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ORIGINAL ARTICLE

Effects of storage on seed germination and viability for three native tree species of Ecuador

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Abstract
Many forests restoration programs and efforts depend on seeds. Particularly in the Andes, further information regarding seed germination requirements and seed storage behavior is necessary. The aim of this study was to evaluate the effects of storage conditions on the germination percentage, the viability and the coefficient of velocity of germination for seeds of three native tree species (*Cedrela montana*, *Weinmannia fagaroides* and *Oreocallis grandiflora*). Under controlled conditions, the seeds were exposed to three levels of seed moisture content and storage temperatures (5 °C, 10 °C and room temperature at approx. 19 °C) for 3, 6 and 12 months. The results showed that at 3–6 months of seed storage under temperatures of 5 and 10 °C, the seeds had a high percentage of germination, viability and germination speed for *C. montana* and *W. fagaroides* compared to those stored at room temperature. At 12 months of storage, there was a marked reduction in seed germination in all treatments for both species. Furthermore, the seed germination and viability of *O. grandiflora* was not influenced by any of the above storage treatments. However, at the end of the experiment a slight decrease was observed, hence this species might be tolerant to medium- and long-term storage conditions. Though limited to just three co-occurring species, the study provided insight into the variability in responses to storage, with preliminary indications of appropriate storage conditions to maximize storability of seeds for restoration programs. Importantly, the study demonstrated the need for empirical testing of storage responses (temperature and duration) of seeds before subjecting untested species to a particular storage regime.

Keywords Ex situ conservation · Seed storage behavior · Desiccation tolerance · Seed management · Reforestation · Andes

Introduction

Globally, tropical mountain forests are under pressure from deforestation, land-use conversion and habitat fragmentation (Soh et al. 2019). This is problematic, considering that most montane tropical forests are in biodiversity hotspots with high levels of endemism (Mittermeier et al. 2011). Thus, there is a growing need to collect and store seeds of forest tree species for subsequent restoration programs to achieve local and global targets for ecosystem restoration (FAO 2014a; Broadhurst et al. 2016; Shaw 2019). Sufficient knowledge of seed storage conditions is scarce for tropical tree species. This knowledge is a fundamental tenet for successful ecological restoration programs, especially considering the variety within tropical forests from dry forests, highlands mountain forests, humid forest, rainforests to cloud forests. To ensure a sustainable supply of planting stock required in reforestation programs, research on the appropriate seed storage conditions is needed for maintaining seed germination capacity through controlling moisture content, storage temperature and storage duration (Colville 2017; Kim 2018). A key constraint to storability is the ability of seeds to tolerate desiccation. Orthodox seeds are tolerant to desiccation, withstand low temperatures and can be stored for long time periods. Recalcitrant seeds, however, are intolerant to desiccation and cannot withstand low temperatures as intact seeds with attenuated storage capacity (Schmidt 2000; Simpson et al. 2004). Intermediate seeds exhibit intermediate storage characteristics (Walters 2015) as they can be dried to a

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low moisture content, but are sensitive to low temperatures with seeds being sustained in storage for only a few years (Bonner 2008). According to Kettle (2012), Umarani et al. (2015) and Kainer et al. (1999), 44% of neo-tropical tree species are recalcitrant. Likewise, Wyse and Dickie (2018) predicted that 10% of the seed plants from Ecuador produce desiccation-sensitive seeds and, based on a local study in a mountain forest, three of seven species investigated possessed seed desiccation sensitivity (Palomeque et al. 2017). In general, seed banking has several challenges for maintaining viable seeds over time, FAO (2014b) has been outlining standards for seed drying based on medium-term storage conditions at 5–10 °C and a relative humidity of 15 ± 3%. For long-term storage, other conditions are needed, including −18 ± 3 °C of temperature. However, long-term storage could result in a loss in viability and seedling vigor due to ageing physiology (Ratajczak et al. 2015). Moreover, the ageing process is known to be accelerated in conditions of high temperature and high seed moisture content (McCormack et al. 2014; Murthy et al. 2003). One of the methods to prolong seed viability is to maintain seeds in a constant state of dryness to reduce the metabolic rate (Bonner 2008). Some studies have reported that orthodox seeds stored for several months or years increased the germination performance. This is the case for *Rhynchospora alba,* a species found in peatlands, whose seed germination increased after 12 months of dry storage (Bourgeois et al. 2019). The same pattern was found for *Kumara plicatilis*, a tree from African montane habitats, which had a higher cumulative percentage germination after being stored at room temperature for 9 months of storage, although these seeds had the slowest speed of germination (Cousins et al. 2014). Whether storage stimulates embryo maturation and/or breaks endogenous seed dormancy or after-ripening is likely species dependent (Bentsink and Maarten 2008; Penfield 2017). In this study, we investigated seed storage behavior of three key tree species of the Andean montane forest as a basis for promoting best practice in community and government, as they require seeds for their ecological restoration programs and to provide a germplasm storage capacity to safeguard species with ecological and socioeconomic importance (Cousins et al. 2014).

We investigated the following questions (1) What are the differences in the germination percentage and viability of seeds of three native tree species stored under different levels of seed moisture content, temperature and duration of storage? (2) What is the germination rate, here measured as coefficient of velocity of germination (CVG), depending on the alterations in moisture content, temperature and duration of storage? CVG can be used as an indication whether some of the experimental settings and conditions are able to impair seed vigor, which in turn enables us to develop appropriate seed production practices.

Materials and methods

Species selection and seed sources

The three framework species, which were selected, *Cedrela montana* (Cedro), *Oreocallis grandiflora* (Gañal) and *Weinmannia fagaroides* (Sarar), are wind-dispersed trees that have ecological and socioeconomic importance in the southern region of Ecuador. The seeds were collected in two natural forests, Llaviuco and Mazan, in the province of Azuay (Fig. 1), within an elevation range of 3.000 to 3.300 m asl. According to the Llaviucu weather station, mean precipitation is 1282 mm (Instituto Nacional de Meteorología e Hidrología de Ecuador, INAMHI), mean average temperature is 10.4 °C, while mean maximum temperature is 15.4 °C and mean minimum temperature is 6.6 °C (Empresa Pública Municipal de Telecomunicaciones, Agua Potable, Alcantarillado y Saneamiento de Cuenca, ETAPA). In each forest, at least 10 mother trees per species were identified based on a marked distance between trees. Their fruits were collected in different months in 2016, depending on peak fructification periods.

Experimental design

The seeds were transported to the laboratory at the University of Cuenca for cleaning and visually inspected for evidence of infestation by fungi or insects. Only clean and intact seeds were then immediately incorporated into the seed storage experiments. The total seed pool was mixed and split in equal portions for: (1) determination of the initial seed moisture content to define the type of seed (orthodox, intermediate and/or recalcitrant), (2) subjection of seeds to varying moisture content and (3) seed storage using different treatments, which includes three moisture contents depending on the species, temperature (5 °C, 10 °C and room temperature) and duration of storage (3, 6 and 12 months). Table 1 shows the three different experimental levels for each treatment.

For the determination of initial seed moisture content, two samples of 100 seeds per species were used, except for *W. fagaroides,* where a total of 200 seeds in each sample were analyzed. The sample of the species were exposed to 103 °C for 17 h according to ISTA regulations (ISTA 2007) (Eq. 1). The seed size of the three species was < 7 mm. They were oven-dried directly after collection (ISTA 2007). A calibrated curve of desiccation by the oven-drying method (Rao et al. 2006) for each species was drawn to identify the time (hours and minutes) at a constant temperature of 45 °C until the seeds reached the two levels of moisture content without causing embryo

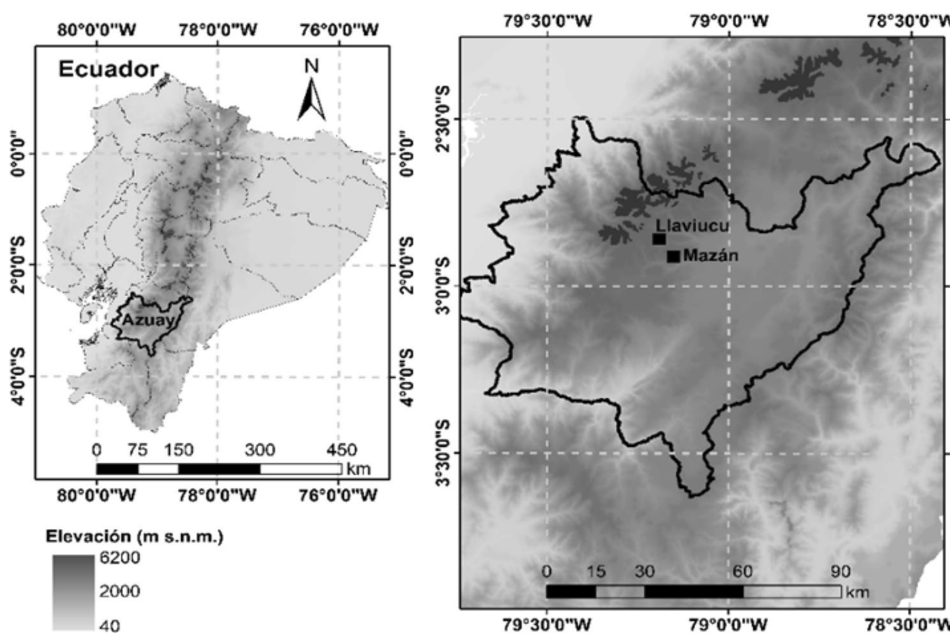

Fig. 1 Seed collection at Mazán and Llaviucu Forests, located in the Azuay province, Southern Ecuador

Table 1 Summary of the seed storage treatments with their respective moisture content levels applied to three native tree species

Species	Family	Seed moisture content (%)	Storage temperature	Duration of storage
Cedrela montana	Meliaceae	(1) 19 (Initial) (2) 10 (3) 15	5 °C, 10 °C, room temperature	3, 6, 12 months
Oreocallis grandiflora	Proteaceae	(1) 11 (Initial) (2) 5 (3) 8	5 °C, 10 °C, room temperature	3, 6, 12 months
Weinmannia fagaroides	Cunoniaceae	(1) 22 (Initial) (2) 10 (3) 15	5 °C, 10 °C, room temperature	3, 6, 12 months

damage or death. This calibrated curve was based on a previous test on thermal tolerance of the embryo axis. The seed moisture levels were calculated by weighing the seed pool before and after oven drying (Eq. 1).

$$\mathrm{CH} = (\mathrm{m2} - \mathrm{m3}) \times \frac{100}{(\mathrm{m2} - \mathrm{m1})} \quad (1)$$

CH: Moisture Content (%), *m*1**:** Weight in grams of the container, *m*2**:** Weight in grams of the container, and the seeds before drying ,*m*3**:** Weight in grams of the container, and the seeds after drying.

Once the seeds reached the two previously determined levels of moisture content, they were wrapped immediately in aluminum foil and placed in Ziploc bags with silica gel and stored in hermetic containers. The temperatures at 5 °C and 10 °C were calibrated in individual refrigerators, while the room temperature was based on the following ambient laboratory conditions: mean temperature 19 °C, minimum temperature 16ºC, maximum temperature 25ºC and an average relative humidity of 64%.

For each treatment, including the control, a total of four replicates with 25 seeds each were used for *C. montana* and *O. grandiflora*, while 50 seeds were used for *W. fagaroides* due to their smaller seed size. The seeds were sown in Petri dishes with inserts of moistened paper, previously sterilized in the oven to avoid contamination. The seeds were disinfected using a liquid soap, sprayed with alcohol (70% solution), and thoroughly rinsed with distilled water. Humidity was maintained with distilled water during the germination period, which was about 50 days for *C. montana* and *O. grandiflora* and about 70 days for *W. fagaroides*. The germination was recorded daily. A seed was considered germinated when embryonic leaves appeared and a radicle had emerged (ISTA 2007).

Once germination had stabilized, non-germinating seeds were evaluated through a viability test using 2,3,5-triphenyltetrazolium chloride (TTC); therefore, two categories post germination were identified as viable and non-viable seeds. Finally, CVG was calculated to indicate the total time of seed germination (Baskin and Baskin 2014) (Eq. 2), where a high value of the CVG indicates rapid seed germination.

$$CVG = \frac{100(A1 + A2 + Ax)}{A1T1 + A2T2 + AxTx)} \quad (2)$$

$A1 + A2$ and Ax = number of seeds germinated on the first, second and final days. $T1$, T2 and Tx = number of days between sowing and first, second and final times.

Statistical analysis

At the beginning of the experiment, a one-way ANOVA test followed by a Tukey comparison was performed for each species, using seed moisture content as a single factor applied to all dependent variables. Here, assumption of normality and homogeneity of variance was achieved. Other analyses were conducted separately for each species using generalized linear models. The fixed factors were duration of storage, temperature, moisture content, and their interactions. The evaluated variables were germination, viability, non-viable seeds and CVG. If the model did not present a normal distribution of residual error, then they were considered as a Poisson distribution. Model selection was based on the lowest values of Akaike information criterion (AIC) and Bayesian information criterion (BIC). Analyses were performed with the R package ''nlme'' (Pinheiro et al. 2018).

Results

Effect of moisture content, temperature and duration of storage on percentage germination, viable and non-viable seeds

In *C. montana,* a clear pattern of higher cumulative germination (above 50%) was observed at 3 and 6 months of seed storage at 5 and 10 °C (Fig. 2a, b). However, the different levels of moisture content did not influence germination ($p > 0.05$) (Table 5). Room temperature substantially and negatively affected seed germination at the beginning of storage, with less than 25% germination under all levels of moisture content (Fig. 2a,b). At 12 months, seed germination was considerably reduced in all treatments (Fig. 2c). The interaction between duration of storage, temperature and moisture content was statistically significant ($p < 0.05$) (Table 5). The results of seed storage showed a similar percentage of germination to the control, except for seeds exposed to 10% of moisture content and seeds at room temperature which both showed low germination outcomes (Table 2). This species possessed a high percentage of non-viable seeds which increased with storage time (Table 3); storage conditions and their interactions with temperature and time had high statistical significance ($p < 0.05$) (Table 5). Both control and the treatments had high proportions of non-viable seeds (post germination) from 6 months of storage (Tables 2, 3). *O. grandiflora* had high seed germination, which approached 100% under all storage conditions evaluated in the experiment (Fig. 2d, f). There was also no statistical significance in the combination of the three factors of moisture, temperature and duration of storage ($p > 0.05$) (Table 5). Nevertheless, at 12 months of storage at room temperature and under all levels of moisture content, the percentage of germination showed a slight reduction (75%) (Fig. 2f). The results of germination in the storage experiment were comparable to the control, except for room temperature at 12 months (Table 2, Fig. 2f). This species showed also a high viability post germination during storage. However, at 12 months under room temperature, non-viable seeds had increased slightly (Table 3). For viable and non-viable seeds, most of the factors tested and their interactions, were statistically significant ($p < 0.05$) (Table 5). In comparison to control treatment, the behavior of the variables was similar to the treatments of storage experiment (Table 2).

W. fagaroides had low germination at all storage conditions tested in the experiment (Fig. 2 g,h,i). At 3 and 6 months, the percentage of germination was similar among all moisture contents and temperatures, ranging between 15 and 39% (Fig. 2 g, h). At 12 months, the room temperature treatment had the lowest germination (< 5%) (Fig. 2i). Statistically significant ($p < 0.05$) was found for the interaction of all the factors (Table 5). In comparison to the control treatment, the percentage of germination was also low (Table 2). This species demonstrated a high percentage of non-viable seeds across all treatments and during storage (Table 3). The interaction of three factors had a statistical significance ($p < 0.05$) (Table 5). Similar results were observed in the control treatment (Table 2).

Effect of seed moisture content, temperature and duration of storage on CVG

C. montana had a decline in seed germination speed over storage time (Table 4) ($p < 0.05$), except with the interaction of three factors (Table 5). In comparison to the control treatments, there were comparable values in the seed storage experiment (Table 2). *O. grandiflora* showed a pattern, where the CVG increased at 6 months in comparison to seed stored for 3 months in almost all treatments. However, at 12 months, there was a reduction in germination rate in the room temperature treatment (Table 4). All factors and their interactions were statistically significant ($p < 0.05$) (Table 5). The results of CVG obtained in the control treatment were comparable to the values found in the seed storage

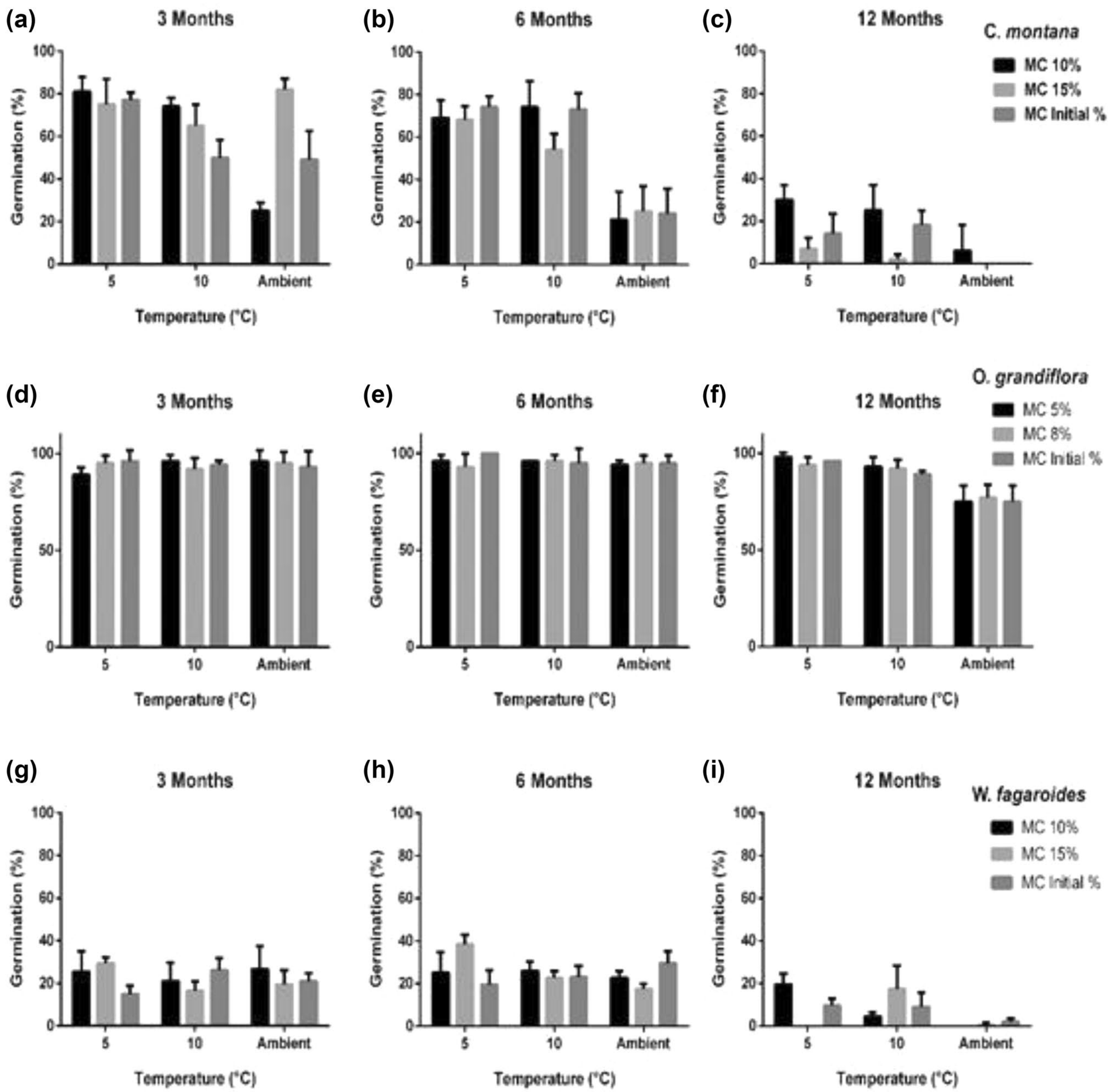

Fig 2 Germination (%) of *Cedrela montana* (**a–c**), *Oreocallis grandiflora* (**d–f**) and *Weinmannia fagaroides* (**g–i**) seeds stored at 5 °C, 10 °C and room temperature, at three levels of moisture content and stored for 3, 6 and 12 months. Bars depict the standard error, $n=4$

experiment (Table 2). *W. fagaroides* had a notable decrease in the values of CVG at room temperature and at 12 month storage, exhibiting the most significant impact on rate of germination (Table 4). All treatments were statistically significant ($p<0.05$), but moisture content did not influence CVG (Table 5). The results found in the storage experiment were similar to the control treatment although the seeds stored at 12 months had a lower CVG (Table 2). Accumulative germination percentage for all treatments across time (3, 6 and 12 months of storage) is shown in Appendix A

Discussion

A major finding of this study is the response of the seeds of native tree species to different storage conditions, which depended on the initial seed moisture content. Seeds of *C. montana* and *W. fagaroides* exhibit intermediate storage behavior due to their tolerance to desiccation after 12 month storage, although the seeds did not perform well at room temperature storage. In terms of potential storage duration, *C. montana* and *W. fagaroides* began to

Table 2 Initial percentage of germination, viable seeds using 2,3,5-triphenyl-tetrazolium chloride (TTC), non-viable (at post germination, PG) and coefficient of velocity of germination (CVG) without storage (control) at three moisture content levels for the three studied native species.

Species	Seed moisture content .(%)	Initial germination (%)	PG viable (TTC) (%)	PG non viable (%)	CVG
Cedrela montana	10	55ab	0a	45ab	3.65b
	15	47b	1a	52a	4.32 a
	19 (I)	69a	1a	30b	4.19a
Oreocallis grandiflora	5	95a	0a	5a	4.58a
	8	90a	4a	6a	4.29b
	11 (I)	93a	0a	7a	3.29c
Weinmanna fagaroides	10	26a	0	74a	3.15a
	15	22a	0	78a	3.44a

(*I*) Initial seed moisture content

Different letters correspond to significant differences according to the Tukey test ($p<0.05$), ($n=4$)

decline in seed germination, viability and velocity after 6 months of storage. In the case of *O. grandiflora,* we found a low initial seed moisture content indicating orthodox seed behavior. This species also tolerated a reduction in moisture content under all storage conditions of temperature and duration with a high tolerance to 12 months of storage time.

Table 3 Percentage of viable seeds using 2,3,5-triphenyl-tetrazolium chloride (TTC) and non-viable seeds (at post germination, PG) for the three studied native species stored at three levels of seed moisture content and temperature (5 °C, 10 °C and room temperature) for 3, 6 and 12 months

Species	Factors (treatments)	3 Months		6 Months		12 Months	
		PG viable (TTC) (%)	PG non-viable (%)	PG viable (TTC) (%)	PG non-viable (%)	PG viable (TTC) (%)	PG non-viable (%)
C. montana	5 °C 10% MC	1	18	1	30	10	60
	5 °C 15% MC	0	25	1	31	6	87
	5 °C initial MC	2	21	2	24	7	79
	10 °C 10% MC	0	26	3	23	0	75
	10 °C 15% MC	4	31	5	41	0	96
	10 °C Initial MC	6	44	3	24	0	82
	Ambient °C 10% MC	1	74	1	78	1	99
	Ambient °C 15% MC	1	17	4	71	3	97
	Ambient °C initial% MC	0	51	7	69	9	91
O. grandiflora	5 °C 5% MC	0	11	2	2	1	1
	5 °C 8% MC	0	5	0	7	5	1
	5 °C initial MC	0	4	0	0	2	2
	10 °C 5% MC	2	2	0	4	5	2
	10 °C 8% MC	3	5	0	4	4	4
	10 °C initial MC	5	1	1	4	8	3
	Ambient °C 5% MC	0	4	3	3	7	18
	Ambient °C 8% MC	1	4	0	5	2	21
	Ambient °C initial% MC	0	7	0	5	5	20
W. fagaroides	5 °C 10% MC	7	68	2	74	6	90
	5 °C 15% MC	5	66	3	59	7	76
	5 °C initial MC	8	78	5	76	6	86
	10 °C 10% MC	3	77	0	80	0	81
	10 °C 15% MC	4	80	3	75	1	99
	10 °C initial MC	4	71	4	74	0	91
	Ambient °C 10% MC	3	71	2	76	0	100
	Ambient °C 15% MC	2	79	5	78	0	100
	Ambient °C initial% MC	9	71	3	68	6	92

Table 4 Mean coefficient velocity of germination for the native species *C. montana, O. grandiflora* and *W. fagaroides* stored at three levels of seed moisture content and temperature (5 °C, 10 °C and room temperature) for 3, 6 and 12 months

Species	Factors (treatments)		3 Months	6 Months	12 Months
Cedrela montana	5 °C	10% MC	4.65	4.34	4.49
	5 °C	15% MC	4.69	4.53	3.07
	5 °C	Initial MC	4.69	4.46	4.38
	10 °C	10% MC	4.59	3.82	4.16
	10 °C	15% MC	3.87	3.85	2.06
	10 °C	Initial MC	4.27	3.80	3.97
	Ambient °C	10% MC	4.71	4.50	NG
	Ambient °C	15% MC	4.42	4.20	NG
	Ambient °C	Initial% MC	3.37	3.99	NG
Oreocallis grandiflora	5 °C	5% MC	4.30	4.66	4.07
	5 °C	8% MC	4.56	4.94	4.47
	5 °C	Initial MC	4.31	4.64	3.99
	10 °C	5% MC	4.39	4.71	4.25
	10 °C	8% MC	3.89	4.34	4.14
	10 °C	Initial MC	3.15	4.40	4.12
	Ambient °C	5% MC	4.31	4.54	3.81
	Ambient °C	8% MC	4.51	4.68	3.51
	Ambient °C	Initial% MC	4.14	3.82	3.15
Weinmannia fagaroides	5 °C 10% MC	10% MC	4.15	3.42	2.01
	5 °C 15% MC	15% MC	4.08	3.38	NG
	5 °C	Initial MC	3.93	3.35	2.79
	10 °C	10% MC	3.68	3.23	2.71
	10 °C	15% MC	3.75	3.44	3.02
	10 °C	Initial MC	2.98	3.27	1.69
	Ambient °C	10% MC	3.98	3.00	NG
	Ambient °C	15% MC	3.74	2.73	0.56
	Ambient °C	Initial% MC	1.68	1.82	1.33

NG non-germination

The percentage of seed germination and velocity of the intermediate species decreased substantially at 12 months. This result suggests that the seeds may be subject to deleterious physiological ageing. Seed ageing occurs as a result of (1) Maillard reactions through the chemical modification of macromolecules that leads to a gradual reduction in metabolic capability (Murthy et al. 2003), (2) accumulation of Active oxygen species (AOS) and free radicals that collectively degrade plant cell components (Bailly 2004; Smirnof 1993), and (3) changes in the activities of proteolytic enzymes over time (Ratajczak et al. 2015). The seeds of *C. montana* lost germination capacity at 6 months, as found for *Jatropa curcas* L. from the Atlantic rain forest region in Brazil, which also had a short viability period with loss of germination capacity after less than 6 months at moderate temperatures (25 °C) (Moncaleano-Escandon et al. 2012). Our results for *C. montana* are also similar to *C. fissilis*, a native tree species from Brazil, where seed storage at high temperature and high moisture content resulted in the lowest germination outcome with the species sensitive to standard seed storage conditions (Martins and Lago 2008).

Furthermore, the high levels of moisture content and storage time could provide optimum conditions for fungal proliferation, which occurred for *C. montana*, with a high percentage of decayed seeds in this study (non-viable seeds, post germination). The latter finding has been shown for *Leucaena leucocephala* after 6 month storage (Lezcano et al. 2015). On the other hand, at room temperature, all species including *O. grandiflora*, had a reduction in germination. This is a significant finding as many seed collectors and restoration operations have limited storage capacity beyond ambient conditions. In addition, ambient storage needs to avoid damage by insect and fungal attack, and keep the integrity of embryos (Bonner 2008). Moreover, the combination of high temperature and high seed moisture content contribute to a substantial loss of seed viability and vigor. In this context, our finding differs from the results of Ribeiro and Costa (2015), who showed that *Myrsine*

Table 5 Statistical evaluation of the effect of duration, temperature and moisture content (MC) on the percentage of seed germination, viability, non-viability seeds and coefficient of velocity of germination coefficient (CVG) according to Generalized Linear Models of the native tree species *C. montana, O. grandiflora* and *W. fagaroides*

Species	Factors	DF	Germination		PG viable seeds (TTC)		PG non-viable seeds		CGV	
			F	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value
Cedrela montana	Time	2	383	< 0.001	17	< 0.001	471	< 0.001	114	< 0.001
	Temperature	2	115	< 0.001	0	0.577	158	< 0.001	66	< 0.001
	MC	2	1	0.248	13	< 0.001	1	0.538	7	0.002
	Time:temperature	4	18	< 0.001	14	< 0.001	41	< 0.001	50	< 0.001
	Time:MC	4	13	< 0.001	3	0.012	20	< 0.001	5	0.001
	Temperature:MC	4	15	< 0.001	15	< 0.001	21	< 0.001	4	0.01
	Time:temperature:MC	8	5	< 0.001	5	< 0.001	10	< 0.001	2	0.135
Oreocallis grandiflora	Time	2	26	< 0.001	64	< 0.001	31	< 0.001	209	< 0.001
	Temperature	2	19	< 0.001	18	< 0.001	79	< 0.001	352	< 0.001
	MC	2	0	0.908	2	0.101	2	0.091	195	< 0.001
	Time:temperature	4	19	< 0.001	13	< 0.001	41	< 0.001	340	< 0.001
	Time:MC	4	0	0.751	9	< 0.001	3	0.017	232	< 0.001
	Temperature:MC	4	1	0.3	8	< 0.001	4	0.004	91	< 0.001
	Time:temperature:MC	8	1	0.531	3	< 0.001	6	< 0.001	72	< 0.001
Weinmannia fagaroides	Time	2	103	< 0.001	6	0.004	94	< 0.001	81	< 0.001
	Temperature	2	6	0.003	2	0.172	6	0.004	23	< 0.001
	MC	2	1	0.426	6	0.005	0	0.875	3	0.08
	Time:temperature	4	3	0.017	9	< 0.001	6	< 0.001	5	0.002
	Time:Mc	4	1	0.647	1	0.619	1	0.628	6	< 0.001
	Temperature:Mc	4	5	< 0.001	3	0.041	5	< 0.001	6	< 0.001
	Time:temperature:Mc	8	8	< 0.001	2	0.055	6	< 0.001	5	< 0.001

parvifolia, a native tree species from Brazil's coastal plain responded positively to storage at 15 and 20 °C. In addition, Oyekale et al. (2012) mentioned that with a higher seed moisture content, germination capacity declines more rapidly over time. *O. grandiflora* is widespread in the Andean region (Hazelhurst et al. 2018) and it is located in a wide topographical range from high to less humidity uplands in the Azuay province (tropicos.org 2019). Such ecological breadth and the absence of seed dormancy could contribute to the ability of the species to respond positively to a range of storage conditions. As a pioneer tree, *O. grandiflora* demonstrated major longevity over the first year of evaluation in comparison to other two study species. According to the seed information database from the Royal Botanic Gardens (RBG), Kew, several species of the Proteaceae family were categorized as orthodox and with high viability under different storage conditions (RBG Kew 2019). For instance, the *Embothrium coccineum* proves a storage behavior of 47% viability following drying to moisture contents in equilibrium with 15% of relative humidity and freezing for 5 years at −20 ºC (RBG Kew 2020). In addition, *Swietenia senegalensis* (syn. genus *Khaya*) was evaluated over 4 years of storage, and showed that after 1 year, seed germination capacity decreases at room temperature but at 5 °C the germination was sustained at 80% (Danthu et al. 1999). Lozano-Isla et al. (2018) showed that the germination of the tropical tree, *Jatropha curcas* L., was not significantly affected after 12 months of storage compared to fresh seeds that had not been stored. All of these findings coincide with the results found in this study. However, long-term storage (> 12 months) must be established if *O. grandiflora* is indeed an orthodox seed.

During post germination evaluation we observed that *W. fagaroides* had a high number of empty seeds (in the category of non-viable seeds) which may be related to breeding systems. Perea et al. (2013) found that a proportion of 50% empty seeds reduces seed predation by 26% for *Ulmus laevis*, suggesting that empty seeds are an ecological strategy for some species to efficiently reduce predation losses for the tree. In addition, environmental stress at the time of seed maturation can substantially decrease plant reproductive output through premature seed abortion (Coello and Martínez-Barajas 2016; Egli 2017). However, this species is a vigorous tree present in a wide variety of disturbed and pristine habitats. Therefore, climatic conditions would be a likely cause for any decline in seed quality.

Conclusions

A long storage duration and specific temperatures had negative effects on the germination percentage and viability of seeds of two framework tree species, *C. montana* and *W. fagaroides,* particularly at 12 months storage at room temperature. On the other hand, *O. grandiflora* maintained a high germination percentage and viability across time independent of seed storage treatments. The CVG for all species showed a reduction across time, although *W. fagaroides* had a slower marked germination at 12 months of storage at room temperature. Information on the germination rate as well as the synchronicity of germination of native species is very useful for planning plant production. This study in the Andean region showed that seed characteristics and storage capability are highly variable. Therefore, storage under standard conditions is not ideal for all of the species in this study. Additional research is needed across a variety of species which represent a large ecotonal range and phylogenetic diversity. Only with this information are we likely to ensure that Andean forest restoration will have a reliable and long-term supply of suitable species for ecological restoration programs.

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Author contribution statement XP obtained the funds to support the study. XP and MP planned and carried out the experiment. XP, CP and FM analysed the data. XP, CP, FM and BS wrote, revised and edited the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Fig. 3

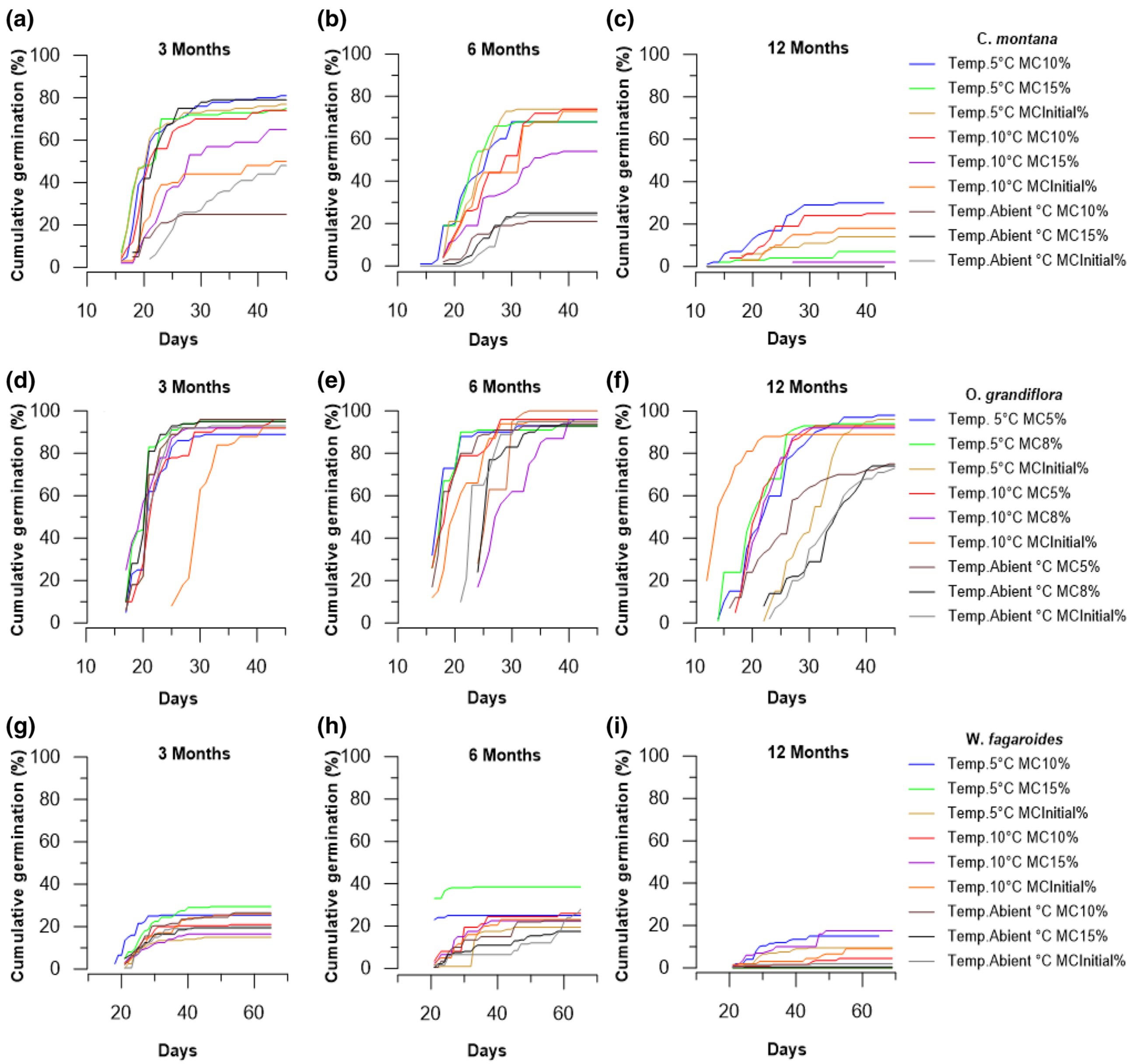

Fig. 3 Cumulative seed germination of *Cedrela montana* (**a–c**), *Oreocallis grandiflora* (**d–f**) and *Weinmannia fagaroides* (**g–i**) stored at three levels of moisture content (%) and temperature (5 °C, 10 °C and room temperature) for 3, 6 and 12 months

References

Bailly C (2014) Active oxygen species and antioxidants in seed biology. Seed Sci Res. 14:93–107. https://doi.org/10.1079/SSR2004159

Baskin CC, Baskin JM (2014) Seeds ecology, biogeography, and evolution of dormancy and germination. Lexington, Kentucky, USA

Bentsink L, Maarten K (2008) Seed dormancy and germination. Arab B 27(17):R874–R878. https://doi.org/10.1016/j.cub.2017.05.050

Bonner F (2008) Storage of Seeds. In: Bonner F, Karrfalt R (eds) The woody plant seed manual. Mississippi State, Mississippi, pp 85–96

Bourgeois B, Lemay MA, Landry T et al (2019) Seed storage behaviour of eight peatland pool specialists implications for restoration. Aquat Bot. 152:59–63. https://doi.org/10.1016/j.aquabot.2018.09.008

Broadhurst LM, Jones TA, Smith FS et al (2016) Maximizing seed resources for restoration in an uncertain future. Bioscience 66:73–79. https://doi.org/10.1093/biosci/biv155

Coello P, Martínez-Barajas E (2016) Changes in nutrient distribution are part of the mechanism that promotes seed development under severe nutrient restriction. Plant Physiol Biochem. 99:21–26. https://doi.org/10.1016/j.plaphy.2015.11.022

Colville L (2017) Seed storage. In: Murray BG, Murphy D (eds) Thomas B. Encyclopedia of applied plant sciences 2, Amsterdam, pp 335–339

Cousins SR, Witkowski ETF, Mycock DJ (2014) Seed storage and germination in *Kumara plicatilis*, a tree aloe endemic to

mountain fynbos in the Boland, south-western Cape. S Afri J Bot. 94:190–194. https://doi.org/10.1016/j.sajb.2014.07.003
Danthu P, Gaye A, Sarr A (1999) Long term storage of *Khaya senegalensis* seeds. Int Tree Crop J 10:93–100. https://doi.org/10.1080/01435698.1999.9752996
Egli DB (2017) Seed biology and yield of grain crops. Oxfordshire, UK
FAO (2014a) The state of the world's forest genetic resources. Italy, Rome
FAO (2014b) Genebank standards for plant genetic resources for food and agriculture. Italy, Rome
Hazelhurst J, Cardenas S, Tinoco B, Karubian J (2018) Pollination ecology of *Oreocallis grandiflora* (Proteaceae) at the northern and southern ends of its geographic range. J Pollinat Ecol 19:71–80. https://doi.org/10.26786/1920-7603(2016)4
ISTA (2007) International rules for seed testing. International Seed Testing Association, Bassersdorf
Kainer KA, Duryea ML, de Malavasi M et al (1999) Moist storage of Brazil nut seeds for improved germination and nursery management. For Ecol Manage 116:207–2017. https://doi.org/10.1016/s0378-1127(98)00461-7
Kettle CJ (2012) Seeding ecological restoration of tropical forests priority setting under REDD+. Biol Conserv. 154:34–41. https://doi.org/10.1016/j.biocon.2012.03.016
Kim DH (2018) Extending populus seed longevity by controlling seed moisture content and temperature. PLoS ONE 8:1–16. https://doi.org/10.1371/journal.pone.0203080
Lezcano JC, Alonso O, Navarro M (2015) Fungal population associated to the germination process of stored seeds of *Leucaena leucocephala* R6cv. Peru Pastos y Forrajes 38:226–230
Lozano-Isla F, Campos MLO, Endres L et al (2018) Effects of seed storage time and salt stress on the germination of *Jatropha curcas* L. Ind Crops Prod. 118:214–224. https://doi.org/10.1016/j.indcrop.2018.03.052
Martins L, Lago A (2008) *Cedrela fisilis* seed preservation seed water content and ambient temperature. Rev Bras Sementes. 30:161–167. https://doi.org/10.1590/S0101-31222008000100020
McCormack M, Lavely E, Zeqing M (2014) Fine-root and mycorrhizal traits help explain ecosystem processes and responses to global change. New Phytol. 204:455–458. https://doi.org/10.1111/nph.13023
Mittermeier RA, Turner WR, Larsen FW et al (2011) Global biodiversity conservation: the critical role of hotspots. In: Zachos F, Habel J (eds) Biodiversity hotspots. Springer, Berlin, Heidelberg, pp 3–22
Moncaleano-Escandon J, Silva BC, Silva SR et al (2012) Germination responses of *Jatropha curcas* L. seeds to storage and aging. Ind Crops Prod. 44:684–690. https://doi.org/10.1016/j.indcrop.2012.08.035
Murthy UMN, Kumar PP, Sun WQ (2003) Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. J Exp Bot 54:1057–1067. https://doi.org/10.1093/jxb/erg092
Oyekale KO, Daniel IO, Ajala MO, Sanni LO (2012) Potential longevity of maize seeds under storage in humid tropical seed stores. Nat Sci 10:114–124
Palomeque X, Maza A, Iñamagua JP et al (2017) Variabilidad intraespecífica en la calidad de semillas de especies forestales nativas en bosques montanos en el sur del Ecuador: Implicaciones para la restauración de bosques. Rev Ciencias Ambient. 51:52–72. https://doi.org/10.15359/rca.51-2.3
Penfield S (2017) Seed dormancy and germination. Curr Biol 27:R874–R878. https://doi.org/10.1016/j.cub.2017.05.050
Perea R, Venturas M, Gil L (2013) Empty seeds are not always bad simultaneous effect of seed emptiness and masting on animal seed predation. PLoS ONE 88(6):e65573. https://doi.org/10.1371/journal.pone.0065573
Pinheiro J, Bates D, DebRoy S, Sarkar D (2018) Linear and nonlinear mixed effects models. R Package Version 3(5):1
Rao NK, Hanson J, Dulloo ME et al (2006) Manual of seed handling in genebanks manual of seed handling in genebanks. Italy, Rome
Ratajczak E, Kalemba EM, Pukacka S (2015) Age-related changes in protein metabolism of beech (*Fagus sylvatica* L.) seeds during alleviation of dormancy and in the early stage of germination. Plant Physiol Biochem 94:114–121. https://doi.org/10.1016/j.plaphy.2015.06.003
RBG Kew (2019) Royal Botanic Gardens, Kew - Seed Information Database, Family Proteaceae. IOP Publishing PhysicsWeb. https://data.kew.org/sid/SidServlet?Clade=&Order=&Family=Proteaceae&APG=off&Genus=&Species=&StorBehav=0. Accessed 20 Sept 2019
RBG Kew (2020) Royal Botanic Gardens, Kew - *Embothrium coccineum*. IOP Publishing PhysicsWeb. https://data.kew.org/sid/SidServlet?ID=8621&Num=PSB. Accessed 19 May 2020
Ribeiro JNS, Costa CSB (2015) The effect of temperature regulation on seed germination of the tropical tree *Myrsine parvifolia* a DC near its southern limit. S Afric J Bot 98:128–133. https://doi.org/10.1016/j.sajb.2015.02.012
Schmidt LH (2000) Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, UK
Shaw TE (2019) Plant diversity species diversity in restoration plantings: important factors for increasing the diversity of threatened tree species in the restoration of the araucaria forest ecosystem. Plant Divers 41:84–93. https://doi.org/10.1016/j.pld.2018.08.002
Simpson JD, Wang BSP, Daigle BI (2004) Long-term seed storage of various Canadian hardwoods and conifers. Seed Sci Technol. 32:561–572. https://doi.org/10.15258/sst.2004.32.2.25
Smirnof NF (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58
Soh MCK, Mitchell NJ, Ridley AR et al (2019) Impacts of habitat degradation on tropical montane biodiversity and ecosystem services a systematic map for identifying future research priorities. Front For Glob Chang 2:1–18. https://doi.org/10.3389/ffgc.2019.00083
Tropicos.org (2019) Missouri botanical garden. IOP Publishing PhysicsWeb. https://www.tropicos.org/Name/26500121. Accessed 12 Jun 2019
Umarani R, Kanthaiya Aadhavan E, Mohamed Faisal M (2015) Understanding poor storage potential of recalcitrant seeds. Curr Sci 108:2023–2034
Walters C (2015) Orthodoxy, recalcitrance and in-between describing variation in seed storage characteristics using threshold responses to water loss. Planta 242:397–406. https://doi.org/10.1007/s00425-015-2312-6
Wyse SV, Dickie JB (2018) Taxonomic affinity, habitat and seed mass strongly predict seed desiccation response a boosted regression trees analysis based on 17 539 species. Ann Bot. 121:71–83. https://doi.org/10.1093/aob/mcx128