Feasibility of using glyphosate to control beach evening primrose *Oenothera drummondii* in heavily invaded coastal dunes, Odiel Marshes, Spain

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SUMMARY

Beach evening primrose *Oenothera drummondii* is a perennial plant native to the southern USA and adjacent parts of Mexico that invades coastal habitats in several countries. There are currently no accepted control methods. We conducted a seven-month controlled field trial using the glyphosate herbicide Roundup® Ultra Plus in the Odiel Marsh Nature Reserve, Huelva Province, southern Spain. Different herbicide concentrations were tested by knapsack spraying. We estimated the costs of treating an entire invaded nature reserve in southern Spain where *O. drummondii* has invaded 123 ha of land. A dose of 20 g active ingredient/litre was the minimum effective dose for this species in coastal dunes. As new seedlings appeared after a single herbicide treatment, periodic treatments would be necessary to maintain the population level below an impact threshold. However, the total glyphosate input (710 kg active ingredient/year) to the Reserve for an indefinite period may give rise to social rejection, and demands for the assessment of ecotoxicological impact on native fauna, adjacent habitats and site uses before initiating control actions at full scale. The control costs of the entire 123 ha invaded area for two herbicide applications/year were estimated at €162,000/year (€1,317/ha/year). This includes materials (30% of total costs) and workers (70% of total costs). The study highlights the difficulties and constraints of controlling advanced stages of invasions.

BACKGROUND

Biological invasions are considered one of the main causes of biodiversity loss. Current invasions are a consequence of the deliberate or accidental introductions of new alien species associated with global trade, or the continuing spread of established invasive organisms. General management guidelines tend to prioritise the eradication of incipient invasions or newly introduced species over the control of large, established and widespread populations (e.g. Genovesi & Shine 2004). Given limited financial resources (the most common scenario), invasive species that are widely distributed or are responsible for large invasions may be left unmanaged. Moreover, the application of this prioritisation principle over time can lead to the paradox of "common" exotic invaders being considered as non-problematic or even perceived as "native" (Bardsley & Edwards-Jones 2006). However, both small- and large-scale invasions result in the same outcome: the progressive loss of natural surface and potential negative consequences on ecosystem functions and services. For advanced invasions, containment or control may be the only feasible strategy to maintain essential ecological processes, habitat characteristics, biological communities and species. Larger populations are also likely to contribute proportionally more to long-distance dispersal than small populations (Hulme 2003). Therefore, large invasions that continue to expand pose a challenge for management.

For a growing spectrum of invasive species worldwide, effective management methods are often unknown. This is the case with the beach evening primrose *Oenothera drummondii* (Onagraceae), a biennial or short-lived perennial plant whose native range occurs from Campeche, Mexico, to North Carolina, USA (Dietrich & Wagner 1988). *O. drummondii* is naturalised in North Africa, Israel, East Asia, South Africa, Argentina and Peru (Dietrich 1997, Dufour-Dror 2013) and has been found as an invasive in China (Xu *et al*. 2012), southwest Australia (Heyligers 2008) and Spain (García-de-Lomas *et al*. 2015). There are a few isolated experiences of elimination by hand-pulling or chemical treatment (recorded only by a few notes on websites) with varying results reported, and little information on the methods used (e.g. timing, frequency of treatment, resources needed). Therefore, in practice the available information seems to be of little help to guide management of this species.

The recent expansion of *O. drummondii* observed in southern Spain demands the evaluation of effective control methods before planning large-scale control actions. Herbicide spraying may be a solution to treat areas widely and densely invaded by alien plants, since it is faster and cheaper than manual methods (Tu *et al*. 2001). Moreover, hand-pulling in sandy habitats disturbs the soil and may enhance reinvasion (Ogden & Rejmánek 2005). In this work, we test the efficacy of chemical treatment in a small-scale field trial. Specifically, we aim to answer the following questions: (i) What is the minimum effective dose of glyphosate to control *O. drummondii*? (ii) Is the minimum effective dose dependent on the size of the plant? (iii) What is the cost of the chemical treatment? (iv) Is it feasible to apply a full-scale herbicide-based control in the long term? The results will be used to plan a strategy to control the southernmost invasive population of *O. drummondii* in Spain.

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ACTION

Study area and target species: The experiment was carried out in the Odiel Marshes Natural Reserve (37.15ºN; 6.91ºW), located near the city of Huelva (southern Spain) (Figure 1). The Reserve is included in the European Union Natura 2000 network. The aim of the Natura 2000 network is to ensure the long-term survival of Europe's most valuable and threatened species and habitats, listed under the Directives 79/409/EEC, 2009/147/EC ("Birds Directive") and the Council Directive 92/43/EEC ("Habitats Directive"). The Reserve covers an area of 6632 ha and is adjacent to the Atlantic Ocean and the mouths of the rivers Tinto and Odiel. The climate is Mediterranean, with hot, dry summers and mild, wet winters. Mean temperature and rainfall at the Huelva meteorological station (11 km from the study area) for the 1981-2010 series are 18.2°C and 525 mm respectively (Aemet 2015). The geographical location and the combination of marshes (971.6 ha) and sand dunes (406.8 ha) in the Reserve provide high ornithological value, serving as a staging, feeding, breeding and resting area for migratory shorebirds. Habitats of Community Interest listed in the European Council Directive 92/43 include shifting dunes with *Ammophila arenaria* (code 2120), fixed dunes with herbaceous vegetation (code 2130), coastal dunes with *Juniperus* spp. (code 2250) and wooded dunes with *Pinus pinea* (code 2270). The invasion of *Oenothera drummondii* is located in the sandy habitats of the Reserve (Figure 1), mainly on shifting and fixed dunes (yellowand grey dunes) dominated by *Ammophila arenaria*, *Achillea maritima*, *Lotus creticus*, *Malcolmia littorea*, *Pancratium maritimum*, *Echium gaditanum*, *Armeria pungens*, *Artemisia crithmifolia* and *Calystegia soldanella*, some of which are regionally endangered (Cabezudo *et al*. 2005). The total invaded area is 123 ha, with maximum densities of 65 individuals/m^2 (García-de-Lomas *et al*. 2015).

O. drummondii mainly flowers in spring–summer (Campos & Herrera 2009), but flowering individuals can be found all year round (Lonard & Judd 1989). It also shows the ability to flower and fruit early (García-de-Lomas, pers. obs.) as well as other features related to invasiveness (small seeds, resprouting ability, burial tolerance) (Kolar & Lodge 2001).

Herbicide treatments: Different herbicides have been used for controlling invasive alien plants (Tu *et al.* 2001). In this trial, we chose glyphosate (N-(phosphonomethyl)glycine), a broad-spectrum, fast-action herbicide, without pre-emergence action (Duke & Powles 2008), for testing the minimum effective dose for *O. drummondii*. Glyphosate as an active ingredient is cheaper (€6-9/L) than other herbicides such as Triclopir (approximately €30/L), and has been proven effective against a number of invasive plants (e.g. Mateos-Naranjo *et al*. 2009, van Dinther *et al*. 2015). In this study, we used Roundup Ultra Plus®, a commercial compound containing glyphosate as

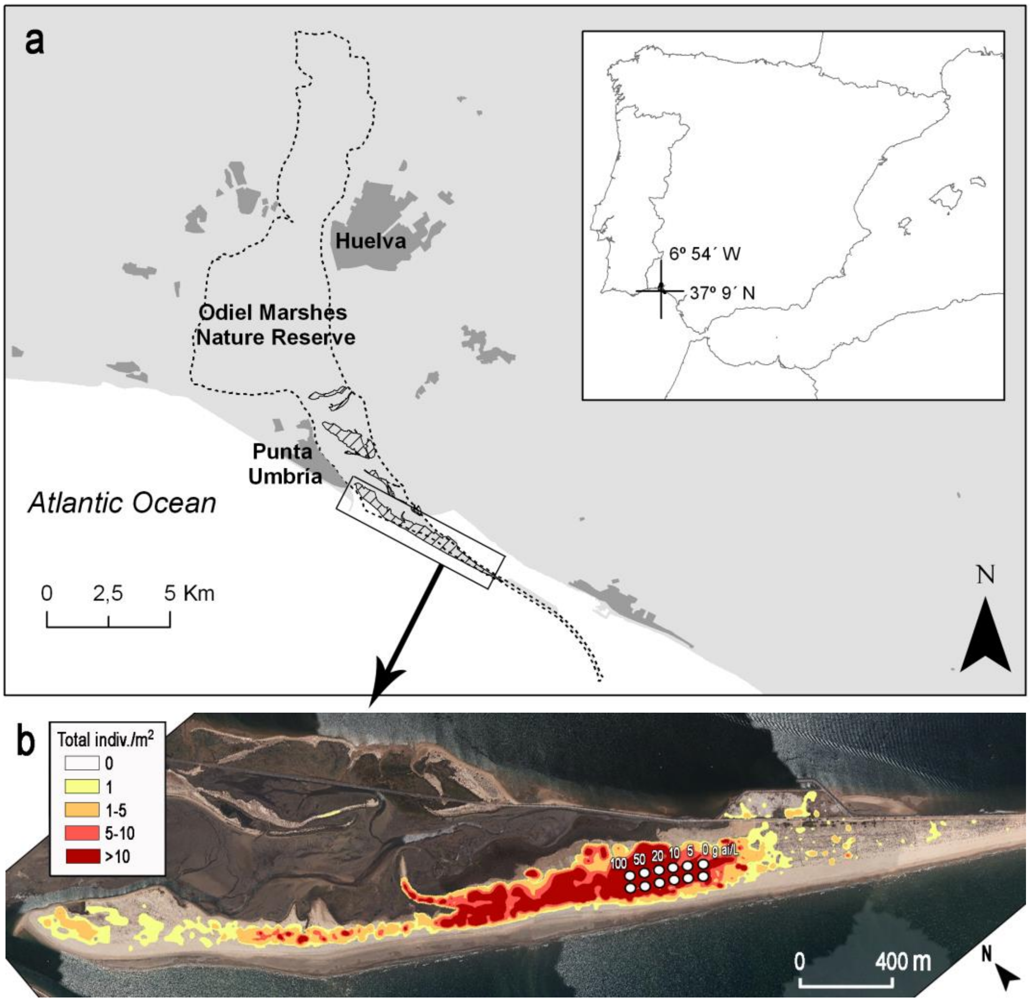

Figure 1. Area of study: a) location of the Odiel Marshes Nature Reserve (Huelva, southern Spain). The cross-hatched areas show the sandy environments, which are favourable habitats for *Oenothera drummondii*. b) Density of *O. drummondii* in the Reserve (modified from García-de-Lomas *et al*. 2015). The white dots show the plots for testing the minimum effective dose of the herbicide Roundup Ultra Plus® (the concentration of the active ingredient is indicated).

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potassium salt at a concentration of 360 g active ingredient/L (hereinafter referred to as g ai/L). This initial concentration was used to prepare further dilutions.

Plots highly invaded by *O. drummondii* (density >10 individuals/m^2) were selected and treated with different herbicide doses on 3 December 2014. We used a knapsack sprayer (capacity 16 L) in order to reproduce the usual conditions of herbicide application. As proved in preliminary trials, low pressure applications lead to a considerable loss of herbicide over the sand. Therefore, we sprayed at high pressure (micro-spraying) using short "shots" to reduce herbicide consumption. This technique is only recommended at low wind speeds to prevent the herbicide from reaching non-target plants. The final doses tested were 5, 10, 20, 50 and 100 g ai/L. Two 5×5 m^2 plots were established in the field for each treatment. The plots were separated by at least 10 m to prevent possible herbicide translocation between treatments (Marrs & Frost 1997). Each plot was treated with a herbicide solution including: (i) the active ingredient (potassium salt of glyphosate from Roundup Ultra Plus®); (ii) a water-soluble blue dye (5 mL/L of Bluemark®) that helps the worker to identify treated plants, thus preventing leaving untreated individuals; and (iii) an adjuvant based on paraffin oil (Velezia®) (15 mL/L). The solution was completed with tap water to a final volume of 1 L/plot. The density of *O. drummondii* was similar for all plots. We used a similar volume of solution for each plot. The use of a dye allowed a homogenous application among treatments. Two additional plots were treated with a solution containing tap water and blue dye (control plots) (it was assumed that the "chemical" treatment included the herbicide and the adjuvant).

One month after treatment (8 January 2015), 20 adult plants/plot and three stalks/plant were selected (n = 120 samples/dose). Additionally, we collected n = 40 seedlings per dose trialled and measured their water loss after treatment, a measure of herbicide effect. Water content was used as an indicator of wilting, according to Hernández *et al*. (2010).

We recorded the density of plants (number of plants/m^2) before and seven months after (30 July 2015) the herbicide treatment. This sampling served to evaluate the degree of invasion within the treated plots and to validate the minimum effective dose previously estimated. We counted all plants of *O. drummondii* within the plots by placing 25 quadrats of 1 m^2 throughout the plot systematically (i.e. 50 quadrats/dose).

Cost of treatment: To estimate the amount and cost of herbicide that would be required to treat the entire invasive population, we used the density data of the invasive plant (García-de-Lomas *et al*. 2015; Table 1), the price of supplies (herbicide, blue dye and adjuvant), including purchase tax, average market prices of equipment, materials and workers (Tragsa 2015) and the minimum effective dose obtained in this study. We also included the total cost of water supply (€0.85/m^3), rental and transportation of water tanks and periodic filling of water tanks, vehicles for worker and material transportation (ca. €45/day), equipment for herbicide handling and application (protective clothing, safety glasses, gloves masks) (about €2000/year), fencing and signs. Worker requirements include two herbicide applicators (€141/worker/day), an expert to provide technical assistance and a field work supervisor (€185/worker/day).

Table 1 Volume of herbicide needed (commercial compound Roundup Ultra Plus®) to treat the entire area occupied by *Oenothera drummondii* in Odiel Marshes Nature Reserve. The plant density distribution was obtained from García-de-Lomas *et al*. (2015).

Plant density (indiv./m^2)	Area (ha)	Herbicide consumption (L/ha)	Total herbicide supply (L)
0	283.99	-	-
1	63.60	2.7	198
2	9.89		
2-5	10.33	5.5	57
5-10	12.32	11.1	137
>10	26.81	22.2	595
Total supply needed (for one application)			987

Statistical analysis: The minimum effective dose was calculated as that for which the rate of water loss reached an asymptote, by logarithmic adjustment curves and the Michaelis-Menten model. For plant density data, normality and homoscedasticity were evaluated by the Kolmogorov-Smirnov test and Levene's test, respectively. We used t-tests to compare plant density data in sampling plots before and after herbicide treatment.

CONSEQUENCES

Seven months after the herbicide treatment, all treated plants with doses ≥ 20 g ai/L were completely withered. No resprouts were observed in treated plants with doses ≥ 20 g ai/L. Therefore, the plants present in these plots (Table 2) were entirely due to regeneration from seed and a significant decrease in invasive plant density was observed seven months after treatment in the ≥ 20 g ai/L treatments (Table 2). Below 20 g ai/L (Figure 2) plants were affected but did not die en masse. The stems turned partially yellow or red and remained on the plant, suggesting only partial damage. The absence of significant differences in the density of plots treated with doses < 20 g ai/L suggests that mortality was offset by recruitment. This is confirmed by the fact that the water content reached an asymptote, above which increased dose had no effect on herbicidal activity, for a dose between 20 and 50 g ai/L (Figure

Table 2. Mean density (± S.E.) of *Oenothera drummondii* in the sampling plots before and seven months after the herbicide treatment. P-values and t-statistics obtained from t-tests are shown, n = 50, * indicates significant differences.

	Glyphosate dose (g ai/L)					
	0	5	10	20	50	100
Before treatment	6.9 ± 0.5	4.1 ± 0.3	4.2 ± 0.3	5.6 ± 0.4	4.2 ± 0.2	4.6 ± 0.3
After treatment	6.1 ± 0.5	4.1 ± 0.4	4.2 ± 0.4	3.6 ± 0.4	0.9 ± 0.1	1.2 ± 0.2
t statistic	1.19	0.04	0.00	3.84	12.95	9.38
p-value (two-tailed)	0.24	0.97	1.00	<0.0001*	<0.0001*	<0.0001*

Figure 2. *Oenothera drummondii* plants one month after treatment with different doses of glyphosate herbicide (8 January 2015)

3). The minimum effective dose obtained in seedlings is relatively consistent with that obtained in adult plants, especially at the highest doses tested (Figure 3).

The volume of herbicide solution used in this trial was 400 L/ha at a concentration of 20 g ai/L. This gives 8000 g ai/ha. The commercial compound (Roundup Ultra Plus®) contains 360 g ai/L, therefore resulting in 22.2 L of commercial compound/ha. We assumed that if a density of 10 plants/m^2 requires 22.2 L of undiluted commercial compound, half the density will require half this amount of product (11.1 L), and so on. Considering the distribution of *O. drummondii* in the Odiel Marshes Nature Reserve (García-de-Lomas *et al*. 2015, Table 1, Figure 1), a total of 987 L of Roundup Ultra Plus® would be needed for a single application of the entire invaded area (123 ha). As the invasive plant persisted after the herbicide treatment (Table 2), more than one application would be needed. One applicator takes approximately 5 minutes to treat 50 m^2, two applicators (working 8 h/day and 22 days/month) will take roughly 5.4 months to complete an application cycle across the invaded area (over 120 ha). Therefore, a maximum of two applications per year could be done, involving the following costs: the costs of herbicide, blue dye and adjuvant are €32,000/year. The costs of water supply, protective clothing, fencing and car rental are €14,295/year. The time spent on herbicide application was 16 h/hectare, which would result in 1,968 hours needed for the treatment of the entire invaded area. This would imply an investment of €70,000/year for two herbicide applicators and €45,695/year for technical assistance and field supervision. This suggests that in total approximately €162,000/year (€1,317/ha) would be needed to control

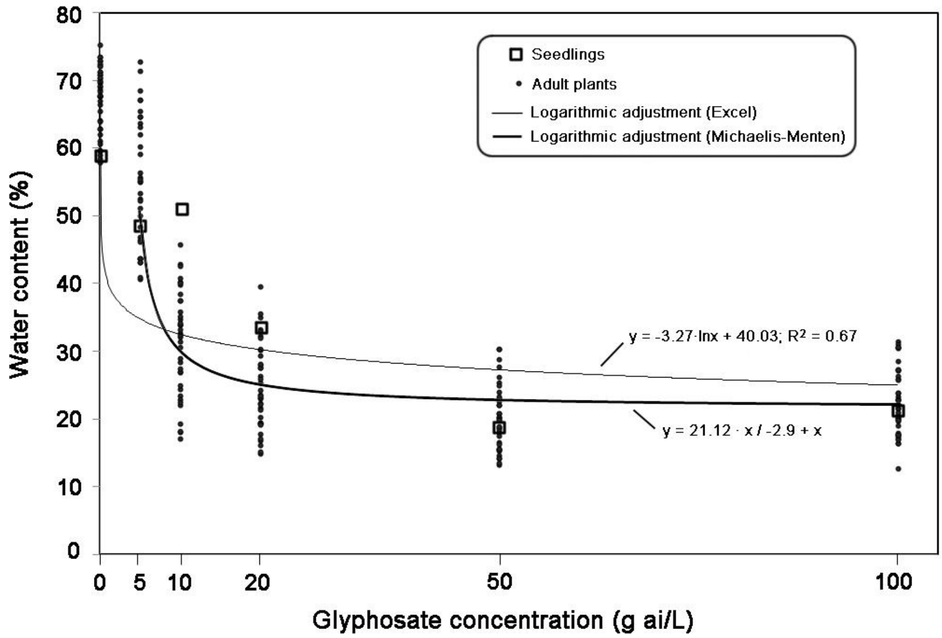

Figure 3. Water content of *Oenothera drummondii* plants treated with different glyphosate concentrations (Roundup Ultra Plus®). The points show the water content in adult plants (n = 40 samples per treatment) and the open squares indicate the water content of the seedlings (one integrated measure of n = 40 seedlings for each herbicide concentration). Logarithmic adjustment curves for adult plant data are shown (i) obtained automatically by Excel and (ii) according to the Michaelis-Menten model (obtained by the iterative method available at http://biomodel.uah.es/metab/enzimas/MM-regresion.htm).

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O. drummondii in the Odiel Marshes Nature Reserve. This treatment involves a total annual input of 710 kg of potassium salt of glyphosate to the system.

DISCUSSION

Effectiveness of the method: Our results show that glyphosate is effective in eliminating *O. drummondii* at a dose of 20 g ai/L. This concentration is within values reported for other perennial exotic plants invading coastal dunes that were treated with Roundup® Ultra Plus, such as the ice plant *Carpobrotus* spp. (minimum dose = 50 g ai/L, Hernández *et al*. 2010) and acacia *Acacia saligna* (minimum dose = 20–50 g ai/L, García-de-Lomas *et al*. 2014). The minimum effective dose reported here exceeds the manufacturer's recommendation for "developed perennial plants" (5.4–9 g ai/L; https://www.roundup.es/productos_landing.php?pagina=3). In the case of Roundup® Ultra Plus, the manufacturer does not specify the doses recommended for *Oenothera drummondii* or other well-known invasive plants in Europe such as *Acacia* spp. and *Opuntia* spp. Our results reveal the importance of conducting pilot-scale trials as part of management planning (García-de-Lomas *et al*. 2014) to allow herbicide concentration to be adapted to each species and specific site conditions.

The differences in glyphosate-based formulations (active ingredients and surfactants) that are commercially available may lead to varying levels of effectiveness (Mann & Bidwell 1999). Therefore, the results obtained are "trademark-specific" and should not be extrapolated to other formulations that include glyphosate as the active ingredient.

The reinvasion of treated plots demonstrates the need for repeated treatments. This is not surprising considering: (i) previous *O. drummondii* records in close localities (Silvestre 1980) and the recent evolution of the habitat invaded (Morales *et al*. 2004) suggest it has been resident for around 35 years; (ii) the small seed size, which is related to the invasion potential of alien plants (Kolar & Lodge 2001); (iii) seed production may be high: individual capsules collected in the area of study showed up to 200 seeds/capsule, which is within values reported for the genus *Oenothera* (Harte 1994, Dietrich 1997); (iv) seed persistence may be high: although specific data for *O. drummondii* are unknown, some species of the genus (*O. biennis*) have remained viable after 80 years (Darlington & Steinbauer 1961, Baskin & Baskin 1994); and (v) the size of the invaded area and the density of invasion: data reported in García-de-Lomas *et al*. (2015) estimate the population size at 2.8 million adult plants and 4.2 million seedlings and young plants (diameter < 10 cm). In sum, small seeds produced by thousands to millions of plants for about 35 years suggest that the seed bank may be involved in reinvasion.

The theoretical absence of pre-emergence activity of glyphosate could promote the recovery of invaded areas by native plants. However, the significant decrease of invasive plant density in plots treated with more than 20 g ai/L (Table 2) suggests a possible unexpected pre-emergence activity, and may be positive for control of *O. drummondii*. Glyphosate absorption is related to the content of iron and aluminum amorphous oxides and organic matter in soil (Morillo *et al*. 2000). The substrate invaded by *O. drummondii* here is dominated by quartz and bioclastic debris (mainly Mollusca) (Borrego *et al*. 2000); thus, activity loss by absorption may be lower. Our finding agrees with the persistence of glyphosate and its degradation products in the soil several months after the herbicide application (Feng & Thompson 1990). Further studies will be required to assess whether this apparent residual effect applies equally to native and alien seeds.

Our results did not show any difference in the minimum dose between adult plants and seedlings. This was rather surprising, as herbaceous seedlings were expected to be more sensitive to chemical treatment than adult plants. According to the manufacturer's recommendations, doses for plants in the "early stage" are about half that for adult plants. One possible explanation is that the small size of seedlings mean that detection is more difficult for the herbicide applicator, and the amount of herbicide reaching seedlings was lower than for adult plants.

Costs: The feasibility of actions related to the management of biological invasions strongly depends on the resources available in the short and long term, although few studies include this evaluation (Kettenring & Adams 2011). Cost estimations are essential for planning control actions, especially in heavily invaded situations, since resources are required for an indefinite period. In highly invaded situations, there is some debate about whether it is more advantageous to focus upon larger core populations (Hulme 2003) or satellite populations (Mack & Lonsdale 2002), but financial constraints could tip the balance towards small-scale actions, even when the theoretical models may recommend otherwise.

The cost estimates (€1,317/ha/year) may be perceived as high but are relatively lower than other control exercises reported in the literature. The average cost for *Heracleum mantegazzianum* control in the UK in 1989–90 was US $1,500/ha for materials alone (Sampson 1994); for *Rhododendron ponticum*, costs reached US $90,000/ha (Compton & Key 1998) and cumulative costs for large areas can total US $70 million (Gritten 1995). Control costs for aquatic species such as *Eichhornia crassipes*, which infested 75 km of the Guadiana River (southwest Spain), rose to €8 million in 2004-2005 (Ruiz-Téllez *et al*. 2008) and accumulated costs between 2006 and 2012 have reached €21.7 million (Cifuentes 2012).

Importance of scale: Our results show that glyphosate is effective against *O. drummondii*. This result may be useful to control satellite populations. However, the assessment of this glyphosate-based strategy at full scale (including costs and feasibility) highlights the complexity of managing advanced invasions. A full-scale control strategy would involve an annual input of 710 kg/year glyphosate over 123 ha. Potential impacts for the accompanying biological community (e.g. terrestrial invertebrates, birds, hares) and for the adjacent marine environment should be considered. This is especially relevant in a protected area. Three factors could theoretically counteract the negative effects of the herbicide or its degradation products on the terrestrial environment of Odiel Marshes: (1) semidiurnal tides (low-mesotidal regime; with mean tidal range of 2.02 m); (2) an eastward longshore current (Benavente *et al*. 2005); and (3) the mouths of several rivers. These characteristics favour the dilution and water renewal, and therefore a low retention time for glyphosate residues that may eventually reach the marine environment. However, on the precautionary principle, the existence of a shellfish fishery (primarily cockles and clams) means that the potential effects of chemical treatment on the marine environment should be evaluated. Given these constraints, full-scale control is currently unfeasible until sufficient knowledge and resources are available and the hypothesised impact risks are understood. Alternatively, the containment of the invasion outside the area frequented by the

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public (swimmers and tourists) should be a priority, to reduce the risk of translocation to neighbouring locations.

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