



# Seed germination of five species from the subalpine shrubland of a mountainous oceanic island with high conservation value (Reunion Island)

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## ABSTRACT

Little is known about the seed ecology of native plants from subalpine and alpine vegetation zones on oceanic islands, although their biodiversity is unique and vulnerable (e.g. facing fires and biological invasions). This study of seed germination focuses on five species, which are representative of the subalpine shrubland of Reunion Island. The main objectives are to identify the presence of dormancy and light requirements, and to evaluate whether GA<sub>3</sub> or a smoke-infused water could substitute for the light requirement for seed germination. Over one month, germination tests were performed under different conditions at three temperatures (15, 20 and 25 °C), in light (12-hours light/12-hours dark) and in dark. Seeds were also treated using a single spraying of gibberellic acid (GA<sub>3</sub> at 1000 ppm) and a smoke-infused water (named Smoke Water in the study). No dormancy was detected for *Stoebe passerinoides*. For *Erica reunionensis*, *Agarista buxifolia*, *Hubertia tomentosa* and *Hypericum lanceolatum* subsp. *angustifolium*, a potential non-deep physiological dormancy (with a conditional dormancy state) was detected. Moreover, for *Agarista buxifolia* and *Erica reunionensis* (Ericaceae), light is required for seed germination. Gibberellic acid (GA<sub>3</sub> at 1000 ppm) substitutes for light, except at 25 °C for *E. reunionensis*. Smoke Water does not improve seed germination, except at 15 °C in dark for *E. reunionensis*. A better understanding of seed germination for species from subalpine and alpine vegetation zones will mean better threat management and restoration actions.

## 1. Introduction

A small number of oceanic islands of the world have subalpine and alpine vegetations (Juvik et al., 2014; Leuschner, 1996). Their limits and terminology may vary depending on the island because of biogeographical singularities (Elias et al., 2016; Juan et al., 2000; Strasberg et al., 2005). Generally, subalpine and alpine vegetation zones are characterized by (1) low temperatures and high thermal amplitudes (Cadet, 1977; Juvik et al., 1993; Lacoste et al., 2014), (2) a low vegetation (0.5–2 m in height for shrubs of subalpine zones) (Leuschner, 1996), and (3) high biodiversity with a high level of endemism, especially in oceanic islands (Ah-Peng et al., 2014; Strasberg et al., 2005). Some human disturbances such as introduction of herbivores or fires can threatened endemic species, notably

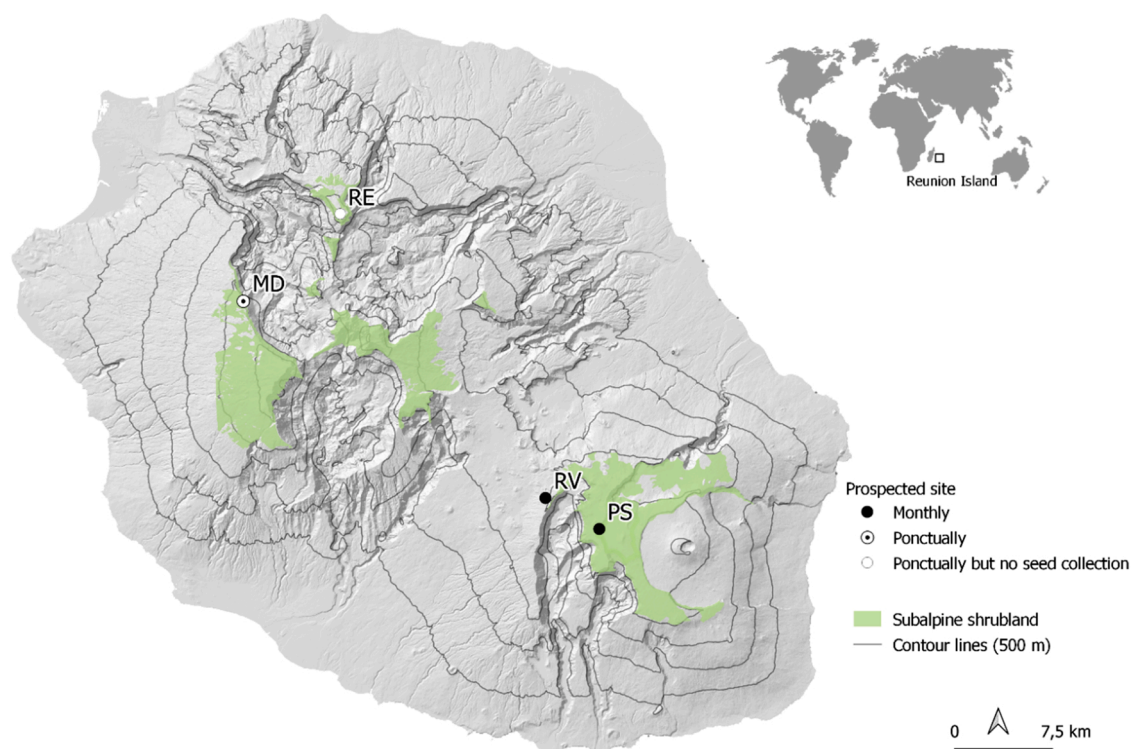
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seedling establishment, e.g. La Palma, Canary Islands (Irl et al., 2012, 2014), Hawaii (Steven and Hess, 2016), Reunion Island (UNESCO WHC, 2013). Fires can also promote germination and seedling establishment of some invasive alien species (Payet et al., 2015; Udo et al., 2017). Globally, invasive alien species which are historically one of the main threats to biodiversity on oceanic islands (Hughes et al., 1991; Payet et al., 2015; Russell and Kueffer, 2019). To these factors can be added new threats such as climate change (Juvik et al., 2014; Pouteau et al., 2010). Indeed, numerous studies have shown that subalpine and alpine ecosystems are among the most sensitive to climate change (e.g., Diaz et al., 2003). There is growing evidence that high mountain environments are warming more rapidly than lower elevations (Pepin et al., 2015). For islands, the threat of climate change is compounded by the fact that native species are often poor competitors to introduced species, their migration potential is limited due to the surrounding ocean, and for species at the highest elevations, higher migration is not possible (Harter et al., 2015). Climate change impacts on high islands may therefore result in a much greater overall loss of global biodiversity than elsewhere (Frazier and Brewington, 2020).

Despite their conservation importance, the seed ecology of subalpine vegetation zones seems to be among the least studied in the world (Baskin and Baskin, 2014; Juvik et al., 2014). However, studying seed germination is crucial because the transition from seed to seedling is a critical step for the successful recruitment of a given plant population and species in a given period (Baskin and Baskin, 2014; Donohue, 2002). Seed germination and emergence, and the establishment of seedlings are determined by morphological and physiological traits at the seed scale, and also by environmental, genetic and physiological factors at the mother-plant scale (Cotado et al., 2020; Fernández-Pascual et al., 2013; Luzuriaga et al., 2006). If the germination of a viable seed is blocked, despite favorable thermal and gas conditions, the seed is considered dormant (Benech-Arnold et al., 2000; Bewley, 1997). To determine the presence of dormancy, seeds need to be exposed to a set of environmental conditions, including temperature, which is an important factor for seed germination (Baskin and Baskin, 2014; Fernández-Pascual et al., 2021). Some studies in tropical subalpine and alpine vegetation zones reveal that the majority of species seem to have physical dormancy (48 %) or no dormancy (35 %), according to a review on 25 species (Baskin and Baskin, 2014).

Light can also stimulate or induce germination (Baskin and Baskin, 2014; Flores et al., 2006; Leperlier et al., 2018). Light requirements increase with decreasing seed mass and mother-plant height (Flores et al., 2011, 2016; Koutsovoulou et al., 2014; Milberg et al., 2000). For example, light requirements for seed germination in alpine habitats in Japan were also reported for 86 % of 27 studied species, including Ericaceous shrubs with very small seeds (< 1 mg) (Shimono and Kudo, 2005). In addition, interactions between phytochromes and phytohormones with regulation of abscisic acid (ABA) and increasing of biosynthesis of gibberellins (GAs) upon light activation for seed germination are reported (Lymperopoulos et al., 2018; Oh et al., 2006; Seo et al., 2009). Without light, added gibberellins (GA<sub>3</sub> at 500–1000 ppm) or smoke exposure could be an alternative treatment to improve seed germination (Alcorn and Kurtz, 1959; Ortega-Baes and Rojas-Aréchiga, 2007; van Staden et al., 1995; Todorović et al., 2007).



**Fig. 1.** Prospected sites in our study of subalpine shrubland (Reunion Island), with RE: Roche Écrite, MD: Maïdo, RV: Route du Volcan and PS: Pas des Sables. Map and data from BD TOPO REUNION IGN-MNT Data Base and “Milieux Naturels Remarquables” shape (Geographic Information System shape from administration.carmencarto.fr).

Reunion Island is an appropriate natural laboratory to study the seed ecology of subalpine habitats, with elevations from 1700 m to 3070 m and the presence of small-seeded species (Bosser et al., 1976; Cadet, 1977). Ericaceae and Asteraceae dominate and structure subalpine shrubland landscapes (Lacoste et al., 2014; Lacoste and Picot, 2011; Strasberg et al., 2005). Species richness of these habitats (i.e. 30 species) is lower than for other habitats on the island, but endemism can reach up to 83 % in shrubland of lapilli habitats (Cadet, 1977; Lacoste and Picot, 2011; Strasberg et al., 2005). These habitats are often considered as protected from human transformation. In reality, endemic species persistence and seedling establishment may be vulnerable when a disturbance occurs, e.g. arson and accidental fires that burn from 760 to 4500 ha depending on the year (Payet et al., 2015; Strasberg et al., 2005). But, even if wildfires could be frequent in Réunion Island, their magnitude is generally very low. However, it clearly appears that the persisting drought over the coming years will increase the vulnerability of vegetation to fires (Brou, 2019). In addition, there are other potential pressures to manage, such as tourism and local planning, which could also facilitate the introduction of exotic species seeds and others bioaggressors (UNESCO WHC, 2013).

Seed ecology data can help to target conservation and restoration programs. To our knowledge, no scientific data about seed germination of native and endemic species in subalpine habitats of Reunion Island have been published. As a consequence, this study aims:

- (1) To identify germination requirements at three temperatures and two photoperiods for five species (*Agarista buxifolia*, *Erica reunionensis*, *Stoebe passerinoides*, *Hubertia tomentosa* and *Hypericum lanceolatum* subsp. *angustifolium*);
- (2) To evaluate whether GA<sub>3</sub> or a smoke-infused water can substitute for the light requirement for seed germination of Ericaceae species.

## 2. Materials and methods

### 2.1. Study sites

A transect of 12.8 km, with an elevation from 1931 to 2350 m on the Fournaise Massif in the South of Reunion Island (from Route du Volcan, RV, 21° 12' 33" S; 55° 36' 34" E to Pas des Sables, PS, 21° 13' 53" S; 55° 38' 55" E) was monitored monthly in 2019 and 2020. At an elevation of approximately 2200 m, on the Piton des Neiges Massif, the site of Maïdo (MD, 21° 4' 15" S; 55° 23' 13" E) in the West of the island and the site of Roche Écrite (RE, 21° 0' 39" S; 55° 27' 36" E) in the North were punctually prospected in May and June 2020 (Fig. 1). Air temperatures at these sites vary from −2 °C to 15 °C, with a mean annual temperature ranging from 6° to 8 °C (Meteo France, 2022). In addition, the maximum temperature recorded is 25 °C (Meteo France, 2022).

### 2.2. Study species and seed collection

Achenes or capsules of five species (Table 1; Fig. 2) were randomly hand-collected, directly from the plant (authorization from local authorities: National Park of Reunion Island). Five to twenty-five shrubs were selected for sampling, depending on the availability of seeds. Collected fruits were packaged in paper bags for transport. Seeds were extracted and stored at ambient room temperature (±26.6 °C) for one to 45 days (depending on lots) before germination tests.

### 2.3. Germination tests

Seeds were selected using a binocular microscope (Leica – Wild M3B), according to morphological characteristics shown in Fig. 3 and Table 2. Germination tests were completed in laboratories of the Pole of Protection des Plantes located in the South of Reunion Island (21° 19' 16" S; 55° 29' 06" E). Five replicates of 20 seeds were sown in plastic Petri dishes (60 × 15 mm, Greiner Bio-One International GmbH) on a flat disc of cotton, which was moistened with water until saturation. Due to the environmental conditions of

**Table 1**

Study species and seed lot details. The sites PS, RV and MD correspond respectively to Pas des Sables, Route du Volcan (high and low area of the transect located on the Fournaise Massif) and Maïdo. ‘# of shrubs’ is the number of shrubs from which the seeds were harvested. ‘Age’ is the number of days between the seed collection date and the sowing date for the germination test.

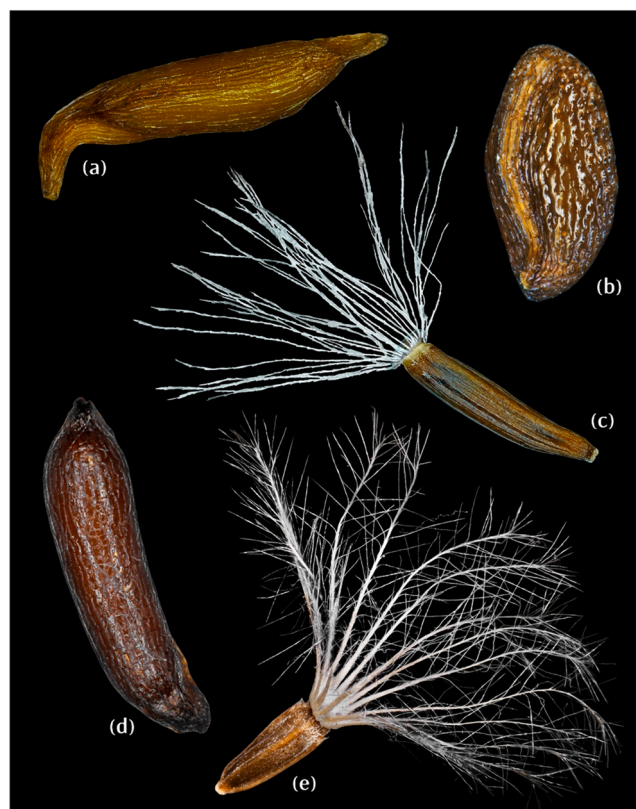
Family	Species	Site	# of shrubs	Collection date	Age (days)
Ericaceae	<i>Agarista buxifolia</i>	PS	6	15/01/2019	7
	(Comm. ex Lam.) G. Don				
	<i>Erica reunionensis</i>	RV	10	06/04/2019	12
Asteraceae	E.G.H. Oliv.	MD	20	16/05/2020	45
	<i>Hubertia tomentosa</i>	RV	20	03/06/2020	2
	Bory	MD	12	10/07/2020	11
		RV	20	20/07/2020	1
	<i>Stoebe passerinoides</i>	PS	20	05/02/2019	2
	(Lam.) Willd.	RV	27	20/07/2020	3
Hypericaceae		RV	25	03/06/2020	2
	<i>Hypericum lanceolatum</i> subsp. <i>angustifolium</i>	PS	5	23/01/2019	2
	(Lam.) N. Robson	MD	22	10/07/2020	7





**Fig. 2.** Studied species in their natural habitats, with their estimated height (m). (a) general view of subalpine shrubland vegetation zones (Pas des Sables, 2350 m, in the South of Reunion Island), (b) *Hubertia tomentosa* (2 m), (c) *Hypericum lanceolatum* subsp. *angustifolium* (4 m), (d) *Stoebe passerinoides* (0.5 m), (e) *Agarista buxifolia* (0.5 m) and (f) *Erica reunionensis* (1 m). Note: all species are endemic to Reunion Island, except *A. buxifolia* (endemic to Madagascar and the Mascarene Archipelago).





**Fig. 3.** Seeds of study species. (a) *Agarista buxifolia*, (b) *Erica reunionensis*, (c) *Hubertia tomentosa*, (d) *Hypericum lanceolatum* subsp. *angustifolium*, and (e) *Stoebe passerinoides*. The characteristics of the studied seeds are shown in Table 2.

**Table 2**

Characteristics of studied seeds. Letters are shown in Fig. 3. Seed size refers to the mean of the longest dimension ( $\pm$  sd). Measurements were made using ImageJ 1.53e software (Schneider et al., 2012) on pictures of 20 seeds per species acquired using an AZ100 Multizoom Microscope (Nikon, Japan), except the measurement on Fig. 5b for *Stoebe passerinoides*. Seed mass ( $\pm$  sd) were measured using a precision balance (readability 0.01 mg; Sartorius, Cubis©) on three lots of 20 seeds per species.

Letters	Species	Seed size (mm)	Seed mass (mg)
(a)	<i>Agarista buxifolia</i>	$1.50 \pm 0.19$	$0.052 \pm 0.008$
(b)	<i>Erica reunionensis</i>	$0.64 \pm 0.04$	$0.022 \pm 0.003$
(c)	<i>Hubertia tomentosa</i>	$2.17 \pm 0.31$	$0.140 \pm 0.044$
(d)	<i>Hypericum lanceolatum</i> subsp. <i>angustifolium</i>	1.0	$0.060 \pm 0.005$
(e)	<i>Stoebe passerinoides</i>	$1.24 \pm 0.14$	$0.120 \pm 0.009$

the greenhouse and nursery sites operated for the restoration programs, replicates were placed in growth chambers (Sanyo, MLR 350) at 3 different temperatures (15, 20, or 25 °C), with 80 % relative humidity, under 12 h of light (white fluorescent lamps) or in darkness (in a black box). Two treatments were also tested because they are easy to produce in a greenhouse and nursery: sprinkling the cotton disc with a solution of gibberellic acid (GA<sub>3</sub>) at 1000 ppm (Duchefa Biochemie) or smoke-infused water (term simplified to ‘Smoke Water’) according to Laperlier et al. (2018) (Supplementary data). Germination was detected by the emergence of the radicle ( $> 1$  mm) if possible, otherwise by a more advanced stage (Figs. 4 and 5). Seeds were checked weekly for four weeks (only on the second and the fourth week for the black boxes) and Petri dishes were watered as necessary.

#### 2.4. Data analyses

Data analyses were performed using R version 4.1.2 (R Core Team, 2021), and figures were created with the package “ggplot2” (Wickham, 2016).

For each seed lot by species, we calculated the cumulative germination (total number of seeds = 100), in the fourth week after sowing, at three temperatures, in light or in dark, and after exogenous treatment in the case of *A. buxifolia* and *E. reunionensis*. We calculated also an exact binomial confidence interval with the binom.test function. We adopted an optimistic approach, considering



**Fig. 4.** From the imbibed seed to the seedling for (a) *Hypericum lanceolatum* subsp. *angustifolium*, (b) *Agarista buxifolia* and (c) *Erica reunionensis*. The second stage represents seed germination *sensu stricto* with the emergence of the radicle, followed by the growth and development of the radicle. The fourth stage is the emergence of the cotyledons, still bound to the tegument. For the fifth stage, the cotyledons are fully visible. Pictures were taken using an AZ100 Multizoom Microscope (Nikon, Japan). Some pictures are the result of an assembly of several views and digital work.

the cumulative germination added to the upper bound of the exact binomial confidence interval. For tropical species in given temperature and light conditions, a seed lot was recorded as having dormant seeds if, the cumulative final germination percentage (plus the upper bound of the confidence interval) was less than 50 % in the fourth week after sowing (Baskin and Baskin, 2014; Ng, 1978).

To compare germination conditions (*i.e.* temperature, light and/or exogenous treatment before sowing), we built a Generalized Linear Model on binomial data (with a logit link function), including interactions between fixed effects. For *A. buxifolia* and *E. reunionensis*, we built a Generalized Linear Mixed Model (GLM, “lme4” package, Bates et al., 2015), including replicates as a random





**Fig. 5.** From the imbibed seed to the first leaves for (a) *Hubertia tomentosa* and (b) *Stoebe passerinoides*. The pappus of *S. passerinoides* seeds has been removed. The same stages are represented as those described in Fig. 4, except the sixth stage which represents the first true leaves. Pictures were taken using an AZ100 Multizoom Microscope (Nikon, Japan). Some pictures are the result of an assembly of several views and digital work.

effect. We validated the GLMM after the analysis of deviance table between GLM and GLMM based on a Chi-square distribution. We tested the fixed effects by a deviance test based on a Chi-square distribution. We used the package “emmeans” (Russel, 2021) to obtain Estimated Marginal Means (EMMs) for germination probabilities and corrected Confidence Intervals (95 %) and to make pairwise post-hoc comparisons based on Odds-Ratios ( $H_0$ : OR = 1), integrating a Bonferroni-like correction (Benjamini and Hochberg, 1995). For analyses of *A. buxifolia* and *E. reunionensis*, we made comparisons for light and dark separately (considering interactions between factors).

### 3. Results

#### 3.1. Germination at three temperatures in light and in dark

For *A. buxifolia*, photoperiod and temperature had a statistically significant effect on cumulative germination (Deviance test,  $P < 2.2 \times 10^{-16}$ ). In light, cumulative germination of the seed lot from PS were greater than 50 %, considering the upper bound. From the pairwise comparisons tests, the highest cumulative germination of 95 % and 97 % were observed in light at 20 °C and 25 °C, respectively, and were not significantly different ( $P = 0.0585$ ), but were different from that at 15 °C ( $P < 0.001$ ). In dark, no germination was observed at 15 °C and the cumulative germinations at 20 °C and 25 °C were lower than 50 %, with the upper bounds  $< 43$  % (Table 3, Supplementary data).

For *E. reunionensis* (seed lot from RV), a significant interaction between light and temperature conditions was detected (Deviance test,  $P < 2.2 \times 10^{-16}$ ). From the pairwise comparisons tests, the highest cumulative germinations were observed in light at 20 °C (82 %) and 25 °C (87 %) and were not significantly different ( $P = 0.3540$ ), but were different from that at 15 °C (42 %,  $P < 0.001$ ). In dark, all the cumulative germinations were lower than 50 %, with the upper bound  $< 38$  % (Table 3). At 15 °C, a difference to qualify was detected ( $P = 0.0449$ ) between light (42 %) and dark (28 %). However, no germination was observed for *E. reunionensis* seeds from MD (Table 3, Supplementary data).

For *H. tomentosa*, only the seed lot from RV had a cumulative germination greater than 50 % in light at 25 °C, with a cumulative germination observed of 46 % with the upper bound at 56 %.

For *S. passerinoides*, all seed lots had cumulative germinations greater than 50 %, except in three different conditions (in light at 15 °C and 20 °C for the lot from PS in February 2019, and in light at 25 °C for that from RV in June 2020, Table 3, Supplementary data). A significant interaction between temperature, light and seed lot was detected (Deviance test,  $P = 0.01081$ ). The analyses of each seed lot revealed that, firstly, for the site of PS (in February 2019) the highest cumulative germinations, 73 %, was at 20 °C in the dark (significantly higher than other percentages,  $P \leq 0.0046$ ). Secondly, for the site of RV (in June 2020), the highest percentages were observed at 20 °C (65 % in dark and 64 % in light, respectively) and were not significantly different ( $P = 0.8875$ ). Thirdly, for the site of RV (in July 2020), no significant effects were detected between all final germination percentages ( $P \geq 0.3306$ ).

For *H. lanceolatum* subsp. *angustifolium*, seed lots from MD and SM had cumulative germinations considerably lower than 50 %, with the upper bounds  $< 19$  % (Table 3, Supplementary data).

#### 3.2. Germination of *Agarista buxifolia* and *Erica reunionensis* after $GA_3$ and smoke water treatments

In light for *E. reunionensis* (Fig. 6a), the three estimated final germination percentages (EFGP) with  $GA_3$  (62 % at 15 °C, 87 % at 20 °C and 7.5 % at 25 °C) were significantly different ( $P \leq 0.0191$ ). At 15 °C, the EFGP were not different, 41 % without  $GA_3$  (Control) and 62 % with  $GA_3$  ( $P = 0.1654$ ). At 20 °C, they were also not different, 84 % without  $GA_3$  and 87 % with  $GA_3$  ( $P = 0.6654$ ). At 25 °C, they were different, 88 % without  $GA_3$  and 7.5 % with  $GA_3$  ( $P < 0.0001$ ). For the treatment with Smoke Water, no differences were observed between the highest EFGP, 78 % at 20 °C and 74 % at 25 °C ( $P = 0.7264$ ). However, they were different from that at 15 °C (16 %,  $P < 0.0001$ ). At 15 °C, the EFGP of 41 % without Smoke Water (Control) and 16 % with Smoke Water were significantly different ( $P = 0.0313$ ). The EFGP with or without Smoke Water, respectively 84 % and 78 % at 20 °C, and 88 % and 74 % at 25 °C, were not different ( $P \geq 0.1460$ ).

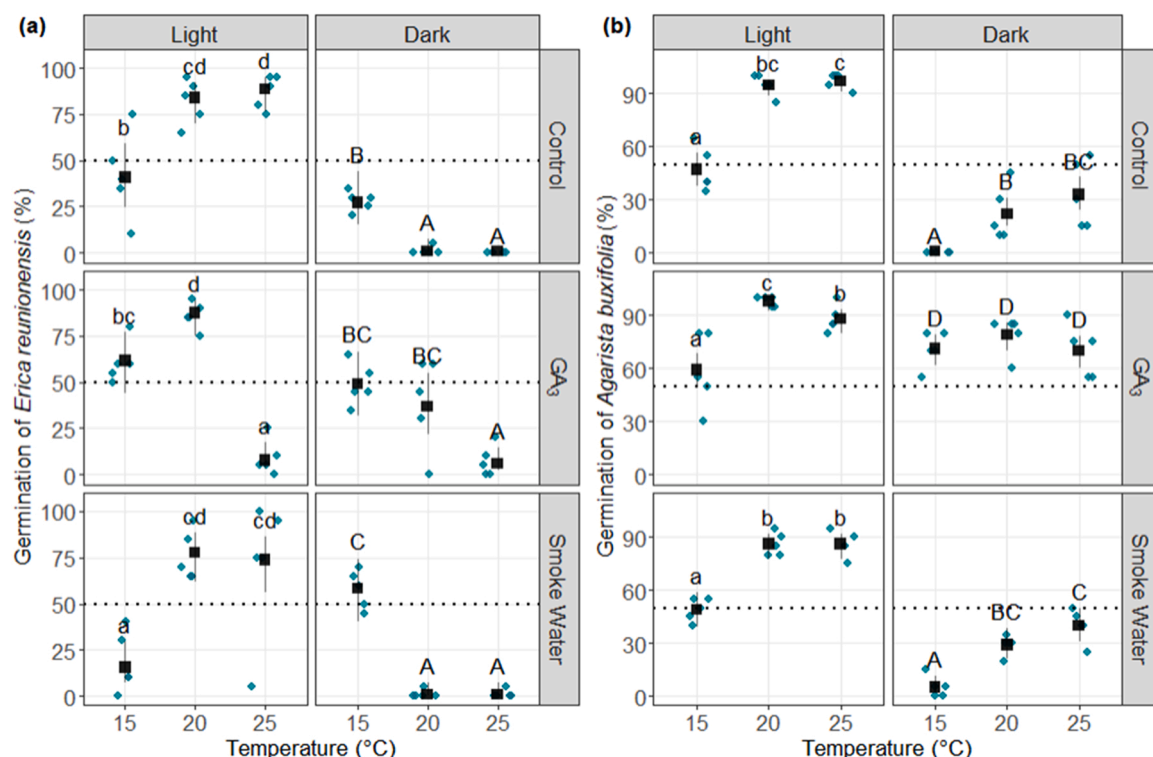
In dark for *E. reunionensis*, with  $GA_3$ , the EFGP at 15 °C (49 %) and 20 °C (37 %) were not different ( $P = 0.4420$ ), but they were different from that at 25 °C (5.8 %,  $P \leq 0.0012$ ). At 15 °C, the EFGP were not different, 27 % without  $GA_3$  and 49 % with  $GA_3$  ( $P = 0.1228$ ). At 20 °C, they were different, 0.8 % without  $GA_3$  and 37 % with  $GA_3$  ( $P = 0.0010$ ). For the treatment with Smoke Water, the highest EFGP was 58 % at 15 °C. This was also the highest value for all conditions in the dark. It was different from those at 20 °C and 25 °C, which were close to 0 ( $P < 0.0001$ ). At 15 °C, the EFGP, 27 % without Smoke Water and 58 % with Smoke Water, were

**Table 3**

Cumulative germination (upper bound of exact binomial confidence interval with 95 %-confidence) in the fourth week after sowing, for the five species, by site and collection date (Coll. date), in light (12 h out of 24) or in dark, at three temperatures (15, 20 and 25 °C). The upper bounds are only shown for values lower than 50 %. The upper bound for the value 0 is 4.

Species	Site	Coll. date	Light			Dark		
			15 °C	20 °C	25 °C	15 °C	20 °C	25 °C
<i>A. buxifolia</i>	PS	01/2019	47 (57)	95	97	0	22(31)	33 (43)
<i>E. reunionensis</i>	RV	04/2019	42 (52)	82	87	28(38)	1(5.4)	0
	MD	05/2020	0	0	0	0	0	0
<i>H. tomentosa</i>	RV	06/2020	27(37)	26(36)	46 (56)	2(7.0)	13(21)	6(12)
	RV	07/2020	8(15)	10(18)	6(13)	3(9)	7(14)	0
	MD	07/2020	0	0	1(5.4)	0	0	0
<i>S. passerinoides</i>	PS	02/2019	35(45)	38(48)	46 (56)	51	73	47(57)
	RV	06/2020	49(59)	64	31(41)	48(58)	65	46(56)
	RV	07/2020	47(57)	49(59)	46(56)	44(55)	60	53
<i>H. lanceolatum</i>	PS	01/2019	4(10)	5(11)	0	1(5.4)	0	0
	MD	07/2020	11(19)	10(18)	0	0	0	0





**Fig. 6.** Estimated final germination percentages (black squares), Confidence Intervals (gray lines) (adjusted by models), observed percentages by replicate (blue points), in light (12 h per 24 h) and in dark, without treatment (Control), with GA<sub>3</sub> (1000 ppm) and Smoke Water, at three temperatures (15, 20 and 25 °C), for two species (a) *Erica reunionensis* and (b) *Agarista buxifolia*. The black dotted line represents the 50 %-threshold used to define dormancy in the data analyses (Baskin and Baskin, 2014; Ng, 1978). Pairwise comparisons with a Bonferroni-like correction (Benjamini and Hochberg, 1995) were made by light condition (lower-case letter in light and upper-case letter in dark). Means with the same letter are not significantly different at the 95 % level (Supplementary data). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

different ( $P = 0.0216$ ). At 20 °C and 25 °C, the EFGP with and without Smoke Water were very low (close to 0) and not different ( $P = 1$ ).

In light for *A. buxifolia* (Fig. 6b), with and without GA<sub>3</sub>, the highest EFGP were at 20 °C and 25 °C. The three EFGP with GA<sub>3</sub> (59 % at 15 °C, 98 % at 20 °C, and 88 % at 25 °C) were different ( $P \leq 0.0229$ ). At 20 °C, the EFGP 95 % without GA<sub>3</sub> and 98 % with GA<sub>3</sub> were also not different ( $P = 0.3173$ ). At 25 °C, the EFGP, 97 % without GA<sub>3</sub> and 88 % with GA<sub>3</sub> were different ( $P = 0.0375$ ). For the treatment with Smoke Water, no differences were observed between the highest final germination percentages at 20 °C and 25 °C, both 86 %, but they were different from that at 15 °C (49 %,  $P < 0.0001$ ). At the three temperatures, all pairwise comparisons for final germination percentages with or without Smoke Water were not significantly different ( $P \geq 0.0515$ ).

In dark for *A. buxifolia* with GA<sub>3</sub>, the three final germination percentages (71 % at 15 °C, 79 % at 20 °C and 25 °C) were not different ( $P \geq 0.1695$ ). All pairwise comparisons for final germination percentages with and without GA<sub>3</sub> for a given temperature indicated a difference ( $P < 0.0001$ ). For the treatment with Smoke Water, the final germination percentages at 20 °C (29 %) and 25 °C (40 %) were not different ( $P = 0.1277$ ), but they were different from that at 15 °C, 5 % ( $P < 0.0001$ ). All pairwise comparisons for final germination percentages with and without Smoke Water for a given temperature did not indicate a difference ( $P \geq 0.1622$ ).

## 4. Discussion

### 4.1. Identification of germination requirements for the five species

For *Agarista buxifolia* and *E. reunionensis* (Ericaceae), light is required for seed germination. This trait is thought to be an adaptive germination strategy for small-seeded species to ensure germination only when seeds are close to soil surface in gaps (Bliss and Smith, 1985; Woolley and Stoller, 1978). These light requirements may also vary depending on temperature (Heschel et al., 2007). For instance, for *E. reunionensis*, cumulative germination is higher at 15 °C than the others temperatures in dark. Perhaps, there is a specific interaction between phytochrome and temperature, as observed for *Arabidopsis thaliana* (Dechaine et al., 2009). Photoreceptors PHYB promote germination under cold temperature, while photoreceptors PHYA suppress it. Concerning detection of dormancy for these species, the threshold of 50 % in the fourth week after sowing is reached in light at three temperatures. Therefore, we assume that the

seeds of *Agarista buxifolia* and *E. reunionensis* have non-deep physiological dormancy, with a state of conditional dormancy in our study (Baskin and Baskin, 2004, 2014). In other words, seeds germinate under a narrow range of conditions, i.e. only in light for these three temperatures. To confirm these results, it would be interesting to use a series of alternating temperature regimes and a wider range of conditions (Baskin et al., 2006). In addition, despite our objective to confirm this result in *E. reunionensis* by another replication, at another site, our second seed lot (from MD) was not conclusive. For collection seeds, there is a high risk that these seeds were not yet mature, according to our additional phenology studies (data not shown).

For Asteraceae, for *S. passerinoides*, no dormancy was detected in seeds. In contrast, for *H. tomentosa*, the optimal temperature for seed germination is 25 °C in our study. However, it is not possible to conclude on the kind of dormancy in this species because of the impossibility to do a reliable viability test before sowing, to evaluate initial viability rate, and at the end of the test (Baskin and Baskin, 2014). Indeed, for this species, it was not possible to have an intact embryo during the cuts. To evaluate seed viability is important, in particular for species in the Asteraceae. For example, some species have recalcitrant seeds, e.g. in the Hawaiian Islands (Chau et al., 2019; Walters et al., 2005; Yoshinaga and Walters, 2003). Nevertheless, we can assume the presence of a non-deep physiological dormancy, with a change of dormancy state for *H. tomentosa*, as for *A. buxifolia* and *E. reunionensis* (Baskin and Baskin, 2004, 2014). In addition, temperature requirements appear to be narrower for *H. tomentosa* (probably, 25 °C) than for *A. buxifolia* and *E. reunionensis*.

For *H. lanceolatum* (Hypericaceae), like for *H. tomentosa*, it is not possible to confirm the kind of dormancy. However, it is likely that *H. lanceolatum* seeds exhibits physiological dormancy, based on reports in other scientific publications. For instance, *H. perforatum*, a medicinal plant recognized in Europe and *H. aviculariifolium* subsp. *depilatum* var. *depilatum*, a Turkish endemic species, have dormant seeds caused by a chemical inhibitor in the capsule (Baskin and Baskin, 2014; Campbell, 1985; Çirak et al., 2007). This chemical dormancy considered by Nikolaeva (1969) corresponds to a part of physiological dormancy in the classification of Baskin and Baskin (2004). Light is also required for seed germination in these two species.

#### 4.2. Germination of *Agarista buxifolia* and *Erica reunionensis* after GA<sub>3</sub> and smoke water treatments

Seed germination is controlled by several parameters such as light, temperature and phytohormones (Lymperopoulos et al., 2018; Ortega-Baes and Rojas-Aréchiga, 2007). In our study, inhibition and improvement of Ericaceae seed germination were investigated for GA<sub>3</sub> and Smoke Water, in light and in dark.

The presence of gibberellins (GA<sub>3</sub> at a concentration of 1000 ppm) in light, did not influence seed germination of *A. buxifolia*. However, for *E. reunionensis*, the effect of GA<sub>3</sub> is different according to temperature. The importance of gibberellins and temperature is also highlighted for seed germination of another Ericaceae, *E. junonia* (Small et al., 1982). For *E. reunionensis*, the final germination percentage was slightly improved at 15 °C. No effect on seed germination was detected at 25 °C. To explain this, our hypothesis is based on the regulation of gibberellins by the interaction between light quality, phytochrome and temperature (Dechaine et al., 2009; Lymperopoulos et al., 2018; Oh et al., 2006; Seo et al., 2009). To this regulated endogenous biosynthesis is added exogenous GA<sub>3</sub> (1000 ppm) which affects seed germination by raising the concentration of gibberellins in the seed (Cerabolini et al., 2004; Razmjoo et al., 2009). In dark, GA<sub>3</sub> could be a substitute for light for seed germination of *A. buxifolia* and *E. reunionensis*, except at 25 °C.

In the presence of Smoke Water, seed germination may be affected differently, with no germination, improvement or inhibition, depending on species. This was observed by Schwilck and Zavala (2012) for 11 of 15 grassland species, which showed no response or inhibition of seed germination. In light, for the two species studied, it seems that there is no major inhibition of seed germination (neutral effect). In comparison to other Ericaceae, Smoke Water significantly improves seed germination of numerous species from the fynbos of South Africa (33 of 53 studied species) and does not affect seed germination of 10 species (Brown and Botha, 2004). In dark, no major effect of Smoke Water was observed, except for an improvement at 15 °C for *E. reunionensis*. To complete the study on the effect of temperature, the authors propose to do a further replication on another lot and to add lower and alternating temperatures. According to Cadet (1977), low temperatures in dry seasons and the absolute thermal amplitudes could have a significant effect, even more than average values on the ecology of species. For seed germination, a case study of 445 species from the subalpine and alpine vegetation zones of the eastern Tibet Plateau in China has shown no effects of alternating temperatures (Liu et al., 2013). However, small-seeded species are more sensitive to alternating temperatures than large-seeded species. This is an interesting point to study in the subalpine and alpine zones of Reunion Island. Seed germination may also depend on the composition and the concentration of smoke extract and species (Brown, 1993). Concerning the Smoke Water in our study, we know that cyanide, one compound that can improve germination was not present (Brown and van Staden, 1997; Chiwocha et al., 2009; Kulkarni et al., 2007; Leperlier et al., 2018). It would be interesting to identify the chemical compounds in the Smoke Water of our study and to test another Smoke Water made by burning subalpine shrubland plants.

#### 4.3. Implications for threat management and conservation

Subalpine and alpine vegetation zones are unique due to their limited area and high endemism (Juvik et al., 2014; Strasberg et al., 2005). However, plant colonization is slow in subalpine shrubland, in particular on lapilli habitats (Cadet, 1977). On Reunion Island, fire is an additional factor that may increase the colonization time of native and endemic plants contrary to invasive alien species. On islands, invasive alien species are among the major threats to biodiversity (Russell and Kueffer, 2019). A study supervised by the National Park in Maïdo has shown that post-fire vegetation cover by alien species tends to increase compared to that of endemic species, including *E. reunionensis* (Payet et al., 2015). These results agree with seed germination data acquired in our study for light requirements. In protected areas, *E. reunionensis* is dominant, while in impacted areas, even with low impact, *E. reunionensis* cover decreases. This is explained by the significant presence of species, such as *Pteridium aquilinum* and *Ulex europaeus*, which deprive seeds



of light, required for germination. In particular, *U. europaeus*, one of the world's 100 worst invasive species seems to be more competitive than native species on Reunion Island (Lowe et al., 2000). This species has a physical dormancy, but seeds from Reunion Island are better able to germinate without scarification (Udo et al., 2017). Great variations in germination between populations and according to temperature are also reported, ranging from 10 % to 60 %. In addition, little is known about the species' conservation in the soil seed bank and the competition with endemic species, including those in our study. For *U. europaeus*, Hill et al. (2001) reported that its soil seed bank is perennial but its dynamics depend on site.

Temperature is one of the determinant factors for recruitment and community persistence in mountainous areas, such as subalpine and alpine vegetation zones in particular in the context of climate change (Buytaert et al., 2011; Juvik et al., 2014; Pouteau et al., 2010). Seed germination may be the most sensitive stage of plant regeneration to climate change (Walck et al., 2011; Wu et al., 2019). Responses to increase in temperature are variable depending on species (Ooi et al., 2011; Pérez-Sánchez et al., 2011). More generally, climate change could influence other plant life-history traits and determine species distribution and persistence in the ecosystem (Parmesan and Hanley, 2015; Walck et al., 2011; Wu et al., 2019).

Thus, a better understanding of native and endemic species seed germination in the presence and absence of disturbances, such as fires and biological invasions (in our case) is relevant to target restoration actions (Kildisheva et al., 2020). For instance, Payet et al. (2015) proposed studying the potential of reseeding burnt areas. This would involve acquiring more data about germination ecology and more generally about mother-plant phenology such as fruit maturation (Côme and Corbineau, 2006). In addition, this study must include soil factors and the impact of fires on life-history traits.

## 5. Conclusion

To our knowledge, these first explorations are the first data officially published on seed germination from subalpine shrubland on Reunion Island. For the first objective, to identify the presence of dormancy and light requirements, our results suggest that light is required for seed germination for *E. reunionensis* and *A. buxifolia*. Without the possibility to test seed viability, we are not able to confirm the kind of dormancy for *H. tomentosa* and *H. lanceolatum* ssp. *angustifolium*. However, we hypothesise that these four species have non-deep physiological dormancy with a conditional dormancy state. Seeds of *S. passerinoides* have no dormancy.

Concerning the second objective, to evaluate whether GA<sub>3</sub> and Smoke Water could substitute for light for seed germination of Ericaceae species, GA<sub>3</sub> (1000 ppm) substitutes for light, except at 25 °C for *E. reunionensis*. With regard to the Smoke Water of our study, this solution does not improve seed germination, except at 15 °C in dark.

This study adds more data on the germination ecology of species of tropical oceanic islands. In particular, on Reunion Island, the need for data is important for ecological restoration actions in disturbed areas where biodiversity is exceptional and vulnerable. This is particularly true in the case of subalpine shrubland, which only exists on a small number of islands in the world. In the context of disturbances by fires and biological invasions, every seed of native and endemic species is important for the conservation of biodiversity.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2022.e02269](https://doi.org/10.1016/j.gecco.2022.e02269).

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