The impact of the herbicide glyphosate on leaf litter invertebrates within Bitou bush, *Chrysanthemoides monilifera* ssp *rotundata*, infestations

Elizabeth A Lindsay* and Kris French

School of Biological Sciences, University of Wollongong, Northfields Ave, Wollongong, New South Wales, Australia 2500

Abstract: *Chrysanthemoides monilifera* ssp *rotundata* (L) T Norl (Bitou bush) is a serious environmental weed along the southeast coast of Australia. The herbicide glyphosate is commonly used to control *C* *monilifera* on the New South Wales coastline, but there have been few studies examining the effects of this herbicide on invertebrate communities in the field, especially on sand dunes. Control and impact sites were selected in coastal hind dunes heavily infested with *C* *monilifera*, and the impact sites were sprayed with a 1:100 v/v dilution of glyphosate-isopropyl 360 g AE litre$^{-1}$ SL (Roundup Biactive). Leaf litter invertebrates were sampled before spraying and after spraying by collecting fixed areas of leaf litter in both the control and impact sites. Samples were sorted for particular invertebrates involved in leaf litter decomposition and some of their predators. This study did not identify any significant direct or indirect effects on leaf litter invertebrate abundance or community composition in the four months following herbicide application. The litter invertebrate assemblages were highly variable on a small spatial scale, with abiotic factors more strongly regulating leaf litter invertebrate numbers than glyphosate application. These results conflict with previous studies, indicating that the detrimental indirect effects herbicide application has on non-target litter invertebrates may depend upon the application rate, the vegetation community and structure and post-spray weather.

Keywords: *Chrysanthemoides monilifera*; glyphosate; herbicide; leaf litter invertebrates

1 INTRODUCTION

The South African shrub *Chrysanthemoides monilifera* ssp *rotundata* (L) T Norl (Family Asteraceae) (Bitou bush) is a common environmental weed along the southeast coast of Australia. *Chrysanthemoides monilifera* was planted extensively between 1946 and 1971 to stabilise coastal sand drifts, and to revegetate dunes after mining operations. The use of *C* *monilifera* as a dune stabiliser was halted when it became apparent that it competed with native coastal plant communities and dune erosion occurred as infestations aged. *Chrysanthemoides monilifera* now covers more than 36 000 ha of the New South Wales coastline in Australia, being the dominant species on over 400 km of headlands and dunes. Many techniques have been used to control *C* *monilifera*, including physical removal, burning, slashing and biological control agents. Currently chemical control is the most successful method for removing large infestations. The herbicide glyphosate [N-(phosphonomethyl)glycine] is the most widely used herbicide, applied via aerial application or high pressure spraying equipment. Glyphosate is a broad-spectrum, non-selective water-soluble systemic herbicide. It is considered to be non-toxic to animals and not to bioaccumulate. The half-life depends on the soil type, and can vary from days to years. Glyphosate can be inactivated in the soil by becoming bound to clay particles and when desorption occurs it can be degraded by soil micro-organisms.

Many herbicides have had detrimental effects on soil fauna. Glyphosate has been found harmful to certain arthropods in the field and in the laboratory. Application of glyphosate to field margins was found to decrease the abundance of spiders, carabid beetles and bugs for four months, with the abundance decreasing as the glyphosate concentration increased. However laboratory toxicity tests and field trials have often produced different results. For example, glyphosate
had no short-term toxicity to the spider *Leptophantes tenus* (Blackwall) in laboratory tests, but in the field numbers were significantly reduced at the same application rates.\(^{25}\) It is thought that herbicides can affect fauna negatively through direct toxicity effects and/or indirect effects through habitat modification. As the treated vegetation dies the decreased canopy cover can change the micro-climate. The invertebrates often become more exposed to the desiccating sun and wind, accompanied by an increase in surface temperature and decrease in soil moisture.\(^9,^{26,27}\)

There have been few studies examining the effects of this herbicide on leaf litter invertebrate communities in the field. This study aims to determine whether glyphosate affects the abundance and composition of non-target leaf litter invertebrates within *C. monilifera* infestations. In particular, invertebrates involved in leaf litter decomposition and some of their predators were investigated, as the loss of these species from sites may influence the nutrient cycling following weed removal.

### 2 EXPERIMENTAL

#### 2.1 Study sites

The experiment was carried out on the hind dunes of Caves Beach (35°16′S, 150°66′E), within Boudderee National Park, Jervis Bay, NSW. *Chrysanthemoides monilifera* was introduced to this area around 1968. All sites were heavily infested with *C. monilifera* (>75% cover) and had a sparse overstorey of *Bankia integrifolia* Lf, *Eucalyptus botryoides* Smith and *Leptospermum laevisatum* F Muell.

Jervis Bay has a mean maximum day temperature of 19.9 °C, a minimum of 13.6 °C and a mean annual rainfall of 1244 mm (Australian Bureau of Meteorology). The soil is a fine Aeolian sand with low amounts of carbonates. It is slightly acidic with low fertility and high permeability.\(^{28,29}\)

Two plots, close in proximity, were chosen which had not previously been sprayed with herbicide. At one plot two control quadrats were randomly allocated and at the other three treatment quadrats were randomly allocated. The control quadrats were 20 × 10 m and the herbicide treatment quadrats were each 10 × 10 m. All quadrats were at least 5 m apart from each other. Treatments were not randomly allocated to plots as we were constrained by the management objectives of the national park.

#### 2.2 Invertebrate sampling

All quadrats were sampled initially for leaf litter invertebrates 4 days prior to herbicide application in January 2002. Five samples were collected within each site, with each sample consisting of four 0.16 × 0.16 m ground leaf litter scraping. Samples were transported in moistened cotton sacks, and extracted within 12 h of collection. No area was ever sampled twice, and a following sample was never taken adjacent to a previous sample.

The treatment quadrats were sprayed with a 1:100 v/v aqueous dilution of a commercial glyphosate-isopropylammonium 480 g litre\(^{-1}\) (360 g AE litre\(^{-1}\)) SL (Roundup Biactive, Monsanto) at a rate of 3–4 litre ha\(^{-1}\) (10.8–14.4 g AE ha\(^{-1}\)). This was done with a high volume sprayer (Quickspray Model 9TBE) from the back of a vehicle. This is a common concentration and application rate used to control *C. monilifera* and other environmental weeds in Australia (B Rafferty, pers comm). All sites were sampled for litter invertebrates two, four, eight and sixteen weeks after spraying. Sampling stopped after this as the *C. monilifera* had died and seasonal changes could alter the invertebrate population and make interpretation of results difficult.

All samples were extracted within 48 h of collection, using a modified Tulglen Funnel. The leaf litter was extracted for 5 days into 75% monoethylene glycol and the invertebrates stored in 70% ethanol. Samples were sorted using a biconcave microscope for the following taxa: Amphipoda (landhoppers), Araneae, Blattodea (cockroaches), Haplotaxida (earthworms), Isopoda, Pseudoscorpionida, the millipede orders Julida, Sphaerotheriida and Spirobolida, and the centipede orders Scolopendrida, Geophilida and Lithobiida.

#### 2.3 Statistical analysis

##### 2.3.1 Change in abundance

An unreplicated repeated measures analysis of variance was performed\(^{30}\) to test for differences in abundance and richness in relation to site (random factor), time (random factor), control/impact (fixed factor) and before/after treatment (fixed factor).\(^{31}\) The data were also analysed with SYSTAT\(^{32}\) without the before/after factor to test for the assumption of sphericity (assumption of independent sampling across time).\(^{33}\) All data except Amphipoda met this assumption, and therefore the degrees of freedom were corrected by the Huynh–Feldt Epsilon value.\(^{34}\)

These analyses were performed for total abundance, number of taxa and abundance of Isopoda, Amphipoda and Araneae. There were insufficient numbers in the other groups for them to be analysed individually, so analyses also investigated differences in Diplopoda, decomposers (Amphipoda, Blattodea, Julida, Sphaerotheriida, Spirobolida, Haplotaxida, Isopoda, Scolopendrida) and predators (Pseudoscorpionida, Araneae, Scolopendrida, Geophilida and Lithobiida). All groups except number of taxa, were log\((x+1)\) transformed prior to analysis to improve normality and homogeneity of variances.

##### 2.3.2 Community composition

To determine whether the herbicide treatment affected the invertebrate assemblage, multivariate analysis was performed using the statistical software PRIMER.\(^{35}\) Following calculation of the Bray–Curtis indices of similarity, data were ordinated using non-metric
Impact of glyphosate on leaf litter invertebrates

To determine initially whether the three impact quadrats and the two control quadrats could be grouped together, a nested analysis of similarity (ANOSIM) with site nested in spray/control was undertaken. The assemblages at each site within each treatment were significantly different (Site: Global $R = 0.153$, $P = 0.001$). Therefore, each quadrat could be seen as a replicate. A two-way ANOSIM was then performed to determine differences between the five time periods and the control/impact samples. When significant differences were found, similarity percentage analysis (SIMPER) was used to reveal which taxa changed between times and sites (% dissimilarity). Analyses were undertaken on square-root-transformed and presence/absence data to distinguish whether differences were due to the abundance of a particular taxa or the presence of specific taxa. Results presented are for square-root-transformed data unless otherwise stated.

3 RESULTS

3.1 Changes in abundance of taxa

The herbicide was effective at killing $C$ monilifera plants as expected. Two weeks after application, the $C$ monilifera leaves had started to turn yellow and by eight weeks all $C$ monilifera plants were dead. A total of 11 630 invertebrates were collected during the study. The amphipods were the most abundant order in the impact sites (Fig 1, Fig 2b) whereas the spiders and amphipods had a similar high abundance in the control sites (Fig 1, Fig 2c).

There was no significant short or long-term decrease in invertebrate abundance following herbicide application. The short-term impacts are indicated by a significant control/impact $\times$ time (before/after) interaction. This interaction was only significant for the amphipods ($F_{3,7} = 13.3$, $P = 0.003$) (Table 1). However, there was an increase, not a decrease, in abundance of amphipods two, four and sixteen weeks after impact compared to the pre-impact samples. Longer-term impacts are indicated in the control/impact $\times$ before/after interaction. This interaction was not significant for any of the groups analysed.

The control and impact sites were different in total abundance, despite their close proximity ($F_{1,3} = 32.2$, $P = 0.011$) (Fig 2). This could be due to the high

<table>
<thead>
<tr>
<th>Source</th>
<th>d f</th>
<th>$F$</th>
<th>$P$</th>
<th>$F$</th>
<th>$P$</th>
<th>$F$</th>
<th>$P$</th>
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<th>$P$</th>
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<td>Site+before/after (control/impact)</td>
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<td>0.539</td>
<td>1.18</td>
<td>0.371</td>
<td>1.29</td>
<td>0.336</td>
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<td>Control/impact</td>
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<td>0.055</td>
<td>32.2</td>
<td>0.011*</td>
<td>2.49</td>
<td>0.213</td>
<td>41.0</td>
<td>0.008*</td>
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<td>Before/after</td>
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<td>28.3</td>
<td>0.013*</td>
<td>24.8</td>
<td>0.016*</td>
<td>1.77</td>
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<td>45.4</td>
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<td>0.100</td>
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<td>0.003</td>
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<td>11.8</td>
<td>0.002*</td>
<td>13.7</td>
<td>0.001*</td>
<td>3.81</td>
<td>0.052</td>
<td>17.5</td>
<td>0.000*</td>
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<td>0.195</td>
<td>0.897</td>
<td>9.14</td>
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* Significant values are marked with an asterisk; $P < 0.05$ significance level.

**Table 1.** Summary of the unreplicated repeated measures ANOVA for the abundance of each group analysed

**Figure 1.** Mean ($\pm$S.E) abundance of each group in the control and herbicide impact sites across all sample times.
Figure 2. Mean total abundance (±SE) of invertebrates and of the dominant groups, before and after glyphosate application for the herbicide impact and control sites.
abundance of amphipods in the impact sites (Fig 1) which also had a significant control/impact effect ($F_{1,3} = 264, P = 0.007$).

There was considerable variation in abundance of most taxa between sampling times for the control and impact sites (Fig 2). The smallest numbers were collected in both the control and impact sites before spraying, and again eight weeks after spraying. These sampling times received the lowest rainfall (Fig 3). The spiders, pseudoscorpions and centipedes were all present in similar numbers in the control and impact sites, and numbers fluctuated in a similar pattern during sampling (Fig 2c, d and h).

3.2 Community composition
There were significant differences in the invertebrate assemblages between sampling times (Global $R = 0.109$, $P = 0.001$) and the control and impact sites (Global $R = 0.796$, $P = 0.001$) when these two factors were analysed using an ANOSIM. The ‘before’ samples were significantly different from all post-treatment samples, except at sixteen weeks (Table 2). These after-impact samples had an average dissimilarity of 53.4% to the before samples, with the amphipods (32.9%) and isopods (15.7%) contributing most to the dissimilarity.

The average dissimilarity amongst sites within the impact group was 40.6% and for the control group 59.6%. The average dissimilarity between the impact and control samples was high at 73.7% (Fig 4). SIMPER analysis of abundance data indicated the earthworms (12.3%) and amphipods (42.3%) consistently contributed to the dissimilarity between the two groups. However, for presence/absence data only the earthworms (15.2%) had a high contribution to the dissimilarity. They were consistently present in all the impact sites, but only occasionally in the control sites.

4 DISCUSSION
The application of glyphosate to $C$ monilifera had no direct or indirect effect on leaf litter invertebrate abundance or community composition in the four months following application. The litter invertebrate assemblages were highly variable on a small spatial scale (tens of metres) and were sensitive to micro-environmental changes. Rainfall and temperature appeared more important in regulating invertebrate numbers than glyphosate application.

4.1 Direct toxic effects
Glyphosate has been shown to be toxic to invertebrates in laboratory tests, but this study does not support that it is toxic in the field when applied at the standard rate and concentration for controlling $C$ monilifera. Direct toxic effects would be indicated by a decrease in abundance in the initial post-application samples. There was only a small decrease in spiders and pseudoscorpions two weeks post-impact (Table 1). This decrease was not significant and could be due natural fluctuation in the communities.

The amount of herbicide that reached the leaf litter and mineral soil is unknown. Consequently, we do not

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Table 2. Results of the analysis of similarity, comparing the before impact time with the four after impact sampling times, for impact and control sites

<table>
<thead>
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<th>Test</th>
<th>R</th>
<th>P</th>
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<td>Global test</td>
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</tr>
<tr>
<td>Pairwise 2 weeks</td>
<td>0.183</td>
<td>0.001</td>
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<tr>
<td>Pairwise 4 weeks</td>
<td>0.230</td>
<td>0.001</td>
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<tr>
<td>Pairwise 8 weeks</td>
<td>0.073</td>
<td>0.025</td>
</tr>
<tr>
<td>Pairwise 16 weeks</td>
<td>0.045</td>
<td>0.091</td>
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</table>

* Data were square-root transformed. $P < 0.05$ significance level.
know the amount of direct exposure the invertebrates had to the herbicide. Glyphosate has been shown to decay only slowly in sandy soils, and has been detected in sandy loam 120 days after application.\textsuperscript{38} The slow decay could increase the chance of contact. However, \textit{C monilifera} is a dense shrub, and the abundant foliage could prevent large amounts of spray drift contacting the ground.

\subsection*{4.2 Indirect effects}
The negative impact of glyphosate on invertebrate communities in the field is generally due to indirect effects.\textsuperscript{21,24,25,39} Indirect effects include changes in vegetation structure, micro-climate changes, loss of food source and decrease in habitat quality. No significant indirect effects were detected in this study. Invertebrate numbers did not decrease up to sixteen weeks after impact (Table 1, Fig 2), and changes in community composition appear to be due to abiotic factors. Samples were collected for four months following impact, even though the \textit{C monilifera} plants were dead by eight weeks. The large sudden leaf input following herbicide application can alter leaf litter invertebrate communities.\textsuperscript{19} However, the dying \textit{C monilifera} lost few leaves, and it has been observed that the leaves usually stay on herbicide-sprayed plants until they are uprooted or are physically removed (B Rafferty, pers comm). The leaf litter under the \textit{C monilifera} also contained native leaves from surrounding remnant vegetation. These are much slower to decompose,\textsuperscript{40} and could have provided a safe habitat and food source for the invertebrates once the \textit{C monilifera} was dead and the existing \textit{C monilifera} leaves had decomposed.

The results of this study conflict with those found by Haughton \textit{et al}\textsuperscript{24} and House \textit{et al}\textsuperscript{39} but both experiments used higher application rates of glyphosate than the 10.8–14.4 g AE ha\textsuperscript{−1} used to control \textit{C monilifera}. In field margins treated with glyphosate at 360 g AE ha\textsuperscript{−1} there was a significant decrease in total invertebrate abundance (31\%) and Araneae abundance (18\%) in treated areas,\textsuperscript{24} while in wheat fields treated with 1.57 kg AE ha\textsuperscript{−1} macroarthropod abundance decreased, with the significance varying with season.\textsuperscript{39} In both cases, the decrease in abundance was thought to be due to a decline in habitat quality and/or loss of food source. The vegetation structure and microclimate of these sites would have been considerably different from those of the \textit{C monilifera} infestations investigated in this study, which were not isolated vegetation patches in an agricultural landscape. The remnant native vegetation could have protected the invertebrates from desiccation and temperature extremes. Larger mobile invertebrates could also have used the adjacent areas to forage. This indicates that the detrimental indirect effects herbicide application has on non-target litter invertebrates may depend upon the application rate, the vegetation community and structure and the post-spray climate.

\subsection*{4.3 Rainfall and temperature}
The invertebrate communities were highly variable at a small spatial scale, with assemblages differing between spray sites and between the spray and control sites before impact. The same taxa were present in the control sites as in the impact sites, but in lower abundance. Many abiotic factors, including temperature, rainfall and soil moisture, can regulate invertebrate populations.\textsuperscript{41} The control area was closer to the beach and was more exposed to onshore winds. The litter microclimate, especially the moisture level, would have been different, possibly making the protected impact site a more favourable habitat.

Invertebrate assemblages were different between the majority of sampling times. The large increase in some taxa with increasing rainfall (Figs 2 and 3) appears to have caused most of this difference. The rainfall fluctuated during the study, but was average for this time of the year.

Sampling times with lower rainfall (before spraying and at eight weeks) had a lower abundance of Haplotaxida, Chilopoda, Diplopoda and Amphipoda. The abundance of these animals has been correlated with rainfall and/or moisture.\textsuperscript{42–45} In Australia, earthworms (Haplotaxida) are limited in their activity and abundance by their need for moisture.\textsuperscript{45} When it is dry they spend less time in the leaf litter and more time in the soil to avoid desiccation.\textsuperscript{44} The Amphipoda prefer moist conditions, and can occur in large numbers when the conditions are right.\textsuperscript{46} Unlike earthworms, Amphipods and many other arthropods cannot burrow deeply into the soil and are not highly mobile, therefore when it becomes dry or hot they may die.\textsuperscript{42,47}

There was more rainfall during the final sampling (sixteen weeks, 16 mm) than the previous sample (eight weeks, 2 mm), yet there was a decrease in isopods, spiders, and centipedes, especially in the control sites. The final samples were collected in autumn, whereas the experiment had begun in summer, and the mean air temperature had decreased by 7.3 °C from 22.4 to 15.3 °C (Fig 3). The abundance and surface activity of many arthropods changes with season\textsuperscript{42,44} and the abundance of these taxa has been correlated with temperature.\textsuperscript{46} The combined affect of rainfall and temperature on invertebrate abundance and composition could have overridden any indirect affects of the herbicide.

\section*{5 CONCLUSION}
Application of dilute Roundup Biactive in summer had no detectable short-term impact on the abundance of leaf litter invertebrates examined in this study. Further studies are needed to determine the effects of glyphosate and the various commercial formulations on the long-term life history of the litter invertebrates, especially their growth rate, behaviour and reproduction.
Abiotic factors appeared more significant in regulating leaf litter invertebrates numbers than glyphosate application. House et al. drew the same conclusion when examining microarthropod populations in agricultural field sites. The response to glyphosate could depend on the season of application, current rainfall and temperature. This is the first study looking at invertebrates and glyphosate application to an environmental weed. These results conflict with the other limited studies in agricultural systems, indicating that the impact could depend upon the vegetation community and structure.

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