

Leaf litter and irrigation can increase seed germination and early seedling survival of the recalcitrant-seeded tree *Beilschmiedia miersii*

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Abstract Although regeneration of recalcitrant-seeded tree species can be affected by prolonged drought, especially in Mediterranean regions, little is known about the response of such species to varying site conditions. A field experiment was performed to determine the effect of irrigation and leaf litter cover on seed germination and early seedling survival of the vulnerable recalcitrant-seeded tree *Beilschmiedia miersii* (Lauraceae). Two levels of irrigation (non-irrigated and irrigated units) and three levels of leaf litter depth (0, 5 and 12 cm) were applied to 72 groups of 30 seeds along a wet ravine of the Coastal Range of Central Chile, equally distributed across sites with different levels of canopy cover. Seed germination was significantly increased by irrigation only under closed-canopy cover, and by leaf litter cover (>5 cm) under all canopy conditions. The effect of leaf litter on germination increased with canopy openness, while the effect of irrigation did not show any tendency. Meanwhile, early seedling survival was significantly increased by irrigation under intermediate canopy cover, and by leaf litter (>5 cm) under closed-canopy cover. As a result of its overall positive effect on germination, leaf litter should be maintained within *B. miersii* communities, particularly under intermediate to closed-canopy conditions, where it can also increase early seedling survival, and both seed germination and early seedling survival might be increased through additional water inputs. The presence of leaf litter might help retaining such inputs, prolonging their effect on regeneration of *B. miersii* communities. We see this as a baseline assessment of regeneration and persistence that needs further testing on species with similar traits, given the expected increase in the frequency and length of dry periods into the future.

Key words: irrigation, leaf litter, recalcitrant seeds, seed germination, seedling survival.

INTRODUCTION

Water availability is frequently a critical factor for tree species regeneration, often determining seed viability and germination (Fenner & Thompson 2005) as well as seedling survival and establishment (Facelli & Pickett 1991). Among other metabolic processes needed for successful plant survival and establishment, seed germination and embryo development are disrupted when internal moisture drops below critical levels (Farnsworth 2000). However, similar to other biotic and abiotic factors, critical moisture thresholds for seed germination and seedling establishment vary considerably between species (Hong & Ellis 1992; Wilson & Witkowski 1998; Baskin 2017). Differences in seed germination strategies

between species are an example of such variation. Most flowering plant species (approximately 92%) produce orthodox seeds. These are seeds for which internal desiccation is a common phase in inducing dormancy (Tweddle *et al.* 2003). This allows seeds to survive dry periods until favourable conditions arise (Farnsworth 2000; Kermodé & Finch-Savage 2002; Fenner & Thompson 2005). However, recalcitrant seeds (also termed non-orthodox and desiccation-sensitive) cannot tolerate large amounts of water loss, which cause deleterious impacts on cell structure, mitotic growth, and other biochemical and biophysical abnormalities. This in turn causes a loss of viability unless the seeds remain hydrated (Farnsworth 2000; Greggains *et al.* 2001; Pammenter & Berjak 2014). Recalcitrant seeds commonly show little dormancy, due to their structure and lack of certain hormones. As such, they proceed directly to germination after shedding, allowing a faster

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transition between seed and seedling stages, (Farnsworth 2000). This is thought to provide fitness advantage through reducing the risks of predation and maximizing embryo development during favourable environmental conditions (Farnsworth 2000; Tweddle *et al.* 2003). However, recently germinated seedlings of recalcitrant-seeded species that reproduce copiously might still undergo massive mortality after germination when facing adverse conditions (Farnsworth 2000). Although plants producing recalcitrant seeds are most common in moist, unseasonal habitats, representing more than 40% of woody angiosperms in some tropical rainforests, they also occur in temperate-seasonal and sometimes dry habitats, where they can reach up to 4% of flowering tree and shrub species (Tweddle *et al.* 2003; Pammenter & Berjak 2014; Joët *et al.* 2016). In such environments, desiccation sensitivity may be a high-risk strategy, since prolonged periods between shedding and the emergence of favourable conditions for germination may result in the death of whole seed cohorts (Joët *et al.* 2016).

Particularly in Mediterranean regions, increasing winter drought might be detrimental for recalcitrant-seeded species (Joët *et al.* 2016). Extreme higher temperatures might also influence drying rates, thus reducing seed viability (Berjak *et al.* 1994). This might be the case for *Beilschmiedia miersii* (Gay) Kosterm (Lauraceae), a Neotropical tree species with recalcitrant, oval seeds of 2–3 cm diameter and 4 cm long (Nishida 1999; Cabello 2013). *B. miersii* is a vulnerable, evergreen tree, endemic to Central Chile, which occurs in subtropical, semiarid vegetation (Nishida 1999) with a Mediterranean climate characterized by winter rainfall and summer drought (Armesto *et al.* 2007). This shade-tolerant, dominant non-pioneer species is currently restricted to small populations within humid and shaded environments in the Chilean Mediterranean region (Novoa 2004). Such areas are threatened by agriculture, livestock and, potentially, drought and leaf litter removal (Henríquez & Simonetti 2001; Becerra *et al.* 2004). Seedlings and saplings are scarce beneath *B. miersii* dominated forests, presumably due to a pulse-based regeneration dynamic dependent on high precipitation years during which seed germination and seedling survival and establishment are feasible. However, even after abundant seed production, little seedling survival is generally observed (Henríquez & Simonetti 2001; Cabello 2013; Brito-Rozas & Flores-Toro 2014).

Together with precipitation, temperature has been shown to be one of the most relevant climate factors determining this species distribution (Pliscoff & Fuentes-Castillo 2011), and both factors are expected to significantly change in the future with a reduction in precipitation, longer droughts, and higher

temperatures (Pliscoff & Fuentes-Castillo 2011; Quintana & Aceituno 2012; Fernández & Morales 2016).

In this sense, leaf litter might play a relevant role in the regeneration of *B. miersii*. The effects of litter on vegetation development are frequently negative, since it often reduces the quality and quantity of light reaching seeds and seedlings, and it can act as a physical barrier that prevents seedlings from reaching the soil. It might also release allelochemicals that inhibit seed and seedling development (Xiong & Nilsson 1999; Loydi *et al.* 2013). However, under high-stress conditions, leaf litter tends to have positive effects. Particularly in dry environments, low to moderate amounts of leaf litter can help maintain soil moisture and moderate air temperature, avoiding desiccation (Loydi *et al.* 2013). A reduction in leaf litter cover in central Chile, caused by reduction in woody plant cover, fire, livestock and direct removal (Fuentes *et al.* 2013), might be hampering *B. miersii* regeneration, as it has been found under laboratory conditions (Becerra *et al.* 2004). Additionally, as a shade-tolerant species, more open environments might restrict the establishment and growth of *B. miersii* when not enough water is available (Donoso 1974). Sites with lower canopy cover should be in general less appropriate, due to the likely higher soil temperatures and evaporation rates, leading to higher risks of desiccation (Breshears *et al.* 1998; Hastwell & Facelli 2003; Binkley & Fisher 2012; Novak 2012; Loydi *et al.* 2013).

Even though the distribution of recalcitrant-seeded species has proven to be highly determined by environmental stress, empirical field studies regarding their regeneration persistence in relation to environmental factors under varying site conditions are rare (Tweddle *et al.* 2003; Joët *et al.* 2016). Such information is particularly relevant for species growing in temperate, seasonal regions, where non-dormancy can be especially disadvantageous under changing climate conditions (Hill & Edwards 2010). Thus, the aim of this study is to determine the effects of irrigation and leaf litter depth on seed germination and early seedling survival of *B. miersii* under different levels of canopy protection, through a manipulative field experiment.

Germination and seedling growth and establishment require specific conditions that do not necessarily coincide (mechanical impediment from litter, in fact, could become limiting only after germination) (Eriksson 1995; Fenner & Thompson 2005). Even if germination shows a positive response to irrigation or to leaf litter, both variables could have a null or negative effect on seedling performance, counteracting the initial positive effect. Therefore, in this study we assess the effects of irrigation and litter both on seed germination and early seedling survival.

Based on the knowledge on the ecology of recalcitrant-seeded species and particularly of *B. miersii*, we hypothesize that (i): irrigation and application of leaf litter cover facilitate seed germination and early seedling establishment, and (ii): the positive effect of irrigation and leaf litter on seed germination and seedling survival increases under decreasing canopy cover. In order to test these hypotheses, we conducted a field experiment at three different sites along a gradient of canopy cover.

MATERIALS AND METHODS

Study area

The study was conducted in a northeast-facing ravine on the western slope of the Coastal Range of Central Chile (33°51'1''S; 71°0'28''W; Fig. 1), 54 km away from Santiago. The region is characterized by a warm temperate climate, with a 6- to 8-month dry season (from October to April). The mean annual precipitation is 502 mm, with rainfall concentrated in winter. Mean monthly air temperature ranges from 8 to 18.1°C, reaching the highest values in summer (Uribe *et al.* 2012). Vegetation is mainly composed of second-growth sclerophyllous trees and shrubs, such as *Cryptocarya alba* (Mol.) Looser (Lauraceae), *Peumus boldus* Mol. (Monimiaceae), *Lithraea caustica* (Mol.)

Hook. et Arn. (Anacardiaceae) and *Quillaja saponaria* Mol. (Quillajaceae). However, in ravines and shaded environments along water courses, the forest develops a continuous canopy dominated by *B. miersii* and *C. alba* (Armesto *et al.* 2007).

Three stands (A, B, and C) were selected, with differential canopy openness and varying levels of dominance of *B. miersii*. Stand A (± 570 m above sea level (m a.s.l.)) is a closed-canopy forest (1.8% visible sky (VS)) with a total basal area of $35.6 \text{ m}^2 \text{ ha}^{-1}$ and is dominated by *B. miersii* (51.2% of the total basal area). Stand B (± 540 m a.s.l.) is an interface between the closed-canopy forest and the sclerophyllous shrubland (3.5% VS and $35.3 \text{ m}^2 \text{ ha}^{-1}$ basal area, of which *B. miersii* represents 29.5%), and stand C (± 500 m a.s.l.) is a sclerophyllous shrubland (9.8% VS and $11.1 \text{ m}^2 \text{ ha}^{-1}$ basal area), without any *B. miersii* trees in the canopy (Fig. 2). The proportions of visible sky were estimated from hemispherical photographs, which were taken from 12 systematically distributed points within each stand, using a Canon EOS 60D digital camera fitted with a Sigma 4.5 mm f/2.8 EX DC circular fisheye lens, at approximately 1.3 m above the ground. The resulting digital images were analysed using HemiView version 2.1.

Experimental design

In each stand, 24 experimental units (EUs) were established in April 2013, at the end of the seed fall period of

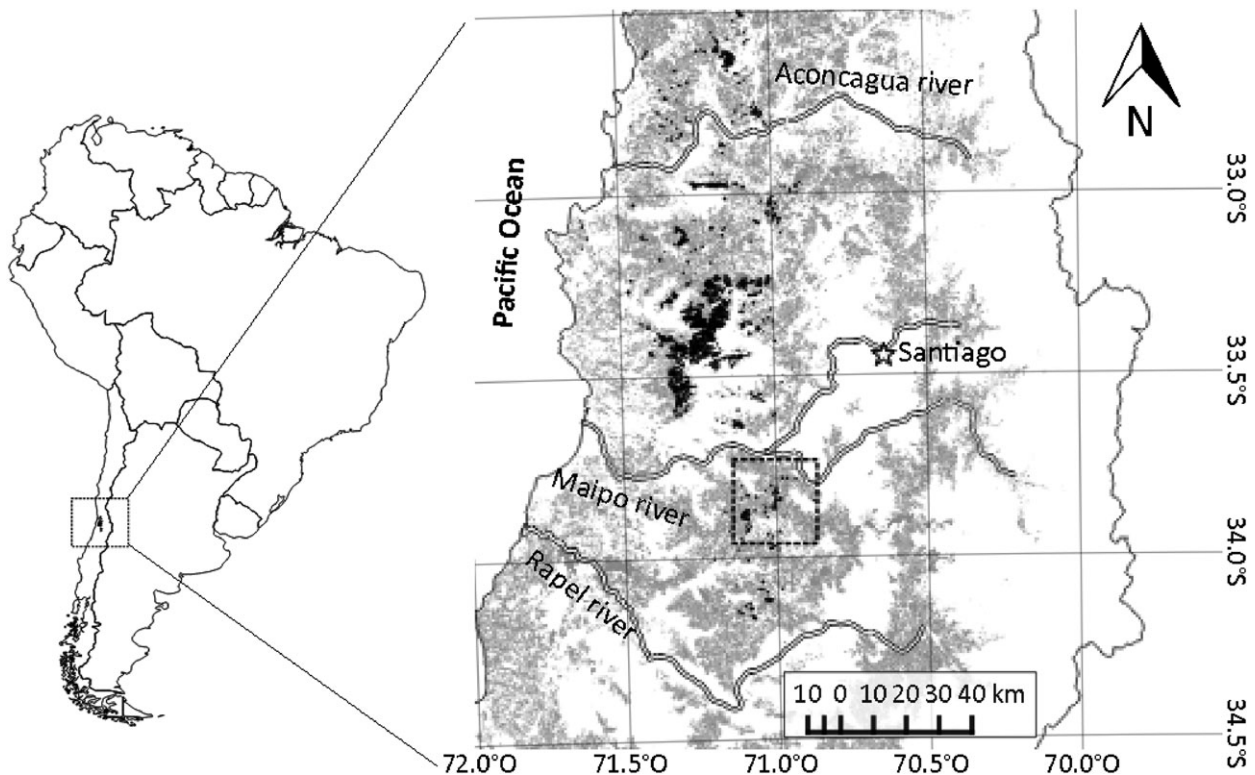


Fig. 1. Location of the study area. Areas in grey show the distribution of the sclerophyllous shrubland and forest, while areas in black represent the distribution of the communities in which *B. miersii* is present. The dotted square encloses the population where the study took place (based on CONAF 2017).

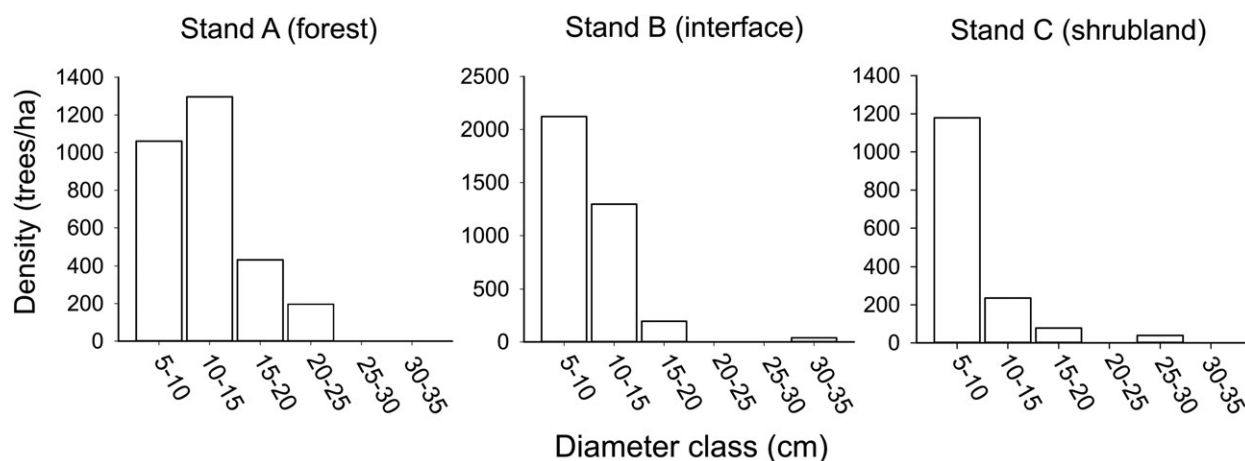


Fig. 2. Tree frequency per 5 cm diameter class for trees ≥ 5 cm dbh (diameter at breast height) in the three stands used for the study.

B. miersii (Cabello 2013). Each EU consisted of 30 seeds that were systematically distributed on 0.2 m². They were sown on bare organic soil and covered with a wire-mesh cage with 1 cm² apertures to exclude vertebrate herbivores. EUs were located at a distance of 2 m from one another, and arranged in a 2 × 3 completely randomized factorial experiment, with four replicates per treatment. Treatments consisted of combinations of two levels of irrigation (non-irrigated and irrigated EUs) and three levels of leaf litter depth (0, 5 and 12 cm). The seeds (89% viable after flotation method) were collected from different trees within the study area 1 week before sowing. The leaf litter used to cover the seeds was obtained from the three stands and then mixed.

EUs with irrigation treatment were watered every 2 weeks during the germination period, from April to October (Cabello 2013). The amount of water added to each EU was twice the mean monthly precipitation estimated for the study area (Uribe *et al.* 2012), and was therefore adjusted each month accordingly (Engelbrecht *et al.* 2005). The total monthly amount was divided into two doses, each of them provided every 2 weeks. Seed germination was monitored every 2 weeks in EUs without leaf litter. A seed was considered germinated when cotyledons were visible. At the end of October, when no new germinated seeds were found in EUs without leaf litter cover, the germination period was considered finished, and germinated seeds were counted in all EUs (with 0, 5 and 12 cm leaf litter cover). In EUs with leaf litter cover, the latter was carefully removed to count germinated seeds, after which they were covered again with the same litter in order to continue assessing seedling survival under constant leaf litter conditions. Therefore, from November 2013 to April 2014, EUs with irrigation treatments were supplied with 5 L of water per month. Finally, in April 2014, all living seedlings were counted.

Statistical analysis

For each stand separately, two-way ANOVA tests were used to evaluate the effects of irrigation (non-irrigated and

irrigated EUs) and leaf litter cover (0, 5 and 12 cm) on the percentage of seed germination (number of germinated seeds, in relation to the 30 seeds initially distributed in each EU). When significant effects were found, multiple least significance difference tests (LSD) were performed as post-hoc tests for multiple comparisons between the different levels of both variables (Sokal & Rohlf 2012). Meanwhile, the non-parametric Scheirer-Ray-Hare test was used to evaluate the effects of the same factors on early seedling survival (number of surviving seedlings in April 2014, in relation to the number of germinated seeds in each EU), since this variable did not meet the homogeneity of variances assumption, even after multiple transformations. In this case, multiple Mann-Whitney *U*-tests were used for post-hoc multiple comparisons (Sokal & Rohlf 2012).

RESULTS

Seed germination

Overall, 66.5, 70.4 and 66.2% of the seeds germinated in the closed-canopy forest (stand A), the interface (stand B) and the shrubland (stand C), respectively.

In the three stands, seed germination was significantly higher when seeds were covered by leaf litter than when not (Table 1; Fig. 3). Leaf litter cover showed an increasing effect size (η^2) along the gradient of canopy openness: Compared to seeds without leaf litter cover, mean germination of seeds covered by leaf litter was more than three times higher in stand A ($\eta^2 = 0.822$), more than four times higher in stand B ($\eta^2 = 0.869$), and more than five times higher in stand C ($\eta^2 = 0.904$). Only in the interface, was there a significant difference between EUs with 5 cm and those with 12 cm leaf litter cover. Meanwhile, although almost all treatments with irrigation showed a higher mean percentage of seed

Table 1. Two-way ANOVA for mean percentage of germinated seeds per experimental unit (\pm SE), in the three stands with different canopy cover, with irrigation and leaf litter cover as the main factors. Stand A is a closed-canopy forest, stand B is an interface between the closed-canopy forest and the sclerophyllous shrubland, and stand C is a sclerophyllous shrubland

Factor	Stand A	Stand B	Stand C
Irrigation			
Non-irrigated	57.1 \pm 36.2 a	69.5 \pm 35.3	64.0 \pm 36.9
Irrigated	74.0 \pm 25.3 b	71.4 \pm 34.2	69.0 \pm 36.7
F(p)	6.683 (0.019)*	0.721 (0.407)	2.346 (0.143)
η^2	0.061	5.29×10^{-3}	0.012
Leaf litter depth			
0 cm	27.5 \pm 19.3 a	20.0 \pm 9.0 a	17.9 \pm 8.9 a
5 cm	85.0 \pm 11.1 b	86.7 \pm 8.0 b	86.9 \pm 10.3 b
12 cm	86.3 \pm 11.5 b	96.3 \pm 4.2 c	94.7 \pm 4.6 b
F(p)	41.569 (<0.001)*	59.741 (<0.001)*	84.380 (<0.001)*
η^2	0.756	0.862	0.891
Interaction			
F(p)	1.050 (0.371)	0.193 (0.826)	0.133 (0.876)

Different letters (a, b and c) indicate statistical differences between treatments as a result of multiple comparisons (LSD test). F(p) = Fisher's test statistic. η^2 = Eta squared, indicating the size of the effect.

*Significant differences at $P < 0.05$.

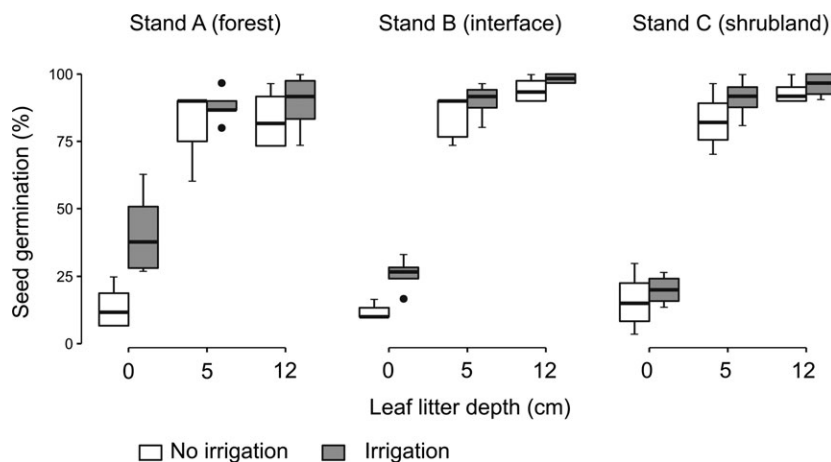
germination in the different stands (Fig. 3), seed germination was significantly increased through irrigation only in stand A, where the mean proportion of germinated seeds was 1.3 times higher in irrigated EUs than in non-irrigated ones (Table 1). The effect size of irrigation (η^2) was the highest in stand A, intermediate in stand C and the lowest in stand B, not showing any clear tendency along the canopy openness gradient. There was no interaction between irrigation and leaf litter cover on germination percentage (Table 1).

Early seedling survival

Overall, 21.8, 7.7 and 0.9% of the seedlings from germinated seeds survived after the first summer

season in the closed-canopy forest (stand A), in the interface (stand B), and in the shrubland (stand C), respectively.

Although almost all treatments with irrigation showed higher mean percentages of seedling survival in the different stands (Fig. 4), irrigation only had a significant effect in stand B, where mean seedling survival of irrigated EUs was 4.3 times higher than that of EUs without irrigation (Table 2). Seedling survival was positively affected by leaf litter cover in the closed-canopy stand (Table 2). In this stand, survival without leaf litter cover was null, and increased to an average of 30.3% with 5 cm litter cover, and to 35.1% with 12 cm litter cover (Table 2; Fig. 4). There was no significant difference between EUs with 5 cm leaf litter cover and those with 12 cm. Since no effect size estimates are provided by the

**Fig. 3.** Mean seed germination percentage per experimental unit (\pm SE) for the different treatments, in stands A (closed-canopy forest), B (interface between closed-canopy forest and sclerophyllous shrubland) and C (sclerophyllous shrubland).

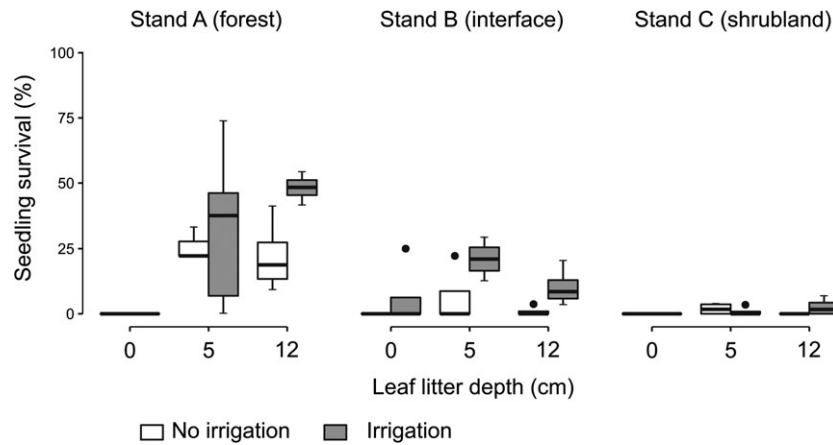


Fig. 4. Mean percentages of early seedling survival per experimental unit (\pm SE) for the different treatments, in stands A (closed-canopy forest), B (interface between closed-canopy forest and sclerophyllous shrubland) and C (sclerophyllous shrubland).

Table 2. Scheirer-Ray-Hare test for mean percentage of surviving seedlings after the first summer season (April 2014) per experimental unit (\pm SE), in response to irrigation and leaf litter cover as main factors, in the three stands with differential canopy cover. Stand A is a closed-canopy forest, stand B is an interface between the closed forest and the sclerophyllous shrubland, and stand C is a sclerophyllous shrubland

Factor	Stand A	Stand B	Stand C
Irrigation			
Non-irrigated	15.1 \pm 14.6	2.9 \pm 6.6 a	0.6 \pm 1.5
Irrigated	27.5 \pm 26.8	12.5 \pm 10.8 b	1.2 \pm 2.3
H(p)	0.852 (0.356)	6.244 (0.012)*	0.200 (0.655)
Leaf litter depth			
0 cm	0.0 \pm 0.0 a	3.6 \pm 9.5	0.0 \pm 0.0
5 cm	30.3 \pm 23.4 b	12.8 \pm 11.4	1.4 \pm 1.9
12 cm	35.1 \pm 17.1 b	5.6 \pm 7.1	1.3 \pm 2.6
H(p)	14.752 (<0.001)*	5.948 (0.051)	3.334 (0.189)
Interaction			
H(p)	1.222 (0.543)	0.475 (0.789)	3.496 (0.174)

Different letters (a, b and c) indicate statistical differences between treatments as a result of multiple comparisons (multiple Mann-Whitney *U*-tests). H(p) = Kruskal-Wallis test statistic.

*Significant differences at $P < 0.05$.

Scheirer-Ray-Hare test, no comparison of the effect size of irrigation and of leaf litter cover on seedling survival could be performed.

DISCUSSION

Seeds of *B. miersii* were able to germinate in the three stands, from the closed-canopy forest to the sclerophyllous shrubland. In all of them, seed germination was assisted by leaf litter cover, while irrigation only showed a significant positive effect in the closed-canopy stand. Meanwhile, early seedling survival was favoured by leaf litter cover only in the closed-canopy stand and was positively influenced by irrigation in the interface between closed-canopy forest and the sclerophyllous shrubland. Almost no

seedlings survived to 1 year in the shrubland (overall mean 0.9%).

This experiment suggests that leaf litter cover is more important for seed germination than for early seedling survival, as proposed by Becerra *et al.* (2004) based on their laboratory experiment. However, neither the influence of litter nor of irrigation is neutral for early survival – as suggested by Becerra *et al.* (2004) – since the litter cover showed a positive effect on survival under high canopy cover, and the irrigation under intermediate canopy cover. This underlines the importance of considering varied environmental conditions of the field to determine the effect of leaf litter and irrigation on seed germination and early seedling survival. In addition, unlike the experiment of Becerra *et al.* (2004), in our study, seedlings were subjected to a longer observational

period before survival assessment (1 year instead of 6 months), during which the lack of water in the field might have been particularly limiting, leading to greater differences in survival between protected (covered by leaf litter) and non-protected (uncovered) seedlings.

Effects of leaf litter and irrigation on germination

The constant positive effect of leaf litter cover on seed germination despite the varying canopy conditions across the different stands, proves that leaf litter cover is an important factor during this phase of the regeneration under both closed and open canopies. Generally, the effect of leaf litter on seed germination depends on overall environmental conditions: whereas it exerts a negative effect on seedling emergence in wetlands and flooded grasslands, positive effects – brought about by maintaining soil moisture and thus reducing seed desiccation (Loydi *et al.* 2013) – are common in dry environments. However, under the generally dry conditions of the study area, which are exacerbated by the persistence of droughts in central Chile (Boisier *et al.* 2016), environmental humidity might have been a limiting factor even in the closed-canopy forest. This explains the positive effects of litter on seed germination also obtained under such conditions. Nevertheless, in agreement with our predictions, the effect of leaf litter on germination was the lowest in the closed-canopy forest, and it increased with canopy openness, showing an intermediate value in the interface, and the highest value in the sclerophyllous shrubland. Thus, according to Loydi *et al.* (2013), the positive effect of leaf litter seems to be higher in open, presumably drier areas. In this sense, the fact that irrigation had a significant effect on germination exclusively in the closed-canopy forest, contrary with our predictions, is rather surprising. This could be explained by the presumably cooler conditions of the closed-canopy forest, which might have allowed for a longer permanence of the water provided by irrigation, adequately compensating for water outputs, and allowing seeds to remain hydrated until germination. On the contrary, in sites with greater exposure, higher solar radiation, higher air temperature and drier soil conditions may have caused increased evaporation and infiltration of the water provided artificially, thereby leading to seed desiccation before germination succeeded. However, measurement of air and soil temperature and moisture, as well as seed water content (before and after the germination experiment) would certainly allow more accuracy in these findings. Different irrigation frequencies and amounts might lead to different results. Therefore,

further experiments addressing these issues might be needed to confirm these interpretations.

Effects of leaf litter and irrigation on early seedling survival

Extreme dry conditions during the summer season are usually a determining factor of seedling mortality in Chilean Mediterranean vegetation (Fuentes *et al.* 1984). Unlike seeds, seedlings of *B. miersii* in our experiment had to withstand the adverse drier conditions of summer. This could, in turn, explain the different responses of seed germination and of early seedling survival to leaf litter cover. Although survival was also shown to be positively affected by leaf litter under closed canopy conditions, the protective effect of litter against evaporation was probably not enough to prevent seedling desiccation in sites with higher exposure (stands B and C), where leaf litter was shown to have no significant effect on survival. It is also worth considering that at the moment of seedling survival evaluation in stand B and particularly in stand C, the leaf litter used to cover the seeds was clearly more fragmented and decomposed than in the highly shaded stand (stand A). This more advanced state of decay of the litter in such stands, presumably due to their lower canopy cover (Mlambo & Mwenje 2010), might also have contributed to higher water losses.

The higher evaporation rates in the stands with lower canopy cover might have been compensated through irrigation, which had a positive effect on seedling survival in the interface (stand B), where leaf litter cover was neutral. This positive influence of irrigation, which did not appear in the closed-canopy forest (stand A), might be due to a compensating effect of lower leaf litter availability due to its more advanced state of decay. A higher water loss without the protection of leaf litter and under intermediate canopy cover would have been compensated through the water provided by irrigation. However, the extreme dry conditions and higher evaporation rates in the sclerophyllous shrubland (stand C) might have outweighed such a compensating effect of irrigation, which in turn could explain its null effect on seedling survival in this stand. Neither leaf litter nor irrigation could increase the low survival rates in the sclerophyllous shrubland, which makes it an inappropriate environment for regeneration of this species.

Implications for management and future research

The results outlined so far contribute to an improved understanding of seed persistence and seedling

establishment of *B. miersii* in the field. It is widely recognized that recalcitrant seeds have better chances to survive when shed into humid and/or cooler environments, where water losses are reduced (Berjak *et al.* 1989). Nevertheless, besides providing empirical data for a threatened species, this study highlights the importance of considering general environmental conditions prior to management decisions. We cannot obviate the fact that the varying effects of litter and irrigation on seed and seedling performance under different environments might be due to multiple factors unrelated to canopy structure and composition, since we have only sampled one site for each canopy condition. However, our findings suggest that the effects of litter and irrigation primarily depend on environmental conditions. When considering sowing, planting or promoting natural regeneration of this species, a significant leaf litter cover should be maintained to facilitate germination. Nevertheless, such efforts should be optimized by prioritizing sites with high to intermediate canopy cover where leaf litter can also positively affect early seedling survival and, additionally, both seed germination and early seedling survival can be increased through irrigation. It is worth mentioning that for *B. miersii*, leaf litter addition might have a double effect, since leaf litter cover has also been observed to significantly decrease seed predation in this species (Galindo *et al.*, 2016, unpublished data).

The brief transition time between seed and seedling stages is generally thought to represent a fitness advantage of recalcitrant-seeded species in seasonal environments, since it maximizes growth and establishment during favourable environmental conditions (Farnsworth 2000; Tweddle *et al.* 2003). Based on our experience, however, the increasing drought and the shortened wet season of the Chilean Mediterranean climate might be disrupting this eventual benefit of the non-dormancy trait, as suggested by the high seedling mortalities even after abundant seed germination. The low seedling survival and the positive effect of treatments under certain circumstances, highlight the importance of focusing not only on germination of recalcitrant-seeded species when promoting their regeneration, but also on the later stages of early establishment, especially in drier regions, where other recalcitrant-seeded species might be experiencing environmental changes that limit the favourable season for seedling establishment.

In this study, we did not control air temperature nor water content of soil and seeds. These data, and particularly the water content of both germinated and dead seeds before and after the experiment, might provide a much deeper look into the effects of litter and irrigation on the regeneration of *B. miersii*, by explaining the processes behind the patterns

observed. Additionally, it is worth addressing the question of whether our results on the effects of irrigation would vary with higher frequency and quantity of water addition. Given the expected increase in the frequency and length of dry periods, and the high sensitivity of regeneration of desiccation-sensitive seeded trees to climate change (Hill & Edwards 2010; Joët *et al.* 2016), it is a matter of scientific and practical importance to further address these questions not only to develop sound conservation practices for this species, but also to deepen the knowledge on the ecological requirements of species with non-dormant seeds for seed germination and seedling establishment.

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