error	t value	P
(Intercept)	18.8092	0.8141	23.104	7.22e-08
Weevils	**0.4825**	**0.1787**	**2.699**	**0.03067**
Leaf damage	**0.1659**	**0.0317**	**5.235**	**0.00121**
Observations	10			

Log Likelihood	-16.873
AIC	39.746
Dispersion	1.7408

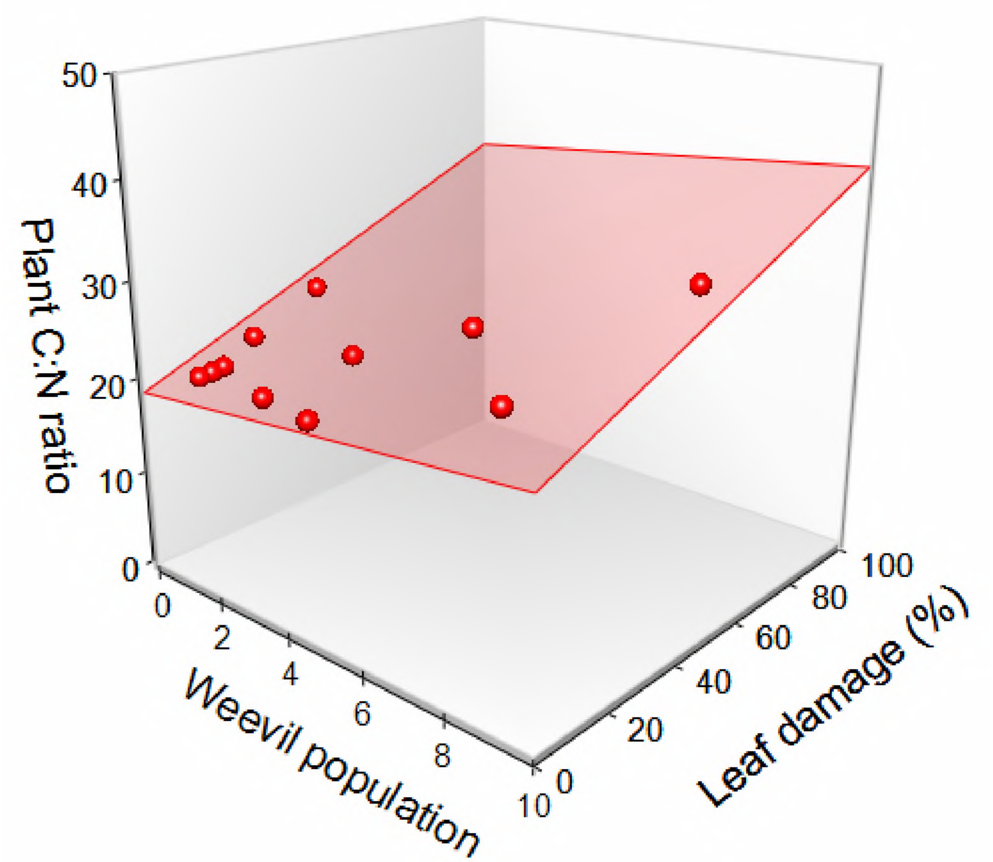


Figure 3.17: Surface response planes for the influence of leaf damage (%) and weevil population (per m2) on plant C:N. Points represent observations, n=11.

3.2.6 Weevil population

Weevil population numbers were not significantly different between full sun and shade $t_{(5)}$=2.2316, P= 0.0755 (Fig. 3.18). A post hoc power analysis was conducted for shade/sun treatment where v_1= 1, v_2= 9, k= 2, N=11, α=0.05, f= 0.038 and 1-β was set at 0.80 and the resultant power was 0.051. This indicates that the test had insufficient statistical power, therefore the null hypothesis was false. While a similar result was found in the greenhouse, it can be concluded that due to the low power of the field statistical analysis, the results of the two studies were consistent with each other, albeit inaccurate. Furthermore, in the greenhouse weevils were not offered any choice but to utilize the mesocosm provided while in the field weevils were free to move.

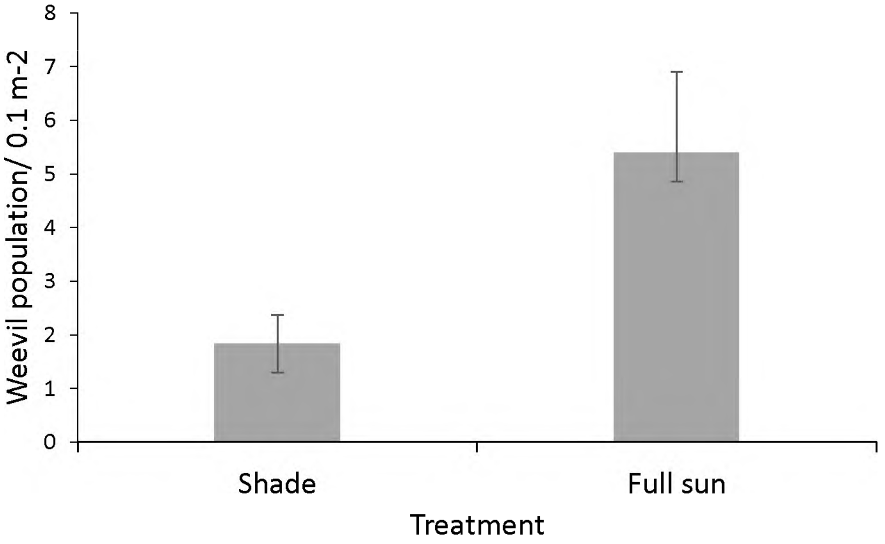


Figure 3.18: Weevil population numbers on *Salvinia molesta* plants in quadrats in full sun or shade. Means were compared using a *t* test, error bars represent S.E.

Variables with the lowest AIC values and delta scores of less than 3 were used to construct the best fit model. The model selection process excluded water ammonium and nitrate, temperature and treatment due to collinearity and high VIF. A dispersion parameter close to 1 validated model fit (Table 3.12). Plant C:N had a significant effect on weevil population size and this was also observed in the greenhouse study (Table 3.12, Table 3.6). Weevil population had a weak positive correlation with plant C:N ratio, however this correlation was not significant (Fig. 3.19).

Table 3.12: Generalized Linear Modelling of water nitrate and plant C:N ratio and their influence on weevil population. Values in bold are significant.

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	-2.23006	1.10529	-2.018	0.0436
C.N	**0.10035**	**0.04290**	**2.339**	**0.0193**
NO_3	0.14139	0.09185	1.539	0.1237
Observations		10		
Log Likelihood		-18.876		
AIC		46		
Dispersion		1.5107		

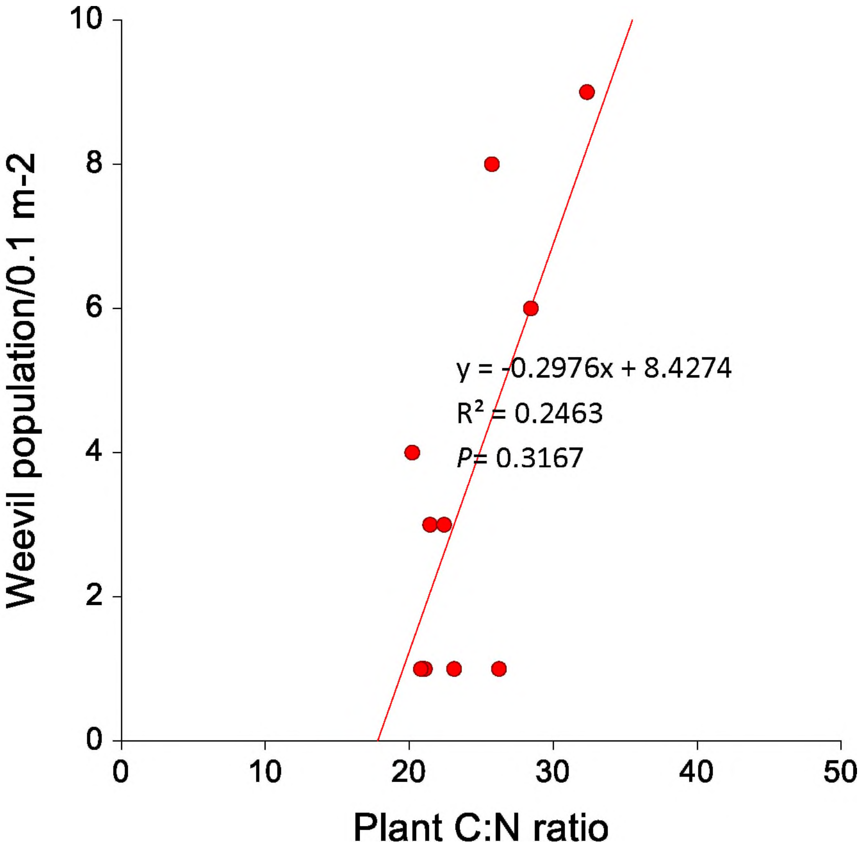


Figure 3.19: Relationship between plant quality (C:N) and weevil population size. Points indicate observations, n= 11.

3.2.6 Leaf damage

Neither treatment (shade/full sun) significantly affected leaf damage, $t_{(6)}$= 1.2296, P= 0.2624 (Fig. 3.20). Because sample size was small (n=5), the lack of differences could have been the result of low statistical power, as leaf damage appeared to be less in the shade. Therefore, a post hoc power analysis was conducted for shade/sun treatment where v_1= 1, v_2= 9, k= 2, N=11, α=0.05, f= 0.37 and 1-β was set at 0.80, resulting in a power of 0.4742. Therefore, the lack of significant differences in leaf damage between the shade/sun treatments was due to low power emanating from a small sample size.

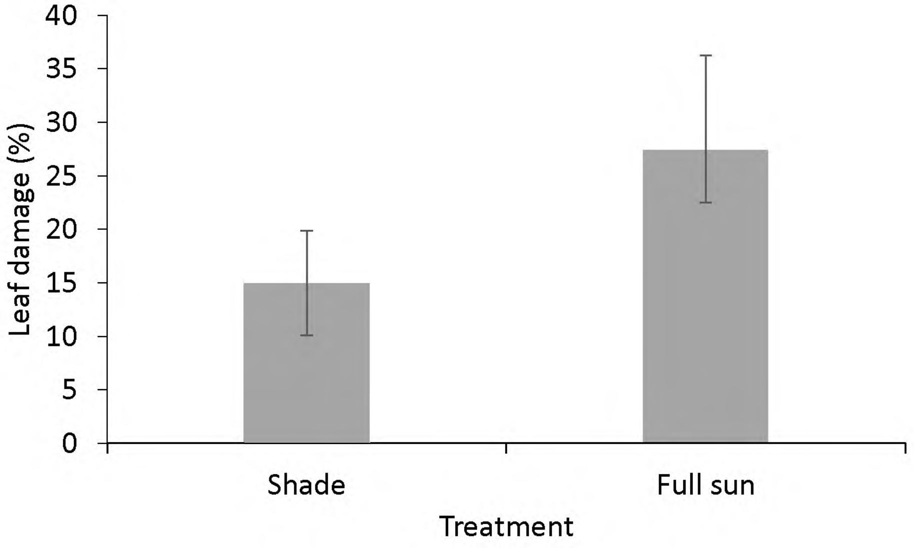


Figure 3.20: Leaf damage under shade and full sun. Means were compared using One-Way ANOVA error bars represent S.E., means were not significantly different.

The final GLM was constructed from variables that had the lowest AICs and delta scores of less than 3. The model selection process excluded water nitrate and ammonium, temperatures and treatment (shade/ full sun) due to collinearity and high VIF values. After running the model, a dispersion parameter close to 1 validated the model fit (Table 3.13). Both plant C:N and weevil population had a significant effect on leaf damage (Table 3.13). These results are similar to the greenhouse study as both experiments showed that plant C:N ratios had a significant effect on leaf damage (Table 3.13, Table 3.8). However, unlike in the greenhouse, weevils showed a significant effect on leaf damage in the field (Table 3.13). Plant C:N was positively correlated with leaf damage, and more damage was inflicted by *C. salviniae* at high C:N values (Fig. 3.21). Weevil feeding was therefore stimulated by the high C:N ratio in plants, leading to greater damage to plants.

Table 3.13: Generalized linear model of percentage leaf damage predicted by weevil population and plant C:N. Values in bold are significant.

Coefficients:	Estimate	Std. Error	z value	*P*
(Intercept)	-2.03994	0.90760	-2.248	0.0246
Weevils	**-0.10652**	**0.05411**	**-1.969**	**0.0490**
C:N	**0.21877**	**0.04189**	**5.223**	**1.76e-07**
Observations	10			
Log Likelihood	-33.560			

AIC	73.119
Dispersion	1.2937

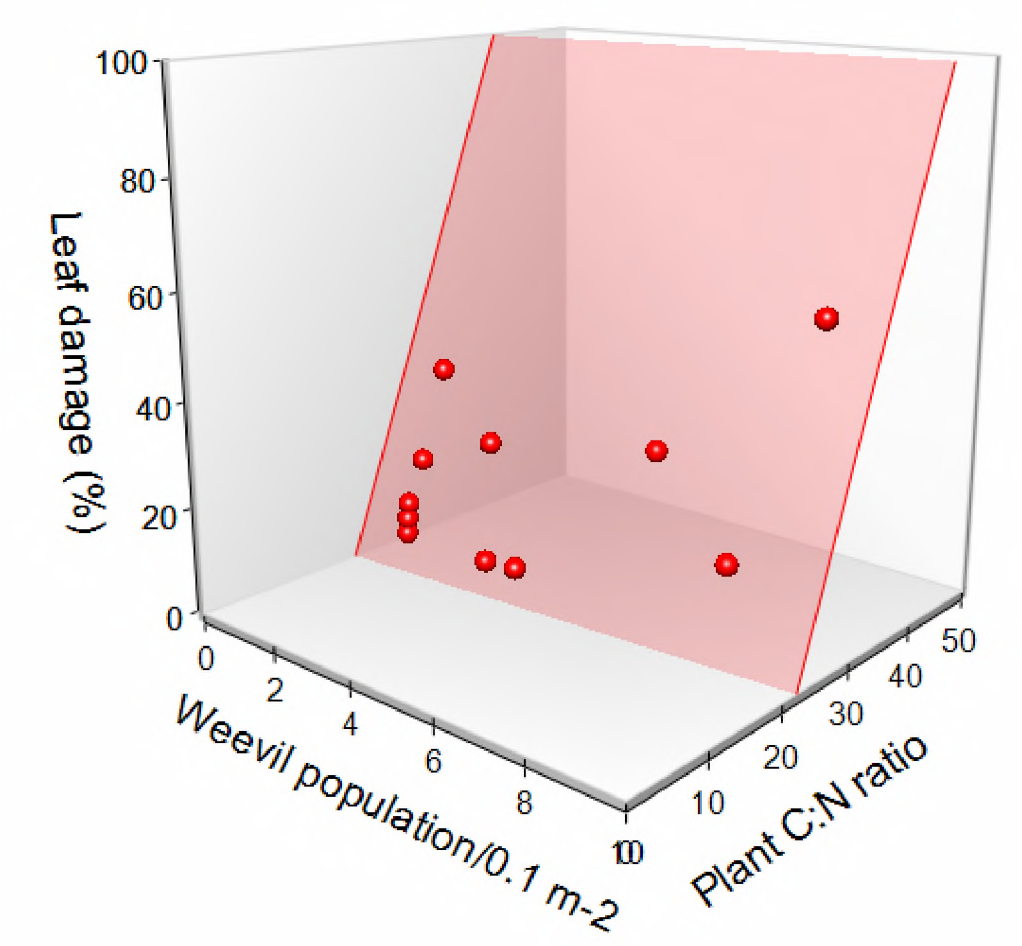


Figure 3.21: Surface response planes for the response of leaf damage to plant C:N ratio and weevil population in the shade and full sun. Points are observations, n=11.

3.2.1 Principal Component Analysis

Principal Component Analysis revealed a distinct separation between the shade and full light variables. However, the PERMANOVA test showed that this distinction was not significant with nutrient treatments (low/ high nutrients), neither was it significant between site treatments (shade/ full sun) (Table 3.14). Despite the statistical lack of significance, the spatial distribution of the variables was clearly separate between the shade and sun, this could be explained by the low statistical power of the experiment as was revealed in preceding sections. PAR and water nitrate proved to be the major drivers of variation in the full sun treatment (Fig. 3.22); this was in sharp contrast to the greenhouse where PAR and ammonium were the major predictors and low shade levels. In the shade, there was no clear indication of what influenced variation, though some of the values were inclined towards water ammonium. In the full sun, both low and high nutrients were closely related and clustered around PAR and nitrate, a similar trend was seen in the shade, although there is clear separation from the environmental predictors (water ammonium and nitrate, PAR). The lack of significant differences within the nutrient treatments can be interpreted as being due to the effect of homogeneity of growth conditions as water nutrients could have uniformly diffused through the water column. Separation between the shade and full sun was significant as there were differences in water nitrate between the two sites. This difference may be caused by differing depths of water between the two.

The BEST function revealed the best possible combination of variables to explain variation, and these included water ammonium, nitrate and PAR (r =1, P = 0.001). In full sun there was separation as points were loosely spread (Fig. 3.11). The first 2 principal components accounted for a cumulative total of 92% of the variation. PERMANOVA revealed that there were no significant effects of both treatment (shade/ full light) and nutrient levels (high/low) (Table 3.10). ANOSIM test for differences showed that there were no significant differences between treatments (r = -0.113, P = 0.7) and also between nutrient levels (r = 0.155, P = 0.15) most likely due to low sample size. Similar to the greenhouse study, PAR explained most of the variation found in the study, especially in full sun (Fig. 3.11, Fig. 3.20).

Table 3.14: Summary of PERMANOVA test of significance for the effect of environmental variables on biological variables.

Source	**DF**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Permutations**
Treatment	1	113.49	113.49	2.1968	0.155	985
Nutrients	1	155.28	155.28	3.0057	0.063	989
Treatment*Nutrients	1	26.752	26.752	0.51782	0.64	987
Residual	7	361.64	51.662			
Total	10	662.23				

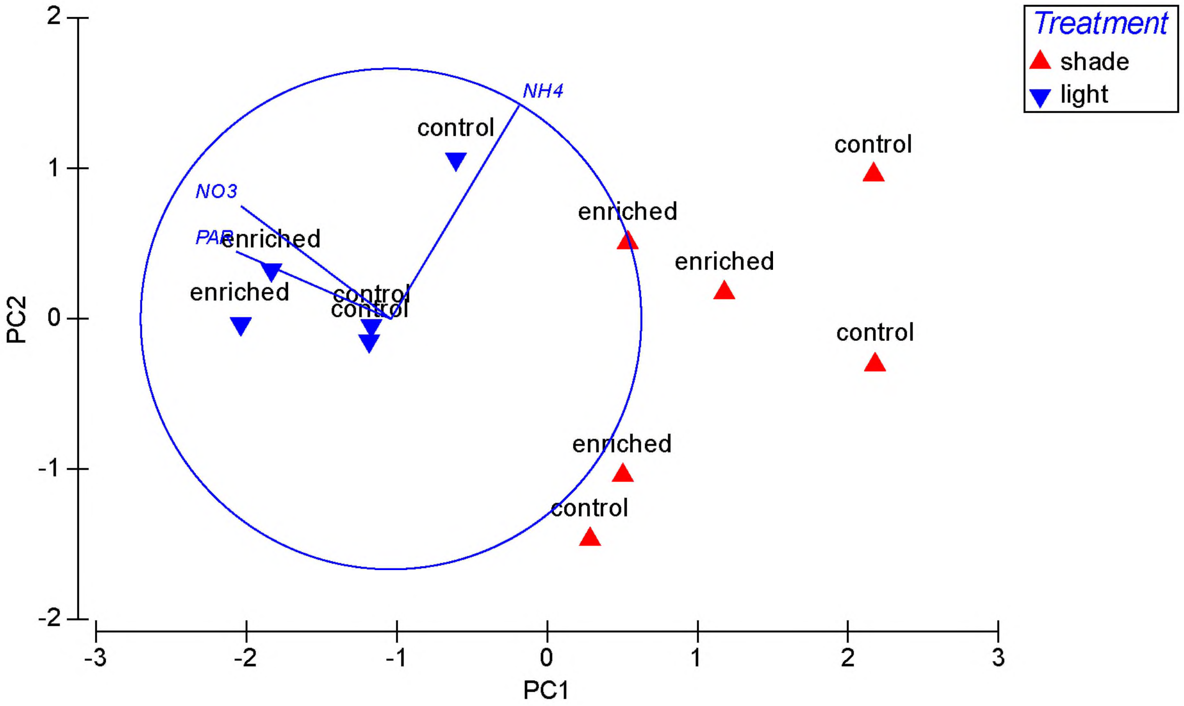


Figure 3.22: Principal Component Analysis of the effect of environmental variables on biological data in the shade and full sun at low and high nutrient conditions.

3.3 Summary

Analysis revealed some corresponding as well as differing results of the two studies (Table 3.15). There was significantly higher plant biomass under high nutrient conditions, and consequently, higher plant damage. Biomass however, did not show any response to either the sun or the shade in all instances, neither did it respond to herbivory. Despite the increase in biomass and plant damage with higher nutrient availability, weevil populations did not respond to higher nitrogen availability, neither did plant quality. However, weevil populations responded to higher plant quality, suggesting that there was stimulated reproduction and faster weevil developmental rates due to more available nitrogen for weevils in the plants. There was poor plant quality in the absence of weevils, while herbivory by weevils induced higher plant quality. The higher plant quality may have been a compensatory response by plants to herbivory probably leading to more allocation of nitrogen in the plant tissues for growth to counter losses from herbivory. Low quality plants suffered more damage as herbivores grazed with greater intensity in a bid to satisfy nitrogen requirements. Plants growing in the sun suffered greater damage than those in shade. Again, in the shade plants recorded relatively higher plant quality compared to the sun exposed plants, thereby underlying the importance of shade in determining plant quality.

Table 3.15: Summary of effects and relationships of all tested variables in both the greenhouse and field studies. +ve indicates a significant effect, 0 indicates a non-significant effect. Bold values indicate greenhouse study (**+ve**), while the normal values indicate the field study (+ve).

	Biomass	**Plant quality**	**Plant damage**	**Weevils**
Nutrients	**+ve**	**0**	**+ve**	**0**
Site	**0**	**+ve**	**+ve**	**0**
	0	0	0	0
Herbivory	**0**	**+ve**	**0**	
	0	0	+ve	
Plant quality	**+ve**		**+ve**	**+ve**
	0		+ve	+ve

Chapter 4 : Discussion

4.1 Introduction

The ability of *Salvinia molesta* to re-invade from the shaded parts of water bodies after initial control by the weevil *Cyrtobagous salviniae* prompted this study as differential success in control between the shade and sun has been reported in some parts of the world, including Australia, USA and South Africa (van Oosterhout, 2006; Hennecke and Postle, 2006; Owens *et al.*, 2011). However, no research has been conducted to investigate the underlying mechanism of how sunlight, particularly Photosynthetically Active Radiation (PAR) influences plant growth and persistence, and how it affects weevil development and population growth. This study therefore sought to investigate if shade improved plant growth or negated weevil impact on the weed.

4.2 Influence of PAR on plants and herbivores

Under low light conditions (normally in the shade) photosynthesis is reduced, while higher photosynthetic rates are realised in the sun as a result of increased stomatal conductance and greater photosynthetic capacity of the mesophyll due to high concentrations of RUBISCO (Givnish, 1988). Plants acclimated to high irradiance have higher photosynthetic rates when compared to those acclimated to low irradiance (Givnish, 1988). However, although photosynthesis increases with PAR, a saturation level is always reached (Usha Rani and Bhambie, 1983). A study conducted in the Amazon on chlorophyll fluorescence of mahogany (*Sweitenia macrophylla* King (Meliaceae)) and tonka bean (*Dipteryx odorata* Aublet (Fabaceae)) revealed that chlorophyll accumulation (chlorophyll *a, b,* and *tot*) was greater in shade leaves in a bid to maximize light harvesting (Gonçalves *et al.*, 2001). Similarly, another study in the Amazon on rosewood (*Aniba rosaeodora* Ducke (Lauraceae)) also revealed that there was greater chlorophyll concentration in the shade plants compared to those growing in full sunlight (Gonçalves *et al.*, 2005). *Salvinia molesta* is likely to have a similar adaptation to the plants in these examples, given that its origins are in the Amazon basin. Shading by forests in the margins of the Amazon River where *S. molesta* grows as an understory species, imposes light limitations on the growth of floating macrophytes (Sioloi, 2000). Here, *S. molesta* receives reduced PAR which filters through the foliage of the tall trees, and it has therefore adapted to these light limited shade conditions in its native range. Poorter (1999) reported that in tropical rainforests, plants at the lower end of the light spectrum are light limited; where only 1-2% of the light received by the canopy reaches the forest floor, and plants on the forest floor have evolved to increase light harvesting efficiency by having large leaf areas as well as chlorophyll accumulation. In the current study, both the greenhouse and field studies yielded similar results in that *S. molesta* plants in the shade received less PAR than those in the sun. This result meant that plants growing in the shade had light limitations which may have led to reduced photosynthetic capabilities compared to those in the open due to limitations in the amount of light they receive. In response, *S. molesta* in the shade accumulated chlorophyll to capture the reduced amount of PAR that it received, where the results of the study showed that in both the greenhouse and the field, *S. molesta* had significantly more chlorophyll in the shade compared to the full sun. The ability of chlorophyll accumulation is consistent with the evolutionary history of *S. molesta* as it evolved under the low light conditions of the Amazon Basin.

Previous studies undertaken in India and the USA revealed higher growth rates of *S. molesta* in the sun compared to the shade (Usha Rani and Bhambie, 1983; Mitchell and Tur, 1975; Owens *et al.*, 2011). A study by Givnish (1988) revealed that irrespective of the level of irradiance received by leaves, most plant species are able to acclimate to certain levels of irradiance and perform optimally under those conditions; while Muthuchelian *et al.* (1989) reported that light induced limitations to photosynthetic rates lower plant productivity hence resulting in smaller plants. In the current study, while there were significant differences in PAR between shaded and full sun sites, there were no corresponding differences in biomass of plants grown under these conditions for both laboratory and field experiments. These results are consistent with the findings by Givnish (1988), and contrary to those of Usha Rani and Bhambie (1983), Mitchell and Tur (1975), and Owens *et al.* (2011) who found greater growth in the sun in India and the USA.

When plants receive excess light, photo-inhibition occurs, resulting in a halt in growth and possibly mortality (White, 1984). High PAR beyond saturation point can thus lead to a decline in plant growth due to an acceleration in plant senescence where plant tissues begin to break down (Gliessman, 2007). Previous research revealed that by the end of a growing season, maize (*Zea mays* L. (Poaceae)) and oilseed rape (*Brassica napus* L. (Brassicaceae)) lose up to 10% of their biomass as a result of photo-inhibition (Long *et al.*, 1994). At high levels of PAR, low growth even with adequate nutrition for the plants could be the result of plants reaching light saturation as has been documented for *S. molesta* (Usha Rani and Bhambie, 1983). In the current study, *S. molesta* biomass in the sun and shade was similar. It is possible that despite potentially greater biomass accumulation associated with high light intensities, these gains may have been nullified by photo-inhibition and solar induced senescence, leading to reduced net biomass, implying that PAR exceeding optimum values may have inhibited growth and also led to plant mortality, consistent with reports by Gliessman (2007). In the shade however, due to low irradiance and limited senescence, *S. molesta* maintained standing biomass with minimal plant mortality, and achieved similar net biomass as plants in the sun. Such a scenario has implications for the biological control of *S. molesta* in the shade and sun as it may mean that in the sun, *C. salviniae* herbivory is complemented by solar induced senescence, hence greater plant mortality, with suppressed recovery, ultimately leading to improved biological control of *S. molesta*.

Plant quality was influenced by PAR and inorganic nitrogen supply in the current study. Lüttge (1997) reported that shade plants generally have greater total plant nitrogen when compared to the sun plants, as in the shade, plants invest more heavily in chlorophyll production in a bid to compensate for light limitation (Gonçalves *et al.*, 2001). However, in the shade, leaves possess less soluble nitrogen and this is detrimental to insect herbivores, hence the lower preference for shade leaves (Evans, 1989; Lüttge, 1997; Lambers et al, 2008). Again some studies have revealed that for photosynthetic reasons, some plants invest heavily in leaf nitrogen in the form of chlorophyll and more importantly in the production of biological catalysts, the most abundant being RUBISCO, which tends to raise leaf nitrogen content (Chapin, 1980; Hahne and Schuch, 2004; Lambers, 2008). Sun plants normally have greater soluble nitrogen concentrations probably due to high enzymatic activity as well as nitrogen mobilisation and reallocation in high irradiance, leading to more soluble nitrogen in the sun exposed leaves during senescence (Evans, 1989). It is likely that this occurs in *S. molesta* where there is greater soluble nitrogen in sun exposed leaves. However, previous work revealed that the persistence of soluble nitrogen abundance is subject to fluctuations between periods of high and low irradiance (Lüttge, 1997). The greenhouse trials in the current study revealed that shade grown plants were of higher quality, consistent with past research. Low plant quality in the plants exposed to full sun in the greenhouse was consistent with previous reports that there is loss of tissue nitrogen at high irradiances. Again, the higher concentrations of chlorophyll recorded in shade plants explains the higher quality of plants in the shade, as has been highlighted previously that some plants invest in chlorophyll when there are light constraints. There is a close relationship between chlorophyll and tissue nitrogen content, because nitrogen is a major component of chlorophyll (Evans, 1983; Bojović and Marković, 2009). However, the same experiments conducted in the field indicated similar plant quality for both the shade and the sun despite the differences in chlorophyll content between the shade and sun.

Roháček *et al.* (2008) reported that low levels of solar radiation result in reduced nitrogen mobilisation and reallocation, hence higher tissue nitrogen concentrations for prolonged periods of time. Other studies highlighted that at high light intensities, protein synthesis is inhibited, while tissue breakdown is accelerated, leading to reduced plant nitrogen (Mattson, 1980; White, 1984; Heard and Winterton, 2000). High irradiance induces inactivation of the photochemical reaction centre where the D1 protein in photosystem II becomes irreversibly damaged, leading to the loss of nitrogen (Gonçalves *et al.*, 2005). In this study, at low PAR levels, *S. molesta* plants were able to maintain higher quality because of little or no solar degradation of plant tissues, hence even in the low supply or depleted levels of inorganic nitrogen, plant tissues remained rich in nitrogen. At higher PAR intensities however, plant quality declined as evidenced by *S. molesta* mortality caused by light saturation and photo-inhibition leading to senescence, hence loss of tissue nitrogen and ultimately a reduction in plant quality. Plant quality in the field was however uniform at both low and high PAR which was contrary to the hypothesis that there is higher quality in the shade. These results suggest that there may have been another factor which determined plant quality in the field.

A study by Wyatt and Brown (1977) on four aphid species, *Myzus persicae* Sulzer (Aphididae), *Macrosiphoniella sanborni* Gillette (Aphididae), *Brachycaudus helichrysi* Kaltenbach (Aphididae) and *Aphis gossypii* Glover (Aphididae), revealed that light has an influence on insect reproduction. Low light coupled with short days negatively impacted reproduction of *M. persicae* and *M. sanborni*; while even in long days, low light intensity hampered reproduction of *M. sanborni*. Under high light intensities, there was accelerated population growth for all aphid species (Wyatt and Brown, 1977). Roháček *et al.* (2008) reported that there is high survival of aphids, coccids and some Lepidoptera on sun exposed plants compared to the shade, thus concurring with the research by Wyatt and Brown (1977). High quality plant material is required by insects for growth and development and plants in the sun periodically have lots of available nitrogen unlocked from plant tissues during mobilisation and reallocation at high irradiances (Mattson, 1980; White, 1984; Roháček *et al.*, 2008). Furthermore, high quality plant material has a positive influence on insect herbivore fecundity as female phytophagous insects have been reported to preferentially lay their eggs in high quality plant material, especially young meristematic tissues (White, 1984; Forno and Bourne, 1985; Room, 1990). There is lower occurrence of meristematic tissue in *S. molesta* growing in the shade which then negatively affects *C. salviniae* populations due to lack of oviposition sites (Forno and Bourne, 1984; Calder and Sands, 1985; Room, *et al.*, 1989; Schooler *et al.*, 2011). In the current study, there were no significant differences in weevil populations in both greenhouse and field trials, regardless of PAR levels, contrary to the expectations that there would be higher populations in the sun owing to the better control that has been measured throughout South Africa. Lack of differences in the field was revealed to have been due to low statistical power of the test, which means that it could not be concluded that there were no differences. Again, a longer duration of the experiment would have revealed more information, but due to the unexpected crash of the weed mat, the trials were terminated prematurely (9 months into the trials). Furthermore, greater replication of the experiment and additional study sites would have been a safe guard against such stochastic events.

Field trials yielded results that were contradictory to the greenhouse study where, though not significant, trends revealed higher weevil populations in the sun. There is the possibility that in the field, weevils dispersed from the shady to the sunny conditions, to reach more favourable conditions in the sunny sites as there may have been more favourable conditions for egg laying. This is in line with the findings of Schooler *et al.* (2011) who demonstrated that since shade plants have few terminal buds, weevil population turnover is reduced because of lack of suitable egg laying sites. This could explain the failure of *C. salviniae* to effectively control *S. molesta* in the shade. Greater weevil populations in the sun supported results of previous studies which have highlighted greater survival and reproduction of insects on plants growing in the sun as such plants provide better conditions for insect reproduction and survival (Roháček *et al.*, 2008).

Findings by Room (1990) revealed that reproduction of *Samea multiplicalis* was successful on *S. molesta* with more than 2% dry matter nitrogen, while high larval mortality was recorded in plants with low nitrogen. Insects need high quality food to complete their life cycles and successfully reproduce and it has been reported that in the sun, plants possess greater concentrations of soluble nitrogen which is more available to insects to complete their development (Room *et al.*, 1989; Roháček *et al.*, 2008). Again, female *C. salviniae* have been observed to preferentially lay their eggs in the young buds on growing tips due to their high nitrogen content essential for development, further highlighting the importance of plant quality (Julien *et al.*, 2009). Accelerated reproduction of *C. salviniae* has been recorded where there is high tissue nitrogen in *S. molesta* (Forno and Bourne, 1984; Forno and Bourne, 1985; Room *et al.*, 1989; Julien *et al.*, 2009). A study by Throop and Lerdau (2004), revealed that female insects select plants with the highest quality to lay eggs as these will enhance the chances of survival of the offspring to adulthood. The current study shows that insect survival and reproduction is highly dependent on nutrition where besides PAR, plant quality played a significant role in determining weevil population in both the greenhouse and field studies. Although there were no differences, *C. salviniae* attained similar population sizes between shade and sun sites, trends from the greenhouse revealed higher populations in the low PAR treatment due to high quality plants.

Room *et al.* (1984) reported that *S. molesta* plants with high tissue nitrogen incurred half the damage sustained by those with low tissue nitrogen. Forno *et al.* (1983) also reported that *C. salviniae* only increased consumption with increase in temperature, while increase in plant tissue nitrogen did not stimulate herbivory. Insect herbivores tend to keep their consumption of high quality plants at optimum levels in order to avoid nitrogen toxicity which has a negative feedback on their performance (Throop and Lerdau, 2004). Kingsolver and Woods (1998) further stated that low plant quality may negatively affect the reproductive performance of insect herbivores while also stimulating increased herbivory to compensate for low dietary protein. Plants in the sun incurred greater damage from *C. salviniae* and this was consistent with expectations that control is more successful in the sun due to greater herbivory intensity, as has been observed in the field prior to this current study (Sullivan and Postle, 2010). However, though no significant differences were recorded due to low statistical power, trends in both greenhouse and field studies revealed that *S. molesta* plants incur greater damage in the sun. Again, results from the greenhouse study revealed that leaf damage was affected by plant quality which most likely had a bearing on *C. salviniae* feeding. The greatest damage was incurred by plants exposed to high PAR intensities, which subsequently recorded the lowest plant quality. In this case, it is likely that due to the low plant quality in the sun, *C. salviniae* were stimulated to consume greater amounts of plant material to compensate for the low nutritional value. Plants growing in the shade showed less damage and higher quality (tissue nitrogen content), consistent with previous research (Throop and Lerdau, 2004) which found that insects tend to limit their consumption rates on high quality plant material in an effort to avoid nitrogen toxicity. This therefore means that *C.*

salviniae had a greater affinity for sun exposed leaves most probably due to the lower plant quality which was recorded in the sun than the shade; and this would be consistent with findings of previous research by Room *et al.* (1984) and Lemoine *et al.* (2014).

4.3 Effects of nutrient supply on plants and insect herbivores

In this study, while PAR did not affect biomass, an increase in water nutrient availability translated into an increase in plant biomass consistent with the report that ammonium and nitrates provide nitrogen for plant tissue formation needed for growth of plants (Sabir *et al.*, 2013). A study by Heard and Winterton (2000) on *Eichhornia crassipes* revealed that biomass accumulation was accelerated by increase in nutrient supply. Similarly, investigations on *Myriophyllum spicatum* revealed greater plant biomass when nutrients were in high supply (Cao *et al.*, 2012). Previous studies on *S. molesta* have also shown that plant growth and survival is greatly accelerated when there is high nutrient supply (Cary and Weerts, 1983; Sands *et al.*, 1983; Room *et al.*, 1989; McFarland *et al.*, 2004; Madsen and Wersal, 2008). In Australia, nitrogen-rich run-off led to increased nutrients in lake water, which consequently accelerated the growth of *S. molesta* (Mitchell *et al.*, 1980; Room, 1990). In this study, in the greenhouse, plant growth was significantly enhanced by high water nutrient concentration, where greater biomass was recorded with high nutrient supply. In the field however, there was no difference in plant biomass despite enrichment of some quadrats with fertiliser, and this was attributable to diffusion of nutrients to the rest of the water body, therefore making the nutrient conditions uniform for all plants in the water body. The difference in results between the greenhouse and field is because in the greenhouse, the study plots were individual mesocosms independent from each other and were able to maintain a set of growth conditions in each mesocosm while in the field, the study plots were floating cages, and ultimately were all connected.

A study on a congeneric species, *Salvinia natans* (L.) All. (Salviniaceae), revealed that plant biomass was greater on plants grown on ammonium nitrogen compared to nitrate (Jampeetong and Brix, 2009). Ammonium has been shown to be the more preferred form of inorganic nitrogen since it is relatively inexpensive to acquire by the plant compared to nitrate which needs to be reduced once it has been taken up (Reddy and Tucker, 1983; Hahne and Schuch, 2004; McFarland *et al.*, 2004). In the current study, higher concentrations of nitrate than ammonium were recorded in both the field and greenhouse; and in the greenhouse, the abundance of nitrate led to increased biomass accumulation. It is possible that in the current study, *S. molesta* preferentially depleted ammonium to very low levels and thereafter the high concentrations of nitrate became more influential on plant growth; this was consistent with a study which revealed that *E. crassipes* readily absorbed ammonium leaving nitrate in the water (Reddy and Tucker, 1983).

A study on *M. spicatum* that focussed on nitrogen uptake and reallocation when plants were exposed to herbivory, revealed that more nitrogen was found in meristematic tissues to allow for continued growth while the plants were under herbivore attack. A decline in nitrogen content for stems was recorded as nitrogen was translocated to plant sections less vulnerable to herbivory (Rothhaupt *et al.*, 2015). *Salvinia molesta* may retain senescing sections of the weed mat in low nutrient conditions in a bid to reallocate nutrients to other sections of the weed mat and this indicates the importance of nutrient supply to the growth and persistence of *S. molesta* (Mitchell and Tur, 1975; McFarland *et al.*, 2004).

Inorganic nitrogen (ammonium and nitrate) is taken up by plants and used for organic nitrogen formation which is essential for manufacture of proteins for plant growth (Sabir *et al.*, 2013). Chapin (1980) also stated that plants subjected to high nutrient supply achieve greater plant quality. A study on *Nasturtium officinale* Aiton (Brassicaceae) revealed that with an increase in the nutrient concentration, foliar nitrogen correspondingly increases (Fernandez-Going *et al.*, 2012), similarly this current study revealed a correlation between *S. molesta* plant quality and ammonium supply. Increase in inorganic nitrogen leads to increased protein yields due to increased cell division and elongation (Mattson, 1980). The greenhouse study showed that there was higher plant quality in the shade compared to the sun when there were high nutrient conditions. Because the causative factor is not obvious here, both of these factors (PAR and nutrient supply) are jointly responsible for the determination of plant quality.

In terms of effects of nutrients on herbivores, and more specifically biological control agents, a study on *E. crassipes* grown at high nutrient conditions revealed that more *Neochetina bruchi* Hustache (Coleoptera) weevils were supported compared to plants grown at medium nutrient concentration (Heard and Winterton, 2000). Similarly, Forno and Bourne (1984) demonstrated that there is greater fecundity of female *C. salviniae* that feed on high nitrogen *S. molesta* plants, enabling rapid population turnover. In addition, Room (1990) reported that although *C. salviniae* adults have little behavioural response to plant nitrogen, they have the ability to respond numerically to nitrogen availability by increased fecundity when there is high nitrogen supply. In the greenhouse study, trends revealed more weevils on plants grown at high nutrient conditions although this was not significant. This was enabled by the assimilation of inorganic nitrogen by plants resulting in significantly higher plant quality, which enabled weevils to reproduce faster. In the field however, there was uniform distribution of ammonium which has been shown in previous research, to be the preferred source of nitrogen by plant (Reddy and Tucker, 1983; Hahne and Schuch, 2004), resulting in similar plant quality across the whole water body; and therefore nutrient enrichment did not influence weevil populations in the field, converse to what was observed in the greenhouse. The field trials however revealed that there was higher nitrate in the full sun sites compared to the shade, but the abundance of nitrate did not correlate with the resultant plant quality, which would ultimately have influenced weevil population. Again, as in the greenhouse study, weevil populations in the field study were influenced by plant quality as influenced by water nutrients. Therefore, nutrient supply plays a crucial role in determining plant quality, and in turn, weevil population growth.

When plants are subjected to stress factors such as deficiencies in mineral nutrition and moisture stress, they become more vulnerable to insect herbivore attacks (White, 1984). In high nutrient supply, biological control of aquatic invaders such as *E. crassipes* becomes increasingly challenging; especially since plants quickly recover from herbivory while actively growing (Coetzee *et al.*, 2011). *Salvinia molesta* damage was significantly influenced by plant quality which was consequently affected by nutrient supply, with ammonium being the determinant for plant quality in the greenhouse trials. Trends in the greenhouse however, revealed that there was significantly more damage to *S. molesta* plants in the low nutrient treatment which consequently had significantly lower tissue nitrogen values. This suggests that *C. salviniae* consumed more *S. molesta* in the low nutrient treatment, in a bid to meet their nitrogen nutritional requirements. However, in the high nutrient treatments, there was less damage inflicted on plants, as weevil nutritional requirements were met with minimal herbivory. In the field, water nutrients distribution resulted in similar plant quality, and no significant differences in damage were recorded as a result. Again the lack of differences in damage for the field study was the result of low statistical power of the experiment. The two studies therefore yielded similar results as they both did not register significant differences in plant damage with nutrients regimes. However, the trends in both studies revealed that low quality *S. molesta* plants suffer more damage compared to high quality plants.

4.4 Comparisons between greenhouse and field studies

Most research done in biological control tends to be laboratory or greenhouse based as experimental conditions are easier to manipulate (Usha Rani and Bhambi, 1983; Room *et al.*, 1984, Forno and Bourne, 1984; Owens *et al.*, 2011, Harms *et al.*, 2009). However, the results obtained from laboratory studies do not always give a true representation of what really occurs in the field where in natural systems, the plant-herbivore interaction is subject to the dynamics of weather and factors such as competition, predation and parasitism (Gurevitch *et al.*, 1992). The value of laboratory studies therefore lies in that they show what happens when all conditions are constant or even optimum, while experimentation in field studies can be regarded as having a stronger link to the natural systems being investigated (Gurevitch *et al.*, 1992). Again laboratory studies are more secure, giving greater chances of completing an experiment with less chances of disturbance which are often experienced in field experiments.

Both the greenhouse and field studies here produced similar results with regards to PAR, where in both trials there was less PAR in the shade compared to the sun. This was in-line with the expectations that *S. molesta* plants grown in the shade receive less PAR than those grown in the sun, which had implications for both the plants and insects. Again, in the greenhouse, less PAR was received in full sun treatments compared to the field, where the greenhouse material absorbed and reflected about 50% of PAR in comparison to what is received in the field. As in the field, higher levels of nitrate compared to ammonium were found in greenhouse experiments, probably because plants took up ammonium much faster since it is the more preferred form of inorganic nitrogen (Lüttge, 1997; Glass *et al.*, 2002; Hahne and Schuch, 2004). However, in the field, water nutrients were not significantly different between the enriched and non-enriched quadrats, hence led to uniform *S. molesta* growth across the water body. This was likely because nutrients added to the enrichment quadrats diffused evenly across the water body; however, differences in nitrate were recorded between the shade and full sun sites while ammonium concentration was uniform. Despite differences in the abundance of nitrate, plant biomass was similar in both the shade and full sun. In the greenhouse, due to the more restrictive environment provided by mesocosms, water nutrients between the nutrient treatments remained different, leading to differences in plant biomass with nutrients. Comparing nutrient concentrations from the two studies, on average, the greenhouse study had less nitrate (2.03mg/L) and ammonium (0.07mg/L) in the shade compared to the field study (5mg/L nitrate; 0.45mg/L ammonium); while in the sun, the greenhouse study recorded less nitrate (1.98mg/L) but higher ammonium (0.19mg/L) compared to the field study (8.12mg/L nitrate; 0.15mg/L ammonium). This result may be because in the greenhouse, plants took up nutrients much faster probably due to optimal environmental conditions which aided growth.

Lack of differences in plant biomass for both studies indicated that biomass accumulation by *S. molesta* was not affected by the amount of PAR they receive and therefore another environmental factor was at play, and in this case, nutrient availability explained variation in biomass. Again the results showed that when there is uniform nutrient supply, despite different levels of radiation, *S. molesta* can still perform optimally with varying PAR regimes. The greenhouse study therefore revealed that plant growth is not only affected by different PAR regimes, but that nutrient supply was important as well; as such greenhouse studies provided a platform for simulations of what can occur in the field when all conditions are held constant. The differences in biomass with nutrient supply in the greenhouse indicate that nutrients were more important than PAR and herbivory in determining biomass accumulation; furthermore, these results are consistent with previous research (Bryant *et al.*, 1983; Cary and Weerts, 1983a; Tilman, 1986; McFarland *et al.*, 2004; Madsen and Wersal, 2008; Sabir *et al.*, 2013).

The factors affecting plant quality in both studies were similar where herbivory explained the variation in plant quality, except that in the greenhouse, ammonium also influenced plant quality. It is likely that these differences were again instigated by the uniformity of water ammonium distribution in the field site; while in the greenhouse nutrient treatments remained independent. It is therefore likely that in both instances, herbivory determined uptake of inorganic nitrogen which was then assimilated into plant tissue, giving rise to the resultant plant quality. Plants have been recorded to have compensatory responses to herbivory and *S. molesta* is no exception with the ability to reallocate nutrient resources (Mitchell and Tur, 1975; Heard and Winterton, 2000; McFarland *et al.*, 2004; Julien *et al.*, 2009). Furthermore, in the greenhouse study, *S. molesta* growing in the shade had higher plant quality compared to shade plants in the field study. In terms of plants growing in the sun, again greenhouse plants had higher quality, with field plants recording half as much tissue nitrogen compared to the greenhouse plants.

Weevil populations were not significantly different between shade and full sun in both studies even though trends in the greenhouse revealed that there were more weevils with higher plant quality, however in the field, weevil population sizes were similar due to uniform plant quality. Again, the lack of differences in the field was probably due to low replication of the experiment, leading to low statistical power. Even with the lack of significant differences, the two studies revealed contrasting results where weevil populations were higher in sites in the sun for the field, while in the greenhouse, populations were higher in the shade. This difference therefore underlines the importance of plant quality in determining weevil populations. Again, the study showed that weevil development is dependent on plant quality as was shown in both the field and greenhouse confirming the findings by Room (1990). Lower weevil populations in the shade for the field trial may have been due to dispersal of the insects to the sun. It is possible that the sun plays a crucial role in disease control in the insects via the UV-B rays, such as control of entomopathogens like *Helicosporidium* species (Hembree, 1981; Fernandes *et al.*, 2015), and this warrants further investigation.

In the greenhouse, leaf damage was affected by plant quality, water nutrient levels and PAR levels, while in the field study, plant quality and weevil populations resulted in significantly more plant damage. Under both conditions, when plant quality was low, plants suffered more damage and this was especially true at high PAR intensities. Greater damage on low quality plants could be due to compensatory feeding by *C. salviniae*, consistent with previous research (Kingsolver and Woods, 1998). Low damage to better quality plant material observed in the low PAR treatment in the greenhouse could have been a response by weevils to avoid nitrogen toxicity, and this would be in line with the study by Throop and Lerdau (2004), where they stated that consumption is maintained at an optimum to avoid toxicity which would otherwise have a negative feedback on herbivore performance.

For the purposes of this study, greenhouse trials however did not reveal what may be actually occurring in the field especially since the two studies yielded converse results. It can therefore be concluded that the greenhouse study only provided a reference point to observe how plants and insects respond when they are in a stable environment, unlike in the field where experiments are prone to a variety of perturbations such shown in this study. The greenhouse studies therefore helped to clearly demarcate and distinguish between shade and full sun in order to test the effects separately in an effort to reveal the dynamics which may be occurring in the field. A number of experiments have been done in greenhouses in research efforts to combat *S. molesta* and have proved instrumental in showing how plants and insects perform when in a stable environment, which is rarely ever the case in the field (Owens *et al.*, 2011; Harms *et al.*, 2009). Work done by Coetzee and Hill (2012) also stressed the importance of laboratory studies in biological control of weeds where it was revealed that nutrient supply is a more important factor compared to herbivory in the biological control programmes. In terms of the current study, the greenhouse trials gave more conclusive results than the field as there was a more stable environment to complete the experiments.

Chapter 5: Conclusion and Recommendations

5.1 Conclusion

This study has highlighted that the plant herbivore interaction was more nutrient-mediated than shade-influenced. Plant growth in either the shade or full sun is more dependent on nutrient supply compared to the photosynthetic capabilities of plants at either PAR regime. Nutrient supply therefore plays a greater role in determining growth and biomass accumulation of *S. molesta* in comparison to the amount of light plants receive, as has been previously reported (Mitchell, 1974; Forno and Bourne, 1984; Room, 1988; Julien *et al.*, 2009; Owens *et al.*, 2011; Harms *et al.*, 2009). In shade conditions, mineral nutrition complemented by herbivory greatly influences plant quality of *S. molesta*. The rate of consumption by *C. salviniae* determined the amount of tissue nitrogen in *S. molesta*, where previous studies have also shown that insect herbivores have a profound influence on tissue nitrogen concentrations of plants (Heard and Winterton, 2000). This was evident in that plants grown in dense shade possibly compensated for herbivory damage inflicted on them especially when there is an abundant nutrient supply, while plants in the sun failed to compensate.

Insect population growth and reproduction is dependent on plant quality as was revealed in both studies in the field and greenhouse. Therefore, greater plant quality, causes higher insect survival. However, the conflicting results in terms of insect populations in the sun and shade of the field and greenhouse studies will need to be investigated further. Furthermore, it could not be decisively concluded whether insects favour sunny areas compared to the shade because of the conflicting population results from the two studies. However, based on the field study which had uniform plant quality across the shade and sun, dispersal of *C. salviniae* from shaded to sunny areas may be triggered, because sunny areas are more favourable to insect development (White, 1984; Rohaceck *et al.*, 2008; Schooler, 2011). In both studies though no significant differences were obtained, patterns showed that plant damage was higher in the sun compared to the shade, and this explains why better control in sunny areas in comparison to the shade was recorded in the field. The question still remains, why is control difficult in the shade compared to the sun? Further testing and studies still need to be done to investigate this problem as this study, particularly the greenhouse trials, concluded that nutrients are the major determinants of the interaction between plants and herbivores; and it is possible that since shaded sites are on the margins of water bodies, they receive more allochthonous inputs and are hence more nutrient rich.

5.2 Recommendations and further research

There were some shortcomings in the study, in particular there was low replication and thus low power of statistics for the field study. There may also be need to conduct field studies in semi-isolated sites to avoid anthropogenic disturbances as was the case in the field study where study plots were vandalised. In future research, it will be important to carry out a *priori* power analysis to determine a viable sample size to avoid low statistical power.

Based on the findings of this study, it would be important to apply site specific nutrient enrichment on shaded areas as this may likely have some positive results for weevil population turnover, where high nutrient supply may induce production of more oviposition sites (growth terminals) for *C. salviniae*. Nutrient enrichment was shown to be effective in encouraging weevil establishment, in that *C. salviniae* established much faster where there was high nutrient supply for *S. molesta* in the field (Room and Thomas, 1986; Room *et al.*, 1989).

Furthermore, inundative releases of *C. salviniae* in the shade could enhance control, in the event of adults dispersing from the shade, as enough eggs to produce larvae could augment the earlier efforts of their folivorous parents before dispersal. Inundative releases are now largely possible due to mass rearing efforts carried out in various research stations which then ensures a high supply of the biological control agents. Initial high densities of *C. salviniae* in the shade will therefore impose high herbivory pressure on *S. molesta*, which will possibly hamper the regenerative capabilities of the weed, thereby preventing it from re-invading water bodies after initial control has been achieved. In the case of very high populations of weevils in the shade, especially in warm temperature conditions and high plant quality in the shade, it may be possible to achieve control of *S. molesta.*

Plant morphology and its effects on the weevils will also need to be investigated. It is suggested that plant rhizome sizes be investigated in both the shade and sun. From the observations during the course of the greenhouse study, plants in the shade tend to persist for longer in the primary growth form, which negatively influences development of the later instars of the weevils, hence leading to poor or failed control of the weed. Plant size preferences of the insects will also need to be correlated with plant sizes in the shade and sun.

If it is the case that plant size is reduced in the shade, then there may be a need to explore the use of an alternative agent which can complete its life cycle on the small plants such as the Florida biotype of *Cyrtobagous salviniae* which is known to utilize *Salvinia minima* Baker (Salviniaceae), a species of salvinia which is smaller than *S. molesta* (Parys and Johnson, 2013). It is possible that since the Florida biotype of *Cyrtobagous salviniae* can utilize *S. minima*, it can survive and complete development on small *S. molesta* plants growing in the shade.

It would also be useful to investigate further and test why insects showed preference for the sun in the field. It may be possible that solar radiation helps insects control the parasite, *Helicosporidium parasiticum* as well as other parasites like the Sporozoan Gregarinida which affect the gut of most Coleoptera (Crowson, 1981; Hembree, 1981). The role of solar radiation in insect development will also be of major interest, where comparisons of development between shade and sun will need to be investigated.

Biological control of *S. molesta* has continued to keep the weed under control despite the problems associated with the shade. This study has helped identify some of the factors that may be hindering complete control of *S. molesta* in the shade. In conclusion, it will be useful to approach biological control in the shade by means of inundative and augmentative releases as high populations have a greater efficacy in the control of *S. molesta*. Timing of the releases of *C. salviniae* is also crucial, where release during the warmer seasons ensures greater chances of establishment and ultimately, continued success of the biological control of *S. molesta*.

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