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RESEARCH ARTICLE

Impacts of a sub-lethal dose of glyphosate on water hyacinth nutrients and its indirect effects on *Neochetina* weevils

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A sub-lethal dose of a herbicide under field conditions was applied to determine if it stimulates an increase in water hyacinth nutrients, thereby increasing feeding intensity by *Neochetina* spp. weevils used as biocontrol agents of the weed. Nitrogen (N) and carbon (C) were measured and compared between sprayed plants and control plants. At one site (Delta Park), N levels were lower in the sprayed plants compared to the control plants both in the leaves and the crown. At the second site (Farm Dam), leaf N was also lower in the sprayed plants than in the control plants, while no difference was found in crown N. Mean number of feeding scars per cm^2 at Delta Park was significantly higher on the sprayed plants compared to the control plants, while no significant difference was found at Farm Dam. At Delta Park, there was no correlation, however, between the number of weevil feeding scars and leaf N or C:N ratio in sprayed plants. In conclusion, the sub-lethal dose of glyphosate did not directly result in an increase in weevil feeding intensity but it can be recommended in an integrated control system to retard water hyacinth growth while conserving the weevil population.

Keywords: integrated pest management; *Neochetina* weevils; glyphosate; feeding; nitrogen; C:N ratio

Introduction

Water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), a South American Amazonian plant, is one of the most damaging invasive aquatic weeds in the world, having negative effects on the environment and economies of many tropical and subtropical countries (Julien, Griffiths, & Wright, 1999). It was first recorded in South Africa in the early 1900s (Cilliers, 1991) and has been a research topic of many studies seeking ways to control its spread (Coetzee, Hill, Byrne, & Bownes, 2011). In South Africa, the biological control programme against water hyacinth was initiated in 1973 with the release of the weevil *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae) (Cilliers, 1991), which together with *N. bruchi* Hustache (Coleoptera: Curculionidae) are regarded as the most successful biocontrol agents against water hyacinth (Julien et al., 1999). Although there is a high number of species of established water hyacinth biocontrol agents in South Africa, the

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success achieved through biocontrol has been variable and not always satisfactory to land users (Hill & Olckers, 2001). Integrated pest management, combining herbicides and biocontrol agents, has proven to be more effective in controlling water hyacinth than either of the control methods used alone (Jones & Cilliers, 1999; van Wyk & van Wilgen, 2002). Herbicides used in the traditional way (i.e. at lethal concentrations) interfere with the biocontrol agents, either directly by killing them (Hill, Coetzee, & Ueckermann, 2012) or indirectly, through habitat destruction (Ainsworth, 2003).

To avoid the negative effect of herbicides on the biocontrol agents, the use of sub-lethal doses of herbicide was proposed (Ainsworth, 2003). A laboratory trial established that *N. eichhorniae and N. bruchi* fed more on water hyacinth plants sprayed with a sub-lethal dose of glyphosate (0.8% glyphosate concentration) compared to unsprayed plants (Jadhav, Hill, & Byrne, 2008). In a similar laboratory experiment, however, there was no significant difference in the performance (survival and feeding habit) of the mirid, *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) between sprayed and control plants (Katembo, 2008). The sub-lethal dose of glyphosate was found not to be detrimental to these biocontrol agents, but remained untested in the field.

Growth and reproduction of herbivores depends mainly on the availability of carbon (C) (digestible carbohydrate) and protein nitrogen (N) levels (Bezemer & Jones, 1998). Many studies have indicated that N is an essential measure of host plant quality for insect herbivores (e.g. Awmack & Leather, 2002; Mattson, 1980). Plants with a high N content have been found to improve the survival and growth rate of immature insects (Wheeler, 2003). For example, *Neochetina* spp. weevils reared on water hyacinth plants grown under three different fertiliser levels, low (1.7–2.0% N), intermediate (2.1–3.5% N) and high (3.5–4.5% N), showed an increase in reproduction rates and population numbers as fertiliser levels increased (Center & Dray, 2010). Center (1994) showed that egg production in water hyacinth weevils was influenced by the quality of host plant (in terms of leaf N), with greater egg production on >4.5% leaf tissue N than on leaves with approximately 2% leaf tissue N. Lower levels of N would be expected to cause a proportional increase in the C:N ratio.

Alterations in plant C content will not only have a negative effect on plant growth, but may also affect their associated herbivores through habitat and food reduction. High levels of C reduced foliar water and N concentrations in *Plantago lanceolata* L. (Plantaginaceae), resulting in slow larval growth of *Junonia coenia* (Nymphalidae) (Fajer, Bowers, & Bazzaz, 1989).

The present study sought to address the following questions: (1) Which water hyacinth plant part (leaves, petioles or crowns) has the highest nutrient content? (2) How do glyphosate-induced changes in plant nutrients affect *Neochetina* species feeding? (3) How does a 0.8% glyphosate dose change the N and C content in water hyacinth leaves and crowns?

Materials and methods

Study site description

This study was conducted in spring of 2008, at two sites in Johannesburg (Gauteng, South Africa), namely Delta Park (26°07′S 28°00′E) and Farm Dam (26°02′S 27°27′E).

Both sites were infested with water hyacinth and had populations of *Neochetina* spp., with lower weevil numbers at Farm Dam compared to Delta Park (Byrne et al., 2010). Both sites have had more than five-year-long-term monitoring histories of water hyacinth biological control.

Experiments

At each site, a strip of water hyacinth mat approximately 12 × 3 m was divided into two plots (6 × 3 m each): a sprayed plot and a control plot. The plots were adjacent to each other and separated by a cable fitted with floating buoys (10 cm diameter) to prevent the movement of plants from one plot to the other. Each plot was further subdivided into three 2 × 3 m blocks as replicates, also separated with buoyed cables to prevent plant movement. Before spraying, the control blocks were covered with plastic sheets to prevent spray drift.

The treatment plot at each site was sprayed with 0.8% of a broad spectrum, glyphosate-based herbicide, 'Roundup' (active ingredient: 360 g/L, Monsanto Pty. Ltd. South Africa) at a spray volume of 140 L/ha, giving an active ingredient of 0.11 g/m^2 (Jadhav et al., 2008). A 12-V battery operated boom sprayer (supplied by Multispray, Midrand/South Africa), fitted with three spray tips (TeeJet even flat: TP65015E), was used to spray the water hyacinth from a motor boat at a speed of 4 km/h. Spraying was conducted once in spring 2008.

N and C measures

Two weeks after spraying, four plants were randomly collected from each block in the sprayed and the control plots. The plants were broken up into separate samples consisting of leaves, petioles and crowns. Center and Wright (1991) noted that adult *Neochetina* spp. preferred feeding on younger leaves than on older ones because of their high N content. In this study, only the three youngest leaves (leaf 1, leaf 2 and leaf 3) and their respective petioles were analysed, the rest were discarded. These samples were oven dried at 60°C for 18 hours, before being sent to BemLab (Stellenbosch, South Africa) for N and C analysis, using the 'combustion analysis method'. Results from the first analysis (two weeks after spraying) were used to compare nutrient levels between the youngest three leaves and their respective petioles and plant crowns. Two weeks later (four weeks after spraying), a second set of samples were collected and treated as above. In these samples, leaves 1, 2 and 3 were pooled together before analysis. Petioles were not included because the first analysis showed no significant difference in nutrient levels between control petioles and crowns.

Plant and weevil measures

An additional 18 whole plants were randomly collected; three from each block both in sprayed plots and control plots. Sampling was conducted weekly for four weeks and was commenced in October 2008. The following parameters were measured: (1) weevil feeding was indirectly measured by counting weevil feeding scars per cm^2 on leaf two. Leaf area was measured with a leaf area metre (Model: LI-3100 Area metre/LI.COR, inc. Lincoln, NE, USA) and the number of scars on the leaf was divided by the area of the leaf; (2) number of larvae present, by splitting the petioles and crowns; (3) leaf turnover rate, referring to the measure of leaf production over time, measured by tagging the second youngest leaf and following its successive position on the plant every two weeks. Weevil performance is narrowly defined as feeding and larval count in this work.

Statistical analysis

A factorial analysis of variance (ANOVA) followed by a least significant difference (LSD) post-hoc test was performed to compare N content in different plant parts (leaves, petioles and crowns), the C:N ratio in leaves and crowns, number of weevil feeding scars per leaf per cm^2, number of larvae per plant and the leaf turnover rate per plant between sprayed and control plants. A two sample *t*-test was used to compare C and N between sprayed and control plants. All analyses were conducted at a critical P level of 0.05 using STATISTICA, version 6.

Results

N content in different plant parts – two weeks after spraying

At Delta Park, in the control plants alone, N was significantly greater in the leaves than in the petioles and the crown, but there were no differences between leaves 1–3 ($F_{(6,28)} = 7.62$, $P < 0.01$) (Figure 1a). There were no differences in N between the petioles and the crown, with the exception of petiole 1, which was higher than the rest ($F_{(6,28)} = 7.62$, $P < 0.01$). The same trend was observed at Farm Dam, with the exception of N in petiole 2 and the crown, which was higher than that in petiole 3, but not different to petiole 1 ($F_{(6,28)} = 8.85$, $P < 0.01$) (Figure 1b).

At Delta Park, N was significantly greater in the leaves than in the petioles and the crown both in the control and sprayed plants ($F_{(6,28)} = 7.62$, $P < 0.01$). Both in the control and sprayed plants, there was no significant difference in N between leaves 1–3. N content in the control leaves was significantly greater than in the sprayed leaves and N in the control petioles was also greater than in the sprayed petioles ($F_{(6,28)} = 7.62$, $P < 0.01$) (Figure 1a). There was no difference in N in the control plants between petioles and the crown, with the exception of petiole 1, which was greater than the rest. In the sprayed plants, there was also no significant difference between petioles and the crown, with the exception of petiole 3, which was lower than the rest ($F_{(6,28)} = 7.62$, $P < 0.01$) (Figure 1a). There was no significant difference between sprayed and control N crown.

At Farm Dam, both in the control and sprayed plants, N was significantly greater in the leaves compared to the petioles and the crown ($F_{(6,28)} = 8.85$, $P < 0.01$). However, both in the control and sprayed plants, there was no significant difference in N between leaves 1–3. N in control leaves was significantly greater than in the sprayed leaves ($F_{(6,28)} = 8.85$, $P < 0.01$) (Figure 1b). In the control plants, there was no significant difference between petioles and the crown, with the exception of petiole 3, which was lower compared to petiole 2 and the crown. In the sprayed plants, N in petiole 1 was significantly greater than in petioles 2 and 3, but significantly lower than in the crown ($F_{(6,28)} = 8.85$, $P < 0.01$). There was no significant difference in N between petioles 1 and 3 in sprayed or control plants. N in petiole 2, however, was greater in control plants compared to sprayed plants, while

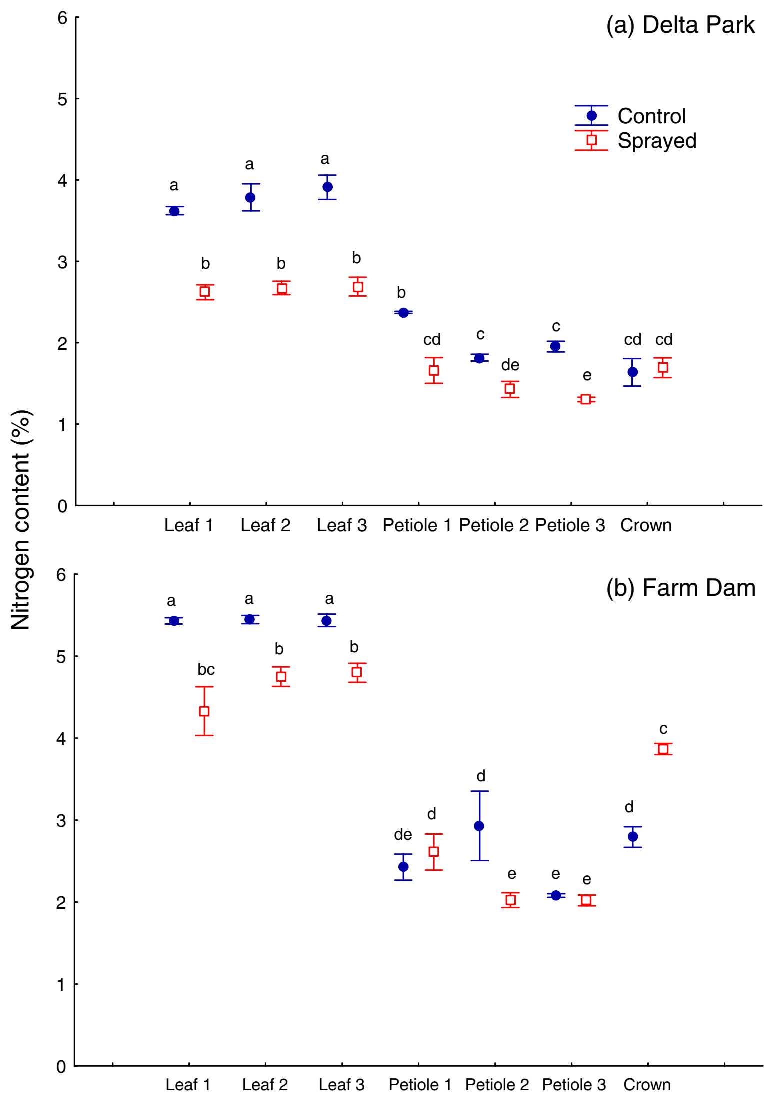

Figure 1. (Colour online) Nitrogen content of water hyacinth plant parts at two field sites (Johannesburg, South Africa), two weeks after spraying with 0.8% glyphosate (140 l/ha spray volume). Error bars represent standard error of the mean. Means with different letters are significantly different from each other $P < 0.05$.

N in the crown was greater in sprayed plants compared to control plants ($F_{(6,28)} = 8.85$, $P < 0.01$) (Figure 1b).

Nutrient content of leaves and crowns – four weeks after spraying

At Delta Park, there was no significant difference in C between the sprayed and the control leaves ($t = 0.77$, $P = 0.48$) (Figure 2a), whereas N was significantly greater in the control leaves than in the sprayed leaves ($t = 9.03$, $P < 0.01$) (Figure 2a). N was

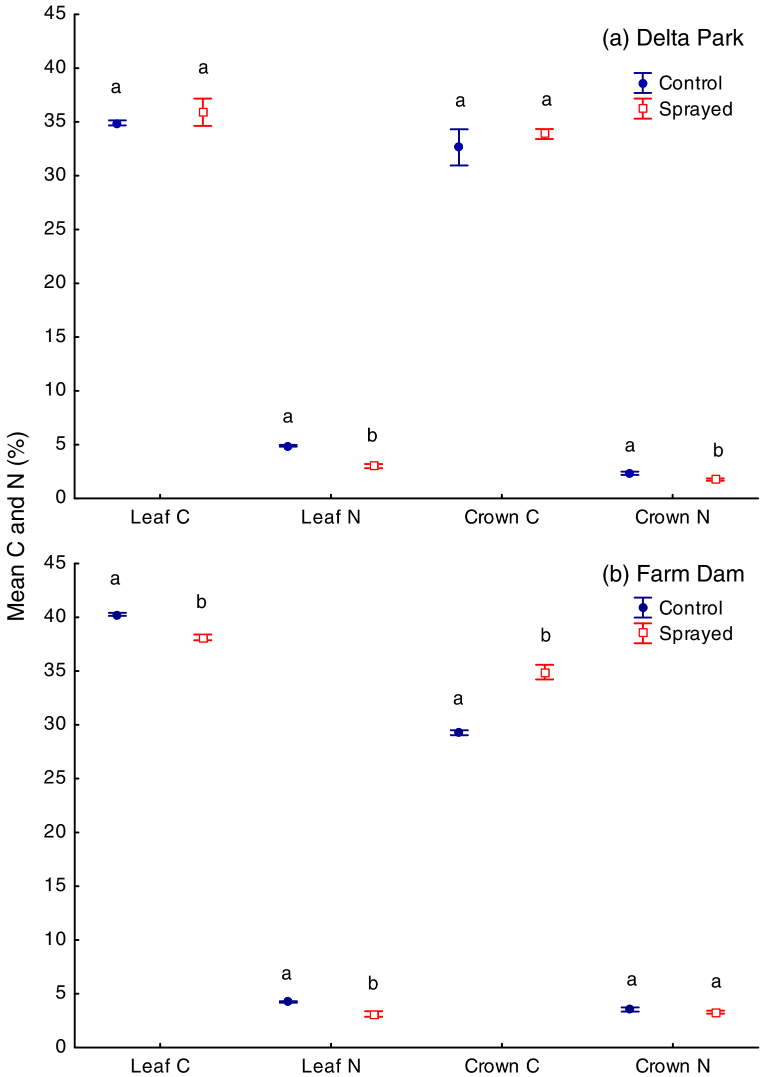

Figure 2. (Colour online) Effect of 0.8% glyphosate (140 l/ha spray volume) on C and N in water hyacinth leaves and crown at two sites, (a) Delta Park and (b) Farm Dam, four weeks after spraying with 0.8% glyphosate (140 l/ha spray volume). Error bars represent standard error of the mean. Control/sprayed pairs with different letters denote significant difference at $P < 0.05$.

also significantly greater in control crowns compared to sprayed crowns (t = 3.14, P = 0.03) (Figure 2a). However, no significant difference was found in C between sprayed and control crowns (t = 0.70, P = 0.51) (Figure 2a).

At Farm Dam, C and N were significantly greater in control leaves compared to sprayed leaves (t = 7.26, P < 0.01; t = 4.26, P = 0.01) (Figure 2b). N was not significantly different between sprayed and control crowns (t = 1.06, P = 0.34) (Figure 2b). However, C was significantly greater in the sprayed crowns compared to the control crowns (t = 7.82 P < 0.01) (Figure 2b).

C:N ratio of water hyacinth leaves and crowns

The C:N ratio in water hyacinth was significantly higher for both leaves and crowns in sprayed plants than in the controls at both sites, Delta Park ($F_{(3,8)}$ = 27.418, P < 0.01) and Farm Dam ($F_{(3,8)}$ = 9.026, P < 0.01) (Figure 3).

Effect of 0.8% glyphosate on weevil feeding

At Delta Park, weevil feeding on leaf 2 was significantly higher on sprayed plants compared to the control plants from week 2 through to week 4 after spraying, but not significantly different in weeks 0 and 1 ($F_{(9,80)}$ = 2.058, P = 0.04) (Figure 4a). At Farm Dam, the weevil feeding activity was very low and there were no differences either within weeks or between sprayed and control plants, with the exception of week 4, where more feeding was observed on control plants than sprayed plants ($F_{(9,80)}$ = 1.080, P = 0.38) (Figure 4b).

Number of larvae per plant – four weeks after spraying

There was no significant difference in the number of larvae per plant either between Delta Park and Farm Dam or control and sprayed plants ($F_{(3,32)}$ = 0.972, P = 0.41) (Figure 5).

Leaf production rate (leaf turnover rate)

Leaf turnover rate was only recorded at Delta Park. At week 4, leaf production was significantly greater in control plants compared to sprayed plants ($F_{(1,32)}$ = 100.00, P < 0.01) (Figure 6).

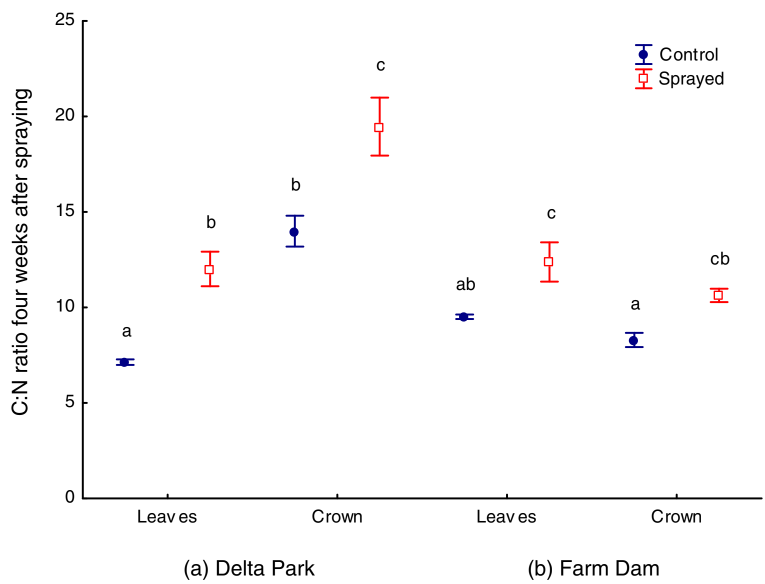

Figure 3. (Colour online) Effect of 0.8% glyphosate (140 l/ha spray volume) on C:N ratio in water hyacinth at two sites, (a) Delta Park and (b) Farm Dam, four weeks after spraying. Error bars represent standard error of the mean. Means with different letters are significantly different from each other P < 0.05.

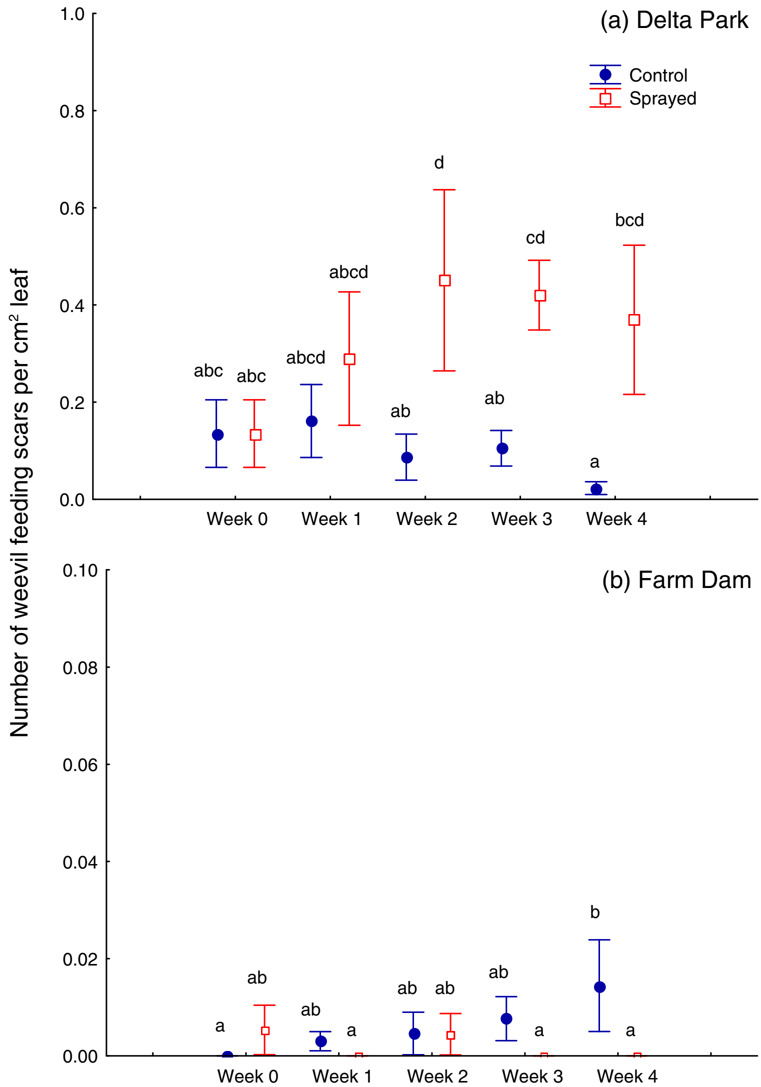

Figure 4. Effect of 0.8% glyphosate (140 l/ha spray volume) on *Neochetina* weevil feeding on leaf 2 on water hyacinth at two sites (a) Delta Park and (b) Farm Dam. Error bars represent standard error of the mean. Means with different letters are significantly different from each other $P < 0.05$.

C:N ratio and the number of weevil feeding scars

At Delta Park, there was a significant positive correlation between the C:N ratio and the number of feeding scars in the control plants (Figure 7), whereas no correlation was found in the sprayed plants. There was no correlation between N or C and the number of feeding scars in the control or the sprayed plants (Table 1). At Farm Dam, both in the control and the sprayed plants, there was no correlation between N, C or C:N ratio and the number of weevil feeding scars (Table 1).

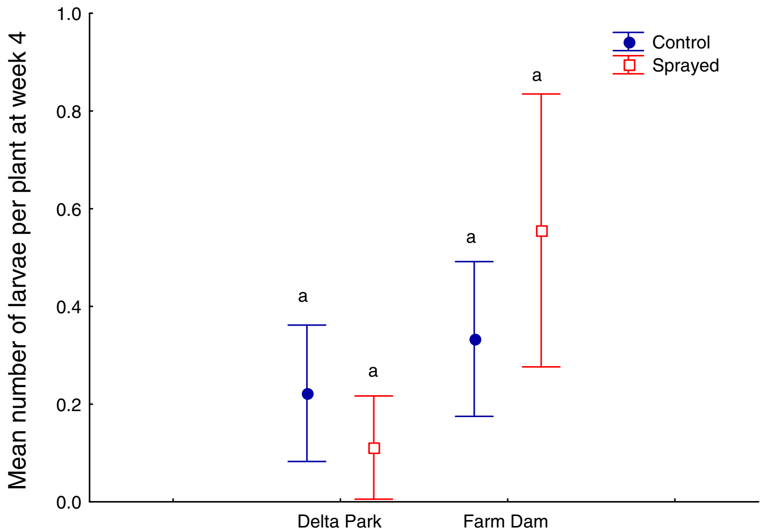

Figure 5. Effect of 0.8% glyphosate (140 l/ha spray volume) on *Neochetina* weevil larvae in water hyacinth at two sites, Delta Park and Farm Dam, four weeks after spraying. Error bars represent standard error of the mean. Means with different letters are significantly different from each other $P < 0.05$.

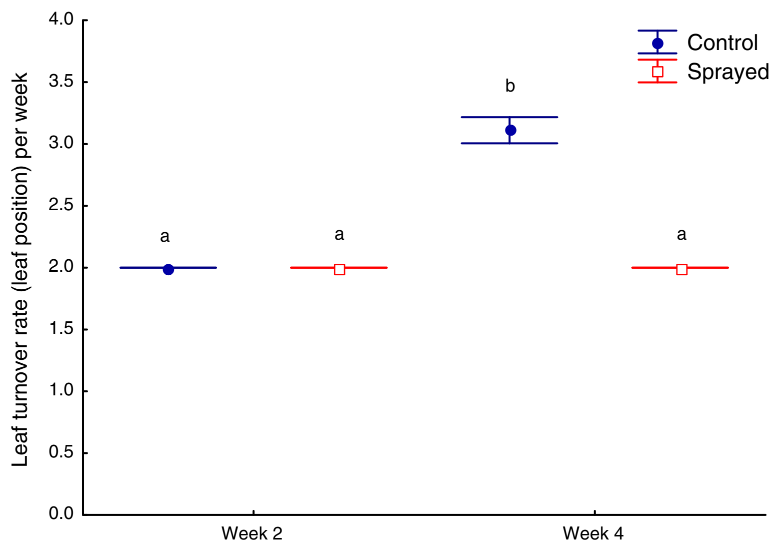

Figure 6. Effect of 0.8% glyphosate (140 l/ha spray volume) on leaf production at Delta Park four weeks after spraying. Error bars represent standard error of the mean. Means with different letters are significantly different from each other $P < 0.05$.

Discussion

Spraying water hyacinth with glyphosate caused a general decrease in N in both leaves and crowns, resulting in an increase in the C:N ratio in plants from two different field sites. This reduction in N was associated with an increase in adult

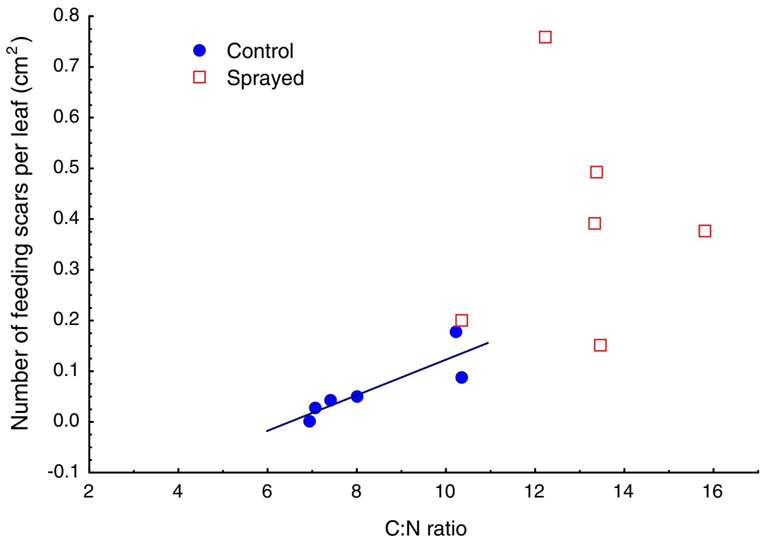

Figure 7. Correlation between C:N ratio in water hyacinth leaves and the number of weevil feeding scars per cm^2 in the control and sprayed plants at Delta Park.

weevil feeding scars at one site, but not at the second, which had a smaller weevil population. This increase in feeding scars could not be explained by scars accumulating on plants with a low leaf turnover, because it occurred before any differences in leaf production were noted, but it could be caused by compensatory feeding on a low N diet. The number of larvae was unaffected by the herbicidal spray over the course of the experiment. These results support those of Jadhav et al. (2008) where an increase in feeding scars was found on sprayed plants (but only after 60 days as compared to 14 days here), and no difference in the number of larvae was revealed for early instars between sprayed and control plants.

Spencer and Ksander (2004) also found that N was highest in water hyacinth leaves, followed by petioles and crowns. Leaf N content can be an indicator of food quality for phytophagous insects (Awmack & Leather, 2002; Mattson, 1980). Adult *Neochetina* spp. prefer feeding on young leaves (Moran, 2004), whereas the petiole

Table 1. Correlation between N, C and C:N ratio in water hyacinth leaves and the number of weevil feeding scars in control and sprayed plants.

	N and feeding scars			C and feeding scars			C:N and feeding scars		
Sites	*N*	R^2	*P*	*N*	R^2	*P*	*N*	R^2	*P*
Delta Park									
Control	6	0.5448	0.09	6	0.5623	0.08	6	0.7524	0.02*
Sprayed	6	0.0318	0.73	6	0.2892	0.27	6	0.0025	0.92
Farm Dam									
Control	6	0.1711	0.4150	6	0.1410	0.4632	6	0.1913	0.3858
Sprayed	6	0.3602	0.2078	6	0.2300	0.3358	6	0.2657	0.2953

*Indicates significant correlation.

serves mainly as a channel allowing adult weevils and larvae to travel from the leaves to the crown for refuge and larval development (Julien et al., 1999). van Lenteren and Noldus (1990) reported that most insect species have the ability to choose plant parts that are most suitable for their feeding and oviposition. The reason why weevils choose leaves as their oviposition site could be explained by a higher N content in the leaves compared to that in other plant parts. Center and Dray (2010) suggested that female *Neochetina* spp. lost their reproductive capacity as a result of a reduction in leaf N content. Adequate N in the crown is also important for larval development as that is the section of the plant where larvae spend their final instar before moving through to the roots for pupation (Center, 1987). So it is reasonable to assume that *Neochetina* spp. are sensitive to N and C levels in the plant.

Lindgren, Gabor, & Murkin (1999) assessed the compatibility of a 2% glyphosate concentration on the oviposition and survival of *Galerucella calmariensis*, a biocontrol agent for *Lythrum salicaria*. Adult *G. calmariensis* were exposed to glyphosate directly (by spraying them) and indirectly (by spraying host plants with adults on them), and in both situations glyphosate did not affect their oviposition or survival (Lindgren et al., 1999). The development time of *G. calmariensis* from larvae to adult was however longer in glyphosate-treated plants compared to control plants (Lindgren et al., 1999). A direct application of glyphosate at the recommended dose (3%) on water hyacinth weevils (*Neochetina)* did not reduce adult weevil survival (Hill et al., 2012) but did kill the plant eventually, and consequently both adult and larval weevils (Jadhav et al., 2008). In the present study, results suggest that larval numbers were not hindered by a sub-lethal dose (0.8%) glyphosate. However, longer-term experiments are needed to measure any effect, such as delay in development, or reduced fecundity caused by the herbicide.

The present study showed that the decreased leaf N induced by glyphosate resulted in an increased C:N ratio in the sprayed leaves and crowns. Generally C levels did not change but N content declined, increasing the C:N ratio. A high C:N ratio or low N concentration in plants often results in a low food nutritive value for herbivores (Lincoln, Couvet, & Sionit, 1986). The feeding of leaf chewing insect herbivores reared on plants grown under elevated CO_2 conditions is often low because of the consequential reduction in the leaf N, increase in the leaf toughness and sometimes an increase in defence compounds (e.g. phenolics) (Hunter, 2001; Zvereva & Kozlov, 2006). The present results show that the number of weevil feeding scars per leaf (scars/cm^2) was increased by the elevated C:N ratio. A water hyacinth leaf C:N ratio of $\leq$17 is believed to be favourable to its aquatic herbivores (McMahon, Hunter, & Russell-Hunter, 1974; Spencer & Ksander, 2004). In the present study, the C:N ratio was generally within favourable ranges, except the crowns from Delta Park, implying that 0.8% glyphosate may not have altered plant nutrient enough to the point of impeding the performance of *Neochetina* spp. However, this should be tested over the complete egg to pupal period (about 50 days) (Center, 1987) to arrive to a definite conclusion.

The increased C:N ratio in the sprayed plants cannot fully explain the trend in weevil feeding because there was no correlation between C or C:N ratio in the sprayed plants and the number of feeding scars per cm^2. On the contrary, a positive correlation was found between C:N ratio in control plants and the number of feeding scars. Alternative explanations for the tendency of weevils to feed more on sprayed plants could include: (1) a reduction in the leaf production rate (leaf turnover) in the sprayed plants. Jadhav et al. (2008), in laboratory trials, found that leaf production rate was slower in plants that were sprayed with 0.8% glyphosate compared to unsprayed plants. The high feeding scar count on the sprayed leaves found only at one site may have been as a result of an accumulation of scars on the sampled leaf, but only after adult feeding had already increased on those plants. (2) An elevated C content in the sprayed plants would mean that the weevils might have had to feed more to get the necessary N. In a study measuring the potential effects of elevated carbon dioxide on forest leaf-feeding insects, increased larval feeding was explained as a compensatory response for poorer plants' quality (Wang, Ji, Wang, & Liu, 2006; Wheeler & Slansky, 1991). Compensatory feeding by adult weevils in response to an increased C:N ratio cannot be ruled out in the present study. (3) Changes in plants' secondary chemistry can have major implications for insect herbivores feeding on them (Scriber & Slansky, 1981). Herbicide-induced reduction of plant secondary metabolites (Bentley & Haslam, 1990) could explain why the weevils performed well on sprayed plants, because glyphosate may have impaired the plants' defence mechanisms. Glyphosate is known to inhibit a key enzyme involved in the biosynthesis of aromatic amino acids, 5-enolpyruvylshikimate-3-phosphate synthase and that of aromatic secondary metabolites (Ainsworth, 2003; Bentley & Haslam, 1990). This hypothesis of a herbicide-induced reduction of plant secondary metabolites would be worth further investigation in water hyacinth.

In conclusion, a sub-lethal glyphosate concentration, administered under field conditions, resulted in a decrease in the leaf N content of the plant without changing its C content, leading to an increased leaf C:N ratio. The weevil's feeding and numbers of larvae were not negatively affected by the alteration in plant nutrients. This suggests that although the sub-lethal dose of glyphosate reduced the N content of the plant, the weevils coped with the change. Herbivores are able to adapt to low nutrient conditions by increasing their food intake, prolonging development time and reducing growth rates and food conversion efficiency (Roth & Lindroth, 1995). In the present study, it is argued that the sub-lethal dose of glyphosate had a tendency to cause compensatory feeding; however, this finding is inconclusive as the observation occurred at only one site. Although limited attention has been directed towards investigating the responses of herbivorous insects to plants treated with sub-lethal dosages of herbicide (Center, Dray, Jubinsky, & Grodowitz, 1999; Kjær & Elmegaard, 1996), this work has shown that a 0.8% glyphosate concentration, which constitutes about 26.6% of the recommended dose, did not have any detrimental effect on the water hyacinth *Neochetina* spp. in field conditions. Thus, a sub-lethal dose of glyphosate can be recommended to retard water hyacinth growth (Jadhav et al., 2008), particularly in an integrated control system where the weevil population is required to be conserved. By taking out the centre of a water hyacinth infestation with a lethal dose of glyphosate, the borders will receive spray drift at a sub-lethal dose, which will retard the growth of water hyacinth while providing a refuge for its biological control agents.

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