Residual Effects of Glyphosate Herbicide in Ecological Restoration

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Abstract

This study assesses the risks in ecological restoration arising from transplanting into soil containing glyphosate residues. Four Australian restoration species were grown for 60 days in nonadsorbing media treated continuously with glyphosate to establish threshold concentrations for damage. Visual signs of injury were observed in three species, and severe effects on root growth in all species, at solution concentrations as low as 18 mg/L. Only the perennial grass *Themeda* sp. died at this concentration, with other species surviving at concentrations in the range 36–360 mg/L, beyond which all plants died. Fourteen days exposure followed by removal of glyphosate from root media produced similar effects. Field and glasshouse experiments with the relatively tolerant tree species *Angophora costata* showed that application rates in the range 10–50 L/ha of herbicide product (360 g/L) would be needed to sustain damage to young plants transplanted into soil typical of local restoration sites. The volume of spray delivered using a hand-operated sprayer varied between operators by 5- and 10-fold to complete the same tasks, at the high end presenting a potential risk to the most tolerant species under field conditions, even when spray concentrations follow label instructions. For all but the most sensitive species, the risk of glyphosate residues in ecological restoration should be minimized by training operators of unregulated applicators to deliver controlled volumes of herbicide when spot spraying prior to transplanting.

Key words: ***Acacia*** **sp.,** ***Angophora*** **sp., bushland restoration, glyphosate,** ***Lomandra*** **sp.,** ***Themeda*** **sp.**

Introduction

Since the introduction of glyphosate in 1976, the herbicide has enjoyed a reputation for low toxicity to mammals, birds, fish, insects, and most bacteria and for low impact on nontarget vegetation (Giesy et al. 2000). Broad-spectrum activity and low cost make glyphosate an important chemical in ecological restoration programs (e.g., Wilkins et al. 2003). However, unless stem injection is used, some herbicide inevitably reaches the soil when applied to weeds. This article is concerned with the possible effects of glyphosate residues in soil when used in bushland restoration.

Glyphosate residues are generally rapidly and strongly adsorbed, although subsequent microbial degradation may take many months in soils with low microbial activity (Sprankle et al. 1975*b*; Torstensson 1985). The soil factors most closely associated with adsorption are broadly those related to phosphate-sorption capacity (Torstensson 1985; Glass 1987). Glyphosate adsorption is inhibited by the addition of P to soil (Hance 1976). Because of rapid adsorption, manufacturers usually make statements on the label to the effect that "the herbicide is inactivated quickly in soil and provides no residual weed control." Although it was known for many years that roots can absorb glyphosate (Sprankle et al. 1975*a*, 1975*b*; Haderlie et al. 1978; Penn & Lynch 1982), it was believed that strong adsorption would preclude root uptake (e.g., Malik et al. 1989) except at very high rates of application (Baird et al. 1972). This belief persisted until Eberbach (1989), Cornish (1992), and Eberbach and Douglas (1993) showed that plant damage could occur under field conditions from root uptake of glyphosate applied at recommended rates to soils with apparently low glyphosate-sorption capacity.

Where residues are not strongly adsorbed, the risk of planting into soil depends on the time since herbicide application and application rate (Cornish 1992), as well as the sensitivity of the species sown (Cornish et al. 1996). Application rate may be an issue in restoration work where unregulated spraying equipment is used for spot application. This equipment includes backpacks and pressurized handguns where herbicide concentrations are controlled but not the spray volumes applied, resulting in unregulated application rates per unit area. This can lead to both excessive herbicide application and run-off from leaves to soil. With respect to species sensitivity, there is little published information on the susceptibility of woody plants to glyphosate, and none that we know of for species typically used in ecological restoration.

Because early research appeared to establish that the risk of crop injury from glyphosate residues was low, subsequent work focused on modes of foliar uptake and action in the plant, together with work on commercial applications. Because soil residues have now been shown

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to be potentially phytoactive, there is a need for more information about the behavior of glyphosate in soil and plant responses to the residues. This study concentrates on responses by species used in ecological restoration and on the spray application technology with a risk of excessive herbicide delivery rates. It reports experiments that aimed to establish the risks of using glyphosate in restoration work by determining (1) threshold concentrations for damage in soil solution for four species used in bushland restoration in the east coast of Australia; (2) factors affecting the phytotoxicity of glyphosate in soil; and (3) variation in application rates by different individuals using backpack sprayers.

Materials and Methods

There were eight experiments. The first determined threshold concentrations for glyphosate damage in four Australian native species used in restoration work: two herbaceous perennials, *Themeda australis* (a grass) and *Lomandra longifolia*; and two tree species, *Acacia longifolia* and *Angophora costata*. Young plants were exposed continuously for 60 days to glyphosate in the glasshouse, using nonadsorbing growth media to ensure constant dose rate and to enable comparison with published data for other species studied under comparable conditions. In subsequent experiments, *A. costata* was the only native used because the major need was for information on susceptibility of woody plants. A second experiment in nonadsorbing media simulated the field situation where glyphosate concentrations diminish with time, so plants were grown for 48 days and exposed to glyphosate for 14 or 48 days. Three pot experiments then examined key factors that may influence plant response to glyphosate residues: soil texture, addition of P fertilizer, and time between herbicide application and transplanting (i.e., the "plant-back" period). A field experiment examined the responses of *A. costata* transplanted under practical field conditions following weed control with glyphosate. Finally, two experiments quantified variation in spray application rates between operators simulating weed control for restoration work. All the glasshouse experiments used a 20/25°C night/day regime.

Herbicide Formulation

Glasshouse experiments used the herbicide formulation Roundup (Monsanto Australia Ltd., Melbourne, Australia), whereas the field experiment used Nufarm Glyphosate 360 (Nufarm Ltd., Melbourne, Australia), both containing the active ingredient (ai) glyphosate (360 g ai/L) and a surfactant. The product was used, rather than active ingredient alone, so that data were provided on the material used in practice. Moreover, the surfactant has small and usually nonsignificant effects on the parameters of interest in this study, compared with much larger effects of glyphosate (Sprankle et al. 1975*a*). Treatments and results are reported as product (containing 360 g ai/L) per unit area (viz L product/ha), except for studies of threshold concentrations for plant damage in nonadsorbing growth media, which are reported as responses to the concentration of active ingredient in solution, to be comparable with other published data.

Description of Experiments

To determine species sensitivity, the four species were compared at 0; 18; 36; 360; 1,800; and 3,600 mg glyphosate/L in the nutrient solution added to the growth media. There were eight replicates, each comprising 1 plant/pot. Concentrations in the range 0–36 mg ai/L were of the order found to affect shoot growth of crop plants (Cornish et al. 1996). The highest concentration was the typical midrange recommendation for use in backpack sprayers (10 mL product/L). Plants were purchased as tube stock established in a sand/compost mixture. They were transplanted into 75-mm-diameter × 150-mm-high pots containing 800 g steam-sterilized, coarse river sand, then irrigated to drainage point with nutrient solution containing a hydroponic nutrient mix (Simple Grow, Sydney, Australia) (2 g/L) plus 100 mg/L P as NaH_2PO_4. P was added to saturate possible glyphosate-sorption sites. Upon commencing treatments, solutions of the required glyphosate concentration were applied twice daily to drainage for 3 days, again to ensure that all sorption sites for glyphosate were occupied in both the potting mix and sand media. Simple Grow was added one to two times weekly to replace leaching losses, and glyphosate treatments were reapplied weekly. Daily observations were made of symptoms for 60 days, when the experiment was terminated. Symptoms varied with species, but the most common first symptom was a distinctive chlorosis at the base of youngest leaves, loss of turgor in young tissue followed by necrosis, and in woody species, a tendency to proliferate new shoots as old growing points died. We report mortality (%) after 60 days, the time taken for deaths to occur (average for the plants that died), severity of symptoms in surviving plants (1–5 scale, with "1" no symptoms through to "3" extensive chlorosis and "5" death), and weight of roots and shoots after 6-week exposure to herbicide. With *Acacia* and *Angophora*, shoot weight was for "new" shoots produced after the imposition of treatments. Measuring only the new shoots increased the power of the experiment to detect growth responses that were small relative to the initial weight of the test plants. New shoots were identified as tissue arising above a water-based dye placed below the growing point on the day treatments commenced. These shoots were removed, dried, and weighed. It was not possible to differentiate between new and "old" shoots in *Lomandra* and *Themeda*, so these data are for whole shoots. The weight of new roots of all the species was determined by first carefully washing the sand from the root mass to reveal the tube stock potting mix that was kept intact with old (dark) proliferating roots.

New roots, which were lighter in color and found outside the "root ball," were trimmed from the old roots, dried at 80°C for 24 hours, and weighed.

In the species comparison above, symptoms of damage in *A. costata* appeared up to 6 weeks after the treatment with glyphosate commenced, but under field conditions, it is likely that microbial degradation will greatly reduce concentrations in this time. This raised the question of whether short-term exposure would result in the longer-term effects observed in the first experiment. So the second experiment aimed to determine threshold concentrations in solution when plants of *A. costata* were exposed for either 14 or 48 days after transplanting. Plants were initially exposed to glyphosate concentrations of 0, 18, 36, and 360 mg ai/L for 14 days. No symptoms of injury were observed at this time. Then, two new treatments were established in which half the pots in each initial concentration continued to receive glyphosate at the initial rate, whereas the other half received no glyphosate. Pots were flushed with either deionized water or fresh glyphosate solution to remove the initial glyphosate application from the soil solution, by passing three lots of water or glyphosate (as required) through each pot over a 24-hour period. After flushing, the plants were fertilized (Simple Grow) and subsequently watered with either water or glyphosate solution, according to treatment. Soil pre-treatment to saturate potential glyphosate-sorption sites, and plant culture, were the same as in the first experiment. There were three replicates of each of the eight treatments. Plants were harvested 5 weeks after flushing the pots. The dry weight of new roots and all shoots was measured.

In the third experiment, the response of *A. costata* to glyphosate residues was evaluated in two soils that were representative of sites commonly chosen for restoration in the Sydney region. The soils differed in clay content, which potentially affects glyphosate adsorption similarly to P sorption. The soils were a loamy sand (73% sand, 22% clay) from the 0- to 10-cm surface of a soil derived from sandstone near Somersby, 80 km north of Sydney, and a sandy loam (91% sand, 6% clay) sampled from the surface of an alluvium near Richmond, 60 km west of Sydney. Neither soil had a significant known history of fertilizer use. Treatments included soil type and rate of herbicide applied to the surface (equivalent to 0, 2, 10, 25, 50, and 100 L product/ha). Two species were used, *A. costata* and Tomatoes (*Lycopersicon esculentum*). Tomatoes were included as a sensitive benchmark species for comparison with *A. costata* (Cornish 1992). There were four replications. To establish the experiment, pots containing 1.5 kg of air-dried soil were brought to field capacity, then allowed to dry for 2 days in the glasshouse. The herbicide was then sprayed on the surface. One day later, one *A. costata* and two Tomato seedlings were planted per pot. Seedlings were treated with a complete nutrient solution before transplanting, but the soil was not fertilized. Plants were watered daily to field capacity. Plants were harvested after 4 weeks. The entire shoot of each species was harvested, and roots were carefully washed from the soil and old and new roots separated. The dry weight of new roots and all shoots was measured. In the analysis of variance (ANOVA), soil type and herbicide rate were treated as main plots and species were treated as subplots in a split-plot design.

The fourth experiment examined the interaction between glyphosate and P fertilizer added to the soil. Treatments comprised P fertilizer rates in factorial combination with herbicide application rates. There were six replications. The unamended "Somersby" soil used in the experiment was low in P (1.9 mg P/kg soil, Bray extraction). Phosphorus as superphosphate was added at three rates (0, 50, and 100 mg P/kg) to air-dried soil, mixed, and filled into pots containing 1.5 kg soil. The soil was brought to field capacity and left for 2 days before surface application of herbicide at 0, 2, 10, and 50 L product/ha. Seedlings of *A. costata* were transplanted, one per pot, 24 hours later. Pots were watered daily to field capacity. Plants were harvested 35 days later, and total root and shoot dry weight was determined.

The risk of glyphosate injury from soil residues reduces with increasing time between spraying for weed control and transplanting into the treated area. The fifth experiment aimed to determine a plant-back interval for *A. costata* in Somersby soil to which 100 mg P/kg had been added. The experiment was essentially a bioassay for phytoactive residues in soil. Herbicide was applied at four rates (0, 2, 10, and 50 L/ha) onto the surface of pots containing 1.5 kg soil at intervals of 4, 2, 1, and 0 weeks before transplanting. Tube stock of *A. costata* was planted on the day 1, 24 hours after the last spray application, providing four treatments differing in time since herbicide application (0, 1, 2, and 4 weeks). There were four replications. Pots were watered daily to field capacity. Plants were harvested after 35 days, and the weight of new shoots and roots was determined.

The experiments described above were under glasshouse conditions, with herbicide sprayed onto bare soil. The sixth experiment tested for field effects of glyphosate residues on transplanted seedlings of *A. costata*. The experiment was conducted on "Richmond" alluvial soil (8% sand, 4% silt, 11% clay) at the University of Western Sydney, in an unfertilized area of native grass pasture used for grazing cattle. The area for the experiment had not been grazed for some time, so it was mown to about 20-mm height and the clippings removed. Unlike the experiments under controlled conditions, the soil was protected from direct spray contact by a layer of vegetation and litter. Treatments comprised herbicide applied by a backpack sprayer at rates equivalent to 0, 5, 10, 50, and 100 L/ha of product (360 g ai/L). The volume of made-up spray was 100 mL/m^2. There were four replicates of each treatment arranged in a randomized complete block design. Plots were 1.5 m^2 and contained eight plants, each at 30-cm spacings. The site received about 40 mm of rain 1 day before spraying plots with glyphosate. Plants from

the NSW State Forestry Commission were transplanted into the soil the day after spraying (10 January). For transplanting, a small hole was dug to enable seedlings to be planted with the minimum soil disturbance. Plants each received 100 mL water after transplanting, and 200 mL per plant was applied 2 days later. Thereafter, no watering was applied, and 50 mm rain was received over the subsequent 2 months prior to harvest. Mean daily temperature through this period was 26°C. No weed control was applied, other than hand clipping of weeds from around control plants. Plots receiving glyphosate were weed free. Plants were observed for visible symptoms of damage 13 days after transplanting, and dug from the soil on 17 April at which time roots were inspected and shoots were dried at 80°C, weighed, and analyzed for total Kjeldahl nitrogen.

The final two experiments examined the variation in application rates between individual operators of unregulated sprayers. In the first experiment, nine "operators," all staff and postgraduate student volunteers, were asked to spray a 0.5-m-wide strip along 20 m of fence line using a backpack, hand-operated sprayer containing a known volume of water. Operators were familiarized with the equipment but otherwise provided with no instructions, other than those on the herbicide label. Time to complete spraying was recorded with a stopwatch, and the volume of water applied (mL) was recorded. In the second experiment, 12 operators were asked to approach and spray a circular area of 1 m^2 around a "target weed." The time taken to apply the spray and the total volume applied were recorded. Seven petri dishes were distributed within the target area to capture spray so that variation in delivery volumes could be estimated. After each operator completed their task, the dishes were sealed and returned to the laboratory and weighed to determine the water deposited at each site in the target area.

Statistical Treatment of Data

All the experiments except the tests of operator performance were randomized complete block designs subjected to ANOVA using SPSS 10.0 for Windows software (SPSS, Inc., Chicago, IL, U.S.A.). In the experiment to determine species sensitivity, data for plant symptom severity required square root transformation and shoot and root dry weight data required log transformation. Retransformed data are presented in tables. Means were compared using Duncan's new multiple range test for unplanned comparisons between multiple means. In the comparison of four species, the data for mortality and time to die could not be statistically analyzed because of the small numbers (eight plants per treatment).

Results

All the species showed visual symptoms of injury from glyphosate at concentrations in solution down to 18 mg ai/L (36 mg/L in *Lomandra*), the lowest tested. *Themeda* was most sensitive by this criterion. Plants of all species died at the highest concentrations (1,800 and 3,600 mg/L) and most also died at 360 mg/L (Table 1). On the basis of total mortality, the tree species *Angophora* appeared to be the most tolerant and the grass species *Themeda* the least tolerant, its seedlings dying first. Root weight was reduced ($p < 0.05$) in all species at the lowest concentration (18 mg/L), with little root growth occurring after treatments commenced, except in the case of *Lomandra*. Shoot growth was less sensitive than root growth. Data for *Lomandra* and *Themeda* shoot weight included material that was present when treatments commenced; therefore, data from the other two species provide a better indication of glyphosate injury, with *Acacia* showing substantial shoot weight reductions beyond 36 mg/L and *Angophora* at the lowest concentration of 18 mg/L.

In the experiment examining plant responses to short-term exposure, the main effect of exposure time (viz 14 and 48 days) was not significant ($p \geq 0.05$), showing that short-term exposure was equally damaging as longer-term exposure (data not tabulated). Root growth was approximately halved at the lowest concentration of 18 mg ai/L, and no new roots were produced during the experiment when concentrations were 360 mg/L. Shoot growth was reduced, but only at the highest concentration (360 mg/L), in which there was no new shoot growth compared with 1.93 g/plant in the controls.

In the experiment examining the effect of soil texture on plant response to soil-applied glyphosate (Table 2), the interaction between soil type and herbicide rate was significant ($p < 0.05$) for Tomato shoots, but it was not significant for either roots or shoots in *Angophora*. Thus, glyphosate residues were more phytoactive in the lighter textured Richmond soil, but this was detected only when Tomato was the bioassay plant. In Richmond soil, root and shoot growth of *Angophora* was significantly reduced at 25 and 50 L product/ha, respectively, compared with the control, whereas Tomatoes suffered reductions at 10 and 25 L/ha for roots and shoots, respectively.

With respect to the effect of adding P fertilizer on the response to glyphosate residues, neither the main effect of P nor its interaction with herbicide was significant ($p \geq 0.05$), although the main effect of herbicide was significant ($p < 0.05$) (data not tabulated). Shoot weight was reduced from 5.5 g/plant in the control to 4.3 g/plant when 50 L/ha of herbicide was applied to the soil surface.

In the bioassay experiment that aimed to determine the safe plant-back interval in Somersby soil, the main effects of concentration and time, and their interaction, were significant ($p < 0.05$) for shoot weight (Table 3). Time and the interaction between time and concentration were significant for root weight ($p < 0.05$). When transplanting occurred 24 hours after application of glyphosate to the soil surface (time zero), 50 L product/ha reduced shoot growth, whereas 2 L/ha or more reduced root growth. Transplanting 2 weeks after application resulted in no statistically significant effects of glyphosate.

Table 1. Responses by four species to glyphosate added at six[a] concentrations to nutrient solution in nonadsorbing growth media.

Glyphosate (mg ai/L)	*Acacia*	*Angophora*	*Lomandra*	*Themeda*	$\overline{X}$
Deaths at 60 days (%)					
0	0	0	0	0	
18	0	0	0	87.5	
36	12.5	0	12.5	100	
360	87.5	50.0	75.0	100	
1,800	100	100	100	100	
Time to die (days)[b]					
0	n.d.	n.d.	n.d.	n.d.	
18	n.d.	n.d.	n.d.	35.3	
36	50.0	n.d.	56.0	27.6	
360	23.0	27.0	29.3	14.6	
1,800	19.6	22.3	27.3	14.0	
Symptoms rating after 60 days[c]					
0	1.4^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.1^{a}
18	2.5^{b}	2.6^{b}	1.4^{a}	4.9^{b}	2.7^{b}
36	3.0^{b}	3.0^{b}	3.1^{b}	5.0^{b}	3.4^{c}
360	4.9^{c}	4.5^{c}	4.5^{c}	5.0^{b}	4.7^{d}
1,800	5.0^{c}	5.0^{c}	5.0^{c}	5.0^{b}	5.0^{d}
Root weight (mg/plant)[d]					
0	504^{a}	1088^{a}	199^{a}	1060^{a}	664^{a}
18	17^{b}	172^{b}	96^{b}	33^{b}	80^{b}
36	10^{b}	96^{b}	47^{bc}	13^{b}	41^{bc}
360	0^{b}	0^{b}	19^{c}	7^{b}	7^{cd}
1,800	0^{b}	0^{b}	6^{c}	13^{b}	5^{d}
Shoot weight (mg/plant)[d]					
0	905^{a}	925^{a}	478^{a}	633^{a}	735^{a}
18	471^{ab}	599^{b}	519^{a}	391^{ab}	495^{b}
36	571^{ab}	425^{c}	405^{ab}	401^{ab}	457^{b}
360	90^{b}	28^{d}	311^{ab}	284^{b}	178^{c}
1,800	6^{b}	11^{d}	183^{b}	353^{ab}	138^{c}

Means with different superscript letters, within species and parameter, are significantly different ($p < 0.05$).
[a] There were no responses from 1,800 to 3,600 mg ai/L, so data from the higher concentration are not presented.
[b] Average of the plants that died. n.d. indicates no deaths up to 60 days.
[c] 1–5 scale. Analysis based on square root transformation.
[d] Values are $\log_{10}$ transformation, for posttreatment ("new") growth in *Acacia* and *Angophora* and for whole shoots in *Lomandra* and *Themeda*.

The field experiment examined responses to glyphosate residues under conditions simulating practical restoration. There were symptoms of glyphosate injury 14 days after transplanting but only in plants sown into soil sprayed with 100 L product/ha. Of the 32 plants in all replicates of this treatment, 5 plants had died, 4 others appeared to be dying, and 14 showed chlorosis and leaf-edge necrosis. Eleven plants eventually died in this treatment, compared with only one plant per treatment in each of the 5 and 10 L/ha treatments and none in the 0 and 50 L/ha treatments. Data for dry weight 2 months after transplanting are given in Table 4. Controls had grown poorly, apparently due to

Table 2. Comparative responses of *Angophora* and Tomato to herbicide[a] applied to two soil types before transplanting.

	Somersby Soil (73% sand, 22% clay)				*Richmond Soil (91% sand, 6% clay)*			
	Angophora		*Tomato*		*Angophora*		*Tomato*	
Rate (L/ha)[b]	*Roots*[b] *(g)*	*Shoots (g)*	*Roots*[b] *(g)*	*Shoots (g)*	*Roots*[b] *(g)*	*Shoots (g)*	*Roots*[b] *(g)*	*Shoots (g)*
0	0.48^{a}	1.29^{a}	0.14^{a}	0.89^{ab}	0.21^{a}	0.84^{bc}	0.10^{a}	0.73^{a}
2	0.25^{b}	1.08^{ab}	0.15^{a}	1.06^{a}	0.15^{a}	1.17^{ab}	0.11^{a}	0.83^{a}
10	0.16^{c}	1.15^{ab}	0.02^{b}	0.92^{ab}	0.19^{a}	1.26^{ab}	0.01^{b}	0.72^{a}
25	0.23^{bc}	1.51^{a}	0.01^{b}	0.75^{b}	0.07^{b}	1.37^{a}	0^{b}	0.33^{b}
50	0.15^{c}	1.09^{ab}	0^{b}	0.40^{c}	0.06^{bc}	0.89^{bc}	0^{b}	0^{c}
100	0.11^{c}	0.76^{b}	0^{b}	0.38^{c}	0.01^{c}	0.63^{c}	0^{b}	0^{c}

Data are in g/plant. Means within plant parts and species with different superscript letters are significantly different ($p < 0.05$).
[a] Product containing 360 g ai/L.
[b] Roots produced between transplanting and harvest.

Table 3. Growth of *Angophora costata* over 35 days in the glasshouse.

Interval after Spraying (Weeks)	Herbicide Application Rate (L/ha)			
	0	*2*	*10*	*50*
Shoot weight (g/pot)*				
0	1.32	1.66	1.57	1.03[a]
1	1.48	1.75	1.57	0.95[a]
2	1.65	1.37	1.44	1.21
4	1.48	1.71	1.72	1.78
Root weight (g/pot)*				
0	0.48	0.12[a]	0.15[a]	0.04[a]
1	0.69	0.55	0.41	0.33
2	0.64	0.45	0.29	0.37
4	0.36	0.28	0.36	0.57

Seedlings were transplanted at different intervals after spraying Somersby soil with herbicide containing glyphosate (360 g ai/L). Means with superscript are significantly different from means with no superscript, within plant part ($p < 0.05$).
*Weights are for whole shoots and roots produced over the 35 days of treatment.

competition from weeds that had been inadequately controlled by hand weeding in this treatment. Control plants contained significantly less N (2.1%) than all other treatments (3.1–3.6%) ($p < 0.05$). Weeds were completely controlled in all treatments where glyphosate was applied (5–100 L/ha), and in these treatments, plant dry weight was reduced at 10 and 50 L/ha rate compared with 5 L/ha, and further reduced at 100 L/ha. Optimal growth and survivorship under the conditions of this experiment was obtained with 5 L product/ha.

Two experiments examined variation in delivery between spray operators. In the first experiment, nine operators took from 24.3 to 112.7 seconds to complete the task of spraying a 20 × 0.5–m strip along a fence ($\overline{X}$: 48 ± 30 seconds). They delivered from 320 to 1,500 mL ($\overline{X}$: 550 ± 373 mL) spray. Applications in the range of 320–1,500 mL over the test area of 10 m^2 are equivalent to 320–1,500 L/ha. With the recommended spray concentration of 10 mL/L (product with 360 g ai/L), application rates would range from 3.2 to 15 L product/ha. In the second of these experiments, 12 operators spraying a 1-m^2 area took between 4.5 and 45 seconds (16 ±11 seconds) and delivered from 20 to 640 mL/m^2 ($\overline{X}$: 235 ± 163.4 mL/m^2), equivalent to 200–6,400 L/ha of spray, or 2–64 L product/ha (at 10 mL product/L). The mean application rate of product was equivalent to 23.5 L/ha. Application varied greatly within the target area, with coefficients of variation ranging from 0.24 to 0.68 ($\overline{X}$: 0.48).

Table 4. Shoot dry weight (g/plant) of surviving *Angophora costata* 2 months after transplanting into glyphosate-treated soil in the field.

Herbicide Application Rate (L product/ha)				
0	*5*	*10*	*50*	*100**
1.85[c]	5.15[a]	3.98[b]	2.97[b]	1.73[c]

Means with different superscripts are significantly different ($p < 0.05$).
*Eleven plants of the 32 transplanted died in this treatment. The mean is for surviving plants.

Discussion

Glyphosate is intended for use as a foliar spray, but some of the herbicide inevitably contacts soil, leaving residues. This may occur where there is incomplete ground cover at the time of application, or where rainfall occurs following application, noting that less than half the herbicide applied to leaves is absorbed (Sprankle et al. 1975*b*), even up to 12 days after application (Schultz & Burnside 1980). The rest is available for wash-off. Any remaining residues on leaves may be incorporated in soil at the time of planting, if this involves any soil disturbance. Moreover, this study is concerned with the possible effects of glyphosate residues in soil when it is applied as a spray in ecological restoration, a situation where the common spray application technology has a risk of high herbicide delivery rate, regardless of whether the concentration used conforms to the label recommendation or not. High delivery volumes will result in run-off from leaves to soil.

These experiments confirm that glyphosate residues in soil may be taken up through roots of a range of species used in restoration projects. All the species showed visual symptoms of injury, despite the fact that aboveground plant parts were never exposed to the herbicide. Quite low concentrations in the soil solution (18 mg ai/L) led to visual signs in foliage and substantially reduced root growth in all species. Reductions in shoot growth were mostly small at this low concentration, although significant in two species. *Themeda australis* died at the lowest concentration of 18 mg/L. On the basis of both visual symptoms and total mortality, the tree species *Angophora costata* was the most tolerant and the grass species *Themeda australis* the least tolerant of those tested. In soil culture, *Angophora* was more tolerant than the sensitive benchmark species Tomato, with about twice the herbicide rate required to cause damage than with Tomato, depending upon the soil type and plant part examined.

Greater sensitivity of root growth compared with shoots is consistent with crop species (Cornish 1992; Cornish et al. 1996), although the concentrations required to reduce growth were generally higher. With root growth of the restoration species, the lowest concentration used was too high to give precise estimation of threshold concentrations for safety, but root growth was severely reduced at 18 mg/L compared with around 2 mg/L for crop species (Cornish et al. 1996). Shoot growth data for *Acacia* and *Angophora* suggest that growth was halved in the range 18–36 mg/L, whereas the crop species studied by Cornish et al. suffered 50% reductions in shoot growth at around 4 mg/L of glyphosate in the soil solution. The capacity to grow and survive, despite severe root damage, may reflect the availability of nutrients and water in this glasshouse

experiment. Effects on shoots may be more severe in the field, given the same degree of damage to roots as in the glasshouse, because of nutrient and water stress and plant competition. These longer-term effects were not evaluated.

As a guide to the field relevance of the concentrations at which damage occurred in solution culture, we estimated potential soil solution concentrations. At the recommended application rate of 9 L/ha for hard-to-kill weeds (360 g ai/L product), the soil solution concentration would be 32 mg ai/L, assuming no adsorption or degradation, and uniform mixing to 5-cm depth in sandy loam soil at field capacity (20% volumetric water content). Thus, in a soil with no adsorption capacity for glyphosate, expected concentrations in soil solution are within the range causing effects on root growth of all species tested in our experiments. The question is whether damaging concentrations ever occur in soil, and particularly under field conditions as reported by Eberbach (1989) and Cornish (1992) for pastures and Tomatoes. Young plants of *Angophora*, the most tolerant of the species tested, consistently showed damage in pot experiments with two soil types when transplanted into soil previously treated with glyphosate, but generally when the application rate of herbicide product (360 g ai/L) was 50 L/ha or greater. The selection of treatments in the pot experiments did not allow detection of any damage in the range 25–50 L/ha. However, in the one field experiment, shoot growth of *Angophora* was significantly reduced when application rate was increased from 5 to 10 L/ha, and further reduced at 50 and 100 L/ha, although plants died only when 100 L/ha had been applied. It is important to note in this experiment that competition was a factor in poor growth when glyphosate was not applied at all, with the best survival and growth being obtained when glyphosate at 5 L (product)/ha had been applied, emphasizing the valuable role of herbicides in controlling competition in restoration work.

The risk of plant damage under field conditions will be greatest in soils with low adsorption capacity for glyphosate, typically sandy soils and possibly those having received high rates of P-containing fertilizer (Sprankle et al. 1975*a*; Hance 1976; Torstensson 1985; Glass 1987; Cornish 1992). Although damage to Tomatoes did occur at lower application rates in the soil with lower clay content and presumably lower sorption capacity (Table 2), damage nevertheless occurred in both soils, neither of which had been heavily fertilized with P, with one containing significant clay (22%). This suggests that the sorption capacity for glyphosate may not always be easy to predict. Although theoretical considerations and some pot experiments have suggested that adding P may increase the phytotoxicity of glyphosate residues (Sprankle et al. 1975*a*; Glass 1987), neither the present experiment nor work on sandy soils near Sydney (Cornish 1992) has demonstrated this.

Because even relatively short exposure (14 days) to high concentrations of glyphosate was damaging, microbial degradation cannot be relied upon as a safety mechanism for dealing with nonadsorbed residues, without knowing the rates of degradation or conducting a bioassay. In the bioassay experiment (Table 3), delaying transplanting for 2 weeks after spraying eliminated damage to the bioassay plant, *A. costata*. The delay required in other situations will depend upon the application rate, the degree of glyphosate adsorption, the degradation rate, and the species sown.

The significance of these findings depends on the likelihood of high application rates, estimated at around 50 L product/ha for the more tolerant species (e.g., *Angophora*) in the soils examined, but noting in the one field experiment, where the soil was protected from direct spray contact by a layer of vegetation and litter, that growth was significantly reduced at 10 L/ha. In broadacre applications, rates of 50 L/ha would not occur if registration requirements were observed. However, backpack and handgun sprayers do not regulate spray volume, although the operator controls the concentration. Two experiments showed that individual operators vary widely in the volume of spray used to perform identical tasks. For the more profligate operators, application rates would exceed 50 L/ha, the rate required to cause damage to the relatively tolerant *Angophora* in both the glasshouse and the field. This high rate would occur even if the spray were made-up according to label. Presumably, less tolerant species than *Angophora*, not tested in the field, could be damaged at lower application rates.

The level of susceptibility in even the most tolerant species, together with variation between operators, suggests that damage could result from preplant weed control with glyphosate. On the other hand, some operators are quite miserly and may not achieve effective weed control. We therefore recommend that users of handguns and backpack-type sprayers be trained to carefully regulate volumes applied per unit area. With care, the risk of damage to transplanted restoration species will be minimized and better weed control will result.

To conclude, effects of glyphosate residues on plants are most likely under a fairly specific set of conditions, being soils with low glyphosate-sorption capacity, combined with high rates of glyphosate, planting soon after spraying, and planting a sensitive species. In practice, these conditions will not coincide very often, so widespread failure due to glyphosate residues would not be expected and have not been reported. For woody species, operator error delivering excessive dose rates appears to present the only real problem. Although rates of glyphosate at the low end (2 L/ha) had some effect on roots, in no experiment was this effect large. Large effects only occurred at higher rates. More sensitive species such as *Themeda* will be more susceptible to damage, but any risks should be manageable through attention to spray application rates and, in high risk situations (soil with low glyphosate sorption), a delay between spraying and planting or thorough mixing of soil at the site of planting to

increase sorption of residues. Local research will be needed to establish actual risks and management options where there is significant potential risk.

Glyphosate has proven to be an effective tool in bushland restoration. The present results do not change this assessment, with the results of the field experiment clearly demonstrating the value of controlling weed competition. Rather, they stress the need for caution with susceptible species on soils with low sorption capacity for glyphosate, and, in particular, they emphasize the need for operator training to ensure that the chemical is used safely and effectively.

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