INTEGRATED CONTROL OF WATER HYACINTH USING A RETARDANT DOSE OF GLYPHOSATE HERBICIDE

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A thesis submitted to the Faculty of Science, University of Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2011

Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or any other examination in any other University.

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8th day of December 2011

Abstract

Eichhornia crassipes (Martius) Solms-Laubach (Pontederiaceae) (water hyacinth), a neo-tropic noxious weed of South American origin, is counted among the "big five" aquatic weeds in South Africa. The weed causes dramatic ecological and economic losses in infested areas. Its control is facilitated by the release of biocontrol agents, mainly *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae). Control efforts via biocontrol are hampered, mainly by the climate incompatibility of the agents, aggravated further by the indiscriminate use of lethal doses of glyphosate based herbicides. The lethal doses interfere with the successful establishment and persistence of the biocontrol agents, thus undermining their impact. Continued use of herbicide kills the water hyacinth mat and as a result, the immature stages of the agents are killed. If biocontrol is to succeed as a control strategy, then low doses of the herbicide need to be advocated. It was hypothesized that a low dose will constrain the vegetative and reproductive capacity of the weed, while maintaining the habitat for the biocontrol agents. Consequently, this study was conducted to identify a retardant dose of glyphosate herbicide and test its effect on the *Neochetina* weevils. A concentration of 0.8% (0.11g m^{-2} or 2880mg a.i /L) glyphosate based herbicide, sprayed at 150 L ha^{-1} was proved to retard the vegetative and the reproductive growth of the weed, in terms of leaf and ramet production. Further, the retardant dose did not have any detrimental effects on the adult weevils and its larval stages. Weevil herbivory was also enhanced by the retardant dose. Furthermore, the retardant dose did not have any detrimental effects on 'plant quality' as evidenced by % nitrogen level in plant tissues such as crown and leaves. Contrary to expectation however, the combined effects of the retardant dose and *Neochetina* herbivory (0.8%+Ne) did not result in the production of lower number of ramets or leaves than water hyacinth plants dosed with 0.8% herbicide alone. Water hyacinth biocontrol agents in South Africa are subjected to frosty winters with low temperatures which cause the biocontrol agents to decline to an overwintering larval population that fails to catch up with the weed as it rebounds from the frost in spring.

This hypothesis was tested in this study at 12 water hyacinth infested sites, which were grouped as temperate and sub-tropical sites. At both the temperate and subtropical sites, water hyacinth plants produced ramets (daughter plants) through autumn and increased biomass during summer. However, weevil numbers were very low at these sites, as evidenced by adult counts and feeding scars, indicating a marked seasonal asynchrony between the phenologies of the weevils and water hyacinth. Hence, intervention by seasonal applications of the herbicide is crucial to constrain weed growth. Herbicidal applications during autumn and spring inhibited the growth of the weed without adversely affecting the adult weevils or immature, immobile stages. Continued use of herbicides raises concerns of effect on non-target species, such as amphibians. Results from this study indicate that a direct application of a retardant dose of glyphosate did not kill or affect the growth of the *Xenopus* larvae, as determined by survival and body lengths. However, under laboratory conditions, this study has shown for the first time that an invasive aquatic weed (water hyacinth) was more lethal to an aquatic vertebrate (*Xenopus* larvae) than a herbicide advocated for its control. This study conclusively shows that retardant dose of glyphosate herbicide can be integrated with biocontrol to provide a sustainable and eco-friendly technique with which to combat water hyacinth infestations in South Africa.

Acknowledgements

Thanks especially to my family and friends, who provided inspiration and encouragement.

The University of Witwatersrand and Water Research Commission, South Africa, are gratefully acknowledged for their financial assistance with this PhD.

Thanks to Profs Marcus Byrne and Martin Hill for their supervision, motivation and for their encouragement towards this project.

Arne Witt, Andre Rensburg, Anton Swanepoel, Rael Hughes, Kathleen Saunders, Fritz, Daleen, Dr. Jim Findlay and Alecia Kirton are acknowledged for their help and suggestions.

Thanks also to Dr. Mark Robertson, Dr. Graham Alexander, Hardi Oberholzer, Angela Bownes, Lori Pillman, Erika Vercuiel, Jacobeth and Chilesha Mphele and Donald McCallum for their help.

Thanks to my colleagues who assisted me in the field on numerous occasions.

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General Introduction

1.1 Water Hyacinth: Origin and spread

Eichhornia crassipes (Martius) Solms-Laubach (Pontederiaceae) or water hyacinth, an invasive aquatic plant of neo-tropical origin is considered one of the world's worst weeds (Holm *et al*., 1977). Owing to its beautiful purple flowers, garden enthusiasts distributed it around the world, and consequently, in the absence of a full suite of natural enemies, water hyacinth has gained notoriety worldwide as the worlds' most important aquatic weed (Julien *et al*., 1996). Water hyacinth was first introduced into the African continent via Egypt at the end of the nineteenth century and has subsequently spread to large parts of tropical and subtropical Africa (Gopal, 1987). In South Africa, water hyacinth was first recorded in KwaZulu-Natal around 1910 (Edwards and Musil, 1975), and is now distributed throughout the country, excluding the Karoo region (Henderson, 2001) (Figure 1.1).

In the introduced range, the invasive potential of water hyacinth is attributed mainly to its propensity for rapid growth by vegetative reproduction. In addition, a viable seed bank, absence of natural enemies (Harley *et al*., 1996) and highly eutrophic waterways of South Africa, where phosphorus (P) levels vary from 0.01-2.8 mg/L and nitrogen (N) levels vary from 0.33-4.9 mg/L (Byrne *et al*., 2010) have enabled the weed to proliferate and reach invasive proportions.

([†]A part of this chapter is published in the journal, Outlooks on Pest Management: Jadhav *et al*., 2007. Integrated weed control using a retardant dose of glyphosate: a new management tool for water hyacinth? Outlooks on Pest Management, 18, 213-216)

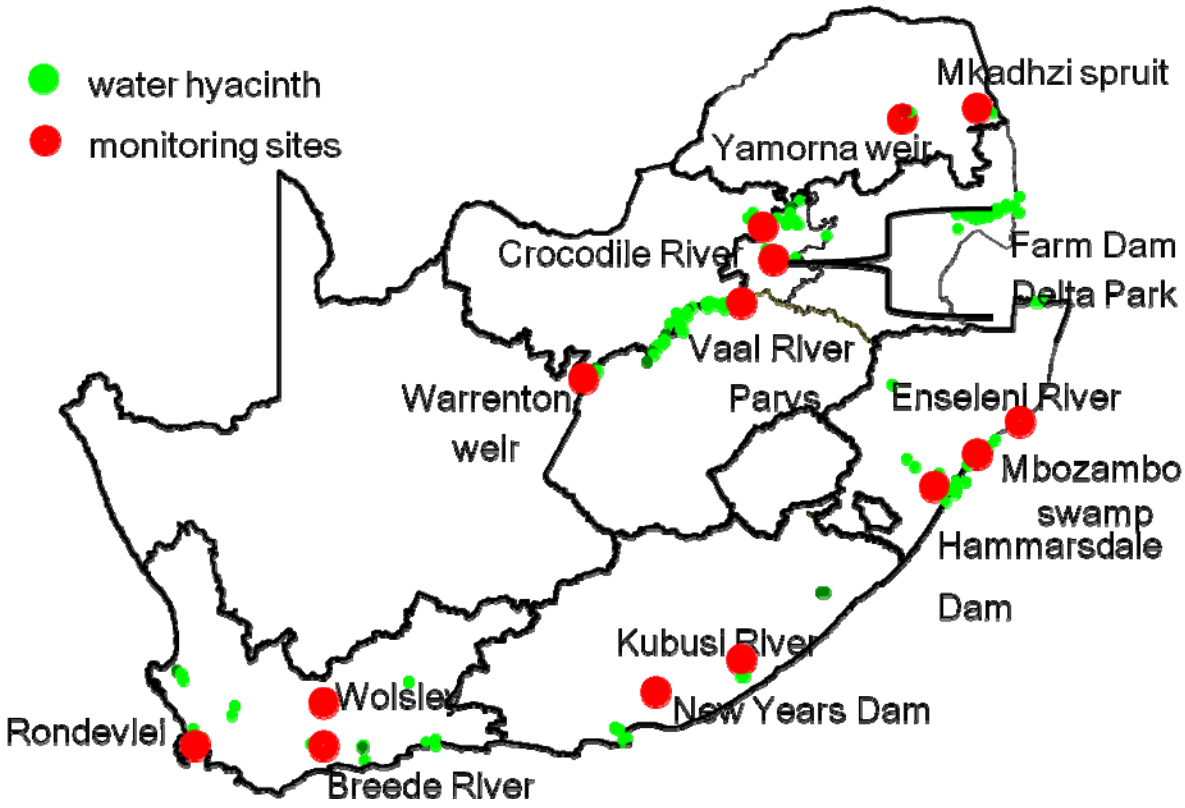

Figure 1.1: Geographical distribution of water hyacinth sites in South Africa (green) from SAPIA (Henderson, 1998). Water hyacinth sites (red) were monitored for a two year period in order to obtain data for assessing seasonal phenology of water hyacinth and the *Neochetina* weevils. Map modified from Byrne *et al*., 2010.

1.2 Biology of water hyacinth

Water hyacinth is a free floating, stoloniferous perennial herb which grows well in high nutrients (Reddy *et al.*, 1989) and shows marked intolerance to salinity (Haller *et al.*, 1974). Optimal growth conditions include a water temperature of 30°C (Knipling *et al.*, 1970) and a pH range of 4 to 10 (Haller and Sutton, 1973). In addition, the plants survive frost provided the rhizome does not freeze. The buoyant leaves show phenotypic plasticity with uncrowded growing conditions leading to the production of short, bulbous leaf petioles, while crowded growing conditions lead to the production of elongated petioles (Center and Spencer, 1981). Flowers are

produced in clusters of up to 23 on a single spike and the seed capsules contain about 50 seeds each (Barrett, 1980). The seeds are small and remain viable for 15 -20 years (Matthews, 1967; Gopal, 1987). Reproduction is mainly by vegetative means wherein stolons are produced by the mother plant (Gopal, 1987). The ability of water hyacinth to persist, dominate and out compete other weeds and coupled with its dispersal ability allows for unprecedented economic and ecological damage.

1.3 Problems associated with water hyacinth

Rapid and unrestricted growth of water hyacinth causes expansive colonies of interwoven plants which obstruct navigation (Gownloch and Bajkov, 1948; Zeiger, 1962), block drainage and irrigation pumps, and reduce water quality due to bad odour and colour. In addition, water supply and flood control projects can be adversely impacted. An increase in the populations of vectors of human and animal diseases is also associated with water hyacinth infestations, and there may be an increase in water loss through evapotranspiration (Harley *et al*., 1996). Aquatic species richness and biodiversity is impacted owing to reduced light penetration. Dense mats also deplete dissolved oxygen resulting in reduced phytoplankton communities, thereby altering the composition of invertebrate communities (Toft *et al*., 2003; Jones, 2009; Midgley *et al*., 2006). In addition, water hyacinth infestations reduce access to potable water which impacts communities in South Africa that rely on water resources for their livelihood (Jones, 2001; Jones, 2009).

The sheer biomass of water hyacinth infestations, however, may provide possibilities for its utilization. Attempts have been made to utilize the weed as a fertilizer and fodder (Gopal, 1987) albeit on a small scale. Water hyacinth has the ability to accumulate heavy metals such as cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in the root tissues of the plant (Muramoto and Oki, 1983) and it has also been utilised for removal and recovery of silver from industrial wastewater. Although these

attributes make water hyacinth an attractive tool for decontamination or bioremediation of affected waterways (Muramoto and Oki, 1983), there is a risk associated with potential spread of the weed infestation to other areas.

1.4 Water hyacinth management strategies

Mechanical harvesters to control water hyacinth plants have been used in some areas such as Port Bell and Owen Falls Dam on the Ugandan side of Lake Victoria and at Benoni Lake in South Africa. However, operational costs are high (Hill, 1999) (for example, the mechanical harvester in use at Benoni Lake costs R 8 million). Moreover, mechanical removal of water hyacinth interferes with the successful establishment of the biocontrol agents (Center *et al*., 1999a). Non-target organisms in the environment are also damaged as a result of mechanical control (Cilliers, 1991). In addition, manual removal is extremely labour intensive and ineffective in larger infestations (Mallya, 1999). Furthermore, neither the use of mechanical harvesters or manual removal can solve the problems of re-growth from seed and re-infestation from hyacinth populations' upstream.

Herbicidal control offers a quick solution to a pressing problem and currently water hyacinth infestations in South Africa are sprayed (either aerially or by using knapsack sprayers) at label-recommended doses (2% to 4%) with registered glyphosate formulations such as Mamba (Dow Agro Sciences, South Africa), Tumbleweed (Enviro Weed Control, South Africa) or Roundup (Monsanto Pty. Ltd. South Africa), or a glyphosate trimesium formulation, Touchdown Plus (Zeneca, South Africa). In addition, terbutryn and diquat (Trade name, Midstream) (Zeneca, South Africa) are also registered for use in South Africa. However, it is interesting to note that 2-4,D and glyphosate formulations such as AquaMaster, AquaPro and Rodeo (routinely used on aquatic systems in USA) are not registered for weed control in South Africa (See: A guide to the use of herbicides of bush encroachment, noxious plants and

aquatic weeds. The Registrar, Act number 36 of 1947. Food safety and Directorate. Quality assurance, sub directorate Production, Agricultural input, 2007). One of the earliest documented reports of herbicidal usage in South Africa is from the late 1970's, where a severe water hyacinth infestation on the Hartebeespoort Dam was brought under control using the terbutryn herbicide Clarosan 500FW (Ashton *et al*., 1979). Despite the apparent success of herbicides, its usage is limited as it has to be re-applied making it one of the most expensive control methods. For example, chemical control at water hyacinth infested sites in South Africa, Nseleni and Mposa rivers, amounted to South African Rand (ZAR) 737/ hectare (ha) annually (Jones, 2009). Moreover, most of the glyphosate formulations contain a toxic surfactant, polyethoxylated tallowamine (POEA) which has been implicated in amphibian deaths (Relyea, 2005 a,b,c). These considerations have led to research and implementation of a third management strategy namely, biological control.

1.5 Biological control

Classical biological control is defined as a method wherein exotic natural enemies, obtained from the country of origin of the weed, are deliberately introduced into new environments in an attempt to limit the density of any invasive species (DeBach, 1974). The potential risk to non-target species presented by such a deliberate introduction is usually low since insects are thoroughly tested for host specificity before release. For example, the two most commonly used species on water hyacinth, the weevils, *Neochetina eichhorniae* (Warner) and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) were tested against 274 plant species in 77 families worldwide (Julien *et al*., 1999; Julien, 2000). Biological control offers a low cost, long-term sustainable control option with no negative environmental impacts (Julien *et al*., 1996). Research into the biological control of water hyacinth was first initiated by the United States Department of Agriculture (USDA) in 1961, and the first control agents were released in the USA ten years later (Harley and Forno, 1990). The impact

of biocontrol on water hyacinth infestations have been measured at numerous locations (Bashir and Bennett, 1984; Cofrancesco *et al*., 1984; Goyer and Stark, 1984; van Theilen *et al*., 1994) and the time taken for effective control using the two weevil species is a long-term process, ranging from three to over ten years (Julien *et al*., 1996).

1.5.1 Biological control agents

A suite of biocontrol agents have been researched and released against water hyacinth worldwide. These include the two weevils, *Neochetina eichhorniae*, *Neochetina bruchi*, a moth- *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae), a mirid, *Eccritotarsus catarinensis* Carvalho (Heteroptera: Miridae), the phytophagous mite, *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), and the fungus, *Cercospora piaropi* Tharp. (Hyphomycetes) (Julien *et al*., 1999; Julien *et al*., 2001). Insect herbivory facilitates infection by fungal pathogens, hence various fungi have also been assessed as control agents (Martyn and Freeman, 1978; Charudattan, 1990; Shabana *et al*., 1995) and it has been suggested that fungal agents may complement the existing insect biological control agents (Charudattan, 1990). In addition, work is progressing on several new insect agents such as the leaf feeder, *Cornops aquaticum* (Orthoptera: Acrididae) (Bownes, 2010). However, to date, the success of biological control around the world is attributed mainly to the *Neochetina* species (Julien, 2000). The life cycles of the agents released against water hyacinth are briefly described below.

The *Neochetina* species are nocturnal and feed on water hyacinth leaves forming characteristic feeding scars. Adults lay eggs in the leaf blades and petioles, and upon hatching, the larvae tunnel down the petiole into the crown of the plant, causing major damage to the plants. As a result, the plants in the mat lose their buoyancy, and if heavily damaged, eventually cause the mat to sink. The larval stages pupate underwater in the roots (Center, 1984). *Neochetina bruchi* is more dependent on

better quality plant material, such as those grown in high nutrient conditions, and offers better control than *N. eichhorniae* (Heard and Winterton, 2000). Different preferred oviposition and feeding sites are recorded for the two species. Inter- specific competition is not seen and generally, the two species complement each other, and the control of water hyacinth is enhanced (Julien *et al*., 1999).

The adult mirids (*E. caterinensis*) are two to three mm long and are highly mobile. Females insert their eggs into the leaf tissue and four nymphal instars are recorded. They undergo nymph-to-adult development for a period of about 23 days at optimal temperatures (25°C). The adult's life span is 50 days and both the adults and the nymphs feed gregariously on the leaf tissue, sucking chlorophyll, resulting in chlorosis of the leaf lamina. Damaged leaves turn yellow to brownish in colour, thus interfering with the photosynthetic capacity of the leaves (Hill *et al*., 1999; Coetzee *et al*., 2009).

The adult moths (*N. albiguttalis*) are yellowish coloured with brown markings with the body lengths ranging from 9.3- 10.1 millimeter. The females usually insert their eggs in the spongy aerenchyma cells of the water hyacinth leaves. The eggs are creamy white, ovoid, and are devoid of obvious markings. The newly hatched larva tunnel through the leaf petiole and appear to feed singly, although clusters of larvae maybe found in one petiole. A total of five instars are found and each instar stage requires about three to four days for development. The larvae feed predominantly on the soft petioles and they usually do not enter the plant crown. A correlation between leaf hardness and larval entry was established by Wright and Bourne (1986). They concluded that leaf hardness and additional factors such as cuticle thickness or content of phenolics in the epidermal cells determined successful entry of the larvae for feeding purposes. The larvae do not function as vectors for plant pathogens, but the extensive feeding damage does facilitate invasion by saprophytic and facultative microorganisms (Deloach and Cordo, 1978).

Adult mites (*Orthogalumna terebrantis*) are shiny, dark brown and heavily sclerotised and are usually found on the lamina and the upper parts of the leaves, and more often, are found clustered in the feeding scars made by the *Neochetina* species (Cordo and Deloach, 1976). The ovipositing females cut a hole in the aerenchyma region of the leaf where the eggs are laid. The eggs are yellowish and shiny and the duration for the larval hatching ranges from seven to eight days. The three nymphal stages are proto-, deuto-, and trito nymphs and are not distinguished easily. Continued feeding produces characteristic galleries on the leaf surface and these galleries usually reach an average length of 3.5mm (Cordo and Deloach, 1976).

1.5.2 Biological control of water hyacinth in Africa and South Africa

Biological control initiatives in South Africa first commenced with the release of *N. eichhorniae* in 1973 (re-released in 1985) (Cilliers, 1991). Biocontrol agents established in South Africa include *N. bruchi*, first released in 1990, *Orthogalumna terebrantis, Niphograpta albiguttalis, Eccritotarsus catarinensis* and a fungus, *Cercospora piaropi*.

The use of biocontrol agents to control water hyacinth has yielded successful outcomes in some parts of Africa, while in South Africa, success has been variable (Hill, 2003). In Africa, successful water hyacinth biocontrol initiatives have been reported on Lake Victoria (Albright *et al*., 2004) and Lake Kariba and the Shire River in Malawi and in Benin, Niger, Ghana and Cote d'Ivoire (Cilliers *et al*., 2003). Successful establishment of the *Neochetina* species in Africa is mainly attributed to favourable mean temperatures ranging from 23°C to 32°C that maintain high developmental rates, allowing the weevils' to reach damaging population densities (up to 32 adult weevils per plant) (Cilliers *et al*., 2003). In addition, the oligotrophic or mesotrophic nutrient status of waterways with phosphorous levels below 0.1mg/L appears to limit the growth of the weed, as a result of which the biocontrol agents are able to suppress hyacinth growth (Cilliers *et al*., 2003).

Despite researching and implementing biocontrol in South Africa for more than three decades, success rates remain unsatisfactory, and are confined to infestation sites such as New Year's Dam in the Eastern Cape Province and at Clairwood Quarry in Kwa-Zulu Natal Province (Hill, 2003). At other sites in Kwa-Zulu Natal Province, such as Hammarsdale Dam, biological control has suppressed water hyacinth to such an extent that it has been overrun by emergent aquatic plant species such as *Ludwigia* spp. (personal observation). Hill and Cilliers (1999) and Hill and Olckers (2001) indentified several constraining factors responsible for inadequate biocontrol of water hyacinth in South Africa. Climate has a significant influence on the efficacy of the biocontrol agents (Clarke, 1996), as evidenced by successful biocontrol initiatives which are limited to tropical and sub tropical areas of the world. In South Africa, water hyacinth grows in a wide range of climatic conditions, typified by (a) temperate summer rainfall areas at high altitudes where frosting occurs during winter (May to August) (b) coastal Mediterranean winter rainfall regions devoid of frost and (c) coastal sub tropical summer rainfall areas (Byrne *et al*., 2010). Biocontrol agents, native to tropical and subtropical regions of South America, are unable to establish or flourish at infestation sites where the plant canopy temperatures are very low (Byrne *et al*., 2010). For example, the water hyacinth mirid, *Eccritotarus catarinensis* is cold sensitive, with a critical thermal minimum (CT_{min}) of 1.2°C and lethal temperature (LT_{50}) of -3.5°C and has repeatedly failed to establish at one high altitude, regularly frosted, cold site (Coetzee *et al*., 2007b). The *Neochetina* weevils, however, are able to establish at high altitude sites due to favourable CT_{min} values ranging from 3.3−4.3°C and a LT_{50} of -7.4°C (Byrne *et al.*, 2004), but decline to an overwintering larval population that fails to catch up with the weed as it rebounds from the winter frost, in spring (Byrne, *et al*., 2010). This seasonal time lag severely undermines the success of biological control (Hill and Olckers, 2001) (See Chapter 3). Additionally, cold, and frosty winters cause browning and death of emergent parts of the plant which removes habitat for the adult and immature stages of the biocontrol agents, thus decimating their population numbers.

The cold temperatures not withstanding, the hydrology of infested water bodies interferes with the success of biocontrol (Hill and Olckers, 2001). Often, the water bodies are small and shallow and in the absence of stress due to wave action, the plants grow unhindered. In deeper, larger water bodies, however, wind and wave action cause the water hyacinth mats to fragment resulting in the sinking of insect damaged plants (Hill and Olckers, 2001).

The inadvertent removal of both the weed and its control agents by periodic flooding results in variable results in biocontrol of hyacinth (Hill and Cilliers, 1999). In the absence of agents, water hyacinth resurges to pre-biocontrol levels (Hill and Olckers, 2001).

Eutrophic waterways in South Africa are enriched with nitrates and phosphorous levels are above 0.1mg/L (Thornton and Walmsley, 1982) due to run-off from industrial and sewage effluents, as a result of which the weed proliferates rapidly. Water hyacinth growth is directly correlated with water nutrient concentrations, particularly nitrogen and phosphorous (Heard and Winterton, 2000). High concentrations of phosphorous result in increased biomass accumulation, ramet production, shoot: root ratio and plant height (Reddy *et al*., 1989 and 1990), and while this increase in growth was not proportional to increase in P, deficiency in P was found to be a limiting factor for growth and reproduction of water hyacinth. Reddy *et al*., (1990) showed that the biomass yield of water hyacinth to be highest with an increase of P up to 1.06 mg/L. At low concentrations of 0.06mg P/L, plant biomass decreased by 50%. Haller and Sutton (1973) found that if concentrations of P dropped below 0.1 mg/L, active growth of water hyacinth stopped but concentrations above this allowed for growth as well as uptake of nutrients in excess of the plant requirements. These values thus represent the upper and lower limits within which growth of water hyacinth can be predicted. Below 0.06 mg P/L, the plants would be expected to die; between 0.06 mg/L and 0.1 mg P/L, the plants would survive but not grow. Between the range of 0.1 mg P/L and 1.06 mg/L, water hyacinth will actively

grow, but above 1.06 mg/L, additional growth is not expected to occur (Reddy *et al*., 1990).

Maximum growth of water hyacinth is achieved at 21 mg/L of nitrogen (Reddy *et al*., 1989). Chadwick and Obeid (1966) showed that an increase in N concentration from 1 to 25mg/L increased the number of plants and total dry weight of the plants. Conversely, Reddy *et al*., (1989), showed that in N limited water bodies, water hyacinth plant tissue biomass decreased by 75% within 4 weeks of growth.

The trophic status of waterways in South Africa as categorized by Resource Quality Services- Department of Water Affairs and Forestry (DWAF) is presented in Table 1.1.

Table 1.1: Criteria used for categorizing the trophic status of water bodies in South Africa as per South African Water Quality Guidelines, (DWAF 1996).

Status of water-body	**Nitrogen Concentration mg/L**	**Phosphorus Concentration mg/L**
Oligotrophic	<0.5	<0.005
Mesotrophic	0.5 – 2.5	0.005 – 0.025
Eutrophic	2.5 – 10	0.025 – 0.250
Hypertrophic	>10	> 0.250

Most of the hyacinth infested water bodies in South Africa are eutrophic (Byrne *et al*., 2010) and water hyacinth plants found in these waterways are often tall and vigorously growing, forming dense infestations. As a result, the biocontrol agents such as the mirid, are unable to combat the massive growth (Coetzee *et al*., 2007a), thereby necessitating the need for intervention with herbicides.

These constraints described above have therefore led to extensive research into development of integrated weed management strategies (See Chapter 2) wherein herbicides and biological control methods are integrated as a management tool.

1.6 General aspects of herbicide formulations and application

Some general aspects of herbicides such as information on formulations and adjuvants and processes involved with spraying are considered below.

Herbicide formulations contain many other compounds called adjuvants besides the active ingredients (a.i.). Adjuvants are defined as ‘‘an ingredient in the pesticide prescription, which aids or modifies the action of the principal active ingredient’’ (Foy, 1989). In a general sense, adjuvants are added to enhance the effectiveness (bioavailability) of the pesticide formulation by enhancing the solubility, or the compatibility of the active ingredients. Other functions can be to enhance adsorption, penetration and translocation of the active ingredients into the target, increase rain fastness, and alter selectivity of the active ingredient toward different plants (Foy, 1989).

Adjuvants can be divided into two general types: (1) formulation adjuvants and (2) spray adjuvants. The first type consists of adjuvants, which are part of the formulation, while the second type of adjuvant is added along with the formulated product to the water in the tank of the spray equipment before application. Spray adjuvants are sometimes called tank mixing additives or just adjuvants, whereas the formulation adjuvants are called additives or inerts (Foy, 1989).

1.6.1 Surfactants

The most common types of activator adjuvants employed are surfactants (Chapter 4). The primary purpose of a surfactant or surface active agent is to reduce the surface tension of the spray solution to allow more intimate contact between the spray droplet and the plant surface. Any substance that brings a pesticide into closer contact with the leaf surface has the potential to aid absorption.

Surfactant molecules may also alter the permeability of the cuticle. Surfactants form a bridge between unlike chemicals such as oil and water or water and the wax on a leaf surface. Although there are many different types of surfactants, in general, they are constructed of a long chain hydrocarbon group on one end that is considered lipophilic (fat loving) and a more hydrophilic (water loving) group of atoms on the other end.

The influence of the surfactant on herbicide performance can be species specific because leaf wax composition varies. For some herbicides, surfactant preference is also herbicide dependent. For example, Roundup® (glyphosate) is a more water-soluble herbicide that requires a more polar type of surfactant (such as the ethoxylated fatty amines; POEA) to improve activity.

Surfactants are classified as nonionic, anionic, or cationic. Nonionic surfactants have no electrical charge and are generally compatible with most pesticides. An anionic surfactant possesses a negatively charged functional group and is most often used with acids or salts. Anionic surfactants are more specialized and sometimes used as dispersants or compatibility agents. Cationic surfactants are used less frequently, but one group (ethoxylated fatty amines) has been frequently used with the herbicide Roundup® (White, 1993; Curran *et al*., 1999).

1.6.2 Application of herbicides

Spray application is a composite process involving a series of transfer stages (i.e. droplet formation at the nozzle, travel to the plant surface, droplet impaction and retention on the leaf surface, deposit formation, uptake of the a.i. into the leaf tissue and the biological response) (Brazee *et al*., 1991). Weed control with foliage-applied herbicides requires spray droplet impaction on the weed (Hislop, 1987). Additionally, a crop canopy may have to be penetrated. Hence, efficiencies of canopy penetration and droplet impaction are related to herbicide performance. Low efficiency may result from spray droplets that (1) do not reach the crop canopy, owing to spray drift, (2) are filtered out by the crop canopy or (3) penetrate the crop canopy, but fail to impact on the weed. Spray droplets impacting on the weed may be retained or may bounce. For foliage-applied herbicides, the efficiency of spray retention determines the quantity of a.i. potentially available for uptake into the leaf (Kudsk, 1988). Efficiency of spray retention depends on the wetting characteristics of the plant, and spray application and solution factors. Generally, smooth leaf surfaces devoid of crystalline epicuticular wax and hydrophobic trichomes are easy-to-wet (Holloway, 1993). On smooth, easy-to-wet leaf surfaces droplet retention is high and is little affected by application and solution characteristics (Holloway, 1993). Difficult-to-wet leaf surfaces are covered with crystalline epicuticular wax or hydrophobic trichomes (Holloway, 1993). On difficult-to-wet surfaces, retention is related to droplet size and speed, the surface tension of the spray droplet solution at the moment of impaction (dynamic surface tension), and the impaction angle of spray droplets with the leaf surface. Generally, it is assumed that herbicide performance is positively related to the amount of a.i. taken up by the target plant. Consequently, for a given amount of herbicide in the leaf, performance would be at maximum if the herbicide was distributed uniformly within the leaf, which is particularly crucial for contact herbicides. On the other hand, systemic herbicides such as glyphosate may be redistributed within the leaf following foliar uptake and thus are expected to be less dependent on even coverage (Knoche, 1994).

1.6.3 Environmental factors affecting foliar-applied herbicide uptake

It is well known that the activity of foliar-herbicide sprays is influenced by the environmental conditions at the time of spraying. Among the many environmental factors that can affect herbicide uptake, two of the most important are temperature and humidity, with optimal uptake being favoured by warm, humid conditions (Muzik, 1976).

Temperature can affect herbicide uptake by changing the viscosity of cuticle waxes, the rate of diffusion, and in conjunction with humidity, cuticle hydration (Price, 1983). Increased diffusion of solutes into cuticles with increasing temperature has been explained as a result of lower partition coefficients as temperature increases (Schonherr *et al*., 1999). While higher temperatures increase diffusion of herbicides across the cuticle, increased efficacy is not always observed (Devine *et al*., 1983). This may be due to reduced herbicide availability caused by rapid drying of droplets to solid deposits in warm conditions (Price, 1983). It was suggested by Price (1983) that effective herbicide uptake at high temperature requires high humidity to prevent rapid droplet drying. While this was indeed the case for acifluorfen efficacy in common ragweed (*Ambrosia artemisiifolia*) (Ritter and Coble, 1981), the absorption of glyphosate into quackgrass (*Elytrigia repens*) has been shown to be unaffected by temperature within a normal range (Devine *et al*., 1983).

Several researchers who have investigated the impact of both temperature and humidity found that humidity has a greater influence on herbicide efficacy than temperature (Coetzer *et al*., 2001). After evaluating glufosinate ammonium efficacy on barley (*Hordeum vulgare* L.) and green foxtail (*Setaria viridis* L.) at various combinations of high and low humidity (0.1 and 1.35 kPa) and daytime temperatures of 8, 15, and 22°C, Anderson *et al*., (1993) reported that humidity had a greater impact on efficacy than did temperature. Similarly, Skuterud *et al*., (1998) found that

when spraying a mixture of ioxynil and dichlorprop, humidity was more important than wind or temperature for predicting herbicide efficacy.

In contrast to the direct physical effects that temperature has on cuticular components, the effects of humidity on herbicide uptake are usually related to its impact on cuticle hydration and the rate of herbicide- droplet drying (Price, 1983; Muzik, 1976). The effect of these two variables can be separated by their temporal relationship with spray events. If increased cuticle hydration is the mechanism that improves efficacy at high humidity then the humidity must be high for some period before spraying to hydrate the cuticle. In contrast, if high humidity improves efficacy through delayed droplet drying then the humidity after spraying should be more important than humidity before spraying. Although the timing of high humidity exposure, either before or after spraying, has received little attention, several investigators have suggested that uptake and efficacy are affected more by high humidity after spraying than before (Ramsey *et al*., 2005). This suggestion supports the theory that delayed droplet drying is more likely the mechanism responsible for high efficacy at high humidity (Ramsey *et al*., 2005).

1.7 Integrated weed management

Center *et al*., (1999a) and Haag (1986 a,b) have shown that herbicides can be integrated with biocontrol agents in water hyacinth control programmes, with the judicious application of herbicides, where an island of water hyacinth mat is left unsprayed to facilitate the migration of the adult *Neochetina* weevils from the herbicide sprayed plants to unsprayed plants. In South Africa, this type of an integrated water hyacinth control programme was initiated in 1995 by incorporating existing chemical and biological control options. The Nseleni River (Kwa-Zulu Natal) was divided into eight management units (MU) which were sprayed with a lethal dose of registered glyphosate herbicide, whilst selected water hyacinth

"islands" were left unsprayed to support biocontrol agent populations. This integrated approach was instrumental in clearing the weed from 22km of the river, and the cleared units now only require occasional follow-up herbicidal sprays, two or three times a year to control any re-growth (Jones and Cilliers, 1999; Jones, 2009). A high annual mean plant canopy temperature of 23°C (Byrne *et al*., 2010) and the mesotrophic status of the water may have also contributed to its success at this site.

Integration of biocontrol with lethal doses of herbicide could however interfere with the long-term successful establishment of the biocontrol agents. Firstly, herbicides such as Midstream® at label recommended lethal doses, cause insect mortality or reduced feeding intensities of the water hyacinth biological control agents (Ueckermann and Hill, 2001). At recommended doses, 100% mortality of water hyacinth mirids (after 48 hours), and a significant percent (35%) mortality of the weevils (after 120 hours) were observed. On the other hand, the use of Roundup® herbicide resulted in significantly lower mortality of the mirid and did not cause any mortality of adult weevils (Ueckermann and Hill, 2001). Hence, Roundup® herbicide was used in Chapters 2, 3 and 4 of this study.

Secondly, lethal doses of herbicides also destroy large mats of water hyacinth and remove habitat and food source of the biocontrol agents (Haag, 1986 a,b; Hill, 2003). Unsprayed, healthy plants that were missed during a herbicide application, or seeds germinating due to increased light availability, can quickly grow to re-infest the open water created by the loss of the water hyacinth mat (Center *et al*., 1999a). The biological control agents, however, take much longer to recover and any biological control achieved previously at the site is compromised (Haag, 1986 a,b; Hill and Cilliers, 1999). Most of the water hyacinth biological control agents, for example, *Neochetina* weevils, have largely sessile lifecycle stages, such as the larvae. The larvae are found in the petioles of the plant and the pupae are enclosed in a cocoon which is attached to a root below the surface of the water. These immobile or slow moving stages sink with the dead plants resulting in reduced numbers (Haag, 1986 a,b).

These challenges can be circumvented by coupling biocontrol with sub-lethal or retardant dosages of glyphosate (Ueckermann and Hill, 2001; Wright and Bourne, 1990; Wilson *et al*., 2006) (See Chapter 2). If the weed growth (e.g. production of daughter plants and leaves) can be constrained by the application of a retardant dose of glyphosate (e.g. Roundup®), an environmentally friendly, cost effective and low-management control method is envisaged, whereby the biocontrol agent populations (e.g. *Neochetina* species) survive herbicidal sprays (Chapter 2) and persist to hold back the weed to acceptable levels.

In order to address aforementioned constraints and challenges, a project (Number: K5/1487) funded by the Water Research Commission (WRC) of South Africa was initiated in 2004 with a team of researchers and students from University of Witwatersrand, Rhodes University and University of Pretoria. The main aim of the project was to ascertain factors affecting the successful control of water hyacinth. In particular, effects of low or retardant doses herbicide (laboratory trials), temperature and nutrients (field measures) on water hyacinth were researched. Monthly data pertaining to these effects (temperature and nutrients) were collected, over a two year period, from 14 water hyacinth sites around the country (Figure 1.1), which were chosen to represent the climatic and the nutrient conditions prevalent in South Africa. In addition, data on plant and insect phenologies were collected. Part of the phenology data (of both plants and insects) from 12 field sites including site descriptions are presented in this thesis (See Chapter 3). Information on phenology of the weed and the weevils will help indicate under which circumstances, herbicide intervention, using retardant doses, will be required.

Continued use of herbicides, however, raises concerns about its non-target effects on flora and fauna. The eco-toxic impacts of glyphosate are considered in Chapter 4.

This thesis is one of the outcomes of the WRC project, and is the first report to identify a retardant dose of a glyphosate based herbicide and to test its effect on water hyacinth and its biocontrol agents (Chapter 2).

1.8 Thesis outline

The primary aim of this thesis is to identify a retardant or sub-lethal dose of a glyphosate herbicide and test its effects on water hyacinth populations and two of its most important biocontrol agents, *Neochetina eichhorniae* and *N. bruchi*. To this end,

Chapter 2 reports on a retardant dose of glyphosate and tests its effects on (a) reproduction of water hyacinth plants (b) plant quality as indicated by nitrogen and phosphorous levels in plant tissue, and (c) survival and feeding capacity of the two *Neochetina* species.

Seasonal fluctuations resulting from cold, frosted winters are implicated in undermining the success of biocontrol in South Africa, and because the success of integrated control depends on the optimal seasonal timing of herbicidal sprays (Ainsworth, 2003), Chapter 3 aims to (a) determine the phenology of water hyacinth and two of its most important biocontrol agents, the *Neochetina* weevils, and (b) identify a seasonal spray regime that is conducive to the persistence of the biocontrol agents and the control of the plants.

In view of the apparent global decline of many amphibian species (Houlahan *et al*., 2000), the eco toxic impacts of glyphosate is of particular concern. Chapter 4, therefore, investigates the detrimental effects (if any) of the sub lethal dose of glyphosate on *Xenopus laevis* tadpoles.

Chapter 5 consolidates the findings in a general discussion and conclusion, and the implications of advocating a retardant dose of glyphosate for management of water hyacinth infestations are discussed.

The figures and tables are numbered in sequence for each chapter and not for the complete thesis. One reference section is given at the end of the thesis, including references used in the published chapters (1 and 2).

1.9 Publications arising from this study

A part of Chapter 1 is published in the journal, Outlooks on Pest Management.
A part of Chapter 2 is published in the journal, Biological Control.

Chapter 2[†]

Identification of a retardant dose of glyphosate and its effect on *Neochetina* weevils and plant quality

2.1 Introduction

Many success stories across the world in terms of weed control have been attributed to classical biocontrol (Julien and Griffiths, 1998), even if this success occurred 10-20 years after release of the agents (McFadyen, 2000). With biological control, unless the results are as remarkable as the one noted for *Azolla filiculoides* (McConnachie *et al*., 2004), any less notable effects such as, for example, a significant reduction in biomass of water hyacinth or even a remaining 20% cover of any water body is not deemed "successful". Water managers, facing an environmental catastrophe perpetuated by water hyacinth infestations and a judgmental public, often cannot and will not wait for such a long period of time and resort to "quick" means of fixing the problem by spraying recommended or lethal doses of herbicides. Efforts to manage water hyacinth infestations by using lethal doses of herbicides often result in weed mats sprayed in their entirety. The herbicide causes death of the sprayed plants resulting in decline in the size of the treated weed mats. Consequently there is a severe and abrupt loss of habitat and food source for all life stages of the weevil populations (Haag and Habeck, 1991). Moreover, lethal doses of herbicides such as 2,4-D, Midstream (diquat) and paraquat causes death and interfere with feeding capacity of the bioagents of water hyacinth such as the mirid (Ueckermann and Hill, 2001). However, the weevil species were found to be tolerant to the glyphosate based herbicide exposure (Ueckermann and Hill, 2001).

([†] A part of Chapter 2 is published in the journal, Biological Control: Jadhav *et al*., 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth *Eichhornia crassipes* (Mart.) Solms-Laubach. Biological Control, 47, 154-158).

Post herbicide application, weevil populations have a much slower rate of increase than water hyacinth populations, and as a result, re-growth of weed mat after spraying will be favoured until the insect population can reach effective levels and suppress weed growth (Grodowitz and Pellessier, 1989). Therefore a cycle of repetitive herbicide application using lethal doses at infested sites may thus preclude effective biological control by the weevils (Center and Durden, 1986; Center, *et al*., 1982). Considering that several published papers emphasize the development of strategies for integrated control of water hyacinth (Center *et al*., 1982; Wright and Center, 1984; Ueckermann and Hill, 2001; Charudattan, 1986; Charudattan *et al*., 1978; Haag and Habeck, 1991), the integration of biological and herbicidal controls could offer a more sustainable, long-term benefit (Center *et al*., 1999b), more so, if the use of reduced or sub-lethal or retardant dosages of glyphosate is researched as recommended by Ueckermann and Hill, 2001; Wilson *et al*., 2006; Wright and Bourne, 1990. Therefore, the first objective of this chapter is to identify a retardant dose of glyphosate which will not kill the water hyacinth mat but will retard the vegetative growth, in terms of ramet (daughter plant) and leaf production, so that the bioagent population can persist. In this study, the most commonly used glyphosate herbicide, Roundup® was used to identify a retardant dose and test its effects on the plants.

2.1.1 Effects of herbicides on plants

The most prominent impact of herbicides usually sprayed at recommended or lethal doses, ranging from 2% to 4%, is undoubtedly through adverse lethal effects on plants via changing plant species composition and diversity, and sublethal or hormetic impacts by way of modifying plant development, growth and morphology (Boutin, 1999). Hormesis is defined as the stimulatory effect of herbicides on plant and insect growth parameters.

Herbicides are known to interfere with the functioning of the photosynthetic pigments in higher plants, which form the material base for photosynthesis. The content of chlorophyll can indicate the growth status and the photosynthetic ability of the plant (Liu *et al*., 2006). The positive correlation between the decrease of Chl-*a* content and the exposure concentration of the herbicides implies that herbicides might have harmful effects to the growth of aquatic macrophytes and terrestrial weeds. The loss in chlorophyll content could be due to peroxidation of chloroplast membranes mediated by herbicides via increased production of free radicals (Mishra *et al*., 2006; Sharma and Dubey, 2005). For example, photosynthetic processes in duckweed were strongly inhibited by flumioxazin. Duckweed exposed to concentrations ranging from 0.5, 1, 3, 5, 10, 20, and 50 and to 100 mg a.i /L of flumioxazin showed variation in chlorophyll fluorescence kinetics (Geoffroy *et al*., 2004).

Herbicides such as glyphosate inhibit the production of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase and thus block the shikimic acid pathway which is involved in the formation of aromatic amino acids, which are required for plant growth.

The use of low doses of (as low as 0.0001mg a.i /L) herbicides have been shown to have stimulatory effect on plant and insect growth parameters (Cedergreen, 2008a). This phenomenon is termed as “hormesis” and such low dose stimulations have been noted for a wide variety of chemicals and across all types of organisms from animals and plants to bacteria and fungi (Calabrese, 2005). The hormesis hypothesis states that most, if not all, chemical and physical agents, such as radiation, have the capacity to stimulate biological effects such as growth parameters, at doses below the toxicity threshold, while causing toxicity at doses above the threshold. When the presence or application of an external stressor agent (e.g., pollutant exposure, herbicides) challenges the adaptive capacity of any biological system, the system typically compensates for the initial disruption and/or damage, by initiating a stimulatory (i.e. hormetic) response. For example, low concentrations of 60 μg/L (or 0.06mg a.i /L;

0.6g/ha) of 2,4-D were found to change leaf morphology of *Myriophyllum spicatum* (Christopher and Bird, 1992) while an increase in flower production in *Myriophyllum sibiricum* and in tuber production in *Potamogeton pectinatus* were attributed to the effect of low doses of 2,4-D (10 µg/ L; 0.01mg a.i /L; 0.1g/ha) (Forsyth *et al*., 1997). Low doses of glyphosate (ranging from 1.8 to 36g/ha) resulted in growth stimulation of *Eucalyptus grandis*, in terms of shoot and total dry weights (Velini *et al*., 2008).

A few studies have attempted to investigate the mechanisms involved in plant growth stimulation by low doses of herbicides, but these were largely inconclusive (Allender *et al*., 1997; Morre, 2000) possibly because experiments were not stringently monitored or that there were trade-offs between traits such as leaf area, plant height or shoot weight to minimize fitness reduction post herbicide application (Cedergreen *et al*., 2007; Cedergreen, 2008b). Plants are sessile organisms and therefore cannot physically escape from hostile conditions. However, they can allocate their resources in ways to optimise their growth under stressful conditions (Cedergreen *et al*., 2007). In the present study, the possibility of hormesis was anticipated, in addition to a retardant effect, as low doses (0.1%, 0.3%, 0.5%; 360mg a.i /L, 1080mg a.i /L and 1800mg a.i /L respectively, a.i: active ingredient) of glyphosate herbicide were applied to water hyacinth plants. It is also probable that the plants may compensate for herbicidal injury by either producing increased number of daughter plants (ramets) or leaves when compared to unsprayed plants.

2.1.2 Effects of herbicides on plant quality

Plant quality is one of the factors on which a biocontrol agent is dependent in order to survive and complete its life cycle. So how does herbicide mediated alteration of weed quality affect biocontrol agents?

Herbicides cause biochemical disruptions in plants which lead to consequences for plant processes such as photosynthesis, resource partitioning, growth and secondary

metabolism and these changes may be of a short or long duration relative to the development time of the biocontrol agents. Disruption of the normal physiology of plants, caused by herbicides with different modes of action, may be comparable with those caused by other stresses and may either have beneficial or adverse effects on herbivore feeding, due to altered plant quality.

The plant stress hypothesis proposes that changes in plants which are stressed, improve their quality as a resource for herbivores, because this stress causes an increase in the amount of nitrogen available in the tissues (White, 1984). The alternative plant vigour theory predicts that insects which have a link between their oviposition and feeding sites, and whose larval development is intricately linked with the plant growth processes, will exhibit ovipositional preference for enhanced performance on vigourous plants (Price, 1991). Later studies found that responses to stress were affected by plant species and the particular stress involved (Ainsworth, 2003). Koricheva *et al*., (1998) reviewed studies involving woody plants and reported that most of the variation in the extent and direction of insect responses were attributed to differences amongst insect feeding guilds. In general terms, boring and sucking insects performed better on stressed plants, since they are better adapted to exploit sensescing tissue, whereas plant stresses adversely affected gall makers and chewing insects, such as *Neochetina* weevils, who exemplify flush feeders. Flush feeders are prone to suffer if the stress reduces or terminates plant growth.

Post glyphosate herbicide application, levels of amino acids may increase and protein synthesis in the plant is disrupted. Similarly, the synthesis of important secondary metabolites, which are feeding deterrents, is disrupted. Consequently, a potentially beneficial effect on insects that feed on sprayed plants is expected. Glyphosate has also been found to reduce levels of indole acetic acid (IAA) leading to changes in plant tissues similar to senescence, which might prove to be beneficial to boring and sucking insects (Westwood and Biesboer, 1986). Glyphosate is known to interfere with nitrogen (N) metabolism in plants. Consequently, insect herbivores which depend on nitrogen as their major source of food nutrient are negatively impacted

(White, 1993) (for example, water hyacinth weevils). Depleted nitrogen resources post herbicide application may have consequences for insect survival and capacity to reproduce because insect herbivory on its own has been found to decrease nitrogen content in leaves (for example: water hyacinth; Heard and Winterton, 2000; Center and Van, 1989). Therefore, the second objective of this chapter is to determine if a sublethal dose of glyphosate influences the nutritive quality of water hyacinth plants. To this end, nitrogen and phosphorous (P) content in glyphosate treated plants were measured to determine its effect on the nutritive value of water hyacinth.

Plants high in nitrogen content have been found to improve survival and growth rate of immature insects (Myers and Post, 1981; Wheeler, 2003) as well as reproduction of adults (Awmack and Leather, 2002).

Phosphorous is an important micronutrient required for important metabolic processes such as photosynthesis, respiration protein synthesis and carbohydrate inter-conversions (Ripley *et al*., 2006) and its deficiency can have negative impacts on plant growth and vigour, as a consequence of which, the performance of the biocontrol agents on P deficient plants maybe impeded.

2.1.3 Effects of herbicides on insects

In the context of biocontrol, the inappropriate use of herbicides is often implicated in the failure of biological control (Moran and Zimmermann, 1991; Olckers *et al*., 1998; Ireson *et al*., 2000). Center *et al*., (1999a) concluded that the impact of biological control on water hyacinth is difficult to assess because of interference of chemical control. So in what way can an herbicidal spray regime affect the biocontrol agents?

Herbicides effect major changes (either beneficial or adverse), either directly or indirectly, on the physiology of the weeds and therefore, its biocontrol agents.

The toxic nature of herbicides is influenced by the chemical formulation of the herbicide and by the presence and type of the wetting agent, or other additives such as surfactants. For example, the toxicity of glyphosate formulations generally depends on the specific type of surfactants added and therefore, formulations may vary in their toxicity toward biocontrol agents and non-target organisms such as amphibians (Gisey *et al*., 2000).

Olckers *et al*., (1998) implicate the use of paraffin, commonly used as a carrier agent in herbicide formulations, in the death of cochineal insects on cactus weed thereby creating the impression that biocontrol was ineffective in managing *Opuntia* infestations (Zimmermann and Neser, 1999). Haag (1986a) concluded that an inverting oil was highly toxic to *Neochetina* weevils, whilst the herbicides being tested (2,4-D, diquat and glyphosate) were non-toxic, corroborating other studies that found 2,4-D to be non-toxic to biocontrol agents (Roorda *et al*., 1978; Trumble and Kok, 1980; Haag, 1986b; Wright and Skilling, 1987; Rees and Fay, 1989). However, a study by Ueckermann and Hill (2001) found that 2,4-D was highly toxic to the water hyacinth mirid, *E. caterinensis*. The use of picloram and triclopyr, either separately or in mixtures, were reported to be non-toxic to *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), a biocontrol agent of purple loosestrife (Lindgren *et al*., 1998). One of the most widely used herbicides, glyphosate, has been found to have low toxicity to biocontrol agents (Ding *et al*., 1998; Lindgren *et al*., 1999; Ueckermann and Hill, 2001).

Direct toxic effects of herbicides on biocontrol agents are mainly attributed to the penetration of the chemical thorough the insect cuticle, uptake via the respiratory system or by ingestion of herbicide sprayed material (Ainsworth, 2003). Consequently, many physiological and physicochemical processes such as protein synthesis are affected (Duke and Powles, 2008).

Insect larvae maybe generally more susceptible than adults to the toxic effects of herbicides, given their higher relative surface area and lower mobility, although the

boring nature of some larvae makes them less exposed to herbicide sprays. Considering that herbicides are often applied to weed infestations with the intention of killing the plants, it is likely that the indirect effects of herbicide, via host plant death, would be more important to the immature stages of biocontrol agents than the direct toxic effects (Ainsworth, 2003).

Consequences of host plant death for biocontrol agents depend on the rate of plant death, the proximity of healthy, unsprayed plants, the timing of the herbicide spray in relation to insect's life cycle and the ability of the relevant biocontrol life stages to disperse (Ainsworth, 2003). For example, Haag (1986a) showed that *Neochetina* weevils could move from dying herbicide treated plants onto untreated healthy plants.

An important aspect of herbicide effects on biocontrol agents concerns oviposition in the time interval between herbicide application and subsequent weed death. A short time interval is preferable because it reduces wasted oviposition (Ainsworth, 2003). However, when substantial oviposition has already occurred at the time of herbicide application, a slower weed death rate is desirable as it allows the biocontrol agents to complete their development and disperse (Ainsworth, 2003). Density of eggs on unsprayed weeds could be increased if there is avoidance of sprayed plants; unaffected if there is no preference, or decreased if sprayed plants are preferred (Ainsworth, 2003). Ainsworth (1999) and Ainsworth and Morris (2000) found that a biocontrol agent of *Marrubium vulgare* L (Lamiales: Lamiaceae) laid fewer eggs on 2,4-D treated plants in choice tests. Ainsworth and Holtkamp (1999) recorded fewer eggs laid by *Mesoclanis polana* Munro (Diptera: Tephritidae), on glyphosate dosed *Chrysanthemoides monilifera* L. (Asterales: Asteraceae). Speight and Whittaker (1987) noted a trend of preferential oviposition by the leaf feeding beetle *Gastrophysa viridula* DeGeer (Coleoptera: Chrysomelidae) on unsprayed rather than sprayed plants. Similar conclusions were drawn from studies conducted by Trumble and Kok (1979) and Stoyer and Kok (1989), who recorded low rates of oviposition on herbicide treated *Carduus* thistles (Asterales: Asteraceae). Similarly, a study by Ding *et al*., (1998) concluded that glyphosate (2.5%) had an adverse effect on the hatching

rates of *Neochetina* eggs. However, the larval and adult survival was not compromised by the herbicide concentration and an increased feeding rate was observed on glyphosate dosed leaves (Ding *et al*., 1998). While all these studies indicate that herbicide application has a negative effect on the oviposition capacity of the biocontrol agents, a study by Hayes (2000) concluded that application of 2,4-D, dicamba and picloram on *Senecio jacobaea* L. (Asterales: Asteraceae) resulted in increased rates of oviposition by the ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae).

While most studies exemplified above have been carried out on terrestrial weed systems and a few on aquatic systems using recommended or lethal rates of herbicides, no studies have been carried out to test the effects of low or sublethal doses of herbicides on biocontrol agents of aquatic weed systems (e.g. water hyacinth). Therefore, the third objective of this chapter is to test the effects of the retardant dose of glyphosate herbicide on the survival of the biological control agents, *N. eichhorniae and N. bruchi* and determine if the low dose has any positive or negative effect on the biocontrol populations. It is expected that the low dose will not kill the biocontrol agents nor interfere with the feeding and reproductive capacities.

2.2 Materials and Methods

Section 2.2.1 describes the experimental setup used to identify the retardant herbicide dose (Objective 1) and to test its effects on water hyacinth and its biocontrol agents, the *Neochetina* weevils (Objective 3). Section 2.2.4 describes the experimental setup used to test the effect of the sublethal dose of glyphosate and *Neochetina* herbivory on N and P levels in water hyacinth leaves and crown (Objective 2). It must be noted that the environmental conditions such as temperature and humidity and additionally the quality of water as determined by phosphates and nitrates prevalent at the time of

spray determines the spray volume. Therefore, the calibrated spray volumes recorded in this study and Chapters 3 and 4 are 140 and 150 L ha^{-1}.

2.2.1. Experimental water quality and herbicide dosages

In each of the experiments described below, four medium sized water hyacinth plants, two of which were tagged with plastic labels on leaf-one (i.e. the innermost, youngest leaf), were placed in circular 50 L (52-cm diameter) plastic tubs, containing 42 L of water, outdoors at the University of the Witwatersrand, Johannesburg, South Africa. The plants were medium to tall phenotypes and formed 100% cover of the water surface in the tubs and were devoid of any ramets. The nutrient levels of the water in the tubs were adjusted to 1.5 mg N L^{-1} (as ammonium nitrate) and 0.22 mg P L^{-1} (as potassium di-hydrogen orthophosphate). These levels approximated those typically found under local conditions during country-wide surveys of water quality performed by the South African Institute for Water Quality Service. The herbicide treatment consisted of applications of a broad spectrum, glyphosate-based herbicide, Roundup® (active ingredient, 360 g L^{-1} glyphosate, containing 480 g isopropylamine salt of glyphosate L^{-1}) with the surfactant polyethoxylated tallowamine, supplied by Monsanto Pty. Ltd., South Africa, which was sprayed on the hyacinth plants at the prescribed dosages. A buffer (2% ammonium sulphate) was added to the spray solution to maintain pH at between 5 and 5.5. A battery operated (12 V) pressurized spray rig (Multispray, South Africa) was calibrated to spray 150 L ha^{-1} using Tee Jet TP (TP11020) nozzles (Tee Jet Technologies, USA). The recommended lethal dose for Roundup® on water hyacinth is 3%.

2.2.2. Identification of a retardant dose of glyphosate for the plants

Trials were carried out during autumn of 2005. Twenty-one tubs were set up, as described above, and divided into seven groups of three. At the outset, glyphosate

was applied at concentrations of 0.1%, 0.3%, 0.5%, 0.8%, 1% and 1.5% with active ingredient values (g m^{-2}) of 0.01, 0.04, 0.07, 0.11, 0.14 and 0.21 respectively. Three tubs were used as controls and were not sprayed with glyphosate. The plants used in this experiment did not contain insects and were free of insect damage.

Over a period of eight weeks, weekly measurements were made on the tagged water hyacinth plants to record: total number of leaves; position of leaf-one and total number of ramets. Endpoint analysis, using One-Way Analysis of Variance and student's t-test (STATISTICA, version 6, StatSoft, Southern Africa) was carried out on each of the plant parameters measured at the end of the experiment and the results between control and herbicide treatments were considered significant at the 0.05 probability level.

2.2.3. Effect of glyphosate on *Neochetina eichhorniae* and *Neochetina bruchi*

Trials were carried out during spring of 2005. Twenty four tubs were set up as described above, 12 for *N. eichhorniae* and 12 for *N. bruchi*. Each set was sub-divided into four groups of three. Three of the groups were treated with herbicide (0.8%, 1.5% and 2%, details above) while one group served as a control which was not treated with herbicide, but contained insects. Four pairs of adult weevils were released onto the plants in each tub, giving an initial weevil density of two weevils per plant. Each tub was then enclosed in a net canopy (mesh size: 0.8 mm x 0.5 mm) to confine the weevils which were then allowed to acclimatize for one week, after which glyphosate was sprayed on the plants (day zero). Two water hyacinth rosettes were randomly chosen in each of the tubs and tagged, so that fortnightly measurement of the feeding intensity on the second-youngest leaf could be measured by counting the number of feeding scars. The lamina area of the second-youngest leaf was measured by scanning and digitizing an outline of the leaf drawn on paper in order to determine the leaf area, which was then used to calculate the number of feeding scars cm^{-2}. The number of petioles mined and the number of adult weevils

(both *N. eichhorniae* and *N. bruchi*) and larvae found were counted by dissecting the tagged plants within each tub at the end of the experiment. The crown of the plant was not cut open to count or recover any late-instar larvae. The experiment ran for eight weeks. Student's t-tests, using STATISTICA program, version 6 (StatSoft, Southern Africa), was carried out on each of the parameters measured. Means obtained for insect parameters were considered significant at the 0.05 probability level. Analysis of Covariance (ANCOVA) was used to test the effect of a covariate (number of leaves) on the number of feeding scars. A contingency table analysis (StatSoft, Southern Africa) was used to compare the proportions of petioles mined between the treated and unsprayed (control) plants.

2.2.4. Effect of 0.8% glyphosate and *Neochetina* herbivory on ramet, leaf and biomass production and N and P levels in water hyacinth

Trials were carried out during summer of 2010. Six, medium sized water hyacinth plants, three of which were tagged with plastic labels on leaf-one (i.e. the innermost, youngest leaf), were placed in circular 50 L (52-cm diameter) plastic tubs, containing 42 L of water, outdoors at the University of the Witwatersrand, Johannesburg, South Africa. The plants were medium to tall phenotypes and formed 100% cover of the water surface in the tubs. The nutrient levels of the water in the tubs were adjusted to 1.5 mg N L^{-1} (as ammonium nitrate) and 0.22 mg P L^{-1} (as potassium di-hydrogen orthophosphate).

Twelve tubs were set up, and were divided into the following treatments: 0.8% (sub-lethal dose of herbicide spray only), 0.8%+Ne (sub-lethal dose of herbicide spray plus *Neochetina eichhorniae*), Ne (*Neochetina eichhorniae* only) and Control (no *Neochetina eichhorniae* or 0.8% herbicide spray) treatments.

Glyphosate was applied at a concentration of 0.8% to the treatments labeled as 0.8% and 0.8%+Ne. The herbicide treatment consisted of application of a broad spectrum,

glyphosate-based herbicide, Roundup® (active ingredient, 360 g L^{-1} glyphosate, containing 480 g isopropylamine salt of glyphosate L^{-1}) with the surfactant polyethoxylated tallowamine, supplied by Monsanto Pty. Ltd., South Africa. A knapsack spray rig (Multispray, South Africa) was calibrated to spray 140 L ha^{-1} using Tee Jet (8003 E) nozzles (Tee Jet Technologies, USA).

Six pairs of *Neochetina* weevils were released onto treatments labeled as Ne and 0.8%+Ne. The control treatment tubs were neither treated with glyphosate nor were insects released onto them.

Over a period of four weeks, weekly measurements were made on the tagged water hyacinth plants to record: total number of leaves; position of leaf-one and total number of ramets. At week zero and week four, wet weights were measured and recorded. These measurements would indicate if the combined effects of the retardant dose and *Neochetina* herbivory (0.8%+Ne) would impact the production of leaves and ramets in treated plants. Weevil feeding scars were recorded at weeks zero, two and four. The number of feeding scars recorded was used to determine if the treatments had any effect on weevil herbivory.

Endpoint analysis, using One-Way Analysis of Variance and student's t-test (STATISTICA, version 6, StatSoft, Southern Africa) was carried out on each of the plant parameters measured across all treatments and the results were considered significant at the 0.05 probability level. End point analysis at week four using Linear regression was conducted across all treatments to discern if there was a relationship between % N level in the water hyacinth leaves and the number of weevil feeding scars.

Plant tissue N and P analyses

At week two and week four, three plants (each week) from each tub across all treatments were collected and leaf 1, leaf 2 and leaf 3 from each treatment were pooled together because a study by Katembo (2010) indicted that the nitrogen (N) level between leaf 1, leaf 2 and leaf 3 did not differ significantly from each other. The plant crown from each treatment was collected and was pooled together. Both leaf and crown samples were then oven dried at 60°C for 16 hours. The oven dried samples were sent to Bemlab, Stellenbosch, South Africa, for nitrogen (N) and Phosphorous (P) analysis using the combustion analyzer method (Refer Bemlab, Stellenbosch, South Africa).

Endpoint analysis, using One-Way Analysis of Variance and student's t-test (STATISTICA, version 6, StatSoft, Southern Africa) was carried out on N and P values for leaf and crown samples and the results were considered significant at the 0.05 probability level.

2.3 Results

2.3.1 Identification of a retardant dose of glyphosate

The retardant effect of the applied herbicide dosages were measured in terms of production of ramets and leaves. A mean (± SE) of 1.5 (±0.80) ramets per plant were produced on plants treated with a 0.8% concentration of herbicide, over a period of eight weeks. This was significantly lower than the mean number of ramets produced by the unsprayed, control plants ($t_{10} = 2.19$; $P = 0.05$) (Fig. 2.1). Ramet production [i.e the total number of ramets produced by tagged plants across the sampling period (56 days)] (Fig. 2.1) and ramet turnover, [i.e number of new ramets produced per plant per week] (Fig. 2.2) in 1% and 1.5% herbicide treatments declined as the

mother plant lost condition. However, the mean number of ramets produced by plants treated with 0.5% and 0.3% concentrations of herbicide were not significantly different from the unsprayed, control plants ($F_{2, 15} = 1.02$; $P = 0.38$).

The mean number of leaves produced by plants treated with a 0.8% concentration of herbicide was significantly lower ($t_{10} = 8.62$; $P < 0.0001$) than the unsprayed, control plants (Fig. 2.3). Although less than the control, plants treated with 0.5% concentration of herbicide produced significantly more leaves than those treated with 0.8% and 1% concentrations ($F_{2, 15} = 9.51$; $P = 0.002$).

Plants treated with a 1.5% concentration lost all their leaves and died (Fig. 2.4). After adding one leaf, the plants treated with the 0.8% concentration did not produce any more new leaves and maintained their leaf numbers throughout the sampling period, as shown by the position of the tagged leaf (Fig. 2.5), which remained at leaf number two position. Plants treated with a 0.5% concentration continued to add leaves during the study, while those treated with the 1% and 1.5% concentrations, initially added a leaf, in some cases two, and then lost leaves (Fig. 2.4).

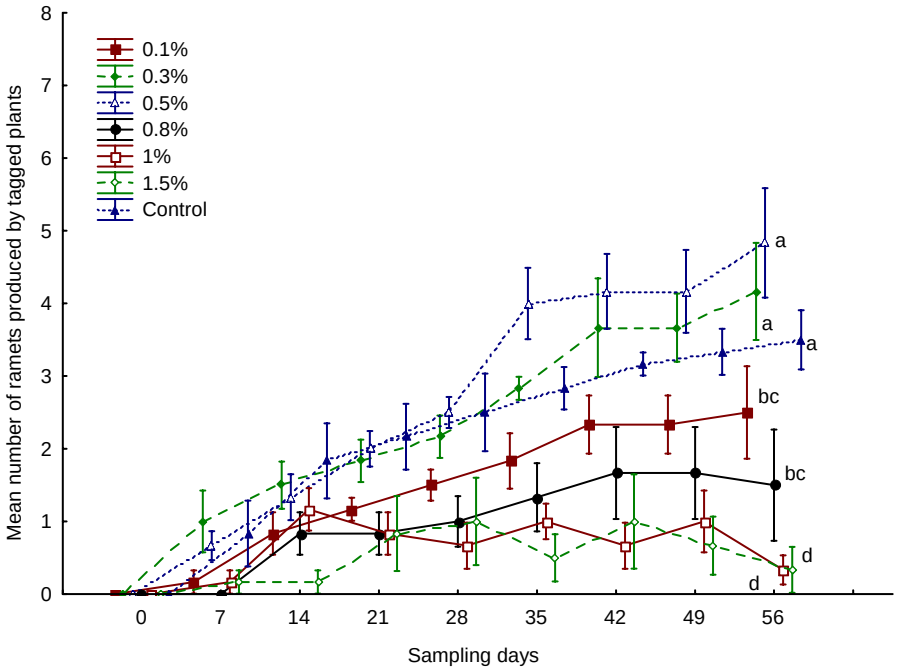

Figure 2.1: Mean number of ramets produced by water hyacinth plants treated with different doses of glyphosate herbicide at 140L/ha. Error bars = standard error of the mean, n=6. Different letters indicate significant differences at P< 0.05 between herbicide and control treatments.

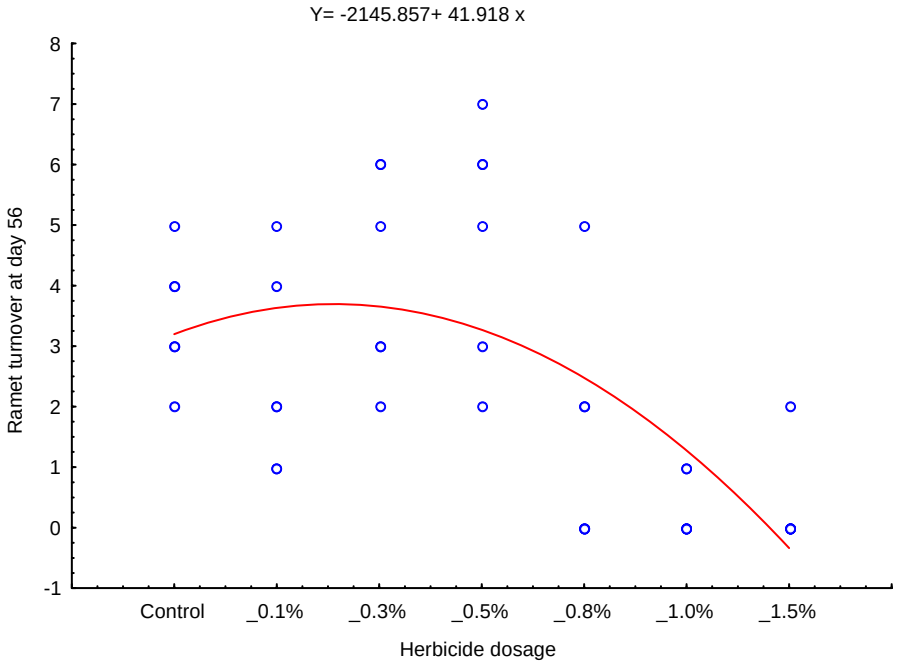

Figure 2.2: Ramet turnover in water hyacinth plants treated with different doses of glyphosate herbicide at 140L/ha, 56 days after treatment.

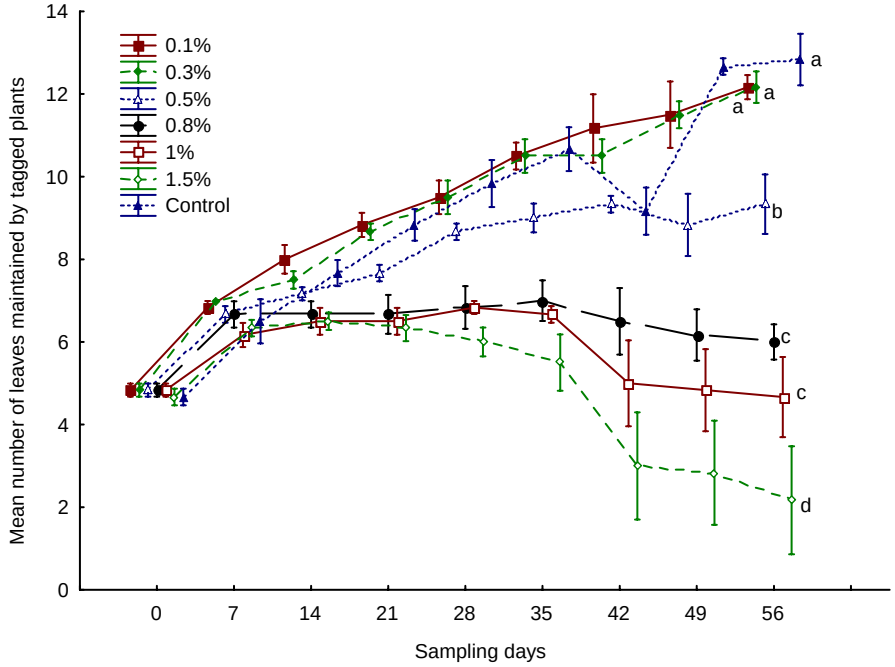

Figure 2.3: Mean number of leaves produced by water hyacinth plants treated with different doses of glyphosate herbicide at 140L/ha. Error bars = standard error of the mean, n= 6. Different letters indicate significant differences at P< 0.05 between herbicide and control treatments.

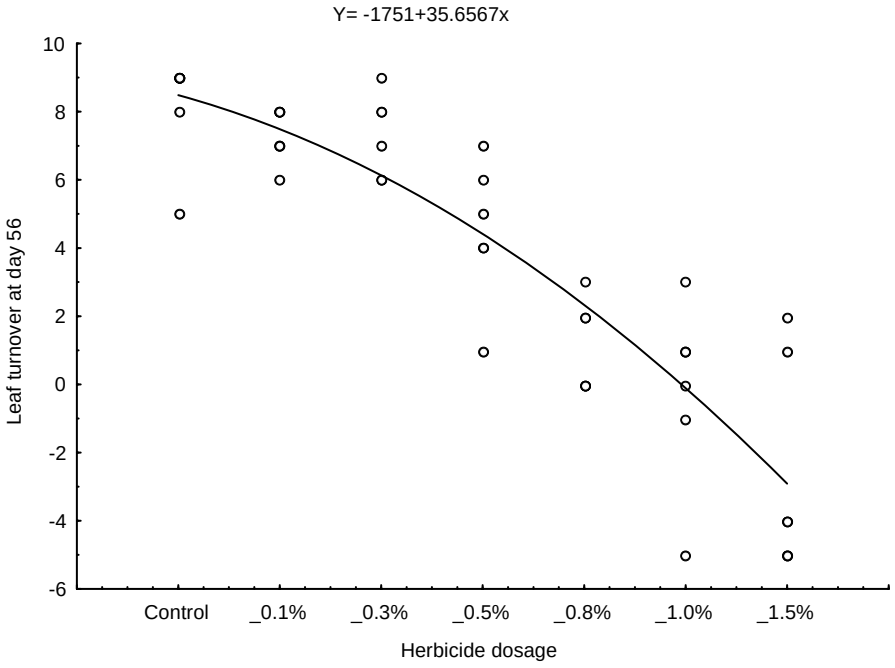

Figure 2.4: Leaf turnover in water hyacinth plants treated with different doses of glyphosate herbicide at 140L/ha, 56 days after treatment.

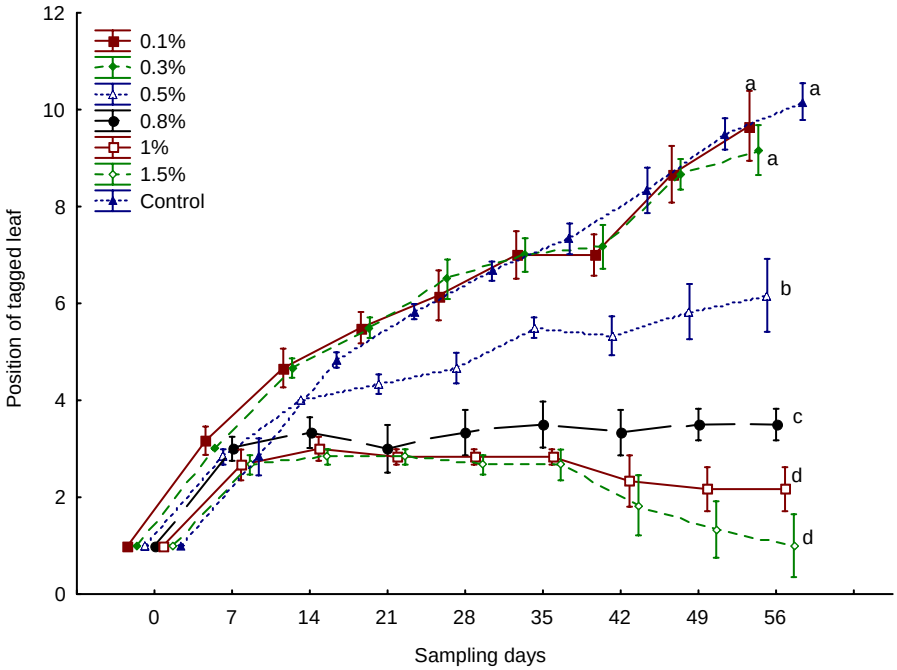

Figure 2.5: Position of tagged leaf in water hyacinth plants treated with different doses of glyphosate herbicide at 140L/ha. Error bars = standard error of the mean, n=6. Different letters indicate significant differences at P< 0.05 between herbicide and control treatments.

2.3.2 Effect of a retardant dose of glyphosate on *Neochetina eichhorniae* and *Neochetina bruchi*

Glyphosate applied at doses of 1.5% and 2% killed the weed 60 days after treatment (DAT), and resulted in the demise of the weevils as their host plant disappeared. The water hyacinth plants treated with a 0.8% concentration of glyphosate were still alive 60 DAT and only these (treated) plants along with the control (unsprayed) plants were considered for further analysis. There were no significant differences, at day 60, between the mean numbers of adults found on the treated (0.8%) and control plants (Fig. 2.6) (for *N. eichhorniae*, $t_{10} = 2.076$, $P = 0.06$, and for *N. bruchi*, $t_{10} = 2.07$; $P = 0.065$).

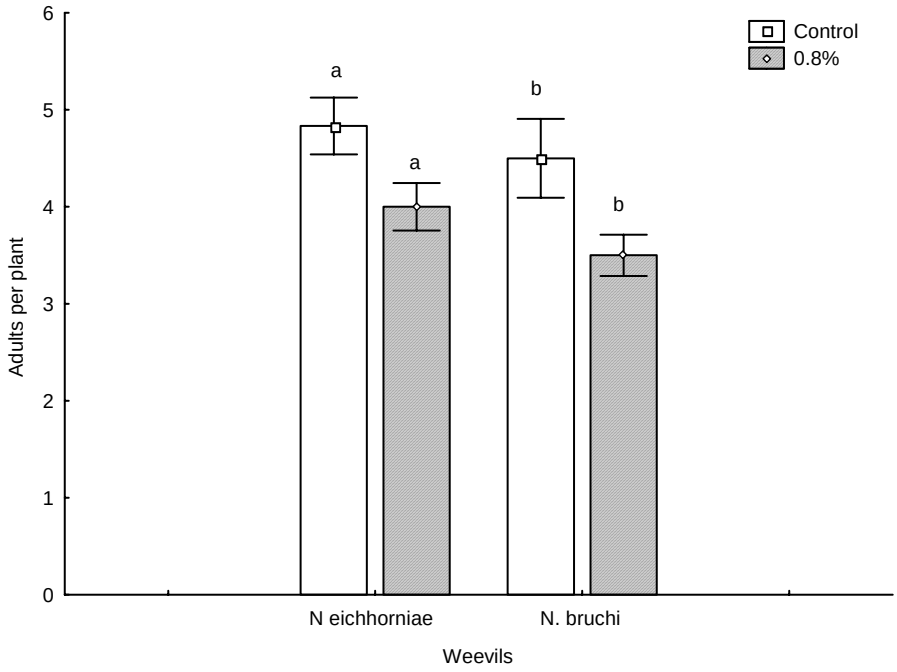

Figure 2.6: Mean (± SE) number of *Neochetina eichhorniae* and *Neochetina bruchi* adults harvested from water hyacinth plants treated with 0.8% glyphosate herbicide at 140L/ha, 60 days after treatment. Error bars = standard error of the mean. Different letters indicate significant differences at P< 0.05 between control and sprayed (0.8%) treatments.

The total number of feeding scars cm^{-2} was significantly higher on the treated plants than the control plants at day 60 (Figs. 2.7 A and 2.7 B) (for *N. eichhorniae,* $t_{10} = -5.83$; $P = 0.0001$; and for *N. bruchi*, $t_{10} = -3.59$; $P = 0.004$) but not on the earlier sample days. ANCOVA results showed that the number of leaves was not a significant covariate ($F_{1, 8} = 0.037$, $P = 0.85$) and hence, the increase in number of feeding scars was the effect of the treatment alone.

The proportion of petioles mined by weevil larvae was significantly greater on the treated plants than on the control plants for both *N. eichhorniae* ($X^2 = 4.51$, $P = 0.03$) and *N. bruchi* ($X^2 = 6.02$, $P = 0.01$) (Table 2.1).

Table 2.1: The percentage of petioles with mining damage caused by *Neochetina* spp. larvae on treated and unsprayed (= control) *Eichhornia crassipes* plants.

	N. eichhorniae	*N. bruchi*
Treated	71.6	75.0
Unsprayed	45.0	41.6

There were no significant differences between the mean numbers of first- and second-instar larvae obtained from the treated plants and control plants at the end of the experiment for both *N. eichhorniae* (1^{st} instars: $t_{10} = 1.53$, $P = 0.15$; 2^{nd} instars: $t_{10} = -0.62$, $P = 0.54$) and *N. bruchi* (1^{st} instars: $t_{10} = 0.0$, $P = 1.00$; 2^{nd} instars: $t_{10} = -1.58$, $P = 0.144$) (Figs 2.8 A and 2.8 B). No third-instar larvae were found on treated plants, possibly due to movement of the instars into the crown to avoid competition as food resources became depleted (Center, 1987).

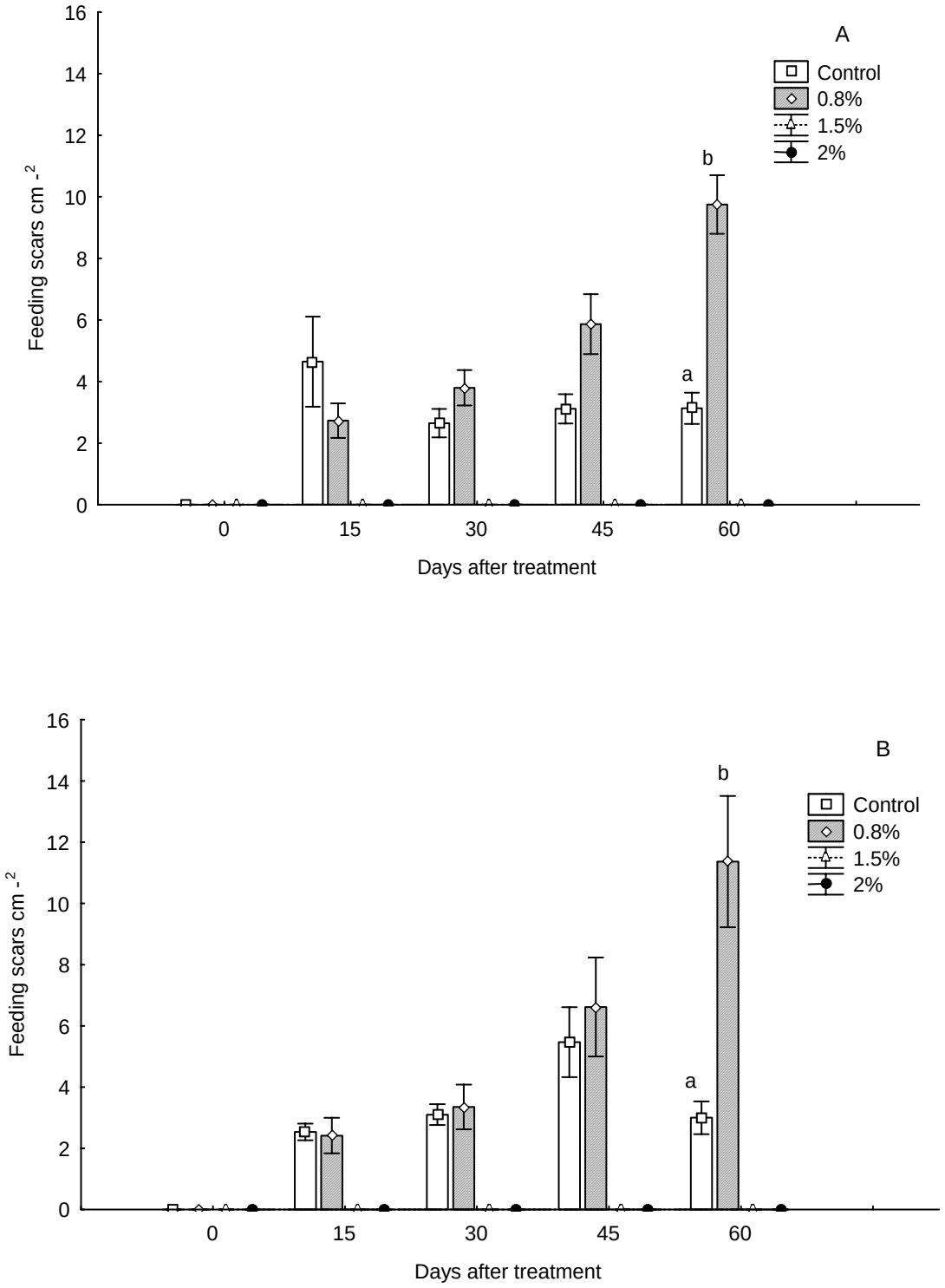

Figure 2.7: Mean (± SE) number of feeding scars cm^{-2} recorded on leaf two of water hyacinth plants harbouring *N. eichhorniae* (A) and *N. bruchi* (B) and treated with 0.8% herbicide at 140L/ha, 60 days after treatment. Error bars = standard error of the mean. Different letters indicate significant differences at $P< 0.05$ between control and sprayed (0.8%) treatments.

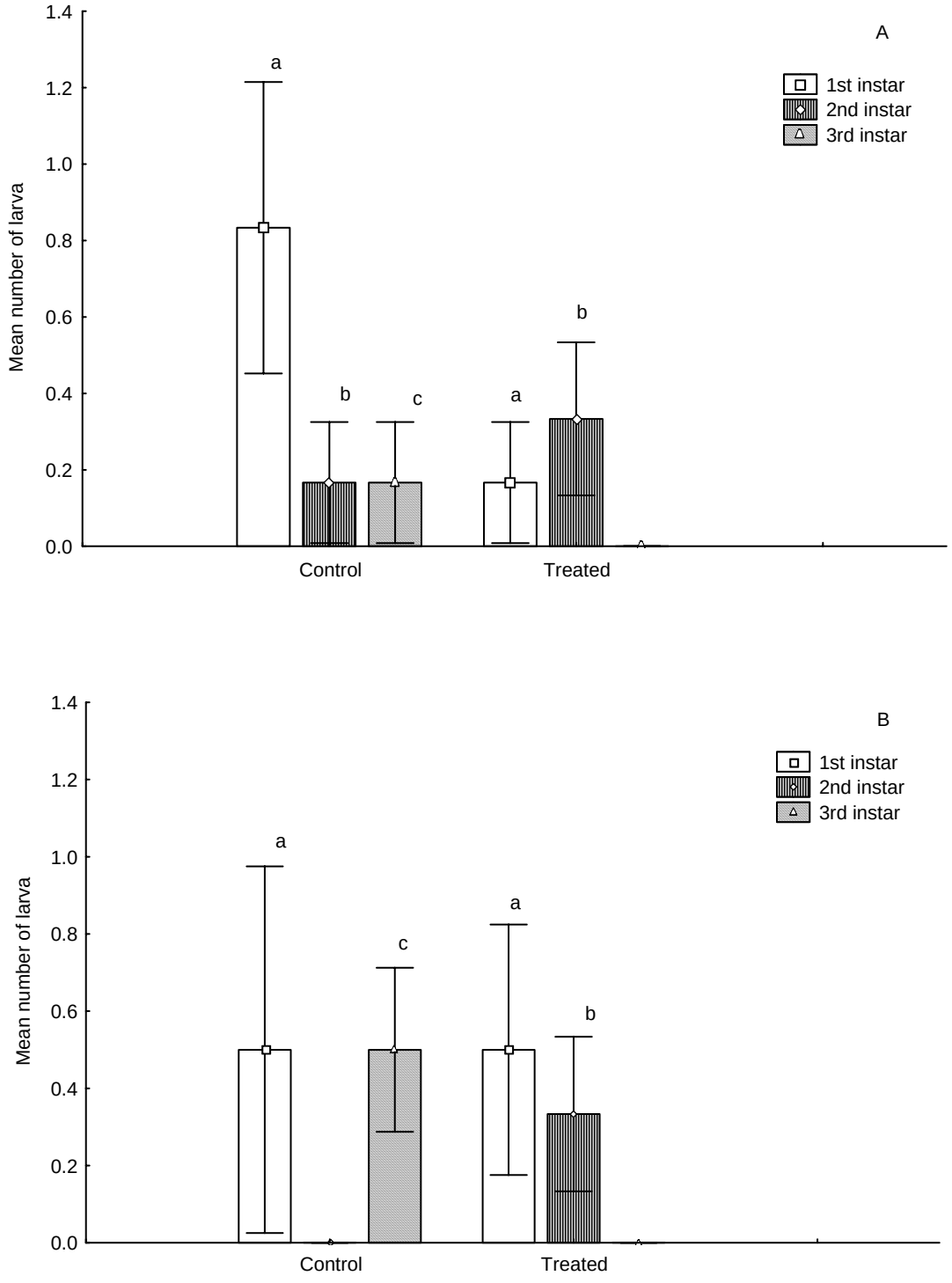

Figure 2.8: Mean (± SE) numbers of first-, second- and third-instar larvae of *N. eichhorniae* (A) and *N. bruchi* (B) found on water hyacinth plants treated with 0.8% herbicide at 140L/ha, 60 days after treatment. Error bars = standard error of the mean. Different letters indicate significant differences at $P< 0.05$ between control and 0.8% herbicide treated regimes.

2.3.3 Effect of 0.8% glyphosate and *Neochetina* herbivory on ramet, leaf and biomass production

The combined effect of the retardant herbicide dosage and *Neochetina* herbivory were measured in terms of production of ramets and leaves.

A mean (± SE) of 0.33 (±0.16) ramets per plant were produced on plants treated with a 0.8% concentration of herbicide, over a period of four weeks. This was significantly lower than the mean number of ramets produced by the unsprayed, control plants ($F_{3, 32} = 12.21$; $P = 0.00$) (Fig. 2.9). Ramet production in the plants treated with a combination of 0.8% and Ne (0.8%+Ne treatment) averaged 1.11±0.48 and there was no significant difference between 0.8% and (0.8%+Ne) ($t_{16} = -1.5$; $P = 0.14$). Ne and control treatments produced 2.88±0.30 and 2.66±0.37 ramets, respectively.

The mean number of leaves produced by plants treated with 0.8% concentration of herbicide and (0.8%+Ne) treatment was significantly lower than the unsprayed, control plants (0.8%= $t_{16} = 4.95$; $P = 0.00$; (0.8%+Ne) = $t_{16} = 2.38$; $P = 0.02$) (Fig. 2.10). Plants treated with Ne produced significantly more leaves than those treated with 0.8% and (0.8%+ Ne) treatment ($F_{3, 32} = 4.45$; $P = 0.01$) (Fig. 2.10).

Plants treated with the 0.8% glyphosate did not produce new leaves between week 2 and 4 as shown by the position of the tagged leaf (Fig. 2.11), which remained at leaf number two position. There was no significant difference between the mean number of leaves produced by 0.8% and (0.8%+Ne) treatments ($t_{16} = 0.24$; $P = 0.8$).

Significant differences at week 4 were noted in terms of biomass production between 0.8%, (0.8%+Ne) and Ne treatments when compared to control treatment ($F_{3, 8} =$

92.50; *P*= 0.00) (Fig. 2.12). However, no significant difference was noted between control and Ne treatments (t_4 = -2.12; *P*= 0.10) (Fig. 2.12).

Significant differences between treatments were not noted for the mean number of weevil feeding scars recorded ($F_{2, 24}$= 0.82; *P*= 0.45) (Fig. 2.13), indicating that the herbicide treatment did not interfere with the feeding capacity of the weevils.

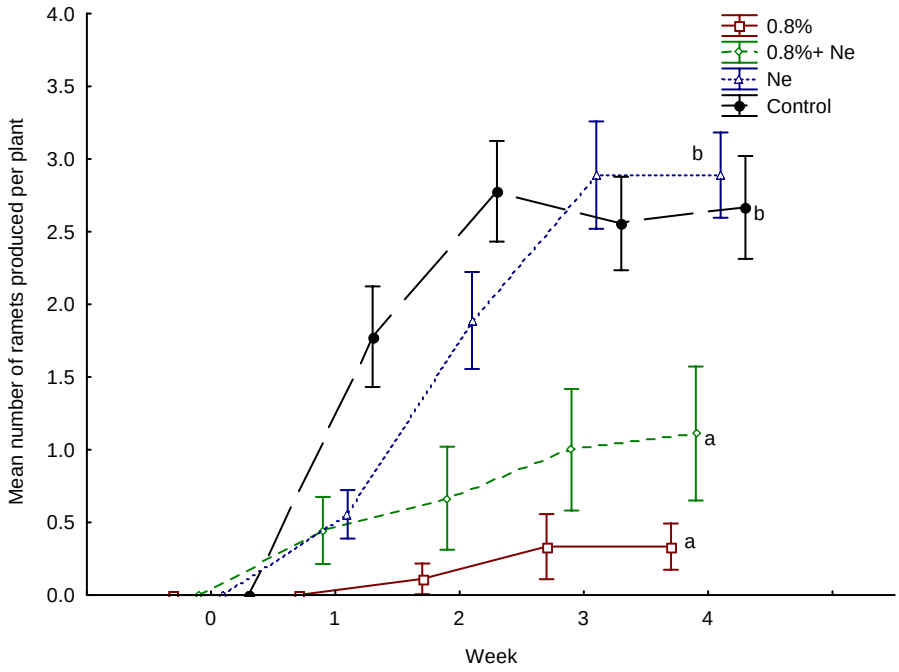

Figure 2.9: Mean number of ramets produced by water hyacinth plants under different treatment regimes. 0.8% dose of glyphosate herbicide was sprayed at 140L/ha. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+ Ne) treatment regimes.

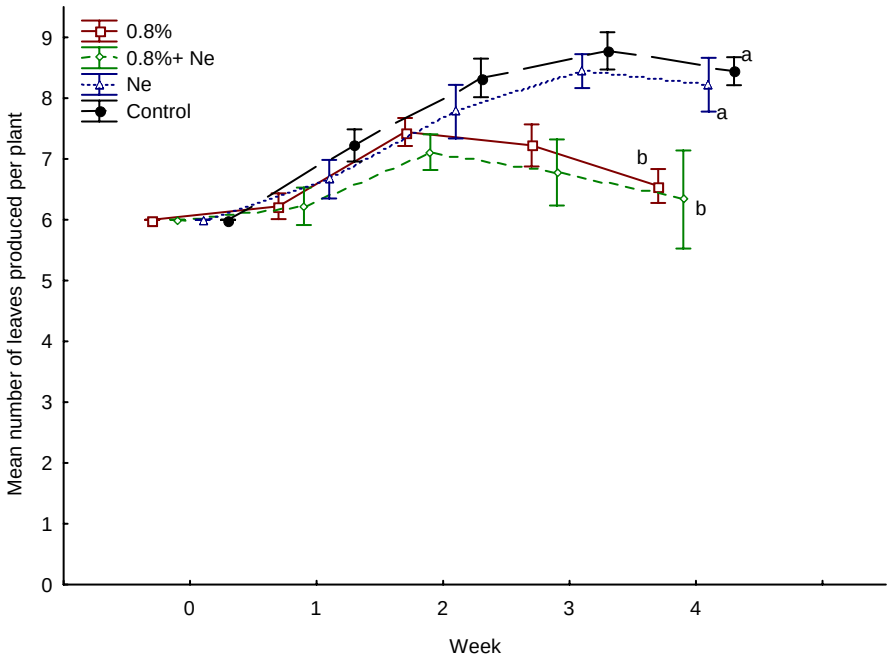

Figure 2.10: Mean number of leaves produced by water hyacinth plants under different treatment regimes. 0.8% dose of glyphosate herbicide was sprayed at 140L/ha. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+ Ne) treatment regimes.

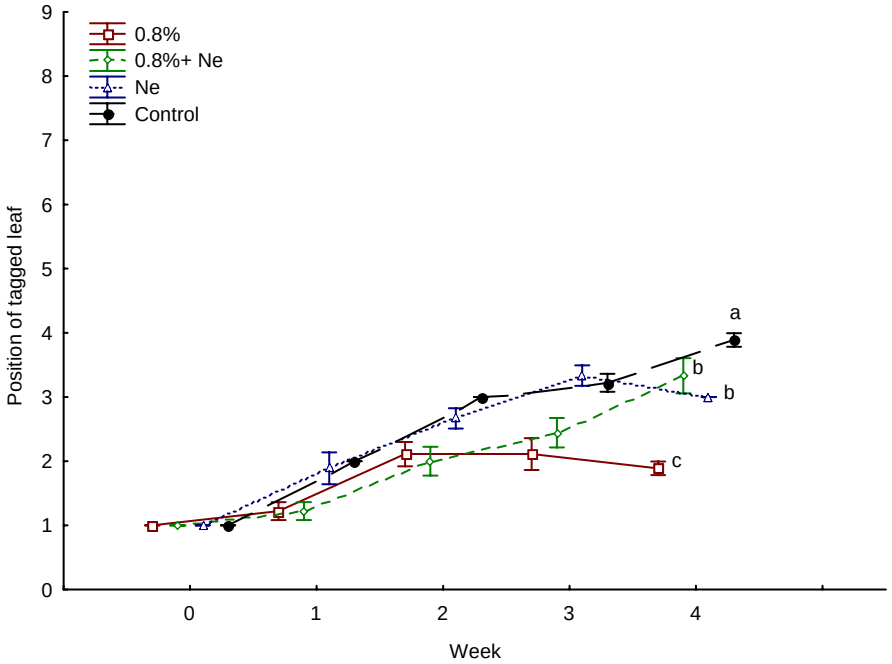

Figure 2.11: Position of tagged leaf in water hyacinth plants under different treatment regimes. 0.8% dose of glyphosate herbicide was sprayed at 140L/ha. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+ Ne) treatment regimes.

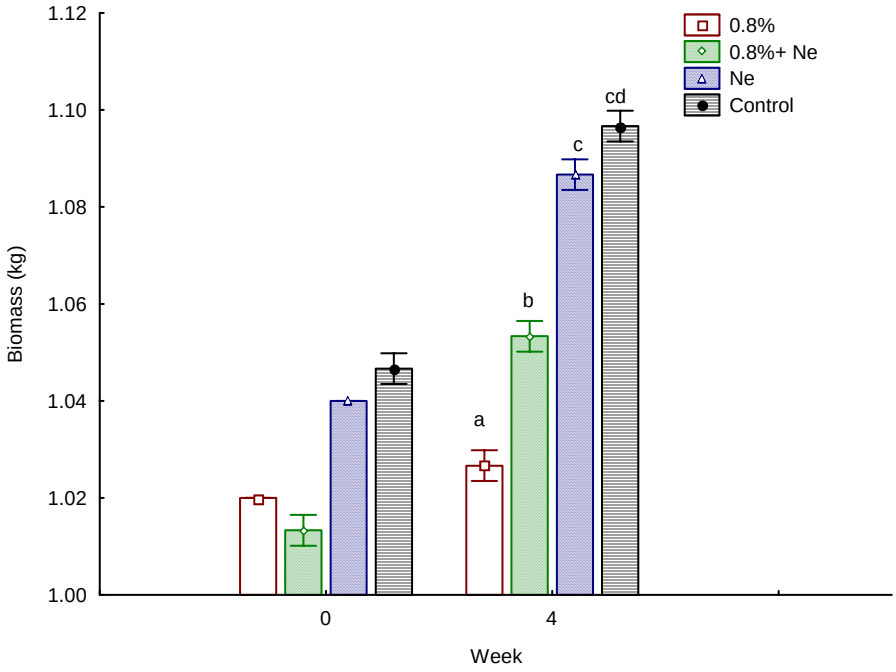

Figure 2.12: Biomass (kg) of water hyacinth plants under different treatment regimes. 0.8% dose of glyphosate herbicide was sprayed at 140L/ha. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+ Ne) treatment regimes at week 4.

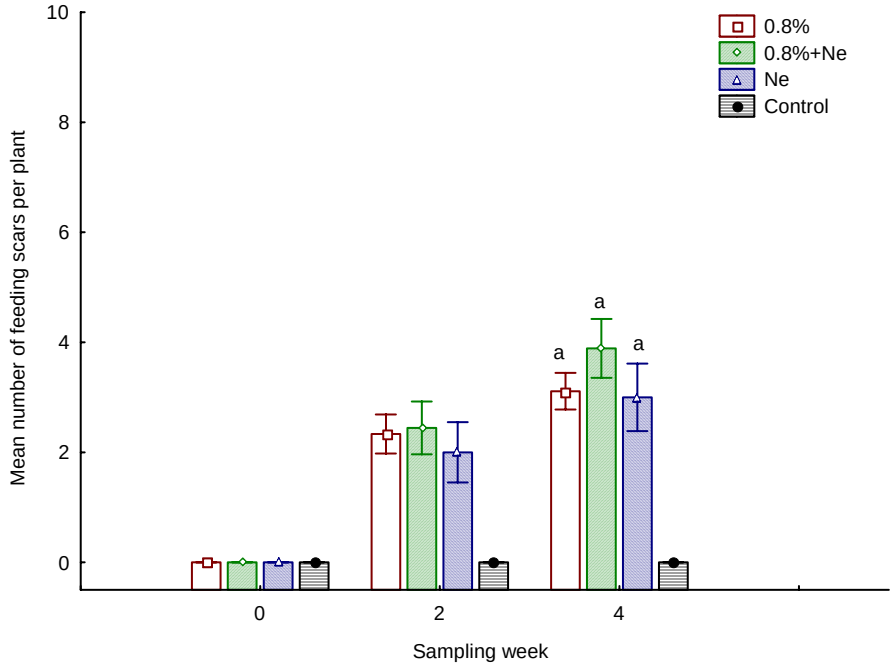

Figure 2.13: *Neochetina* feeding scars on water hyacinth plants under different treatment regimes. 0.8% dose of glyphosate herbicide was sprayed at 140L/ha. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between 0.8% herbicide, *Neochetina* (Ne) and (0.8%+ Ne) treatment regimes at week 4.

2.3.4 Effect of 0.8% glyphosate and *Neochetina* herbivory on N and P levels in water hyacinth

The nitrogen levels (%) between water hyacinth leaf samples ($F_{3, 8} = 3.01$; $P = 0.09$) (Fig. 2.14 A) and crown samples ($F_{3, 8} = 0.68$; $P = 0.58$) (Fig. 2.14 B) were not significantly different, four weeks post herbicide spray.

Similarly, the phosphorous levels (%) between water hyacinth leaf samples ($F_{3, 8} = 2.07$; $P = 0.18$) (Fig. 2.15 A) and crown samples ($F_{3, 8} = 0.51$; $P = 0.68$) (Fig. 2.15 B) were not significantly different, four weeks post herbicide spray.

2.3.5 Relationship between %N level and *Neochetina* feeding scars

Linear regression analyses across all the treatments [0.8%, (0.8+Ne) and Ne] indicated that there was no positive correlation between % N level in leaf samples and feeding scars at week four (Fig. 2.16). The control treatment was excluded from the analysis as no feeding scars occurred in the absence of the weevils.

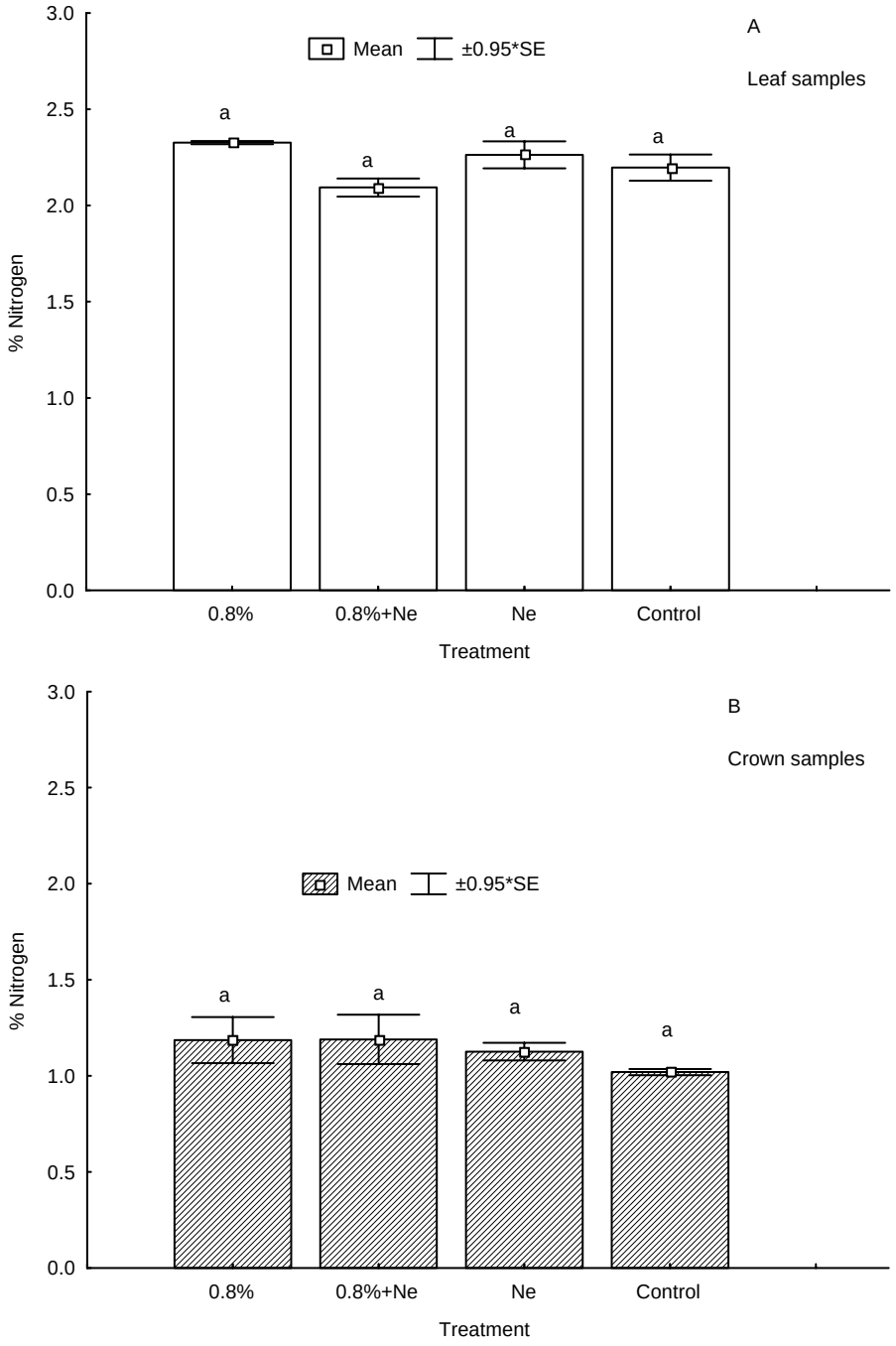

Figure 2.14: Effect of herbicide treatment and *Neochetina* herbivory on Nitrogen content in (A) water hyacinth leaves and (B) water hyacinth crown samples four weeks after spraying. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+Ne) treatment regimes.

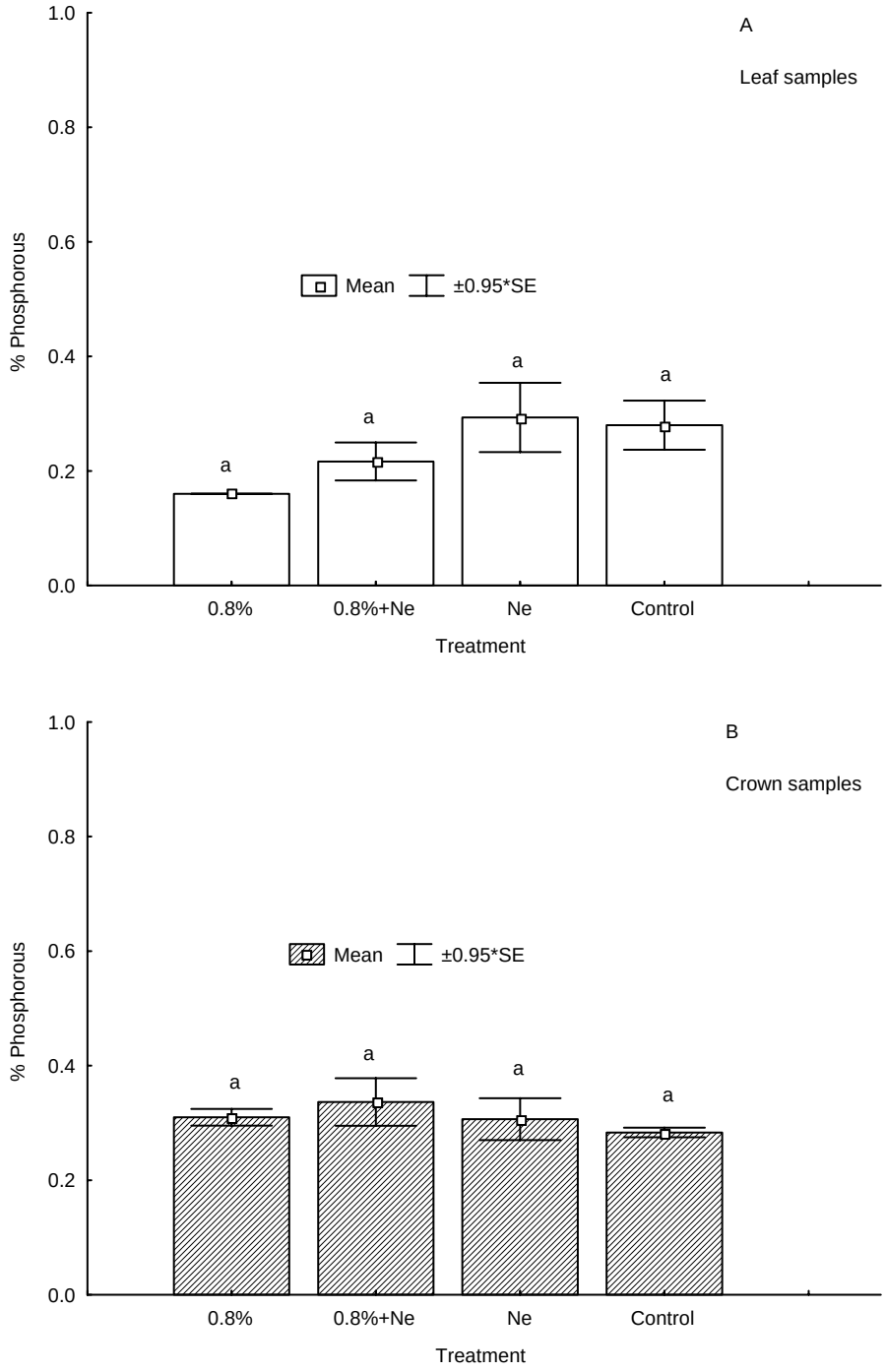

Figure 2.15: Effect of herbicide treatment and *Neochetina* herbivory on Phosphorous content in (A) water hyacinth leaves and (B) water hyacinth crown samples four weeks after spraying. Error bars = standard error of the mean, n= 9. Different letters indicate significant differences at P< 0.05 between control, 0.8% herbicide, *Neochetina* (Ne) and (0.8%+Ne) treatment regimes.

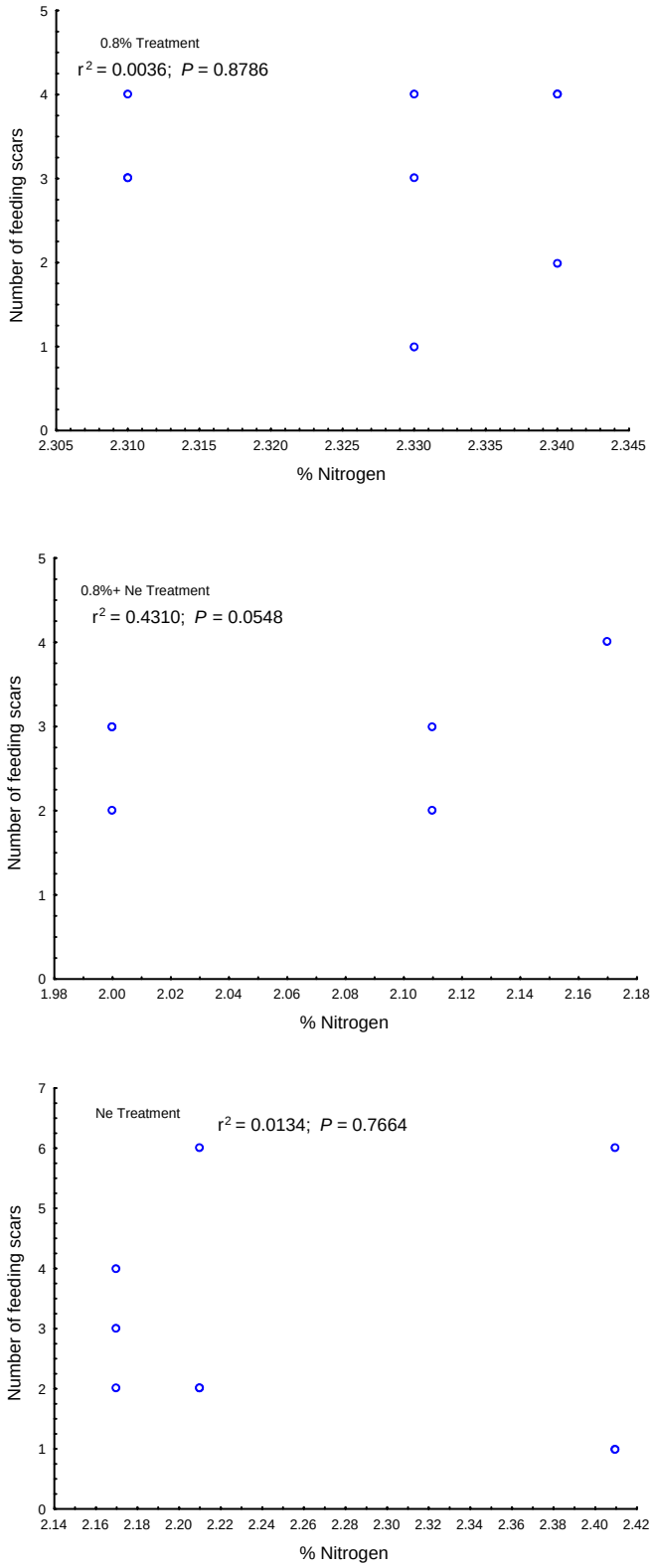

Figure 2.16: Correlation between % Nitrogen in water hyacinth leaves and number of feeding scars in 0.8%, (0.8%+ Ne) and Ne treatments.

Discussion

2.4.1 Identification of a retardant dose of glyphosate

In this study, the retardant effect of the glyphosate herbicide was measured in terms of production of ramets and leaves or the lack of thereof. A 0.8% dosage of glyphosate sprayed at 150 L ha^{-1} was identified as the retardant dose and its application resulted in the production of a low number of ramets and leaves by the treated plants, essentially freezing plant growth. The ramets that were produced were smaller in size when compared to the ramets produced by the unsprayed plants. The retardant effect of the low dose prevailed over a period of eight weeks and did not kill the plants. Although 0.3% and 0.5% glyphosate doses appeared to have a stimulatory effect on water hyacinth plants in terms of production of ramets, it was not statistically significant when compared to the ramet production by unsprayed plants. Furthermore the stimulatory effect was only evident between weeks five and six. Studies carried out on barnyard grass, *Eucalyptus* and *Ceratophyllum*, to test the stimulatory effects of glyphosate and quinclorac conclude that the increase in growth rates primarily took place within the first week after spraying after which it declined to levels slightly below the control plants (Schabenberger *et al*., 1999; Duke *et al*., 2006; Calabrese and Baldwin, 2002).

The low dose tested in this study interfered with the production of leaves. Center (1980) concluded that water hyacinth plants maintained a constant number of six to eight leaves per plant. Results from this study indicate that unsprayed plants produced 12 leaves at the end of eight weeks (56 days), while the 0.8% herbicide dosed water hyacinth plants produced about six leaves over a period of eight weeks, resulting in 50% reduction in the number of leaves produced.

Leaf production rates of water hyacinth noted in this study were consistent with published reports. Center and Van (1989) reported that leaf production rates varied

considerably among water hyacinth colonies, ranging from 0.087 to 0.18 leaves per rosette per day. In this study, the unsprayed, control plants produced 0.16 leaves per day (or 1.12 leaves per week), while the 0.8% dosed plants produced 0.03 leaves per day (or 0.25 leaves per week).

2.4.2 Effect of a retardant dose of glyphosate on *Neochetina eichhorniae* and *Neochetina bruchi*

While the low dose of glyphosate (0.8% or 1.1kg/ha) retarded the production of ramets and leaves it did not affect the survival of the *Neochetina* species as evidenced by adult and larval counts. Studies conducted on terrestrial weed systems corroborate these findings. The larvae of leafy spurge hawk moth, *Hyles euphorbiae* L. (Lepidoptera: Sphingidae), a biocontrol agent of leafy spurge, survived sprays of 2.2 kg /ha 2, 4-D and picloram (Rees and Fay, 1989), while applications of 2,4-D, imazethapyr and picloram plus 2,4-D at 1.1kg, 0.28kg and 0.28+1.1kg/ha respectively, did not affect the long term establishment of *Spurgia esulae* Gagne (Diptera: Cecidonyiidae) larval populations, another biocontrol agent released to control leafy spurge (Lym and Carlson, 1990). The survival capacity and the overwintering fitness of another leafy spurge biocontrol agent, *Aphthona nigriscutis* Foudras (Coleoptera: Chrysomelidae) populations by application of Picloram plus 2,4-D at 0.56 and 1.1kg/ha was not compromised (Nelson and Lym, 2003). Low doses of the systemic, broadleaf herbicide, triclopyr amine, applied at a rate of 12 kg/ha (Lindgren *et al*., 1998) did not affect adult survival of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), a biocontrol agent of purple loosestrife.

The reproductive capacity of the weevils was not compromised by the low dose of herbicide. The newly hatched larvae were able to establish in the petioles, as evidenced by the mined petioles and the early and late instar larval counts. Larval survival has positive implications for biocontrol as larval feeding by both species of

Neochetina results in more damage and reduction in the plants compared to feeding by adults alone (Grodowitz *et al*., 1991). Therefore the larvae that survive the low dose herbicide spray should contribute to additional feeding stress. For example, published reports indicate that four larvae of *N. bruchi* per plant reduced leaf and daughter plant production by 56% and 31% respectively, while the same number of *N. eichhorniae* larvae reduced leaf and ramet production by 29.7% and 24.3% (Bashir *et al*., 1984). *Neochetina bruchi* larvae appear to have more devastating effect on the plants since they usually feed on the central buds of the plants thereby damaging the young leaves. This form of attack is not evident in *N. eichhorniae* larval tunneling as they tended to feed on tissues of mature petioles (Bashir *et al*., 1984).

Herbicides can affect petiole hardness and soften them which make them more susceptible to larval tunneling. For example, 2,4-D improved water hyacinth plant quality for the larval stages of the moth, *Niphograpta albiguttalis* by lowering the petiole hardness of the youngest and second youngest leaves (Wright and Bourne, 1990). In this study, the percentages of petioles mined (71.6% and 75.0% for *N. eichhorniae* and *N. bruchi*, respectively) were higher in glyphosate herbicide treated plants when compared to the unsprayed plants.

The low dose of glyphosate appeared to have a stimulatory effect on the feeding intensity of the adult weevils, as evidenced by feeding scars (Fig. 2.7), probably owing to increased palatability of the glyphosate treated plants. Wright and Bourne (1990) have shown that application of 2,4-D to water hyacinth increased the levels of nitrogen in leaves, making the plants more palatable. It is also possible that a very low dose of glyphosate increases the sugar content in the sprayed plants (Su *et al*., 1992; Dusky *et al*., 1986) thereby increasing its palatability. Lignin synthesis is dependent on the shikimic acid pathway and low doses of glyphosate may inhibit lignification sufficiently enough to allow more carbon to be portioned into sucrose (Velini *et al*., 2008). Increased feeding levels by the *Neochetina* spp. could also be due to glyphosate-induced inhibition of the synthesis of phenylalanine derived

phenols and secondary metabolites that act as feeding deterrents in many plants (Ainsworth, 2003). The presence of high number of feeding scars on glyphosate treated plants noted in this study has several benefits. They serve as oviposition sites for other biocontrol agents of water hyacinth such as *Niphograpta albiguttalis* and *Orthogalumna terebrantis* (Ajuonu *et al*., 2007) and the feeding scars also serve as entry points for pathogens. In addition, adult mirids were found to crowd onto leaves with fresh *Neochetina* scars, indicating that the mirids profited by the nutrients exuded from the scars (Ajuonu *et al*., 2007).

2.4.3 Effect of 0.8% glyphosate and *Neochetina* herbivory on ramet and leaf production

Center and Van (1989) noted that persistent herbivory by weevil population strongly influences leaf production in water hyacinth plants. In this study, however, contrary to expectation, the combined effects of the retardant dose (0.8%) and *Neochetina* herbivory (0.8%+Ne) did not result in the production of lower number of ramets or leaves than the water hyacinth plants dosed with 0.8% herbicide alone, underscoring the importance of persistent herbivore pressure by the weevils. For example, four *N. bruchi* adult males per plant reduced new leaf and ramet production by 32.7% and 8.8% respectively while the same number of *N. eichhorniae* adults per plant caused a reduction of 3.2% and 4.4% in leaf and ramet production (Bashir *et al*., 1984). Forno (1981) however, found that a density of 10 pairs of *Neochetina eichhorniae* per plant were required to effect a reduction in the number of leaves produced per plant. In this study, however, only one pair of *Neochetina* weevil was present per plant and therefore, the leaf and ramet production in (0.8%+Ne) treatment was not significantly different from the plants dosed with 0.8% herbicide. In this study, significant differences in terms of ramet and leaf production were not noted for Ne and control treatments which are in agreement with the findings of Soti and Volin (2010) who found that water hyacinth is able to compensate for low levels of simulated weevil feeding.

2.4.4 Effect of 0.8% glyphosate and *Neochetina* herbivory on N and P levels in water hyacinth

Growth and reproduction of biocontrol agents are affected by many factors such as disease, predators and plant quality as a consequence of which, their performance and establishment is hindered (Newman *et al*., 1998; Room and Thomas, 1985; Room *et al*., 1989). With respect to water hyacinth, in terms of plant quality, tissue nitrogen levels are especially important as it determines the growth, reproduction and abundance of water hyacinth weevils. In this study, the most important finding is that the application of 0.8% of glyphosate or the combined effect of 0.8% glyphosate and *Neochetina* herbivory did not affect the nitrogen and phosphorous levels in herbicide treated water hyacinth leaves and crown samples. These results bode well for biocontrol and biocontrol agents. Center and Wright (1991) reported that laboratory populations of water hyacinth weevils preferred leaves with high nitrogen concentration. However the weevils preferred to feed more on leaf tissues with nitrogen levels of 3.6% than on tissues with nitrogen levels between 2% and 3% (Center and Wright, 1991). This could explain why a positive significant correlation was not found between nitrogen concentration and feeding scars in this study, wherein the nitrogen levels in leaf samples were 2.5%.

Nitrogen rich (about 4.5%) water hyacinth plants harboured most reproductively active weevil populations (Center and Dray, 1992) and profoundly influenced egg production by *Neochetina eichhorniae* (Center, 1984), whereas plants with low tissue N (1.7%) did not harbor reproductive females (Center and Dray, 2010). However, Heard and Winterton (2000) reported that both species of *Neochetina* weevils reduced growth of water hyacinth with low tissue N although *N. bruchi* had a significantly greater impact on growth of water hyacinth with high tissue N. They attributed this greater impact on high N plants to quicker development, higher survival, and higher fecundity of *N. bruchi* on high N plants.

The results from this study however contradict the findings of Katembo (2010) who showed the application of 0.8% glyphosate herbicide decreased leaf N resulting in high C:N ratio in sprayed plants, possibly owing to differences in herbicide application methods. In this study, the herbicide dosage applied to experimental plants was carefully monitored in terms of walking speed and nozzle height from the top of the plant canopy, resulting in precise application. However, Katembo (2010) applied the herbicide dosage from a boat, at a speed of 4km/hour which may have affected the even coverage of the herbicide, resulting in under application or a patchy application of the herbicide.

A high C:N ratio or low N concentrations in plants, as recorded by Katembo (2010), often results in reduced nutritive quality to herbivores (Lincoln *et al*., 1986). However, Katembo (2010) concluded that the weevil performance in terms of feeding, survival and reproduction was neither affected nor benefited by the elevated C:N ratio or by the decreased N content in plants because there was no significant difference in % nitrogen recorded in crown samples of both sprayed and unsprayed plants as a result of which, the instar development of the weevil larvae would not be affected.

2.5 Conclusion

Considering that herbicide application at water hyacinth infested sites is usually detrimental to water hyacinth weevils due to precipitous loss of habitat, results from this chapter show that the application of a low dose of glyphosate herbicide (0.8%) onto a water hyacinth mat, in addition to effecting major reductions in leaf and ramet production, will not kill the plants, thereby maintaining the habitat and food source for all stages of the weevil population. Furthermore, it is concluded that the low dose of glyphosate herbicide does not kill adult *Neochetina* weevils or its immature stages

and is therefore compatible with biocontrol. The palatability and suitability of the treated plants allowed the weevils to persist and complete their life cycle despite fewer oviposition sites being available to them due to reduced numbers of leaves. Wilson *et al*., (2006) indicated that early larval stages of *N. eichhorniae* experienced density-dependent mortality when larval densities were high and there was disruption of leaf dynamics. It is predicted that when populations of *Neochetina* spp. reach damaging levels, severe damage to the plants by larval feeding may lead to cannibalism among the crowded larvae which in turn may cause a decline in adult weevil numbers, but a total loss of the weevil population is not expected. Furthermore, the integration of 0.8% with *Neochetina* weevils (0.8%+Ne treatment) does not affect the nutritive quality of the plants in terms of tissue nitrogen levels nor does it interfere with the feeding capacity of the *Neochetina* weevils.

Results from this chapter show that chemical and biological control of water hyacinth need not be mutually antagonistic but instead can be potentially integrated to provide the desired level of weed suppression. However, one must note that the density of weevils is important and high weevil numbers is are required for the combination of biocontrol and retardant dose of herbicide to have any greater effect than glyphosate alone. One of the key aspects for successful implementation of an integrated management control is the optimal timing of the herbicide application (Cullen, 1996). Chapter 3 therefore, attempts to determine the seasonal phenology of the weed and *Neochetina* weevils and advocate the best seasonal spray regime to manage water hyacinth infestations in South Africa.

Chapter 3

The seasonal effect of a retardant dose of glyphosate on the growth of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach and its biocontrol agents, *Neochetina eichhorniae* and *N. bruchi*

3.1 Introduction

Establishment and efficacy of introduced biocontrol agents against invasive alien weed species are often limited by low temperatures (Wagner *et al*., 1984; Byrne *et al*., 2004). Cold climates limit the impact of biocontrol in two ways: Firstly, low temperatures hamper the efficacy of natural enemies by impacting on the behavioural and physiological attributes of insects such as metabolic rate, flight activity, nutrition, developmental growth rates, oviposition and longevity (Liu *et al*., 1995; Lombardero *et al*., 2000; McAvoy and Kok, 1999; McClay, 1996). For example, temperature estimates at which the developmental rates and oviposition capacity is arrested in *Neochetina* weevils, biocontrol agents released against water hyacinth are presented in Table 3.1. These temperatures are often exceeded during winter in South Africa, which is characterised by a wide range of climatic zones such as high-altitude regions subjected to cold, dry winters with frequent frosts, Mediterranean winter rainfall areas where frost is absent, and coastal subtropical summer rainfall areas. Temperature extremes experienced by these regions, especially the high altitude areas have hampered the establishment of some biological control agents (Byrne *et al*., 2004) as well as limited their impact (McClay, 1996; Hill and Olckers, 2001).

Table 3.1: Thermal tolerance data for the biological control agents *Neochetina eichhorniae* and *Neochetina bruchi*, which are released against water hyacinth in South Africa. 1) Coetzee (2003). 2) (DeLoach and Cordo, 1976a,b) 3. (Julien, 2001). 4) Estimates compiled from existing literature. Table courtesy: Byrne *et al*., 2010.

Temperature criteria	*Neochetina eichhorniae*	*Neochetina bruchi*
Lower LT_{50}	-7.4°C [1]	-
Upper LT_{50}	41.68°C [1]	41.57°C [1]
CT_{min}	4.3°C [1]	3.3°C [1]
CT_{max}	50.97°C [1]	48.84°C [1]
Lower oviposition threshold	10°C [2]	-
Lower developmental threshold	~11.9°C [4]	15°C [3] ~6.8°C [4]
Degree-day requirements (egg to adult)	~195 DD [4]	~240 DD [4]
Optimum temperature for feeding/ oviposition	30°C [3]	30°C [3]

Secondly, low temperature extremes result in insect habitat destruction. For example, water hyacinth plants grow at an optimum temperature of 25°C to 30°C and the plants cease to grow at temperatures below 10°C and above 40°C (Gopal, 1987). Low temperature limits of water hyacinth were extensively investigated by Owens and Madsen (1995) who concluded that exposure of water hyacinth plants to -16°C for less than 12 hours did not produce significant stem mortality, whereas exposure to constant temperatures below 5°C for at least two to three weeks resulted in significant stem base mortality, as a result of which the crowns become submerged. Additionally, aerial parts of the plant exposed to low temperatures and frost brown off and die back (Owens and Madsen 1995). Consequently, the loss of habitat and food supply leads to an increase in mortality of immobile stages of natural enemies overwintering on aerial parts of the

plant. For example, Grodowitz *et al*., (1991) found that deterioration of water hyacinth plants due to low temperatures prevalent in winter resulted in the reduction of *N. eichhorniae* larvae and pupae (< 25 larvae/m^2 when compared to 60 larvae/m^2 found in spring). Reduced larval survival was attributed to a decrease in available leaf biomass and changes in the nutritional status of the plant. Decline in pupation was attributed to the degraded nature of the root material which was speculated to interfere with oxygen uptake by the pupae (Grodowitz *et al*., 1991). Frost has also been shown to influence the geographical distribution of other insect species and has even been implicated in local extinctions (Inouye, 2000), for example, Ehrlich *et al*., (1972) described the local extinction of the butterfly *Glycopsyche lygdamus,* caused by the loss of host plants as a direct result of heavy frosting. Extrapolating to a South African context, air temperatures are never cold enough for a sustained duration to cause stem base mortality in the field (Byrne *et al*., 2010). Consequently, at a population level, water hyacinth has a greater tolerance for low temperatures while the capacity of the insect populations to survive and develop under low temperature regimes is very limited. This mismatch of tolerances increases the likelihood of a lack of synchrony between water hyacinth and its biocontrol agents in infestation areas characterised by low temperatures, as the weed can recover from overwintering individuals, while the third instar larvae, which are the only life stages to successfully overwinter must pupate before the new generation adults can emerge. This asynchrony may offer an opportunity for a planned intervention with an integrated management strategy relying on application of low doses of glyphosate herbicide (Ueckermann and Hill, 2001; Wilson *et al*., 2006; Wright and Bourne, 1990).

Integrated control typically aims at integrating conventional means of control such as herbicide usage with biological control. Scientists originally assumed that classical biological control and chemical weed control techniques were incompatible (Harris, 1991). However, interactions between herbicides and biological control agents are not necessarily unfavourable, as evidenced from results presented in Chapter 2 of this thesis, which showed that the retardant dose of glyphosate based herbicide was not detrimental to the weevils in terms of feeding and reproduction capacities and their integration may result in improved weed biological control programmes, agreeing

with other published reports such as Harris, 1991; Lindgren *et al*., 1999; Messersmith and Adkins, 1995. Cullen (1996) described a method, termed 'ecological integration', in which weed biological control may be integrated with other methods of weed management. Ecological integration requires that biological control agent populations be preserved and complete eradication of the weed is usually not an objective. To implement an ecological integration method, information on (a) toxicity of herbicides to biocontrol agents (Chapter 2) and (b) season mediated phenology of the biocontrol agents and the weed (this chapter) are required. Therefore, the first objective of this chapter was to study the seasonal phenology of the weed and its biocontrol agent, *Neochetina* weevils, at 12 weed infested sites in South Africa, with differing climatic and nutrient conditions (Table 3.2).

3.1.1 Timing of herbicide sprays

Herbicidal applications, as part of an integrated management strategy are known to be effective only when the biocontrol agents are compatible with herbicide usage, and this compatibility depends on herbicide rates and timing of application, either seasonal or in relation to the phenologies of the weed and its biocontrol agents (Jacobs *et al*., 2000; Cullen, 1996).

In terms of timing of seasonal sprays, spring application of low doses of 2,4-D and picloram and 2,4-D combined with picloram did not affect *Urophora affinis* Frauenfeld (Diptera: Tephritidae) and *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae) (biocontrol agents released against spotted knapweed) larval or pupal mortality rates, and the herbicides did not interfere with the re-establishment of *Urophora* spp. (McCaffrey and Callihan, 1988; Jacobs *et al*., 2000). However, application of 2,4-D and clopyralid to *C. achates* during autumn resulted in larval mortality, owing to loss of habitat (Story and Stougaard, 2006). Conversely, studies carried out on *Aphthona* species (Coleoptera: Chrysomelidae), a biocontrol agent of leafy spurge conclude that the application of 2,4-D

and picloram herbicides during autumn did not interfere with the survival capacity and the overwintering fitness of the biocontrol agent (Nelson and Lym, 2003; Lym and Nelson, 2002), while a spring application of herbicides eliminated the adult food source due to desiccation of leafy spurge foliage resulting in poor establishment of the biocontrol agent (Lym and Nelson, 2002).

Herbicide timing relative to the phenology of the agent can also be crucial in limiting antagonism between herbicides and biological control. Application of herbicides at immature stages of insect development such as egg development and early larval growth can adversely affect insect survival (Messersmith and Adkins, 1995). For example, mortality of *Rhinocyllus conicus* Frol (Coleoptera: Curculionidae) thistle head weevil on musk thistle was greater when 2,4-D and dicamba were applied to plants within 48 hours of oviposition than when applied one to three weeks after oviposition (Trumble and Kok, 1979; Tipping, 1991). Application of herbicides such as Brush Off (sulfonyl urea), Starane (fluroxypyr) and Graslan (tebuthiuron) at the first and second larval instars stage of *Neurostrota gunniella* Busck (Lepidoptera: Gracillariidae) (a biocontrol agent released against *Mimosa pigra* L.) interfered with their development and emergence, while an application of herbicides at the sixth–eighth instar larval stages were considered optimal, as there were no significant difference in the proportion of *N. gunniella* that emerged from plants treated with herbicides and the control plants (Paynter, 2003). Applications of glyphosate and triclopyr amine herbicides to the third instar larval stage of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) did not affect the ability of the larvae to pupate to new generation adults (Lindgren *et al*., 1998, 1999).

Herbicide timing relative to the phenology of the weed can be critical for the establishment of biocontrol agents. For example, survival of *Urophora affinis*, *U. quadrifasciata* Meigen and *Agapeta zoegana* L. (Lepidoptera: Cochylidae), biocontrol agents of spotted knapweed were not compromised when sprayed with 2,4-D and clopyralid at the rosette stage of the weed, while herbicide sprays applied at the bud stage reduced the emergence of flies (Story *et al*., 1988; Story and Stougaard, 2006). Dicamba (0.5%) applied at the full bloom or senescence stage of *Carduus* thistle did not affect *R.*

conicus survival, whereas hexazinone (0.06%) reduced *R. conicus* survival when applied at all growth stages (bud, bloom, or senescence)(Tipping, 1991).

These examples conclude that proper timing of herbicidal intervention is crucial, either as seasonal applications; or in relation to the life cycle of the biocontrol agent; or in terms of phenology of the weed, to ensure that the bioagents survive and establish on the target weed. Appropriate timing ensures that a precipitous loss of habitat or food source does not occur, thereby providing the biocontrol agents enough time to complete their life cycle. Therefore, the second objective of this chapter is to test the seasonal effects of the retardant dose of glyphosate on water hyacinth and its biocontrol agents, *N. eichhorniae* and *N. bruchi* and delineate a spray regime which is beneficial for the biocontrol agents. Data gathered from this study will provide information on seasonal phenology of the weed and its natural enemies and will help advocate herbicidal sprays at an optimal time of the year/ season.

Environmental conditions under which plants grow significantly affect the activity and the effect of foliage applied herbicides (Kudsk and Kristensen, 1992). For example, plants grown under low humidity tend to have smaller leaves with thicker cuticles, more epicuticular wax and this might interfere with the interception, retention and penetration of the herbicide (Wanamarta and Penner, 1989). Weeds growing vigorously have been shown to absorb herbicide more readily (Legg, 1983) resulting in rapid movement of the herbicide along with the photosynthates away from the leaf, as a result of which the herbicide concentration gradient is maintained on both sides of the leaf, which is crucial for rapid and effective penetration of the herbicide (Muzik, 1976). At the time of herbicide spray and post herbicide application, herbicide retention, uptake and translocation is also affected by climatic conditions (Gerber *et al*., 1983). Pre- and post herbicide spray temperatures determine the efficacy of herbicides. For example, a study conducted on spruce seedlings by Lund- Hoie (1983) indicted that a high temperature of 24°C maintained for at least 2-3 days after application of glyphosate resulted in 77% of the seedlings showing

phytotoxic symptoms, conversely, low temperatures (12°C) resulted in spruce seedlings exhibiting tolerance to glyphosate.

In the case of water hyacinth, plant density declines with the advent of freezing temperatures in winter (Center *et al*., 1982), indicating poor growth conditions, which may impede the uptake of herbicides. Monsanto Pty. Ltd, South Africa, recommends that glyphosate herbicide should be ideally applied when the weed (water hyacinth) is growing vigourously, the wind speed does not exceed 10km/h (in order to avoid spray drift), temperature is between 15°C- 20°C and the relative humidity is around 60%. It is also recommended that the plants should not be sprayed under wet conditions or when damaged by frost. Therefore this study did not attempt to test the effects of the herbicide during the winter season.

3.2 Materials and Methods

3.2.1 Seasonal plant and insect phenology at sites with different climate and nutrient regimes

Water hyacinth sites monitored in this study (Table 3.2) were selected from over 3000 sites which were monitored for water quality by Resource Quality Services (Table 3.2) and characterised using Principal Components Analysis (PCA) (Byrne *et al*., 2010). Environmental variables such as daily maximum and minimum temperature, average number of days with frost, and altitude were used in the PCA. The sites chosen for this study combined the climatic (temperate and sub-tropical) and nutrient (eutrophic and mesotrophic) ranges over which water hyacinth grows in South Africa.

Additionally, continuous measurement of temperature (every 30 minutes) facilitated by temperature buttons, were recorded. Temperature measures of three microsites included water temperature and air temperature within the water hyacinth canopy,

which was facilitated by a floating buoy, and ambient air temperature. Daily maximum and minimum temperatures recorded were averaged and plotted on a monthly basis to give an indication of seasonal trends

Table 3.2: Water hyacinth infested field sites selected in this study for presentation of seasonal phenology data. These sites were selected from known water hyacinth monitoring sites in South Africa based on their climatic and nutrient characteristics (See Fig. 1.1). Climates are summarized as: Temperate (T); Sub tropical (ST). Nutrient status as described in Byrne *et al* (2010). KZN: Kwa Zulu Natal province.

Site Number	**Site Name**	**Province**	**Latitude**	**Longitude**	**Climate**	**Nutrient status**
1	Breede river	Western Cape	33° 18 ’S	20° 35 ’E	Temperate	Eutrophic
2	Crocodile river	Northwest	25° 39 ’S	27°47’E	Temperate	Eutrophic
3	Delta Park	Gauteng	26° 07 ’S	28°00’E	Temperate	Eutrophic
4	Farm Dam	Gauteng	26° 02 ’S	27°27’E	Temperate	Eutrophic
5	Feesgronde	Free State	26° 52 ’S	27°28’E	Temperate	Eutrophic
6	Hammarsdale	KZN	29° 48 ’S	30°39’E	Sub-tropical	Eutrophic
7	Mkadhzi spruit	Limpopo	23° 49 ’S	31°37’E	Sub-tropical	Mesotrophic
8	Nseleni	KZN	28°40’S	32°02’E	Sub-tropical	Eutrophic
9	New Years’ Dam	Eastern Cape	33°17’S	26°07’E	Temperate	Mesotrophic
10	Princess Vlei	Western Cape	34° 02 ’S	18°29’E	Temperate	Eutrophic
11	Warrenton Weir	Northern Cape	28° 07 ’S	24°56’E	Temperate	Eutrophic
12	Wolseley	Western Cape	33° 25 ’S	19°59’E	Temperate	Eutrophic

In order to gather data on insect and plant phenology, ten plants per site were randomly selected and plant growth parameters such as the number of ramets and leaves produced were counted and recorded, after which the plants were destructively sampled to record insect parameters such as number of weevils present, weevil feeding scars on the second youngest leaf, and petioles mined. These measures were used to infer adult and larval weevil populations at each site. Plant biomass was taken

from wet weight measures of above- water live plant material in three 0.5x0.5 m quadrats, removed from the water hyacinth mat. Both plant and insect data were gathered monthly for a period of 24 months.

A linear mixed effects model was used to delineate seasonal (autumn, winter, summer, and spring) differences in terms of plant and insect parameters between sites and the models were fitted to the data gathered in order to determine which factors were influencing the plant and insect response parameters. In this model, year 2006 was used as the base category for the year term and winter was used as the base category for the season term. The effects of these categories are contained in the intercept term. The parameter estimates for the remaining categories of year (2004 and 2005) and season (autumn: April, spring: September- October, and summer: January) represent the deviation away from the base category. The plant and insect response variables considered include the number of ramets, the number of leaves, above water biomass, number of petioles mined, the number of feeding scars and the number of weevil adults. These responses were modeled against parameters for time to account for the temporal changes in the responses. The predictor variables included in the analysis included temperature, season, year and month. The changes in insect and plant responses were not linear since a monthly time step was used. Therefore a polynomial for month needed to be included in the model which required a squared and cubed terms for month to account for the seasonality of both the plant and insect phonological responses, which would allow these responses to be cyclical through time. SAS (ver. 9.1) was used to fit the linear mixed effects models.

3.2.2 Seasonal effect of the retardant dose of glyphosate on water hyacinth and its biocontrol agents, *Neochetina eichhorniae* and *Neochetina bruchi*

Results from Section 3.3.1 indicate that water hyacinth plants reproduce during autumn and winter and the plants accumulate living biomass in spring. Hence this experiment was designed to test the seasonal effects of the retardant dose of herbicide

on water hyacinth and its biocontrol agents, the *Neochetina* weevils. Trials were carried out during the autumn (April) and spring (mid October) seasons of 2007. The methods described below apply to both the seasonal trials.

Two experimental pools (3 meter diameter) were maintained outdoors at the Weeds Division, Plant Protection Research Institute, Pretoria, South Africa. A plastic rope divided each pool into two equal sections. One section was sprayed with a concentration of 0.8% glyphosate at 140 L/ha (a.i. = 0.11 g m^{-2}). The other half was covered during spraying and formed the control. These pools were maintained as part of a mass rearing programme at Plant Protection Research Institute and thus contained a healthy population of adult weevils, *Neochetina eichhorniae* and *N. bruchi* at all developmental stages.

Six medium sized water hyacinth plants (for the autumn trial) and five water hyacinth plants (for the spring trial) per pool, per section were randomly selected and tagged. The plants were monitored weekly during the sampling period (four weeks for the autumn trial and three weeks for the spring trial).

A broad spectrum, glyphosate based herbicide, Roundup® (active ingredient isopropylamine salt (360g/L) with the surfactant polyethoxylated tallowamine (POAE), supplied by Monsanto Pty Ltd, South Africa, was sprayed at the above mentioned concentration using a knapsack sprayer (Multispray, South Africa) calibrated at 140L/ha, using Tee Jet nozzles (8003E) (Tee Jet Technologies, USA).

The following parameters were recorded on the tagged plants over a period of three and four weeks (spring and autumn seasons, respectively): total number of ramets and leaves, number of adult weevils, weevil instars, and petioles mined. Endpoint analysis, using student's t-test (STATISTICA, version 6; StatSoft, Southern Africa) was carried out on each of the parameters measured and the results were considered

significant at the 0.05 probability level between 0.8% herbicide sprayed and unsprayed plants.

3.3 Results

3.3.1 Seasonal plant and insect phenology at sites with different climate and nutrient regimes

The *P*-values in Table 3.3 indicate that temperature is a significant predictor of the number of ramets. Interestingly, the coefficient is negative, indicating that the number of ramets decreases with increasing temperature (Fig. 3.1). The month terms are significant, indicating that there is a cubic relationship between month and the number of ramets. The *P*-value for 2005 is significant, indicating that the number of ramets in 2005 was significantly different compared to 2006. The *P*-value for spring is significant, indicating that the number of ramets was greater in spring compared to other seasons.

Table: 3.3: Mixed effects linear model data for number of ramets at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	P value	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			2.697	0.656	473	4.11	<0.000	1.408	3.986
Temperature			-0.061	0.018	1633	-3.27	0.001	-0.097	-0.024
Month			1.078	0.222	2094	4.84	<0.000	0.641	1.514
Month_^{2}			-0.226	0.040	2089	-5.64	<0.000	-0.304	-0.147
Month_^{3}			0.011	0.002	2094	5.61	<0.000	0.007	0.015
Year		**2004**	-0.052	0.193	2100	-0.27	0.786	-0.432	0.327
Year		**2005**	-0.357	0.075	2100	-4.72	<0.000	-0.505	-0.208
Season	**Autumn**		-0.349	0.188	2090	-1.86	0.063	-0.718	0.019
Season	**Spring**		0.581	0.173	2090	3.35	0.000	0.240	0.921
Season	**Summer**		0.064	0.237	2100	0.27	0.786	-0.401	0.530

Temperature is a significant predictor of the number of leaves (Table 3.4), with a positive coefficient, indicating that the number of leaves increases as temperature increases (Fig. 3.2). The month terms are significant indicating that there is a cubic relationship between month and the number of leaves. The year term was not significant, indicating that there was no significant difference in the number of leaves from year to year. Autumn and spring have significant *P*-values indicating that the number of leaves in these seasons were significantly greater than summer.

Table: 3.4: Mixed effects linear model data for number of leaves at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	p value |	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			4.894	0.763	702	6.41	<0.000	3.395	6.393
Temperature			0.083	0.022	917	3.74	0.000	0.039	0.126
Month			0.854	0.270	2096	3.16	0.001	0.324	1.384
Month_^{2}			-0.175	0.048	2089	-3.61	0.000	-0.271	-0.080
Month_^{3}			0.008	0.002	2096	3.54	0.000	0.003	0.013
Year		**2004**	0.251	0.234	2083	1.07	0.284	-0.208	0.711
Year		**2005**	0.069	0.091	2081	0.76	0.449	-0.110	0.248
Season	**Autumn**		0.524	0.228	2090	2.30	0.021	0.077	0.972
Season	**Spring**		0.442	0.210	2091	2.10	0.035	0.029	0.855
Season	**Summer**		0.506	0.287	2095	1.76	0.078	-0.057	1.070

Mean monthly water temperatures recorded at 12 water hyacinth monitoring sites remained well over 9.5°C (Fig. 3.3) which is indicated as the threshold temperature for water hyacinth plant growth (Byrne *et al*., 2010), demonstrating that the leaf and ramet production were correlated to water temperatures.

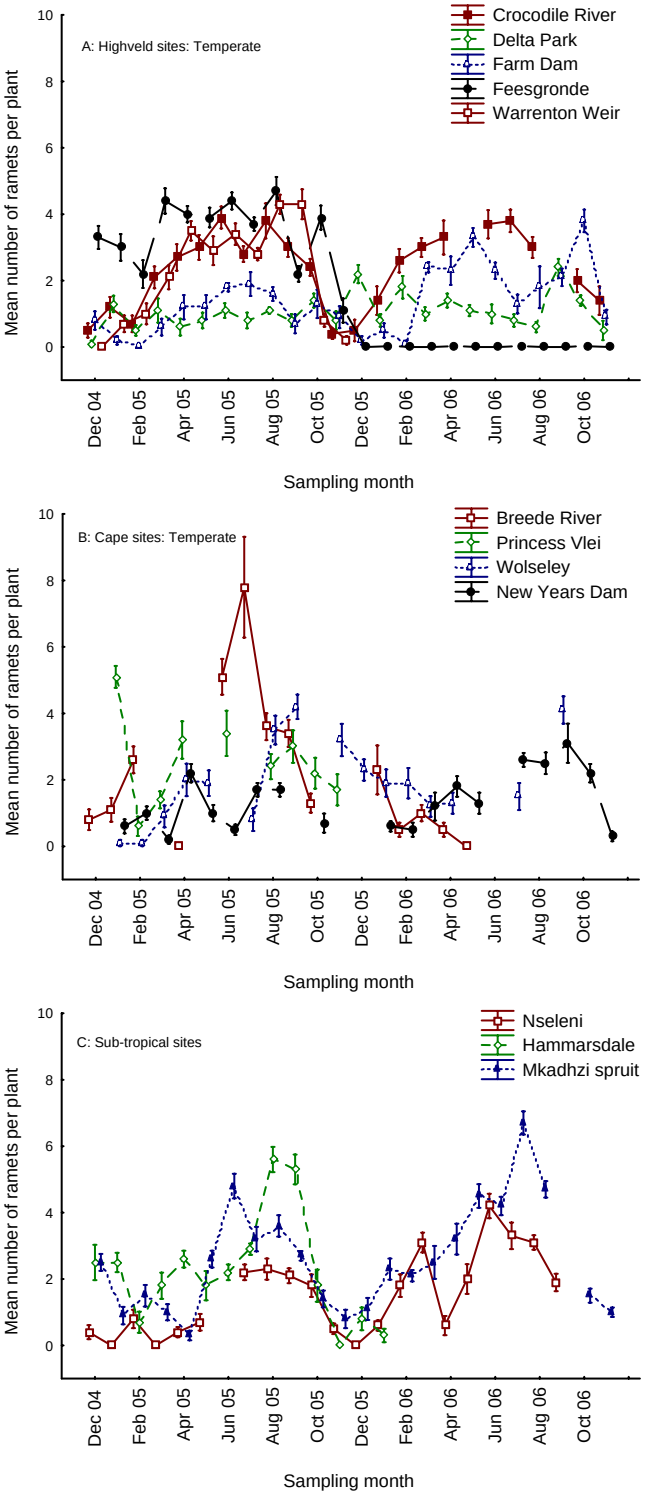

Figure 3.1: Water hyacinth ramet production at water hyacinth infested sites with different climatic (A: Highveld:Temperate; B: Cape: Temperate, C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

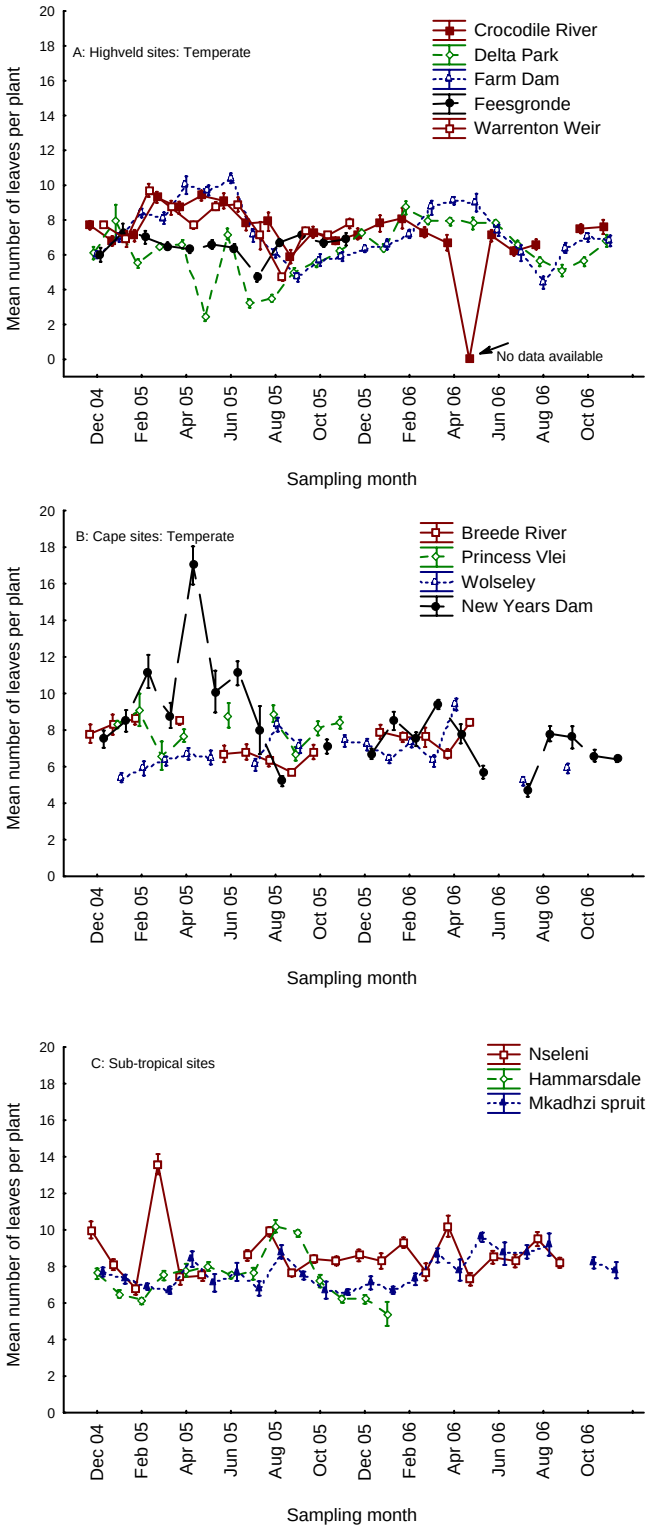

Figure 3.2: Water hyacinth leaf production at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

In terms of biomass response variable, the *P*-value for temperature (Table 3.5) indicates that it is not a significant predictor of biomass. The cubic term for month is significant, indicating a cubic relationship between month and biomass. The *P*-values for year are not significant. The season term are also not significant at the 5% level, and only summer has the smallest *P*-value of 0.05, indicating that the biomass in summer is greater from spring and autumn (Fig. 3.4).

Table: 3.5: Mixed effects linear model data for biomass at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	p value	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			2.610	0.933	261	2.79	0.005	0.771	4.449
Temperature			0.016	0.026	601	0.63	0.531	-0.034	0.067
Month			0.222	0.307	598	0.72	0.469	-0.380	0.826
Month_^{2}			-0.096	0.055	597	-1.75	0.080	-0.204	0.011
Month_^{3}			0.006	0.002	598	2.29	0.022	0.000	0.012
Year		**2004**	-0.088	0.277	602	-0.32	0.750	-0.634	0.457
Year		**2005**	0.061	0.105	602	0.58	0.560	-0.145	0.268
Season	**Autumn**		0.054	0.260	597	0.21	0.834	-0.457	0.567
Season	**Spring**		0.048	0.238	597	0.20	0.840	-0.420	0.517
Season	**Summer**		0.627	0.328	600	1.91	0.056	-0.017	1.271

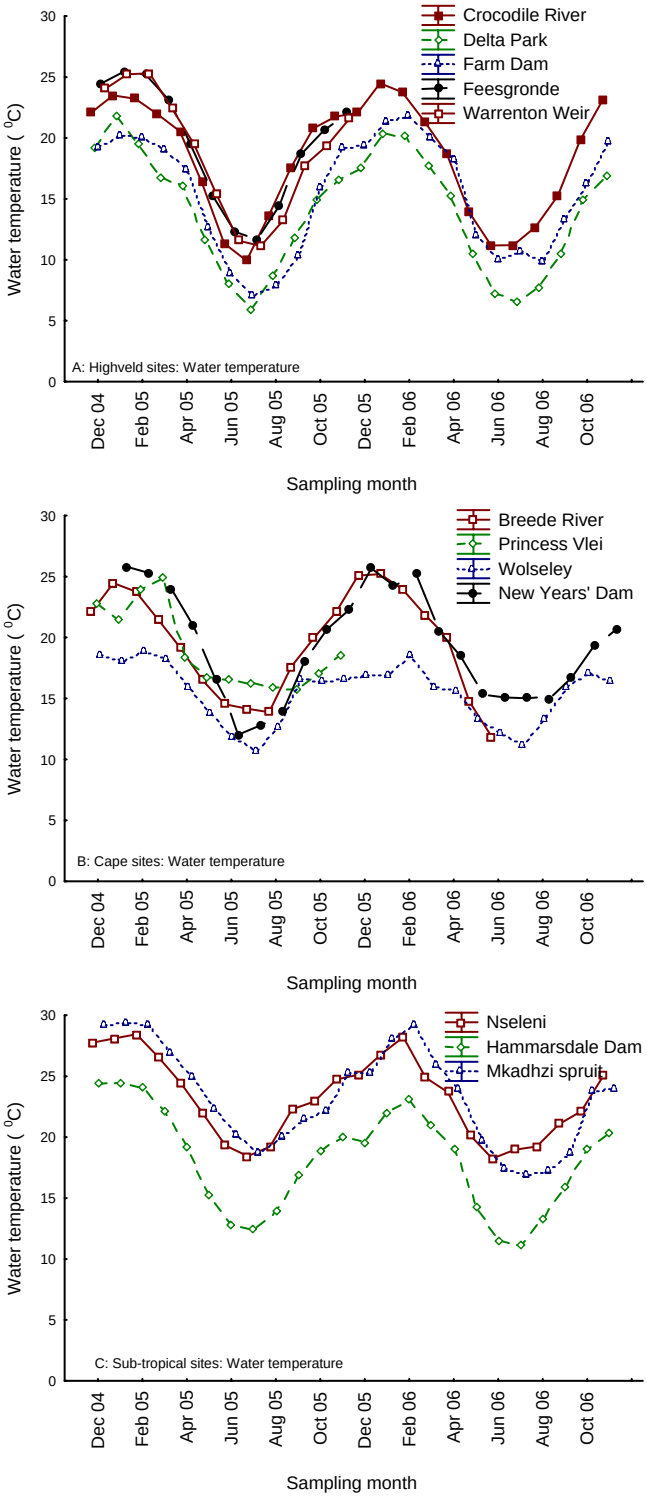

Figure 3.3: Mean monthly water temperature recorded at water hyacinth infested sites with different climatic (A: Highveld: Temperate; B: Cape: Temperate; C: Sub-tropical) regimes.

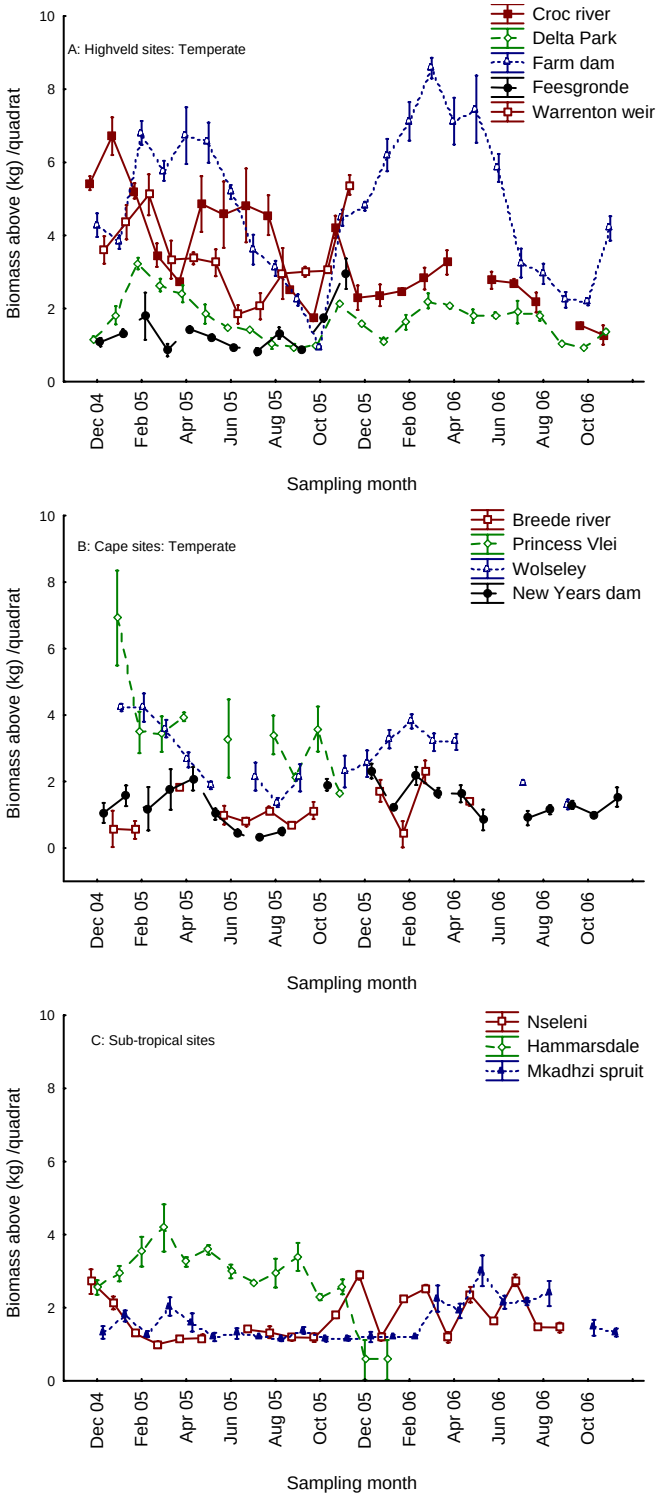

Figure 3.4: Water hyacinth biomass (above) production at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean.

Table 3.6 indicates that there is a significant temperature effect, with a positive coefficient, indicating that the number of adult weevils increases with increasing temperature. The squared and cubed terms for month were not significant, and so were excluded from the model, and only the linear term for month was included in the model analysis. The year factor was also not significant. Autumn gave a significant *P*-value, indicating that the number of adult weevils in autumn was significantly less compared to spring and summer (Fig. 3.5 and 3.6).

Table: 3.6: Mixed effects linear model data for number of weevil adults recorded at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	p value	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			-0.394	0.306	117	-1.29	0.201	-1.001	0.212
Temperature			0.038	0.014	1487	2.66	0.007	0.010	0.066
Month			0.023	0.011	2093	2.15	0.031	0.002	0.045
Year		**2004**	-0.051	0.173	2102	-0.30	0.766	-0.391	0.288
Year		**2005**	0.019	0.069	2098	0.28	0.777	-0.117	0.156
Season	**Autumn**		0.254	0.114	1974	2.23	0.026	0.030	0.479
Season	**Spring**		-0.030	0.108	2079	-0.28	0.780	-0.243	0.183
Season	**Summer**		0.055	0.123	2096	0.45	0.654	-0.187	0.298

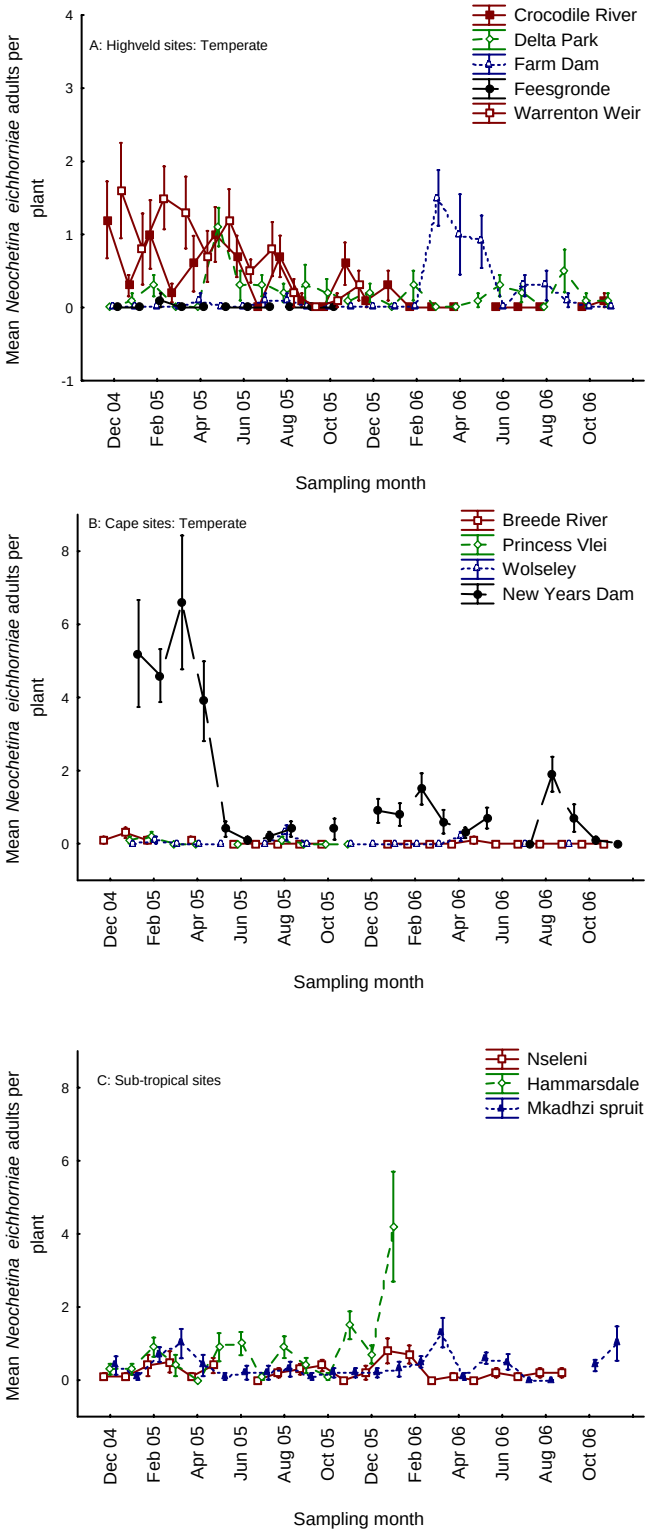

Figure 3.5: Mean number of *Neochetina eichhorniae* adults recorded at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

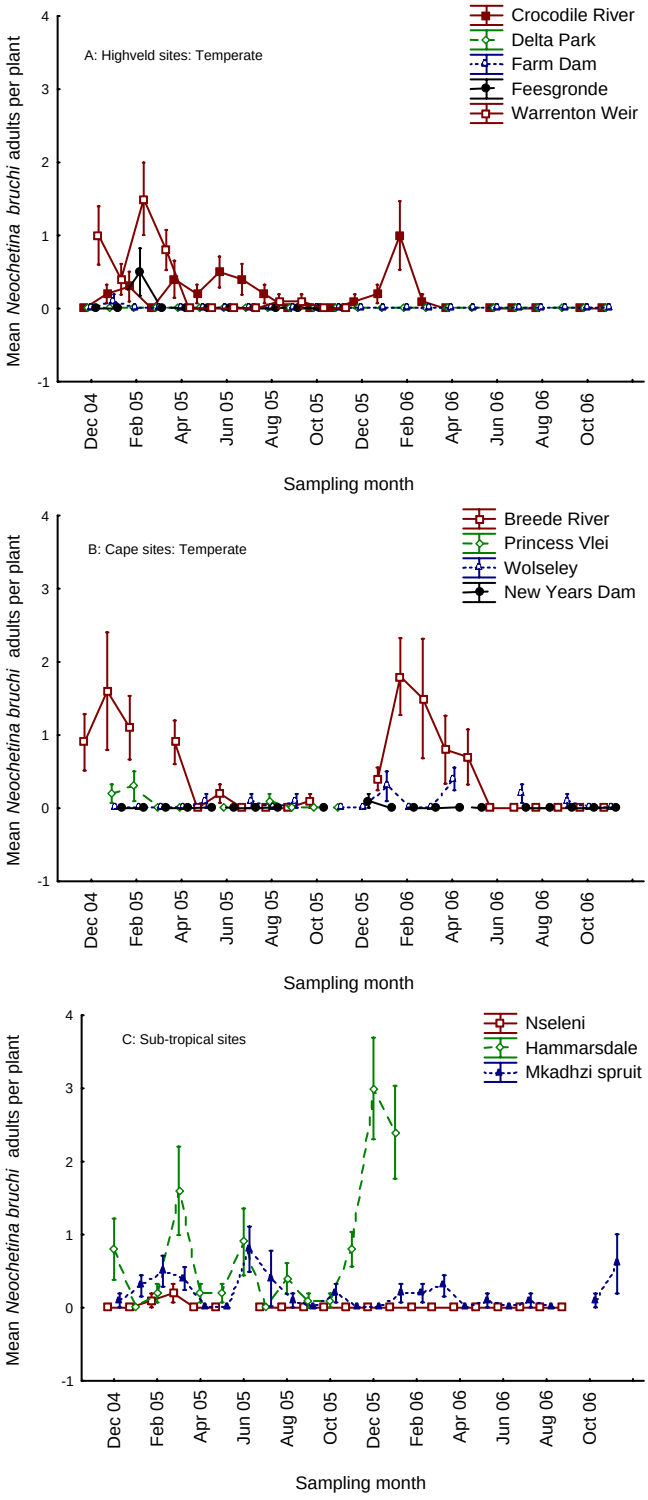

Figure 3.6: Mean number of *Neochetina bruchi* adults recorded at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

Results in Fig. 3.7 indicate that temperature has a significant effect on the mean number of feeding scars recorded and indicates an increasing trend. However, the negative coefficient of temperature in Table 3.7 indicates that the number of feeding scars decreases with increasing temperature (possibly because the number of leaves increases with increasing temperature, and hence the feeding scars are more dispersed, consequently, the number of feeding scars per unit area will be less). The cubic term for month is significant indicating that there is a cubic relationship between month and the number of feeding scars. The *P*-values for year are not significant. The *P*-values for summer and spring are significant, indicating that the number of feeding scars in these seasons is significantly greater than those recorded for autumn.

Table: 3.7: Mixed effects linear model data for number of feeding scars recorded at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	p value	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			77.378	14.619	262	5.29	<0.000	48.593	106.16
Temperature			-0.829	0.400	1940	-2.07	0.038	-1.615	-0.042
Month			-12.284	4.739	2080	-2.59	0.009	-21.578	-2.990
Month_^{2}			-0.216	0.851	2077	-0.25	0.799	-1.886	1.452
Month_^{3}			0.098	0.044	2080	2.23	0.025	0.012	0.185
Year		**2004**	-0.942	4.115	2087	-0.23	0.818	-9.012	7.128
Year		**2005**	-0.453	1.613	2087	-0.28	0.778	-3.617	2.710
Season	**Autumn**		-7.045	4.010	2078	-1.76	0.079	-14.909	0.818
Season	**Spring**		8.365	3.684	2078	2.27	0.023	1.139	15.590
Season	**Summer**		20.816	5.048	2085	4.12	<0.000	10.915	30.717

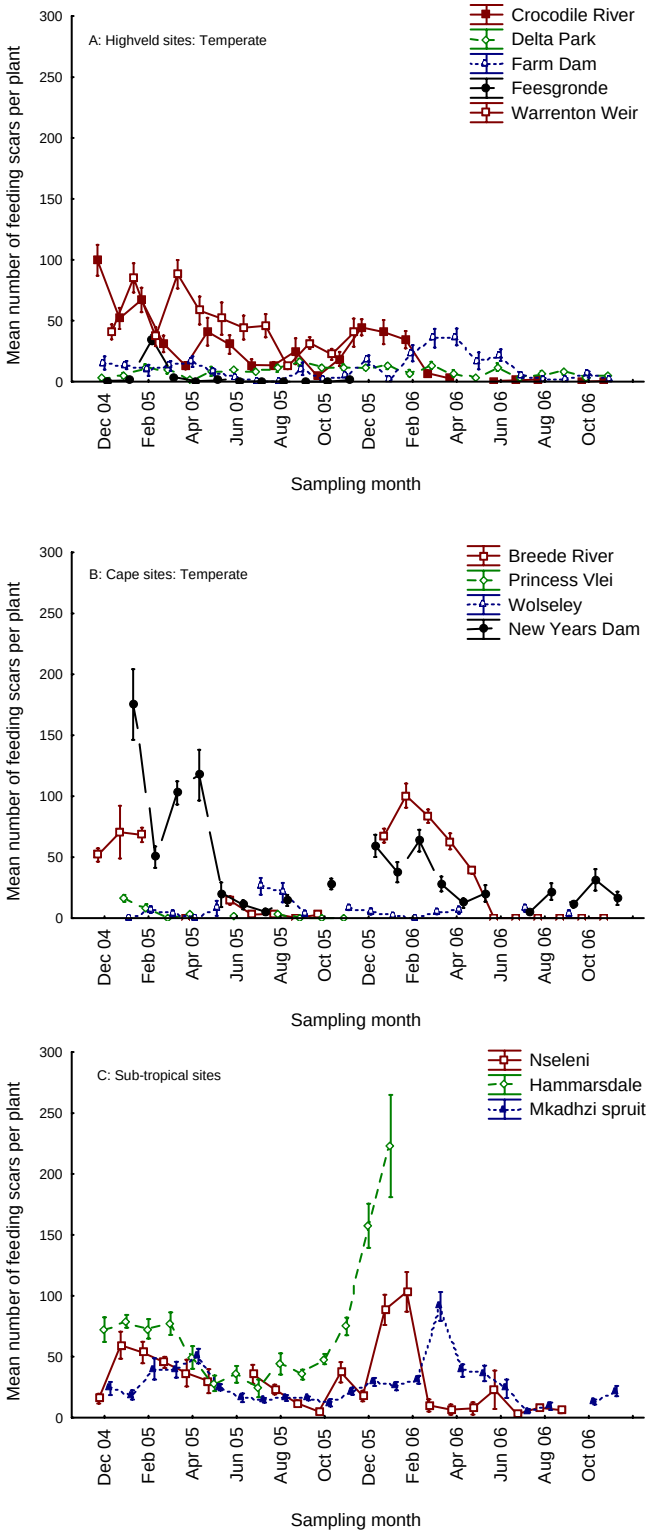

Figure 3.7: Mean number of *Neochetina* feeding scars recorded at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

Temperature is a significant predictor of the number of petioles mined (Table 3.8), with a positive coefficient, indicating that the number of petioles mined increases with increasing temperature. The squared and the cubed terms for month were not significant, and were excluded. Therefore, the model was run with only a linear term for month. The *P*-value for 2004 is significant, indicating that the number of petioles mined in 2004 was significantly greater compared to 2006. The *P*-value for spring and autumn are significant, indicating that the number of petioles mined in autumn is less than the petioles mined during spring and summer (Fig.3.8).

Table: 3.8: Mixed effects linear model data for number of petioles mined at 12 water hyacinth sites in South Africa. DF: degrees of freedom.

Effect	Season	Year	Estimate	Standard Error	DF	t Value	p value	Lower 95% confidence limit	Upper 95% confidence limit
Intercept			0.519	0.497	24.5	1.04	0.306	-0.506	1.546
Temperature			0.051	0.015	2095	3.27	0.001	0.020	0.082
Month			0.022	0.011	2091	1.90	0.058	-0.000	0.046
Year		**2004**	0.824	0.186	2094	4.41	<0.000	0.458	1.191
Year		**2005**	0.082	0.075	2096	1.10	0.273	-0.065	0.230
Season	**Autumn**		0.505	0.124	2102	4.06	<0.000	0.261	0.749
Season	**Spring**		-0.300	0.117	2099	-2.55	0.010	-0.531	-0.069
Season	**Summer**		-0.016	0.133	2097	-0.13	0.899	-0.279	0.245

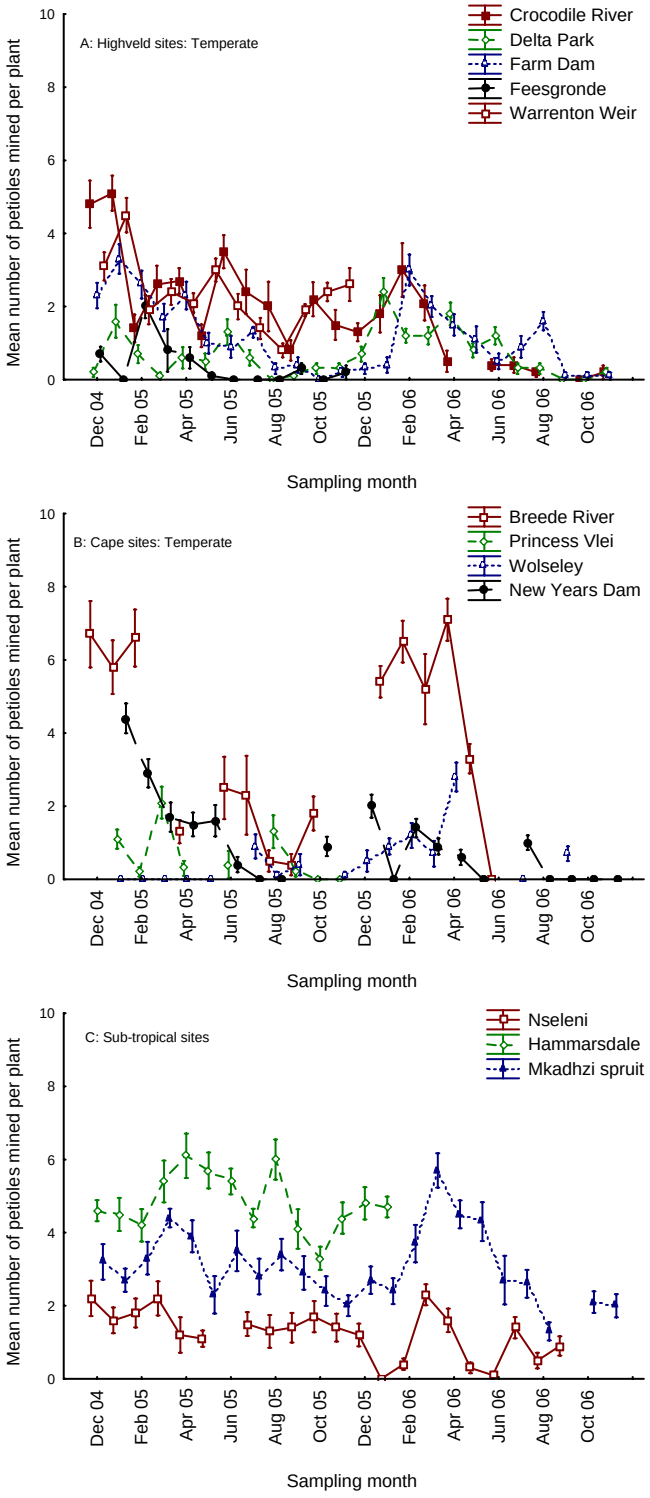

Figure 3.8: Mean number of petioles mined recorded at water hyacinth infested sites with different climatic (A: Highveld sites: Temperate; B: Cape sites: Temperate; C: Sub-tropical) regimes. Error bars = standard error of the mean. (Mean of 10 plants ±SE).

3.3.2 Seasonal effect of the retardant dose of glyphosate on water hyacinth and its biocontrol agents, *Neochetina eichhorniae* and *Neochetina bruchi*

Application of a 0.8% glyphosate herbicide to water hyacinth plants in a semi-field experiment resulted in reduced number of ramets and leaves produced, corroborating the results from Chapter 2 of this study. Furthermore, the low dose did not kill the adult weevils or the immature instar stages of *Neochetina* weevils. Both the seasonal spray regimes (autumn and spring) tested in this study interfered with the reproductive capacity of the water hyacinth plants, but did not have any negative effects on the weevil adult and instar populations.

End point analysis using a student's t-test showed that the mean number of ramets produced by sprayed plants, during autumn (2.9±0.25 SE) and spring (1.2±0.35 SE) were significantly lower (autumn: t_{22}= 2.916; *P*= 0.008; spring: t_{18}= 6.77; *P*= 0.000) than the mean number of ramets produced by the unsprayed, control plants (autumn: 5.1±0.72 SE; spring: 4.1±0.23 SE (Figs 3.9 A and B respectively).

In addition, the mean number of leaves produced by the sprayed plants in autumn (12.5±0.25 SE) and spring (7.3±0.39 SE) were significantly fewer (autumn: t_{22}=2.22; *P*= 0.03; spring: t_{18}=3.28; *P*= 0.004) compared to the unsprayed, control plants (autumn: 14.8±0.9 SE; spring: 9±0.33 SE) (Figs. 3.10 A and B).

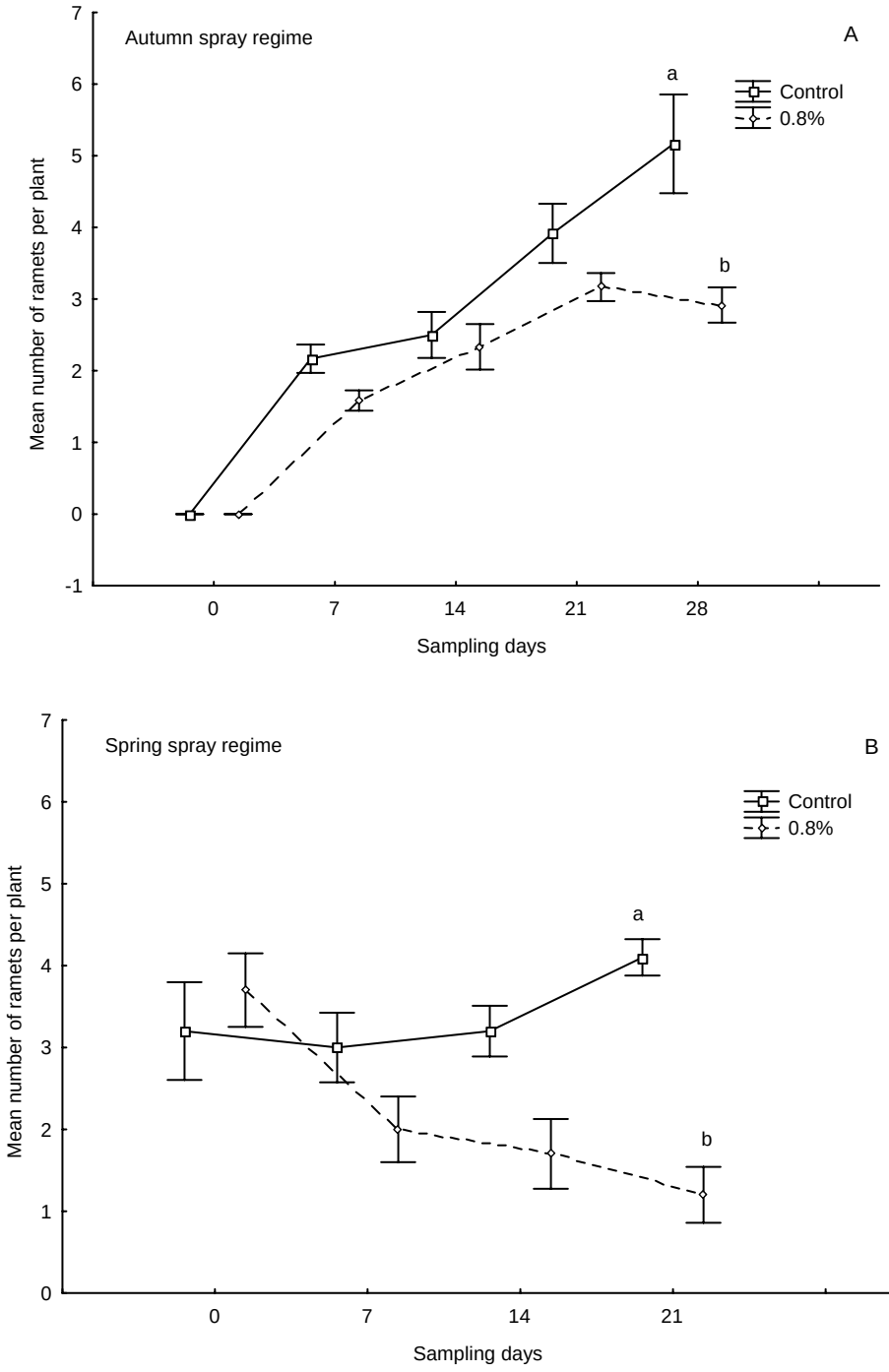

Figure 3.9: Mean number of ramets on water hyacinth plants with *Neochetina eichhorniae* and *N. bruchi* weevils and sprayed with 0.8% glyphosate herbicide at 140L/ha during autumn (A) and spring (B). Error bars = standard error of the mean. Means followed by the different letters indicate significant differences between sprayed and control plants at $P< 0.05$.

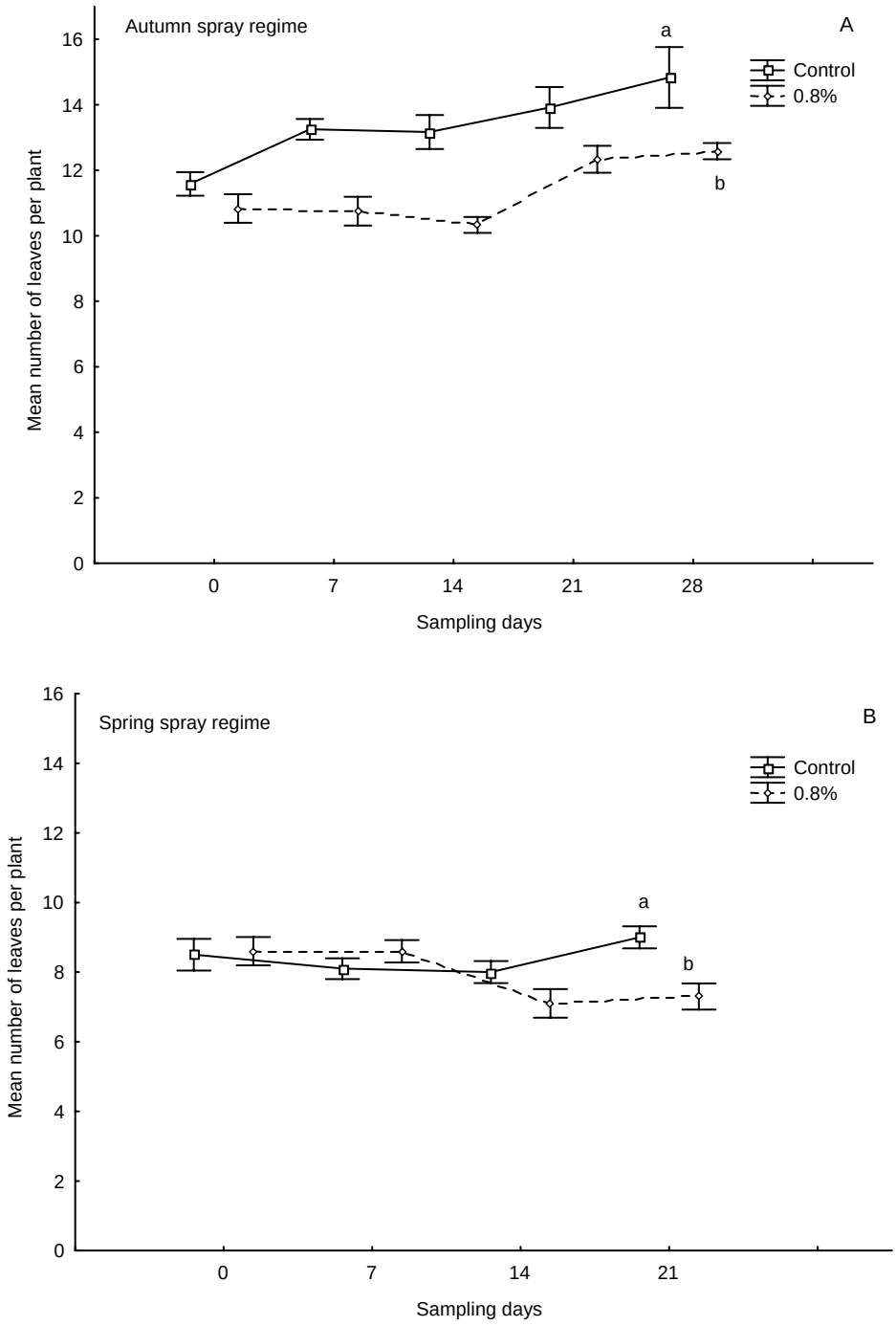

Figure 3.10: Mean number of leaves produced by water hyacinth plants with *Neochetina eichhorniae* and *N. bruchi* weevils and sprayed with 0.8% glyphosate herbicide at 140L/ha during autumn (A) and spring (B). Error bars = standard error of the mean. Means followed by the different letters indicate significant differences between sprayed and control plants at $P< 0.05$.

There were no significant differences between the mean numbers of weevil larvae found in the 0.8% sprayed plants compared to the unsprayed, control plants at the end of the autumn (control: 0.9±0.35 SE; sprayed: 0.75±0.21 SE) or spring season (control: 0.2±0.20 SE; sprayed: 0.8±0.41 SE) (autumn: t_{22}= 0.397, *P*= 0.69; spring: t_{18}= -1.29, *P*= 0.21) (Figs. 3.11 A and B).

There was no significant difference between the mean number of petioles mined on sprayed (4.41±0.62 SE) and control plants (4.08±0.57 SE) during autumn (t_{22}= -0.395, *P*= 0.69). However, the mean number of petioles mined during spring was significantly higher in the sprayed plants (3.2±0.24 SE) compared to the unsprayed control plants (1.8±0.29 SE) (t_{18}= -3.65, *P*= 0.001) (Figs. 3.12 A and B).

There was no significant difference between the mean number of adult weevils found on sprayed and unsprayed plants during autumn (t_{22}= 0.61, *P*= 0.54). The mean number of adult weevils on sprayed and unsprayed plants was 2.5± 1.18 SE and 3.6±1.4 SE respectively (Fig. 3.13 A).

During spring, low numbers of adult weevils were found on the sprayed (0.6±0.22 SE) and unsprayed plants (0.8±0.41 SE) following recovery from winter frost, compared to the mean number of adults found during autumn. However, there was no significant difference between the mean numbers of adults found on the sprayed and unsprayed plants (spring: t_{18}= 0.42, *P*= 0.67) (Fig. 3.13 B).

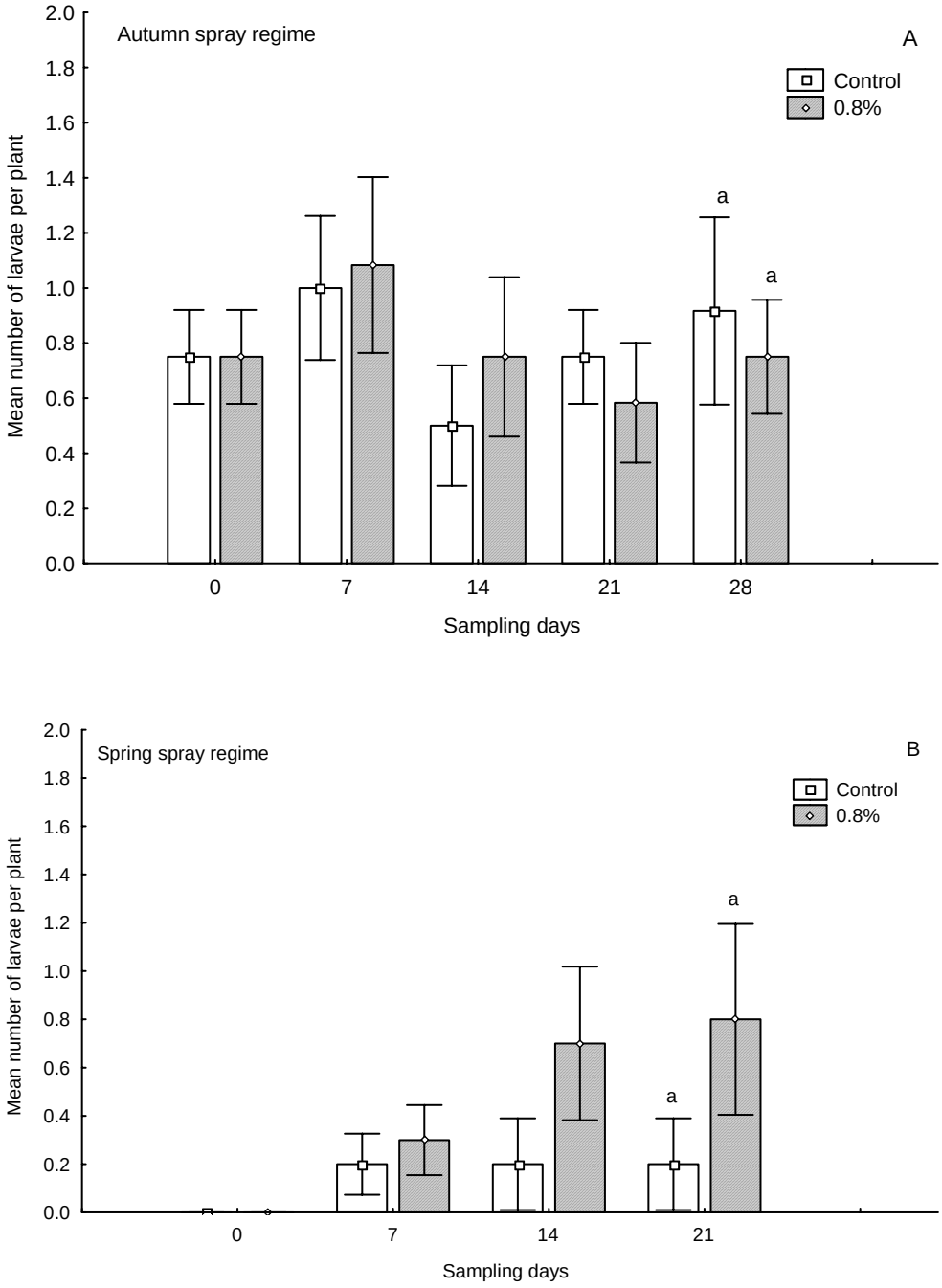

Figure 3.11: Mean number of larvae found on water hyacinth plants containing *Neochetina eichhorniae* and *N. bruchi* weevils and sprayed with 0.8% glyphosate herbicide at 140L/ha during autumn (A) and spring (B). Error bars = standard error of the mean. Means followed by the different letters indicate significant differences between sprayed and control plants at $P< 0.05$.

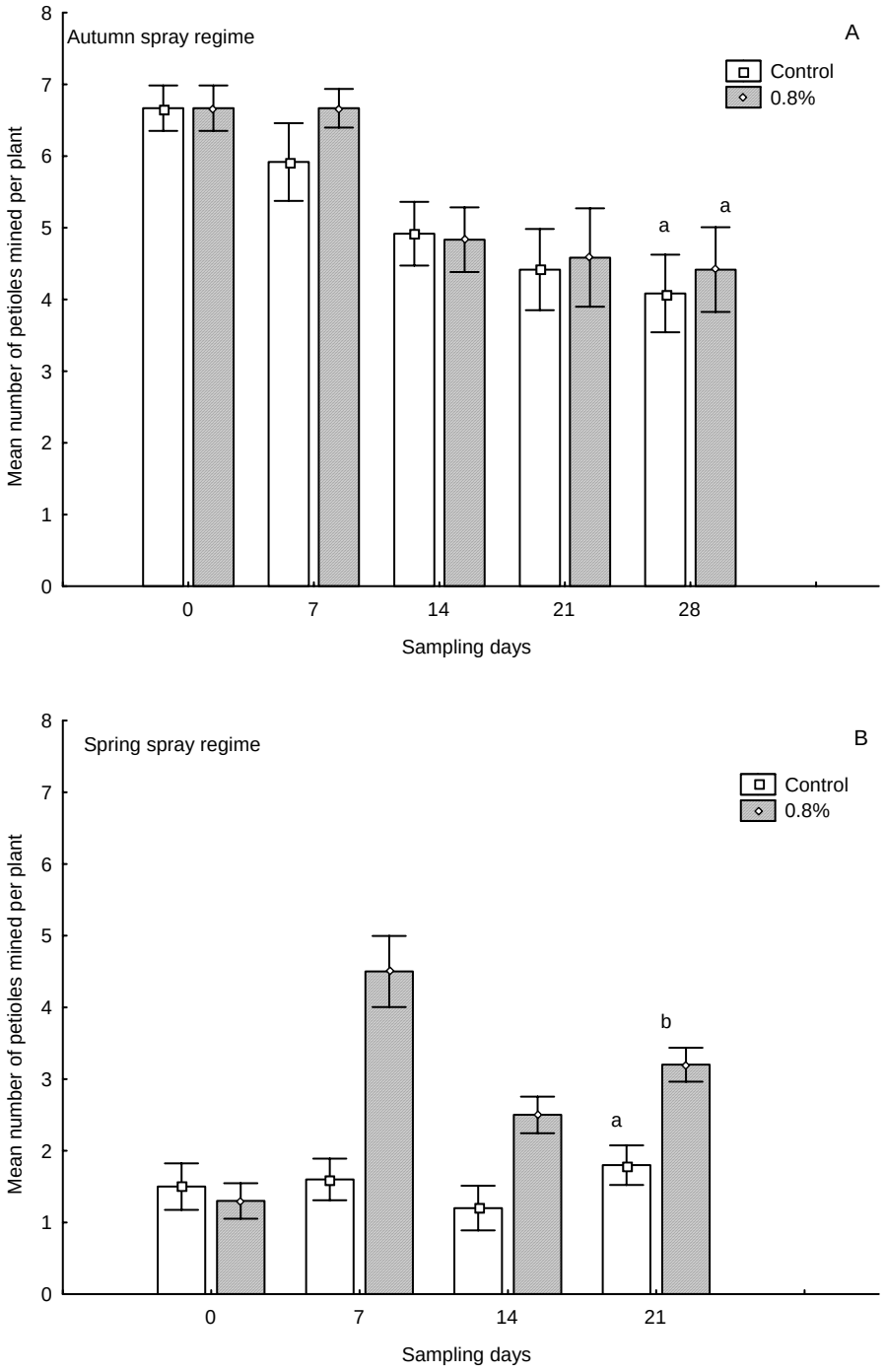

Figure 3.12: Mean number of petioles mined in water hyacinth plants containing *Neochetina eichhorniae* and *N. bruchi* weevils and sprayed with 0.8% glyphosate herbicide at 140L/ha during autumn (A) and spring (B). Error bars = standard error of the mean. Means followed by the different letters indicate significant differences between sprayed and control plants at $P < 0.05$.

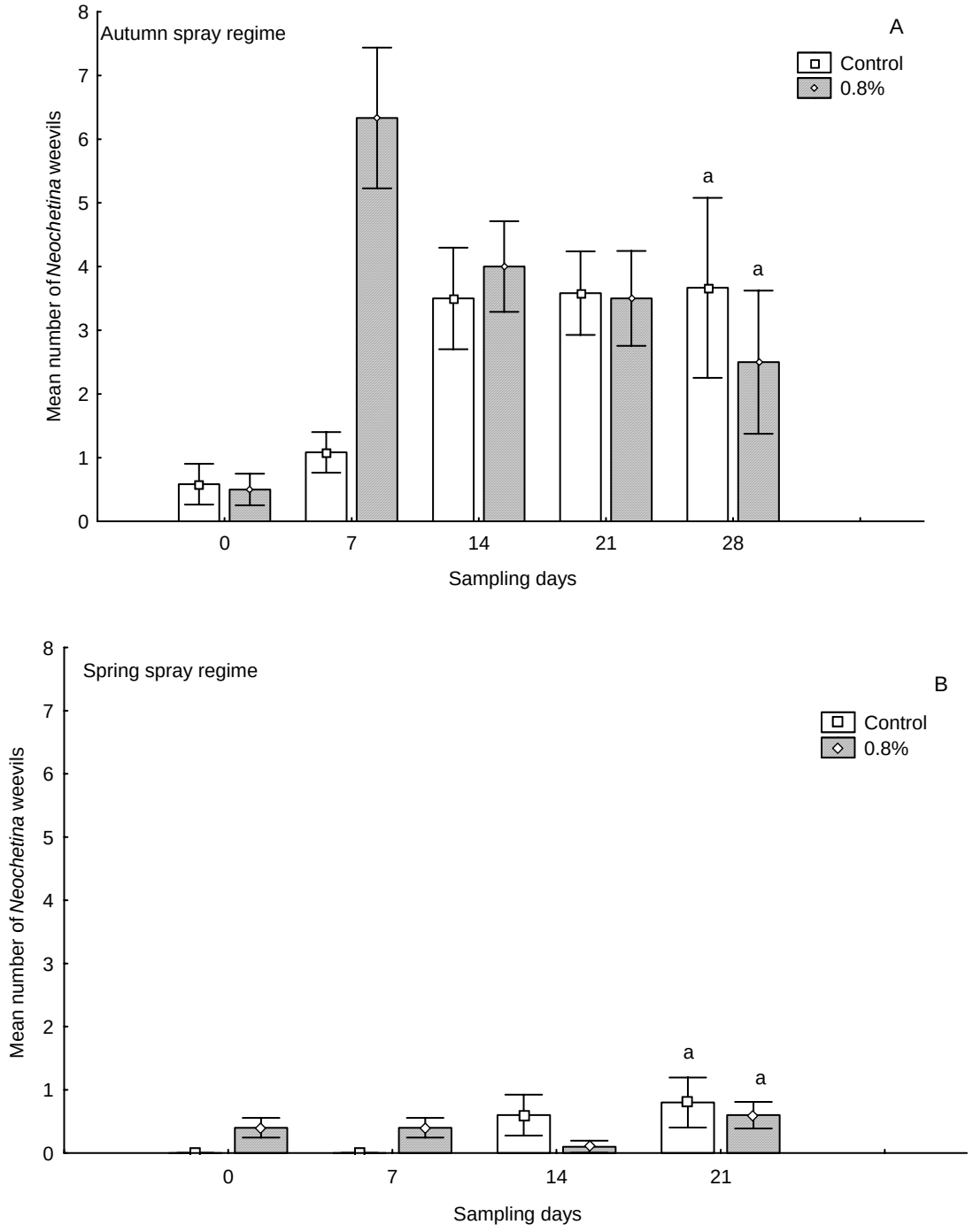

Figure 3.13: Mean number of *Neochetina* adult weevils found on water hyacinth plants sprayed with 0.8% glyphosate herbicide at 140L/ha during autumn (A) and spring (B). Error bars = standard error of the mean. Means followed by the different letters indicate significant differences between sprayed and control plants at $P< 0.05$.

3.4 Discussion

Successful integrated control requires an understanding of the ecology of the target weed (McFadyen, 1998) and the biocontrol agent. However, a variety of ecoclimatic factors result in differing natural enemy and target weed ecologies, which leads to seasonal asynchrony which in turn, allows a weed to escape early season regulation so that it reaches damaging levels before control agents can accumulate in sufficient numbers to have any measure of impact (Muller *et al*., 1990). Results from this chapter show limited, seasonally mediated growth patterns between the weed and its most important natural enemy, *Neochetina eichhorniae* at all the 12 field sites (with differing climate and nutrient regimes) monitored. However, the increase in ramets, leaves and biomass were not consistent between the years.

At both the temperate and subtropical sites, water hyacinth plants reproduced asexually by production of ramets during autumn leading into winter, a trend also recorded by Center and Spencer (1981) in North-Central Florida Lake, United States. At all the sites monitored in this study, the water temperatures during winter season remained well over 9.5°C -10°C, which is indicted as the growth threshold for plant growth (Gopal, 1987, Byrne *et al*., 2010). With the onset of summer, plants accumulated new living biomass.

Increase in ramet reproduction is also thought to be triggered by increased light penetration through the plant canopy (Methy and Roy, 1993) and reduced plant density (Center and Spencer, 1981). Light is a major morphogenetic factor in water hyacinth populations (Richards and Lee, 1986). Plant canopy shade reduces the photosynthetic photon flux density (PPFD) and the ratio of red to far-red light (z) (Richards and Lee, 1986; Morgan, 1981). These effects cause clonal plants such as water hyacinth to increase potential for light acquisition and interception by producing new ramets in less shaded microsites (Methy *et al*., 1990). Center and

Spencer (1981) found that with a decline in the total daily solar radiation associated with autumn and winter seasonal regimes, the average size of the plants also decreased resulting in the dense canopy 'opening up' allowing for light availability which prompted them to conclude that "ramet production is contingent upon light penetration beyond the uppermost leaves and is not able to be sustained under a dense high mono-layered canopy". Highveld winters in South Africa result in frosting and low water temperatures as a consequence of which, small cohorts of plants are thought to be lost, thereby causing a decrease in plant biomass (Byrne *et al*., 2010) which results in thinning of the dense canopy. Moreover, leaf loss due to frost results in the dense canopy being lost, allowing for efficient light penetration, which triggers ramet production.

The numbers of leaves produced at both the temperate and subtropical sites during spring were consistent with the observations made by Center (1980), who recorded eight leaves per plant during spring season. However, during autumn season, about nine leaves per plant were recorded at the sites monitored in this study, compared to six leaves per plant, recorded by Center (1980). The production of leaves is thought to be triggered by favourable temperatures during and post winter (Center and Spencer, 1981). As solar radiation increases, the plants respond by drawing on stored energy reserves in the rhizome, as a result of which, reapportioning of the biomass distribution occurs (Center and Spencer, 1981).

The mixed effects model data indicates that the biomass during summer was significantly greater than either spring or winter. Center and Spencer (1981) indicate that with increasing temperatures, water hyacinth plants respond by producing new leaves. This causes crowding and intense competition among plants for light and space as a result of which water hyacinth plants respond by increasing their petiole lengths to better intercept available light. This elongation of petioles and production of new leaves adds to biomass increase.

The weevil populations at both the temperate and subtropical sites were susceptible to low temperatures, as evidenced by adult numbers and feeding scars. The population growth of the weevils depends on reproductive ability, reflected as the percentage of reproductive females in the population (Center and Dray, 2010). In this study, weevil numbers lagged behind summer increases in plant biomass and showed no response to winter mediated ramet production at either site. Low weevil numbers recorded could be attributed to the low reproductive ability of the weevils during autumn and winter (Byrne *et al*., 2010), agreeing with Grodowitz *et al*., (1997) who found that the percentage of weevils with functioning ovaries was low during late autumn and early winter. The number of ovarian follicles was minimal during winter and attained maximal levels during spring before rapidly decreasing.

A recent study by Center and Dray (2010) concludes that temperature is not the only force driving populations of *Neochetina* weevils and that plant quality as determined by Nitrogen content should also be taken into consideration. Minimal tissue N concentrations needed for positive population growth is about 3% for *N. bruchi* and about 2% for *N. eichhorniae* (Center and Dray, 2010). Results from analysis of plant tissue (leaf and crown samples) by Katembo (2010) indicate that the nitrogen content in leaf samples at two Highveld sites, Delta Park and Farm Dam were 3.5% and 5% respectively. Although these values are greater than the threshold values indicated by Center and Dray (2010), very low weevil numbers were recorded at these sites. These observations suggest that the reproductive biology of *N. eichhorniae* and *N. bruchi* and hence, its numbers in the field is governed by both temperature and plant quality.

A low number of petioles mined at all the water hyacinth infested sites reveal that the immature stages of the weevil are susceptible to cold and frosted winters, as a result of which, the larval populations that survive the winter are very low and consequently, the weevil population goes through a bottleneck each winter. Grodowitz *et al*., (1991) found that with the onset of winter, the deterioration of water

hyacinth plants, in terms of decline in total biomass and low number of living leaves, resulted in reduction in numbers of *N. eichhorniae* pupae and larvae.

Reduced larval survival at all water hyacinth sites is attributed to a decrease in available larval habitat area and changes in the nutritional status of the plant. Only third instar larvae, normally occupying the crown (DeLoach and Cordo, 1976a,b) and thus spared from the ill effects of frost and low temperatures, are able to successfully overwinter in meaningful numbers, and therefore be able to contribute to a post winter weevil population. Thus, the water hyacinth plants free from early season herbivory and aided by ramet production through winter are able to recover quickly from winter and outpace the effects of weevil herbivory well into the growth season. Therefore, prioritising herbicide intervention using a retardant dose of glyphosate herbicide during these periods (autumn and spring) should delay early season growth and accumulation of increasing biomass, respectively, and allow it to overlap with higher levels of weevil mediated herbivory during the start of summer in the new season.

Semi-field application of the retardant dose of glyphosate (0.8%) during autumn and spring seasons interfered with the reproductive and the vegetative growth of the weed, largely mirroring the results obtained in Chapter 2.

Both the seasonal spray regimes did not interfere with the reproductive capacities of the weevils as evidenced by the larval counts, corroborating with several demonstrated cases where optimal timing of application of herbicides did not affect insect herbivores used as biological control agents of different terrestrial invasive plant species (McCaffrey and Callihan, 1988; Jacobs *et al*., 2000; Nelson and Lym, 2003; Lym and Nelson, 2002).

Results from this chapter also show that the herbicide sprays applied during autumn and spring did not impede larval feeding, as evidenced by petiole mines. Continued

larval feeding on the crown of water hyacinth prior to pupation, is believed to reduce the production of leaves or ramets or both (Grodowitz *et al.*, 1991) as the meristematic tissues are damaged.

Both the seasonal spray regimes did not kill the adult *Neochetina* weevils and this bodes well for biocontrol, because herbivory during autumn, particularly, hinders the ability of the plants to store carbohydrate reserves before winter months as a result of which, spring re-growth is slowed down (Center and Spencer, 1981).

Very low numbers of adult weevils, however, were recovered from both sprayed and unsprayed plants during spring. This is not surprising because very few adults would have survived through winter. A spray application at this point in time will retard the plants, but will not kill any remaining adults, which can now oviposit and contribute toward building up the next generation. However, it should be noted that female beetles which have overwintered as adults will have a lower reproductive capability than newly emerged females that overwintered as third instar larvae (Byrne *et al*., 2010).

3.5 Conclusion

Results from this chapter show that in temperate and subtropical climatic regimes of South Africa, water hyacinth plants do not remain dormant during winter, but produce ramets and accumulate living biomass during warmer summer months. However during winter, as the temperature declines, the number of new *Neochetina* weevils (in the form of eggs) being introduced into the weevil population and the number of surviving adults will fall. This population decline results in fewer weevils available to exert herbivory pressure on water hyacinth populations. With the onset of warmer temperatures (summer), the weevil population will re-establish itself from older females that have overwintered, or much later, from third instar larvae that have

completed development, pupated then emerged and become sexually mature. This is biologically advantageous for the weevils but disadvantageous for biocontrol due to asynchrony between the reproduction of the plant and the weevils. This asynchrony however, offers an opportunity for a tactical intervention with glyphosate herbicide in autumn, when plant reproduction can be halted without suppressing beetle numbers, and a second spray in spring to freeze new plant growth, which will allow the new season's (summer) adults to persist and produce a F_1 generation, which would suppress further plant growth, as the weed recovers from the herbicide.

Before advocating the use of herbicides, particularly glyphosate, which has garnered bad press for its non-target ecological impacts (Relyea, 2005 a,b,c), it is important to test its effects on non-target organisms such as amphibians. Therefore, the primary aim of Chapter 4 was to discern if low dose of glyphosate has a detrimental effect on the larvae of a local amphibian species.

Chapter 4

Effect of a retardant dose of glyphosate on *Xenopus laevis* larvae

4.1 Introduction

4.1.1 Glyphosate

Glyphosate is currently the worlds' most widely applied agrochemical, used to control terrestrial and aquatic weeds (Baylis, 2000). The introduction of the first glyphosate based herbicide, Roundup® by Monsanto in the early 1970s has resulted in "freeing millions of people from the drudgery of manual weed control" (Baylis, 2000).

The glyphosate molecule was first synthesised in 1970 by Henri Martin of a small Swiss pharmaceutical company (Cilag) but was not tested or patented for herbicidal use, until John E. Franz of Monsanto synthesised and tested glyphosate as a herbicide for commercial purposes in 1974 (Franz *et al*., 1997) and it was patented for herbicide use (Grossbard and Atkinson, 1985). Glyphosate is anionic at physiological pH levels and is active as a salt with various cations (for example, the sodium or isopropylamine salts) (Duke and Powles, 2008). It is often combined with a surfactant, polyethoxylated tallowamine (POEA) (a mixture of polyethoxylated long chain alkylamines synthesized from animal-derived fatty acids; Gisey *et al*., 2000) to facilitate active penetration of the herbicide into the leaf cuticles.

The mode of action of glyphosate is unique in that it is the only molecule that is highly effective at inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway in plants (Duke and Powles, 2008).

Glyphosate is a transition state analog of phosphoenylpyruvate, one of the substrates for EPSPS. Inhibition of EPSPS leads to reduced feedback inhibition of the pathway, resulting in massive carbon flow to shikimate-3-phosphate, which is converted to high levels of shikimate (Duke, 1988). The high levels of shikimate that rapidly accumulate in glyphosate treated plant tissues was the clue that led Amrhein and his co-workers to discover EPSPS as the molecular target site of glyphosate (Steinrucken and Amrhein, 1980). Though, how glyphosate induced inhibition of the shikimate pathway actually kills the plants is not entirely clear, researchers believe that insufficient aromatic amino acid production necessary to maintain protein synthesis is the primary effect (Duke and Powles, 2008). Other researchers have produced evidence to support the view that the increased carbon flow to the shikimate pathway by deregulation of the pathway by inhibiting EPSPS results in shortages of carbon for other essential pathways (Siehl, 1997). The rapid cessation of carbon fixation in glyphosate treated sugar beet is better explained by this mechanism than by reduction in aromatic amino acid pools (Servaites *et al*., 1987).

4.1.2 Environmental profile

The fate of glyphosate in aquatic environments is an important consideration. Glyphosate is completely biodegraded in water by bacteria such as *Pseudomonas* sp strain PG 2982 or *Flavobacterium* sp strain GD1 (Balthazor and Hallas, 1986). One of the major metabolites of this biodegradation is aminomethylphosphonic acid (AMPA) which is non toxic (Balthazor and Hallas, 1986). Glyphosate also rapidly dissipates from both flowing and standing surface waters within a period of few days to two weeks (Gisey *et al*., 2000). Dissipation is usually aided through processes such as adsorption to particulate matter or sediments. Glyphosate herbicides are effective only when applied directly to the plant surface, and residual glyphosate that enters the soil is essentially unavailable to the plants due to its very high affinity to soil (Gisey *et al*., 2000). The half lives of glyphosate and POEA are 7 to 70 days and 21 to 28 days respectively (Giesy *et al*., 2000). The maximum concentration of glyphosate

recorded in a water body is 3.7mg active ingredient (a.i)/L (Giesy *et al*., 2000) and in natural habitats, Roundup® has been detected at concentrations of 0.1 to 2.3 mg a.i/L (Relyea, 2005 c). An eco-toxic risk assessment carried out by Gisey *et al*., (2000) for Roundup® concluded that glyphosate does not bio-concentrate in fish or other animals and is safe for use in aquatic habitats. However, the authors conclude, on a cautionary note, that aquatic organisms and amphibians are likely to be subjected to very high concentrations of Roundup® as a result of drift and run-off, resulting in mortality. Factors such as degradation and sorption potential of the herbicide, and its interception by target vegetation may mitigate any potential detrimental effects to aquatic fauna.

4.1.3 Glyphosate based herbicides and their eco-toxic effects

Amphibian declines are of huge concern because, as Reeder *et al*., (2005) surmise, "these species are important grazers, prey species and predators in aquatic and terrestrial ecosystems and serve as valuable sentinels of ecologic integrity". Recent studies have shown that amphibian recruitment and species richness maybe significantly reduced due to coverage by submerged macrophytes, and by the use of pesticides and herbicides (Nystrom *et al*., 2007). Additionally, combinations of factors such as pH, UV light, temperature and predator induced stress can affect the lethality of the herbicides, thereby causing mortality of amphibians (Relyea, 2003; Zaga *et al*., 1998; Lohner and Fischer, 1990). Exposure to herbicides not only alters population dynamics of the amphibians, it also alters community dynamics due to differences in species-specific mortality rates (Smith, 2001). In terms of susceptibility of developmental stages of test organisms, larval amphibians have been found to be more sensitive to herbicides than their embryos or adults. Reasons for such disparity include lack or insensitivity of target organs and/or protection offered by the presence of the perivitelline membrane in embryos (Edington *et al*., 2004).

Numerous studies have investigated the LC_{50} (Lethal concentration of herbicide required to kill 50% of the test population) effects of label recommended rates of glyphosate formulations on larval amphibians. For example, studies carried out by Mann and Bidwell (1999) on variety of taxa of amphibians indicate that the LC_{50} values for Roundup® ranged from 3.9 to 15.5mg a.i/L (LC_{50} at 48 hours) for four species of Australian tadpoles while LC_{50} values (at 96 hours) for *Xenopus* larvae L. were 12.4mg a.i/L (Perkins *et al*., 2000). Lajmanovich *et al*., (2003) surmised that LC_{50} value (at 48 hour) for a formulation of glyphosate based herbicide, GLYFOS, tested on South American tadpoles, is 1.74 mg a.i/L. Relyea (2005 a,b) concluded that concentrations of Roundup® ranging from 1.3 mg a.i/L to 3.8mg a.i/L severely compromised the survival capacity of several species of North American frogs and he further estimated that the 16-day LC_{50} value for tree frogs was 1.4 mg a.i/L and 2.5 mg a.i/L for American toads and leopard frogs. However, it is important to note that Relyea (2005a,b) used the commercial form of Roundup® called Roundup Weed and Grass that contained POEA surfactant. In United States of America, this formulation is registered for turf use and not for aquatic use. Therefore his results are not consistent with the results of most other studies discussed in this chapter. Edington *et al*., (2004) estimated LC_{50} value (at 96 hours) for glyphosate based Vision herbicide to be 1.5- 4.7mg a.i/L). These studies implicate the surfactant, POEA to be the cause for the toxicity of glyphosate based formulations. Wide-ranging LC_{50} values derived from these studies can be attributed to the duration of the studies, formulations of glyphosate tested which includes the nature of the surfactant used, and the sensitivity of the amphibian populations used in the study. While most studies implicate POEA alone for the toxicity of Roundup®, a study by Tsui and Chu (2003) implicated both the isopropyl amine salt (IPA) of glyphosate and POEA. They concluded that organisms such as algae, which are photosynthetic and possess similar metabolic pathways to higher plants (e.g. aromatic amino acids synthesis), were therefore also susceptible to the herbicidal effect of IPA salt of glyphosate.

The toxicity of surfactants varies with temperature, pH, species and the developmental stages of the animal exposed (Howe *et al*., 2004). For example, the

glyphosate herbicides Vision and Roundup Original and its surfactant MON 0818 show high toxicity at elevated pH levels to rainbow trout and *X. laevis* embryos (Edington *et al*., 2004). MON 0818 is a tertiary amine blend with one fatty alkyl group and two polyoxyethylene groups attached to a nitrogen atom which presumably is responsible for the weak base interaction with pH (Edington *et al*., 2004). Gill uptake and accumulation of MON 0818 surfactant occurs readily at high alkalinities.

Non ionic surfactants such as POEA exhibit their negative effects by disrupting the respiratory surfaces of aquatic organisms (Lindgren *et al*., 1996). For example, they interfere with gill morphology of tadpoles causing lysis of epithelial cells leading to asphyxiation or loss of osmotic stability (Partearroyo *et al*., 1991). Several non ionic surfactants (i.e nonyl- and octylphenols) may also act as endocrine disrupting compounds (EDCs). These EDCs have various endocrine and reproductive effects, such as: mimicking effects of endogenous hormones such as oestrogen and androgen, antagonizing the effects of normal, endogenous hormones, altering the pattern of synthesis and metabolism of natural hormones and modifying hormone receptor levels (Routledge and Sumpter, 1996; Aneck-Hahn *et al*., 2005).

Acute and chronic toxicity of various glyphosate based formulations are often tested on amphibian species because of their dependence on aquatic sites for reproduction and early development which makes them susceptible to toxic exposure to herbicides. Amphibians are also ideal test organisms to detect the effects of EDCs due to their typical aquatic larval development and hormonally dependent metamorphosis and sexual differentiation (Howe *et al*., 2004). Parameters such as survival, growth, or reproduction are routinely used to characterize the toxicity of Roundup® (Gisey *et al*., 2000). No studies to date have tested the effects of a retardant dose of glyphosate on *Xenopus laevis*. Hence, the aim of the present study is to test the effects of a low or retardant dose of glyphosate based formulation Roundup® which has been developed in this study, on *Xenopus laevis* (more commonly known as the Platanna or the African clawed frog) larval growth and survival capacity. *Xenopus laevis* is an ideal test organism because of its fecundity and the ability to obtain embryos throughout

the year. Unlike other amphibians with annual breeding cycles, *Xenopus laevis* can be induced to breed throughout the year by intraperitoneal injection of human chorionic gonadotrophin (HCG) (Zhanfen and Xiaobai, 2006). In addition, *Xenopus* occurs throughout South Africa (Passmore and Carruthers, 1995; Channing, 2001) and is entirely aquatic. It is expected that the retardant dose, either sprayed directly or intercepted by vegetation, in this case, by water hyacinth plants, will not affect the survival capacity of the larvae.

4.2 Materials and Methods

4.2.1 Animal care

Housing and husbandry of *Xenopus laevis* adult frogs and larvae were done under the supervision of the Central Animal Services (CAS) of The University of the Witwatersrand and care and treatment of the test animals were in accordance with the guidelines of the Animal Ethics Committee of The University of the Witwatersrand. Experimental procedures used were cleared by the Animal Ethics Screening Committee of the University of the Witwatersrand under the following animal ethics number: 2008/58/2A.

Xenopus laevis mating and ovulation was induced by injecting a single priming dose of 300 i.u. (i.u: international unit) of Folligon (Intervet South Africa Pty. Ltd) into the dorsal lymph sac of ten adult, female *X. laevis*. Two days later, a second dose of 750 i.u) human chorionic gonadotropin (Adcock Ingram Ltd, South Africa) was injected in order to induce egg laying. Ten males were injected with 200 i.u. human chorionic gonadotropin on the day of the females' second dose, for gonadotropin stimulation. Males and females were then paired up and placed in 10 litre polythene breeding tanks filled with dechlorinated water. Tanks were fitted with wire grating held approximately 30 mm off the bottom of the tank to allow fertilised eggs to drop through. Amplexus, egg laying and fertilization occurred within 24 h of injections, in

a darkened room. After amplexus, the frogs were removed from the breeding tanks and the eggs were allowed to hatch. Tadpoles used for the experiment were at Gosner-stage 25 in their development (Gosner, 1960).

4.2.2 Experimental design

Trials were carried out in October, 2007, outdoors at the University of the Witwatersrand, Johannesburg, South Africa. A total of nine tubs (50L plastic tubs with a diameter of 52 cm containing 42L of water) were set up containing five medium- sized water hyacinth plants each (henceforth referred to as WH). The tubs were sub- divided into two herbicidal treatments: WH 0.8% (retardant dose) and WH 3% (lethal or full dose) with active ingredient values (a.i.) (gm^{-2}) of 0.11g and 0.41g respectively. Three replicates were used for each herbicide treatment. Three tubs were established as control treatments and were not sprayed with glyphosate (WH 0%). A second experimental set-up without water hyacinth plants (henceforth referred to as NWH) was established for herbicidal treatments, 0.8% (NWH 0.8%) and 3% (NWH 3%). Three replicates were established as control treatments and were not sprayed with glyphosate (NWH 0%). Five tadpoles were released into each of the experimental tubs at week 0 (five tadpoles per tub, therefore, three tubs contained 15 tadpoles, n=15, unless otherwise indicated). The tadpoles fed on algae inoculated into the water from a mature pond.

Glyphosate based herbicide, Roundup® (active ingredient, 360g/L glyphosate (acid equivalent a.e) /L, containing 480g isopropylamine salt of glyphosate/ L) with the surfactant polyethoxylated tallowamine (POAE), supplied by Monsanto Pty. Ltd. South Africa, was sprayed onto the experimental tubs at the above spray dosages (week 0), using a knapsack sprayer (Multispray, South Africa) calibrated at 140L/ha, using Tee Jet nozzles (8003E) (Tee Jet Technologies, USA).

The experiment ran for a total of three weeks, with week one designated as week 0 on Figs. 4.1 and 4.2. On a weekly basis, five tadpoles were collected from each tub and fixed using 4% alcohol. The body lengths of the tadpoles were measured using a clear ruler. At the end of the experiment (week 2), remaining tadpoles were collected using a sieve, and were measured and counted.

One-way ANOVA and a Student t-test (STATISTICA program, version 6, StatSoft, Southern Africa) were used to test the effects of herbicide concentrations and water hyacinth on the survival and body lengths of the tadpoles and the means obtained were considered significant at the 0.05 probability level.

4.3 Results

4.3.1 Survival of tadpoles

At week one, significantly fewer tadpoles were collected from WH 0.8% and WH 3% treatments compared to NWH 0.8% and NWH 3% treatments (0.8%: $t_4 = 2.75$, $P = 0.05$; 3%: $t_4 = 2.75$, $P = 0.05$) (Fig. 4.1).

Fewer live tadpoles were collected from the NWH 3% treatment (2.66±1.45 SE) at week two, but not significantly less than the NWH 0% (5±0.0 SE) and NWH 0.8% (5±0.0 SE) treatments ($F_{(2,6)} = 2.57$, $P = 0.15$) (Fig.5.1). No live tadpoles were found in WH treatments (0%, 0.8% and 3%) at week 2 (Fig. 4.1).

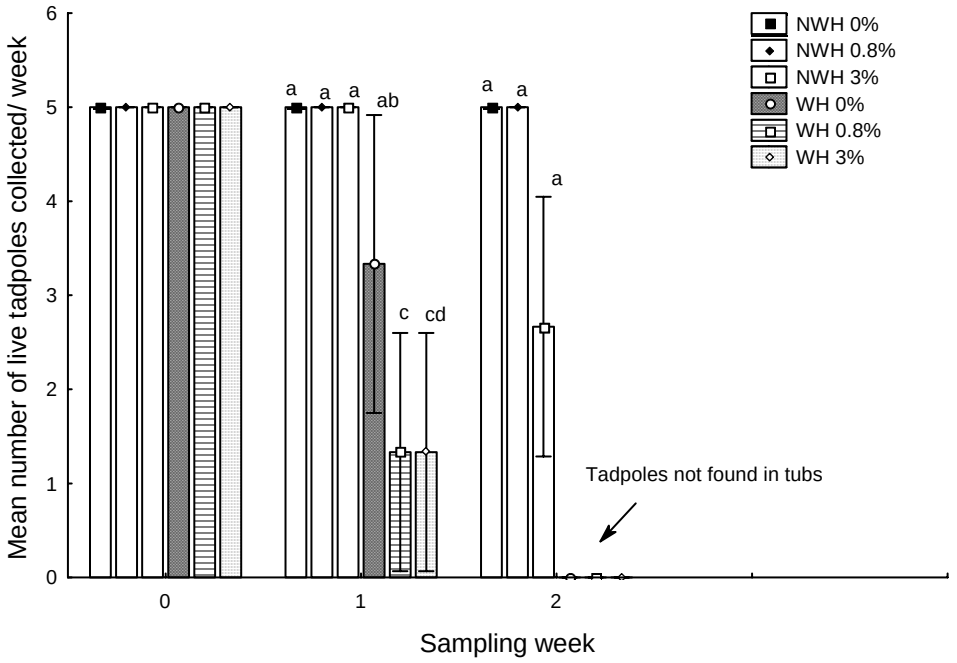

Figure 4.1: Mean number of live tadpoles collected from tubs treated with 0%, 0.8% and 3% glyphosate herbicide. NWH: water hyacinth plants absent from tubs; WH: water hyacinth plants present in the tubs. Error bars = standard error of the mean. Different letters indicate significant differences between treatments at P< 0.05.

4.3.2 Body lengths

NWH treatments:

At week one, there were no significant differences between the mean body lengths of the tadpoles collected from the NWH 0% (1.69±0.08 SE), NWH 0.8% (1.65±0.06 SE) and NWH 3% (1.54±0.09 SE) ($F_{(2,42)} = 0.98$, $P = 0.38$) (Fig.4.2).

At week two, a significant difference in mean body length (shorter) was noted for NWH 3% treatment (1.62±0.42 SE) compared to NWH 0% (3.54±0.13 SE) and NWH 0.8% (3.77±0.09 SE) ($F_{(2,42)} = 20.22$, $P = 0.000$).

There were no significant differences between the mean body lengths of NWH 0% and NWH 0.8% treatments (t_{28}= -1.35, *P*= 0.18) (Fig.4.2).

WH treatments:

The WH treatment (WH0%) showed significant differences in mean body lengths measured at week one, compared to WH 0.8% and WH 3% treatments ($F_{(2,42)}$ = 2.80, *P*= 0.07) (Fig. 4.2). The mean body lengths measured were as follows: WH 0% (0.69±0.13 SE), WH 0.8% (0.29±0.12 SE) and WH 3% (0.31±0.14 SE).

There were no significant differences between the mean body lengths of WH 0.8% and WH 3% treatments (t_{28}= 0.39, *P*= 0.69) (Fig.4.2).

No live tadpoles in any WH treatments (WH 0%, WH 0.8% and WH 3%) were found at week two. Hence no body length measurements could be made.

NWH vs WH treatments:

Significant differences in mean body lengths were noted at week one between NWH (0%, 0.8% and 3%) and WH treatments (0%, 0.8% and 3%) [(0%: (t_{28}= 6.48, *P*= 0.00); 0.8%: (t_{28}= 9.52, *P*= 0.00); 3%: (t_{28}= 7.33, *P*= 0.00)].

At week two, live tadpoles were not found in tubs containing water hyacinth (WH 0%, 0.8% and 3% treatments). Hence, comparisons could not be made between NWH and WH treatments.

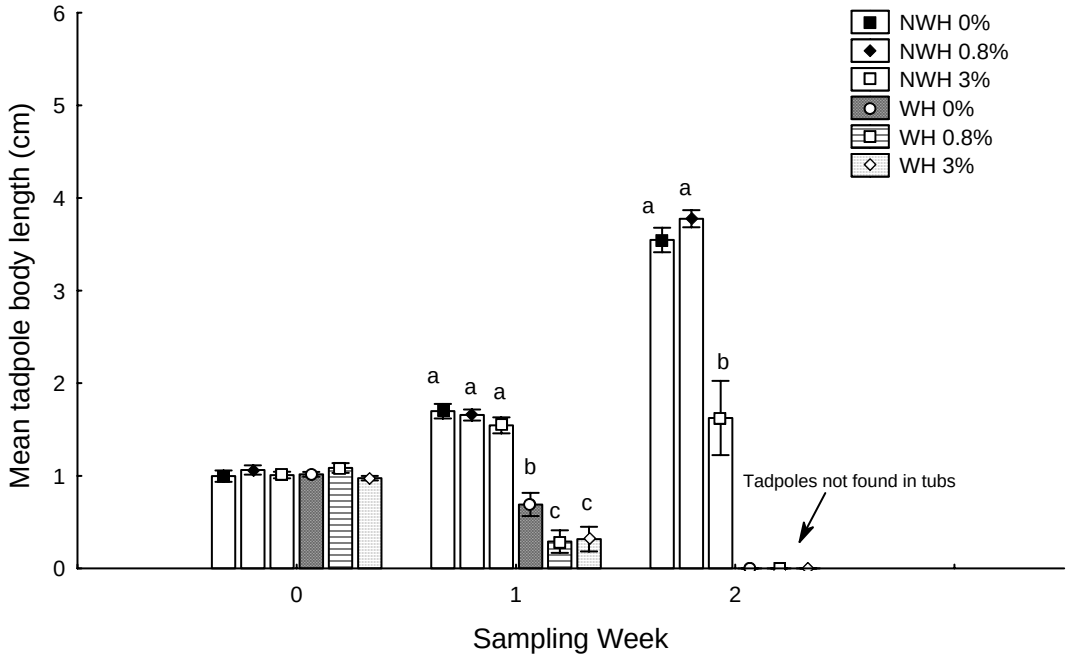

Figure 4.2: Mean (± SE) body lengths of live tadpoles collected from tubs treated with 0%, 0.8% and 3% glyphosate herbicide. NWH: water hyacinth plants absent from tubs; WH: water hyacinth plants present in the tubs. Error bars = standard error of the mean. Different letters indicate significant differences between treatments at $P < 0.05$.

4.4 Discussion

Direct effects of herbicides on non-target plants and organisms are expected consequences of weed management programmes (Gisey *et al*., 2000). This study indicates that water hyacinth, alone or coupled with an application of glyphosate herbicide, is potentially lethal to aquatic amphibians. All *Xenopus* larvae died in the treatments containing water hyacinth, regardless of whether they were unsprayed, or sprayed with a retardant dose or a lethal (to the plant) dose of glyphosate. The detrimental effects of alien weeds on insects has been documented for terrestrial weeds (Kinvig and Samways, 2000), and water hyacinth has been shown to suppress aquatic invertebrate fauna in South Africa (Midgley *et al*., 2006; Jones, 2009). Canopies of aquatic macrophytes influence the amount and quality of habitat available to aquatic organisms (Frodge *et al*., 1990). Water hyacinth canopy results in

lower dissolved oxygen (DO), higher water temperatures and lower pH (Perna *et al*., 2005; Ultsch, 1973).Therefore, mortalities of anurans observed in WH treatments could also be due to depressed levels DO within the water. This study, under laboratory conditions, has shown for the first time that an invasive aquatic weed was more lethal to an aquatic vertebrate than the herbicide advocated for its control. Significant mortality, however, was not recorded for the 0.8% herbicide treatment devoid of water hyacinth (NWH0.8%). Only 47% of the inoculated tadpoles were collected from tubs treated with a label recommended dose (3%) (NWH3%) of glyphosate corroborating the findings of several authors who conclude that the label recommended application of the formulations of glyphosate such as Roundup® "Weed and Grass killer" are toxic to several species of aurans such as *Rana pipiens*, *Rana sylvatica*, *Bufo americanus*, *Scinax nasicus*, tree frogs, toads, leopard frog tadpoles (Relyea, 2005 a, b, c; Relyea *et al*., 2005; Lajmanovich *et al*., 2003; Howe *et al*., 2004). The conclusions drawn from these studies were that Roundup® reduced survival of the amphibians, while Roundup Original (MON 78078) formulation and another formulation of glyphosate, GLYFOS® (containing 48% GLY as isopropylamine salt and POEA surfactant) resulted in 100% mortality of the test species. Additionally, glyphosate has been shown to reduce phytoplankton population (Hildebrand *et al*., 1980), which is a food source for filter feeders such as tadpoles. Therefore, the effect of glyphosate in this trial could have been direct toxicity or indirect via the food source.

Water hyacinth affects water bodies by lowering the temperature, bicarbonate alkalinity and dissolved oxygen content and increasing the pH, free carbon dioxide content, biological oxygen demand (BOD) and nutrient levels (Gopal, 1987; van Wyk and van Wilgen, 2002). Application of herbicide alters the pH content in water bodies either through direct spray or drift. For example, Edington *et al*., (2004) have shown that the toxicity of glyphosate based Vision herbicide to *Xenopus laevis*, *Rana clamitans*, *R. pipiens* and *Bufo americanus* is enhanced by an increase in pH. Therefore the combined effect of the water hyacinth plants and the lethal herbicide dose can be attributed to the mortality of the larvae in WH treatments. Additionally,

glyphosate photodegrades (Tu *et al*., 2001) and disappears from water within three days of treatment when exposed to sunlight (Wang *et al*., 1994). However, the shading produced by the water hyacinth canopy may have interfered with the photo-degradation process of the herbicide, thereby negatively impacting the *Xenopus* larvae in the WH treatments. Observed mortality of the anurans in this study (both WH and NWH treatments) could also be attributed to the fact that glyphosate and the surfactants such as POEA cause slower growth (lower metabolic rate) of anuran larvae or damage respiratory surfaces (Edginton *et al*., 2004), accounting for mortality and smaller size (Cauble and Wagner, 2005). It is critical to note that application of a glyphosate based herbicide Rodeo which is devoid of POEA was non- toxic to aquatic invertebrates such as *Chironomus* (Diptera: Chironomidae), *Hyalella azteca* Saussure, *Stagnicola elodes* Say, *Nephelopsis obscura* Verrill and fish at label recommended rates (Mitchell *et al*., 1987), which further lends credibility to the fact that surfactants such as POEA is more toxic than glyphosate, as substantiated by Folmar *et al*., (1979) who found that glyphosate contributed only a small percentage of toxicity to Roundup® and that the surfactant was the primary toxic agent.

Larval growth rate and size (in terms of bodylength) of anurans is related to food quality (Dash and Dei, 1998) and the physical, chemical and biological characteristics of sprayed sites undoubtedly will influence the quality and quantity of food available to the larvae, and interactions of these parameters with herbicides could affect the growth patterns of larval amphibians living in herbicide exposed environments (Wojtaszek *et al*., 2004). Results from this study show that the mean bodylengths of tadpoles were shorter for 0%, 0.8% and 3% sprays in treatments containing water hyacinth plants (WH). Reduced tadpole lengths could be due to the presence of allelochemicals (biochemicals that influence the growth and development of other organisms) released by water hyacinth plants (Sun *et al*., 1993; Sharma *et al*., 1996) which interfere with the growth of organisms, including algae which serve as food source for tadpoles. In addition, it has been noted that low dissolved oxygen levels influence the anuran community. Madsen (1997) found that water hyacinth infested

areas had the lowest dissolved oxygen levels compared to milfoil, hydrilla, and pondweed. Dissolved oxygen levels in water hyacinth infestations were below 5 mg/L which is notable because it represents the level at which many fish and possibly tadpoles start to experience oxygen stress, with consequences for larval development (Madsen, 1997). Mean bodylengths of tadpoles in NWH treatments were not significantly different between 0%, 0.8% and 3% at week one, indicating that the presence of water hyacinth plants exerted a more severe effect than did the herbicide sprays.

4.5 Conclusion

The results from this chapter show that advocating low doses of herbicides have obvious advantages for integrated weed control and anuran populations. However, it is prudent to keep in mind that environmental factors can either attenuate or exacerbate chemical toxicity (Kimball and Levin, 1985), for example, Gisey *et al*., (2000) indicated that factors that help mitigate the potential effects of glyphosate in shallow waters include degradation, sorption and interception by vegetation. At any given site of herbicide application, interactions that can occur among the active ingredient, surfactant blend, physical, chemical and biological characteristics of the aqueous environment, water chemistry and biological tissues of the affected organism in turn affect the fate, persistence and bioavailability of herbicides (Wojtaszek *et al*., 2004; Wan *et al*., 1989). Nonetheless, Gisey *et al*., (2000) conclude that aquatic habitat restoration using Roundup® will not lead to adverse effects on the environment and amphibians, and the use of sub-lethal doses of herbicide will reduce the concentration of the herbicide and POEA (60% less than the label recommended dosage) thereby minimising any damage to non-target flora and fauna.

Chapter 5

General Discussion

Control of invasive weeds around the world, has been mainly attempted with herbicides, but chemical and application costs required to control weeds on large infestations can become prohibitive (R 1481/ha as opposed to R 309/ha for biocontrol) (van Wyk and van Wilgen, 2002). Therefore, the use of biological control agents is considered more cost effective than herbicides for large scale weed control programmes mainly because it is a less resource-intensive solution (van Wyk and van Wilgen, 2002). Classical biological control of invasive weeds has been attempted on many species and examples exist where both single- and multiple-agent introductions successfully controlled target invasives (Briese, 1997; Hosking *et al*., 1988; Pemberton and Turner, 1990). In South Africa, Hoffmann (1995) indicates that 83% of weed projects undertaken are under complete or substantial control. Despite these successes, the use of biocontrol agents alone to control weeds has been effective in only about 30% of the attempts (Syrett *et al*., 2000; McFadyen, 1998) mainly because the effectiveness of biocontrol relies heavily on the successful release of control agents and the ability of biocontrol agents to thrive under a range of climatic conditions. With the average annual spread of invasive weeds ranging from 8 to 30% worldwide (Duncan *et al*., 2004) and the long time-frame (up to 20 years) required by biological control to manage weeds to acceptable levels (McFadyen, 2000), it seems intuitive that long-term weed control programmes would be most successful if all available methods of control were used (Lym, 2005). Theoretically, integration of other weed control methods with biological control agents can reduce a weed infestation more quickly than insects alone and may also increase the effectiveness of marginally successful agents (Messersmith and Adkins, 1995).

In case of water hyacinth, classical biocontrol via release of agents such as *Neochetina* weevils, a mirid, the moth and the mite has not resulted in consistent

success (Hill, 2003). This variability in performance of biocontrol agents is due to interference from herbicidal control (Center *et al*., 1999a), climatic incompatibility of released biocontrol agents such as the mirid (Byrne *et al*., 2004; Coetzee *et al*., 2007b, 2009) and variations in plant quality (Center and Wright, 1991; Center and Dray, 1992; Center and Dray, 2010). In general, weevil population growth has seemed more satisfactory on higher quality plants (Center and Wright, 1991; Heard and Winterton, 2000; Center and Dray, 2010). However higher quality plants are often associated with eutrophic conditions and exhibit rapid growth rates. Even though weevil populations do well under these circumstances, their impact is mitigated by profuse plant growth (Coetzee *et al*., 2007a). As a result, resource managers, unable to wait long periods for measurable results, will resort to using herbicides. However, label recommended doses of herbicides kill the weed, as intended, resulting in habitat loss for both the adults and immature stages which decimates their population, thereby reducing their impact on the weed (Wilson *et al*., 2006; Hill, 2003). The dead weed mat decomposes, aided by microbial processes and physical leaching (Gupta *et al*., 1996), releasing significant amount of nutrients (nitrogen and phosphorous) which accumulate in the sediment-water interface and contribute to an increasing nutrient load of the water body (Reddy and DeBusk, 1991), which further stimulates fresh water hyacinth growth via remaining plants and germination of viable seeds (Gupta *et al*., 1996). Thus, manifold economic and environmental losses between the introduction of biocontrol agents and the suppression of the target invasive, varied success rates of biocontrol and the long duration of time associated with successful biocontrol (McFadyen, 2000), coupled with negative effects of the lethal doses of herbicides on biocontrol, has necessitated research towards integrating control strategies (Kok and Kok, 1982). A successful integrated weed management regime depends on procedures that reduce weed population density, interfere with weed vigour and restrict weed reproduction (Blackshaw *et al*., 2006). Therefore, the main objective of this study was to identify a low or retardant dose of glyphosate-based formulation, Roundup®, and test its effect on the weed and its biocontrol agents, *Neochetina eichhorniae* and *Neochetina bruchi*. Water hyacinth plants increase rapidly by vegetative reproduction through the

production of ramets (Gopal, 1987), so any reduction in their production will have negative effects on the rate of spread and the competitive ability of the water hyacinth plants. It was envisaged that the weed growth could be constrained by a low, non-lethal dose of glyphosate and the insect populations would survive herbicidal sprays to persist in high population numbers and suppress the weed growth.

Results from Chapter 2 identified the retardant dose of glyphosate herbicide as 0.8% which did not kill the water hyacinth mat but retarded the weed growth, in terms of ramet and leaf production. The retardant dosed plants do not produce new leaves, which mean that the complement of leaves that are already present on the plants would age, as noted by leaf position. It is expected that the presence of older leaves on the sprayed plants will not interfere with weevil oviposition or larval mobility. Center (1987) concluded that the selection of a leaf for oviposition by a gravid female weevil is based on leaf age and this appears to be related to two factors, Firstly, stipules subtending older leaves were more likely to be loose, thus enabling the weevils to aggregate between the stipule and the leaf petiole. Eggs were seldom deposited on the youngest leaves. Eggs are found among the profusion of feeding scars at the base of the petiole, or in the succulent stipule itself. Secondly, older leaves were more likely to have softer necrotic spots in the petioles and many eggs are found in these softer areas. Center (1987) also tested larval dispersion because leaf age affects location of larvae after an appropriate developmental period. Not surprisingly, larvae were found on the oldest leaves, considering that older leaves were preferred for oviposition. However, as the leaves started to senesce, the larvae, mainly first and second instars, showed a tendency to move downwards and often burrow into the rhizome. Third instars were largely unaffected by the change in leaf dynamics and moved about within the shoot. In terms of plant dynamics, it must be noted, however, that while the retardant dose did not kill the plants, premature leaf loss may reduce translocation of nutrients and adversely affect the nutrient dynamics of the rosette. Furthermore, reduced longevity of leaves may affect the buoyancy and stability of the mat (Center and Van, 1989).

The survival of weevil populations post herbicide applications is crucial. The presence of weevils might regulate the ability of water hyacinth to colonize a site or reinvade following control operations (Center *et al*., 1999a). In the presence of large number of weevils, the water hyacinth mat expands slowly, because plants subjected to *Neochetina* herbivory do not show normal vigorous growth, and the reduction in the amount of spongy mesophyll associated with the petioles causes the plants to sink (Center *et al*., 1999a). Results from Chapter 2 prove that the retardant dose of glyphosate was not lethal to *N. eichhorniae* or *N. bruchi,* which is in agreement with the findings by Haag (1986a,b) and Ueckermann and Hill (2001). The survival of weevils has positive implications for biocontrol. Weevil numbers as low as two pairs of weevils per plant resulted in a slower growth rate of the weed, in terms of reduced plant height, root length, number of live leaves and total plant dry weight (Forno, 1981; Goyer and Stark, 1984), as compared to weevil free plants (Center *et al*., 1982). Though Chapter 2 did not test the effects of the low dose on the sap sucking mirid, there is evidence that positive herbivore population responses to sub-lethal dosages of herbicides have mainly been found for sap sucking herbivores and insects feeding on meristamatic tissues (Kajer and Elmegaard, 1996, Katembo, 2007). Katembo (2007) found that an application of low dose of herbicide (0.8%) did not affect the feeding or the reproductive capacity of the mirid. The reason for this maybe that sap sucking insects may derive higher nutritional value from their food when the host plant is stressed. Concentrations of free amino acids are consistently found to increase in plants subjected to stressors such as herbicide treatments (Kajer and Elmegaard, 1996). Therefore, a sub-lethal dose of glyphosate is compatible with other biocontrol agents of water hyacinth such as the mirid. The mirid, which lives entirely on the water hyacinth leaves would be a useful additional agent (Ajuonu *et al*., 2007) under conditions where the weevils are not effective, such as water hyacinth infestations that are seasonally rooted in mud (Ajuonu *et al*., 2003).

Another aim of Chapter 2 was to determine the combined effects of herbivory and herbicide spray on the productivity of water hyacinth plants, in terms of ramet and

leaf production. While the 0.8% dosed plants showed a retardant effect, surprisingly the (0.8%+Ne) or *Neochetina* treated plants did not show any marked decrease in the production of ramets or leaves. This is possibly because of low weevil numbers (one pair per plant) used in this study to simulate field conditions, wherein < 2 weevil per plant were found in most of the South African water hyacinth infested field sites (Chapter 3). These results are in agreement with the findings by Soti and Volin (2010) who concluded that water hyacinth plants compensate for low levels of herbivory.

Herbicide application and insect herbivory have implications for host plant quality which in turn can influence the ability of insects to develop and reproduce (Moran, 2004). The major nutrient required by insects is protein, which is often the limiting factor for optimal growth of insects. The level of protein nitrogen is therefore an adequate measure of 'plant quality'. Plant quality is variable both in time and space (Simpson and Simpson, 1990) and this effect of nutrition has implications for success of biological control. Nitrogen is considered a crucial component of host plant quality for insect herbivores (Mattson, 1980; Awmack and Leather, 2002). As a key element in the physiology of all organisms, nitrogen often is a limiting factor that affects the performance of individuals and the dynamics and interactions of populations (Mattson, 1980). Furthermore, White (1993) concludes that the shortage of nitrogenous food, i.e. low plant quality, particularly for immature animals, limits the abundance of most herbivores. High or increased plant nitrogen has been reported to improve the survival and growth rate of immature insects (Wheeler, 2001; 2003), and the fecundity of the resultant adults (Heard and Winterton, 2000; Awmack and Leather, 2002; White, 1993). For example, providing nitrogen fertilizer was found to have assisted in the establishment of *Cyrtobagous salviniae* on *Salvinia molesta* and improved rate of control (Room and Thomas, 1985). In terms of *Neochetina* weevils, their effectiveness against water hyacinth varies but appears to be related to plant quality and the differential preferences shown by the two weevil species for plants of different phenologies (Center and Wright, 1991; Center and Dray, 1992; Center and

Dray, 2010). Pronounced herbivory by weevils has an impact on the physiology and biochemical composition of the leaves. High or low weevil numbers decreased N levels in high plant densities when compared to no weevils (Center and Van, 1989). Phosphorous concentrations were significantly higher in leaves from plants subjected to high numbers of weevils than in leaves from plants devoid from weevils or subjected to low numbers of weevils. Total available carbohydrates in leaves were reduced in plants subjected to herbivory, regardless of density of weevils, as compared to plants not exposed to weevils (Center and Van, 1989). Additionally, *Neochetina* herbivory resulted in uniform plant stature, leaf size and leaf shape (Center and Durden, 1986). Results from Chapter 2 indicate that the application of 0.8% glyphosate or the combination of 0.8% glyphosate and *Neochetina* did not affect the nitrogen levels in the leaf and crown samples of sprayed water hyacinth plants, thus maintaining "plant quality", nor did it interfere with weevil feeding. Therefore, it is expected that biocontrol agents such as *N. bruchi* will respond positively to high quality plants and will have a high growth rate for both the adult and larval stages. Generally, under field conditions, *N. bruchi* is more sensitive to plant quality than *N. eichhorniae* and a higher proportion of *N. eichhorniae* was found on mature plants whereas *N. bruchi* preferred lush plants growing in nutrient enriched water and previously unstressed by herbivory (Center and Dray, 1992). Additionally, the most reproductively active populations of both weevil species, but particularly *N. bruchi* were found on highest quality plants probably because *N. bruchi* has a higher nitrogen requirement due to its higher fecundity (Center and Dray, 1992; Center and Dray, 2010).

Results from Chapter 2 show that feeding activity by *Neochetina* weevils did not decrease on sprayed plants, instead the weevils fed more on sprayed plants (Fig. 2.7; trial carried out in spring, 2005). Increased feeding could be due to glyphosate-induced inhibition of the synthesis of phenylalanine derived phenols and secondary metabolites that are feeding deterrents (Ainsworth, 2003). It is also possible that a very low dose of glyphosate increased the sugar content in the sprayed plants, thereby

making the plants more palatable (Su *et al*., 1992). However, results from the study testing the combined effects of 0.8% and Ne herbivory (0.8%+ Ne) did not show increased feeding intensity by the weevils (Fig. 2.13). These variations in feeding intensities could be due to the seasonal fluctuation in the content of tissue nitrogen. Weevils were shown to feed more on leaf tissues greater than 3% nitrogen (Center and Wright, 1991) The (0.8+ Ne) study was carried out in summer, 2010, and published report by Tucker and DeBusk (1983) indicate that nitrogen content in water hyacinth plants was minimal during summer (2.5%), which corroborates with the finding in Chapter 2 (Fig. 2.14 A), and peaked during spring (3.8%), explaining why the summer trial did not have increased feeding (Fig. 2.13), while the spring trial did.

Leaf hardness is another component of plant quality which may act as a physical barrier to normal feeding or oviposition by herbivores (Wright and Bourne, 1990). Wright and Fuller (1984) described the use of a penetrometer which consisted of a dial gram gauge, with removable probes. The probes were made from stainless steel pins or rods with the apex ground flat, each 12 mm long, soldered at right angles onto a modified 3 mm electrical spade receptacle. Probes were slid over the flat feeler tip of the gauge and could be easily changed if different probe diameters were required for testing leaves of varying toughness. The gauge (Chatillon AG 50) was used for experiments with *S. japonica* and was calibrated to 0-50 g but gauges with 150, 300 and 500 g capacities are available for use on plants with greater leaf toughness.

Herbivory by *Neochetina* weevils increases leaf hardness, but it does not negatively affect the adult weevil or larval feeding. However, increase in leaf hardness had negative consequences for *Niphograpta albiguttalis* populations as the plant quality decreased (Wright and Boland, 1989). Wright and Bourne (1986) have shown that once the hardness of water hyacinth leaf exceeded a particular limit, newly hatched larvae of the moth were unable to tunnel through the petiole. However, application of 2,4-D amine and glyphosate have been shown to increase petiole softness (Wright and Bourne, 1990). Softer petioles favour the adult and the immature stages of

biocontrol agents. For example, shoot feeding beetle *Lochmaea suturalis* Thomson (Coleoptera: Chrysomelidae), a biocontrol agent of heather was reported to feed more and live longer on heather sprayed with triclopyr and picloram, suggesting favourable changes in plant quality resulting from herbicide sprays (Hayes, 1999). Similar comparisons can be drawn from this study; the low dose of glyphosate did not interfere with *Neochetina* larval tunneling as evidenced by a higher percentage of petioles that were mined in sprayed plants when compared to unsprayed plants.

There is strong evidence that glyphosate predisposes plants to infection by facultative pathogens (Levesque and Rahe, 1992). Positive interactions between insect herbivores and plant pathogenic fungi are potentially useful in biological control (Moran, 2005). High numbers of insect feeding scars noted in this study could serve as entry points for fungal pathogens and insects can deliver fungal inoculum on their cuticular surfaces or in digestive excreta (Caesar, 2003). Field studies have revealed a positive association between *Neochetina* feeding and *Cercospora piaropi* Tharp infection (Charudattan, 1990). However, feeding scars of *Neochetina* affect the survival of the adult mirids (Ajuonu *et al*., 2007). High mortality of mirids was noted on water hyacinth plants with old weevil feeding scars, possibly due to the reduction of tissue nutrients due to adult weevil feeding (Ajuonu *et al*., 2007). However, mortality of adult mirids was lower on fresh feeding scars by *Neochetina* weevils. Increased number of feeding scars found in this study may well have a negative effect on the mirid population, but considering that the mirids are highly mobile in the water hyacinth canopy, it is expected that the adults would seek out plants or leaves with less feeding damage (Ajuonu *et al*., 2007).

Timing of herbicide sprays requires information on plant and insect phenology (Cullen, 1996). The impact of biocontrol agent herbivory depends not only on the feeding rate of the insects and its persistence (Ainsworth, 2003), but is also dependent on the growth rate of the plants involved, both of which are affected differently by

temperature (Van der Heide *et al.,* 2006). In terms of water hyacinth, Hill and Olckers (2001) hypothesised that possible asynchrony of population growth brought on by differential response of water hyacinth and its biocontrol agents to low temperature and frost will compound control efforts, especially in colder areas of South Africa. Biocontrol agents in the Highveld regions of South Africa undergo a population bottleneck wherein the adults are reduced to an overwintering larval population which fails to build up high levels of numbers until the following summer, whilst the weed recovers rapidly, post winter. This hypothesis was tested in Chapter 3. Results from Chapter 3 substantiate this hypothesis and indicate that, at all water hyacinth infested field sites (both temperate and subtropical climatic regimes), water hyacinth plants produced ramets through autumn and winter (Fig. 3.1), while increasing biomass during summer months (Fig. 3.4). Low temperatures were shown to affect the development of *Neochetina* weevils, as evidenced by feeding scars and petioles mined. However, *N. eichhorniae* was the more common and most abundant agent found, despite *N. bruchi's* reputation of tolerance to cold weather (DeLoach and Cordo, 1983; Hill and Cilliers, 1999) and high nutrients (Heard and Winterton 2000; Hill and Cilliers, 1999), averaging at 0.55 weevils per plant, with a maximum of 23 weevils found at Hammarsdale Dam (Byrne *et al*., 2010). However, it was noted that populations of the weevils did not show any marked increase in numbers through successive generations within a season (Byrne *et al*., 2010).

The weevils overwintered as third instar larvae and had to complete their development and lay eggs at the onset of summer, before they could contribute to significant plant damage through larval mining of petioles (Chapter 3; Wilson *et al*., 2005). This resulted in a herbivory free season for plants, which start to accumulate living biomass in terms of leaves and ramets, with the onset of favourable conditions during spring. This time lag results in asynchronous growth patterns between the weed and its natural enemies at all the study sites (Byrne *et al*., 2010), thereby offering a window of opportunity to prioritize seasonal herbicidal spray regimes during these periods (autumn and spring).

Results from Chapter 3 showed that both the seasonal spray regimes interfered with the vegetative and the reproductive growth of the weed, corroborating the findings in Chapter 2. Moreover, the spray regimes did not adversely affect the reproductive capacity of the biocontrol agents as evidenced by larval counts and petioles mined, corroborating with the results of several published reports which conclude that the optimal timing of herbicidal sprays, either seasonal or relative to the phenology of the biocontrol agent, did not affect insect herbivores used as biological control agents against terrestrial invasive plants (McCaffrey and Callihan, 1988; Jacobs *et al*., 2000; Nelson and Lym, 2003; Lym and Nelson, 2002; Lindgren *et al*., 1999). To summarise, seasonal application of glyphosate herbicide in autumn and spring reduced ramet production by 40 and 75% respectively, while leaf production was reduced by nearly 20% in autumn and spring, over a period of three weeks. Additionally, new living biomass accrual was affected but the beetle numbers and their reproductive capacities were not harmed. Thus, the new season's (summer) adults are expected to be able to persist and produce a new generation, which would suppress further plant growth. Forno (1981) has shown that *Neochetina eichhorniae* adults fed extensively on leaves throughout the summer season resulting in reductions in plant growth. Additionally, larval herbivory of rhizome reserves during autumn might stress the plants as the plants evidently draw upon rhizome reserves to compensate for environmental stress due to low temperatures (Grodowitz *et al*., 1991). Thus, the stresses effected by the retardant dose of the herbicide early in the season coupled with herbivore pressure later in the season are expected to reduce the growth of water hyacinth plants.

Literature indicates that frequent use of herbicides has detrimental effects on the environment and its associated fauna, both aquatic and terrestrial. Glyphosate has been shown to have non-target toxic effects on several species of amphibians (Relyea, 2005 a,b,c). Therefore, Chapter 5 tested the effects of the retardant dose of glyphosate herbicide (Roundup® SL, 360g/L) on *Xenopus laevis* tadpoles. Roundup® (such as Roundup® SL, Roundup® Max, Mamba and Mamba Max are registered for use on

aquatic systems in South Africa (See: A guide to the use of herbicides of bush encroachment, noxious plants and aquatic weeds. 2007/ The Registrar, Act number 36 of 1947, Food Safety and Directorate). The current study did not attempt to identify LC_{50} concentration of the glyphosate nor did it test the effects of a more extensively used herbicide in USA, 2,4-D, simply because in South Africa, 2,4-D is not registered for either terrestrial or aquatic weed control in South Africa.

Surprisingly, results show that water hyacinth plants were potentially more lethal to aquatic amphibians than an application of glyphosate herbicide A direct application of the retardant dose of glyphosate to the water alone, however, did not kill the amphibian larvae nor did it compromise the developmental integrity of the larval populations as evidenced by bodylengths. The application of low dose of glyphosate correspondingly decreased the amount of the surfactant (POEA) used, thereby decreasing the lethality of the commercial formulation (Wan *et al.*, 1989). However, it is prudent to note that additional stresses faced by aquatic organisms such as low dissolved oxygen concentrations, low water levels, onset of reproduction or immaturity at the time of herbicide application or predatory stress (Relyea, 2005a) may exacerbate the effect of herbicide mediated toxicity.

Published results indicate that the surfactants, rather than the active ingredient in herbicides may be responsible for observed mortalities of amphibians (Gisey *et al*., 2000; Folmar *et al*., 1979; Mitchell *et al*., 1987; Perkins *et al*., 2000; Relyea, 2004; Relyea *et al.*, 2005; Relyea, 2005 a, b, c; Howe *et al.*, 2004; Mann and Bidwell, 1999). Application of a low dose of herbicide means that low concentrations of Roundup or POEA are eventually found in water bodies. Maximum concentration of Roundup found in a 15 cm deep water body is estimated to be 3.7mg ai/L (Giesy *et al*., 2000). Based on probit regression analyses, 3.7mg ai/L would kill 90% to 100% of the amphibians (Relyea, 2005a). In terms of water bodies infested with water hyacinth, it is likely that the water body would be deeper than 15 cm and if the low dose of 0.8% herbicide is applied at a rate of 140L/ha, then the amount of active ingredient deposited on the plants and the water body would be 0.0028mg/L. Post

application, this concentration of the active ingredient will be subjected to normal plant physiological processes such as uptake, translocation and assimilation. The likelihood that any glyphosate is still remaining in the water body following these processes, at concentrations toxic to the amphibians is doubtful. Although, this study did not test the effects of the low dose on algae, published reports by Wojtaszek *et al*., (2004) and Relyea *et al*., (2005) conclude that algae have a wide range of sensitivities to glyphosate and exposure to glyphosate can alter algal species richness, biodiversity and primary productivity. However, exposure to moderate (low) doses of glyphosate has shown to induce increased proliferation of some algal species, leading to positive growth effects on anuran larval development (Wojtaszek *et al*., 2004).

In conclusion, the main aim of the thesis, to investigate the possibility of integrating a low dose of herbicide with biocontrol, has been shown. South African water hyacinth ecosystems have been characterized by unstable 'boom-and-bust' water hyacinth populations which preclude the build up of damaging numbers of biocontrol agents. Integration of a retardant dose of glyphosate with biological control offers a potential tool with which to manage water hyacinth. The low dose does not kill the water hyacinth mat and should preserve the habitat for immature and immobile stages of the weevils, allowing the adult populations to persist at damaging levels. The herbicide-induced curb on season mediated vegetative growth of the water hyacinth plants will result in disproportionate levels of damage by the weevils and further suppression of the weed through continued herbivory. Jones and Cilliers (1999) have successfully implemented an integrated management system for water hyacinth using a full (3%), lethal dose of glyphosate by sub-dividing the infestation into smaller areas and maintaining herbicide-free, insect refuges. This however, requires a level of management not currently available for many water hyacinth infested African waterways, and requires a high dose of glyphosate, which is under scrutiny for its detrimental effects on other aquatic organisms (Relyea, 2005a,b,c). The use of a sub-lethal dose of glyphosate did not have detrimental effects on non-target organisms like amphibians and therefore provides water managers with a sustainable and environmentally friendly integrated management tool for control of water hyacinth.

The integrated approach is already regarded as cost effective in terms of per hectare weed infestation cleared, where the monetary investment has been calculated to be R 277/ha, in contrast to solely herbicidal control where the cost would be R 1481/ha because of expenditure on herbicides and follow up regimes involved (van Wyk and van Wilgen, 2002). Furthermore, given the eutrophic nature of South African water bodies, laboratory studies have shown that the low dose will retard the plant growth over a wide range of water nutrient levels (Kirton, 2005). That study indicated that the nutrient levels do not override the retardant effects of the herbicide and this type of integrated approach may thus contribute to control of unrestrained water hyacinth growth at eutrophic sites. Additionally, the sub- lethal dose of glyphosate will exert a very weak selection pressure, if any, for resistance selection in water hyacinth; coupled with herbivore pressure from biocontrol agents, herbicide resistance is not expected to evolve in water hyacinth in an integrated management programme.

Before the integrated technique can be recommended for use on a large scale, field testing of both the spray dosage and the seasonal spray regimes is strongly recommended. Other established biocontrol agents such as the moth and the mite should be tested for their compatibility with the retardant dose of glyphosate. Furthermore, the field trips that were undertaken to gather data for Chapter 3 were expensive (for example: trips to Kwa-Zulu Natal sites costed around R 6500 per trip) and time consuming in terms of travel time. Therefore, future work on monitoring water hyacinth should consider the use of hyperspectral remote sensors (RS) to detect the extent of water hyacinth infestation (Santos *et al*., 2009; Robles *et al*., 2010). The use of hyperspectral RS will allow for the detection of vigorously growing plants, and determine where insect damage is the greatest, and whether or not the shape of the mat affects these trends. The high spectral resolution of hyperspectral imagery will also enable the detection of plant pigment concentrations in the plants which act as an indication for health status of the weed. Hyperspectral RS could also potentially allow for the detection of nutrient status of the plants, and thus, an inference could be made to establish the nutrient status of the water body (Fisher *et al*., 2006). With this information, a decision can be made on whether or not the water body is eutrophic, and if biocontrol

or integrated control, using a retardant dose of glyphosate should be considered as a control option.

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