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INTEGRATED PEST MANAGEMENT OF THE WATER HYACINTH

DISSERTATION
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By
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DECLARATION

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

(Signature of candidate)

__Tuesday the 15th ____day of __June______________2010__________

Abstract

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) is the most damaging water weed in South Africa. Biological control has had varied success and therefore attention has shifted toward integrated management, using insects and herbicides. The objective of this work was to find out how a sub-lethal dose of glyphosate herbicide can be used in conjunction with the *Neochetina* weevils in the control of water hyacinth in the field. Plants infested with the weevils, *Neochetina eichhorniae* and *N. bruchi*, were sprayed with a sub-lethal dose of herbicide (0.8% glyphosate concentration at 140 l/ha spray volume) at two sites, Delta Park and Farm Dam (Johannesburg). Plant parameters (plant biomass, number of leaves, and number of ramets) and insect parameters (reproduction, survival, and feeding) were compared between the sprayed plants and the unsprayed plants. Results showed that some aspects of plant growth (leaf production and biomass accumulation) were reduced, whereas the performance of the weevils was not impaired. Delta Park plants were found to be more susceptible to the herbicide compared to Farm Dam plants. The effect of glyphosate on water hyacinth nutritive quality was also analysed by testing N, C, and P contents of the plant. Generally the N content of the plant decreased resulting in an increased C:N ratio. In conclusion the combination of a sub-lethal dose of glyphosate and the *Neochetina* weevils is feasible in the field, however may not be an ideal control method for large infestations where radical reduction of water hyacinth mat is required.

To the one and only God be all the glory and honour, for carrying me through this journey.

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CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

1.1.1 The Origin of Water Hyacinth

Water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach: Pontederiaceae), is a perennial, herbaceous, free floating aquatic plant originating from the Amazonian basin in South America (Center, 1994). The plant has proven to be one of the most damaging invasive aquatic weeds, having negative consequences both for the environment as well as economies in many tropical and subtropical parts of the world (Julien *et al.*, 1999). This attractive plant has been called a beautiful devil (Vietmeyers, 1975) owing to its magnificent lilac violet flowers arranged in spikes (Gopal, 1987). McLean (1922) referred to *E. crassipes* as a pest and terror, and De Groote *et al.* (2003) called it "dollar weed" because billions of dollars have been and are being spent in the effort to control it (Gopal, 1987, Byers *et al.*, 2001). By the end of the nineteenth century the plant, which was by then distributed around the world for ornamental purposes and botanical curiosity, was declared a nuisance (Gopal, 1987). This was after it became a menace to the environment and started infesting open water bodies, hence interfering with water related benefits, such as fishing, recreational activities, navigation, and hydroelectric power generation (van Wyk *et al.*, 2006).

Eichhornia crassipes was introduced into South Africa in the early 1900s (Cilliers, 1991). It is believed that the weed was first recorded in South Africa on the Cape Flats in 1908 (Stent, 1913). Water hyacinth is one of the five major aquatic weeds in South Africa. The other four include *Pistia stratiotes* Linnaeus (Araceae) (water lettuce), *Salvinia molesta* D.S. Mitchell (Salviniaceae) (salvinia), *Myriophyllum aquaticum* (Vellozo Conceição) Verdcourt (parrot's feather), and *Azolla filiculoides* Lamarck (Azollaceae) (red water fern). Of these, water hyacinth is the most significant and damaging weed. It is widespread throughout South Africa and impinges on rivers in the Western and Eastern Cape, KwaZulu-Natal, Mpumalanga and on the Vaal River in the Gauteng and Free State provinces (Richardson & van Wilgen, 2004).

1.1.2 Water hyacinth morphology

A detailed account of water hyacinth structure and physiology was presented by Gopal (1987) and later by Julien *et al.* (1999) among other authors. Water hyacinth's leaves consist of a petiole, isthmus (thin part between petiole and blade) and a blade. The petiole is sheathed at its base and carries a large membranous stipule which forms a sheath around the next youngest leaf. Depending on habitat factors such as nutrient availability, depth of the water body and horizontal space, water hyacinth petioles can either be tall and elongated, or short, horizontal and inflated with a bulbous form (Gopal, 1987). Upright slender petioles usually occur within dense and crowded infestations, while horizontal bulbous petioles are usually found in plants which occur in open water (Julien *et al.,* 1999).

Water hyacinth roots are adventitious, fibrous, and have conspicuous root caps (Gopal, 1987). Difference in root length is attributed to the water nutrient status as well as the depth of water bodies. Plants growing in nutrient rich waters have short roots (less than 20 cm) whereas those growing in nutrient poor waters tend to have long roots (more than 60 cm) (Knipling *et al.,* 1970). This is because in nutrient rich waters the plant does not need to extend its roots to absorb the readily available nutrients. Short roots are also observed in plants growing in muddy water systems (Gopal, 1987).

Water hyacinth reproduces by seed and vegetatively (Julien *et al.*, 1999). Vegetative propagation occurs through the formation of short runner stems (stolons) that branch out from axillary buds, situated at the base of the plant, forming daughter plants (offshoots). These are referred to as ramets (Gopal, 1987). Ramets eventually break off and develop into new plants (Julien *et al.*, 1999). Rapid spread and colonization of new water bodies is achieved through vegetative propagation (Gopal, 1987), hence control methods targeting the sexual reproductive aspect have received less attention in the fight against water hyacinth. During sexual reproduction of water hyacinth, the plant flowers profusely and a large number of fruits and seeds are produced (Barrett, 1980). Water hyacinth has bluish purple flowers with a yellow center. Hitchcock *et al.* (1950) reported massive seed production; but the development from seedlings to mature plants was limited by unfavourable growing conditions, including humidity and temperature, rather than conditions unsuitable for germination. Sexual reproduction becomes very important in the sense that water hyacinth seeds which are dropped in the water can lie dormant for a period of up to twenty years, over which time germination is possible (Gopal, 1987). This attribute guarantees the perpetuation of the weed even after the adult population has been eradicated. This is an important point to consider when contemplating control measures.

1.1.3 Influence of temperature, light and nutrients on water hyacinth

Temperature has an important and pervasive influence on the distribution and abundance of organisms through its effects on physiological processes such as photosynthesis and nutrient fixation (Somero, 2002). Therefore water hyacinth reproduction and development is also greatly influenced by temperature. The weed has been observed to actively grow under optimum temperatures of 25 to 27.5^{o}C (Gopal, 1987). At temperatures below 10^{o}C and above 40^{o}C, the plants cease to grow. However it has been observed that even at temperatures below 10^{o}C, ramets could still be produced (Gopal, 1987); and the plant can survive freezing temperatures ranging from 0 to -16 ^{o}C for at least 24 hours (Owens & Madsen, 1995).

Light quality and quantity have a significant effect on water hyacinth morphological growth (Méthy *et al.*, 1990). The plant’s leaves form a canopy which lessens photosynthetic photon flux density and the ratio of red to far-red light throughout the rest of the plant (Méthy *et al.*, 1990). Therefore, like most canopy shaded plants, water hyacinth has the ability to increase its potential to intercept light by elongating its petioles upwardly and subsequently increasing leaf area and through horizontal growth by positioning new ramets laterally (Smith 1982). Nevertheless, the production of ramets has been noted to decrease under low light conditions (Méthy & Roy, 1993)

High nutrient content in water bodies can contribute to the rapid proliferation of water hyacinth. Heard and Winterton (2000) found that there was a direct correlation between water nutrient concentrations, particularly nitrogen and phosphorus and water hyacinth growth. Many water bodies in South Africa have a N:P (nitrate: phosphate) ratio of 7:1

(Byrne *et al.*, 2010) which according to Wilson (2002), is ideal for water hyacinth growth. A positive correlation exists between water nutrient concentration, especially nitrate and phosphate which are the principal macronutrients responsible for eutrophication in water bodies (Petrucio & Esteves, 2000), and water hyacinth growth (Heard & Winterton, 2000). Ripley *et al.*, (2006) found an increase in water hyacinth biomass, ramet production, and plant height resulting from increasing concentrations of nitrate and phosphate. This fast growth of water hyacinth renders its biological control methods ineffective (Hill & Cilliers, 1999) because of the weed's propensity to compensate for herbivory damage (Ripley *et al.*, 2006).

1.1.4 Problems associated with water hyacinth invasion

Alien invasive weeds cause serious problems in natural, semi-natural, terrestrial and freshwater ecosystems around the world (Richardson & van Wilgen, 2004). They are also widely recognized as one of the largest global threats to biodiversity (Macdonald *et al.*, 1986), with results such as transformation of ecosystems by using excessive amounts of resources, notably water, light and oxygen (Richardson & van Wilgen, 2004). In many parts of the world, governments are mobilizing both financial and human resources in efforts to control alien species, preventing their menacing impacts and repairing systems already damaged (Byers *et al.*, 2001).

Water hyacinth forms dense mats that have the potential to completely cover entire water surfaces, hence interfering with many water-dependent activities, such as navigation, irrigation, fishing and power generation (Julien *et al.*, 1999). Water hyacinth mats competitively exclude native submersed and floating-leaved plants resulting in the displacement of indigenous fauna through habitat modification (Macdonald *et al.*, 1986). The dense floating mats impede water flow and create good breeding conditions for vectors of animal and human diseases (Grodowitz, 1998), such as malaria, encephalitis, filariasis, schistosomiasis, river blindness and possibly cholera (Gopal, 1987; Richardson and van Wilgen, 2004). It is argued that the weed is responsible for the loss of water from impoundments due to high rates of evapotranspiration (Lallana *et al.*, 1987). Water hyacinth mats also increase the incidence of flood events by slowing down the normal water flow of rivers (Center *et al*., 2002).

This fast growing and damaging alien plant can be utilised in the manufacturing of paper, handicrafts and furniture, in the treatment of wastewater or for mineral nutrient removal from polluted water bodies, as fodder, compost and fertilizer (Edwards & Musil, 1975). Unfortunately these uses are outweighed by the threats and problems the weed represents to the ecosystem and the economy of affected countries (Julien *et al*., 2001). That is why under the South African legislation, water hyacinth is a declared weed under category one, which means it must be controlled (Henderson, 2001).

1.1.5 Water hyacinth control methods

In the effort to control the weed, three management strategies are often used which include mechanical control, herbicide control, and biological control. Recently the importance of integrated management has been emphasized (Cilliers *et al*., 1996; Ainsworth, 2003).

Mechanical control

The use of mechanical harvesters to control water hyacinth has shown to be effective in some areas. Some examples include Port Bell and Owen Falls Dam on the Ugandan side of Lake Victoria (Center *et al.*, 1999). However, the purchase cost as well as the operational cost of these harvesters is extremely high (Julien *et al*., 2001). Apart from the running costs of harvesters, mechanical removal further reduces the natural enemy population in water bodies where biocontrol agents are established (Center *et al.,* 1999). Non-target organisms in the environment are also destroyed as a result of mechanical control (Cilliers, 1991).

In some cases cables fitted with buoys are anchored on riverbanks to maintain designated areas free from weeds and in other cases they serve to reduce down-stream spread of an infestation. However, more often than not, cables break due to the accumulated pressure exerted by the retained dense mats of the weed.

Manual removal, on the other hand, is extremely labour-intensive and ineffective in larger infestations (Mallya, 1999). In addition, manual removal can be a risky exercise as some infested rivers are inhabited by crocodiles and hippopotamuses.

Herbicidal control

Herbicidal control has been practiced against water hyacinth since the early 1900's with chemicals such as dichlorophenoxyacetic acid (2,4-D), Clarosan and glyphosate (Ueckermann & Hill, 2001). In South Africa, water hyacinth control has largely depended on the use of herbicides since the 1970's (Julien *et al.*, 1999). Examples include the successful control of a water hyacinth infestation on the Hartebeespoort Dam with terbutryn herbicide (Ashton *et al.*, 1979). Herbicides such as 2,4-D and diquat work effectively in controlling water hyacinth infestations (Gopal, 1987). However they are not accepted for use in most water bodies (Julien *et al.*, 1999) because of their non-selective characteristics, especially in areas where communities are using untreated water for domestic use (Julien *et al.*, 1999). People are sceptical about herbicide control, as they have stigmatized herbicides as being “poisonous” (Relyea, 2005). In addition, the use of chemicals on water hyacinth provides a temporary control as re-infestation from the seed bank as well as individual plants missed by the herbicide during spraying is inevitable; thereafter requiring repeated herbicide spray (Center *et al.*, 1999). Repeated herbicide applications result in massive plant kills which may lead, through the process of plant decomposition, to water pollution or algal blooms, as was the case at Hartebeespoort Dam, South Africa (Bartram *et al.*, 1999).

Herbicides used in the traditional way (recommended doses), will interfere with the biocontrol agents where the two control methods occur in combination on water hyacinth (Ueckermann & Hill, 2001), since these herbivorous arthropods are totally dependent on the weed for food and habitat. If the weed is sprayed with a lethal dose of herbicide, it will die and subsequently all the biocontrol agents will eventually die as well. However, from its thousands of dormant seeds, the weed will re-infest previously cleared water bodies (Gopal, 1987) whenever conditions (temperature, light intensity) are favourable (Edwards & Musil, 1975). In the absence of its natural enemies, the weed will proliferate

(Center *et al.*, 1999). It is for this reason that the concept of setting aside “refuges” for biocontrol agents’ sustainability was proposed (Center *et al.*, 1999; Hill & Olckers, 2001). Another method of ensuring the survival of arthropod biological agents is the use of sub-lethal doses of herbicide in water hyacinth management programmes where biocontrol agents are used in juxtaposition with herbicides (Di Tomaso, 2007). A sub-lethal herbicide concentration will retard plant growth so as to give the biocontrol agents a competitive advantage over the plant (Wright & Bourne, 1990). Therefore using herbicides and biocontrol synergistically could offer a more consistent control provided that the weed is sprayed with a sub-lethal dose of herbicide.

Biological control

Different authors have defined the concept of biocontrol differently. For example, McFadyen (2000) opted to use the definition given by DeBach (1964): “the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence.” Louda *et al.* (2003) defined classical biological control as an exercise where exotic natural enemies are deliberately released into new environments in an attempt to limit the density of an invasive species. Bringing new organisms to act as pest control agents into new environments may present a danger to non-target species, therefore creating a new problem rather than solving the existing one. However in biological control, the potential risk to non-target species is generally low since pest control agents used are thoroughly tested and proven host specific before they are released (Ernest, 2005).

Around 1961, studies on a number of arthropods to be used as water hyacinth biocontrol agents began in North America (Center, 1994). In South Africa, the biological control program against water hyacinth was initiated in 1973 with the release of the weevil *Neochetina eichhorniae* (Cilliers, 1991). Currently South Africa relies on six established biocontrol agents, *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), *N. bruchi* Hustache (Coleoptera: Curculionidae), *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae), *Eccritotarsus catarinensis* Carvalho (Heteroptera: Miridae), *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), and *Cercospora piaropi*

Tharp. (Deuteromycetae: Melanconiales: Dematiaceae: Scolecosporae). Three other biocontrol agents are still under investigation, these include the grasshopper *Cornops aquaticum* Bruner (Orthoptera: Acrididae), the planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), and the mining flies *Thrypticus sp* (Diptera: Dolichopodidae) (Oberholzer & Hill, 2001).

Biocontrol using pathogens

Fungal pathogens used as water hyacinth biocontrol agents in South Africa include, *C. piaropi*, *Acremonium zonatum* (Sawada) Gams, and *Alternaria eichhorniae* (Jones, 2009). Other examples of fungal pathogens suitable as bioherbicides of weeds include, *Uredo eichhorniae*, *Myrothecium roridum*, *Rhizoctonia solani*, *Fusarium pallidoroseum* (Cooke) Sacc., and *C. rodmanii*, Conway (Hyphomycetes) (Charudattan, 2001; Praveena *et al.*, 2007). Pathogens are reported to induce injuries such as leaf spots, leaf necrosis, and secondary root rot (Conway, 1976). Similar to arthropod biocontrol agents, pathogens alone will not effectively reduce water hyacinth biomass; combination with other control strategies however, such as chemical or/and insect biocontrol agents may enhance the efficiency of weed control (Rayachhetry & Elliott, 1997; Caesar, 2000). For example, *C. rodmanii* was seen to perform well in terms of reduction in plant height, number of ramets, as well as biomass when applied in conjunction with 2,4-D at 5 and 154 ppm (Charudattan, 1986). Nevertheless, high herbicide concentrations have been reported to impede fungus growth and sporulation (Praveena *et al.*, 2007). In an experiment under *in vitro* conditions, cultures of *F. pallidoroseum* (a bioherbicide used to control *Hydrilla verticillata*) placed in conical flasks containing a liquid media (100 ml of Czapek's (Dox) broth) were exposed to different herbicides at different concentrations. After inoculation and incubation, it was found that high herbicide concentrations (2,4-D: 1.00 and 0.25 kg a.i ha^{-1}; and Paraquat: 0.75 and 0.19 kg a.i ha^{-1}) inhibited fungal growth and sporulation while low concentrations (2,4-D: 0.06 and 0.02 kg a.i ha^{-1}; and Paraquat: 0.05 and 0.01 kg a.i ha^{-1}) were less inhibiting. On the other hand, glyphosate did not prevent fungal sporulation at all concentrations (Glyphosate: 0.80, 0.20, 0.05, and 0.01 kg a.i ha^{-1}). The above results suggest that herbicides can potentially be used in conjunction with pathogens provided they are applied at low concentrations. However, combinations should to be tested in the field before concluding on feasibility.

Early work by Charudattan (1986) found that water hyacinth shoot height was significantly reduced when *C. rodmanii* was combined with the two *Neochetina* weevils, compared to when the pathogen or the weevils were used alone. In a 7 ha water body with 3 ha of water hyacinth coverage, Jiménez and Balandra (2007) also recorded a fresh weight reduction of 29% as well as a 59% diminution in the number of plants per square meter in an integrated weed management, combining *C. piaropi* and *A. zonatum* with *Neochetina* weevils. In another laboratory experiment, *Hydrilla verticillata* ((L. f.) Royle) shoots were significantly damaged when a combination of leaf-mining larvae of *Hydrellia pakistanae* Deonier (Diptera; Ephydridae) with *F. pallidoroseum* was used (Shabana *et al.*, 2003). It was noted that leaf minings by *H. pakistanae* facilitated fungal infection of the weed.

The most successful biocontrol agents against water hyacinth weed have been the two *Neochetina* weevils, *N. bruchi* Hustache (Coleoptera: Curculionidae) and *N. eichhorniae* Warner (Coleoptera: Curculionidae) (Julien *et al.*, 1999) (Fig. 1.1), and these are the biocontrol agents that were used in this work. *Neochetina*, originally from South America, are both host specific to the Pontederiaceae family. Julien *et al.* (1999) described the biology and life cycle of the weevils.

Figure 1.1 *Neochetina bruchi* and *Neochetina eichhorniae* (photo courtesy USDA)

They spend their entire life cycle on water hyacinth. In contrast to adult *E. catarinensis* which are diurnal, adult *Neochetina* are nocturnal, and lay their eggs in older leaves and petioles, from where the hatching larvae burrow down into the stem base where they inflict most damage. The larvae move onto the roots of the plants for pupation, and the emerging adults feed on the leaves (Cordo & De Loach, 1976). *Neochetina bruchi* appear to be more dependent on healthier plant material than are *N. eichhorniae* (Heard & Winterton, 2000). Wherever the two weevils co-occur, control of water hyacinth is enhanced because the two complement each other (Julien *et al.*, 1999).

South Africa's water hyacinth biocontrol programme has the highest number of established agents, yet the success achieved through the programme (biocontrol) has been variable (Hill & Olckers, 2001). Successful biocontrol has only been reported at a few sites such as New Year's Dam in the Eastern Cape Province and at Clairwood Quarry in the Kwa-Zulu Natal Province (Hill, 2003). The cold winters (Hill & Cilliers, 1999) coupled with the eutrophic status of most water bodies (Wilson, 2002; Ripley *et al.*, 2006) are reported to impede biological control of water hyacinth. Unsuccessful results in a biocontrol programme can also be accredited to a poor match between biocontrol agents' native climate range and the local climatic conditions in the area of introduction (Byrne *et al.,* 2002). Therefore the need for an integrated control programme is emphasized. Integrated weed management becomes an option because none of the above control methods have always proven to be effective on their own in controlling water hyacinth.

Integrated Pest Management (IPM)

Di Tomaso (2007) referred to IPM as a practice that includes combinations of two or more of the following control techniques: mechanical, cultural, biological and chemical control. The weed equivalent of IPM is integrated weed management (IWM) and similarly to IPM, IWM is defined as the integrated use of an array of techniques, including physical, chemical, and biological methods without solely depending on any one method alone (Powles & Matthews, 1992). The choice of combinations of strategies has to be tailored to the site, economics, and management intentions (DiTomaso, 2007).

It is suggested that in the same way a raging wild fire can't be fought with only one method, this is applicable to invasive weeds. In this work two control measures, biocontrol and herbicidal control were combined to combat water hyacinth. Herbicides used in the traditional way, i.e. at lethal concentrations, have been reported to interfere with biocontrol as herbicides may kill insects directly or indirectly through habitat destruction (Ueckermann & Hill, 2001); therefore to avoid this, the use of low herbicide concentrations was investigated by Jadhav *et al*. (2008).

1.1.6 Sub-Lethal Dose of Glyphosate

Previous studies have suggested that the best way of integrating herbicide control with biological control is by applying sub-lethal concentrations of a recommended herbicide (Wright & Bourne, 1990). Sub-lethal concentrations of herbicide serve to weaken the plant's defence mechanism and retard its growth, thereby rendering it more susceptible to other stresses such as herbivory (Center *et al.*, 1999).

Jadhav *et al.*, (2008) found that 1.5% and 2% glyphosate concentrations sprayed on water hyacinth under *N. eichhorniae* and *N. bruchi* herbivory resulted in mortality of the two biocontrol agents; whereas Ueckermann and Hill (2001) found no effect on the weevils but rather recorded high mortality of the mirid, *Eccritotarsus catarinensis*. Nevertheless, it is important to note that the mortality recorded in Ueckermann and Hill (2001) was a result of agents' direct exposure to the herbicide, as opposed to Jadhav *et al.* (2008), where mortality was a result of plant annihilation and therefore habitat destruction. Therefore, the need to find a concentration of herbicide which is neither lethal to the plant nor to the biocontrol agents arose. Not only will sub-lethal doses of herbicide prevent annihilation of the weed but they will also reduce the cost of herbicides that will be used; and hopefully reduce the frequency of herbicide applications, as biocontrol agents will exert some pressure on the weed such that the interval between subsequent sprays will be prolonged (Kirton, 2005).

Jadhav *et al.* (2008), after testing several herbicide concentrations, found 0.8% roundup concentration (glyphosate formulated herbicide), administered at a spray volume of 140 l/ha, to be sub-lethal to water hyacinth as it retarded its vegetative growth. In addition, they also found that the performance (survival, reproduction and feeding) of the weevil *N. eichhorniae* and *N. bruchi* was not jeopardized at this concentration. Another experiment was conducted to examine survival of *E. catarinensis* released on water hyacinth plants sprayed with 0.4%, 0.8%, and 1% glyphosate. Results showed that reproduction and survival of *E. catarinensis* was not impeded at any of these low herbicide concentrations (Katembo, 2008).

Important factors to consider when applying sub-lethal dosages herbicide

Factors affecting herbicide performance, such as plant phenotype, weed growth stage, and herbicide application techniques, need to be considered when applying herbicides, especially at reduced doses. Plants at different growth stages respond differently to herbicide applications. It is reported that weed growth stage is an important factor influencing herbicide effectiveness (Steckel *et al*., 1997; Jordan *et al*., 1997). Auskalnis (2003) proposes that when herbicides are applied at reduced doses, plants should be targeted when they are at a very early growth stage. In the case of water hyacinth, Jadhav *et al*. (2008) proposed spraying when the plants are actively growing, in autumn so as to retard the production of ramets and subsequently in spring in order to suppress biomass accumulation as plants recover from winter frost.

Herbicide application techniques, which involve the selection of nozzle type and size, nozzle pressure and volume rate, are also to be considered when reducing herbicide doses. For example, plants are found to be more susceptible to herbicides when applied as small droplets than when applied as larger droplets (Knoche, 1994). However the prerequisite while minimizing herbicide doses is to ensure a uniform herbicide distribution when spraying (Kudsk, 2008).

Unfortunately, Jadhav *et al*. (2008) in their laboratory study did not make provision for the disparity in plant phenotypes from one site to another, nor did they consider the seasonal plant size variations even within the same site. Water hyacinth grows in different phenotypes mainly depending on factors such as water nutrient (nitrogen and phosphorus in particular) (Heard & Winterton, 2000), stage of invasion, and open versus closed canopy. Therefore a sub-lethal dose of glyphosate could have a specific effect on particular water hyacinth phenotypes, while having a much lower effect or no effects at all on other water hyacinth phenotypes purely because of size differences between the plants.

1.1.7 Effect of herbicides on plant quality for arthropod herbivores

Herbicides have the propensity to change the quality of plants as food source for arthropod herbivores (Wright & Bourne, 1990; Kjær & Heimbach, 2001); as a result the performance of herbivorous arthropods tends to vary when feeding on herbicide treated plants. Kjær & Elmegaard (1996) showed that the performance of a chrysomelid beetle, *Gastrophysa polygoni* L. was reduced upon chlorsulfuron treatment of its host plant, the black bindweed, *Polygonum conuoluulus* L. In many other instances, biocontrol agent populations have adversely been affected though host plant death (Ainsworth, 2003). For example, Center *et al.* (1999) observed that the population of the two water hyacinth weevils, *N. eichhorniae and N. bruchi* declined after the water hyacinth weed was sprayed with a lethal dose of 2,4-D. This suggests that herbicide treated plants could be detrimental to arthropod herbivores.

Although, in general, the performance of herbivorous arthropods on herbicide treated plants tends to decline, the contrary has also been observed. Oka & Pimental (1976) have noted that corn leaf aphids, corn borers, and corn leaf blight were more abundant on 2,4-D treated corn than they were on untreated corn. The performance of the water hyacinth biological control agent *Niphograpta albiguttalis* has also been observed to increase following 2,4-D application on the weed (Wright and Center, 1984). Such reports suggest that plant quality is seemingly enhanced by herbicides therefore favouring some herbivorous arthropods. According to Ainsworth (2003), boring and sucking insects are believed to perform better on herbicide-stressed plants than on unstressed ones. In an experiment by Jadhav *et al.* (2008), the water hyacinth weevils *N. bruchi* and *N. eichhorniae,* survived better on water hyacinth plants that had been sprayed with a retardant dose of glyphosate than on unsprayed plants. Similar results were obtained with the water hyacinth mirids, *E. catarinensis* (Katembo, 2008). In both experiments, leaf feeding was also higher in sprayed plants than in unsprayed ones. These results suggested that investigations should be conducted to find out why herbicide treated plants appeared to have been fed upon more than untreated ones.

Three reasons are proposed to explain the recorded improved insect performance on herbicide treated plants:

a) Herbicide-induced reduction in plant hardness. Wright and Bourne (1990) noted that 2,4-D improved water hyacinth plant quality by decreasing leaf and petiole hardness.

b) Herbicide-induced increased plant nutritive value. White (1984) reported that herbicide-stressed plants had an increased nutrient content, especially nitrogen; consequently favouring feeding by sap-sucking insects. Denno and McClure (1983) reported that piercing and sucking insects are favoured by an increase in the soluble nitrogen component of their food.

c) Herbicide-induced reduction of plant secondary metabolites (Bentley, 1990), could be one reason why insects have performed well in some instances. In other words, herbicides may render plants vulnerable to insect attacks by impairing its defense mechanism.

1.2 Research Outline

A pilot test of a water hyacinth management strategy, which consisted of combining biocontrol (using *Neochetina* weevils) in conjunction with a sub-lethal dose of glyphosate (0.8% herbicide concentration), was conducted by Jadhav *et al*. (2008) under laboratory conditions. Hence the basis of the present work was to test the same management strategy under field conditions. The outcome of this work will inform environmental managers and farmers about the feasibility of using a low dose of glyphosate herbicide in conjunction with biological control agents *Neochetina* weevils to control water hyacinth in the field.

This work comprises five chapters. Chapter one gives a general literature review of topics discussed with regard to water hyacinth and its management strategies. Chapter two investigates the feasibility of combining a sub-lethal dose of herbicide with biocontrol agents to control water hyacinth in the field. In chapter three, the relationship between leaf surface area and plant mass is tested in light of its implications on the effect of herbicide on plants of different phenotypes. In chapter four, the nutritive value of treated plants is determined. Water hyacinth's nitrogen, carbon, and phosphorus levels are analyzed to find out how herbicides affect them and thereby how any change influences herbivory. Finally chapter five draws together a general discussion and conclusion from all the findings; closing with some recommendations.

1.3 Research Questions

- How can a sub-lethal dose of glyphosate be used in conjunction with biocontrol agents in integrated management control of water hyacinth?

- How does a sub-lethal dose of Roundup change the quality of plants as a food source for arthropod herbivores?

1.3.1 Research aims

- To assess the suitability of combining a sub-lethal dose of herbicide (0.8% Roundup) with two biocontrol agents (*N. eichhorniae and N. bruchi*) to control water hyacinth in the field.
  - By comparing insect populations on sprayed and unsprayed plants.
  - By comparing plant growth of sprayed and unsprayed plants.

- To determine the relationship between water hyacinth leaf surface area and plant mass, with regard to its effect on herbicide uptake.
  - By examining the leaf surface area to plant mass relationship among four different plant phenotypes.

o To assess how a sub-lethal dose of Roundup can change the quality of water hyacinth plants as a food source for biocontrol agents.

- By comparing nitrogen, carbon, and phosphorus contents of sprayed and unsprayed water hyacinth plants.
- By comparing agent performance (feeding, reproduction and survival) on sprayed and unsprayed water hyacinth plants.

CHAPTER TWO: INTEGRATING *NEOCHETINA* WEEVILS AND GLYPHOSATE TO CONTROL WATER HYACINTH

2.1 Introduction

The basic premise for integrating different control measures is to fill in the gaps in one control method by using another control method, in an effort to fight against a given invasive species. Bisignanesi & Borgas (2007) defined integrated pest management (IPM) as the adoption of a linked set of strategies, ranging from monitoring and the limited strategic use of chemicals, to the use of refuges for beneficial insects used as control agents. The choice of combination of control methods has not only to be tailored to the site, economics, and management intentions (Di Tomaso *et al*., 2006) but is also based on the nature of the weed to be controlled.

Often, integrated approaches of weed control combined with biological control methods involve either prescribed burning (Fellows & Newton, 1999; Lym, 2005), addition of competitive, desirable vegetation (DiTomaso *et al*., 2006), or the use of herbicides (Ainsworth, 2003; Jadhav *et al*., 2008). This present work looked at the combination of herbicides and biocontrol as a management strategy to control water hyacinth. Although South Africa's biocontrol programme has the highest number of established agents, the success achieved through this programme has not been satisfactory (Hill & Olckers, 2001). Since the control of water hyacinth cannot rely solely on biological control, it is therefore paramount to integrate some herbicides to the management strategy in order to reduce the infestations to levels that are acceptable to land owners and that can be easily maintained (Cilliers *et al*., 1996).

2.1.1 Integrating biocontrol agents with herbicides

The combination of classical biological control and chemical control of invasive alien weeds was originally believed to be incompatible (Harris, 1991). However, now many other studies have recognized that the two approaches may result in improved weed control programmes (Messersmith & Adkins, 1995; Lindgren *et al*., 1999; Di Tomaso, 2007). In South Africa, the first water hyacinth integrated control management commenced in 1995 on the Nseleni River (Kwa Zulu Natal). This programme consisted of spraying water hyacinth with a lethal dose of herbicide while leaving some unsprayed plants to serve as refuge for the biological control agents (Jones & Cilliers, 1999).

Some biological control programmes alone have yielded successful results in controlling invasive aquatic weeds, for example: the weevil *Stenopelmus rufinasus* Gyllenhal (Curculionidae) on *Azolla filiculoides* Lamarck (Azollaceae) (red water fern) (Hill, 2003); the leaf-feeding beetle, *Lysathia* sp. (Chrysomelidae) on *Myriophyllum aquaticum* (Vellozo Conceição) Verdcourt (parrot’s feather) (Cilliers, 1999); the weevil *Cyrtobagous salviniae* Calder and Sands on *Salvinia molesta* D.S. Mitchell (Salviniaceae) (salvinia) (Cilliers, 1991). Other examples with terrestrial weeds include the cactus-boring moth *Cactoblastis cactorum* on *Opuntia stricta* (Hoffmann *et al*., 1998), and the cinnabar moth *Tyria jacobaeae* on *Senecio jacobaea* (Pemberton & Turner, 1990). Nonetheless, the integration of herbicide applications with biological control agents is necessary in many instances especially when rapid suppression of a weed infestation is required (Ainsworth, 2003). McFadyen (2000) reported that in some cases biocontrol agents take up to twenty years before results become evident, therefore other control techniques such as moderate herbicide intervention may be indispensable if weed infestations are to be contained. Lym (2005) suggested that the integration of biological control with herbicide control could reduce weed density below the economic threshold more rapidly than any control method used singly (Fig. 2.1). For example, the combination of a sub-lethal concentration of fluridone with a hydrilla specific fungal pathogen (*Mycoleptodiscus terrestris*) resulted in a reduction of more than 90% weed biomass than when the pathogen or the herbicide was used alone (Netherland & Shearer, 1996). Boydston & Williams (2004) found that the vegetative growth of field bindweed plants (*Convolvulus arvensis)* was reduced more, by combining the gall mite (*Aceria malherbae)* with either 2,4-D or glyphosate application, than only using mites or herbicides alone. Center *et al*. (1999) found that the two biocontrol weevils (*N. bruchi* and *N. eichhorniae*) were instrumental in maintaining water hyacinth population at considerably lower density after it had been sprayed with 2,4-D. However water hyacinth populations in Center *et al.* (1999), were reduced primarily through applications of 2,4-D herbicide at recommended concentrations, therefore the weevils simply prevented the rapid re-growth of resurgent plants.

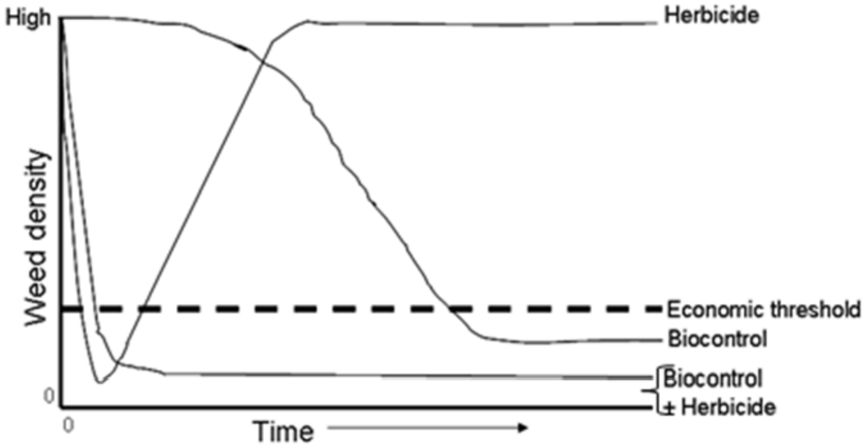

Figure 2.1 Relative efficacy of herbicide, biocontrol agent, and herbicide plus biocontrol agent for weed control over time (Lym, 2005).

Cullen (1996) differentiated three ways in which biological control may be integrated with herbicide applications: a “purpose-specific approach”, an “ecological approach”, and a “physiological approach”.

- Purpose-specific approach: in this approach biological control and herbicide control, although integrated, are not used simultaneously on the whole infested site. For instance, herbicide applications may be used to control important (or central) weed infestations while relying on biological control agents to suppress peripheral infestations on the very same site (Lym, 1998). This approach is similar to the one used at Nseleni River (Jones & Cilliers, 1999)
- Ecological integration approach: this refers to cases where weed infestations are controlled through chemical and biological management consecutively. The purpose here is to consistently decrease weed infestation using herbicides, then allowing biological control agents to maintain the weed population at an acceptable level (Zimmermann *et al.*, 2004).
- Physiological integration approach: this is similar to the ecological approach. The only difference is that in the physiological approach sub-lethal doses of herbicide are used in lieu of lethal doses. Consequently the aim here is not to decrease weed

infestations primarily using herbicides but to allow both arthropod agents and herbicides to work synergistically towards suppressing the infestation (Cullen, 1996). Physiological integration approach could be one solution to the challenges that biocontrol-herbicide management programmes for water hyacinth have at present because a sub-lethal dose of herbicide will not kill the weed but it will only retard its growth while allowing biocontrol agents to exert more pressure on the weed.

Such an integrated approach was tested by Jadhav *et al.* (2008) in the control of water hyacinth in a laboratory set up. They found that a sub-lethal dose of glyphosate (0.8% glyphosate concentration; 140 l/ha spray volume) resulted in retarding water hyacinth growth (ramet and leaf production) while not interfering with *Neochetina* weevils' performance (reproduction, survival and feeding). The present work repeated this approach in the field and results are presented later in this chapter.

2.1.3 Drawbacks of combining biocontrol agents with herbicides

One of the major drawbacks of IPM lies in the interactive complexity among different selected management strategies (Bisignanesi & Borgas, 2007). Early reports also highlight a lack of basic knowledge on the interactions between plant, pest, climate and natural enemies (Burn *et al*., 1987). There have been cases where the integration of biocontrol agents with herbicide applications proved not to be an ideal weed control technique. For example, the use of herbicide to control *Opuntia aurantiaca* (Cactaceae) in South Africa was observed to interfere with the biocontrol agent *Dacylopius austrinus* by reducing its population (Zimmermann & Neser, 1999). Toxicity of herbicides such as 2,4-D to biocontrol agents at field rates has been reported in many studies (Ueckermann and Hill, 2001). The population of *Aphthona* spp. biological control agents of leafy spurge (*Euphorbia esula*) was seen to decline in plots treated with 2,4-D and picloram compared to untreated plots (Larson *et al*., 2007). Nevertheless, in most cases the damage to biocontrol agents has been a result of the destruction of their host plants rather than direct herbicide toxicity on the agents (Di Tomaso, 2007). Another important point to note in an integrated management programme combining herbicide spray with biocontrol agents is the timing of chemical application (Di Tomaso, 2007). Untimely glyphosate application on purple loosestrife (*Lythrum salicaria*, Lythraceae) resulted in the destruction of the plant which is the food source for the biocontrol agent, leaf beetle *Galerucella calmariensis* (Lindgren *et al*., 1999).

2.1.3 Rationale for this chapter

There have been relatively few studies focusing on the integration of arthropods and herbicides as a way of managing aquatic weeds (Lym & Nelson, 2002). Of the few herbicide-arthropods studies that have been conducted, often the focus was directed to the effect of herbicides on the arthropods rather than the combined effect of herbicides and arthropods in controlling weeds (Paynter, 2003). In addition, most of these studies were conducted under laboratory conditions (Boydston & Williams, 2004; Jadhav *et al*., 2008). Therefore it is the purpose of this work to test the feasibility of combining a sub-lethal dose of glyphosate with *Neochetina* weevils under field conditions in the control of water hyacinth.

In Jadhav *et al*.'s (2008) laboratory work, several glyphosate herbicide concentrations were sprayed on water hyacinth to find out which concentration will not be lethal to the weed or the *Neochetina* agents, but one which will only result in the retardation of the vegetative growth of the weed. The different tested herbicide concentrations included 0.1%, 0.3%, 0.5%, 0.8%, 1%, and 1.5%. Their results concluded that 0.8% glyphosate concentration sprayed at140 l/ha spray volume (0.11 g/m^2 active ingredient) retarded ramet and leaf production, while not killing the agents.

2.2 Research questions addressed in this chapter

- What is the impact of a combination a sub-lethal dose of glyphosate (0.8% glyphosate concentration, 0.11 g/m^2 a.i) and the *Neochetina* weevils on water hyacinth in the field?
- How does a sub-lethal dose of glyphosate impact the performance of water hyacinth weevils (*N. eichhorniae* and *N. bruchi*) in the field?

2.3 Materials and methods

Trials were carried out at Delta Park and Farm Dam, Johannesburg. Both sites are infested with water hyacinth and have populations of *Neochetina* weevils; however there is lower weevil infestation at Farm Dam compared to Delta Park (Byrne *et al.*, 2010). Farm Dam is situated in the North Riding area, whereas Delta Park is located in the Randburg area. Farm Dam is characterized by big plants (Fig. 2.2) with relatively short roots; whereas Delta Park has small plants with long roots (Fig. 2.3). Table 2.1 describes the nutrient and temperature profiles plus a geographical situation of these sites. Both sites are prone to frost during winter.

Figure 2.2 Water hyacinth plants at Farm Dam, with large leaves and long petioles (spring, 2008)

Figure 2.3 Water hyacinth plants at Delta Park, with small leaves, and short, bulbous petioles (spring, 2008)

Table 2.1 Description of Delta Park and Farm Dam; median values of water nutrients, and Minimum/maximum temperature are given (extracted from Byrne *et al.*, 2010)

Site name	Total N	Total P	Latitude	Longitude	Temperature
Delta Park	4.80 (mg/L)	0.71 (mg/L)	$26^o07'S$	$28^o00'E$	1 – 16 ºC
Farm Dam	4.10 (mg/L)	0.68 (mg/L)	$26^o02'S$	$27^o57'E$	1 – 17 ºC

These two sites were chosen because they have more than five years worth of history of water hyacinth biological control. At each site a strip of water hyacinth plants, approximately 12 x 3 meters, was restrained between two cables fitted with buoys. Cables perpendicular to these divided the strip into two equal plots of 6 x 3 meters, comprising a treatment plot and a control plot. Both plots were each further subdivided into three blocks of 2 x 3 meters, forming three replicates per plot. 1 – 17 ºC

The treatment plots at each site were sprayed with 0.8% of a broad spectrum, glyphosate based herbicide, Roundup (active ingredient: 360 g /L, Monsanto Pty. Ltd. South Africa) at a spray volume of 140 l/ha giving an a.i (active ingredient) of 0.11 g/m^2 (Jadhav *et al.*, 2008), while the control plot was kept herbicide free. A 12v battery operated boom sprayer (Multispray, Midrand / South Africa), fitted with three spray tips (TeeJet even flat: TP65015E), was used to spray water hyacinth from a motor boat at a speed of 4 km/h. Spraying was conducted in three seasons, once per season, in autumn 2008, spring 2008 and summer 2009.

Because the action of Roundup is visible from two weeks after application (Roundup instruction booklet by Monsanto Europe S.A., Nov. 2006 (L.D.M.)), samples were taken every week for five weeks, from the second week after spraying. Base measures were taken before herbicide application. Due to a flood event that occurred during summer of 2009, Farm Dam site was washed out and destroyed. Therefore for this site only autumn 2008 and spring 2008 data are presented. However at Delta Park, data for three seasons autumn 2008, spring 2008 and summer 2009, are presented.

2.3.1 Comparison of plant growth between sprayed and control plants

The following parameters were recorded: number of ramets per plant, number of leaves per plant, leaf-two petiole length, leaf turnover (only in summer at Delta Park), and above-water biomass (kg). A 0.25 m^2 PVC quadrat was randomly thrown one time onto each of the three blocks (three replicates), and above-water biomass per quadrat was recorded from all of the plants enclosed within each quadrat (kg). To determine plant susceptibility to the 0.8% glyphosate herbicide between Delta Park and Farm Dam, growth measures (above-water biomass per quadrat and number of leaves per plant) from unsprayed (control) plants were subtracted to those from sprayed plants and the difference was compared between sites. The above-water biomass was correlated to the number of ramets per plant to find out if there was any correlation between biomass accumulation and ramet production.

2.3.2 Comparison of weevil populations between sprayed and control plants

The following parameters were used to estimate agent populations from each plot: number of weevil larvae per plant, number of adult weevils per plant, and number of adult weevil feeding scars per cm^2 on leaf two (the second youngest leaf).

Plant and insect parameters (with the exception of above-water biomass) were recorded from nine plants randomly collected from each plot (treatment and control plots). All the measures were taken through destructive sampling.

2.3.3 Statistical analysis

The computer programme employed for statistical analysis was STATISTICA, version 6. Plant and insect parameters were analyzed using Factorial ANOVA followed by the Tukey`s HSD Post-Hoc test for comparisons between sprayed and control plants over time. A linear regression was performed to show the relationship between the number of ramets and plant above-water biomass. All analyses were conducted at a critical *P* level of 0.05.

2.4 Results

2.4.1 The combined effect of 0.8% glyphosate and *Neochetina* weevils on water hyacinth

Above-water biomass measures

At Delta Park, in autumn 2008, above-water biomass was not significantly different between control and sprayed plants ($F_{(4, 20)} = 0.99$, $P = 0.43$) (Fig. 2.4.a). In spring 2008 and summer 2009 respectively, above-water biomass was significantly higher in control plants than in the sprayed plants ($F_{(4, 20)} = 6.50$, $P < 0.001$; $F_{(4, 20)} = 10.69$, $P < 0.001$) (Fig. 2.4.b.c).

At Farm Dam, in autumn 2008, there was no significant difference in above-water biomass between control and sprayed plants ($F_{(4, 20)} = 1.57$, $P = 0.21$) (Fig. 2.5.a). In spring 2008, above-water biomass was significantly higher in control plants than in sprayed plants ($F_{(4, 20)} = 5.01$, $P < 0.001$). This difference was observed on the second and third date after herbicide application, and towards the end of the sampling season (Fig. 2.5.b).

In autumn 2008, apart from the first and the fourth week, where above-water biomass was higher in Farm Dam plants compared to Delta Park plants, there was no statistical difference between the two sites ($F_{(4, 20)} = 2.08$, $P = 0.12$) (Fig. 2.6.a). In spring 2008, above-water biomass was significantly higher in Farm Dam plants compared to Delta Park plants ($F_{(4, 20)} = 23.13$, $P < 0.001$) (Fig. 2.6.b).

In autumn 2008, there was no significant difference in the control minus spray above-water biomass between Delta Park plants and Farm Dam plants ($F_{(4, 20)} = 1.67$, $P = 0.19$) (Fig. 2.7.a); however during spring 2008, this difference was significant at Farm Dam compared to Delta Park ($F_{(4, 20)} = 4.11$, $P = 0.01$) (Fig. 2.7.b). This is because, unlike Farm Dam plants, Delta Park plants took longer to accumulate biomass (see Fig. 2.6).

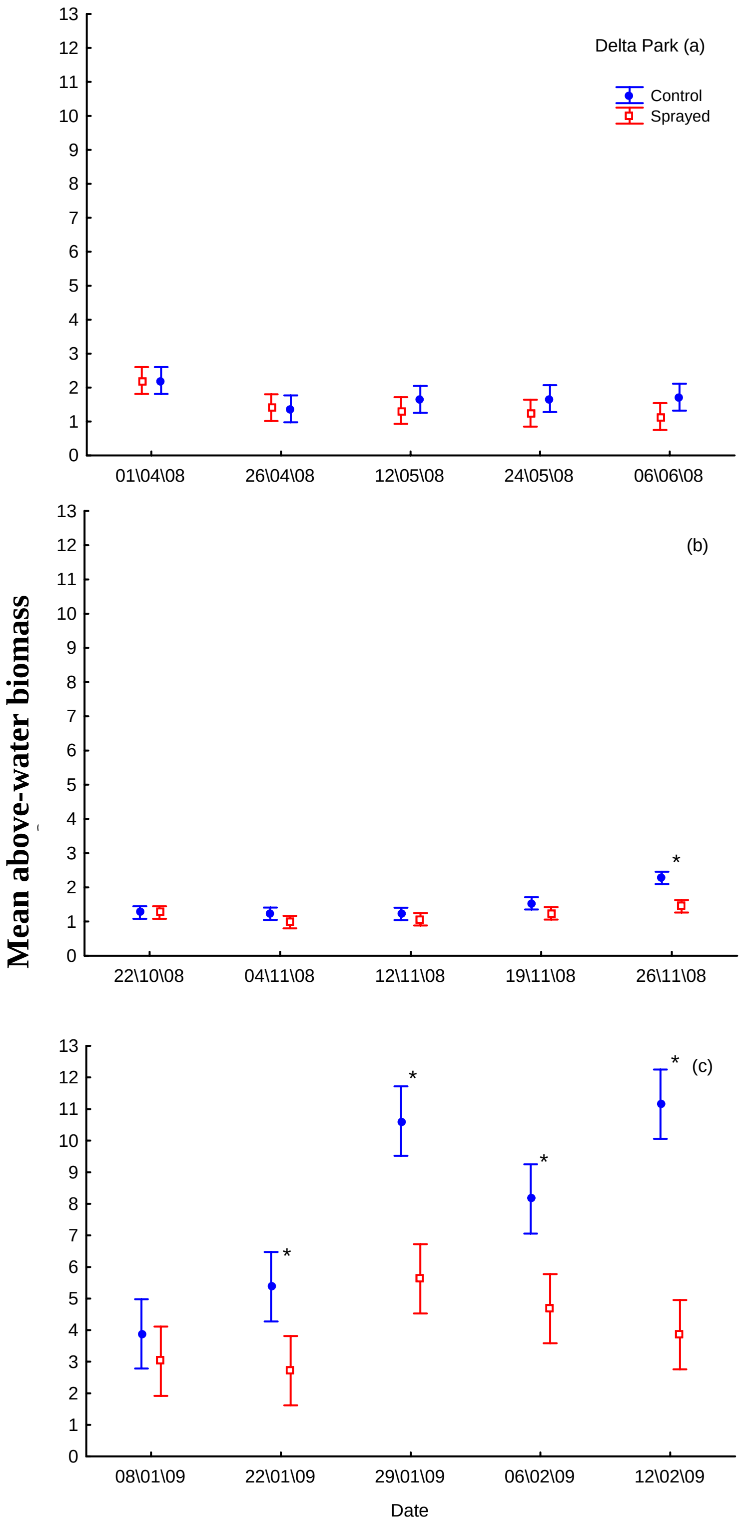

Figure 2.4 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on water hyacinth above-water biomass at Delta Park in (a) autumn 2008, (b) spring 2008, and (c) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

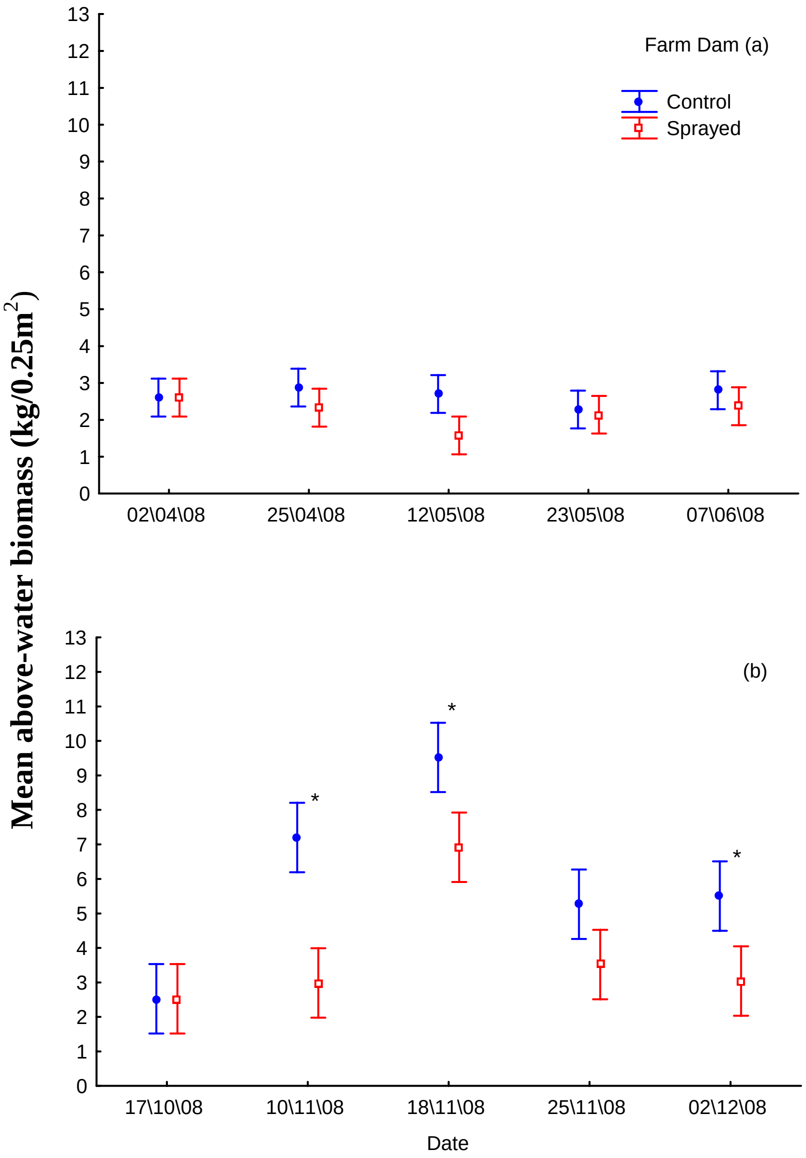

Figure 2.5 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on water hyacinth above-water biomass at Farm Dam in (a) autumn 2008, and (b) spring 2008. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

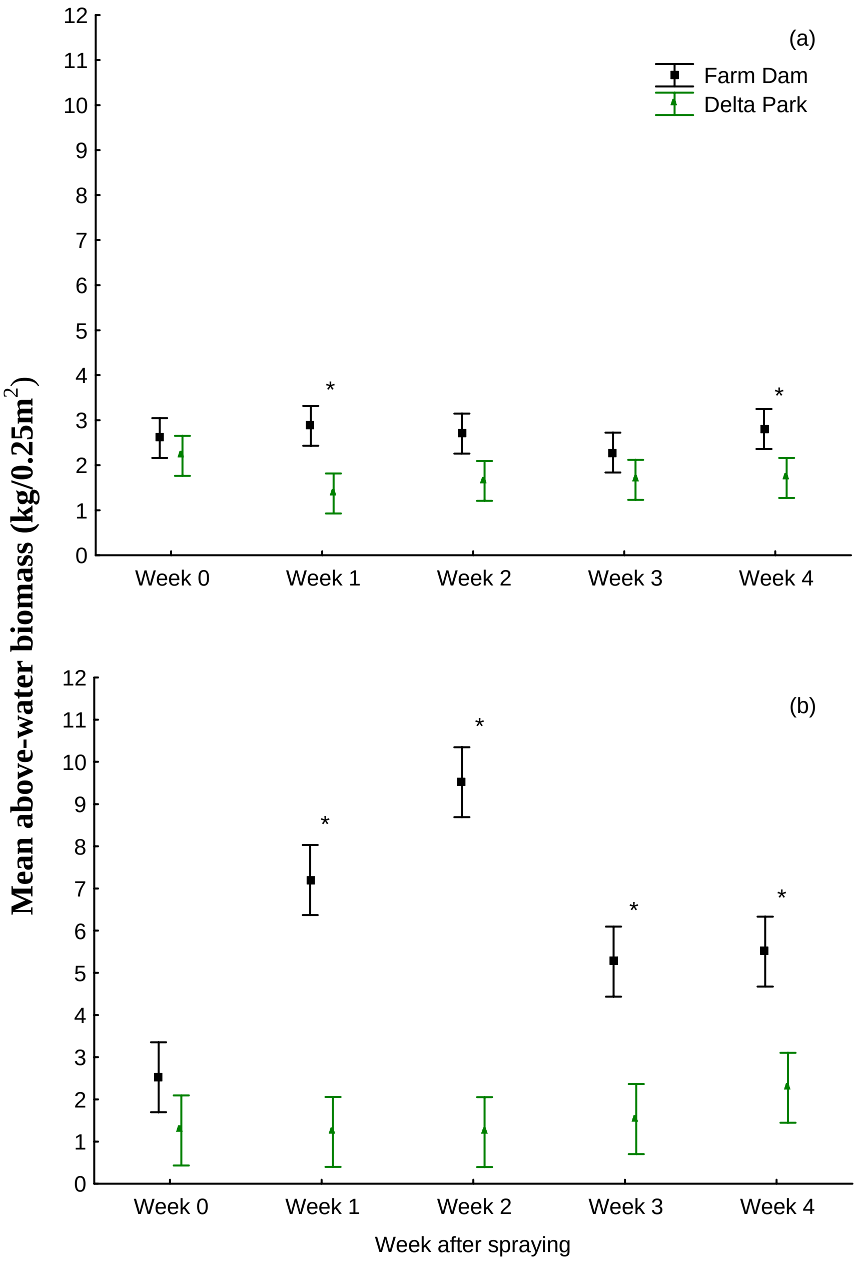

Figure 2.6 Comparison of water hyacinth above-water biomass in (a) autumn 2008 and (b) spring 2008 between Farm Dam and Delta Park unsprayed plants. Error bars represent standard error of the mean. * between Farm Dam/Delta Park pairs denote significant difference at $P < 0.05$.

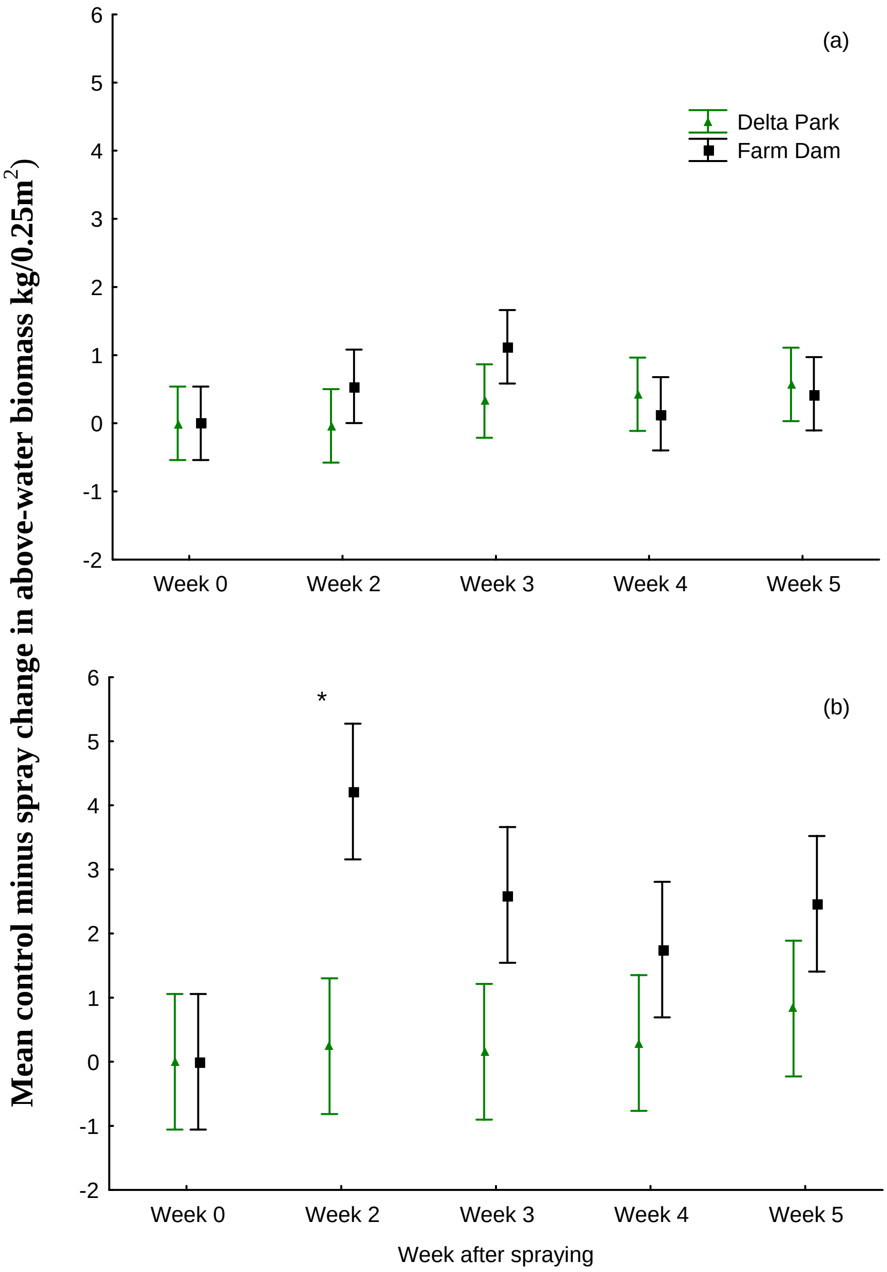

Figure 2.7 Comparison of control-spray difference in above-water biomass in (a) autumn 2008 and (b) spring 2008 between Farm Dam and Delta Park unsprayed plants. Error bars represent standard error of the mean. * between Farm Dam/Delta Park pairs denote significant difference at $P < 0.05$.

Number of ramets per plant

At Delta Park, in autumn 2008, there were significantly more ramets on sprayed plants than on control plants on one occasion ($F_{(4, 90)} = 3.35$, $P = 0.01$) (Fig. 2.8.a). However the general trend showed no difference throughout the whole season with the exception of second last sampling date. In spring 2008, there was no significant difference in the number of ramets per plant between control and sprayed plants ($F_{(4, 80)} = 2.07$, $P = 0.09$) (Fig. 2.8.b). In summer 2009, there was no significant difference in the number of ramets per plant between control plants and sprayed plants ($F_{(4, 80)} = 1.94$, $P = 0.11$) throughout the whole season (Fig. 2.8.c).

At Farm Dam, in autumn 2008, there were significantly more ramets on the control plants compared to the sprayed plants ($F_{(4, 90)} = 5.04$, $P < 0.001$) (Fig. 2.9.a), however this difference was only observed on the last sampling date. In spring 2008, on the other hand, the number of ramets per plant was significantly higher in the sprayed compared to the control plants ($F_{(4, 80)} = 6.49$, $P < 0.001$) (Fig. 2.9.b).

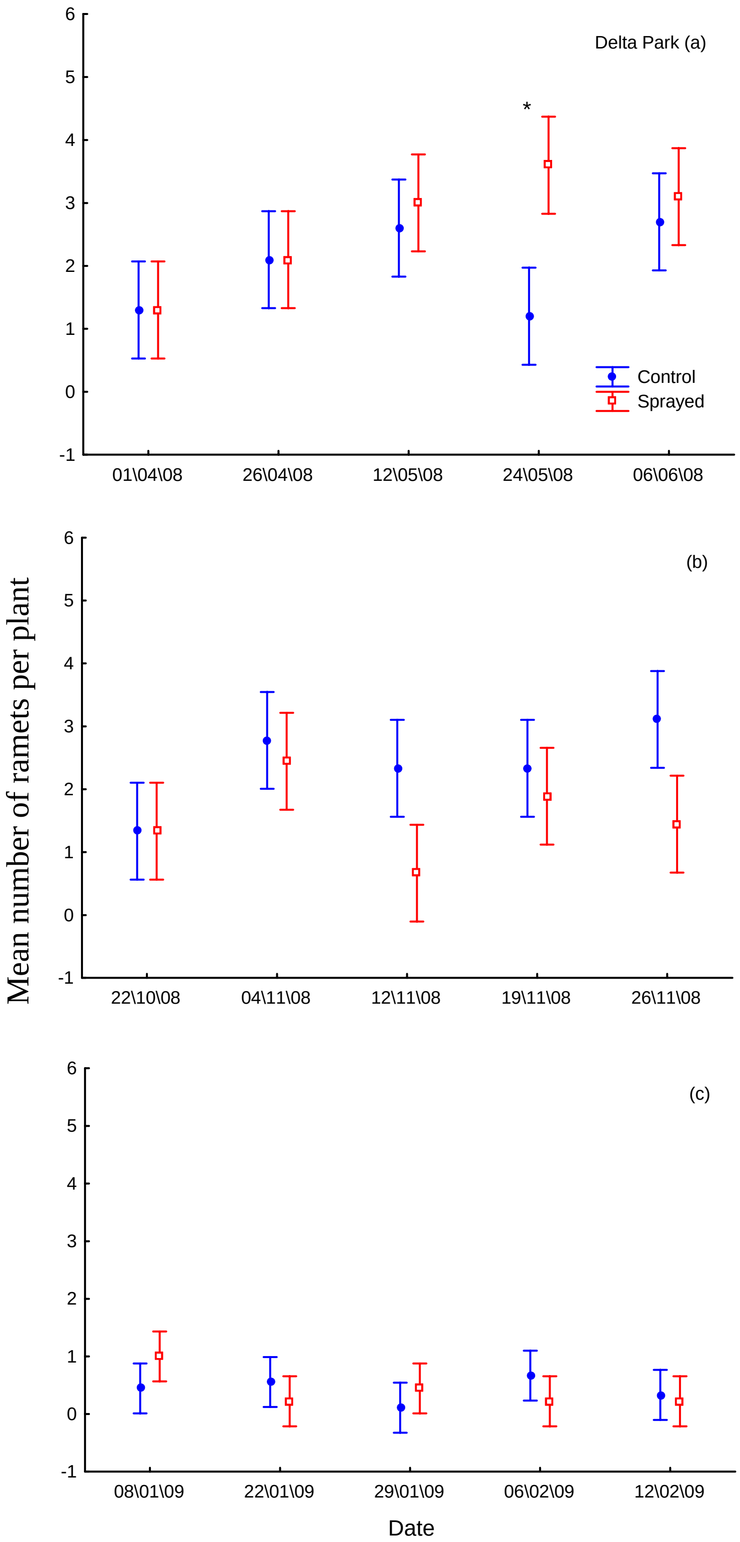

Figure 2.8 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number water hyacinth ramets at Delta Park in (a) autumn 2008, (b) spring 2008, and (c) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

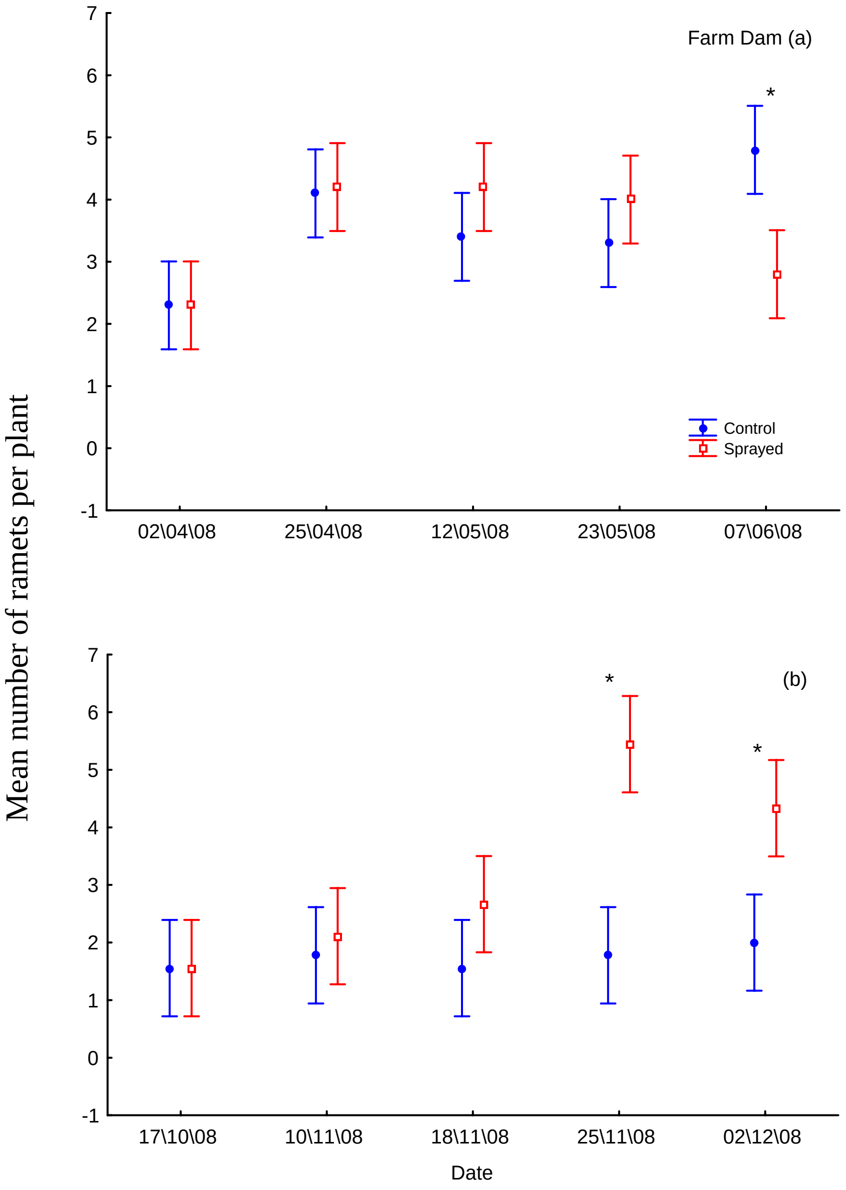

Figure 2.9 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number water hyacinth ramets at Farm Dam in (a) autumn 2008 and (b) spring 2008. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

Relationship between the number of ramets and above-water biomass

There was a significant negative correlation at Delta Park in autumn 2008 between the number of ramets and the above-water biomass in the sprayed plants. However, for both Delta Park and Farm Dam, there was no significant correlation between the number of ramets and the above-water biomass during the rest of the year (Fig 2.10; Fig 2.11).

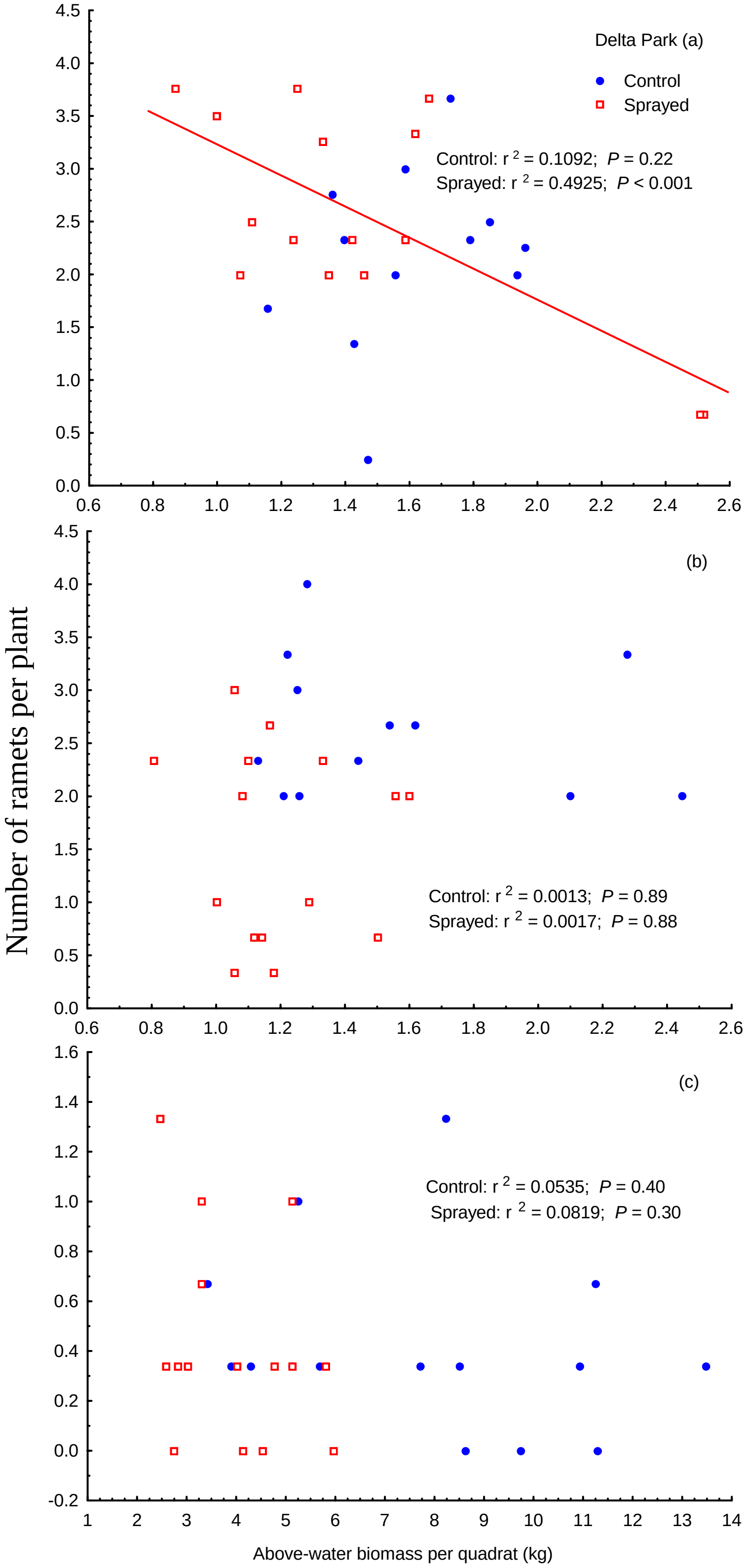

Figure 2.10 Relationship between the above-water biomass per quadrat and the number of ramets per plant at Delta Park in (a) autumn, (b) spring and (c) summer

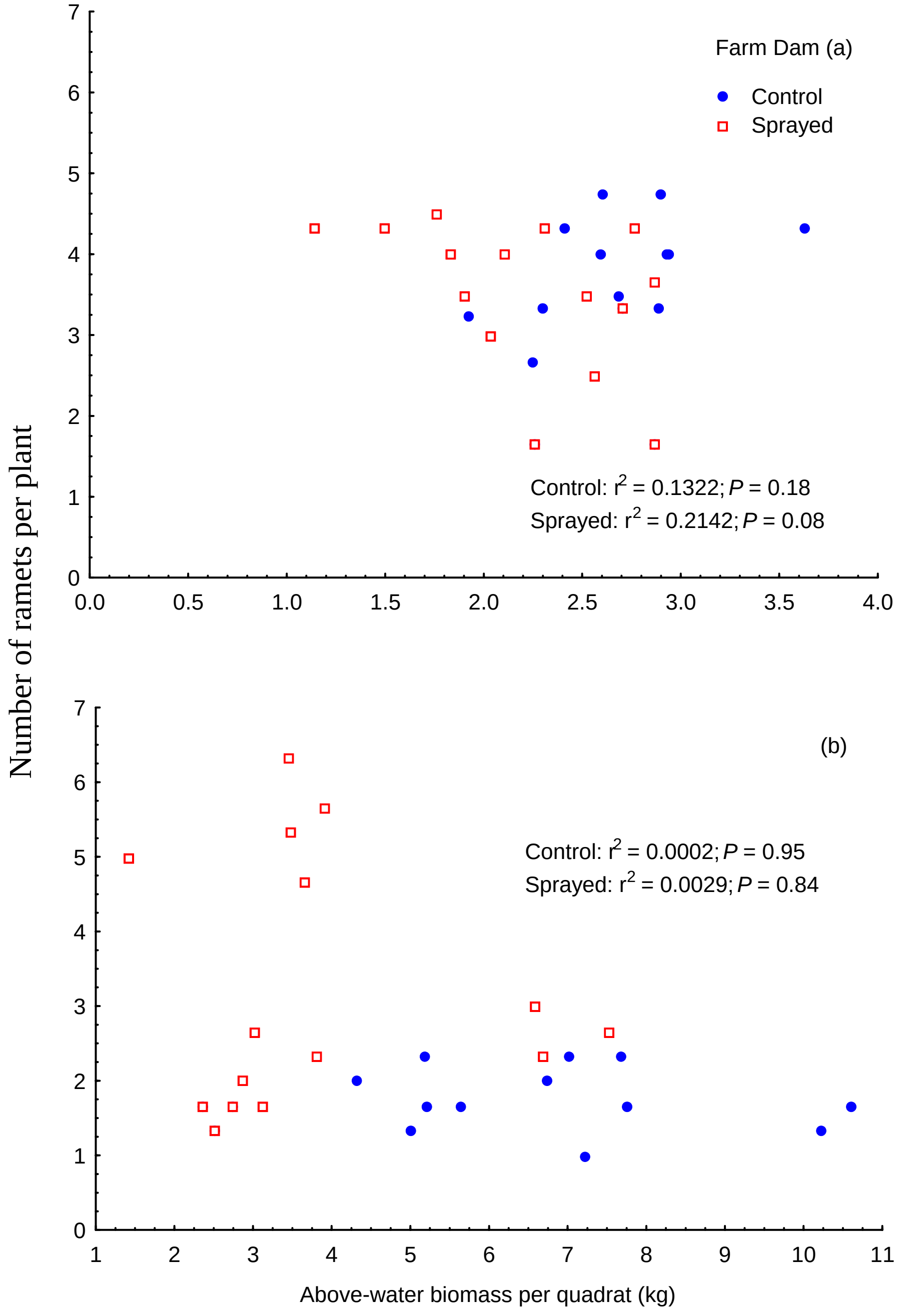

Figure 2.11 Relationship between the above-water biomass per quadrat and the number of ramets per plant at Farm Dam in (a) autumn and (b) spring

Number of leaves per plant

At Delta Park, in autumn 2008, spring 2008 and summer 2009 respectively, the number of leaves per plant was significantly higher in the control plants than in the sprayed plants ($F_{(4, 90)} = 3.33$, $P = 0.01$; $F_{(4, 80)} = 7.19$, $P < 0.001$; $F_{(4, 80)} = 21.10$, $P < 0.001$) (Fig. 2.12.a,b,c).

Similarly at Farm Dam, in autumn 2008, and spring 2008 respectively, the number of leaves was significantly higher in the control than in the sprayed plants ($F_{(4, 90)} = 13.32$, $P < 0.001$; $F_{(4, 80)} = 3.93$, $P < 0.001$) (Fig. 2.13.a,b). However plants at Delta Park are seen to be more susceptible to the 0.8% glyphosate than Farm Dam plants as shown in figure 2. 14.

In autumn 2008 and spring 2008 respectively, the control minus spray difference in the number of leaves was significantly greater at Delta Park compared to Farm Dam ($F_{(4, 90)} = 2.66$, $P = 0.03$; $F_{(4, 80)} = 3.81$, $P < 0.001$) (Fig. 2.14.a,b).

Leaf-two petiole length (cm)

At Delta Park, leaf-two petiole was longer in the control plants compared to the sprayed plants in autumn 2008, spring 2008 and summer 2009 respectively ($F_{(4, 90)} = 2.99$, $P = 0.02$; $F_{(4, 80)} = 43.70$, $P < 0.001$; $F_{(4, 80)} = 4.50$, $P < 0.001$) (Fig. 2.15.a,b,c). However in autumn there was no difference in leaf-two petiole length between control and sprayed plants except from one sampling date and in spring the difference was only seen during the last two sampling dates.

At Farm Dam, in autumn 2008 and in spring 2008 respectively, leaf-two petiole was significantly longer in the control plants compared to the sprayed plants ($F_{(4, 90)} = 8.12$, $P < 0.001$; $F_{(4, 80)} = 64.82$, $P < 0.001$) (Fig. 2.16.a,b).

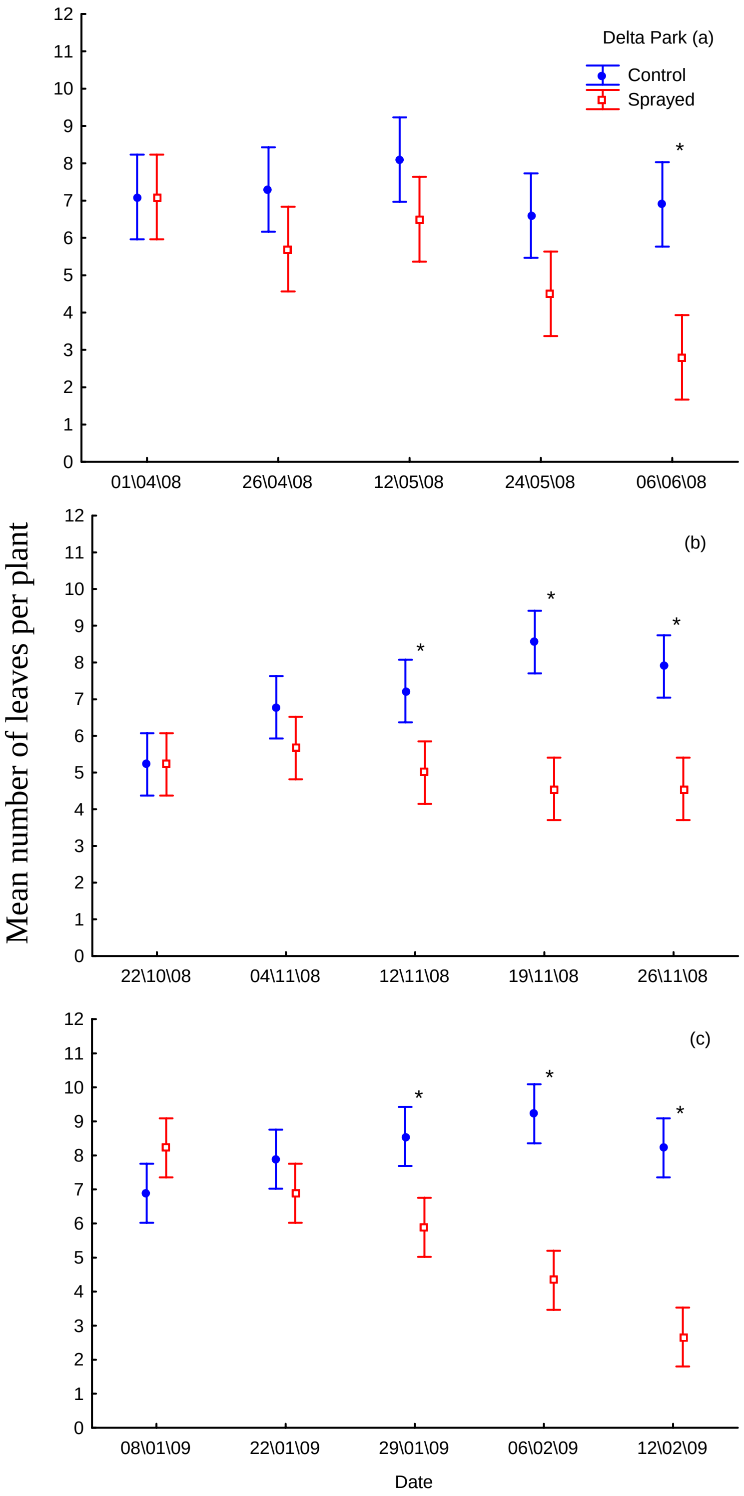

Figure 2.12 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number water hyacinth leaves at Delta Park in (a) autumn 2008, (b) spring 2008, and (c) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

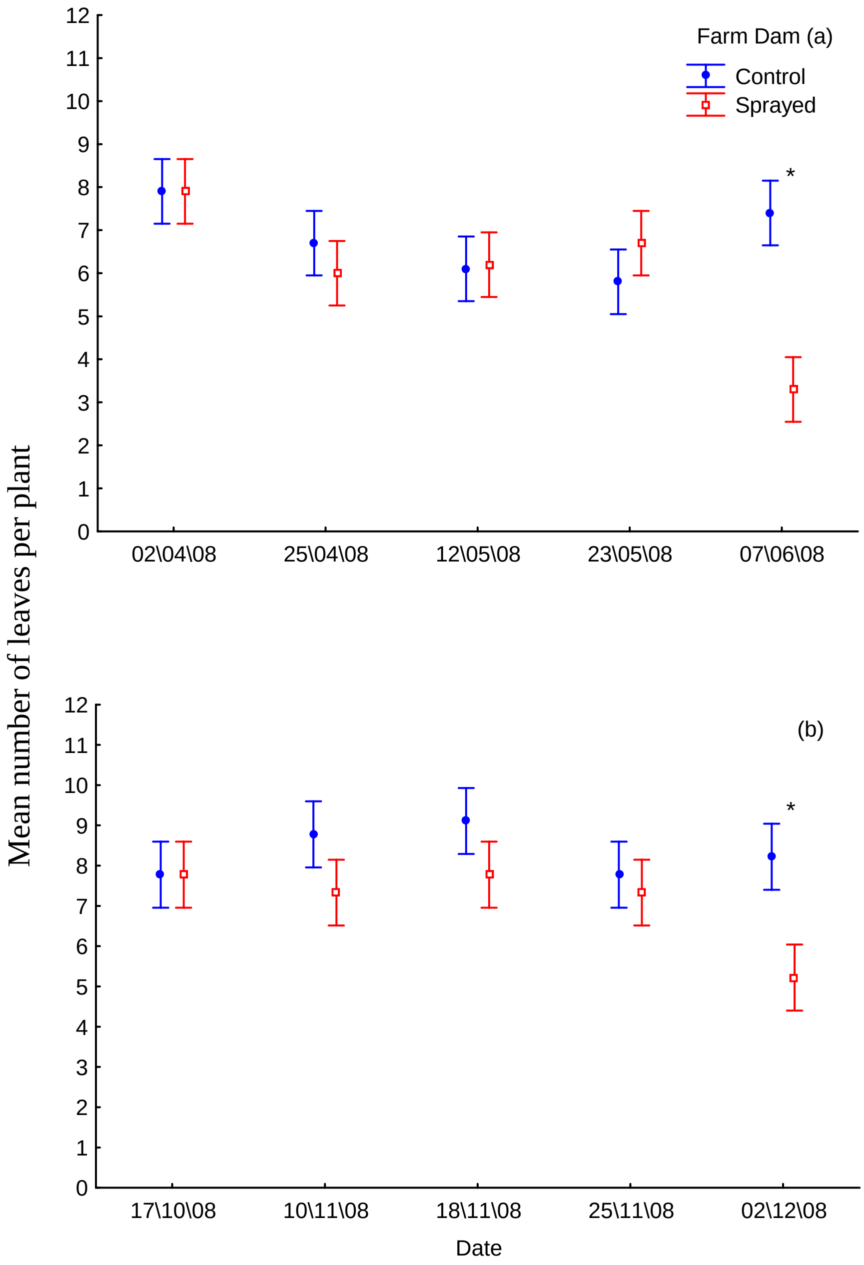

Figure 2.13 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number water hyacinth leaves at Farm Dam in (a) autumn 2008 and (b) spring 2008. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at *P* < 0.05.

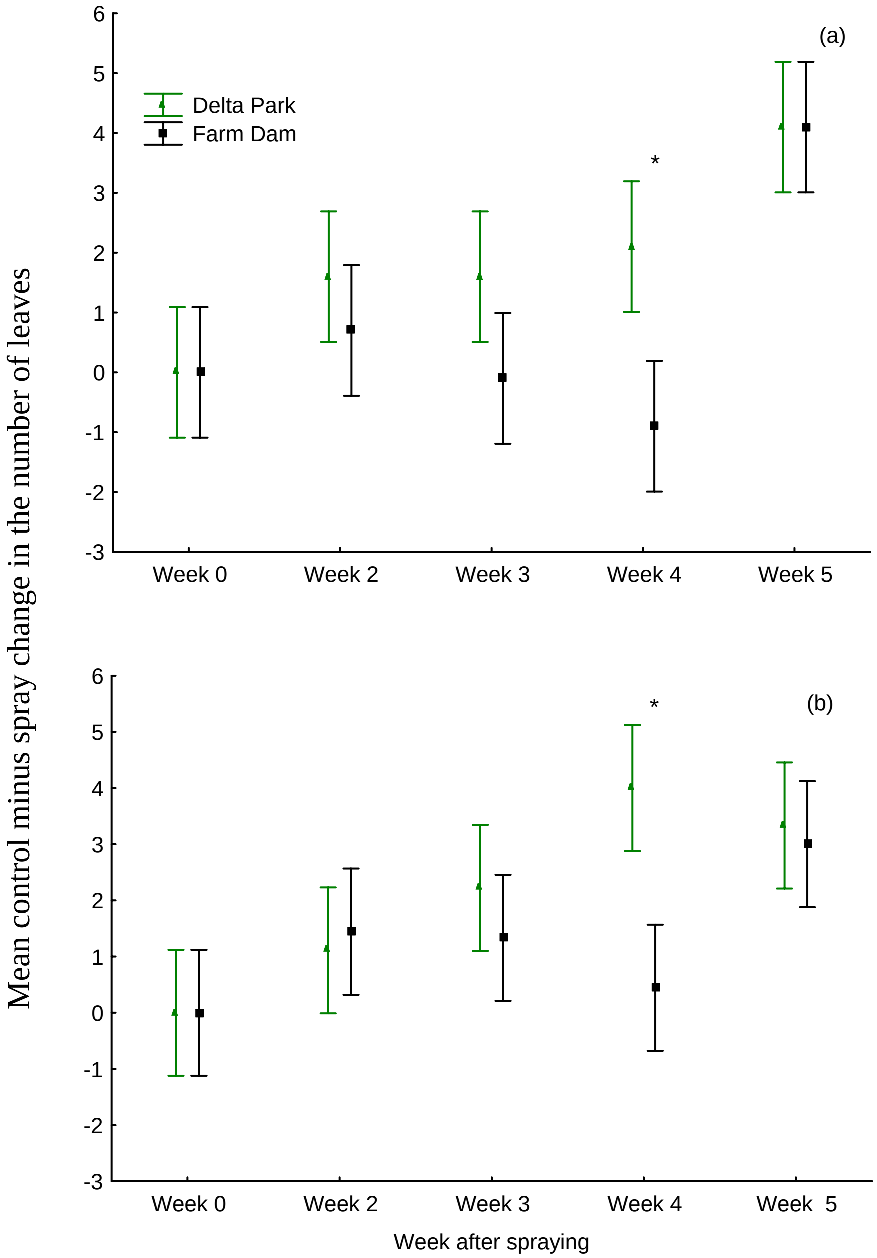

Figure 2.14 Comparison of control-spray difference in the number of leaves in (a) autumn 2008 and (b) spring 2008 between Farm Dam and Delta Park. Error bars represent standard error of the mean. * between Farm Dam/Delta Park pairs denote significant difference at *P* < 0.05.

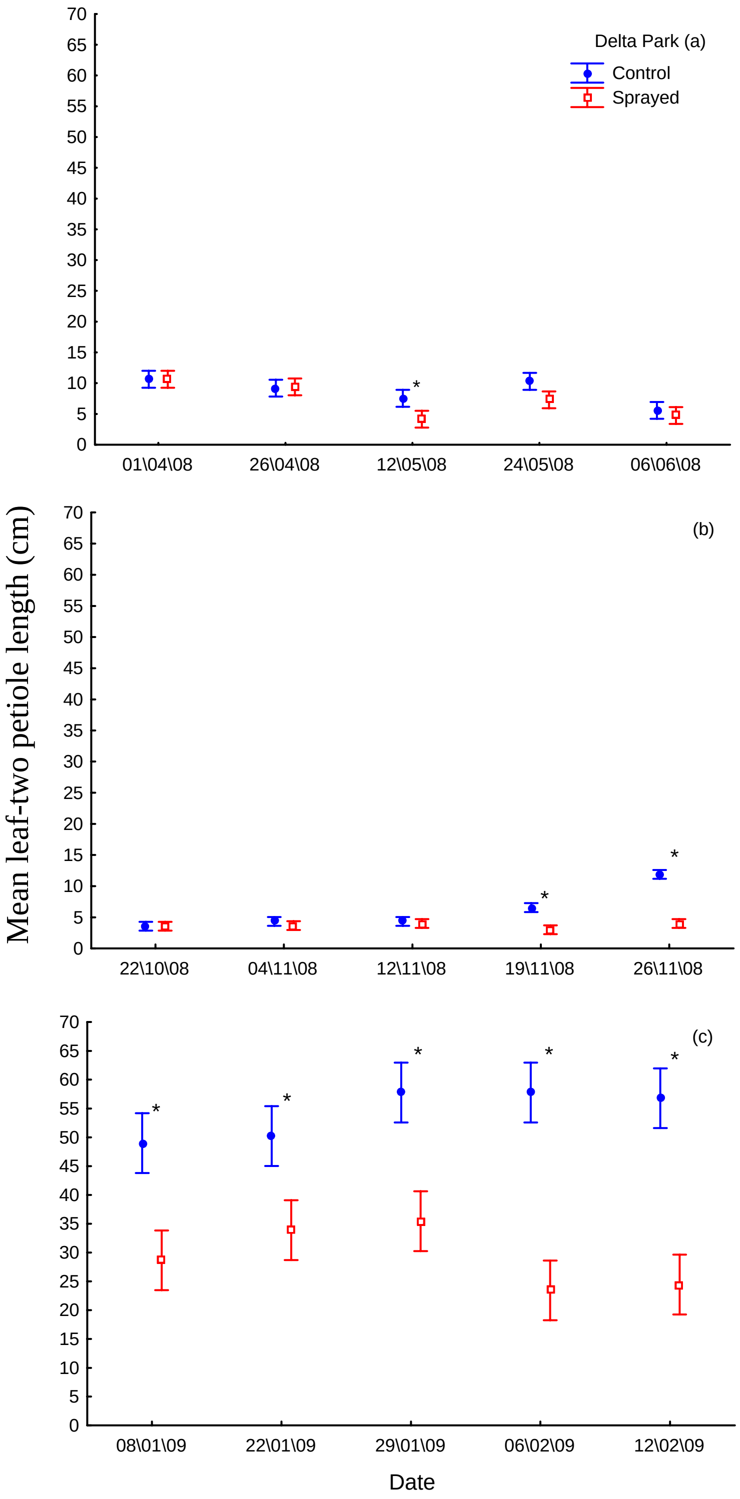

Figure 2.15 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on water hyacinth leaf-two petiole length at Delta Park in (a) autumn 2008, (b) spring 2008, and (c) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at *P* < 0.05

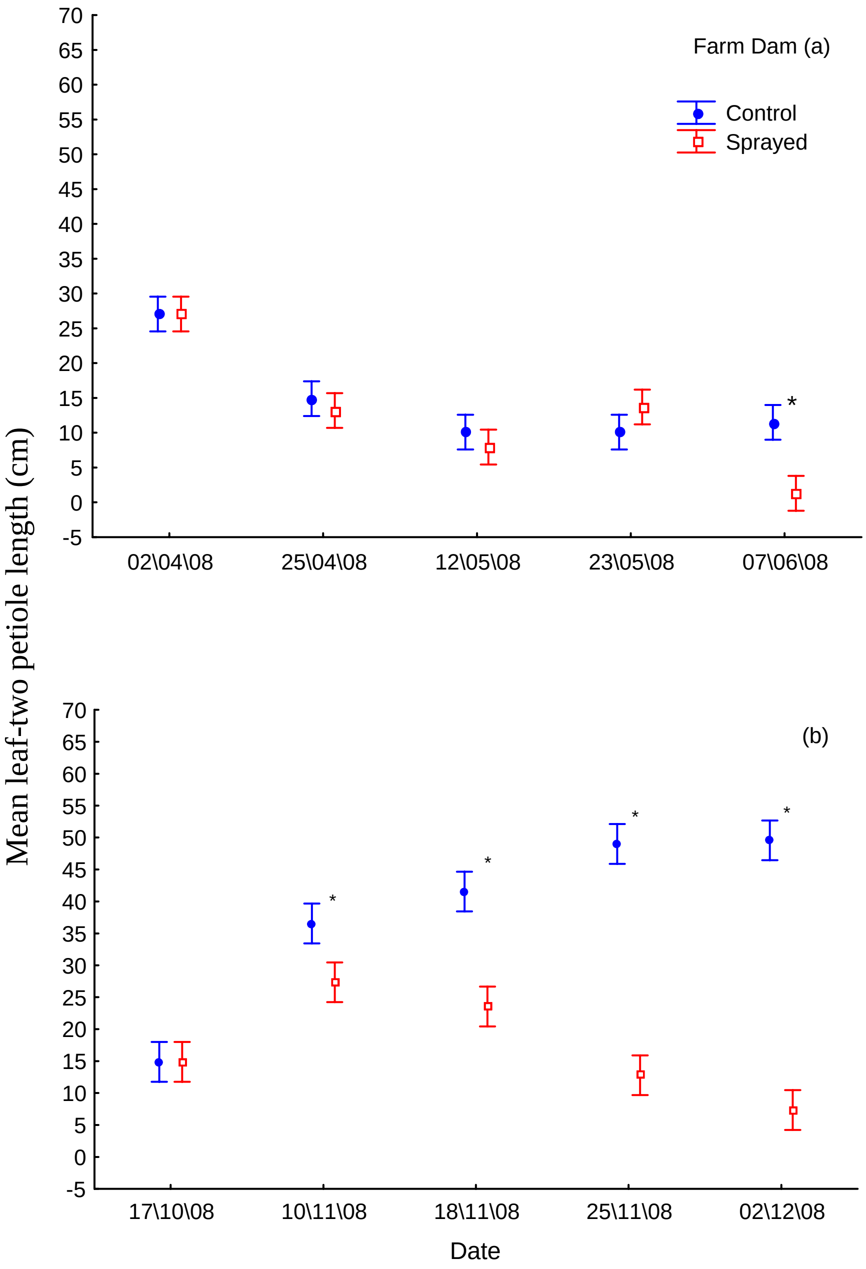

Figure 2.16 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on water hyacinth leaf-two petiole length at Farm Dam in (a) autumn 2008 and (b) spring 2008. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$

Leaf turnover rate (leaf production over time)

At Delta Park, in summer 2009, in the interval of one week (from week 3 through week 4), control plants produced new leaves while sprayed plants did not ($F_{(1,32)} = 100$, $P < 0.001$) (Fig. 2.17).

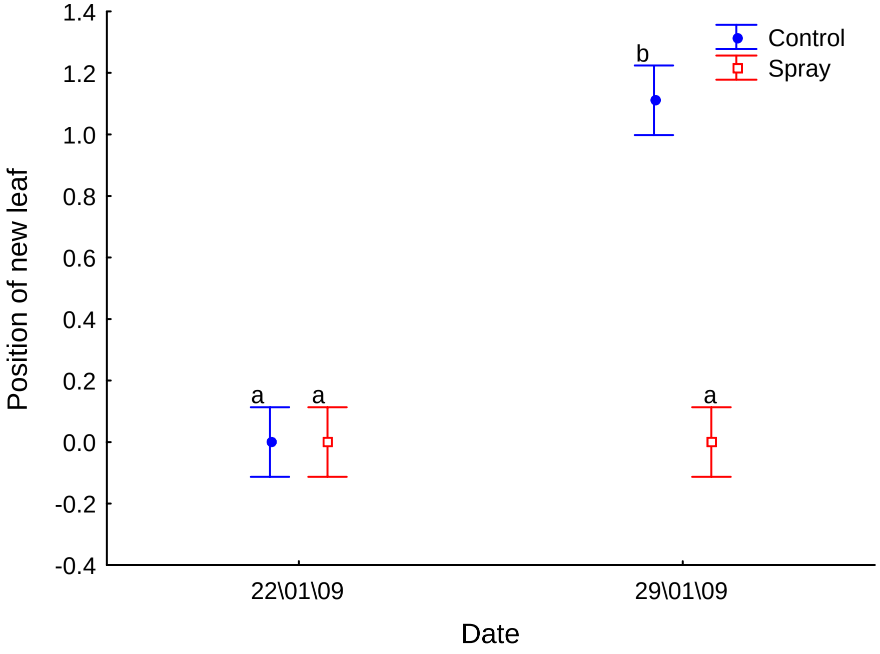

Figure 2.17 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on leaf turnover rate in summer 2009, at Delta Park. Error bars represent standard error of the mean. Bars with the same latter are not significantly different at $P = 0.05$

2.4.2 Insect performance on sprayed and control water hyacinth plants

Number of *Neochetina* larvae per plant

At Delta Park, in autumn 2008, spring 2008 and summer 2009 respectively, the number of *Neochetina* larvae per plant was not significantly different between the control and sprayed plants ($F_{(4, 90)} = 0.20$, $P = 0.93$; $F_{(4, 80)} = 0.36$, $P = 0.83$; $F_{(4, 80)} = 1.06$, $P = 0.37$) (Fig. 2.18.a,b,c).

At Farm Dam, no larvae were found on either sprayed or control plants in autumn 2008. In spring 2008, there was no difference in the number of larvae between the control and sprayed plants ($F_{(4, 80)} = 1.63$, $P = 0.17$ (Fig. 2.19).

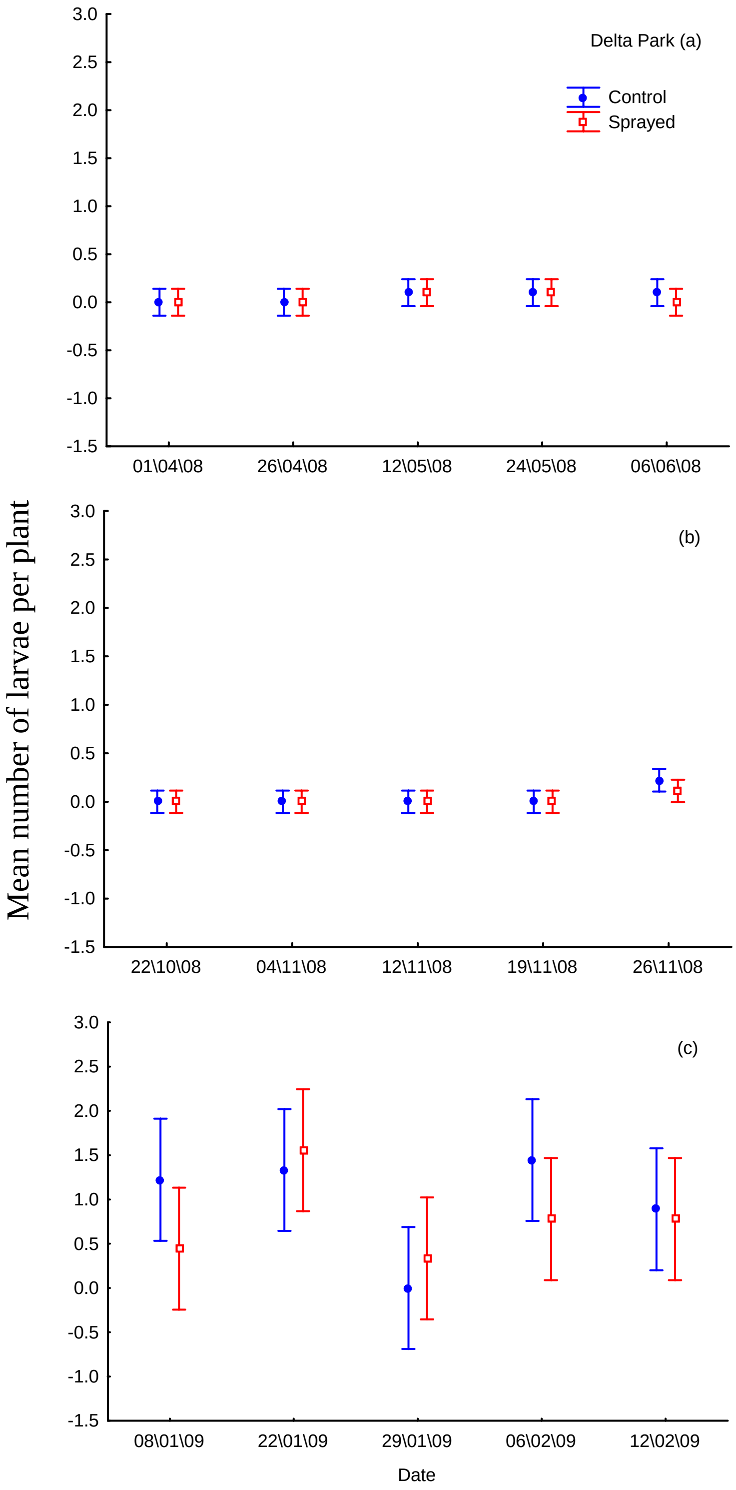

Figure 2.18 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number of *Neochetina* larvae at Delta Park in (a) autumn 2008, (b) spring 2008 and (c) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$

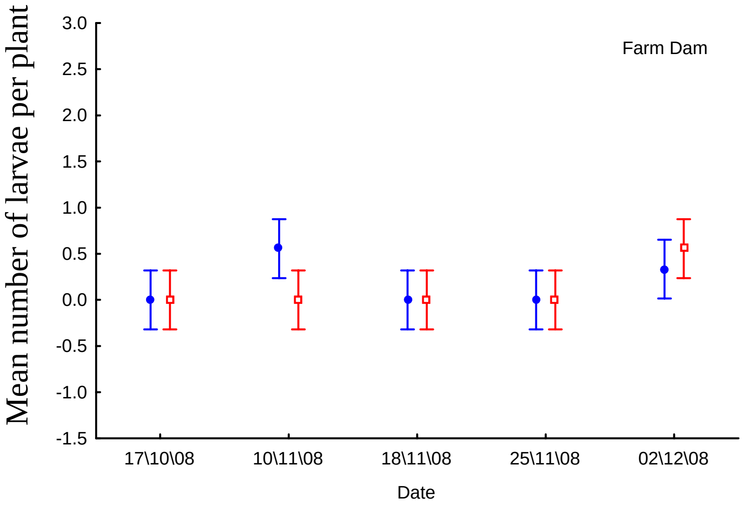

Figure 2.19 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number of *Neochetina* larvae collected from water hyacinth plants at Farm Dam in spring 2008. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$

Number of adult *Neochetina* per plant

At Delta Park, in autumn 2008 and in summer 2009 respectively, the number of adult *Neochetina* weevils per plant was not significantly different between control and sprayed plants ($F_{(4, 90)} = 1.60$, $P = 0.17$; $F_{(4, 80)} = 1.62$, $P = 0.17$) (Fig. 2.20.a,b). In spring 2008, no adult weevils were found on either sprayed or control plants.

At Farm Dam, no adult *Neochetina* were found in either autumn 2008 or in spring 2008 on sprayed or control plants.

Number of weevil feeding scars per cm^2

At Delta Park, in autumn 2008, spring 2008, and summer 2009 respectively, the number of feeding scars on leaves was not significantly different between control and the sprayed plants ($F_{(4, 90)} = 1.48$, $P = 0.21$; $F_{(4, 80)} = 1.13$, $P = 0.34$; $F_{(4, 30)} = 0.81$, $P = 0.52$) (Fig. 2.21.a,b,c). However in spring 2008, there appeared to be a slight but not significant increase in the number of feeding scars on the sprayed plants compared to the control plants.

At Farm Dam, in autumn 2008 and spring 2008 respectively, the number of feeding scars on leaves was not significantly different between the control and the sprayed plants ($F_{(4, 90)} = 0.35$, $P = 0.84$; $F_{(4, 80)} = 1.44$, $P = 0.22$) (Fig. 2.22.a,b).

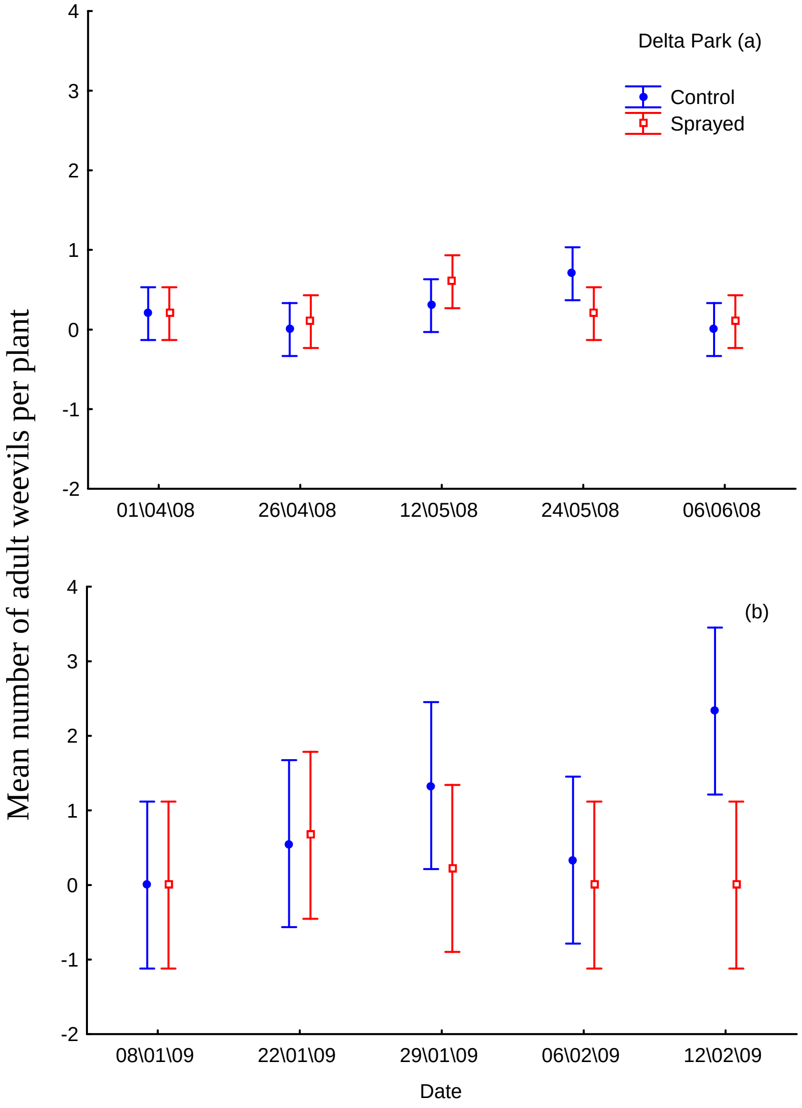

Figure 2.20 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number of adult *Neochetina* on water hyacinth plants at Delta Park in (a) autumn 2008 and (b) summer 2009. Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$

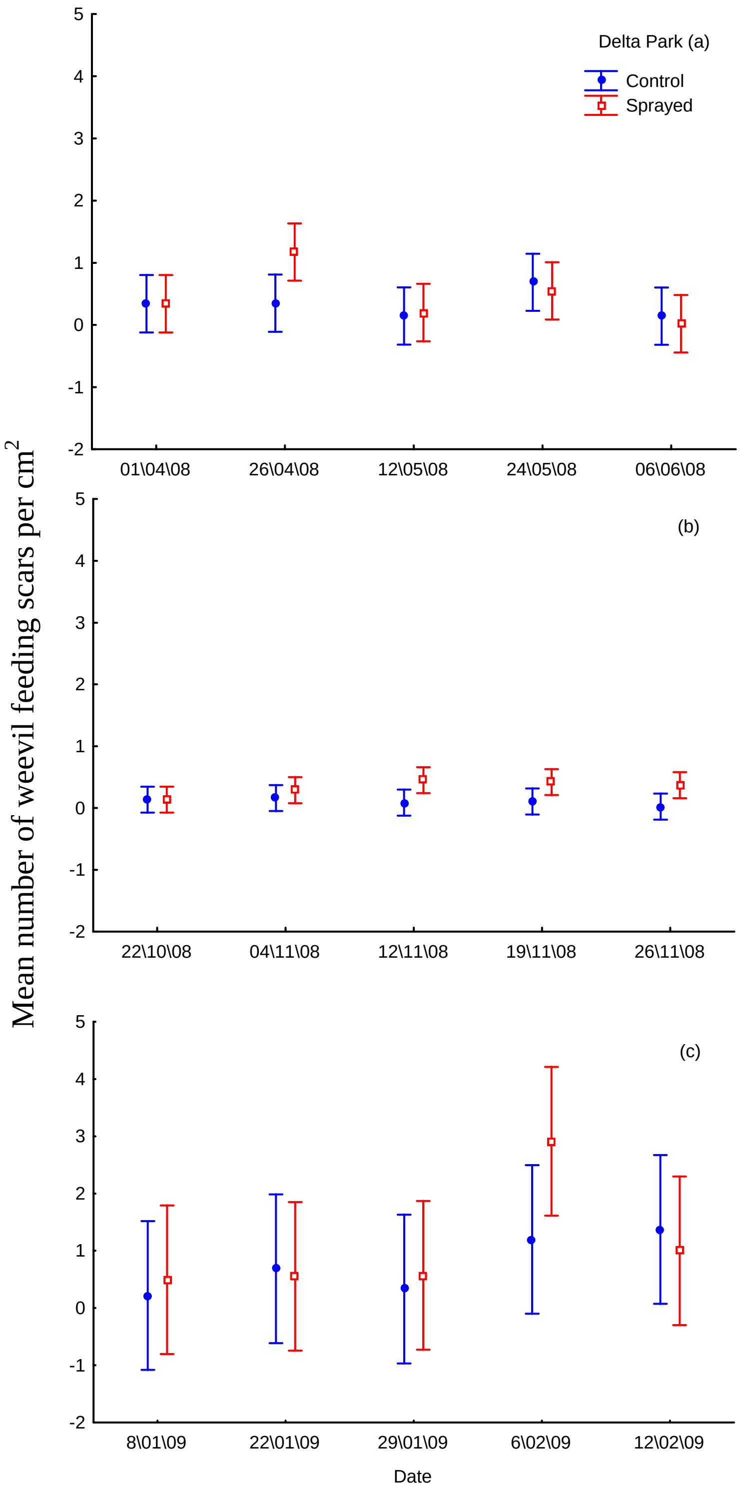

Figure 2.21 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number of feeding scars on water hyacinth leaves at Delta Park in autumn 2008 (a), spring 2008 (b) and summer 2009 (c). Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$.

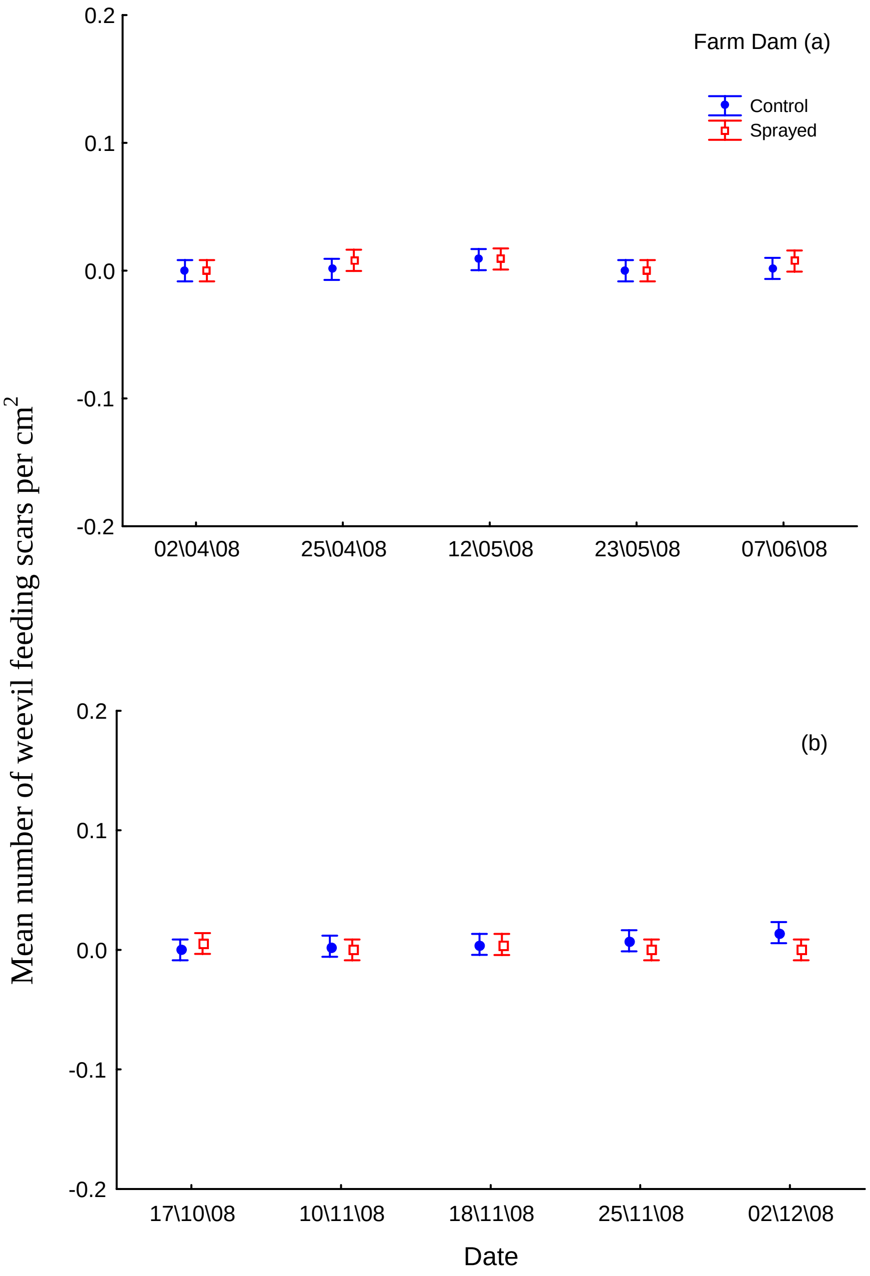

Figure 2.22 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on the number of feeding scars on water hyacinth leaves at Farm Dam in autumn 2008 (a) and spring 2008 (b). Error bars represent standard error of the mean. * between control/sprayed pairs denote significant difference at $P < 0.05$

2.4 Discussion

This study has shown that 0.8% glyphosate herbicide dose can be used in conjunction with the *Neochetina* weevils to control water hyacinth in the field. However, contrary to laboratory findings (Jadhav *et al*. 2008), the sub-lethal dose of glyphosate did not enhance weevil feeding proclivity.

The above-water biomass was significantly higher in control plants compared to sprayed plants in spring and summer, but insignificantly so in autumn plants at both sites. While the reduction of biomass accumulation in the sprayed plants in all seasons can be interpreted as a result of the effect of 0.8% glyphosate; the proximity of the winter season could also explain why the control plants in autumn did not increase as much as spring and summer plants did. The control minus spray difference in the above-water biomass between Farm Dam plants and Delta Park plants was used to determine which site was more susceptible to the sub-lethal glyphosate herbicide. From the spring results, it seemed like Farm Dam plants were more susceptible to the sub-lethal glyphosate herbicide than Delta Park plant because their control minus spray difference was greater than that at Delta Park. However in reality it is biomass accumulation of unsprayed plants that was very slow at Delta Park as opposed to Farm Dam, resulting in a small difference in biomass between the herbicide retarded sprayed plants and the slow growing control plants.

Jadhav *et al*.'s (2008) laboratory trials concluded that 0.8% glyphosate concentration retarded ramet production. Apart from autumn results at Farm Dam, which showed a retarding effect on ramet production at the last sampling date, results from the other seasons were very varied and sometimes contradictory to Jadhav *et al.* (2008). The trend that emerged from our work suggested that 0.8% glyphosate stimulated ramet production in autumn at Delta Park and in spring at Farm Dam, while not having any effect at all in other seasons. The production of ramets was almost halted in the control plants in spring and summer, and this might have been caused by the crowding effect brought about by the constriction of actively growing plants within cables. These cables not only prevented the spread of plants onto the entire water body but they also stimulated upward growth to the detriment of lateral (ramet production) expansion. Conversely, because sprayed plots were low in plant density, this gave room for more ramets to be produced. In the present study, there was a significant negative correlation between the number of ramets and plant biomass in the sprayed plants, in autumn; showing that bigger plants had less ramet compared to smaller plants.

Other studies have found that keeping water hyacinth density low promoted ramet production (Geber *et al*., 1992; Center, *et al*., 1999). Cofrancesco (1982) recorded a significant reduction in water hyacinth biomass from herbivory by the water hyacinth moth *Bellura densa* larvae (Lepidoptera, Noctuidae); however while plant biomass decreased on one hand, there was an increase in ramet production on the other hand, to the point where there was no actual reduction in the surface coverage of the weed. Center *et al*. (1999) found that herbicide treated sites showed a greater capacity for clonal growth by means of ramet production compared to untreated sites. This, however was simply because herbicide treated sites had more room for expansion after recovering from the treatment whereas herbicide-free sites had a compacted dense population, and therefore poor light penetration through the canopy. There is a negative correlation between levels of far-red radiation and ramet production, i.e. few ramets are produced under low light conditions (Méthy & Roy, 1993).

The difference in the experimental procedures between the present filed work and Jadhav *et al.*'s (2008) laboratory work may also explain the different responses of the sub-lethal glyphosate herbicide on the production of ramets. For example, in Jadhav's experiment, plants were kept in cylindrical 50 L (52 cm diameter) plastic tubs, where they were evenly sprayed at a constant walking speed. Conversely in my work, plants were sprayed from a motor boat, thereby causing difficulties keeping a constant speed and maintaining the spray rig at a constant height. This possibly resulted in uneven or patchy spray and even more importantly in a reduction in the herbicide dose reaching the plants, which may have encouraged ramet production. Jadhav *et al*. (2008) found that ramet production was higher in plants sprayed with 0.3% and 0.5% glyphosate concentration compared to control plants; Katembo (2008) showed high numbers of ramets from plants sprayed with a low (0.4%) glyphosate concentration.

In all the seasons at both sites, 0.8% glyphosate had a retarding effect on the number of leaves produced. Control plants had significantly more leaves compared to sprayed plants. However, Farm Dam plants looked less susceptible to 0.8 glyphosate such that the number of leaves between control plants and sprayed plants was significantly different only on the last sampling date. When comparing the control minus spray difference in the number of leaves between Delta Park and Farm Dam, results showed that this difference (between leaves in the control plants and those in the sprayed plants) was greater at Delta Park compared to Farm Dam. The lower effect of 0.8% glyphosate on the number of leaves per plant at Farm Dam as compared to Delta Park can be explained in two ways. Firstly, Farm Dam plants by virtue of their large size might have been less susceptible to 0.8% glyphosate than small plants at Delta Park if there is an allometric size difference (Refer Chapter three). Secondly, the lack of pressure from a very low population of biocontrol agents at this site could explain the reduced susceptibility of Farm Dam plants. Herbicides are more effective when applied to plants under some form of pressures (e.g. predation) (Boone & Semlitsch, 2002). At Delta Park, in summer for example, the juxtaposition of a low dose of glyphosate and biocontrol agents resulted in the reduction in leaf production as well as in biomass accumulation. However at Farm Dam, during the entire sampling period no adult *Neochetina* weevils were found except for a few larvae in spring which did not exert any real pressure on the weed. Richardson *et al*. (2008) argued that the inadequacy of integrated methods, combining herbicides and biocontrol agents could be attributed to the biocontrol agents' failure to establish a population high enough to reduce the density of weeds. This, however, is not entirely true in the case of biocontrol of water hyacinth in South Africa. In many circumstances, biocontrol agents are not so much of a limiting factor for a successful water hyacinth control as nutrient enrichment of waters coupled with cold winters (Byrne *et al.*, 2010).

The 0.8% sub-lethal glyphosate concentration used in the field had neither a detrimental nor beneficial effect on the reproduction of water hyacinth weevils, as no difference was found in the number of larvae, between sprayed and control plants. These results corroborate Jadhav *et al*. (2008) laboratory findings, that 0.8% glyphosate did not affect weevil reproductive capacity, and Katembo (2008) who found no difference in mirid (*Eccritotarsus catarinensis*) reproduction between water hyacinth plants sprayed with a sub-lethal glyphosate concentration compared to unsprayed ones. Center *et al*. (1999) found that there were less reproductive female weevils at herbicide free sites (33% reproductive females) compared to herbicide treated sites (55% reproductive females). This was largely because plants in herbicide treated sites in Center et al.'s study, constituted a re-growth population emerging from previously sprayed populations; and were high in nitrogen. However they also found no significant difference in the number of larvae between sites.

While plant biomass and leaf production were variably reduced by a 0.8% glyphosate concentration spray, there was no significant difference between adult weevil survival on the sprayed plants and the control plants. Nevertheless, the trend in spring at Delta Park showed a slight, but not significant increase in the number of weevils in control plants compared to sprayed plants. Larson *et al*. (2007) found that populations of both *Aphthona lacertosa* and *A. nigriscutis* were lower in herbicide sprayed leafy spurge (*Euphorbia esula*) plots than in herbicide free plots. Messersmith and Adkins (1995) suggested that plants that survived herbicide application could provide fewer nutrients to herbivores than unsprayed plants. These findings imply that unsprayed plants are expected to sustain a bigger weevil population than herbicide sprayed plants because they might be of high nutritive quality (Refer Chapter four).

No adult weevils were found at Farm Dam during the whole sampling period. It has been observed that Farm Dam always had a very small population of biocontrol agents (Byrne *et al.*, 2010) for unknown reasons. However the instability of the Farm Dam site through flooding events and the manual removal of the weed from the Dam could explain the absence of weevils on this site. Center and Durden (1986) noted that sites where water hyacinth populations were regularly disturbed through mechanical control were likely to have insignificant weevil damage. It has been noted that water hyacinth weevil scarring and *Cercospora piaropi* fungal necrosis accumulated on leaves at low disturbance sites

(Center & Durden, 1986; Moran, 2004). However the low numbers of weevils recorded even at Delta Park in this study are not surprising because most of the water hyacinth sites monitored in South Africa experience disturbance in one form or another (e.g. Flooding, frost, herbicide). This explains the observed general low numbers of weevils on these sites (Byrne *et al*., 2010).

Other studies have found arthropod herbivore populations to increase on herbicide treated plants (Campbell 1988; Oka & Pimentel 1976). Boydson and Williams (2004) found more galls per plants on field bindweed (*Convolvulus arvensis*) under control by a sub-lethal glyphosate concentration and a gall mite, *Aceria malherbae*, than in plants exclusively under *A. malherbae* control. However in the present work, the application of a sub-lethal dose of glyphosate did not affect the number of weevils per plot.

Center *et al*. (1999) found that weevil feeding intensity was much higher in unmanaged sites (control sites) compared to managed sites (sprayed sites). Managed sites, having being characterized by re-growth plants, were expected to have less insect feeding scars. This is also largely driven by the lower number of weevils at these sites compared to unmanaged sites. In this study, results on the number of feeding scars were inconsistent, ranging from trends showing an increase, but not significant, in the number of scars in sprayed plants compared to control plants, to trends showing no significant difference between control plants and sprayed plants. Katembo (2008) found no significant difference in the extent of feeding by *E. catarinensis* between sprayed water hyacinth plants and control water hyacinth plants. Jadhav *et al*. (2008) arrived at the same conclusion with *Neochetina* weevils. However in both studies the trend seemed to have suggested more insect feeding on the sprayed plants than on control plants. This behaviour in feeding preference has prompted investigations, which are dealt with in chapter four, to find out the effect that 0.8% glyphosate concentration may have on water hyacinth nutrients for herbivory by the weevils.

Findings on leaf production suggest another explanation of the observed slight increase in the feeding on leaves of the sprayed plants. The 0.8% glyphosate resulted in slowing the rate of water hyacinth leaf production (referred to herein as leaf turnover), concurring with Jadhav *et al.* (2008). Since leaf turnover rate was very slow in the sprayed plants, there might have been accumulation of feeding scars on the sampled leaf (leaf two) compared to control plants which produced a new leaf every week. On the other hand, the number of feeding scars was very low at Farm Dam, reflecting the low number of adult weevils at this site. It has been noted that water hyacinth weevil scarring and *Cercospora piaropi* fungal necrosis accumulated on leaves at low disturbance sites (Center & Durden, 1986; Moran, 2004).

In conclusion, results obtained from the combination of *Neochetina* weevils and a sub-lethal dose of glyphosate differed from those presented by Jadhav *et al.*'s (2008) study and varied within seasons and between both sites. Delta Park plants were more susceptible to the 0.8% glyphosate as seen in the reduction of leaf and biomass production in spring and summer; whereas Farm Dam plants proved to be less susceptible to this control method. It is believed that Farm Dam plants by virtue of their large size, almost double that of Delta Park plants, may have received a relatively low dose of herbicide per plant mass. This difference in plant response to the herbicide between Farm Dam and Delta Park prompted investigations that are dealt with in the next chapter; which looks at the relationship between leaf surface area and plant mass. It was also concluded that 0.8% glyphosate concentration was neither beneficial nor detrimental to the weevils' survival and reproduction. However a trend emerged in the number of feeding scars which suggested weevils preferred feeding more on the sprayed plants compared to the control plants. This is in agreement with studies that found herbicide-induced alteration of plant physiology to be beneficial to biocontrol agents' performance (Boydson & Williams, 2004). Therefore chapter four seek to determine the effect of 0.8% glyphosate on the nutritive quality of water hyacinth as food for the *Neochetina* species.

CHAPTER THREE: THE RELATIONSHIP BETWEEN LEAF SURFACE AREA AND PLANT MASS IN WATER HYACINTH

3.1 Introduction

In the previous chapter, the effect of 0.8% glyphosate on plant growth and reproduction was patchy and unpredictable, in contrast to Jadhav *et al.* (2008). To explain this, assumption was made that herbicide delivery system was variable, coupled with variation in plant size between laboratory plants and field ones. Same support for this was suggested by the differences in susceptibility of plants to the herbicide between seasons when they were different in size, and between sites, where Delta Park plants were of a different (smaller) phenotype to Farm Dam plants. This was clearly seen in the “control minus spray” difference in the number of leaves which was significantly greater at Delta Park compared to Farm Dam (Refer Chapter two, Fig. 2.14). One suggestion to explain the susceptibility of Delta Park plants to the 0.8% glyphosate dose compared to Farm Dam plants is the difference in relative plant phenotypes between the two sites. Depending on its habitat, water hyacinth occurs in a wide range of sizes and shapes, referred to as phenotypes (Gopal, 1987). Cooley *et al.* (1979) recognized three water hyacinth biotypes exhibiting different plant phenotypes, which they named: superhyacinths, small or stunted, and normal.

Plant surface area also plays an important role in herbicide phytotoxicity, especially in foliar applied herbicides, such as glyphosate (Wang & Liu, 2007). Foliar uptake of herbicides depends on a suite of factors including, leaf surface characters (thickness or fineness of leaf cuticle), physicochemical properties of the active ingredient (molecular size and lipophilicity), types and concentration of the additives, and the environmental conditions under which herbicide is applied (Wang & Liu, 2007). It has been shown that foliar uptake of glyphosate in plants is positively correlated to the concentration of the active ingredient in the spray mixture, i.e. the higher the active ingredient concentration the greater the uptake (Cranmer & Linscott, 1991; Duncan Yerkes & Weller, 1996). Furthermore, Ramsdale *et al*. (2003) reported that uptake of glyphosate on plants is negatively correlated to the spray volume. However, it is the amount of active ingredient absorbed per leaf surface area that influences the effectiveness of foliar applied herbicides (Liu, 2004).

Knowing leaf surface area or plant mass alone may not be informative in determining herbicide effectiveness on a plant because, although glyphosate is applied to the foliage, it is translocated through the whole plant. Thus it is the surface area to plant mass ratio that influences herbicide effectiveness. If leaf surface area is small relatively to a large plant mass, dilution of the herbicide may occur. Therefore, in theory, if the relationship between leaf surface area and plants mass is isometric (Fig. 3.1.a), i.e. every increase in the x axis (plant mass) corresponds to an equal increase on the y axis (leaf surface area); there will be no need to adjust herbicide concentration with regard to different water hyacinth phenotypes. However in the case of allometric relationships two scenarios are to be noted.

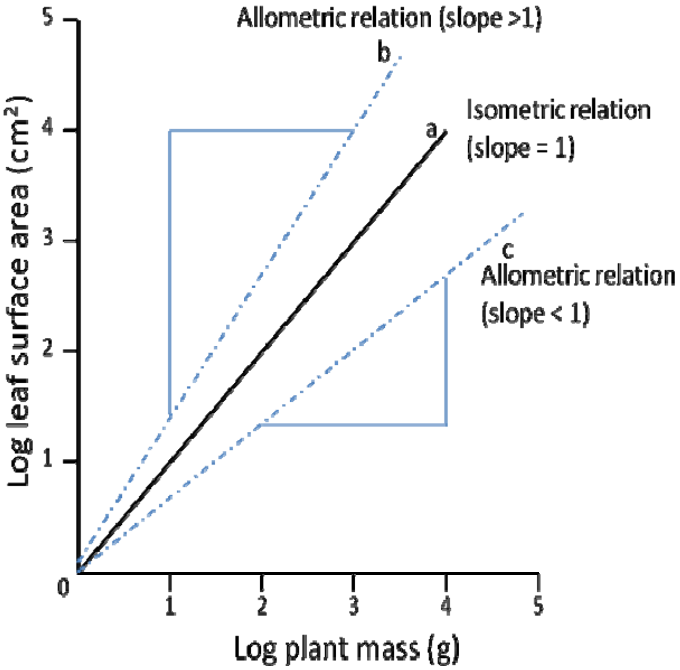

Figure 3.1 Hypothetical relationships between leaf surface area and plant mass of three plant phenotypes with different leaf areas and plant masses

Firstly, if the slope of leaf surface area - plant mass relationship is above one, the effect of herbicide spray on the plants should increase with increasing mass because of the increasingly large leaf surface area from which herbicide is being translocated through a relatively smaller plant biomass (Fig. 3.1.b). Secondly, if leaf surface area - plant mass slope is below one, the effect of herbicide spray on the plant is expected to decrease with increasing mass because the herbicide will be translocated from a relatively smaller leaf surface area to act on a relatively larger plant biomass (3.1.c).

Sher-Kaul *et al*. (1995) compared plant biomass to surface area relationships of six different submerged aquatic plant species, *Elodea canadensis* Michx., *Myriophyllum spicatum L, Nitellopsis obtusa* (Desv.) J.Gr., *Potamogeton lucens L., Potamogeton pectinatus* L. and *Potamogeton perfoliatus* L. They found that for the same plant biomass, plant surface area was different for each of the six plants, implying an allometric relationship. Based on the theory illustrated above, if these different plant species were to be sprayed with the same herbicide dose, plant species with the smallest surface area to plant mass ratio would be the least susceptible to the treatment because herbicide will be absorbed from a relatively very small surface area to act on a relatively large plant biomass. And consequently plant species with a larger surface area to plant mass ratio would be more susceptible compared to the rest. If the different water hyacinth phenotypes were to be considered as different plant species as in the previous example, to achieve the same herbicide response on the different plant phenotypes, different herbicides doses would have to be formulated for different plant phenotypes, unless an isometric relationship exists between all plant phenotypes. This example reiterates the importance of knowing the leaf surface area to plant mass ratio of plants of different phenotypes if the same herbicide concentration is to be applied on each of these plants phenotypes. To the best of our knowledge, no other study has investigated the relationship between water hyacinth leaf surface area and plant biomass.

3.1 Research question addressed in this chapter

What is the relationship between leaf surface area and plant mass in water hyacinth, and how can it be used to predict the susceptibility of plants to herbicides?

3.2 Materials and methods

Laboratory plants

Four different water hyacinth plant phenotypes were collected from water hyacinth plant cultures at Wits University, South Africa. Plant sizes included tiny plants (mean petiole length 5.3 cm), small plants (9.9 cm), medium plants (22.6 cm) and large plants (53.2 cm). The petiole length was measured on leaf-two. Ten replicates were used per plant phenotype. Plants from each size category were weighed using an electronic balance (Type: BL-320H / Capacity: 320g; accuracy: 0.001g). After weighing, all living leaves were removed from each plant, and the leaf surface area measured using a leaf area meter (cm^2) (Model: LI-3100 Area Meter / LI.COR, inc. Lincoln, Nebraska USA). Using a linear regression, the ratio of leaf surface area to plant biomass of all plant phenotypes was calculated.

Field plants

Laboratory measures of leaf surface area to plant biomass ratio were compared with those from the field using data from spring plants (Chapter two):

- Leaf area per plant was obtained by multiplying the leaf-two area by the number of leaves per plant.
- Biomass per plant was obtained by dividing the sum of above-water and below-water biomass per quadrat by the number of plants per quadrat.

3.2.1 Statistical analysis

A linear regression was performed to test the correlation between leaf surface area and plant mass of all plant phenotypes. One way ANOVA followed by the Tukey Post-hoc test was used to compare some plant parameters (biomass per plant and leaf-two petiole length) between laboratory plants and field plants (Farm Dam plants and the Delta Park plants). The computer programme employed for statistical analysis was STATISTICA, version 6.

3.3 Results

3.3.1 Plant biomass per plant phenotype

Plant biomass was significantly greater at Farm Dam compared to Delta Park, but smaller than that of large plants ($F_{(5, 54)} = 36.94$, $P < 0.001$) (Fig. 3.2). No difference was found between the tiny plant phenotype, small phenotype, and the Delta Park plants.

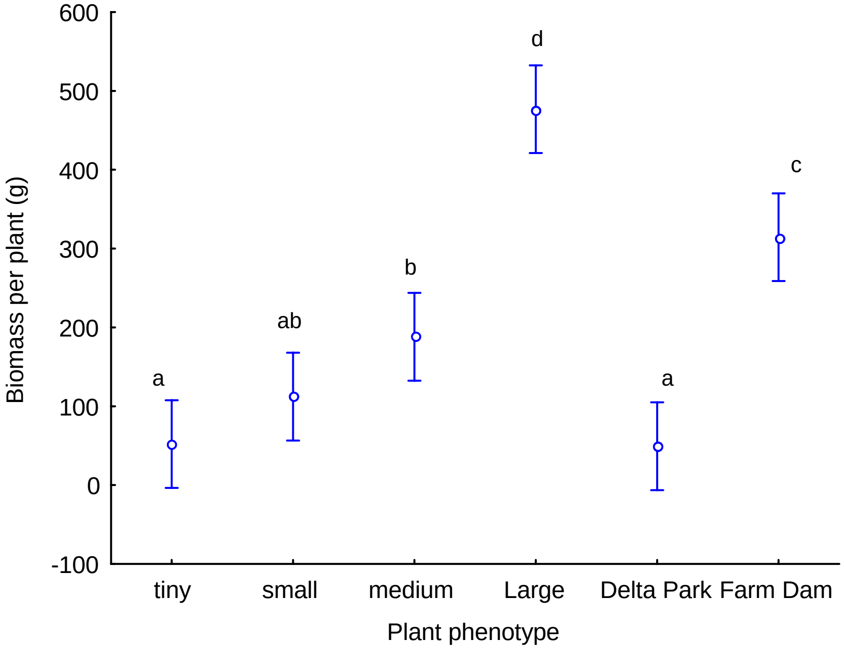

Figure 3.2 Comparison of plant biomass between different plant phenotypes. Error bars represent standard error of the mean. Bars followed by different letters denote significant difference at $p < 0.05$

3.3.2 Correlation between leaf surface area and plant biomass

There was a significant positive correlation between plant biomass and total leaf surface area of all plant phenotypes (Fig. 3.3). Similar result was found for Delta Park plants and Farm Dam plants (Fig. 3.4) using leaf-two to estimate total leaf surface area. However, the relationship slope in figure 3.3 was 0.9290, which is very close to one as compared to 0.5010 in figure 3.4.

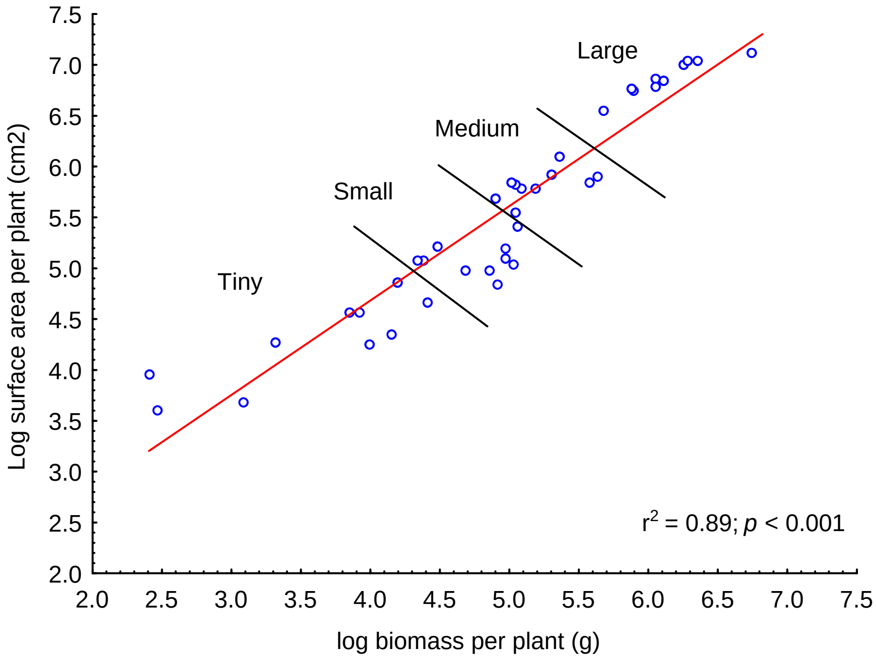

Figure 3.3 Correlation between leaf surface area (using all leaves on the plant) and plant biomass of four different plant phenotypes. Equation line: y = 0.956432445 + 0.929041196x

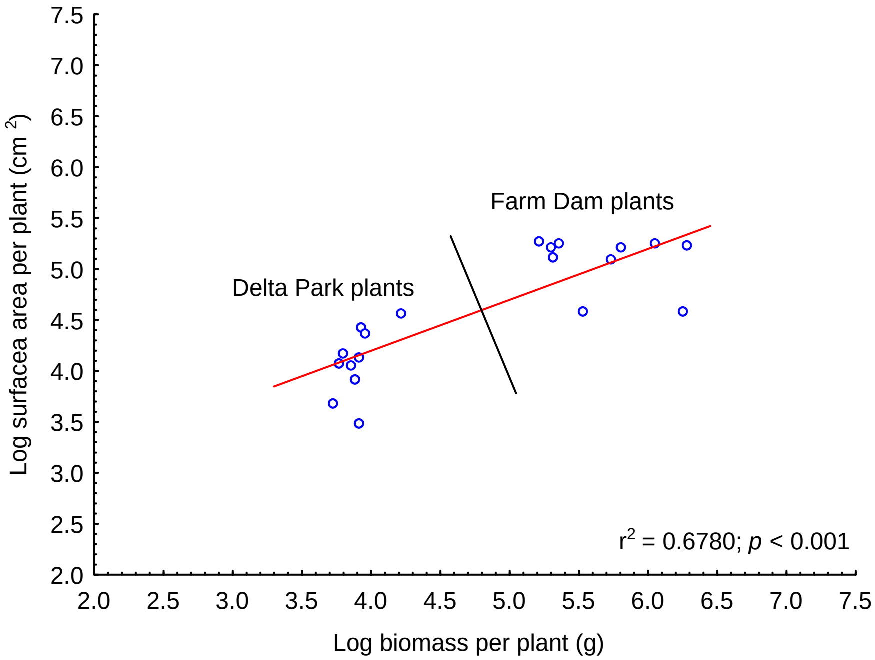

Figure 3.4 Correlation between leaf surface area (using leaf-two to estimate total leaf surface area) and plant biomass of Delta Park plants and Farm Dam plants. Equation line: y = 2.19672206 + 0.501012274x

3.3.3 Leaf-two petiole length per plant phenotype

Petiole length was significantly different between Delta Park plants and Farm Dam plants ($F_{(5, 54)} = 380.42$, $p < 0.001$) (Fig. 3.5). However no difference between Farm Dam plants and large plant phenotypes was found, or between Delta Park plants and medium plant phenotypes.

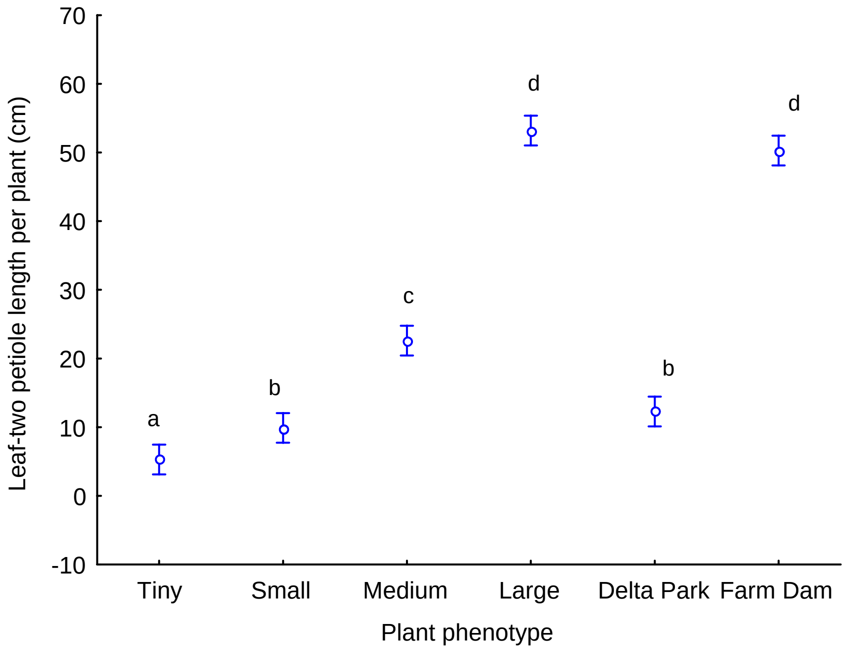

Figure 3.5 Comparison of leaf-two petiole length per plant between different plant phenotypes. Error bars represent standard error of the mean. Means followed by different letters denote significant difference at $p < 0.05$

3.4 Discussion

It was clear that Farm Dam plants were significantly larger compared to Delta Park plants, which resuscitated the question shouldn’t herbicide dose be adjusted accordingly? From the theory stated early in this chapter, herbicide can only be adjusted if the leaf surface area to plant biomass relationship is allometric, but if this relationship is isometric no herbicide adjustment will be required. In the present work, the relationship slope of the four plant phenotypes was 0.9290 which is close to one, implying an isometric relationship. Consequently, if the theory were true, herbicide response or plant susceptibility to a given herbicide dose should be the same across all phenotypes, and therefore there will be no need to adjust the herbicide dose. This is so because when spraying at a constant speed, the number of droplets falling on the leaf surface will be proportional to the leaf area of the sprayed plants, i.e. the larger the leaf area the more droplets it will receive.

The relationship between leaf surface area and plant biomass in the field plants (Delta Park and Farm Dam) was allometric, with a relationship slope of 0.5010; which is substantially below one. Therefore larger plants will be less susceptible to the herbicide. Because Farm Dam plants were bigger than Delta Park plants, this may explain why they were less susceptible to the treatment compared to Delta Park plants.

Farm Dam plants could have been less susceptible to the herbicide simply by virtue of their size. In addition to the larger mass, they had very long petioles compared to Delta Park plants; therefore their canopy was more closed than that at Delta Park. Although glyphosate application was to the foliage, in an open canopy the chemical can easily spread to the petioles and the crown, unlike in a closed canopy. This may explain observations by Haller and Tag el Seed (1979) who noted that long-styled water hyacinth plants were less susceptible to 2,4-D.

There are other factors influencing herbicide injury to a plant such as plant growth rate and leaf surface cuticle permeability. Cedergreen *et al*. (2003) found that the difference in sensitivity of aquatic plants to the herbicide metsulfuron-methyl was due to variations in growth rates rather than to variations in exposed leaf area. Riemens *et al*. (2008) also found that herbicide toxicity was lower in older plants than in younger ones, arguing that the difference in the thickness or fineness of their cuticles was a possible explanation. This is because foliar uptake of herbicides is a diffusion process across the epicuticular wax, the cuticle, and the plasma membrane of epidermal cells (Wang & Liu, 2007). Maybe the leaf cuticles of Farm Dam plants were thicker compared to Delta Park those in Delta Park plants, therefore rendering them less prone to herbicide injury. However this possibility was not investigated.

In conclusion, Delta Park plants were expected to be more susceptible to the herbicide because they were smaller than the Farm Dam plants. For future herbicide applications, especially when a low dose is used, the leaf surface area to plant biomass relationship should be considered to ensure herbicide effectiveness on the sprayed plants. This consideration however, may not be important in the case where herbicide is used at doses recommended by the manufacturer, for example 3% in the case of glyphosate (Jadhav *et al.*, 2008), because these doses are high enough to kill plants regardless of factors such as weed flora, weed growth stage, crop competitiveness, or climatic conditions (Kudsk, 2008).

In the next chapter, the effect of a sub-lethal dose of herbicide (0.8% glyphosate) on plant nutrients is investigated. Also investigated in that chapter is the change in plant nutrients between plants at Delta Park (more susceptible to herbicide) and plants at Farm Dam (less susceptible to herbicide).

CHAPTER FOUR: IMPACT OF GLYPHOSATE ON WATER HYACINTH NUTRIENTS

4.1 Introduction

In chapter two, it was concluded that glyphosate herbicide sprayed at a sub-lethal dose (0.8% glyphosate concentration) did not kill the water hyacinth but reduced its growth in terms of biomass accumulation and leaf production. However, these results were patchy and variable as explained in chapter three. In addition, it was found that the performance of the biocontrol agents, *Neochetina* weevils, was not impaired by the herbicide, instead their feeding intensity was slightly increased. Therefore the present chapter was aimed at finding out how a sub-lethal dose of glyphosate would change the nutritive quality of the water hyacinth as a food source for the weevils. This aim was mainly based upon Jadhav *et al.*'s (2008) study, which showed the *Neochetina* weevil feeding levels to significantly increase on herbicide sprayed plants compared to the unsprayed ones.

The nineteenth century saw the advent of the agricultural revolution, which preceded the formulation of a number of weed killers also called herbicides. Glyphosate is one of the most widely used broad-spectrum herbicides. It inhibits a key enzyme involved in the biosynthesis of aromatic amino acids, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and that of aromatic secondary metabolites (Bentley, 1990). Glyphosate falls into the category of amino acid synthesis inhibitors (Table 4.1).

Table 4.1 Categories of some herbicides and their respective mode of action (adapted from Gower, 2002)

Category of herbicides	**Mode of action**	**Associated chemicals**
Amino acid synthesis inhibitors	Prevent production of essential amino acids by inhibiting a specific enzyme	- Sulfonamides: cloransulam_methyl - Amino acid derivatives: glyphosate
Lipid synthesis inhibitors	Prevent the formation of fatty acids, which are essential for lipid formation	- Cyclohexanediones: clethidim - Aryloxyphenoxypropionates: quizalifop
Photosynthetic inhibitors	Block photosynthetic process, electro transport	- Triazines: atrazine, metribuzin - Benzothiadiazoles: bentazon

Since most herbivorous arthropods depend on plants for their food and habitat, herbicides that interfere with the plant's normal growth and metabolic processes will directly or indirectly have negative impacts on insect herbivores. Some studies have found 2,4-D applied at a recommended dose to be toxic to biocontrol agents (Hayes, 2000); while others have found it (2,4-D) moderately to completely non toxic (Rees & Fay, 1989; Ainsworth, 1999; Nelson & Lym, 2003). Ainsworth (2003) reported that in many cases herbicides themselves are not toxic to arthropods; but it is the additives formulated with them, such as wetting agents and surfactants, that are toxic. Indirectly, herbicides kill biocontrol agents via habitat destruction (Ainsworth, 2003). Norris and Kogan (2000) reported that glyphosate was nontoxic to several insects, but destruction of vegetation indirectly affected insect populations by altering the quantity and quality of food supply. Boydston and Williams (2004) found a sub-lethal dose of 2,4-D to be detrimental to the biocontrol agent *Aceria malherbae* (Acari: Eriophyidae) through the stunting of field bindweed, *Convolvulus arvensis* L. (Convolvulaceae); which constitutes the agent's source of food and habitat.

Other literature has shown that herbicide applications, especially at low concentrations, do not hinder but rather improve insect performance by changing the chemistry of their food plants. Sap-sucking insects were found to derive higher nutritional value, with special reference to nitrogen, from herbicide-stressed plants than from non stressed ones (White, 1984). Ainsworth (2003) noted an improvement in the performance of boring and sucking insects living on herbicide treated plants. Mirids, *Eccritotarsus catarinensis* (Katembo, 2008) and water hyacinth *Neochetina* weevils (Jadhav *et al*., 2008) respectively, were observed to feed more on sprayed water hyacinth plants compared to unsprayed plants. The water hyacinth moth *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae) populations have been seen to increase following 2,4-D treatment, and it was found that the increased moth population was brought about as a result of 2,4-D reducing leaf hardness while increasing plant nitrogen content (Wright & Bourne, 1990). In this work nitrogen, carbon, and phosphorus were used to examine the effect of a sub-lethal dose of glyphosate on the nutritive value of water hyacinth plants for the biocontrol agents, *Neochetina* species. These nutrients were chosen because they are very important for both insects and plants. Phosphorus and nitrogen were particularly shown to have a positive correlation with water hyacinth growth, such as ramet production and biomass accumulation (Reddy *et al.,* 1989).

4.1.1 Nitrogen

Many studies have indicated that nitrogen (N) is an essential constituent of host plant quality for insect herbivores (Mattson, 1980; Awmack & Leather, 2002). It has been shown unequivocally that nitrogen is required by herbivorous arthropods for their reproduction, development and survival (Mattson, 1980; Schoonhoven *et al*., 1998; Center & Dray, 2010). High nitrogen content plants have been found to improve survival and growth rate of immature insects (Myers & Post, 1981; Wheeler, 2003), as well as reproduction in adults (Awmack & Leather, 2002; Center & Dray, 2010). Denno and McClure (1983) reported that piercing and sucking insects are favoured by an increase in the soluble nitrogen component of their food, thus some nitrogen forms are more easily accessible to herbivorous arthropods than others. Karley *et al.* (2002) suggested that free amino acids and amides are a better source of nitrogen than proteins because they are absorbed into insects' guts free from interference by proteinase inhibitors, and they are also soluble and mobile (Cockfield, 1988).

Glyphosate is known to inhibit nitrogen metabolism in plants by interfering with the shikimate pathway (Bentley, 1990; Taiz & Zeiger, 1991); thus potentially exerting negative impacts on the performance of insect herbivores, which depend on nitrogen as their main food nutrient (White, 1993). On the other hand, Center, *et al.* (1999) found that nitrogen content was higher in 2,4-D treated water hyacinth populations compared to the untreated populations. This can be explained by the fact that these herbicide treated water hyacinth were not directly exposed to the herbicide but were freshly growing from previously sprayed populations which had decomposed elevated the nitrogen levels in the water (Center & Dray, 2010). Awmack and Leather (2002) reported that protein or nitrogen concentrations are usually higher in younger tissues of many plants compared to older tissues. In addition, these re-growth plants were not as heavily weevil-stressed as were plants on untreated sites. This has an implication for the nitrogen availability because weevil feeding on its own has been found to decrease the nitrogen content in water hyacinth leaves (Heard & Winterton, 2000; Center & Van, 1989).

4.1.2 Carbon and Carbon/Nitrogen ratio

Carbon (C) content in plants is regulated by the rates of photosynthetic reactions (Kasige & Takashi, 2008), thus photosynthetic inhibitor herbicides, such as atrazine, have a negative impact on the C in plants (Gower, 2002). Alterations in plant carbon content will not only have a negative effect on plant growth but may also impede their associated herbivores (Lincoln *et al.,* 1986). In an experiment by Fajer *et al.* (1989), larvae of the buckeye, *Junonia coenia* (Nymphalidae), reared on, *Plantago lanceolata* (Plantaginaceae), were grown into two different CO_2 concentrations, 350 ppm (low concentration) and 700 ppm (high concentration). Larvae reared on high CO_2 foliage had a slow growth rate compared to those reared on low CO_2 foliage, although no difference was observed in their survival. The slow larval growth on high CO_2 foliage may have been caused by the reduced foliar water and nitrogen concentrations in their host plants. Carbon and nitrogen contents seem to work antagonistically to each other in plants, i.e. as the C in plants increases on one hand the N decreases on the other. Sionit (1983) showed that leaves of soybean plants grown under high CO_2 conditions had high carbohydrate levels while their nitrogen levels were low. A high C/N ratio in plants indicates a relatively reduced concentration of leaf protein and therefore reduced nutritive value to herbivores (Lincoln *et al.,* 1986). Consequently, it has been noted that herbivorous arthropods feeding on low nitrogen diets either increase their feeding rates, or reduce their growth rate to compensate for low nutrients (Scriber & Slansky 1981; Di Giulio & Edwards, 2003).

4.1.3 Phosphorus

Phosphorus and nitrogen are the two main nutrients responsible for eutrophication in many water bodies, encouraging rapid proliferation of many water weeds (Hill & Olckers, 2001). Early studies have found a positive correlation between the nitrogen and phosphorus content in water hyacinth tissues and that in the water bodies they grow in (Gosset & Norris, 1971; Reddy *et al.,* 1990; Heard & Winterton, 2000). Phosphorus is a very important nutrient in plants as it is required for a large number of metabolic processes, including photosynthesis, respiration and generation of high energy bonds, protein synthesis, carbohydrate inter-conversions, to mention but a few (Ripley *et al*., 2006). Consequently its deficiency has been shown to have serious negative impacts on plant growth and vigour (Ripley *et al*., 2006). However these authors also reiterated that P and N together have a more significant effect on plant growth than P alone. This is because P uptake in plants has usually been found to be dependent upon the availability of N (Reddy *et al*., 1989).

4.1.4 Rationale for this chapter

There is mounting evidence that low doses of herbicide improve plant nutritive quality for some arthropod herbivores (Wright & Bourne, 1990; Ainsworth, 2003). The number of feeding scars per cm^2 at Delta Park in chapter two, showed a trend that suggested that weevils preferred feeding more on herbicide treated water hyacinth plants than on untreated ones. However, in general there was no significant difference in insect performance between herbicide treated plants and untreated plants. In contrast, Jadhav *et al*. (2008) found high *Neochetina* weevil feeding levels on herbicide treated plants compared to the untreated ones. This chapter seeks to ascertain how a sub-lethal dose of glyphosate can influence the nutritive quality of water hyacinth plants as food source for the biocontrol agents *Neochetina eichhorniae* and *N. bruchi*. Nitrogen, carbon, and phosphorus contents in water hyacinth plants were compared between sprayed plants and control plants to determine which plants are higher in nutrient content.

Larval and adult activities of *Neochetina* weevils generally occur in leaves, petioles and crowns. *Neochetina* adults have been reported to prefer feeding upon younger leaves (Center, 1985; Center and Wright 1991), therefore only the three youngest leaves, their respective petioles, and the crowns were considered for nitrogen, carbon and phosphorus analysis.

4.2 Research questions addressed in this chapter

- Which water hyacinth plant part has the highest nutrient content, comparing leaves, petioles, and crowns; and how does that dictate weevil performance on the plant?
- How does 0.8 % of glyphosate changes N, C, and P content in water hyacinth leaves and crowns?
- How does any glyphosate induced change in plant nutrients affect *Neochetina* feeding intensity?

4.3 Materials and methods

In spring 2008, Farm Dam and Delta Park plants were set up as described in chapter two, and sprayed with 0.8% glyphosate concentration at 140 l/ha spray volume. Two weeks after spraying, four plants were collected per block in each plot (each plot had three blocks). The plants were broken up into seven samples with three replicates: leaf one, leaf two, leaf three, petiole one, petiole two, petiole three, and crown. Samples were then oven dried at 60ºC for 18 hours. Samples from both sites were sent to BemLab, Stellenbosch, South Africa, for nitrogen (N), carbon (C), and phosphorus (P) analysis using the combustion analyzer method (Refer BemLab, Stellenbosch / South Africa). Results from the first analysis (two weeks after spraying) were used to compare N, C, and P within the three leaves, their respective three petioles and the plant crowns. It is reported that the effects of Roundup become visible from two weeks after its application (Roundup instruction booklet by Monsanto Europe S.A., Nov. 2006 (L.D.M.)), therefore another batch of samples constituting leaves and crowns only was prepared at week four after spraying, and then sent to Bemlab for analysis. In the preparation of the second batch of samples the three leaves (leaf one, leaf two, and leaf three) were pooled together. To compare plant nutrient response to the glyphosate between week two and week four after spraying, the control to spray ratio of C, N, and P was calculated. This ratio was calculated by dividing plant nutrients (C, N, P) in the control plants by those in the sprayed plants. A high control:spray ratio should indicate that nutrients in the sprayed plants had declined, i.e. the response of the plant to glyphosate was high.

4.3.1 Statistical analysis

To compare N, C and P content between water hyacinth leaves, petioles and crowns, as well as the difference in these nutrients between sprayed plants and control plants, a Factorial ANOVA followed by the Tukey Post-hoc test was performed.

A two sample t-Test was conducted to compare C:N ratio and N:P ratio between the control plants and the sprayed plants four weeks after spraying. A linear regression was conducted to explore the relationship between C:N ratio in water hyacinth leaves and the number of weevil feeding scars on leaves of sprayed and control plant.

All analyses were conducted at a critical *P* level of 0.05.

4.4 Results

4.4.1 Nitrogen, carbon and phosphorus in water hyacinth plant parts

At Delta Park, two weeks after the plants had been sprayed, N content was significantly higher in the three youngest water hyacinth leaves than it was in their respective petioles and in the plant's crown ($F_{(6,28)} = 7.62$, $P < 0.001$) (Fig. 4.1.a). Nitrogen in leaf one, two and three was not significantly different to each other. There was no significant difference in the carbon content ($F_{(6,28)} = 0.47$, $P = 0.81$) (Fig. 4.1.b) or in the phosphorus content ($F_{(6,28)} = 1.86$, $P = 0.12$) (Fig. 4.1.c) between the three youngest leaves, their respective petioles, and the crown.

At Farm Dam, the same patterns were observed in the nitrogen content between leaf one, two and three ($F_{(6,28)} = 8.85$, $P < 0.001$) (Fig. 4.2.a), carbon content ($F_{(6,28)} = 0.78$, $P = 0.58$) (Fig. 4.2.b) and phosphorus content ($F_{(6,27)} = 2.67$, $P = 0.03$) (Fig. 4.2.c).

The effect of glyphosate comparing week two and week four after spraying

At Delta Park, there was no significant difference in the effect of glyphosate on water hyacinth leaf nutrients between week two and week four after spraying ($F_{(2, 12)} = 1.62$, $P = 0.23$) (Fig. 4.3.a), however N and P were higher at week four after spraying.

At Farm Dam the P content was significantly higher at week four compared to week two after spraying ($F_{(2, 11)} = 5.65$, $P = 0.02$) (Fig. 4.3.b).

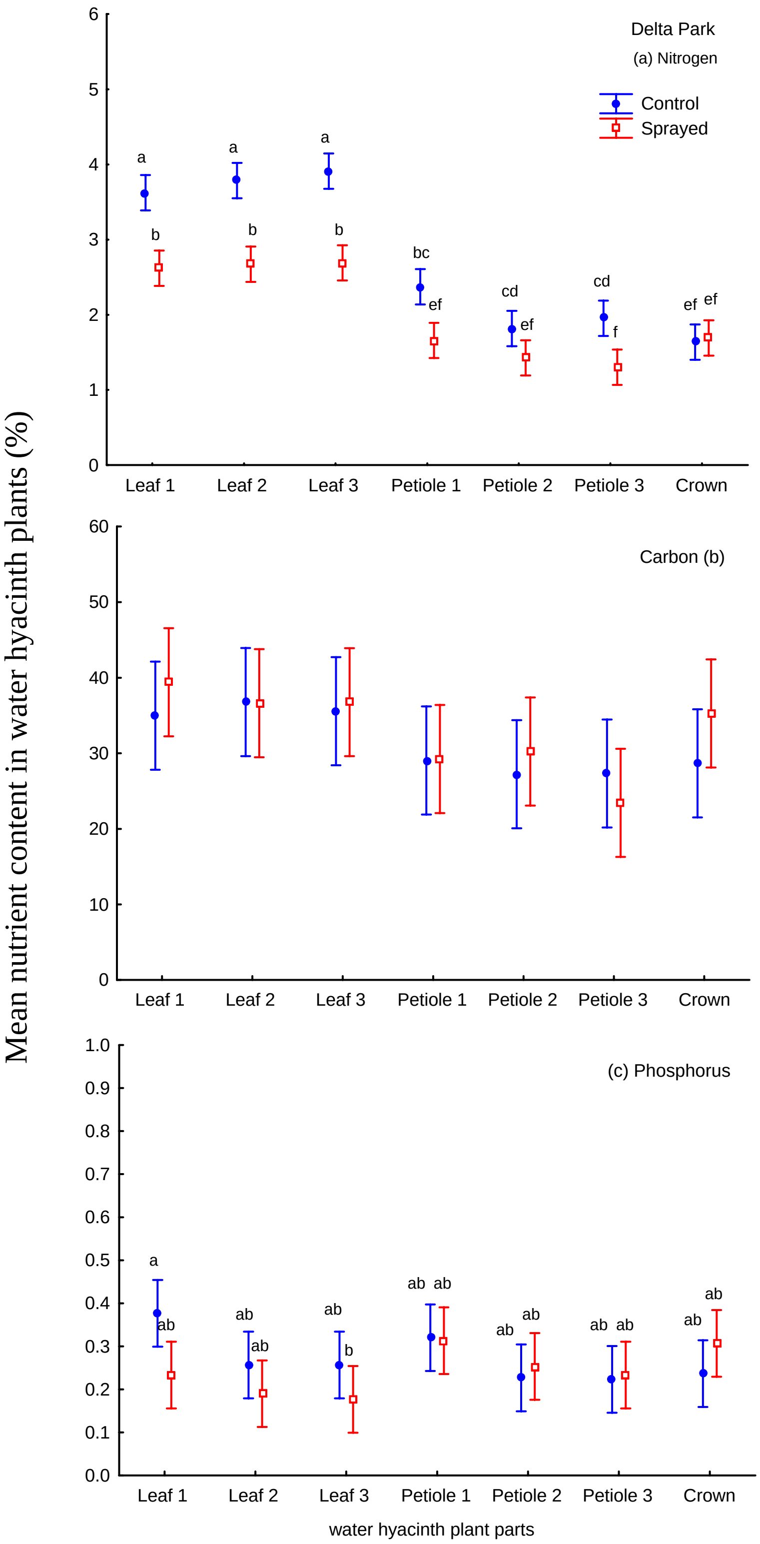

Figure 4.1 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on (a) nitrogen, (b) carbon, and (c) phosphorus content in water hyacinth plants at Delta Park, two weeks after spraying. Error bars represent standard error of the mean. Means with different letters are significantly different to each other between control/sprayed pairs and across the whole data set at *P* < 0.05

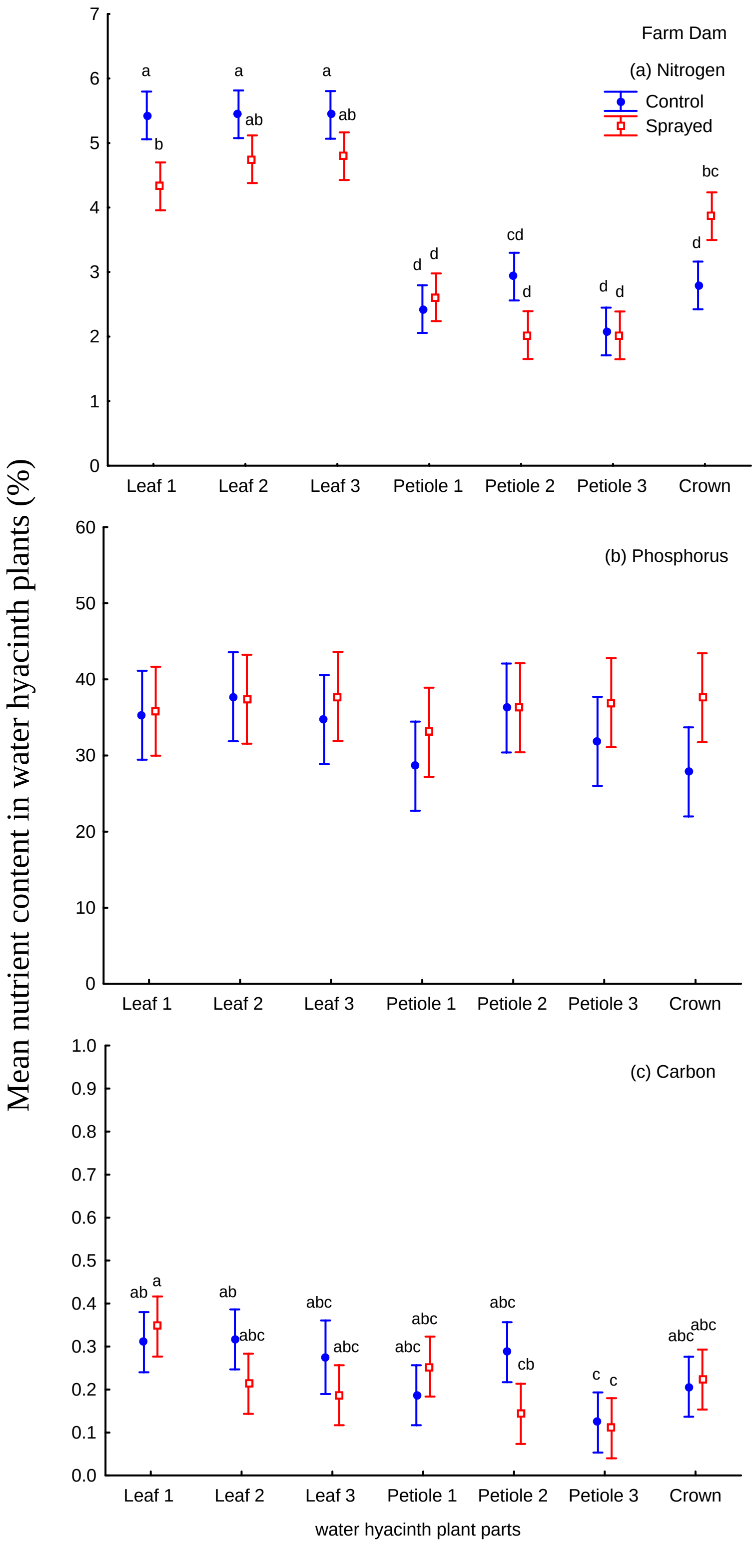

Figure 4.2 Effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on (a) nitrogen, (b) carbon, and (c) phosphorus content in water hyacinth plants at Farm Dam, two weeks after spraying. Error bars represent standard error of the mean. Means with different letters are significantly different to each other between control/sprayed pairs and across the whole data set at $P < 0.05$

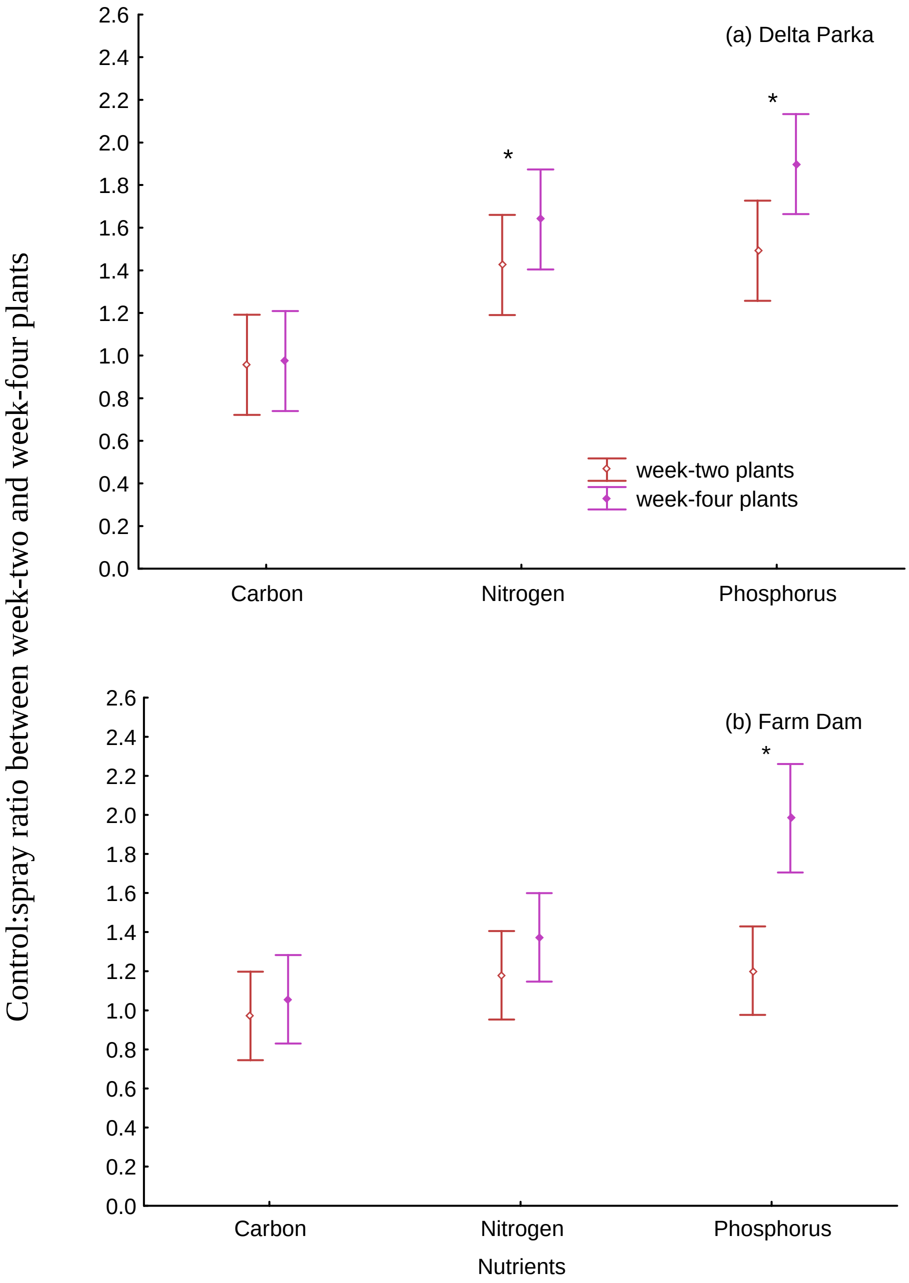

Figure 4.3 The effect of 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) on water hyacinth leaf N between week two and week four after spraying at (a) Delta Park and (b) Farm Dam. Error bars represent standard error of the mean. * indicates significance between week-two/week-four pairs at $P < 0.05$

4.4.2 Nitrogen, carbon and phosphorus content in control and sprayed plants

Plants analysed four weeks after spraying were used in the rest of the analysis because the effect of glyphosate on the nutrients of pooled water hyacinth leaves was more pronounced in plants from the fourth week than those from the second week of spray.

At Delta Park, N content in water hyacinth leaves was significantly greater in the control plants than in the sprayed plants, while C and P between control plants and sprayed plants were not different ($F_{(2,12)} = 3.66$, $P = 0.05$) (Fig. 4.4.a). In water hyacinth crowns, no difference was found in N, C and P between control plants and sprayed plants ($F_{(2,12)} = 0.85$, $P = 0.45$) (Fig. 4.4.b).

At Farm Dam, N and C in water hyacinth leaves were significantly greater in control plants compared to sprayed plants ($F_{(2,11)} = 15.91$, $P < 0.001$) (Fig. 4.5.a), whereas no difference was found in their P content. In water hyacinth crowns, N and P were not different between control plants and sprayed plants, however C content in water hyacinth crown was significantly greater in sprayed plants compared to control plants ($F_{(2,12)} = 57.68$, $P < 0.001$) (Fig. 4.5.b).

Comparison of N, C, and P between Delta Park and Farm Dam

There was no significant difference in C, N, and P in the leaves or in the crowns between Delta Park plants and Farm Dam plants ($F_{(2, 30)} = 2.61$, $P = 0.09$; $F_{(2, 30)} = 1.08$, $P = 0.35$) (Fig. 4.6).

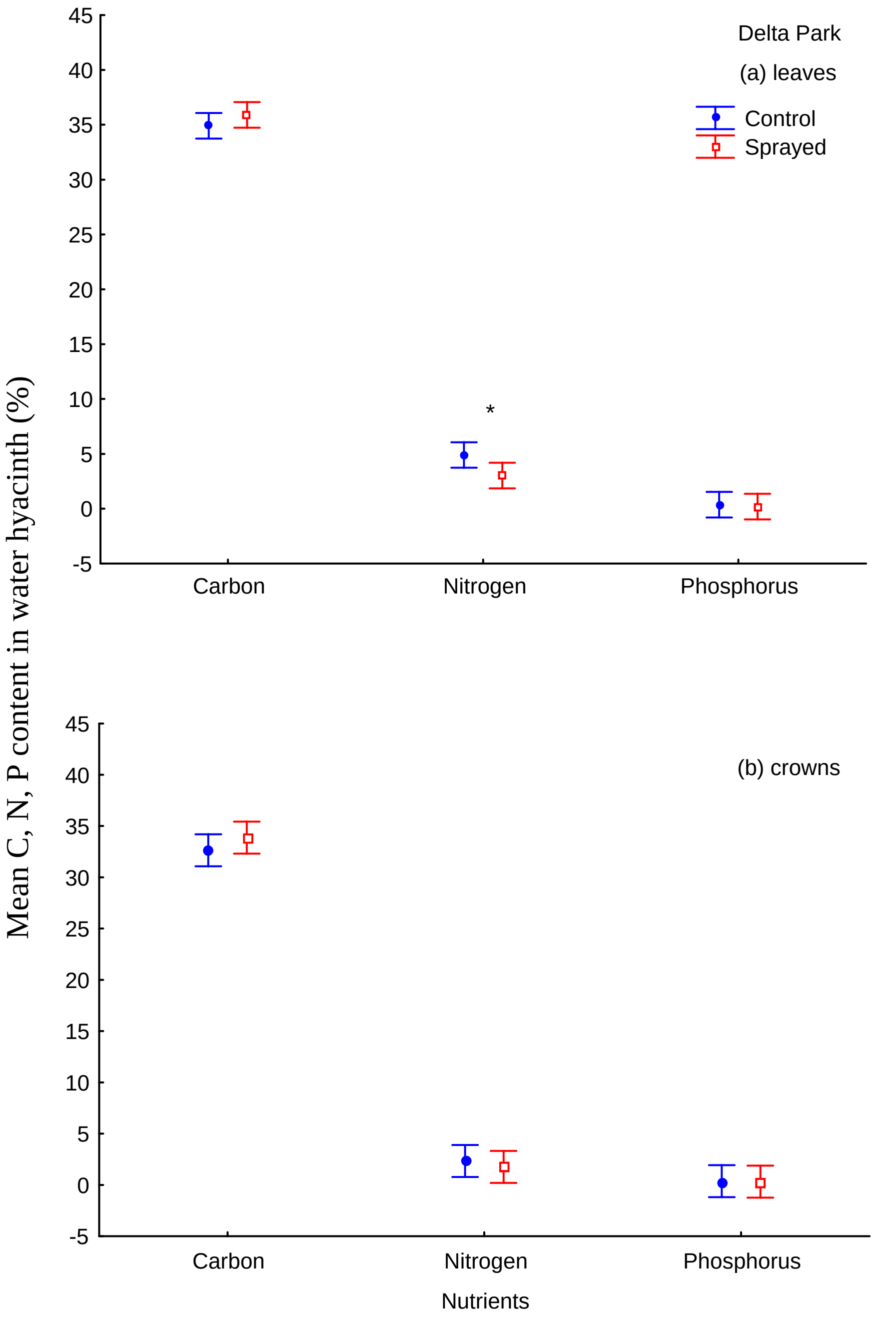

Figure 4.4 Effect of 0.8% herbicide glyphosate (140 l/ha spray volume) on N, C and P content in (a) water hyacinth leaves and (b) crowns between the control plants and the sprayed plants four weeks after spraying at Delta Park. Error bars represent standard error of the mean. Asterisks between control / sprayed pairs denote significant difference at $P < 0.05$

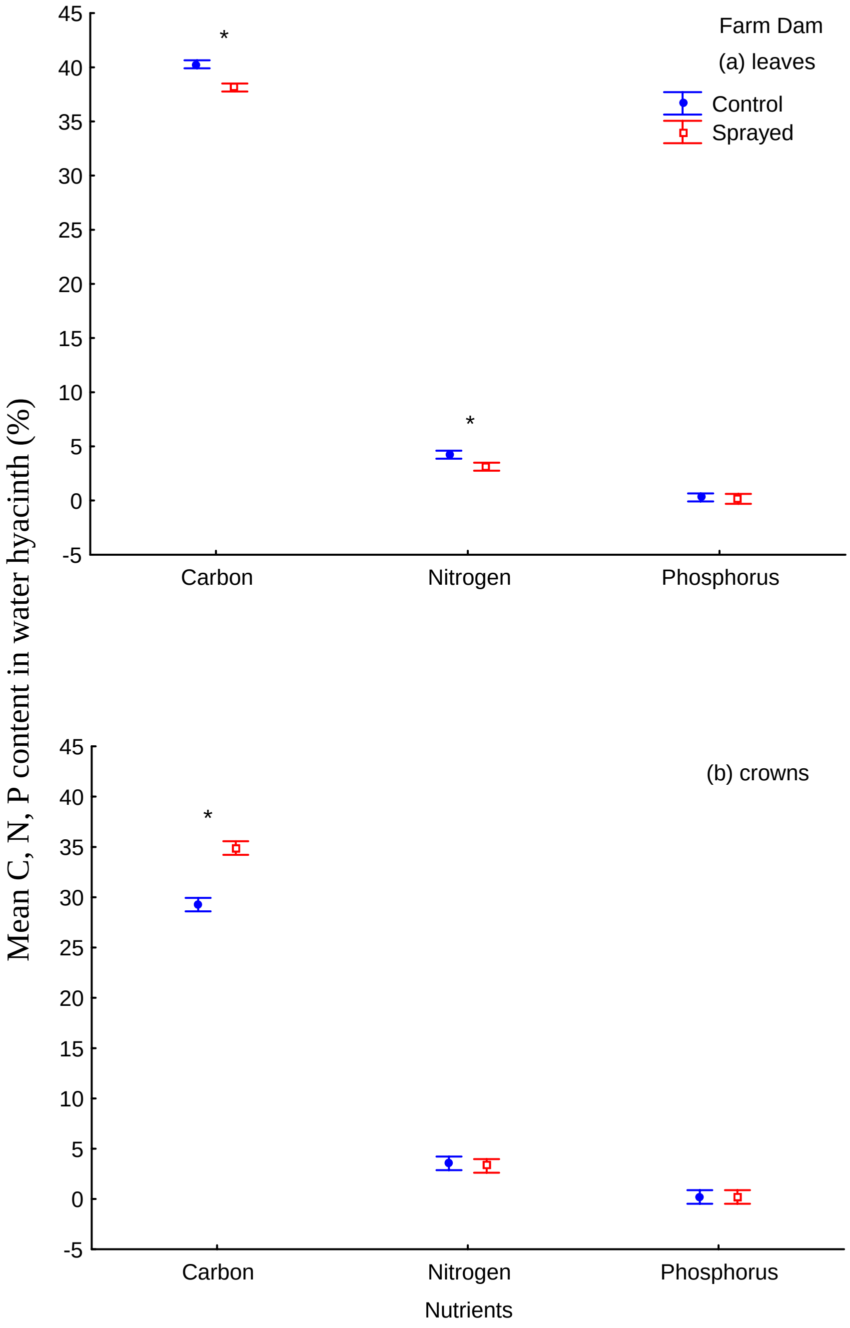

Figure 4.5 Effect of 0.8% herbicide glyphosate (140 l/ha spray volume) on N, C and P content in (a) water hyacinth leaves and (b) crowns between the control plants and the sprayed plants four weeks after spraying at Farm Dam. Error bars represent standard error of the mean. Asterisks between control / sprayed pairs denote significant difference at $P < 0.05$

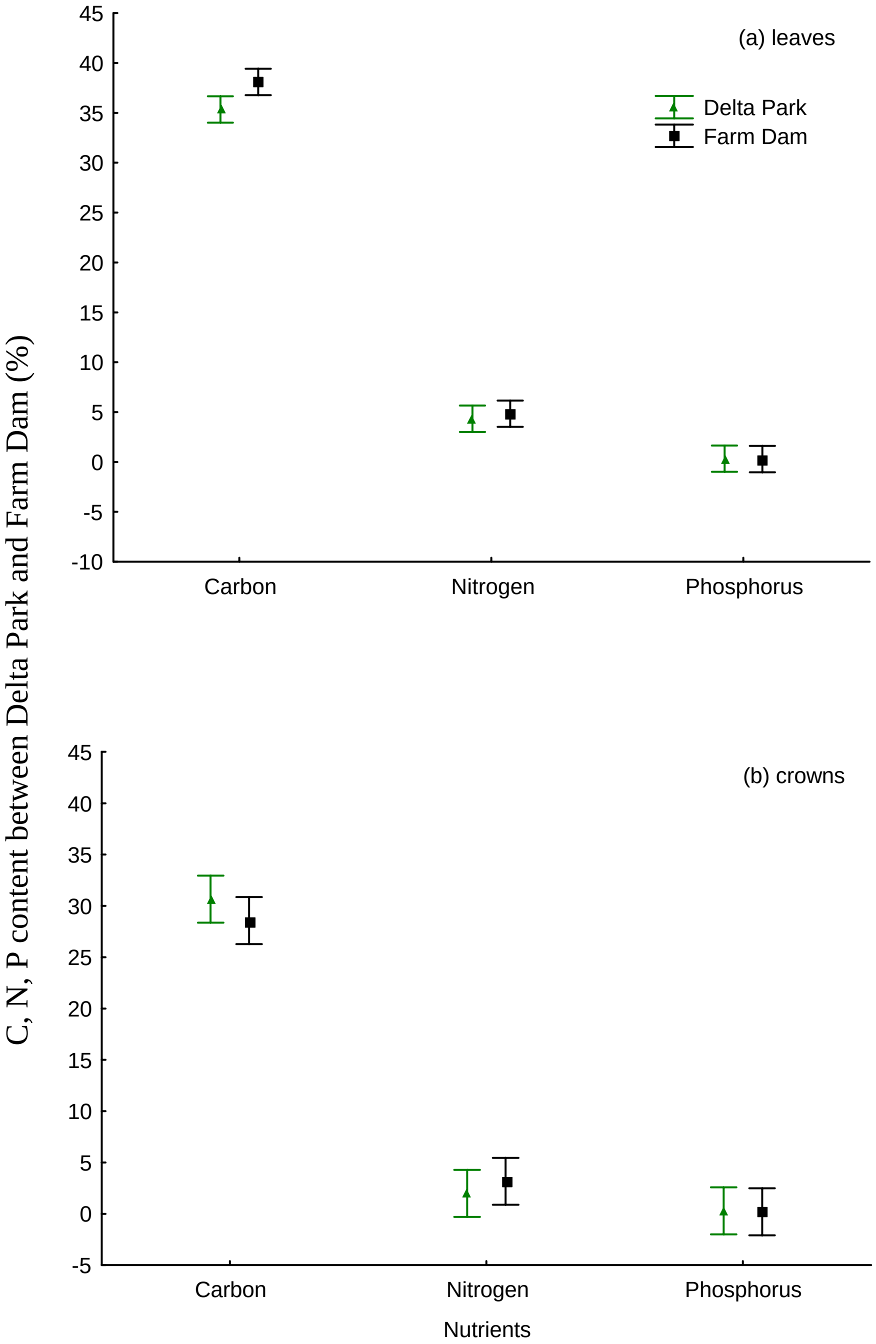

Figure 4.6 Different between C, N, and P content in (a) water hyacinth pooled leaves and (b) water hyacinth crowns between Delta Park plants and Farm Dam plants. Error bars represent standard error of the mean, *P* = 0.05

4.4.3 C:N ratio and N:P ratio in water hyacinth crowns and leaves

C:N ratio in water hyacinth leaves and crowns at Delta Park was significantly higher in sprayed plants than in control ones (t = 5.32, $P < 0.001$; t = 3.18, $P = 0.03$) (Fig. 4.7.a).

At Farm Dam, the C:N ratio in water hyacinth leaves was slightly, but statistically insignificantly higher in sprayed plants compared to control plants ($t_3 = 2.76$, $P = 0.05$). However in water hyacinth crowns C:N ratio was significantly higher in sprayed plants than in control plants ($t_3 = 4.56$, $P < 0.01$) (Fig. 4.7.b).

At Delta park, N:P ratio was not significantly different between control and sprayed plants both in water hyacinth leaves and crowns respectively ($t_3 = -2.24$, $P = 0.08$; $t_3 = 1.62$, $P = 0.18$) (Fig. 4.8.a). The same pattern was observed at Farm Dam ($t_3 = -1.63$, $P = 0.20$; $t_3 = 1.00$, $P = 0.37$) (Fig. 4.8.b).

Relationship between C:N ratio and the number of weevil feeding scars

At Delta Park, there was a significant positive correlation between the C:N ratio in the control plants and the number of feeding scars; while no correlation was found in the sprayed plants (Fig. 4.9). On the other hand, there was no correlation between the C content in the control leaves and the number of feeding scars nor was there any correlation in the sprayed leaves (Fig. 4.10). Similarly, there was no correlation between the N content in the control leaves and the number of feeding scars or the N content in the sprayed leaves and the number of feeding scars (Fig. 4.11).

At Farm Dam, there were no weevil feeding scars on the control or the sprayed plants; therefore no correlation was established between plant nutrients and the number of feeding scars at this site.

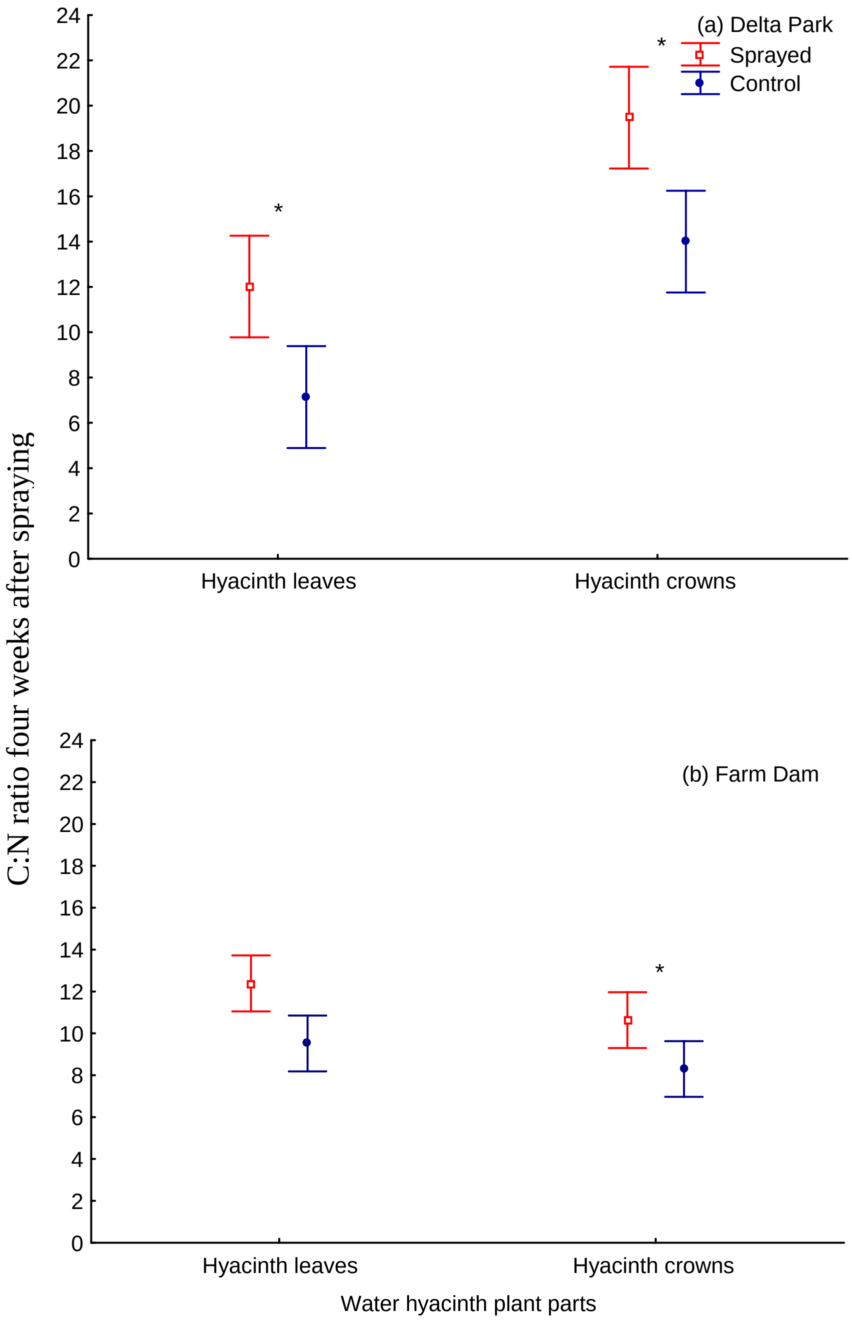

Figure 4.7 Effect of 0.8% herbicide glyphosate (140 l/ha spray volume) on C:N ratio in water hyacinth leaves and crowns between control plants and the sprayed plants from (a) Delta Park and (b) Farm Dam four weeks after spraying. Error bars represent standard error of the mean. Asterisks between control / sprayed pairs denote significant difference at $P < 0.05$

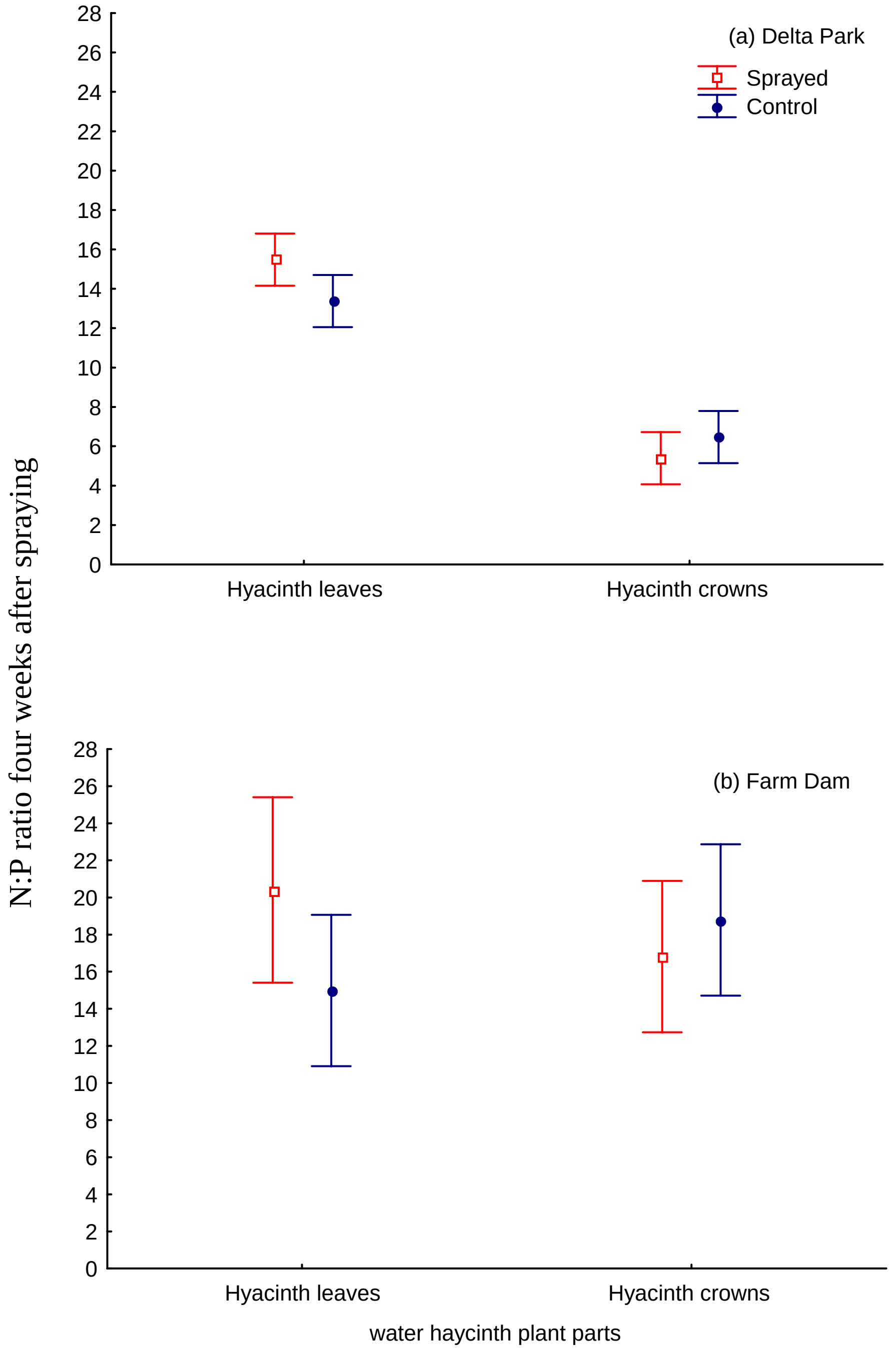

Figure 4.8 Effect of 0.8% herbicide glyphosate (140 l/ha spray volume) on N:P ratio in water hyacinth leaves and crowns between control plants and the sprayed plants from (a) Delta Park and (b) Farm Dam four weeks after spraying. Error bars represent standard error of the mean. Asterisks between control / sprayed pairs denote significant difference at $P < 0.05$

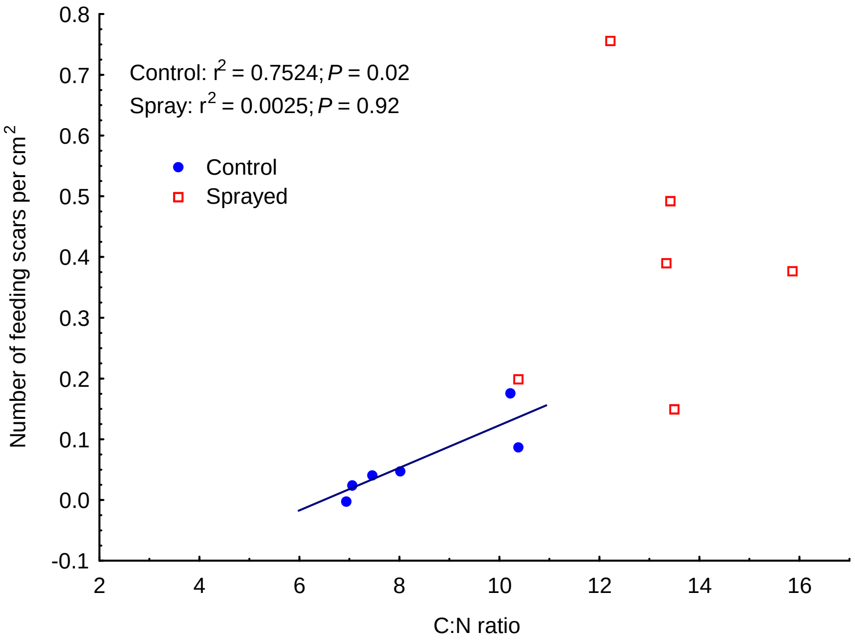

Figure 4.9 Correlation between C:N ratio in the water hyacinth leaves and the number of weevil feeding scars per cm^2 in the control and the sprayed water hyacinth plants at Delta Park

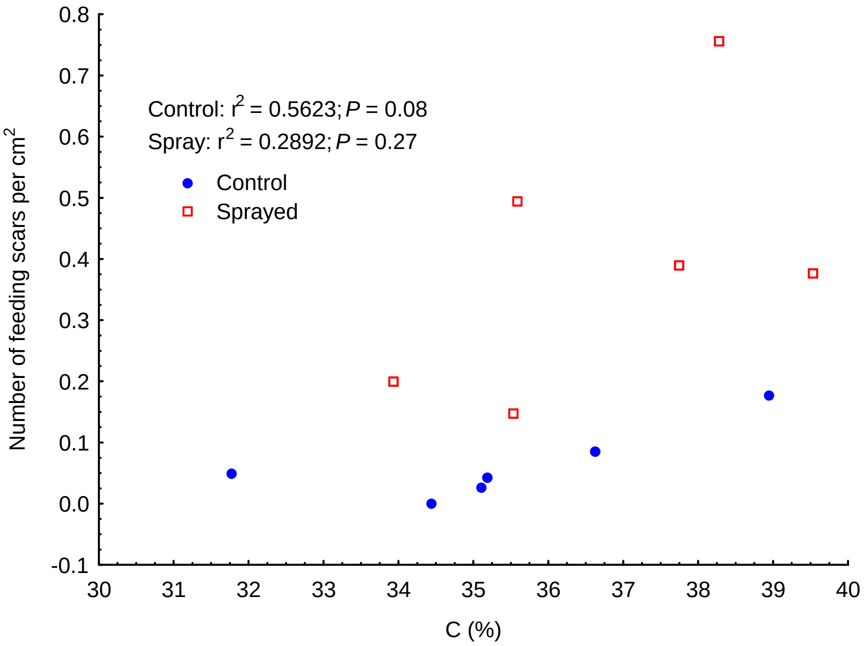

Figure 4.10 Correlation between C content in the water hyacinth leaves and the number of weevil feeding scars per cm^2 in the control and the sprayed water hyacinth plants at Delta Park

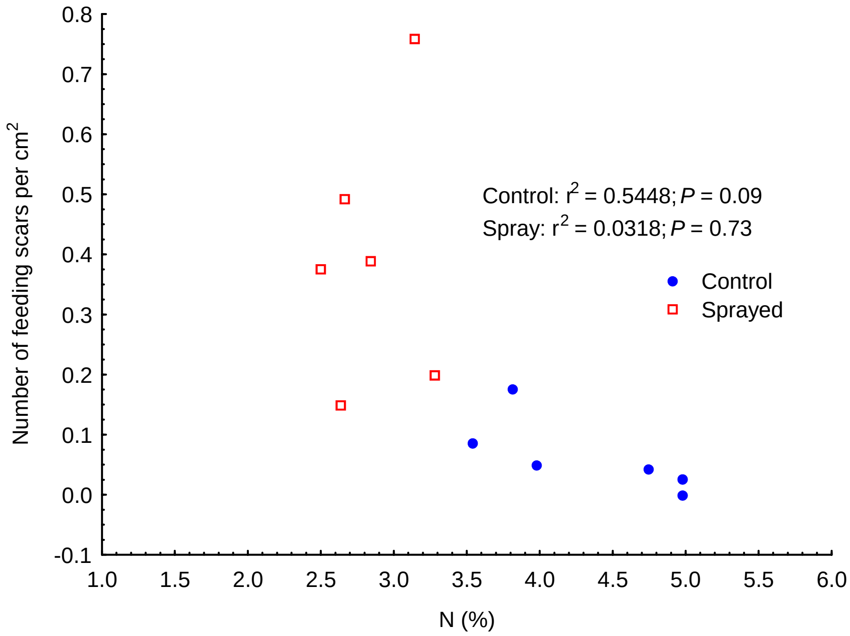

Figure 4.11 Correlation between N content of water hyacinth leaves and the number of weevil feeding scars per cm^2 in the control and the sprayed water hyacinth plants at Delta Park

4.5 Discussion

Not many studies have looked at the effect of herbicides on the nutritional quality of plants as food source for arthropod herbivores. Early studies have suggested that plants exposed to stressors such as herbicides should have increased free amino acids (Fedtke, 1973); but this phenomenon was argued to simply be a reallocation of resources from storage tissues to actively growing ones rather than an increase in the whole plant (Kjær & Elmegaard, 1996). The present study has clearly shown general decline in the leaf N in herbicide sprayed plants compared to unsprayed plants.

The nutrient contents, nitrogen in particular, have been found to occur in different concentrations in different plant parts. Nitrogen content was higher in the leaves, followed by the petioles and then the crowns. The same pattern was observed both at Delta Park and Farm Dam. Previous studies have pointed out that leaf N content is an indicator of food quality for phytophagous insects (Mattson, 1980; Awmack & Leather, 2002). Park *et al*. (2009) noted that *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) preferred laying eggs on the upper plant section of cherry tomato cultivars, *Lycopersicon* *esculentum* Miller; which had leaves with high nitrogen content. This could explain why adult *Neochetina* weevils prefer feeding on the leaves; whereas petioles serve only as a channel allowing adult weevils and larvae to travel from the leaves to the crown for refuge and larval development respectively (Julien *et al.*, 1999). van Lenteren and Noldus (1990) reported that most insect species have the ability to chose plant parts that are most suitable for feeding and oviposition.

Results on the comparison of the plant nutrient response to 0.8% glyphosate (140 l/ha; 0.11 g/m^2 a.i) between week two and week four after spraying revealed that the nutrient change was more pronounced at week four after spraying than at week two. According to Monsanto, the action of Roundup on plants is visible from two weeks after application (Roundup instruction booklet, Nov. 2006 (L.D.M.)). These results explain why the rest of the analysis was based on plants analysed four weeks after being sprayed.

Nitrogen content was significantly higher in the control water hyacinth leaves compared to the herbicide sprayed leaves, however in the crown there was no significant difference between herbicide sprayed and unsprayed plants at both sites. This could explain why, at Delta Park, in spring 2008, there was no difference in the number of larvae between the herbicide sprayed and the unsprayed plants although in general the mean number of larvae per plant was less than one at both sites (Chapter 2). Since N is considered to be indicative of plant nutritional quality (Mattson, 1980; Center & Dray, 2010), the instar development of weevil larvae would not be jeopardized because the N content in the crown is not depleted.

It was noted that Farm Dam plants and Delta Park plants were not significantly different in their nutrient contents. In addition, sprayed plants at both sites showed a significant decrease in the leaf N. These results are interesting because from their different phenotypes, one would expect Farm Dam plants (large plants) to be higher in nutrient content compared to Delta Park plants (small to medium plants). Heard and Winterton (2000) reported that there was a strong correlation between water hyacinth growth and the water nutrient concentrations (particularly N and P) from where the plant occurs. The water nutrient content at Farm Dam was in the same range as that at Delta Park (Byrne *et al.,* 2010). Therefore the only proposed explanation for the difference in phenotypes between Farm Dam plants and Delta Park plants would be that these plants are of two different biotypes.

This study showed that the decreased leaf N resulted in a high C:N ratio in the plant leaves and crowns. Generally (except for Farm Dam) C didn't change but N went down, therefore N content decrease is driving the C:N ratio more than C content rising. A high C:N ratio or low nitrogen concentration in plants often results in reduced nutritive value to herbivores (Lincoln *et al*., 1986). Some studies reported that herbivore selection for quality plants was determined by the plant's nutrient contents, with special reference to high nitrogen and a low C:N ratio (Villalba & Provenza, 1999; Pe´rez-Harguindeguy *et al.,* 2003). For example, Evju *et al.* (2009) found that sheep preferred grazing on large, late-flowering herbs with low C:N ratios in leaves, i.e. they preferred high N to low N herbs. Leaf chewing herbivores generally increase tissue consumption through compensatory feeding when feeding on plants grown under elevated CO_2, which are low in nutritional quality (Williams *et al.,* 1994). It is also reported that the feeding of leaf chewing insect herbivores reared on plants grown under elevated CO_2 conditions is often low because of the consequential reduction in the leaf N, increase in the leaf toughness and sometimes an increase in defence compounds (e.g. phenolics) (Lincoln *et al.,* 1993; Hunter 2001; Zvereva and Kozlov 2006). However, the present results show that weevil performance (feeding, survival, and reproduction) was neither affected nor benefitted by the elevated C:N ratio or by the decreased N content in plants. This indicates that the 0.8% glyphosate, although it changed the nutrient status of the plants, it did not affect the nutritive quality of these plants for the *Neochetina* weevils.

McDonald *et al.* (2001) found that glyphosate applied at a low dose increased the sucrose content in sugar cane. In the present work, the observed tendency of weevils to feed more on herbicide sprayed plants than on unsprayed plants, in spring 2008, at Delta Park, could not be explained as a result of glyphosate increasing the plant's soluble sugars since the leaf C content of the plants was not increased by the herbicide. The increase in the C:N ratio in the plants cannot explain the trend in weevil feeding either because there was no correlation between the C or the high C:N ratio in the sprayed plants and the number of feeding scars per cm^2. On the contrary, a positive correlation was found between the C:N ratio in the control plants and the number of feeding scars. The alternative explanation for the tendency of weevils to feed more on the sprayed plants is the delay in leaf production (leaf turnover) in the sprayed plants. Jadhav *et al.* (2008) found that leaf turnover was stopped in plants that were sprayed with 0.8% glyphosate. In the present work, similar results as Jadhav *et al.*'s (2008) were found in the field. The suggested implication of these results is that the number of feeding scars on the sprayed leaves must have accumulated on the sampled leaf-two since it did not change its position over time; thus resulting in a higher feeding scar count in the sprayed plants as compared to the control plants.

In conclusion, the sub-lethal glyphosate concentration generally resulted in a decrease in the leaf N content of the plant while not changing its C content, hence resulting in an increased C:N ratio. The weevil's survival, feeding and, reproduction was not affected by the alteration in plant nutrients.This suggests that although the herbicide treatment changes the nutritional value of the plant, the beetles may have compensated for any such changes since they were not adversely affected. Although limited attention has been directed towards investigating the responses of herbivorous insects to plants treated with sub-lethal dosages of herbicide (Kjær & Elmegaard, 1996), this work has shown that the 0.8% glyphosate concentration, which constitutes about 26.6% of the recommended dose, did not have any beneficial or detrimental effect on the water hyacinth *Neochetina* weevils. These findings reiterate the possibility of combining a sub-lethal dose of herbicides and biocontrol agents in the battle against water hyacinth, without killing or impeding the biological control agents.

CHAPTER FIVE: GENERAL DISCUSSION AND CONCLUSION

It’s been close to a century from the time water hyacinth (*Eichhornia crassipes*) was first recorded in South Africa (Cilliers, 1991), and the fight against this weed has been ongoing for more than three decades (Cilliers 1991). Yet this weed is still the most invasive water weed in South Africa, with a recent infestation observed on the Benoni Lake, South Africa (personal observation). There have been numerous efforts concentrated towards controlling this weed both locally and internationally, and the commonly used control methods include mechanical control, herbicidal control, biological control, and integrated control. The primary aim of this work was to assess the suitability of integrating a sub-lethal concentration of glyphosate herbicide with the biocontrol agents *Neochetina* weevils to control water hyacinth in the field. The decision to use a sub-lethal dose of glyphosate in lieu of a recommended dose was to avoid killing the plant which is not only the food source of the biological control agents but also serves as its habitat (Ainsworth, 2003; Jadhav *et al.*, 2008). Ainsworth (2003) noted the need to study the interactive effects of herbicide and biological control on invasive plant species rather than concentrating simply on effects that herbicides may have on biocontrol agents.

Jadhav *et al.* (2008) through laboratory trials found that a 0.8% glyphosate concentration was not lethal to the biocontrol agent *Neochetina* weevils, neither was it lethal to the plants; and the highlight of their findings was that spraying plants with a sub-lethal dose of glyphosate resulted in the freezing of ramet and leaf production. The present work repeated Jadhav *et al.*’ (2008) experiment in the field and these results revealed that the sub-lethal dose of glyphosate (0.8% glyphosate, 140 l/ha spray volume; 0.11 g/m^2 a.i) led to some reduction in water hyacinth growth, with particular reference to leaf production and biomass accumulation. At this herbicide concentration *Neochetina* weevils’ reproduction, survival and feeding intensity were not jeopardized, concurring with Jadhav *et al.* (2008).

Jadhav *et al.* (2008) found more weevil feeding scars on sprayed plants compared to control plants. The performance of the water hyacinth biological control agent *Niphograpta albiguttalis* has also been observed to increase following 2,4-D application on the weed (Wright, 1984). Although weevil feeding in the present study was not significantly influenced by the herbicide, there was a trend that showed slightly more feeding scars on the sprayed plants compared to the control plants. This could be explained by a possible weakening of the plants' defence mechanism by the herbicide. Glyphosate has been reported to inhibit the synthesis of secondary metabolites in plants (Ainsworth, 2003). Another proposed explanation is by Wright and Bourne (1990) who found that the herbicide 2,4-D amine decreased water hyacinth leaf and petiole hardness, thereby rendering it more palatable for larval stages of the moth, *Niphograpta albiguttalis* and the *Neochetina* weevils.

Unfortunately the observed increase in weevil feeding cannot be explained as a result of increased plant nutrient content because the N content in the sprayed plants was reduced by the herbicide. However, both in Jadhav *et al.* (2008) and in the present work, the 0.8% glyphosate resulted in retardation of water hyacinth leaf turnover. Consequently, there must have been an accumulation of feeding scars in the sprayed plants since the sampled leaf (leaf-two) did not change positions over time compared to control plants which produced a new leaf every week.

There were two major noticeable differences between Farm Dam plants and Delta Park plants. Firstly, the weevil performance (especially feeding) was very low at Farm Dam compared to Delta Park. This was largely attributed to the fact that Farm Dam site was often disturbed through floods and manual removal of plants, hence disturbing the stability of the weevil population. However in spring 2008, when plant nutrients were analysed, Farm Dam site did not encounter any form of disturbance, yet it had a very low weevil activity. One would think that a low level of nutrients at this site may be the reason that weevils did not do as well at Farm Dam as they did at Delta Park. However results show that water hyacinth nutrients were not significantly different between Farm Dam plants and Delta Park plants. Therefore the poor performance of the *Neochetina* weevils at Farm Dam compared to Delta Park raised the question of whether or not Delta Park plants and Farm Dam plants are of different biotypes. This question was not answered in this work. Nevertheless, the biocontrol of *Lantana camara* L. in South Africa offers a classical example of a poor biocontrol agent establishment due to the plant's variety in biotypes (Heystek, 2006, Winder *et al.,* 1984; Neser & Cilliers 1990). Baars and Neser (1999) noted that there were several types of *Lantana camara* in South Africa varying in their morphology, physiology and genotype. It is then speculated that the unsuccessful establishment of some biocontrol agents on *Lantana* could be the result of a mismatch between the agents used and the type of biotypes that occur in South Africa (Day & Neser, 2000). Cooley *et al.,* (1979) categorized three water hyacinth biotypes according to plant size and disposition to feeding by *Neochetina eichhorniae*, these phenotypes are namely: superhyacinths, small to stunted plants, and normal plants. It was then observed that feeding by *Neochetina eichhorniae* was different in each of these phenotypes, with superhyacinths or longstyled water hyacinth being particularly more resistant to insect attack (Haller & Tag el Seed, 1979).

Secondly, it was found that Farm Dam plants were less susceptible to the 0.8% glyphosate compared to Delta Park plants. That may be explained by the low numbers of adult *Neochetina* weevils at Farm Dam. On the contrary, at Delta Park, the combination of a well established weevil population coupled with the 0.8% glyphosate may have been responsible for significantly reducing water hyacinth leaves and biomass accumulation. Studies have shown that water hyacinth under biocontrol agent management grow more slowly and attain smaller sizes than agent-free plants (Center, 1994; Grodowitz *et al*., 1997).

The difference in phenotype between Farm Dam plants and Delta Park plants could also explain why Farm Dam plants were less susceptible to the herbicide compared to Delta Park plants. The experiment reported in chapter three attempted to explain how plants of different phenotypes would respond differently to the same dose of herbicide. In that experiment, it was theorized that in an isometric relationship there will not be a need to adjust herbicide doses regardless of different plant phenotypes. However, if the relationship were allometric, the herbicide doses would have to be adjusted accordingly. Farm Dam and Delta Park plant phenotypes showed an allometric relationship, with a slope less than one; thus Farm Dam plants were expected to be less susceptible to the herbicide by virtue of their large size. To the best of our knowledge, no other study has investigated the relationship between water hyacinth leaf surface area and plant biomass.

The significant increased weevil feeding from sprayed plants reported in Jadhav *et al.* (2008), together with the trend observed in chapter two, showing slightly more feeding scars from the sprayed plants compared to the control plants, prompted experiments which looked at herbicide-induced plant nutrient alterations. The aim of that experiment was to find out how a sub-lethal dose of glyphosate could affect the nutritive quality of plants, thereby enhancing the performance of biological control agents. Room *et al.* (1989) and Spencer and Ksander (1999) suggested that assessing plant nutrients is important if one wants to understand interactions between plant damage, and insect growth and reproduction. Some studies have reported an increase in N content in water hyacinth upon herbicide application (White, 1984; Wright & Bourne, 1990), whereas the present study showed the contrary. It was found that spraying a sub-lethal dose of glyphosate resulted in decreasing the N content in water hyacinth while keeping the C content unchanged and subsequently resulting in an increased C:N ratio. Moran (2006) and Lincoln *et al*. (1986) found a negative relationship between N content and damage by water hyacinth weevils, while the contrary was found by Center and Wright (1991), who noted that adult weevils preferred feeding on young leaves than on old ones because of their high nitrogen content. Nevertheless, in this work the glyphosate-induced change in plant nutrient did not reduce the feeding intensity of the weevils neither did it influence their reproduction or survival. These results indicate that the high weevil feeding intensity reported in Jadhav *et al.* (2008) might not have been as a result of a sub-lethal dose of glyphosate enhancing the nutritive value of plants as food source for the weevils; however the accumulation of weevil feeding scars on the leaf-two as a result of a slow leaf turnover rate seems to offer a better explanation.

In conclusion, the present work has shown that a sub-lethal dose of glyphosate and the *Neochetina* weevils can be combined to control water hyacinth in the field, however more work needs to be done in the formulation of sub-lethal doses in relation to different water hyacinth plant phenotypes. Cilliers *et al*. (1996) stated that: “Integrated control should be implemented in such a way that the different control methods supplement each other and where possible have an additive effect”. In the case of the present work however, the performance of the weevils was not improved nor was it impaired by the sub-lethal dose of glyphosate; but the synergistic pressure exerted by both stressors (biocontrol agents and herbicide) resulted in a significant decline in the production of leaves as well as accumulation of biomass. Ainsworth (2003) argued that even if performance of the biocontrol agents was reduced through herbicide spray, the overall effect of integrating a sub-lethal herbicide and biocontrol would still be worthwhile. This is because plant growth in the presence of biocontrol agents is not as fast as in their absence (Hill & Olckers, 2001).

Recommendations

Depending on the extent of a water hyacinth infestation and/or the uses of a particular water system, spraying a sub-lethal dose of herbicide may not always be the best option to land owners who would want to see their water system cleared in a minimum time. Aerial spray using lethal herbicide concentrations is definitely the best option for reducing water hyacinth mat size especially when dealing with very large infestations where manual removal will be practically impossible. However to keep biocontrol agents in the system, it is recommended to strip spray along the middle section of the infested water body to radically reduce the weed’s population while keeping peripheral plants unsprayed to act as refuges for the agents. This strategy was seen to yield successful results on the Nseleni River, in South Africa (Jones, 2009).

In light of the above recommendation, future research should examine:

- The effect of spray drift on indigenous vegetation and/or crops and determine how far from the bank can one spray to reduce the damage if any.
- Whether *Neochetina* weevils are capable of moving from the herbicide affected plants to the peripheral unsprayed ones.

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