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NEOTROPICAL TREE PRODUCTION: INSIGHTS INTO GERMINATION, GROWTH AND OUTPLANTING FOR *Maytenus boaria*

Keywords:
High mountain forests
Management
Mycorrhizal inoculation
Reforestation

ABSTRACT: Knowledge about tree production practices is essential to support forest restoration projects, but is still lacking for many tree species. *Maytenus boaria* is a neotropical tree distributed across the temperate and subtropical South American mountains. In central Argentina, it is mainly restricted to the most preserved forest remnants. Attempts to plant this species have had little success due to difficulties in seedling production and low seedling survival. We set up four trials aiming to identify the constraints of seedling production and outplanting. Under greenhouse conditions, we evaluated (i) pre–germination treatments and (ii) seedling response to inoculation with arbuscular mycorrhizal fungi (AMF). In the field, we planted *M. boaria* saplings as well as saplings of the most abundant tree in our study site and recorded (iii) survival and height for 10 years. Finally, (iv) we quantified natural recruitment in an attempt to determine *M. boaria* regeneration niche. Germination varied from 13.1 to 29.2% among treatments. Depulped seeds stratified at 5 °C showed the highest germination (29.2%). Shoot phosphorus concentration in AMF-treated seedlings was significantly higher (45%) than in non-inoculated seedlings. Survival of *M. boaria* saplings was similar to that of the most abundant tree in our study site, but their lower height suggested limited growth. We recorded low abundance of *M. boaria* seedlings in the field; therefore, we were unable to identify the characteristics of its regeneration niche. Reforestation activities should include seed depulping and stratification at 5 °C to improve germination. The capacity of AMF to enhance nutrition should be evaluated under field conditions.

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PRODUÇÃO DE ÁRVORE NEOTROPICAL: PERCEPÇÕES SOBRE A GERMINAÇÃO, CRESCIMENTO E PLANTIO DE *Maytenus boaria*

Palavras chave:
Florestas de alta montanha
Manejo
Inoculação de micorrizas
Reflorestamento

RESUMO: Conhecimento sobre as práticas de produção da árvore é essencial para apoiar projetos de restauração florestal, mas é ainda insuficiente para muitas espécies arbóreas. Maytenus boaria é uma árvore neotropical distribuída pelas montanhas temperadas e subtropicais da América do Sul. No centro da Argentina, é majoritariamente restrita nos remanescentes de floresta mais preservados. Tentativas de plantar esta espécie tiveram pouco sucesso devido às dificuldades de produção de mudas e baixa sobrevivência das plântulas. Montamos quatro ensaios com o objetivo de identificar as restrições à produção de mudas e plantio de campo. Sob condições de estufa, avaliamos os tratamentos de (i) pré-germinação e (ii) resposta das plântulas à inoculação com fungos micorrízicos arbusculares (FMA). No campo, nós plantadas mudas de *M. boaria*, bem como mudas da árvore mais abundante em nosso local de estudo, e avaliamos a (iii) sobrevivência e altura por 10 anos. Finalmente, (iv) nós quantificamos o recrutamento natural em tentativa de determinar o nicho de regeneração da *M. boaria*. A germinação variou de 13,1 a 29,2% entre os tratamentos. Sementes sem a polpa estratificadas a 5 °C obtiveram a maior germinação (29,2%). Concentração de fósforo na parte aérea das mudas tratadas com FMA foi significativamente maior (45%) do que em mudas não inoculadas. Sobrevivência das mudas de *M. boaria* foi semelhante ao da árvore mais abundante em nosso local de estudo, mas sua baixa altura sugeriu crescimento limitado. Nós gravamos baixa abundância de plântulas de *M. boaria* no campo, portanto, não fomos capazes de identificar as características do seu nicho de regeneração. Atividades de reflorestamento devem incluir a retirada da polpa das sementes e estratificação a 5 °C para melhorar a germinação. A capacidade de FMA para melhorar a nutrição deve ser avaliada em condições de campo.

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INTRODUCTION

Mountain forests provide important ecosystem services worldwide, such as biodiversity maintenance, clean water supply, carbon sequestration, and protection from soil erosion (KAUFFMAN et al., 2003). Knowledge about tree production practices to help forest restoration projects is important but still lacking for many tree species (RENISON et al., 2011). *Maytenus boaria* Mol. (Celastraceae) is a South American tree exhibiting a disjunct distribution, including three regions: the south of Brazil, the central and southern Andes of Chile and Argentina, where it is most widely distributed, and the high mountains of central Argentina (CORREA, 1988). *Maytenus boaria* is commercially produced for reforestation purposes in the central and southern Andes of Chile and Argentina, where most research on this species has been conducted; however, there are no reports indicating difficulties or failures in plant production and planting establishment (e.g. DONOSO, 2006; REID; ARMESTO, 2011a; REID; ARMESTO 2011b). Conversely, forest restoration projects within the *M. boaria* range in the high mountains of central Argentina have faced several production difficulties due to the low germination rates of this species. In addition, an outplanting trial evaluating *M. boaria* performance in an altitudinal gradient ranging from 900 to 2,700 m a.s.l. also reported low survival and growth rates for central Argentina, especially under livestock grazing (MARCORA et al., 2013). The authors observed that, after three years, survival was on average 3 and 16 % at sites with and without livestock, respectively, showing the highest survival between 1,500 and 2,400 m a.s.l. These low performance values hinder the use of *M. boaria* in forest restoration projects in its central range. Due to the lack of knowledge about how to improve outplanting of this particular species at high altitudes, *M. boaria* represents an interesting study species to evaluate different techniques in order to enhance seedling performance.

The disjunct distribution of *M. boaria* in relatively different climates prevents us from making inferences about the species growth in central Argentina. For example, more than 120 days of cold stratification enhanced *M. boaria* seed germination (DONOSO; WENDLER, 1985). In central Argentina, *M. boaria* trees are mainly restricted to the *Polylepis australis* Bitt. high mountain forests (RENISON et al., 2011), but it also occurs at lower altitudes (1,000 m a.s.l.) together with Chaco Serrano forest species. In this region, seeds of *M. boaria* are often consumed by frugivorous birds that could act as dispersers (REID; ARMESTO, 2011b); however, no specific study has confirmed frugivory nor has seed depulping been tested as a procedure to break seed dormancy in *M. boaria*.

Furthermore, seed germination could be influenced by soil physicochemical characteristics, and seedling establishment might be hampered by neighboring vegetation and herbivores (TORRES et al., 2008). In particular, belowground organisms, including mutualists and pathogens, could be influencing seedling survival and growth. Arbuscular mycorrhizal fungi (AMF), including the species *Rhizophagus intraradices* (Schenck and Smith) Walker and Schüβler, have been widely used to promote host development and protection against root pathogens (e.g. MARRO et al., 2014).

Our aim was to provide insights into the biology of this high altitudinal tree species by identifying the main constraints to *M. boaria* seedling production in central Argentina via experiments under greenhouse conditions and further establishment in the field. Specifically, we addressed whether (i) germination percentage of *M. boaria* differs among seeds from three sources and under different treatments (depulping and two stratification temperatures), (ii) the AMF *R. intraradices* affects seedling growth and nutrient concentration, (iii) natural regeneration is influenced by topography, soil erosion, and vegetation characteristics, and (iv) sapling survival and growth in height are similar to those of saplings of other tree species under the same conditions in a field transplant experiment.

MATERIAL AND METHODS

Study site and species

The study area is located in the high mountains of central Argentina (up to 2,790 m a.s.l.). Mean temperatures at 2,100 m a.s.l. are 5 and 14.4 °C for the coldest and warmest months, respectively, with no frost–free period. Mean annual precipitation is 920 mm, with 83% of the rainfall being concentrated in the warmest months (October to April) (CABIDO, 1985).

Vegetation consists of a mosaic of tussock grasslands and pastures, intermingled with granite outcrops and rocky surfaces exposed due to soil erosion. Interspersed among the grasslands are *P. australis* shrublands and woodlands that often contain *M. boaria* trees, as a scarce subdominant species, shrubs, and fern communities (CINGOLANI et al., 2004).

Maytenus boaria is a South American tree of up to 20 m in height. The leaves of this species are a valuable fodder source for livestock (DONOSO, 2006). Since the beginning of the 17th century, livestock rearing has been the main economic activity in the high mountains of central Argentina. Livestock management traditionally included controlled fires to restrict forest expansion and promote grass regrowth. These practices have created different vegetation types, including forests varying in their structural complexity (CINGOLANI et al., 2004; RENISON et al., 2011). At present, the most conserved mature forest stands are represented by small remnants restricted to ravines or rocky outcrops with steep slopes, where the impact of livestock and fires is low (RENISON et al., 2015). These forest relicts commonly contain isolated *M. boaria* individuals, revealing the potential susceptibility of this species to anthropogenic influence (RENISON et al., 2011).

Greenhouse assays

Germination

Seeds were collected in Quebrada del Condorito National Park (1,900 m a.s.l.; 31°40`S, 64°42`W) from three different sources: (1) bird droppings (n= 1,500), (2) pooled seeds from seven randomly selected individuals (n = 1,400), and one individual tree with outstanding seed productivity (n = 6,000). The latter source was subjected to a pre-germination treatment; for that purpose, we divided it into two subsamples of 3,000 seeds each: (3) a group of seeds was depulped (seed aril was mechanically removed by washing the seed thoroughly with tap water in a sieve), and (4) the other group of seeds was not depulped and was used as a control treatment. These four groups of seeds were divided in two subsamples and placed to germinate at two stratification temperatures in germination chambers (5 °C and 15 °C for 90 days), thus totaling eight seed treatments. Seeds were sown in seedbeds containing sterilized sand and were allowed to germinate for 49 days; they were watered daily. Cotyledon emergence was used as germination criterion.

The different seed sources were chosen after field observations in the study area. Bird droppings are very commonly observed away from parent trees, suggesting that birds are the most effective dispersers of the target tree species. Isolated individuals with high seed production are also found in the area.

AMF inoculation

Spores of *R. intraradices* and leek roots colonized by the same AMF species (origin: La Plata, Herbario Spegazzini (LPS), Tierra del Fuego 28) were used for the inoculation experiment. To prepare spore inoculum, *R. intraradices* was propagated on alfalfa (*Medicago sativa* L.) plants grown in a soilless medium in 190-mm^3 pots containing a mixture of sterile perlite and vermiculite (1:1) for 6 months. Plants were watered three times a week with the following nutrient solution: $MgSO_4{\cdot}7H_2O$, 0.75 mM; $NaNO_3$, 1 mM; K_2SO_4, 1 mM; $CaCl_2{\cdot}2H_2O$, 2 mM; $Na_2HPO_4{\cdot}12H_2O$, 3.2 μM; FeNa-EDTA, 0.025 mM; $MnSO_4{\cdot}4H_2O$, 5 μM; $CuSO_4{\cdot}5H_2O$, 0.25 μM; $ZnSO_4{\cdot}7H_2O$, 0.5 μM; H_3BO_3, 0.025 mM, $NaMoO_4{\cdot}2H_2O$, 0.1 μM. For root inoculum, the fungus was cultured for 6 months in pots containing the same substrate, as described previously, but using leek (*Allium porrum* L.) as host plant. Inoculum of AMF consisted of 0.25 g of leek roots with 62.5% colonization, as proposed by McGonigle et al. (1990), and 5 g of the perlite–vermiculite mixture containing 1,800 spores.

Seeds of *M. boaria* were surface-sterilized with 10% NaClO for 5 minutes, then washed with sterile water and sown in Petri dishes containing sterilized sand at 5 °C for 90 days. When emerged, seedlings were placed in trays containing a substrate of a sterile soil and sand mixture (3:1). Soil physicochemical properties were: pH: 6.58, organic matter: 14.68%, organic carbon: 8.52%, N: 0.68% and P: 86.5 ppm. Two–month old seedlings were transplanted to containers (190 mm^3 volume, 20 cm long x 4 cm wide) and grown under greenhouse conditions (24/18° C day/night, 16/8 h day/night photoperiod) for six months. Two treatments with five replicates each were used: (i) plants inoculated with *R. intraradices* (hereafter called "AMF- treated") and (ii) plants not inoculated with *R. intraradices* (hereafter called "control"). For the AMF-treated plants, substrate was placed in the container up to two thirds of its volume; then a homogeneous layer of the fungal inoculum (roots and spores) was placed 3 cm below the substrate, *M. boaria* seedlings were planted and roots were covered with the same substrate. For the control treatment, 0.25 g of non–mycorrhizal leek roots and 5 g of sterilized perlite–vermiculite mixture were added.

After six months, plants were harvested and shoot and root length (to the nearest mm), and dry weight (to the nearest g) were measured. Phosphorus concentration (percentages of P per g of dry weight) was determined by the acid dry digestion method (JONES et al., 1991). In order to assess root colonization, plant roots were cleared with 10 % KOH (15 min at 90° C), then acidified with 1 % HCL (1 min at room temperature) and stained in 0.05 % aniline blue, according to Phillips and Hayman (1970). To determine root colonization percentage, five permanent slides per plant were mounted and 100 line intersections per root sample were quantified under Nikon–E200 light microscope at 400x magnification, according to the magnified intersection method of McGonigle et al. (1990).

Field assays

Survival and height

To produce *M. boaria* for the outplanting assay, we collected approximately 10,000 seeds from several forest fragments in April 2001. Seeds were sown in germination trays in a greenhouse after 90 days of stratification at 5 ºC (Donoso and Wendler 1985); 3 months later all emerged seedlings (i.e. individuals up to 5 cm in height) were transplanted to pots (5 x 15 cm) containing field soil and sand (3:1). Saplings (i.e. individuals > 5 cm in height) were transplanted at 6–7 months of age. In the field, saplings were spaced 3 to 12 m apart and well-watered at planting. The same procedure was used to produce *Polylepis australis* Bitt. saplings, the dominant native tree in the forests where *M. boaria* is present in central Argentina. *P. australis* seeds, which do not require stratification, were used as a control for comparison with the target tree species.

Saplings (n = 104 per species) were outplanted to "Los Gigantes" (31º25`S, 64º48`W), in a 22-ha livestock exclosure at the beginning of the summer (December 2001). They were spaced at least 10 m apart and watered abundantly only on the day of planting; each individual was assigned a number that was indicated in a metal pin. Sapling survival and height of both species were measured at planting (December 2001) and every winter (June–August) until 2006 and again in 2011 (10 years after planting), totaling seven measurement dates.

Regeneration niche

To evaluate natural regeneration, two river basins with different disturbance histories were chosen: Los Molles (relatively well-preserved basin with 15% forest and 0.21 human settlement/km^2; 31°58`S, 64°56`W) and Mina Clavero (degraded basin with 7% forests, 0.65 settlement/km^2, and 34% of exposed rock surfaces due to extensive soil erosion; 31° 58`S, 64° 56`W) (CINGOLANI et al., 2004; 2013; RENISON et al., 2011). Permanent plots of 30 m^2 (30 at Los Molles and 26 at Mina Clavero) were used to assess seedling occurrence. Within each plot, 10 1-m^2 quadrats were placed to record: (1) soil erosion, considering three classes: 0 = absence of erosion, 1 = less than 50 % exposed soil, and 2 = more than 50% exposed soil; (2) local slope inclination; (3) sun incidence visually estimated as the sun's trajectory (º) that directly illuminates the quadrat, i.e. in a flat plateau with no obstacles the value would be 180º; height (cm) of (4) litter, (5) dead, and (6) live vegetation at four randomly selected points; and proportion of (7) mosses; (8) ferns; (9) thick tussocks (*Poa stuckertii* (Hack.) Parodi), (10) thin tussocks (*Festuca* spp., *Deyeuxia hieronymii* (Hack.) Türpe and *Stipa* spp.), (11) *M. boaria* seedling occurrence, and (12) number of adults near the quadrat.

Data analysis

We evaluated the effects of the different treatments on seed germination (bird droppings + random individuals + depulped seeds x two stratification temperatures) using a Pearson's Chi–squared test. For pair comparisons, Yates continuity correction was applied.

An analysis of variance (ANOVA) with treatment as fixed factors (two levels: AMF-treated and control) was performed to determine the effect of inoculation on *M. boaria* growth, shoot P concentration, and root colonization.

For the outplant experiment, the probability of sapling survival was analyzed using Kaplan–Meier survival estimation curves of the Survival (THERNEAU, 2014) package in R (R CORE TEAM, 2013). Survival curves were compared between species using χ^2 test. Differences in sapling height among dates and between species were detected by performing a repeated measures ANOVA, with dates as within factor (seven levels: planting year and years 1, 2, 3, 4, 5 and 10 after planting) and species (two levels: *M. boaria and P. australis*) as between factor. A Tukey post-hoc test was applied to determine differences among dates and between species. Height was previously log-transformed to achieve normality of error distributions.

For niche characterization, we performed a principal component analysis (PCA) to select the variables that were best correlated to the components explaining at least 70 % of variance. Seedling occurrence (presence–absence) was regressed against the selected variables using binomial regressions, which were fitted using the *glm* function with a logit link. The significance of the fitted model was determined using the Akaike information criterion (AIC) and the P value with a significance level of 0.05.

All statistical analyses were performed using the R software; and in all cases, significance level was 0.05, and residuals were previously tested for normality and homoscedasticity using Shapiro–Wilks and Levene's tests, respectively.

RESULTS

Greenhouse assays

Germination

Germination of seeds in the different treatments varied from 13.1 to 29.2% (Table 1). Seeds collected from one *M. boaria* tree with outstanding seed productivity showed the highest germination percentage when subjected to depulping and stratification at 5 °C (29.2 %). Seeds depulping and stratification at 5 °C significantly improved seed germination compared to depulped seeds at 15 °C ($\chi^2 = 6.81$, $P = 0.01$); the remaining treatments had no significant effect on germination percentage (Table 1).

TABLE 1 Germination percentages of *Maytenus boaria* seeds under different treatments. Lowercase and uppercase letters indicate significant differences among rows and among columns, respectively ($p < 0.05$).

Sources	Stratification temperature	
	5 °C	15 °C
bird droppings	16.2 aA	16.6 aA
random individuals	21.7 aA	26.0 aA
depulped seeds[1]	29.2 aA	13.1 aB
non-depulped seeds[1]	21.9 aA	22.0 aA

[1]Seeds for these treatments were collected from one *M. boaria* tree showing outstanding seed productivity.

AMF inoculation

Control plants did not show root colonization, demonstrating the lack of contamination during the greenhouse experiment. Seedlings of *M. boaria* inoculated with *R. intraradices* showed *Arum*–type colonization (i.e. arbuscules and intercellular hyphae, Figure 1). Root colonization varied from 74.3 to 92.6 % (mean = 81.5 ± 3.2). The highly branched exchange organs (i.e. arbuscules) were the most abundant intraradical structures (mean = 62.4 ± 3.7), ranging from 39.8 to 84.6 %. AMF vesicle colonization ranged from 12.5 to 20.4 % (mean = 16.4 ± 1.7), hyphae from 6.2 to 11.9 % (mean = 12.4 ± 1.5) and entry points from 0 to 17.9 % (mean = 2.3 ± 0.6).

After 6 months under greenhouse conditions, height of *M. boaria* seedlings did not show significant differences among treatments (shoot length: $F = 2.9$, $P = 0.1$; root length: $F = 3.4$, $P = 0.1$) (Figure 2a, 2b). AMF-treated seedlings showed a non-significant trend of higher values in root and shoot dry weight, as well as root to shoot ratio, than control plants (root dry weight: $F = 1.1$, shoot dry weight: $F = 0.4$, $P = 0.5$; $P = 0.3$; root/shoot: $F = 1.6$, $P = 0.2$) (Figure 2c, 2d, 2e). Phosphorus concentration was significantly higher in AMF-treated plants than in control ones ($F = 10.9$, $P = 0.01$; Figure. 2f).

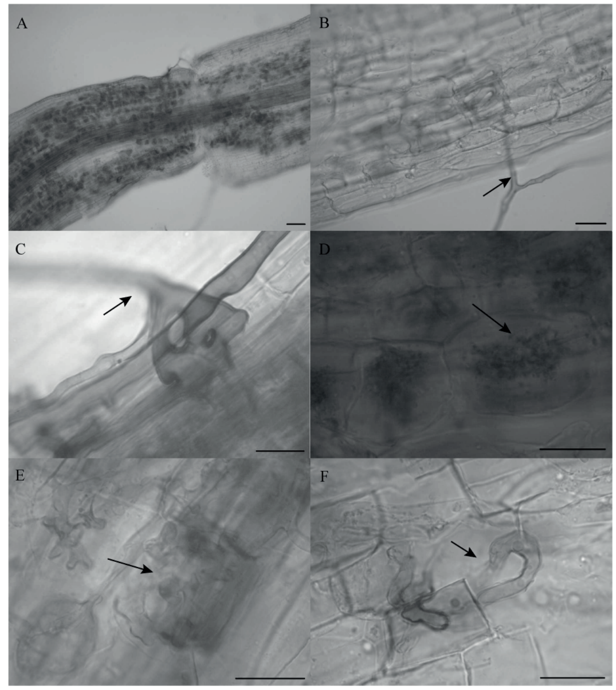

FIGURE 1 Roots of *Maytenus boaria* colonized by *Rhizophagus intraradices*. A: General view of a colonized root. Arrows indicate B and C: entry point, D and E: arbuscule, and F: coil. Scale bars: 50 μm.

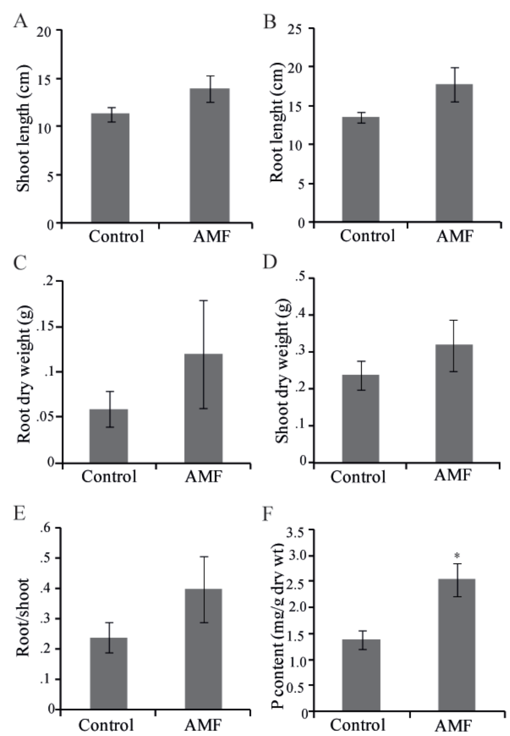

FIGURE 2 Mean ± standard error of A: Shoot length, B: Root length, C: Root dry weight, D: Shoot dry weight, E: Shoot/root ratio, and F: P concentration (percentage/g dry weight) of *Maytenus boaria* inoculated (AMF) and not inoculated (Control) with *Rhizophagus intraradices*.

Field assays

Survival and height

Average survival of outplanted saplings was 30 % for *M. boaria* and 45 % for *P. australis* over the 10 years of measurements, with no significant differences being detected between species (χ^2= 2.3, *P* = 0.125; Figure 3a). Average height for *M. boaria* saplings did not increase from year 1 through 5, but doubled in the next period until year 10, reaching a height of 12 ± 2 cm. *Polylepis australis* seedlings steadily grew on average 5 cm/year, reaching a height of about 50 ± 7 cm in year 10 (Figure 3b). These different height patterns were reflected in a significant interaction between species height and dates of measurements (*F* = 76.60, *P*< 0.001), as well as in significant differences among dates for both species (*M. boaria: F* = 55.85, *P*< 0.001; *P. australis: F* = 32.85, *P*< 0.001). On dates 0 (at planting) and 1 (after the first year), *M. boaria* saplings were significantly taller than *P. australis* saplings (at planting: *F* = 332.6, *P*< 0.001; year 1: *F* = 19.93, *P*< 0.001). In the following two years, both species were similar in height (year 2: *F* = 1.29, *P* = 0.257; year 3: *F* = 0.59, *P* = 0.445). However, after the fourth year and even after 10 years from outplanting, *M. boaria* saplings were significantly smaller than *P. australis* saplings (year 4: *F* = 5.60, *P*< 0.05; year 5: *F* = 10.5, *P*< 0.01; year 10: *F* = 13.77, *P*< 0.001).

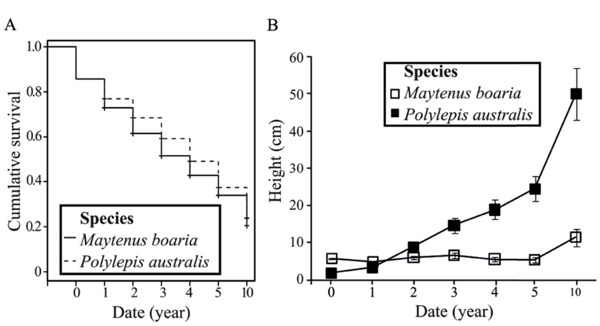

FIGURE 3 A: Kaplan–Meier survival estimation curves, and B: Mean height ± standard error of *Maytenus boaria* and *Polylepis australis* seedlings outplanted to the high mountains of central Argentina and monitored for 10 years (0: planting date; 1, 2, 3, 4, 5 and 10: years after planting (n = 104).

Regeneration niche

In the most degraded river basin (Mina Clavero), no seedlings were recorded in any of the 260 quadrats. In the most preserved river basin (Los Molles), a total of 52 seedlings were recorded in only 6 of the 300 quadrats (Figure S1). The first two PCA axes explained 75 % of the variation in the quadrat site data and were correlated to proportion of fern and sand, thin tussocks, height of both dead and live vegetation, and tree cover. The quadrats were characterized by a proportion of ferns from 0.15 to 1.0 (mean = 0.41, s.e. =0.13) and of thin tussocks from 0 to 0.15 (mean = 0.04, s.e. =0.03). Proportion of dead vegetation ranged from 0.25 to 0.77 (mean=0.47, s.e. =0.87) and of live vegetation from 0.2 to 0.7 (mean=0.46, s.e.=0.85). However, binomial regressions of seedling occurrence against these variables were not significant (Table 2).

TABLE 2 Binomial regression between seedling occurrence and the most relevant variables.

	Regression coefficient	AIC	P
quadrats (1 m²)			
fern proportion	0.875	18.25	0.38
sand proportion	–0.004	18.05	0.99
thin tussock proportion	–0.544	18.66	0.59
dead vegetation height	–1.039	16.44	0.30
live vegetation height	–0.796	19.69	0.43
plots (30 m²)			
tree cover	–0.005	17.50	0.99

DISCUSSION

Mountain forests are being reduced due to anthropogenic influences (ELLENBERG, 1979). Logging, fires and livestock rearing are reducing the extensions of forests, therefore affecting the ecosystem services they provide (HENSEN, 2002). In this context, it is highly relevant to evaluate suitable techniques for improving forest management. In this study, we observed disparate responses of *M. boaria* to different greenhouse and field techniques commonly used for reforestation practices.

As a first approach to the study of seed ecology of *M. boaria* in the high mountain forests of central Argentina, we compared the germination of different seed sources, including pooled seeds from randomly selected trees as a control treatment. The highest germination percentage values were obtained with mechanical removal of the seed aril together with stratification at 5 °C. This effect is not individual–specific, since seeds from the same tree without depulped seeds or at a different stratification temperature evidenced lower germinability. The combination of seed depulping and stratification in other bird-dispersed species was also frequently reported as the most suitable pre-germination treatment (e.g. KAYE; KUYKENDALL, 2001). Moreover, previous studies on *M. boaria* in Chile showed that successful germination requires stratification at 5 °C for at least 30 days (DONOSO; WENDLER, 1985). Although cold stratification is an adaptation for germination after the winter in Mediterranean-type climates with wet winters, cold stratification was also necessary for achieving germination in the monsoonal climate of central Argentina. Probably, the typical dry winters with irregular rains are adequate for a successful germination of *M. boaria* seeds. Nevertheless, further research in germination success of the species in central Argentina is needed, since the best performing treatment reached only 29.2 % germination, which is still low compared to germination values of up to 87.5 % reported for central Chile by Reid and Armesto (2011a).

In a previous study, seeds of *M. boaria* forests from central Chile obtained from bird droppings showed similar germination to that of manually depulped seeds (REID; ARMESTO, 2011a). A similar trend was observed in our study when comparing seeds from bird droppings and manually depulped seeds. However, contrary to results of Reid and Armesto (2011a) these treatments showed lower germination values than intact fruits. Gut digestion of *M. boaria* seeds by avian frugivores in the high mountain forests of central Argentina (mainly by *Turdus chiguanco* Lafresnaye and d'Orbigny) may not be as successful in stimulating seed germination as digestion by Chilean dispersers (*Colorhamphus parvirostris* Gould and Gray) and *Xolmis pyrope* Kittlitz) (REID; ARMESTO, 2011b). Seed digestion may sometimes change the compounds of fruit pulp, thus altering seed microenvironment and reducing germinability (SAMUELS; LEVEY, 2005). Further research that combines treatments simulating passage through bird guts with different types of stratifications might yield higher germination rates for *M. boaria* of central Argentina.

Seedling inoculation with AMF before outplanting might promote establishment and growth. Previous works revealed the presence of mycorrhizal colonization in *M. boaria* roots (FONTENLA et al., 1998). In this study we tested whether *R. intraradices* AMF helps to improve the target species growth and P nutrition. Usually late–successional trees, such as *M. boaria*, have been depicted as highly mycotrophic and dependent on the association with AMF for establishment and growth (JANOS, 1980). Although growth parameters were not significantly influenced by AMF inoculation, at least during the first six months of seedling development, a trend to an increase in root and shoot length and biomass of AMF-treated plants compared to control ones was observed. Moreover, shoot P uptake was probably promoted by the association with AMF, since our inoculated seedlings showed higher P concentration than control plants, in agreement with results of Smith et al. (2011). It would be interesting to evaluate possible variations of the response of trees to AMF inoculation during later stages of their life cycle. In addition, whether seedling growth is promoted under nutrient–poor soils or even by inoculation with other AMF taxa should be further evaluated.

The outplanting experiment of *M. boaria* compared with *P. australis*, which is a relatively more abundant tree endemic to the high mountains of central Argentina, revealed similar survival for both species. However, for *M. boaria*, it was almost three times higher than previously reported for the study area (MARCORA et al., 2013). We identified that shoot growth of *M. boaria* was especially low compared with that of *P. australis*, which is being used for restoration projects in the area (RENISON et al., 2013). Interestingly, for *M. boaria* we found almost no growth during the first 5 years, whereas an increase in height was detected between years 5 and 10. Probably, after year 5, the vegetation of the plantation area may have been tall enough to facilitate *M. boaria* growth through nurse effect via amelioration of harsh abiotic environmental conditions prevailing in the area. The fact that *M. boaria* in the mountains of central Argentina is often found only in mature forests (RENISON et al., 2011) also supports our hypothesis. Therefore, we suggest studying facilitation by nurse plants to promote seedling growth, as observed for other shade–tolerant woody species.

Precise microsite characterization of *M. boaria* regeneration niche was not possible in this study because of the low abundance of recorded seedlings. The low seedling regeneration would not be explained by a lack of seed sources, since *M. boaria* adult density was 6.8 and 19.3 trees·ha^{-1} in the degraded and preserved basin, respectively (re-calculated from RENISON et al., 2011), and while performing the field studies, we often found bird droppings containing *M. boaria* seeds. Our data set does show that regeneration is very low and aggregated, and that the number of seedlings was about five times lower for *M. boaria* than for *P. australis* (TORRES et al., 2008). Tree recruitment and seedling distribution in bird–dispersed species is highly dependent on frugivores (HERRERA et al., 1994). Future studies should focus on determining the role of native bird dispersers in *M. boaria* regeneration to evaluate if this process is being constrained by the decreasing abundance of specific bird dispersers. Moreover, seed predators (e.g. rodents) could be limiting target species recruitment, which should be further investigated.

CONCLUSION

Maytenus boaria reforestation activities should include manually depulped seeds (i.e. mechanical removal of seed aril) together with stratification at 5 °C to promote seed germination. Moreover, tree growth under field conditions should be evaluated to confirm a positive effect of AMF in later developmental stages, since P uptake was found to be promoted by AMF inoculation in the first months of seedling growth. We suggest testing facilitation by nurse plants. Our study addressed for the first time the response of *M. boaria* to different techniques that could overall be implemented to evaluate outplanting strategies for different tree species.

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