LANKESTERIANA 25(3): 177–199. 2025. doi: http://dx.doi.org/10.15517/rwsfdp60

RECRUITMENT BOTTLENECK IN APHYLLOUS *VANILLA* SEEDLINGS FACING DROUGHT CONDITIONS

Alemao Botomanga[1], Mamisitraka T. G. Dinaharilala[1],
Johnson G. Andrianantenaina[1], Hoby N. Nomenjanahary[1],
Vololoniaina H. Jeannoda[1], Nicola Fuzzati[2] & Aro Vonjy Ramarosandratana[1,3]

[1]University of Antananarivo, Department of Plant Biology and Ecology, 101 Antananarivo, Madagascar.
[2]Chanel Parfums Beauté, Innovation Research & Development Laboratories, 93694 Pantin, France.
[3]Author for correspondence: arovonjy.ramarosandratana@univ-antananarivo.mg

Abstract. Seedling survival is strongly dependent on forest environmental conditions, which in Madagascar have been heavily degraded. Rising temperatures and declining rainfall further exacerbate the vulnerability of these ecosystems. We investigated seedling recruitment across ecologically diverse sites to identify the key factors influencing germination and seedling survival in aphyllous *Vanilla* species. *In situ* seedling monitoring and *in vitro* seed germination trials were conducted to assess tolerance to water stress at various developmental stages. Among the 13 study sites, only three showed positive recruitment rates, with two sites exhibiting rates exceeding 50%. Recruitment was influenced by factors such as fruiting success, soil acidity, high silt content, and clay-rich soil composition. After 12 months of monitoring, approximately 85% of seedlings were lost following severe drought conditions. *In vitro* asymbiotic germination assays revealed two distinct peaks: rapid germination of immature white seeds after four months, followed by delayed germination of mature black seeds eight months later. The assessment of drought tolerance across protocorm developmental stages exposed to a high-concentration gelling agent revealed that advanced-stage protocorms had increased resistance to water stress. However, no developmental stage was capable of surviving a prolonged six-month drought. Due to the absence of seedling recruitment in several locations, aphyllous vanilla recruitment may benefit from assisted propagation through seed cultures and the subsequent reintroduction of young plantlets into natural habitats. Moreover, conservation and restoration programs should incorporate research on developing soil conditions that facilitate vanilla seedling recruitment.

Keywords: aridity, aridez, deforestación, deforestation, establecimiento de plántulas, mortalidad de plántulas, orchids, orquídeas, protocorm, protocormo, seedling establishment, seedling mortality

Introduction. Understanding species recruitment mechanisms and limitations is crucial for the conservation and restoration of orchid populations (Phillips *et al*., 2020), particularly regarding the factors that control seed germination and seedling establishment (McCormick & Jacquemyn, 2014; McCormick *et al*., 2012; Rasmussen *et al*., 2015). Seed and microsite limitations are the two primary mechanisms that restrict species recruitment (Dalling *et al*., 2002; Eriksson & Ehrlén, 1992; McCormick & Jacquemyn, 2014). Microsite limitations occur when recruitment is hindered by the quantity and quality of suitable sites for establishing new plants. Conversely, seed limitation arises when seeds fail to reach appropriate sites or when seed production is insufficient to occupy potential recruitment sites, even if dispersal is possible (Nathan *et al*., 2000). The recruitment rate of orchids in their natural habitats generally remains low (Bell, 2021; Hens *et al*., 2017; McCormick *et al*., 2012; Rasmussen *et al*., 2015). This is primarily due to the difficulty of seed germination, which is often constrained by various types of dormancy. Three main types of dormancy have been identified: (1) morphological dormancy, caused by underdeveloped embryos (Arditti & Ghani, 2000; Bewley & Black, 2013; Prutsch *et al*., 2000; Yeung, 2017); (2) physical dormancy, due to a thick and impermeable seed coat (Cameron & Chase, 1998; Nishimura & Tamura, 1993; Yeh *et al*., 2021);

ORCID of the Author: AB, HNN, AVR
Received 2 April 2025; accepted for publication 10 November 2025. First published online: 4 December 2025.

and (3) physiological dormancy, resulting from the accumulation of inhibitors in mature seeds (Lee *et al*., 2015; Xu *et al*., 2020).

Given that orchid seeds typically lack an endosperm (Arditti & Ghani, 2000; Yeung, 2017), their reliance on fungal partners during germination is widely recognized (Gao *et al*., 2019; Li *et al*., 2021; Rasmussen, 1992; Sousa *et al*., 2019; Yoder *et al*., 2000). Symbiotic fungi provide most of the minerals, nutrients, vitamins, and water necessary for seed germination and seedling development (Herrera *et al*., 2019; Li *et al*., 2021; McCormick *et al*., 2018). Increased mycorrhizal inoculum significantly enhances the germination and recruitment of young seedlings (McCormick *et al*., 2016; Těšitelová *et al*., 2022).

The availability of symbiotic fungi alone does not guarantee successful orchid seed germination; other environmental factors are often overlooked (Fay *et al*., 2015; Izuddin *et al*., 2019; McCormick & Jacquemyn, 2014; Rasmussen & Whigham, 1998; Stuckey, 1967; Yang *et al*., 2017). Abiotic factors, such as temperature and humidity, also play important roles in germination (Izuddin *et al*., 2019, Rasmussen *et al*., 2015). Also, light is crucial for orchid recruitment and influences various stages of the life cycle. It affects reproductive success (Horth, 2019; Kirillova & Kirillov, 2020) and influences seed germination (Kartzinel *et al*., 2013; Kirillova & Kirillov, 2019; Mahdavi *et al*., 2023; Sorgato *et al*., 2020) and the survival of adult plants and seedlings (Fritsche *et al*., 2022; Scade *et al*., 2006). Soil structure, which determines the ability of seeds to reach an optimal depth, may affect germination (Kinderen, 1995; McCormick *et al*., 2013; Wright *et al*., 2007). Several studies have also indicated that germination success can be associated with the presence of litter (Higaki *et al*., 2017; Li *et al*., 2022), lower pH (Batty *et al*., 2001; Higaki *et al*., 2017), and increased soil moisture and organic matter content (Batty *et al*., 2001; Diez, 2007; Rasmussen *et al*., 2015).

Host trees may influence seedling establishment due to the roughness of certain bark structures that can provide better support and moisture retention, thereby enhancing germination rates (González-Orellana *et al*., 2024; Timsina *et al*., 2016; Zarate-García *et al*., 2020). It is estimated that 69% of orchid species are epiphytes (Zotz, 2013; Zotz *et al*., 2021a), and their life cycle is intricately connected to that of phorophytes. In addition to survival, the distribution of orchids and the organisms that interact with them is also influenced by climatic factors such as temperature, precipitation, and humidity (Swarts & Dixon, 2009). These factors can affect orchid distribution, particularly regarding climate change (Fay, 2015) or specific changes following forest degradation, such as alterations in light levels and quality (Abeli *et al*., 2013; Falara *et al*., 2013; Kindlmann *et al*., 2014) and soil characteristics (Hempel *et al*., 2013).

The aphyllous vanillas of Madagascar are orchids derived from an African ancestor that have diversified on the island, with seven known morpho-species. They are sold as medicinal plants (Rakotoarivelo *et al*., 2019; Randriamiharisoa *et al*., 2015). Like many climbers, they may also serve as connections between trees for primates and arboreal animals (Dunn *et al*., 2012; Montgomery & Sunquist, 1978; Rendigs *et al*., 2003; Yanoviak, 2015). As crop wild relatives, they may provide a potential gene pool for breeding new varieties to tackle climate uncertainties (Flanagan *et al*., 2018; Maxted & Kell, 2009; Pimentel *et al*., 1997). Understanding their recruitment dynamics under increasing drought conditions is critical for developing effective conservation and restoration strategies. Aphyllous vanillas are located in the humid forests of the north and east, as well as the dry forests of the west and south of the island (Andriamihaja *et al*., 2022). They are nomadic vines because their seeds germinate in the soil before the plant climbs and develops on a host tree (Zotz *et al*., 2021b). Their reproduction is both sexual and vegetative (Botomanga *et al*., 2024a; Gigant *et al*., 2014, Petersson, 2015).

Spontaneous seedling establishment has rarely been reported in aphyllous vanillas, and the conditions favorable for *in situ* germination and seedling development remain unknown. However, habitat degradation and severe drought episodes have been noted to cause recruitment bottlenecks in several species (Gale *et al*., 2018; Garnier *et al*., 2021; Muñoz-Rojas *et al*., 2016; Phillips *et al*., 2020). Drought is expected to further challenge the survival and recruitment of new orchid seedlings (Ackerman, 2021; Evans *et al*., 2020; Janissen *et al*., 2022). Rampant deforestation (Rafanoharana *et al*., 2024; Suzzi-Simmons, 2023), coupled with increasing climate risks in Madagascar (Hending *et al*., 2022; Tadross *et al*., 2008; Weiskopf *et al*., 2021), suggests that aphyllous *Vanilla* Mill. species may have low *in situ* recruitment rates.

The primary objective of this study was to quantify natural recruitment in aphyllous *Vanilla* species by recording seedling abundance *in situ* across multiple forest sites. We examined how indicators of forest degradation such as forest structure, soil physicochemical properties, and litter quantity, influence seed germination, and we identified microsite characteristics associated with successful recruitment. Seedling survival was monitored throughout the dry season to evaluate sensitivity to drought prior to the onset of rains. Because protocorms are extremely small and difficult to assess under natural conditions, we complemented field observations with *in vitro* assays, exposing different protocorm developmental stages to media with reduced water availability to evaluate their drought responses under controlled conditions.

Materials and methods. *Study sites*.— In November 2019, fieldwork was conducted at 13 sites across different regions of Madagascar (Fig. 1, Table 1). The country's landform features an elevated central plateau that gently slopes toward coastal plains in the west, south, and north, separated from the eastern littoral region by steep escarpments. This topographical diversity, combined with winds and maritime currents, has created different regional climates (Table 1), as shown by Donque (1972) and Cornet (1974). The eastern regions experience a rainy climate, without a marked dry season due to the "*Alizé*" winds that bring high humidity from the Indian Ocean (Cornet, 1974). Manompana (MAN), a study site in the east, has an average annual precipitation of 3042 mm (Table 1). In contrast, the western regions experience moderate precipitation, with about eight dry months annually (Cornet, 1974) and a mean temperature of 26 °C (Table 1). The central plateau generally has cooler temperatures. The Andringitra (AND) mountains in the highlands have an annual mean temperature of 20 °C (Table 1). Rainfall is irregular, with the dry season mitigated by frequent fog and light rain (Cornet, 1974). In the south and southwest, the climate is semi-arid to arid, characterized by low precipitation and a long dry period. The mean annual temperature is 25 °C (Table 1). This considerable climatic variation has resulted in diverse vegetation types, ranging from evergreen rainforests with closed canopies in the east, to deciduous forests in the west, and spiny thickets with gallery forests in the south (Moat & Smith, 2007).

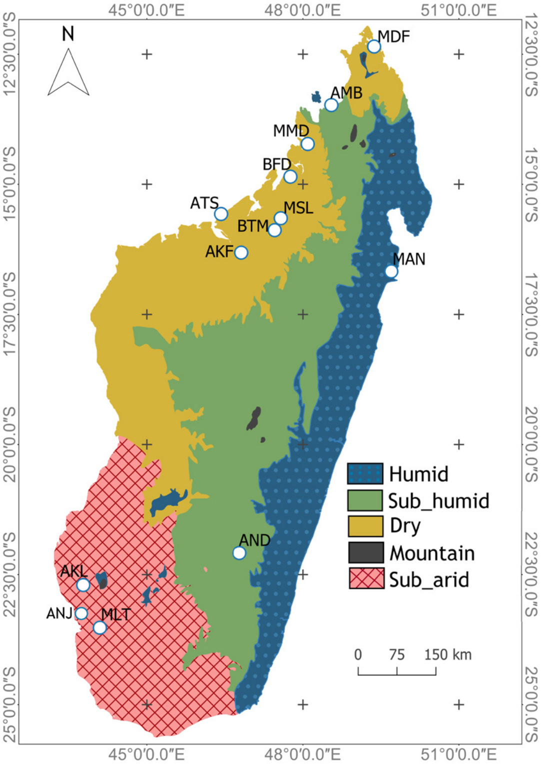

Figure 1. Map showing the five bioclimatic zones of Madagascar after Cornet (Madagascar Catalogue, 2023). Circles indicate the geographic positions of the 13 study sites.

Study species.— Seven aphyllous *Vanilla* species have been documented in Madagascar: *V. bosseri* L.Allorge and *V. decaryana* H.Perrier, *V. humblotii* Rchb.f., *V. madagascariensis* Rolfe, *V. perrieri* Schltr, *V. allorgeae* C.F.Andriam. & Pailler, and *V. atsinananensis* C.F.Andriam. & Pailler (Allorge-Boiteau, 2005, 2013; Andriamihaja *et al*., 2022; Cribb & Hermans, 2009; Portères, 1954). The aphyllous habit (Fig. 2A), has a polyphyletic origin and is thought to have evolved independently at least three times across Africa, Asia, and the Americas as a convergent adaptation to drought conditions following coastal establishment and island colonization (Bouetard *et al*., 2010). Flowers are produced once a year (Fig. 2B), with colors ranging from

TABLE 1. Soil parameters and climatic information of 13 study sites in Madagascar. Climatic data were obtained from https://www.worldclim.org (data for 1970 – 2000) and soil data were obtained from SoilGrids™ (https://soilgrids.org)

	AMB	AND	ANJ	AKF	AKL	ATS	BFD	BTM	MLT	MAN	MMD	MSL	MDF
Bioclimat	Sub humid	Sub humid	Sub arid	Dry	Sub arid	Dry	Dry	Dry	Sub arid	Humid	Dry	Dry	Dry
Litter (cm)	1.3	6.5	2	6.5	1.8	1	1.5	3	4	7.8	1.2	1.3	1.5
pH	5.4	6	6.5	6.2	6.3	5.9	6.2	6.2	6.6	5.9	5.8	6.2	6.1
Sand (g/kg)	391	542	518	531	555	424	571	557	540	511	498	571	505
Clay (g/kg)	327	277	291	253	257	293	218	252	257	270	281	243	262
Silt (g/kg)	281	181	191	216	188	283	211	190	202	210	221	186	233
OC (g/kg)	40.6	24.6	28.1	64.3	34.3	75.7	29.2	33.9	26.1	38.8	42.1	36.7	33.6
BIO1 (mm)	2089	963	575	1442	671	1683	1552	1474	614	3042	1797	1496	1139
BIO2 (°C)	26	20	25	26	24	26	26	26	25	24	26	25	25
BIO3 (°C)	21	14	18	20	17	21	20	19	17	20	21	19	20
BIO4 (°C)	31	26	32	32	30	32	32	33	32	28	31	32	29
BIO5 (kPa)	2.2	1.69	2.2	1.9	2.4	2.3	2.2	2.5	2.1	2.3	2.4	2.2	2.3
BIO6 (%)	15.6	42.1	11.6	51.3	28.5	17.8	26	21	39	61.8	16.3	22	25.5

Study sites: AMB: Ambanja, AND: Andringitra, ANJ: Anja, AKF: Ankarafantsika, AKL: Ankililoaka, ATS: Antsianitia, BFD: Befandrama, BTM: Betaramahamay, MLT: Mahaleotse, MAN: Manompana, MMD: Maromandia, MSL: Marosely, MDF: Montagne des Français

Environmental variables: Litter: Litter thickness, pH: Soil pH in $H_2$0 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

white to yellow, and their sizes vary depending on the species (Andriamihaja *et al*., 2022). Flowering times differ accross species and regions (Andriamihaja *et al*., 2020). Based on taxonomic studies by Andriamihaja *et al*. (2021, 2022) and considering the geographical origin of the plant materials used in this study, the *Vanilla* species found at the 13 study sites are as follows: *V. madagascariensis* at both Ambanja (AMB) and Maromandia (MMD), *V. humblotii* at Montagne des Français (MDF), *V. allorgeae* at Andringitra (AND) and Anja (ANJ), *V. atsinananensis* at MAN, *V. decaryana* at Mahaleotse (MLT), *V. perrieri* at Befandrama (BFD) and Betaramahamay (BTM), *V. bosseri* at Antsianitia (ATS) and Marosely (MSL), and hybrids of *V. bosseri* and *V. perrieri* at Ankarafantsika (AKF) and Ankililoaka (AKL).

Characterization of vanilla plants and seedlings.— A 50 × 20 m plot was established in an area with homogeneous vegetation where vanilla vines were present according to the Braun-Blanquet method (Braun-Blanquet, 1932). Three plots were created at each site. The number of vanilla plants in each plot was recorded, along with the number of flowers and fruits for each individual. The seeds of aphyllous vanilla plants are so small that observing the protocorms is extremely challenging. Therefore, we counted seedlings at advanced developmental stages by carefully inspecting surfaces conducive to seed germination, including the soil, tree trunks, and rocks. The physical characteristics of the seedlings, such as size (length and diameter) and color, were also recorded. Additionally, the distances between the nearest neighboring seedlings and between each seedling and the nearest adult individual were measured to understand the mode of dispersal. The fruiting rate of vanilla plants was calculated as the percentage of fruit-bearing individuals relative to the total number of individuals per plot. Similarly, the recruitment rate of aphyllous vanilla plants was calculated as the percentage of young vanilla seedlings relative to the total number of individuals per plot.

Forest vertical structure.— Gautier's method (Gautier *et al*., 1994) was used to investigate the vertical structure of the vegetation. Briefly, a transect line was

Figure 2. Recruitment of aphyllous *Vanilla*: (A). Aphyllous vanilla individual; (B). Flower visited by an insect; (C). Mature fruit blackening at the capsule tip; (D). Spontaneous seedlings under litter; (E). Seed baits placed in the soil; (F). Seedling characterized by the presence of tubers (arrow) in the underground part. Scale bar = 5 cm. Photographs by Botomanga A.

drawn through the center of the plot along its length. We divided the transect line into 50 sampling points spaced at regular intervals of 1 m and erected a vertical graduated pole at each point. Thereafter, we measured the height of all plants that came into contact with the pole, as well as those present within a one-meter width on both sides of the transect line. The data collected from the height contact points of each vegetation facies enabled us to characterize the vertical structure of each plot and analyze the cover by stratum, which corresponded to the average percentage of height classes (Fig. S1). The scale for forest cover, which corresponds to the stratum openness established by Godron *et al*. (1983), was used to interpret the results (Fig. S1).

In situ germination.— To identify microsites favorable for the germination and establishment of aphyllous vanilla seedlings, seed baits were prepared using 6 cm diameter cotton cleansing disks. Seeds were extracted from fully mature *V. madagascariensis* pods (Fig. 2C), collected in December 2019 at AMB and MMD sites. One hundred seeds were placed between two thin layers of cotton disks and wrapped in mesh fabric with a pore size of 100 µm to protect the seeds while allowing air and moisture exchange (Fig. 2E). Five seed baits were placed in each of the tested microsites, including tree trunks, rocks, dead wood debris, under leaf litter, and 5 cm deep topsoil. Each bait location was tagged to facilitate monitoring. Baits were inspected with a magnifying glass every two months over a one-year period to observe germination. The germination rate was calculated by dividing the number of newly formed protocorms by the total number of seeds per disk and multiplying by 100.

Seedling development monitoring.— We monitored the development of *V. madagascariensis* seedlings at the AMB and MMD sites. Sixty seedlings, with an initial average size of approximately 5 cm (Fig. 2F), were observed. Every two months, over the course of 18 months, we collected data on stem diameter, length, color, and mortality rate of the seedlings to assess their growth and survival dynamics. Mortality rate was calculated as the percentage of dead seedlings relative to the total number of seedlings.

Soil analysis.— Soil profiles up to a depth of 50 cm were established to evaluate the characteristics of each horizon and the thickness of the leaf litter, which is important for understanding the environmental conditions that support vanilla plant development. Five topsoil variables, namely pH, sand content, clay content, silt, and organic carbon (Table 1), were extracted from raster data at a 250-meter resolution provided by SoilGrids 2.0 (Poggio *et al.*, 2021).

Seedling in vitro germination.— Five green pods of *V. madagascariensis*, likely resulting from flowers that were naturally pollinated earlier in the year, measured approximately 15 cm in length and 4 cm in diameter. They were harvested along with their flower stalks in December 2019 in the MMD forest. After harvesting, the pods were transported and stored at room temperature for four days. First, the pods were cleaned with a detergent and then thoroughly rinsed with tap water. Next, they were immersed for one hour in a fungicide solution (Mancolaxyl 720 WP containing 6% mancolaxyl (64% mancozeb + 8% metalaxyl)) combined with detergent, and then rinsed five times with distilled water. Surface sterilization continued with a 2.6% sodium hypochlorite solution for 30 minutes, followed by five washes with distilled water. The pods were then immersed in 70° ethanol for ten seconds and briefly flamed with a Bunsen burner. Afterward, the pods were opened under sterile conditions, and the seeds were carefully extracted and placed on sterile filter paper.

The germination medium, hereafter referred to as MS medium, consisted of half-strength Murashige and Skoog (1962) medium, supplemented with 30 g/L sucrose. The pH was adjusted to 5.7 before adding 8 g/L agar (Type E bacteriological agar, Biokar Diagnostics, France). Culture media were sterilized by autoclaving at 120°C and 1.5 bar for 20 minutes, then dispensed into 9 cm diameter Petri dishes. The green pods varied in maturation age, resulting in the extraction of both immature white and mature black seeds (Fig. S2A). During the culture period, immature seeds were separated from mature seeds (Fig. S2B, C). For each pod, approximately 100 seeds were placed on the medium in five replicates, resulting in a total of 25 Petri dishes containing around 2500 seeds. The cultures were incubated for 18 months without any subculturing in a controlled growth room maintained at 25°C, under direct exposure to a light intensity of 3000 lx and a photoperiod of 16 hours of light and 8 hours of darkness. Culture monitoring was conducted every two weeks using a binocular microscope with digital image capture. MESURIM software (Madre, 2011) was utilized to count the germinated seeds, while the dimensions (length and width) of seeds and the various developmental stages of seedlings were measured and analyzed using ImageJ software (Schneider *et al.*, 2012). The germination percentage was determined by calculating the ratio of seeds with a ruptured seed coat to the total number of cultured seeds, which was then multiplied by 100.

Effects of low water availability medium on seed germination and protocorm survival.— To mimic drought conditions in the natural environment of *V. madagascariensis*, we observed protocorm responses to limited water availability by transferring protocorms at different developmental stages onto a medium with a high concentration of a gelling agent. The excess agar does not draw water quickly from plant tissue but increases the matric potential of the medium to reduce free water availability (Klimaszewska & Smith, 1997; Owens & Wozniak, 1991), and thus mimicking the desiccating conditions encountered by seeds and protocorms during drought periods. We extended the water restriction experiment to 24 weeks to simulate the dry season in northwestern Madagascar (April – October) while monitoring the responses and survival of the protocorms. The drying medium (M4) is similar to the MS medium but is supplemented with 16 g/L agar. Protocorms derived from previous germination experiments were used in this study. The staging of protocorm development was adapted from Fang *et al.* (2021) with slight modifications (Fig. S3). These include Stage 1 (P1: white protocorm), Stage 2 (P2: chlorophyllous protocorm), Stage 3 (P3: protocorm with a single leaf primordium), and Stage 4 (P4: protocorm with two leaf primordia). Thirty mature seeds or 20 protocorms were placed in each Petri dish. The experiment was conducted using 10 Petri dishes, repeated five times for mature seeds and for each protocorm developmental stage, resulting in a total of 50 Petri dishes per developmental stage. Subsequently, five seeds or protocorms from different boxes were transferred to the MS medium every two weeks for six months, totaling 50 seeds or protocorms per treatment. Seed and protocorm development were monitored on both culture media for an additional six months following transfer. Monitoring included counting germinated seeds and assessing protocorm size and color.

The size of the protocorms was evaluated by calculating the sum of their lengths and widths, dividing by two. A protocorm was considered dead when it exhibited complete necrosis and was unable to resume development after being transferred to MS medium. The survival rate of the protocorms was determined by calculating the ratio of protocorms that continued their growth to the total number of transferred protocorms and then multiplying by 100.

Analysis of climate data in Maromandia and Ambanja.— Using historical monthly weather data from the WorldClim 2 database (Fick & Hijmans, 2017), we analyzed variations in average monthly precipitation (Fig. S4) and maximum temperatures (Fig. S5) by comparing two distinct periods: before 2000 (1960–1999) and after 2000 (2000–2019). This analysis was conducted at two study sites, where we monitored the development of young vanilla seedlings to better understand potential changes in rainfall and temperature patterns and their impact on the growth and recruitment of these species.

Statistical analysis.— The data were analyzed after confirming normal distribution using the Shapiro-Wilk test. All analyses were performed with R version 4.3.1 (R Core Team, 2023). The germination percentages of immature and mature seeds were analyzed separately. For each two-week period, the average germination rate was determined using a one-way ANOVA. Multiple comparisons of means were conducted using Tukey's post hoc test with the "glht" function from the R package "multcomp" (Hothorn *et al*., 2015) at a significance level of $p < 0.05$. A similar analysis assessed the variation in protocorm size across MC and M4 culture media. The half-life (T50) required for protocorm necrosis was evaluated separately for each developmental stage. Similarly, the survival rate was assessed independently for each developmental stage. To assess the distance between the seedlings and aphyllous adult vanilla plants, we combined data from the two sites. Principal component analysis (PCA) was performed using the FactoMineR package (Lê *et al*., 2008) to explore the relationship between aphyllous vanilla recruitment and the environmental factors at the study sites. The factoextra package (Kassambara & Mundt, 2017) was used to enhance the visualization of the PCA results. A Pearson correlation test was conducted to evaluate the relationship between environmental factors and the recruitment of aphyllous vanilla plants.

Results. *Description of recruitment capacities in different ecoregions*.— Across the 13 study sites, the density of adult aphyllous vanilla plants varied significantly ($p < 0.001$), ranging from 33 individuals per hectare in MAN to 2036 individuals per hectare in BTM. Almost all sites exhibited fruiting vanilla plants, except for MAN and AKF (Table 2). Fruiting rates ranged from 1.3% to 35.6% for MDF and MMD, respectively (Table 2). *Vanilla* seedlings were recorded at only three sites in northwestern Madagascar, with moderate rainfall. MMD and AMB, featuring *V. madagascariensis* populations, exhibited the highest recruitment rates of 53.5% and 51.7%, followed by ATS, where *V. bosseri* is present, at 13.2% (Table 2). Interestingly, AMB was the only site in the study where *V. planifolia* was grown, and positive recruitment of aphyllous vanilla was also observed. The seedlings displayed a green hue and were found growing within the soil, with heights ranging from 0.5 to 6.7 cm (Fig. 2D, F). The underground portions of the seedlings consisted of white tubers (Fig. 2F). The average distance between an adult individual and a young vanilla seedling was 0.95 ± 0.5 m, with a maximum distance of 2.42 m. Seedlings were distributed at an average distance of 0.18 ± 0.12 m apart, with maximum spacing of 0.52 m.

Assessment of environmental factors contributing to aphyllous vanilla recruitment.— Principal Component Analysis (PCA) of the environmental variables classified the 13 sites into four distinct groups (Fig. 3). Group 1 (G1) comprised the single-site AND, characterized by low monthly temperatures (20.1 ± 0.7 °C), soil with low organic carbon content (24.6 g/kg), and a thick litter layer (6.5 cm) (Table 3). Group 2 (G2) included AKF and MAN, exhibiting a semi-open canopy, zero fruiting rate, very high monthly precipitation (186.8 ± 30 mm), thick litter layer (7.1 ± 0.6 cm), and soils rich in organic carbon (51.5 ± 12 g/kg) (Table 3). Group 3 (G3) consisted of AKL, ANJ, BFD, BTM, MLT, MDF and MSL, characterized by low fruiting rates, low

TABLE 2. Population size, fruiting rate, and recruitment rate of aphyllous *Vanilla* species across 13 study sites in Madagascar.

Sites	Code	Species	Vanilla number	Fruiting (%)	Recruitment (%)
Ambanja	AMB	*V. madagascariensis*	137	30.2	51.7
Andringitra	AND	*V. allorgeae*	127	4.5	0
Anja	ANJ	*V. decaryana*	513	3.7	0
Ankarafantsika	AKF	*V. bosseri-perrieri*	42	0	0
Ankililoaka	AKL	*V. bosseri-perrieri*	423	5.4	0
Antsianitia	ATS	*V. bosseri*	241	7.4	13.2
Befandrama	BFD	*V. perrieri*	529	3.7	0
Betaramahamay	BTM	*V. perrieri*	611	2.1	0
Maleotsy	MLT	*V. decaryana*	89	1.6	0
Manompana	MAN	*V. atsinananensis*	10	0	0
Maromandia	MMD	*V. madagascariensis*	173	35.6	53.5
Marosely	MSL	*V. bosseri*	236	2.2	0
Montagne des Français	MDF	*V. humblotii*	159	1.3	0

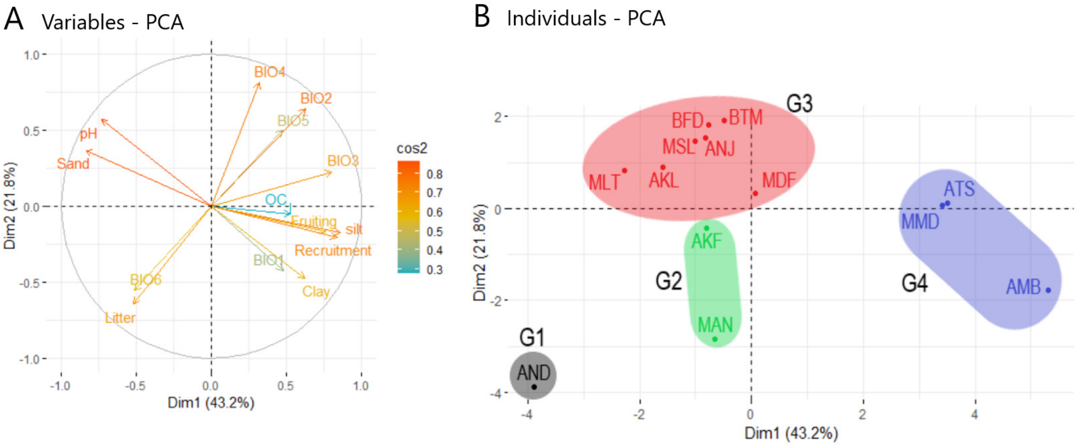

FIGURE 3. Factorial plans of Principal Component Analysis. (**A**) Variables consisting of abiotic and vegetation characteristics of the study sites. pH – Soil pH in H$_2$0 at 5 cm depth, Sand – Quantity of sand in g/kg, at 5 cm depth, Clay – Quantity of clay in g/kg at 5 cm depth, Silt – Quantity of silt in g/kg at 5 cm depth, OC – Quantity of organic carbon in g/kg at 5 cm depth, BIO1 – Annual precipitation, BIO2 – Average annual temperature, BIO3 – Minimum annual temperature, BIO4 – Maximum annual temperature, BIO5 – Water vapor pressure, BIO6 – Canopy cover rate. (**B**) Factor map showing the distribution of localities. Ambanja – AMB, Anja – ANJ, Andringitra – AND, Ankarafantsika – AKF, Ankililoaka – AKL, Antsianitia – ATS, Befandrama – BFD, Betaramamay – BTM, Maleotsy – MLT, Manompana – MAN, Maromandia – MMD, Marosely – MSL, Montagne des Français – MDF. The variables are colored according to their Cos2, and color gradients are used to highlight their degree of correlation: variables with low, mid, and high Cos2 values are colored in blue, nanking yellow, and red, respectively.

monthly precipitation (89.5 ± 13.2 mm), and sandy soils (545.2 ± 9.6 g/kg) (Table 3). Group 4 (G4) included the AMB, ATS, and MMD sites, featuring a very open canopy, high fruiting rates (24.4 ± 8.6%), a thin litter layer (1.1 ± 0.8 cm), average monthly precipitation (154.6 ± 25.7 mm), and clayey-silty soils (Table 3). Recruitment showed strong positive correlations with fruiting rate ($r = 0.9$, $p < 0.001$), soil silt content ($r = 0.59$, $p < 0.05$), and soil clay content ($r = 0.64$, $p < 0.05$) (Fig. S6). In contrast, it

Table 3. Comparison of ten environmental parameters (mean ± SE) among the four PCA groups. Means followed by the same superscript within a column are not significantly different ($p > 0.05$), according to Tukey's post-hoc test.

Group	Canopy closure (%)	Fruiting (%)	Litter (cm)	Precipitation (mm)	Temperature (C°)	pH	OC (g/kg)	Clay (g/kg)	Silt (g/kg)	Sand (g/kg)
1	42.1^{ab}	4.5^{b}	6.5^{a}	80.2 ± 25.6^{ab}	20.1 ± 0.7^{c}	6.0^{ab}	24.6^{a}	277^{ab}	181^{b}	542^{ab}
2	56.5 ± 5.2^{a}	0 ± 0^{b}	7.1 ± 0.6^{a}	186.8 ± 30.0^{a}	25.0 ± 0.4^{ab}	6.1 ± 0.1^{ab}	51.5 ± 12^{a}	261.5 ± 8.5^{ab}	213 ± 3^{ab}	521 ± 10^{ab}
3	24.8 ± 3.1^{b}	2.8 ± 0.5^{b}	2.1 ± 0.3^{b}	89.5 ± 13.2^{b}	25.1 ± 0.2^{b}	6.3 ± 0.1^{a}	31.7 ± 1^{a}	254.2 ± 8.2^{b}	200.1 ± 6^{b}	545.2 ± 9.6^{a}
4	16.5 ± 0.6^{b}	24.4 ± 8^{a}	1.1 ± 0.8^{b}	154.6 ± 25.7^{ab}	26.2 ± 0.2^{a}	5.7 ± 0.3^{b}	52.8 ± 11^{a}	300.3 ± 13.7^{a}	261.6 ± 20^{a}	437.7 ± 31^{b}

Group 1: AND; Group 2: AKF and MAN; Group 3: AKL, ANJ, BFD, BTM, MDF, MLT and MSL; Group 4: AMB, ATS, and MMD.

Environmental variables: pH: Soil pH in H20 at 5 cm depth, OC: Quantity of organic carbon in g/kg at 5 cm depth, Clay: Quantity of clay in g/kg at 5 cm depth, Silt: Quantity of silt in g/kg at 5 cm depth.

was negatively correlated with soil pH ($r = -0.75$, $p < 0.01$) and sand content ($r = -0.68$, $p < 0.05$) (Fig S6). When the MAN and AKF sites, where no fruiting was recorded, were excluded from the analysis, precipitation showed a significant positive correlation with recruitment ($r = 0.69$, $p < 0.05$) (Fig. S7). Notably, no recruitment was observed at the southern Madagascar sites, which are characterized by low annual precipitation, despite significant fruit production (Table 2).

Responses of seed baiting.— After 12 months of monitoring, the seed baits placed on different substrates, including those buried in soil, showed no signs of germination.

Seasonal growth and mortality of young seedlings.— Throughout one year, the increments in length and diameter of young vanilla seedlings exhibited significant variations correlated with precipitation (Fig. 4A–C). From January to June, seedling length increased by 3.6 ± 0.9 cm at AMB and by 2.4 ± 0.3 cm at MMD, while diameter increased by 0.67 ± 0.14 mm at AMB and 0.57 ± 0.16 mm at MMD (Fig. 4A–C). During the dry season (June – October), the length increment was limited to 1.2 ± 0.3 cm at AMB and 0.9 ± 0.1 cm at MMD, while seedling diameter decreased to 0.63 ± 0.25 mm at AMB and 0.57 ± 0.22 mm at MMD (Fig. 4A–C). During this period, the seedlings turned yellow (Fig. 5C, D, E). From November through February, during the heavy rainy season, the surviving seedlings showed a marked increase in length (5.8 ± 1.8 cm at AMB and 5.4 ± 2.8 cm at MMD) and diameter (1.1 ± 0.4 mm at AMB and 0.7 ± 0.4 mm at MMD) (Fig. 4A, C). Over the year, young aphyllous vanilla plants demonstrated a total length growth of 10.5 ± 2.7 cm (Fig. 5A, B). However, a high mortality rate was observed, with 24/30 (80%) of the seedlings at AMB and 27/30 (90%) at MMD not surviving through December; the majority of losses occurred between October and December (Fig. S8). Between 2000 and 2019, there was a significant decrease in annual precipitation ($p < 0.05$), with a reduction of 151 mm at AMB and 109 mm at MMD compared to the 1970–1999 period. Simultaneously, maximum temperatures increased by 0.5 °C at AMB and 0.6 °C at MMD.

In vitro asymbiotic germination.— The highest cumulative germination percentage occurred in immature seeds, reaching 98% after 210 days of cultivation (Fig. 6A), while mature seeds displayed a cumulative germination rate of 25% after 390 days (Fig. 6B). Germination peaked at 105 days after cultivation (DAC) for immature seeds (Fig. 6A) and at 270 DAC for mature seeds (Fig. 6B). Asymbiotic germination of aphyllous vanilla is initiated by seed imbibition and subsequent rupture of the seed coat (Fig. 7A, B), followed by the release of a whitish embryo (Fig. 7C). Non-chlorophyllous protocorms emerged from immature seeds at 75 DAC on MS medium (Fig. 6A), whereas germination was noted only

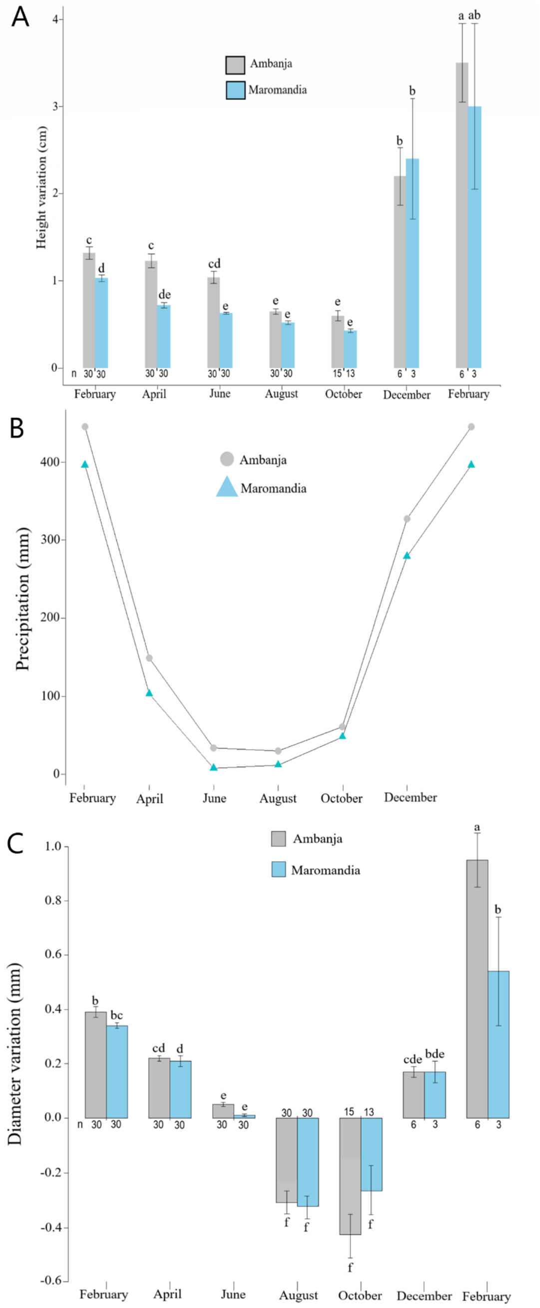

FIGURE 4. Annual variations in precipitation, seedling height, and seedling diameter at the Maromandia and Ambanja sites. (**A**) Variation in seedling height (cm); (**B**) average monthly precipitation (https://www.worldclim.org); (**C**) variation in seedling diameter (mm). (n = Sample sizes); bars with different letters are significantly different after Tukey post hoc test with P < 0.05.

at 195 DAC in mature seeds (Fig. 6B). Initially, white protocorms enlarged and gradually developed green pigmentation (Fig. 7D) after an average period of 3 ± 1.2 weeks post seed coat rupture (WPSCR). However, a few protocorms (1.2%) exhibited atypical development, remaining white before turning brown, and subsequently dark brown at 21 ± 2.2 WPSCR. Chlorophyllous protocorms continued to grow, forming a cotyledon (Fig. 7E) at 8 ± 1.6 WPSCR, and developing the first leaf (Fig. 7F) at 15 ± 2.3 WPSCR. The protocorm developed into a young seedling with roots (Fig. 7G) at 34 ± 3.2 WPSCR. At 390 DAC, the germination of some mature seeds was still observed (Fig. 6B).

Effect of the M4 drying medium on seed germination and protocorm development.— After 24 weeks of culture in M4 medium, there was no germination observed in the mature seeds. In contrast, seeds transferred to the MS medium had a cumulative germination rate of 6% over the same period. Regarding growth, the size of the protocorms in the M4 medium remained unchanged after 24 weeks of cultivation (Table 4). Conversely, all protocorms cultured in MS medium showed a significant increase in size compared to the beginning of the cultivation period (Table 5). Over several weeks in M4 medium, the protocorms gradually turned brown (Fig. S2D), although the time required for this color change varied depending on the stage of the protocorms. P1, initially white, turned brown after 6 ± 1.5 weeks in the M4 medium, and then dark brown after 10 ± 1.7 weeks (Fig. S9). P2, P3, and P4, which were initially green, turned dark brown after 14 ± 1.5, 17 ± 2, and 21 ± 2 weeks, respectively, in the M4 medium (Fig. S9). The transfer of protocorms from M4 to MC revealed differing capacities to resume development after water stress, which is influenced by both the duration of exposure to the M4 medium and the developmental stage of the protocorms. For the P1, P2, P3, and P4 protocorms, the times required to reach 50% mortality (T50) in M4 medium were 10, 12, 14, and 16 weeks, respectively (Fig 8). Complete mortality, with no protocorms surviving after transfer to the MS medium, was observed after 14, 16, 18, and 22 weeks (Fig 8).

Discussion. *Fruiting rate and soil properties are important factors controlling seedlings recruitment*.— Across 13 sites, aphyllous vanilla seedlings were observed at

Figure 5. Seedling evolution. (**A–B**) Healthy seedlings observed at the beginning of the year (**A**) and after one year of monitoring (**B**). (**C–D**) Dehydrated seedlings observed in August (**C**) and an advanced stage of dehydration leading to necrosis of the upper parts (**D**). (**E**) Close-up of a necrotic seedling stem caused by severe dehydration. Scale bar: (A, C) = 1 cm; (B, D) = 2 cm. Photographs by Botomanga A.

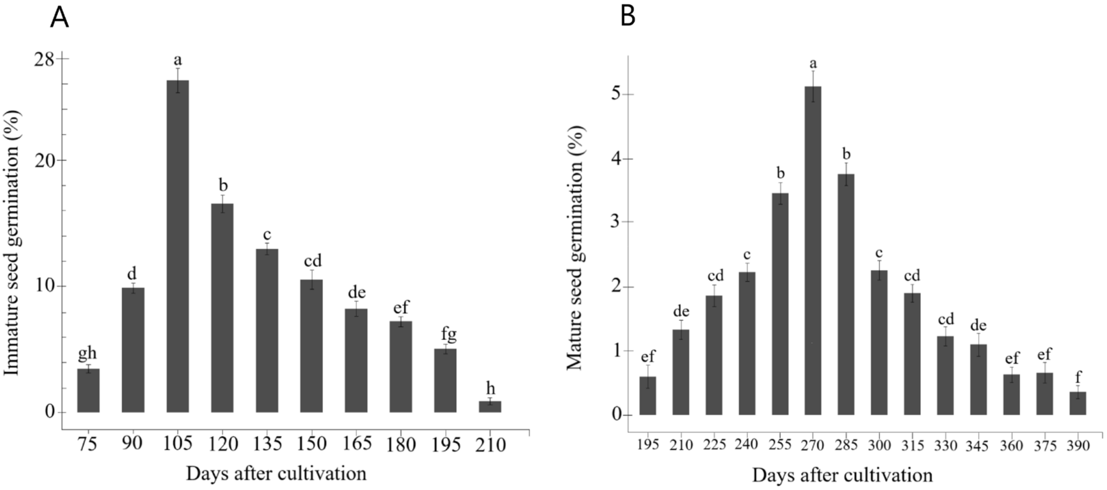

Figure 6. Percentage of seed germination of aphyllous vanilla in MC culture medium over 390 days. (**A**) Germination of immature seeds; (**B**) Germination of mature seeds. Bars correspond to the standard error (SE) of means. Sample sizes were as follows: immature seeds (n = 1000), mature seeds (n = 1500). Bars with different letters are significantly different after Tukey post hoc test with $P < 0.05$.

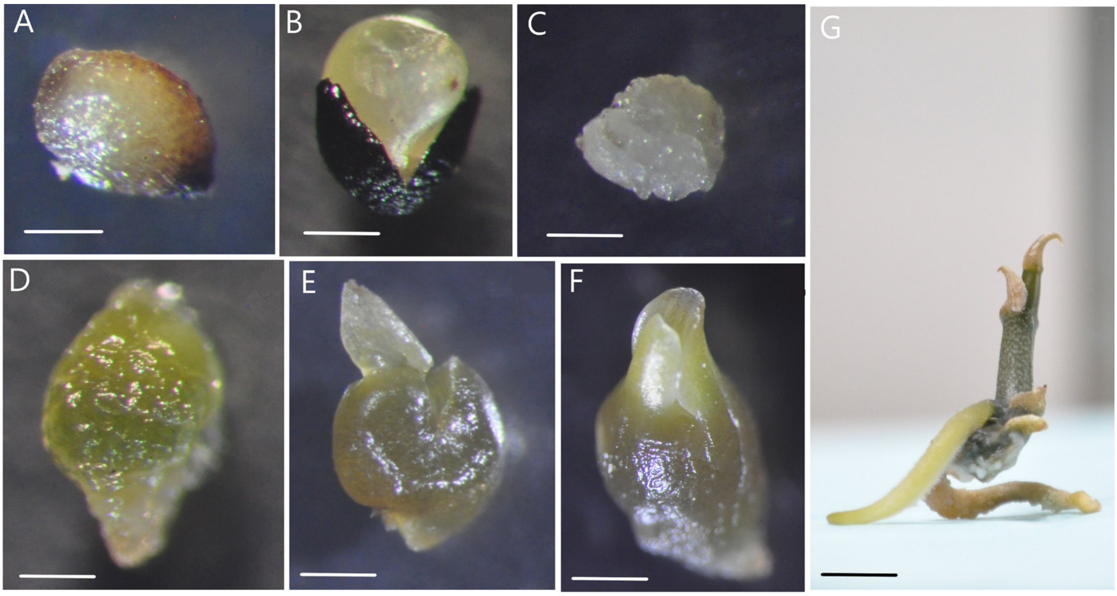

Figure 7. Development of protocorms and growth of aphyllous vanilla seedlings: (**A**). Imbibed seed; (**B**). Protocorm emerging from the seed coat; (**C**). White protocorm after seed coat rupture; (**D**). Green protocorm (4 weeks after seed coat rupture); (**E**). Protocorm with cotyledon (8 weeks after seed coat rupture); (**F**). Protocorm with the first leaf (14 weeks after seed coat rupture); (**G**). Seedlings with healthy roots (34 weeks after seed coat rupture). Scale bars: (A, B, D) = 0.1 mm; (C) = 0.2 mm; (E) = 0.25 mm; (F) = 1 mm; (G) = 1 cm. Photographs by Botomanga A.

only three northwestern sites (AMB, ATS, and MMD), highlighting the stringent recruitment requirements for orchid species under natural conditions (Batty *et al*., 2001; Gill, 1996; Hens *et al*., 2017). These three sites likely provide environmental conditions conducive to both germination and seedling growth, including a high fruiting rate, high silt content, high clay content, and acidic soil. In contrast, at the other 10 sites, particularly those in southern Madagascar, we assume that recruitment in the aphyllous vanilla occurs exclusively through vegetative reproduction via stem branching.

Fruit production, which is essential for seed availability, is crucial for orchid recruitment (Ackerman *et al*., 1996; Hens *et al*., 2017; Nathan & Muller-Landau, 2000). Our results suggest that higher fruiting rates correlate with a greater likelihood of encountering wild seedlings. In populations influenced by density-dependent factors such as suitable microsite availability, individuals producing more seeds have a better chance of locating an appropriate microsite than those producing fewer seeds (Hens *et al*., 2017; Shefferson *et al*., 2020).

Seedling occurrence was also associated with lower pH. Soil acidity can directly or indirectly influence orchid seed germination (Diez, 2007). Due to their reliance on mycorrhizae for germination, factors affecting fungal distribution indirectly influence germination success, and soil pH significantly affects the distribution of mycorrhizal fungi (Janowski & Leski, 2022). Soil fungi tend to be more diverse in low-pH environments (Blagodatskaya & Anderson, 1998; Rousk *et al*., 2010). Furthermore, HCl solutions are commonly used to mimic animal digestive acids and to break down the impermeable seed coats of endozoochorous plants (Jaganathan *et al*., 2019; Kleyheeg *et al*., 2018; Šoch *et al*., 2023), suggesting that soil acidity may directly impact seed germination by acting similarly to that of orchid seeds.

The silt and clay contents of soils play a critical role in geological and environmental processes because of the fine particle size and porous structure of these components (Huntley, 2023; Li *et al*., 2018). Soil pore space governs the movement of air and water through its porosity and permeability, which in turn determine the availability of these resources to plant roots (Matus, 2021). These physical properties endow soils with high water retention capacity and substantial potential for carbon and nitrogen stor-

Table 4. Variation in protocorm size (mean ± SE) on drying M4 medium after 24 weeks of culture. Means followed by the same superscript in a column were not significantly different ($p > 0.05$) according to Tukey post-hoc test.

Temps (T)	P1 (mm)	P2 (mm)	P3 (mm)	P4 (mm)
T0	0.37 ± 0.03^{e}	0.81 ± 0.02^{d}	1.14 ± 0.08^{c}	2.98 ± 0.26^{b}
T1	0.41 ± 0.01^{e}	0.80 ± 0.08^{d}	1.12 ± 0.11^{c}	3.04 ± 0.13^{b}
T2	0.32 ± 0.03^{e}	0.71 ± 0.06^{d}	1.10 ± 0.12^{c}	3.12 ± 0.71^{ab}
T3	0.30 ± 0.05^{e}	0.63 ± 0.04^{d}	1.08 ± 0.11^{c}	3.08 ± 1.1^{b}

Table 5. Variation in protocorm size (mean ± SE) on germination media after 24 weeks of culture. Means followed by the same superscript in a column were not significantly different ($p > 0.05$) according to Tukey post-hoc test.

Temps (T)	P1 (mm)	P2 (mm)	P3 (mm)	P4 (mm)
T0	0.35 ± 0.02^{j}	0.83 ± 0.07^{i}	1.15 ± 0.30^{h}	2.94 ± 0.37^{e}
T1	0.77 ± 0.02^{i}	1.52 ± 0.09^{g}	2.39 ± 0.21^{f}	4.7 ± 0.38^{c}
T2	1.36 ± 0.04^{g}	2.47 ± 0.11^{f}	3.11 ± 0.41^{d}	7.2 ± 1.3^{b}
T3	2.85 ± 0.04^{ef}	3.2 ± 0.14^{de}	4.2 ± 0.35^{c}	13.7 ± 2.4^{a}

P1: stage 1 protocorm, P2: stage 2 protocorm, P3: stage 3 protocorm, P4: stage 4 protocorm, T0: culture initiation, T1: 8 weeks after initiation, T2: 16 weeks after initiation and T3: 24 weeks after initiation.

age (Li *et al*., 2018; Matus, 2021; Moreno-Maroto & Alonso-Azcárate, 2018; Rabot *et al*., 2018). Although we did not observe a direct correlation between precipitation and aphyllous vanilla plant recruitment, G4 sites received an average of 154 mm of rainfall per month, ensuring consistent moisture. The clayey soils at these sites may be instrumental in maintaining moisture, facilitating germination by preventing seed desiccation, and promoting water imbibition. The negative charge of clay particles enables them to attract and retain essential cations such as calcium, potassium, and magnesium (Kumari & Mohan, 2021; Schoonheydt *et al*., 2018). These nutrients, which are vital for plant growth, are gradually released by clay and serve as reservoirs for seedling root development. Loamy soils, characterized by intermediate particle size and low compaction, provide favorable conditions for the development of fungal networks (Pauwels *et al*., 2023). The genus *Vanilla* is frequently associated with various fungal partners, such as *Rhizoctonia solani* J.G.Kühn, *Tulasnella bifrons* Bourdot & Galzin, *Tulasnella deliquescens* Juel (Juel), and *Ceratobasidium cornigerum* (Bourdot) D.P.Rogers (Porras-Alfaro & Bayman, 2007; Sathiyadash *et al*., 2020). These fungi display a variety of ecological strategies ranging from saprotrophic growth to symbiotic associations with plant roots. They colonize root tissues and extend their hyphae beyond the rhizosphere, facilitating the uptake and transfer of water and nutrients to host plants (Bahadur *et al*., 2019; Cheng *et al*., 2021).

An additional biotic factor in orchid recruitment is the positive influence of seedling recruitment near adult individuals. We found an average distance of less than 1 m between seedlings and adults, corroborating results that indicate germination and successful recruitment often occur preferentially near parental or conspecific plants (Diez, 2007; González-Orellana *et al*., 2024; McCormick *et al*., 2018). This spatial proximity may be attributed to the enhanced availability of mycorrhizal fungi and optimal microsite conditions in the vicinity of adult specimens (Diez, 2007; Fernández *et al*., 2023; Petrolli *et al*., 2022). Read *et al*. (2024) confirmed that adult plants transfer carbon to seedlings via mycorrhizal fungal networks that connect the roots. They suggested that this mechanism may play a crucial role in orchid establishment and development in natural habitats.

Delayed germination of mature seeds to align with favorable environmental conditions.– The immature seeds of the aphyllous *Vanilla* species exhibited nearly complete germination, while only 25% of the mature seeds germinated in the culture medium. This indicates that seed coat impermeability and the deposition of inhibitory substances occur late in seed development, as observed in many orchid seeds (Šoch *et al*., 2023; Yamazaki & Miyoshi, 2006; Yeung *et al*., 2018; Zhang *et al*., 2013). The results indicated that the germination of mature seeds peaked at approximately nine months and persisted for just over one year. In their natural habitat, the pods of *V. madagascariensis* reach ma-

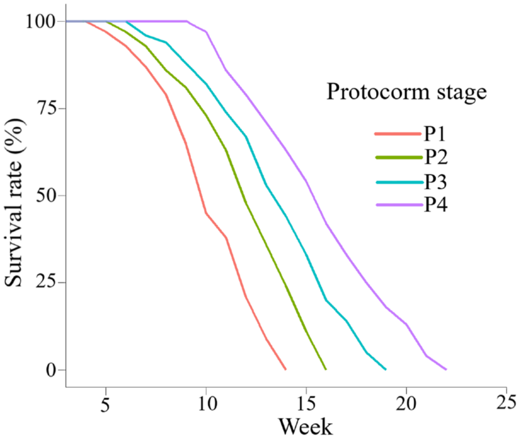

Figure 8. Survival rates of various protocorm stages after several weeks of culture on M4 medium. For each protocorm developmental stage, n = 600.

turity between June and August. They likely dehisce between September and November in response to the high temperatures and dryness typical of that period, thereby releasing seeds at the onset of the rainy season, between November and January. In line with the observed results, germination began around mid-August with a peak recorded in early October, which historically marked the beginning of the rainy season (Fig. S4). Due to a prolonged interval between the onset of germination and seed coat rupture, germination likely occurs during dry periods, and protocorm development coincides with the following rainy season, which is favorable for their growth. However, under current climate change conditions in Madagascar, the first rains tend to occur later, often in December. This could affect the survival rate of developing protocorms. The peak germination rate of the mature seeds was 5%, which is low. However, considering the number of seeds in an aphyllous vanilla pod, this still represents a significant number of seedlings. Consequently, it is not surprising that our seed-baiting experiment failed to yield positive results, as the experiment was terminated prematurely. In certain orchid species, as reported by Rasmussen & Whigham (1998), seed germination was not observed within 20 months following bait installation but only in the subsequent year.

High seedling mortality occurs as the dry season intensifies.– Our findings revealed that 85% of the young seedlings perished during the 18-month monitoring period in their natural habitats. This high mortality rate was primarily attributed to excessive dehydration, characterized by a progressive reduction in seedling diameter, yellowing of the stem, and ultimately, collapse of the inner tissues. The elevated mortality observed at the onset of the rainy season suggests that the adverse conditions during the dry season inflicted irreversible damage on the seedlings. An analysis of climate data over the past six decades has indicated a decline in annual rainfall and a rise in temperature in AMB and MMD. These results corroborate a recent analysis that incorporated controlled and homogenized data from 28 meteorological stations in Madagascar between 1950 and 2018 (Randriamarolaza *et al*., 2022). The rising temperatures likely led to increased evaporation and surface drying, thereby intensifying drought conditions caused by the lack of rainfall (Gebrechorkos *et al*., 2025). Such dual effects of water limitation and extreme heat were found to cause seedling mortality among different pine species, with rising temperatures exacerbating moisture stress (Hankin *et al*., 2025). Furthermore, recruitment is strongly affected by climate change even among species that have adaptive traits to withstand drought (Félix-Burruel *et al*., 2025; Milton *et al*., 2024).

Responses of protocorms to low water availability are dependent on their developmental stages.– In the drying medium, all stages of protocorms ceased development, as indicated by the lack of significant size variation over the 24-week culture period. Water restriction limits the growth and development of germinating embryos (Bazalar, 2020; Klimaszewska *et al*., 2000). Prolonged water restriction results in plant death (Huang & Song, 2013; Ntuli, 2012; Wojtyla *et al*., 2020), as shown in this study. However, we observed that drought tolerance varied depending on the developmental stage of protocorms. P1-stage protocorms are more sensitive to drought than P4-stage protocorms, supporting the hypothesis that body size is a key life-history trait influencing drought survival in plants (Midgley & Van Der Heyden, 1999; Milton *et al*., 2024). The P4 protocorms, which were significantly larger than P3, P2, and P1, exhibited greater drought resilience, with a notable delay in mortality. This increased resistance is likely due to the higher water storage capacity of the well-developed tissues. This mechanism enhances survival probability in water-limited environments (Midgley & Van Der Heyden, 1999; Ripley *et al*., 2013). In the present study, no new seedling recruitment was observed at any of the sites located within ecoregions characterized by low precipitation. This absence of recruitment is concerning as it highlights the vulnerability of *Vanilla* species in increasingly arid environments. These findings underscore the urgency of prioritizing conservation actions for vanilla populations inhabiting drought-prone regions, where natural recruitment appears to be critically limited.

Conclusion and implications to conservation. The recruitment of young plants and successful sexual reproduction play crucial roles in maintaining high genetic diversity and promoting effective gene flow within populations (Berry *et al*., 2019; Paulo *et al*., 2019; Salgotra & Chauhan, 2023). The absence or low recruitment rate observed in this study, combined with the high clonality found in certain aphyllous vanilla populations (Botomanga *et al*., 2024a), may further constrain seedling recruitment. Indeed, reduced genetic diversity and increased inbreeding are associated with lower germination rates and decreased seedling fitness at early developmental stages (Booy *et al*., 2000; Capblancq *et al*., 2021). This highlights the need for corrective action, such as translocation strategies, including reintroduction and assisted colonization, to support species recovery (Phillips *et al*., 2020; Shao *et al*., 2017; Zhao *et al*., 2021). The results presented here lay the groundwork for developing habitats conducive to the recruitment of aphyllous *Vanilla* within a restoration program. Such approaches have proven successful for some orchid species (Wright *et al*., 2007). To preserve and enhance the genetic diversity of aphyllous *Vanilla* orchids, seed germination success may be enhanced using tissue culture techniques (Jolman *et al*., 2022). The resulting seedlings can then be reintroduced into their natural habitat once they attain sufficient robustness to withstand drought.

The aphyllous vanilla species found in Madagascar have demonstrated a remarkable capacity to adapt to heterogeneous environments, including humid and dry forests. Interspecific comparisons of root anatomical traits revealed an increased number of aerenchyma and vascular bundles in low-rainfall regions (Botomanga *et al*., 2024b). Such attributes could be used to select drought-tolerant *V. planifolia* genotypes to help farmers facing climate change. Interestingly, two aphyllous species, *V. perrieri* and *V. bosseri*, displayed a wide distribution, spanning different bioclimatic regions. In contrast, they are candidates for genome–environment associations (GEA) studies at the intraspecific level to identify genomic signatures of drought adaptation that are potentially useful in future breeding programs (Ravelonanosy *et al*., 2025).

Acknowledgements. Our work received research permit no. 004/17/MEEF/54/DGF/DSAP/SCB.Re. from the Ministry of Environment and Sustainable Development of Madagascar. We express our gratitude to Nivohanintsoa Ravoniarison for granting access to the Tissue Culture Laboratory. The vanilla conservation program was supported by Chanel Parfums Beauté. We extend our heartfelt thanks to Hanitriniaina Razaiarimanana for her valuable technical expertise and support.

Author contributions. Writing review & editing: AB, M.T.G.D, JGA, HNN, VHJ, NF, AVR. Writing original draft: AB, VHJ, AVR. Methodology: AB, M.T.G.D, JGA, HNN, VHJ, AVR. Validation: AB, M.T.G.D, JGA, HNN, VHJ, AVR. Formal Analysis: MTGD., AB. Investigation: AB, MTGD, JGA, HNN, AVR. Data curation: JGA, HNN, AB. Supervision: VHJ, AVR. Conceptualization: AB, VHJ, NF, AVR. Funding acquisition: NF, AVR. Visualization: AVR. Resources, Project administration: AVR.

Funding. This research received financial support from Chanel Parfums Beauté.

Conflict of interest. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Literature cited

Abeli, T., Jäkäläniemi, A., Wannas, L., Mutikainen, P., & Tuomi, J. (2013). Pollen limitation and fruiting failure related to canopy closure in *Calypso bulbosa* (Orchidaceae), a northern food-deceptive orchid with a single flower. *Botanical Journal of the Linnean Society*, 171(4), 744–750. https://doi.org/10.1111/boj.12014

Ackerman, J. D., Sabat, A., & Zimmerman, J. K. (1996). Seedling establishment in an epiphytic orchid: an experimental study of seed limitation. *Oecologia*, 106, 192–198. https://doi.org/10.1007/BF00328598

Ackerman J.D. (2021). Orchids on the move: go forth & proliferate! In: Wang Y-T, Chang Y-CA, Lee Y-I, Chen F-C, Tsai W-C (Eds.), *Proceedings of the 2021 Virtual World Orchid Conference* (pp. 262–266). Tainan City: Taiwan Orchid Growers Association.

Allorge-Boiteau, L. (2005). Les vanilles succulentes de Madagascar. *Succulentes*, *2*, 3–11.

Allorge-Boiteau, L. (2013). Une nouvelle espèce de vanille à Madagascar. *Hommes Et Plantes*, *85*, 4–5.

Andriamihaja, C. F., Botomanga, A., Misandeau, C., Ramarosandratana, A. V, Grisoni, M., Da Silva, D., & Besse, P. (2022). Integrative taxonomy and phylogeny of leafless *Vanilla* orchids from the South-West Indian Ocean region reveal two new Malagasy species. *Journal of Systematics and Evolution*, 61(1), 80–98. https://doi.org/10.1111/jse.12858

Andriamihaja, C.F., Ramarosandratana, A.V., Grisoni, M., Jeannoda, V.H., Besse, P., (2021). Drivers of population divergence and species differentiation in a recent group of indigenous orchids (*Vanilla* spp.) in Madagascar. *Ecology Evolution*, 11, 2681–2700. https://doi.org/10.1002/ECE3.7224.

Andriamihaja, C. F., Ramarosandratana, A. V., Grisoni, M., Jeannoda, V., & Besse, P. (2020). The leafless *Vanilla* species-complex from the south-west Indian ocean region: A taxonomic puzzle and a model for orchid evolution and conservation research. *Diversity*, 12(12), 1–25. https://doi.org/10.3390/D12120443

Arditti, J., & Ghani, A. K. A. (2000). Numerical and physical properties of orchid seeds and their biological implications. *The New Phytologist*, 145(3), 367–421. doi:10.1046/j.1469-8137.2000.00587.x

Bahadur, A., Batool, A., Nasir, F., Jiang, S., Mingsen, Q., Zhang, Q., Pan, J., Liu, Y., & Feng, H. (2019). Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *International Journal of Molecular Sciences*, 20(17), 4199. https://doi.org/10.3390/ijms20174199

Batty, A. L., Dixon, K. W., Brundrett, M., & Sivasithamparam, K. (2001). Constraints to symbiotic germination of terrestrial orchid seed in a mediterranean bushland. *New Phytologist*, 152(3), 511–520. https://doi.org/10.1046/j.0028-646X.2001.00277.x

Bazalar, V. P. F. (2020). Effects of water deficit on the physiology, biochemistry and morphology of the protocorms of the epiphytic orchid *Laelia lobata* Lindl. Phd Thesis. Universidade de São Paulo.

Bell, S. A. J. (2021). Successful recruitment following translocation of a threatened terrestrial orchid (*Diuris tricolor*) into mining rehabilitation in the Hunter Valley of NSW. *Ecological Management & Restoration*, 22(2), 204–207. https://doi.org/10.1111/emr.12473

Berry, A. S. F., Salazar-Sánchez, R., Castillo-Neyra, R., Borrini-Mayori, K., Chipana-Ramos, C., Vargas-Maquera, M., Ancca-Juarez, J., Náquira-Velarde, C., Levy, M., & Brisson, D. (2019). Sexual reproduction in a natural *Trypanosoma cruzi* population. *PLoS Neglected Tropical Diseases*, 13(5), e0007392. https://doi.org/10.1371/journal.pntd.0007392

Bewley, J. D., & Black, M. (2013). Germination, Structure, and Composition. In: Springer Science & Business Media (Eds), *Seeds: physiology of development and germination* (pp. 1–33). New York: Plenum Press. DOI 10.1007/978-1-4899-1002-8

Blagodatskaya, E. V, & Anderson, T.-H. (1998). Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO2 of microbial communities in forest soils. *Soil Biology and Biochemistry*, 30(10–11), 1269–1274. https://doi.org/10.1016/S0038-0717(98)00050-9

Booy, G., Hendriks, R. J. J., Smulders, M. J. M., Van Groenendael, J. M., & Vosman, B. (2000). Genetic diversity and the survival of populations. *Plant Biology*, 2(04), 379–395.

Botomanga, A., Andriamihaja, C. F., Besse, P., Jeannoda, V. H., Grisoni, M., Ny Aina Ranaivoson, S. E. F., Antsonantenainarivony, G., Fuzzati, N., & Ramarosandratana, A. V. (2024a). Continuous Degradation of Forest Structure and Composition Causes a Shift in Phorophytes and Population Genetics in Aphyllous *Vanilla Species*. *Tropical Conservation Science*, 17. https://doi.org/10.1177/19400829241301920

Botomanga, A., Jeannoda, V. H., Fuzzati, N., & Ramarosandratana, A. V. (2024b). Morpho-anatomical responses of leafless *Vanilla* spp. roots to drought and habitat degradation. *Flora*, 152562. https://doi.org/10.1016/j.flora.2024.152562

Bouetard, A., Lefeuvre, P., Gigant, R., Bory, S., Pignal, M., Besse, P., & Grisoni, M. (2010). Evidence of transoceanic dispersion of the genus *Vanilla* based on plastid DNA phylogenetic analysis. *Molecular Phylogenetics and Evolution*, 55(2), 621–630. https://doi.org/10.1016/j.ympev.2010.01.021

Braun-Blanquet, J. (1932). Plant sociology. The study of plant communities. 1st ed. McGraw-Hill Book Co., Inc., New York and London

Cameron, K. M., & Chase, M. W. (1998). Seed morphology of vanilloid orchids (Vanilloideae: Orchidaceae). *Lindleyana*, 13(3), 148–169.

Capblancq, T., Munson, H., Butnor, J. R., & Keller, S. R. (2021). Genomic drivers of early-life fitness in *Picea rubens*. *Conservation Genetics*, 22(6), 963–976. https://doi.org/10.1007/s10592-021-01378-7

Cheng, S., Zou, Y.-N., Kuča, K., Hashem, A., Abd_Allah, E. F., & Wu, Q.-S. (2021). Elucidating the mechanisms underlying enhanced drought tolerance in plants mediated by arbuscular mycorrhizal fungi. *Frontiers in Microbiology*, 12, 809473.

https://doi.org/10.3389/fmicb.2021.809473

Cornet, A. (1974). *Essai de cartographie bioclimatique à Madagascar*. Orstom Paris.

Cribb, P., & Hermans, J. (2009). *Field guide to the orchids of Madagascar.* Royal Botanic Gardens.

Dalling, J. W., Muller-Landau, H. C., Wright, S. J., & Hubbell, S. P. (2002). Role of dispersal in the recruitment limitation of neotropical pioneer species. *Journal of Ecology*, 90(4), 714–727. https://doi.org/10.1046/j.1365-2745.2002.00706.x

Diez, J. M. (2007). Hierarchical patterns of symbiotic orchid germination linked to adult proximity and environmental gradients. *Journal of Ecology*, 95, 159–170. https://www.jstor.org/stable/4495966

Donque, G. (1972). The climatology of Madagascar. In: Battistini, R., Richard-Vindard, G. (Eds), *Biogeography and ecology in Madagascar* (pp. 87–144). Dordrecht: Springer. https://doi.org/10.1007/978-94-015-7159-3_3

Dunn, J. C., Asensio, N., Arroyo-Rodríguez, V., Schnitzer, S., & Cristóbal-Azkarate, J. (2012). The ranging costs of a fallback food: liana consumption supplements diet but increases foraging effort in howler monkeys. *Biotropica*, 44(5), 705–714. https://doi.org/10.1111/j.1744-7429.2012.00856.x

Eriksson, O., & Ehrlén, J. (1992). Seed and microsite limitation of recruitment in plant populations. *Oecologia*, 91, 360–364. https://doi.org/10.1007/BF00317624

Evans, A., Janssens, S., & Jacquemyn, H. (2020). Impact of Climate Change on the Distribution of Four Closely Related *Orchis* (Orchidaceae) Species. *Diversity*, 12(8), 312. https://doi.org/10.3390/d12080312

Falara, V., Amarasinghe, R., Poldy, J., Pichersky, E., Barrow, R. A., & Peakall, R. (2013). The production of a key floral volatile is dependent on UV light in a sexually deceptive orchid. *Annals of Botany*, 111(1), 21–30. https://doi.org/10.1093/aob/mcs228

Fang, L., Kong, X., Wen, Y., Li, J., Yin, Y., Li, L., Ma, G., Wu, K., & Zeng, S. (2021). Characterization of embryo and protocorm development of *Paphiopedilum spicerianum*. *Plant Physiology and Biochemistry*, 167, 1024–1034. https://doi.org/10.1016/j.plaphy.2021.09.001

Fay, M. F. (2015). British and Irish orchids in a changing world. *Curtis's Botanical Magazine*, 32(1), 3–23. https://www.jstor.org/stable/48505622

Fay, M. F., Pailler, T., & Dixon, K. W. (2015). Orchid conservation: making the links. *Annals of Botany*, 116(3), 377–379. https://doi.org/10.1093/aob/mcv142

Félix-Burruel, R. E., Larios, E., González, E. J., & Búrquez, A. (2025). Population decline of the saguaro cactus throughout its distribution is associated with climate change. *Annals of Botany*, 135(1–2), 317–328. https://doi.org/10.1093/aob/mcae094

Fernández, M., Kaur, J., & Sharma, J. (2023). Co-occurring epiphytic orchids have specialized mycorrhizal fungal niches that are also linked to ontogeny. *Mycorrhiza*, 33(1), 87–105. https://doi.org/10.1007/s00572-022-01099-w

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302–4315. https://doi.org/10.1002/joc.5086

Flanagan, N. S., Chavarriaga, P., & Mosquera-Espinosa, A. T. (2018). Conservation and sustainable use of *Vanilla* crop wild relatives in Colombia. *Handbook of Vanilla Science and Technology*, 85–109. https://doi.org/10.1002/9781119377320.ch6

Fritsche, Y., Pinheiro, M. V. M., & Guerra, M. P. (2022). Light quality and natural ventilation have different effects on protocorm development and plantlet growth stages of the *in vitro* propagation of *Epidendrum fulgens* (Orchidaceae). *South African Journal of Botany*, 146, 864–874. https://doi.org/10.1016/j.sajb.2022.02.019

Gale, S. W., Fischer, G. A., Cribb, P. J., & Fay, M. F. (2018). Orchid conservation: bridging the gap between science and practice. *Botanical Journal of the Linnean Society*, 186(4), 425–434. https://doi.org/10.1093/botlinnean/boy003

Gao, Y., Guo, S., & Xing, X. (2019). Fungal diversity and mechanisms of symbiotic germination of orchid seeds: a review. *Mycosystema*, 38(11), 1808–1825.

Garnier, S., Giordanengo, E., Saatkamp, A., Santonja, M., Reiter, I. M., Orts, J., Gauquelin, T., & Meineri, E. (2021). Amplified drought induced by climate change reduces seedling emergence and increases seedling mortality for two Mediterranean perennial herbs. *Ecology and Evolution*, 11(22), 16143–16152. https://doi.org/10.1002/ece3.8295

Gautier, L., Chatelain, C., & Spichiger, R.-E. (1994). Presentation of a releve method for vegetation studies based on high resolution satellite imagery. In: J. H. Seyani & A. C. Chikuni (Eds.), *Proceedings of XIII th plenary meeting of AETFAT* (pp. 1339–1350). National Herbarium and Botanic Gardens of Malawi.

Gebrechorkos, S. H., Sheffield, J., Vicente-Serrano, S. M., Funk, C., Miralles, D. G., Peng, J., Dyer, E., Talib, J., Beck, H. E., & Singer, M. B. (2025). Warming accelerates global drought severity. *Nature*, 1–8. https://doi.org/10.1038/s41586-025-09047-2

Gigant, R. L., De Bruyn, A., Church, B., Humeau, L., Gauvin-Bialecki, A., Pailler, T., Grisoni, M., & Besse, P. (2014). Active sexual reproduction but no sign of genetic diversity in range-edge populations of *Vanilla roscheri* Rchb. f.(Orchidaceae) in South Africa. *Conservation Genetics*, 15(6), 1403–1415. https://doi.org/10.1007/s10592-014-0626-8

Gill, D. E. (1996). The natural population ecology of temperate terrestrials: Pink lady's slippers, *Cypripedium acaule*. *North*

American Native Terrestrial Orchids conference: Propagation and Production, 91–106.

Godron, M., Daget, P., Long, G., Sauvage, C., Emberger, L., Le Gloch, E., Poissonnet, J., & Wacquant, J. P. (1983). Relevé méthodologique de la végétation et du milieu, code et transcription sur carte perforée. *CNRS, Paris, 281p*.

González-Orellana, N., Mendoza, A. S., Tremblay, R. L., & Ackerman, J. D. (2024). Host suitability for germination differs from that of later stages of development in a rare epiphytic orchid. *Lankesteriana*, 93–114. https://doi.org/10.15517/lank.v24i1.59617

Hankin, L. E., Barrios-Masias, F. H., Urza, A. K., & Bisbing, S. M. (2025). Lethal combination for seedlings: extreme heat drives mortality of drought-exposed high-elevation pine seedlings. *Annals of Botany*, 135(1–2), 293–304. https://doi.org/10.1093/aob/mcae064

Hempel, S., Götzenberger, L., Kühn, I., Michalski, S. G., Rillig, M. C., Zobel, M., & Moora, M. (2013). Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. *Ecology*, 94(6), 1389–1399. https://doi.org/10.1890/12-1700.1

Hending, D., Holderied, M., McCabe, G., & Cotton, S. (2022). Effects of future climate change on the forests of Madagascar. *Ecosphere*, 13(4), e4017. https://doi.org/10.1002/ecs2.4017

Hens, H., Pakanen, V.-M., Jäkäläniemi, A., Tuomi, J., & Kvist, L. (2017). Low population viability in small endangered orchid populations: Genetic variation, seedling recruitment and stochasticity. *Biological Conservation*, 210, 174–183. https://doi.org/10.1016/j.biocon.2017.04.019

Herrera, H., García-Romera, I., Meneses, C., Pereira, G., & Arriagada, C. (2019). Orchid mycorrhizal interactions on the Pacific side of the Andes from Chile. A review. *Journal of Soil Science and Plant Nutrition*, 19, 187–202. https://doi.org/10.1007/s42729-019-00026-x

Higaki, K., Rammitsu, K., Yamashita, Y., Yukawa, T., & Ogura-Tsujita, Y. (2017). A method for facilitating the seed germination of a mycoheterotrophic orchid, *Gastrodia pubilabiata*, using decomposed leaf litter harboring a basidiomycete fungus, *Mycena sp*. *Botanical Studies*, 58, 1–7. https://doi.org/10.1186/s40529-017-0214-6

Horth, L. (2019). Understanding the impact of plant–arthropod interactions, pollination, and canopy light on the rare orchid, small whorled pogonia (*Isotria medeoloides*). *Plant Ecology*, 220, 563–576. https://doi.org/10.1007/s11258-019-00936-x

Hothorn, T., Bretz, F., & Westfall, P. (2015). Package multcomp: Simultaneous Inference in General Parametric Models. *Published Online in the CRAN Repository*.

Huang, H., & Song, S. (2013). Change in desiccation tolerance of maize embryos during development and germination at different water potential PEG-6000 in relation to oxidative process. *Plant Physiology and Biochemistry*, 68, 61–70. https://doi.org/10.1016/j.plaphy.2013.02.029

Huntley, B. J. (2023). Soil, water and nutrients. In Ecology of Angola: Terrestrial biomes and ecoregions. *Springer*, 127–147. https://doi.org/10.1007/978-3-031-18923-4_6

Izuddin, M., Yam, T. W., & Webb, E. L. (2019). Germination niches and seed persistence of tropical epiphytic orchids in an urban landscape. *Journal of Plant Research*, 132(3), 383–394. https://doi.org/10.1007/s10265-019-01110-0

Jaganathan, G. K., Li, J., Biddick, M., Han, K., Song, D., Yang, Y., Han, Y., & Liu, B. (2019). Mechanisms underpinning the onset of seed coat impermeability and dormancy-break in *Astragalus adsurgens*. *Scientific Reports*, 9(1), 9695. https://doi.org/10.1038/s41598-019-46158-z

Janissen, B., Lawrie, A. C., & Huynh, T. (2022). Warm stratification and optimised temperatures improve conservation of the endangered orchid, *Caladenia robinsonii* (Orchidaceae). *Australian Journal of Botany*, 70(4), 275–291. https://doi.org/10.1071/BT21085

Janowski, D., & Leski, T. (2022). Factors in the distribution of mycorrhizal and soil fungi. *Diversity*, 14(12), 1122. https://doi.org/10.3390/d14121122

Jolman, D., Batalla, M. I., Hungerford, A., Norwood, P., Tait, N., & Wallace, L. E. (2022). The challenges of growing orchids from seeds for conservation: An assessment of asymbiotic techniques. *Applications in Plant Sciences*, 10(5), e11496. https://doi.org/10.1002/aps3.11496

Kartzinel, T. R., Trapnell, D. W., & Shefferson, R. P. (2013). Critical importance of large native trees for conservation of a rare Neotropical epiphyte. *Journal of Ecology*, 101(6), 1429–1438. https://doi.org/10.1111/1365-2745.12145

Kassambara, A., & Mundt, F. (2017). Package 'factoextra.' Extract and Visualize the Results of Multivariate Data Analyses, 76(2). *Published Online in the CRAN Repository*

Kinderen, G. van der. (1995). A method for the study of field germinated seeds of terrestrial orchids. *Lindleyana*, 10 (2), 68–73

Kindlmann, P., Meléndez-Ackerman, E. J., & Tremblay, R. L. (2014). Disobedient epiphytes: colonization and extinction rates in a metapopulation of *Lepanthes rupestris* (Orchidaceae) contradict theoretical predictions based on patch connectivity. *Botanical Journal of the Linnean Society*, 175(4), 598–606. https://doi.org/10.1111/boj.12180

Kirillova, I. A., & Kirillov, D. V. (2020). Effect of illumination conditions on the reproductive success of *Epipactis helleborine* (L.) Crantz (Orchidaceae). *Russian Journal of Ecology*, 51, 389–393. https://doi.org/10.1134/S1067413620040098

Kirillova, I. A., & Kirillov, D. V. (2019). Effect of lighting conditions on the reproductive success of *Cypripedium calceolus* L. (Orchidaceae, Liliopsida). *Biology Bulletin*, 46, 1317–1324. https://doi.org/10.1134/S1062359019100157

Kleyheeg, E., Claessens, M., & Soons, M. B. (2018). Interactions between seed traits and digestive processes determine the germinability of bird-dispersed seeds. *PLoS One*, 13(4), e0195026. https://doi.org/10.1371/journal.pone.0195026

Klimaszewska, K., Bernier-Cardou, M., Cyr, D. R., & Sutton, B. C. S. (2000). Influence of gelling agents on culture medium gel strength, water availability, tissue water potential, and maturation response in embryogenic cultures of *Pinus strobus* L. *In Vitro Cellular & Developmental Biology-Plant*, 36, 279–286. https://doi.org/10.1007/s11627-000-0051-1

Klimaszewska, K., & Smith, D. R. (1997). Maturation of somatic embryos of *Pinus strobus* is promoted by a high concentration of gellan gum. *Physiologia Plantarum*, 100(4), 949–957. https://doi.org/10.1111/j.1399-3054.1997.tb00022.x

Kumari, N., & Mohan, C. (2021). Basics of clay minerals and their characteristic properties. In: Gustavo Morari Do Nascimento (Ed.), *Clay and Clay Minerals* (pp 15–43). London: IntechOpen. DOI: 10.5772/intechopen.97672

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, *25*, 1–18.

Lee, Y.-I., Chung, M.-C., Yeung, E. C., & Lee, N. (2015). Dynamic distribution and the role of abscisic acid during seed development of a lady's slipper orchid, *Cypripedium formosanum*. *Annals of Botany*, *116*(3), 403–411. https://doi.org/10.1093/aob/mcv079

Li, J., Ramirez, G. H., Kiani, M., Quideau, S., Smith, E., Janzen, H., Larney, F., & Puurveen, D. (2018). Soil organic matter dynamics in long-term temperate agroecosystems: Rotation and nutrient addition effects. *Canadian Journal of Soil Science*, *98*(2), 232–245. https://doi.org/10.1139/cjss-2017-0127

Li, T., Yang, W., Selosse, M.-A., & Gao, J. (2021). Progress and prospects of mycorrhizal fungal diversity in orchids. *Frontiers in Plant Science*, *12*, 646325. https://doi.org/10.3389/fpls.2021.646325

Li, Y.-Y., Boeraeve, M., Cho, Y.-H., Jacquemyn, H., & Lee, Y.-I. (2022). Mycorrhizal switching and the role of fungal abundance in seed germination in a fully mycoheterotrophic orchid, *Gastrodia confusoides*. *Frontiers in Plant Science*, 12, 775290. https://doi.org/10.3389/fpls.2021.775290

Madre, Jean François (2011). Mesurim un logiciel destiné à faire différents types de travaux sur les images numérisées, ENS, ACCES, Lyon, France. https://svt.ac-amiens.fr/040-vue-d-ensemble-des-logiciels-de-m-madre.html

Mahdavi, Z., Daylami, S. D., Fadavi, A., & Vahdati, K. (2023). Artificial seed production of *Phalaenopsis* orchid: effect of encapsulation materials, temperature, light spectra, and storage period. *Plant Cell, Tissue and Organ Culture*, 155(3), 797–808. https://doi.org/10.1007/s11240-023-02600-9

Matus, F. J. (2021). Fine silt and clay content is the main factor defining maximal C and N accumulations in soils: a meta-analysis. *Scientific Reports*, 11(1), 6438. https://doi.org/10.1038/s41598-021-84821-6

Maxted, N., & Kell, S. P. (2009). Establishment of a global network for the *in situ* conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy, 266.

McCormick, M. K., Lee Taylor, D., Juhaszova, K., Burnett, R. K., Whigham, D. F., & O'Neill, J. P. (2012). Limitations on orchid recruitment: Not a simple picture. *Molecular Ecology*, 21(6), 1511–1523. https://doi.org/10.1111/j.1365-294X.2012.05468.x

McCormick, M. K., Parker, K. L., Szlavecz, K., & Whigham, D. F. (2013). Native and exotic earthworms affect orchid seed loss. *AoB Plants*, 5, plt018. https://doi.org/10.1093/aobpla/plt018

McCormick, M. K., & Jacquemyn, H. (2014). What constrains the distribution of orchid populations? *New Phytologist*, 202(2), 392–400. https://doi.org/10.1111/nph.12639

McCormick, M. K., Taylor, D. L., Whigham, D. F., & Burnett Jr, R. K. (2016). Germination patterns in three terrestrial orchids relate to abundance of mycorrhizal fungi. *Journal of Ecology*, *104*(3), 744–754. https://doi.org/10.1111/1365-2745.12556

McCormick, M. K., Whigham, D. F., & Canchani-Viruet, A. (2018). Mycorrhizal fungi affect orchid distribution and population dynamics. *New Phytologist*, *219*(4), 1207–1215. https://doi.org/10.1111/nph.15223

Midgley, G. F., & Vander Heyden, F., (1999). Form and function in perennial plants. In: Dean, W. R. J., Milton, S. J. (Eds.), *The Karoo: Ecological Patterns and Processes* (pp. 90–106). Cambridge, Cambridge University Press.

Milton, S. J., Clark, C., Hundermark, C. R., Hurt, C., & Van der Merwe, H. (2024). Population trends in an endemic dwarf succulent over two decades: rainfall, elevation, microsite and landuse effects. *Journal of Arid Environments*, 223, 105181. https://doi.org/10.1016/j.jaridenv.2024.105181

Moat, J., & Smith, P. P. (2007). *Atlas of the vegetation of Madagascar*. Royal Botanic Gardens, Kew.

Montgomery, G. G., & M. E. Sunquist., (1978). Habitat selection and use by two-toed and three-toed sloths. In: G. G. Montgomery (Eds), *The ecology of arboreal foliovores* (pp. 329–359). Smithsonian Institution Press, Washing-ton, D.C., USA.

Moreno-Maroto, J. M., & Alonso-Azcárate, J. (2018). What is clay? A new definition of "clay" based on plasticity and its impact on the most widespread soil classification systems. *Applied Clay Science*, 161, 57–63. https://doi.org/10.1016/j.clay.2018.04.011

Muñoz-Rojas, M., Erickson, T. E., Martini, D. C., Dixon, K. W., & Merritt, D. J. (2016). Climate and soil factors influencing seedling recruitment of plant species used for dryland restoration. *Soil*, 2(2), 287–298. https://doi.org/10.5194/soil-2-287-2016

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3).

Nathan, R., & Muller-Landau, H. C. (2000). Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution*, 15(7), 278–285. DOI: 10.1016/S0169-5347(00)01874-7

Nishimura, G., & Tamura, M. (1993). Seed coat formation in *Apostasia nipponica*. *Journal of Japanese Botany*, 68(4) Doi:10.5555/19940311215

Ntuli, T. M. (2012). Drought and desiccation-tolerance and sensitivity in plants. In: John Mworia (Ed.), *Botany* (pp. 29–60). Croatie: InTech.

Owens, L. D., & Wozniak, C. A. (1991). Measurement and effects of gel matric potential and expressibility on production of morphogenic callus by cultured sugarbeet leaf discs. *Plant Cell, Tissue and Organ Culture*, 26, 127–133. https://doi.org/10.1007/BF00036116

Paulo, D., Diekmann, O., Ramos, A. A., Alberto, F., & Serrão, E. A. (2019). Sexual reproduction vs. clonal propagation in the recovery of a seagrass meadow after an extreme weather event. *Scientia Marina*, 83(4), 357–363. https://doi.org/10.3989/scimar.04843.06A

Pauwels, R., Graefe, J., & Bitterlich, M. (2023). An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. *Mycorrhiza*, 33(3), 165–179. https://doi.org/10.1007/s00572-023-01106-8

Petersson, L. (2015). Pollination biology of the endemic orchid *Vanilla bosseri* in Madagascar. Master Thesis. Uppsala University, Disciplinary Domain of Science and Technology, Biology Education Center.

Petrolli, R., Zinger, L., Perez-Lamarque, B., Collobert, G., Griveau, C., Pailler, T., Selosse, M., & Martos, F. (2022). Spatial turnover of fungi and partner choice shape mycorrhizal networks in epiphytic orchids. *Journal of Ecology*, 110(11), 2568–2584. https://doi.org/10.1111/1365-2745.13986

Phillips, R. D., Reiter, N., & Peakall, R. (2020). Orchid conservation: from theory to practice. *Annals of Botany*, 126(3), 345–362. https://doi.org/10.1093/aob/mcaa093

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T., & Cliff, B. (1997). Economic and environmental benefits of biodiversity. *BioScience*, 47(11), 747–757. https://doi.org/10.2307/1313097

Poggio, L., De Sousa, L. M., Batjes, N. H., Heuvelink, G. B. M., Kempen, B., Ribeiro, E., & Rossiter, D. (2021). SoilGrids 2.0: producing soil information for the globe with quantified spatial uncertainty. *Soil*, 7(1), 217–240. https://doi.org/10.5194/soil-7-217-2021

Porras-Alfaro, A., & Bayman, P. (2007). Mycorrhizal fungi of *Vanilla*: diversity, specificity and effects on seed germination and plant growth. *Mycologia, 99*(4), 510–525. https://doi.org/10.1080/15572536.2007.11832545

Portères, R. (1954). Le genre *Vanilla* et ses espèces : Le Vanillier et la Vanille dans le Monde. *Encyclopedia of Biology*, 46, 94–290.

Prutsch, J., Schardt, A., & Schill, R. (2000). Adaptations of an orchid seed to water uptake and-storage. *Plant Systematics and Evolution*, 220, 69–75. https://doi.org/10.1007/BF00985371

Rabot, E., Wiesmeier, M., Schlüter, S., & Vogel, H.-J. (2018). Soil structure as an indicator of soil functions: A review. *Geoderma*, 314, 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009

Rafanoharana, S. C., Andrianambinina, F. O. D., Rasamuel, H. A., Waeber, P. O., Wilmé, L., & Ganzhorn, J. U. (2024). Projecting forest cover in Madagascar's protected areas to 2050 and its implications for lemur conservation. *Oryx*, 58(2), 155–163. https://doi.org/10.1017/S0030605323001175

Rakotoarivelo, N. H., Randrianarivony, T. N., Rakotoarivony, F., & Randrianasolo, A. (2019). "Mangidy": Malagasy Folk Beverages Sold in Madagascar's Market Places. *Ethnobotany Research and Applications*, 18, 1–14. https://ethnobotanyjournal.org/index.php/era/article/view/1565

Randriamarolaza, L. Y. A., Aguilar, E., Skrynyk, O., Vicente-Serrano, S. M., & Domínguez-Castro, F. (2022). Indices for daily temperature and precipitation in Madagascar, based on quality-controlled and homogenized data, 1950–2018. *International Journal of Climatology*, 42(1), 265–288. https://doi.org/10.1002/joc.7243

Randriamiharisoa, M. N., Kuhlman, A. R., Jeannoda, V., Rabarison, H., Rakotoarivelo, N., Randrianarivony, T., Raktoarivony, F., Randrianasolo, A., & Bussmann, R. W. (2015). Medicinal plants sold in the markets of Antananarivo, Madagascar. *Journal of Ethnobiology and Ethnomedicine*, 11, 1–13. https://doi.org/10.1186/s13002-015-0046-y

Rasmussen, H. N. (1992). Seed dormancy patterns in *Epipactis palustris* (Orchidaceae): Requirements for germination and establishment of mycorrhiza. *Physiologia Plantarum*, *86*(1), 161–167. https://doi.org/10.1111/j.1399-3054.1992.tb01325.x

Rasmussen, H. N., Dixon, K. W., Jersáková, J., & Těšitelová, T. (2015). Germination and seedling establishment in orchids: a complex of requirements. *Annals of Botany*, *116*(3), 391–402.

Rasmussen, H. N., & Whigham, D. F. (1998). Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae). *American Journal of Botany*, *85*(6), 829–834. https://doi.org/10.2307/2446418

R Core Team (2023). R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Read, D. J., Haggar, J., Magkourilou, E., Durant, E., Johnson, D., Leake, J. R., & Field, K. J. (2024). Photosynthate transfer from an autotrophic orchid to conspecific heterotrophic protocorms through a common mycorrhizal network. *New Phytologist*, 243(1), 398–406. https://doi.org/10.1111/nph.19810

Rendigs, A., Radespiel, U., Wrogemann, D., & Zimmermann, E. (2003). Relationship between microhabitat structure and distribution of mouse lemurs (*Microcebus* spp.) in northwestern Madagascar. *International Journal of Primatology*, 24, 47–64. https://doi.org/10.1023/A:1021494428294

Ripley, B. S., Abraham, T., Klak, C., & Cramer, M. D. (2013). How succulent leaves of Aizoaceae avoid mesophyll conductance limitations of photosynthesis and survive drought. *Journal of Experimental Botany*, 64(18), 5485–5496. https://doi.org/10.1093/jxb/ert314

Ravelonanosy R., Andriamihaja C., Grisoni M., Ramarosandratana A. V., Rafidison V., Fuzzati N., Besse P. (2025). Leafless *Vanilla* species from Madagascar as potential models to study bioclimatic adaptation. In: Tikam Dakal (Ed.), *Advances in Genetic Diversity - Recent Advancements, Challenges and Prospects*. IntechOpen. DOI: 10.5772/intechopen.1012448

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal*, 4(10), 1340–1351. https://doi.org/10.1038/ismej.2010.58

Salgotra, R. K., & Chauhan, B. S. (2023). Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*, 14(1), 174. https://doi.org/10.3390/genes14010174

Sathiyadash, K., Muthukumar, T., Karthikeyan, V., & Rajendran, K. (2020). Orchid mycorrhizal fungi: structure, function, and diversity. *Orchid Biology: Recent Trends & Challenges*, 239–280. https://doi.org/10.1007/978-981-32-9456-1_13

Scade, A., Brundrett, M. C., Batty, A. L., Dixon, K. W., & Sivasithamparam, K. (2006). Survival of transplanted terrestrial orchid seedlings in urban bushland habitats with high or low weed cover. *Australian Journal of Botany*, 54(4), 383–389. https://doi.org/10.1071/BT04025

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. doi: 10.1038/nmeth.2089

Schoonheydt, R. A., Johnston, C. T., & Bergaya, F. (2018). Clay minerals and their surfaces. *Developments in clay science*, 9 (1), 1–21). https://doi.org/10.1016/B978-0-08-102432-4.00001-9

Shao, S.-C., Burgess, K. S., Cruse-Sanders, J. M., Liu, Q., Fan, X.-L., Huang, H., & Gao, J.-Y. (2017). Using *in situ* symbiotic seed germination to restore over-collected medicinal orchids in Southwest China. *Frontiers in Plant Science*, 8, 888. https://doi.org/10.3389/fpls.2017.00888

Shefferson, R. P., Jacquemyn, H., Kull, T., & Hutchings, M. J. (2020). The demography of terrestrial orchids: life history, population dynamics and conservation. *Botanical Journal of the Linnean Society*, 192(2), 315–332. https://doi.org/10.1093/botlinnean/boz084

Šoch, J., Šonka, J., & Ponert, J. (2023). Acid scarification as a potent treatment for an *in vitro* germination of mature endozoochorous *Vanilla planifolia* seeds. *Botanical Studies*, 64(1), 9. https://doi.org/10.1186/s40529-023-00374-z

Sorgato, J. C., Soares, J. S., Damiani, C. R., & Ribeiro, L. M. (2020). Effects of light, agar, activated charcoal, and culture medium on the germination and early development of'*Dendrobium*'seedlings. *Australian Journal of Crop Science*, 14(4), 557–564.

Sousa, K. C. I., Araújo, L. G. de, Silva, C. de S., Carvalho, J. C. B. de, Sibov, S. T., Gonçalves, L. de A., Pereira, M. C., Gonçalves, F. J., & Filippi, M. C. da C. de. (2019). Seed germination and development of orchid seedlings (*Cyrtopodium saintlegerianum*) with fungi. *Rodriguésia*, *70*, e02302016. https://doi.org/10.1590/2175-7860201970004

Stuckey, I. H. (1967). Environmental factors and the growth of native orchids. *American Journal of Botany*, 54(2), 232–241. https://doi.org/10.1002/j.1537-2197.1967.tb06914.x

Suzzi-Simmons, A. (2023). Status of deforestation of Madagascar. *Global Ecology and Conservation*, *42*, e02389. https://doi.org/10.1016/j.gecco.2023.e02389

Swarts, N. D., & Dixon, K. W. (2009). Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, *104*(3), 543–556. https://doi.org/10.1093/aob/mcp025

Tadross, M., Randriamarolaza, L., Rabefitia, Z., & Zheng, K. Y. (2008). Climate change in Madagascar; recent past and future. *World Bank, Washington, DC*, 18, 1771–1790.

Těšitelová, T., Klimešová, L., Vogt-Schilb, H., Kotilínek, M., & Jersáková, J. (2022). Addition of fungal inoculum increases germination of orchid seeds in restored grasslands. *Basic and Applied Ecology*, *63*, 71–82. https://doi.org/10.1016/j.baae.2022.04.001

Timsina, B., Rokaya, M. B., Münzbergová, Z., Kindlmann, P., Shrestha, B., Bhattarai, B., & Raskoti, B. B. (2016). Diversity, distribution and host-species associations of epiphytic orchids in Nepal. *Biodiversity and Conservation*, *25*, 2803–2819. https://doi.org/10.1007/s10531-016-1205-8

Weiskopf, S. R., Cushing, J. A., Morelli, T. L., & Myers, B. J. E. (2021). Climate change risks and adaptation options for Madagascar. *Ecology and Society*, 26(4), 36. https://doi.org/10.5751/ES-12816-260436

Wojtyla, Ł., Paluch-Lubawa, E., Sobieszczuk-Nowicka, E., & Garnczarska, M. (2020). Drought stress memory and subsequent drought stress tolerance in plants. In: Mohammad A. H., Fulai L., David J. B., Masayuki F., Bingru H. (Eds.), *Priming-mediated stress and cross-stress tolerance in crop plants* (pp. 115–131). Academic Press. https://doi.org/10.1016/B978-0-12-817892-8.00007-6

Wright, M., French, G., Cross, R., Cousens, R., Andrusiak, S., & McLean, C. B. (2007). Site amelioration for direct seeding of *Caladenia tentaculata* improves seedling recruitment and survival in natural habitat. *Lankesteriana*, *7*(1–2), 430–432. https://doi.org/10.15517/lank.v7i1-2.19653

Xu, X., Fang, L., Li, L., Ma, G., Wu, K., & Zeng, S. (2020). Abscisic acid inhibits asymbiotic germination of immature seeds of *Paphiopedilum armeniacum*. *International Journal of Molecular Sciences*, 21(24), 9561. https://doi.org/10.3390/ijms21249561

Yamazaki, J., & Miyoshi, K. (2006). *In vitro* asymbiotic germination of immature seed and formation of protocorm by *Cephalanthera falcata* (Orchidaceae). *Annals of Botany*, 98(6), 1197–1206. https://doi.org/10.1093/aob/mcl223

Yang, F.-S., Sun, A.-H., Zhu, J., Downing, J., Song, X.-Q., & Liu, H. (2017). Impacts of host trees and sowing conditions on germination success and a simple *ex situ* approach to generate symbiotic seedlings of a rare epiphytic orchid endemic to Hainan Island, China. *The Botanical Review*, 83, 74–86. DOI: 10.1007/s12229-017-9178-1

Yanoviak, S. P. (2015). Effects of lianas on canopy arthropod community structure. *Ecology of Lianas*, 343–361. https://doi.org/10.1002/9781118392409.ch24

Yeh, C.-H., Chen, K.-Y., & Lee, Y.-I. (2021). Asymbiotic germination of *Vanilla planifolia* in relation to the timing of seed collection and seed pretreatments. *Botanical Studies*, 62(1), 6. https://doi.org/10.1186/s40529-021-00311-y

Yeung, E. C. (2017). A perspective on orchid seed and protocorm development. *Botanical Studies*, 58(1), 33. https://doi.org/10.1186/s40529-017-0188-4

Yeung, E. C., Li, Y.-Y., & Lee, Y.-I. (2018). Understanding seed and protocorm development in orchids. In: Lee, YI., Yeung, ET. (Eds), *Orchid Propagation: From Laboratories to Greenhouses—Methods and Protocols* (pp 3–26). New York: Humana Press. https://doi.org/10.1007/978-1-4939-7771-0_1

Yoder, J. A., Zettler, L. W., & Stewart, S. L. (2000). Water requirements of terrestrial and epiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. *Plant Science*, 156(2), 145–150. https://doi.org/10.1016/S0168-9452(00)00246-6

Zarate-García, A. M., Noguera-Savelli, E., Andrade-Canto, S. B., Zavaleta-Mancera, H. A., Gauthier, A., & Alatorre-Cobos, F. (2020). Bark water storage capacity influences epiphytic orchid preference for host trees. *American Journal of Botany*, *107*(5), 726–734. https://doi.org/10.1002/ajb2.1470

Zhang, Y., Lee, Y.-I., Deng, L., & Zhao, S. (2013). Asymbiotic germination of immature seeds and the seedling development of *Cypripedium macranthos* Sw., an endangered lady's slipper orchid. *Scientia Horticulturae*, *164*, 130–136. https://doi.org/10.1016/j.scienta.2013.08.006

Zhao, D.-K., Selosse, M.-A., Wu, L., Luo, Y., Shao, S.-C., & Ruan, Y.-L. (2021). Orchid reintroduction based on seed germination-promoting mycorrhizal fungi derived from protocorms or seedlings. *Frontiers in Plant Science*, *12*, 701152. https://doi.org/10.3389/fpls.2021.701152

Zotz, G. (2013). The systematic distribution of vascular epiphytes–a critical update. *Botanical Journal of the Linnean Society*, *171*(3), 453–481. https://doi.org/10.1111/boj.12010

Zotz, G., Weigelt, P., Kessler, M., Kreft, H., & Taylor, A. (2021a). EpiList 1.0: a global checklist of vascular epiphytes. *Ecology*, 102(6), e03326. https://doi.org/10.5167/uzh-202729

Zotz,G., Almeda, F., Arias, S., Hammel, B., & Pansarin, E. (2021b). Do secondary hemiepiphytes exist? *Journal of Tropical Ecology*, *37*(6), 286–290.

Supporting Information: Additional material related to this article is available in the online Supporting Information section.

Figure S1. Stratum closure rates for the thirteen study sites.

Figure S2. Aseptic culture of aphyllous vanilla seeds. (**A**). Immature (white) and mature (black) seeds of aphyllous vanilla. (**B**) Germination of immature seeds after 38 weeks of culture. (**C**) Germination of mature seeds after 38 weeks of culture. (**D**) Necrotic protocorms after 18 weeks of culture on M4 medium. Bar (A) = 1 mm; Bar (B, D) = 5 mm; Bar (C) = 2 mm. Photographs by Botomanga A.

Figure S3. Description of the developmental stages of *V. madagascariensis* protocorms cultivated in vitro. (**A**). Stage 1 : protocorm barely emerged from the seed coat, white in color, with an average size of 0.35 ± 0.02 mm. (**B**). Stage 2 : protocorm having acquired a green coloration, with an average size of 0.83 ± 0.07 mm. (**C**). Stage 3 : protocorm with a cotyledon (arrow) and an average size of 1.15 ± 0.30 mm. (**D**) Stage 4 : protocorm with a cotyledon and one leaf primordial (arrows) and an average size of 2.94 ± 0.37 mm. Bar (A) = 0.2 mm; Bar (B) = 0.5 mm; Bar (C) = 1 mm; Bar (D) = 1 cm. Photographs by Botomanga A.

Figure S4. Variations in precipitation between the 1960–1999 period (40-year average) and the 2000–2019 period (20-year average) at Maromandia and Ambanja.

Figure S5. Differences in maximum temperatures between the 1960–1999 period (40-year average) and the 2000–2019 period (20-year average) at Maromandia and Ambanja.

Figure S6. Pearson correlation among the environmental factors studied. Environmental variables: Litter: Litter thickness, pH: Soil pH in H20 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

Figure S7. Pearson correlation Analysis of environmental variables excluding MAN and AKF. Environmental variables: Litter: Litter thickness, pH: Soil pH in H20 at 5 cm depth, Sand: Quantity of sand at 5 cm depth, Clay: Quantity of clay at 5 cm depth, Silt: Quantity of silt at 5 cm depth, OC: Quantity of organic carbon at 5 cm depth, BIO1: Annual precipitation, BIO2: Annual Mean Temperature, BIO3: Annual Mean Min Temperature, BIO4: Annual Mean Max Temperature, BIO5: Annual mean Water vapor pressure, BIO6: Canopy closure.

Figure S8. Seedling mortality rate after 12 months of development monitoring at the Maromandia and Ambanja sites. Sample sizes were as follows: Ambanja (n = 30), Maromandia (n = 30).

Figure S9. Change in coloration of protocorms to black (necrosis) over time on the M4 culture medium. Histogram colors accurately represent the browning of protocorms. For each developmental stage, n = 200.