RESEARCH ARTICLE

Toxic effect of herbicides used for water hyacinth control on two insects released for its biological control in South Africa

M.P. Hill[a]*, J.A. Coetzee[a] and C. Ueckermann[b]

[a] *Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa,* [b] *Department of Botany, University of Pretoria, Pretoria, 0001, South Africa*

*Corresponding author address: m.hill@ru.ac.za

Abstract

The integrated control of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) has become necessary in South Africa, as biological control alone is perceived to be too slow in controlling the weed. In total, seven insect biological control agents have been released on water hyacinth in South Africa. At the same time, herbicides are applied by the water authorities in areas where the weed continues to be troublesome. This study investigated the assumption that the two control methods are compatible by testing the direct toxicity of a range of herbicide formulations and surfactants on two of the biological control agents released against water hyacinth, the weevil, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and the water hyacinth mirid, *Eccritotarsus catarinensis* (Carvahlo) (Hemiptera: Miridae). A number of the formulations used resulted in significant mortality of the mirid and the weevil. Products containing 2,4-D amine and diquat as active ingredients caused higher mortality of both agents (up to 80% for the mirid) than formulations containing glyphosate. Furthermore, when surfactants were added to enhance herbicide efficiency, it resulted in increased toxicity to the insects. We recommend that glyphosate formulations should be used in integrated control programmes, and that surfactants be avoided in order to reduce the toxic nature of spray formulations to the insect biological control agents released against water hyacinth.

Keywords: *Eccritotarsus catarinensis, Eichhornia crassipes*, integrated control, *Neochetina eichhorniae*

Introduction

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), is a perennial, herbaceous, free-floating, aquatic plant native to the Amazon Basin of South America (Center 1994). This species is now widespread throughout the tropical and subtropical areas of the world (Holm et al. 1977), and is invasive in South Africa where dense mats of the weed degrade aquatic ecosystems (Midgley et al. 2006) and severely limit their use (Hill 2003). Biological control of water hyacinth has been highly successful in some areas of South Africa, where a suite of seven biological control agents have been used to reduce populations of the weed (Coetzee et al. 2011). However, in colder areas of the country (Hill and Olckers 2001) and in eutrophic waters, biological control has been less successful (Coetzee et al. 2007; Coetzee and Hill 2012). In these areas, biological control is considered to be inadequate, or too slow acting and attempts have been made to integrate different control methods to achieve the benefits of both short-term and sustainable, long-term control (Cilliers et al. 1996; Coetzee et al. 2012). The integration of biological and herbicide control is currently the most widely advocated method. However, this approach relies on the assumption that these two methods are compatible and especially that the herbicides themselves are not toxic to the biological control agents (Hill and Olckers 2001).

There have been a number of studies investigating the direct effect of herbicides on some of the biological control agents released on water hyacinth (e.g. Roorda et al. 1978; Center et al. 1982; Haag 1986a, b; Haag et al. 1988; Wright and Skilling 1987; Grodowitz and Pellessier 1990; Jianqing et al. 1999, Jadhav et al.2008). Generally, these studies found that the herbicide increased mortality of the biological control species. It also was found that it was not always the active ingredient in the herbicide that made it toxic to the agents, but rather the surfactants and other additives (e.g. drift retardants) that caused mortality (Affeld et al. 2004). In addition, the rapid loss of habitat through the sinking of the water hyacinth mat after herbicide application decimated biological control agent populations, and required re-inoculation once the mat had regenerated through seedling recruitment (Center et al. 1999).

In an attempt to improve integrating herbicides with water hyacinth biological control agents, the toxicity of a number of herbicides registered for use on the weed in South Africa and elsewhere in the world was tested on two of the species released as biological control agents. The two agents chosen were the water hyacinth weevil, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), which has been released in at least 30 countries around the world (Julien and Griffiths 1998), and the leaf-sucking mirid, *Eccritotarsus catarinensis* (Carvahlo) (Heteroptera: Miridae), which was released on water hyacinth in South Africa in 1996 (Hill et al. 1999). These two agents differ in that the adult weevils are nocturnal, hiding amongst the youngest, rolled water hyacinth leaves during the day and the larvae feed within the plant tissue (DeLoach and Cordo 1976), whereas *E. catarinensis* nymphs and adults feed externally on the leaf surface during the day (Hill et al. 1999).

The approach to integrated control of water hyacinth in South Africa employed in the larger systems, such as the Vaal River, is to spray 75% of the weed infestation with herbicides and leave a 25% “reserve” of the weed onto which the biological control agents can move (Cilliers et al. 1996). This approach also has been suggested in the USA (Haag et al. 1988) and has the benefit of providing both short- and long-term control. However, this method relies on the assumptions that the biological control agents can and do disperse and not sink with the sprayed plants. Grodowitz et al. (1997) showed that adult *N. eichhorniae* and its congener *N. bruchi* (Hustache) frequently had reduced wing musculature and would thus not be capable of flight, and the sessile stages of all of the agents would sink with the mat. Secondly, the method assumes that the natural enemy populations in the “reserve” are large enough to curb the spread of the weed, and the reserve itself will not become the source of re-infestation. Lastly, it assumes that the herbicides themselves are not directly toxic to the biological control agents. It is the last assumption that was tested in this study.

Materials and methods

Herbicide selection

In this study, a series of herbicide formulations (Table 1) was applied directly to the two insect test species. Diquat, glyphosate and glyphosate-trimesium are registered for use on water hyacinth in South Africa (Vermeulen et al. 1998), and although 2,4-D amine is not, it was included as it is popular in neighbouring countries due to its effectiveness and affordability. For each product, a range of concentrations was tested, from below to far above the manufacturers’ recommended rates for water hyacinth control. In addition to these herbicides, two surfactants also were tested; Agral®, which is recommended for use with Midstream® (diquat) and Add-2®, which is recommended for use with Touchdown® (glyphosate-trimesium). Combinations of Midstream® with Agral®, and Touchdown® with Add-2®, were tested to determine whether adding surfactants to the herbicides affected toxicity.

Table 1. Specifications of the herbicides and surfactants selected for testing (formulation composition and recommended dosages), and dosages tested on two agents released for the biological control of water hyacinth in South Africa.

Brand name	Manufacturer	Content		g a.e/l	Recommended dosages (% vol/vol)	Dosages tested (% vol/vol)
2,4-D amine	Sanachem	2,4-Dichlorophenoxyacetic acid 480 amine		480	2.00–6.00	1, 3, 9, 18
Mamba 360 SL[a]	Sanachem	Glyphosate SL (IPA salt)		360	2.00–6.00	3, 4, 12, 24
Midstream[a]	Zeneca	Diquat (dibromide salt)		373.5	3.75–5.00	1, 2, 3.75, 4, 5
Mon 52276	Monsanto	Glyphosate (IPA salt)		360	2.00–6.00	3, 4, 12, 24
Muster[a]	Zeneca	Glyphosate (Trimethyl sulfonium salt)		330	2.00–3.00	2, 3, 12, 24
Rodeo[a]	Monsanto	Glyphosate (IPA salt)		480	4.50–9.00	1.5, 3, 4, 9, 18
Roundup[a]	Monsanto	Glyphosate (IPA salt)		360	2.00–6.00	1, 2, 3, 4, 12, 24
Roundup Ultra[a]	Monsanto	Glyphosate		360	2.00–6.00	3, 4, 12, 24
Touchdown[a]	Zeneca	Glyphosate (Trimethyl sulfonium salt)		480	2.00	2, 4, 8, 20
Tumbleweed[a]	Enviro Weed Control	Glyphosate (IPA salt)		240	2.00–6.00	1, 4, 12, 24, 50
Surfactants		Description				
Add-2[a]	Zeneca	Spreader adjuvant (with Touchdown)	Polysaccharide (hexitan ester)	600	0.20–0.30	0.2, 0.3, 0.6, 1.0
Agral[a]	Zeneca	Wetting and sticking adjuvant (with Midstream)	Alkylated phenol-ethylene oxide concentrate	940	0.75	0.05, 0.75, 1

g a.e/l, gram acid equivalent per litre.
[a]Registered under Act 36 (1947) for control of water hyacinth.

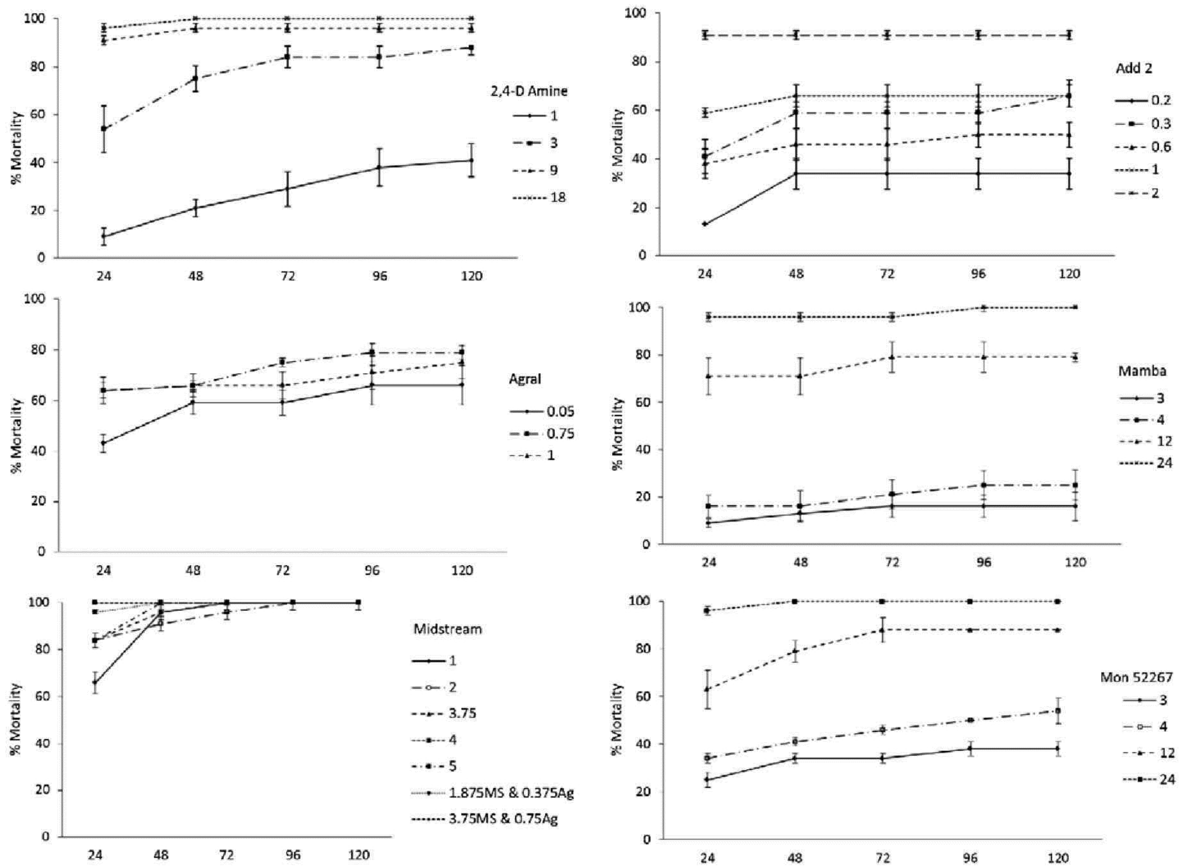

Figure 1. Mean percentage mortality of *Eccritotarsus catarinensis* as a result of direct treatment with 12 selected herbicides and herbicide/adjuvant combinations, at various concentrations over five time intervals. For comparison, mirid mortality in the control treatment was low: 24 hours – 8%; 48 hours – 10%; 72 hours – 15%; 96 and 120 hours – 17%. Legends represent % concentration (%vol/vol) of herbicide applied. Error bars represent 95% CI.

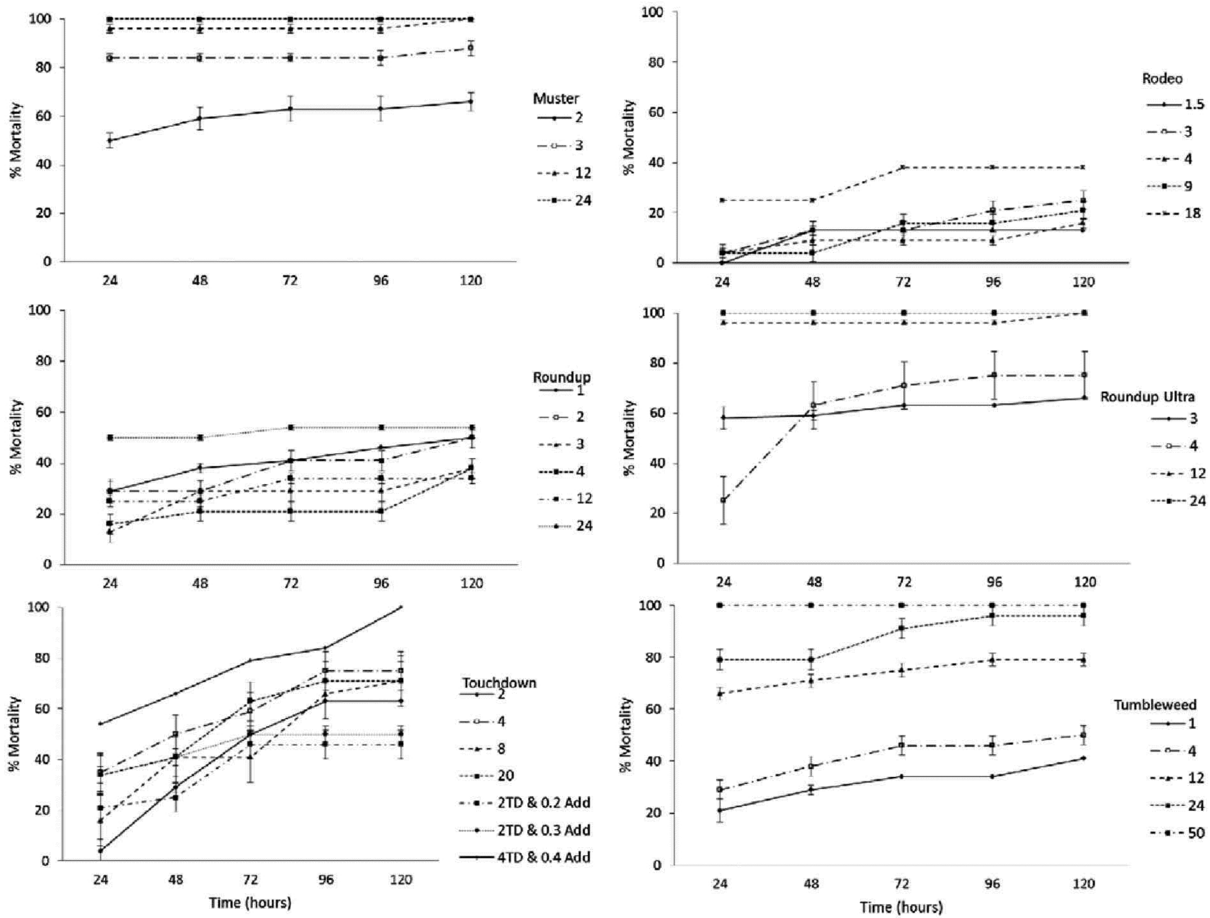

Figure 1. *Continued*

Table 2. LC_{50} values, after 24 and 120 hours of exposure to herbicides and surfactants registered for use in the control of water hyacinth, and the rating of each according to the effect that they had on the control agents, *Eccritotarsus catarinensis* and *Neochetina eichhorniae*, during acute toxicity tests.

		E. catarinensis			*N. eichhorniae*		
		$LC_{50} \pm SE$ (χ^2 value, $*P < 0.05$)			$LC_{50} \pm SE$ (χ^2 value, $*P < 0.05$)		
Herbicide[a]	Dosages (%) used in practice[b]	24 h	120 h	Rating[c]	24 h	120 h	Rating[c]
2,4-D amine	2.0–6.0	4.3 ± 0.02 ($\chi^2 = 192.0*$)	0.7 ± 0.04 ($\chi^2 = 120.9*$)	Toxic			Safe
Add-2	0.2–0.3	0.81 ± 0.11 ($\chi^2 = 135.3*$)	0.38 ± 0.11 ($\chi^2 = 62.4*$)	Safe			Safe
Agral	0.8	0.3 ± 0.18 ($\chi^2 = 11.2*$)	< 0.01 ($\chi^2 = 3.18$)	Toxic	0.69 ± 0.66 ($\chi^2 = 119.4*$)	0.63 ± 0.58 ($\chi^2 = 138.2*$)	Toxic
Mamba	2.0–6.0	10.1 ± 0.01 ($\chi^2 = 242.8*$)	7.8 ± 0.02 ($\chi^2 = 246.8*$)	Safe			Safe
Midstream	3.8–5.0	< 0.05 ($\chi^2 = 18.3*$)	NA (100% mortality)	Toxic			Safe
Mon 52276	2.0–6.0	8.56 ± 0.01 ($\chi^2 = 144.4*$)	4.2 ± 0.02 ($\chi^2 = 142.3*$)	Hazardous			Safe
Muster	2.0–3.0	< 0.01 ($\chi^2 = 91.2*$)	1.46 ± 0.20 ($\chi^2 = 83.9*$)	Toxic	10.73 ± 0.02 ($\chi^2 = 420.0*$)	8.54 ± 0.03 ($\chi^2 = 516.2*$)	Safe
Rodeo	4.5–9.0	> 18 ($\chi^2 = 44.2*$)	> 18 ($\chi^2 = 15.7*$)	Safe			Safe
Roundup	2.0–6.0	> 26 ($\chi^2 = 23.9*$)	> 26 ($\chi^2 = 0.9$)	Safe			Safe
Roundup ultra	2.0–6.0	4.38 ± 0.02 ($\chi^2 = 172.3*$)	< 2 ($\chi^2 = 93.8*$)	Toxic	> 26 ($\chi^2 = 22.2*$)	18.89 ± 0.01 ($\chi^2 = 59.4*$)	Safe
Touchdown	2.0	> 20 ($\chi^2 = 11.34*$)	< 0.01 ($\chi^2 = 0.28$)	Toxic[d]			Safe
Tumbleweed	2.0–6.0	10.48 ± 0.01 ($\chi^2 = 214.1*$)	3.61 ± 0.01 ($\chi^2 = 170.5*$)	Hazardous			Safe

[a]Formulation or surfactant.
[b]Refer to product labels for specified dosages and application instructions.
[c]Safe, LC_{50} never fell within recommended dosage range; Hazardous, LC_{50} fell within or below the recommended dosage range at any time during 120 hours; Toxic, LC_{50} fell within or below the recommended dosage range from 24 hours.
[d]Considered toxic as it caused high mortality from 72 hours onwards.

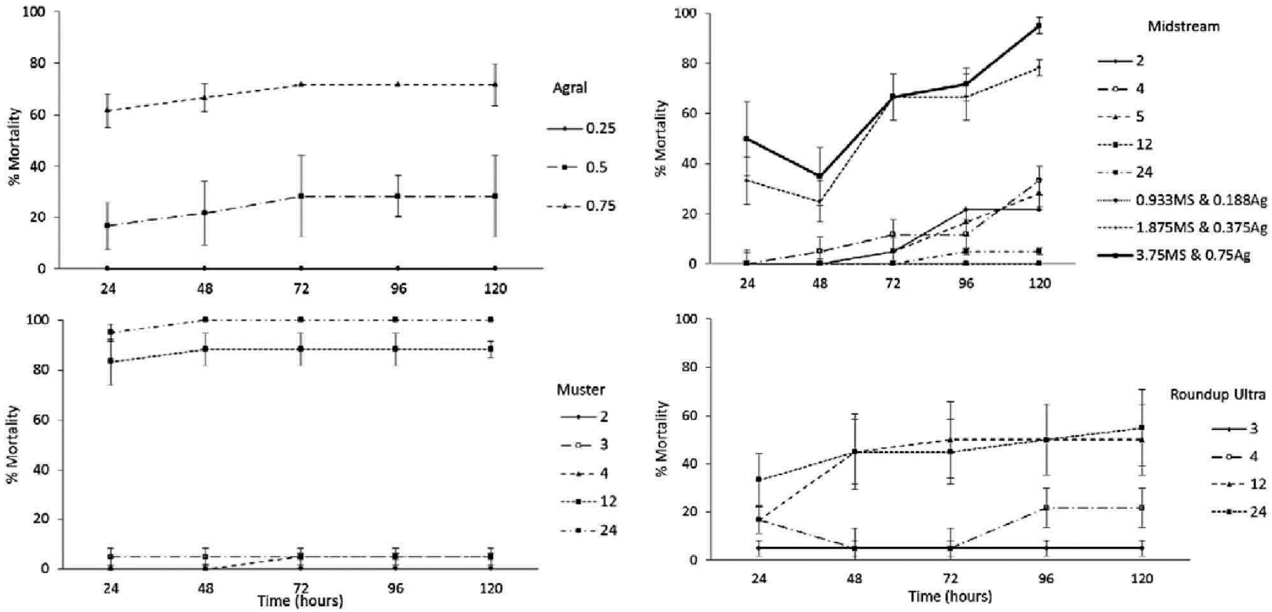

Figure 2. Mean percentage mortality of *Neochetina eichhorniae* as a result of direct treatment with four selected herbicides and herbicide/adjuvant combinations, at various concentrations over five time intervals. Control mortality of the weevils was 0% at all of the time intervals. Legends represent % concentration (%vol.vol) of herbicide applied. Error bars represent 95% CI.

Test organisms

1. *Eccritotarsus catarinensis*

Insects were collected approximately 2 days before treatment from ponds of water hyacinth at the Plant Protection Research Institute in Pretoria, South Africa, and kept in tubs with recently severed water hyacinth leaves from plants maintained at the Institute. Insects were sorted into groups of eight adults (four males and four females) and placed in small Petri-dishes (65 mm diameter) lined with moist filter paper, 1 day before treatment in order to acclimatise. Fresh dilutions of test chemicals were mixed less than 1 hour before application. A 20 µl droplet of chemical was applied dorsally to each insect. A chemical-free control also was set up, whereby a 20 µl droplet of distilled water was applied to each insect. Insect mortality was monitored every 24 hours for up to 120 hours after application of the chemical. Water hyacinth leaf pieces (20 x 30 mm) were provided as food every 24 hours and the humidity in the Petri-dish was maintained by moistening the filter paper with water. Petri-dishes were exposed to a light regime of 16 hours light, 8 hours darkness and temperatures varied between 22 and 28°C. Three replicates of eight insects were used for each of the chemical treatments and the control.

2. *Neochetina eichhorniae*

Adult weevils were collected from a field site (Bon Accord Dam 25°38'15.4"S/ 28°11'01.6"W), just north of Pretoria, approximately 2 weeks before initiation of the experiment and kept in plastic tubs with recently severed water hyacinth leaves, from plants maintained in pools at PPRI. Six weevils (three male and three female) were placed in 500 ml plastic containers lined with moistened filter paper and aerated through small holes in the top, at least 48 hours before commencement of the experiment. Insects were exposed to different chemical concentrations by applying a 20 µl droplet dorsally to the elytra of the insect. Again, a distilled water control was set up. Food was provided daily in the form of a severed water hyacinth leaf. The experiment was conducted under 16 hours light, 8 hours darkness and temperatures varied between 22 and 28ºC. Mortality was noted every 24 hours for up to 120 hours after chemical application. Three replicates of six insects each were used for each treatment and the control.

Statistical analyses

A lethal concentration (LC_{50}) value for each herbicide and/or adjuvant was obtained for each insect species, after 24 hours and 120 hours exposure, by deriving regressions of insect mortality against chemical concentration, using Probit regression analysis in Statistica v10 (StatSoft, Inc. 2011). The safety of each chemical was then rated based on its toxicity to the biocontrol agents, and recommended dosages used in practice.

Results

The two biological control agents responded differently to the chemical applications: the mirid was far more susceptible to the chemicals than the weevil. Generally, higher mortality of the mirid was recorded at higher concentrations, which increased over time until 72 hours, after which mortality was constant (Figure 1). The herbicides Rodeo® and Roundup® were the only two that did not cause 100% mortality of the mirid at any of the concentrations tested. Midstream® caused high mortality at all concentrations over the entire time period, while the addition of the surfactant, Agral®, caused 100% mortality at both concentrations tested. All of the other herbicides tested induced high mortality, particularly at higher dosages. The surfactant Agral® used alone was toxic to the mirid at all concentrations tested, but mortality of the mirid exposed to Add 2®, the other surfactant tested, was less than 60% at all of the dosages, except the highest dosage used, where mean mortality was 91% ± 0.93 across the time period. Mirid mortality remained between 8 and 17% across the time period for the control treatment.

The LC_{50} values were generally higher after 24 hours exposure than 120 hours exposure to the chemicals tested, implying that toxicity increased with time (Table 2). In certain cases (Midstream®, Muster®, Rodeo®, Roundup® and Touchdown®), the LC_{50}s could not be calculated for either or both the 24 and 120 hour periods, because they fell outside of the range of concentrations tested.

Based on the LC_{50} values and the recommended doses of each chemical, all the herbicides that were rated as safe for use at recommended dosages with *E. catarinensis* were glyphosate-based, *viz.* Mamba®, Rodeo® and Roundup®. The surfactant Add-2® also was rated safe, whereas the two glyphosate-trimesium products, Muster® and Touchdown®, and 2,4-D amine, were both rated toxic. Applying Add-2® (41.7 % mortality) with Touchdown® (4.2 % mortality) increased the toxicity of Touchdown® (33.3 % mortality) (Figure 1). The diquat-based herbicide Midstream® was the most toxic herbicide tested against *E. catarinensis*, 100% mortality was recorded for all concentrations of Midstream® at 96 hours after treatment, confirming its toxic nature (Figure 1). The surfactant Agral® also was considered toxic on its own (62.5 % mortality), and when combined with Midstream® (93.3 % mortality) (Table 2, Figure 1).

The weevil *N. eichhorniae* was less susceptible to the toxic effects of the herbicides and/or adjuvants used than *E. catarinensis*. Weevil mortality was low overall and only Agral®, Muster® and Roundup Ultra® resulted in high mortality (Figure 2). Zero mortality was experienced for the control treatment. The surfactant Agral® was the only chemical rated toxic to the weevil and all the other chemicals were found to be safe at recommended dosages (Table 2).

Discussion

This study showed that many of the herbicides/surfactants used in the control of water hyacinth resulted in significant mortality of two of the agents released as biological control agents. The mirid *E. catarinensis* was more susceptible to the toxic effects of the chemicals than the weevil *N. eichhorniae.* Herbicides containing glyphosate as an active ingredient were less toxic, whereas formulations with surfactants were more toxic. The study also showed that toxicity increased at higher concentrations. These results are consistent with other studies (e.g. Roorda et al. 1978; Center et al. 1982; Haag 1986a; Wright and Skilling 1987; Grodowitz and Pellessier 1990; Jianqing et al. 1999) that showed some level of toxicity of herbicide formulations on water hyacinth biological control agents.

Most herbicides are thought to have low toxicity to animals because the active ingredients have been developed to act on plant pathways such as the shikimic acid pathway (Franz et al. 1997). However, the surfactants added to herbicide formulations are designed to break down the surface tension of the herbicide and increase coverage. They also play a role in dissolving the waxy cuticle of plants, increasing the uptake of herbicide (Affeld et al. 2004). Therefore the surfactants also may be harmful to insects by destroying the waxy cuticle of the exoskeleton. Wax removal would lead to water loss and dehydration, or possibly liquid flooding into the trachea via the spiracles, impeding gas exchange and, although not tested in this study, direct toxicity could occur due to ingestion of herbicide contaminated food (Ainsworth 2003).

Herbicide application causes biochemical changes that might alter the palatability of water hyacinth plants, making them either more, or less attractive to the biological control agents. Wright and Bourne (1990) showed that the application of 2,4-D amine onto water hyacinth decreased the laminar hardness and increased the nitrogen content of the plants, thereby improving plant quality for *N. eichhorniae*, *N. bruchi* and the moth *Niphograpta albiguttalis* Warren (= *Sameodes albiguttalis*(Warren)) (Lepidoptera: Crambidae), whereas applications of glyphosate-based products do not appear to change the plant quality (Jadhav et al. 2008).

Extrapolation of these results from the laboratory to the field must take the behaviour of the insect species into consideration. The weevil is nocturnal, hiding in the base of the petioles during the day when herbicide application occurs, feeding on the leaves at night. The weevil is therefore highly unlikely to come into direct contact with the herbicide, and if it does, this study has shown that it will not be greatly affected. The mirid, however, feeds on the leaves during the day (Hill et al. 1999) and is therefore likely to come into contact with droplets of the herbicide. Thus, mirid populations will be negatively impacted by herbicide application, not only through the loss of habitat, but also acute toxicity.

Populations of all of the agents thus far released on water hyacinth are negatively affected by herbicide interventions due to mortality of sessile stages that are unable to disperse from a sinking mat. In an attempt to better integrate herbicide and biological control, Haag (1986b) suggested that water hyacinth mats should be sprayed in strips leaving unsprayed sections of the mat for the weevils to move on to, but this still resulted in mortality of the eggs, larvae and pupae in the sprayed sections. Van and Center (1994) showed that the growth retardant, paclobutrazol, acted synergistically with *N. eichhorniae* to control water hyacinth, although this was never implemented in the field. In a more recent study, Jadhav et al. (2008) showed in the laboratory that by spraying water hyacinth with a sub-lethal dose of glyphosate (0.8%) as opposed to the recommended dose of 3%, the plants did not die, but did stop growing, flowering and producing daughter plants. The low dose preserved the habitat for the immature stages of the *Neochetina* weevils, allowing them to build up high populations, but would still result in high mortalities of the more susceptible species such as *E. catarinensis*.

For the integrated management of water hyacinth to succeed in South Africa, site specific plans need to be developed that take into consideration compatibility of the insects with the herbicides to be used. Some herbicide formulations are better suited to integrated control where insects are already established. For example, in systems where *E. catarinensis* populations are to be maintained, herbicides with glyphosate as an active ingredient, a low surfactant content, and preferably at low concentrations should be used. In systems where *N. eichhorniae* is present, the choice of herbicide formulation is less vital. Timing and mode of application also are critical to successful integrated control of water hyacinth. In South Africa, the weevils, *N. eichhorniae* and *N. bruchi* overwinter as third instars in the crown of the plant (Byrne et al. 2010), thus herbicide applications in early spring (September and October) should be avoided as these insect stages are unable to move onto unsprayed plants. The timing of releases of agents could also be manipulated where susceptible agents such as the mirid could be released after herbicide application onto mats left unsprayed. Most of the agents used as biological control agents against water hyacinth in the world can easily be mass-reared (Julien et al 1999; 2001) and could be used in this way. Although the direct application of herbicides to several of the biological control agents released against *E. crassipes* has been shown to be toxic, a thorough understanding of the ecology of the weed and the arthropods used in its control, and the formulations of herbicides has shown that chemical and biological control of this weed can be integrated in a compatible manner.

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