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https://doi.org/10.1080/11263504.2021.1884621

The use of glyphosate for *Carpobrotus* eradication in sand dune ecosystems: evaluation of the potential effects on the reintroduction of native plants

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ABSTRACT

Glyphosate application avoids some problems associated with manually controlling *Carpobrotus*. However, before it can be extended, we must understand whether residual glyphosate affects the restoration of natural vegetation by sowing or planting. Thus, we sprayed glyphosate on plots with 100% *Carpobrotus* coverage at 10 times the maximum recommended dose (4 g/m^2) and directly on sand at 0.3 and 4 g/m^2. Sand, sifted sand without *Carpobrotus* litter and sand with *Carpobrotus* litter were subsequently collected 15, 30, and 60 days after applications, and were used as substrates to evaluate seedling emergence of four native dune species sown in trays. We also assessed the development of two species when grown in pots. Seedling emergence of *M. marina*, *L. creticus* and *O. ramosissima* was not affected in sand without *Carpobrotus* litter; *A. arenaria* emergence was reduced by 35%. Seedling emergence of four dune species was inhibited when glyphosate had been directly sprayed on sand. Effect of dose and time after spraying was observed. The presence of *Carpobrotus* litter reduced the emergence of *L. creticus, O. ramosissima* and *A. arenaria,* and inhibition seemed be caused by the allelopathic properties of the *Carpobrotus* litter. No significant effects of glyphosate spraying were observed in growth tests.

ARTICLE HISTORY
Received 19 May 2020
Accepted 19 January 2021

KEYWORDS
Carpobrotus; chemical control; dune restoration; glyphosate; native plant reintroduction

Introduction

The Mediterranean Basin is more sensitive to the problem of invasive species because of its rich biodiversity and narrow endemism, especially in terms of plant species. Moreover, climatic conditions in the Mediterranean Basin favour the establishment of subtropical plants introduced as ornamental species or for other purposes (Brunel et al. 2013). The succulent genus, *Carpobrotus* N. Br. (*Aizoaceae*), includes some of the main invasive species now present in Mediterranean coastal ecosystems (Hulme 2004; Andreu et al. 2010; Campoy et al. 2018; Chenot et al. 2018; Lazzaro et al. 2020). In Spain, *Carpobrotus* is present in all coastal peninsular and insular provinces (Castroviejo et al. 1990, Sanz-Elorza et al. 2004). Invasive *Carpobrotus* sp. pl. is native to South Africa, but has now been introduced into five different continents and has become widely naturalised in many coastal habitats (Campoy et al. 2018). In the past, *Carpobrotus* was widely used as an ornamental species and to stabilise dunes or to control erosion. However, this species has now dispersed from these planted areas into natural ecosystem such as dunes, coastal scrub and rocky coast. *Carpobrotus* grows horizontally and radially in all directions and forms zones of monospecific carpets in which it attains near-dominance (D'Antonio 1990, 1993; Sanz-Elorza et al. 2004; Suehs et al. 2004a, 2004b; Travaset et al. 2008; Roiloa et al. 2014; Campoy et al. 2018). Numerous studies have shown that *Carpobrotus* affect native plant and animal species and also influence soil characteristics (D'Antonio and Mahall 1991; D'Antonio 1993; Palmer et al. 2004; Moragues and Traveset 2005; Vilà et al. 2006; Zedda et al. 2010; Santoro et al. 2011; Chenot et al. 2014; Novoa and González 2014; Campoy et al. 2018; Chenot et al. 2018; Souza-Alonso et al. 2020). Furthermore, *Carpobrotus* branches and leaves decompose slowly and form a thick litter (Conser and Connor 2009) that can prevent the germination of native species (Novoa et al. 2012).

Several *Carpobrotus* management and eradication programmes have been implemented all around the world and in different European countries (Ruffino et al. 2015; Campoy et al. 2018). Indeed, Spanish environmental administrations have developed different strategies for *Carpobrotus* management, including direct control and/or complete eradication, as well as prevention, education or communication activities (Campoy et al. 2018). Mechanical removal by pulling out individual plants by hand is the most commonly used method for *Carpobrotus* eradication (Andreu et al. 2010; Brunel et al. 2013; Ruffino et al. 2015; Campoy et al. 2018; Chenot et al. 2018). This method is generally effective, but the reappearance of new individuals has been observed (Chenot et al. 2014, 2018) and long-term monitoring of cleaned areas may be necessary to prevent reintroduction (Ruffino et al. 2015). Of note, this mechanical removal

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generates large amounts of plant material that retain the potential to act as asexual propagules (Souza-Alonso and González 2017; Souza-Alonso et al. 2019, 2020), and moreover, the transport and disposal of this plant material can also create logistical problems (Campoy et al. 2018).

A chemical method based on glyphosate application has also been employed for *Carpobrotus* eradication in California (Albert 1995), Spain, Portugal, Ireland (Campoy et al. 2018) and Italy (Lazzaro et al. 2020). Glyphosate-based herbicides are among the most widely used broad spectrum herbicides in the world (Henderson et al. 2010; Myers et al. 2016). Glyphosate, or N-(phosphonomethyl) glycine, is a non-selective herbicide that inhibits plant growth by interfering with the production of essential aromatic amino acids which do not share biosynthetic pathways with members of the animal kingdom (Henderson et al. 2010). Glyphosate is assimilated by leaves and other green plant tissue, and is then rapidly translocated via the phloem through the entire plant, including to the roots (Henderson et al. 2010). The large-scale use of glyphosate is currently restricted or banned by legislation in many European countries (Lazzaro et al. 2020) but the context and scale must be considered when applying such bans on a small scale, such as for the purposes of invasive plant control (Pergl et al. 2020).

Glyphosate was recently used for *Carpobrotus* eradication in a natural protected area of local interest on the Tuscan coast in Italy (Lazzaro et al. 2020). These reported results confirmed that glyphosate application is an efficient method to decrease *Carpobrotus* cover, which also promotes the rapid recovery and increased richness and diversity of native species. However, to extend the use of glyphosate as a general method for the eradication of invasive plants, and in particular, for the eradication of *Carpobrotus* in coastal areas, the potential negative effects after its application must first be identified and understood. For this purpose, we studied in laboratory conditions the emergence of seedling from four native Mediterranean dune species using dune sand collected after glyphosate spraying on *Carpobrotus* or on sand under different doses and time conditions. We also evaluated the effect of glyphosate on the survival and development of two species of adult native Mediterranean dune plants grown in pots with sand collected after glyphosate spraying. The application of this herbicide simulated different conditions of glyphosate spraying during eradication or control campaing.

Materials and methods

Glyphosate application assays and substrate collection

The natural area for glyphosate applications was located on the coast in two locations in the province of Valencia in Spain: Oliva (38° 55′10″ N, 0° 07′16″ W) and Tavernes de La Valldigna (39° 04′18″ N, 0° 16′04″ W). These areas are included in 'Dunes of *La Safor*' listing in the Sites of Community Importance in the Valencian Community (July 19, 2006, European Commission). The natural habitats at this site include the following: (1) embryonic shifting dunes 2110, (2) shifting dunes along the shoreline with *Ammophila arenaria* 2120, (3) *Crucianellion maritimae* fixed beach dunes 2210, (4) *Malcolmietalia* dune grasslands 2230 and (5) *Cisto-Lavenduletalia* dune sclerophyllous scrubs 2260. The coastal areas for study were selected with the supervision of environmental officers from the Government of the Valencian Region and the Spanish Ministry of Environment.

The glyphosate applications were carried out under controlled conditions on 1-m^2 experimental plots (Figure 1, D0). Glyphosate was applied on plant leaves in six plots with 100% *Carpobrotus* coverage and on beach sand in three plots without vegetation in each locality. Glyphosate (200-ml per plot) was applied using a hand-held spray bottle at a height of 50 – 60 cm above the *Caprobrotus* plants or sand. Glyphosate (36 g/l, as ammonium salt) was sprayed on the *Carpobrotus* at doses of 4 g/m^2 or 0.3 g/m^2 and on the beach sand at a dose of 0.3 g/m^2 (Table 1). A non-ionic surfactant was added at 0.1% v/v in all cases. The concentration of 4 g/m^2 is around 10 times the maximum recommended dose while 0.3 g/m^2 falls within the recommended dose range (European Food Safety Authority (EFSA) 2017). The glyphosate applications started in May 2007 and continued until January 2008.

Either 15 or 60 days after the glyphosate spraying (at 4 g/m^2), dry and decomposed *Carpobrotus* leaves, branches and litter were removed, and beach sand without *Carpobrotus* litter was sifted with a 1-mm pore size (Table 1). Dry and decomposed *Carpobrotus* leaves and together with beach sand were also collected 60 days after glyphosate application (at 4 g/m^2) (Table 1). Finally, beach sand was collected and sifted 30 days after glyphosate spraying (at 0.3 g/m^2) on plots without vegetation (Table 1). A sand layer approximately 5 cm deep was collected in every case.

Glyphosate was also sprayed (as previously described) under laboratory conditions on trays containing one litre of sifted, washed and sterilised beach sand at the equivalent of either at 4 or 0.3 g/m^2 (Table 1).

Seedling emergence test to evaluate the effect of glyphosate applications on dune species

We evaluated the effect of glyphosate application on seedling emergence for four native species of Mediterranean dune ecosystem: *Medicago marina* L., *Lotus creticus* L., *Ononis ramosissima* Desf. (*Fabaceae*) and *Ammophila arenaria* (L.) Link. (*Poaceae*). These species are very common and abundant among the natural vegetation of the Valencian coast. Seeds were supplied by the seed bank at the Centre for Forest Research and Experimentation (CIEF, Generalitat Valenciana). Laboratory emergence tests were performed in trays with one litre of different sand substrates, obtained as described above (Table 1) and using sterilised beach sand as a control. The substrates used for the tests were obtained with equivalent parts of the samples collected in both locations. One hundred seeds were sown per tray (with three repetitions per dune species and substrate). The seeds of *L. creticus* and *O. ramosissima* were sown either non-scarified or previously scarified (15 minutes in H_2SO_4) (Table 2). The seedling emergence tests were carried out in a chamber under controlled conditions (16 h light/8 h darkness, 20° C, 60% relative

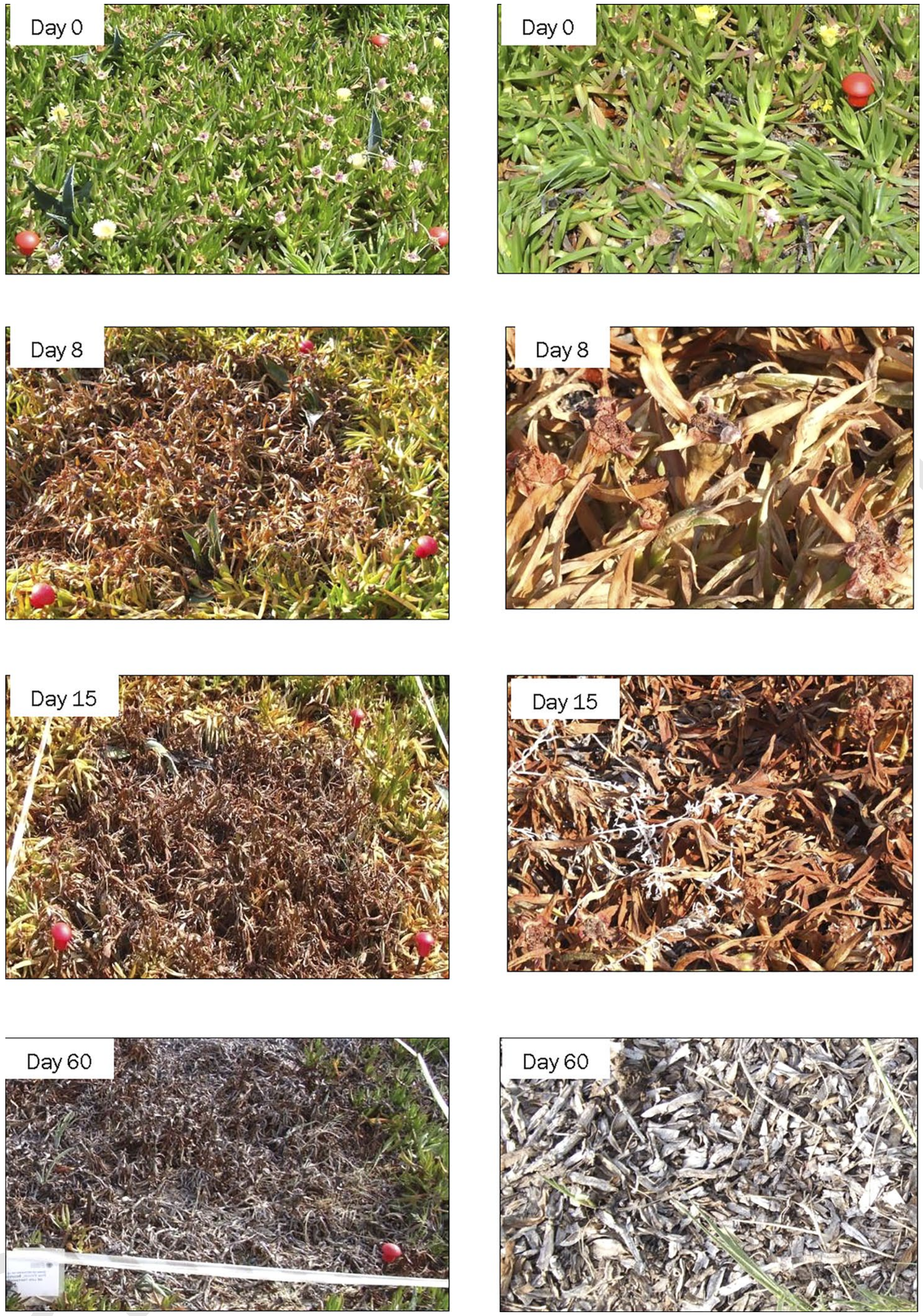

Figure 1. Effects of glyphosate application at a dose of 4 g/m^2 on *Carpobrotus* plants 8, 15 and 60 days after manual spraying in experimental plots.

humidity). The emergence of the aerial part of seedlings was monitored for at least 60 days (Figure 2).

Growth tests to evaluate the effect of glyphosate application

For the growth tests, glyphosate was sprayed at a concentration of 0.3 g/m^2 in experimental plots with 100% coverage of *Carpobrotus* and in plots without vegetation (Table 1). Glyphosate applications (as previously described) were carried out in December 2007 and January 2008. About 15, 30 and 60 days after the glyphosate spraying, beach sand without *Carpobrotus* litter was collected, sifted and used as a potting substrate (Table 1). One-litre plastic pots were filled with sand and used to plant *L. creticus* and *A. arenaria* plants. The glyphosate was also applied in the pots (with 1 litre of sterilised beach sand) under laboratory conditions (at 0.3 g per one m^2 equivalent surface area; Table 1). Theses sprayed pots were used in the growth tests 15 days after the glyphosate application. Pots filled with autoclave sterilised beach sand were used as the control (Table 1), and 12 plants per species for treatment were tested. *Lotus creticus* and *A. arenaria* plants used in these tests were supplied by Cultidelta nurseries (Amposta, Tarragona and Spain). Vegetative development was evaluated 4, 9 and 13 weeks after planting.

Table 1. Substrates used to evaluate the effect of prior glyphosate spraying on seedling emergence and growth assays with native Mediterranean dune species.

Glyphosate spraying	Glyphosate doses (g/m²)	Substrate collected and used after spraying	Evaluation assays after glyphosate spraying	Evaluated effect	Evaluated species
Carpobrotus on experimental plot	4.0	Sifted sand without *Carpobrotus* litter	Seedling emergence 15 and 60 days after	Total emergence	*Mm–Lc–Or–Aa*
Carpobrotus on experimental plot	4.0	Sand with *Carpobrotus* litter	Seedling emergence 60 days after	Total emergence	*Mm–Lc–Or–Aa*
Beach sand on experimental plot	0.3	Sifted sand	Seedling emergence 30 days after	Total emergence	*Mm–Lc–Or–Aa*
Trays with sterilised sand–laboratory spraying	4.0	Trays 4.0 g/m² glyphosate	Seedling emergence 15 and 60 days after	Total emergence	*Mm–Lc–Or–Aa*
Trays with sterilised sand–laboratory spraying	0.3	Trays 0.3 g/m² glyphosate	Seedling emergence 30 days after	Total emergence	*Mm–Lc–Or–Aa*
Unsprayed trays with sterilised sand	0.0	Trays control	Seedling emergence 15, 30 and 60 days after	Total emergence	*Mm–Lc–Or–Aa*
Carpobrotus on experimental plot	0.3	Sifted sand without *Carpobrotus* litter	Plantation 15, 30 and 60 days after	Survival and growth	*Lc–Aa*
Beach sand on experimental plot	0.3	Sifted sand	Plantation 15, 30 and 60 days after	Survival and growth	*Lc–Aa*
Pots with sterilised sand–laboratory spraying	0.3	Pot 0.3 g/m² glyphosate	Plantation 15 days after	Survival and growth	*Lc–Aa*
Unsprayed pots with sterilised sand	0.0	Pot control	Plantation 15, 30 and 60 days after	Survival and growth	*Lc–Aa*

Mm – Medicago marina; Lc – Lotus creticus; Or – Ononis ramosissima; Aa – Ammophila arenaria.

Table 2. Seedling emergence of *Medicago sativa*, *Lotus creticus*, *Ononis ramosissima* and *Ammophila arenaria* seeds in laboratory conditions with substrates collected after glyphosate spraying under different conditions, days (D) and doses.

Medicago marina

	Start date of laboratory seedling emergence trials					
	15 D after spraying		30 D after spraying		60 D after spraying	
Substrate evaluated	**Emergence**	**%**	**Emergence**	**%**	**Emergence**	**%**
Control tray 0 glyphosate	49 ± 8 a	100	46 ± 5 a	100	46 ± 5 a	100
Sifted sand without litter 4.0 g/m² glyphosate	55 ± 12 a	112			43 ± 5 a	94
Sand with *Carpobrotus* litter 4.0 g/m² glyphosate					43 ± 12 a	94
Tray 4.0 g/m² glyphosate	0 ± 0 b	0			8 ± 3 b	17
Tray 0.3 g/m² glyphosate			16 ± 3 b	35		
Sifted sand 0.3 g/m² glyphosate			24 ± 7 b	52		

Lotus creticus

	Start date of laboratory seedling emergence trials					
	15 D after spraying		30 D after spraying*		60 D after spraying*	
Substrate evaluated	**Emergence**	**%**	**Emergence**	**%**	**Emergence**	**%**
Control tray 0 glyphosate	4 ± 2 ab	100	53 ± 12 a	100	53 ± 12 ab	100
Sifted sand without litter 4.0 g/m² glyphosate	8 ± 0 a	200			71 ± 9 a	134
Sand with *Carpobrotus* litter 4.0 g/m² glyphosate					35 ± 11 b	66
Tray 4.0 g/m² glyphosate	0 ± 0 b	0			0 ± 0 c	0
Tray 0.3 g/m² glyphosate			2 ± 2 b	4		
Sifted sand 0.3 g/m² glyphosate			40 ± 10 a	75		

Ononis ramosissima

	Start date of laboratory seedling emergence trials					
	15 D after spraying		30 D after spraying*		60 D after spraying*	
Substrate evaluated	**Emergence**	**%**	**Emergence**	**%**	**Emergence**	**%**
Control tray 0 glyphosate	2 ± 1 a	100	16 ± 5 a	100	16 ± 5 a	100
Sifted sand without litter 4.0 g/m² glyphosate	6 ± 1 b	300			13 ± 3 ab	81
Sand with *Carpobrotus* litter 4.0 g/m² glyphosate					8 ± 2 ab	50
Tray 4.0 g/m² glyphosate	0 ± 0 a	0			3 ± 1 b	19
Tray 0.3 g/m² glyphosate			3 ± 1 b	19		
Sifted sand 0.3 g/m² glyphosate			4 ± 1 b	25		

Ammophila arenaria

	Start date of laboratory seedling emergence trials					
	15 D after spraying		30 D after spraying		60 D after spraying	
Substrate evaluated	**Emergence**	**%**	**Emergence**	**%**	**Emergence**	**%**
Control tray 0 glyphosate	34 ± 2 a	100	14 ± 5 a	100	14 ± 5 a	100
Sifted sand without litter 4.0 g/m² glyphosate	22 ± 3 b	65			9 ± 3 a	64
Sand with *Carpobrotus* litter 4.0 g/m² glyphosate					1 ± 1 b	7
Tray 4.0 g/m²	0 ± 0 c	0			0 ± 0 b	0
Tray 0.3 g/m² glyphosate			6 ± 3 b	43		
Sifted sand 0.3 g/m² glyphosate			8 ± 2 ab	57		

Final seedling emergence (mean ± SD) and percentage emergence compared to the control (%) after 60 days. One hundred seeds per tray and three trays per substrate were studied. *Seeds scarified prior to sowing. In each column, different letters denote statistically significant differences according to Tukey multiple comparison test ($p < 0.05$).

Statistical analysis

The seedling emergence of four native species under laboratory conditions with different substrates collected after the application of glyphosate was analysed using an analysis of variance (ANOVA) via Statgraphics plus software (5.1 for Windows, 1994, Statistical, Corporation, Warrenton, VA). The statistical analysis of the data was performed independently for each species and for each substrate collection time point after the glyphosate application (15, 30 and 60 days). For each species and for each time, we carried out a simple factorial ANOVA using the type of substrate collected after application of glyphosate as the main factor. Tukey multiple comparison test was used for each ANOVA to determine the significance of any differences in the seedling emergence between the different substrates collected on the same day (15, 30 or 60 days) after glyphosate application with respect to the control substrate ($p < 0.05$).

The vegetative development of two native species in plastic pots with different substrates collected after the application of glyphosate was also analysed by ANOVA using the previously mentioned statistical software. The statistical analysis of the data was performed independently for each species and each week after planting (4, 9 and 13 weeks). For each species and each week after planting, a simple factorial ANOVA was carried out using the type of substrate collected after application of glyphosate as the main factor. Tukey multiple comparison test was used for each ANOVA to determine the significance of any differences in vegetative development between the substrates collected after glyphosate application with respect to the control substrate ($p < 0.05$).

Results

The effectiveness of glyphosate at a dose of 4 g/m² for the eradication of Carpobrotus

Eight days after glyphosate spraying (4 g/m^2), the experimental plots had clearly been affected. Although the leaves and branches still maintained the typical appearance of a succulent, the *Carpobrotus* plants presented a yellowish-brown colouration and all the flowers had been affected (Figure 1, D8). In addition, the *Carpobrotus* plants about 15 cm outside of the plots had also been slightly affected and showed a light yellowish-green colouration (Figure 1, D8). The plants on complete experimental plots showed a dark brown colour, and some *Carpobrotus* plants even showed grey colouration. Most of leaves and branches had a dry appearance 15 days after the glyphosate application (Figure 1, D15), and by 60 days after glyphosate application, the plants were a grey-black, and the leaves and branches appeared dry, were completely fragmented and were broken (Figure 1, D60).

Seedling emergence tests in trays

There was around 50% emergence of *M. marina* seedlings in control trays (Figure 2, M trays; Table 2), and their emergence in sand without *Carpobrotus* litter after glyphosate spraying (4 g/m^2, 15 and 60 days) and in sand with *Carpobrotus* litter after glyphosate spraying (4 g/m^2, 60 days) was also not significantly different from the control (Table 2). The emergence of seedlings was completely inhibited when glyphosate (4 g/m^2) was applied directly onto the trays (15 days), but it was only partially inhibited 60 days after glyphosate application (Table 2, tray 4 glyphosate). Emergence was also significantly reduced by spraying glyphosate directly on sand, both in experimental plots and under laboratory condition (0.3 g/m^2, Table 2).

Emergence of *L. creticus* seedlings (Figure 2, L trays) in sand without *Carpobrotus* litter after glyphosate spraying (4 g/m^2, 15 and 60 days) was not significantly different from the control (Table 2). However, their emergence was significantly reduced in sand with *Carpobrotus* litter after glyphosate application (4 g/m^2, 60 days; Table 2). Glyphosate spraying (4 g/m^2) directly into the trays completely inhibited the emergence of *L. creticus* seeds even 60 days after the application (Table 2) and when applied on sand in the trays (0.3 g/m^2) it significantly inhibited *L. creticus* seed germination. In contrast, seedling emergence was not significantly affected when the same dose of glyphosate was sprayed on sand in the experimental plots (Table 2).

Emergence of *O. ramosissima* seedlings (Figure 2, O trays) was not decreased in sand without *Carpobrotus* litter after glyphosate application (4 g/m^2, 15 and 60 days; Table 2), and emergence in sand with *Carpobrotus* litter after glyphosate application (4 g/m^2, 60 days) was not significantly different from the control. However, glyphosate spraying on sand (4 g/m^2, trays) completely inhibited *O. ramosissima* emergence after 15 days, although after 60 days the inhibition was not 100% (Table 2). Similar results were obtained for *O. ramosissima* seedling emergence at the lower dose of glyphosate dose (0.3 g/m^2) when applied directly on sand (Table 2).

The overall emergence rate of *A. arenaria* seedlings (Figure 2, A trays) in sand without *Carpobrotus* litter after glyphosate spraying (4 g/m^2, 15 and 60 days) decreased by 35% compared to the control, but no significant difference was observed 60 days after spraying (Table 2). Moreover, the presence of *Carpobrotus* litter in the sand (4 g/m^2, 60 days) significantly decreased the emergence of *A. arenaria* seeds, and their emergence was completely inhibited when glyphosate (4 g/m^2) was applied directly on the trays (15 and 60 days), although it was only partially inhibited after application at lower doses (0.3 g/m^2, 30 days). Nonetheless, the emergence was not significantly affected when glyphosate was applied at the lower dose on the sand plots (Table 2, sifted sand, 0.3 g/m^2 glyphosate).

Additionally, we also observed seedlings from nine native species in emergence test trays when we used sand without *Carpobrotus* litter (Figure 2, centre trays) and sand with *Carpobrotus* litter (Figure 2, right trays) collected 60 days after glyphosate spraying as substrates. The native species identified were *Ammophila arenaria*, *Centaurea seridis* L., *Euphorbia paralias* L., *Lotus creticus, Malcolmia littorea* (L.) W.T.Aiton, *Senecio vulgaris* L., *Scabiosa atropurpurea* L., *Sonchus oleraceus* L. and *Sonchus tenerrimus* L. In addition*, Carpobrotus* seedlings were mainly detected in trays with sand without *Carpobrotus* litter (Figure 2, centre trays).

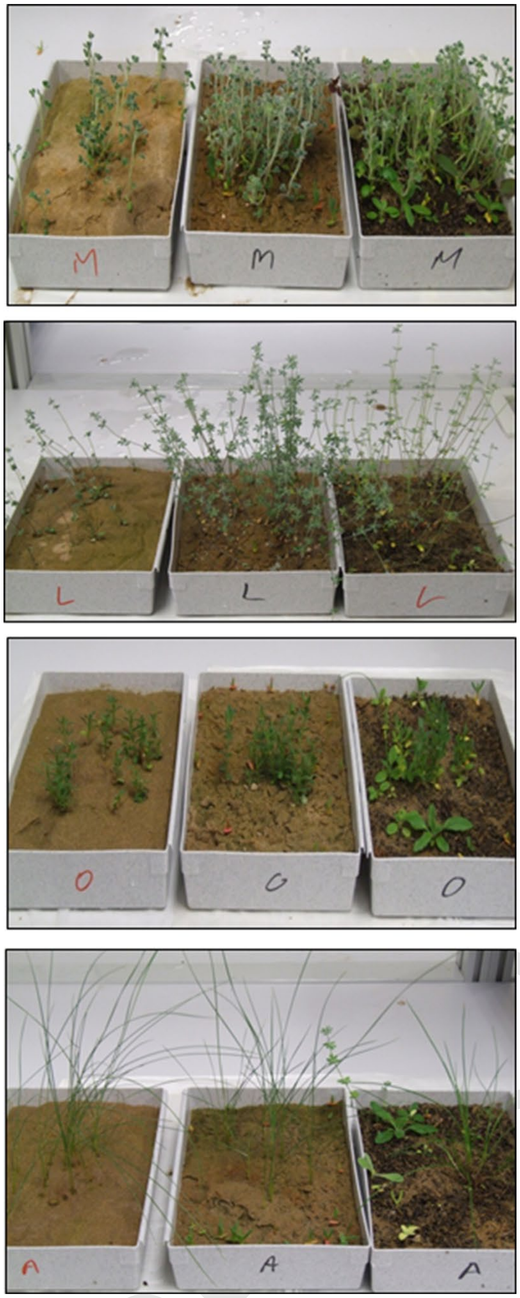

Figure 2. Seedling emergence in trays sown under laboratory conditions to evaluate the effect of glyphosate application on four native species. M – Medicago marina, L – Lotus creticus, O – Ononis ramosissima and A – Ammophila arenaria. Left – control trays. Centre – sifted sand without *Carpobrotus* litter collected 60 days after glyphosate spraying (4 g/m^2). Right – sand with *Carpobrotus* litter collected 60 days after glyphosate spraying (4 g/m^2).

Growth tests

The survival of *L. creticus* and *A. arenaria* plants was 100% in all the substrates used in the growth tests after glyphosate application (0.3 g/m^2). Mortality was only observed in one of *L. creticus* plants when glyphosate was sprayed directly onto the pot under laboratory conditions.

Four weeks after planting, the growth of the *L. creticus* plants was significantly lower with respect to the control in four of the seven substrates tested (Figure 3). Nine weeks after planting, the lower growth of *L. creticus* plants could only be observed in the two substrates used 15 days after glyphosate spraying (Figure 3, Pot 0.3 and sifted sand without *Carpobrotus* litter); 13 weeks after planting, poorer growth was only observed in *L. creticus* plants cultivated in sifted sand without *Carpobrotus* litter collected 15 days after glyphosate spraying (Figure 3).

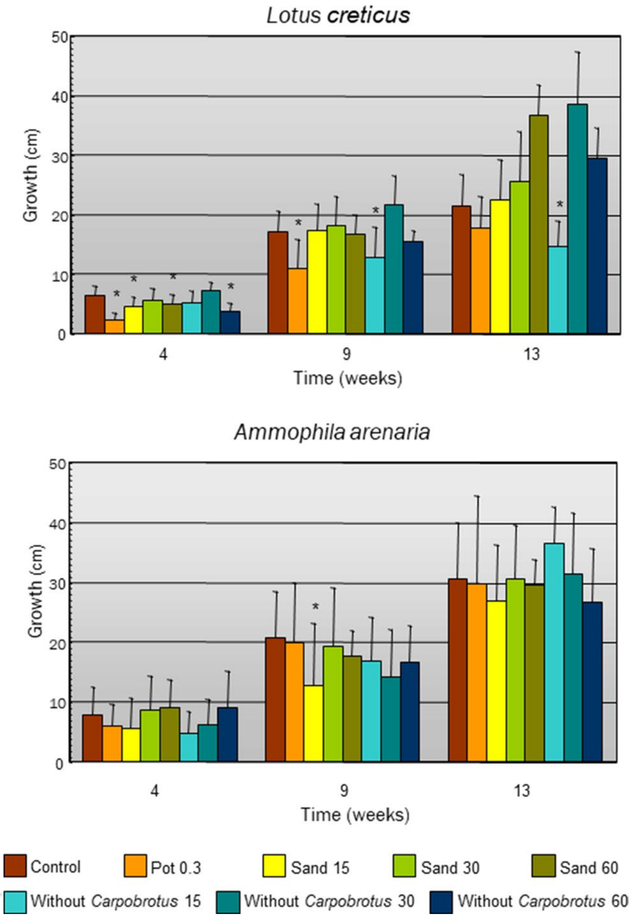

Figure 3. Average growth of *Lotus creticus* and *Ammophila arenaria* plants 4, 9 and 13 weeks after transplanting them to pots. (X ± SE, *significant differences compared to the control $p < 0.05$).

At 4 and 13 weeks after planting, no significant differences in the growth of *A. arenaria* plants were observed between the control substrate and all the substrates on which glyphosate had been applied (Figure 3). Finally, at 9 weeks after planting, poorer growth was only detected in *A. arenaria* plants cultivated in sifted sand collected 15 days after glyphosate spraying on beach sand (Figure 3).

Discussion

The results obtained describe the effects and symptoms exhibited over time by *Carpobrotus* plants after glyphosate spraying (Figure 1). They also show the rapid and high effectiveness of glyphosate application in *Carpobrotus* eradication. Targeted spraying reduced the damage to the surrounding areas, and only *Carpobrotus* plants directly sprayed by the glyphosate solution were affected, even when using a dose 10 times higher than the maximum recommended concentration (Figure 1). These observations show that the effect of this herbicide was therefore limited to direct foliar contact (Henderson et al. 2010). Targeted glyphosate spraying has been reported to cause significant reductions in the invasive plant *Sarracenia purpurea,* without adversely affecting the surrounding vegetation (Walker et al. 2016). The data we present here confirm that the use of glyphosate is an efficient method for *Carpobrotus* eradication, in agreement with previous descriptions (Albert 1995; Campoy et al. 2018; Lazzaro et al. 2020). Glyphosate-derived herbicides have also been successfully employed in the management and control of other invasive plant species in protected areas. Indeed, glyphosate applications have recently been used in eradication campaigns against *Opuntia dillenii* Haw. and *Agave americana* L. (Arevalo et al. 2015), *Pennisetum purpureum* Schumach. (Grey et al. 2015), *Andropogon gayanus* Kunth. (Luck et al. 2019), *Brachypodium pinnatum* (L.) P.Beauv. (Redhead et al. 2019), *Poa annua* (L.) (William et al. 2019) and *Oxalis pes-caprae* L. (Lazzaro et al. 2019).

In the case of *Carpobrotus,* the most widely used method in restoration projects is mechanical removal while the most efficient way was the carpet rolling technique in which the *Carpobrotus* mat is rolled up from one side and the roots are severed underneath with shovels (Andreu et al. 2010; Brunel et al. 2013; Ruffino et al. 2015; Campoy et al. 2018; Chenot et al. 2018), although some drawbacks have been reported for this latter method (Ruffino et al. 2015; Souza-Alonso and González 2017; Campoy et al. 2018; Chenot et al. 2018; Souza-Alonso et al. 2019, 2020). Glyphosate application avoids some problems derived from manually controlling *Carpobrotus*. First, it prevents accumulation of large amounts of fresh material generated by mechanical methods in the surroundings which would otherwise have to be removed. Second, glyphosate application causes complete drying and breaking of plants (Figure 1), thereby preventing their possible vegetative regrowth via stem fragments. For example, *C. edulis* rapidly emits adventitious roots after fragmentation, regardless of oxygen availability (Lechuga-Lago et al. 2016; Roiloa et al. 2016; Roiloa and Retuerto 2016). Third, the effectiveness of glyphosate spraying in coastal areas circumvents the movement of sand–soil associated with manual extirpation of roots and rhizomes thereby avoiding its potentially erosive effect in protected areas (Chenot et al. 2018). Fourth, as opposed to the usual massive emergence of *Carpobrotus* seedlings and opportunist species after mechanical control interventions (Magnoli et al. 2013; Novoa et al. 2013; Fried et al. 2014; Novoa and González 2014), glyphosate application results in extremely limited emergence of *Carpobrotus* seedlings in invaded areas (Lazzaro et al. 2020). Fifth, the chemical control of *Carpobrotus* would minimise the post-eradication and monitoring activities that usually accompany the manual management of *Carpobrotus* in invaded areas (Ruffino et al. 2015; Chenot et al. 2018, Lazzaro et al. 2020). Taken together, all these factors suggest that the chemical control of *Carpobrotus* will require less logistical planning and clearly has a lower economic cost and execution time than mechanical eradication.

However, the use of glyphosate has also been questioned because of its possible effect on both environmental and human health. Glyphosate-based herbicides are the most heavily applied herbicide in the world, and usage continues to increase because they are highly efficacious, cost-effective, practically non-toxic and degrade readily in the environment (Henderson et al. 2010). But, glyphosate is now authoritatively classified as a probable human carcinogen in the United States and the European Union (Myers et al. 2016; European Food Safety Authority (EFSA) 2017). Nonetheless, the current consensus statement indicates that the regulatory estimate of tolerable daily intakes is based on outdated science and so new epidemiological, biomonitoring and toxicology studies are required (Myers et al. 2016).

Nevertheless, the rapid establishment recovery of native dune species similar to that observed after the manual eradication (Vilà et al. 2006) was recently detected after using glyphosate in a *Carpobrotus* eradication campaign (Lazzaro et al. 2020). Six months after glyphosate application, 17 species (including *Carpobrotus*) were sampled in a *Carpobrotus*-invaded area, and a total of 32 species were detected after three years of glyphosate application (Lazzaro et al. 2020). In this current work, we detected the emergence of seedling from a total of nine native species (plus *Carpobrotus*) collected along with the sand used as the substrate for the emergence tests. The presence of seedlings was mainly observed in sand with *Carpobrotus* litter (Figure 2, right trays). Of note, viable seeds from different native species were present even though glyphosate had been sprayed at a dose ten times higher than the maximum recommended concentration. Thus, these results reinforce the idea that the application of glyphosate for *Carpobrotus* eradication does not affect subsequent recovery of native vegetation in invaded coastal dunes.

Although complete restoration of invaded areas will always not be possible, creating an ecosystem with features that resemble the original habitat should be beneficial (Marchante et al. 2011). Simply removing invasive species does not seem to be sufficient to completely restore ecosystems and so additional interventions are likely to be required (King and Hobbs 2006). Proposal for such beneficial activities includes planting desirable species, removing the litter created by invasive species and depleting their seed banks, and the introduction of seeds from native species in order to increase biological competition (Holmes 2002; Luck et al. 2019). Importantly, the success of these actions can be affected by the previous management of invasive species, and therefore, it is a key to evaluate the possible negative effects of the application and herbicide must also be evaluated.

Our results indicate that overall seedling emergence was not significantly affected for three native plant species when using sand without *Carpobrotus* litter collected 15 or 60 days after glyphosate spraying at 10 times the maximum recommended dose (European Food Safety Authority (EFSA) 2017). In contrast, dramatic seedling emergence inhibition (80% to 100%) was observed in all the tested species when glyphosate was sprayed directly on sand under laboratory conditions at the same dose (Table 2). *Carpobrotus* plants form dense mats (up to 50 cm deep) with prostrate nodes and internodes (Campoy et al. 2018) that prevent the glyphosate reaching the sand and therefore from inhibiting seed emergence. These results reflect the importance of meticulousness in herbicide application tasks in order to avoid spraying outside the area occupied by *Carpobrotus* plants, as previously observed in the eradication of the invasive plant *S. purpurea* (Walker et al. 2016).

In addition, the inhibitory effect of glyphosate on seeding emergence seemed to decline over time. Total inhibition of *M. marina* and *O. ramosissima* seeds was observed after 15 days after spraying versus 20% emergence 60 days after spraying on sand under laboratory conditions at 10 times the maximum recommended dose (Table 2). The median half-life of glyphosate in soil has been widely studied and values between 2 to 197 days have been reported, but a typical field half-life of 47 days has been suggested for field conditions (Henderson et al. 2010). As expected, the inhibition of seedling emergence by glyphosate was clearly associated with the application dose. The emergence of *M. marina, L. creticus* and *A. arenaria* seeds was higher at the 0.3 g/m^2 dose (after 30 days) with respect to 4 g/m^2 dose (after 60 days; Table 2). Moreover, the seedling emergence of four native species was superior in sand collected in experimental plots (0.3 g/m^2) than under laboratory conditions (0.3 g/m^2) for the same time after glyphosate spraying (Table 2). These results suggest that climate conditions (wind, solar radiation, rain, etc.) reduce glyphosate persistence in soil (Henderson et al. 2010), therefore reducing glyphosate toxicity in sand collected from experimental plots.

Seedling emergence was reduced in the case of two native species (*L. creticus* and *O. ramosissima*), while in *A. arenaria,* it only reached 7% of the control levels (Table 2) when the substrate was collected 60 days after glyphosate spraying (4 g/m^2) and contained *Carpobrotus* litter. This inhibitory effect on seedling emergence could have been caused by residual glyphosate present in the *Carpobrotus* litter. Nevertheless, it has been reported that glyphosate had a median half-life of eight to nine days in leaf litter from red alder (*Alnus rubra* Bong.) and salmonberry (*Rubus spectabilis* Pursh) (Henderson et al. 2010). In this case, sand with *Carpobrotus* litter was collected 60 days after glyphosate spraying. Thus, another potential cause of this inhibitory effect on seedling emergence might have been the allelopathic properties of *Carpobrotus* litter. The high production and accumulation of *Carpobrotus* litter and its slow decomposition (Conser and Connor 2009) might have been sufficient to ensure the accumulation of high levels of secondary metabolites with allelopathic potential that could be inhibited the germination and early root growth of other species, as previously described for *Malcolmia littorea* seeds (Novoa et al. 2012).

Planting of native species is an alternative strategy aimed at the reintroduction of original vegetation in invaded areas (Hartman and McCarthy 2004; Galatowitsch and Richardson 2005). Planting will probably be the most successful strategy because these introduced plants will have a height advantage over the invasive seedlings. However, such actions entail elevated costs associated with plant production, transport and manual labour, and therefore, it is important to understand the possible effects of the previous application of herbicides in the planting area. The experiments described in this current work simulated native plant reintroduction by planting into *Carpobrotus*-invaded coastal dunes after chemical eradication by glyphosate spraying. Our results indicate that the survival rates and development of *L. creticus* and *A. arenaria* native coastal dune plants were not affected when they grew in pots with sand collected at different times after glyphosate applications at the recommended dose of 0.3 g/m^2 (Figure 3), even before reaching the median half-life of glyphosate in soil (Henderson et al. 2010). Similar survival rates of *Acacia holosericea* A.Cum. ex. G.Don plants were observed when they were planted in experimental plots three months after the application of herbicides including glyphosate to eradicate *Andropogon gayanus* (Luck et al. 2019). In this case, it is interesting to note that direct glyphosate application onto the sand affected the emergence but not the survival of transplanted plants.

In conclusion, targeted glyphosate spraying has been shown to be an efficient method for *Carpobrotus* eradication that could be implemented as an alternative to manual removal. The use of glyphosate was also a suitable method for *Carpobrotus* eradication when active management actions are directed towards the promotion of original vegetation in protected areas like coastal dunes. Thus, seed sowing and planting of native dune species are potential methods to ensure recolonisation with native plants after glyphosate-mediated eradication of invasive species.

Acknowledgements

The authors would like to thank of the Valencian Region Government environmental officers, Gabriel Ballester and Vicente Deltoro for their logistic support, as well as the seed bank at the Centre for Forest Research and Experimentation (CIEF, Generalitat Valenciana) for supplying the native species seeds. Finally, we thank Dr. Eduardo Rojas and Dr. Rafael Sirera for their critical review of this manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was made possible through significant support from the Conselleria de Territori i Habitatge, Generalitat Valenciana (grant Number T621700) and from the Conselleria d'Educació, Generalitat Valenciana (project GVPRE/2008/134).

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