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IMPACT OF HERBICIDES USED IN WATER HYACINTH CONTROL ON NATURAL ENEMIES RELEASED AGAINST THE WEED FOR BIOLOGICAL CONTROL

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EXECUTIVE SUMMARY

INTRODUCTION

Water hyacinth is South Africa's most damaging aquatic weed. It is widely distributed throughout the country and mats of the weed impact all aspects of water utilization. Several control options are practiced in South Africa including large scale herbicide control, limited mechanical control and manual removal, biological control and aspects of nutrient control. More recently, attempts have been made to try and integrate a number of the control methods to achieve that highest level of control. The integration of biological and herbicide control is currently the most widely advocated control method. However, this relies on the assumption that these two methods are compatible.

OBJECTIVES

To test the assumption that the herbicides used in water hyacinth control are not toxic to two arthropod species, the weevil *Neochetina eichhorniae* and the water hyacinth bug *Eccritotarsus catarinensis*, released as biological control agents for the weed in South Africa.

METHODOLOGY AND RESULTS

Of the two insect species tested, the bug (E. catarinenis) was most susceptible to direct herbicide application. Midstream (diquat) and a combination of Midstream and Agral (surfactant), caused high mortality while Muster (glyphosate-trimesium), Roundup Ultra (glyphosate), 2,4-D amine and Touchdown (glyphosate-trimesium) caused some mortality of the mirid bug. The surfactant Agral applied separately also caused mortality at recommended dosages. The weevil, N.eichhorniae was less suceptable to herbicide surfactants although Agral, Muster and Roundup Ultra caused some mortality, while Midstream seemed to be less toxic to the weevils. A combination of Midstream and Agral caused higher mortality in weevils than did the same concentration of Agral cause when applied separately. Weevils exposed to herbicides showed decreased feeding in the case of Midstream and Agral combined, but not separately. No other decreases or increases in feeding behaviour were noted.

Weevils fed on Midstream (diquat) treated water hyacinth leaves showed a significant decrease in feeding and significant mortality after 120 hours of feeding. When fed on 2,4-D amine treated leaves, weevils also fed significantly less but little mortality occurred within the 144 hours test period.

Movement studies in small ponds showed that weevils migrated from herbicide treated water hyacinth to untreated plants. The mean number of feeding scars in the unsprayed area was significantly higher than in the sprayed area, indicating that herbicide treated plants become unpalatable to the weevil.

IMPLICATIONS FOR INTEGRATED CONTROL

To ensure successful integrated control management of water hyacinth in South Africa each site needs to be investigated thoroughly. A management plan must take into consideration the degree of control required, the type and concentration of herbicide and the biological control agent used.

FUTURE RESEARCH NEEDS

Sub-lethal effects of herbicides on natural enemy physiology and on the weed community; spray patterns for herbicide application to allow the highest number of natural enemies to vacate sinking plants; the concepts of leaving refugia for natural enemies; and the re-innoculation of water hyacinth regrowth with large numbers of natural enemies, should be investigated.

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THESAURUS

2,4-D (2,4-dichlorophenoxy) acetic acid is a chemical that is generally

formulated as a salt or ester for use as a herbicide. Physicalchemical properties of this substance varies depending on the acid form. A 2,4-D amine formulation was used in this study. Ester variations of this chemical tend to be more toxic than amine

formulations.

Acid equivalent/ae The theoretical yield of parent acid from a pesticide active ingredient

which has been formulated as a derivative.

Active ingredient
The chemical in a herbicide formulation primarily responsible for its

phytotoxicity, and which is identified as the active ingredient on the

product label.

Acute toxicity The potential of a substance to cause injury or illness shortly after

exposure.

Add Add-2 (refer to Table 2.1)

wetters, foaming agents and evaporation agents. (Includes

surfactants)

Herbicide For the rest of this report, the use of the word herbicide indicates a

formulation or combination of formulation and surfactant as further

specified.

IPA salt Isopropylamine salt

LC 50 Lethal concentration 50 tests are toxicity tests that are performed to

determine what concentration of a substance cause 50 % mortality of

the test organisms in a population.

Mon Mon 52276 (refer to Table 2.1)

MS Midstream (refer to Table 2.1)

Must Muster (refer to Table 2.1)

RU Roundup (refer to Table 2.1)

RUU Roundup Ultra (refer to Table 2.1)

Surfactant A substance added to the formulation to improve the emulsifying,

dispersing, spreading, wetting or other properties of a liquid by

modifying its surface characteristics. (Also an additive)

TD Touchdown (refer to Table 2.1)

LIST OF PUBLICATIONS

Parts of the research presented in this study have been published in the following format:

Paper presented at conferences, symposia and workshops:
 Ueckermann, C., Hill, M.P & Reinhardt, C.F. 2000. Sensitivity towards selected herbicides of two insect biocontrol agents for water hyacinth. Combined

Congress of SAVO/SAWSS, 17-20 January 2000, Bloemfontein. (Published as abstract).

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Posters presented at conferences, symposia and workshops:

Ueckermann, C., Hill, M.P., Reinhardt, C.F. & Van Rooyen, M.W. 2000.
Suggestions towards integrated control of water hyacinth. 26th Annual Conference of the South African Association of Botanists. 10-14 January 2000, Potchefstroom. (Published as an abstract).

Hill, M.P. & Ueckermann, C. 2000. The impact of herbicides used in water hyacinth control on the natural enemies released against the weed for biological control. *Third International Weed Science Congress, Foz do Iguassu, Brazil, 6-11 June 2000.* (Published as an abstract).

· MSc Thesis:

Ueckermann, C. 2000. Susceptibility towards selected herbicides of two insect biocontrol agents for water hyacinth. MSc Thesis. University of Pretoria.

STORAGE OF DATA

Raw data used for the purposes of this study is lodged at the Plant Protection Research Institute at Rietfontein^a and is available in electronic format.

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1. Introduction

1.1 BACKGROUND

Water hyacinth, Eichhornia crassipes (Mart.) Solms-Laub. (Pontederiaceae), is a perennial, herbaceous, free floating aquatic plant originating in the Amazon Basin, South America (Center 1994). The leaves are arranged in a rosette and the petioles vary from spongy and bulbous to slender and non-bulbous. The latter are typical of plants in dense infestations whereas the former are typical of open water and spreading infestations. The weed produces lilac to blue flowers and can set up to 300 seeds per flower. The seeds sink following release from the seed capsule and remain viable for 5-20 years (Penfound & Earl 1948). The plant also reproduces vegetatively through the rapid production of daughter plants.

Water hyacinth is regarded as the world's most damaging aquatic weed. The lack of natural enemies and invariably the presence of nutrient-enriched waters in the regions of introduction have certainly contributed to its establishment and subsequent weedy status. Water hyacinth has established on slow moving water bodies, lakes and swamps in most countries of the world lying between 40° N and 45° S (Holm et al. 1977).

Water hyacinth was first recorded in South Africa shortly after 1900 (Gopal 1987) and was rapidly dispersed by gardeners, aquarium owners and boating enthusiasts (Jacot Guillarmod 1979). The weed is now established in river systems and dams throughout the country with the exception of the more arid, Karoo region (Figure 1.1).

1.2 IMPACT OF WATER HYACINTH

Thick mats of water hyacinth have a serious impact on aquatic ecosystems and cause problems for all aspects of water utilization. These impacts are socio-economic and environmental, and include: a reduction in the quality of drinking water caused by bad odours, taste, colour and turbidity; promotion of the development of waterborne, water based and water related diseases (e.g. malaria, encephalitis and filariasis); increase in siltation of rivers and dams; reduction of useful water surface area for

fishing and water transport; deterioration of aquatic biodiversity; clogging of irrigation canals and pumps, and enhancing flood damage to road and rail bridges and hydroelectric power generation schemes.

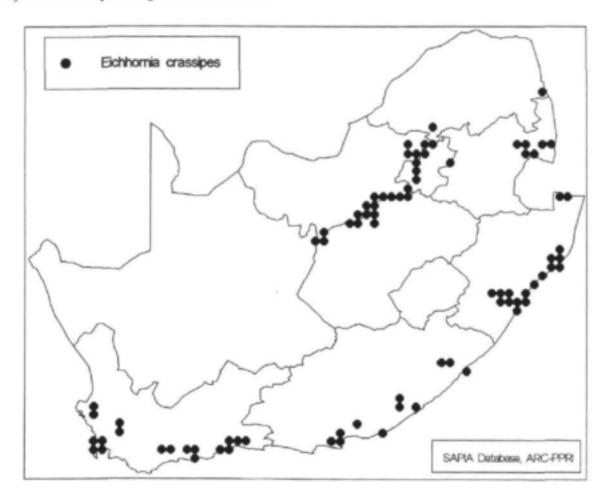


Figure 1.1 Distribution of Eichhornia crassipes, water hyacinth in South Africa (Drawn by L. Henderson, Plant Protection Research Institute)

Economic and environmental losses due to water hyacinth are high. However, the breadth of its impact means that it is almost impossible to estimate. Fish and wildlife losses due to the weed in the southern states of the USA in 1947 were estimated at US\$ 4 million (Center 1994). Holm et al. (1969) estimated that water hyacinth infestations in Florida, Mississippi, Alabama and Louisiana caused losses of US\$ 43 million in 1956. The US Army Corps of Engineers control program on water hyacinth is estimated to have provided a benefit of nearly US\$ 14 million in the USA in 1965 (Gordon & Coulson 1974). The most recent estimate available claims that US\$ 43 1880 000 was spent in the control water hyacinth and water lettuce combined in Florida state between 1980 to 1991 (Schmitz et al. 1993).

1.3 CONTROL OF WATER HYACINTH

Traditionally the control of water hyacinth has fallen into one of three broad categories: mechanical control, herbicide control and biological control. More recently the emphasis has moved to an integration of the three methods (Cilliers et al. 1996).

1.3.1 Mechanical Control

Mechanical control or the manual removal of water hyacinth is invariably the initial control option exercised once an infestation of the weed has become problematic. However, these control options are impractical in infestations larger than about one hectare because of the rapid rate of increase of the weed and the fact that it is in excess of 90% water (Penfound & Earl 1984). While manual removal using rakes and pitchforks can be successful, it is extremely labour intensive and ineffective in larger infestations.

Mechanical harvesters have been advocated for water hyacinth control with varying levels of success. Often these have been modifications of machines used for algal control in the canals of Europe, Japan and the USA and are inefficient against water hyacinth plants, which can be up to 1.2 m in height. However, mechanical harvesters have been successful in some areas. Examples of this are Port Bell and the Owen Falls Dam on the Ugandan side of Lake Victoria, where they have provided temporary access to the port for ships and cleared the water intake pumps for the hydroelectric power generation at the dam. The disadvantage to mechanical harvesters is that they are very expensive. The harvester currently being used in Port Bell, cost in excess of US\$ 1 million. In addition to the initial cost of the machine, operating costs and the cost of removal of the water hyacinth material are higher (Hill 1999).

1.3.2 Herbicide Control

Herbicide control has been practiced against water hyacinth since the early 1900's. Sodium arsenate was first used in Florida in the USA until 1905 when it was discontinued after problems arose concerning the poisoning of cattle (Center 1975). Since then a number of other herbicides have been used. Water hyacinth is very susceptible to diquat, the amine and acid formulations of 2,4-D and glyphosate (Divakar & Manoharan 1979; Wright & Purcell 1995). These have proved very successful in the control of this plant in small, single-purpose water systems such as irrigation canals and dams (Wright & Purcell 1995).

Application of herbicides includes aerial spraying from helicopter, fixed wing and microlight aircraft. High pressure motorized units mounted on boats are used in smaller infestations in navigable waters and knapsack sprayers are used along river banks in limited areas.

Herbicide control of water hyacinth depends on skilled operators who maintain a long-term follow up programme to continually control reinfestation from scattered plants and those germinating from seed. Therefore any herbicide programme against the weed requires a commitment to an ongoing operation of unlimited duration. It is the lack of a rigorous follow-up regime that has often lead to the failure of herbicide control programs.

The negative impacts of herbicides on the environment have often been used to promote alternative control methods for water hyacinth. These negative impacts include the potential threat to human life in rural areas of the world where communities use untreated water for domestic use; the threat of herbicide residues in the aquatic ecosystem; the threat of massive de-oxygenation of the water column following decomposition of large mats of water hyacinth following treatment; and the general perception that herbicides are poison. These concerns have been overrated. The majority of herbicides currently being used in water hyacinth control, whilst not ecologically benign, impact the environment considerably less than mats of water hyacinth. However, the concern of spray drift onto non-target vegetation is real and demands responsible use of herbicides by highly trained personnel.

1.3.3 Biological Control

While mechanical and herbicide control are viewed as the short-term or immediate control options, biological control is perceived as the long-term or sustainable control option for this weed. The United States Department of Agriculture in 1961 initiated biological control of water hyacinth. Since this time there have been a number of surveys of water hyacinth in South America (Center 1994) with the purpose of identifying natural enemies (phytophagous insects, mites and pathogens) which might be suitable for release on the weed in its introduced range as biological control agents. The first biological control agent was released on the weed in 1971 and since then a further seven natural enemies have been released around the world (Julien & Griffiths 1998).

The two weevil species, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) are the most widely used agents having been released in 29 and 26 countries respectively (Juilen & Griffiths 1998). The biologies of both species are well documented (Center 1994), rearing and releasing techniques have been refined and a universal post-release evaluation method has been developed. These two weevils have contributed to the control of the weed worldwide: examples of successful complete control using only these species are Papua New Guinea (Julien & Orapa 1999) and on Lake Kyoga in Uganda (Ogwang & Molo 1997). The recent introduction of the two weevil species to Lake Victoria is also starting to reap rewards as they have established at a number of sites, and in some areas of the lake, a dramatic decline in the weed population has been reported (Murphy 2000). This success has prompted authorities to reject the use of herbicides on the lake in favour of biological control. This success contradicts the report by Pearce (1998) which suggested that biological control would be ineffective on Lake Victoria and that the large scale use of 2,4-D would be the only solution to the water hyacinth problem on the lake.

The moth, Niphograpta albiguttalis Warren (Sameodes albiguttalis) (Lepidoptera: Pyralidae) has also been introduced to a number of countries and, in conjunction with the two weevil species has contributed to the control of water hyacinth in Australia (Julien & Stanley 1999) and the USA (Center 1994). Another moth, Xubida infusellus (Walker) (Lepidoptera: Pyralidae), the leaf feeding mirid, Eccritotarsus catarinensis (Carvalho) and the water hyacinth mite, Orthogalumna terebrantis Wallwork

(Acarina: Galumnidae) have also been released in a few countries. A number of pathogens have been recorded from water hyacinth, but *Cercospora rodmanii* Conway (Fungus: Hyphomycetes) (= *Cercospora piaropi* Tharp. (Morris¹ pers. comm.)) is the only one intentionally introduced as a biological control agent.

The biological control programme against water hyacinth in South Africa was initiated in 1973 and the weevil, N. eichhorniae was released in 1974 (Cilliers 1991; Julien & Griffiths 1998). The programme was terminated in 1977, but restarted in 1985 when the weevil was re-released (Cilliers 1991). However, N. eichhorniae was deemed unlikely to achieve the desired level of control throughout the weed's geographical range in South Africa and additional natural enemies were collected, tested and released on the weed. The biological control of water hyacinth programme in South Africa currently relies on the two weevil species, N. eichhorniae and N. bruchi, the pyralid moth, N. albiguttalis, the water hyacinth bug, E. catarinensis, the galumnid mite, O. terebrantis and the fungal pathogen, C. piaropi. These species have been released and have established at a number of sites throughout South Africa. Biological control of water hyacinth has been very effective in some areas of the country. Examples of this are at New Years Dam, Alicedale (Eastern Cape Province) where the release of N. eichhorniae in 1991 resulted in an 80% reduction of the water hyacinth mat by 1994, and in the insect "reserve" sites along the Vaal River where the water hyacinth mats, infected with N. eichhorniae and N. albiguttalis have been shown to collapse within four years if left undisturbed (Cilliers², unpublished data).

In spite of these successes, the results of the biological control program have been variable. Hill and Cilliers (1999) have suggested several factors that might have constrained the impact of the biological control agents. Firstly, it could be the inability of some of the species to become abundant and control the weed in the range of climatic conditions under which water hyacinth grows. Successful control of water hyacinth elsewhere in the world has been limited to areas with tropical and/or subtropical climates. In South Africa, many of the water hyacinth infestations occur in high elevation areas characterised by cold winters and frequent frosts. This means that the active growing period of the plant and therefore the biological control agents

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is limited to about six months of the year. In addition, the weevil, N. eichhorniae has been seemingly ineffective in regions with Mediterranean climates.

Secondly, many of the aquatic ecosystems that are invaded by the weed in South Africa are enriched with nitrates and phosphates (Bosman³, pers. comm.) allowing the weed to proliferate at a high rate, which the insects are unable to suppress. An example of this is Hammarsdale Dam (KwaZulu-Natal Province) where the water hyacinth mirid and N. eichhorniae have attained very high population levels but appear to have had little impact on the plants which were fertilized by enriched effluent from a nearby waste water treatment plant. An essential feature of integrated management of the weed in areas such as these, is the control of water quality.

Finally, regular removal of water hyacinth infestations and their natural enemy populations through flooding and herbicide and mechanical control programs has also had an effect on the biological control of water hyacinth. Re-infestation of cleared or treated sites from seeds and scattered plants left untreated rapidly occurs and populations of the plant proliferate in the absence of natural enemies (Center et al. 1999).

The variable success of the biological control program on water hyacinth has prompted two courses of action. Firstly to expand the present suite of natural enemies to include species that might be more cold tolerant and be able to affect a greater level of control over the short growing period of the plant. Secondly, to investigate an integrated control approach for the weed, which would incorporate aspects of biological, herbicide, mechanical, hydrological and nutrient control options on a site specific basis. The management plan formulated by Cilliers et al. (1996) for the Vaal River provides an example of how local water hyacinth problems should be addressed.

³ Bosman, H.H. DWAF, Private Bag X313, Pretoria, 0001, South Africa

1.3.4 Integrated Control

(a) Background

A definition of integrated control could be: the use of two or more methods to achieve the optimum control of water hyacinth in a given area. The abundance of water hyacinth on dams and rivers in South Africa is a threat to infrastructure and supply of potable water for a burgeoning population. Together with the perceived slowness and variable results of biological control, this has resulted in an integrated approach to controlling water hyacinth in this country.

The main focus of integrated control in South Africa currently, is combining biological and herbicide control. The upgrading of waste water treatment plants and investigation into industrial effluent and therefore nutrient control is an ongoing process, while no large scale mechanical control is practiced and at this stage. Little attention is paid to hydrological control.

Integrated control has been very successful on the Vaal River in South Africa. This takes the form of cables spanned across the river which assist in the accumulation of isolated plants moving down the system into manageable infestations which are cost effective to treat with a herbicide by air or boat (Cilliers et al. 1996). Floating booms have also been successfully used to prevent water hyacinth entering hydroelectric water intake pumps from where they can be manually removed (e.g. Kafue Gorge Dam, Zambia).

One of the approaches to integrated control of water hyacinth is to inoculate an infestation with all of the natural enemy species, and then, treat 75% of the infestation with herbicide, leaving a 25% "reserve" of the weed for the natural enemies to disperse onto. This approach has the benefit of both long- and short-term control. However, it relies on certain assumptions:

The insects can and do disperse and not sink with the plants. Grodowitz et al.
 (1991) showed that the Neochetina adults frequently had much reduced wing musculature and were therefore not able to disperse by flight.

- ii) The natural enemy population in the "reserve" is large enough to curb the spread of the weed and the "reserve" does not itself become the source of reinfestation of the treated area.
- iii) The herbicides themselves are not toxic to the natural enemies.

It is the last assumption that is tested in this study.

(b) Literature review4

There have been several studies investigating the impact of herbicides used in the control of water hyacinth on the biological control agents in an attempt to integrate biological and herbicide control. Some of these are summarized below.

Roorda et al. (1978) investigated the direct toxicity of several commercially available herbicide formulations on the water hyacinth mite, O. terebrantis. The herbicides used by them in the study were, 2,4-D (2,4-dichlophenoxyacetic acid), glyphosate (isopropyl amine salt), diquat and paraquat. The mites were submerged in different concentrations of the herbicides for two minutes and then monitored for mortality. Diquat was found to be toxic to the mites resulting in up to 95% mortality after 48 hours. The other three herbicides also caused some mortality. However, this experiment was conducted without controls so it is difficult to put the mortality caused by the herbicides into context.

Although biological control of water hyacinth is effective, the effect is manifested in a gradual decline of the weed, which is not rapid enough for many control purposes. Therefore, Center et al. (1982) investigated the affects of the weevil, N. eichhorniae and a water hyacinth growth retardant, EL-509. They found that the retardant had no impact on water hyacinth on its own and that the combination of the retardant and the weevils had a greater impact than just using the weevils. However, there was a lack of clear evidence for retardant effects and weevil/retardant synergism. This paper concluded that chemical treatments causing catastrophic decline of the plant population would eliminate the weevils and reduce control effects. They recommended that the proper timing of growth retardant application and careful monitoring of the weevil and water hyacinth populations are important factors in the integration of biological and herbicide control.

One of the concerns with the integration of biological and herbicide control, is that although the active ingredient in the herbicide formulation might not be toxic to the insects, the additive or surfactants applied with the herbicide are toxic or increase the toxicity of the herbicide. Haag (1986 a) showed that although several herbicides were not toxic to the water hyacinth weevils, the inverting oil, I'VOD which was often added to the herbicides was toxic.

⁴ Consult original articles for more information on the specific active ingredients and formulations used

Haag (1986a) also tried to quantify the sub-lethal and behavioural effects of herbicides on N. eichhorniae and N. bruchi. In a series of experiments, mats and plots of water hyacinth infested with the weevils were sprayed with 2,4-D (2,4-dichlorophenoxyacetic acid) and the movement of the weevils from treated to untreated plants was monitored (Haag 1986a, b; Haag et al. 1988). These studies showed that there was some movement of adult weevils to untreated plants as the water hyacinth plants started to decay, but that mortality of eggs, larvae, pupae and adults with the sinking plants occurred. Furthermore, wing muscle development increased after spray application which is typical of adult Neochetina weevils under conditions of stress. Haag (1986a) also concluded that the timing and pattern of application is more important than the type of herbicide used.

Wright and Skilling (1987) also looked at the affect of 2,4-D on N. eichhorniae. They dipped the weevils into several herbicide formulations at concentrations recommended on the label and found that although 2,4-D and its surfactant (not named) were not toxic to the insects in isolation, the formulation of active ingredient and surfactant was toxic. Therefore, when Wright and Bourne (1990) investigated the effect of 2,4-D Amine on plant quality for biological control agents, they used only the active ingredient. 2,4-D Amine induced changes in plant quality, including decreased leaf and petiole hardness and an increase in nitrogen content. These factors improved plant quality for the larval stages of the moth, N. albiguttalis and the two weevil species and they recommended that spraying water hyacinth with sub-lethal dosages of 2,4-D Amine without its surfactant would enhance the control of water hyacinth.

In the only study found on pathogens, Liyanage and Gunasekera (1989) showed that low rates of 2,4-D (2,4-dichlorophenoxyacetic acid) application improved the success of the local pathogen *Myrothecium roridum* Tode ex Fr. in Sri Lanka.

Grodowitz and Pellessier (1990) suggested that herbicide control was the most important factor affecting the success of biological control of water hyacinth in the southern States of the USA. They applied several herbicides (2,4-D (dichlorophenoxyacetic acid), diquat and glyphosate) and surfactants (X-77, Nalcotrol II, Sta Put, Cide Kick and a combination of Nalcotrol II and X-77) to Neochetina

weevils and monitored mortality and changes in feeding behaviour. They found that X-77 and diquat caused significant mortality to the weevils and that while feeding rates of the weevils treated with the herbicides remained fairly constant, there was a movement of weevils from treated plants to untreated plants.

In a parallel study to this one, Jianqing et al. (1999) investigated the impact of recommended concentrations and formulations of paraquat, Londex 10WP (active ingredient not specified), Roundup (41% IPA salt of glyphosate) and Coaganlin (10% IPA salt of glyphosate, produced in China) on all growth stages of N. eichhorniae. Paraquat was toxic to the adult weevils, producing 100% mortality after 70 days while mortality of the control weevils was 3 %. They also found high adult mortality of the weevils after they had been fed with leaves sprayed with high concentrations (13.67 mg/ml) of Roundup.

The conclusions of many of these studies were that although the herbicides themselves (with the exception of diquat and paraquat) were not directly toxic to the insects, the loss of large numbers of immatures with a sinking mat changes the population structure of the weevils. Although the adults move onto untreated plants, considerable time is required for the recovery of sufficient numbers of immatures to significantly impact the plants (Haag et al. 1988; Grodowitz & Pellessier 1990; Van & Center 1994). As a result, in the absence of sufficient numbers of biological control agents, the water hyacinth quickly reinfests the site. Grodowitz and Pellessier (1990) were able to show with a simple model the decline in the weevil population and rapid increase in plant production following herbicide application and concluded that in the USA, at that time (1990) herbicide control technologies were not compatible with biological control programs.

Other important issues were that the physiological and reproductive status of the weevils should be assessed before spraying (Haag 1986a; Grodowitz & Pellessier 1990). It was also suggested that large unsprayed areas with small boundry areas (small area for rapid expansion of the mat through daughter plants) be left to harbour large populations of the biological control agents (Center et al. 1982; Haag 1986a, b). Further more, drift retardants and surfactants should be closely monitored because their requirements for registration are not as strict as for the active ingredients (Henry et al. 1994).

1.4 AIMS OF THIS STUDY

In this study, the impact of several herbicide formulations on two species released for the biological control of water hyacinth was tested. The first species tested was the weevil, *N. eichhorniae*, which was selected because it is presently the most wide spread of the agents established on water hyacinth in South Africa and because the results on this species could be extrapolated to its congeneric *N. bruchi*. The other species tested was the water hyacinth mirid bug *Eccritotarsus catarinensis*. The bug was selected because it is the most recently released natural enemy on water hyacinth in southern Africa, and because it is the only agent thus far released on water hyacinth where both the immature and adult stages feed externally on the plant.

A series of four experiments were conducted to investigate the impact of several herbicide formulations on these two species:

- The acute toxicity of several concentrations of several herbicide formulations to the weevil and the mirid bug were tested (Chapter 2).
- The feeding behaviour of the weevil after being exposed to several concentrations of several herbicide formulations was tested (Chapter 3).
- The feeding behaviour of the weevil on water hyacinth leaves treated with several concentrations of several herbicide formulations was tested (Chapter 4)
- iv) The behaviour of weevil populations on water hyacinth mats sprayed with two herbicide formulations was tested (Chapter 5).

This study forms part of an ongoing series of studies on water hyacinth with the ultimate aim of improving the integrated management of water hyacinth in South Africa. The implications of this study for this ultimate goal are discussed in Chapter 6.

2. Acute toxicity of selected herbicides to

Eccritotarsus catarinensis and Neochetina eichhorniae.

2.1 INTRODUCTION

The purpose of this experiment was to determine whether herbicides used on water hyacinth are toxic to the natural enemies released for biological control. Secondly, we set out to determine the relative toxicity of herbicide formulations and surfactants registered in South Africa for use on water hyacinth, to the natural enemies. Toxicity tests were performed on two insect species to indicate relative toxicity and to make recommendations for the future use of herbicides on water hyacinth.

2.1.1 Standardized Tests

The purpose of toxicity tests is to asses the potential dangers of chemical compounds to the environment prior to its wholesale use. A variety of standard toxicity tests exist, such as those available from the Federal Register, published by the United States Environmental Protection Agency (Landis & Yu 1995). These tests aim to highlight potentially harmful substances, by testing a few representative species of the different components of the biological system. Some experiments are designed to suit certain test species with the two water flea species, Daphnia magna and Daphnia pulex, probably the most popularly used arthropod species in toxicity tests.

Standard tests have the advantage of repeatability and comparability, and often simplicity in design. Acute toxicity tests typically continue for 24, 48 or 96 hour periods, dependant on the generation time of the test species. Other factors to consider are test organism age, size, sex and health; environmental factors such as light intensity, photo-period temperature and humidity; food quality; and exposure scenarios.

2.1.2 Exposure Scenario

The method of exposing insects to a toxin will determine uptake efficiency and ultimate effect on the insect. In the vapour phase, exposure can occur through the cuticle, by inhalation into tracheal system and through oral ingestion into the digestive system (Gullan & Cranston 1994). In many toxicology tests the toxin is injected into certain areas in the body (Landis & Yu 1995). Another method is feeding on a diet incorporating different concentrations of the toxin, but this occasionally leads to insects avoiding feeding because the substance is unpalatable (Landis & Yu 1995). Aquatic insects can be exposed by submergence into water treated with toxin for the entire or part of the test period (Silva-Finha & Regis 1997).

During this experiment, tests were designed to become a standard method by which a selection of herbicides (Table 2.1) could be arranged according to their toxicity. The droplet application method was designed to portray a possible field scenario and not to overestimate the potential toxicity of the herbicides as a complete emergence in the solution might have. Weevils and bugs were exposed by applying a 20 µl droplet onto the dorsal surface of the insect. Droplets sized 300 to 400 µl are specified by some herbicide labels (Anonymous 1994).

2.1.3 Organism Health and Population Composition

The condition of the test organism is vital. Aspects of concern are the health of test organisms, genetic composition (culture history), sex, age and availability.

The use of clonal organisms has been widely debated. Cloning means that certain strains are inbred, in order to eliminate variability that result from genetic differences within the test population. The advantage of using cloned organisms in studies, is that environmental and genetic factors can be separated and manipulated to determine its importance for resistance to the toxin (Forbes & Depledge 1993). Another advantage of using clones is the repeatability of experiments (Baird 1993), but this must not interfere with the relevance of the test (Forbes & Depledge 1993). The toxicity of substances to

the organism can be highly genotype dependent (Forbes & Depledge 1993). Baird (1993) demonstrated that no single genotype could ever represent the response of the whole species, and that no "generally resistant" or "generally sensitive" genotype exists.

To try and best mimic the situation in the field, insects from natural populations of the test organisms were collected randomly, for this experiment. Unfortunately this precluded us from knowing the age of the insect, but the test period of approximately 5 days was short in comparison to the adult longevity of 120 days for *N. eichhorniae* (DeLoach & Cordo 1976) and 50 days for *E. catarinensis* (Hill et al. 1999).

Egg, larval, nymphal and pupal mortality was not tested, but it is expected that most of the immature insects who live inside the water hyacinth plant material and are sedentary, will be killed when plants die as a result of herbicide application (Haag 1986b).

2.1.4 Tolerance and Resistance

Tolerance is the ability of an individual insect to survive exposure to a poison or toxin, while resistance is achieved through selection of insect genotypes that are able to survive exposure to a toxin. Mechanisms of resistance include increased behavioural avoidance (repellent); physiological changes such as reduced cuticle permeability, accelerated excretion and specialized tissue to cope with compounds; biochemical detoxification (metabolic resistance); and decreased sensitivity (target-site resistance) (Gullan & Cranston 1994). Phytophagous and especially polyphagous herbivore insects tend to develop resistance more rapidly than predatory insects. This may be a result of natural detoxifying abilities for secondary chemical compounds encountered while feeding on plants (Gullan & Cranston 1994).

2.1.5 Time

Some substances are absorbed quicker and have an immediate effect while others may lead to toxic responses after a time lapse (Landis & Yu 1995). Toxicity tests are classified according to the experiment length relative to life span of organism and complexity of the biological community. Acute toxicity tests usually last for between 24 to 48 hours for insects, rats, fish and birds. Chronic and sub-lethal tests last for a significant portion of an organism's life expectancy or for more than one generation, and can include monitoring of reproductive output of the test organism. Growth may be assessed by determining biomass production.

Field toxicity studies are costly and logistically difficult. Because of the complexity of biological systems and temporal and spatial heterogeneities, they are difficult to control and measure, and make replication and comparisons difficult.

The acute toxicity experiments done during this study continued for 120 hours, but the more accurate results are expected for the first 72 hours.

2.1.6 LC50 Tests

LC50 tests are often used to interpret toxicity of chemicals to living organisms. Although this criterion possesses recognizable limitations, it provides the first and most statistically reliable measure of the toxic effect a chemical may impose on an organism (Forbes & Forbes 1993). The LC50 test is also called population tolerance distribution (Forbes & Forbes 1993), and is a widely used and trusted method to determine what concentration of a substance causes 50% of the organisms in a test population to die. With the help of computer programs such as (LSTATS) P/PROBAN only a few concentrations need to be tested to determine lethal doses (Van Ark 1983).

The response of LC50 tests does not have to be mortality (Jagers op Akkerhuis et al. 1999), but quantifying any response other than death is difficult. The accuracy of a LC50 test depends on the sample size and replicates, number of endpoints observed, number of concentrations, measurement ability at endpoints, intrinsic variability within experimental population and statistic methodologies (Landis & Yu 1995).

2.2 MATERIALS AND METHODS

2.2.1 Eccritotarsus catarinensis

In the acute toxicity test, the water hyacinth bug was directly exposed to different herbicide concentrations (Table 2.2) and mortality was noted. A group of eight unsexed adult insects were kept in a small petri-dish (35 ml), lined with moist filter paper. Food discs sized 2x3cm were provided every 24 hours, and moisture was checked regularly to avoid desiccation. Temperatures varied between 22 and 28 °C and they were exposed to a light regime of 16 hours light, 8 hours dark.

The bugs were cooled in the fridge at about 5°C for less than a minute before application of herbicide and/or surfactant, as they tend to be very active and fly or run off. Fresh concentrations of herbicides were made up less than an hour before application. A 20 µl droplet of herbicide was applied dorsally on each insect. Within minutes after application the insects were checked to ensure no death had occurred as a result of drowning in herbicide. Insects trapped in herbicide were removed carefully with a fine, clean brush. Insect mortality was monitored every 24 hours up to 120 hours. There were three replicates of 8 insects.

2.2.2 Neochetina eichhorniae

Insects were directly exposed to different herbicide concentrations (Table 2.2) by applying a 20 µl droplet dorsally onto the elytra of the insect. Groups of 6 individuals (three male and three female) were kept in plastic containers (500 ml), lined with moist filter paper and aerated through small holes in the top. A fresh water hyacinth leaf was provided daily. Temperatures varied between 22 and 28 °C and they were exposed to a light regime of 16 hours light, 8 hours dark. Mortality was noted every 24 hours up to 120 hours and there were three replicates of six insects.

Table 2.1 Information on herbicides selected to include in tests: composition and recommended dosages.

Brand name	Manufacturer		Content		Recommended
				g a.e/l	Dosage (%)
2,4-D amine	Provided by Sanachem	2,4-D 480 Amine		480	2-6(12)
Mamba 360 SL*	Sanachem	Glyphosate SL (IPA salt)	salt)	360	2 - 6(12)
Midstream	Zeneca	Diquat (Dibromide salt)	alt)	373.5	3.75 - 5
Mon 52276	Monsanto	Glyphosate (IPA salt)		360	2 - 6(12)
Muster	Zeneca	Glyphosate (Trimethyl sulfonium salt)	yl sulfonium salt)	330	2-3
Rodeo	Monsanto	Glyphosate (IPA salt)		480	4.5 - 9
Roundup	Monsanto	Glyphosate (IPA salt)		360	2 - 6(12)
Roundup- Ultra	Monsanto	Glyphosate		360	2 - 6(12)
Touchdown	Zeneca	Glyphosate (Trimethyl sulfonium salt)	yl sulfonium salt)	480	2
Tumbleweed.	Enviro Weed Control	Glyphosate (IPA salt)		240	2 - 6(15)
Surfactants					
		Description			
Add-2	Zeneca	Spreader adjuvant	Polysaccharide	009	0.2 - 0.3
		(with Touchdown)	(hexitan ester)		
Agral	Zeneca	Wetting and	Alkylated phenol-	940	0.75
		sticking adjuvant	ethylene oxide		
		(with Midstream)	concentrate		

* Registered under Act 36 (1947)

Table 2. 2 Concentrations of herbicide formulations and surfactants used during acute toxicity tests on two insect species.

Herbicide	Active ingre	dient		C	oncen	trat	ion t	teste	d in t	oxicit	y tests	(%)	hhorniae							
		gΛ	E	Eccritotarsus catarinensis					1	Veoch	etina e	ichho	rniae							
2,4 D	2,4-D amine	480	1	3	9	18			1	3	4	9	12	2						
Add-2	Adjuvant		0.2	0.3	0.6	1	2		0.2	0.3	1	2.4								
Agral	Adjuvant		0.5	0.75	1				0.25	0.5	0.75									
Mamba	Glyphosate	360	3	4	12	24			3	4	12	24								
Midstream	Diquat	374	1	2	3.75	4	5		2	4	5	12	24							
Mon 52276	Glyphosate	360	3	4	12	24			3	4	12	24								
Muster	Glyphosate-	330	2	3	12	24			2	3	4	12	24							
,	trimesium																			
Rodeo	Glyphosate	480	1.5	3	4	9	18		1.5	3	4	9	12	13						
Roundup	Glyphosate	360	1	2	3	4	12	24	1	2	3	4	12	2						
Roundup-Ultra	Glyphosate	360	3	4	12	24			3	4	12	24								
Touchdown	Glyphosate-	480	2	4	8	20			2	4	8	12	24							
	trimesium																			
Tumbleweed	Glyphosate	240	1	4	12	24	50		1	4	12	24								
Combinations			Eccritotarsus catarinensis							Neochetina eichhorniae										
2 % TD* & 0.2 % Add			X						X											
2 % TD & 0.3 %	6 Add				X															
4 % TD & 0.2 %	6 Add										X									
4 % TD & 0.4 %	6 Add				\mathbf{X}				l		X									
8% TD & 0.8 % Add									X											
0.9375 % MS &	0.1875% Ag										х									
1.875 % MS &	0.375 % Ag		x						x											
3.75 % MS & 0.	75 % Ag				X						X									

^{*} TD = Touchdown; Add = Add2; MS = Midstream; Ag = Agral

2.2.3 Statistical procedures

The Proban analysis program obtained to calculate LC50 values for this experiment, draws a regression of mortality over concentration of formulation in water (Figure 2.1). Concentration values are transformed to logarithms, a transformation called normalization considered standard practice in toxicity testing (Van Ark 1983). Normalizing the data is a way to compensate for the a-symmetrical population reaction curve, usually a result of the high tolerance exhibited by a few insects (Van Ark 1983). The log concentration value that cause 50 % mortality can then be anti-logged to give a predicted LC50 value, the concentration that is expected to cause 50 % mortality.

The LC50 value indicated on figure 2.1 gives the concentration expected to result in mortality of 50 % of the population, at 48 hours after exposure to the toxin. A linear correlation coefficient (r) gives the accuracy of the estimated regression line (■), or the fit of the line to the observed mortality (◆).

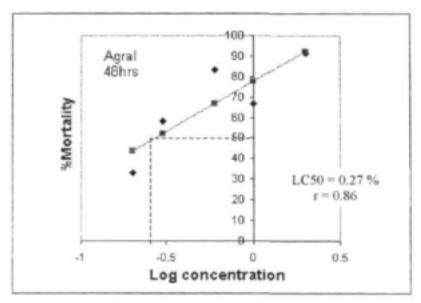


Figure 2.1 The regression line obtained for *Eccritotarsus catarinensis* after 48 hours exposure to the surfactant Agral. The estimated LC50 value (percentage of formulation in water) and linear correlation coefficient (r) are indicated.

- = predicted mortality
- = observed mortality

2.3 RESULTS

2.3.1 Eccritotarsus catarinensis

(a) Determining LC50 values

The percentage mortality of adult *E. catarinensis* treated with herbicides are illustrated in figure 2.2, and means and standard deviations appear in table 2.3.

Estimated LC50 values for *E. catarinensis* were determined after exposure to a range of herbicides and are listed in table 2.4. These concentrations are expected to cause 50 % mortality of the population after the indicated period of exposure. Both Proban analysis (P) and regression (R) values are listed, with linear correlation coefficient values (r) where available, in brackets. The Proban analysis values are considered more accurate because the program takes into account the natural mortality of control treatments. However, the program is also sensitive to the amount of concentrations tested and could not be used in all cases.

When comparing the more accurate Proban values with those of the regressions in table 2.4, they clearly show the same trends but are in most cases slightly higher. As the regression graphs could be drawn for all herbicides throughout the experiment, it will be used as a measure of comparison during further discussion, keeping in mind that it slightly overestimates the toxicity of all products tested.

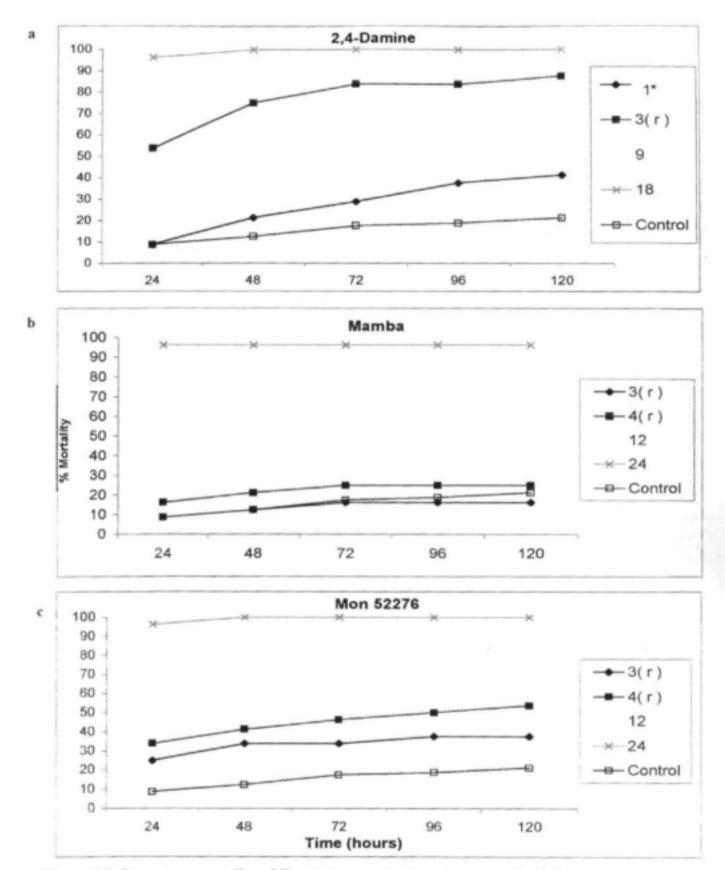


Figure 2.2 Percentage mortality of Eccritotarsus catarinensis as a result of direct treatment with selected herbicides.

- (r) a recommended concentration of specified herbicide
- * legend entries are percentage concentrations of specified herbicide

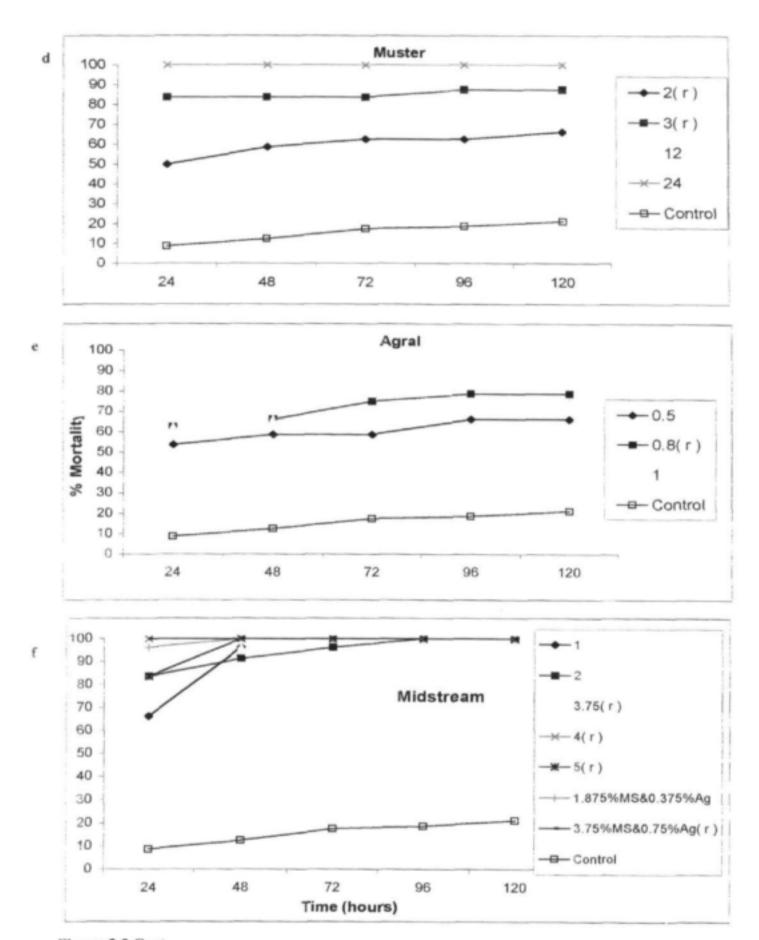


Figure 2.2 Cont.

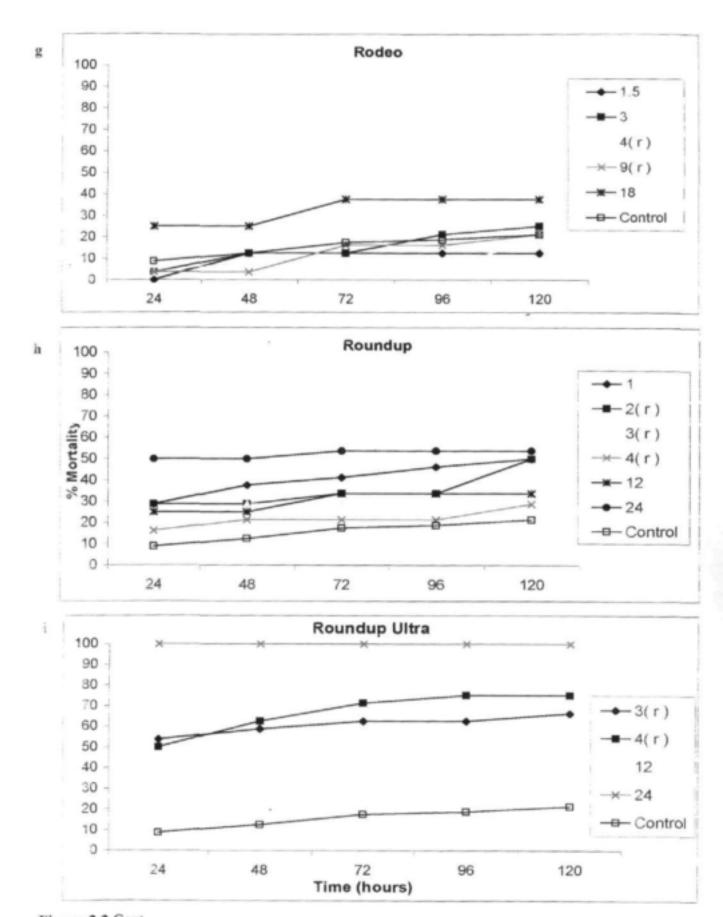
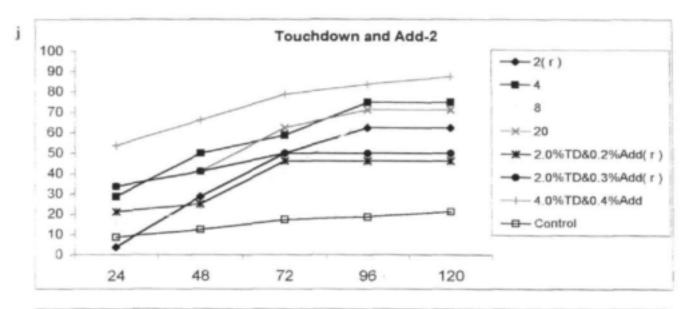
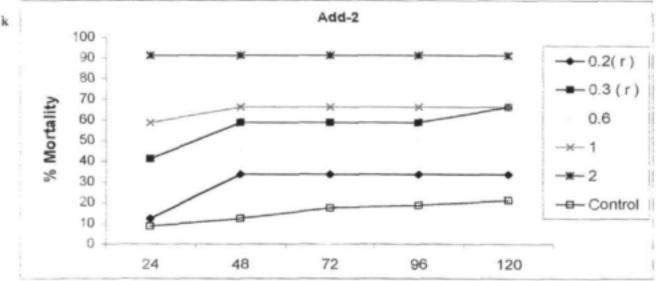


Figure 2.2 Cont.





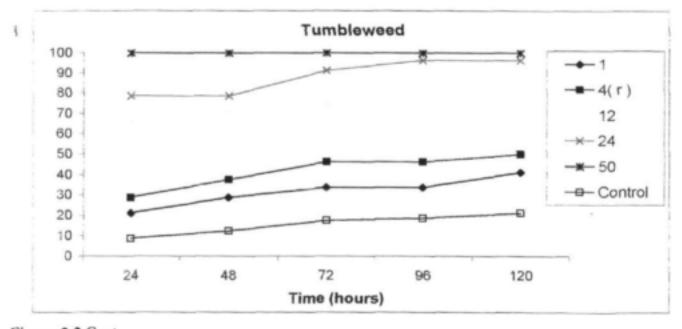


Figure 2.2 Cont.

Table 2.3 Mortality of *Eccritotarsus catarinensis* after direct exposure to selected herbicides (standard deviation in brackets).

Herbicide	Concentration	n	Mean number of adults dying							
			24 ²	48	72	96	120			
2,4-D amine	1.0%	3	0.7 (1.2)	1.7 (1.2)	2.3 (2.3)	3 (2.6)	3.3 (2.3			
	3.0%	3	4.3 (3.2)	6 (1.7)	6.7 (1.5)	6.7 (1.5)	7 (1)			
	9.0%	3	7.3 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)			
	18.0%	3	7.7 (0.6)	8(-)	8(-)	8(-)	8(-)			
Add-2	0.2%	3	1(-)	2.7 (2.1)	2.7 (2.1)	2.7 (2.1)	2.7 (2.1)			
	0.3%	3	3.3 (2.3)	4.7 (1.5)	4.7 (1.5)	4.7 (1.5)	5.3 (2.1)			
	0.6%	3	3 (2)	3.7 (2.1)	3.7 (2.1)	4 (1.7)	4 (1.7)			
	1%	3	4.7 (0.6)	5.3 (1.5)	5.3 (1.5)	5.3 (1.5)	5.3 (1.5)			
	2%	3	7.3 (0.6)	7.3 (0.6)	7.3 (0.6)	7.3 (0.6)	7.3 (0.6)			
Agral (Ag)	0.5%	3	4.3 (1.2)	4.7 (1.5)	4.7(1.5)	5.3 (2.5)	5.3 (2.5)			
	0.75%	3	5 (1.7)	5.3 (1.5)	6 (1.7)	6.3 (2.1)	6.3 (2.1)			
	1.0%	3	5 (1)	5.3 (0.6)	5.3 (0.6)	5.7 (1.2)	6 (1)			
Mamba	3.0%	3	0.7 (0.6)	1 (1)	1.3 (1.5)	1.3 (1.5)	1.3 (1.5)			
	4.0%	3	1.3 (1.5)	1.7 (2.1)	2 (2)	2 (2)	2 (2)			
	12.0%	3	5.7 (2.5)	5.7 (2.5)	6.3 (2.1)	6.3 (2.1)	6.3 (2.1)			
	24.0%	3	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)			
Midstream (MS)	1.0%	3	5.3 (1.5)	7.7 (0.6)	8(-)	8 (-)	8 (-)			
	2.0%	3	6.7 (1.5)	7.3 (0.6)	7.7 (0.6)	8(-)	8 (-)			
	3.75%	3	6.7 (1.5)	7.7 (0.6)	8(-)	8(-)	8(-)			
	4.0%	3	8(-)	8(-)	8(-)	8(-)	8(-)			
	5.0%	3	6.7 (1.2)	8(-)	8(-)	8(-)	8(-)			
Mon 52276	3.0%	3	2 (1)	2.7 (0.6)	2.7 (0.6)	3 (1)	3 (1)			
	4.0%	3	2.7 (0.6)	3.3 (0.6)	3.7 (0.6)	4 (-)	4.3 (0.6)			
	12.0%	3	5 (2.6)	6.3 (1.5)	7 (1.7)	7 (1.7)	7 (1.7)			
	24.0%	3	7.7 (0.6)	8 (-)	8 (-)	8 (-)	8(-)			
Muster	2.0%	3	4 (1)	4.7 (1.5)	5 (1.7)	5 (1.7)	5.3 (1.2)			
	3.0%	3	6.7 (0.6)	6.7 (0.6)	6.7 (0.6)	7 (1)	7 (1)			
	12.0%	3	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	8 (-)			
	24.0%	3	8(-)	8 (-)	8 (-)	8 (-)	8(-)			
Rodeo	1.5%	3	0(-)	1 (1)	1 (1)	1 (1)	1 (1)			
	3.0%	3	0.3 (0.6)	1 (1)	1 (1)	1.7 (0.6)	2 (1)			
	4.0%	3	0.3 (0.6)	0.7 (1.2)	0.7 (1.2)	0.7 (1.2)	1.3 (1.5			
	9.0%	3	0.3 (0.6)	0.3 (0.6)	1.3 (1.5)	1.3 (1.5)	1.7 (2.1			
	18.0%	3	2 (1.7)	2 (1.7)	3 (2)	3 (2)	3 (2)			
Roundup	1.0%	3	2.3 (2.1)	3 (2.6)	3.3 (4.7)	3.7 (3.5)	4 (3.6)			
	2.0%	3	2.3 (4)	2.3 (4)	2.7 (3.7)	2.7 (3.7)	4 (3.6)			
	3.0%	3	1 (1.7)	2.3 (3.2)	2.3 (3.2)	2.3 (3.2)	3 (2.6)			
	4.0%	3	1.3 (2.3)	1.7 (2.9)	1.7 (2.9)	1.7 (2.9)	2.3 (2.5			
	12.0%	3	2 (1.7)	2 (1.7)	2.7 (1.5)	2.7 (1.5)	2.7 (1.5			
	24.0%	3	4 (2.6)	4 (2.6)	4.3 (3.1)	4.3 (3.1)	4.3 (3.1			
Roundup-Ultra	3.0%	3	4.3 (2.5)	4.7 (2.5)	5 (2.6)	5 (2.6)	5.3 (2.1			
	4.0%	3	4 (2)	5 (2)	5.7 (1.5)	6 (1)	6 (1)			
	12.0%	3	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	7.7 (0.6)	8 (-)			
	24.0%	3	8(-)	8(-)	8(-)	8(-)	8(-)			

¹ Mean number of adults dying (eight per replicate)
² Hours after application

Table 2.3 Cont.

Herbicide	Concentration	n	Mean number of adults dying						
			24	48	72	96	120		
Touchdown(TD)	2.0%	3	0.3 (0.6)	2.3 (1.2)	4 (1.7)	5 (3)	5 (3)		
	4.0%	3	2.3 (4)	4 (3.6)	4.7 (3.1)	6 (2)	6 (2)		
	8.0%	3	1.3 (1.2)	1.7 (1.5)	3.3 (2.3)	5.3 (3.1)	5.7 (3.2)		
	20.0%	3	2.7 (0.6)	3.3 (0.6)	5 (1.7)	5.7 (2.1)	5.7 (2.1)		
Tumbleweed	1.0%	3	1.7 (2.1)	2.3 (2.5)	2.7 (2.1)	2.7 (2.1)	3.3 (1.5)		
	4.0%	3	2.3 (0.6)	3 (1)	3.7 (1.5)	3.7 (1.5)	4(2)		
	12.0%	3	5.3 (2.1)	5.7 (1.5)	6 (1.7)	6.3 (2.1)	6.3 (2.1)		
	24.0%		6.3 (0.6)	6.3 (0.6)	7.3 (0.6)	7.7 (0.6)	7.7 (0.6)		
	50.0%	3	8(-)	8 (-)	8(-)	8(-)	8(-)		
Combinations	2.0%TD&0.2%Add	3	1.7 (1.5)	2 (1.7)	3.7 (2.3)	3.7 (2.3)	3.7 (2.3)		
	2.0%TD&0.3%Add	3	2.7 (2.5)	3,3 (3.1)	4 (2.6)	4 (2.6)	4 (2.6)		
	4.0%TD&0.4%Add	3	4.3 (3.2)	5.3 (3.1)	6.3 (1.5)	6.7 (1.2)	1 (1)		
	1.875%MS&0.375%Ag	3	7.7 (0.6)	8(-)	8 (-)	8(-)	8(-)		
	3.75%MS&0.75%Ag	3	8(-)	8(-)	8(-)	8(-)	8(-)		
Control	H ₂ O	41	0.7 (0.8)	1 (1)	1.4 (1.2)	1.5 (1.2)	1.7 (1.2)		

Table 2.4 LC50 values determined for Eccritotarsus catarinensis using a normal regression program and the Proban analysis program, with linear correlation coefficient values where available.

	24*		48		72		96		120	
	R	P	R	P	R	P	R	P	R	P
2,4-D amine	3.15(1)		2.02(1)	2.24	1.5(0.9)	1.96	1.17(0.9)	1.75	0.93(0.9)	1.58
Add-2	0.63(0.9)	0.73	0.38(0.9)	0.51	0.38(0.9)	0.60	0.36(0.9)	0.59	0.33(0.8)	
Agral	0.33(0.9)	0.48	0.27(0.9)	0.32	0.22(0.6)	0.43	0.04(0.4)	0.15	0.12(0.7)	0.29
Mamba	8.04(1)	9.06	7.66(1)	9.29	6.91(1)	8.68	6.91(1)	8.80	6.91(1)	9.02
Midstream	0.38(0.9)	0.63	9.8E-7 (0.59)	0.003	4.8E-29 (0.3)					
Mon	6.75(1)	7.55	5.05(1)	5.74	4.51(1)	5.55	4.15(1)	5.22	3.92(1)	5.17
Muster	0.95(0.9)	1.80	0.6(0.9)	1.58	0.16(0.9)	1.55	0.35(0.9)	1.44	0.26(0.9)	1.78
Rodeo	723.43 (0.8)		5.96E+5 (0.40)		148.79 (0.8)		191.41 (0.7)		146.99 (0.8)	
Roundup	61.04 (0.7)	51.06	2.3E+3 (0.32)	3124	195.57 (0.5)	337.5	521.8 (0.3)	707.02		
RoundupUltra	2.96(1)		2.03(1)	3.15	0.61(0.9)	2.82	1.16(1)	2.74	0.95(1)	2.82
Touchdown	112.6 (0.7)	120.8	1.2E+5 (0.1)	1.3E+6			0.00103	0.11	0.00284 (0.5)	0.20
Tumbleweed	6.15(1)	8.33	5.14(1)	6.63	3.14(1)	5.76	2.98(1)	5.57	2.21(1)	4.60

^{*} Hours after application of substance R = Regression values (Microsoft Windows Excel)

P = Proban analysis values () = r (linear correlation coefficient)

(b) The occurrence of non-linear relationships

All herbicides increased the mortality of the bug in comparison to the control, which was treated with distilled water. For most of the herbicides, higher concentrations increased mortality, but for Roundup, Rodeo and Touchdown this was not the case. These three formulations showed a non-linear relationship between herbicide concentration and mortality, meaning mortality did not show a linear increase with increased concentration. A very high LC50 or great variation of the value over time is a result of such a non-linear relationship.

Observations made during experiments suggest that non-linear relationships may be a result of droplet surface tension that increase with increased herbicide concentration in the solution. This caused some droplets to roll off the insect, not coming into contact with the insect's body. An even higher concentration became sticky possibly inhibiting movement or breathing and having a different "toxic" effect on the insect, than a much lower concentration would have. The reason for these non-linear relationships is not certain, but for the purpose of this investigation it will be sufficient to know what happens at recommended concentrations of these three herbicides.

(c) Relative toxicity

Bugs treated with herbicides and control insects showed increased mortality over time (Figure 2.2, Table 2.3). The mortality of control insects increased from 8.75 % mortality at 24 hours to 21.25 % at 120 hours. This may simply be natural mortality, but could be a result of increased stress of confinement as well.

LC50 values are also illustrated in figure 2.3, with recommended dosages indicated as concentrations. In figure 2.3 a, Agral and Midstream show a LC50 value lower than the recommended dosage range. This means that at the recommended concentrations, more than 50 % mortality can be expected. For Roundup Ultra in the same graph, some of the recommended dosages will cause 50 % mortality, other lower recommended dosages will

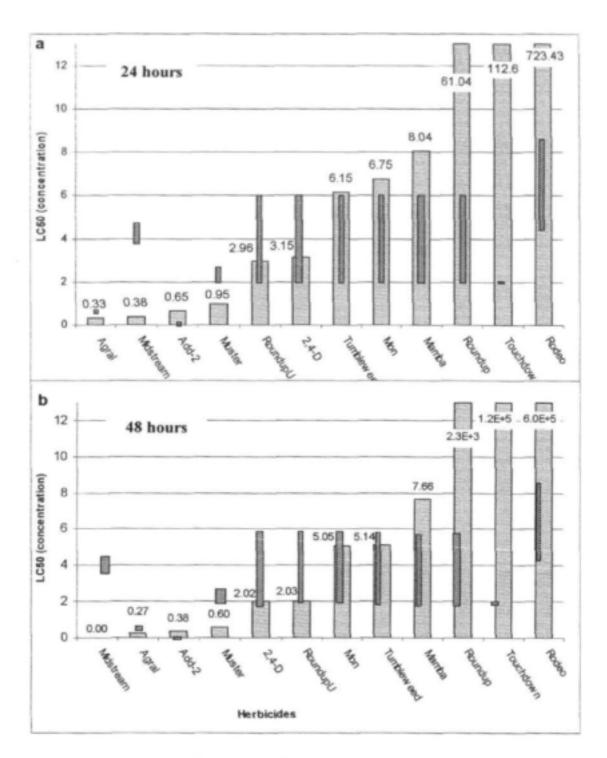


Figure 2.3 LC50 values calculated for *Eccritotarsus cata*rinensis after acute toxicity testing with selected herbicides after, 24 hours (a) and 48 hours (b).

- LC50 values
- Recommended dosage range

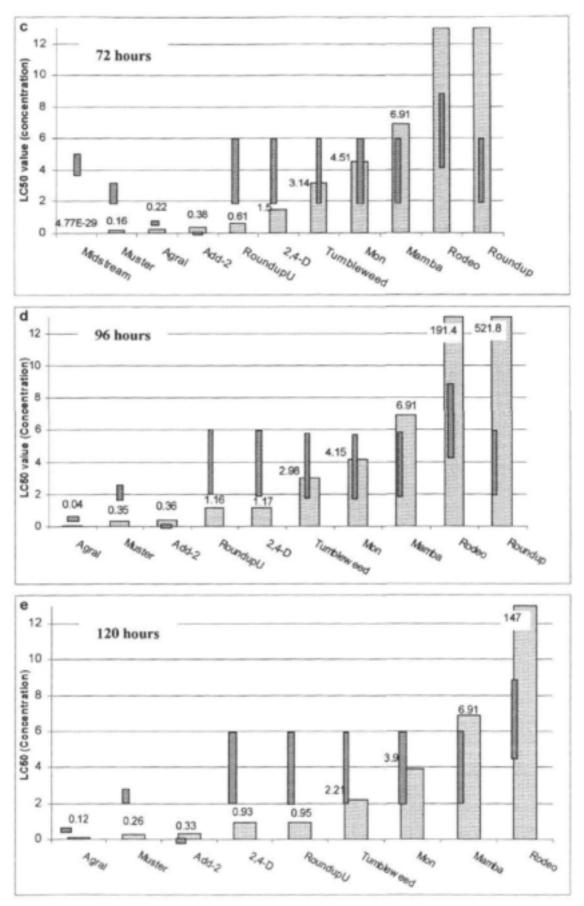


Figure 2.3 Cont. 72 hours (c), 96 hours (d) and 120 hours (e) after exposure.

cause less. For Mamba, all recommended concentrations will cause less than 50 % mortality because the recommended dosages lie well below the LC50 value.

Table 2.5 describes the overlap that exists between the LC50 value and the recommended dosage for each substance. This description or rating is based on the results obtained during this study and limited to the specific species. It is however expected to be a good indication of relative toxicity for other insect species as well. This relative scale indicates that 2,4-D amine has a toxic effect on *E. catarinensis*, when used at recommended dosage, while Roundup is considered safe at recommended dosages.

Table 2.5 Herbicides rated according to the effect they have on *Eccritotarsus* catarinensis during lethal toxicty tests.

HERBICIDE ¹	DOSAGE (%)	LC50 V	ALUES ³	RATING ⁴
	USED IN PRACTICE ²	24 hrs	120 hrs	
2,4-D amine	2 - 6 (to 12)	3.15	0.93	Toxic
Add-2	0.2 - 0.3	0.63	0.33	Safe
Agral	0.75	0.33	0.12	Toxic
Mamba	2 - 6 (to 12)	8.04	6.91	Safe
Midstream	3.75 - 5	0.38		Toxic
Mon 52276	2 - 6 (to 12)	6.75	3.92	Hazardous
Muster	2 -3	0.95	0.26	Toxic
Rodeo	4.5 - 9	23.43	146.99	Safe
Roundup	2 - 6 (to 12)	61.04		Safe
Roundup Ultra	2 - 6 (to 12)	2.96	0.95	Toxic
Tumbleweed	2-6 (to 12)	6.15	2.21	Hazardous
Touchdown	2	112.6	0.00284	Toxic

Formulation or surfactant

²Refer to product labels for specified dosages and application instructions

³ Regression values (Excel)

Safe = LC50 never falls within recommended dosage range;

Hazardous = LC50 falls within or below the recommended dosage range at some time during the 120 hours;

Toxic = LC50 falls within or below the recommended dosage range from 24 hours

The surfactant Agral, showed a LC50 value lower than 0.75%, its recommended dosage, throughout the 120 hours. Its effect on the species is therefore described as toxic (Table 2.5). The other surfactant Add-2 also exhibit a low LC50 value between 0.63 and 0.33%, but as this never overlaps with the recommended dosage range of 0.2% to 0.3%, it is labeled safe (Table 2.5).

Midstream was the most toxic herbicide throughout the 120 hours. Its LC50 value of 0.38 % at 24 hours fall far below the lowest recommended dosage of 3.75% (Figure 2.3 a). Midstream is labeled toxic in table 2.5. It would have been insightful to test another diquat based herbicide as this could indicate whether the toxic nature of the herbicide is mostly a result of active ingredient or additives.

Of the glyphosate based herbicides, Rodeo was by far the least toxic, which may be a result of the absence of additives in the formulation. Very high LC50 values for Roundup suggest it is very safe, but as pointed out earlier, this is mostly a result of a non-linear relationship between concentration and mortality. Roundup caused between 29 % (24hrs) and 50 % (120hrs) mortality at 1 and 2 % concentration. Roundup is however considered to be relatively safe. Mamba, Mon 52276 and Tumbleweed all exhibit an average toxic effect. Mamba being least toxic of the three and never reaching a LC50 value that falls within the recommended dosage range. Tumbleweed was the most toxic of the three and is described as hazardous (Table 2.5).

Glyphosate-trimesium based Muster is considered toxic, as the estimated LC50 values fall below the recommended dosages throughout the experiment (Figure 2.3 a-e). Another glyphosate-trimesium based herbicide Touchdown, showed an initial high LC50 value, as a result of the non-linear relationship described earlier. Bugs treated with Touchdown however started to die only after 48 hours only, and the herbicide is considered toxic (Table 2.5).

2,4-D amine is considered toxic with LC50 values between 3.15% and 0.93% and recommended at 2 to 6 % concentration.

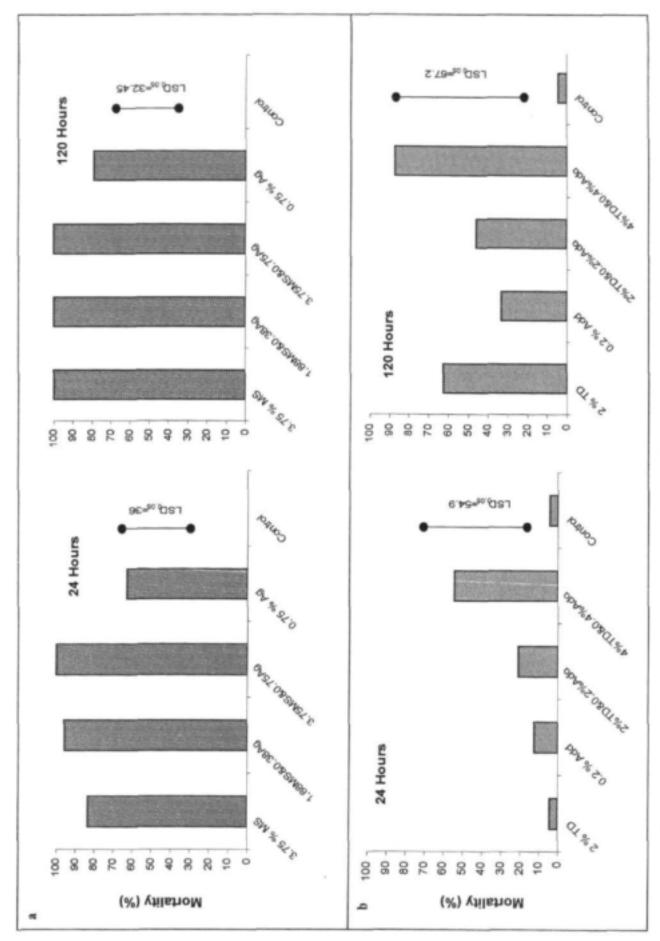


Figure 2.4 Percentage mortality of Eccritotarsus catarinenensis after direct treatment with two formulations, with and without surfactants added (a) Midstream and Agral, (b) Touchdown and Add-2

Combinations of Midstream with the surfactant Agral were more toxic than they were when applied separately (Figure 2.4 a). Touchdown combined with 0.2 % Add-2, resulted in less mortality than 2 % Touchdown alone (Figure 2.4 b). Only double the recommended dosage of 4 % Touchdown and 0.4 % Add-2 increased the mortality of the combination significantly.

At concentrations that are applicable to those used in the weed control practice, Midstream, Muster, Roundup Ultra, Touchdown and the surfactant Agral can be considered toxic to this species.

2.3.2 Neochetina eichhorniae

The percentage of mortality for adult N. eichhorniae are illustrated in figure 2.5 (a – l), and means and standard deviations appear in table 2.6. In several of the graphs the mortality of the weevils was zero, which means that concentration lines overlap.

The weevil was less susceptible to herbicide application than the bug. Only Agral (Figure 2.5 b), Midstream (Figure 2.5 c), Muster (Figure 2.5 g) and Roundup Ultra (Figure 2.5 j) produced significant mortality. Regressions could only be drawn, and LC50 values calculated, for Agral, Muster and Roundup Ultra, because of the relatively high mortalities they caused (Table 2.7). Proban analysis LC50 values could only be determined for Muster and Roundup Ultra (Table 2.7), again giving slightly lower LC50 values but correlating very well with the regression LC50 values.

Considering the LC50 values in table 2.7, Agral is again the most toxic substance. It is followed by Muster which should not cause considerable mortality when applied at the recommended dosage of 2 to 3 % (Table 2.6). Roundup Ultra caused slightly higher mortality than Midstream, but neither will result in significant mortality when applied at recommended concentrations.

When combining Midstream with the surfactant Agral, significant mortality was caused to the insect population (Figure 2.5 c). The lowest recommended dosage of 3.75 % Midstream with 0.75% Agral resulted in 95 % mortality after 120 hours (Figure 2.6 a). Even half the recommended dosage for Midstream and Agral combined, caused up to 78 % mortality (Figure 2.6 a). Agral at 0.75 % caused up to 72 % mortality and contributed mostly to the toxic effect for this species (Figure 2.6 a).

Touchdown combined with Add-2, another adjuvant, did not cause any significant mortality (Figure 2.5 d, k; Figure 2.6 b), furthermore, Add-2 and Touchdown separately did not cause significant mortality (Figure 2.6 b). It is interesting to note that the Touchdown is glyphosate-trimesium based, as is Muster which resulted in much higher mortality.

No mortality occurred after treatment with up to 24% Rodeo, while the other glyphosate based herbicides resulted in very little mortality (Table 2.6). 2,4-D amine showed very low mortality overall (Table 2.6).

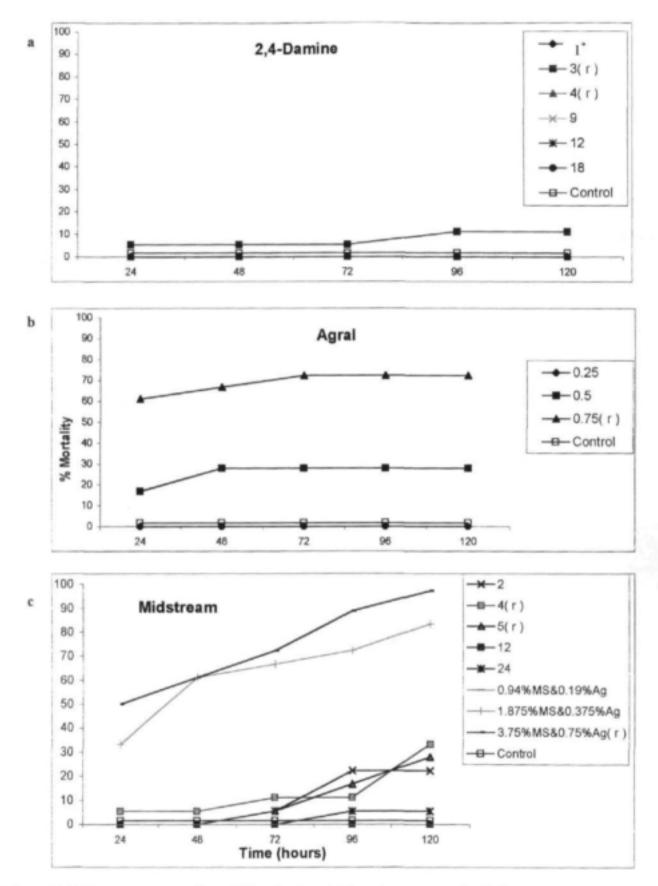
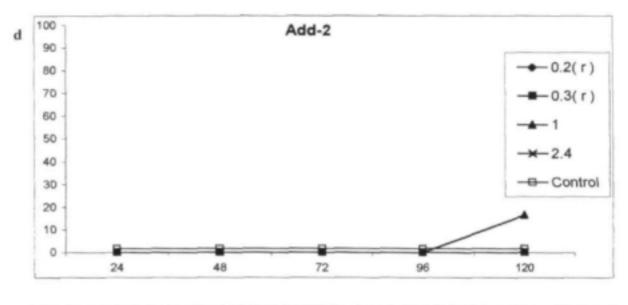
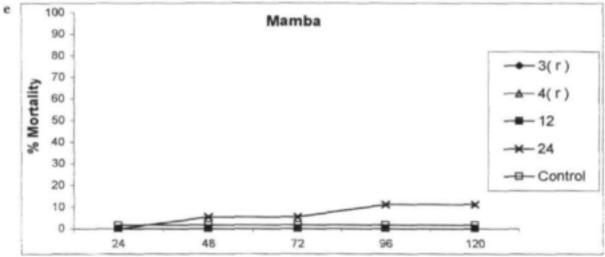


Figure 2.5 Percentage mortality of Neochetina eichhorniae as a result of direct treatment with selected herbicides.

(r) a recommended concentration of specified herbicide

^{*} legend entries are percentage concentrations of specified herbicide





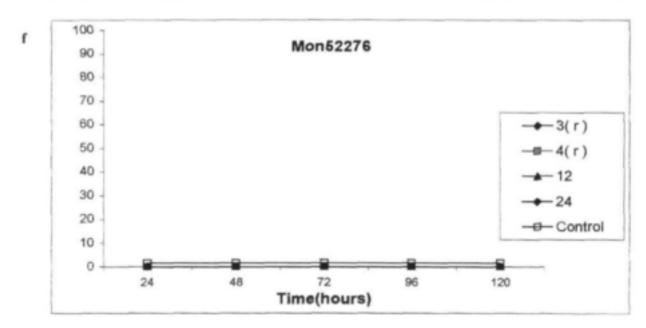


Figure 2.5 Cont.

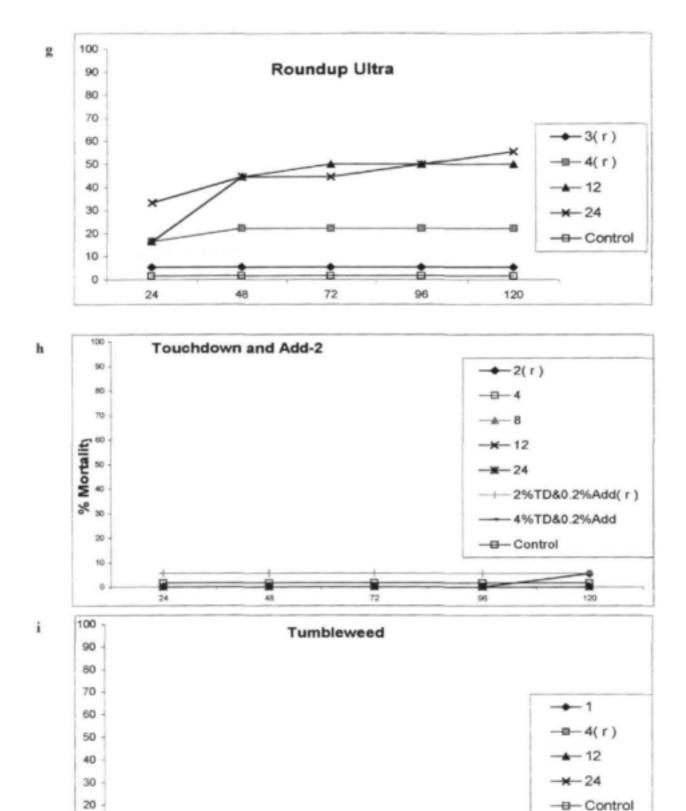
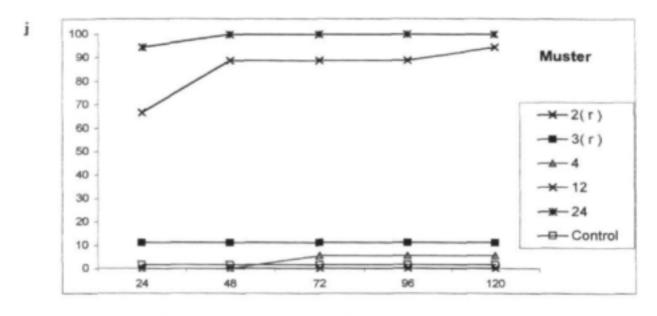
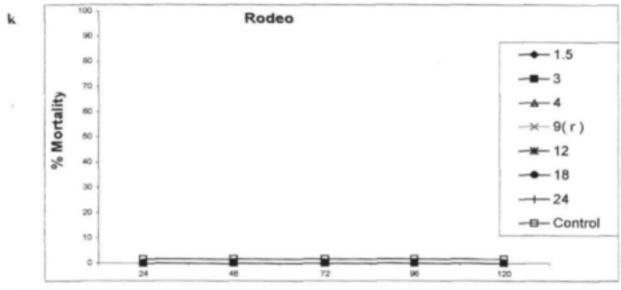


Figure 2.5 Cont.

Time(hours)





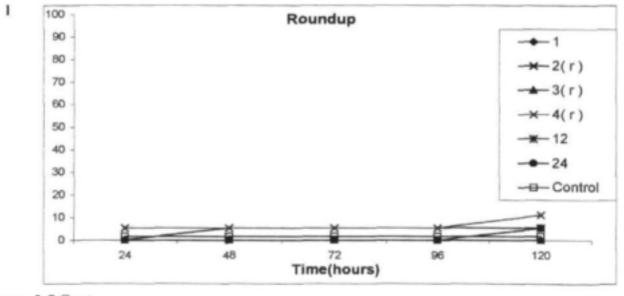


Figure 2.5 Cont.

Table 2.6 Mortality of Neochetina eichhorniae after direct exposure to selected herbicides (standard deviation in brackets).

Herbicide	Concentration	n		Mean number of adults dying ³					
			244	48	72	96	120		
2,4-D amine	1.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	3.0%	3	0.3(0.6)	0.3(0.6)	0.3(0.6)	0.7 (0.6)	0.7 (0.6)		
	4.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	9.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	18.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
Add-2	0.2%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	0.3%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	1%	3	0(-)	0(-)	0(-)	0(-)	0.3 (0.6)		
	2.4%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
Agral (Ag)	0.25	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	0.5%	3	1 (1.7)	1.3 (2.3)	1.7 (2.9)	1.7 (2.9)	1.7 (2.9)		
	0.75%	3	3.7 (1.2)	4 (1)	4.3 (1.5)	4.3 (1.5)	4.3 (1.5)		
Mamba	3.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	24.0%	3	0.7(1.2)	1 (1)	1 (1)	1.3 (1.2)	1.3 (1.5)		
Midstream (MS)	2.0%	3	0(-)	0(-)	0.3 (0.6)	1.3 (2.3)	1.3 (2.3)		
	4.0%	3	0(-)	0.3 (0.6)	0.7 (0.6)	0.7 (0.6)	2 (2.6)		
	5.0%	3	0(-)	0(-)	0.3 (0.6)	1 (1)	1.7 (2.1)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	24.0%	3	0(-)	0(-)	0(-)	0.3 (0.6)	0.3 (0.6)		
Mon 52276	3.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	24.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
Muster	2.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	3.0%	3	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)		
	4.0%	3	0(-)	0(-)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)		
	12.0%	3	5 (1.7)	5.3 (1.2)	5.3 (1.2)	5.3 (1.2)	5.7 (0.6)		
	24.0%	3	5.7 (0.6)	6(-)	6(-)	6(-)	6(-)		
Rodeo	1.5%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	3.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	9.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	18.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
B	24.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
Roundup	1.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	2.0%	3	0(-)	0.3 (0.6)	0.3 (0.6)				
	3.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%	3	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)		0.7 (0.6)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0.3 (0.6		
Damedon IIII	24.0%	3	0(-).	0(-)	0(-)	0(-)	0.3 (0.6		
Roundup-Ultra	3.0%	3	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6		
	4.0%	3	1 (1)	0.3 (1.5)	0.3 (1.5)	1.3 (1.5)	1.3 (1.5		
	12.0%	3	1 (1)	2.7 (2.9)	3 (2.7)	3 (2.7)	3 (2.7)		
	24.0%	3	2 (2)	2.7 (2.5)	2.7 (2.5)	3 (2.7)	3.3 (2.9)		

³ Mean number of adults dying (six per replicate)
⁴ Hours after application

Table 2.6 Cont.

Herbicide	Concentration	n	Mean number of adults dying						
			24	48	72	96	120		
Touchdown(TD)	2.0%	3	0(-)	0(-)	0(-)	0(-)	0.3 (0.6)		
	4.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	8.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	24.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
Tumbleweed	1.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%	3	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3(0.6)		
	12.0%	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	24.0%	3	0(-)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)		
Combinations	2.0%TD&0.2%Add	3	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)	0.3 (0.6)		
	4.0%TD&0.2%Add	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	4.0%TD&0.4%Add	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	8.0%TD&0.8%Add	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	0.938%MS&0.188%Ag	3	0(-)	0(-)	0(-)	0(-)	0(-)		
	1.875%MS&0.375%Ag	3	2 (1.7)	3.7 (1.5)	4 (1.7)	4 (1.7)	4.7 (0.6)		
	3.75%MS&0.75%Ag	3	3 (2.7)	3.7 (2.1)	4 (1.7)	5.3 (1.2)	5.7 (0.6)		
Control	H ₂ O	39	0.1 (0.3)	0.1 (0.3)	0.1 (0.3)	0.1 (0.3)	0.1 (0.3)		

Table 2.7 LC50 values obtained for Neochetina eichhorniae treated with selected herbicides.

	24*		48		72		96		120	
	R	P	R	P	R	P	R	P	R	P
Agral	0.72		0.62		0.59		0.59		0.59	
	(0.92)		(0.97)		(0.96)		(0.96)		(0.96)	
Muster	8.57	7.96	7.27	5.96	7.11	5.96	7.11	5.96	6.92	5.94
	(0.97)		(0.96)		(0.97)		(0.97)		(0.96)	
Roundup Ultra	160	83.19	24.1	22.71	21.2	20.53	17.9	17.78	15.6	15.59
	(0.89)		(0.93)		(0.9)		(0.94)		(0.97)	

⁼ hours after application
() = r (linear correlation coefficient)
R = Regression (Excel)
P = Proban analysis

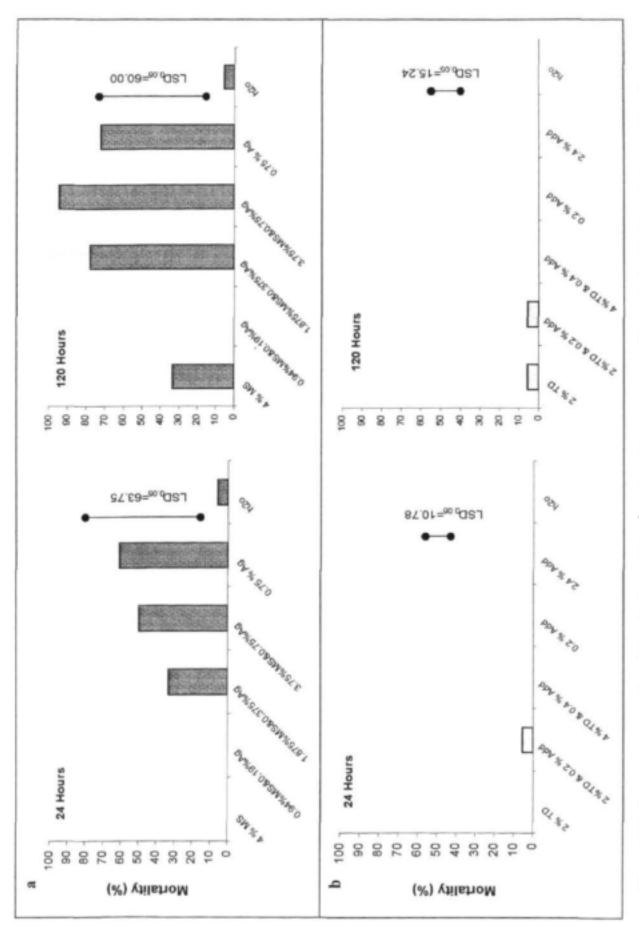


Figure 2.6 Percentage mortality of Neochetina eichhorniae after direct treatment with two herbicide formulations, with and without surfactants added (a) Midstream and Agral, (b) Touchdown and Add-2

2.4 DISCUSSION

In this study we tested the hypothesis that the herbicides used in the control of water hyacinth in South Africa are not toxic to two of the biological control agent species. Based on the results, we would have to reject this hypothesis and say that certain of the herbicides used on water hyacinth, at certain concentrations are acutely toxic to N. eichhorniae and E. catarinensis. However, we have to put these results into context. These trials were conducted in the laboratory, under very confined conditions and in many cases the concentrations of herbicides used, far exceeded that recommended by the manufacturer. Therefore, these results should be considered to be conservative and any extrapolation from the petri-dishes to the field should be made with care.

2.4.1 Susceptability

In spite of the conservative nature of the tests, the results showed quite clearly that the water hyacinth bug is susceptible to herbicides and that their populations are likely to be affected by herbicide application. The weevil on the other hand proved to be tolerant to herbicide application at reasonable concentrations.

2.4.2 Role of surfactants

Several authors (Haag 1986 a; Wright & Skilling 1987; Grodowitz & Pellessier 1990) suggested that while the active ingredients of the herbicides themselves might not be toxic to the insects, the additives/adjuvants/surfactants might be toxic. One of the aims of this study was to test this hypothesis.

The surfactant Add-2 does not result in serious mortality if used at recommended dosages. Add-2 combined with Touchdown (glyphosate-trimesium), was even less toxic than they were separately on *E. catarinensis*, at recommended dosages. However, the surfactant Agral was especially toxic, when used alone, and when combined with Midstream (diquat), it greatly increased the toxicity of the formulation. Grodowitz and Pellessier (1990) also tested a range of surfactants on *N.eichhorniae* and concluded that one surfactant combined with diquat caused significant mortality of the weevils.

The contribution of additives to the toxicity of a formulation, is emphasized when comparing the mortality caused by Rodeo and other glyphosate based herbicides. Rodeo, with practically no surfactants added (Findlay⁵, pers. comm.), caused the least mortality of all substances in both the weevil and bug populations. Rodeo will not be applied without surfactant when used in practice, and it has to be stressed that the choice of surfactant will finally determine the toxicity of the applied solution towards the insect population.

Muster and Touchdown, both 720 g a.e/l glyphosate-trimesium, also differed in toxicity. For E. catarinensis from 24 to 48 hours, Touchdown seemed non-toxic. However, at 96 hours onwards the LC50 value for Touchdown fell far below that of Muster (Table 2.3). For N. eichhorniae, Muster was toxic and Touchdown seemed not to be toxic at all. It is possible that the effect of Touchdown is delayed, and will it cause mortality in time as it did with E. catarinensis, or that the weevil is tolerant to this substance.

2.4.3 Rating herbicides

Table 2.4 describes the expected effect on E. catarinensis of the tested formulations and surfactants. 2,4-D amine, Agral, Midstream, Muster, Roundup Ultra and Touchdown have a toxic effect on this species. The most toxic substance for N. eichhorniae is Agral, followed by Muster and Roundup Ultra. Midstream is not highly toxic to the weevil, unless it is combined with Agral, at recommended dosages.

The glyphosate based herbicides were the least toxic group with Roundup Ultra exhibiting uncharacteristic toxic behaviour. Glyphosate-trimesium based herbicides varied in toxicity but tend to be toxic, depending on the susceptibility of the insect species. Diquat was highly toxic to the more susceptible bug, but not especially toxic to the weevil. Diquat was also found to be slightly toxic to adult *Orthogalumna terebrantis*, another natural enemy also released in South Africa, in tests comparing 2,4-D amine, glyphosate, diquat and paraquat formulations (Roorda et al. 1978).

⁵ Findlay, J. Agricultural Resource Consultants, P.O.Box 3474, Parklands, 2121, South Africa

2,4-D amine was also labeled toxic to the bug, but was not toxic to the weevil. This active ingredient is popular in many countries. It was found in Australia that only combined with a surfactant, did it cause significant mortality of N. eichhorniae (Wright & Skilling 1987).

Experiments by Haag (1986 a) indicate that N. eichhorniae and N. bruchi treated with 2,4-D amine, diquat, glyphosate and some additives showed no significant mortality. This agrees with our results, for the weevil and concurs that this species is not highly susceptible to herbicide toxicity. Jianqing et al. (1999) found that paraquat was toxic to adult weevils but Roundup had no significant effect, except when ingesting it through feeding on treated leaves.

2.5 CONCLUSION

Extrapolation of these results from the laboratory to the field must take the behaviour of the insect species into consideration. The weevil is nocturnal, sheltering at the base of the petioles during the day, feeding on the leaves at night. It is therefore very unlikely that they would come into direct contact with a droplet of herbicide. Therefore the impact of water hyacinth treated with herbicides on the feeding behaviour and the population dynamics of the weevil is likely to be more important and is investigated in chapters 3 and 4.

The mirid on the other hand feeds externally during the day and is therefore likely to come into contact with droplets of herbicide. In this experiment we only tested adult *E. catarinensis* but we could assume that the nymphs would be at least as susceptible to herbicides, as the adults. Mirid populations are therefore likely to be negatively impacted by herbicide application, not only through the loss of habitat but also through mortality caused by acute toxicity. Therefore the choice of herbicide formulation and the species of insect in the system are important factors to consider in the integrated control of water hyacinth.

Feeding behaviour of Neochetina eichhorniae weevils treated with selected herbicides

3.1 INTRODUCTION

Neochetina eichhorniae exhibited low susceptibility to the herbicides during acute toxicity tests (Chapter 2). The species was only sensitive to one glyphosate-trimesium based herbicide, Muster, and a surfactant Agral alone, and combined with a diquat based herbicide, Midstream.

There are several other methods to measure the toxic effects of substances on insects, besides acute toxicity. Sub-lethal effects such as fecundity and changes in behaviour, which includes feeding behaviour, are considered effective ways to monitor the health of treated subjects (Yokoyama & Pritchard 1984; Jagers op Akkerhuis et al. 1999).

The aim of this study was to monitor the feeding of *Neochetina eichhorniae* on water hyacinth leaves after the weevils had been treated with several herbicide formulations, in an attempt to investigate the possible sub-lethal and chronic impact of these herbicides on the weevils.

3.2 MATERIALS AND METHODS

A 20 µl droplet was applied to the elytra of each weevil. Six weevils (three male and three female) were placed in 500 ml plastic containers, lined with moist filter paper and aerated through small holes in the top. Fresh leaves were provided daily for food, care was taken to select medium sized leaves (approximately 12 x 8 cm) of the same colour, and therefore presumably the same nutrient content. Temperature varied between 22 and 28 °C with a light regime of 16 hours light and 8 hours dark. Leaves were removed every 24 hours and replaced with fresh leaves and scars counted twice, at 48 and 96 hours after treatment. A

feeding mark of approximately 4 x 3 mm in size was considered a scar. The experiment was repeated three times. A one way analysis of variance was used to analyze the data.

3.3 RESULTS

The mean number of feeding scars made by weevils after exposure to 4 % and 12 % concentrations of a range of herbicides are illustrated in figure 3.1. For the 4 % treatments at 48 hours after exposure, no significant difference between the control and any other treatment occurred (Figure 3.1 a). After 96 hours the combination of Midstream and Agral caused a significant decrease in feeding from the control treatment (p = 0.01) (Figure 3.1 b), while Midstream alone did not.

Concentrations of 12 % are higher than are usually applied in practice. Muster seemed to decrease feeding at 12 % relative to the control, but this was not significant at 48 or 96 hours (Figure 3.1 c, d).

Very few significant differences could be detected, probably a result of great variation in feeding of the weevils in general. Another factor may be that only three replicates were used, more replicates would result in less variance and trends becoming clearer. Determining the feeding before treatment of the insects may act as another control and indicate any radical increases or decreases in feeding after treatment.

Herbicides that seem to cause increased feeding are Mon 32276, Rodeo, Touchdown and Tumbleweed at 4 %, although this is not a significant increase (Fibure 3.1 a, b). Mon 32276 and Tumbleweed also result in increased feeding at 12 % (Figure 3.1 c, d). Herbicides that tend to cause decreased feeding were 2,4-D amine, Roundup and Roundup Ultra at 4 %, while Mamba, Midstream, Muster, Roundup and Roundup Ultra led to decreased feeding at 12 % (Figure 3.1 a-d).

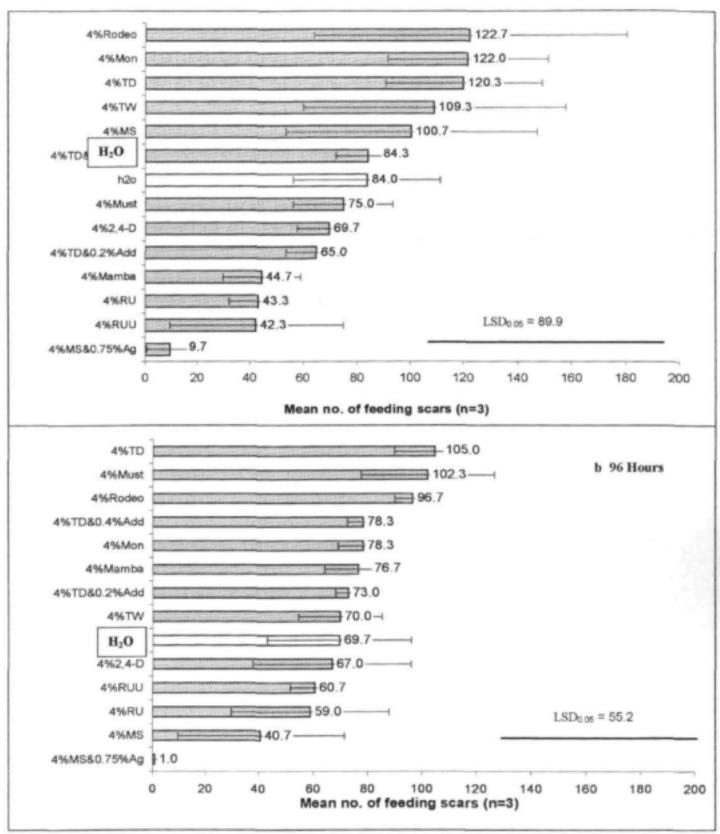


Figure 3.1 Mean number of feeding scars as an indicator of health for Neochetina eichhorniae, after direct treatment with 4 % of selected herbicides.

Feeding scars
Standard deviation

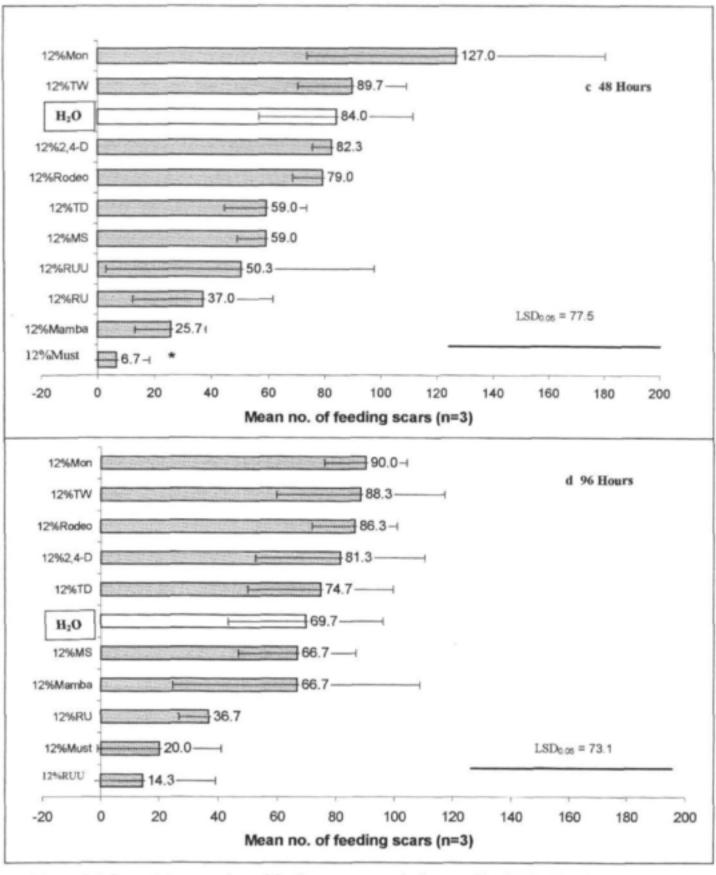


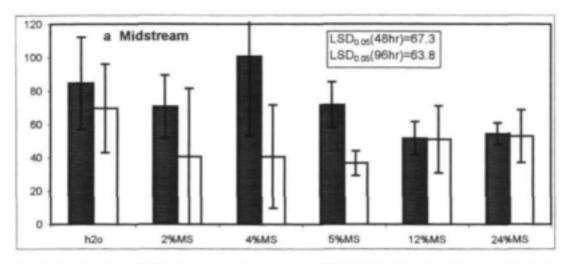
Figure 3.1 Cont. Mean number of feeding scars as an indicator of health for Neochetina eichhorniae, after direct treatment with 12 % of selected herbicides.

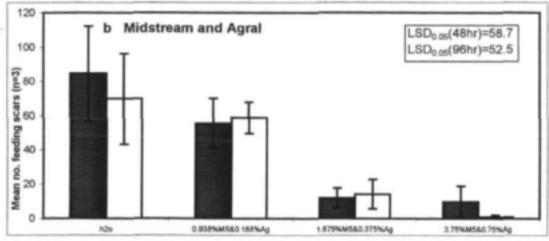
The trends that were identified for some of the herbicides are illustrated in figure 3.2.

These herbicides showed decreased feeding with increase in concentration while the herbicides not included here showed no definite trend.

Insects treated with Midstream showed no significant increase or decrease in feeding with increased concentration (Figure 3.2 a). When Midstream and Agral were combined at the lowest recommended dosage (3.75 % MS and 0.75 % Ag), and even half the lowest recommended dosage, it decreased feeding significantly (p = 0.01) (Figure 3.2 b). Exposure to high concentrations of Muster led to a significant decrease in feeding, but only at 48 hours (p = 0.05) (Figure 3.2 c).

For the glyphosate based herbicides that exhibited some trend, Mamba seem to cause decreased feeding at higher concentrations, but this was not significant (Figure 3.2 d). Roundup led to decreased feeding at higher herbicide concentrations, and feeding at 12 % and 24 % was significantly less (p = 0.05), but only at 48 hours (Figure 3.2 e). Roundup Ultra also caused significant decreases in feeding at 12 % and 24 % but only after 96 hours (Figure 3.2 f).





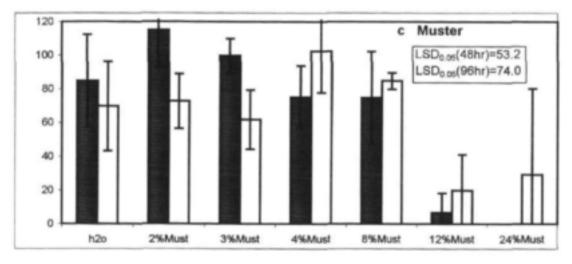


Figure 3.2 Mean number of feeding scars produced by groups of six Neochetina eichhorniae, after direct exposure to selected herbicides and surfactants (n=3).

Mean number of feeding scars after 48 hours

Mean number of feeding scars after 96 hours

LSD_{0.05} Least significant difference at p = 0.05

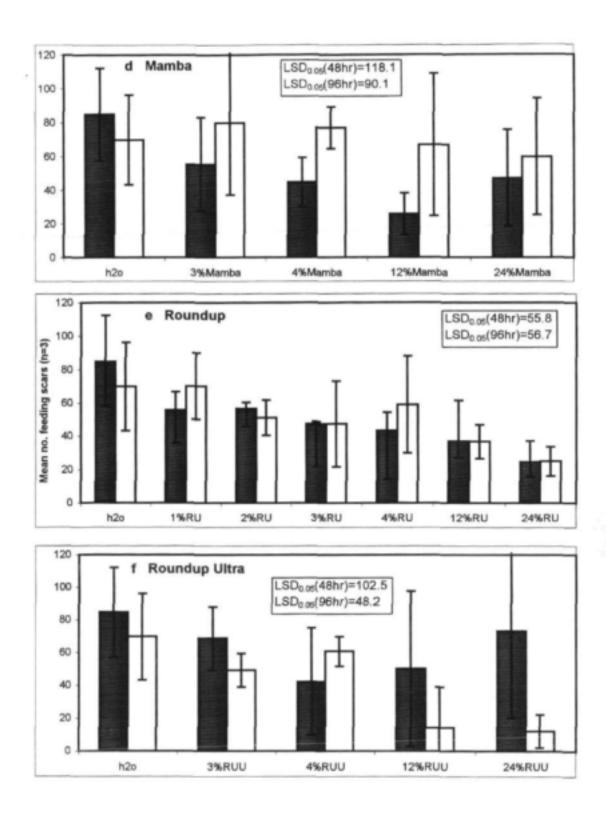


Figure 3.2 Cont.

3.4 DISCUSSION

Midstream combined with the surfactant Agral at recommended dosages decreased feeding significantly (p < 0.01) from that of the control. No further significant increases or decreases in feeding could be noted, a result of high natural variance in feeding and too few replicates. The herbicides could not be rated based on these results.

Direct treatment with concentrations of "Midstream-Agral combination", and high concentrations of Muster, Roundup and Roundup Ultra led to decreased feeding by the weevils. The results do not describe the effect of recommended dosages relative to the control because of very high variance, and too few replicates. It does however, warn against the use of high concentrations of herbicides.

3.5 CONCLUSION

As in the previous experiment, application of a droplet of herbicide to N. eichhorniae does not represent the situation the weevil will be exposed to in the field. The effect on the weevil having fed on herbicide treated water hyacinth would represent a more realistic scenario and is investigated in the next chapter. The experiment did however show that, as with the acute toxicity tests (Chapter 2) very high concentrations of some herbicides have a negative impact on weevil feeding and therefore negatively impact weevil populations.

4. Feeding behaviour of *Neochetina eichhorniae* on water hyacinth leaves treated with selected herbicides

4.1 INTRODUCTION

The previous two chapters have shown that direct application of herbicides to the weevil Neochetina eichhorniae did have some effect on its mortality and feeding behaviour. This effect was usually not significantly different from the control and showed the weevils to be resistant to, and tolerant of most of the herbicides tested. In addition, the expected design of the previous two chapters was not indicative of what the weevils are exposed to in the field. As the weevil is nocturnal, sheltering at the base of the petioles during the day it is unlikely to receive direct contact from the herbicides. However, the weevils will feed on water hyacinth that has been sprayed with herbicides.

The effect of herbicide treated plants on weevil feeding behaviour have not been studied well. Jianqing et al. (1999) claimed that feeding on plants treated with high concentrations of Roundup had some toxic effects on the weevil. Water hyacinth plants exposed to 2,4-D amine have softer petioles and increased nitrogen content, which may favour herbivore attack (Wright & Bourne 1990). Another study by Pellessier (1988) showed that feeding by N.eichhorniae and N. bruchi decreased on 2,4-D amine and diquat treated leaves.

Here we test the hypothesis that water hyacinth sprayed with herbicides negatively effect the feeding behaviour of adult N. eichhorniae.

4.2 MATERIALS AND METHODS

Groups of five adult weevils were kept in plastic containers (500ml), lined with moist filter paper and aerated through small holes in the top. Young water hyacinth leaves were painted with a freshly made-up solution of either Roundup (glyphosate), 2,4-D amine or Midstream (diquat) at a 6 % concentration (Table 2.1).

Leaves were replaced every 24 hours. Number of feeding scars per leaf and mortality of weevils was recorded every 24 hours. The experiment continued for 144 hours and was repeated six times. Temperature varied 22 and 28°C, and the light regime was 16 hours light, 8 hours dark. A one way analysis of variance was used to analyze the data.

4.3 RESULTS

4.3.1 Appearance of Leaves

Control leaves painted with distilled water, displayed no visible signs of deterioration or wilting. Roundup treated leaves appeared normal, sometimes discolouring slightly and becoming yellowish. Midstream treated leaves exhibited uneven, brown discolourations which was possibly a result of uneven coverage because of high surface tension of the formulation without added surfactant. Leaves treated with 2,4-D amine turned brown and slightly sticky on the surface, and were often infected with a powdery white fungus.

4.3.2 Mortality

At 120 hours after starting to feed, insects fed on 6 % Midstream treated leaves showed significant mortality, between 57 % at 120 hours and 70 % at 144 hours, in comparison to the control (Figure 4.1). 2,4-D amine also resulted in some mortality but this was not significant in comparison to the control (Figure 4.1). No control insects or insects fed on Roundup treated leaves died (Figure 4.1).

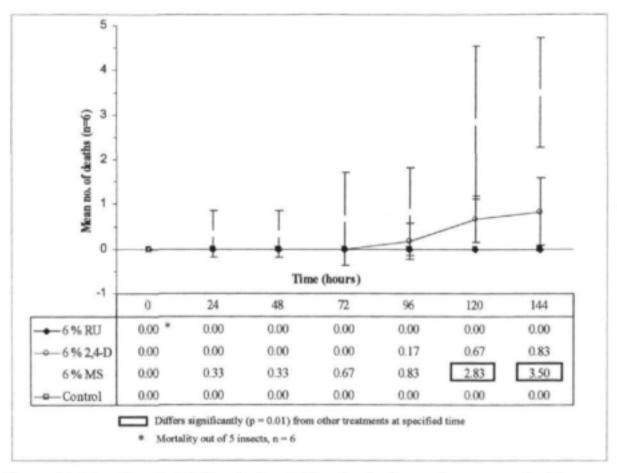


Figure 4.1 Mortality of adult Neochetina eichhorniae feeding on leaves treated with three herbicides over a six day period.

Ingestion of water hyacinth leaf material treated with a 6 % concentration of Midstream caused higher mortality in the weevils than direct exposure to the herbicide (Chapter 2). The highest mortality documented for *N.eichhorniae* after direct toxicity treatment with Midstream, was at a 4 % concentration after 120 hours when 33 % of the test population died (Table 2.6). Ingestion of treated leaf material resulted in 57 % mortality at 120 hours, after a 6 % Midstream treatment.

The highest mortality caused by 2,4-D amine during acute toxicity tests was at a 3 % concentration after 120 hours, when 12 % of the population died (Table 2.6). Similar mortality of 13 % was noted in this experiment, after ingestion of leaf material treated with a 6 % solution after 120 hours (Figure 4.1).

Roundup at 6 % resulted in negligible mortality of the weevils whether they were directly exposed to the herbicide or ingested food treated with it (Table 2.6, Figure 4.1)

4.3.3 Feeding

There was an overall decrease in feeding over time, the control insects' feeding dropped from a mean of $66.0 (\pm 13.3)$ scars at 24 hours to $51.7 (\pm 22.0)$ scars at 144 hours (Table 4.1). The control insects' feeding decreased by 14.3 scars; Roundup treatment decreased by 20 scars; 2,4-D by 34.7 scars and Midstream by 46.6 scars over 144 hours (Figure 4.2).

Feeding documented at 0 hours (24 hours of feeding on untreated leaves) show no significant difference as was expected (Figure 4.3 a). After 24 hours of feeding on leaves treated with Midstream and 2,4-D, the weevils showed a significant decrease in feeding activity in comparison to the control (p = 0.01) (Figure 4.3 b). This decreased feeding activity continued for the rest of the 144 hours, for both Midstream and 2,4-D (Figure 4.3 c-g).

Table 4.1 Mean number of feeding scars produced by a group of five Neochetina eichhorniae, after feeding on treated water hyacinth leaves (STD = Standard deviation).

TREATMENT	TIME (Hours)											
	n	0	24	48	72	96	120	144				
6 % Roundup	6	58.2	58.0	52.8	39.2	32.2	27.2	38.0				
	STD	(22.1)	(17.4)	(20.4)	(13.4)	(6.0)	(13.8)	(10.3)				
6 % 2,4-Damine	6	52.5	26.7	31.3	25.0	15.5	15.2	17.8				
	STD	(18.6)	(7.1)	(11.3)	(11.2)	(8.8)	(5.7)	(10.6)				
6 % Midstream	6	50.8	27.0	17.8	10.5	10.8	5.7	4.2				
	STD	(8.2)	(8.5)	(8.4)	(2.9)	(10.6)	(4.8)	(9.7)				
Control	6	66.0	67.3	73.3	62.8	56.0	41.3	51.7				
	STD	(13.3)	(17.3)	(9.5)	(27.6)	(23.7)	(21.0)	(22.0)				

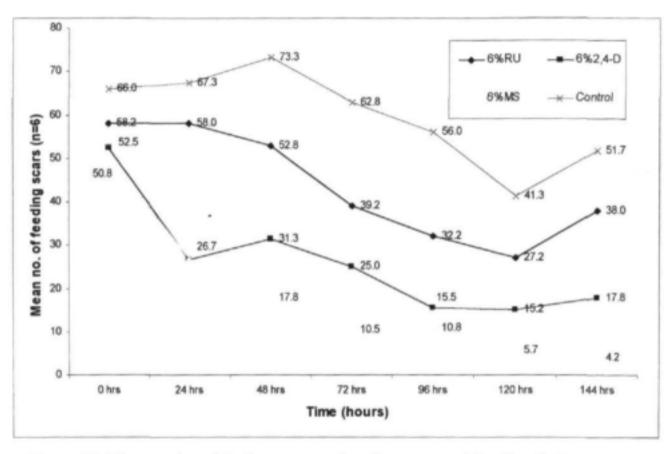
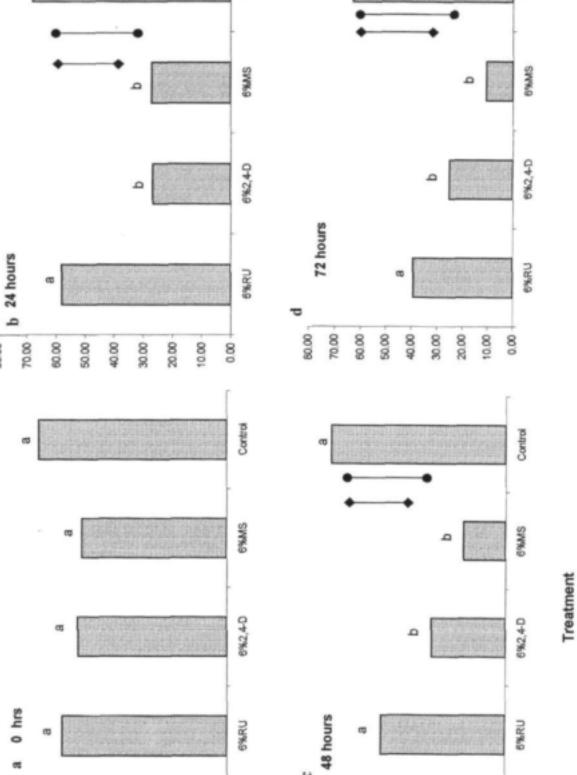


Figure 4.2 Mean number of feeding scars produced by a group of five Neochetina eichhorniae, during 144 hours of feeding on treated water hyacinth leaves (n=6).



70.00

30.00

50.00

80.00

90.00

70.00

90.00

50.00

40.00

30.00

10.00

20.00

800

Feeding scars per group of five weevils (n=6)

Control

Figure 4.3 Mean number of feeding scars produced by a group of five Neochetina eichhorniae after feeding on treated leaves over a period of six days

Control

 after feeding on treated leaves for 24 hours; d. after feeding on treated leaves for 72 hours. c. after feeding on treated leaves for 48 hours; a. after 24 hours feeding on untreated leaves;



19

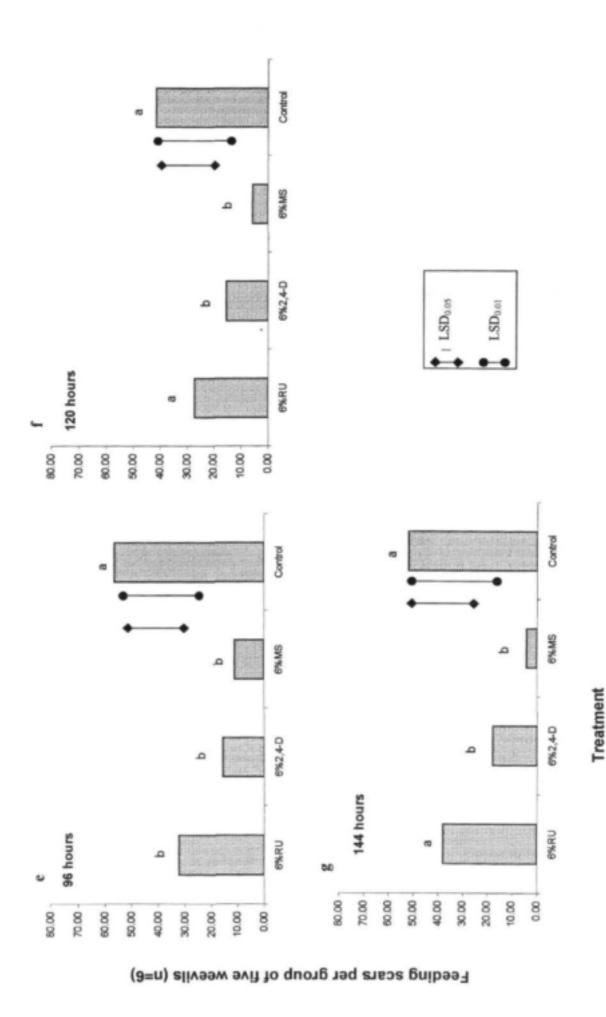


Figure 4.3 Cont.

e. after 96 hours of feeding on treated leaves;

g. after 144 hours of feeding on treated leaves.

f. after 120 hours of feeding on treated leaves;

4.4 DISCUSSION

Ingestion of leaves treated with Midstream (diquat) is more toxic than leaves treated with 2,4-D amine and Roundup (glyphosate). The results suggest that ingestion of diquat may be more toxic than direct exposure. The effect of Roundup on feeding is negligible.

Similar to this experiment, decreases in weevil feeding occurred on plants treated with diquat and 2,4-D amine, and not for the control population in tests by Pellessier (1988). Diquat treated plants showed significant decreases in ether extractable compounds and ash content. Both 2,4-D and diquat treated plants showed significantly reduced crude protein levels and increase in nitrogen-free extracts. Calcium content in 2,4-D treated plants increased greatly. Pellessier (1988) also found correlations between the changes in feeding on diquat treated plants, with changes in ether extractable compound and moisture content.

Wright and Bourne (1990) found that 2,4-D treated plants had softened petioles and increased nitrogen content that might increase feeding by herbivores. It has been shown that glyphosate can lead to increased feeding by mammal herbivores, when applied at low concentrations (Sullivan 1985). Insects did not show increased feeding on treated, severed leaves in this experiment or the work of Pellessier (1988), but the results may be different if tests are performed on whole plants. Herbicide treated plants transport toxins to other parts of the plant body, i.e. glyphosate is transported to the roots and apical meristem to be stored or metabolized (Bariuan *et al.* 1999). This transport is not possible in severed leaves possibly changing the chemistry of leaves more than it would an intact plant, or be available at a much higher level to be ingested by herbivores.

If whole plants show the same intensity of degradation resulting in reduced feeding as seen after treating leaves with 2,4-D amine and diquat, weevils will prefer to move away from treated plants. If no untreated plants are available or insects are unable to move away, it will result in poor feeding and reduced egg laying, because of metabolism of fat reserves as a consequence of starving. The result will be a smaller insect population to re-colonize the re-growth of the weed.

4.5 CONCLUSION

A sudden change in plant condition as would occur after treatment with diquat or 2,4-D, will lead to a decrease in feeding followed by a mass migration of adult weevils. It is also possible that a very rapid degradation in plant condition may cause the mat to sink rapidly, killing a large insect population.

Slow degradation as would occur as a result of glyphosate application, may give insects more time to escape the dying mat. It may also be so slow and undetectable that insects continue feeding and laying eggs in the plant material, while feeding on treated plants and consuming more toxins that could have a chronic effect on the insects.

Once again, the solution of the herbicide to be used is vital in the integrated control water hyacinth.

5. Behaviour of adult Neochetina eichhorniae populations on water hyacinth mats treated with selected herbicides

5.1 INTRODUCTION

In the integrated control of water hyacinth, it has been suggested that an area of unsprayed plants be left to act as a reservoir for insects to move onto, as plants that have been sprayed start to die and sink (Haag 1986 b). This relies on the assumption that insects have the ability to disperse and are not directly affected by the herbicide, and we have shown that some herbicide formulations do increase mortality and decrease the feeding rate of *N. eichhorniae* and *E. catarinensis* while other herbicide formulations have less of an affect (Chapters 2 to 4). It also relies on the assumption that the insects used in the biological control of water hyacinth are not attracted to the plants that have been sprayed, especially as Delfosse and Perkins (1977) showed that damaged water hyacinth plants released kairomones that stimulated feeding and oviposition by *N. eichhorniae* and *O. terebrantis*.

Haag (1986 b) and Pellessier (1988), however, noted some movement by adult weevils from sprayed to unsprayed water hyacinth plants. Pellessier (1988) found that plants that had been sprayed with diquat degraded rapidly leading to rapid migration of weevils to unsprayed plants, but also resulting in high mortality of larvae and pupae. She suggested that low dosages or slower acting herbicides may allow more pupae to emerge while females would lay fewer eggs as the plants lost condition, resulting in less of an impact on the insect population. However, Wright and Bourne (1990) showed that the moth, *N. albiguttalis*, was attracted to water hyacinth plants sprayed with 2,4-D amine as this herbicide softened the petioles and increased the nitrogen content of the plants.

The hypothesis tested in this experiment is that adult *N. eichhorniae* will migrate from water hyacinth plants treated with herbicides to untreated plants.

5.2 MATERIALS AND METHODS

These experiments were conducted in rectangular asbestos trays (2100mm long, 900mm wide and 150mm deep). The trays were divided in half across the tray using twine. They were then filled with water and 25 g of the slow release fertilizer and 2.5g of chelated iron was added to the water (this amounts to a concentration of 0.1 g/l for the fertilizer and 0.01 g/l for the iron chelate). Fifty young water hyacinth plants were added to each half of each tray and left for seven days to acclimatise.

Adult N. eichhorniae were collected from the rearing facility at the Rietondale Experimental Farm. The weevils were marked using the corrective fluid, Tippex, a drop of which was applied to the right elytrum. For each tray, 50 weevils were marked white and 50 were marked pink. The pink weevils were added, one per plant, to the half to be sprayed with herbicide and the white weevils to the half to be left unsprayed. The trays were left overnight for the weevils to acclimatise.

One day following the release of the weevils, one half of the tray was sprayed with a herbicide. The application of the herbicide took place in the morning, before 11h00, ensuring that there was sufficient sunlight hours left to allow effective uptake of the herbicide. The 50 plants to be sprayed were removed from the tray and placed in another container and returned to the tray once they had been sprayed. This was to ensure that there was no spray drift onto the unsprayed plants, either through the air, or the shared water body. Herbicides were applied with a hand held atomiser and every effort was made to ensure an even spray of the herbicide. After spraying, the plants were carefully replaced in the tray. The control comprised a tray, set up identically to the experimental tray with the exception that one half was sprayed with distilled water.

Only two herbicides were used in these experiments, Roundup, applied at 3% (15 ml spray volume) and Midstream (diquat) applied at 3.75% combined with 0.75% Agral (15 ml spray volume).

The Roundup treated plants were harvested 12 days after application, but the Midstream treated plants had to be harvested 6 days after application as the plant quality decreased more rapidly. We attempted to harvest the plants once they had started to discolour, but before they had died in order to determine if the weevils would be attracted to sprayed plants. During harvesting, the locality of the marked weevils was recorded and the number of feeding scars on the first fully expanded leaf were counted. There were four replicates for each herbicide.

5.3 RESULTS

5.3.1 Appearance of Leaves

After treatment with Roundup the plants turned yellow after a few days and any flowers that emerged turned white. The plants treated with this low dosage did not seem to rot within the 12 days of monitoring, except for some of the smaller plants and older leaves. Midstream treated plants turned brown within days and started to rot rapidly. By the sixth day these plants had all turned brown and it was decided to terminate the experiment at this stage.

5.3.2 Movement of Weevils

According to the distribution of weevils at the time of collection the weevils moved from treated to untreated plants (Figure 5.1, Table 5.1). The movement of control insects was probably a result of natural dispersal, 21 % of the surviving pink weevils had moved to the unsprayed section, while 35 % of the surviving white weevils had moved to the distilled-water sprayed section.

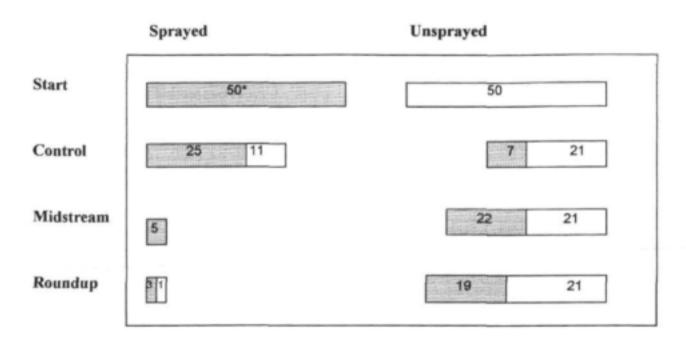


Figure 5.1 Movement of Neochetina eichhorniae between herbicide sprayed and unsprayed water hyacinth plants in a pond (n=4).

* Number of weevils released (at start) or captured

Table 5.1 Movement and feeding of Neochetina eichhorniae between herbicide sprayed and unsprayed water hyacinth plants in a pond.

	Mean num of weevils		ber	Mean number of feeding scars ¹
	N	Pink	White	
ROUNDUP				
Sprayed	4	2.8 (2.2)	1.3 (0.5)	9.9 (4.5)
Unsprayed	4	19.3 (5.7)	20.5 (3.7)	40.2 (5.0)
MIDSTREAM				
Sprayed	4	5.3 (5.1)	0 (0)	8.6 (2.2)
Unsprayed	4	21.5 (5.0)	20.5 (3.7)	36.6 (3.5)
CONTROL				
Sprayed	2	24.5 (9.2)	11(0)	35.0 (10.4)
Unsprayed	2	6.5 (3.5)	20.5 (9.2)	35.5 (8.8)

On youngest open leaf

Pink = Insects released in sprayed area

White = Insects released in unsprayed area

() = Standard deviation

The migration of surviving weevils during the Midstream treatment was 80 % towards the unsprayed area, and 0 % towards the sprayed area. Movement of weevils after treatment with Roundup was also towards the unsprayed area, 87 % of the surviving pink weevils had moved to the unsprayed area and 6 % of the surviving white weevils had moved onto plants in the sprayed area.

The weevil movement towards Roundup treated plants (6 % of the surviving insects) was less than the normal movement by the control insects (35 % of the surviving insects) towards the distilled water sprayed section.

Only 49 % of the released population was re-captured for the whole experiment (Table 5.2). Unfortunately the trays were not covered so we are unable to determine if the reduction in the total number of weevils was due to death, or as is more likely the case, dispersal. There was no evidence of excessive mortality. In addition, the numbers collected are likely to be an underestimate due to the cryptic behaviour of the weevils. The first trial showed lower survival overall, for both control and Roundup treated ponds (Table 5.2). This could be as a result of leaving this trial for twice as long as the Midstream trial, thereby allowing more time for dispersal of the insects.

Table 5.2 Survival rate for Neochetina eichhorniae on water hyacinth plants sprayed with the herbicide Roundup (glyphosate) and Midstream (diquat).

	Survival Rate (%)
Roundup	36.6
Control	54
Total	39.5
Midstream	53.4
Control	82
Total	58.2
	Control Total Midstream Control

5.3.3 Feeding

The feeding damage (as expressed as number of adult feeding scars on the first fully expanded leaf) was very similar in both halves of the control trays (Table 5.1, Figure 5.2). However, in the herbicide treated trays a significantly higher number of feeding scars were recorded on the unsprayed half of the tray than on the sprayed half (one way analysis of variance, p< 0.01) (Table 5.1, Figure 5.2). This indicates that the water hyacinth plants treated with herbicides become unpalatable to the weevils and they move to the unsprayed plants. However, the number of feeding scars on the unsprayed halves of the herbicide treated trays was not significantly higher that either half of the control trays, suggesting that the weevils are dispersing away from the tray rather than to the adjacent, unsprayed water hyacinth within the same tray.

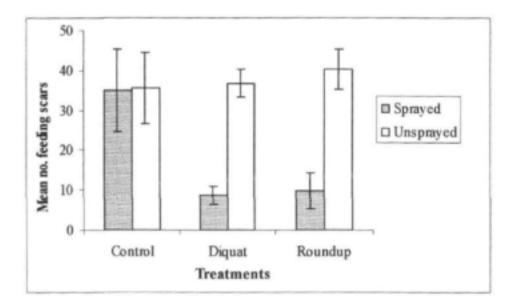


Figure 5.2 Feeding by Neochetina eichhorniae on herbicide sprayed and unsprayed water hyacinth plants.

5.4 DISCUSSION

In this experiment, we attempted to simulate a field scenario where a section of a water hyacinth mat would be sprayed with a herbicide, while other sections would be left as "reserves" for biological control agents, as proposed in the integrated management plan for the Vaal River (Cilliers et al. 1996). To further investigate this idea, we tested the hypothesis that adult N. eichhorniae would migrate from water hyacinth that had been sprayed with a herbicide, to unsprayed plants. The results obtained in this experiment suggest that we should accept this hypothesis.

The results of the behavioural experiments using marked weevils showed that adult weevils will crawl to adjacent healthy plants from sprayed, dying plants. In addition to which, they would migrate through flight to other mats of the weed, although this was not tested in this experiment. This laboratory evidence is supported with observations from the field where populations of all biological control agents become large and concentrated on the unsprayed fringe of water hyacinth plants that remain after mats on dams are controlled chemically.

In a parallel study, Haag (1986 a, b) sprayed a series of water hyacinth plots with 2,4-D and monitored the movement of the two weevil species, N. eichhorniae and N. bruchi. Here results were very similar to ours in that she showed that the weevils would move from sprayed to unsprayed plants, at least over a distance of 4m. She suggested that it might be possible to spray mats of water hyacinth selectively and thereby herd adult weevils to unsprayed plants (Haag 1986 b) and that portions of the mats should be left unsprayed and contained in distant areas where their economic impact would be small.

This was really a preliminary experiment and it would be useful to repeat it using a wider range of herbicides, a wider range of biological control agents and, ultimately conducting it in a field situation where the results are less likely to be affected by edge effects, as they were no doubt affected in this experiment.

5.5 CONCLUSION

We have shown that the weevils used in the biological control of water hyacinth will migrate from sprayed to unsprayed plants. Therefore, in the integrated control of water hyacinth, areas of mats of the weed need to be left unsprayed as reserves for biological control agents. However, we need more research to determine the recovery rates of both the weed and the insect populations in order to determine the size and number of reserves to be left unsprayed.

6. General discussion

6.1 SUMMARY OF RESULTS

This study essentially showed five aspects to be important in the integrated control of water hyacinth in South Africa.

6.1.1 Eccritotarsus catarinensis

The herbicides used in the control of water hyacinth are, at least to some extent, toxic to the insect most recently released on water hyacinth in South Africa, the mirid, Eccritotarsus catarinensis. This species is still not well established on water hyacinth in South Africa, and it begs the question, in view of what we have shown, should this species be released in areas that are targeted for herbicide application?

Recently, Bon Accord Dam, just north of Pretoria was spayed, presumably with Roundup. The herbicide application was successful and two months after the application there was a fringe of water hyacinth around the dam. The mirid was well established at this site and the sinking of the mat served to herd the mirids onto the remaining water hyacinth plants. There is no doubt that the herbicide would have caused significant mortality of the adult mirids and the sinking of the mat would have caused mass mortality of the eggs and nymphs, but it still served to concentrate the adult insects on the remaining plants. What impact the high population of mirids will have on the regrowth of the water hyacinth at this highly eutrophic site is uncertain and should be investigated. This also afforded us the opportunity to collect and redistribute mirids to several other sites around South Africa.

6.1.2 Neochetina eichhorniae

The adult N. eichhorniae weevils are unaffected by most of the herbicides formulations used in the control of water hyacinth in South Africa. We feel confident that the results obtained for this insect can be extrapolated to its congeneric, N. bruchi. We have shown that the weevils will also move from sprayed and sinking mats to unsprayed plants, at least over a short distance. However, we did not investigate the impact of the herbicide treatment on the physiological state of the weevils. We also did not investigate the impact that a sinking mat with all of the sessile stages of the weevil would have on weevil populations.

It is generally believed that the mortality of eggs, larvae and pupae for the two weevil species, in herbicide treated water hyacinth plant material, is high (Cillers 1991; Haag & Habeck 1991). One study has shown it might be overestimated, as direct exposure of weevil eggs to glyphosate and paraquat based herbicides, resulted in normal hatching rates (Jianqing et al. 1996). The hatching rate for eggs inside leaf matter, treated with the herbicides (indirect treatment), was only reduced in the case of paraquat (Jianqing et al. 1996). The larval stage may be more susceptible as it has a very permeable body wall unlike the casing of eggs. No more literature is available on the effect of herbicides on immature stages of water hyacinth natural enemies.

6.1.3 Role of Active Ingredient

We showed that choice of active ingredient seemed to be important in the integrated control of water hyacinth. We showed that herbicides with the glyphosate as the active ingredient seemed to be the least toxic to the insects, those with glyphosate-trimesium and 2,4-D amine showed an intermediate toxicity, while the herbicides which have diquat as an active ingredient were generally toxic to both the mirid and the weevil.

6.1.4 Role of Surfactants

Although we probably did not fully investigate the insecticidal role of surfactants in the herbicide formulations used, we do have sufficient evidence to suggest that they do play a role in increasing mortality of the insects. We found that Rodeo, which is reputed to have the lowest surfactant content of the glyphosate herbicides used in this study, caused the least mortality in both the mirid and the weevil. In addition, we found that the two surfactants we tested, Add-2 and Agral did cause mortality of the mirid. A surfactant combined with an active ingredient can cause higher mortality than when used separately, at the same concentrations, as in the case of Midstream and Agral for both insect species, but not necessarily as in the case of Touchdown and Add-2.

6.1.5 Role of Herbicide Concentration

As expected, the concentrations of the herbicides are important and we found that at higher concentrations (usually far higher than recommended for water hyacinth control) increased mortality of both the weevil and mirid could be expected.

6.2 IMPLICATIONS FOR INTEGRATED CONTROL

If the integrated control management of water hyacinth is to succeed in South Africa, the control options for each site infested with the weed need to be thoroughly investigated and the control strategy needs to be well planned, taking into consideration the needs of water resource managers and biological control practitioners. We now know that some herbicides are better suited to integrated control where insects are involved, e.g. in systems where E. catarinensis is to play a role, a herbicide containing glyphosate as the active ingredient with very low surfactant content and preferably at low concentrations, should be applied. We are also starting to understand that insect refugia should be maintained, the size and number of which will depend on the insect species involved, the climate of the area and the function of the water body.

Insect populations on water hyacinth, in the long-term, serve to lessen the plant vigor and reduce the vegetative and sexual reproductive potential of the plants. This suggests that, providing the herbicide treatments are not incompatible with biological control, the water hyacinth mats might not recover as quickly in areas where the insects are present in comparison to where they are absent. Slower recovery times means that treatment using herbicides could be required less frequently resulting in savings to the water resource managers.

While it has been accepted that an integrated pest management approach is likely to achieve the best short- and long-term control of water hyacinth, the integrated control management plan for water hyacinth in South Africa, as currently practiced is largely a herbicide treatment plan. Far more emphasis has to be placed on the requirements of the biological control agents. Hopefully, this study has gone some way to achieving a better understanding of what is required to ensure that herbicide control programs are compatible with biological control programs to achieve the long-term goal of reducing the impact of water hyacinth on South Africa's aquatic ecosystems.

6.3 CONCLUSIONS

The integration of herbicides and biological control must rely on highly trained personnel. The results from this study indicate that the type and concentration of the herbicide to be used is vital. Therefore, instructions on the labels, regarding concentrations should be adhered to strictly and no other additives should be mixed with the herbicides. The type of biological control agents might further dictate the type and concentration that can be safely used without causing mass mortality of the biological control agents. The spray pattern is also vital. In essence, it should be decided what level of control is desired in each situation, and the dynamics of the control program should be designed based on sound scientific evidence. It is therefore recommended that management plans for each water hyacinth infestation be designed taking into consideration all control options and desired level of control.

6.4 FUTURE RESEARCH NEEDS

This project only considered two of the 5 arthropod species released as biological control agents on water hyacinth in South Africa. Although we can assume that the behaviour of the other weevil species, N. bruchi is likely to be the same as N. eichhorniae, the impact of herbicides on the mite, Orthogalumna terebrantis and the adults of the moth, Niphograpta albiguttalis still needs to be investigated.

The concept of leaving refugia to harbor biological control agents after herbicide application in the hope that they will maintain the weed population at a desired level needs to be revised. The practice of spraying 75% of the weed population, leaving 25% as a reserve for the insects in the hope that they will then maintain the population at 25% relies on too many assumptions. What is more likely to happen is that there will be a high percentage mortality of the biological control agents with the 75% reduction in habitat and herbicide toxicity (e.g. Eccritotarsus catarinensis). We have shown both in the laboratory and in the field, that there will be migration of adult weevils from treated to untreated plants. However, the water hyacinth along the edge of the mat will go into a rapid growth phase, due in part, to the release of nutrients into the water column from the decaying mat of the weed, and in part to the newly created area into which the plant can expand. This rate of expansion could exceed the rate of increase of the biological control agents and mat expansion into the cleared area is likely to occur. Obviously this scenario depends on many factors, including climate, population size of the natural enemies, physiological state of the natural enemies and nutrient status of the water. Therefore, refugia, or pockets of water hyacinth left untreated to harbor biological control agents will not preclude the need for follow-up treatments, but might reduce the frequency of sprays. Therefore, a thorough investigation into the population dynamics of both the weed and the natural enemy species needs to be undertaken.

In this study, we suggest that the use of herbicides which cause a slow die-off of the water hyacinth might be preferable to herbicides that rapidly kill the plant. The rationale behind this concept is that, we have shown that *N. eichhorniae* adults migrate from treated plants to untreated plants fairly soon after application of the herbicide, suggesting that the herbicides render the plants unpalatable to the weevils. Applying a slow acting herbicide to the plants would allow the adults to migrate to untreated plants, refrain from laying eggs on and inside treated plant material and also allow the late instar larvae and pupae to develop and move from the sinking mat. The same would apply to the other natural enemies released on water hyacinth, which have shorter life cycles than *N. eichhorniae*. A further avenue of research might be directed towards the use of sub-lethal doses of herbicides, which could serve to reduce the vegetative vigour of the water hyacinth plants, while allowing the natural enemies the opportunity to migrate.

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