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Germination of *Oenothera odorata*, endemic ruderal Onagraceae from Argentina

Germinación de *Oenothera odorata*, onagráceae ruderal endémica de Argentina

Chichizola GA, AE Rovere, SL Gonzalez

Abstract. The Onagraceae are a well-defined family of flowering plants much used by man. Many representatives of this group, which contains 75 species belonging to 8 genera, are indigenous to Argentina, and 19% of these are commonly found throughout the country. Many Onagraceae are ruderal and promote the subsequent establishment of other species, thus contributing to the restoration of degraded areas. Since it is important to know the characteristics of seeds to be used in restoration projects, we studied the basic germination physiology of *Oenothera odorata* Jacq., a ruderal plant indigenous to Argentina. Material was collected from roadsides and embankments along National Highway 40 in the municipality of San Carlos de Bariloche, which lies within the Patagonian phytogeographical Province. Road construction generates degraded areas where *O. odorata* could be used to initiate revegetation and consolidate the substrate. We evaluated percentage germination (G), mean germination time (MGT) and time until initiation of germination (TUG) of a control (C) and of the following pre-germination treatments: 45 days cold moist stratification (CMS) and mechanical scarification with a scalpel (MS). Germination percentages were high (98% CMS, 96% MS, 93% C), with differences recorded only between CMS and C. MGT was lower in the CMS treatment than the control, but similar to MS. TUG was 4 days for all treatments. In conclusion, this species lacks dormancy mechanisms under these experimental conditions, which, together with the fact that it grows in disturbed sites, is endemic, and captures heavy metals, makes it suitable for use in the ecological restoration of areas affected by transport systems.

Keywords: Dormancy; Steppe; Roadside; Restoration.

Resumen. Onagraceae es una familia bien definida de plantas con flores y muy utilizada por el hombre. En Argentina posee numerosas representantes indígenas, distribuidas en 8 géneros y 75 especies, siendo el 19% de las especies endémicas. Muchas onagráceas son ruderales y facilitan el establecimiento de especies más tardías, siendo útiles para la restauración de áreas degradadas. Dada la importancia de conocer las características de las semillas y de la germinación para su utilización en proyectos de restauración, se indagó sobre aspectos fisiológicos básicos de la germinación de *Oenothera odorata* Jacq., hierba ruderal endémica de Argentina. El material se colectó de taludes y bordes de la Ruta Nacional 40, dentro del éjido municipal de San Carlos de Bariloche, en la Provincia Fitogeográfica Patagónica. La construcción de rutas genera áreas degradadas denudadas de vegetación, donde la especie *O. odorata* podría utilizarse para iniciar los trabajos de revegetación y consolidar el sustrato. Se evaluó el porcentaje de germinación (G), el tiempo medio de germinación (MGT) y el tiempo hasta el inicio de la germinación (TUG) de un control (C) y de tratamientos pre-germinativos: estratificación húmeda fría de 45 días (CMS), escarificación mecánica con bisturí (MS). Los porcentajes de germinación fueron elevados (98% CMS, 96% MS, 93% C), solo se registraron diferencias entre CMS y C. El MGT fue menor en el tratamiento de CMS, respecto al C, pero fue similar al de MS. El TUG fue de 4 días para todos los tratamientos. En conclusión, la especie carece de mecanismos de dormición bajo las condiciones experimentales, lo que sumado al hecho de crecer en ambientes disturbados, ser endémica, y capturar metales pesados, la convierte en una especie propicia para utilizarse en trabajos de restauración ecológica en áreas afectadas por el transporte.

Palabras clave: Dormición; Estepa; Bordes de caminos; Restauración.

Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA, CONICET-UNCOMA). Quintral 1250. San Carlos de Bariloche (8400), Río Negro, Argentina.
Address correspondence to: Sofía González, Laboratorio Ecotono, Universidad Nacional del Comahue. Quintral 1250. 8400. San Carlos de Bariloche, Río Negro, Argentina., *e-mail:* sofia903@gmail.com
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INTRODUCTION

The Onagraceae family comprises 22 genera and approximately 657 species, they are found in both hemispheres (Raven, 1988; Zuloaga & Morrone, 1999; Wagner et al., 2007). The taxa of this family present different life forms, including shrubs, subshrubs and herbs, and different adaptations both in land and marsh environments (Romeo et al., 2008). It is a well-defined family, of great use to man (Dimitri, 1972). Various species are cultivated for ornamental purposes, such as those belonging to the *Fuchsia* L., *Godetia* Spach and *Oenothera* L. genera (Dimitri, 1972); the *Fuchsia*, and *Ludwigia* L. genera are used for the extraction of dyes (Romeo et al., 2008); and the *Oenothera* for medicinal purposes (Gambino & Vilela, 2011; Sing et al., 2012). Other species are used for remediation of soils contaminated with Cu, such as *Oenothera affinis* Cambess. (Gonzalez et al., 2008) and *Oenothera picensis* Phil. in Chile (Cornejo et al., 2017).

Many representatives of this family, 75 species belonging to 8 genera, are indigenous to Argentina, and 14 species are common in the country (Zuloaga & Morrone, 1999). *Oenothera* L. (1753), with 145 species, is the second biggest genus in Onagraceae, and although it is native only to America (Wagner et al., 2007), many of its species are widely naturalised in different areas of the planet (Dietrich, 1977; Hall et al., 1988; Mihulka et al., 2003; Wagner et al., 2007). The genus *Oenothera* contains annual, biennial and perennial plants, many of which are found in open habitats, such as abandoned fields, dunes, riversides and roadsides (Dietrich, 1977), and are considered ruderal plants (Crisafulli et al., 2013). Some of these species act as pioneers, naturally colonising disturbed environments (Mihulka et al., 2003) such as waste ground, roadsides (Hall et al., 1988) and rocky slopes (Bowers, 1987).

Within the *Oenothera* genus 8 species have been described which are found in more or less open communities in Patagonia (Cabrera, 1994). They frequently grow on the sea coast, on dunes and other sandy, stony areas, grassy and shrubby plains, riversides, roadsides and degraded areas (Cabrera, 1994), behaving as ruderal plants. Native ruderal species are suitable for use in the revegetation of degraded areas (Masini & Rovere, 2015), as is the case of *O. odorata* which occurs frequently in altered environments (Ezcurra & Brion, 2005). This species has been registered as spontaneously colonising abandoned quarries, together with abundant exotic species, in Chubut province, Argentina (Arce et al., 2015), and roadsides in Neuquén and Río Negro provinces (Pirk & Farji Brener, 2012).

In other countries where *O. odorata* is exotic, its use has been evaluated for remediation (Kim et al., 2009; Son et al., 2012). *Oenothera odorata* is a naturalised species that is widely distributed in Korea, including mining areas (Kim et al., 2009), and has been described as a potential species for the phytoremediation of soils in Korean mines contaminated with heavy metals (Kim et al., 1999; Son et al., 2012). Due to this particular characteristic the species is highly appropriate for use on roadsides, where the deposit of heavy metals from vehicle exhaust fumes is common (Atkins et al., 1982).

Roads are important in modern society, given that they offer corridors for the transport of goods and people through different landscapes, and increasing interest is therefore being shown in the effect of roads on local and regional environments (Rencht et al., 2005). The use of ecological restoration techniques employing native species on roadsides not only helps to stabilise the soil, forming a ground cover that protects against erosion (Tormo et al., 2007; Bochet et al., 2010a), but also helps to conserve local biological diversity. There are several advantages to using native species: they are already adapted to the environmental conditions, provide suitable habitats for other species of plants and animals, improve natural colonisation, and most importantly, their use can be transformed into a strategy to increase appreciation of their value and promote their conservation (Bochet et al., 2010b; Sabatino et al., 2015; Masini et al., 2016). Given native plant adaptation to local environmental conditions, the establishment of new populations of these species is one of the main strategies used in restoration (Abella et al., 2012; Sabatino et al., 2015; Masini et al., 2016). For this reason native species, and especially endemic ruderal species, should be used for the stabilisation of embankments and the revegetation of bare roadsides, ensuring not only traffic safety but also conservation of local biodiversity.

Different methods of active restoration can be used for the recuperation of plant communities in degraded sites, such as direct sowing in the field, the use of soil seed banks or the reintroduction of nursery-grown plants (Bainbridge, 2007). However, in order to implement any of these techniques, the germination strategies and appropriate cultivation techniques of the plants must been known (Ulian et al., 2008; Lai et al., 2016), since certain species may present mechanisms of seed dormancy, making reproduction more difficult to achieve (Baskin & Baskin, 2008).

With regard to dormancy, it is known that in environments where the wet season occurs in winter, like Patagonia (Paruelo et al., 1998), species that disperse their seeds in summer-autumn may present physiological dormancy (Figueroa & Jaksic, 2004). The seeds of plants with this characteristic should be exposed to stratification treatment, cold or warm, in order to end this latent state (Baskin & Baskin, 2004). This physiological dormancy mechanism is present in most temperate forest species (Rovere, 2006), such as *Fuchsia magellanica* Lam., whose seeds require cold, humid stratification in order to germinate (Figueroa et al., 1996). In arid environments physical dormancy is also frequently observed in some botanical families (e.g., Fabaceae and Chenopodiaceae) and can be broken by means of chemical or mechanical scarification (Baskin & Baskin, 2004; Beider, 2012).

The objective of this work was to characterise the size and weight of *Oenothera odorata* seeds, and determine the presence of physiological or physical dormancy in their germination.

MATERIALS AND METHODS

Species description. *Oenothera odorata* (Fig. 1), a species indigenous to Argentina, is widely distributed throughout Patagonia in Neuquén, Río Negro, Chubut and Santa Cruz provinces. It is also found in the provinces of Buenos Aires, La Pampa, Mendoza, the east of Córdoba, the south of San Juan and in Chile (Zuloaga et al., 2008). This species generally grows between 0 and 1000 m a.s.l., reaching an altitude of 2800 m only in the cordillera of Mendoza (Dietrich, 1977). It is present in altered environments of Nahuel Huapi National Park (Ezcurra & Brion, 2005), in sandy or stony ground, and is frequently found at roadsides in the Patagonian steppe (Green & Ferreyra, 2012) and at the edges of forests (Bisheimer, 2012). The plant is annual (Green & Ferreyra, 2012), biennial, or short-lived perennial (Kiesling, 2003), from 25 to 80 cm in height, and forms a simple rosette or has a main stem and lateral stems, either prostrate or arched, which ascend from the rosette (Dietrich, 1977; Kiesling, 2003). The basal leaves are linear or narrowly oblanceolate, while the upper leaves are linear, narrowly lanceolate, with a short leafstalk (Green & Ferreyra, 2012). The corolla is yellow, actinomorphic, and over 1 cm in depth; the nectar has a high sugar concentration and proportion of sucrose, which corresponds to the flower type visited by nocturnal insects (Chalcoff et al., 2006). The fruit is a cylindrical capsule (Bisheimer, 2012) of 3-5 cm x 0.3-0.4 cm, with numerous light brown ellipsoid seeds (Dimitri, 1972).

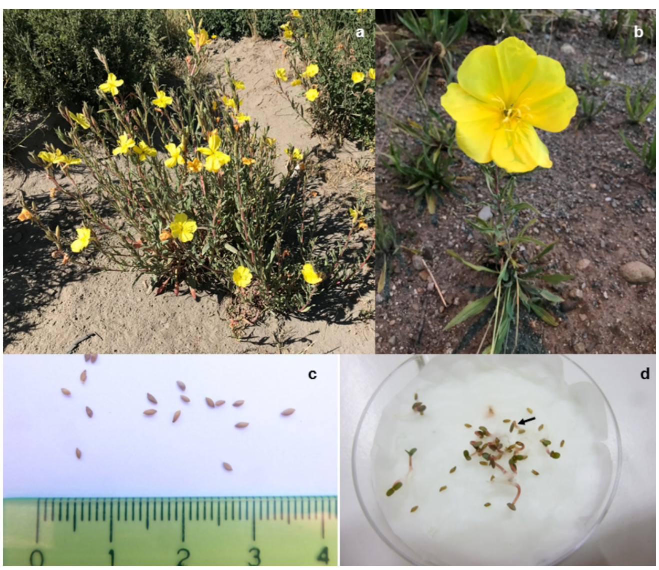

Fig. 1. *Oenothera odorata* (a) Individual during flowering, (b) flower, (c) seeds with a cm scale, (d) germinated seed (arrow) during germination assay.

Fig. 1. *Oenothera odorata* (a) Ejemplar durante la floración, (b) detalle de la flor, (c) semillas junto a una escala en cm, (d) semillas germinadas (indicada con flecha) durante el ensayo de germinación.

In popular medicine this plant is used to treat rheumatism, stomach pain and diarrhoea (Green & Ferreyra, 2012), and dermatological uses have also been investigated (Choi et al., 2010). It is an ornamental species (Dimitri, 1972) suitable for arid sites, due to its large, luminous yellow flowers, its extensive flowering period during summer and its perfume (Green & Ferreyra, 2012). Its use in xero-gardening has been registered in Mendoza (Dalmasso et al., 2009).

The authors of the genera and species mentioned in this publication were abbreviated according to The International Plant Names Index (2012).

Study site. The study was carried out on the eastern side of Nahuel Huapi National Park, in the north of Patagonia, Argentina (41° 07´ 12" S, 71° 13´ 23" W). Sampling took place in a steppe zone, on both sides of National Highway 40 (2 ha). Disturbance of the area is principally due to the presence of this road. The sampling site is homogenous in terms of vegetation within the area, and also altitude, with an average of 838 m a.s.l. The annual average temperature in the area is 8 °C and annual average precipitation is 600 mm. The vegetation presents both native species typical of the area (e.g., *Pappostipa speciosa* (Trin. & Rupr.) Romasch., *Mulinum spinosum* Pers., and *Oenothera odorata*), and exotic herbs (e.g., *Rumex acetosella* L., *Bromus tectorum* L., *Carduus thoermeri* Weinm. and *Verbascum thapsus* L.) (Correa, 1969-1999).

Collecting, cleaning and storage of seeds. Mature seed capsules from 30 *Oenothera odorata* plants were collected in March 2016. The seeds were removed in the laboratory and left to dry at room temperature. Once dry, all the seeds were gathered into one lot and stored for 6 months in a labelled paper bag in a refrigerator at 5 °C, in the dark, until the germination assay was carried out. Seeds that seemed viable by appearance were used for the measurements, weights and assays described here, while those which appeared empty (flattened shape) were discarded.

Seed dimensions, shape and weight. Using digital callipers we measured the maximum width, thickness, and maximum length of each of a sample of 100 seeds (Sanchez et al., 2002). Seed dimensions (length, width and thickness) were used to determine seed shape; that is, the variance in the three dimensions. Each value was divided by the highest of the three values. The variance has a minimum value of 0 in seeds which are perfectly spherical in shape, and a maximum value of 1 in needle- or disc-shaped seeds (Thompson et al., 1993; Pérez-Harguindeguy, 2013). In order to determine seed weight, a total of 10 lots of 100 seeds were weighed using a precision balance.

Germination assay and seed viability. Before conducting the germination assay, two pre-germination treatments were applied: (1) cold moist stratification for 45 days (CMS), the seeds being placed between layers of cotton wool moistened with a fungicide solution of copper oxychloride, then put inside hermetically sealed plastic bags and kept in a refrigerator at 5 °C, in darkness; (2) mechanical scarification with a scalpel (MS), involving cutting of the tegument without damaging the endosperm. For each treatment and the control (C) 10 repetitions of 30 seeds were carried out, seeds having been chosen at random from the lot. The seeds were disinfected with a 2% sodium hypochlorite solution for 2 minutes, and then rinsed under running water for 2 minutes before being put in transparent plastic Petri dishes. The seeds were placed on filter paper discs and cotton wool, both of which had been moistened with distilled water and fungicide solution. The assay was conducted in a germination chamber under controlled light and temperature conditions: 12 h light/12 h darkness, at 20/10 °C, respectively. This assay lasted 56 days and germination was checked every 4 days, when seeds were watered with a solution of distilled water and fungicide. A seed was considered to have germinated when at least 2 mm of radicle had emerged from the tegument.

At the end of the germination assay a viability test was carried out on the seeds which had not germinated, by means of a cut test, where a transversal cut was made with a scalpel and the state of the internal tissues observed in order to distinguish those which were definitely not viable, or dead, from those which were probably viable (Gosling, 2003). The seeds were classified as empty, attacked by fungi, infected (attacked by insects) and viable (turgid).

Data analysis. In order to evaluate the pre-germination treatments applied, the final germination percentage was determined (G) and the mean germination time (MGT) for each treatment and the control. The final germination percentage for each repetition was calculated using the formula G=g/ (g+vs+f), where g is the number of germinated seeds, vs is the number of viable seeds according to the cut test and f is the number of seeds attacked by fungi (Gosling, 2003). The seeds which did not have germination potential were not taken into account in this calculation; seeds which appeared to be empty or infected (attacked by insects) in the cut test were excluded.

Mean germination time (MGT) was calculated for each treatment, which indicates the average number of days taken for one seed to germinate. The equation used to obtain MGT was based on the proposal made by Khajeh-Hosseini et al. (2003):

$$\text{MGT} = \frac{\sum_{(i=1)}^{n} fi\ xi}{\sum_{(i=1)}^{n} xi}$$

Where *fi* is the number of days since the beginning of the germination assay, and *xi* is the number of seeds that germinated within certain consecutive intervals of time (4 days).

Also calculated was time until germination (TUG), taken as the number of days that passed until germination began, for the control and the different treatments carried out (Méndez, 2007).

Since the data for the variables G and MGT did not comply with the assumptions of normality and homogeneity of variance, even when the data were arcsine transformed, the treatment means were compared using the Kruskal-Wallis non-parametric test. In the cases where significant differences were found, pairwise multiple comparisons of the means (Siegel & Castellan, 1995) were performed using the SPSS 23 package for Windows. For the TUG data no statistical analysis was carried out, since the same values were found for the treatments and their repetitions.

Table 1. Seed dimensions (mean ± SD, N=100) and weight of 100 seeds (N=10) of *Oenothera odorata*.
Tabla 1. Dimensiones de una semilla (media ± DE, N=100) y peso de un lote de 100 semillas (N=10) de *Oenothera odorata*.

	Oenothera odorata
Length (mm)	1.84 ± 0.19
Width (mm)	0.79 ± 0.08
Thickness (mm)	0.70 ± 0.08
Weight (g)	0.044 ± 0.002

RESULTS

Seed dimensions, shape and weight. Seeds dimensions and weight are shown in Table 1. Variance for the seeds was 0.08, a value close to 0, indicating that the seeds are ovoid in shape, more spherical than flattened.

Germination. Significant differences were found between treatments, both for percentage germination (H = 7.050; P = 0.029) and MGT (H = 9.388; P = 0.009), (Fig. 2, Fig. 3). For percentage germination and MGT the only treatments that differed from each other were C and CMS, the highest percentage germination value having been obtained for CMS and the lowest mean germination time for CMS (Fig. 2, Fig. 3). With regard to TUG, the seeds in the control and in the pre-germination treatments began to germinate after 4 days (Fig. 4).

Germination progress was analysed using cumulative germination curves as a function of time (Fig. 4). The curve shows that germination occurred rapidly during the first 4 days of the assay. Similar progress was found in all treatments: C seeds became stabilised and reached their highest accumulated germination percentage 24 days after the start of the assay, while the CMS treatment reached this point after 4 days, and the MS treatment after 12 days (Fig. 4).

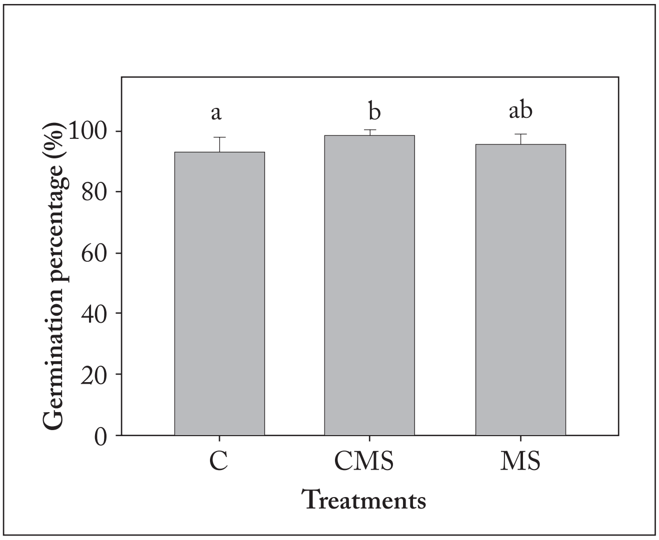

Fig. 2. *Oenothera odorata* germination percentage (mean ± SD) in control (C), cold moist stratification (CMS) and mechanical scarification with scalpel (MS) treatments. Different letters indicate statistically significant differences between treatments (P<0.05).

Fig. 2. Porcentaje de germinación de semillas *Oenothera odorata* (media ± DE), en el control (C), estratificación húmeda fría (CMS) y escarificación mecánica con bisturí (MS). Letras diferentes indican diferencias estadísticamente significativas entre tratamientos (P<0,05).

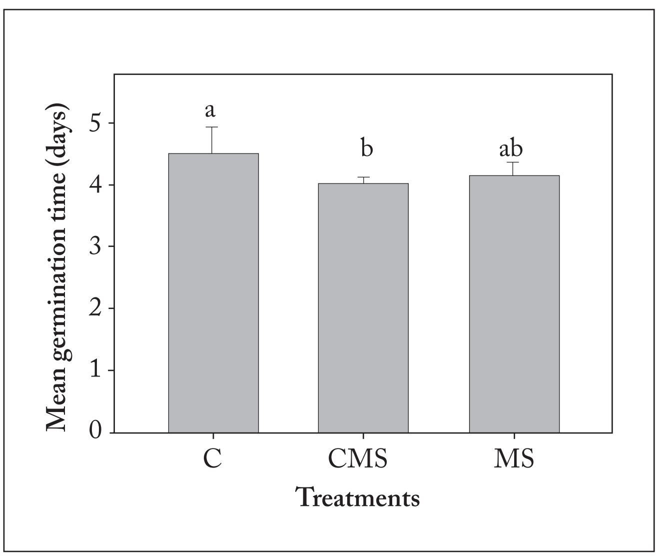

Fig. 3. Mean germination time (MGT) (mean ± SD) of *Oenothera odorata* seeds in control (C), cold moist stratification (CMS), and mechanical scarification (MS) with scalpel treatments. Different letters indicate statistically significant differences between treatments (P<0.05).

Fig. 3. Tiempo medio de germinación (MGT) (media ± DE) de semillas de *Oenothera odorata* en el control (C) y los tratamientos de estratificación húmeda fría (CMS) y escarificación mecánica (MS) con bisturí. Letras diferentes indican diferencias estadísticamente significativas entre tratamientos (P<0,05).

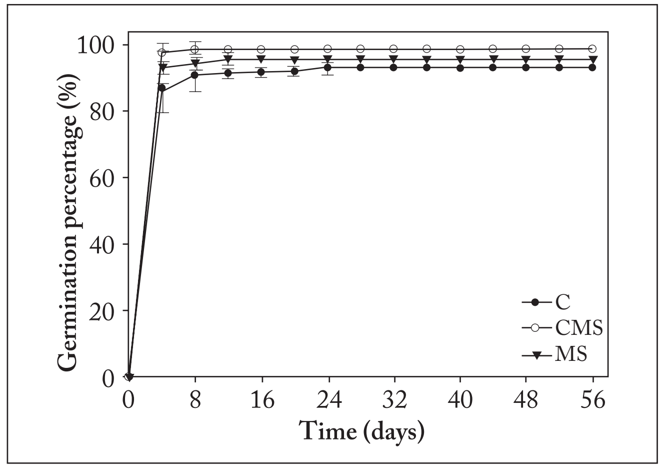

Fig. 4. Cumulative percentage germination of *Oenothera odorata* (mean ± SD) in control (C), cold moist stratification (CMS) and mechanical scarification with scalpel (MS) treatments.

Fig.4. Porcentaje de germinación acumulada de *Oenothera odorata* (media ± DE), en el tiempo para el control (C), estratificación húmeda fría (CMS) y escarificación mecánica con bisturí (MS)

DISCUSSION

Oenothera odorata seeds did not present dormancy mechanisms under the conditions evaluated. Following a cold storage period, and under the pre-germination treatments applied, germination percentages were high, and similar to the control. The scarification treatment presented values similar to the stratification treatment and the control. However, differences were observed in results from the cold moist stratification treatment, which were higher than the control. Also, cold moist stratification accelerated *O. odorata* seed germination making it more uniform over time. Both aspects are important for the development of plants in nurseries since the production and the growing season are maximized (Rovere, 2006).

The absence of dormancy mechanisms and high germination percentages have been found in other *Oenothera* species, such as *O. curtiflora* W.L. Wagner & Hoch, *O. glazioviana* Micheli (Baskin & Baskin, 2014) and *O. affinis* (Royal Botanic Gardens Kew, 2008). Seeds from these species present 100% germination with no specific treatment, and germinate rapidly, in 14 days, whether at constant or alternating temperatures. The high percentage of germination and speed of germination recorded for *O. odorata* have not been documented for other species of the genus. In the cumulative germination curve of *O. odorata,* the stratification treatment achieved the highest percentage of germination in the first week (8 days). Seeds of other *Oenothera* species germinate more slowly, with germination percentages depending on the temperature they are exposed to. An example of this is *O. acaulis* Cav., which achieves 59% germination after 77 days at a constant temperature of 15 °C; 72% after 70 days at a constant temperature of 20 °C and 100% after 70 days at 21 °C (Royal Botanic Gardens Kew, 2008). Other species, such as *O. macrocarpa* Pursh and *O. pilosella* Raf., present physiological dormancy which can be broken with cold stratification, while in *O. laciniata* Hill and *O. drummondii* Walp. dormancy can be broken with moist stratification (Baskin & Baskin, 2014). It has been reported that *O. biennis* presents 93% germination following scarification with scalpel and the application of gibberellins acid at alternating temperatures of 33/19 °C (Royal Botanic Gardens Kew, 2008); other authors have reported that *O. biennis* presents physiological dormancy that can be broken by cold stratification (Baskin & Baskin, 1994). In *O. odorata* the cold moist stratification and alternating temperatures and photoperiod of 20 light /10 °C darkness were adequate to achieve high percentages of germination.

The weight of the *O. odorata* seeds (weight of 1000 seeds extrapolated from the data: 0.44 g) lies within the range documented for other species of this genus, such as *O. affinis* (0.39 g), *O. biennis* (0.40 g) and *O. parviflora* L. (0.54 g) (Royal Botanic Gardens Kew, 2008). Seed weight and shape are key regenerative characteristics and knowledge of these is essential to our understanding of the reproductive biology and establishment strategies of plant species: they provide information on dispersion capacity and mechanisms (Fenner & Thompson, 2005), the risk of predation, and possible interaction with granivorous species (Pirk & López de Casenave, 2010). Lighter seeds tend to be dispersed over a wider distance than heavier seeds, and smaller-sized, more spherical seeds become buried in the soil more easily than larger ones, thus avoiding predation (Thompson et al., 1993).

Oenothera odorata seeds are ovoid in shape, more spherical than elongated, and this, along with their weight, indicates that a persistent seed bank could be formed. The size and shape of seeds are important since these characteristics are related to longevity, and therefore persistence in the seed bank (Thompson et al., 1993; Funes et al., 1999; Traba et al., 2006). This ecological information is relevant to the management and restoration of vegetation, whether in forest environments (van der Valk & Verhoeven, 1988) or the steppe (Gonzalez & Germandi, 2008). Onagraceae seeds are generally orthodox, and remain viable for variable time periods when they are stored (Royal Botanical Gardens Kew, 2008) or form part of soil seed banks (Evans et al., 2006). *Oenothera biennis* seeds can retain viability in soil for over 80 years (Darlington & Steinbauer, 1961), and develop an aerial seed bank consisting of seeds remaining on the plant (Evans et al., 2006). These characteristics are not known for *O. odorata,* and could be the subject of further research.

During this study *O. odorata* seeds presented high germination values under the photoperiod of 12/12 hours of light and darkness. It is known that species of the *Oenothera* genus generally require light for germination, and that seedlings can become established in different types of bare soil (Mihulka et al., 2003). That light is required for germination is a frequent characteristic of species like *O. odorata,* which grow in open or disturbed habitats (Harper, 1977). Several Onagraceae (*Oenothera biennis*, *O. fallax* Renner and *O. glazioviana*) that colonise artificial habitats such as roadsides or train tracks can produce new shoots following severe damage, which in disturbed conditions may represent an alternative strategy to regeneration from the seed bank (Martínková et al., 2006).

Germination is a process of extreme importance, given that it marks the beginning of a lifecycle (Ruiz et al., 2013). Although this work evaluates *O. odorata* germination under laboratory conditions, the results are useful both for plant propagation and sowing in the field. Future research could study conditions for successful establishment in the field. In addition, the *O. odorata* type of taproot (Rovere, personal observation) optimises the capture of resources in sites with high hydric stress, such as roadside embankments (Valladares et al., 2011). The fact that the seeds of this species are orthodox (Royal Botanic Gardens Kew, 2008) and have no dormancy mechanisms means they can be stored in germplasm banks and propagated easily.

Given the importance of integrating degraded areas into the environment or natural landscape, and considering revegetation with native plants and interaction with local fauna (Menz et al., 2011; Sabatino et al., 2015), the use of *O. odorata* for revegetation would afford functional integration due to its interaction with other species, as a food source for the only species of leafcutter ant in Patagonia (Pirk & Farji-Brener, 2012), and for the nocturnal insects that pollinate it (Chalcoff et al., 2006). Furthermore, *O. odorata* has been registered as a valuable species for bees in northwest Chubut (Forcone & Kutschker, 2006; Forcone & Ruppel, 2012), and could therefore be of interest to beekeepers.

Finally, due to the considerable environmental impact of road construction (Garcia Palacios et al., 2010), the lack of basic information on the ecology of areas degraded by road infrastructure (Bradshaw & Huttl, 2001) and the little known of possible strategies to recover these environments (Matesanz et al., 2006), the present study makes a practical contribution to facilitate the use of a native species for this purpose.

CONCLUSIONS

Oenothera odorata presents high percentages of germination, no specific requirements for germination and the capacity to fix heavy metals. These characteristics make it a key species for use in ecological restoration work in degraded sites affected by traffic. As this species is a low herb it does not cause visual interference for vehicles, and at the same time helps to integrate the disruptive element (e.g., road system, train tracks) into the landscape and sustains interactions with local fauna involving herbivory and pollination, thus contributing to restoration of the original habitat and preservation of regional biodiversity. This work constitutes a new contribution to the study of an Onagraceae species that is native to Argentina, and the results can be applied to *in situ* conservation by means of different restoration techniques.

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REFERENCES

Abella, S.R., D.J. Craig, S.D. Smith & A.C. Newton (2012). Identifying native vegetation for reducing exotic species during the restoration of desert ecosystems. *Restoration Ecology* 20: 781-787.

Arce, M.E., V. Walicki, I. Castro, M.F. Valenzuela, A. de Sosa Tomas, M.G. Rack & L. Ferro (2015). Evaluación de la revegetación natural en canteras de áridos en dos sitios de la provincia de Chubut. In: Martínez Carretero, E. and Dalmasso, A.D. (eds), pp. 355-383. Restauración Ecológica en la Diagonal Árida de la Argentina 2. IADIZA, Mendoza, Argentina. 483 p.

Atkins, D.P., I.G. Trueman, C.B. Clarke & A.D. Bradshaw (1982). The evolution of lead tolerance by *Festuca rubra* on a motorway verge. *Environmental Pollution* 27: 233-241.

Bainbridge, D. (2007). A Guide for Desert and Dryland Restoration: New hope for Arid Lands. Island press, Washington. 391 p.

Baskin, C.C. & J.M. Baskin (1994). Germination requirements of *Oenothera biennis* seeds during burial under natural seasonal temperature cycles. *Canadian Journal of Botany* 72: 779-782.

Baskin, C.C. & J.M. Baskin (2014). Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. Elsevier Inc., San Diego. 1586 p.

Baskin, C.C. & J.M. Baskin (2004). A classification system for seed dormancy. *Seed Science Research* 14: 1-16.

Baskin, J. M. & C.C. Baskin (2008). Some considerations for adoption of Nikolaeva's formula system into seed dormancy classification. *Seed Science Research* 18: 131-137.

Beider, A. (2012). Viverización de especies nativas de zonas áridas. *Experimentia - Revista de Transferencia Científica* 2: 9-67.

Bochet, E., P. García-Fayos & J. Tormo (2010a). How can we control erosion of roadslopes in semiarid Mediterranean areas? Soil improvement and native plant establishment. *Land Degradation & Development* 21: 110-121.

Bisheimer, M.V. (2012). Flores de la Patagonia Argentina. Editorial de Autor, Neuquén. 244 p.

Bochet, E., J. Tormo & P. García-Fayos (2010b). Native species for roadslope revegetation: selection, validation, and cost effectiveness. *Restoration ecology* 18: 656-663.

Bowers, J.E. (1987). 100 Roadside Wildflowers of Southwest Woodlands. Western National Parks Association. Tucson, AZ.

Bradshaw, A.D. & R.F. Huttl (2001). Future minesite restoration involves a broader approach. *Ecological engineering* 17: 87-90.

Cabrera, A.L. (1994). Regiones fitogeográficas argentinas. In: Kugler W.F. (ed) pp, 1-85. Enciclopedia Argentina de agricultura y jardinería, Tomo II, Fascículo 1: regiones fitogeográficas Argentinas. ACME, Buenos Aires. 85 p.

Chalcoff, V.R., M.A. Aizen & L. Galetto (2005). Nectar concentration and composition of 26 species from the temperate forest of South America. *Annals of Botany* 97: 413-421.

Choi, M.E., W.H. Woo & Y.J. Mun (2010). Inhibitory Effects of Herb and Seeds of *Oenothera odorata* on the Melanogenesis. *The Journal of Korean Medicine Ophthalmology and Otolaryngology and Dermatology* 23(1): 149-157.

Cornejo, P., S. Meier, S. García, N. Ferrol, P. Durán, F. Borie & A. Seguel (2017). Contribution of inoculation with arbuscular mycorrhizal fungi to the bioremediation of a copper contaminated soil using *Oenothera picensis*. *Journal of Soil Science and Plant Nutrition* 17: 14-21.

Correa, M.N. (1969-1999). Flora Patagónica. Colección científica. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Crisafulli, A., R.M. Picone & A. Soldano (2013). *Oenothera indecora* (Onagraceae) a neophyte new to Italy. *Flora Mediterranea* 23: 87-92.

Dalmasso, A.D., R. Candia & C. Ganci (2009). La Xerojardinería con especies nativas. Fundación CRICYT, Mendoza, Argentina. 80 p.

Darlington, H.T. & G.P. Steinbauer (1961). The eighty-year period for Dr. Beal's seed viability experiment. *American Journal of Botany* 48: 321-325.

Dietrich, W. (1977). The South American species of *Oenothera* sect. *Oenothera* (Raimannia, Renneria; Onagraceae). *Annals of the Missouri Botanical Garden* 64: 425-626.

Dietrich, W., W.L. Wagner & P.H. Raven (1997). Systematics of *Oenothera* section *Oenothera* subsection *Oenothera* (Onagraceae). Sistemática de *Oenothera* sección *Oenothera* subsección *Oenothera* (Onagraceae). *Systematic Botany* 50: 12-34.

Dimitri, M.J. (1972). Enciclopedia Argentina de Agricultura y Jardinería. Tomo 1, Vol. 1. Editorial ACME SACI, Buenos Aires, Argentina. 1161 p.

Evans, M.E., R. Ferriere, M.J. Kane & D.L. Venable (2006). Bet hedging via seed banking in desert evening primroses (*Oenothera*, Onagraceae): demographic evidence from natural populations. *The American Naturalist* 169: 184-194.

Ezcurra, C. & C. Brion (2005). Plantas del Nahuel Huapi. Catálogo de la Flora Vascular del Parque Nacional Nahuel Huapi, Argentina. Universidad Nacional del Comahue-Red Latinoamericana de Botánica, Bariloche, Argentina. 70 p.

Figueroa, J.A. & F.M. Jaksic (2004). Latencia y banco de semillas en plantas de la región mediterránea de Chile central. *Revista Chilena de Historia Natural* 77: 201-215.

Figueroa, J., J.J. Armesto & J.F. Hernández (1996). Estrategias de germinación y latencia de semilla en especies del bosque templado de Chiloé, Chile. *Revista Chilena de Historia Natural* 69: 243-251.

Forcone, A. & A. Kutschker (2006). Floración de las especies de interés apícola en el noroeste de Chubut, Argentina. *Revista del Museo Argentino de Ciencias Naturales nueva serie* 8: 151-157.

Forcone, A. & S. Ruppel (2012). Polen de interés apícola del Noroeste de Santa Cruz (Patagonia Argentina): aspectos morfológicos. *Boletín de la Sociedad Argentina de Botánica* 47: 77-86.

Funes, G., S. Basconcelo, S. Díaz & M. Cabido (1999). Seed size and shape are good predictors of seed persistence in soil in temperate mountain grasslands of Argentina. *Seed Science Research* 9: 341-345.

Gambino, P. & A. Vilela (2011). Morphological traits and allocation patterns related to stress-tolerance and seed-yield in wild and domesticated evening primrose (*Oenothera* L. Onagraceae). *Industrial Crops and Products* 34: 1269-1276.

García-Palacios, P., S. Soliveres, F.T. Maestre, A. Escudero, A.P. Castillo-Monroy & F. Valladares (2010). Dominant plant species modulate responses to hydroseeding, irrigation and fertilization during the restoration of semiarid motorway slopes. *Ecological Engineering* 36: 1290-1298.

Gonzalez, S. & L. Ghermandi (2008). Postfire seed bank dynamics in semiarid grasslands. *Plant Ecology* 199: 175-185.

González, I., V. Muena, M. Cisternas & A. Neaman (2008). Acumulación de cobre en una comunidad vegetal afectada por contaminación minera en el valle de Puchuncaví, Chile central. *Revista Chilena de Historia Natural* 81: 279-291.

Gosling, P.G. (2003). Viability Testing. In: Smith, R.D. Dickie, J.B, Linington, S.H., Pritchard H.W. & Probert R.J. (eds.), pp. 445-481. Seed Conservation: turning science into practice, The Royal Botanic Gardens. Kew, Great Britain. 994 p.

Green, L. & M. Ferreyra (2012). Flores de la estepa patagónica. Vázquez. Mazzini Editores. Buenos Aires, Argentina. 288 p.

Hall, I.V., E. Steiner, P. Threadgill & R.W. Jones (1988). The Biology of Canadian Weeds: 84. *Oenothera biennis* L. *Canadian Journal of Plant Science* 68: 163-173.

Harper, J.L. (1977). Population Biology of Plants. Academic Press, London, UK. 1977 pp.xxii + 892 p.

Khajeh-Hosseini, M., A.A. Powell & I.J. Bingham (2003). The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Science and Technology* 21: 715-725.

Kiesling. R. (2003). Flora de San Juan. República Argentina. Volume II: multiple-leaf dicotyledons (second part: Oxalidaceae and Umbelliferae). Estudio Sigma. 257 p.

Kim, D.Y., H. Park, S.H. Lee, N. Koo & J.G. Kim (2009). Arsenate tolerance mechanism of *Oenothera odorata* from a mine population involves the induction of phytochelatins in roots. *Chemosphere* 75: 505-512.

Kim, J.G., S.K. Lim, S.H. Lee, Y.M. Yoon, C.H. Lee & C.Y. Jeong (1999). Evaluation of heavy metal pollution and plant survey around inactive and abandoned mining areas for phytoremediation of heavy metal contaminated soils. *Korean Journal Environmental Agricultural* 18: 28-34.

Lai, L., L. Chen, L. Jiang, J. Zhou, Y. Zheng & H. Shimizu (2016). Seed germination of seven desert plants and implications for vegetation restoration. *AoB PLANTS* 8: plw031.

Martínková, J., J. Klimešová & S. Mihulka (2006). Vegetative regeneration of biennial *Oenothera* species after disturbance: Field observations and experiment. *Flora-Morphology, Distribution, Functional Ecology of Plants* 201: 287-297.

Masini, A.C.A. & A.E. Rovere (2015). Requerimientos germinativos de *Sphaeralcea mendocina* Philippi (Malvaceae), especie ruderal endémica de Argentina. In: Martínez Carretero, E. & Dalmasso, A.D. (eds), pp. 203-213. Restauración Ecológica en la Diagonal Árida de la Argentina 2. IADIZA, Mendoza, Argentina. 483 p.

Masini. A.C.A., A.E. Rovere & G.I. Pirk (2016). Germinación de *Gutierrezia solbrigii* y *Senecio subulatus*, asteráceas endémicas de Argentina. *Phyton International Journal of Experimental Botany* 85: 314-323.

Matesanz, S., F. Valladares, D. Tena, M. Costa-Tenorio & D. Bote (2006). Early dynamics of plant communities on revegetated motorway slopes from southern Spain: is hydroseeding always needed? *Restoration Ecology* 14: 297-307.

Méndez, E. (2007). Germination of *Denmoza rhodacantha* (Salm-Dyck) Britton & Rose (Cactaceae). *Journal of Arid Environments* 68: 678-682.

Menz, M.H.M., R.D. Phillips, R. Winfree, C. Kremen, M.A. Aizen, S.D. Johnson & K.W. Dixon (2011). Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. *Trends in Plant Science* 16: 4-12.

Mermoz, M., C. Úbeda, D. Grigera, C. Brion, C. Martin, E. Bianchi & H. Planas (2009). El Parque Nacional Nahuel Huapi: sus características y estado de conservación. Editorial Administración de Parque Nacionales, Parque Nacional Nahuel Huapi, San Carlos de Bariloche, Argentina. 80 p.

Mihulka, S., P. Pyšek & J. Martínková (2003). Invasiveness of *Oenothera* congeners in Europe related to seed characteristics. Plant invasions: ecological threats and management solutions. Backhuys Publishers, Leiden, pp. 213-215.

Miller, J.H. & K.V. Miller (2005). Forest plants of the southeast and their wildlife uses. Rev. ed. Athens: University of Georgia Press. 454 p.

Paruelo, J.M., A. Beltrán, E. Jobbágy, O.E. Sala & R.A. Golluscio (1998). The climate of Patagonia: general patterns and controls on biotic processes. *Ecología Austral* 8: 85-101.

Perez-Harguindeguy, N., S. Diaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, et al. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of botany* 61: 167-234.

Pirk, G.I. & A.G. Farji-Brener (2012). Foliar herbivory and its effects on plant growth in native and exotic species in the Patagonian steppe. *Ecological Research* 27: 903-912.

Pirk, G.I. & J.López de Casenave (2010). Influence of seed size on feeding preferences and diet composition of three sympatric harvester ants in the central Monte Desert, Argentina. *Ecological Research* 25: 439-445.

Raven, P.H. (1988). Onagraceae as a model of plant evolution. In: Gottlieb L.D & Jain S.K. (eds), pp. 85–107. In Plant evolutionary biology. Springer, Dordrecht. 414 p.

Rentch, J.S., R.H. Fortney, S.L. Stephenson, H.S. Adams, W.N. Grafton & J.T. Anderson (2005). Vegetation–site relationships of roadside plant communities in West Virginia, USA. *Journal of Applied Ecology* 42: 129-138.

Romeo, R.A., A.C. Sánchez & L. Novara (2008). Onagraceae. *Aportes Botánicos de Salta-Serie Flora* 8: 1-48.

Rovere, A.E. (2006). Cultivo de Plantas Nativas Patagónicas: árboles y arbustos. Editorial Caleuche, San Carlos de Bariloche, Argentina. 54 p.

Royal Botanic Gardens Kew (2008). Seed Information Database (SID). Version 7.1. http://data.kew.org/sid/SidServlet?ID=16340&Num=19X. Viewed: January 10, 2018.

Ruiz, M., A. Golberg & M.L. Molas (2013). From seed to seedling: an ecophysiological point of view. In: Busso, C.A. (ed), pp. 3-26. From seed germination to young plants. Ecology, Growth and environmental influences. Nova Biomedical. New York. 369 p.

Sabatino, C.M., A.E. Rovere & N. Maceira (2015). Germinación de *Eryngium regnellii:* especie clave para la restauración ecológica de las interacciones planta-polinizador en la Pampa Austral (Buenos Aires, Argentina). *Phyton International Journal of Experimental Botany* 84: 434-442.

Sanchez, A.M., F.M. Azcarate, L. Arqueros & B. Peco (2002). Volumen y dimensiones como predictores del peso de semilla de especies herbáceas del centro de la Península Ibérica. *Anales Jardín Botánico de Madrid* 59: 249-262.

Shim, S.R. & J.H. Kim (2006). Vegetation Characteristics of Main Herbaceous Flowers for Ecological Restoration. *Journal of the Korea Society of Environmental Restoration Technology* 9: 64-71.
Siegel, S. & N.J. Castellan (1995). Estadística No Paramétrica: Aplicada a Las Ciencias de La Conducta, Trillas, México. 437 p.
Singh, S., R. Kaur & S.K. Sharma (2012). An updated review on the *Oenothera* genus. *Journal of Chinese Integrative Medicine* 10: 717-725.
Son, K.H., D.Y. Kim, N. Koo, K.R. Kim, J.G. Kim & G. Owens (2012). Detoxification through phytochelatin synthesis in *Oenothera odorata* exposed to Cd solutions. *Environmental and Experimental Botany* 75: 9-15.
The International Plant Names Index (2012). The International Plant Names Index. Disponible en: http://www.ipni.org. Accedido March 1, 2018.
Thompson, K.B.S.R., S.R. Band & J.G. Hodgson (1993). Seed size and shape predict persistence in soil. *Functional Ecology* 7: 236-241.
Traba, J., F.M. Azcárate & B. Peco (2006). The fate of seeds in Mediterranean soil seed banks in relation to their traits. *Journal of Vegetation Science* 17: 5-10.
Tormo, J., E. Bochet & P. García-Fayos (2007). Roadfill revegetation in semiarid Mediterranean environments. Part II: topsoiling, species selection, and hydroseeding. *Restoration Ecology* 15: 97-102.
Ulian, T., A.E. Rovere & B. Muñoz (2008). Taller sobre conservación de semillas para la restauración ecológica. *Ecosistemas* 17: 147-148.
Valladares, F., L. Balaguer, I. Mola, A. Escudero & V. Alfaya (2011). Restauración ecológica de aéreas afectadas por infraestructuras de transporte. Base científica para soluciones técnicas. Fundación Biodiversidad, Madrid, España. 324 p.
van der Valk, A.G. & J.T.A. Verhoeven (1988). The restoration of quaking fens from floating forests: the role of the seed bank and understory species. *Vegetatio* 76: 3-13.
Wagner, W.L., P.C. Hoch & P.H. Raven (2007). Revised classification of the Onagraceae. The American Society of Plant Taxonomists. *Systematic Botany Monographs* 83: 1-240.
Zuloaga, F. & O. Morrone (1999). Catálogo de las Plantas Vasculares de la República Argentina II. *Monographs in Systematic Botany from the Missouri Botanic Garden* 74: 879-891.
Zuloaga, F.O., O. Morrone & M.J. Belgrano (2008). Catálogo de las Plantas Vasculares del Cono Sur. Missouri Botanical Garden, Saint Louis. 3486 p.