1. Introduction

Water hyacinth (*Eichhornia crassipes*) is a South American plant that has spread widely and become one of the most damaging weeds in the world. South African biological control programs on the weed, which have been running for almost 20 years (Cilliers 1991), have been fairly successful. However, constraints such as climate and enriched water bodies restrict the impact of biological control agents (Hill & Cilliers 1999). Chemical control has also been successfully applied to confront the problem (Findlay & Jones 1996), but gives only temporary relief against the rapidly reproducing weed. The idea of applying integrated control by using a combination of chemical and biological methods and eventually phasing out the use of chemicals has been suggested (De Groot 1993).

In order to implement integrated control, information is needed on the susceptibility of natural enemies towards the herbicides used against water hyacinth. The arthropods that have been released as natural enemies on water hyacinth in South Africa are two congeneric weevil species (*Neochetina eichhorniae* and *N. bruchi*), a mirid (*Eccritotarsus catarinensis*), a moth (*Niphograpta albiguttalis*) and a mite (*Orthogalumna terebrantis*) (Julien & Griffiths 1998). Some of these species have undergone tests in the USA and Australia to determine their susceptibility towards herbicides. It has been reported that *N. eichhorniae* (Pellessier 1988) and *O. terebrantis* (Roorda *et al.* 1978) are susceptible toward diquat, while glyphosate is relatively non-toxic.

Herbicides that are registered for use on water hyacinth in South Africa are diquat, glyphosate, glyphosate-trimesium and terbutryn (Vermeulen *et al.* 1998). Another herbicide that is popularly used on water hyacinth is 2,4-D-amine as it results in rapid death of the weed (Divakar & Manoharan 1979). No work has been done in South Africa to determine the toxicity of any of these herbicides towards arthropod species, and little has been done in the rest of the world. Literature on the toxic effects of glyphosate suggests that it is relatively non-toxic towards a variety of tested organisms, although only a few invertebrates have been tested (Sullivan 1988; Tooby

1985). It was, however, found that a formulation of glyphosate was more toxic than the active ingredient alone, possibly as a result of added surfactants (Tooby 1985).

Another consideration for integrated control practices against water hyacinth is the management of arthropod populations subsequent to herbicide applications. Haag (1986a) suggested that natural enemies fleeing treated mats should be provided with healthy untreated plants to serve as a reserve. This will result in higher insect survival when plants die and sink, and better re-establishment when the water hyacinth population resumes its growth. Others suggest that some arthropods might be attracted to treated plants for reasons varying from softened petioles, increased sugar content and the release of a kairomone, which could have dire consequences for the arthropod population (Delfosse & Perkins 1977; Wright & Bourne 1990). The abilities of the different species to successfully escape sinking mats, the size of reserve mats and the distance of reserve mats necessary to implement such a system, have also been questioned (Bennett & Zwolfer 1968; Haag 1986a, b; Haag *et al.* 1988).

Even though we know little about the interaction between herbicides and biocontrol agents, integrated control of water hyacinth is currently pursued in South Africa. It is the aim of this study to make recommendations towards future integrated control practices of water hyacinth. The specific objectives are:

(i) To assess the relative acute toxicity of selected herbicide formulations for the mirid (*Eccritotarsus catarinensis*) and the weevil (*Neochetina eichhorniae*), with the purpose of determining the least toxic, and therefore the prefered herbicide to use during integrated control practices (Chapter 3).

(ii) To use feeding behaviour, i.e. a sub-lethal indicator of relative toxicity, to compare the effect of selected herbicide formulations on the weevil (Chapter 4).

(iii) To establish whether normal feeding continued on treated plant material by determining the feeding behaviour of the weevil on water hyacinth leaves treated with selected herbicide formulations (Chapter 5).

(iv) To investigate the behaviour of weevil populations of water hyacinth mats sprayed with two herbicide formulations, and to determine whether migration away or toward treated plants occurs (Chapter 6).

2. Literature review

THE BIOLOGY OF WATER HYACINTH

2.1.1 Taxonomy

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach (family Pontederiaceae, order Liliales)) originates in the Amazon basin of South America, but has now spread to almost every tropical and subtropical region of the world (Holm *et al.* 1977; Barrett & Forno 1982). The Pontederiaceae is a monocot family that includes six genera, with one of the seven species in the genus, *Eichhornia natans*, being indigenous to Africa and common in Ethiopia (Smith 1898; Divakar & Manoharan 1979; Aweke 1994). About 30 to 35 species of the family are aquatic and indigenous to the Americas and none are considered threatened or endangered in their native habitat (Eckenwalder & Barrett 1986; USDA 1999).

2.1.2 Morphology

Eichhornia crassipes is an erect, herbaceous, floating hydrophyte. The leaves are arranged in a rosette and held above the water while the roots and rhizome are submerged (Figure 2.1). Roots have visible root caps and may be purplish or black in colour if exposed regularly to light because of the presence of anthocyanines (Holm *et al.* 1977; McKnight 1993). Plants that are rooted in mud have white roots and may have a symbiotic relationship with micorhiza (Stent 1913; Penfound & Earl 1948; Wright & Purcell 1995).

The whorled leaves are divided into the base and the petiole, which is often bulbous, the narrow isthmus and the petiole blade, which is not a true lamina but the flattened part of the petiole (Penfound & Earl 1948; Holm *et al.* 1977). At low population densities the petiole bases tend to be swollen with aerenchyma tissue, but plants occurring in thick mats have large petiole blades and slender, elongated petiole bases (Center & Spencer 1981). Blades are heart-shaped and between 40 and 150 mm long and wide, and may act as sails if the plants travel downstream during dispersal (Holm *et al.* 1977).

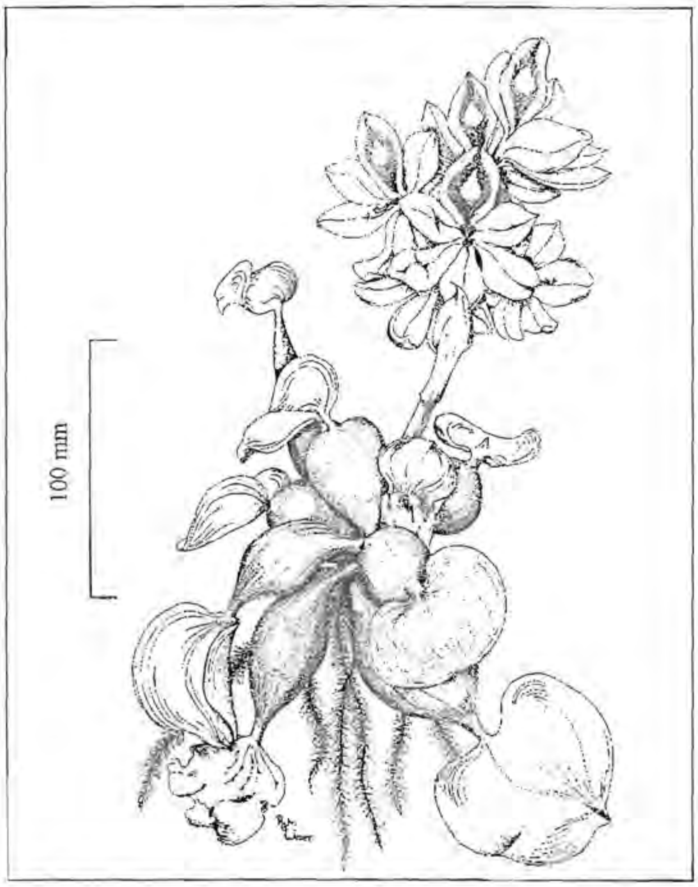


Figure 2.1 Morphology of *Eichhornia crassipes*, (Drawn by R. Weber, NBI, Pretoria).

2.1.3 Flowering and reproduction

Flowering is closely correlated to temperature with night temperatures above 21° required for flowering (Edwards & Musil 1975). The inflorescence is a spike with approximately eight flowers on a 300 mm long rachis. The flowers are white, lilac, blue, to purple and trimeric, with a dark purple and yellow blotch on the upper tepal, apparently serving as an insect nectar guide.

The presence of nectar and a nectar guide suggest that the plant is insect pollinated, but few insects have been observed visiting the flowers (Barrett 1977; 1980a; Wright & Purcell 1995). According to some studies sexual reproduction is limited within the new distribution range, possibly as a result of inefficient pollinators and environmental factors (Barrett 1980a, b). Others suggest that the dry pollen is proof of a self-pollination reproductive strategy, being accomplished when the wilted spike curves downwards into the water (Holm *et al.* 1977; Divakar & Manoharan 1979).

Tristyly, a type of genetic polymorphism, which is found in the flowers of water hyacinth, offers more proof for the sexual reproduction strategy theory (Barrett 1977; Barrett & Forno 1982; Harley 1993). The mid-styled flower form is widespread, the long styled form occurs less often and the short-styled form is scarce, but all three occur in the Amazon area, which suggests that the plant originated from this region (Barrett & Forno 1982). South African populations are of the mid-styled flower form, but long styled forms also occur in this country.

Each flower produces a capsule containing between 200 and 500 seeds that can remain viable for at least 15 years in the mud at the bottom of a pond (Stent 1913; Holm *et al.* 1977; Divakar & Manoharan 1979). Fluctuating water levels are characteristic of the natural habitat of the plant, causing exposure to sunlight which triggers germination (Center & Spencer 1981; Barrett & Forno 1982). Seeds may germinate on beach slimes and other periodically wet areas, and seedlings collected by the incoming tide or rising waters (McKnight 1993).

Although vegetative reproduction is considered the most important method of reproduction for water hyacinth, the potential of seeds to cause infestations has been underestimated. Large-scale herbicide treatment of the plant on the Nile River was followed by high seed germination among the dead plant material, which led to rapid re-infestation (Pettet 1964). In Japan plants are often damaged by low winter temperatures and regenerate mainly through seeds (Ueki & Oki 1979).

2.5.1 Physiology and tolerance

Water hyacinth mats have been reported to increase the loss of water through evapotranspiration by 1.4 to 3.7 times that of open water surfaces (Timmer & Weldon 1967; De Groot 1993; Singh & Gill 1996). Water hyacinth stomata are larger than those of other aquatic weeds investigated, which explains its high rate of evapotranspiration (Penfound & Earl 1948; Lallana *et al.* 1987). However, many of these studies have been questioned due to large edge effects in the experimental plots.

The plant grows best in a pH of 7, but can survive at a pH ranging from 4 to 10 (Penfound & Earl 1948; Wright & Purcell 1995). Aquatic micro environment changes studied by Ultsch (1973), showed that water under water hyacinth mats have a lower pH, lower dissolved oxygen content, higher dissolved carbon dioxide content and lower temperature than neighbouring open water surfaces. These, as well as nutrient changes are the results of the decomposition of high volumes of dead tissue (Stent 1913; Reddy & De Burk 1991).

The plant contains 93 to 96 % water, and has a high nitrogen, phosphate and potassium content (Edwards & Musil 1975; Holm *et al.* 1977; Imaoka & Teranishi 1988). It has the ability to remove and bio-accumulate nutrients and metals from polluted water, which makes it a successful inhabitant of eutrophied waters (Newman & Haller 1988; Zaranyika & Ndapwadza 1995; Mansor 1996). Two major contributing factors toward the water hyacinth problem in South Africa are the high nutrient content of water bodies, combined with hydrological factors such as impounded rivers which create stable water bodies (Edwards & Musil 1975).

Low temperatures limit the distribution range of the weed (Owens & Madsen 1995). Frost may lead to the loss of leaves, but the plant only dies when the rhizome tip freezes (Sastroutomo *et al.* 1978). Rooted plants fare better than free-floating plants when exposed to low temperatures (Owens & Madsen 1995). Forno and Bourne (1978) found that the death of taller leaves due to frost, increased light penetration and the production of shorter, more bulbous leaves.

2.5.2 Ecology

Water hyacinth behaves as a classical r-selected species throughout its native and introduced range. When conditions are suitable it germinates quickly, grows rapidly and sets copious amounts of seed. As the seeds are very long-lived, the plant is able to survive extreme conditions of cold and drought and re-infest the water body when conditions are again suitable. Its ability to survive in eutrophied water bodies increases its competitive abilities. The paucity of indigenous free-floating aquatic macrophyte species in South Africa provides a nearly vacant niche for this fierce competitor. Water hyacinth can facilitate succession by providing a platform for other water plants to germinate and establish, but it is mostly other weed species that take advantage of this opportunity (Penfound & Earl 1948).

2.2 NEGATIVE IMPACT

The negative impacts of water hyacinth in Africa can be divided into its effect on the environment; impact on socio-economy and on human health and recreation.

The weed competes directly with indigenous organisms for light and nutrients, but also changes the aquatic environment by making it unsuitable for native aquatic invertebrates, vertebrates and plants, thereby decreasing biodiversity (Wright & Purcell 1995; Findlay & Jones 1996; Orach-Meza 1997). A reduction in predatory fish; increased problematic algal blooms; and reduced photosynthetic rates by submerged plant species have been reported as a result of water hyacinth infestations (Penfound & Earl 1948; Nichols 1991).

In Africa, India and Sri Lanka, water hyacinth interferes with agricultural practices by blocking irrigation and drainage systems, and increasing the loss of water through evapotranspiration (Divakar & Manoharan 1979; Cilliers 1991; Room & Fernando 1992; Aweke 1994; Abdelgadir 1996). Hydro-electrical power generation is obstructed and sedimentation in rivers and dams is increased, shortening the life of water bodies (Aweke 1994; Wright & Purcell 1995; Findlay & Jones 1996; Watts 1997). Movement of boats is impeded, hindering transport, communication and tourism activities (Abdelgadir 1996; Orach-Meza 1997).

Water hyacinth infested water develops bad odours, taste, colour and turbidity, resulting in reduced quality. More important, it promotes the development of water-borne, water-based and water-related diseases e.g. malaria, encephalitis, filariasis, amoebic dysentry and typhoid fever (Orach-Meza 1997). Water hyacinth mats are the ideal hiding place for crocodiles and snakes that attack animal and human visitors to the water (Findlay & Jones 1996; Gough 1997; Orach-Meza 1997). Mats impede human access to the water, restrict fishing activities from the shore, and prevent bathing, swimming, water-skiing and other water sports (Aweke 1994; Abdelgadir 1996).

2.3 PEST STATUS AND SPREAD

Water hyacinth is considered the worst aquatic weed in South Africa, followed by *Salvinia molesta* D.S. Mitchell and *Pistia stratiotes* L., and two relatively new weed species: *Myriophyllum aquaticum* (Vell.) Verdc. and *Azolla filliculoides* Lam. (Cilliers 1991). In the last 100 years, water hyacinth has spread to almost every tropical and sub-tropical region of the world. Its introduction to some countries is summarised in Table 2.1.

Table 2.1 The introduction of water hyacinth in various countries, with date of introduction and references (adapted from Scott *et al.* 1979)

Country	Date of entry or first report	Reference
USA	1884	Edwards & Musil 1975
Egypt	1879/1893	Scott *et al.* 1979
Australia	1890/1894	Stent 1913; Wright & Purcell 1995
Java (Indonesia)	1894	Edwards & Musil 1975
Siam (Thailand)	1901	Scott *et al.* 1979
Bengal (India)	1902	Divakar & Manoharan 1979
China	1902	Divakar & Manoharan 1979
Ceylon (Sri Lanka)	1905	Edwards & Musil 1975
Fiji Islands	1905	Scott *et al.* 1979
Malaysia	1907	Scott *et al.* 1979
South Africa	1908/1910	Stent 1913; Harley 1993
Burma	1910	Scott *et al.* 1979
Philippines	1912	Scott *et al.* 1979
Reunion and Madagascar	1920	Scott *et al.* 1979
Borneo	1926	Scott *et al.* 1979
Zimbabwe	1937	Chikwenhere 1994
Hawaii	1946	Scott *et al.* 1979
Okinaqa (Ryukyu Islands)	1952	Scott *et al.* 1979
Congo Brazzaville	1952	Scott *et al.* 1979
Sudan	1956	Pettet 1964
Senegal	1964	Scott *et al.* 1979
Ethiopia	1965	Aweke 1994

According to Harley (1993), the first infestations in Africa occurred in Egypt, South Africa and the Congo River (Democratic Republic of Congo). It has recently caused serious problems in Nigeria, Cote d'Ivoire, on Lake Victoria and on Lake Chivero (Zimbabwe) (Harley 1993). Benin, Ghana, Niger and most eastern and southern African countries, except Botswana, Namibia and Lesotho experience problems with the weed (Harley 1993).

Water hyacinth was first recorded in the Western Cape Province of South Africa in 1913, and soon spread and caused problems in Kwazulu-Natal rivers (Figure 2.2) (Edwards & Musil 1975; Cilliers 1991). It has become especially troublesome in the Kwazulu-Natal coastal areas and along the Vaal River (Edwards & Musil 1975). Since its introduction, it has caused problems in the Swartskops River (Eastern Cape Province), Hartebeespoort Dam, Crocodile and Vaal River (Edwards & Musil 1975). Its importance as a weed was acknowledged in 1977, when the infestation on the Hartebeespoort Dam increased to cover 60 % of the water surface and chemical control had to be implemented (Scott *et al.* 1979).

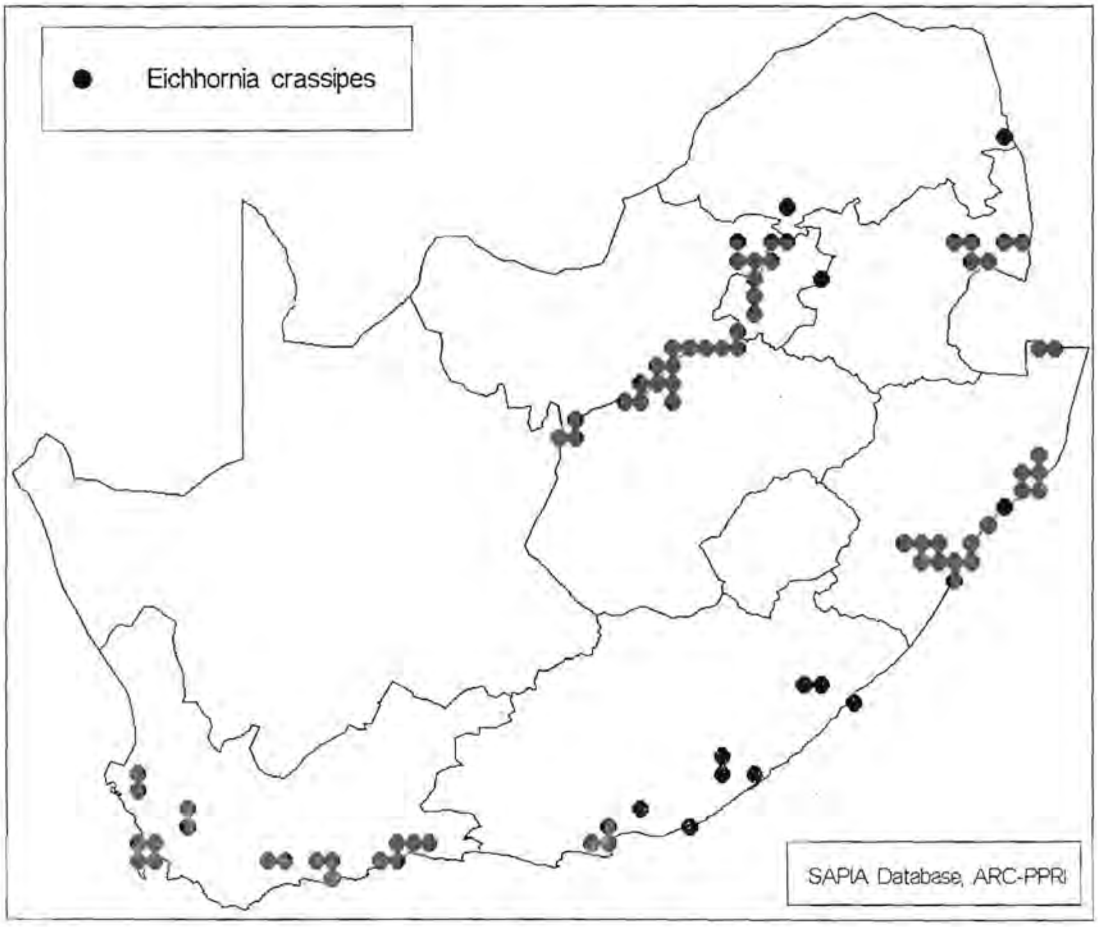


Figure 2.2 Distribution of *Eichhornia crassipes* in South Africa (Drawn by L. Henderson, Plant Protection Research Institute).

Human transport of the plant for aesthetic reasons was originally the main method of distribution. Further distribution within continents and countries occurred through natural water movement, as plants were swept downstream or seeds carried in mud on animal feet (Edwards & Musil 1975). Human transport of the plant still plays a big role in its spread as plants are often used as cushions in boats or are snagged by boats and carried upstream over great distances (Holm *et al.* 1977). In Thailand, China and Japan, the use of water hyacinth for pig fodder has contributed considerably to its dispersal through the region (Edwards & Musil 1975).

Eutrophied water plays an important role in creating the ideal environment for water hyacinth to grow in South Africa. The dumping of sewage and other effluent into rivers, has led to serious infestations in the past (Marshall 1993; Watts 1997). By interfering with the hydrology of rivers and providing stable or slow moving water bodies, we further contribute to its success (Edwards & Musil 1975). In its native range, *E. crassipes* prefers slow moving water bodies with high nutrient content. In South America population densities seldom reach high numbers as natural enemies and competing plant species suppress the weed (Wright & Purcell 1995).

2.4 UTILISATION

Many uses for the weed have been suggested, but none have proved to be very profitable on a large scale or have led to successful control of the weed as yet. A short list of the most popular uses is provided in Table 2.2. For more detail on practical uses for water hyacinth, see Little (1968); Holm *et al.* (1977); Edwards and Musil (1975); Gopal (1987) and Findlay and Jones (1996).

To utilise water-weeds on an economically profitable basis, large industrial developments and a constant source of plants are necessary (De Groot 1993). The role of small-scale use in the spread of the weed also causes concern (De Groot 1993). Removal and transport of the plant material contribute to spreading the seeds and plants that can survive dry periods and infest new areas.

Table 2.2 Some popular uses for water hyacinth

Aquarium plant	Homeopathic medicine (Indians)
Animal food (unpalatable, must add supplements)	Human consumption (itching may occur)
Detergent (use ash)	Insecticide
Cigar wrappers	Paper making
Colour dye	Recovering polluted water
Fertiliser (high nitrogen)	Source of chemicals, carotene, vitamin A
Fish traps	Weaving baskets, mats, ropes
Growth medium for mushrooms	

2.5 CONTROL

2.5.1 Introduction

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

2.5.2 Mechanical control

Mechanical control or the manual removal of water hyacinth is invariably the initial control option exercised once a water hyacinth infestation has become problematic. However, some operators consider this option impractical in infestations larger than about one hectare because of the rapid rate of increase of the weed, and its weight as a result of high water content.

Machines such as harvesters, cutters, triturators, saw-boats and crusher plants are expensive to build, while their running costs are high (Gutierrez *et al.* 1996). The boats that carry these machines are usually too large to enter shallow waters, and patches of weed are left behind in these areas, contributing to regrowth. As a result, these types of harvesters are unsuccessful in African river systems, which are often meandering and shallow. Disposing of waste products after removal is problematic as dumped water hyacinth plants and seeds can cause re-infestation or spread. Drying the plants in the sun followed by burning and burying was suggested, but would be very labour intensive (Du Toit 1938). Mechanical control is labour intensive, slow and of a temporary nature, but can be applied successfully between herbicide applications to contain regrowth as part of an integrated control programme (Cilliers *et al.* 1996; Findlay & Jones 1996; Reinhardt 1997).

2.5.3 Chemical control

Herbicides can control infestations immediately and can be used on large infestations. Disadvantages of herbicide use are the expense associated with continued application and the possibility of negative effects on the environment. However, putting the problem into perspective, water hyacinth control is an expensive long-term management commitment, irrespective of control method or combination thereof. Another consideration is that the majority of herbicides currently used for water hyacinth control, whilst not ecologically benign, impact the environment considerably less than mats of water hyacinth.

Chemical control has been practised against water hyacinth since the early 1900's. Sodium arsenate was first used in Florida in the USA but discontinued after cattle poisoning occurred in 1905 (Center 1975). A number of other herbicides have since been used. Herbicides registered for use on water hyacinth in South Africa are glyphosate, glyphosate-trimesium, diquat and terbutryn (Vermeulen *et al.* 1998), while 2,4-D is still used in many other countries. Water hyacinth is apparently very susceptible to diquat and 2,4-D amine formulations (Divakar & Manoharan 1979; Wright & Purcell 1995).

Glyphosate products have been safely and successfully used in this country, but are relatively expensive compared with 2,4-D amine, diquat and glyphosate-trimesium formulations, which are preferred in many African countries, the USA, Mexico, Australia, Taiwan, India and Malaysia (Wang *et al.* 1994; Wright & Purcell 1995; Guitierrez *et al.* 1996; Mansor 1996; Singh & Gill 1996).

Herbicides are applied from aircraft, boats and from the ground, often with the help of mounted spray equipment (Findlay & Jones 1996). Large infestations are best contained by aerial spraying from fixed wing aircraft, helicopters or micro-light aircraft. Follow-up application can be done from boats or with knapsack sprayers, depending on the size and location of the infestation. Especially during aerial application, care has to be taken to avoid herbicide drift to non-target vegetation. An evaluation of application methods specifically for the control of water hyacinth in South Africa was done for the Department of Water Affairs (Anonymous 1990).

Successful chemical control depends on skilled operators who maintain a long-term follow-up programme, which continually controls re-infestations from scattered plants and germinating seed. Any herbicide programme against the weed requires a commitment to continued operation of unlimited duration. It is the lack of a rigorous follow-up regime that has often led to the failure of chemical control programmes.

For further discussion of the active ingredients and formulations used in this study refer to Appendix 1.

2.5.4 Biological control

(a) Background

While mechanical and chemical control is viewed as short-term, immediate control options, biological control is perceived as a long-term or sustainable control option. Biological control takes a long time and dedication to implement as insect populations have to be released and established, and reach levels that can have an impact on the weed population.

Natural enemies considered for release against water hyacinth include phytophagous insects, mites, pathogens, a snail, herbivorous fish and the manatee (Andres & Bennett 1975; Divakar & Manoharan 1979; Cassani *et al.* 1981). Cattle, hippopotami and even ducks have been observed feeding on the weed, but their contribution toward its control, is doubtful.

Arthropods are considered ideal biocontrol agents because of their specificity towards their host plant, which reduces the risk of the species becoming a pest on other indigenous plant species. Biocontrol agents undergo vigorous testing before release, including starvation and multiple choice tests (Bennett 1977), in addition to extensive data collection from the region of origin and the literature. Selected species must be host-specific feeders and preferably have a short life cycle, reproduce rapidly and be able to adapt to a new environment.

The United States Department of Agriculture in 1961 initiated the biological control programme on water hyacinth. Since that time there have been a number of surveys of water hyacinth in South America (Center 1994), with the purpose of identifying natural enemies 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 water hyacinth in 1971 and since then a further six natural enemies have been released around the world (Appendix 2) (Julien & Griffiths 1998).

It is not necessary for biological control agents to kill whole plants. Many natural enemies cause constant injury to the plant, thereby leading to reduced reproductive rates, decreased leaf size, discarding of leaves and changes in nutrient composition (Center 1980; Center & Van 1989).

(b) Released natural enemies

The two weevil species, *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) are the most widely used agents and have been released in 29 and 26 countries respectively (Julien & Griffith 1998). The biology of both species is well documented (Center 1994), rearing and releasing techniques have been refined and a universal post-release evaluation method has been developed.

The two weevils have contributed to the control of the weed world-wide. The release of only these two species have led to successful control in 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 combination with the two weevil species has contributed to the control of water hyacinth in Australia and the USA (Center 1994). A number of pathogens have been recorded from water hyacinth and is contributing towards control, but *Cercospora rodmanii* Conway (Fungus: Hyphomycetes) (= *Cercospora piaropi* Tharp.) is the only one that has been intentionally introduced as a biological control agent (Julien & Griffiths 1998).

Although these species result in effective biological control of the weed in some areas, results in other areas have been variable. This prompted biological control practitioners to investigate additional natural enemy species. The moth *Xubida infusella*, the mite, *Orthogalumna terebrantis* and the mirid, *Eccritotarsus catarinensis* have also been released in a number of countries (Hill & Cilliers 1999) and further surveys for additional agents are being carried out.

(c) History of biological control in South Africa

The biological control programme against water hyacinth in South Africa was initiated in 1973 and the weevil, *Neochetina 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; Hill & Cilliers 1999). 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 (*Neochetina eichhorniae* and *N. bruchi*) [1], the pyralid moth (*Niphograpta albiguttalis*), the water hyacinth bug (*Eccritotarsus catarinensis*), the galumnid mite (*Orthogalumna terebrantis*) and the fungal pathogen (*Cercospora rodmanii*). These species have been released and have established at a number of sites throughout South Africa.

[1] Consult Appendix 2 for a list of arthropod species released worldwide on water hyacinth, and Appendix 3 for a detailed biology of the arthropod species released in this country.

Biological control of water hyacinth has been very effective in some areas of the country, such as New Years Dam at Alicedale (Eastern Cape Province). The release of *Neochetina eichhorniae* in this area in 1991 resulted in an 80 % reduction of the water hyacinth mat by 1994. In the insect "reserve" sites along the Vaal River, water hyacinth mats infected with *Neochetina eichhorniae* and *Niphograpta albiguttalis,* have been shown to collapse within four years if left unhindered (Hill & Cilliers 1999).

Four more insect species are being considered for release in this country, they are the moth *Xubida infusella* Walker (=*Acigona infusella* Walker), the grasshopper *Cornops aquaticum* Bruner, the planthopper *Megamelus scutelavis* and flies of the genus *Thrypticus* (Hill & Cilliers 1999).

(d) Success of biological control

In spite of the successes discussed above the outcomes of the biological control programme have been variable. Hill and Cilliers (1999) have suggested several factors that might have constrained the impact of the biological control agents. Firstly, the inability of some of the species to proliferate 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 or subtropical climates. In South Africa, many of the water hyacinth infestations occur in areas at high elevation, characterised by cold winters and frequent frosts. This means that the active growing period of the plant is limited, and therefore the population growth of the biological control agents is limited to approximately six months of the year.

Secondly, many of the aquatic ecosystems that are invaded by the weed in South Africa are enriched with nitrates and phosphates that allow the weed to proliferate at such a high rate that insects are unable to suppress it. An example of this is Hammarsdale Dam (KwaZulu-Natal Province) where the water hyacinth mirid (*Eccritotarsus catarinensis*) and the weevil (*Neochetina eichhorniae),* have attained very high population levels. However, the natural enemies appear to have had little impact on the plants, possibly as they are fertilised by enriched effluent from a nearby waste water treatment plant (M.P.Hill[2] Pers. comm.).

Finally, regular removal of water hyacinth infestations with their natural enemy populations through flooding and chemical and mechanical control programmes has also had an effect on the biological control of water hyacinth. Re-infestation of cleared or treated sites by seeds and scattered plants occurs rapidly, and populations of the plant proliferate in the absence of natural enemies.

The variable success of the biological control programme 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 able to achieve a greater level of control over the restricted growing period of the plant. Secondly, to investigate an integrated control approach for the weed, which would incorporate aspects of mechanical, chemical, biological, hydrological and nutrient control 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.

2.5.5 Integrated control

(a) Introduction

Integrated control is the use of a combination of methods to control the weed. The aim is to phase out short-term physical and chemical control and in the long-term rely completely on biological control to suppress the weed (De Groot 1993). The methods chosen depend on the level of control desired, the hydrological nature of the site, time of the year, intensity of the infestation, and resources such as labour and infrastructure available.

An example of integrated control is the management of Lake Chivero in Zimbabwe. Between 1990 and 1991 a serious infestation, partly as a result of eutrophied water, could only be contained by using aerial herbicide application. Natural enemies (*Neochetina* weevils) had been released previously and were released following this

[2] Hill, M.P. PPRI, ARC, Private Bag X134, Pretoria, 0001

herbicide application, while a "floating fence" was used to prevent new plants from entering the lake (Chikwenhere 1994; Watts 1997). Chikwenhere (1994) highlights an important aspect of integrated management, when he emphasises the necessity of a site-specific plan.

(b) Combining herbicides and natural enemies

Charudattan (1986) documented the development of a mycoherbicide containing *Cercospora rodmanii*. He showed that 100 % control can be attained within seven months after applying the mycoherbicide with both weevil species, while used separately neither of them resulted in sufficient control. The best results were obtained when the mycoherbicide was applied and followed three weeks later, by an application of a moderate concentration of the herbicide 2,4-D.

Caunter and Mohamed (1990) found in a laboratory trial that on its own *Neochetina eichhorniae* could not contain water hyacinth significantly when released. However, when combined with the pathogen, *Myrothecium roridum*, good results where obtained after four weeks. When *Myrothecium roridum* was applied with low rates of 2,4-D, it gave better control than when applied without herbicide (Liyanage & Gunasekera 1989). Center *et al.* (1982) suggested that combining a plant growth retardant with the weevil may give successful control of water hyacinth. It has subsequently been demonstrated that paclobutrazol, another growth retardant, also combined with *N. eichhorniae*, can result in effective control (Van & Center 1994).

Delfosse and Perkins (1977) noted the presence of a kairomone released by damaged plants, and suggested it could be a phagostimulant or oviposition stimulant that attracts *Neochetina eichhorniae* and *Orthogalumna terebrantis* to young plants, following herbicide application. Treated plants may also exhibit an increase in sucrose, as found in plants after treatment with glyphosate. Delfosse and Perkins (1977) suggested spraying herbicides at a low concentration and leaving plants to release the kairomone that would attract insects, to control the weed further. Wright and Bourne (1990) suggested that *Niphograpta albiguttalis* preferred feeding on leaves sprayed with 2,4-D amine as it softened the petiole.

When combining chemical and biological control, it is necessary to determine the direct and indirect negative effects that herbicides may have on the insects. It was suggested that reservoirs of unsprayed plants be left for insects to escape to, from sinking mats (Bennett & Zwölfer 1968; Haag 1986a). This recommendation relies on the assumption that the insects have the ability to disperse and are not directly affected by the herbicides. The movement of *Neochetina* weevils from plants sprayed with 2,4-D was tested, and although there was some movement of adult weevils, a high mortality of eggs, larvae and pupae occurred (Haag 1986a, b; Haag *et al.* 1988). The timing and pattern of spraying may be important. If spraying coincides with the migration period, when weevils normally develop wing muscles, better survival may be achieved (Haag 1986b).

3. Acute toxicity of selected herbicides towards *Eccritotarsus catarinensis* and *Neochetina eichhorniae*

3.1 INTRODUCTION

The aim of this study was to test whether the herbicides currently used in the control of water hyacinth in South Africa are acutely toxic, and therefore incompatible with two of the natural enemies released as biological control agents.

The biological control of water hyacinth in South Africa relies on five arthropod species, only *Eccritotarsus catarinensis* (water hyacinth mirid) and *Neochetina eichhorniae* (water hyacinth weevil) were chosen for inclusion in this study. The weevil, *Neochetina eichhorniae* is presently the most widespread of the agents released in South Africa on water hyacinth and the results on this species can be extrapolated to its congeneric *N. bruchi*. The mirid was selected because it is the most recently released natural enemy on water hyacinth worldwide, 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.

Previous studies have shown that adults of another arthropod species used in the biological control program on water hyacinth, the mite *Orthogalumna terebrantis*, is very sensitive to exposure to diquat, but not to 2,4-D, glyphosate or paraquat (Roorda *et al.* 1978). Other studies have shown that active ingredients combined with surfactants can be toxic even when neither caused any mortality when applied separately to the weevil *Neochetina eichhorniae* (Grodowitz & Pellessier 1990; Wright & Skilling 1987).

The ability of natural enemies to survive after exposure to the selected herbicide formulations and surfactants as well as combinations will determine recommendations for integrated control of the weed.

3.2 MATERIALS AND METHODS

3.2.1 Background

Lethal concentration tests (LC_{50} tests) are often used to quantify the toxicity of chemicals to living organisms. Although this criterion possesses recognizable limitations, it usually provides the first and most statistically reliable measure of the toxic effect a chemical may impose on an organism (Forbes & Forbes 1993). The test is a widely used and trusted method to determine the concentration of a substance that causes 50 % of the organisms in a test population to die. Only a few concentrations need to be tested to determine lethal doses with the help of computer programs such as LSTATS P/PROBAN (Van Ark 1983). The response of LC_{50} tests does not have to be mortality (Jagers op Akkerhuis *et al.* 1999), but quantifying any response other than death is less objective and therefore less accurate.

Toxicity tests can be classified according to the experiment duration relative to life span of the organism. Acute toxicity tests usually last between 24 to 48 hours for insects, rats, fish and birds (Landis & Yu 1995). The acute toxicity experiments done during this study, continued for 120 hours and were monitored every 24 hours.

Insects from natural populations of the test organisms were collected randomly for this experiment, to give a test population representative of the genetic composition in the field. Unfortunately this precluded knowledge about 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).

The herbicide concentrations were determined according to recommendations by manufacturers as well as preliminary tests (Table3.1 & 3.2). Manufacturers recommend that Touchdown be used with the surfactant Add-2 while Midstream is recommended with the surfactant Agral. Both were tested in combination with and without this surfactant to determine an increased toxicity as a result of the surfactant.

The exposure scenario determines the uptake efficiency and ultimate effect on the insect. The droplet application method was designed to portray a possible field scenario, while not overestimating the potential toxicity of the herbicides as a complete emergence in the solution might have. Weevils and bugs were exposed to a 20 µl droplet, applied on the dorsal surface of the insect. In practice, droplets sized 300 to 400 µl are specified by some herbicide labels (Sanachem 1998). As large droplets tend to submerge and drown insects during the application process, it was decided to apply smaller droplets, as only the relative toxicity of herbicides was under investigation.

Egg, larval, nymphal and pupal mortality were not tested, but it is expected that most of the sedentary immature life stages inside the water hyacinth plant material, will be killed as a result of deteriorating plant quality and rapid plant death following herbicide application (Haag 1986b).

3.2.2 *Eccritotarsus catarinensis*

In this acute toxicity test, the water hyacinth bug was directly exposed to different herbicides at various concentrations and combinations with surfactants (Table 3.1 & 3.2), and mortality was noted. The insects were collected approximately two days before treatment, from ponds forming part of the breeding program at the PPRI in Pretoria and kept in tubs with an abundance of fresh, severed leaves as food. Insects were sorted in groups of eight unsexed adults in small petri-dishes (65 mm diameter), lined with moist filter paper a day before treatment to acclimatise. The sex of the bugs was not determined as this is a difficult procedure causing further stress to the insects. Food in the form of water hyacinth leaf pieces, sized 20 x 30 mm, 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 darkness.

Table 3.1 Information on herbicides selected for testing: formulation composition and recommended dosages

Brand name	Manufacturer	Content			Recommended
				g a.e/l[1]	Dosage (% vol/vol)
2,4-D amine	Provided by Sanachem	2,4-D 480 amine		480	2.00 – 6.00
Mamba 360 SL[2]	Sanachem	Glyphosate SL (IPA salt)		360	2.00 – 6.00
Midstream[2]	Zeneca	Diquat (Dibromide salt)		373.5	3.75 – 5.00
Mon 52276	Monsanto	Glyphosate (IPA salt)		360	2.00 – 6.00
Muster[2]	Zeneca	Glyphosate (Trimethyl sulfonium salt)		330	2.00 – 3.00
Rodeo[2]	Monsanto	Glyphosate (IPA salt)		480	4.50 – 9.00
Roundup[2]	Monsanto	Glyphosate (IPA salt)		360	2.00 – 6.00
Roundup Ultra[2]	Monsanto	Glyphosate		360	2.00 – 6.00
Touchdown[2]	Zeneca	Glyphosate (Trimethyl sulfonium salt)		480	2.00
Tumbleweed[2]	Enviro Weed Control	Glyphosate (IPA salt)		240	2.00 – 6.00
Surfactants					
		Description			
Add-2[2]	Zeneca	Spreader adjuvant (with Touchdown)	Polysaccharide (hexitan ester)	600	0.20 – 0.30
Agral[2]	Zeneca	Wetting and sticking adjuvant (with Midstream)	Alkylated phenol-ethylene oxide concentrate	940	0.75

[1] g a.e/l = gram acid equivalent per litre
[2] Registered under Act 36 (1947)

Table 3. 2 Concentrations of herbicide formulations and surfactants used in acute toxicity tests on two insect species

Herbicide or Surfactant	Active ingredient		Concentration used in toxicity tests (% vol/vol)											
		g /l	*Eccritotarsus catarinensis*						*Neochetina eichhorniae*					
2,4 D	2,4-D amine	480	1	3	9	18			1	3	4	9	12	24
Add-2	Adjuvant	600	0.2	0.3	0.6	1	2		0.2	0.3	1	2.4		
Agral	Adjuvant	940	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-trimesium	330	2	3	12	24			2	3	4	12	24	
Rodeo	Glyphosate	480	1.5	3	4	9	18		1.5	3	4	9	12	18
Roundup	Glyphosate	360	1	2	3	4	12	24	1	2	3	4	12	24
RoundupUltra	Glyphosate	360	3	4	12	24			3	4	12	24		
Touchdown	Glyphosate-trimesium	480	2	4	8	20			2	4	8	12	24	
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% Add	X	
4% TD & 0.2% Add		X
4% TD & 0.4% Add	X	X
8% TD & 0.8% Add		X
0.9375% MS & 0.1875% Ag		X
1.875% MS & 0.375% Ag	X	X
3.75% MS & 0.75% Ag	X	X

• TD = Touchdown; Add = Add2; MS = Midstream; Ag = Agral

The bugs were cooled in a 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 mortality 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. Three replicates of eight insects were done for each of the treatments.

3.2.3 *Neochetina eichhorniae*

Weevils were captured from the field approximately two weeks before initiation of the experiment and kept in plastic tubs with an abundance of fresh, severed leaves as food. 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, at least 48 hours before commencement of experiment to acclimatise. Insects were directly exposed to different herbicide concentrations (Table 3.2) by applying a 20 µl droplet dorsally onto the elytra of the insect. Food was provided daily in the form of a fresh water hyacinth leaf. Temperatures varied between 22 and 28°C and they were exposed to a light regime of 16 hours light, 8 hours darkness. Mortality was noted every 24 hours up to 120 hours. Three replicates of six insects each were used.

3.2.4 Statistical procedures

The Proban analysis program used to calculate LC_{50} values in this study, determines a regression of mortality against concentration of the formulation in water (Figure 3.1). Concentration values are transformed to logarithms, a normalization procedure considered standard practice in toxicity testing (Van Ark 1983). Normalizing the data is a way to compensate for the asymmetrical population reaction curve, usually a result of the high tolerance exhibited by a few insects (Van Ark 1983). The log concentration value that causes 50% mortality can then be anti-logged to obtain a predicted LC_{50} value, which represents the concentration that is expected to cause 50% mortality.

The LC_{50} value indicated on Figure 3.1 gives the concentration expected to result in mortality of 50% of the population at 48 hours after exposure to the toxin. The linear correlation coefficient (r) gives the accuracy of the estimated regression line (■), or the fit of the line to the observed mortality (υ).

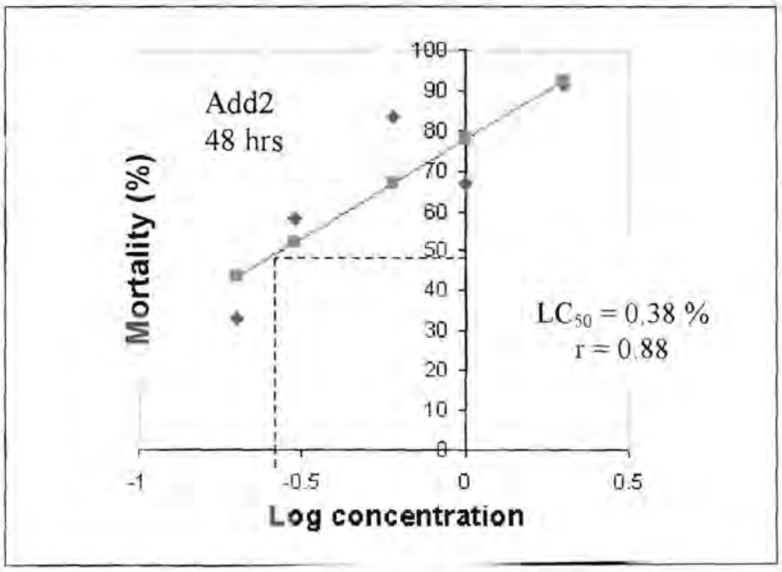


Figure 3.1 The regression line obtained for *Eccritotarsus catarinensis* after 48 hours exposure to the surfactant Add2. The estimated LC_{50} value (percentage of formulation in water) and linear correlation coefficient (r) are indicated.
■ = predicted mortality
υ = observed mortality

3.3 RESULTS AND DISCUSSION

3.3.1 Determining LC_{50} values

Both Proban analysis (P) and linear regression (R) values are listed, with linear correlation coefficient values (r) where available, in Appendix 4. 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 number of concentrations used and could not be used in all cases.

When comparing the more accurate Proban values with those of the regressions, they clearly show the same trends but are in most cases slightly higher. As the regression curves could be fit for all herbicides examined in this study, 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.

3.3.2 *Eccritotarsus catarinensis*

(a) 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 Rodeo, Roundup and Touchdown this was not the case (Figure 3.2h, i, k). 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 LC_{50} or great variation of the value over time is the result of such a non-linear relationship. Roundup at 1 and 2 %, for example, caused more mortality than did 12 % (Figure 3.2i) and resulted in a high LC_{50} value of 61.04 % at 24 hours (Table 3.4). For Touchdown the LC_{50} value dropped from 112.60 % at 24 hours to 0.003 % at 120 hours (Table 3.4), showing great variation over time. For these herbicides the toxicity cannot be fully explained by simply considering the LC_{50} values.

Observations made during experiments suggest that non-linear relationships may be a result of droplet surface tension that increases 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. Higher concentrations were sticky, thus possibly inhibiting movement or interfering with gaseous exchange by covering spiracles and therefore having a different "toxic" effect on the insect than lower concentrations. 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.

(b) Relative toxicity

General

The percentage mortality of adult *Eccritotarsus catarinensis* treated with herbicides is illustrated in Figure 3.2, and means and standard deviations appear in Table 3.3. Estimated LC_{50} values for *E. catarinensis* are illustrated in Figure 3.3. Also see Appendix 4, 5, 6 and 7 for statistical analysis.

Bugs treated with herbicides and control insects showed increased mortality with time (Figure 3.2, Table 3.3). The mortality of control insects increased from 8.75 % at 24 hours to 21.25 % at 120 hours. This could simply be a result of natural mortality, or can be partly attributed to increased stress as a result of confinement.

LC_{50} values are illustrated in Figure 3.3 where herbicides are organized from most to least toxic on the x-axis at the specified time, with recommended dosages indicated for further comparison. Both Agral and Midstream show a LC_{50} value lower than the recommended dosage range (Figure 3.3a). This means that at the concentrations recommended for the control of water hyacinth, more than 50 % mortality of *E. catarinensis* can be expected. For Roundup Ultra (Figure 3.3a), some of the recommended dosages cause 50 % mortality, while other lower recommended dosages cause less. For Mamba, none of the recommended dosages will cause more than 50 % mortality because the recommended dosages lie well below the LC_{50} value (Figure 3.3a-e).

Table 3.4 describes the overlap between the LC_{50} value and the recommended dosage for each herbicide. This description or rating is based on the results obtained during this study and is limited to the specific species. It is however, the first indication of how insects will react to the tested herbicides and therefore the preferred herbicides to use in integrated control. This relative scale indicates for example that 2,4-D amine has a toxic effect on *E. catarinensis*, when used at recommended dosages, while Roundup is considered safe at recommended dosages.

Surfactants

The surfactant Agral, showed a LC_{50} value lower than 0.75 %, its recommended dosage, throughout the 120 hours. Its effect on the species is therefore described as toxic (Table 3.4). The other surfactant Add-2 also exhibits a low LC_{50} value between 0.63 and 0.33 %, but as this never overlapped with the recommended dosage range of 0.2 to 0.3 %, it is labeled safe (Table 3.4).

Formulations

Midstream (diquat) was the most toxic herbicide throughout the 120 hours. Its LC_{50} value of 0.38% at 24 hours fall far below the lowest recommended dosage of 3.75 % (Figure 3.3a). Therefore Midstream is labelled toxic in Table 3.4. It would have been informative to test another diquat-based herbicide as this could have indicated whether the toxic nature of the herbicide could be ascribed to the active ingredient or to the additives of the formulation.

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 LC_{50} 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 29.20 % (24 hours) and 50.00 % (120 hours) mortality at both 1 and 2 % concentration (Table 3.3). Roundup is however considered to be relatively safe as it did not cause more than 50 % mortality at recommended dosages during the test period (Table 3.3). Mamba, Mon 52276 and Tumbleweed all exhibited a moderate toxic effect. Mamba was the least toxic of the three as it never reached a LC_{50} value that falls within the recommended dosage range (Figure 3.3a-e). Tumbleweed was the most toxic of the three and is described as hazardous (Table 3.4).

Glyphosate-trimesium-based Muster is considered toxic, as the estimated LC_{50} values fall below the recommended dosages throughout the experiment (Figure 3.3a-e). Another glyphosate-trimesium-based herbicide, Touchdown, showed an initial high LC_{50} value as a result of the non-linear relationship described earlier, appearing non-toxic. Initially Touchdown appeared to have a low toxicity, but at the recommended dosage of 2 %, it resulted in more than 50 % mortality of the population at 120 hours and is therefore labelled toxic (Figure 3.2k, Table 3.4).

2,4-D amine is considered toxic with LC_{50} value of 3.15 % at 24 hours, reduced to only 0.93 % at 120 hours, with recommended dosages of 2 to 6 % (Table 3.4).

Combinations

Combinations of Midstream with the surfactant Agral were more toxic than when applied separately (Figure 3.4a). At 120 hours, Touchdown at 2 % combined with 0.2 % Add-2 resulted in less mortality than 2 % Touchdown alone (Figure 3.4b). Only double the recommended dosage of 4 % Touchdown and 0.4 % Add-2 increased the toxicity of the combination appreciably.

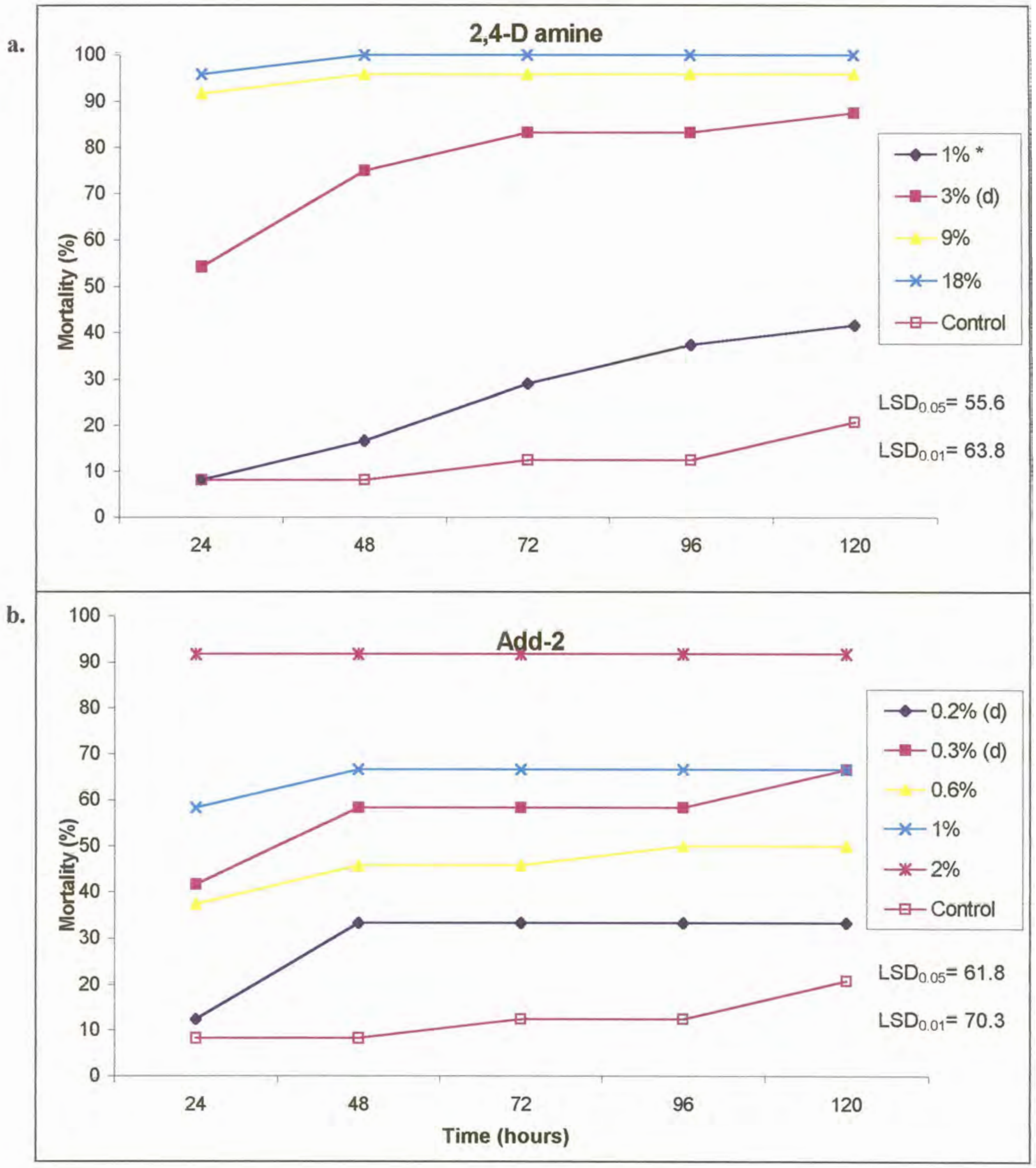


Figure 3.2 Percentage mortality of *Eccritotarsus catarinensis* as a result of direct treatment with selected herbicides

* percentage of specified herbicide applied in water medium

(d) dosage recommended in practice

$LSD_{0.05}$ Least significant difference ($p<0.05$ or <0.01) in %

c.

d.

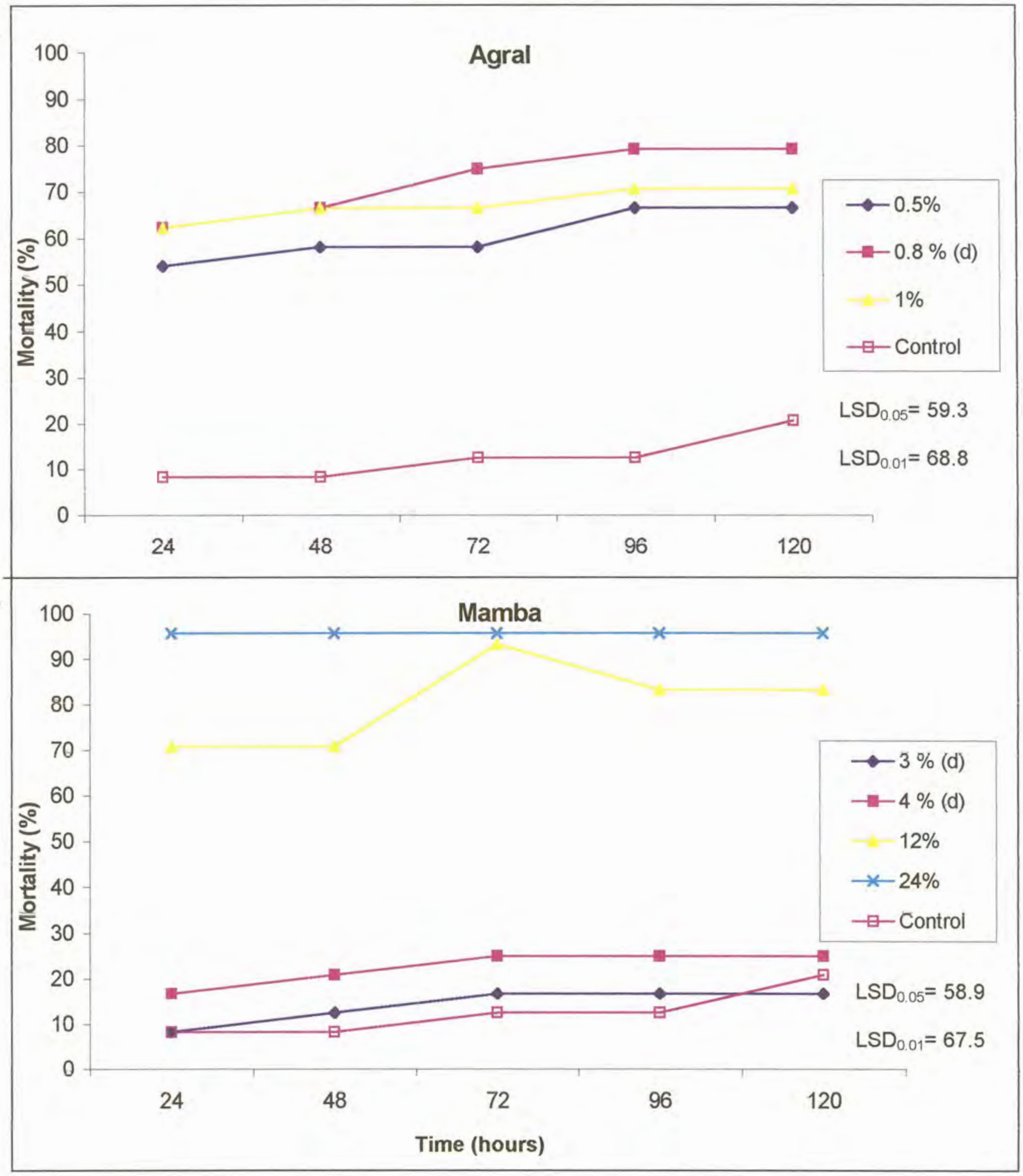


Figure 3.2 Continued

e.
f.

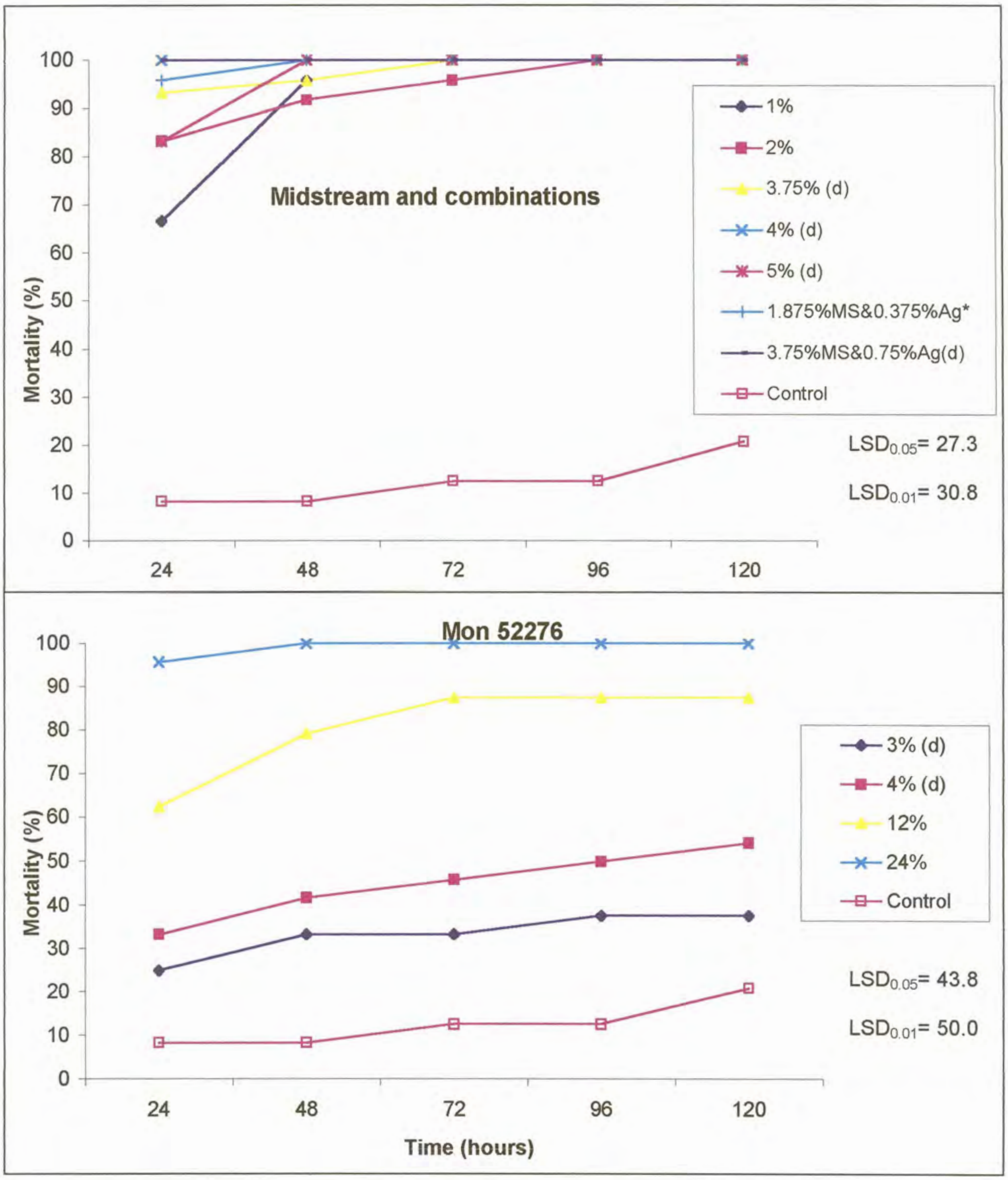


Figure 3.2 Continued
* Ag = Agral; MS = Midstream

g.

h.

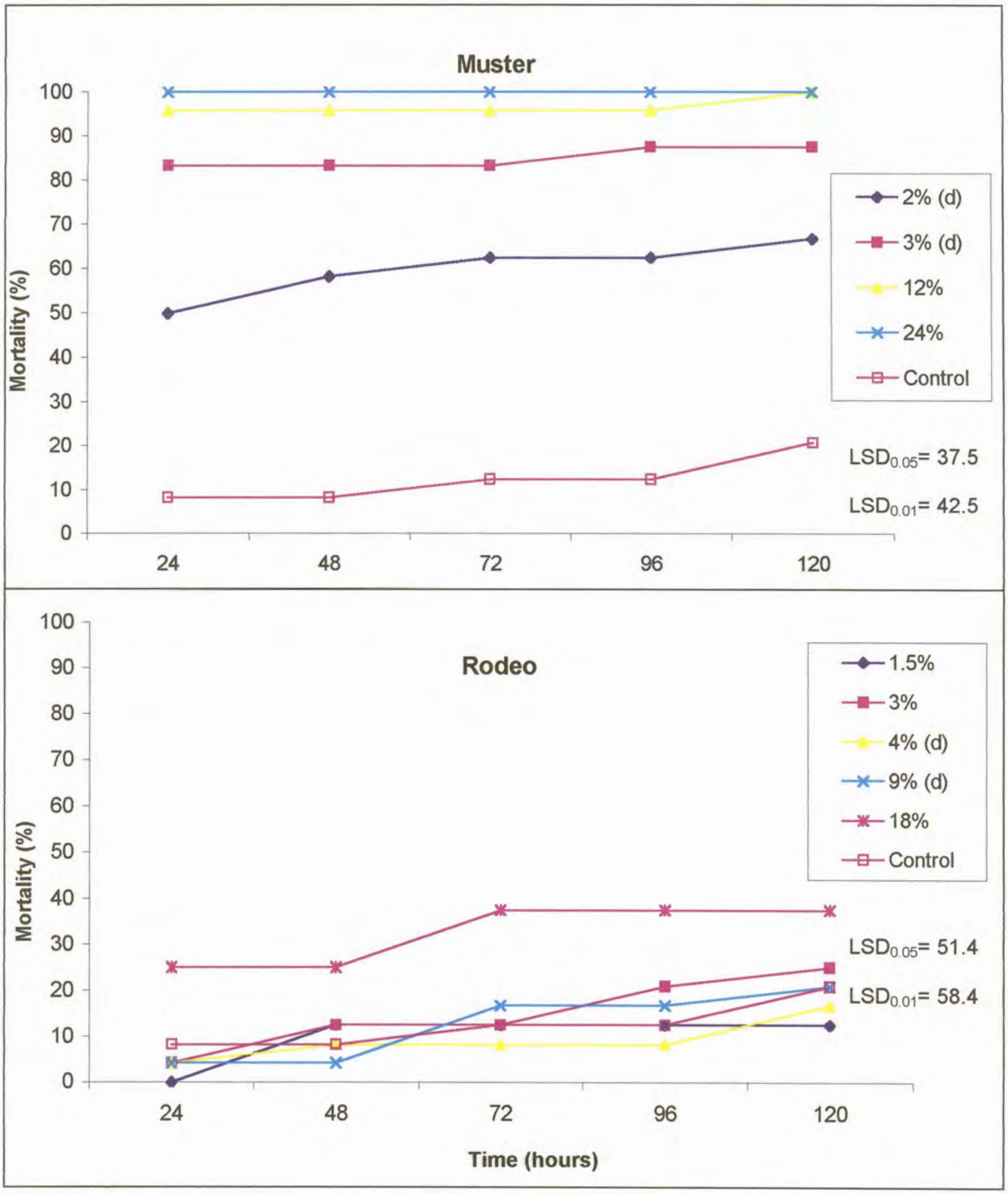
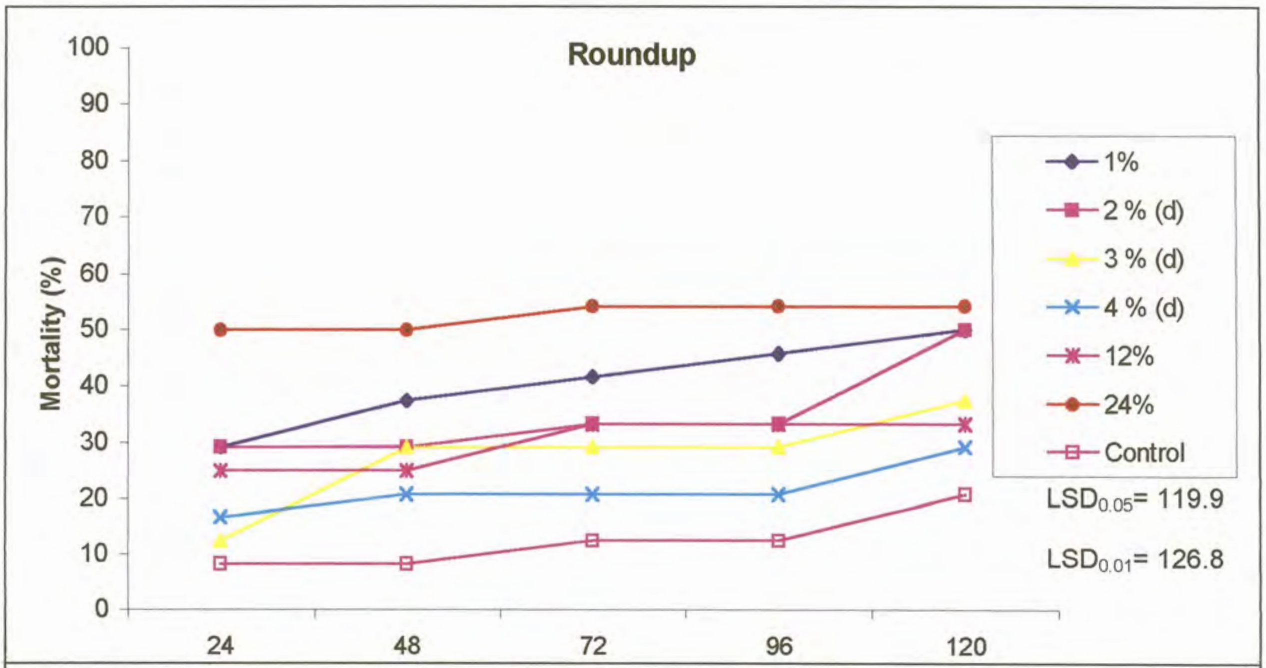


Figure 3.2 Continued

i.



j.

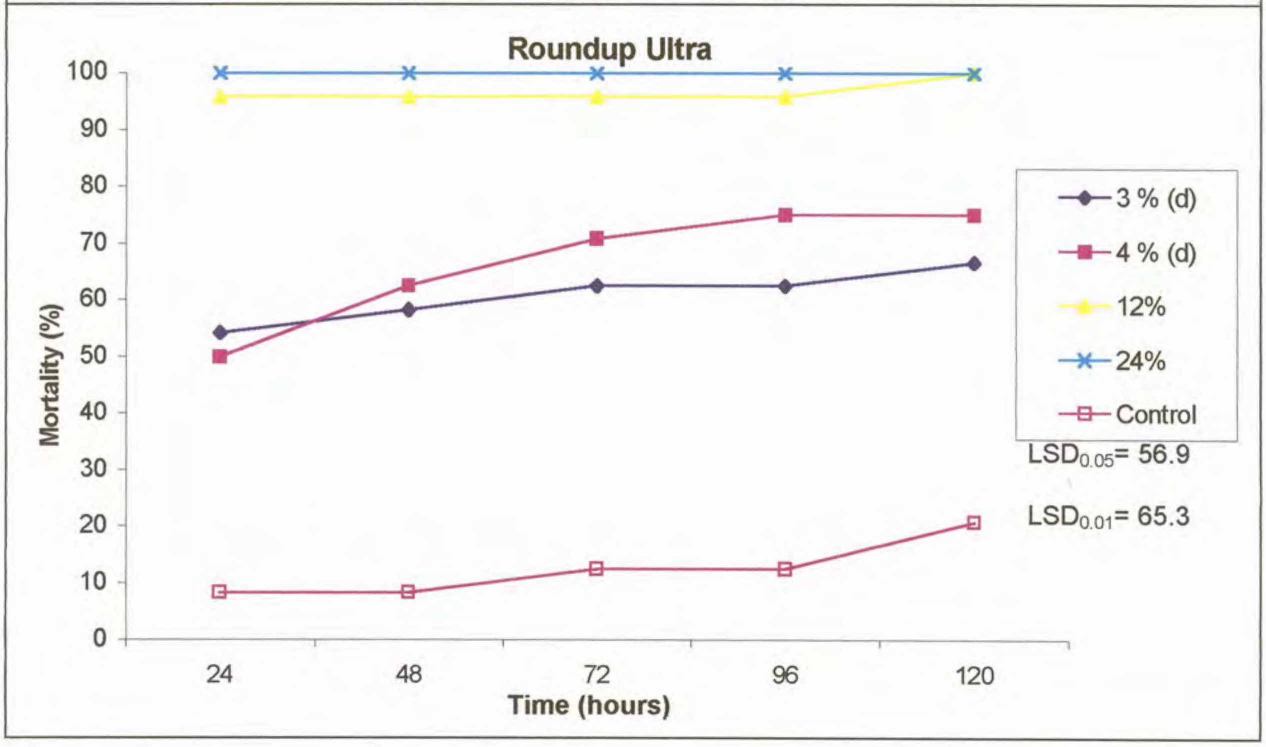
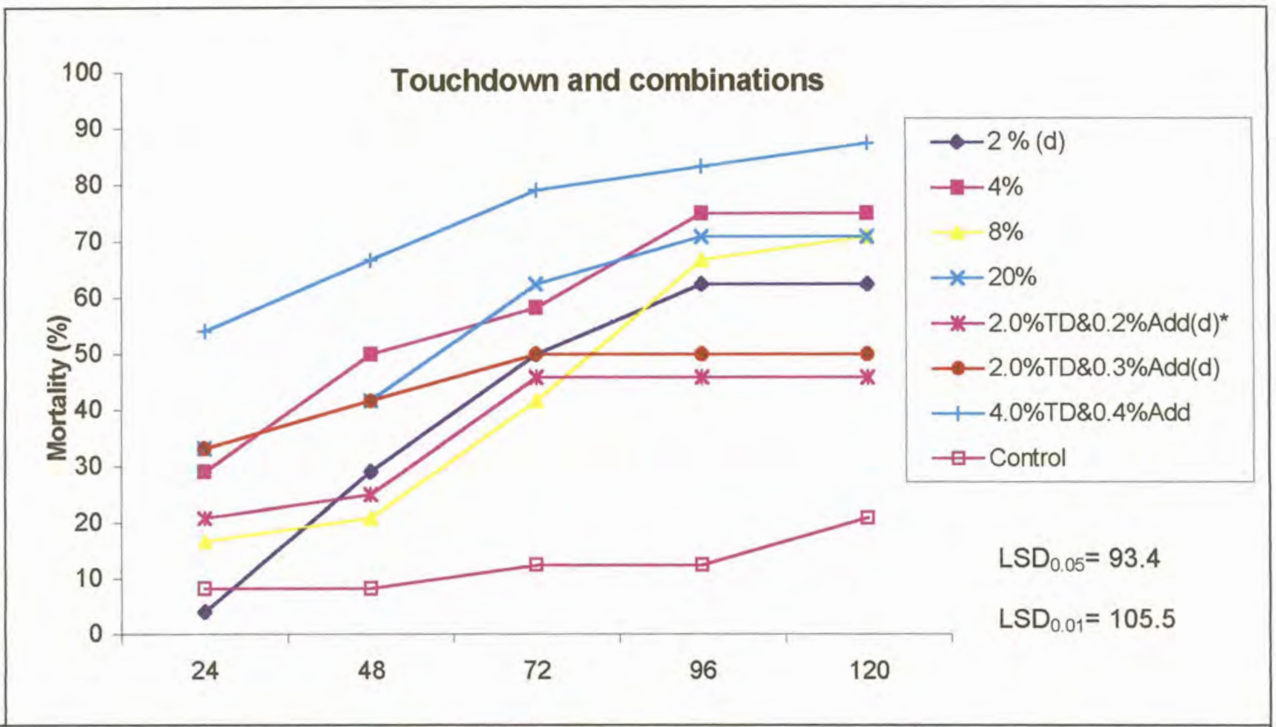


Figure 3.2 Continued

k.



l.

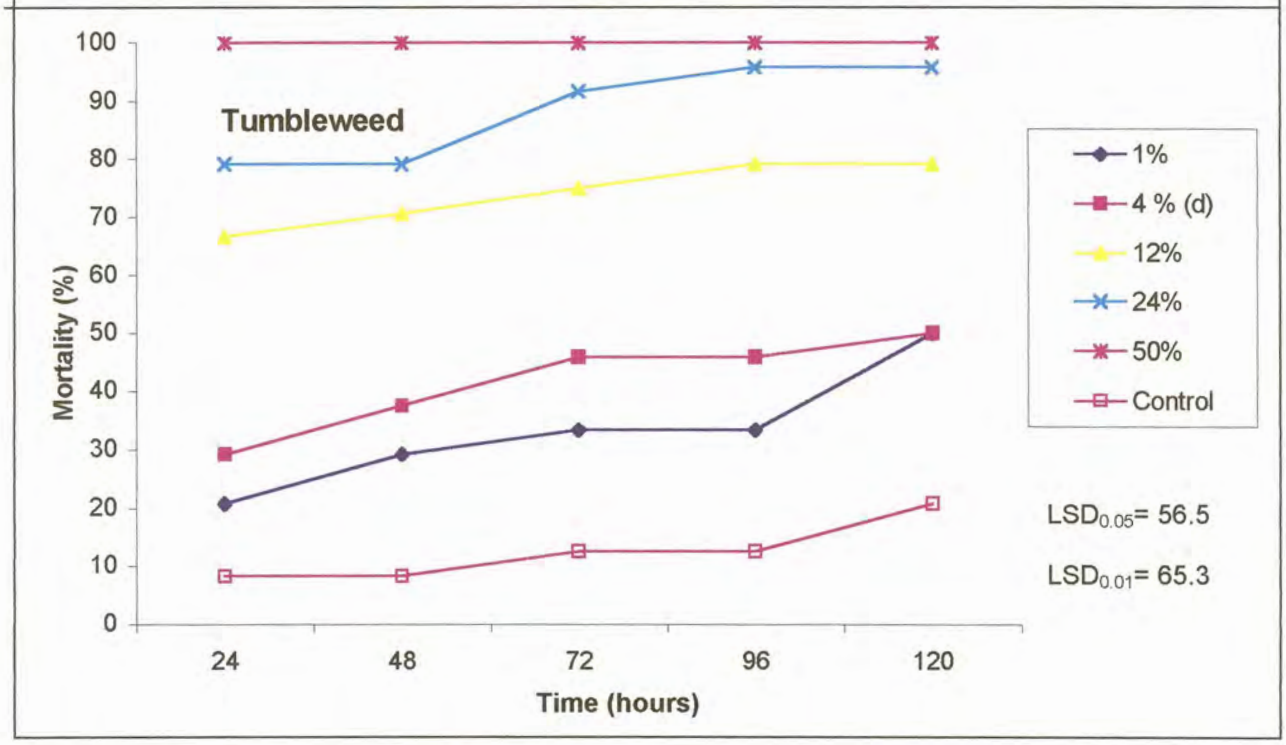


Figure 3.2 Continued
* TD = Touchdown

Table 3.3 Mortality (% and standard deviation) of *Eccritotarsus catarinensis* after direct exposure to the following herbicides

Herbicide	%	n	Mean percentage of adult mortality[1]				
			24[2]	48	72	96	120
			MEAN	MEAN	MEAN	MEAN	MEAN
2,4-D amine	1.0	3	8.3 ± 15.0	16.7 ± 15.0	29.2 ± 28.8	37.5 ± 32.5	41.7 ± 28.8
	3.0	3	54.2 ± 40.0	75.0 ± 21.3	83.3 ± 18.8	83.3 ± 18.8	87.5 ± 12.5
	9.0	3	91.7 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5
	18.0	3	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Add-2	0.2	3	12.5 ± 0.0	33.3 ± 26.3	33.3 ± 26.3	33.3 ± 26.3	33.3 ± 26.3
	0.3	3	41.7 ± 28.8	58.3 ± 18.8	58.3 ± 18.8	58.3 ± 18.8	66.7 ± 26.3
	0.6	3	37.5 ± 25.0	45.8 ± 26.3	45.8 ± 26.3	50.0 ± 21.3	50.0 ± 21.3
	1.0	3	58.3 ± 7.5	66.7 ± 18.8	66.7 ± 18.8	66.7 ± 18.8	66.7 ± 18.8
	2.0	3	91.7 ± 7.5	91.7 ± 7.5	91.7 ± 7.5	91.7 ± 7.5	91.7 ± 7.5
Agral (Ag)	0.5	3	54.2 ± 15.0	58.3 ± 18.8	58.3 ± 18.8	66.7 ± 31.3	66.7 ± 31.3
	0.8	3	62.5 ± 21.3	66.7 ± 18.8	75.0 ± 21.3	79.2 ± 26.3	79.2 ± 26.3
	1.0	3	62.5 ± 12.5	66.7 ± 7.5	66.7 ± 7.5	70.8 ± 15.0	70.8 ± 12.5
Mamba	3.0	3	8.3 ± 7.5	12.5 ± 12.5	16.7 ± 18.8	16.7 ± 18.8	16.7 ± 18.8
	4.0	3	16.7 ± 18.8	20.8 ± 26.3	25.0 ± 25.0	25.0 ± 25.0	25.0 ± 25.0
	12.0	3	70.8 ± 31.3	70.8 ± 31.3	83.3 ± 26.3	83.3 ± 26.3	83.3 ± 26.3
	24.0	3	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5
Midstream (MS)	1.0	3	66.7 ± 18.8	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	2.0	3	83.3 ± 18.8	91.7 ± 7.5	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0
	3.8	3	83.3 ± 18.8	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	4.0	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	5.0	3	83.3 ± 15.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mon 52276	3.0	3	25.0 ± 12.5	33.3 ± 7.5	33.3 ± 7.5	37.5 ± 12.5	37.5 ± 12.5
	4.0	3	33.3 ± 7.5	41.7 ± 7.5	45.8 ± 7.5	50.0 ± 0.0	54.2 ± 7.5
	12.0	3	62.5 ± 32.5	79.2 ± 18.8	87.5 ± 21.3	87.5 ± 21.3	87.5 ± 21.3
	24.0	3	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Muster	2.0	3	50.0 ± 12.5	58.3 ± 18.8	62.5 ± 21.3	62.5 ± 21.3	66.7 ± 15.0
	3.0	3	83.3 ± 7.5	83.3 ± 7.5	83.3 ± 7.5	87.5 ± 12.5	87.5 ± 12.5
	12.0	3	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	100.0 ± 0.0
	24.0	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Rodeo	1.5	3	0.0 ± 0.0	12.5 ± 12.5	12.5 ± 12.5	12.5 ± 12.5	12.5 ± 12.5
	3.0	3	4.2 ± 7.5	12.5 ± 12.5	12.5 ± 12.5	20.8 ± 7.5	25.0 ± 12.5
	4.0	3	4.2 ± 7.5	8.3 ± 15.0	8.3 ± 15.0	8.3 ± 15.0	16.7 ± 18.8
	9.0	3	4.2 ± 7.5	4.2 ± 7.5	16.7 ± 18.8	16.7 ± 18.8	20.8 ± 26.3
	18.0	3	25.0 ± 21.3	25.0 ± 21.3	37.5 ± 25.0	37.5 ± 25.0	37.5 ± 25.0
Roundup	1.0	3	29.2 ± 26.3	37.5 ± 32.5	41.7 ± 58.8	45.8 ± 43.8	50.0 ± 45.0
	2.0	3	29.2 ± 50.0	29.2 ± 50.0	33.3 ± 46.3	33.3 ± 46.3	50.0 ± 45.0
	3.0	3	12.5 ± 21.3	29.2 ± 40.0	29.2 ± 40.0	29.2 ± 40.0	37.5 ± 32.5
	4.0	3	16.7 ± 28.8	20.8 ± 36.3	20.8 ± 36.3	20.8 ± 36.3	29.2 ± 31.3
	12.0	3	25.0 ± 21.3	25.0 ± 21.3	33.3 ± 18.8	33.3 ± 18.8	33.3 ± 18.8
	24.0	3	50.0 ± 32.5	50.0 ± 32.5	54.2 ± 38.8	54.2 ± 38.8	54.2 ± 38.8
Roundup-Ultra	3.0	3	54.2 ± 31.3	58.3 ± 31.3	62.5 ± 32.5	62.5 ± 32.5	66.7 ± 26.3
	4.0	3	50.0 ± 25.0	62.5 ± 25.0	70.8 ± 18.8	75.0 ± 12.5	75.0 ± 12.5
	12.0	3	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	95.8 ± 7.5	100.0 ± 0.0
	24.0	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

[1] Eight insects per replicate
[2] Hours after herbicide application

Table 3.3 Continued

Herbicide	%	n	Mean percentage of adults dying				
			24	48	72	96	120
			MEAN	MEAN	MEAN	MEAN	MEAN
Touchdown (TD)	2.0	3	4.2 ± 7.5	29.2 ± 15.0	50.0 ± 21.3	62.5 ± 37.5	62.5 ± 37.5
	4.0	3	29.2 ± 50.0	50.0 ± 45.0	58.3 ± 38.8	75.0 ± 25.0	75.0 ± 25.0
	8.0	3	16.7 ± 15.0	20.8 ± 18.8	41.7 ± 28.8	66.7 ± 38.8	70.8 ± 40.0
	20.0	3	33.3 ± 7.5	41.7 ± 7.5	62.5 ± 21.3	70.8 ± 26.3	70.8 ± 26.3
Tumbleweed	1.0	3	20.8 ± 26.3	29.2 ± 31.3	33.3 ± 26.3	33.3 ± 26.3	50.0 ± 18.8
	4.0	3	29.2 ± 7.5	37.5 ± 12.5	45.8 ± 18.8	45.8 ± 18.8	50.0 ± 25.0
	12.0	3	66.7 ± 26.3	70.8 ± 18.8	75.0 ± 21.3	79.2 ± 26.3	79.2 ± 26.3
	24.0	3	79.2 ± 7.5	79.2 ± 7.5	91.7 ± 7.5	95.8 ± 7.5	95.8 ± 7.5
	50.0	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Combinations	2.0%TD&0.2%Add	3	20.8 ± 18.8	25.0 ± 21.3	45.8 ± 28.8	45.8 ± 28.8	45.8 ± 28.8
	2.0%TD&0.3%Add	3	33.3 ± 31.3	41.7 ± 38.8	50.0 ± 32.5	50.0 ± 32.5	50.0 ± 32.5
	4.0%TD&0.4%Add	3	54.2 ± 40.0	66.7 ± 38.8	79.2 ± 18.8	83.3 ± 15.0	87.5 ± 12.5
	1.875%MS&0.375%Ag	3	95.8 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	3.75%MS&0.75%Ag	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Control	H_2O	3	8.3 ± 14.4	8.3 ± 14.4	12.5 ± 12.5	12.5 ± 12.5	20.8 ± 19.1
	LSD[3] ($p < 0.05$)		70.0	71.3	71.3	75.0	73.8
	LSD ($p < 0.01$)		77.5	78.8	78.8	82.5	81.3

[3] LSD = Least significant difference, with p-values as specified (Statistical analyses in Appendix 6)

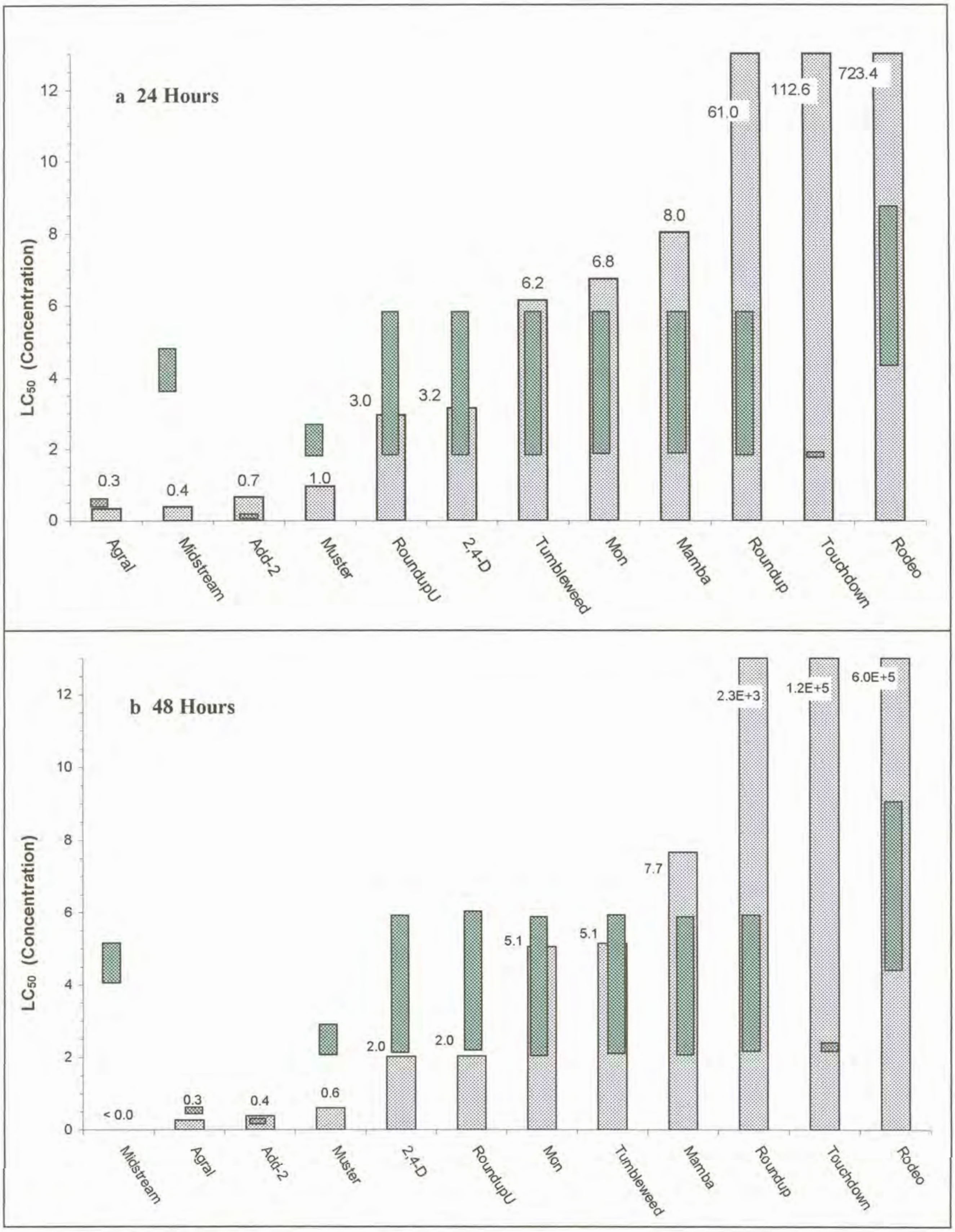


Figure 3.3 LC_{50} values calculated for *Eccritotarsus catarinensis* after acute toxicity testing with selected herbicides after (a) 24 hours; (b) 48 hours; (c) 72 hours; (d) 96 hours; and (e) 120 hours.

LC_{50} values   Recommended dosage range

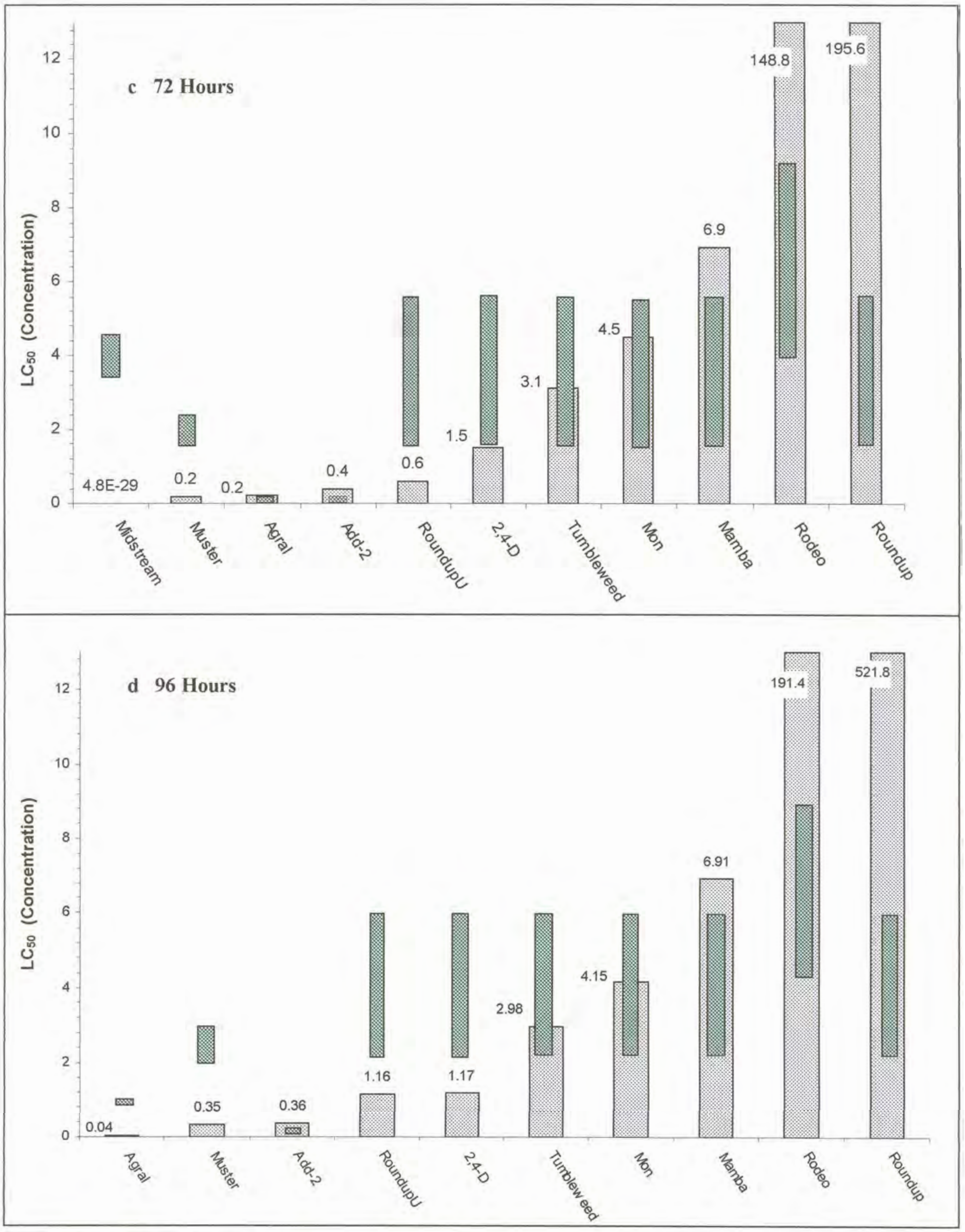


Figure 3.3 Continued

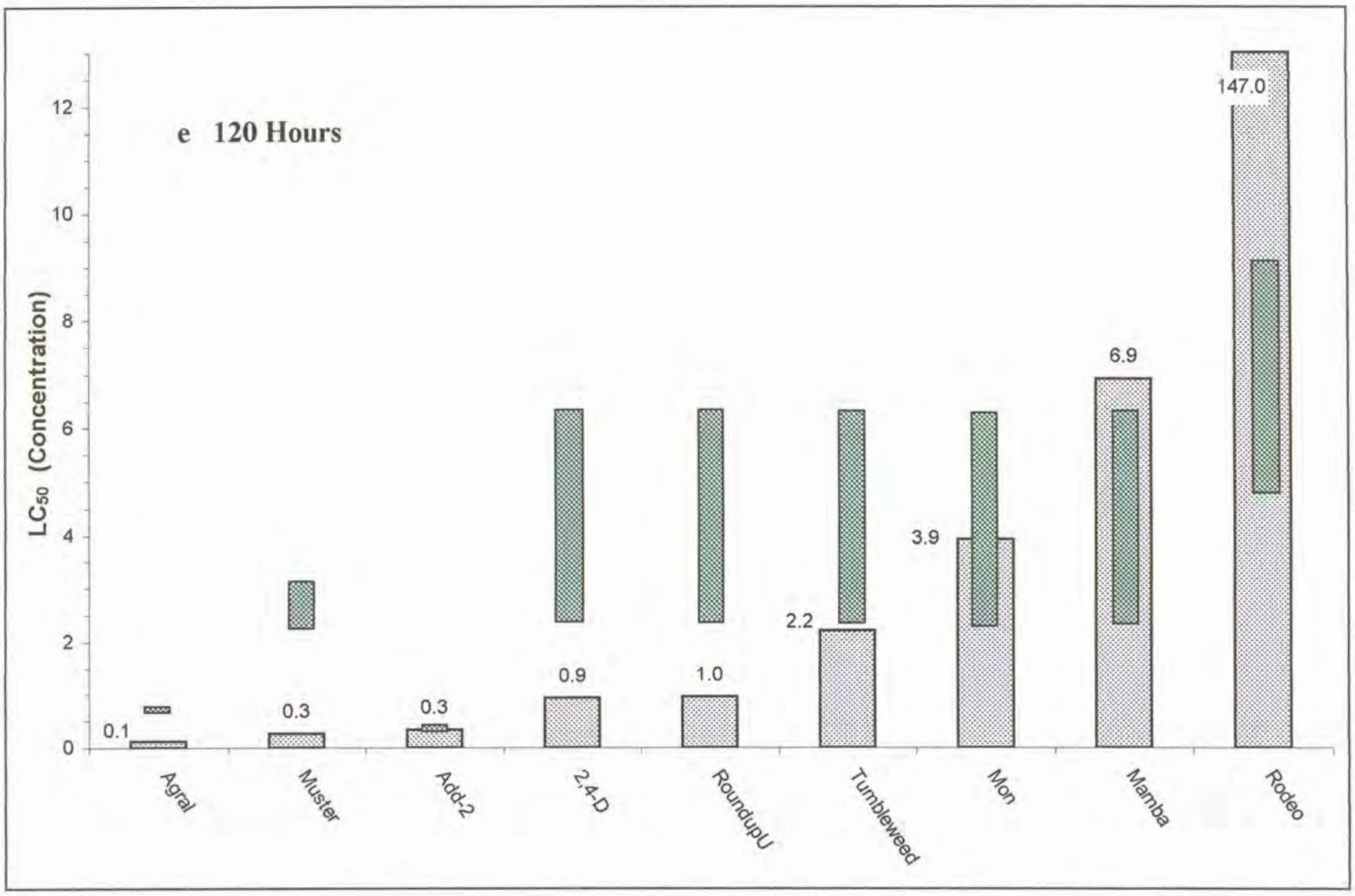


Figure 3.3 Continued

Table 3.4 Herbicides rated according to the effect they have on *Eccritotarsus catarinensis* during acute toxicity tests

HERBICIDE[1]	DOSAGES (%) USED IN PRACTICE[2]		LC_{50} VALUES[3] 24 hrs	120 hrs	RATING[4]
2,4-D amine	2.0 – 6.0	(to 12.0)	3.15	0.93	Toxic
Add-2	0.2 – 0.3		0.63	0.33	Safe
Agral	0.8		0.33	0.12	Toxic
Mamba	2.0 – 6.0	(to 12.0)	8.04	6.91	Safe
Midstream	3.8 – 5.0		0.38	-	Toxic
Mon 52276	2.0 – 6.0	(to 12.0)	6.75	3.92	Hazardous
Muster	2.0 –3.0		0.95	0.26	Toxic
Rodeo	4.5 – 9.0		723.43	146.99	Safe
Roundup	2.0 – 6.0	(to 12.0)	61.04	-	Safe
Roundup Ultra	2.0 – 6.0	(to 12.0)	2.96	0.95	Toxic
Touchdown	2.0		112.6	0.00284	Toxic
Tumbleweed	2.0 – 6.0	(to 12.0)	6.15	2.21	Hazardous

[1] Formulation or surfactant
[2] Refer to product labels for specified dosages and application instructions
[3] Values obtained by linear regression (Excel)
[4] Safe = LC_{50} never falls within recommended dosage range;
Hazardous = LC_{50} falls within or below the recommended dosage range at some time during the 120 hours;
Toxic = LC_{50} falls within or below the recommended dosage range from 24 hours

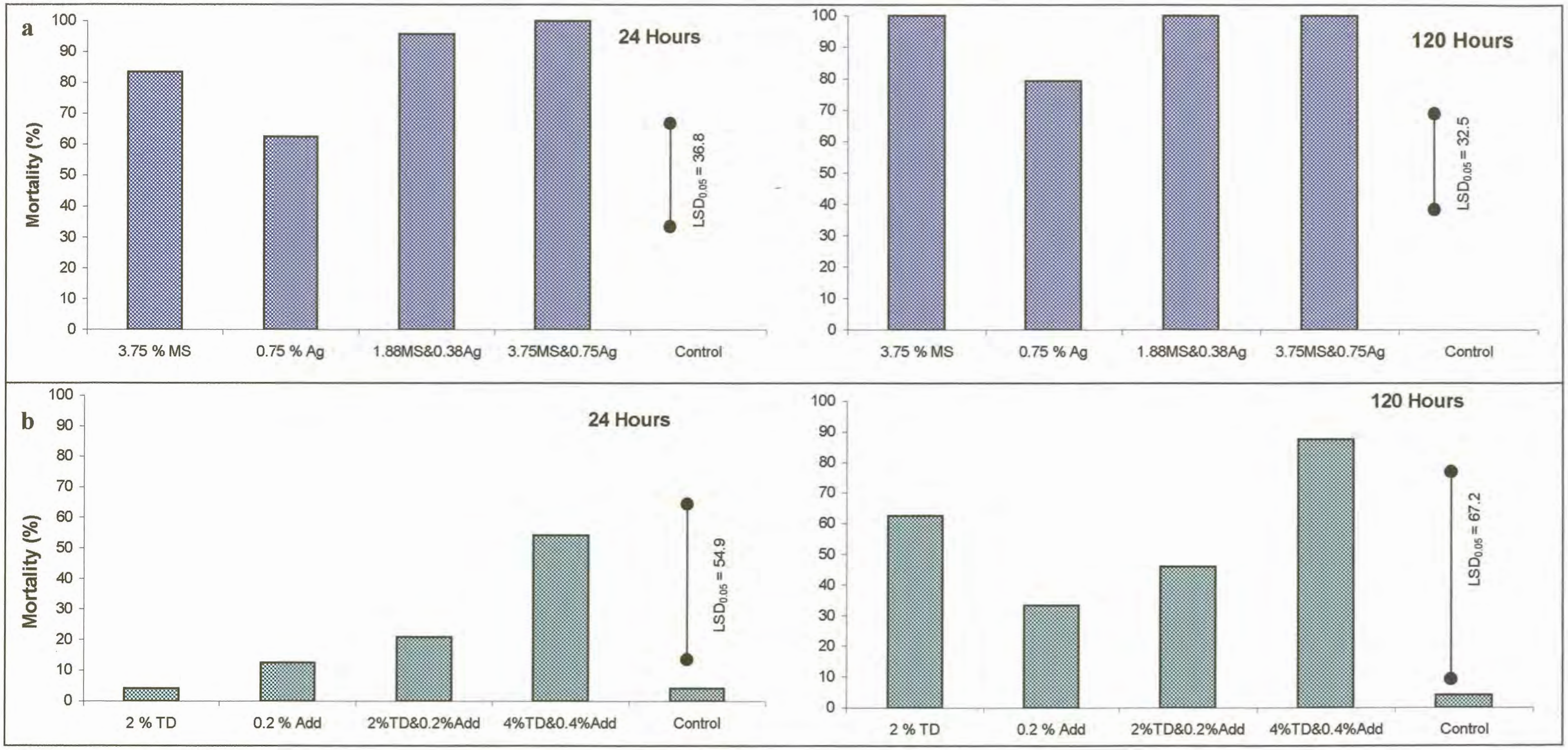


Figure 3.4 Percentage mortality of *Eccritotarsus catarinensis* after direct treatment with two formulations, with and without surfactants at 24 and 120 hours after treatment (a) Midstream (MS) and Agral (Ag), (b) Touchdown (TD) and Add.

3.3.3 *Neochetina eichhorniae*

The percentage mortality for adult *N. eichhorniae* is illustrated in Figure 3.5a–l with means and standard deviations appearing in Table 3.5. In several of the graphs the mortality of the weevils was zero and the concentration lines overlap.

Surfactants and formulations

The weevil was less susceptible to herbicide application than the bug. Only Agral (Figure 3.5c), Midstream (Figure 3.5e), Muster (Figure 3.5g) and Roundup Ultra (Figure 3.5j) produced appreciable mortality. Regressions could only be drawn, and LC_{50} values calculated, for Agral, Muster and Roundup Ultra, because of the relatively high mortalities caused by them (Appendix 4, Table B). Proban analysis LC_{50} values could only be determined for Muster and Roundup Ultra, again giving slightly lower LC_{50} values but correlating well with the regression LC_{50} values.

Considering the LC_{50} values in Table B (Appendix 4), Agral is again the most toxic substance and cause approximately 50 % mortality at the recommended dosage of 0.75 % concentration at 24 hours. It is followed by Muster which should not cause appreciable mortality when applied at the recommended dosage of 2 to 3 % (Table 3.5). Roundup Ultra caused slightly higher mortality than Midstream, but neither will result in significant mortality when applied at recommended concentrations. No mortality occurred after treatment with up to 24 % Rodeo or Mon 52276 while other glyphosate-based herbicides resulted in very little mortality (Table 3.5). 2,4-D amine showed very low mortality overall (Figure 3.5a, Table 3.5).

Combinations

When combining Midstream with the surfactant Agral, significant mortality was caused to the insect population (Figure 3.5e). The lowest recommended dosage of 3.75 % Midstream with 0.75 % Agral resulted in 94.44 % mortality after 120 hours (Figure 3.6a). Even half the recommended dosage for Midstream and Agral combined, caused up to 77.78 % mortality (Figure 3.6a). Agral at 0.75% caused up to 72.22 % mortality and contributed most to the toxic effect on this species (Figure 3.6a).

Touchdown and Add-2, an adjuvant, did not cause any significant mortality when combined or when used seperately (Figure 3.5b, k; Figure 3.6b). It is interesting to note that the Touchdown is glyphosate-trimesium-based, as is Muster which resulted in much higher mortality.

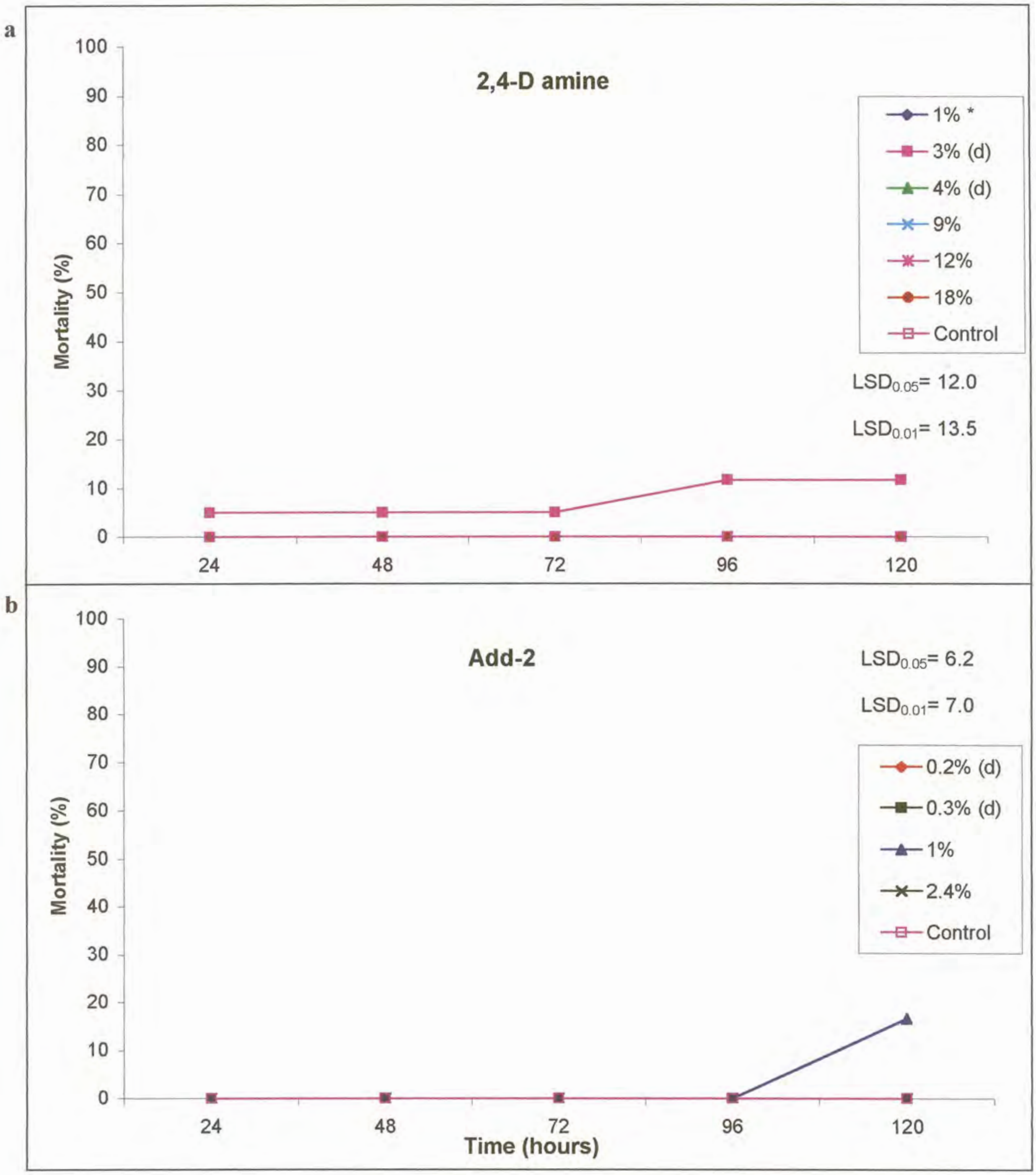


Figure 3.5 Percentage mortality of *Neochetina eichhorniae* as a result of direct treatment with selected herbicides

* percentage of specified herbicide applied in water medium

(d) dosage recommended in practice

$LSD_{0.05}$ Least significant difference ($p<0.05$ or <0.01) as %

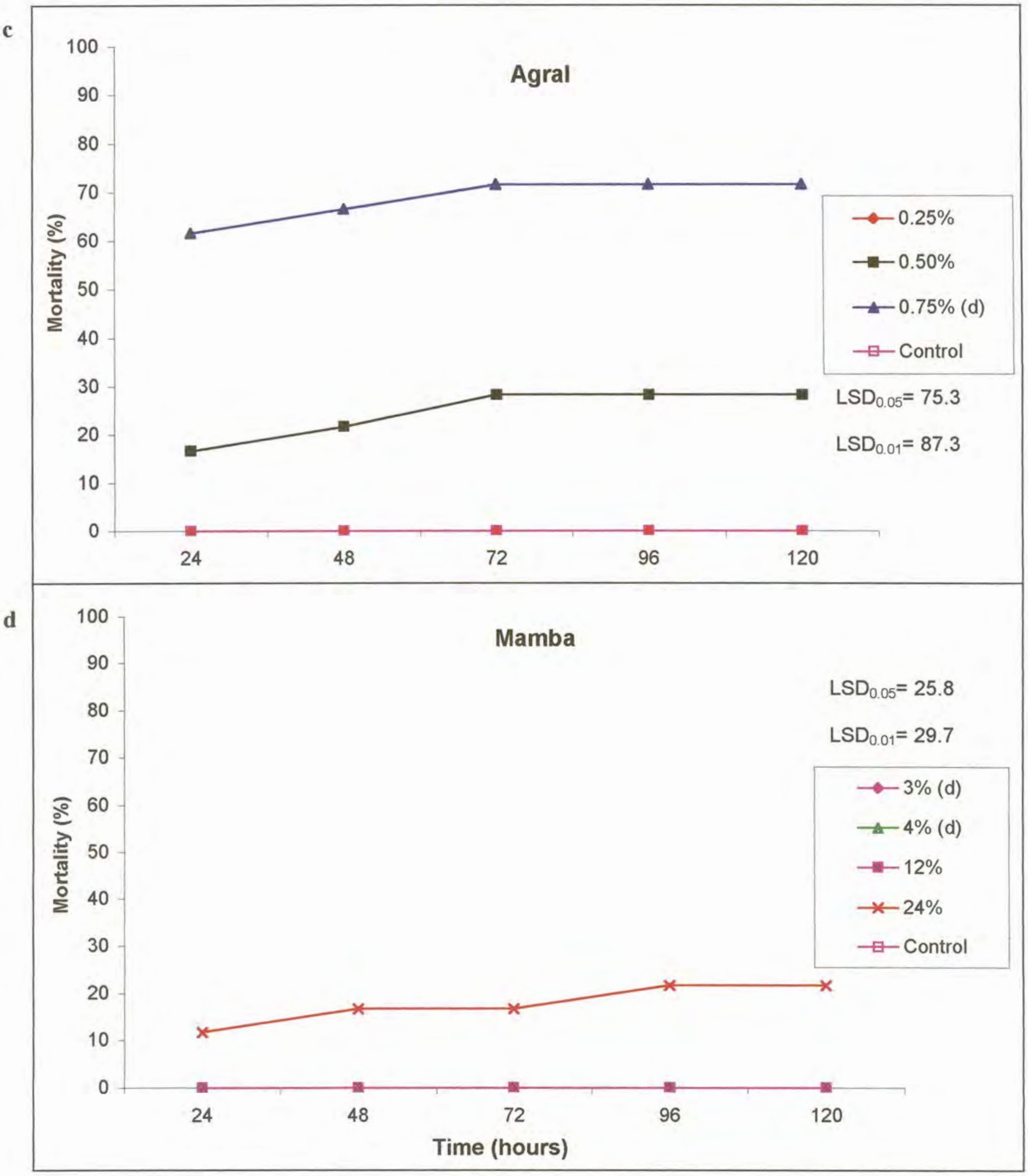


Figure 3.5 Continued

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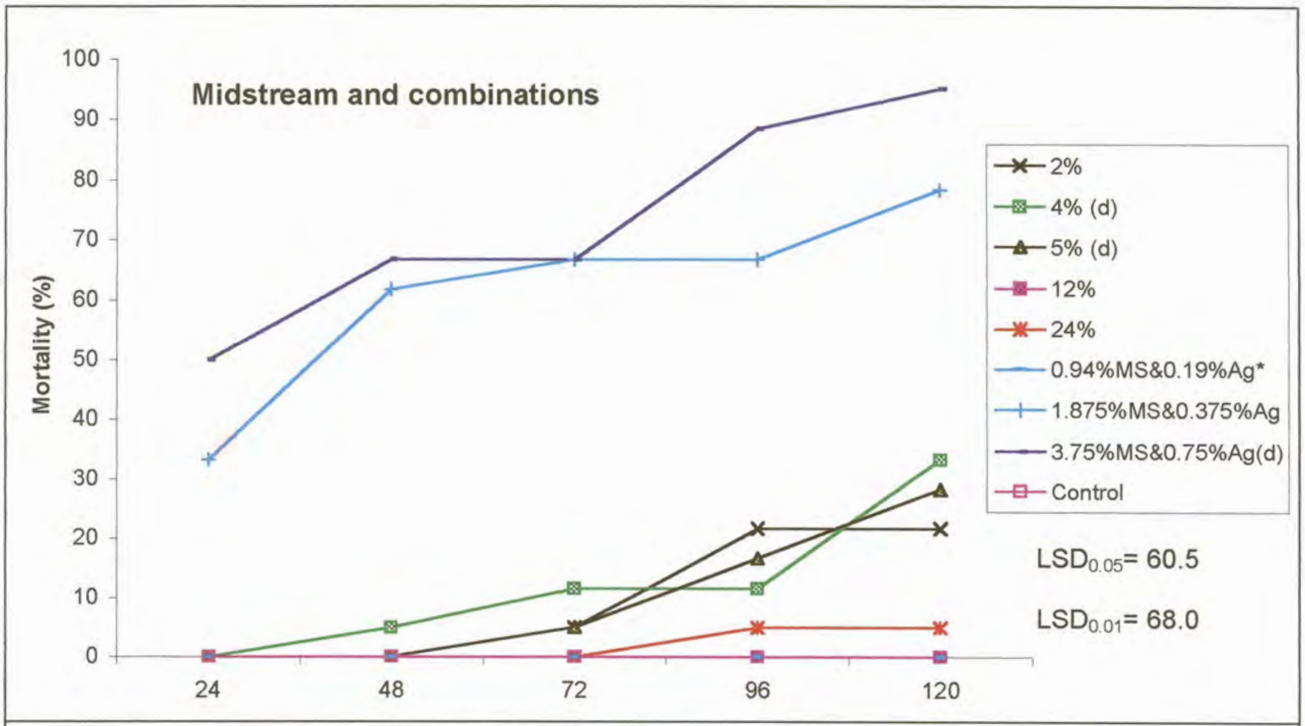

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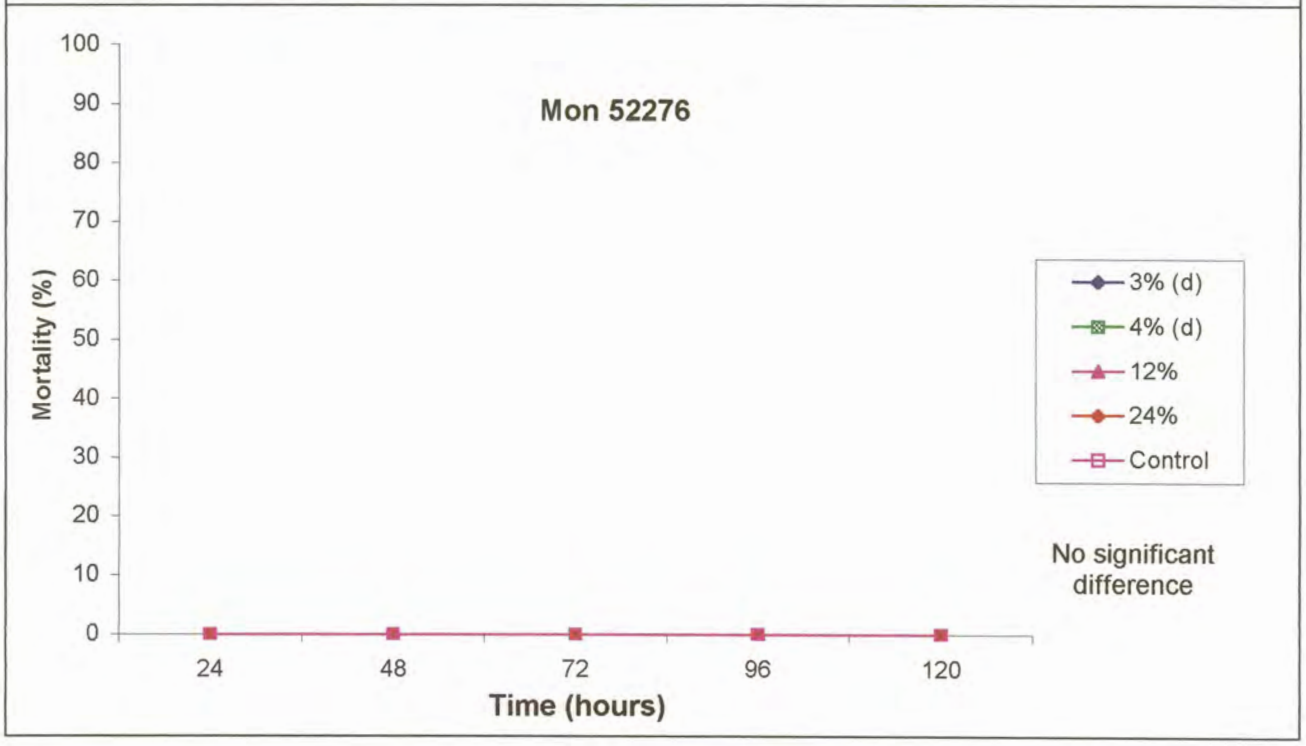

Figure 3.5 Continued

* Ag = Agral; MS = Midstream

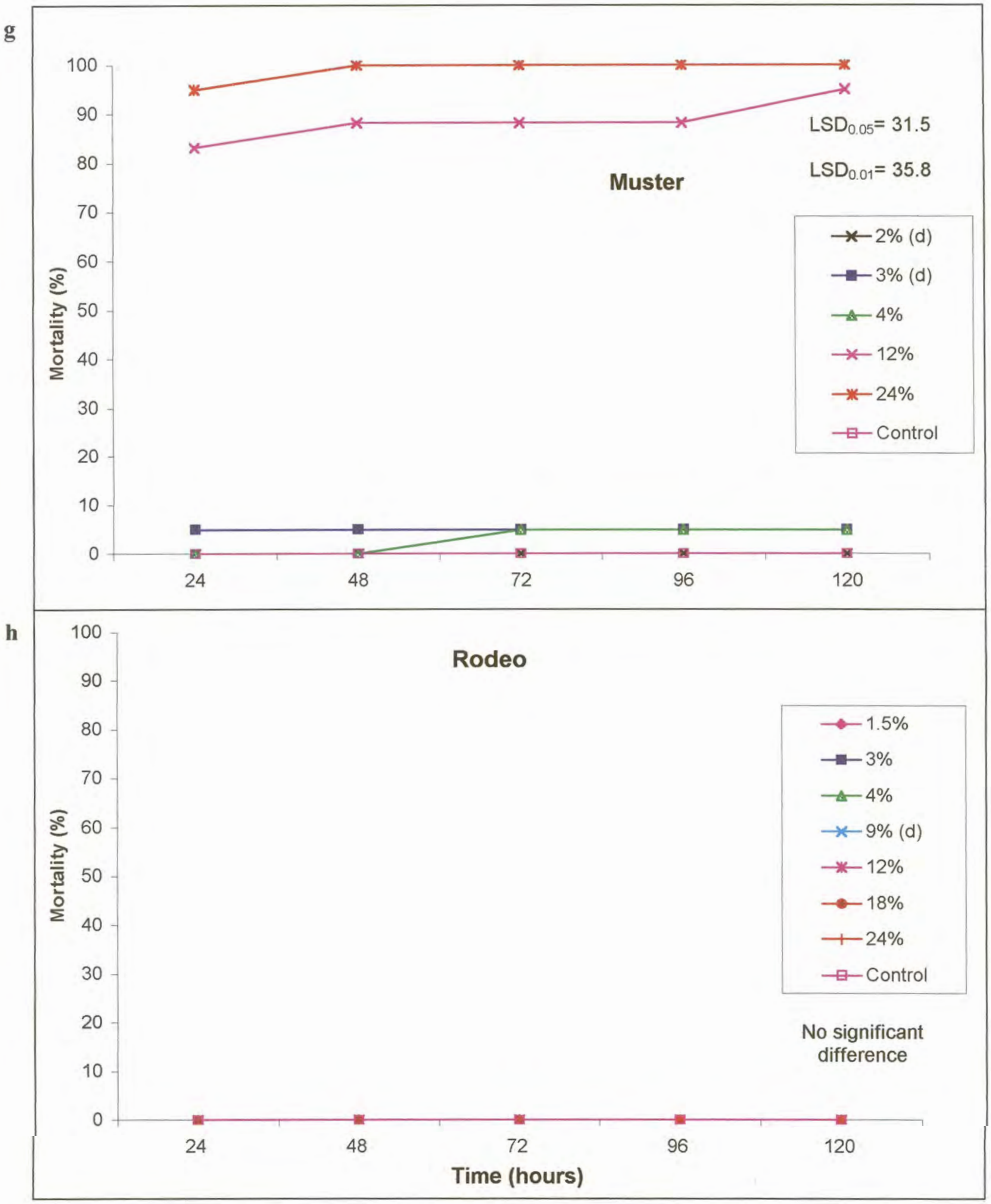

Figure 3.5 Continued

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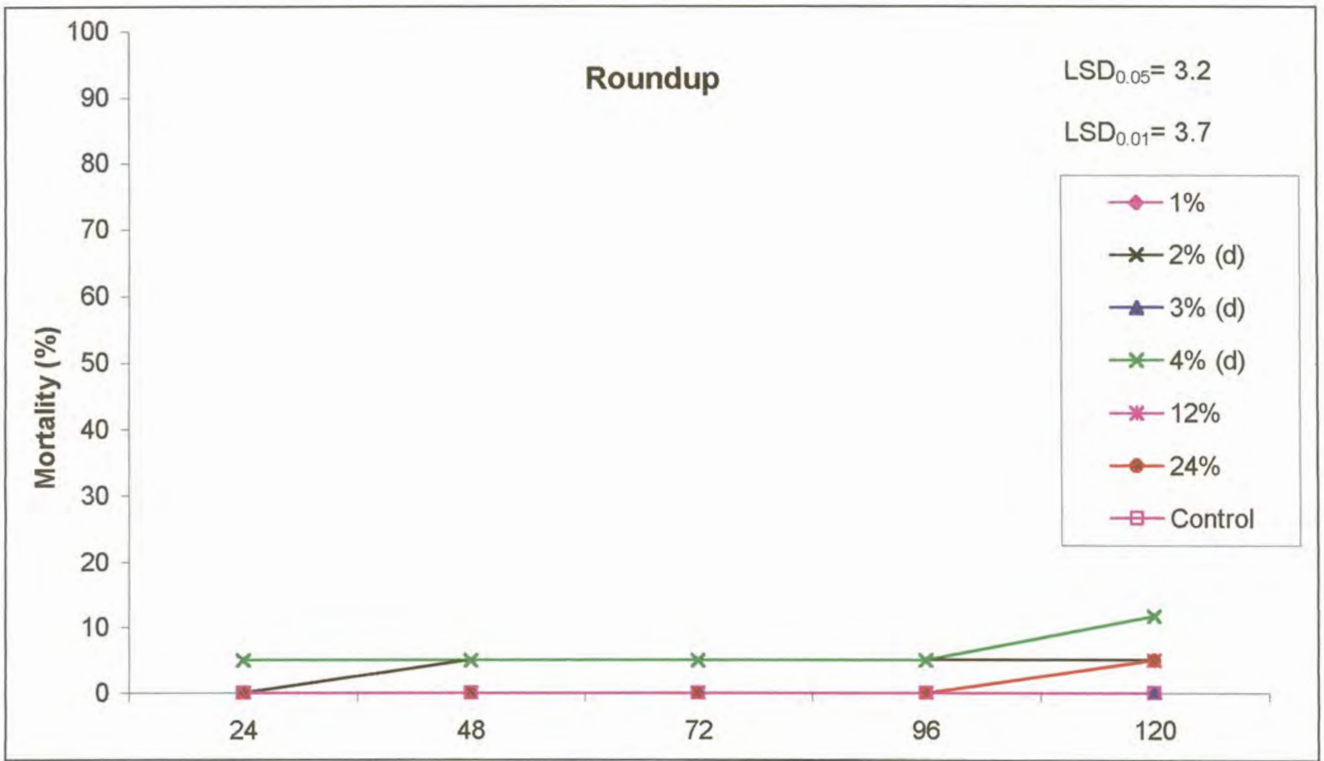

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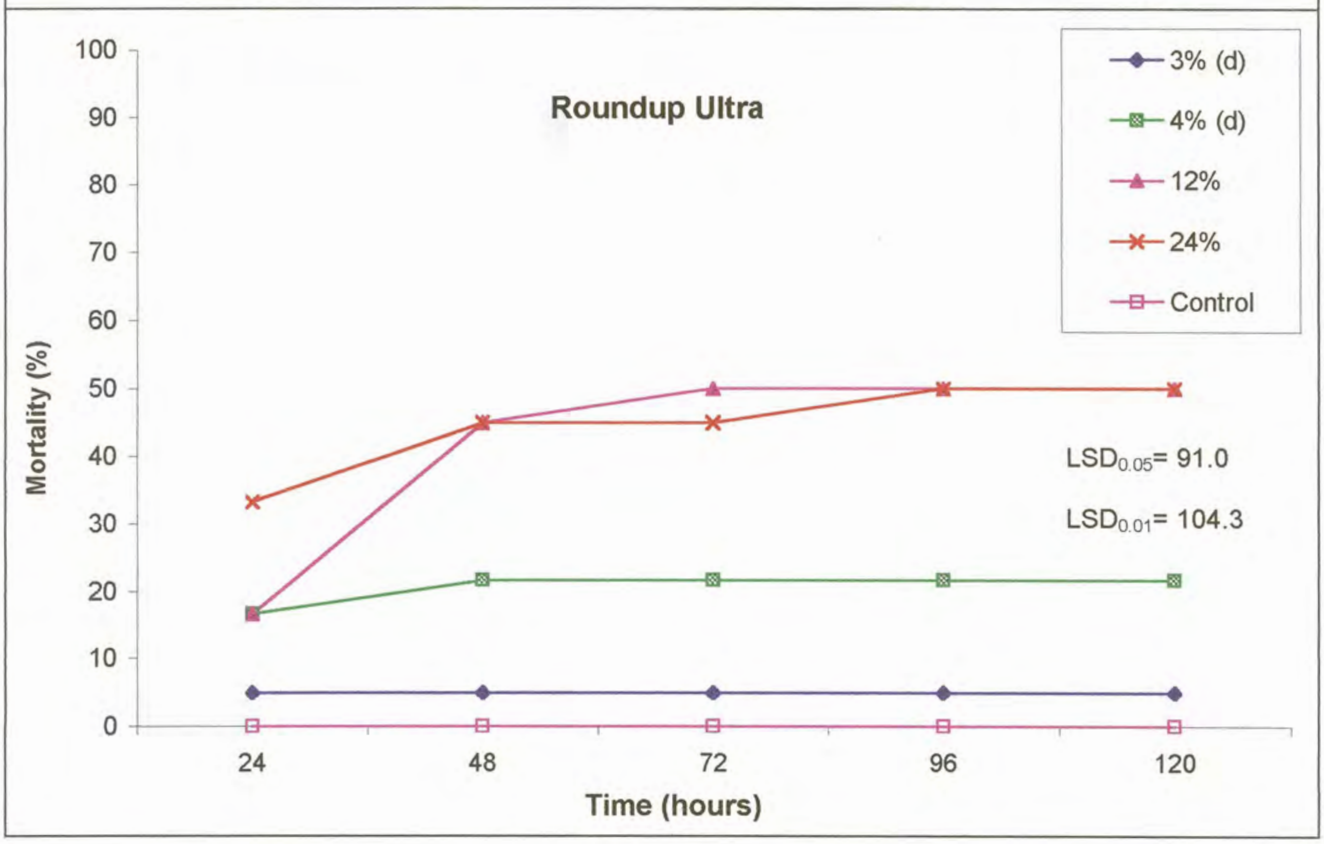


Figure 3.5 Continued

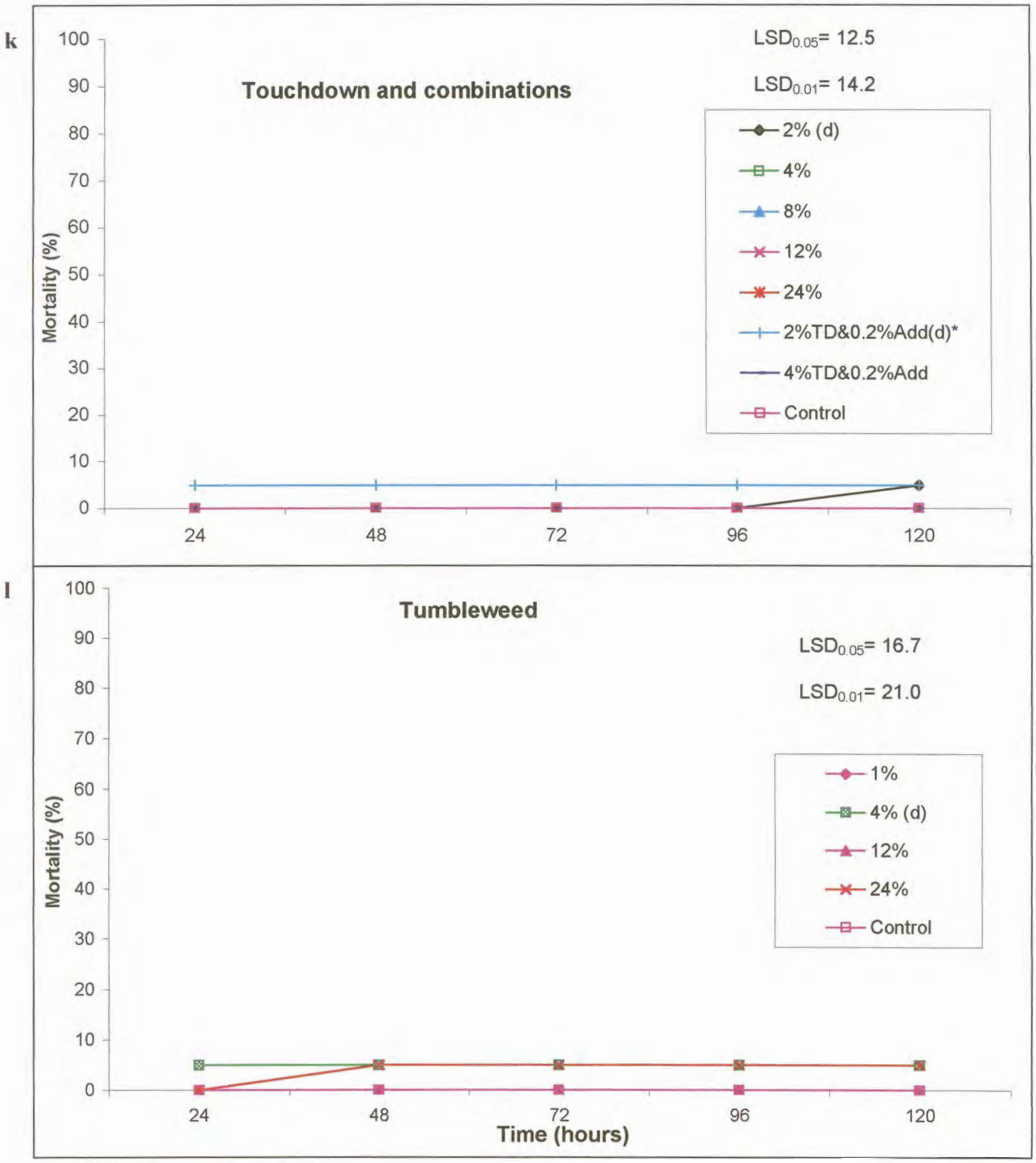


Figure 3.5 Continued

* TD = Touchdown

Table 3.5 Mortality (% and standard deviation) of *Neochetina eichhorniae* after direct exposure to the following herbicides

Herbicide	%	n	24[2] MEAN	48 MEAN	72 MEAN	96 MEAN	120 MEAN
			Mean number of adults dying[1]				
2,4-D amine	1.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	11.7 ± 10.0	11.7 ± 10.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	9.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	18.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Add-2	0.2	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.3	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0
	2.4	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Agral (Ag)	0.3	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	3	16.7 ± 28.3	21.7 ± 38.3	28.3 ± 48.3	28.3 ± 48.3	28.3 ± 48.3
	0.8	3	61.7 ± 20.0	66.7 ± 16.7	71.7 ± 25.0	71.7 ± 25.0	71.7 ± 25.0
Mamba	3.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	11.7 ± 20.0	16.7 ± 16.7	16.7 ± 16.7	21.7 ± 20.0	21.7 ± 20.0
Midstream (MS)	2.0	3	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0	21.7 ± 38.3	21.7 ± 38.3
	4.0	3	0.0 ± 0.0	5.0 ± 10.0	11.7 ± 10.0	11.7 ± 10.0	33.3 ± 43.3
	5.0	3	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0	16.7 ± 16.7	28.3 ± 35.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0	5.0 ± 10.0
Mon 52276	3.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Muster	2.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	12.0	3	83.3 ± 28.3	88.3 ± 20.0	88.3 ± 20.0	88.3 ± 20.0	95.0 ± 10.0
	24.0	3	95.0 ± 10.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Rodeo	1.5	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	9.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	18.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Roundup	1.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	2.0	3	0.0 ± 0.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	3.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	11.7 ± 10.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0
	24.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0
Roundup-Ultra	3.0	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	4.0	3	16.7 ± 16.7	5.0 ± 25.0	5.0 ± 25.0	21.7 ± 25.0	21.7 ± 25.0
	12.0	3	16.7 ± 16.7	45.0 ± 48.3	50.0 ± 45.0	50.0 ± 45.0	50.0 ± 45.0
	24.0	3	33.3 ± 33.3	45.0 ± 41.7	45.0 ± 41.7	50.0 ± 43.3	55.0 ± 48.3

[1] Six insects per replicate
[2] Hours after herbicide application

Table 3.5 Continued

Herbicide	%	n	Mean number of adults dying				
			24	48	72	96	120
			MEAN	MEAN	MEAN	MEAN	MEAN
Touchdown (TD)	2.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 10.0
	4.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	8.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tumbleweed	1.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	12.0	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	24.0	3	0.0 ± 0.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
Combinations	2.0%TD&0.2%Add	3	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0	5.0 ± 10.0
	4.0%TD&0.2%Add	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4.0%TD&0.4%Add	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	8.0%TD&0.8%Add	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.9%MS&0.2%Ag	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1.9%MS&0.4%Ag	3	33.3 ± 28.3	61.7 ± 25.0	66.7 ± 28.3	66.7 ± 28.3	28.3 ± 10.0
	3.8%MS&0.8%Ag	3	50.0 ± 43.3	61.7 ± 35.0	66.7 ± 28.3	88.3 ± 20.0	95.0 ± 10.0
Control	H_2O	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	LSD[3] ($p < 0.05$)		35.2	40.2	42.0	44.8	48.7
	LSD ($p < 0.01$)		41.2	47.2	49.3	52.5	57.2

[3] LSD = Least significant difference, with p-values as specified (Statistical analyses in Appendix 9)

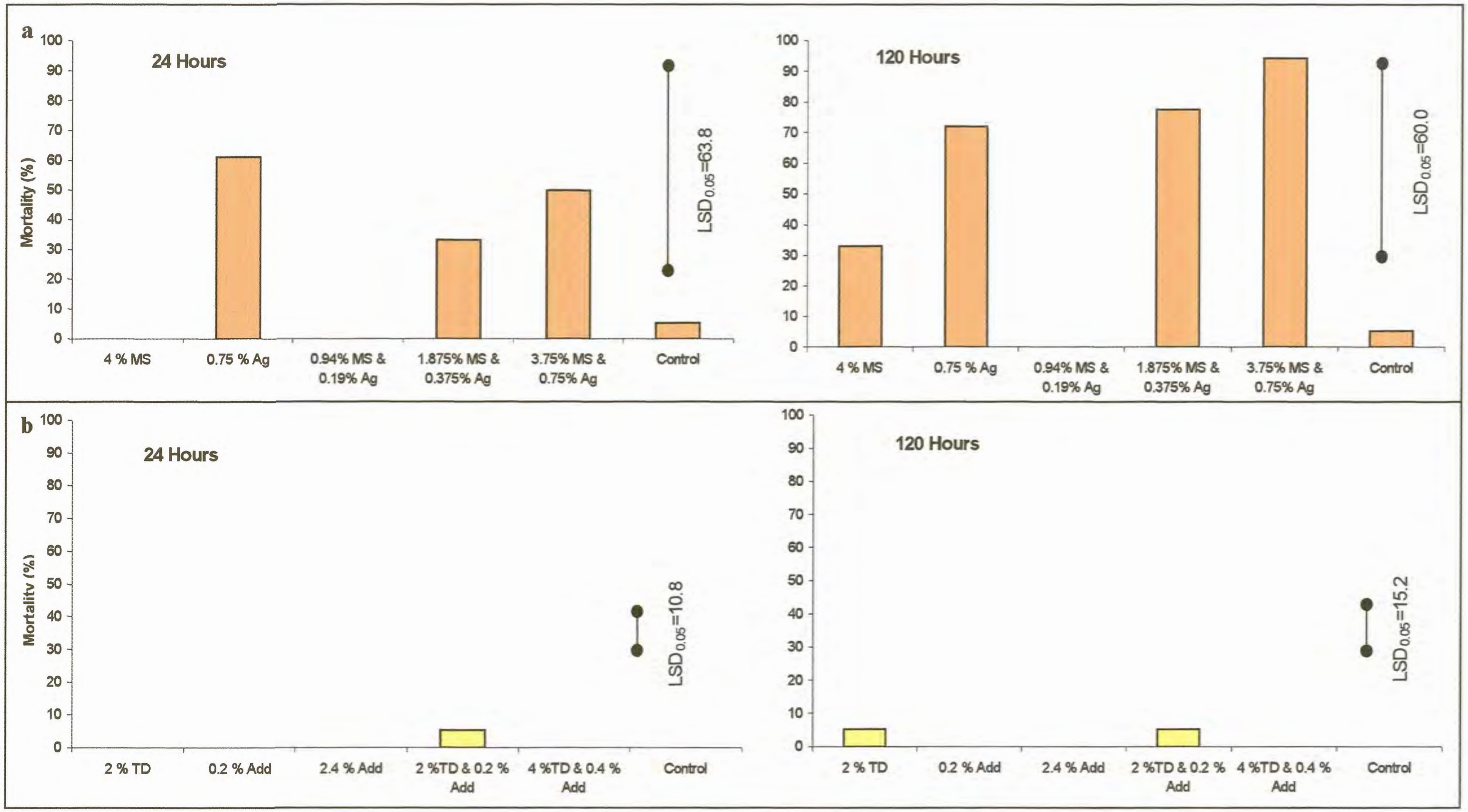


Figure 3.6 Percentage mortality of *Neochetina eichhorniae* after direct treatment with two formulations, with and without surfactants at 24 and 120 hours after treatment (a) Midstream (MS) and Agral (Ag), (b) Touchdown (TD) and Add-2.

3.3 CONCLUSION

The results for *Eccritotarsus catarinensis* give a good indication of the relative toxicity of the formulations that were tested. All the herbicides that were rated "safe" for use with *E. catarinensis* are glyphosate-based. The least toxic substance, Rodeo, is also glyphosate-based and contains the least surfactants.

Glyphosate-trimesium seemed to be acutely toxic to the mirid. However, one glyphosate-trimesium formulation had no toxic effect on the weevil, even at dosages that are much higher than recommended or when combined with a surfactant.

The diquat-based herbicide was the most toxic of all the formulations toward the bug, and when combined with a surfactant it was also toxic to the weevil at recommended dosages. The toxic effects of diquat and low toxicity of glyphosate-based herbicides is echoed in the result of other studies on the mite *Orthogalumna terebrantis* (Roorda *et al.* 1978) and *Neochetina eichhorniae* (Pellessier 1988).

Most substances resulted in high mortality when used at dosages higher than those recommended. Therefore, it is crucial that herbicides are used strictly according to manufacturers' recommendations.

Surfactants definitely contribute to the toxicity of the herbicide formulations, considering the low toxicity of Rodeo (low surfactant content) relative to other glyphosate-based herbicides consisting of varied quantities of various types of surfactants. The increased mortality resulting when diquat (Midstream) is combined with the surfactant (Agral), is further proof of the contribution of surfactants. More work needs to be done on surfactants to rate them according to their toxicity toward natural enemies of water hyacinth and make recommendations for their use. This experiment highlights the importance of choice of surfactants with regard to toxicity towards arthropods used in biological control.

4. Feeding behaviour of *Neochetina eichhorniae* weevils treated with selected herbicides

4.1 INTRODUCTION

Neochetina eichhorniae exhibited low susceptibility to most herbicides during acute toxicity tests (Chapter 3). The species was only sensitive to one glyphosate-trimesium-based herbicide Muster, and the surfactant Agral when applied alone, as well as combined with a diquat based herbicide, Midstream. However, besides acute toxicity, there are several other methods to measure the toxic effects of substances on insects. Sub-lethal effects such as changes in fecundity and 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). Behaviour is a sensitive indicator of stress, fitness and disruptions at physiological level (Slobodkin 1968; Bayne 1980 In: Forbes & Forbes 1993).

Literature on arthropod and insect toxicology is limited and usually concerned with environmental toxicology. In these studies a single species is chosen to represent the effect of a toxin on a whole taxa. In this way, *Daphnia* (Crustacean) and *Chironomus* (midge) species are popularly used to represent insects, arthropods or invertebrates (Forbes & Forbes 1993; Landis & Yu 1995). These arthropods are aquatic, short-lived, small and soft-bodied arthropods relative to the test species used in this study.

It would be impractical to do feeding tests on most insect species, including the bug (*Eccritotarsus catarinensis*), as it is difficult to quantify feeding. However, the weevil leaves visible feeding scars that can be distinguished easily (Figure 4.1). The aim of this study was to monitor the feeding of *Neochetina eichhorniae* on water hyacinth leaves after 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.

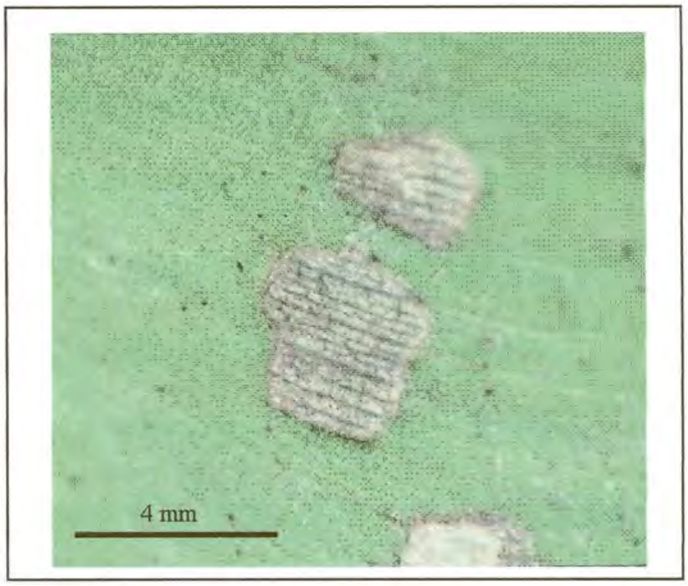


Figure 4.1 The visible and evenly sized feeding scars made by *Neochetina eichhorniae* (weevil) when feeding on water hyacinth leaves.

4.2 MATERIALS AND METHODS

A 20 μl droplet of herbicide solution was applied to the elytra of each weevil. The herbicides listed in Table 3.1 were used at concentrations of 4 % and 12 %, made up freshly with distilled water within an hour before application. Weevils were captured from the field approximately two weeks before initiation of the experiment and kept in plastic tubs with an abundance of fresh, severed leaves as food. 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, at least 48 hours before commencement of experiment to acclimatise. Fresh leaves were provided daily for food and care was taken to select medium sized leaves (approximately 120 x 80 mm) 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 darkness. Leaves were removed every 24 hours and replaced with fresh leaves. Feeding scars were counted on the removed leaves twice, at 48 and 96 hours after treatment. A feeding mark of approximately 4 x 3 mm in size was considered a scar. Three replicates were used. A test for normal distribution showed that the data complied with the requirements for parametric analysis (Ray 1982), and therefore a one-way analysis of variance was used to analyze the data. Results of the statistical analyses are presented in Appendix 11 and 12.

4.3 RESULTS AND DISCUSSION

4.3.1 Herbicide treatments: 4 and 12 %

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 4.2. For the 4 % treatments at 48 hours after exposure, no significant difference occurred between the control and any of the treatments (Figure 4.2a). After 96 hours the combination of Midstream and Agral caused a significant decrease in feeding compared with the control ($p < 0.05$), while Midstream alone did not (Figure 4.2b).

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 4.2c, d). No significant increases or decreases in feeding could be noted, probably as a result of high natural variance in feeding and too few replicates. A more accurate method for determining normal feeding and growth is determining weight changes (Forbes & Forbes 1993), but as with other measurements of the insect body the increased handling contributes to further stress to the test organisms.

Herbicides that caused increased feeding were Mon 52276, Rodeo, Touchdown and Tumbleweed at 4 %, although these increases were not significant (Figure 4.2a, b). Mon 52276 and Tumbleweed also resulted in increased feeding at 12 % (Figure 4.2c, d). Herbicides that caused decreased feeding were 2,4-D amine, Roundup and Roundup Ultra at 4 %, while 2,4-D amine, Mamba, Midstream, Muster, Roundup and Roundup Ultra led to decreased feeding at 12 % (Figure 4.2a-d).

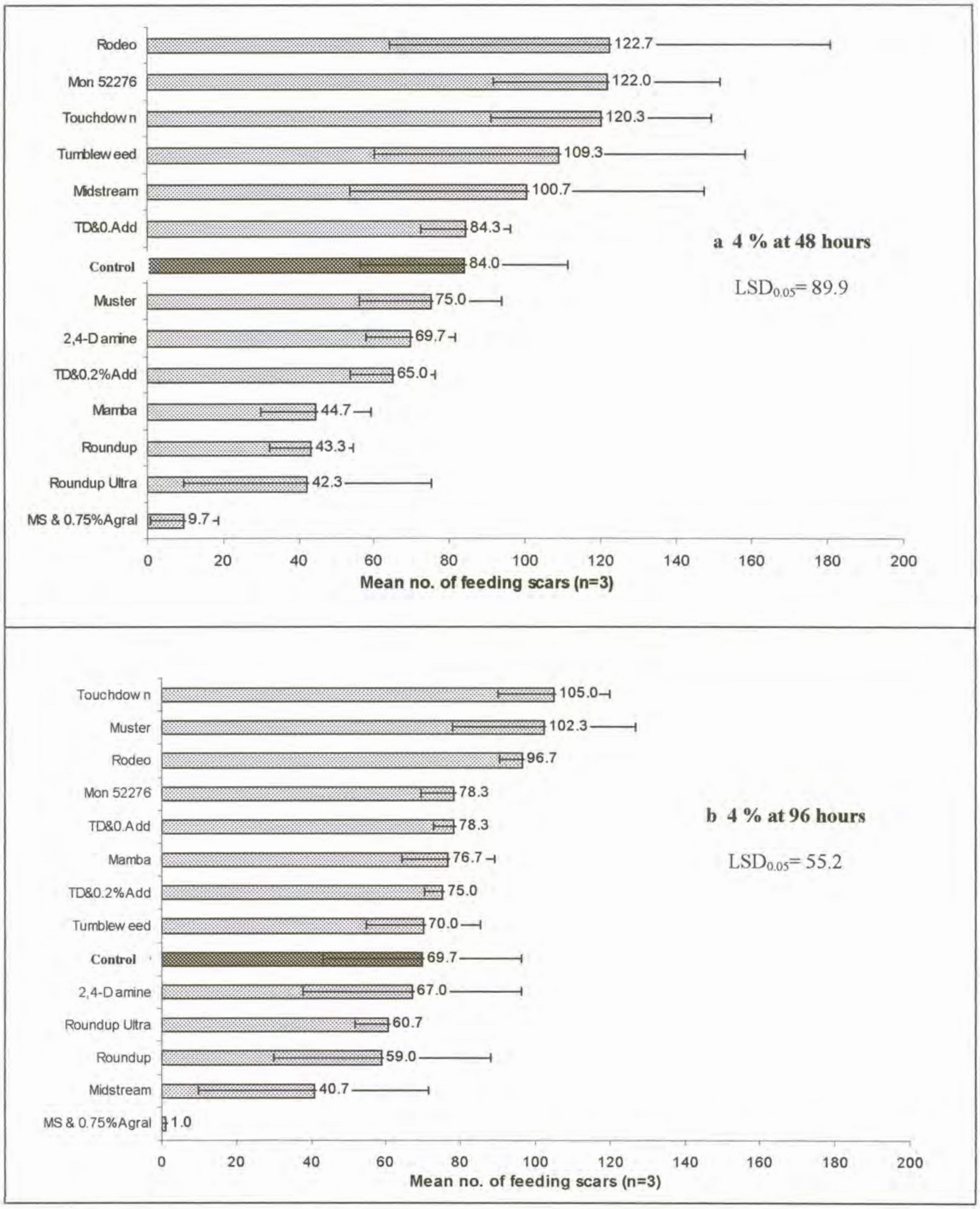


Figure 4.2 Mean number of feeding scars as an indicator of health for *Neochetina eichhorniae*, after direct treatment with 4 % (a, b) and 12 % (c, d) of selected herbicides, at 48 and 96 hours.
Feeding scars ▒ Standard deviation ⊢—⊣

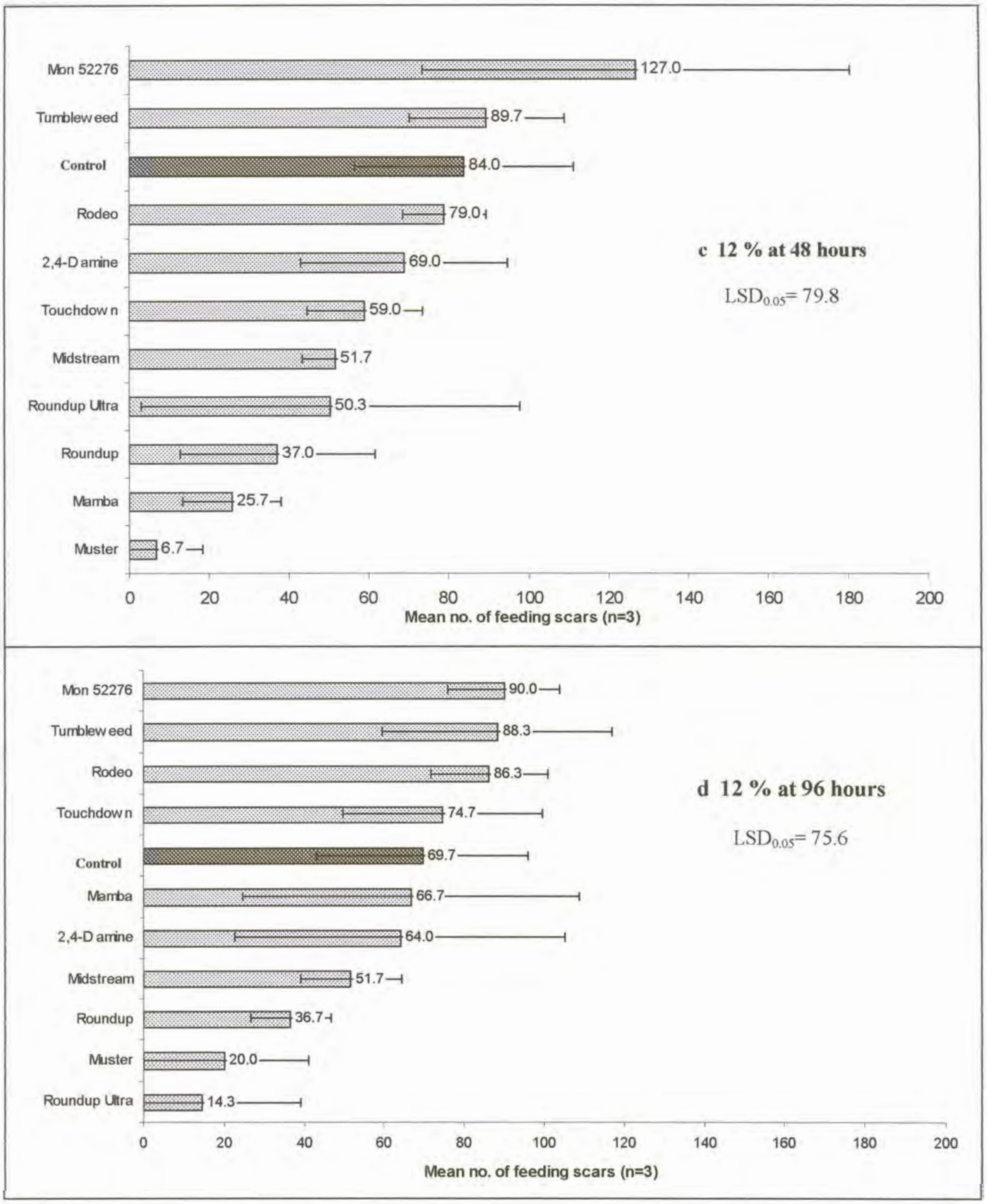


Figure 4.2 Continued

4.3.2 Effect of increased herbicide concentration

The herbicides in Figure 4.3 caused trends of decreased feeding with increases in herbicide concentration, while those not included showed no definite dose-response. Insects treated with Midstream showed no significant increase or decrease in feeding with increased concentration (Figure 4.3a). When Midstream and Agral were combined at the lowest recommended dosage (3.75 % Midstream and 0.75 % Agral), and even half the lowest recommended dosage, it decreased feeding significantly ($p < 0.05$) (Figure 4.3b). Exposure to high concentrations of Muster led to a significant decrease in feeding at 48 hours, but there was no significant difference at 96 hours (Figure 4.3c). Muster, which had previously led to mortality of the weevil (Chapter 3), only decreased feeding significantly at unusually high concentrations of the herbicide.

Of the glyphosate-based herbicides that exhibited a dose-response, Mamba caused decreased feeding at higher concentrations, but this was not significant (Figure 4.3d). Roundup led to decreased feeding at unusually high herbicide concentrations, feeding at 12 % and 24 % was significantly less ($p < 0.05$), but only at 48 hours (Figure 4.3e). Roundup Ultra also caused significant decreases in feeding at unusually high concentrations of 12 % and 24% but only at 96 hours ($p < 0.05$) (Figure 4.3f).

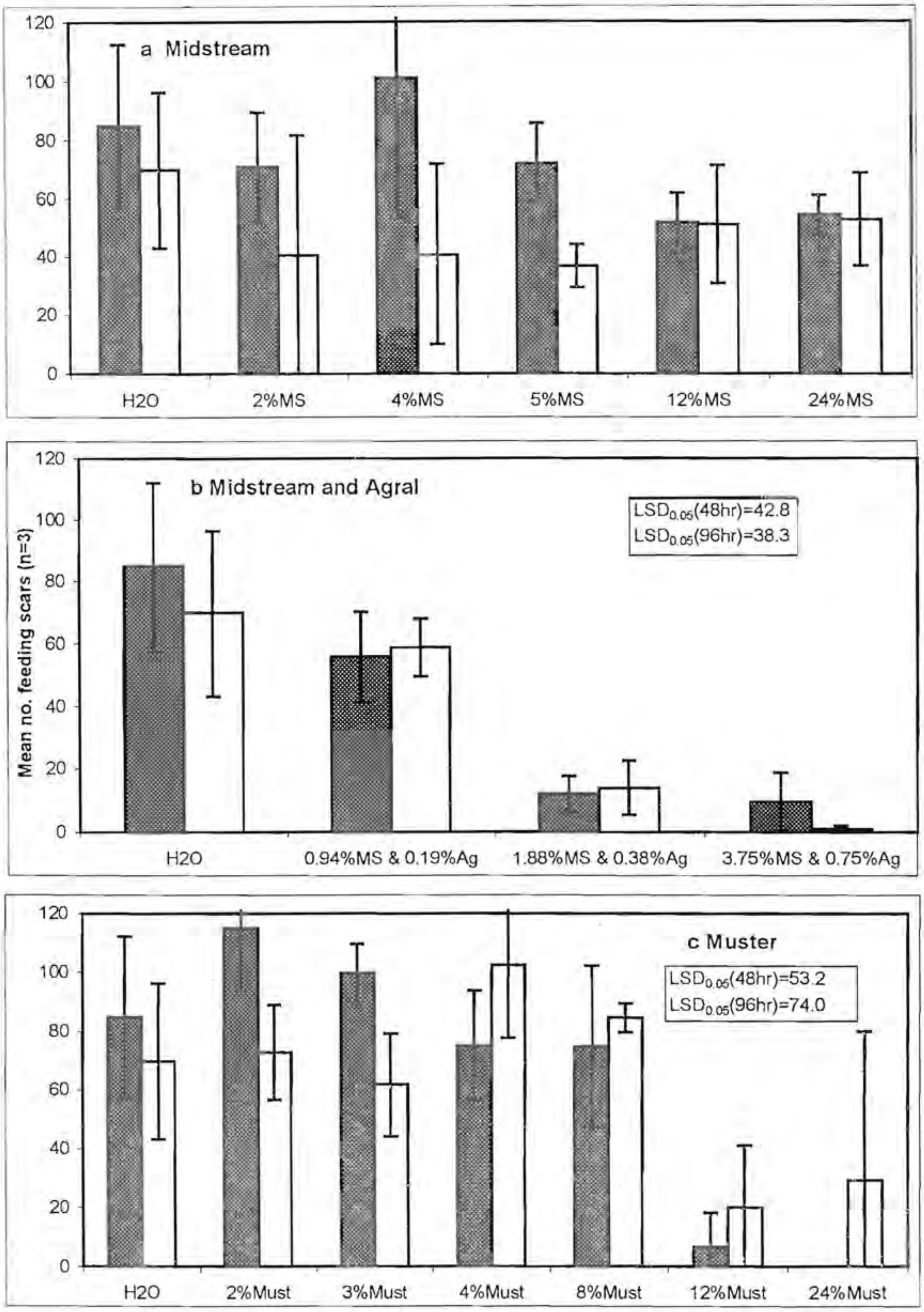


Figure 4.3 Mean number of feeding scars produced by groups of six *Neochetina eichhorniae*, after direct exposure to selected herbicides and surfactants (n=3).

Mean number of feeding scars after 48 hours
Mean number of feeding scars after 96 hours

LSD = Least significant difference; H2O = Control; MS = Midstream; Ag = Agral; Must = Muster

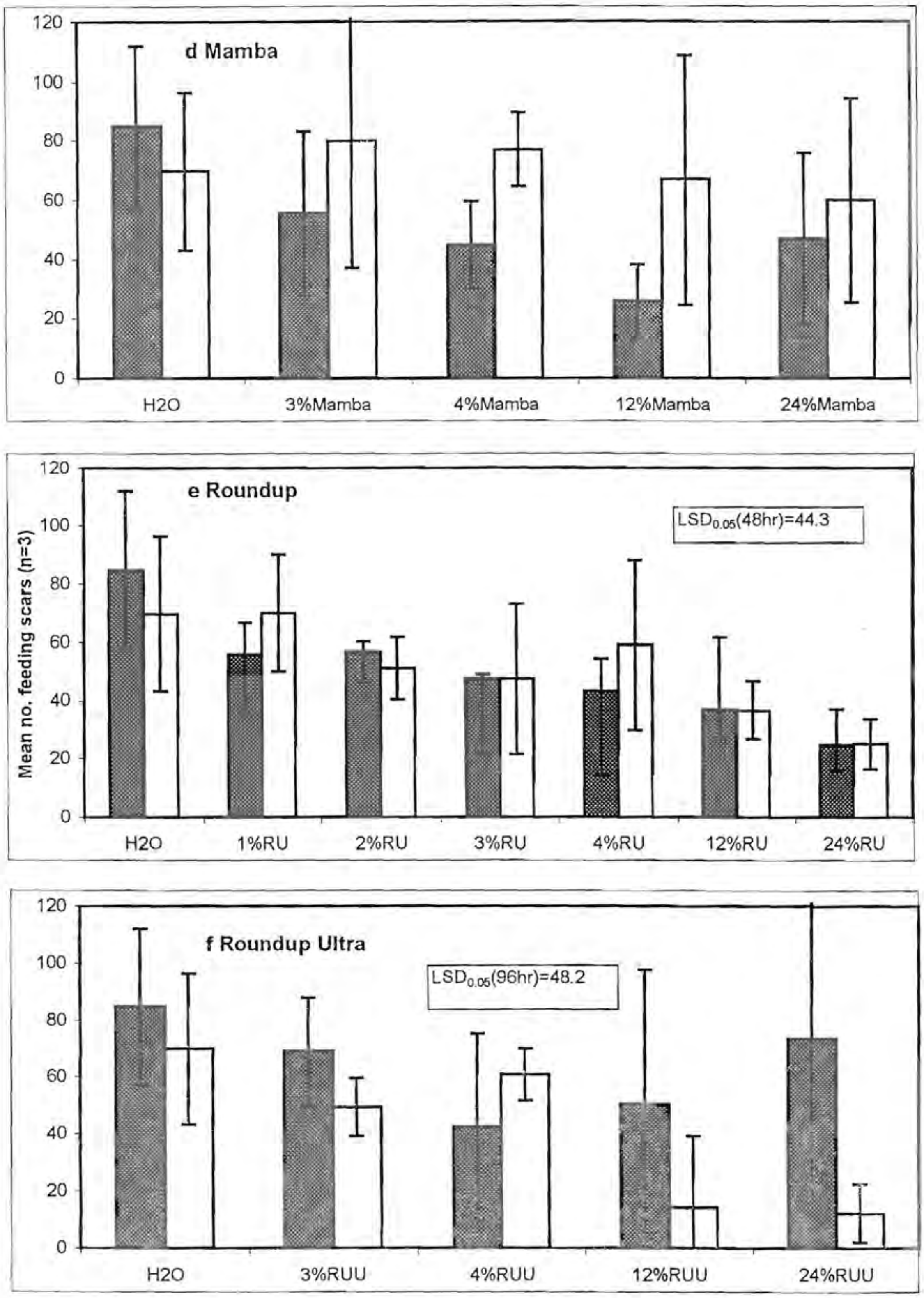


Figure 4.3 Continued

LSD = Least significant difference; H2O = Control; RU = Roundup; RUU = Roundup Ultra

4.4 CONCLUSION

The changes in feeding could not be attributed to a specific active ingredient, as treatment with some glyphosate formulations resulted in increased feeding (including Rodeo and Tumbleweed) and others decreased feeding (including Roundup and Roundup Ultra). For the glyphosate-trimesium formulations, one caused increased feeding (Touchdown) while the other caused decreased feeding (Muster). The 2,4-D amine formulation resulted in decreased feeding. Diquat was the only formulation that resulted in significant changes in feeding, when combined with a surfactant. The herbicides could not be rated based on these results.

It could be that surfactants contribute to the toxic effect of the formulation more than the active ingredient, which explains why different formulations of the same active ingredient, even at the same amount of acid equivalent (Figure 3.1), lead to different effects on feeding.

Exposure to toxins may result in complex metabolic reactions that could not be explained by quantifying feeding by counting feeding scars. Increases in feeding could be the result of increased metabolism through which insects try to rid their bodies of toxins, as it has been shown by Landis and Yu (1995) that fasting results in decreased metabolic rate and toxins stay in the organism's body longer. Decreased feeding may be a strategy to avoid the intake of toxins e.g. the grass carp's (*Ctenopharyngodon idella* Val.) feeding decreased at sub-lethal concentrations of herbicides (Tooby *et al.* 1980).

Application of a droplet of herbicide on the weevil's body does not represent the situation it will be exposed to in the field. A more likely scenario where the weevil come into contact with herbicide treated leaf material during nocturnal feeding, is investigated in the next chapter.

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

5.1 INTRODUCTION

The previous two chapters have shown that direct application of herbicides to the weevil *Neochetina eichhorniae* did have an effect on its mortality and feeding behaviour. This effect was usually not significantly different from the control and showed the weevils to be fairly resistant or tolerant to most of the herbicides tested. In addition, the methodology in the previous two chapters was not a true reflection of what the weevils are exposed to in the field, but a study of the relative toxicity of selected formulations.

As the weevils are nocturnal and shelter at the base of the petioles during the day they are unlikely to come into direct contact with the herbicides during field application. However, the weevils will feed on water hyacinth that has been sprayed with herbicides. The effect of herbicide-treated plants on weevil feeding behaviour has not been studied well. Jianquin *et al.* (1999) claimed that feeding on plants treated with high concentrations of Roundup resulted in some mortality of 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). However, a study by Pellessier (1988) showed that feeding by *N. eichhorniae* and *N. bruchi* decreased on 2,4-D amine and diquat treated leaves relative to the control treatment.

This study investigates the feeding behaviour and mortality of *Neochetina eichhorniae* on water hyacinth leaves treated with three herbicide formulations, each containing a different active ingredient.

5.2 MATERIALS AND METHODS

Groups of five adult weevils were kept in plastic containers (500 ml), lined with moist filter paper and aerated through small holes in the top. A young water hyacinth leaf was removed from the plant and put into each container for a period of 24 hours, after which the leaf was removed and feeding scars and weevil mortality documented (0 hours). This initial period of 24 hours serves as a second control, to ensure that the groups of weevils showed similar feeding capacity.

At 0 hours, a new leaf painted with a freshly made-up solution of either glyphosate (Roundup); 2,4-D amine or diquat (Midstream) made up to a 6 % concentration with distilled water, or a control leaf treated with distilled water, was provided (Table 3.1). Weevils were allowed to feed on the treated leaves for 24 hours after which the feeding scars and mortality were recorded. Treated food was provided in the same manner, every 24 hours and feeding scars and mortality was recorded daily, at 24, 48, 72, 96, 120 and 144 hours (Figure 5.2). The experiment was conducted at temperatures varying between 22 and 28°C, and a light regime of 16 hours light and 8 hours darkness. The experiment was replicated six times. The mortality data did not comply with the requirements for parametric analysis, therefore the Kruskal-Wallis ANOVA by ranks and Dunn's multiple comparison test (Zar 1984) was used (Appendix 13). The feeding data was analized using a one-way analysis of variance for parametric data (Appendix 14).

5.3 RESULTS AND DISCUSSION

5.3.1 Evaluation of methodology

The toxicity may be overestimated because weevils receive freshly treated food daily. A more natural situation would be to feed on an intact plant that has received a single herbicide treatment at the start of the experiment. This would however make regular observation of feeding and mortality difficult. If only one treated leaf is provided, wilting will occur quickly and the experiment could only last for approximately 48 hours. This method was therefore chosen to determine whether insects detect herbicides and refuse to feed, carry on feeding or show increased feeding on treated leaves.

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 and changes in the chemistry of leaves may occur, that would not in an intact plant. Even though severed leaves were used in this study, the results show a similar decrease in feeding that was found by Pellessier (1988) on whole plants.

5.3.2 Appearance of Leaves

Control leaves painted with distilled water, displayed no visible signs of deterioration or wilting. Glyphosate (Roundup) treated leaves appeared normal, sometimes discolouring slightly and becoming yellowish. Diquat (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 infected with a powdery white fungus, as this fast-acting herbicide led to rapid tissue deterioration.

5.3.3 Mortality

From 120 hours insects that fed on 6 % diquat (Midstream) treated leaves showed a significant increase in mortality (56.7 % at 120 hours and 70.0 % at 144 hours) when compared with the control (Figure 5.1, Appendix 13). 2,4-D amine also resulted in some mortality but this was not significantly different from the (Figure 5.1, Appendix 13). No insects feeding on untreated leaves or leaves treated with glyphosate (Roundup) died (Figure 5.1). Feeding on treated leaves lead to a reduction in feeding in some cases that could have led to starvation, but not in the 144 hours that the experiment lasted (Figure 5.2). Ingestion of water hyacinth leaf material treated with a 6 % concentration of diquat (Midstream) caused higher mortality in the weevils than direct exposure to the herbicide (Chapter 3). The highest mortality documented for

5.3.4 Feeding

There was an overall decrease in feeding over time, the control insects' feeding dropped from a mean of 66.0 scars at 0 hours to 51.7 scars at 144 hours (Figure 5.2). The control insects' feeding decreased by 14.3 scars; glyphosate (Roundup) treatment decreased by 20.2 scars; 2,4-D amine by 34.7 scars and diquat (Midstream) by 46.6 scars over 144 hours (Figure 5.2).

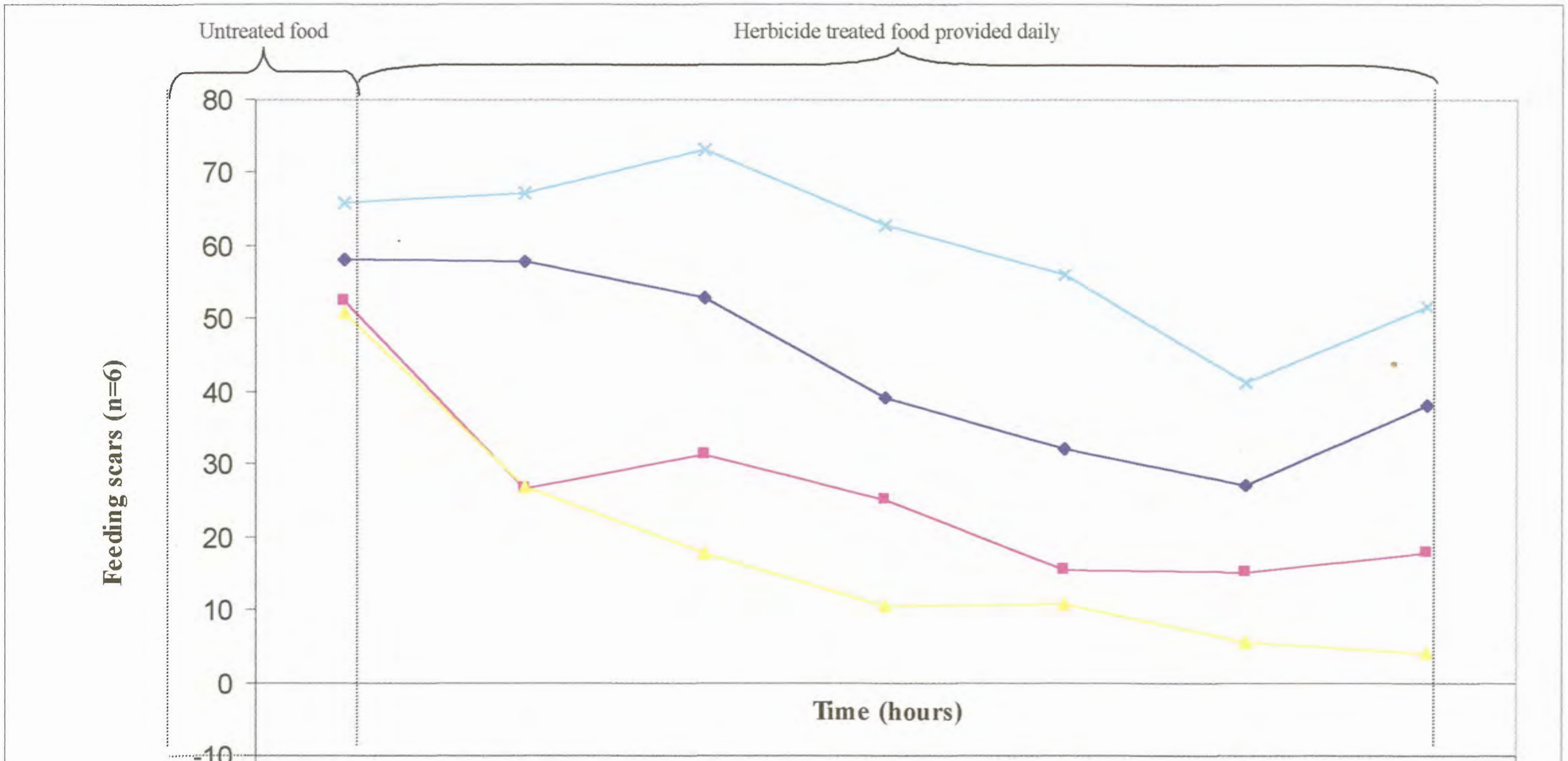
Feeding documented at 0 hours after 24 hours of feeding on untreated leaves, showed no significant difference (Figure 5.3a, Appendix 14). After 24 hours of feeding on leaves treated with diquat and 2,4-D amine, the weevils showed a significant decrease in feeding activity in comparison to the control ($p < 0.05$) (Figure 5.3b, Appendix 14). This decreased feeding activity continued for the rest of the 144 hours, for both diquat and 2,4-D amine (Figure 5.3c-g, Appendix 14).

Feeding on glyphosate (Roundup) treated leaves only reduced feeding significantly at 96 hours.

5.3.5 Literature

Wright and Bourne (1990) found that 2,4-D amine treated plants displayed softened petioles and increased nitrogen content that might induce increased feeding by herbivores. They suggested that it might be the reason for higher activity on the plants of the moth (*Niphograpta albiguttalis*) after herbicide treatment, and suggested that weevils may show the same trend. It has also been shown that glyphosate can lead to increased feeding by mammal herbivores, when applied at low concentrations (Sullivan 1985), possibly a result of increased sucrose.

Pellessier (1988) reported decreased weevil feeding on plants treated with diquat and 2,4-D (Weedar64), relative to a control population. Diquat-treated plants showed significant decreases in ether extractable compounds and ash content (Pellessier 1988). Both 2,4-D- and diquat-treated plants showed significantly reduced crude protein levels and an increase in nitrogen-free extracts, while 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. In this experiment, weevils did not show increased feeding on treated, severed leaves in this study or in the work of Pellessier (1988) on whole plants.



	0	24	48	72	96	120	144
6%Glyphosate	58.2	58.0	52.8	39.2	32.2	27.2	38.0
6%2,4-D amine	52.5	26.7	31.3	25.0	15.5	15.2	17.8
6%Diquat	50.8	27.0	17.8	10.5	10.8	5.7	4.2
Control	66.0	67.3	73.3	62.8	56.0	41.3	51.7

Figure 5.2 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).

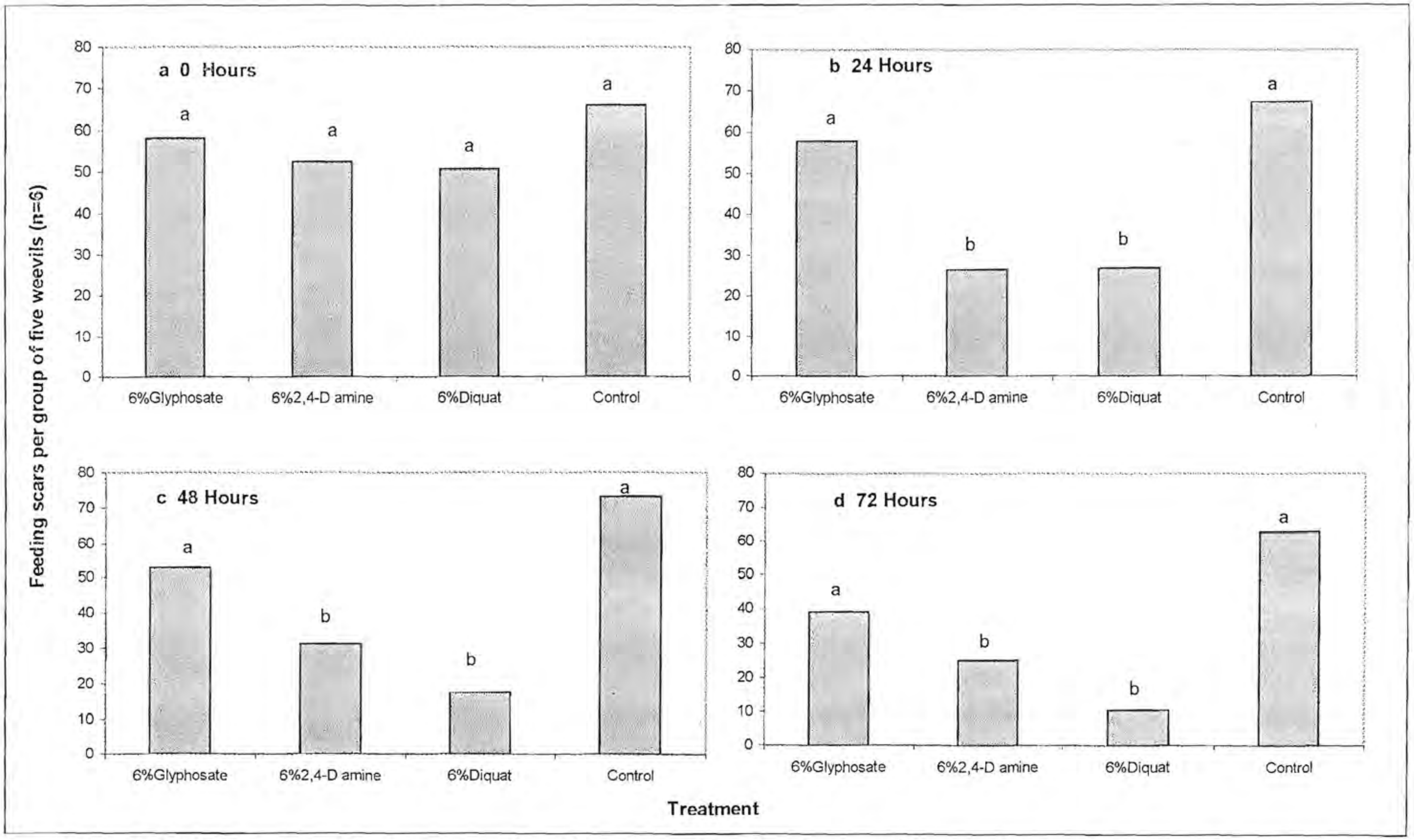


Figure 5.3 Mean number of feeding scars produced by a group of five *Neochetina eichhorniae* after feeding on treated leaves over a period of six days (Different alphabet letters indicate significant differences, also see Appendix 14),
a. After 24 hours of feeding on untreated leaves; b. after 24 hours of feeding on treated leaves;
c. after 48 hours of feeding on treated leaves; d. after 72 hours of feeding on treated leaves;
e. after 96 hours of feeding on treated leaves; f. after 120 hours of feeding on treated leaves;
g. after 144 hours of feeding on treated leaves.

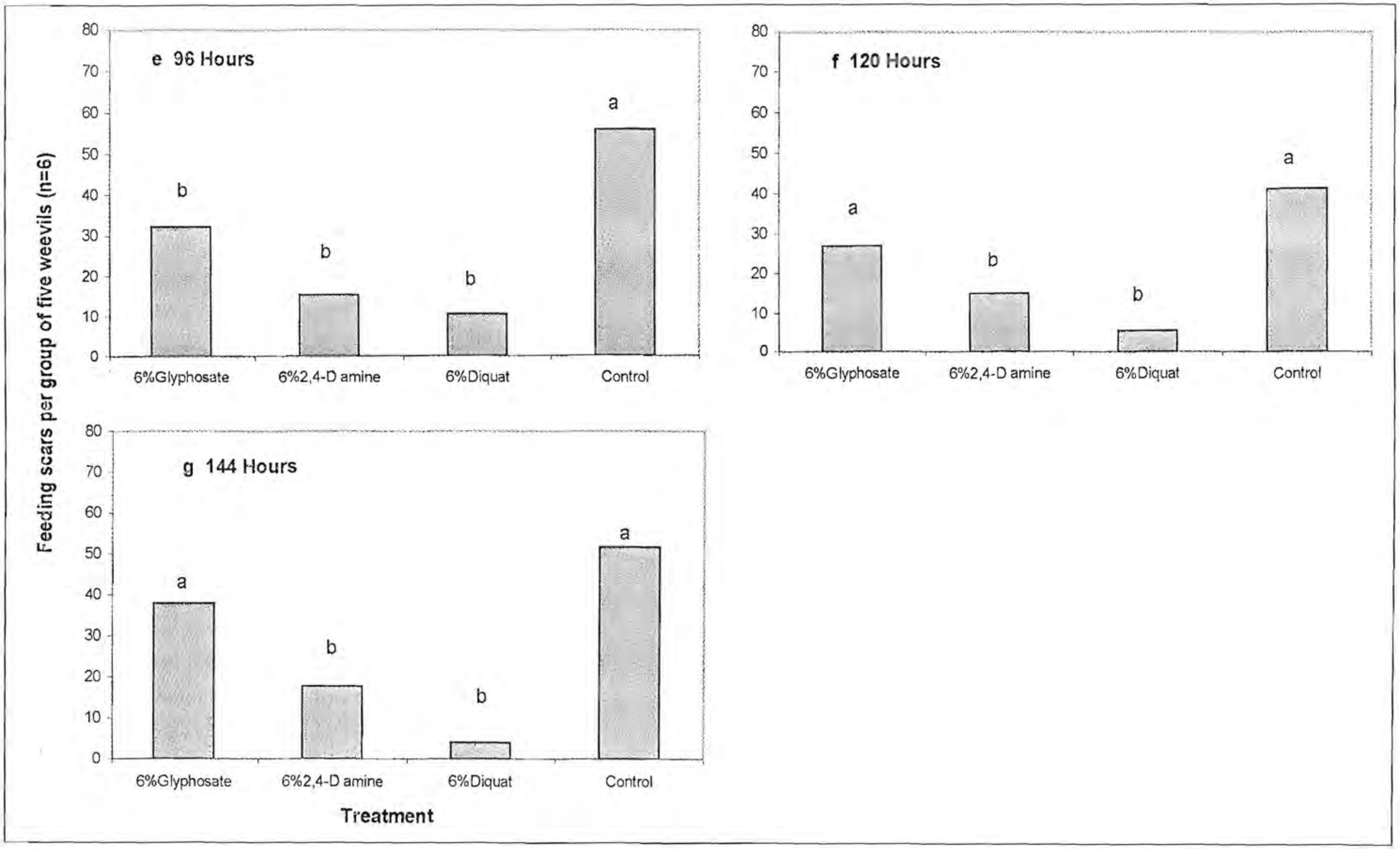


Figure 5.3 Continued

5.4 CONCLUSION

Ingestion of water hyacinth material treated with herbicides caused mortality similar to direct exposure experiments (Chapter 3). Feeding on glyphosate treated leaves resulted in no mortality; 2,4-D amine caused intermediate mortality and only diquat resulted in significant mortality of the weevils. All herbicide treatments led to decreased feeding, but only diquat significantly reduced feeding. Increased nitrogen (Wright & Bourne 1990) or sucrose in herbicide treated plant material did not lead to increased feeding.

This study has shown that weevils do not feed as well on treated plants as they did on untreated plants. It is therefore their ability to migrate that will determine their survival in rapidly degrading plant mats, as they did not exhibit a preference for treated plants. A slow acting herbicide may give insects ample time to flee or may be so undetectable that insects continue feeding and consuming toxins that may lead to chronic effects such as reduced fecundity.

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

6.1 INTRODUCTION

One of the concepts suggested in the integrated control of water hyacinth is the establishment of reservoir areas of unsprayed plants for insects to move onto while sprayed plants die and sink (Haag 1986b). This suggestion relies on the assumption that insects have the ability to disperse and are not directly affected by the herbicide. It has been shown in this study that some herbicide formulations do increase mortality and decrease the feeding rate of *Neochetina eichhorniae* and *Eccritotarsus catarinensis*, and that surfactants can contribute to the toxic nature of formulations (Chapters 3 to 5).

This suggestion 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. It has previously been shown that damaged water hyacinth plants release kairomones that stimulated feeding and oviposition by *Neochetina eichhorniae* and *Orthogalumna terebrantis* (Delfosse & Perkins 1977). The feeding of the moth *Niphograpta albiguttalis* seemed to increase on plants sprayed with 2,4-D amine (Wright & Bourne 1990). It was however shown in the previous chapter that *Neochetina eichhorniae* exhibit decreased feeding on severed, treated water hyacinth leaves.

Haag (1986b) and Pellessier (1988) 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.

Therefore, the aim of this study was to determine the pattern of movement and feeding behaviour of adult *Neochetina eichhorniae* on plants sprayed with formulations of two active ingredients, diquat and the slower acting glyphosate.

6.2 MATERIALS AND METHODS

Experiments were conducted in rectangular asbestos trays (2 100 mm long, 900 mm wide and 150 mm 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 (Osmocote) and 2.5 g of chelated iron was added (amounting 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 *Neochetina eichhorniae* were collected from the rearing facility at the Rietondale Experimental Farm in Pretoria. 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 herbicide was applied in the morning, before 11:00, ensuring that there was sufficient sunlight hours left to allow effective uptake of the herbicide. The 50 plants to be sprayed were lifted carefully from the tray to ensure that the leaves did not get wet, and placed in another water filled container. The group was then sprayed, left for approximately 10 minutes and carefully lifted and returned to the original tray. 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. 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 this experiment, glyphosate (Roundup), applied at 3 % (15 ml spray volume) and diquat (Midstream) applied at 3.75 % combined with 0.75 % Agral (15 ml spray volume). This was 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 under field conditions.

The glyphosate-treated plants were harvested 12 days after application, but the diquat-treated plants had to be harvested 6 days after application as the plant quality decreased rapidly (Figure 6.1). Plants were harvested once they 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 wase counted. There were four replicates for each herbicide. As data complied with the requirements for parametric analysis, a one-way analysis of variance was conducted to analyse feeding data (Appendix 15).

Figure 6.1 Water hyacinth plants six days after treatment with diquat (Midstream at 3.75 %) and a surfactant (Agral at 0.75 %) in the foreground, and untreated plants in the background.

6.3 RESULTS AND DISCUSSION

6.3.1 Appearance of Leaves

After treatment with glyphosate the plants turned yellow within a few days and any flowers that emerged turned white. The plants treated with this low dosage did not rot within the 12 days of monitoring, except for some of the smaller plants and older leaves. Diquat 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.

6.3.2 Movement of Weevils

According to the distribution of weevils at the time of collection the weevils moved from treated to untreated plants (Table 6.1, Figure 6.2). There was some movement of control insects, but this was probably a result of natural dispersal, 21.0 % of the surviving pink weevils had moved from the sprayed to the unsprayed section, while the remainder (79.0 %) of the pink weevils remained in the distilled water sprayed area. At the same time, 34.4 % of the surviving white weevils had moved to the distilled-water sprayed section with 65.6 % remaining in the unsprayed area.

The migration of surviving pink marked weevils in the diquat (Midstream) treatment was 80.2 % towards the unsprayed area, while 0.0 % of the surviving white weevils migrated towards the sprayed area (Table 6.1). Movement of weevils after treatment with glyphosate (Roundup) was also towards the unsprayed area, 87.3 % of the surviving pink weevils had moved to the unsprayed area and 6.0 % of the surviving white weevils had moved onto plants in the sprayed area (Table 6.1).

The weevil movement towards glyphosate treated plants (6.0 % of the surviving insects) was less than the normal movement by the control insects (34.4 % of the surviving insects) towards the distilled water sprayed section (Table 6.1). This does not agree with the suggestion that treated plants attract natural enemies because of an increased nitrogen content, softened petioles or the release of a kairomone associated with injury.

Table 6.1 The mean percentage survival and mean number of feeding scars by *Neochetina eichhorniae* weevils, after release on trays with herbicide treated and untreated plants

		Mean survival (%) of weevils		Mean number of feeding scars[1]
TREATMENT	**n**	**Pink**	**White**	
Glyphosate				
Sprayed	4	5.6 ±4.4	2.6 ±1.0	9.9 ±4.5
Unsprayed	4	38.6 ±11.4	41.0 ±7.4	40.2 ±5.0
Diquat				
Sprayed	4	10.6 ±10.2	0.0 ±0.0	8.6 ±2.2
Unsprayed	4	43.0 ±10.0	41.0 ±7.4	36.6 ±3.5
Control				
Sprayed	2	49.0 ±18.4	22.0 ±0.0	35.0 ±10.4
Unsprayed	2	13.0 ±7.0	41.0 ±18.4	35.5 ±8.8

[1] On youngest open leaf
Pink = Insects released in sprayed area
White = Insects released in unsprayed area
± = Standard deviation

Only 54.7 % of the released population was re-captured for the whole experiment (Table 6.2). As the trays were not covered, the reason for the reduction in the total number of weevils is uncertain and may be a result of mortality, 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 (44.4 %), considering the overall survival of the second trial (65.0 %) (Table 6.2). This could be as a result of leaving this trial for twice as long as the diquat trial, thereby allowing more time for dispersal of the insects. Treated ponds exhibited lower survival, or possibly higher dispersal, than the control pond in each trial (Table 6.2).

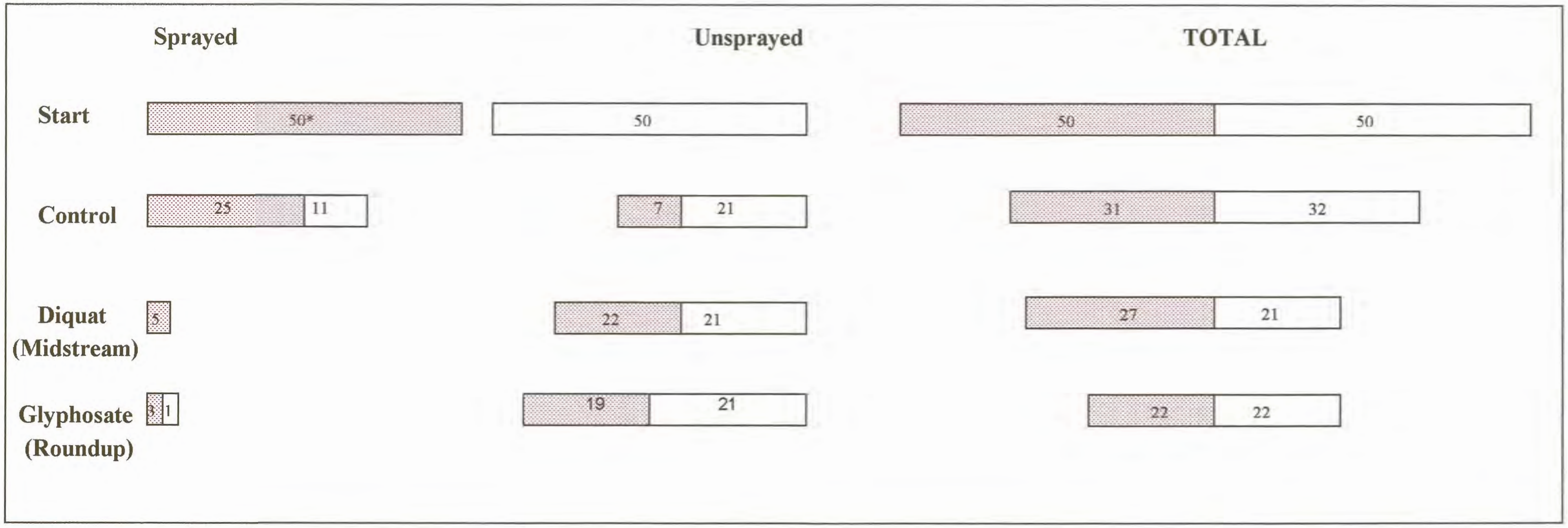


Figure 6.2 Movement of *Neochetina eichhorniae* adults between herbicide sprayed and unsprayed water hyacinth plants in a pond (n=4).

* Number of weevils released (at start) or captured

Pink (Weevils released in sprayed area of tray)

White (Weevils released in unsprayed area of tray)

Table 6.2 Survival rate for *Neochetina eichhorniae* on water hyacinth plants sprayed with the herbicide glyphosate (Roundup) and diquat (Midstream) as a percentage of the released number of insects

		Released	Recovered	Survival Rate (Rec/Relx100)
Trial 1	Treated ponds (glyphosate)	400	175	43.8
	Control	100	47	47.0
	Total	500	222	44.4
Trial 2	Treated ponds (diquat)	400	247	61.8
	Control	100	78	78.0
	Total	500	325	65.0
Total		1000	547	54.7

6.3.3 Feeding

The feeding damage expressed as number of adult feeding scars on the first fully expanded leaf, was similar in both halves of the control trays (Table 6.1, Figure 6.3). 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, $p < 0.05$) (Figure 6.3, Appendix 15). This suggests that the water hyacinth plants treated with herbicides are avoided by the weevils, possibly as it becomes unpalatable. However, the number of feeding scars on the unsprayed halves of the herbicide treated trays was not significantly higher than 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.

As in Chapter 5, feeding decreased significantly on diquat-treated plants, followed by movement away from the plants. Although feeding on glyphosate-treated plants did not result in mortality or seriously decreased feeding in the previous experiment, it seems that weevils still prefer untreated plants and move towards them.

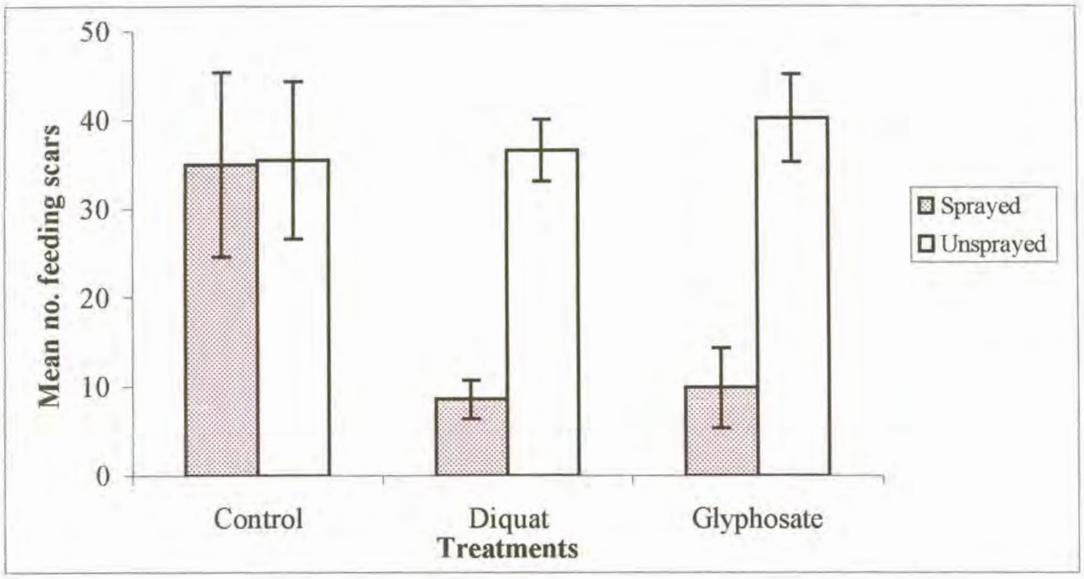


Figure 6.3 Feeding by *Neochetina eichhorniae* on herbicide sprayed and unsprayed water hyacinth plants.

6.4 CONCLUSION

This experiment was an attempt 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 a reservoir for biological control agents, as proposed in the integrated management plan for the Vaal River (Cilliers *et al.* 1996). It was shown that adult *N. eichhorniae* migrate from water hyacinth that had been sprayed with a herbicide, to unsprayed plants. Water hyacinth treated with diquat and glyphosate formulations do not seem to attract weevils when nutrient levels change, as was the case with 2,4-D amine which is suggested to lead to reduced petiole hardness and increased nitrogen content (Wright & Bourne 1990). If glyphosate had indeed led to increased sucrose values in the water hyacinth leaves as it does in crops such as sugar cane or grasses, it did not attract the weevils during this study.

The results of the behavioural experiments using marked weevils showed that adult weevils crawl to adjacent healthy plants from sprayed, dying plants. In addition to which, they could migrate through flight to other mats of the weed, although this was not tested in this experiment. In a similar study, Haag (1986a, 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*. The results of that experiment were similar to those reported in this study, and showed that weevils would move from sprayed to unsprayed plants up to a distance of four meters. The author suggested that it might be possible to spray mats of water hyacinth selectively and thereby herd adult weevils to unsprayed plants (Haag 1986b) and that portions of the mats should be left unsprayed and contained in distant areas where their economic impact would be small.

More research is necessary to determine the recovery rates of both the weed and the insect populations in order to determine the size and number of reserves of unsprayed plants needed.

7. General discussion and recommendations

7.1 AIM

The aim of this study was to determine whether herbicides applied to water hyacinth in South Africa are toxic to two of the insect species released as natural enemies. Furthermore, feeding and movement behaviour of insects in the presence of herbicide treated water hyacinth material was investigated. Two insect species were used in the trials, a weevil (*Neochetina eichhorniae*) and the water hyacinth bug (*Eccritotarsus catarinensis*). The weevil is a well-established natural enemy of the weed in South Africa and many other countries and the mirid is a recently released natural enemy against water hyacinth.

7.2 ACUTE TOXICITY TESTS

Acute toxicity tests encompassed exposure of insects to a droplet of herbicide. Ten herbicide formulations were tested of which all, except the 2,4-D amine formulation, contained one of the active ingredients registered for use on water hyacinth in South Africa. Although herbicide brands already contain surfactants, the efficiency of some formulations increase when more surfactant is added immediately before application. Two such surfactants were tested on their own and in combination with a herbicide formulation, at recommended dosages. Most herbicides were tested at a concentration slightly less that it is recommended at, one or two recommended dosages and one or two higher than recommended dosages.

Increased concentrations of most herbicides resulted in increased insect mortality. The weevil species was less susceptible to the herbicides overall, relative to the bug. The active ingredients could be rated according to the toxic effects on the two insect species tested.

Direct exposure to a diquat formulation, only one diquat formulation was tested, caused the highest mortality of all the formulations, correlating with results from previous toxicity studies (Pellessier 1988; Roorda *et al.* 1978). Glyphosate was the least toxic active ingredient, and the 2,4-D amine formulation was intermediately toxic. Two formulations of the active ingredient glyphosate-trimesium were tested and the toxicity varied from low to hazardous, probably the result of different surfactants in the formulations.

The least toxic substance was one of the five glyphosate formulations tested, and the only glyphosate formulation without any surfactant. It should be noted however, that another glyphosate formulation, Roundup Ultra, was rated as toxic toward the bug. This highlights the contribution of surfactants to the toxicity of a formulation, as even the most harmless active ingredient can result in serious insect mortality when combined with certain surfactants. The toxicity of the diquat formulation also increased when applied with a surfactant. The toxicity of surfactants was not sufficiently investigated during this experiment, and needs attention during further research on the topic.

It is therefore clear that the rating of active ingredients is very much dependant on the final formulation or spray mixture. Surfactants in the formulation or added to the spray mixture must be taken into account when making a choice of herbicide in an integrated control program with insect bio-control agents.

7.3 FEEDING BEHAVIOUR AND MOVEMENT

During the first group of feeding trials, weevils were directly exposed to a range of herbicides at concentrations of 4 % or 12 %, of all ten herbicides and some of the herbicides with added surfactants. Weevil feeding scars were counted at 48 and 96 hours after exposure. Diquat combined with a surfactant at recommended dosages, resulted in a significant decrease in feeding while the diquat formulation alone did not. Exposure to some of the other herbicides also caused increased feeding and others decreased feeding, but these differences were not significant.

The next feeding study tested the feeding of weevils on herbicide treated leaves over a six-day period. Freshly severed leaves treated with recommended dosages of either a diquat, 2,4-D amine or glyphosate formulation, were provided daily. Weevils feeding on diquat treated leaves showed significantly decreased feeding and significant mortality relative to the control treatment. Weevil feeding also decreased significantly on leaves treated with 2,4-D amine but little mortality occurred. Leaves treated with glyphosate had no effect on feeding. The results support the suggestion that glyphosate is the least toxic active ingredient and that diquat is relatively toxic to the weevil.

The last experiment entailed the monitoring of weevil movement on a water hyacinth mat, partly treated with herbicide. Two formulations were tested at recommended dosages, a diquat formulation (Midstream) with a surfactant (Agral), and a slower reacting glyphosate formulation (Roundup). Weevils were shown to migrate from the sprayed half of the mat to the unsprayed half, for both spray mixtures. Contradictory to previous reports, insects were not attracted by treated plants but fled the dying mats (Delfosse & Perkins 1977; Wright & Bourne 1990).

7.4 CONSIDERATIONS AND RECOMMENDATIONS

This study illustrates the important contribution of surfactants toward the toxicity of formulations or spray mixtures for insects. Keeping this in mind, the least toxic active ingredient, and therefore the one preferred for use in future integrated control of water hyacinth, is glyphosate. Formulations of both glyphosate-trimesium and 2,4-D amine can be used safely with insect bio-control agents when adhering to label specifications and concentrations, and careful consideration of formulation and surfactants. Diquat-based herbicides should not be used in integrated control of water hyacinth, as insect mortality will be high.

Contrary to previous reports, this study has shown that weevils, unlike many mammal herbivores, are not attracted to herbicide treated leaves. Even glyphosate treated plants do not attract weevils, although it has been shown that grasses treated with glyphosate become more palatable to cattle and deer prefer feeding on glyphosate treated shrubs in the USA (Sullivan 1985). When weevil populations are forced to continue feeding on leaves treated with diquat, high mortality follows. However, weevils have been shown to move away from sprayed to unsprayed plants, thus aiding integrated control practices.

The suggestion of using reserve mats or "refugia" for insects to flee to after herbicide applications is therefore viable, but needs further investigation. Information concerning the required size and distance of reserve mats, which may vary depending on the natural enemy used at the site, is required.

7.5 CONCLUSION

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.

8. Summary

Water hyacinth is an aquatic plant originating from South America, and recognized as the world's most damaging aquatic weed. Large mats of water hyacinth invade water bodies, thus degrading the aquatic ecosystem and limiting all aspects of water utilization. Although biological control of the weed has been successful it is considered too slow, while herbicide applications can render short-term relief. Therefore, the integrated use of the two methods is expected to produce both successful short-term and long-term control. However, the success of this approach relies on the assumption that the two methods are compatible. This study was designed to determine whether herbicides registered for use on water hyacinth in South Africa are toxic to two insect species released for its biological control. The two agents chosen for this study were the weevil, *Neochetina eichhorniae*, and the mired, *Eccritotarsus catarinensis*. The study also investigated the feeding and migration of the weevil species, following direct and indirect exposure through herbicide treated plant material.

The first experiment tested the mortalities of both species as a result of direct exposure to the herbicides at a range of concentrations. The mirid was very susceptible to herbicide exposure and high mortalities were recorded, especially from the herbicide product with diquat as active ingredient. The surfactant content of herbicides played an important role in determining the toxicity of a formulation. Even though weevils were in general less susceptible to herbicide exposure, treatment with diquat still resulted in significant mortality in the weevil population.

The feeding behaviour of the weevil was quantified after it had been treated with herbicide concentrations of 4 or 12 %. The herbicide containing diquat combined with the surfactant Agral caused a significant reduction in weevil feeding. Of the other herbicides tested, some caused an increase and some a decrease in weevil feeding, but these differences were not significant.

Weevils feeding on diquat- and 2,4-D amine-treated leaves exhibited a significant decrease in feeding over a six-day period. Feeding on diquat-treated leaves also resulted in significantly higher mortality than on 2,4-D amine- and glyphosate-treated leaves.

Movement of weevils was monitored after half a mat of water hyacinth was sprayed with either glyphosate or diquat. In both cases, the weevils migrated away from the sprayed plants to the unsprayed sections of the mat.

The implications for integrated control of water hyacinth in South Africa are that where possible glyphosate-based herbicides with low surfactant content and low active ingredient concentrations should be used. It is vital that reserves or "refugia" be maintained to harbour insect populations, although the required size and distance of reserve areas warrant further investigation. Consultation between herbicide and biological control practitioners, on a site-specific basis is necessary for the successful integrated control of water hyacinth in South Africa and elsewhere where the weed constitutes a problem.

8. Opsomming

Waterhiasint is 'n akwatiese plant wat afkomstig is van Suid Amerika en wêreldwyd beskou word as een van die nadeligste onkruide. Die plant verdring inheemse waterplante om groot matte te vorm wat die akwatiese ekosisteem drasties verander en degradeer. Biologiese beheer van die onkruid was reeds in sommige gevalle baie suksesvol. Die implementering van biologiese beheer neem egter lank en daarom word onkruiddoders steeds gebruik om korttermyn beheer te bewerkstellig. Die beheer van waterhiasinte is tans reeds 'n vorm van geïntegreerde beheer, al is dit onduidelik of die twee metodes mekaar aanvul. Hierdie studie het beoog om vas te stel of die onkruiddoders wat geregistreer is vir gebruik op waterhiasinte, toksies is vir twee van die insekspesies wat as natuurlike vyande in Suid-Afrika vrygelaat is. Die invloed van indirekte blootstelling aan sommige van die onkruiddoders en bymiddels word ook ondersoek, deur insekvoeding en -migrasie te monitor in die aanwesigheid van behandelde plantmateriaal. Die twee biologiese beheeragente wat vir die studie gekies is, is die snuitkewer, *Neochetina eichhorniae*, en 'n besie wat van die familie Miridae, *Eccritotarsus catarinensis*.

Die eerste eksperiment het die mortaliteit van beide spesies bepaal nadat hulle direk blootgestel is aan onkruiddoders teen 'n reeks konsentrasies. Die besie was in die algemeen meer sensitief vir onkruiddoders as die kewer, en veral sensitief vir die onkruiddoder met dikwat as aktiewe bestandeel. Die snuitkewer het lae mortaliteit getoon in die algemeen, terwyl blootstelling aan die dikwat onkruiddoder duidelik die hoogste kewer mortaliteit veroorsaak het. Die bymiddels het wel bygedra tot die toksisiteit van die onkruiddoders.

Die voeding van snuitkewers gemonitor nadat hulle direk blootgestel is aan onkruiddoders teen konsentrasies van 4 of 12 %. Behandeling met die onkruiddoder wat dikwat bevat gekombineer met die bymiddel Agral het 'n betekenisvolle afname in voeding veroorsaak. Ander toenames en afnames in snuitkewervoeding is gevind maar hierdie veranderinge was nie betekenisvol nie.

Die volgende voedingseksperiment het die reaksie van snuitkewers ondersoek, in die aanwesigheid van waterhiasintblare wat afgesny is en daarna met ondkruiddoder behandel is. 'n Dikwat, 2,4-D amien en glifosaat onkruiddoder is gekies en getoets oor 'n tydperk van ses dae. Voeding het betekenisvol afgeneem op dikwat- en 2,4-D amien-behandelde voedsel, en betekenisvolle mortaliteit is aangeteken by snuitkewers wat op dikwat-behandelde plantmateriaal gevoed het.

Die beweging van snuitkewers is bestudeer in 'n dam waar die helfde van die waterhiasintmat behandel is met glifosaat of dikwat teen 'n aanbevole konsentrasie. Die snuitkewers het in beide gevalle migreer vanaf die behandelde na die onbehandelde deel van die plantmat.

Voorstelle vir geïntegreerde beheer wat gedurende hierdie studie na vore gekom het, is dat daar verkieslik van glifosaat onkruiddoders met 'n lae bymiddelinhoud en teen lae aktiewe bestandeel konsentrasies gebruik gemaak moet word. Dit is noodsaaklik dat reserwe plante beskikbaar is tydens onkruiddodertoedienings, maar die grootte en afstand van sulke matte benodig verdere ondersoek. Konsultasie tussen diegene wat chemiese en biologiese beheer toepas is noodsaaklik vir elke infestasie, om suksesvolle geïntegreerde beheer te verseker in Suid-Afrika asook elders waar waterhiasinte probleme veroorsaak.