



Seed bank longevity and germination ecology of invasive and native grass species from Mediterranean wetlands

Tania Company · Pilar Soriano · Elena Estrelles ·
Olga Mayoral

Received: 30 June 2018 / Revised: 26 May 2019 / Accepted: 25 June 2019 / Published online: 16 August 2019
© Institute of Botany, Czech Academy of Sciences 2019

Abstract Most Mediterranean wetlands are overexploited and degraded by different factors and are especially vulnerable to alien and invasive species. Currently, there is still a lack of knowledge on certain aspects of plant invasions, especially from an applied point of view, as key tools in restoration and conservation programmes. In this sense, *Cortaderia selloana* has showed a rapid expansion in the western Mediterranean Basin, threatening habitats of special interest such as wetlands. A comparative study between *C. selloana* and *Saccharum ravennae*, a representative plant of Mediterranean marshlands, was carried out to characterize the biological pattern that has made *C. selloana* a potential threat. The role of these plant species in soil seed banks and the potential competitive displacement of *S. ravennae* were evaluated through seed germination and viability tests under accelerated ageing conditions and seedling root development, considering seed morphological characteristics, seed maturity and plant sexuality as influential factors. Additionally, the potential allelopathic effects of *C. selloana* leaves on *S. ravennae* seed germination were evaluated. The study found differences between the species in all the tested parameters,

seed morphology, germination response and longevity, adsorption isotherm curves, and allelopathic effects. All these factors establish a complex interaction that contributes to the proven invasive character of *C. selloana*. These results provide useful data for eradication and management programmes.

Keywords *Cortaderia selloana* · *Saccharum ravennae* · ageing test · germination · invasiveness · soil seed bank

Introduction

Wetlands have been lost and disturbed more rapidly than other ecosystems, and many of them are seriously degraded (Millennium Ecosystem Assessment 2005). Worldwide, half of their total area has been lost due to anthropogenic pressure (Zedler and Kercher 2005). Specifically, Mediterranean wetlands are overexploited and damaged because of geomorphological alterations, changes in land use, changes in water regime and invasions of alien species (Brunel et al. 2010). Biological invasions are an emergent concern around the world (Strayer 2010) and in particular in Mediterranean areas (Santos et al. 2014). The tangible susceptibility of European inland waters to invasions is a direct consequence of the rapid growth of anthropogenic perturbations, mainly transport, trade and tourism (Genovesi 2007), which seems likely to continue in the future. Currently, the specific invasion strategies are poorly understood, making it difficult to design strategies when considering restoration and conservation programmes.

T. Company · P. Soriano · E. Estrelles · O. Mayoral
Jardí Botànic – ICBiBE, Universitat de València, Quart 80,
46008 València, Spain

O. Mayoral (✉)
Dpto. Didáctica de las Ciencias Experimentales y Sociales,
Facultad de Magisterio, Universitat de València, Avinguda dels
Tarongers, 4, 46022 València, Spain
e-mail: olga.mayoral@uv.es

Invasive plants pose different threats depending on their life history and competitiveness, and methods of their control very much depend on their reproduction, namely seed dispersal and seed bank persistence. The presence of invasive plants in soil seed banks may affect plant community dynamics and methods available for species eradication (Gioria et al. 2014). Some *ex situ* techniques have been proposed to predict the persistence of seeds and to enable land managers to make faster and better-informed decisions in weed management programmes (Panetta et al. 2011). Amongst them, studies related to permanence capacity of seeds in the soil seed bank provide essential data to plan the control and eradication of weeds in natural areas. Regarding *in situ* seed banks, the permanence capacity can be frequently affected by seed maturity at the dispersion moment. This fact is especially important in the Mediterranean context, where strong climatic events, frequent in autumn, can promote premature seed dispersion. This fact affects species with wind dispersed seeds, such as members of the Poaceae, especially when the ripening phase lasts until late autumn.

Regardless of seed persistence in seed banks, the performance of alien plants will depend on their ability to germinate under different conditions (Wainwright and Cleland 2013), which can be enhanced in many cases by allelopathic effects on the natural propagation of native species (Hierro and Callaway 2003).

The main purpose of this research is to understand the seed bank biology of the invasive species *Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn. (pampas grass), a plant from the family Poaceae, considered a potential threat for Mediterranean wetlands, comparing it with *Saccharum ravennae* (L.) P. Beauv., a native species from the same family, living in similar environments and therefore being affected by the expansion of the invasive species (Lambrinos 2001; Bacchetta et al. 2010). Previous studies have focused on seed germination of *C. selloana* (Domènech and Vilà 2008; Bacchetta et al. 2010) and *Saccharum ravennae* (Springer and Goldman 2016), considering abiotic conditions. In this paper we focus on the comparison of seed bank longevity of these species, trying to assess the potential competitive displacement of *S. ravennae* by *C. selloana* in the soil seed bank and during early development. We tested the hypothesis that invasive species *C. selloana* has a competitive advantage over the native species *S. ravennae* through higher persistence in seed banks, higher recruitment or both. We

specifically asked the following questions to test this hypothesis: (1) Does seed morphology among species and sexual morphs influence seed viability? (2) Do the species differ in the performance of their ageing seeds? (3) Do the species differ in their optimal temperature for germination? (4) Is there an allelopathic effect of *C. selloana* leaves on *S. ravennae* seed germination?

Material and methods

Study species

Cortaderia selloana has been introduced mainly for ornamental purposes because of its size and its showy panicles, and nowadays it is considered one of the most invasive plants in Western Mediterranean wetlands. It is a Neotropical species, that has shown a rapid and deep impact in coastal areas of the Eastern Iberian Peninsula in the western part of the Mediterranean basin. It is a gynodioecious species with female and hermaphroditic individuals; however, it is functionally dioecious because the hermaphroditic individuals act as males (Domènech and Vilà 2008; Bacchetta et al. 2010) and produce seeds with low germination ability (pers. obs.). It is anemochorous and its seeds have the ability to disperse over distances of up to 30 km (Basnou 2006). This rapid expansion poses a threat to habitats of interest for conservation such as wetlands. In Spain, *C. selloana* has undergone a major expansion throughout the country's northern territories (Herrera and Campos 2006) and currently it is included in the National Spanish Catalogue of Invasive Species as an invasive species of wetlands and sand dunes.

Saccharum ravennae is a Late Paleotropical species with a Mediterranean-Turanian distribution (Pignatti 1982). In the Mediterranean region it grows specifically in the thermomediterranean and lower mesomediterranean thermotypes (Peris et al. 2010). It inhabits wetlands, ephemeral riverbeds and rocky beds of rivers, sandy areas, and lowlands along the coast with a high water table. Some of these habitats are included in Annex I of the Habitats Directive (92/43/EEC) as 'Humid dune slacks-code 2190'.

Seed collection

Seeds of *C. selloana* and *S. ravennae* were collected in autumn of 2012, prior to their wind dispersion, from a

population growing in Cullera (SE Spain). The soil where the plants grew was classified as an entisol fluvent xerofluvent according to the USDA soil taxonomy (Soil Survey Staff 1999).

To compare the potential different behaviour, four different seed samples were collected: seeds from female (CSf) and hermaphroditic (CSpf) individuals of *C. selloana* and seeds in the initial maturation phase (SRi) from *S. ravennae* on 18 October, and, additionally, fully mature seeds (SRm) from *S. ravennae* on 15 November.

Specifically, single panicles from at least ten individuals were collected at random over the whole area under study. Seeds were removed from inflorescences and placed in a low-temperature and low-humidity storage environment (15°C and 15% RH) until testing to stall the ageing process.

Morphological characterization

The morphological characterization of seeds was carried out on 50 units for each treatment, using an Orion Cahn C-33 microbalance to determine their average weight. Their size was determined from images using the free software Image J 1.45s (<https://imagej.nih.gov/ij>). Differences in weight, length and width between all the samples were compared.

Relative seed longevity under controlled ageing

Relative seed longevity was measured by a controlled ageing test (CAT) following the protocol recommended by Newton et al. (2009). The method is based on the equilibration of seeds at high relative moisture and high temperature, specifically 60% RH and 45°C, which are the standard conditions for accelerated ageing. The humidity was adjusted in a hermetic glass jar with a 30% (w/v) LiCl solution, which was maintained within an incubator with controlled temperature over the course of the CAT.

The very low initial germination percentage of hermaphroditic *C. selloana* ($21 \pm 11.49\%$) made it unsuitable for the controlled ageing test, so we used only seeds of female plants for *C. selloana*. Additionally, the effect of maturity level on seed longevity was evaluated in *S. ravennae*.

An average of twelve replicates of 100 seeds for each treatment was placed in an open vial on a stand, inside a sealed container at 20°C and 47% RH ($370 \text{ g}\cdot\text{l}^{-1}$ of LiCl) for a period of fourteen days to rehydrate the seeds. Then, the seeds were transferred to the ageing environment at 60% RH ($330 \text{ g}\cdot\text{l}^{-1}$ LiCl) in an oven at 45°C. The seeds were germinated at 25°C and a 12-h photoperiod on agar dishes (0.6%), and checked daily. The temperature was selected as the optimal condition not only for germination, but also for root development. The dishes were sealed with plastic film to prevent moisture loss. Seeds with different treatments were examined regularly for signs of fungal infection and transferred to clean plates if required. Seeds that had not germinated within 15 days were dissected to assess viability and empty seeds were subtracted from total seed numbers.

The germination percentages obtained were plotted against the ageing time in days. The results were adjusted by the Johnson-Mehl-Avrami form of the Avrami kinetics equation to generate curves of loss of seed viability versus storage time:

$$\ln\left(\frac{N_0}{N}\right) = \left(\frac{t}{\emptyset}\right)^n,$$

where t is storage time and N_0/N is the reciprocal of percentage germination. The coefficients \emptyset and n describe the shape of the sigmoidal curve (Walters et al. 2004). Longevity parameters were calculated by solving the coefficients of the Avrami equation using a least squares linear fit of the double logarithmic expression:

$$\ln\left(\ln\left(\frac{N_0}{N}\right)\right) = n(\ln(t)) + Y_0,$$

where n is the slope and Y_0 is the y-intercept. The constant N_0 can be calculated as $0.5 +$ the maximum germination percentage. Values for N were the average germination percentages for the time (t) in years. The coefficient \emptyset and the exponential factor n were calculated from the coefficients of the regression line:

$$\emptyset = e^{\left(\frac{-Y_0}{n}\right)}.$$

The P20, P50 and P80 values (time for viability to decline to 20, 50 and 80%, respectively) were obtained

by interpolating the Avrami equation (Walters et al. 2005). Significant differences between mature *S. ravennae* and *C. selloana* and between the two samples of *S. ravennae* with different maturity levels were analysed.

Vitality check of ageing seeds

As a vitality measure of the ageing seeds and an indicator of seed vigour, we used root length measurements. Decreasing seedling root length is a significant indicator widely used to determine seed vigour (Qun et al. 2007). To ensure the optimal development of roots, seeds were sown in straight lines on Petri dishes with agar and the dishes were placed in vertical position. Six days after sowing the seeds, a picture was taken under graph paper. Root length was determined using Image J 1.45s software (<https://imagej.nih.gov/ij>). Variations in root length development caused by maturity (in *S. ravennae*) or by different responses to ageing conditions (between female *C. selloana* and mature *S. ravennae*) were compared.

Sorption isotherm

The water sorption isotherm is a good indicator of seed moisture content and its relationship with relative humidity, relevant for understanding seed quality and the response under accelerated seed ageing tests (McDonald 2007). It was calculated for both species (female *C. selloana* and mature *S. ravennae*) by the gravimetric technique at 20°C according to Gold and Hay (2014). The aim was to verify the results obtained from the controlled ageing test by analysing the different effect of moisture in seeds. The desired range of equilibrium relative humidity (eRH; 11 to 95%) was obtained by introducing a set of saturate saline solutions of LiCl in airtight glass containers (Gold and Hay 2014). Each 100-ml container was filled with 20 ml of a saline solution, labelled with the concentration and RH level. A sample of 20 fully dried seeds of each species was suspended above the solution in all containers during fourteen days to allow them reach equilibrium. Water content was determined at 103°C until constant weight (17 h). Moisture data were plotted against eRH to obtain the sorption isotherm.

Optimal temperature for germination

Germination experiments started one month after the collection of seed samples. Seeds were sown in 55-mm diameter Petri dishes with a 0.6% agar substrate. Four replications of 25 seeds were used for each set of conditions tested. Temperature was tested within a range from 15 to 35°C at intervals of 5°C using daylight fluorescent illumination with a 12-h photoperiod ($100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in climate-controlled cabinets. Germination was assessed daily and germinated seedling were removed from the dishes.

The final germination percentage was calculated after fifteen days, and mean germination time (MGT) was determined, considering the the lower the value, the more rapid the germination. All the obtained data, both in tables and graphs, are expressed as means of the four replications.

With the purpose of comparing thermal responses, thermal time (*S*) was calculated for both species (Trudgill 1995) without considering the velocity values for temperatures above the optimal conditions.

Allelopathic effects

Tests were undertaken with aqueous extracts of *C. selloana* leaves and soil samples, both collected from the sampling plots. Fresh leaves were chopped into pieces of a size of about 2 cm and frozen at -20°C until use. A portion of the leaves was dried under ambient conditions. Leaf extracts were prepared using dry or fresh leaves of *C. selloana*. Leaves were incubated in distilled water ($20 \text{ g}\cdot\text{l}^{-1}$ of plant material for dry leaves and $10 \text{ ml}\cdot\text{g}^{-1}$ for fresh leaves) at 20°C for 72 h. The aqueous extract was filtered through a sheet of Whatman 42 filter paper and stored for 24 h at 4°C until use. To prepare the soil extract, 30 g of air-dried soil was mixed with 200 ml of distilled water in a shaker at 75 rpm for 20 h. The solutions were filtered through a sheet of Whatman No. 1 filter paper.

Saccharum ravennae seeds were placed in 90-mm diameter dishes on filter paper with 5 ml of either dry leaf, fresh-leaf or soil extracts. Distilled water was used as control. For each experiment, four replicates containing 25 seeds were placed in straight lines to allow root length measurements. The dishes were sealed with Parafilm to avoid water loss and cultivated at 25°C under a 12-h photoperiod. The dishes were checked

periodically. The criterion for assessing viability was radicle protrusion and normal root development. Because of fungal contamination of leaf-extract samples, the seeds were cleaned with the anti-fungal agent Captan and reintroduced to the dishes. Root length was measured following the protocol described above.

The effect of *C. selloana* fresh-leaf extract on *C. selloana* seed germination was also tested as a control, applied to four replicates of seeds following the same procedure described previously. Germination percentage ($G\%$) and mean germination time (MGT) were calculated for all the experiments.

Statistical analyses

Depending on the number of treatments and factors compared, different analyses were used to detect significant differences, mainly Student's T test and one-factor analysis of variance (one-way ANOVA). As an additional subgroup analyses, two post hoc tests were utilized: Tukey's test for establishing homogeneous groups and Dunnett's test for detecting deviations from the control treatment.

Results

Morphological characterization of seeds

Samples from *S. ravennae* individuals in different maturation stages were differenced by the colour of caryopses. Those collected in the first stage of maturation had a clearer colour, light yellow, and those collected in the fully mature stage had a darker colour, almost brown. *Saccharum ravennae* seeds were significantly heavier and larger than those of *C. selloana*, and, moreover, significant differences were found in seed weight and length, but not in width between female (CSf) and hermaphroditic (CSpf) individuals (Fig. 1).

Relative seed longevity under controlled ageing

The curves obtained through the Avrami kinetics equation showed the rapid fall of viability in *C. selloana* seeds compared to *S. ravennae* (Fig. 2) under controlled

ageing conditions. The P20, P50 and P80 coefficients (Table 1) obtained from the curve supported this hypothesis. Significant differences were found in seed longevity between *C. selloana* and mature *S. ravennae* ($P < 0.05$), suggesting lower seed persistence of the invasive species in the field.

The same test was applied to seeds in the initial maturation phase of *S. ravennae* (Fig. 2), confirming the lowest seed longevity of this treatment in comparison to the one involving fully mature seeds.

Root length as a measure of ageing

The decrease in root development of the three samples (SRm, SRi and CSf) under ageing conditions confirms the results obtained through the Avrami equation, showing a loss of viability for all samples. It is important to note that there are no measurements of root development for all ageing periods; this is because the ageing period in each treatment was measured in a different way, adapted to the diverse longevities found.

Thus, it can be seen that root length drops significantly from day 18 of ageing ($P < 0.05$) in mature *S. ravennae* (Fig. 3a), in comparison with the control treatment. For the same species, the seeds in the initial maturation phase (Fig. 3b) experienced a significant decrease of its development from day 10 ($P < 0.05$). This proves that maturity also influences the vigour of *S. ravennae* under controlled ageing conditions. In *C. selloana* (Fig. 3c) the reduction in root growth was even faster than in the two samples of *S. ravennae*, falling significantly after seven days ($P < 0.05$) under ageing conditions.

Despite the decrease in root length as ageing time increased in all treatments, there was large variability of this parameter and the presence of atypical data, with a deviation in the normal behaviour of measures, especially on day 28 in SRm and on day 12 in SRi (Fig. 3).

Sorption isotherm as a measure of ageing

Water absorbed by seeds of *C. selloana* and *S. ravennae* exposed to different relative humidity levels varied for the species from 15 to 60% relative humidity, being higher for the invasive species *C. selloana* (Fig. 4).

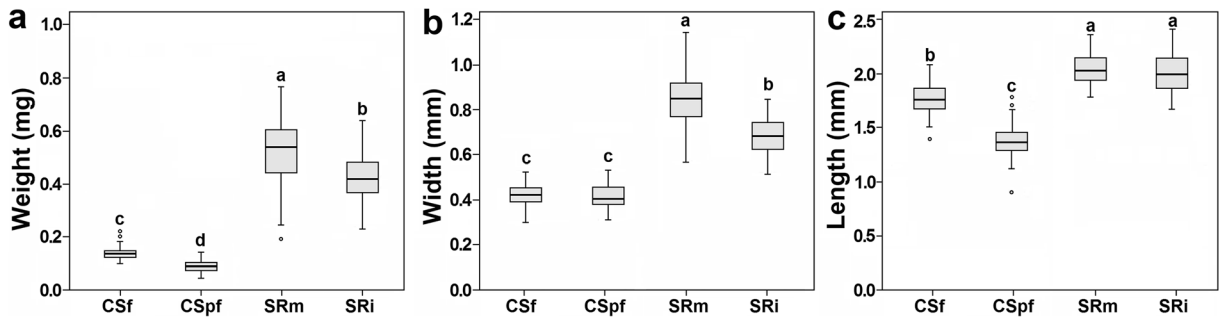


Fig. 1 Seed morphological characterization: **a** – weight, **b** – width, **c** – length. CSf – seeds from female individuals of *C. selloana*, CSpf – seeds from hermaphroditic *C. selloana* individuals, SRm – fully

mature seeds of *S. ravennae*, SRi – seeds in the initial maturation phase of *S. ravennae*; different letters indicate significant differences between groups according to Tukey’s test.

Optimal germination temperature

The germination pattern for the compared species was similar (Fig. 5a,b). No dormancy was detected, high final percentages were reached in all the conditions tested (Table 2), and higher temperatures were preferred by both species studied. The species differed by germination velocity (Table 2). The optimal

temperature for *C. selloana*, calculated from regression lines of germination rates (Fig. 5c), was 30.7°C. Values for calculated thermal time (Fig. 5c) were 27.2°C during the day for *S. ravennae* whereas *C. selloana* only required 19.2°C during the day for optimal germination. From the regression lines plotted in Fig. 5c we observe higher velocity of *C. selloana* over *S. ravennae* from 14.5°C.

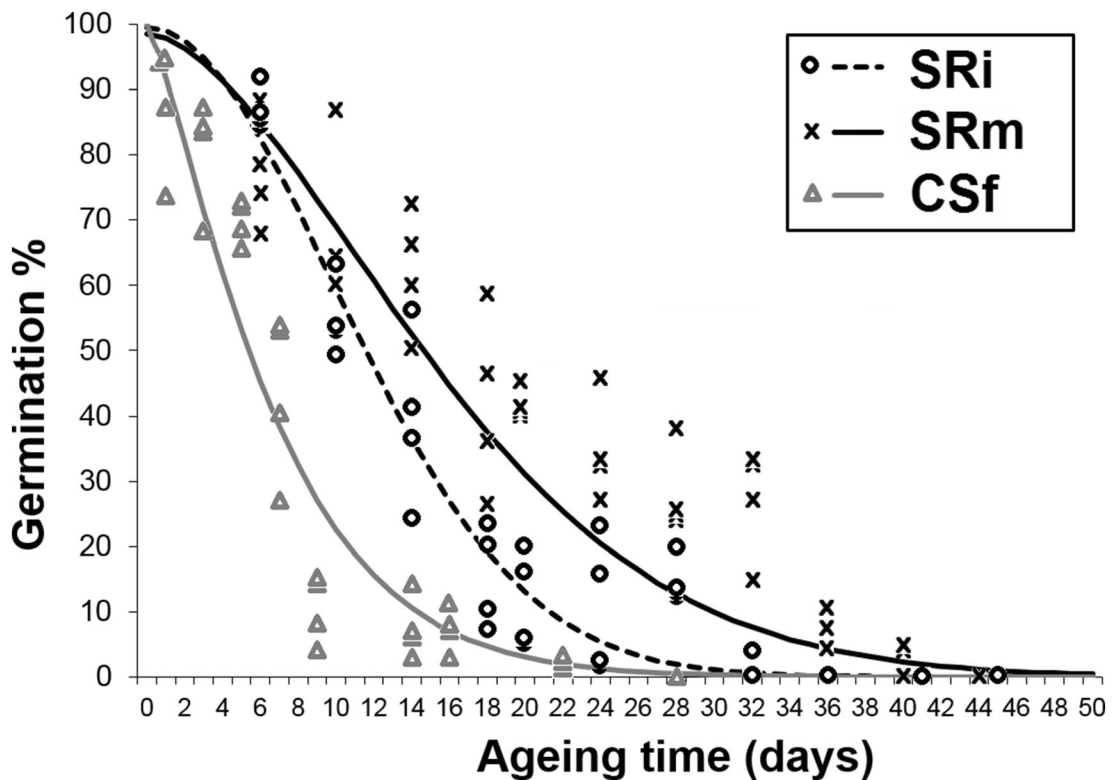


Fig. 2 Ageing curves of seed samples of SRm (fully mature seeds of *S. ravennae*), SRi (seeds in the initial maturation phase of *S. ravennae*) and CSf (seeds from female individuals of *C. selloana*) showing the relation of germination percentage and ageing time.

Table 1 Ageing coefficients for each sample, mature *S. ravennae* (SRm), immature *S. ravennae* (SRi) and female *C. selloana* (CSf)

| | P20 | P50 | P80 |
|-----|--------|--------|--------|
| SRi | 18.1 b | 12.0 b | 6.9 a |
| SRm | 24.7 a | 15.1 a | 7.9 a |
| CSf | 10.0 c | 05.9 c | 02.4 b |

Same letters indicate homogeneous subsets according to Student's T test

Allelopathic effects

The effects of the leaf and soil extracts of *C. selloana* on *S. ravennae* seed germination and root length are shown in Fig. 6. The fresh-leaf extract significantly reduced the germination and root growth of *S. ravennae* in comparison with the control whereas mean germination time was not affected. The dry-leaf extract reduced only root growth and therefore the vigour of future seedlings of *S. ravennae* but not germination percentage or time.

It was verified that the fresh-leaf extract of *C. selloana*, besides affecting negatively the germination and vigour of *S. ravennae*, also affected its own development. The allelopathic effects were obvious from the germination percentage ($P = 0.000$; $F = 69.429$), MGT ($P = 0.013$; $F = 12.058$) and root growth ($P = 0.008$; $F = 15.005$).

Discussion

This study tried to attain new knowledge on the functioning of plant invasions in Mediterranean wetlands, comparing the potential competitive displacement of *S. ravennae* by the invasive species *C. selloana* through

seed behaviour in the soil seed bank. We rejected the hypothesis that *C. selloana* has a competitive advantage over the native species *S. ravennae* in seed bank survival or during early life stages. The invasive species *C. selloana* has smaller seeds and those seeds are less persistent in seed banks than seeds of the native species *S. ravennae*. The species studied showed a similar germination pattern at the different temperatures tested. Whereas the invasive species had a lower optimal temperature for germination, both species germinated very rapidly at high temperatures. The allelopathic effect of the invasive species on the native one was observed after treatment with the fresh-leaf extract, but this same response was also observed on seeds of *C. selloana*. The recent spread of the invasive species *C. selloana* is therefore caused probably by factors other than those studied, probably by seed production (Springer and Goldman 2016) or the better effectiveness in the use of water resources and greater resistance to drought (Vourlitis and Kroon 2013).

Seed size, morphology and survival in the seed bank

Regarding morphological seed characters, particularly seed mass, related to seed longevity, diverse studies have dealt with this question without a clear conclusion, given that the longevity of the seeds is a complex factor. Evidence for the higher persistence of small seeds has been presented (Guzzon et al. 2018), but the contrary strategy has been suggested by other authors (Moles and Westoby 2006). In our study, the species with the larger and heavier seeds, *S. ravennae*, had higher longevity in the seed bank than the species with the smaller seeds *C. selloana*. Moreover, both species had rather short-

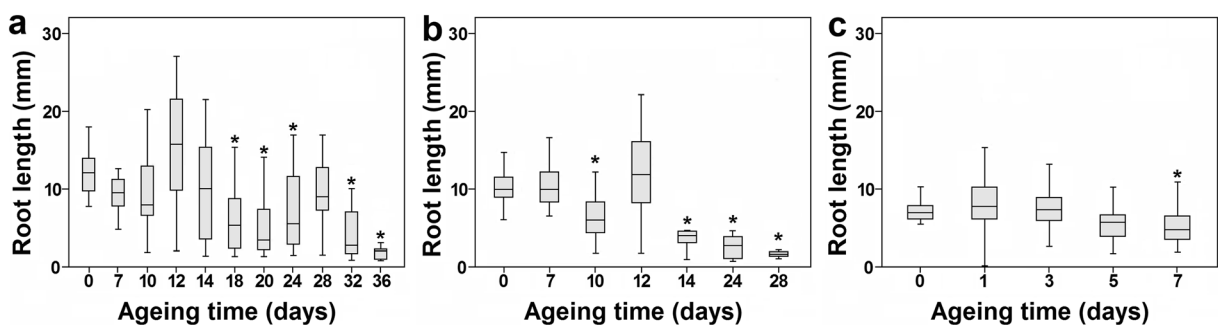


Fig. 3 Root length for different ageing times in: **a** – SRm (fully mature seeds in *S. ravennae*), **b** – SRi (seeds in the initial maturation phase of *S. ravennae*), **c** – CSf (seeds from female individuals

of *C. selloana*). Asterisks indicate significantly shorter roots compared with the control, according to Dunnett's test.

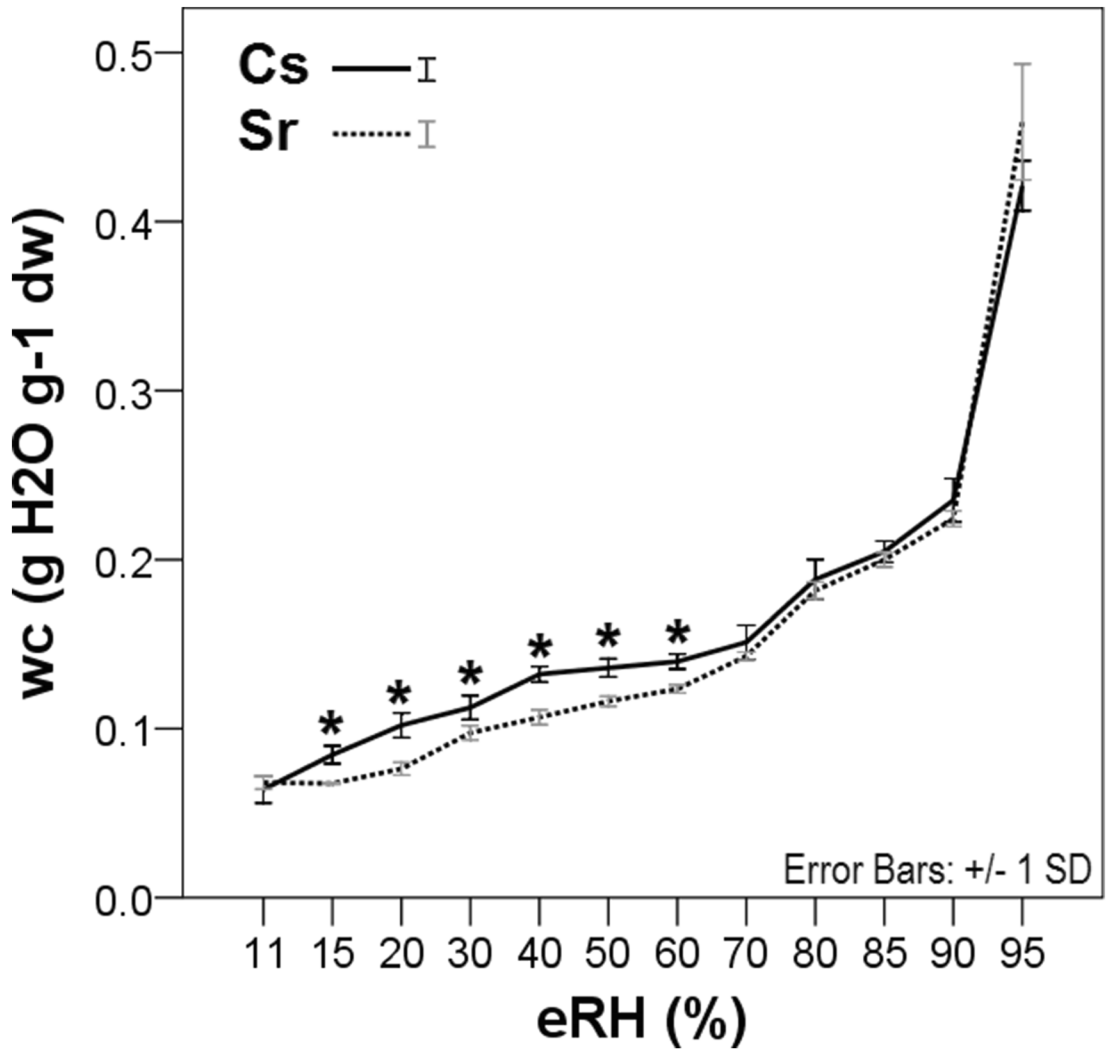


Fig. 4 Adsorption isotherm curves showing seed water content equilibrium under a wide range of relative humidity for mature *S. ravennae* (SRm) and female *C. selloana* (CSf) at 20°C. Asterisks show significant differences according to Student's T test.

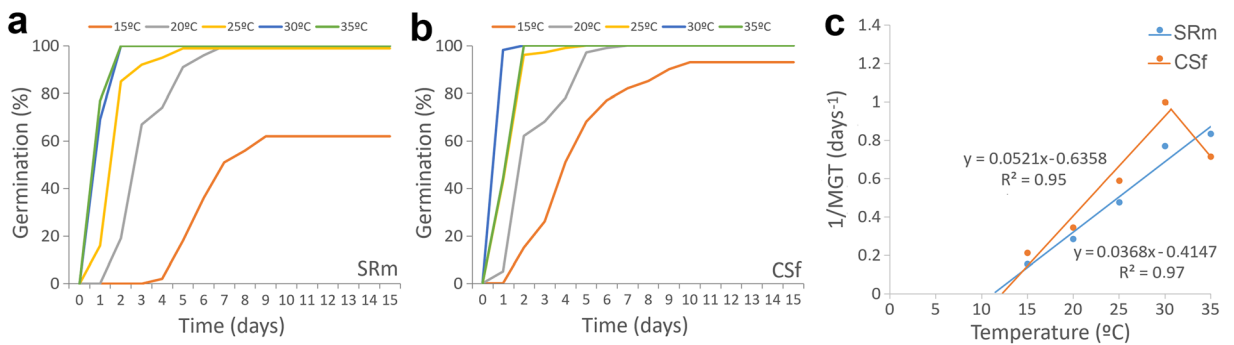


Fig. 5 Accumulative seed germination curves within the temperature range from 15°C to 35°C for: **a** – SRm (fully mature seeds in *S. ravennae*), **b** – CSf (seeds from female individuals of *C. selloana*), and **c** – linear regression of germination rates related to temperature.

Table 2 Seed germination percentages ($G\%$) and mean germination time (MGT) for the species under study, *S. ravennae* (SRm) and *C. selloana* (CSf)

| | | Temperature [°C] | | | | |
|-------|-----|------------------|-------------|--------------|-------------|--------------|
| | | 15° | 20° | 25° | 30° | 35° |
| $G\%$ | SRm | 62.0 ± 9.5 b | 100 ± 0.0 a | 99.0 ± 2.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| | CSf | 93.0 ± 3.8 b | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| MGT | SRm | 6.4 ± 0.2 d | 3.5 ± 0.2 c | 2.1 ± 0.2 b | 1.3 ± 0.1 a | 1.2 ± 0.1 a |
| | CSf | 4.7 ± 0.5 d | 2.9 ± 0.2 c | 1.7 ± 0.1 b | 1.0 ± 0.0 a | 1.4 ± 0.3 ab |

Letters indicate homogeneous groups for germination parameters values within one of the species studied

lived (transient) seed banks, below the mean P50 value for Poaceae (Probert et al. 2009). Nevertheless, our results are based on experimental ageing and although it might be a proxy of seed longevity in the soil seed bank (Long et al. 2008), many diverse biotic and abiotic factors could influence these seed features in nature, providing additional variance to the response of seeds in natural environments.

Longevity and water content in seeds

When seed longevity is studied, moisture content is one of the principal factors determining the degree of seed deterioration in a specific environment, as stated in the viability equation of Ellis and Roberts (1980). The association of seed moisture content and environmental temperature is the main factor influencing seed longevity (Pritchard and Dickie 2003). The greater capacity of *C. selloana* to absorb water in comparison to *S. ravennae* contributes significantly to seed deterioration during ageing (Mira et al. 2015) and is in accordance with the lower seed longevity of *C. selloana*.

Optimal germination temperature

The optimal temperatures, 30°C for *C. selloana* and 35°C for *S. ravennae*, detected in our germination tests were higher than those observed in previous studies (Springer and Goldman 2016; Bacchetta et al. 2010). Although the invasive species was not characterized by fast germination at optimal temperature, it showed faster germination at lower temperatures, which is in accordance with studies done under different field environmental conditions (Domènech and Vilà 2008). This ability may be advantageous to the invasive species in spring, when *C. selloana* may start to germinate earlier than the native species *S. ravennae*.

Allelopathy

The allelopathic effect of *C. selloana* fresh-leaf extracts on seed germination and seedling development of *S. ravennae* has been proved. Nevertheless, we opine that the decrease of *S. ravennae* recruitment observed previously is not a consequence of this factor alone.

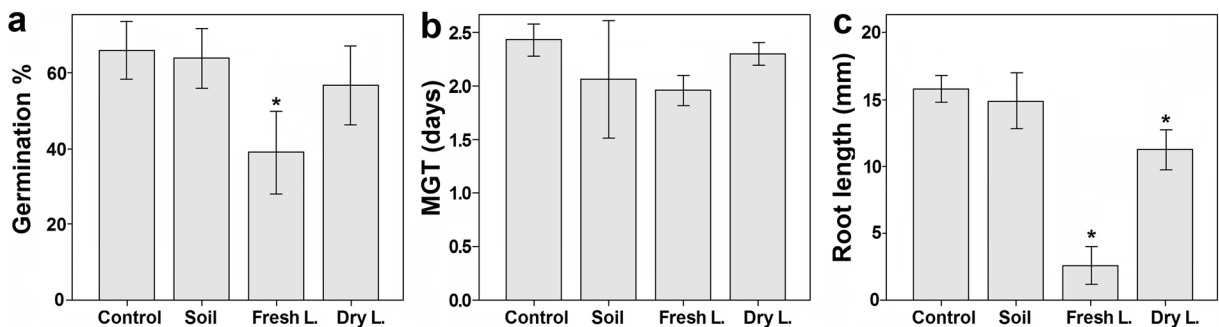


Fig. 6 Allelopathic effects of *C. selloana* fresh-leaf extracts on SRm (fully mature seeds in *S. ravennae*) for each treatment. **a** – germination percentage, **b** – mean germination time, **c** – seedling

root length. Asterisks show that treatments are significantly different from the control according to Dunnett's test.

Many studies have found evidence for potential allelopathic effects of alien invasive plants, but the methodologies applied are frequently controversial (Hierro and Callaway 2003). These authors recommended deep studies, including soil analysis, to determine the specific chemical compounds and the minimum active concentration required for inhibition.

Our results suggest that the inhibition of growth is due to the high concentration of fresh-leaf extract that reduces the vigour of seedlings and promotes the development of saprophytes.

Seed quality and reproductive system

Another aspect confirmed by our research is the lower amount and viability of seeds produced by hermaphroditic individuals of *C. selloana*, previously reported by Domènech and Vilà (2008) and Bacchetta et al. (2010). This fact supports their role as pollen donors and is the reason why we advocate the use of hermaphroditic individuals only for ornamental purposes, as their expected invasive capacity is lower. The development of a fast and cheap method for identifying hermaphroditic individuals in juvenile stages would be of great interest, given the extensive use of *C. selloana* in landscaping projects (Domènech-Carbó et al. 2018). In this sense, *S. ravennae* is a good alternative option for ornamental purposes because it has a similar appearance; in fact it is already used for gardening purposes outside of its native area of occurrence (Springer and Goldman 2016).

Conclusion

The results of our study, showing no special seed longevity for *C. selloana*, provide useful guidance for wetland management and for the success of eradication programmes. This implies that mechanical control of plants and subsequent monitoring do not require long periods. We suggest in a first approach that two years are enough if plants are eliminated before the fructification stage.

Acknowledgements The experiments comply with the current laws of Spain, the country in which they were performed.

References

- Bacchetta G, Dettori CA, Mascia F, Meloni F, Podda L (2010) Assessing the potential invasiveness of *Cortaderia selloana* in Sardinian wetlands through seed germination study. *Pl Biosyst* 144:518–527
- Basnou C (2006) *Cortaderia selloana*, DAISIE project. Available at http://www.europe-aliens.org/pdf/Cortaderia_selloana.pdf (accessed November 2017)
- Brunel S, Schrader G, Brundu G, Fried G (2010) Emerging invasive alien plants for the Mediterranean Basin. *EPPO Bull* 40:219–238
- Domènech R, Vilà M (2008) *Cortaderia selloana* seed germination under different ecological conditions. *Acta Oecol* 33:93–96
- Domènech-Carbó A, Montoya N, Soriano P, Estrelles E (2018) An electrochemical analysis suggests role of gynodioecy in adaptation to stress in *Cortaderia selloana*. *Curr Pl Biol* 16:9–14
- Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. *Ann Bot (Oxford)* 45:13–30
- Genovesi P (2007) Towards a European strategy to halt biological invasions in inland waters. In Gherardi F (ed) *Biological invaders in inland waters: profiles, distribution, and threats*. Springer, Dordrecht
- Gioria M, Jarošík V, Pyšek P (2014) Impact of invasions by alien plants on soil seed bank communities: emerging patterns. *Pl Ecol Evol Syst* 16:132–142
- Gold K, Hay F (2014) *Equilibrating seeds to specific moisture levels. Technical Information Sheet 09*. Millennium Seed Bank Project. RBG, Kew, UK
- Guzzon F, Orsenigo S, Gianella M, Müller JV, Vagge I, Rossi G, Mondoni A (2018) Seed heteromorphy influences seed longevity in *Aegilops*. *Seed Sci Res* 28:1–9
- Herrera M, Campos JA (2006) *El Carrizo de la pampa (Cortaderia selloana) en Bizkaia. Guía práctica para su control*. Instituto de Estudios Territoriales de Bizkaia y Diputación Foral de Bizkaia. Bizkaia
- Hierro JL, Callaway RM (2003). Allelopathy and exotic plant invasion. *Pl & Soil* 256:29–39
- Lambrinos JG (2001) The expansion history of a sexual and asexual species of *Cortaderia* in California, USA. *J Ecol* 89:88–98
- Long RL, Panetta FD, Steadman KJ, Probert R, Bekker RM, Brooks S, Adkins SW (2008) Seed persistence in the field may be predicted by laboratory-controlled aging. *Weed Sci* 56:523–528
- McDonald, MB (2007). Seed moisture and the equilibrium seed moisture content curve. *Seed Technol* 29:7–18
- Millennium Ecosystem Assessment (2005) *Ecosystems and human wellbeing: wetlands and water synthesis*. World Resources Institute, Washington, DC
- Mira S, Estrelles E, González-Benito ME (2015) Effect of water content and temperature on seed longevity of seven Brassicaceae species after 5 years of storage. *Pl Biol* 17: 153–162
- Moles AT, Westoby M (2006) Seed size and plant strategy across the whole life cycle. *Oikos* 113:91–105

- Newton R, Hay F, Probert R (2009) *Protocol for comparative seed longevity testing*. Technical Information Sheet 01. RBG, Kew, London
- Panetta FD, Cacho O, Hester S, Sims-Chilton N, Brooks S (2011) Estimating and influencing the duration of weed eradication programmes. *J Appl Ecol* 48:980–988
- Peris JB, Roselló R, Sanchis E (2010) Listado de táxones destacados de la Comunidad Valenciana en relación con el piso bioclimático, ombroclima y sector corológico. *Toll Negre* 12:35–42
- Pignatti S (1982) *Flora d'Italia 1–3*. Edagricole. Bologna
- Pritchard HW, Dickie JB (2003) Predicting seed longevity: use and abuse of seed viability equations. In Smith RD, Dickie JB, Linington SH, Pritchard HW, Probert RJ (eds) *Seed conservation: turning science into practice*. RBG, Kew, UK, pp 653–722
- Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Ann Bot (Oxford)* 104:57–69
- Qun S, Wang JH, Sun BQ (2007). Advances on seed vigor physiological and genetic mechanisms. *Agric Sci China* 6: 1060–1066
- Santos FD, Stigter TY, Faysse N, Lourenço TC (2014) Impacts and adaptation to climate change in the Mediterranean coastal areas: the CIRCLE-MED initiative. *Regional Environm Change* 14 (Suppl 1):S1–S3
- Soil Survey Staff (1999) *Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys*. 2nd ed. U.S.D.A. Handbook. U.S. Gov. Print. Office, Washington, DC, 436 pp
- Springer TL, Goldman JJ (2016) Germination of *Saccharum ravennae* (L.) L. (*Poaceae*) caryopses and intact spikelets. *Crop Sci* 56:682–688
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interaction with other stressors, and prospects for the future. *Freshwater Biol* 55:152–174
- Trudgill DL (1995) Why do tropical poikilothermic organisms tend to have higher threshold temperatures for development than temperate ones. *Funct Ecol* 9:136–137
- Vourlitis GL, Kroon JL (2013) Growth and resource use of the invasive grass, pampasgrass (*Cortaderia selloana*), in response to nitrogen and water availability. *Weed Sci* 61:117–125
- Wainwright CE, Cleland EE (2013). Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. *Biol Invas* 15:2253–2264
- Walters C, Wheelern L, Stanwood PC (2004) Longevity of cryogenically stored seeds. *Cryobiology* 48:229–244
- Walters C, Wheeler LM, Grotenhuis JM (2005) Longevity of seeds stored in a genebank: species characteristics. *Seed Sci Res* 15:1–20
- Zedler JB, Kercher S (2005) Wetland resources: status, trends, ecosystem services, and restorability. *Annual Rev Environm Resources* 30:39–74

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.