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Herbicidal control of bridal creeper (*Asparagus asparagoides*) in an ecologically sensitive environment

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Abstract. *Asparagus asparagoides* (bridal creeper) is a highly invasive noxious environmental weed in southern Australia. It poses a severe threat to biodiversity and conservation in temperate natural ecosystems. *Pterostylis arenicola*, a threatened terrestrial orchid endemic to South Australia, is directly imperilled by this weed in most of its remnant populations. The coincident growth phenologies of orchid and weed make for an ecologically sensitive environment when considering methods of weed control or eradication. To minimise impact on the orchid and its ecosystem, this paper examines the efficacy of herbicide application for *A. asparagoides* control using the weed wiping technique, comparing it to the conventional spray application method. The most prolonged control of *A. asparagoides* was achieved after a single wipe-application of 1.5 g a.i. (active ingredient) L^{-1} metsulfuron methyl, either alone or in combination with 120 g a.i. L^{-1} glyphosate, both treatments giving significantly better weed control five years after treatment than comparable spray applications. An investigation of the effect of glyphosate on cultures of the mycorrhizal fungus isolated from *P. arenicola* indicated a significant decline in mycelial growth with increasing herbicide concentration over the range 0.5–3.0 kg a.i. ha^{-1}. These results provide further incentive for the use of ecologically sensitive herbicide application techniques, such as weed wiping, in areas of high conservation concern.

Additional keywords: glyphosate, metsulfuron methyl, mycorrhizae, *Pterostylis arenicola*, sandhill greenhood orchid, terrestrial orchid, threatened plants, weed wiper application

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Introduction

Environmental weeds have significant impacts on many plant species and communities through effects on plant competition, the soil environment, ecosystem function and biodiversity (Vilà *et al*. 2011). Fragmentation of native vegetation into smaller remnant patches with large edge/area ratios has been largely responsible for the increase in weed encroachment in many areas (Rose 1997; Kemper *et al*. 1999). Threatened species, especially geophytes and terrestrial orchids, are particularly vulnerable (Cheal 1991; Sorensen and Jusaitis 1995; Morin and Scott 2012), but the risks to these populations can be significantly reduced by timely weed control (Baider and Florens 2011). However, the sensitive ecological environment in which many of these rare species exist makes effective weed management very difficult, particularly when invading plants have temporally similar growth patterns and exist in such close proximity so as to surround or envelop native plants.

Asparagus asparagoides (L.) Druce (bridal creeper) is one such weed that has overrun large areas of native vegetation and has the potential to choke out many native species in its wake (Groves and Willis 1999; Willis *et al*. 2003; Office of Environment and Heritage 2013). Its rapid growth, climbing habit and below-ground dominance make it highly competitive, smothering and eventually eliminating most understorey native plants, and producing numerous effects on many other components of the

natural ecosystem (Downey 2006; Morin *et al*. 2006*a*; Turner *et al*. 2006; Stephens *et al*. 2008). It has been recognised as one of Australia's worst invasive plants and is listed in the top 32 Weeds of National Significance (Australian Weeds Committee 2012; Invasive Plants and Animals Committee 2017).

Downey (2006) quantified the impact of *A. asparagoides* on native vegetation from southern New South Wales with an interim list of 52 species, including several terrestrial ground orchids, potentially threatened by this weed. In South Australia, increasing numbers of terrestrial orchids are also potentially threatened by *A. asparagoides*, including *Caladenia behrii*, *Caladenia* sp. 'Brentwood', *C. macroclavia*, *Pterostylis bryophila*, and *Pterostylis* sp. 'Halbury' from the Lofty Block region (Quarmby 2006), *C. richardsiorum* and *C. calcicola* from the south-east region (Dickson *et al*. 2012), and *C. brumalis* from the Eyre Peninsula (Pobke 2007). Stephens *et al*. (2003), when studying the endangered orchid *P. bryophila*, found no orchids in plots with more than 70% *A. asparagoides* cover, and showed that all other native ground-level plants were completely eliminated when cover exceeded 90%. The weed clearly presents a significant threat to many native plant species, but to herbaceous plants and terrestrial orchids in particular.

One such orchid seriously threatened by *A. asparagoides* is the sandhill greenhood orchid, *Pterostylis arenicola* M.A.Clem. & J.L.Stewart (Obst 2005). This terrestrial orchid is endemic to South Australia and is listed as Vulnerable under the Australian Commonwealth *Environment Protection and Biodiversity Conservation Act, 1999* (EPBC Act), and assessed as Vulnerable using IUCN (2001) criteria (VU B1ab(iii)). Of the nine remnant populations (eight south-west of Tailem Bend and one in the metropolitan area of Adelaide), all but one are less than a hectare in size (area of occupancy) and all but one are threatened by *A. asparagoides* (Obst 2005). Mowantjie Willauwar Conservation Park, near Tailem Bend, protects one of these populations of the orchid and is also home to a large infestation of *A. asparagoides*. Preliminary trials in this park showed that plots with *A. asparagoides* had lower numbers of orchids than those without (Sorensen and Jusaitis 1995). The management plan for the park (Department for Environment and Heritage 2008) and the recovery plan for the orchid (Obst 2005) both include objectives to control introduced plant species (including *A. asparagoides*) in the vicinity of the orchid populations, but fail to detail exactly how this will be achieved.

Control or eradication of *A. asparagoides* has traditionally been by herbicidal means (Pritchard 1991, 2002; Meney *et al*. 2002; Ensbey 2014), although biological control agents have also shown varying levels of success (Kleinjan *et al*. 2004; Morin *et al*. 2006*b*). While biological agents substantially reduce populations of *A. asparagoides*, they do not eradicate them (Morin *et al*. 2006*b*), and supplementary hand-grubbing or herbicides are required to achieve complete and long-lasting control. Hand-grubbing is time and labour intensive and can result in excessive soil disturbance while removing dense mats of underground tubers and rhizomes (Morin *et al*. 2006*a*), potentially affecting the re-establishment of native species (Thomas *et al*. 2000). Herbicides present major difficulties when controlling environmental weeds in native plant populations, particularly if the growth phenologies of weed and native coincide or substantially overlap, as is the case for *P. arenicola* and *A. asparagoides* (Sorensen and Jusaitis 1995). Treatment of weeds invariably leads to off-target damage when non-selective herbicides are used. *A. asparagoides* is a perennial plant that lies dormant as persistent underground rhizomes and storage tubers during the summer months, emerges with new growth during autumn–winter and flowers/fruits in spring before dying down again in summer (Morin *et al*. 2006*a*). The development of *P. arenicola* is largely in synchrony with this life cycle, although short windows of opportunity may exist early or late in the growing season to capture the weed in the absence of the orchid. However, the exact timing of these opportunities can vary from year to year and between populations, making the formulation of specific control recommendations extremely difficult. Furthermore, the weed may not be actively growing or have sufficient fresh foliage at these times to allow adequate uptake of the chemical.

An alternative herbicide application technique aimed at minimising the application of chemical to non-target species is that of weed wiping using ropewick or brush applicators (Davison and Parker 1983; Combellack 1984; Moyo 2008; Harrington and Ghanizadeh 2017). This technique applies chemical only to target plants touched by the applicator, thus avoiding spray drift, reducing the

amount of wasted chemical, and eliminating soil contact (Grekul *et al*. 2005). Although this technique has been recommended as suitable for use on *A. asparagoides* (ARMCANZ *et al*. 2001; Office of Environment and Heritage 2013), published information detailing its efficacy in controlling this weed is lacking. Weed wiping is labour-intensive and realistically feasible only for small infestations, but it could prove very useful in ecologically sensitive populations of threatened plants where conservation value is high and accurate targeting of the weed is critical. Studies focussing on spray application have found glyphosate and/or metsulfuron methyl to be most effective for controlling *A. asparagoides* (Pritchard 1991, 2002; Dixon 1996; Meney *et al*. 2002), although neither are currently registered in Australia for control of *A. asparagoides* using wiper equipment.

Terrestrial orchids such as *P. arenicola* have long been known to be dependent on mycorrhizal fungi for seed germination and growth (Warcup 1981; Clements 1988; Sommer *et al*. 2012). Another disadvantage of spray application in communities of terrestrial orchids is the increased chance of herbicide contacting the soil and potentially affecting soil microorganisms, specifically mycorrhizae. Herbicides are known to affect soil mycorrhizal fungi in various ways, and different fungi can respond quite differently to a given herbicide (Trappe *et al*. 1984; Weidenhamer and Callaway 2010). Glyphosate was found to inhibit growth of mycorrhizal fungi *in vitro* (Chakravarty and Sidhu 1987; Estok *et al*. 1989), in glasshouses (Druille *et al*. 2013; Zaller *et al*. 2014), and in field trials (Druille *et al*. 2015) and thus has the potential to adversely affect recruitment and seedling growth of *P. arenicola* if applied indiscriminately.

This study aimed to compare the efficacy of spray and wipe applications of glyphosate and metsulfuron methyl, applied separately or together, to *A. asparagoides* in a field trial. In addition, the effect of glyphosate on growth of the specific mycorrhizal fungus isolated from *P. arenicola* was examined.

Materials and methods

Herbicide trial design

A herbicide trial was set up in the Mowantjie Willauwar Conservation Park ~6 km south-west of Tailem Bend in the Murraylands region of South Australia. The park is underlain by sheet limestone covered with a deep to shallow layer of reddish brown sand with a neutral to slightly alkaline pH (Department for Environment and Heritage 2008). The vegetation is characterised by a closed forest community of *Callitris gracilis* (southern cypress pine) infested with several introduced plant species, predominantly *A. asparagoides* and *Ehrharta calycina* (perennial veldt grass). The park also provides critical habitat for the vulnerable sandhill greenhood orchid (*P. arenicola*) which appears to be threatened by these weeds (Sorensen and Jusaitis 1995).

The trial was set up in a level area of the park that was fenced to exclude rabbits and kangaroos. Seven treatment plots (each 1 × 1 m) were laid out along each of three replicate transects in areas of dense *A. asparagoides* coverage. Individual plots were separated by a 1-m buffer zone to minimise boundary effects and the seven treatments (Table 1) were allocated randomly to plots along each transect. Commercial formulations of the herbicides Roundup® (Monsanto) containing 360 g a.i. (active ingredient) L^{-1} glyphosate (as the isopropylamine salt) and Brush-Off® (Du Pont) containing 600 g a.i. kg^{-1} metsulfuron methyl were used. Application rates for spray treatments were based on label recommendations for control of *A. asparagoides* (Brush-Off) or perennial weeds (Roundup) respectively. Wiper application rates were based on the recommended rate for wiper equipment on the Roundup label (50-fold higher than the spray rate).

Spray treatments were applied using a Solo® backpack sprayer fitted with a hollow cone nozzle. A non-ionic surfactant (Agral 60®, ICI Australia) was added to these treatments (1 mL L^{-1}) and *A. asparagoides* foliage was sprayed to runoff. Calm weather at the time of spraying ensured that spray drift was minimised. Wipe treatments included 2 mL L^{-1} Pulse Penetrant® (Nufarm) as a surfactant and were applied using a Zero Weeding Brush® (Yates Australia) to dab and brush chemical directly onto exposed foliage of *A. asparagoides* plants. Although it was impossible to cover all foliage by this method, care was taken to ensure that at least a portion of each ramet of *A. asparagoides* received

some chemical application. Treatments were applied when weeds were actively growing in July 1995 and were repeated a year later in August 1996.

Percentage cover of *A. asparagoides* was assessed at approximately monthly intervals during the growing season over the first two years, and then at least annually during the peak of the growing season over the next four years. Cover estimation was facilitated by the use of a 1 × 1 m quadrat subdivided into 100 grids (10 × 10 cm) placed over each plot. A protected Fisher's l.s.d. test was used to separate treatment means, and orthogonal contrasts were constructed to examine between-group comparisons involving 12 groups of treatments. The first set of six contrasts compared each herbicide treatment with the control. A second set of orthogonal contrasts was constructed to compare six treatment groupings (spray versus wipe, glyphosate versus metsulfuron methyl, single versus combination herbicide, glyphosate spray versus glyphosate wipe, metsulfuron methyl spray versus metsulfuron methyl wipe, and combination spray versus combination wipe). Each set of contrasts was analysed for each assessment time. All statistical tests were performed on arcsine-square-root-transformed data to ensure homogeneity of variances and analyses were run using Stata® 12 software (StataCorp 2011).

Glyphosate and growth of mycorrhizal fungus

Mycorrhizal fungus was isolated from fresh *P. arenicola* plants collected from the wild population. Several flowering plants were harvested and returned to the laboratory in a cool, damp state to avoid desiccation. Soil, roots and leaves were removed and the thickened region of underground stem immediately below the leaf rosette (the collar) was excised and washed in running tap water for up to 2 h before sterilising in 20% NaOCl (plus a drop of Triton X-100 non-ionic surfactant to improve wettability) for 5 min, and then rinsed twice in sterile RO (reverse osmosis) water. The collar was trimmed and sectioned longitudinally under a dissecting microscope, and using a fine needle, fungal pelotons were teased out from the cortex into a drop of sterile water. Pelotons were transferred to fungal isolating medium (Clements 1982) in sterile Petri dishes that were then sealed with Parafilm strips and incubated at 20°C in darkness. Pure hyphal filaments emanating from pelotons were subcultured onto oatmeal medium (0.25% blended oatmeal, 1.2% Sigma agar). Symbiotic germination tests with seeds of the orchid using the method of Jusaitis and Sorensen (1993) confirmed that the isolated fungus was mycorrhizal for this species. Cultures of the fungus were maintained on oatmeal medium at 20°C in darkness until required for subsequent experiments.

To examine the influence of glyphosate herbicide on growth of the mycorrhizal fungus in vitro, plates of oatmeal medium containing five levels of glyphosate were prepared. The appropriate level of Roundup (360 g a.i. L–1 glyphosate) for each treatment was added to oatmeal agar before pH adjustment (5.5) and autoclaving. The autoclaved liquid media were dispensed aseptically into 86-mm (internal diameter) plastic Petri dishes (20 mL per dish) and allowed to cool. The concentration of glyphosate was calculated for each treatment based on the surface area of the medium in the Petri dish (0.0058 m2) and the volume of medium dispensed per dish (20 mL). Effectively, the five treatments received 0, 0.87, 1.74, 2.4 or 4.8 µL of Roundup per Petri dish, to yield a final concentration equivalent to 0, 1.5, 3.0, 4.15 and 8.3 L f.p. (formulated product) ha–1 of Roundup, or 0, 0.5, 1.0, 1.5 and 3.0 kg a.i. ha–1 of glyphosate in each dish respectively.

Each dish was inoculated centrally with a small cube of mycorrhizal fungal isolate. Treatments were replicated three times and dishes were sealed with Parafilm and incubated upside down at 23°C in darkness. The diameter of the fungal colony was recorded at intervals over 21 days, each record being the average of three equally spaced diameters per dish, measured to the nearest millimetre. The standard errors of the means (n = 3) at each assessment time were calculated.

Results

Herbicide trial

The effect of the various herbicide treatments on percentage cover of *A. asparagoides* is shown in Fig. 1. The experiment started with ~65–80% *A. asparagoides* covering plots at the time of the first

treatment. Within 2 months, the glyphosate and combination treatments had reduced *A. asparagoides* coverage to below 3.5%, significantly lower than metsulfuron methyl and control treatments at this time. However, by early winter the following year, metsulfuron methyl spray and wipe treatments had reduced *A. asparagoides* coverage to below 1% and 0% respectively, remaining at these levels for the remainder of that year. Meanwhile, the early effects of glyphosate spray were fading, as *A. asparagoides* increased significantly in this treatment during that year.

Reapplication of treatments a year after the first application again resulted in effective control of *A. asparagoides* by glyphosate spray, and all herbicide treatments were effective in reducing *A. asparagoides* to below ~2% compared with control levels of 68% cover. Metsulfuron methyl and combination wipe treatments had no *A. asparagoides* cover at the time of reapplication so they did not receive a second treatment. Monitoring continued for a further three years to determine which treatments gave effective long-term control of *A. asparagoides*. While levels of control remained stable over the fourth and fifth years, by the sixth year significant regrowth of *A. asparagoides* was occurring in some treatments. Regrowth appeared to be from residual rhizomes of resident plants that had not been completely killed by the herbicide treatments. Combination spray and metsulfuron methyl spray showed 29% and 24% *A. asparagoides* coverage respectively, while glyphosate wipe and spray treatments were below 15%. The most lasting control of *A. asparagoides* was seen in the metsulfuron methyl wipe (6%) and combination wipe (3%) plots, each of which received only the single initial application of herbicide. Controls at this time showed coverage of 82% *A. asparagoides* (Fig. 1).

Between-group comparisons reinforced the significance of treatment effects compared with controls (Table 2). Combination treatments and glyphosate wipe were the only treatments to remain significantly below controls for the duration of the experiment. After 1.6 years, all herbicide treatments had significantly less *A. asparagoides* than did controls. Differences between the spray and wipe groups were seen at 1.6 and 5.7 years, indicating that wipe treatments produced a longer-lasting suppression of *A. asparagoides* than did spray treatments. This difference was further reinforced by the metsulfuron methyl and combination spray/wipe comparisons, which showed significantly better control of *A. asparagoides* with wipe rather than spray treatments at Year 5.7 (Fig. 1, Table 2). Glyphosate was faster acting than metsulfuron methyl, but metsulfuron methyl delivered more prolonged *A. asparagoides* control. Glyphosate versus metsulfuron methyl contrasts showed the significance of the former effect early in the experiment and the significance of the latter effect at Year 1.66 (Table 2).

Glyphosate and growth of mycorrhizal fungus

Glyphosate incorporated into the culture medium had a significant effect on the growth rate of the *P. arenicola* mycorrhizal fungal isolate. While mycelial growth covered control Petri dishes (0 glyphosate) within 11 days, those exposed to 0.5 and 1.0 kg ha^{-1} glyphosate took 14 and 21 days respectively to reach the same diameter (Fig. 2). Cultures growing on higher concentrations of glyphosate were slowed progressively further, failing to cover their plates over the period of monitoring. The decline in fungal growth with glyphosate concentration followed an exponential decay curve, with growth at the highest concentration of 3.0 kg ha^{-1} being severely restricted (Fig. 3). Nevertheless, within the timeframe and parameters of this study, the fungus did not appear to be killed by the herbicide.

Discussion

Asparagus weed infestations have traditionally been managed by physically removing plants (by hand weeding, slashing, grubbing, crowning or grazing) or by using herbicides, fire, and/or biological control agents to suppress populations (ARMCANZ *et al*. 2001; Morin *et al*. 2006*a*; Ensbey 2014). Manual control methods are really only practical for removing small plants in relatively small to moderate infestations, or in areas of high ecological sensitivity. Herbicides are a far more efficient method of eradication when infestations are large, or where soil erosion might occur following soil disturbance resulting from grubbing (Office of Environment and Heritage 2013).

One of the major drawbacks of using herbicides in natural ecosystems is the off-target damage that can result from broadacre or spot-spraying (Matarczyk *et al*. 2002; Brown *et al*. 2014; Bohnenblust *et al*. 2016). Moore (1999) tested the tolerance of 39 native tree and shrub species to post-emergent herbicides and found that 87% of the species tested tolerated glyphosate and 49% tolerated metsulfuron methyl when applied at recommended rates. However, the effects of these herbicides on terrestrial orchids and other forbs have rarely been examined and are poorly understood. Brown *et al*. (2002) found that only 1% of native geophytes were affected after spraying with metsulfuron methyl at 5 g ha^{-1}. Dixon (1996) found several non-woody herbaceous survivors in metsulfuron-methyl-treated plots, including the terrestrial orchids *Pterostylis* aff. *nana* and *Caladenia latifolia*. He postulated that a thick litter layer and heavy charcoal deposits following wildfire may reduce passage of the herbicide into the soil. Metsulfuron methyl has been shown to persist in alkaline soils for up to a year (Noy 1996; Hollaway *et al*. 2006), so it is likely that residual activity via root uptake may be possible during this time.

Off-target damage is not limited only to vegetation, but may also include effects on edaphic microflora (Weidenhamer and Callaway 2010; Druille *et al*. 2013, 2015). Glyphosate and its formulations have shown varying degrees of fungicidal activity on a range of fungal species (Morjan *et al*. 2002, and references therein) and have been shown to reduce the growth of several mycorrhizal fungi in culture (Chakravarty and Sidhu 1987; Estok *et al*. 1989; Chakravarty and Chatarpaul 1990) and even to convert a mycorrhizal interaction with a host into a parasitic one (Beyrle *et al*. 1995). Glyphosate acts on the shikimate metabolic pathway in plants (Amrhein *et al*. 1980), a pathway that is also present in fungi and bacteria, so it is not surprising that these microorganisms may also be affected by the herbicide (Lévesque and Rahe 1992).

The present study found a significant effect of glyphosate on the growth rate of mycorrhizal fungus isolated from *P. arenicola*. Fungal growth *in vitro* was inhibited as the concentration of glyphosate in the culture medium increased, suggesting that similar microbial growth inhibition may be possible *in situ* if the herbicide came into contact with fungus in the soil. Glyphosate was tested at rates up to 3.0 kg a.i. ha^{-1} (8.3 L f.p. ha^{-1}), although the Roundup label recommends application rates of up to 9 L f.p. ha^{-1} using boom application for some perennial weeds. Application by handgun or knapsack sprayer could result in even higher concentrations contacting the soil as a result of foliar runoff or off-target application while spaying to wet all foliage. If glyphosate reduced soil fungal activity *in situ*, this could conceivably lead to a decline in the orchid population as a result of reduced availability of the mycorrhizal symbiont to facilitate seed germination and growth. A degree of caution is required when interpreting the results of *in vitro* studies such as the present one, however, as soil-borne fungi have been known to exhibit different physiological responses to glyphosate in pure culture when compared with natural field conditions (Sidhu and Chakravarty 1990; Wardle and Parkinson 1990). Indeed, some studies have found increased soil fungal activity following glyphosate application and these authors have postulated that soil fungi may be using glyphosate as a nutrient and energy source (Araújo *et al*. 2003; Sebiomo *et al*. 2011). Moreover, fungicidal activity of glyphosate may be attributable to formulation components other than the active ingredient (Morjan *et al*. 2002; Cox and Surgan 2006). Glyphosate is known to bind to soil, reducing its movement through the soil profile, and to be readily biodegraded in soil (Sprankle *et al*. 1975; Sviridov *et al*. 2015), all factors that would moderate its exposure to the soil microecosystem.

Although the effect of metsulfuron methyl on soil mycorrhizae was not tested here, evidence suggests that while this herbicide inhibited growth or affected the community structure of some soil bacteria (Ismail *et al*. 1996; Boldt and Jacobsen 1998; Girvan *et al*. 2004; He *et al*. 2006), soil fungal populations were either not affected (He *et al*. 2006) or increased with increasing concentration of metsulfuron methyl (Ismail *et al*. 1996; He *et al*. 2006). Zabaloy *et al*. (2008) found only minor changes to soil microbial populations and activities following treatment with glyphosate or metsulfuron methyl applied at 10 times the recommended label rates, with the effects of glyphosate being more pronounced than those of metsulfuron methyl.

The analysis of herbicide application methods tested in this paper showed, for the first time, that the weed wiping technique using metsulfuron methyl alone or in combination with glyphosate gave effective long-term control of *A. asparagoides* while minimising off-target foliar and soil exposure to

the chemical. An initial weed cover of 65–80% was reduced to 0% one year after these treatments were applied and rose to 3–6% by the fifth year after treatment. Although herbicide treatments were reapplied to *A. asparagoides* in the second year, these two treatments were devoid of *A. asparagoides* at the time of this second treatment, so effectively the weed control recorded at Year 5 was due to the single initial treatment. In fact, much of the cover recorded in these two treatments in Year 5 was due to encroachment of *A. asparagoides* into plots from surrounding buffer zones rather than regeneration from pre-existing rhizomes. This suggests that the initial wipe treatment of metsulfuron methyl alone, or in combination with glyphosate, was effective in destroying *A. asparagoides* rhizomes in these plots, yielding good control of the weed for at least five years. Spray treatments would require reapplication in the second and fifth or sixth years to achieve adequate levels of control, thereby further increasing the risk of off-target damage to the ecosystem.

Therefore, the recommendation for control of *A. asparagoides* in this ecologically sensitive environment is to apply a single treatment of metsulfuron methyl, alone or in combination with glyphosate, during the period of active growth, using the wiping technique and rates outlined for Treatments 4 or 6 in Table 1. Weed pressure should be monitored again after 5–6 years, with reapplication as necessary after that time. The only apparent advantage of adding glyphosate is to achieve a faster knockdown of the weed in the first year, as metsulfuron methyl alone is slow to produce symptoms on *A. asparagoides*, even though its long-term efficacy is higher than that of glyphosate (Pritchard 1991). Also, there is some evidence that applying herbicides in combination can delay the onset of herbicide resistance (Diggle *et al*. 2003), so this could be an additional benefit.

It is worth pointing out that this study was undertaken before the release of *A. asparagoides* rust in South Australia in 2000 (Morin *et al*. 2002). Thus none of the results were affected by the presence of rust and it is uncertain how the presence of rust, particularly heavy infestations where leaf surface area is significantly reduced, may affect herbicidal activity in infected plants.

The main advantage of the weed-wiping technique compared with using traditional spray application is that the former minimises off-target damage to desirable vegetation and soil microorganisms. It is possible that wiped herbicide may still reach the soil by washing off the foliage, plant decomposition, or exudation from roots or tubers (Messersmith and Lym 1985; Moyo 2008; Harrington *et al*. 2016), but these possibilities were not examined here. Another advantage, as demonstrated in this study, is that wipe applications proved more effective than spray applications in terms of longevity of *A. asparagoides* control and, furthermore, they did not require the high frequency of reapplication usually recommended for spray treatments (Pritchard 1996, 2002; Meney *et al*. 2002). This provides further benefits by preventing the development of herbicide resistance that can arise following regular, repetitive use of a single herbicide group (Vencill *et al*. 2012).

The technique of weed wiping is suitable for use in small to moderate infestations of *A. asparagoides*, but may be impractical where infestations are extremely dense or widespread. In such circumstances, if any ground-story plants remain, collection of propagules for propagation and future reintroduction after elimination of *A. asparagoides* by spraying may be the only option (Graham and Mitchell 1996). In any event, weed control alone may not be sufficient to restore severely degraded areas after removal of *A. asparagoides*, and additional restoration activities may be required to speed up the rehabilitation process and ensure that weed substitution is avoided (Buchanan 1991; Turner and Virtue 2006; Turner *et al*. 2008). Studies to determine the long-term effects of infestation of *A. asparagoides* on populations of *P. arenicola* are continuing.

Conflicts of interest

The author declares no conflicts of interest.

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Table 1. Herbicide treatments applied to plots of *Asparagus asparagoides*

Rates for Roundup (360 g a.i. L^{-1} glyphosate) and Brush-Off (600 g a.i. kg^{-1} metsulfuron methyl) are expressed both as formulated product (f.p.) and as active ingredient (a.i.)

Treatment	Roundup		Brush-Off	
	mL f.p. L^{-1}	g a.i. L^{-1}	g f.p. L^{-1}	g a.i. L^{-1}
1. Glyphosate spray	10	3.6	–	–
2. Glyphosate wipe	500	180	–	–
3. Metsulfuron methyl spray	–	–	0.05	0.03
4. Metsulfuron methyl wipe	–	–	2.5	1.5
5. Combination spray	5	1.8	0.035	0.021
6. Combination wipe	333	120	2.5	1.5
7. Control	–	–	–	–

Table 2. Significance of selected orthogonal contrasts using arcsine-square-root-transformed data from Fig. 1

Herbicide treatments were applied at 0.57 and 1.66 years. GP, glyphosate; MM, metsulfuron methyl; Comb., combination of glyphosate and metsulfuron methyl. *F*-ratio: n.s., non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Contrast[A]	Year										
	0.57	0.66	0.70	1.45	1.56	1.66	2.58	2.75	3.59	4.68	5.74
1 versus 7	n.s.	***	***	n.s.	n.s.	***	***	***	***	***	***
2 versus 7	n.s.	***	***	*	**	***	***	***	***	***	***
3 versus 7	n.s.	n.s.	n.s.	**	**	***	***	***	***	***	***
4 versus 7	n.s.	n.s.	n.s.	**	**	***	***	***	***	***	***
5 versus 7	n.s.	***	***	*	**	***	***	***	***	***	***
6 versus 7	n.s.	***	***	**	**	***	***	***	***	***	***
Spray versus wipe	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	**
GP versus MM	n.s.	***	***	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Single versus combination	n.s.	***	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GP spray versus GP wipe	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
MM spray versus MM wipe	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Comb. spray versus Comb. wipe	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**

[A]Numbers refer to treatment numbers listed in Table 1.

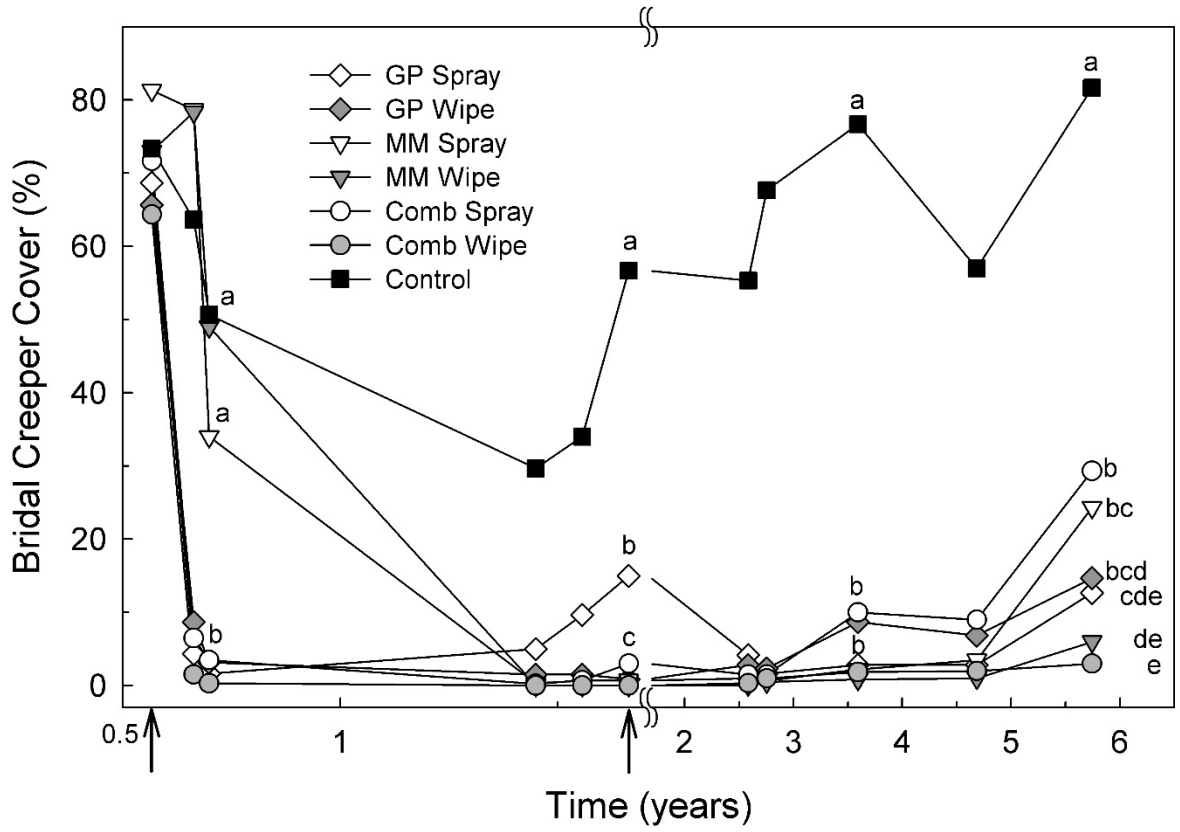

Fig. 1. Time course of *Asparagus asparagoides* cover in response to the various herbicide treatments outlined in Table 1. Means at 0.7, 1.7, 3.6 and 5.7 years were separated by a protected Fisher's l.s.d. test and points with the same letter at each of these times are not significantly different ($P = 0.05$) at that particular time. The two arrows on the *x*-axis indicate the treatment application times. The *x*-axis origin corresponds to 1 July 1995, and note the change in time scale at 1.7 years. GP, glyphosate; MM, metsulfuron methyl; Comb, combination of glyphosate + metsulfuron methyl.

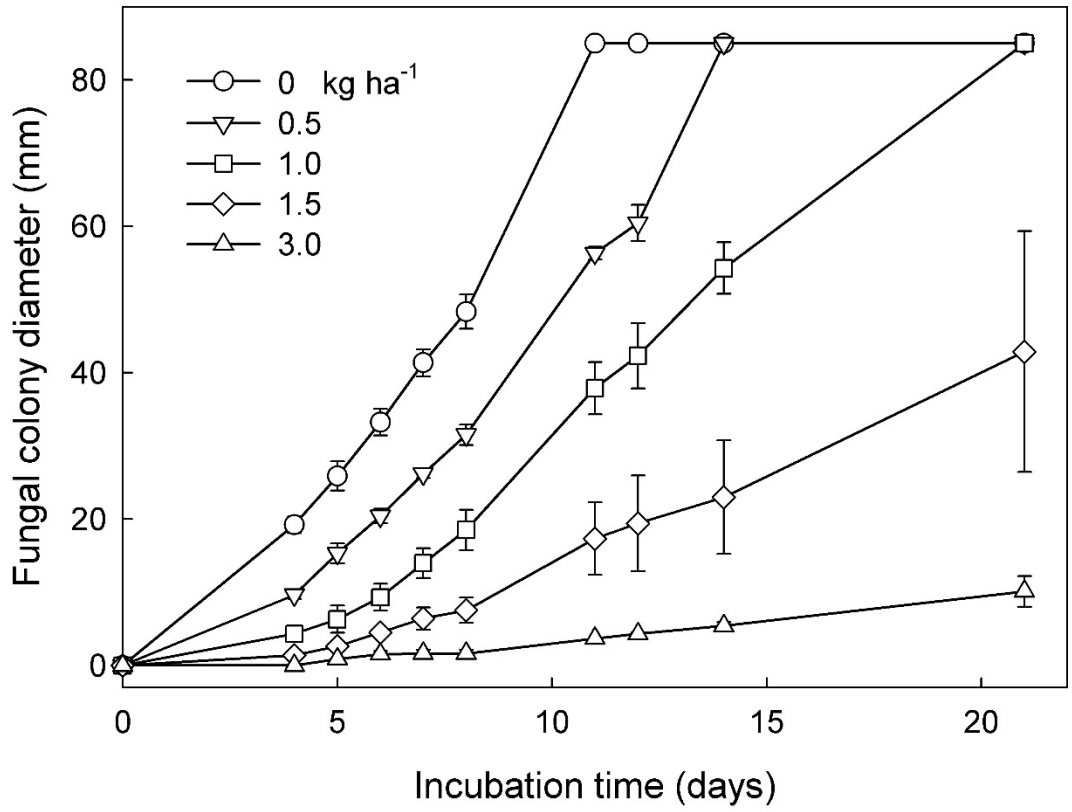

Fig. 2. Growth of mycorrhizal fungal colonies on oatmeal media containing different levels of glyphosate (0–3 kg a.i. ha^{-1}). Vertical bars represent the standard error of the mean (n = 3). Error bars not visible fall within the symbol.

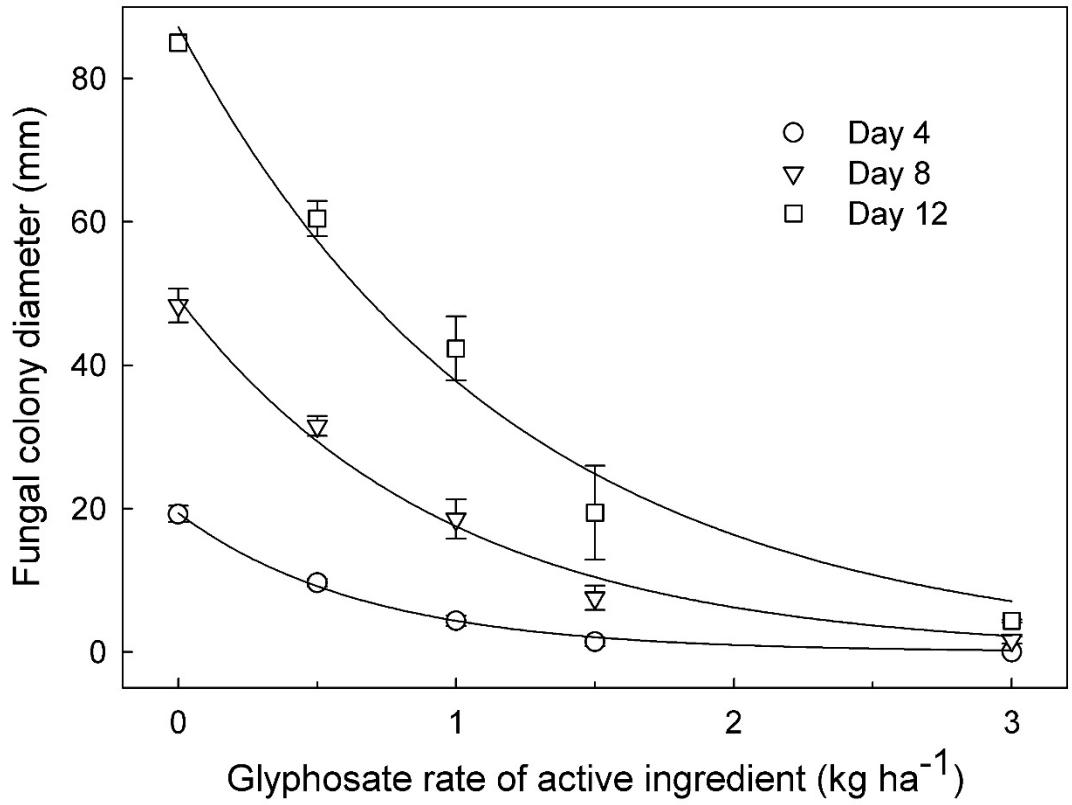

Fig. 3. Decline in fungal colony diameter with increasing glyphosate concentration at 4, 8 and 12 days of incubation. Vertical bars represent the standard error of the mean (n = 3). Error bars not visible fall within the symbol.