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Post-sowing weed control technique can affect woody seedling numbers, with early hand-weeding potentially more beneficial than early spraying

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Declaration of Interest

Declarations of interest: none.

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Summary

Direct seeding is increasingly being used as a cost-effective revegetation technique. Successful outcomes from direct seeding rely on effective weed control, particularly during the vulnerable seedling establishment phase. Post-sowing weed control options are constrained by the need to protect seedlings from damage and few studies have compared the effectiveness of different weed control techniques. We evaluated the effect of preliminary hand weeding (with subsequent spraying), spraying monthly or spraying quarterly with glyphosate, on woody seedling emergence, survival and growth in trials sown either in spring or autumn at a riparian site in Victoria, southeastern Australia. Seedling numbers were recorded monthly for 6 months and at 12 months after sowing, with seedling survival and heights assessed at 12 months. Total seedling numbers were higher in subplots initially hand weeded than in subplots sprayed on either a monthly or quarterly basis, regardless of when direct seeding occurred, but weed control treatment had no effect on seedling survival. These results indicate that post-sowing spraying limited rates of seedling emergence, either directly or indirectly. For direct seeding, hand weeding in the first few months after sowing may maximise rates of woody seedling emergence, while spraying with glyphosate may inhibit seedling emergence.

Key words: direct seeding, glyphosate, hand weeding, post-sowing weed control, riparian restoration, woody seedlings

Introduction

Restoration of degraded landscapes often requires active revegetation, with plants being introduced to sites either as nursery-grown plants or as seeds. The main advantage of using nursery-grown plants is that the highly vulnerable life history stages of germination, seedling emergence and establishment are completed under favourable conditions in the nursery. In contrast, seeds sown directly into a site must complete these stages under conditions that may be highly variable and less than favourable. For this reason, the same seed lot can produce ten time more plants when grown in the nursery than when sown directly (Corr 2003).

Despite this limitation, direct seeding can be more cost-effective than planting (Ede *et al.* 2017; Grossnickle & Ivetić 2017; Raupp *et al.* 2020), particularly in large-scale restoration projects (Pérez *et al.* 2019). Direct seeding is now being used in revegetation projects across a wide range of ecosystems in tropical, temperate and arid zones around the world (e.g. Azam *et al.* 2012; Gibson-Roy & Delpratt 2015; Palma & Laurance 2015; Ceccon *et al.* 2016; Grossnickle & Ivetić 2017).

Successful direct seeding projects require the optimisation of seed, soil and climatic factors, protection from granivores and herbivores, and the preparation of a receptive seedbed with adequate soil moisture (Dalton 1993; Corr 2003; Cuneo *et al.* 2018). Effective weed control is critical, particularly in riparian areas which are highly vulnerable to weed invasion (Richardson *et al.* 2007), with diverse and dense riparian weed communities potentially limiting direct seeding outcomes (Gould 2012). Effective weed control is also important in sites previously used for agriculture, which have high weed seed loads and high potential for weed recolonisation after direct seeding.

Weed seed loads can be reduced by scalping off the topsoil, but scalping is not recommended in riparian areas due to the risk of soil erosion. Herbicide options are also limited in riparian areas due to concerns about off-target impacts on aquatic systems. In Victoria (Australia), the only herbicide registered for use in areas adjacent to a waterway is a glyphosate formulation developed to minimise impacts on aquatic biota (Roundup Biactive®, Bayer Crop Science, Australia). This broad-spectrum, post-emergent herbicide is highly effective pre-sowing, but must be used carefully post-sowing to prevent harm to desirable seedlings.

The requirement to protect desirable seedlings from damage constrains post-sowing weed control options in all direct seeding projects. That such weed control is required to achieve good outcomes for woody seedlings has been demonstrated by experimental studies testing weed control techniques ranging from hand weeding and manual clearing to herbicide applications (e.g., Löf *et al.* 2004; Piiroinen *et al.* 2017; Passaretti *et al.* 2020). Comparative studies assessing different physical control methods (Hooper *et al.* 2002; Pereira *et al.* 2013), different chemical control options (Barron *et al.* 1998; Jinks *et al.* 2006; Semple & Koen 2006; Willoughby & Jinks 2009), and comparing physical and chemical control methods (Löf & Welander 2004) have reinforced the requirement for post-sowing weed control, but not identified the most consistently effective weed control techniques to optimise the establishment of woody seedlings.

There is considerable investment being made in riparian revegetation in many parts of the world, as public agencies and private landholders seek to improve waterway health. However, uncertainty about the effectiveness of direct seeding in riparian areas with high weed loads currently limits the extent of its application. It is particularly important to elucidate effective ways to limit riparian weed competition post-sowing. To address this, we undertook a field experiment to compare different post-sowing weed control techniques and frequency of application at a riparian site sown in either spring or autumn. We conducted the experiment to test the following hypotheses: 1) that the technique of post-sowing weed control does not affect the number of seedlings that establish; 2) that increasing the frequency of weed control in the first months after sowing increases the numbers of seedlings that establish; and 3) that the effects of post-sowing weed control technique and frequency on seedling numbers are independent of season of sowing.

Methods

Study site

Our study was located on a farm adjacent to the Bass River, in southern Victoria, Australia (38°26'17.7" S, 145°33'34.6" E). Prior to trial establishment, the site was grazed by beef cattle. The extant vegetation was dominated by exotic pasture grasses and weedy forbs, with some indigenous woody species present including Silver Wattle (*Acacia dealbata*), Blackwood (*A. melanoxylon*) and Burgan (*Kunzea leptospermoides*). Invasive Willows (*Salix* x *rubens*) were present along the waterway, while indigenous forest dominated the opposite bank.

The study site experiences warm summers and cool, wet winters. Mean maximum temperatures range from 13.6°C in July to 24.6°C in February (Wonthaggi, Site No. 086127, Bureau of Meteorology 2019). Mean annual rainfall at the nearest monitoring site (Glen Forbes, 5 km from the study site), is approximately 880 mm. Monthly rainfall data for the study period is provided in Supplementary Fig. 1.

Site preparation

The site was fenced before sowing, to prevent stock access. However, the site was accessible to other herbivores resident within the riparian zone, with Eastern Grey Kangaroo (*Macropus giganteus*), Swamp Wallaby (*Wallabia bicolor*), Wombat (*Vombatus ursinus*) and Sambar Deer (*Rusa unicolor*) captured by digital infra-red detection cameras (Reconyx Hyperfire™).

Exotic woody species were removed from the site by excavator in 2012. In the 6 months prior to sowing, herbaceous weeds were sprayed three times with Roundup Biactive® (360 g l^{-1} glyphosate; Bayer Crop Science, Australia), with the last spray treatment applied approximately two weeks before sowing. At the time of trial establishment, plots were raked to remove dead vegetative material and prepare a receptive seed-bed.

Experimental treatments

A total of 38 plots were established in the study, 18 of which were sown in September 2014 (the spring trial) with 18 sown in April 2015 (the autumn trial), with 2 plots left unsown. To test the effect of different post-sowing weed control treatments, each plot (6 m x 3 m) was divided into three subplots (2 m x 3 m), with one post-sowing weed control treatment applied randomly to each subplot. The treatments were initial monthly hand weeding (followed by spraying), monthly spraying, and quarterly spraying.

The hand weeded subplots were weeded once a month for 4 months after sowing, then sprayed monthly for 2 months with Roundup Biactive®, (Bayer Crop Science, Australia) and then at quarterly intervals, in a similar way to the spraying treatments described below. In order to minimise soil disturbance during hand weeding, most weeds were cut at the soil surface, while very small weeds were pulled out. Cutting was effective for those species that did not readily resprout but less effective for grasses and some dicotyledon species which regrew between weeding events.

The monthly spraying treatment involved spraying weeds with Roundup Biactive® (Bayer Crop Science, Australia) on a monthly basis for the first 6 months after sowing, and then at quarterly intervals for the remainder of the study, as described below.

The quarterly spraying treatment was first applied 3 months after sowing and then at quarterly intervals for the remainder of the study. This treatment reflected current weed management practices used locally at riparian revegetation sites.

Herbicide solution concentrations ranged from 1% to 1.5% v:v. Herbicide was carefully applied using a knapsack spray unit, with individual weeds spot-sprayed. Each sub-plot was searched thoroughly for emerging and emerged native seedlings prior to spraying, with plastic cups placed over seedlings during spraying to protect them from herbicide, as per Jusaitis & Polomka (2008).

The two unsown plots were included to assess levels of natural recruitment from naturally occurring seed banks. These plots had the same pre-sowing weed control treatments as the other plots. They were established in spring and sprayed on a quarterly basis.

Unweeded sown subplots were not included in the study as it has already been well established in other studies that effective post-sowing weed control is critical for successful direct seeding outcomes.

Seed and sowing

Nine indigenous tree and shrub species were sown, with seed locally sourced (Table 1). The rate of sowing was the same in all plots and the same seed lot was used in both spring and autumn, with all seeds treated and applied in the same way at both sowing times.

Seeds of *Acacia* species were pre-treated by immersion for 1–2 minutes in just-boiled water, then soaked overnight in a cold smoke water solution (a 10% dilution of smoke water concentrate). Seeds were then air-dried in readiness for sowing that day. Seeds of the remaining species were not pre-treated.

Seed was hand-broadcast over plots, using sand as a carrier and 40 ml of smoke water (a 10% dilution of smoke water concentrate) was added to the seed-sand mix prior to sowing. The two unsown plots were treated with an equivalent rate of sand mixed with smoke water (but no seed) to control for the effect of the addition of smoke water.

Monitoring

At monthly intervals for the first 6 months after sowing (just prior to the application of weed control treatments), and at 12 months after sowing, all subplots were surveyed at ground-level for indigenous seedlings. Indigenous seedlings were identified at the cotyledon stage, i.e., at the point of emergence above ground. A plastic plant label was inserted next to each seedling recording a unique identifying number and the date on which the seedling was first found. At each subsequent search, the presence or absence of all tagged seedlings was recorded and new seedlings were tagged and recorded, to give the total number of seedlings present at each survey. Ambiguous seedlings were also tagged, with the tags removed if subsequent monitoring identified them as an unsown species.

In this study, seedling emergence was defined as the point at which cotyledons became visible above ground. Survival was defined as the number of seedlings present at 12 months after sowing as a percentage of the total number of seedlings that emerged during the first 6 months of the study (the period in which the different weed control treatments were applied). Plant heights were measured at 12 months after sowing.

Data Analysis

We assessed the effects of weed treatments on seedling numbers, survival and heights using generalised linear mixed models. Effects on total seedling numbers were assessed using a Poisson regression model (Gelman and Hill 2006) with Season of trial (two levels: spring and autumn), Weeding treatment (three levels: hand weeding, monthly spraying and quarterly spraying) and Month since sowing (seven levels: months 1-6 and month 12) and all potential interactions included as fixed effects, with Plot included as random effect to account for repeated sampling of the same plots.

We assessed effects on seedling survival at 12 months using a beta regression model (Ferrari and Cribari-Neto 2004) with Season of trial (two levels), Weeding treatment (three levels) and Species (four levels: *Acacia* spp., *Eucalyptus* spp., Hop Goodenia (*Goodenia ovata*) and Prickly Teatree (*Leptospermum continentale*) and all potential interactions included as fixed effects. Survival values at the plot level were first transformed ((x*0.9) + 0.05)) to ensure values were >0 and <1 as required for beta regression. Effects on seedling heights at 12 months were assessed using normal linear regression using the same model structure as per seedling survival.

Assessments of seedling survival and heights were done at the genus level for *Acacia* and *Eucalyptus* species because of low numbers of seedlings of some species precluded analysis at the species level.

We present raw means and standard errors and results from Wald chi-square tests of fixed effects from the above models. For all models, residual plots were inspected to confirm assumptions of the analyses were met. All analyses were performed using R v.4.0.2 (R Core Team 2020).

Results

Seedling emergence

A total of 1,412 seedlings emerged over the course of both the spring and autumn trials. Initial hand weeding resulted in more seedlings than spraying either monthly or quarterly in both trials (Fig. 1,

Table 2). This difference was clear 2 months after sowing in the spring trial, and 3 months after sowing in the autumn trial. Seedling numbers peaked in the hand weeded subplots after 3 months in the spring trial (2.5 ± 0.7 seedlings/m^2) and after 5 months in the autumn trial (2.1 ± 0.4 seedlings/m^2). After 12 months, seedling numbers were still significantly higher in the hand weeded subplots in the spring trial than in either the monthly or quarterly sprayed subplots (1.7 ± 0.4, 0.9 ± 0.3 and 0.6 ± 0.2 seedlings/m^2, respectively; $p < 0.001$ for both contrasts with hand weeded subplots), but were similar across the three treatments in the autumn trial (0.5 ± 0.1, 0.4 ± 0.1 and 0.3 ± 0.1 seedlings/m^2; $p > 0.05$ for both contrasts) (Fig. 1). This was due to a steep decline in the number of seedlings in the hand weeded subplots in the last 6 months of the autumn trial (Fig. 1), probably due to very dry conditions in spring (September to November 2015, Supp. Fig. 1). There were no clear differences in total seedling numbers between the monthly and quarterly spray treatments in either trial.

At the species level, patterns were generally consistent with the patterns for total numbers of seedlings (Table 3), with numbers of Prickly Teatree seedlings in particular, higher in hand weeded subplots in both trials.

It is possible that a few *Acacia* seedlings emerging in the field originated from the soil seed bank rather than from sown seed. In one of the unsown control plots, two Silver Wattle seedlings emerged indicating that this species is present in the soil seed bank at the site. No other seedlings of sown species emerged in the control plots.

Seedling survival at 12 months

Weed control treatment had no effect on seedling survival in either the spring or autumn trial (Fig. 2, Table 4). Overall, seedling survival rates were greater in the spring trial (39%) than the autumn trial (19%), regardless of weed control treatment or species (Table 4). Survival rates also differed between species, with *Eucalyptus* seedlings having the lowest rates of survival, particularly in the autumn trial (10%).

Seedling heights at 12 months

Most *Acacia*, *Eucalyptus* and Prickly Teatree plants remained small (<20 cm tall) 12 months after sowing in both the spring and autumn trials (Fig. 3). Many of these seedlings had evidently been browsed and consequently some were growing in a prostrate manner. In contrast, Hop Goodenia plants were not browsed and were considerably taller (>40 cm on average) 12 months after sowing in both the spring and autumn trials (Fig. 3, Table 5).

Pooling across species, seedlings in the spring trial were significantly taller in hand weeded subplots than subplots sprayed quarterly, but not monthly ($p < 0.01$ and $p > 0.05$ for relevant contrasts, respectively), but there were no differences in plant heights between weed control treatments in the autumn trial (Fig. 3, Table 5).

Discussion

In our study, higher seedling numbers were found in the treatment that involved hand weeding in the first months after sowing compared with either treatment that involved spraying alone. This finding was counter to our hypothesis that the technique of weed control would not affect numbers of establishing seedlings. However, seedling survival (i.e., survival at the end of the study of seedlings that emerged during the first six months of the study) was not affected by weed control technique or frequency. These findings indicate that seedling numbers were higher in subplots initially weeded by hand because seedling emergence was greater.

It is possible that some positive effect of hand weeding on seedling emergence in our study may have arisen from rapid removal of weed competition in the early stages of seedling development, although it is unclear how large this effect might be. Negative impacts of the herbicide application process should not be discounted, but it is unlikely that herbicide application directly harmed already emerged seedlings as these were physically protected from spray drift during spraying. It is also unlikely that the accumulation of dead thatch harmed existing seedlings or inhibited seedling emergence, as monthly spraying resulted in very low weed biomass.

Lower rates of seedling emergence in the monthly sprayed treatment may have been the result of frequent, repeated applications of glyphosate harming ungerminated seed or germinated seed pre-emergence. Such damage would be unexpected, given that the manufacturer states that Roundup Biactive® is absorbed by foliage and green stems, is inactivated immediately in the soil and does not provide residual weed control (Bayer Crop Science, Australia 2020). However, it has been shown that glyphosate can remain in soils at varying concentrations (Silva *et al.* 2018); limits seed germination when applied directly to seeds (e.g., Blackburn & Boutin 2003; Gomes *et al*. 2017; Türedi *et al.* 2018); and affects soil and rhizosphere microbial communities in varied and complex ways (Kremer & Means 2009). For example, glyphosate has been found to be toxic to phytosymbiotic pink-pigmented facultative methylotrophic bacteria which promote seed germination in some species (Irvine *et al.* 2013). Likewise, reductions in populations of other key microbial species, including arbuscular mycorrhizae, have been found following repeated glyphosate applications to a native grassland community (Druille *et al.* 2016). Therefore, it is not out of the question that the repeated monthly applications of glyphosate from the outset of the study had some impact on ungerminated seed, germinating seed or pre-emergent seedlings.

The *frequency* of spray applications in the first 6 months after sowing had no effect on seedling numbers, seedling survival rates or seedling heights, with no differences found between subplots sprayed monthly at the outset of the study and those sprayed quarterly. This result was unexpected, particularly as subplots that were sprayed quarterly tended to develop dense weed coverage in the period between spray applications and dense cover is known to inhibit seedling recruitment (e.g., Willoughby & Jinks 2009; Piiroinen *et al.* 2017). Competition from this dense weed cover may explain the lower numbers of seedlings in quarterly sprayed subplots compared with seedling numbers in subplots that were initially hand weeded. If repeated applications of glyphosate in the monthly spray treatment were limiting seedling emergence as discussed above, this would help to explain the lack of treatment difference between the monthly and quarterly spray treatments.

We did not test hand weeding at different frequencies so further studies which include monthly and quarterly hand weeding treatments would be beneficial, particularly given the high labour costs involved with hand weeding (Ede *et al.* 2017). It is possible that at some direct seeding sites, less frequent hand weeding may be equally as effective in limiting weed competition as hand weeding monthly, depending on the weed species present. If so, this would contribute to addressing the resource constraints that hand-weeding may impose on operational projects undertaken at much larger scales (hectares).

Multiple environmental and climatic factors affect the outcomes of direct seeding (e.g., Hallett *et al.* 2014), including seasonal variations in temperature and rainfall. Nonetheless, the results from our study did support our hypothesis that the effect of the weed treatments, both technique and frequency, are independent of sowing season, with one exception: height growth differed between weed treatments in the spring sown trial, but not in the autumn sown trial.

As other studies have found, one of the key drivers of success for direct seeding is the availability of adequate soil moisture during seedling establishment (e.g., Löf & Welander 2004; Hallett *et al.* 2014; Grossnickle & Ivetić 2017), hence the need to reduce weed competition as much as possible. In our study, seedlings establishing from autumn sown seed faced unusually dry conditions in spring (September to November 2015, Supp. Fig. 1), resulting in high levels of seedling mortality between 6 and 12 months after sowing in hand weeded subplots. In this instance, the benefits afforded by hand weeding were insufficient to overcome the conditions imposed by drought.

Herbivory by native macropods and introduced herbivores can also limit the outcomes of revegetation projects in Australia (e.g. Cuneo *et al.* 2018; Moser & Greet 2018). At our study site, seedling growth was severely impacted by ongoing herbivory for all species except Hop Goodenia. We observed that seedlings of palatable species that established in close proximity to Hop Goodenia seedlings were afforded some protection from browsing and had increased rates of survival and growth. Cryptic planting, growing palatable plants in close proximity to unpalatable plants, can be effective in reducing herbivore browsing (Moser & Greet 2018), as we found with Hop Goodenia. This suggests that including unpalatable species into a seed sowing mix may benefit palatable species. However, the extent of browsing in our study indicates that where herbivores are abundant, investment in browsing protection is critical to direct seeding success – without it the benefits of investing in hand weeding are likely to be limited.

The results from our study have been replicated in an operational direct seeding project sown with a similar species mix at different but nearby site, with higher seedling numbers recorded in hand weeded plots than in plots repeatedly sprayed with glyphosate (Ede and Greet, 2017, unpublished data). The results of this second project, to be published separately, appear to lend weight to the significance of our findings that frequent applications of glyphosate post-sowing can inhibit seedling emergence. Given the extensive use of this herbicide in revegetation projects in southeastern Australia and globally, further studies to determine whether this effect is found in other ecosystems with other species would be worthwhile. Practitioners need to be aware that direct seeding projects could potentially be less successful if glyphosate is repeatedly sprayed on sown areas after sowing, regardless of the care taken to protect already emerged seedlings.

In future, it may be possible to protect emerging seedlings from herbicide damage by pelletising seed. Recent studies have shown that encasing seed in pellets containing activated charcoal and superabsorbent polymers protected emerging seedlings from pre-emergent herbicides without compromising rates of seedling emergence and growth (Brown *et al.* 2019; Clenet *et al.* 2019).

In conclusion, our results show that there are likely to be benefits of post-sowing hand weeding over herbicide spraying for the early stage of establishment of woody species. While we did not include an unweeded control in our experiment as it is already well known that effective post-sowing weed control is critical to successful direct seeding outcomes, what has not been well identified previously are the most effective techniques for this weed control. Our results indicate that for revegetation managers, investing in hand weeding may be worthwhile to promote seedling establishment, and that frequent, repeated applications of glyphosate, particularly in the first few months post-sowing, may inhibit seedling emergence.

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Declaration of Interest

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Table 1. Species sown at the Bass River study site in spring (September 2014) and autumn (April 2015)

Species	Common name	Growth form	Weight seed sown/m^2 in field (g)
Acacia dealbata	Silver Wattle	Tree	0.018
Acacia melanoxylon	Blackwood	Tree	0.105
Acacia verticillata	Prickly Moses	Shrub	0.016
Eucalyptus obliqua	Messmate	Tree	0.032
Eucalyptus ovata	Swamp Gum	Tree	0.095
Eucalyptus radiata	Narrow-leaved Peppermint	Tree	0.046
Eucalyptus viminalis	Manna Gum	Tree	0.071
Goodenia ovata	Hop Goodenia	Shrub	0.029
Leptospermum continentale	Prickly Teatree	Shrub	0.042
Total seed sown			0.454

Table 2. Results from Wald chi-square tests for fixed effects of Season of sowing, Weed control treatment, and Month after sowing, from the Poisson regression model of total seedling numbers. ndf = numerator degrees of freedom; treatment effects which are significant at P <0.05 are highlighted in bold.

Fixed term	Wald statistic	ndf	*P*
Season	0.236	1	0.627
Weed	736.001	2	**<0.001**
Month	422.340	6	**<0.001**
Season x Weed	48.550	2	**<0.001**
Season x Month	67.742	6	**<0.001**
Weed x Month	9.896	12	0.625
Season x Weed x Month	46.018	12	**<0.001**

Table 3. Mean number of seedlings present at 12 months after sowing in each weeding treatment, for each species sown in spring and in autumn. *Acacia* spp. represents those seedlings which could not be identified to species. All data presented are raw data

Species	Mean number of seedlings at 12 months after sowing (seedlings/m^2)					
	Spring sown trial			Autumn sown trial		
	Hand weed	Monthly spray	Quarterly spray	Hand weed	Monthly spray	Quarterly spray
A. dealbata	0.44	0.34	0.25	0.12	0.14	0.17
A. melanoxylon	0.05	0.04	0.05	0.07	0.06	0.04
A. verticillata	0.04	0.02	0.00	0.00	0.00	0.00
Acacia spp.	0.19	0.07	0.03	0.01	0.00	0.00
E. obliqua	0.02	0.02	0.01	0.00	0.00	0.01
E. ovata	0.09	0.02	0.03	0.03	0.00	0.04
E. radiata	0.06	0.05	0.02	0.06	0.00	0.00
E. viminalis	0.05	0.02	0.03	0.01	0.01	0.00
G. ovata	0.62	0.28	0.16	0.12	0.03	0.09

L. continentale	0.10	0.03	0.02	0.13	0.01	0.00
All species	1.66	0.88	0.59	0.54	0.25	0.34

Table 4. Results from Wald chi-square tests for fixed effects of Season of sowing, Weed control treatment, and Species from the beta regression model of seedling survival rates. ndf = numerator degrees of freedom; treatment effects which are significant at P <0.05 are highlighted in bold

Fixed term	Wald statistic	ndf	*P*
Season	11.058	1	**<0.001**
Weed	0.215	2	0.898
Species	26.740	3	**<0.001**
Season x Weed	0.097	2	0.953
Season x Species	2.036	3	0.565
Weed x Species	3.309	6	0.769
Season x Weed x Species	2.512	6	0.867

Table 5. Results from Wald tests for fixed effects of Season of sowing, Weed control treatment, and Species from the normal linear regression of mean seedling heights. ndf = numerator degrees of freedom; treatment effects which are significant at P <0.05 are highlighted in bold

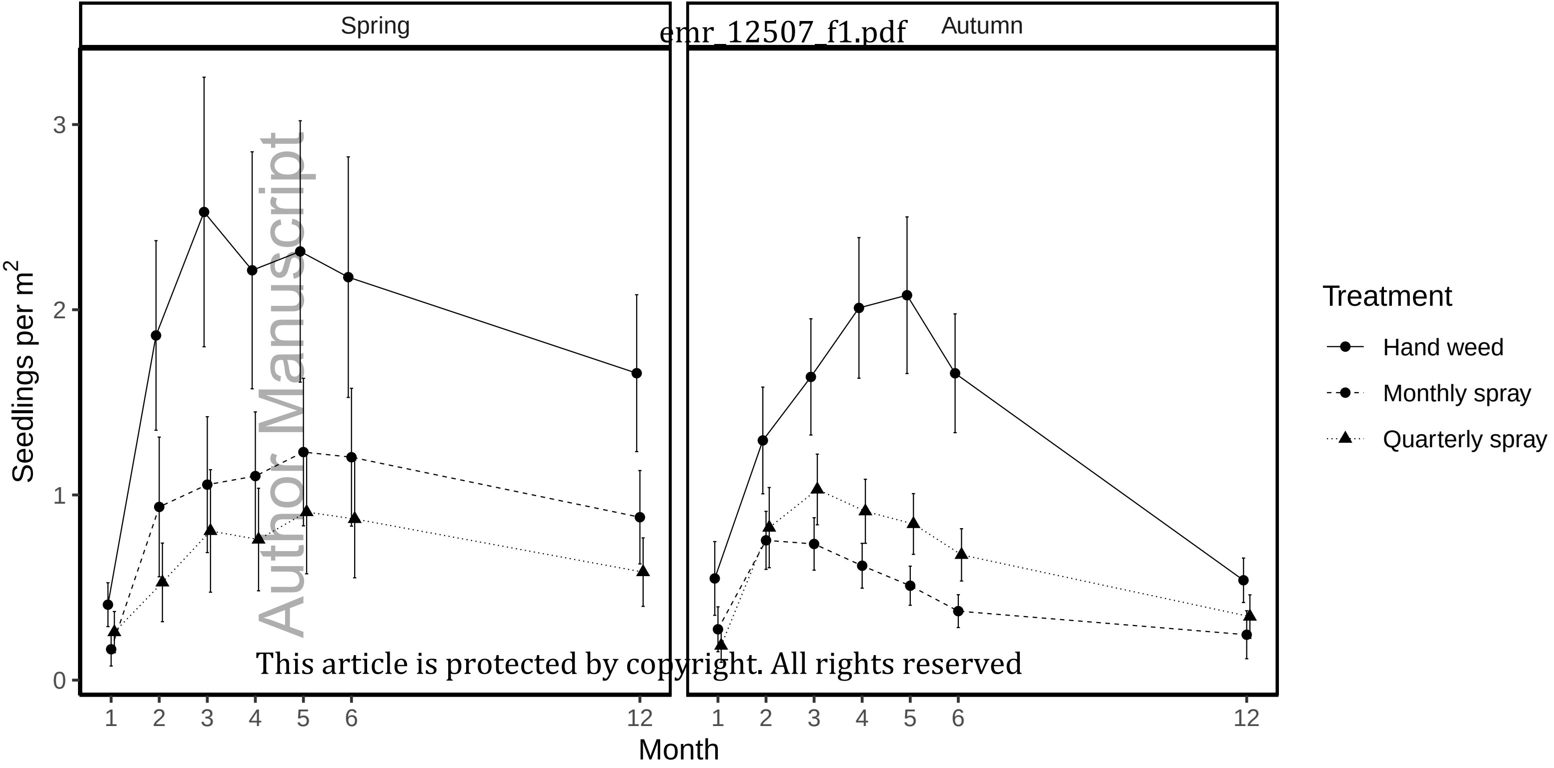
Fixed term	Wald statistic	ndf	*P*
Season	3.695	1	0.055
Weed	13.756	2	**0.001**
Species	516.933	3	**<0.001**
Season x Weed	8.777	2	**0.012**
Season x Species	4.898	3	0.179
Weed x Species	2.008	6	0.919
Season x Weed x Species	5.587	5	0.349

Figure 1. Mean (± SE) seedling numbers per m^2 by month for each of three weed control treatments (monthly hand weeding, monthly spraying and quarterly spraying) for two direct seeding trials, one sown in spring, one sown in autumn.

Figure 2. Mean (+ SE) seedling survival (%) by season of sowing (spring and autumn) and species for the different weed control treatments (monthly hand weeding, monthly spraying and quarterly spraying). Aca = Acacia spp.; Euc = Eucalyptus spp.; Good = Hop Goodenia; Lepto = Prickly Teatree.

Figure 3. Mean (+SE) seedling heights by season of sowing (spring and autumn) and species for the different weed control treatments (monthly hand weeding, monthly spraying and quarterly spraying). Aca = Acacia spp.; Euc = Eucalyptus spp.; Good = Hop Goodenia; Lepto = Prickly Teatree.

Supplementary Figure 1. Monthly rainfall recorded at Glen Forbes monitoring site (5 km from the study site) over the duration of the study.


Spring
Autumn
Seedlings per m2
0
1
2
3
1
2
3
4
5
6
12
Month
Treatment
Hand weed
Monthly spray
Quarterly spray

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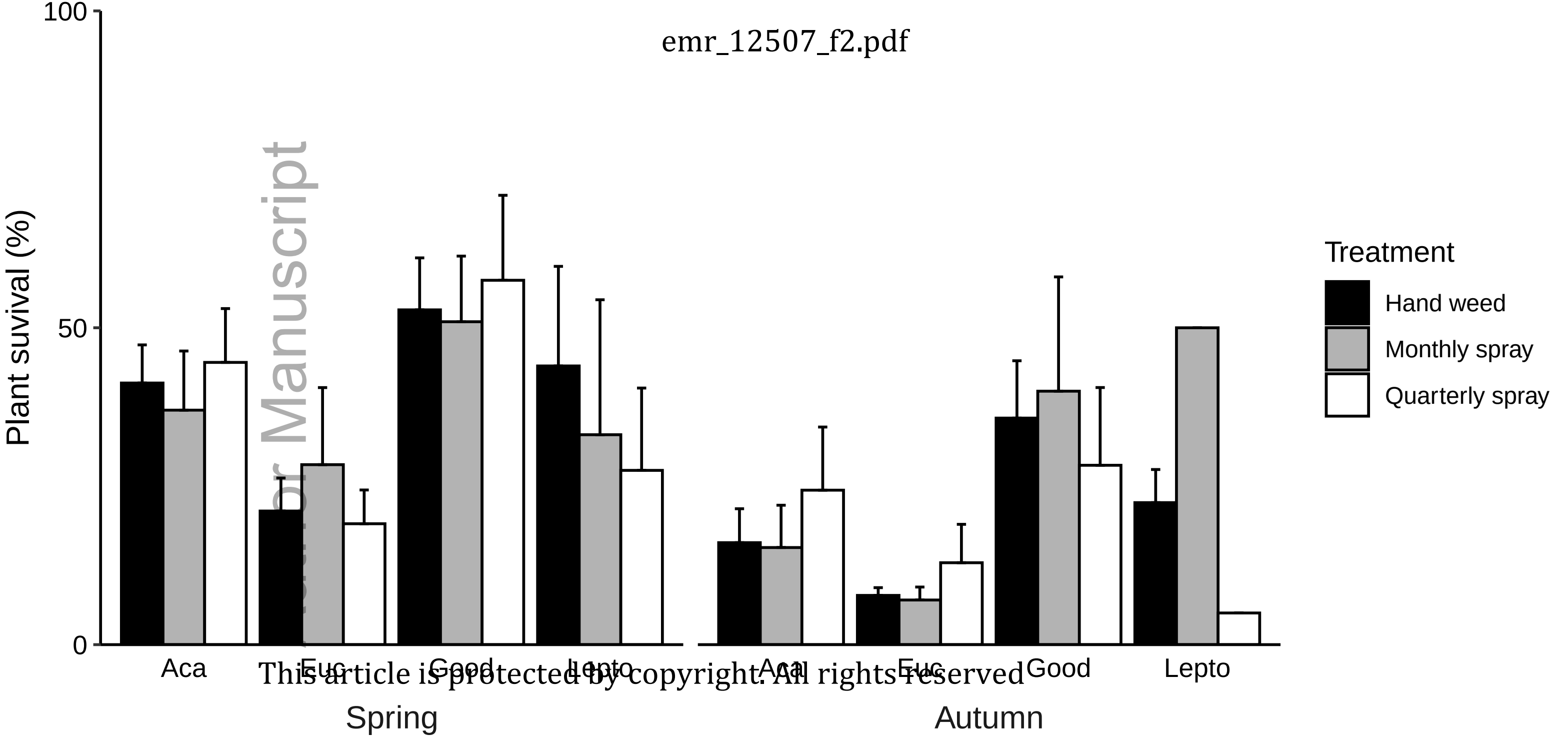

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