EFFECT OF GLYPHOSATE ON GERMINATION AND SEEDLING DEVELOPMENT OF FOUR NATIVE PLANTS OF DUNES IN SPAIN

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ABSTRACT

Using glyphosate is easier than manually controlling the invasive plant *Carpobrotus* in dune ecosystems. However, before it is used its effect on native species in this ecosystem needs to be determined. This study reports the effects in the laboratory of different concentrations of glyphosate (1.0, 0.5, 0.3, 0.1, 0.05, 0.01, and 0.005 g/m^2) on germination and seedling emergence of four species of Mediterranean dune plants, *Lotus creticus*, *Medicago marina*, *Ononis ramosissima* and *Ammophila arenaria,* used in the restoration of this natural ecosystem. Germination was completely inhibited when glyphosate was applied at 0.5, and 1.0 g/m^2. The recommended dose (0.3 g/m^2) also inhibited the germination of *L. creticus* and *M. marina*. The percentage germination of *O. ramosissima* and *A. arenaria* was around 40% and 30% respectively when a dose of glyphosate of 0.1 g/m^2 was used, whereas for seeds of *L. creticus* and *M. marina* it was lower than 5%. The T_{50} at germination of seed incubated with glyphosate increased in all species independently of the dose of glyphosate. Seedling emergence from seeds previously germinated in the presence of glyphosate was only recorded for *O. ramosissima* and *A. arenaria.* The results indicate that seeds of *O. ramosissima* are more tolerant of glyphosate, followed by those of *A. arenaria* and those of *L. creticus* and *M. marina* are the most sensitive.

Keywords: germination; glyphosate; herbicide tolerance; chemical control; dune restoration; native plant reintroduction

Introduction

The invasive non-native succulent plant genus *Carpobrotus* (L.) N.E. Br. (*Aizoaceae*) is one of the most important weeds in Mediterranean coastal ecosystems and numerous studies have shown that it has strong adverse effects on native plants and animals (Campoy et al. 2018). Attempts to eradicate this invasive weed have been implemented in many places around the world (Buisson et al. 2020; Lazzaro et al. 2020, 2023; Fos et al. 2021, 2022) including in Mediterranean habitats (Campoy et al. 2018). Although the most common method of eradicating *Carpobrotus* is removal by hand, the use of herbicides has been an effective means of control. Glyphosate, N-(phosphonomethyl) glycine, was the herbicide most often used (Lazzaro et al. 2020; Fos et al. 2021, 2022).

Glyphosate-based herbicides are among the most widely used broad spectrum herbicides in the world (Henderson et al. 2010; Myers et al. 2016). Glyphosate is a non-selective herbicide that inhibits plant growth by interfering with the production of essential aromatic amino acids, which do not share the same biosynthetic pathways with members of the animal kingdom (Henderson et al. 2010; Velmourougane et al. 2021). Glyphosate is assimilated by leaves and other green plant tissues and then rapidly translocated via the phloem through the entire plant, including the roots (Henderson et al. 2010; Badani et al. 2023). 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 purpose of controlling an invasive plant (Pergl et al. 2020).

However, before deciding to use glyphosate to eradicate invasive plants, and in particular, the eradication of *Carpobrotus* in coastal areas, the potential negative effects of this herbicide must first be identified and understood. For this purpose, the germination and emergence of seedlings of four native plants growing in dunes around the Mediterranean were recorded after their seeds were incubated in solutions of different concentrations of glyphosate and the effect of glyphosate exposure on germination and seedling development evaluated.

Material and Methods

Plant material

The effect of glyphosate on germination and seedling emergence was evaluated for four native plants of dunes in the Mediterranean area: *Lotus creticus* L, *Medicago marina* L, *Ononis ramosissima* Desf. (*Fabaceae*) and *Ammophila arenaria* (L.) Link. (*Poaceae*). These species are very common and abundant in the natural vegetation growing along the Valencian coast and belong to the association *Medicagini mariane-Ammophiletum australis,* found in embryonic dunes and those that are moving. Seed of these species was supplied by the seed bank at the Centre for Forest Research and Experimentation (CIEF, Generalitat Valenciana).

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Tests used to evaluate the effect of glyphosate on dune plants

Germination

The seeds of *L. creticus*, *M. marina*, *O. ramosissima* and *A. arenaria*, which were previously scarified (15 minutes in H_2SO_4), sterilized by placing them in 0.5% sodium hypochloride (NaClO) solution for two minutes and thoroughly rinsed with deionized water before sowing.

These seeds were placed in 9 cm diameter Petri dishes on two layers of filter paper (Whatman nº 1) moistened with 20 ml of distilled water or glyphosate solutions of a particular concentration. Four replicates of 50 seeds were used in each treatment. Seeds were considered to have germinated when approximately 2 mm of the primary root had emerged. Seeds were germinated in a growth chamber (16 h light/8 h darkness, 21 °C, 60% relative humidity).

Glyphosate (36% p/v, as an ammonium salt; Touchdown, Syngenta Iberica, Spain) was used to obtain solutions of the desired concentration for moistening seeds (20 ml/germination box). For each species, seeds were germinated in glyphosate solutions equivalent to doses of 1.0, 0.5, 0.3, 0.1, 0.05, 0.01 and 0.005 g/m^2. The minimum effective glyphosate concentration for controlling *Carpobrotus* is 0.4 g/m^2 (Fos et al. 2021) and the recommended dose of glyphosate is 0.3 g/m^2 (EFSA 2017).

Percentage germination was recorded over a period of 40 days. At the end of the experiment, non-germinated seeds were dissected in order to determinate their viability and only seeds with a complete embryo were considered as viable. Percentage seed germination (G) for each replicate was calculated at the end of the experiment as: $\%G = 100 \times (GS/FS)$, where *GS* is the number of seeds that germinated and *FS* the number of viable seeds. Rate of germination was estimated using T_{50}, which is the time in days to when 50% of the seeds had germinated.

Seedling emergence

Germinated seeds incubated in a solution of glyphosate (0.005 to 0.1 g/m^2) were sown in trays containing 0.5 l of sterilized beach sand to evaluate the effect of glyphosate on seedling emergence and development. For each species and glyphosate treatment twenty germinated seeds were used. The emergence tests were carried out in a chamber under controlled conditions (16 h light/8 h darkness, 20 °C, 60% relative humidity). The emergence and development of the aerial parts of seedlings was monitored for at least 60 days.

Statistical analysis

The analysis of percentage germination of the seed of four native species in solutions of glyphosate equivalent to different doses was done using analysis of variance (ANOVA) and Statgraphics plus software (5.1 for Windows, 1994, Statistical, Corporation, Warrenton, VA). The statistical analysis of the data was done separately for each species using the glyphosate doses as the main factor. Tukey multiple comparison test was used for each ANOVA to determine the significance of any differences in germination between the different glyphosate doses with respect to that on the control substrate ($p < 0.05$).

Results

Effect of exposure to glyphosate on time to and percentage germination of dune plants

Percentage germination of *L. creticus* seeds after 40 days of incubation in the control was higher than 90% (Fig. 1a). It was significantly reduced by up to 50% and 45% at glyphosate doses of 0.005 and 0.01 g/m^2 (Fig. 1a) and less than 10 and 5% for the doses of 0.05 and 0.1 g/m^2 (Fig. 1a). No germination was recorded when incubated with doses of glyphosate equal to or greater than 0.3 g/m^2 (Fig. 1a). The T_{50} for the control was 4 days and increased to over 20 days when incubated in the presence of glyphosate (Fig. 2a). No differences in T_{50} were recorded between the different doses of glyphosate.

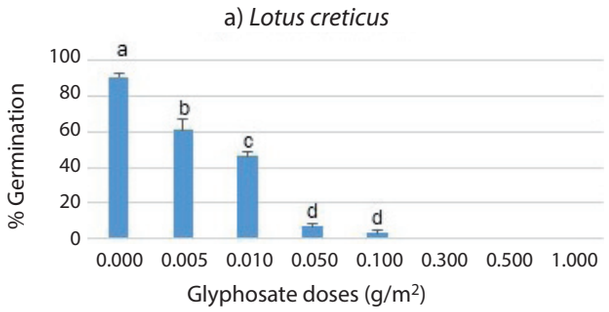
Percentage germination of *M. marina* seeds after 40 days of incubation in the control was approximately 70% (Fig. 1b). It was significantly reduced by up to 75% at glyphosate doses of 0.005 and 0.01 g/m^2 (Fig. 1b) and was less than 3% for doses of 0.05 and 0.1 g/m^2 (Fig. 1b). No germination was recorded when incubated with doses equal to or greater than 0.3 g/m^2 (Fig. 1b). The T_{50} for the control was 8 days and increased to 15–18 days when incubated in the presence of glyphosate (Fig. 2b).

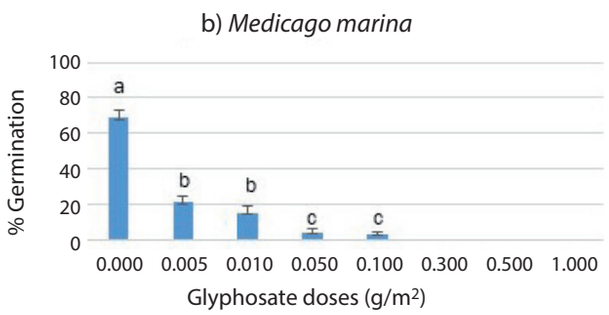
Percentage germination of *O. ramossisima* seeds after 40 days of incubation in the control was approximately 30% (Fig. 1c). It was significantly reduced by 50% at a glyphosate dose of 0.005 g/m^2 and 60% at glyphosate doses between 0.01 to 0.1 g/m^2 (Fig. 1c) and 3% at a dose of 0.3 g/m^2. The T_{50} for the control was 4 days and increased to 16–22 days when seeds were incubated in the presence of glyphosate (Fig. 2c). No differences in T_{50} were recorded for the different doses of glyphosate (Fig. 2c).

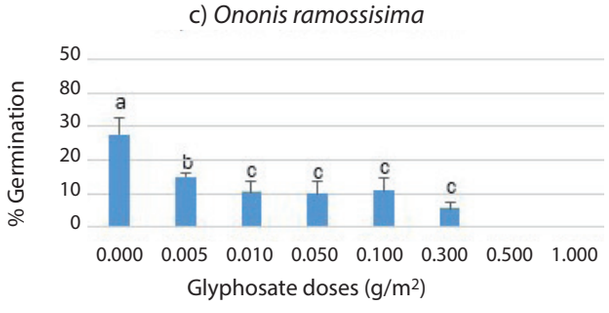
Percentage germination of *A. arenaria* seeds after 40 days of incubation in the control was approximately 42% (Fig. 1d). It was significantly reduced by about 40% compared to control at glyphosate doses of 0.005 and 0.01 g/m^2, about 70% at doses between 0.05 to 0.1 (Fig. 1d) and 1–2% at a dose of 0.3 g/m^2. The T_{50} for the control was 13 days (Fig. 2d) and increased to 20–25 when incubated in the presence of glyphosate (Fig. 2d).

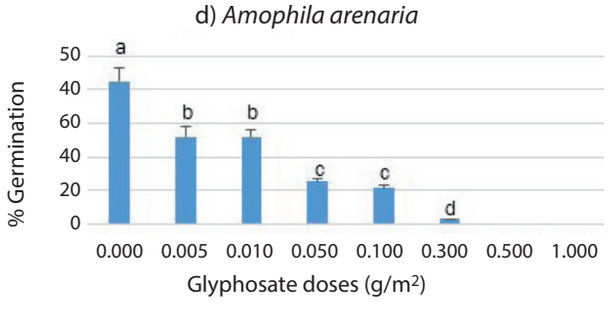
Effect of exposure to glyphosate on seedling emergence and development of dune plants

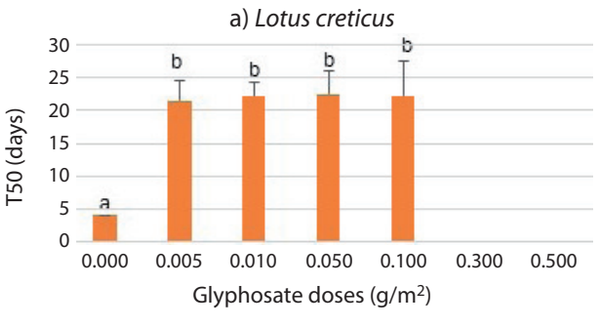
Seedling emergence in the control ranged from 70% for *A. arenaria* and *M. marina,* 75% for *O. ramosissima* and a maximum of 85% for *L. creticus* (Table 1). Seedling emergence of *O. ramosissima* was 40% for doses of 0.005 and 0.01 g/m^2 and 10% for 0.1 g/m^2 (Table 1 and Fig. 3). In the case of *A. arenaria,* it was 10% for a dose

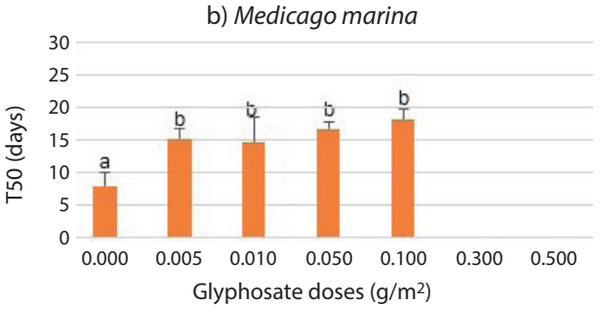


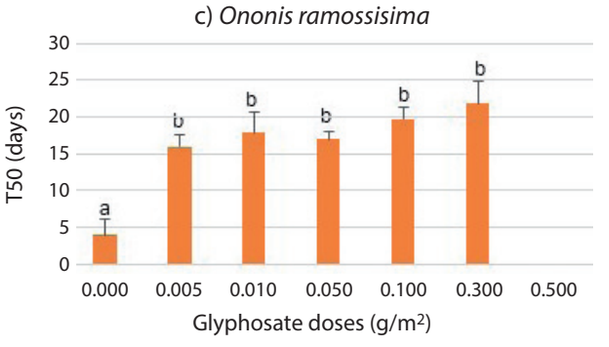












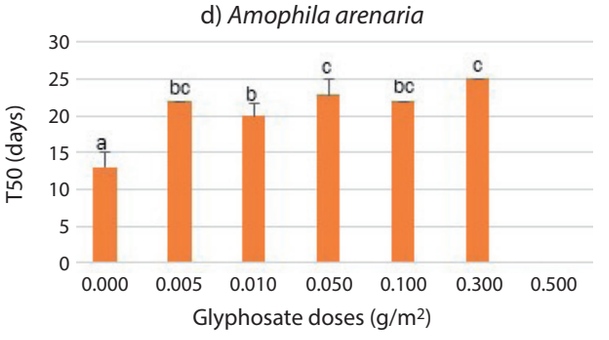


Fig. 1 Percentage germination of the seed of *Lotus creticus*, *Medicago marina, Ononis ramosissima* and *Ammophila arenaria* previously exposed to different doses of glyphosate. Percentage germination was recorded after 40 days (mean values ± standard error). Different letters denote statistically significant differences in the percentage germination at different doses of glyphosate based on Tukey's multiple comparison test ($p < 0.05$).

Fig. 2 Time to germination of seed of *Lotus creticus*, *Medicago marina, Ononis ramosissima* and *Ammophila arenaria* treated with different doses of glyphosate. T_{50} (mean values ± standard error). Different letters denote statistically significant differences based on Tukey's multiple comparison test ($p < 0.05$).

of 0.005 g/m² and 30% for 0.01 g/m² (Table 1 and Fig. 3). No seedling emergence was recorded for *L. creticus* and *M. marina* previously incubated in presence of glyphosate (Table 1).

The height of the seedlings of *O. ramosissima* that germinated from seeds incubated with glyphosate decreased relative to the control from 30% for the dose of 0.005 g/m² to 50% for 0.1 g/m² (Table 1 and Fig. 3). The decrease in development relative to the control was greater for the *A. arenaria* seedlings that germinated from seed incubated with glyphosate, with the height 40% lower for the dose of 0.005 g/m² and 60% lower for the dose of 0.01 g/m² (Table 1 and Fig. 3).

Discussion

In summer in the Mediterranean area, the seeds of many species of *Fabaceae* are dormant, which prevents germination during the dry hot summers and postpones germination to the following spring. Germination of *M. marina* seeds is less than 10% (Scippa et al. 2011) and in the case of *O. ramosissima* it is between 6 and 16% (Devesa et al. 2000). Seed dormancy in these species is associated with the condition of the testa, which after mechanical or chemical scarification, results in almost 100% germination in *L. creticus* (Hajri et al. 2018; Rejili et al. 2009), *M. marina* (Scippa et al. 2011) and *O. ramosissima* (Devesa et al. 2000). The scarification treatment used has been highly effective for *L. creticus* (92%) and *M. marina* (70%) (Fig. 1). The percentage germination of scarified seed of *O. ramosissima* (30%, Fig. 1) was higher than that of non-scarified seed (Devesa et al. 2000). The time taken to complete dormancy may be longer than the time to germination after scarification (Devesa et al. 2000). The percentage germination of the *A. arenaria* seeds that were not exposed to glyphosate was higher than 42% (Fig. 1). This is lower than previously reported for this species (Chergui et al. 2013).

Glyphosate is known to affect germination or seedling quality when applied directly to seed of species of different families, such as: *Poaceae* (Yenish and Young

Table 1 Seedling emergence and development of *Lotus creticus*, *Medicago sativa*, *Ononis ramosissima* and *Ammophila arenaria* from seed incubated in different doses of glyphosate (n = 20). Final seedling emergence and percentage emergence compared to the control (%) after 60 days. Height of seedlings (mean) and percentage height compared to the control (%) after 60 days.

Lotus creticus				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	17	85	13.1	100
0.005	0	0	0	0
0.010	0	0	0	0
0.050	0	0	0	0
0.100	0	0	0	0
Medicago marina				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	14	70	9.3	100
0.005	0	0	0	0
0.010	0	0	0	0
0.050	0	0	0	0
0.100	0	0	0	0
Ononis ramosissima				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	15	75	4.4	100
0.005	8	40	3.1	70
0.010	8	40	2.6	59
0.050	0	0	0	0
0.100	2	10	2.3	52
Ammophila arenaria				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	14	70	13.9	100
0.005	2	10	8.0	57
0.010	6	30	5.6	40
0.050	0	0	0	0
0.100	0	0	0	0

Fig. 3 Seedling emergence and development of *Ononis ramosissima* and *Ammophila arenaria* seeds previously incubated in solutions of different doses of glyphosate.

2000; Piotrowicz-Cieślak et al. 2010) *Fabaceae* (Piotrowicz-Cieślak et al. 2010; Gomes et al. 2017; Mondal et al. 2017) and *Brassicaceae* (Piotrowicz-Cieślak et al. 2010; Kashyap et al. 2023). As expected, based on the widespread use of glyphosate for pre- and post-emergence control, the presence of this herbicide in the incubation medium inhibited seed germination of all the dune plants used in this study (Fig. 1). In addition, the percentage inhibition of germination by glyphosate was clearly associated with the dose (Fig. 1). The germination was completely inhibited at doses of 0.5 and 1.0 g/m^2 (Fig. 1), which are both above the recommended dose of 0.3 g/m^2 (EFSA 2017). The inhibitory effect of exposure to glyphosate decreased progressively for doses equal to or lower than the recommended dose (Fig. 1).

The results indicate that the percentage inhibition of germination depends on the species. Thus, the presence of glyphosate at the recommended dose (0.3 g/m^2, EFSA, 2017) did not completely inhibit the germination of the seed of *O. ramosissima* and *A. arenaria* (Fig. 1), whereas it did for that of *L. creticus* and *M. marina* (Fig. 1). Differences were also recorded for the percentage germination of the seed of the 4 species of dune plants at doses below the recommended dose (Fig. 1). An equivalent dose of 0.1 g/m^2 was used in a glyphosate tolerance screening of wild relatives of crop plants belonging to the *Brassicaceae* (Kashyap et al. 2023). At this dose, the percentage germination relative to the control was reduced by more than 95% for *L. creticus* and *M. marina*, 75% for *A. arenaria* only by 40% for *O. ramosissima* (Fig. 1). The results indicate that *L. creticus* and *M. marina* are more sensitive to glyphosate than the other two species (Fig. 1).

Glyphosate not only reduced percentage germination, but also significantly increased the time to germination (Fig. 2). Relative to the control the T_{50} of glyphosate treated seed of *M. marina* and *A. arenaria* doubled and that of *L. creticus* and *O. ramosissima* increased 4 to 5fold (Fig. 2). The general trend in the 4 species is that the increase in T_{50} is very similar regardless of the dose of glyphosate used (Fig. 2). The results also indicate that germination of seed incubated with glyphosate starts after a similar length of time for all species (Fig. 2). These results might indicate that the time to germination is associated with the loss of glyphosate toxicity. Glyphosate has a half-life of 2 to 14 days in water (Badani et al. 2023), thus the inhibitory effect of glyphosate applied to beach sand on the emergence of dune species would begin to decline after 15 days (Fos et al. 2022).

The results also indicate that glyphosate affected the emergence and development of seeds germinated previously in the presence of this herbicide (Table 1 and Fig. 3). Seedling emergence from germinated seeds previously incubated in the presence of all the doses of glyphosate was not recorded for 2 of the 4 species, *L. creticus* and *M. marina.* These results reinforce the different sensitivity of the species to the inhibiting effect of this herbicide (Table 1) as recorded for the wild relatives of different crop plants belonging to the *Brassicaceae* (Kashyap et al. 2023). In addition, the seeds of *A. arenaria* and *O. ramosissima* were more resistant to glyphosate.

The results, however, are based on laboratory tests under very homogeneous and controlled conditions. The seeds were soaked in a solution containing the herbicide. These conditions do not correspond to those in the dunes where glyphosate is used to control the non-native invasive plant *Carpobrotus.* In a real situation, in which this herbicide is applied by professionals, the seed in the soil seed bank would only be slightly wetted by the herbicide in areas adjacent to monospecific carpets of *Carpobrotus*. In addition, the seeds used in the laboratory tests were not subjected to changing environmental conditions as they would be in the field. Previous results of a study on seedling emergence of Mediterranean dune plants on substrates collected after spraying with glyphosate under real conditions reports higher emergence values (Fos et al. 2022) than those obtained under laboratory conditions (Table 1).

Conclusions

In conclusion, the presence of the herbicide in the incubation medium inhibited seed germination of four species of Mediterranean dune plants and the inhibition was dependent on the dose of glyphosate used. The increase in T_{50} of seeds incubated in the presence of glyphosate was independent of the dose used. Glyphosate also affected the later emergence and development of seedlings from seed germinated previously in the presence of this herbicide. The four dune plants differed in their sensitivity to glyphosate, with *A. arenaria* and *O. ramosissima* the least sensitive to the herbicide. Under field conditions, the negative effect of glyphosate is significantly less than that recorded in the laboratory.

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REFERENCES

Badani H, Djadouni F, Haddad FZ (2023) Effects of the herbicide glyphosate [n-(phosphonomethyl) glycine] on biodiversity and organisms in the soil – a review. Eur J Environ Sci 13: 5–14. doi: 10.14712/23361964.2023.

Buisson E, Braschi J, Chenot-Lescure J, Hess MCM, Vidaller C, Pavon D, Ramone H, Amy-Krebs E, Cottaz C, Passetti A, Aboucaya A, Affre L (2020) Native plant community recovery after *Carpobrotus* (ice plant) removal on an island – results of 10 year project. Appl Veg Sci 24, e12524.

Campoy JG, Acosta ATR, Affre L, Barreiro R, Brundu G, Buisson E, González L, Lema M, Novoa A, Retuerto R, Roiloa SR, Fagúndez J (2018) Monographs of invasive plants in Europe: *Carpobrotus*. Bot Lett 165: 440–475.

Chergui A, Zakariya I, Nejjari R, El Hafid L, Melhaoui M (2013) The effects of temperature, hydric and saline stress on the seedling growth of marram grass (*Ammophila arenaria* L.). Int J Appl Environ Sci 13: 633–642.

Devesa JA, Ruíz T, Buzo G (2000) Ensayos de germinación en especies de *Ononis* L: (*Fabaceae*) del SW de España. Collect Bot 25: 195–205.

EFSA (European Food Safety Authority) (2017) Conclusion of peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA J 15: 4979. doi: 10.2903/j.efsa.2017.4979.

Fos M, Sanz B, Sanchis E (2021) *Carpobrotus* management in a Mediterranean sand dune ecosystem: minimum effective glyphosate dose and an evaluation of tarping. J Ecol Engi 22: 57–66. doi: 10.12911/22998993/138871.

Fos M, Sanz B, Sanchis E (2022) The use of glyphosate for *Carpobrotus* eradication in the sand dune ecosystem: evaluation of the potential effects on reintroduction of native plants. Plant Biosyst 156: 480–489. doi: 10.1080/11263504.2021.1884621.

Gomes MP, da Silva Cruz FV, Bicalho EM, Borges FV, Fonseca MB, Juneau P, García QS (2017) Effects of glyphosate acid and the glyphosate-commercial formulation (Roundup) on *Dimorphandra wilsonii* seed germination: interference of seed respiratory metabolism. Environ Pollut 220: 452–459. doi: 10.1016/j.envpol.2016.09.087.

Hajri R, Ouhibi C, Mechri M, Kourda H, Younes MB (2018) Salinity and water deficit effects on seed germination and recovery of *Lotus* populations from northern Tunisia. Pak J Bot 50: 2085–2090.

Henderson AM, Gervais JA, Luukinen B, Buhl K, Stone D, Strid A, Cross A, Jenkins J (2010) Glyphosate Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/archive/glyphotech.html, accessed 2 March 2023.

Kashyap A, Kumari S, Garg P, Kushwaha R, Tripathi S, Sharma J, Gupta N.C, Kumar RR, Vishwakarma H, Bhattacharya R, Yadav R, Rana JC, Rao M (2023) Morpho-biochemical responses of *Brassica* coenospecies to glyphosate exposure at pre- and post-emergence stages. Agronomy 13: 1831. doi: 10.3390/agronomy13071831.

Lazzaro L, Mugnai M, Ferretti G, Giannini F, Giunti M, Benesperi R (2023) (Not) sweeping invasive alien plants under the carpet: results from the use of mulching sheets for the control of invasive *Carpobrotus* spp. Biol Invasions 25: 2583–2597.

Lazzaro L, Tondini E, Lombardi L, Giunti M (2020) The eradication of *Carpobrotus* spp. In the sand-dune ecosystem at Sterpaia (Italy, Tuscany): indications from a successful experience. Biologia 75: 199–208.

Mondal S, Kumar M, Haque S, Kundu D (2017) Phytotoxicity of glyphosate in the germination of *Pisum sativum* and its effect on germinated seedlings. Environ Health Toxicol 32: e2017011, doi: 10.5620/eht.e2017011.

Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, Hansen M, Landrigan PJ, Lanphear BP, Mesnage R, Vandenberg LN, vom Saal FS, Welshons WV, Nenbrook CH (2016) Concerns over use of glyphosate-based herbicides and risk associated with exposures: a consensus statement. Environ Health 15:19, doi: 10.1186/s12940-016-0117-0.

Pergl J, Härtel H, Pyšek P, Stejskal R (2020) Don't throw the baby out with the bathwater – ban of glyphosate use depends on context. NeoBiota 56: 27–29.

Piotrowicz-Cieślak A, Adomas B, Michalczyk DJ (2010) Different glyphosate phytotoxicity to seeds and seedlings of selected plant species. Pol J Environ Stud 19: 123–129.

Rejili M, Vadel AM, Guetet A, Mahdhi M, Lachiheb B, Ferchichi A, Mars M (2009) Influence of temperature and salinity on the germination of *Lotus creticus* (L.) from the arid land of Tunisia. Afr J Ecol 48: 329–337.

Scippa GS, Petrollini E, Trupiano D, Rocco M, Falco G, Di Michele M, Chiatante D (2011) Dormancy of *Medicago marina* (L.) seed. Environ Exp Bot 73: 320–329.

Velmourougane K, Blaise D, Manikandan A, Savitha S, Waghmare VN (2021) Environmental impacts of herbicide tolerant crops and glyphosate-based herbicides – A review. Appl Ecol Environ Res 19: 3481–3504.

Yenish JP, Young FL (2000) Effect of preharvest glyphosate application on seed and seedling quality of spring wheat (*Triticum aestivum*). Weed Technol 14: 212–217.