Ex Situ Conservation of Orchid Seeds of the *Lycaste* Genus

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María Alejandra Alfaro Pinto

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Abstract

The orchid family (Orchidaceae) is the second largest family in the plant kingdom with about 28000 species. Most orchid species are endangered. Both *in situ* and *ex situ* approaches are necessary for the conservation of endangered and threatened species, including orchids. *In situ* conservation should be the priority, however, *ex situ* conservation can complement initiatives to ensure species survival *in situ*. More information on orchid seed dispersal mechanisms, pollination, and germination biology is needed to support conservation efforts.

This study included three endangered species from Mesoamerica, *Lycaste virginlis, L. cochleata*, and *L. lasioglossa* that should be considered a high conservation priority. The primary intention of the different experiments was to contribute information that favours seed conservation of endangered orchid species from the Mesoamerica biodiversity hotspot. For this, characterization of the seed capsule morphology and seed micro-morphological traits were investigated followed by the assessment of the effect of three media on *in vitro* asymbiotic seed germination and the viability and germination of the seeds under different storage conditions.

The qualitative traits of the seed capsules and the seeds were similar in appearance and colour for the three *Lycaste* species evaluated. However, high variability was found in the quantitative traits of both seed capsules and seeds. Based on the micro-morphological traits' findings, it is possible to speculate that these three epiphytic *Lycaste* species which all grow under dense tropical canopies are dispersed by water drops falling onto the orchid plant from the canopy and by gravity. This suggestion is consistent with their having small-sized seeds, relatively large embryos, and low air volume (in comparison with other epiphytic and terrestrial orchid species). Specifically, those traits may allow the seeds to be dispersed in vegetation-dense and humid natural environments. Further research needs to be done to validate these findings, including more species of the *Lycaste* genus.

For the successful implementation of conservation plans, it is important to understand the specific nutritional requirements for seed germination of the target species. This study assessed three different germination media (Murashige and Skoog (MS), Knudson C, and terrestrial orchid medium BM-1) to identify the most suitable one for asymbiotic *in vitro* germination.

The germination percentages and seedling development significantly varied across the three *Lycaste* species. MS media sustained the highest germination percentages with high-quality plantlets (in stage 4). Still, germination did not reach the potential suggested by the tetrazolium test for viability, meaning that further media optimization is required. Knudson C media was also a good option for the seed germination and seedling development for two of the three species (*L. cochleata* and *L. lasioglossa*).

The effect of different storage conditions (temperature, RH, and time) on seed viability and germinability was tested. The differential scanning calorimetry (DSC) method was used to identify each species' lipid melting and crystallization points. Based on the DSC findings, storage temperatures below -75°C is recommended for the storage of three *Lycaste* species. Still, further research needs to be done by testing more and different storage temperatures to validate these findings.

Keywords: orchid; epiphytic; tropical; seed morphology; micro-morphology; air space volume; viability; asymbiotic *in vitro* germination; cryopreservation; *ex situ* conservation; seedling development.

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Abbreviations

ANOVA	Analysis of variance
ASV	Air space volume
BM-1	Terrestrial orchid medium
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CONAP	National Council of Protected Areas of Guatemala (Consejo Nacional de Areas Protegidas de Guatemala)
EL	Embryo length
EV	Embryo volume
EW	Embryo width
FAUSAC	Agronomy Faculty of the University of San Carlos of Guatemala (Facultad de Agronomía de la Universidad de San Carlos de Guatemala)
GLM	General linear model
ICTA	Guatemalan Institute of Agricultural Science and Technology (Instituto de Ciencia y Tecnología Agrícolas de Guatemala)
LEA	List of Threatened Species of Guatemala (Lista de Especies Amenazadas de Guatemala)
LiCl	Litium Chloride

m a.s.l.	Meters above sea level
MAGA	Ministry of Agriculture, Livestock and Food of Guatemala (Ministerio de Agricultura, Ganadería y Alimentación de Guatemala)
MPI	Ministry of Primary Industries of New Zealand
MS	Murashige and Skoog
MSB	Kew's Millennium Seed Bank
MSBP	Millennium Seed Bank Partnership
NaOCl	Sodium hypochlorite
NPPO	National Plant Protection Organizations
PBI	Plant Biosecurity Index
RH	Relative humidity
SL	Seed length
SV	Seed volume
SW	Seed width
TCC	2,3,5-triphenyl tetrazolium chloride
USAC	University of San Carlos of Guatemala (Universidad de San Carlos de Guatemala)

Chapter 1.: Literature Review

1.1 World Orchid Diversity and Conservation Status

Orchids belong to the family Orchidaceae, with 736 genera and about 28000 species, covering almost 10% of flowering plants (Chase et al., 2015; Christenhusz & Byng, 2016; Gaskett et al., 2018). Orchids are distributed worldwide, except in Antarctica and are distinguished by having attractively flowered species (Lawson et al., 2019). The richness and distribution of the species varies across and within continents, showing a wide diversity of epiphytic, terrestrial, lithophytic, semiaquatic, or subterranean growth forms (Ames & Correll, 1953; Barman & Devadas, 2013). Although Orchidaceae is the second largest family in the plant kingdom (after Asteraceae) it is also highly vulnerable, with most of its species threatened (Fay, 2018). In nature, a factor limiting orchid species persistence is their high reliance on symbionts for pollination and seed germination, in addition to anthropogenic threats such as over-harvesting and habitat destruction.

1.1.1 Orchid Pollination Biology

Orchids display a wide variety of floral forms and pollination mechanisms (Jersakova et al., 2006). Some orchid species are autogamous (self-pollinated), and a few are pollinated by birds, but the majority of species are pollinated by insects (Nilsson, 1992). To ensure insect pollination, orchids have developed highly specialized plant-pollinator interactions that provide either reward (*e.g.*, nectar) or deception (*e.g.*, no floral compensation) to the pollinator (Nilsson, 1992). In the wild, the size of orchid populations may become dependent on the distribution and size of their pollinators (Jersakova et al., 2006).

When being pollinated by insects, the main pollination mechanisms employed by orchids are:

a) Use a food-deceptive strategy to attract pollinators, manipulating the pollinators' feeding and reproductive behaviour without providing floral rewards - around one-third of orchid species use this mechanism (Ackerman, 1986; Jersakova et al., 2006; Nilsson, 1992).
b) Some orchids, especially the majority of neotropical epiphytic species, are pollinated by male *Euglossine* bees. Pollination happens when bees reach the flowers and gather

fragrance compounds that serve as activators and possibly precursors of their reproductive pheromones (Ackerman, 1986).

c) Other orchids produce a limited number of flowers for an extended period containing high-quality rewards for pollinators (Ackerman, 1986; Nilsson, 1992).

1.1.2 Orchid Seed Morphology

Orchid seeds are very small (dust-like) with low weight. A single capsule (the reproductive unit) can produce thousands of seeds (Schiff, 2018). Orchid seeds are formed of a balloon-like seed coat that incorporates an embryo and an internal air space volume (between the seed coat and embryo) (Figure 1) (Arditti & Ghani, 2000; Barthlott et al., 2014). Orchid seed diversity is very high (Barthlott et al., 2014). Orchid seeds and embryos vary in shape, size, colour, structure, and appearance.

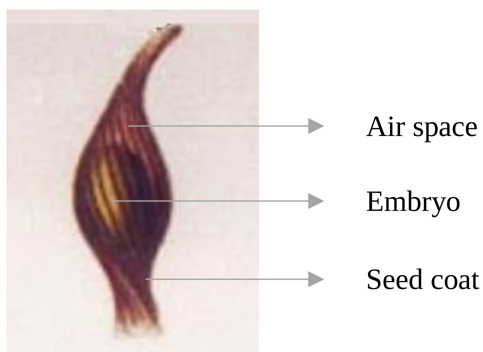

Figure 1. General appearance of Lycaste harrisoniae (Hook.) G.Don ex Loudon. Adapted from Arditti and Ghani (2000).

Orchid seed size is highly variable among species. The length of the seed can range from 100 µm (*Oberonia similis* (Blume) Lindl.) to 6000 µm (*Epidendrum secundum* Jacq.). Following the classification of Barthlott et al. (2014), the seed size can be categorized from very small (100-200 µm), small (200-500 µm), medium (500-900 µm), large (900-2000 µm), and very large (2000-6000 µm). The colour of the seeds in orchids is determined by the seed coat and the embryo, and the variability of colours among orchids is also high. The most common orchid seed colours are whitish, brownish, dark-brownish, black, beige, yellowish, reddish, orange, greenish, or yellow brownish (Barthlott et al., 2014). Orchid seeds also vary in the shape, number, and pattern of cells that form the seed coat.

In seed morphology studies, it is essential to identify the seed and embryo shape before estimating the micro-morphological quantitative traits (*i.e.,* formulas are established for every specific seed shape; seed volume = SV, embryo volume = EV, and percentage of inner air space within the seed = ASV). The formulas to estimate the micro-morphological parameters of seeds were established by Arditti and Ghani (2000). Most orchid species have fusiform seeds (*i.e.,* two cones joined at their bases) (Equation 1). Orchid embryos can be oblate spheroids (Equation 2) (Figure 2a), or prolate spheroids (Equation 3) (Figure 2b). After having estimated the seed and embryo volume, the air space percentage can be estimated using Equation 4 (Arditti & Ghani, 2000).

Equation 1 $$SV = 2\left(\left({}^{SW}/_{2}\right)^2 * \left({}^{SL}/_{2}\right) * 1.047\right)$$

Equation 2 $$EVa = {}^{4}/_{3}\ \pi * \left({}^{EL}/_{2}\right)^2 * \left({}^{EW}/_{2}\right)$$

Equation 3 $$EVb = {}^{4}/_{3}\ \pi * \left({}^{EL}/_{2}\right) * \left({}^{EW}/_{2}\right)^2$$

Equation 4 $$ASV\ (\%) = \left(\frac{SV-EV}{SV}\right) * 100$$

Where: SW = seed width, SL = seed length, EW = embryo width, EL = embryo length.

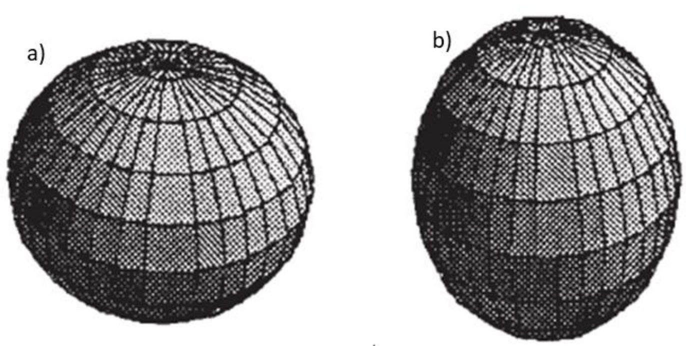

Figure 2. Orchid embryos, a) oblate spheroid, and b) prolate spheroid. Adapted from Arditti and Ghani (2000).

1.1.3 Orchid Seed Dispersal and Germination

In nature, orchid seeds are adapted for wind, water and animal dispersal (Arditti & Ghani, 2000; Royal Botanical Gardens Kew, 2013). To perpetuate the species, both the large number and the physical characteristics of orchid seeds are designed to facilitate a more extensive dispersal (Arditti & Ghani, 2000). However, the orchid strategy is not to disperse over long distances but to spread large amounts of seeds to increase the chances of landing in the ideal place where optimum environment is present for germination (Arditti & Ghani, 2000; Royal Botanical Garden Kew, 2013).

Most orchid seeds need a germination niche to grow, which means they need the ideal combination of biotic (*e.g.,* association with mycorrhizal fungi) and abiotic conditions (*e.g.,* right temperature and moisture) to germinate and grow (Rasmussen et al., 2015). Due to the complex germination system, the germination and regeneration rates of natural orchid populations are very low (Diantina, 2020; Verma et al., 2014).

1.2 Anthropogenic Threats to Orchids

Globally, the main anthropogenic threats for orchid populations are the damage to their natural habitats and unsustainable harvesting (generally illegal and hence undocumented) for horticulture, medicine, or food. Additionally, orchids' complex biology (*i.e.*, high reliance on distinctive pollinators, mycorrhizal fungi, and host trees) makes them more vulnerable to climate change. This includes new pressures on orchids and their symbionts due to the increasing environmental temperatures, such as seasonal changes, increased droughts, intensifications in storms, prolonged fire seasons, rising sea levels, and changes in orthographic wind dew point (Fay, 2018; Fay & Chase, 2009; Koopowitz & Hawkins, 2012; Wraith & Pickering, 2018). Some examples of potential threats to orchid populations are mentioned in a review by Barman and Devadas (2013): in Asia, global warming is manifesting rapidly due to the rapid growth of population and industrial zones, generating pressure on the natural resources and environment. In Eastern Australia, there is an increased risk of drought and fires, which could contribute to the reduction of diversity of several species. In Latin America, there is the risk of significant loss of species because they are restricted to limited habitat ranges.

The future of orchids is a global concern. Hence in the last twenty years, there has been a growing interest in orchid conservation. From 2010 to 2018, over 100 publications were related to orchid conservation issues such as taxonomy and genetics, mycorrhizal relationships, propagation methods, and pollination systems (Wraith et al., 2020). In addition, all orchid species are listed in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to control and regulate their illegal harvest and commerce (Fay, 2018; Lawson et al., 2019). Orchids represent over 70% of the plant species registered in CITES (Fay, 2018).

Table 1 shows the number of orchid species listed on appendices I and II of CITES. Appendix I comprises species threatened with extinction, while appendix II includes species that need limited trade to secure their survival and prevent them from becoming threatened species. In orchids, 176 species are listed in appendix I, and 21696 species in appendix II (0.8%, and 99.2%, respectively).

Table 1. Number of orchid species listed in CITES appendices I and II.

Appendix	**No. species**
I	176
II	21,696
Total	21,872

Source: (Species+/CITES, 2021).

1.3 Orchid Conservation Status in Latin America and Guatemala

Latin America can be defined as the Americas that lie south of the U.S.A. and is usually divided into four geographical regions: North America (Mexico), Central America, the Caribbean, and South America. Latin America is considered a critical region for conservation worldwide as within it are essential biodiversity hotspots for conservation priorities (Myers et al., 2000). The highest diversity of orchid species worldwide is found in the Latin American region, where the variety of pollinators is also high (Meisel et al., 2014). The main threats for orchid (and biodiversity) conservation in Latin America are the high deforestation rate (and hence habitat loss), climate change, overharvesting (Barman & Devadas, 2013; Fay, 2018), and complex and unstable political and social-economic conditions.

The region of central Mexico and Central America (historically recognized as Mesoamerica) is considered one of the seven hotspots for conservation priorities in the Americas (Figure 3). Although Mesoamerica was identified as a biodiversity hotspot due to the high diversity of endemic species, it was also recognized as a very vulnerable region as many species within it (including orchids) are at risk of extinction (Myers et al., 2000).

Figure 3.The seven conservation hotspots of the Americas with Mesoamerica standing out. Adapted from the Conservation Biology Institute (2011).

Guatemala is a developing country located in Mesoamerica and is considered biologically rich. The most diverse plants in Guatemala are orchids, with over 734 identified species (Cameron, 2000). Most of those orchid species are distributed between 800 to 1600 meters above sea level (m a.s.l.). In Guatemala, orchids are mainly used for decoration or religious and ceremonial offerings (*e.g.*, *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins), but also in some cases for food flavouring (*e.g.*, *Vanilla planifolia* Jacks. ex Andrews) (López-Selva Quintana, 2016).

All Guatemalan orchid species are listed in CITES. Furthermore, the Guatemalan authorities have published the List of Threatened Species of Guatemala (LEA – Lista de Especies Amenazadas de Guatemala) through the National Council of Protected Areas (CONAP), that includes 125 orchid species (around 15.62% of the total number of species identified in the country) (CONAP, 2009; López-Selva Quintana, 2016). Eight orchid species are listed in category 1, which indicate that they have a very high threat of extinction (CONAP, 2009). The National Flower of Guatemala 'Monja Blanca' ('White Nun') (*Lycaste virginalis f. alba* (Dombrain) Archila & Chiron – synonym of *L. virginalis* (Scheidw.) Linden), is one of the orchids considered at high threat of extinction by LEA.

For many Guatemalan species, information to develop appropriate conservation plans is incomplete or unavailable (*e.g.*, taxonomy, current distribution, genetics, population dynamics, dispersal system, reproductive behaviour, seed physiology, etc.) (IUCN SSC, 2017). With the overarching goal of contributing to the knowledge base on Guatemalan orchids, this study aimed to explore the seed morphology, seed physical properties, *in vitro* germination assessment, and optimal storage conditions for three Guatemalan orchids:

a) *Lycaste virginalis*,
b) *Lycaste lasioglossa*, and
c) *Selbyana cochleata* - synonym of *Lycaste cochleata*.

These orchid species were selected based on their cultural importance, availability of germplasm resources, threat of extinction, and the absence of data required for their conservation.

1.4 The *Lycaste* Genus and its Importance

Lycaste is a neotropical orchid genus (Ryan, 2001). Their species can be epiphytic, terrestrial, or rock-inhabiting plants with short and thickened pseudobulbs. Flowers are generally large, very attractive, often with a pleasant, sweet scent (Ames & Correll, 1953), developed with three sepals, two petals, and a labellum or lip (Ames & Correll, 1953). *Lycaste* species are generally pollinated by male *Euglossine* bees, which collect and store floral fragrance compounds (Ackerman, 1986; Ryan, 2001).

According to *World Flora Online*, this genera consists of about forty-six (46) species (WFO, 2021) that can be naturally found throughout the south of Mexico, Central America, the north of South America, and the Atlantic Rainforest (Figure 4) (Ames & Correll, 1953; Ryan, 2001). The taxonomic classification of *Lycaste* is (Chase et al., 2015):

Family:	*Orchidaceae*
Subfamily:	*Epidendroideae*
Tribe:	*Cymbidieae*
Subtribe:	*Maxillariinae*
Genera:	*Lycaste* Lindl.

Figure 4. Geographical distribution range of Lycaste. Adapted from Ryan (2001) and Global Biodiverstiy Information Facility (GBIF) (2021).

The species of *Lycaste* are highly desirable in horticulture due to their beauty and different colour variations (Ames & Correll, 1953), which make them more vulnerable to overharvest. For example, *L. virginalis* f. alba (synonym of *L. virginalis*) has high cultural value. It has been a patriotic symbol of Guatemala ever since it was considered an icon of peace, purity, beauty, and fertility of Guatemalan soils. However, it is also at increased risk of extinction (CONAP, 2009).

1.4.1 *Lycaste virginalis* (Scheidw.) Linden

General information: *Lycaste virginalis* is an epiphytic orchid species commonly known as ‘Monja Blanca’. It can grow above 100 cm (Ames & Correll, 1953). It was declared the national flower of Guatemala on February 21, 1934 due to its beauty and uniqueness. It has a high patriotic value within the Guatemalan population. For instance, it appears in the fifty-cent coin issued by Guatemala, stamps, and the logos of several institutions and businesses. This species has many horticultural varieties differentiated by their colour. The forms vary from pure white (f. alba) to deep purple (Ames & Correll, 1953).

Habitat: *Lycaste virginalis* grows in the North of Guatemala, in habitats characterized by high rainfall (therefore with high humidity), at altitudes ranging between 1400 and 2800 m a.s.l. (Archila Morales & Chiron, 2011). They are naturally found in the following habitats: subtropical lower montane rain forest, very humid subtropical forest (cold), and very humid subtropical montane forest (Archila Morales & Chiron, 2011; De la Cruz, 1976).

Conservation Status: *Lycaste virginalis* is listed in category 1 of LEA, which indicates that it is considered as having a significant threat of extinction and that its commercial use is forbidden. Still, samples are allowed to be utilized for research purposes (CONAP, 2009). In CITES, it is listed in appendix II (Species+/CITES, 2021).

Figure 5. Illustration of Lycaste virginalis.
Extracted from Archila Morales and Chiron (2011).

1.4.2 *Lycaste lasioglossa* Rchb.f.

General Information: *Lycaste lasioglossa* is an epiphytic orchid species, up to 60 cm tall (Ames & Correll, 1953). It has showy flowers with elliptical and rounded yellow petals that are around 3.5 to 4 cm long and 1.5 cm wide; the sepals and lip are reddish-brown (Ames & Correll, 1953).

Habitat: *Lycaste lasioglossa* grows in cloud forests at an altitudinal range between 1400 to 1800 m. a.s.l. It is endemic to Mexico, Guatemala, El Salvador, Honduras and possibly Costa Rica (International Orchid Foundation, 2021).

Conservation Status: *Lycaste lasioglossa* is listed in category 3 of LEA, which indicates that the species is not at risk of extinction yet, but it could be if its use and trade are not regulated. Its use is allowed for research, propagation, and commercial purposes with prior authorization from CONAP (CONAP, 2009). In CITES, it is listed in appendix II (Species+/CITES, 2021).

Figure 6. Illustration of Lycaste lasioglossa.
Extracted from Fitch and Hooker (1876).

1.4.3 *Lycaste cochleata* Lindl.

General Information: *Lycaste cochleata* is an epiphytic orchid species. It has several inflorescences, with small flowers for the genus but still very showy. Petals are deep orange, elliptical and obtuse of around 2.2 to 2.5 cm long and 1.1 to 1.2 cm wide. The sepals are greenish-yellow, and the lip is deep orange (Ames & Correll, 1953).

Habitat: *Lycaste cochleata* is distributed in Mexico, Guatemala, El Salvador and Costa Rica (Archila Morales, 2011).

Conservation Status: *Lycaste cochleata* is listed in category 2 of LEA, which includes all the endemic species, their distribution is limited to a specific habitat. Its use is allowed for research, propagation, and commercial purposes with prior authorization from CONAP (CONAP, 2009). In CITES it is listed in appendix II (Species+/CITES, 2021).

Figure 7. Flower of Lycaste cochleata (synonym of Selbyana cochleata). Extracted from Archila Morales (2011).

1.5 Orchid Conservation Strategies

Worldwide, there are many orchids in different growth forms whose diversity is heterogeneously distributed (Gaskett et al., 2018). For instance, the subantarctic region has low orchid diversity (10 *spp.*), whereas Guatemala, in the Latin American region, has high orchid diversity (734 *spp.*) (Cameron, 2000; Gaskett et al., 2018). The variety of niche breadths, range sizes, and dispersal mechanisms within the regions also varies because orchids rely on specific symbionts (*e.g.*, pollinators and fungi) in most genera (Gaskett et al., 2018). Orchid species have experienced dramatic losses in habitat and abundance of individuals in nature, making them highly vulnerable to extinction (Barman & Devadas, 2013).

Some of the potential strategies to conserve orchid diversity include *in situ* (maintaining ecosystems in their natural habitat, *e.g.*, national parks, protected areas *etc.*) and *ex situ* (keeping samples of living organisms outside their natural habitat, *e.g.*, botanical gardens, seed storage, *in vitro* conservation, cryopreservation). Barman and Devadas (2013) suggest the following strategies for efficient conservation of orchids:

a) Restoring and maintaining native ecosystems.
b) Managing native habitats for threatened, endangered, and rare species.
c) Ranking species according to their level of vulnerability.
d) Monitoring the phenology of orchids and pollinators in the long-term.
e) Assisting species migration.
f) Supporting restoration by symbiotic seed germination and seedling growth.
g) Breeding by intra-species hybridization.
h) Using *ex situ* conservation approaches by seed storage and banking.

Both *in situ* and *ex situ* approaches are necessary for an integrated conservation strategy for threatened taxa. *In situ* conservation should be the priority. However, *ex situ* conservation can support initiatives to ensure species' survival (Swarts & Dixon, 2009).

1.5.1 *Ex situ* Conservation

Globally, two in five plant species are threatened with extinction (Antonelli et al., 2020). Plants that are not useful to humans are at higher threat than those plants that are considered useful (Kress & Krupnick, 2022). It has been internationally recognized that the rate of biodiversity loss is increasing. In response to the recognition that plants are indispensable to conservation, the Global Strategy for Plant Conservation (GSPC) was established (Martyn Yenson et al., 2021). This global strategy includes the *ex situ* conservation approach, which should support the international conservation efforts of threatened, endangered, and rare species by providing a backup in case some species no longer exist in the wild (IUCN SSC, 2014). For those specific cases, *ex situ* plant collections are sources of material for translocation or restoration of the plants in their natural habitats (Martyn Yenson et al., 2021).

The orchid family encompasses more species at risk of extinction than any other plant family (Merritt et al., 2014). The implementation of *in situ* and *ex situ* conservation and recovery approaches for orchids is needed. *Ex situ* conservation should not take the place of *in situ* conservation but should be seen as a source of support of plant material resources in the event of species extinction in the wild (Merritt et al., 2014). In orchids, *ex situ* conservation appears to be a suitable approach to support the conservation efforts because they have the smallest seeds in the plant kingdom (an orchid seed is about the size of a dust particle), so large areas are not needed for their storage (Royal Botanical Garden Kew, 2013).

1.5.1.1 Seed Storage Behaviour

Seed storage behaviour is defined by the seed's capability to withstand desiccation (drying) and low temperature storage (Royal Botanical Gardens Kew, 2021). Having knowledge of the seed storage behaviour of species of interest is vital to define if seed storage is a viable approach for conservation, and also to know suitable approaches for seed collection and desiccation (Hong et al., 1996). Based on their storage behaviour, tolerance to low temperatures, and lifespan, seeds can be classified in three categories:

- Orthodox seeds, can be dried to low moisture contents (5% and below) without reduction in viability. In addition, their lifespan increases in a predictable way over storage environments with low moisture contents and temperatures (Hong et al., 1996; Royal Botanical Gardens Kew, 2021).
- Intermediate seeds, can be dried to 10-12.5% moisture contents, but below those moisture contents, seeds viability could be compromised. Intermediate seeds are considered chilling and freezing sensitive when dry and stored at low moisture contents and temperatures (Hong et al., 1996; Royal Botanical Gardens Kew, 2021).
- Recalcitrant seeds, cannot be dried to moisture contents below 15-20% and are also sensitive to low temperatures. Thus, these seeds are considered not amenable to long-term storage (Hong et al., 1996; Royal Botanical Gardens Kew, 2021) under standard storage conditions of low temperature and moisture.

1.5.1.2 Seed Storage of Orchidaceae

Many orchid seeds show orthodox storage behaviour (*i.e.*, are tolerant to desiccation), which allows them to be subsequently stored in extremely dry and cold conditions (generally below zero) (Hay et al., 2010; Priestley et al., 1985). However, the challenge with the storage of orchid seeds is that they tend to live for a short time under internationally recommended seed banking conditions (*i.e.*, dry the seeds to a moisture content that maintains the relative humidity under 25%, and store them at -20°C or less) (Maschinski, 2022), prompting the need to determine how to extend their lifetime by creating species-specific seed banking protocols or improving the existing ones (Hay et al., 2010).

1.5.1.3 Cryopreservation

Biotechnology through cryopreservation can be an innovative opportunity to support orchid seed storage. Cryopreservation techniques allow the preservation of cells or tissues by cooling them below sub-zero temperatures (liquid nitrogen at -196 °C) (Engelmann, 2011). Cryopreservation is a valuable method for the long-term conservation of the germplasm of recalcitrant, rare and endangered species because it enables the maintenance of genetic stability, requires small spaces, and facilitates the exchange and transport of samples (Vendrame et al., 2014). In orchids, cryopreservation of seeds could provide the advantage of prolonging their lifespan because the liquid nitrogen replaces the oxygen within the air space volume around the embryo, reducing the detrimental effects of cell freezing (Diantina, 2020).

The number of publications on cryopreservation of endangered and rare orchid species is still limited, however, in recent decades, various methods for orchid seed storage using cryopreservation have been established. The response of orchid seeds varies among species (Diantina, 2020; Nikishina et al., 2007), suggesting that protocols will need to be developed for individual species. The success of cryopreservation of orchid seeds may be influenced by the seed morphology of the target species (taxa and growth form) combined with environmental factors (living habitats and biogeographical origin) that affect the seed traits (Diantina, 2020).

1.5.1.4 Factors Affecting Seed Longevity in Storage

Seed longevity in storage can be influenced by several factors, such as genetic, seed maturity, seed handling, and environmental factors. Most orchid seeds tend to live for a short time under seed banking conditions (Hay et al., 2010). The short storage life of orchid seeds may be correlated with their natural dispersal strategy. Nonetheless, some hypotheses on why orchid seeds are short-lived include:

- The primary storage reserves of orchid seeds are their fatty acids and protein bodies; it has been shown that seeds containing fatty acids as significant energy storage sources have a shorter lifespan than those composed of proteins or carbohydrates (Ames & Correll, 1953; Nagel & Börner, 2010).
- Orchid seeds have an interior air space around the embryo. Seed deterioration may be due to the oxidation of fatty acids (McDonald, 1999). In orchids, this airspace may be accelerating deterioration due to oxidation of fatty acids (Diantina, 2020).

For these reasons, additional research on seed physiology (properties of the seed components), seed morphology (oxygen volume around the embryo), and chemical composition (lipid content) across a range of orchid species is needed to understand if the short life of orchid seeds under seed bank conditions correlates with any of these factors.

1.5.1.5 Seed Viability Testing

Seed viability testing is vital in plant conservation and research because it helps to assess whether seeds are viable after collection or after being in storage (Hosomi et al., 2012; Pradhan et al., 2022). Chemical (*e.g.*, tetrazolium, Evans blue, fluorescein diacetate tests), physical (*e.g.,* gently pushing or cutting the seed), and non-invasive (*e.g.,* using X-rays) methods can be used to determine seed viability (Halbritter Rechsteiner et al., 2019). In plants with very small size seeds, like orchids, chemical methods are better options to score viability.

Pradhan et al. (2022) found in a study on seed viability testing for research and conservation of epiphytic and terrestrial orchids that the Evans blue test (EB) is a more reliable seed viability test as compared to fluorescein diacetate (FDA) and tetrazolium (TTC) tests. However, TTC test has been most commonly used as it allows to make a rapid assessment of seed viability (ISTA, 2022).

1.5.1.6 Seed Germinability Testing

Seed germinability is usually estimated using emergence tests to specify the germination potential of a seed lot (ISTA, 2022). Germination can be evaluated *in* or *ex vitro*; in orchids, *in vitro* germination is a good alternative as the media utilized can provide the nutrient requirements needed for asymbiotic germination (Dutra et al., 2009; Nadarajan et al., 2011). In nature, most terrestrial orchid seeds have established a symbiotic association with fungi that helps them germinate and grow (symbiotic germination), and these symbionts may not be available to seeds that are stored, contributing for the failure of the seed to germinate post-storage (Ames & Stewart Correll, 1953; Colville et al., 2016).

In the asymbiotic *ex situ* germination, one of the limitations is that the media effectiveness can differ significantly between species, even within species sourced from different habitats within the same geographic regions, as these habitats may have resulted in locally adapted ecotypes (Diantina, 2020; Hufford & Mazer, 2003).

1.6 Objectives of this Study

Considering the literature review and background data, and with the primary intention of contributing with information that favours seed conservation of rare and endangered orchid species from Mesoamerica, this thesis aims to:

a. Characterize the seed morphology of *L. virginalis, L. lasioglossa,* and *L. cochleata.*
b. Perform *in vitro* germination trials on different media to define the most suitable media for seed germination of *L. virginalis, L. lasioglossa,* and *L. cochleata* seeds.
c. Determine the optimal conditions for seed storage by testing different combinations of seed moisture content and storage temperatures, and to correlate this with the seed physical properties.

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Chapter 2.: Morphological Characterization of Three Tropical Orchid Seed Species of the genus *Lycaste*

2.1. Introduction

The Orchidaceae family is the second-largest family of flowering plants, with around 28,000 species (Chase et al., 2015). Globally, orchids are recognized for having floral species that are extremely desired for horticulture, medicine, or food (Lawson et al., 2019). However unsustainable harvesting and the destruction of their natural habitat are anthropogenic threats for orchids, combined with climate change (Fay, 2018), therefore, it is essential initiatives to conserve orchids to avoid their extinction *in situ* are taken.

Taxonomically, Orchidaceae have been considered a challenging group due to their vast diversity and a wide variety of morphological characteristics (Barthlott et al., 2014; Chase et al., 2015). Historically, genera and other taxa have been classified mainly based on flower structure (*e.g.*, column and pollinia). However, flowering traits may be predisposed to homoplasy (*i.e.*, lineage-independent loss or acquisition of traits over the course of evolution), as a result of their co-evolution with pollinators (Barthlott et al., 2014). Over the last 20 years, several phylogenetic studies have been carried out on orchid species. As a result, orchids have been re-classified and subdivided into five subfamilies: Apostasioideae, Cypripedioideae, Epidendroideae, Orchidoideae, and Vanilloideae (Chase et al., 2015).

Seeds are essential for the adaptation, regeneration, distribution, and persistence of the species, playing a significant role in orchid conservation strategies (Diantina et al., 2020b; Verma et al., 2014). In addition, seed morphology traits are associated with dormancy, germination, and storability (Diantina et al., 2020b). However, due to their minuscule size, their specific characteristics remain undescribed for many orchid species. Little is published on orchid seeds, yet existing studies suggest that the variability in micro-morphology traits of orchid seeds across species is remarkably high. The wide seed variety within orchids is observed in their shape, size (*e.g.*, *Oberonia* 0.1 mm, *Epidendrum* 6 mm), and complex seed-coat structure and surface appearance (Barthlott et al., 2014). *In vitro* regeneration studies also suggest that the nutrient necessities for seed germination and seedling development is species-specific (Diantina, 2020).

The study of the seed micro-morphology traits is significant for conservation because it provides information on possible ecological and biological strategies of dispersal, adaptation, and germination for orchids (Diantina et al., 2020b). For instance, Diantina et al. (2020b) accomplished a comparative study of seed morphology of tropical and temperate orchid species with different growth habits and found high variability in the micro-morphological traits and concluded that they are not related to taxonomy, growth habit, or biogeographical origin of the species under study, suggesting that the differences of seed features are ecological adaptations of the species to facilitate their seed dispersal.

This study aims to characterize the seed capsule morphology and seed micro-morphological traits of three threatened epiphytic species of the Guatemalan genus *Lycaste* to contribute knowledgebase information for conservation efforts to preserve orchid species in the Mesoamerican region.

2.2. Materials and Methods

2.2.1. Seed Material

The three *Lycaste* species studied here are identified as threatened by CITES (all listed under appendix II) and by the Guatemalan authorities through LEA. Therefore, before taking plant samples, it was compulsory to obtain research and collection certificates (Appendix A and Appendix B respectively) issued by the Guatemalan Council of Protected Areas (CONAP). To export seed capsules from Guatemala, it was essential to obtain exportation permits issued by CONAP (Appendix C and Appendix D), and then phytosanitary certificates issued by the Guatemalan Ministry of Agriculture, Livestock and Food (MAGA) (Appendix E and Appendix F). The imported seed must meet the import requirements of New Zealand (section 5.2.3 of the Import Health Standard for Dried and Preserved Plant Material and Plant Material for Research issued by the New Zealand Ministry of Primary Industries (MPI)), which is confirmed through a phytosanitary certificate (government to government National Plant Protection Organizations (NPPO's)) from the country of export. New Zealand required no treatment for orchid seed capsules. In addition, the *Lycaste* species used in this study are listed as 'Basic' in the Plant

Biosecurity Index (PBI- https://www1.maf.govt.nz/cgi-bin/bioindex/bioindex.pl), which means that no import certificate is required.

L. cochleata, *L. lasioglossa*, and *L. virginalis* plants were grown at the Experimental Orchid Station Farm of the Archila's Family, located in Cobán, Alta Verapaz, Guatemala. Cobán is part of the very humid tropical forest, with an altitude of 1316 m a.s.l., temperatures ranging from 12.5°C to 27.8°C (Appendix G) and relative humidity from 76% to 91% (Appendix H) (Weather Atlas, 2021). The seed capsules were hand-pollinated and harvested when ripe (*i.e.*, capsules that naturally completed development and were fully grown). Two batches were shipped by courier, the first with ten capsules of *L. lasioglossa* and *L. virginalis* each, and the second with ten capsules of *L. cochleata*, they were packed to protect the seeds as much as possible. The ten capsules of each species were placed in different plastic containers with silica gel to absorb humidity and tissue paper on the top and bottom to minimize movement (Appendix I). The cold shipping package was provided by a Guatemalan supplier (SM Soluciones S.A.) and consisted of a standard duration cooling system of 96 hours at a constant 2 to 5°C (plastic ice gel packs surrounded by polyethylene thermal insulation – see Appendix J).

Upon arrival, the seed capsules were placed to dry in plastic containers in a controlled environment room operating at 20°C and 55% relative humidity (RH) on the Massey University Turitea Campus in Palmerston North. The seed capsules were allowed to open naturally under those conditions and the exposed seeds were then collected and stored in glass vessels under the same conditions (20°C and 55% RH) before being used for experimentation.

2.2.2. Seed Capsules Assessment

To observe the morphological variations of the seed capsules, ten capsules per species were assessed, each capsule was treated as a replicate. The length, top diameter, central diameter, and bottom diameter of each capsule were measured using a vernier. The measurement consisted in putting the seed capsules longitudinally (for length) and transversely (for diameter) between the two vernier scales. In addition, seed shape was visually evaluated and compared, particularly the number of ribs per capsule and the colour of the seed capsules with the Pantone Colour Matching System (Pantone, 2017).

2.2.3. Seed and Embryo Micro-measurements

Prior to the execution of the measurements, seed samples of five different capsules per species were assessed using the tetrazolium test, which allowed viable and non-viable seeds to be distinguished. Viable seeds are the ones in which the embryos are stained red (ISTA, 2022), which for measurement purposes allows distinguishing the embryo from the seed-coat (Hosomi et. al., 2012).

The tetrazolium test was performed following the method employed by (Diantina et al., 2020a). Firstly, seed samples were placed into plastic vials with a sucrose solution at 10% (w/v) in a room at 20°C for twenty-four hours. Afterwards, the sucrose solution was replaced with 2,3,5-triphenyl tetrazolium chloride at 1% (w/v) and then seeds were incubated in a dark incubator at 40°C for another twenty-four hours. Lastly, 10 red stained seeds per capsule (50 seeds per species) were measured for the length and width of both the seed and the embryo under a binocular microscope Olympus SZX7 at magnification 5.6x.

The seed volume (SV), embryo volume (EV), and percentage of air space within the seed (ASV) were estimated using the formulas established by Arditti and Ghani (2000) for seeds with prolate spheroid embryos:

$$SV = 2\left(\left({}^{SW}\!/_{2}\right)^2 * \left({}^{SL}\!/_{2}\right) * 1.047\right)$$

$$EV = {}^{4}\!/_{3}\ \pi * \left({}^{EL}\!/_{2}\right) * \left({}^{EW}\!/_{2}\right)^2$$

$$ASV\ (\%) = \left(\frac{SV - EV}{SV}\right) * 100$$

Where: SW= seed width, SL= seed length, EW= embryo width, EL= embryo length

2.2.4. Data Analysis

The Shapiro-Wilk test was used to confirm the data met the premises of normality ($p > 0.05$). A one-way analysis of variance (ANOVA) with the multivariate general linear model (GLM) was performed to compare the differences in the seed capsules and seed micro-morphology dimensions among the species. Afterwards, means were compared by post-hoc Tukey's test (significance 5%), using the software 'IBM SPSS Statistics' software version 28.0.1.1. (IBM Corp., 2022). In addition, the relationship of the different seed micro parameters studied, SL/SW, EL/EW, and SV/EV, was analyzed as described above.

2.3. Results

2.3.1. Seed Capsule Morphology

The seed capsules of the three species had six longitudinal ribs. The seed capsules were considered ripe when their colour was green yellowish (Pantone 3604-382) (Figure 8). On drying, the capsules turned brown with some small green-yellow spots (Pantone 1255-1265-582-457) (Figure 9) (Pantone, 2017).

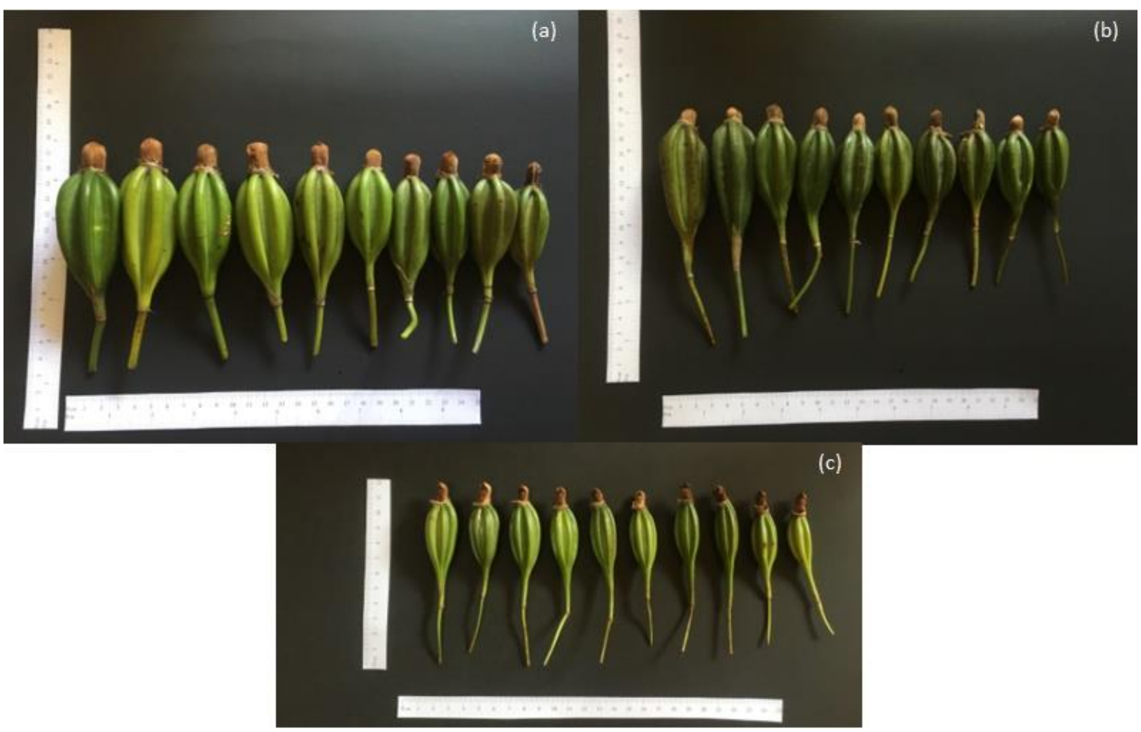

Figure 8. Variability of ripe seed capsules of (a) Lycaste virginalis, (b) L. lasioglossa, and (c) L. cochleata. Scale is a 30 cm ruler.

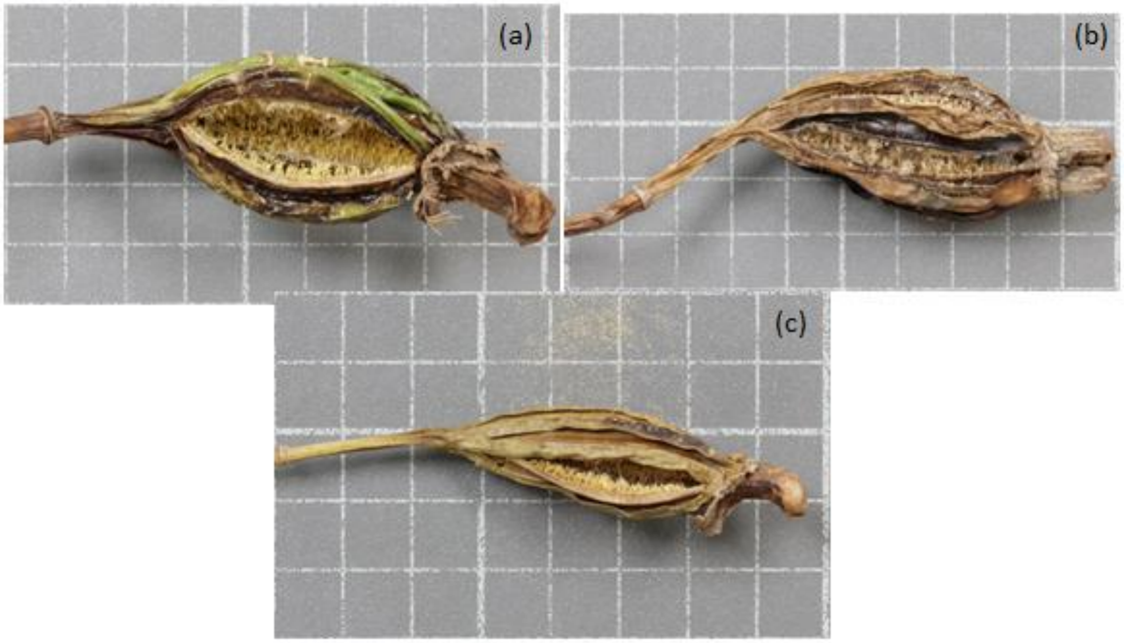

Figure 9. Dry capsules of (a) Lycaste virginalis, (b) L. lasioglossa, and (c) L. cochleata. Each square is 1 x 1 cm (1 cm^2).

A one-way ANOVA using the multivariate general linear model (GLM) revealed that *L. cochleata* capsules were statistically significantly different in mean length ($F (2,27) = 13.28$, $p=0.0$) and for the diameter measured at the top of the capsule ($F (2,27) = 47.88$, $p=0.0$). from *L. lasioglossa* and *L. virginalis*. For the diameter measured at the centre of the capsule, there were no significant differences in means among species ($F (2,27) = 1.5$, $p=0.24$). For the bottom diameter, there was a statistically significant difference in the mean between all three species ($F (2,27) = 28.5$, $p=0.0$) (

Table 2) with *L. virginalis* having the largest diameter and *L. cochleate* the smallest.

Table 2. Variability of the three different Lycaste orchid seed capsule's length, diameters (Ø) and ripening time.

Species	**Capsule measurement (cm)**			
	Length	**Ø Top**	**Ø Central**	**Ø Bottom**
L. cochleata	7.90 ± 0.74 [b]	1.0 ± 0.00 [b]	2.1 ± 2.13	1.0 ± 0.00 [c]
L. lasioglossa	9.80 ± 1.03 [a]	2.1 ± 0.32 [a]	2.9 ± 0.32	1.6 ± 0.52 [b]
L. virginalis	10.3 ± 1.42 [a]	2.2 ± 0.42 [a]	3.0 ± 0.47	2.0 ± 0.00 [a]

For each individual value (mean ± SD) followed by letters that indicate significant differences when different (Tukey test, $p < 0.05$).

2.3.2. Seed Micro-Morphology

Visually, *Lycaste* seeds look like fine yellowish sand, the colour intensity varies slightly between each species (*L. cochleata* Pantone 393, *L. lasioglossa* Pantone 615, and *L. virginalis* Pantone 459) (Figure 10) (Pantone, 2017). Under the microscope, it was possible to observe that a seed-coat and an embryo formed in the seeds, and that the embryos of the three species have a shape defined by Arditti and Ghani (2000) as prolate spheroid (Figure 10).

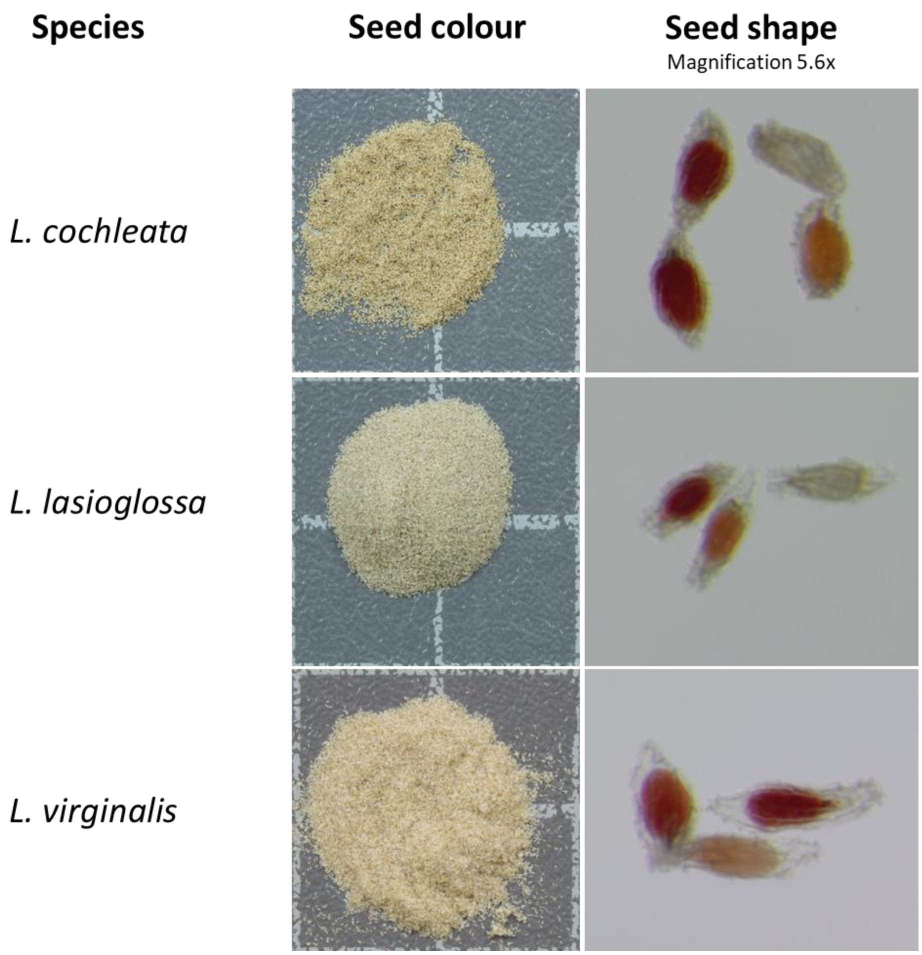

Figure 10. Lycaste seeds with (right photographs) and without (left photographs) magnification. The seeds enlarged at 5.6x are stained with 2,3,5-Tetrazolium Chloride (TTC), seeds red stained are viable. Each square is 1 cm^2.

To compare the micro-morphological dimensions of three *Lycaste* species, a one-way ANOVA using the GLM was performed. The analysis revealed that there was a statistically significant difference in the seed dimensions (SL = seed length, SW = seed width, and SV = seed volume), and significant differences within the embryo dimensions (EL = embryo length, EW = embryo width, and EV = embryo volume) between the three species (Table 3). The data showed that the mean percentage of air space differed significantly between the three species ($F (2, 147) = 105.62$, $p=0.0$) with *L. lasioglossa* having the highest percentage of the seed as air space and *L. cochleata* the lowest. Likewise, the post-hoc comparison using Tukey's test after GLM found that the mean value of the micro-morphological traits was significantly different between the three species, but this differed for individual traits with *L. virginalis* having the longest seed but an embryo equal in length to *L. cochleata*. *L. cochleata* had the largest embryo volume.

Table 3. Morphological micro-characteristics analysis of three Lycaste species.

	Seed dimensions			
Species	**Length (mm)**	**Width (mm)**	**Volume (mm^3)**	
L. cochleata	0.21 ± 0.02 ^{c}	0.11 ± 0.01 ^{a}	0.00063 ± 0.0002 ^{a}	
L. lasioglossa	0.23 ± 0.03 ^{b}	0.09 ± 0.01 ^{b}	0.00051 ± 0.0001 ^{b}	
L. virginalis	0.26 ± 0.02 ^{a}	0.09 ± 0.01 ^{b}	0.00060 ± 0.0002 ^{a}	
	Embryo dimensions			**Air-space %**
Species	**Length (mm)**	**Width (mm)**	**Volume (mm^3)**	
L. cochleata	0.14 ± 0.01 ^{a}	0.082 ± 0.01 ^{a}	0.00051 ± 0.00011 ^{a}	17.4 ± 8.0 ^{c}
L. lasioglossa	0.12 ± 0.01 ^{b}	0.066 ± 0.01 ^{c}	0.00029 ± 0.0007 ^{c}	42.3 ± 8.1 ^{a}
L. virginalis	0.15 ± 0.01 ^{a}	0.073 ± 0.01 ^{b}	0.00041 ± 0.00009 ^{b}	29.7 ± 9.5 ^{b}

For each individual value (mean ± SD) followed by letters that indicate significant differences when different (Tukey test, $p < 0.05$).

Table 4 shows the comparison of the relationship between some of the micro-morphological parameters evaluated, *i.e.*, ratio of embryo length (EL) with embryo width (EW); seed length (SL) with seed width (SW); and seed volume (SV) with embryo volume (EV). The SL/SW and SV/EV differed across all three species.

Table 4. Relationship between seed length and seed width (SL/SW), embryo length and embryo width (EL/EW), and seed volume with embryo volume (SV/EV) of three Lycaste species.

Species	**SL/SW**	**EL/EW**	**SV/EV**
L. cochleata	1.98 ± 0.26 ^{c}	1.78 ± 0.24 ^{b}	1.22 ± 0.13 ^{c}
L. lasioglossa	0.57 ± 0.37 ^{b}	1.86 ± 0.19 ^{b}	1.77 ± 0.25 ^{a}
L. virginalis	2.75 ± 0.38 ^{a}	2.04 ± 0.26 ^{a}	1.45 ± 0.21 ^{b}

Different letters indicate significant differences ($p < 0.05$) between species for each trait (mean± SE) after a Tukey post-hoc test.

2.4. Discussion

In this study, the seed capsule morphology and seed micro-morphology of three epiphytic species of the *Lycaste* genus were characterized. The seed capsule morphological assessment was based on the capsule's length, diameter (*i.e.*, top, middle, bottom), and colour. The seed micro-morphological evaluation consisted of the estimation of seed length (SL), seed width (SW), seed volume (SV), embryo length (EL), embryo width (EW), embryo volume (EV), and air-space ratio within the seeds (ASV). In addition, the relationship between seed length and seed width (SL/SW), embryo length and embryo width (EL/EW), and seed volume with embryo volume (SV/EV) was assessed.

Similarities were found in the qualitative traits of the seed capsules. The seed capsules were very similar in appearance (six ribs per capsule) and colour (Figure 8 and Figure 9). Other studies have reported similarities in seed capsule form, colour, and the number of ribs for other *Lycaste* species (Ryan, 2001; Standley & Steyermark, 1946). The morphological appearance (*e.g.*, the presence of six ridges) and colour of the seed capsules seem to be considered an indicator of the *Lycaste* taxa, however further research needs to be done on the other forty-three species that comprise the taxa to conclude this accurately (Chase et al., 2015; WFO, 2021). In contrast, the seed capsules' length varied within species, *L. cochleata* range from 7 to 9 cm, *L. lasioglossa* from 9 to 12 cm, and *L. virginalis* from 8 to 13 cm. The differences in the seed capsule size within the same species may be due to the genetic basis of the species, availability of water and light of the plants when developing the capsules (*i.e.*, the seed capsules were produced in the same location at the same time, and the collected capsules were positioned in the same location on different plants; however the plants did not grow in a controlled environment), intra-plant competition may have created differences in the seed capsules development (*i.e.*, competition between individuals of the same species for resources, edge effects). Regardless, to objectively conclude on this, more research over several production seasons is required.

The seed capsule dimensions varied across the three species (

Table 2). For length and top diameter, *L. cochleata* was smaller (average length 7.9 cm, and top diameter 1 cm) and statistically different to *L. lasioglossa* (length 9.8 cm, and top diameter 2.1 cm) and *L. virginalis* (length 10.3 cm, and top diameter 2.2 cm). Whereas for the middle diameter, no significant differences were found (*L. cochleata* 2.1 cm, *L. lasioglossa* 2.9 cm, *L. virginalis* 3 cm); and for the bottom diameter, the three species differed (*L. cochleata* 1 cm, *L. lasioglossa* 1.6 cm, *L. virginalis* 2 cm). The seed capsule dimension variability among the species may indicate that those variables can be species-specific traits (Diantina, 2020; Vafaee et al., 2021), and could be an adaptation to their natural environment that facilitate seed dispersal.

The seed micro-morphological dimensions of the seeds also varies among species (Table 3). *L. cochleata* seed volume was equivalent to that of *L. virginalis* (0.0006 mm^3), and the overall embryo volume was the largest compared with the other two species (0.0005 mm^3); therefore, the internal air-space was the smallest when compared with the other two species (17%). For *L. virginalis* the seed volume corresponded to *L. cochleata*, the embryo volumes were medium size when compared with the other two species (0.0004 mm^3), and as a result, the inner air-space was also intermediate (30%). Lastly, *L. lasioglossa* presented the smallest volumes for both seeds and embryos (0.0005 and 0.00029 mm^3, respectively); consequently, the air-space percentage was the largest (42%). A study investigating the seed micro-morphology of tropical epiphytic orchid species (*Dendrobium strebloceras* and *D. lineale*), temperate terrestrial orchid species (*Gastrodia cunnninghamii*, *Pterostylis banksii* and *Thelymitra nervosa*), and temperate epiphytic species (*D. cunninghamii*) found evidence that micro-morphometrical traits among orchid species are variable, independent of the genus, growth form, origin, and habitat (Diantina et al., 2020b).

In this study, the qualitative seed micro traits of the three *Lycaste* seeds are similar; the seed coat is elongated, and the embryos have a prolate-spheroid form (EL/EW ratios above confirm the prolate-spheroid shape of the embryos) (Diantina et al., 2020b; Lavarack et al., 2000). Nonetheless, the micro-morphometric dimensions significantly vary among the three species. Based on these results, it can be concluded that the micro-morphometrical traits among the three species of *Lycaste* are independent of the growth form (the three species are epiphytes), origin (the three species are from the same location in Guatemala), and habitat (the three species are tropical). Other studies have reported variations in seed size and micro-morphometry traits in species within other genera. For example, Diantina et al. (2020b) found that the internal air-space of *D. strebloceras* (92%) and

D. lineale (32%) were significantly different, although both species are from the same habitat (Indonesia – tropical, same as *Lycaste*), epiphytic (same as *Lycaste*) and with similar taxonomic features. Another study by Prasongsom et al. (2016) reported that the air-space volume differed among the epiphytic orchid species *D. schildhaueri* (13%), *D. findlayanum* (36%), *D. chrysotoxum*, *D. parishii* and *D. fimbriatum* (22-25%) collected in the same habitat (Thailand). More research is needed to understand the evolutionary micro-morphological adaptations of orchid species from the same taxa, habitat, and growth form; further research needs to be done across multiple seed production years for the same species and, if possible, the same plants to determine if the production environment is influencing micro-morphological traits.

In orchids, seeds' micro-morphological traits combined with the numerous seeds produced have been suggested as adaptations to maximize fertility, their specific shape targets enhanced dispersal and screens the environment for suitable hosts (Arditti & Ghani, 2000; Eriksson & Kainulainen, 2011). Orchid seeds are very well adapted for wind dispersal; they are very tiny, 'dust-like' seeds, though they still display substantial length differences (Verma et al., 2014). Seed sizes can be categorized based on their length, from very small (100-200 µm), small (200-500 µm), medium (500-900 µm), large (900-2000 µm), and very large (2000-6000 µm) (Barthlott et al., 2014). Based on this classification, *L. cochleata, L. lasioglossa,* and *L. virginalis* are all considered species with small-sized seeds (210, 230, and 260 µm, respectively). These findings correlate with the epiphytic growth form of *Lycaste* species in nature, which grow above the ground in host trees, allowing the small-sized seeds to float for extended periods and disperse in more extensive areas by the wind (Arditti & Ghani, 2000). Other studies have also reported epiphyte orchid species with relatively small size seeds compared with their counterparts (terrestrial, lithophytic, or mycoheterotrophic) (Verma et al., 2014).

Epiphyte orchids also tend to have large-sized embryos as they are dispersed from above the ground (from the top of host trees) to allow the seeds to remain in the air for extended periods and hence disperse across larger areas (Arditti & Ghani, 2000; Verma et al., 2014). In addition, the embryo size has been positively associated with the seed weight; the assumption is that the bigger the embryo is, the heavier the seed will be, and heavier seeds float for longer than lighter seeds (*e.g., Phaius spp*., and *Eulophia spp*.) (Arditti & Ghani, 2000; Dangat & Gurav, 2016; Diantina, 2020). The findings of this research correlate with those assumptions, since the three *Lycaste* species have relatively large embryos, thus low SV/EV ratio (*L. lasioglossa* 1.77; *L. virginalis* 1.45, and *L. cochleata* 1.22). The SV/EV ratio explains the occupancy of the embryo within the seed, the high occupancy of the embryo directly results in low air volume, and for epiphytes small SV/EV ratios have been previously reported. For example, other studies have found small SV/EV ratios for epiphyte orchid species (compared with terrestrial species) (Diantina et al., 2020b; Verma et al., 2014).

Since epiphytes have small size seeds with relatively large embryos, they are also predisposed to have less interior air-space volume (Verma et al., 2014). Nevertheless, the air volume of the *Lycaste* seeds under study ranges widely from 17% to 42% (*L. lasioglossa* 42%, *L. virginalis* 30%, and *L. cochleata* 17%). A hypothesis for seeds with relatively large embryos and small inner air volume is that they may be non-dormant seeds, an adaptation that may allow them to germinate better in natural humid micro-environments (Prasongsom et al., 2016), however in this study, seed dormancy was not evaluated. Still, these assumptions correlate with the natural distribution of these species in very humid tropical habitats. Further research needs to be done, including seed dormancy of the seeds, to validate whether *Lycaste's* seed micromorphological traits are associated with dormancy and if that dormancy is correlated with natural habitat distribution.

Seed morphological adaptation has enabled plants to occupy new ecological niches (Llavata Peris et al., 2010). This study found that the three *Lycaste* species have small-sized seeds, with large comparative embryos and an air volume that occupies less than half the seed space, features that can be adaptations that allow these species to have longer flotation times and therefore enable wider dispersal (which is the case of most epiphyte species) (Arditti & Ghani, 2000; Barthlott et al., 2014; Diantina, 2020). Additionally, those specific seed micro-features may be adaptations of many epiphytic orchid species for better dispersal and germination (especially in humid natural environments) (Barthlott et al., 2014; Prasongsom et al., 2016). This research did not evaluate the dispersal or germination success of the seeds in their natural habitat; however, based on the available information, the natural distribution of these *Lycaste* species is in the very humid tropical forest in Guatemala. Further research needs to be done to evaluate if there is any correlation between the seed characteristics and the dispersal and germination mechanisms of the species.

Overall, the outcome of this study indicates variability in the seed morphology across *Lycaste* species, indicating that in the genus, even closely related species may have different seed morphological features. To validate these findings, more studies including more *Lycaste* species are needed to compare the seed morphology with the natural habitat distribution of the species, seed dispersal mechanisms, and seed germination success.

2.5. Conclusions

Overall, our results show similarities in the qualitative traits of the seed capsules and the seeds of *Lycaste*. High variability in the quantitative traits of the seed capsules and seed-micromorphology was found. More information about the seed dispersal mechanisms of the three species is needed, however, based on our findings, it is possible to speculate that our *Lycaste* species seeds may develop under dense canopies where they do not need to travel very long distances, being dispersed by waterdrops falling onto the orchid plant from the canopy (hydrochore), therefore,small seeds, with relatively large embryos, and low air volume allow them to be dispersed in tropical ecosystems.

We only studied 6.5% of the total species of the *Lycaste* taxa (3 species out of 46); to validate these research findings further research including more *Lycaste* species is necessary.

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Chapter 3.: Effect of Media on *In Vitro* Asymbiotic Seed Germination of Three Tropical Species of the genus *Lycaste*

3.1. Introduction

Orchid species are facing the risk of genetic loss due to their intricate biology (high reliance on distinctive pollinators, mycorrhizal fungi, and host trees), combined with anthropogenic threats (destruction of their natural habitat, over-harvesting and illegal trade), and vulnerability to climate change (Diantina, 2020; Fay, 2018; Wraith & Pickering, 2018). *Lycaste* is a neotropical orchid genus that consists of about forty-six (46) species (Ryan, 2001; WFO, 2021), distributed throughout the south of Mexico, Central America, and the north of South America (Ames & Correll, 1953; Ryan, 2001). Species of the *Lycaste* genus are facing the same risk of genetic loss as the rest of the species of the Orchidaceae family. With the aim of conserving and regulating illegal collection and trading, all orchid species are registered by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Fay, 2018; Lawson et al., 2019) and *in situ* and *ex situ* conservation approaches are being continuously developed (Diantina et al., 2020).

Orchid seeds are extremely small (dust-like) and contain limited food reserves. Therefore, many orchid seeds germinate in nature only after being infected by mycorrhizal fungi (symbiotic germination) that supply the developing embryo with water, minerals, carbohydrates, and vitamins (Kauth et al., 2008; Lekshmi & Decruse, 2018). The interaction between orchid seeds and mycorrhizal fungi can be species-specific, leading to extremely low orchid seed germination and seedling development rates in natural conditions (Diantina, 2020; Verma et al., 2014). The complex germination system of orchid seeds may become a significant limitation for implementing *in situ* regeneration methods. Consequently, it is essential to develop *ex situ* approaches to contribute, not only to the storage of germplasm, but also for the growth and reintroduction of species in their natural habitats (Diantina et al., 2020). In general, the development of seed germination protocols is a fundamental step in conservation plans. Moreover, for plant reintroduction initiatives, seed-propagated orchids are considered more advantageous as they provide more genetic variation in the reintroduced populations than plants that are vegetatively produced (Gogoi et al., 2012; Mala et al., 2017).

Asymbiotic germination consists of sowing seeds in a culture media that includes all the ingredients necessary for germination (macronutrients, micronutrients, sugars, vitamins, and hormones) in the absence of mycorrhizal fungus (Yenson et al., 2021). In orchids, asymbiotic seed germination has been utilized for *ex situ* conservation purposes, and it is considered an approach that could support the multiplication and reintroduction of orchid species into their natural habitat (Gogoi et al., 2012; Kauth et al., 2008). Within the overarching goal of contributing to the global initiatives for orchid conservation, this study aimed to identify a suitable *in vitro* germination media for *Lycaste cochleata*, *L. lasioglossa*, and *L. virginalis*. To achieve this aim, three different media - Murashige and Skoog (MS) (Murashige et. al., 1962), Knudson C (Knudson, 1946), and terrestrial orchid medium BM-1 (Van Waes et. al., 1986) were assessed.

3.2. Materials and Methods

3.2.1. Seed Material

Flowers of *L. cochleata*, *L. lasioglossa*, and *L. virginalis* were hand-pollinated and seed capsules harvested at maturity in the Experimental Orchid Station of the Archila's Family Farm in Cobán, Alta Verapaz, Guatemala. Upon harvesting, the seed capsules were sent by courier (1-2 weeks) to the Massey University Turitea Campus in Palmerston North, New Zealand.

Before the experiments, the seed capsules were placed in partially open plastic containers in a room at 20°C and 55% of relative humidity (RH) to dry and naturally release the seeds (4-5 weeks) (Appendix K). Using a paintbrush, the seeds contained within each capsule were placed in different glass vessels and temporarily stored (1-3 weeks) under the same conditions (20°C and 55% RH). For the experiment, seeds from ten capsules for each species were assessed separately.

3.2.2. Seed Viability Assessment

Seed viability was assessed using the tetrazolium test. The test was performed following the procedure outlined by Diantina et al. (2020). Seed samples were placed in plastic vials with a sucrose solution at 10% (w/v) in a room at 20°C. After twenty-four hours, the sucrose solution was replaced with 2,3,5-triphenyl tetrazolium chloride at 1% (w/v). The pH of the 2,3,5-triphenyl tetrazolium chloride was adjusted to between 6.5 and 7.5 prior to addition to the seed samples. The samples were then placed in a dark incubator at 40°C for another twenty-four hours.

The seeds were scored as viable and non-viable using a binocular microscope Olympus SZX7, and the 'Cell Counter' plugging of 'ImageJ' (Schneider et al., 2012). Viable seeds showed red staining to all the embryo tissue, whereas non-viable seeds were not stained, partially stained, or were identified as empty (no embryo present) (Figure 11). The overall number of seeds per sample was around 200. The viability rate was calculated using this formula:

$$Viability\ (\%) = \frac{Number\ of\ viable\ seeds}{Total\ number\ of\ seeds} \times 100$$

*Figure 11. Viability test assessment using 2,3,5-triphenyl tetrazolium chloride of **a)** Lycaste cochleata, **b)** L. lasioglossa, and **c)** L. virginalis. Magnification at 4x.*

3.2.3. *In Vitro* Seed Germination Assessment

For the *in vitro* seed germination assessment, seeds were folded in filter paper (55 mm diameter) envelopes to facilitate handling. Seeds of the three *Lycaste* species were sown in petri dishes (9 cm diameter) containing three different media: MS, Knudson C, and BM1 (media nutrient composition available in Appendix M). The seed disinfection and sowing procedures were conducted as described by Diantina et al. (2020). The seed disinfection procedure involved immersion of the filter paper envelopes containing seeds in 10% NaOCl (sodium hypochlorite) solution (v/v) for five minutes followed by rinsing in sterile distilled water for two minutes, replacing the distilled water after one minute rinsing, and finally rinsing with 1% (v/v) antimycotic-antibiotic solution (Sigma-Aldrich, New Zealand) for a minute.

After disinfection, seeds envelopes were unfolded on sterile filter paper in a laminar flow hood to air-dry for about one minute, before sowing on the germination media (Appendix L). Four replicates of around 200 seeds from each seed capsule were evenly spread onto Petri dishes containing different germination medium. These plates were then placed in an incubation chamber with 12 hours of photoperiod at 25 °C.

3.2.4. Seed Germination Stages

Seed germination stages were identified following the criteria described by Diantina (2020) (Table 5). Observations on developmental stages during seed germination were undertaken at different intervals after sowing using a binocular microscope operating in a sterile environment at 0.8x magnification. Figure 12 shows the germination stages illustration of orchid seeds for the *Lycaste* genus. The percentage of seedlings was calculated by dividing the number of germinated seedlings in each germination and development stage by the total number of seeds sown for each treatment.

Table 5. Description of the developmental stages during seed germination of epiphytic orchids.

Stage	**Description**
S0	No germination, embryo inside the seed.
S1	Testa intact, embryo enlargement inside the testa.
S2	Appearance of protocorm and rhizoids.
S3	Elongation of protomeristem (initial shoot and root).
S4	Elongation of the of the first leaf.

Modified from Diantina (2020).

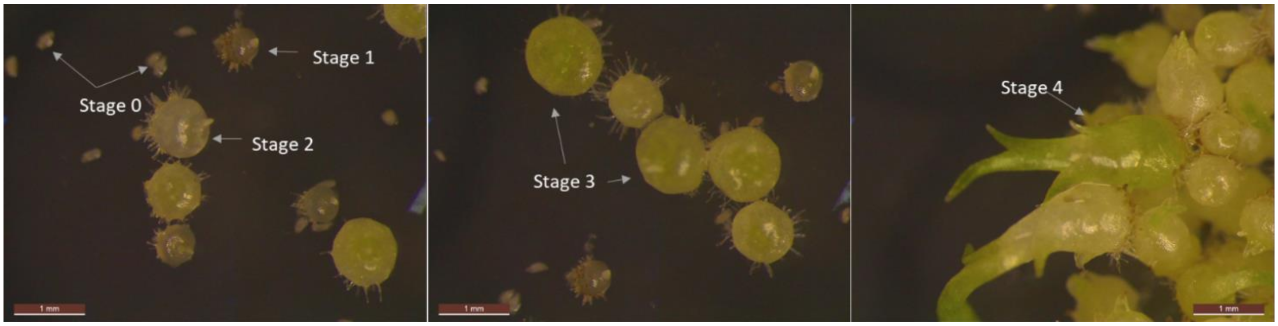

Figure 12. Germination stages of the epiphytic orchid species of the Lycaste genus.

3.2.5. Data Analysis

Comparisons of viabilities and germination rates or germination percentages across treatments were performed using a one-way analysis of variance (ANOVA) with the general linear model (GLM). Means were compared using Tukey's Range Test with $p<0.05$, using the statistical software IBM SPSS Statistics software version 28.0.1.1 (IBM SPSS Statistics, 2022). Graphs on the changes in the seed germination stages were drawn using the media and standard error of the germination stages and weeks in the same way described above.

3.3. Results

3.3.1. Seed Viability

Table 6 shows the seed viability results of ten capsules for each species assessed with the TZ staining method. The results revealed that there was a statistically significant difference in the viability mean between the three species ($F(2, 24) = 4.98$, $p=0.016$).

Table 6. Initial seed viability of seeds of the Lycaste genus.

Species	**Viability (%)**
L. cochleata	77 ± 18.1 [ab]
L. lasioglossa	83 ± 12.2 [a]
L. virginalis	49 ± 35.3 [b]

Percentages values (mean ± SD) followed by letters that indicated that they are not significantly different when they are the same at $p < 0.05$ (Tukey post hoc test).

3.3.2. *In Vitro* Seed Germination

Figure 13 shows a comparison of the seed germination stages of the *Lycaste* species eighteen weeks after sowing in three different germination media (MS, Knudson C, and BM1). *Lycaste cochleata* (Figure 13a; Figure 14a) seeds developed well on MS and Knudson C media. *L. lasioglossa* (Figure 13b; Figure 14b) and *L. virginalis* (Figure 13c; Figure 14c) grew well only in MS media. The three species produced poor-quality plantlets in BM-1.

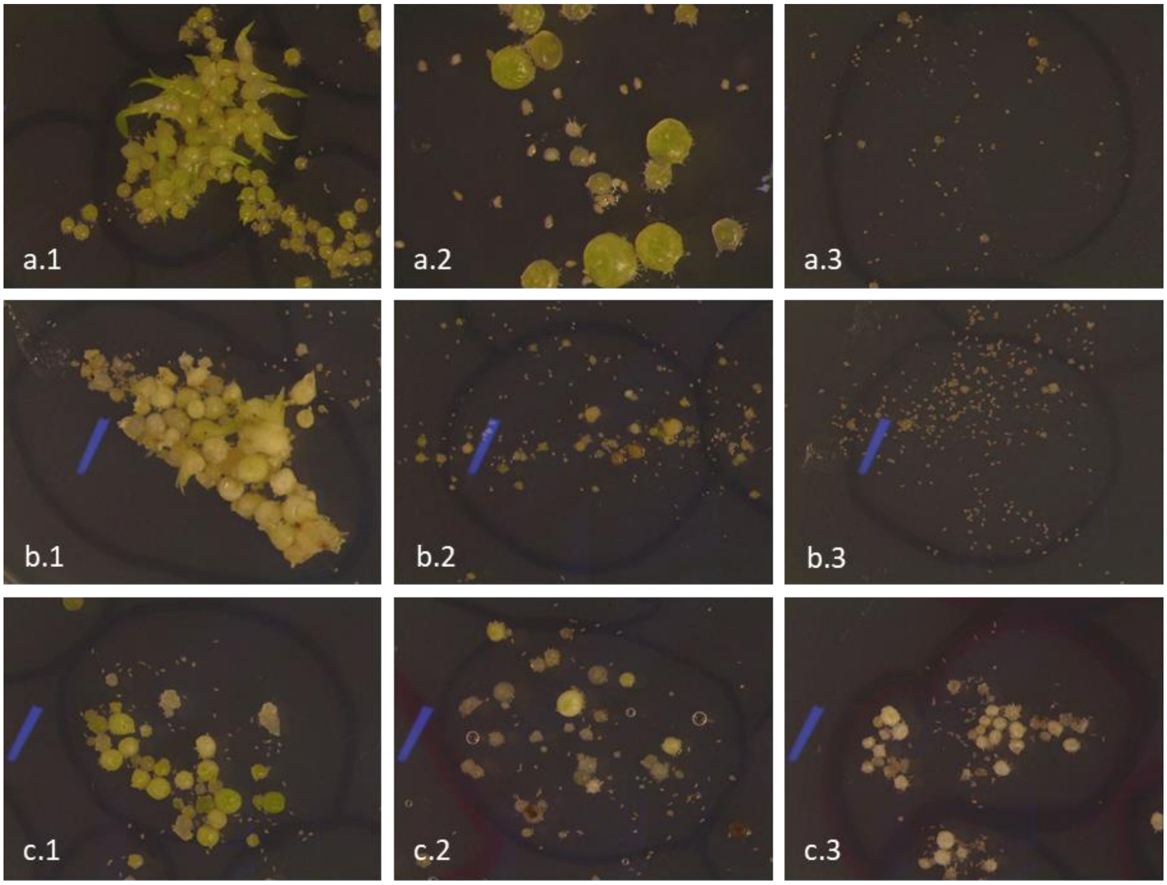

Figure 13. Comparative germination stages development of ***a)*** *Lycaste cochleata,* ***b)*** *L. lasioglossa, and* ***c)*** *L. virginalis, 18 weeks after sowing on different media* ***1*** *MS,* ***2*** *Knudson C, and* ***3*** *BM-1.*

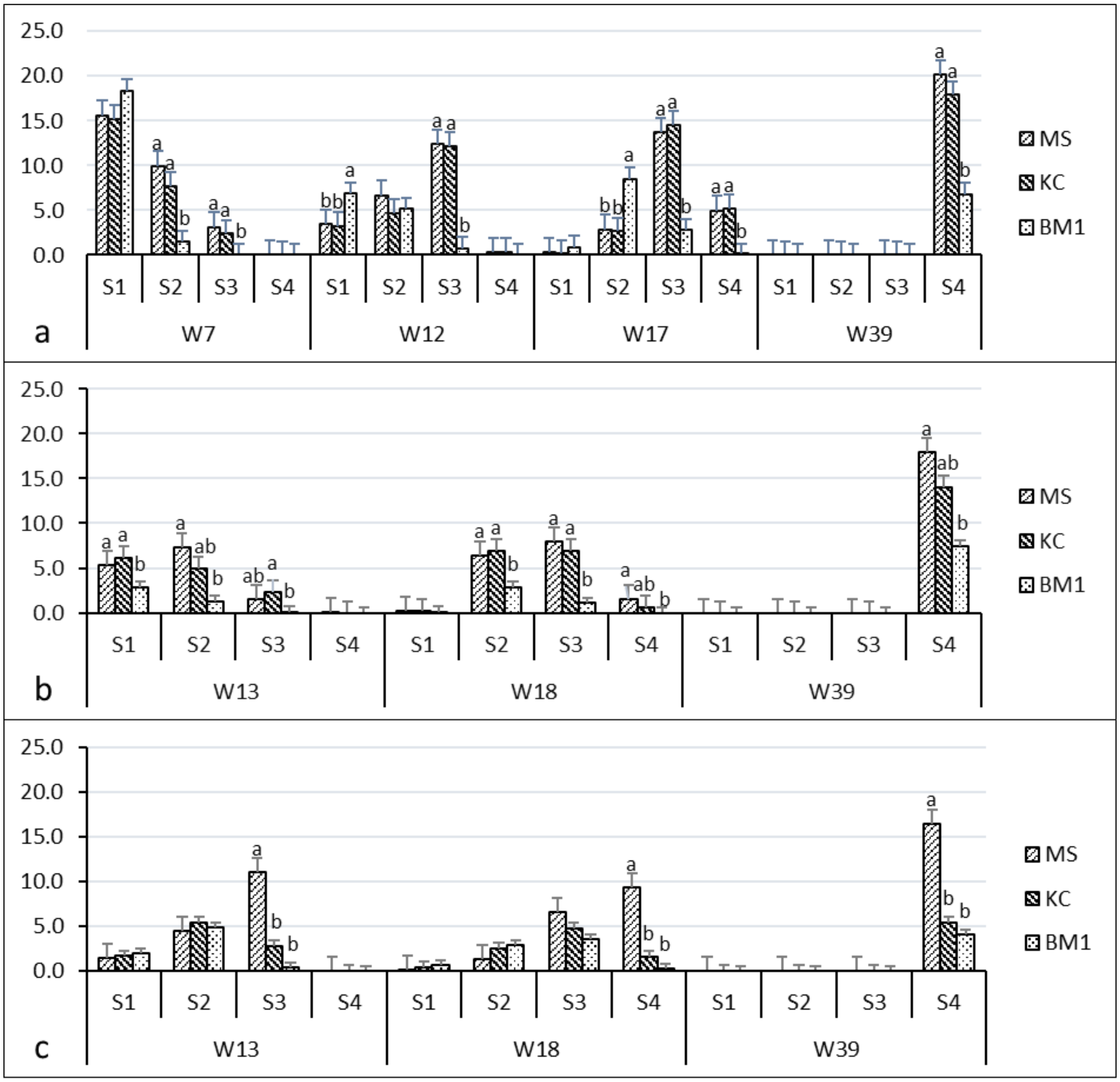

Figure 14. Changes on seed germination stages of ***a)*** *Lycaste cochleata,* ***b)*** *L. lasioglossa, and* ***c)*** *L. virginalis in different germination media: MS, Knudson C, and BM-1. Vertical axes represent percentages. Horizontal axes represent germination stages at different weeks. Bars within each developmental stage and weeks with the same letter are not significantly different (P<0.05 using Tukey's post hoc test). No letter indicates that within a stage development stage was statistically similar.*

Table 7 shows the results of a one-way ANOVA using the GLM, which indicated that there is no interaction between the species and media ($p=0.569$). Among species, *L. virginalis* had statistically significantly lower germination compared with *L. cochleata* and *L. lasioglossa* ($F(2,257) = 8.15$, $p<0.001$). Among the three media treatments there were statistically significant differences ($F(2,257) = 28.28$, $p<0.001$).

Table 7. Effect of media on germination (plantlet establishment (stage 4)) of three species of Lycaste after 39 weeks of sowing.

Germination media	Species		
	L. cochleata	***L. lasioglossa***	***L. virginalis***
MS	20 ± 14.3 $^{a, A}$	18 ± 13.4 $^{a, A}$	16 ± 13.2 $^{b, A}$
Knudson C	18 ± 12.7 $^{a, B}$	14 ± 12.4 $^{a, B}$	5 ± 6.85 $^{b, B}$
BM1	7 ± 5.63 $^{a, C}$	7 ± 6.77 $^{a, C}$	4 ± 3.81 $^{b, C}$

Percentage values (mean ± SD) followed by lowercase letters that indicate significant differences for each species in a column, and capital letters that indicate significant differences for each media in a row (Tukey test, $p < 0.05$).

3.4. Discussion

The asymbiotic seed germination procedure has been used for germinating seed in *ex situ* conservation of threatened, endangered, and rare orchid taxa (Dutra et al., 2009). One of the main advantages of asymbiotic seed germination is that it can allow the reproduction of normal and healthy plants in less time than that reached under natural conditions, making it a valuable alternative not only for conservation purposes but also for commercial ends (Nadarajan et al., 2011). Nevertheless, the success of asymbiotic germination depends on identifying the media that provides adequate nutrients for each species' germination in place of the mycorrhiza found under natural conditions. Due to the wide diversity in Orchidaceae and the mycorrhizal interactions in many orchids, the nutritional composition of the media required for germination can vary significantly within and among taxa (Diantina et al., 2020; Nadarajan et al., 2011).

To assess the success of the germination media used, the viability of the seed sown needs to be known. One of the most common and rapid methods to test viability in seeds is with the tetrazolium test (TZ), which allows for distinguishing viable from non-viable seeds (the embryos of viable seeds will stain red completely, whereas non-viable seeds will not stain or will partially stain; non-viable seeds do not have the capability to germinate even with the ideal germination conditions) (Alomia et al., 2017; ISTA, 2022). The TZ test has been successfully employed to assess the viability of epiphytic orchids (Diantina et al., 2020; Thompson et al., 2001).

In this study, we assessed the initial seed viability of the three *Lycaste* epiphytic species with the tetrazolium test, which indicated that *L. cochleata* and *L. lasioglossa* had high viability (77% and 83%, respectively), and therefore had more than adequate viability to be tested for their *in vitro* germination response on different media. Even though *L. virginalis* viability was lower (49%), some seed (around half) is still viable, meaning it is adequate for assessing germination across the three media (Table 6).

This difference in viability across the three species may be due to the naturally low viability and germination of some species. It may be also due to differences in the level of physiological maturity of the seed capsules when collected (in orchids, it is valuable to collect mature capsules to get seeds with high viability) (Pradhan et al., 2022). Some seed capsules in all three species had fungus outside the capsule on arrival in New Zealand, possibly due to humidity, hence the seed moisture may have been high. If high capsule moisture is the reason only *L. virginalis* viability appears to have been affected by high moisture. Alternatively, some other unknown damage may have occurred when transporting the seed capsules from Guatemala to New Zealand. To confirm any of these suggestions this it is first necessary to understand if there were any differences in the maturity level of the seeds at harvesting across the species, and also any differences in the production environment.

For germination, development at different stages and at different times was assessed (Figure 13 and Figure 14). In addition, the effect of media on the final germination stage (plantlet establishment or stage 4) was analyzed, and significant differences between the media and the species found (Table 7). In our findings, the three *Lycaste* species germinated well and produced normal plantlets (healthy) on MS media compared with the other two media (*L. cochleata* 20%, *L. lasioglossa* 18%, and *L. virginalis* 16%). In Knudson C media, the germination was similar to the germination on MS media for *L. cochleata* and *L. lasioglossa*, but very low for *L. virginalis*, still, healthy (high-quality) plantlets were produced (*L. cochleata* 18%, *L. lasioglossa* 14%, and *L. virginalis* 6%). Conversely, in BM-1 media, the three species had very poor germination, and the quality of the seedlings was poor (*i.e.*, seedling tissues decayed, necrotic, or discoloured) (*L. cochleata* 7%, *L. lasioglossa* 7%, and *L. virginalis* 4%). The three species studied are from the same geographical region (tropical), the same life-form (epiphyte), and the same genus. This suggests that the germination requirements for *Lycaste* can be species-specific since both the germination rate and plantlet development were affected by the species and the asymbiotic culture media.

Diantina et al. (2020) compared seed germination and seedling development in tropical and temperate orchids and found that the nutrient requirements for seed germination are species-specific and that the epiphyte orchid species studied germinated better in full or half-strength MS media (*D. strebloceras* and *D. lineale*), in addition, it was reported that BM-1 was a better option for the seed germination of temperate orchids (*Pterostylis banksii* and *Thelymitra nervosa*) (Diantina et al., 2020). Although there are few studies published on *Lycaste* asymbiotic seed germination, a study by Mata-Rosas et al., 2010 of *in vitro* regeneration from pseudobulb sections for *L. aromatica* also reported that MS media offers a suitable nutritional composition for the genus *Lycaste*.

In the three *Lycaste* species, the germination percentage differed in the three media assessed when compared with the viability percentage (overall viability was higher than germination). The germination percentage indicates the proportion of normal seedlings (*i.e.*, the physical appearance of the basic structures of the plantlet indicates that a plant is going to develop well in suitable natural conditions) produced by a determined number of seeds (ISTA, 2022). Whereas the viability percentage indicates the proportion of viable seeds (*i.e.*, seeds with the capability to grow and develop in a normal seedling) (ISTA, 2022). The difference between germination and viability percentages may be because the germination media that we evaluated did not meet or only partially met the needs that the seeds required to achieve the optimum germination. However, more asymbiotic and symbiotic germination studies are needed to conclude accurately and standardize the germination test for these species.

The *Lycaste* species studied are listed in appendix II of CITES (Species+/CITES, 2021). Furthermore, they are also included on the List of Threatened Species of Guatemala (LEA) (CONAP, 2009). *L. virginalis* is one out of eight orchid species considered to be at a very high threat of extinction in Guatemala (category 1 LEA); followed by *L. cochleata*, an endemic species with limited habitat distribution (category 2 LEA). While *L. lasioglossa* is not considered at risk of extinction at the moment, its trade is regulated to avoid their loss (category 3 LEA) (CONAP, 2009). Seaton et al. (2015) suggested that species that are at immediate risk of genetic loss must be a priority for both *ex situ* conservation (seed banking, living collection in botanical gardens, tissue culture, and cryopreservation) and *in situ* conservation approaches (schemes of reintroduction of plants in their natural habitats). In this context, for these *Lycaste* species, more research is required in optimization of conservation strategies that includes sourcing biological material and standardising plant establishment approaches for growth and survival to increase the long-term sustainability of endangered species in conservation hotspots (Yenson et al., 2021).

3.5. Conclusions

It is important to understand the specific nutritional requirements for seed germination of orchids for the successful implementation of conservation plans. Germination percentages and seedling development of *Lycaste* were affected by the species and the asymbiotic culture media assessed.

MS media supported the highest germination percentages with high-quality plantlets (in stage 4). Still, germination did not reach the potential suggested by the tetrazolium test for viability, suggesting that further optimization of media and the methods are required, and / or possibly the seed samples suffered aging or dormancy, however neither seed aging nor dormancy were evaluated in this investigation.

Knudson C media was also a good option for the seed germination of *L. cochleata* and *L. lasioglossa*, but not for *L. virginalis*. The three species showed very poor germination BM-1 media.

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Chapter 4.: Germination and Viability of Seeds of Three Tropical Species of the *Lycaste* Genus Under Different Storage Conditions

4.1. Introduction

The conservation status of the majority of orchid species is declining; habitat loss is the most pressing threat to orchids (and to biodiversity in general); globally, the leading cause of habitat loss is the change of land use for agriculture, logging, mining, and urban expansion (Hagsater et al., 1996). Other significant threats to orchids are over-harvesting (for horticulture, medicine, or food) and climate change (Fay, 2018; Koopowitz & Hawkins, 2012; Wraith & Pickering, 2018). The orchids' complex biology limits dispersal, reproduction, and establishment, adding a challenge to the survival and conservation of these species. Therefore, there is an urgent need to implement initiatives to ensure the survival of rare and threatened orchid species, including *ex situ* conservation (*e.g.,* collection and preservation of germplasm in seed banks) while simultaneous re-introducing plants in their natural habitat (*in situ* conservation) to mitigate the loss that has occurred already (Hagsater et al., 1996).

Ex situ conservation through seed banking is a method that provides a security backup for the genetic diversity, in addition, seeds are easier to handle than whole plants (Pradhan et al., 2022). Plant species and population diversity can be stored in small areas for a long-term period, and for many species, the loss of viability can be small over the storage period, which makes this approach a very convenient option (Li & Pritchard, 2009; Offord et al., 2004). For *ex situ* conservation purposes, seeds are the best choice for plant propagation (and therefore for storage) because they allow plants to be regenerated from genetically diverse materials; their handling is more straightforward and is a cost-effective method (Li & Pritchard, 2009; Nadarajan et al., 2021). Seed banking is also a source of genetic diversity for breeding programs and complements other forms of biodiversity conservation (Hawkes et al., 2012). However, seed banking has some limitations; for simplicity it requires that seeds can be desiccated to low moisture and the seed germinated during and post storage to monitor viability in store and for use in restoration respectively. There is limited information about the seed storage behaviour, viability and germinability ranges and seed dormancy information for many species (Offord et al., 2004).

Seed storage behaviour includes the capacity of seeds to survive desiccation; knowing this is vital because it allows understanding of how amenable the seeds of different species are for long-term storage (Royal Botanical Gardens Kew, 2021). Other parameters like temperature, and storage time can also markedly affect seed survival and germination (Balešević-Tubić et al., 2010; Kuntal, 2014; Pradhan & Badola, 2012). One factor contributing to viability loss is the degradation of seed lipids, which are important protective and storage components of the seed. Different lipids vary in their cooling and melting points, causing seeds to vary in their temperature tolerance (Diantina et al., 2022). Thermal analysis using Differential Scanning Calorimetry (DSC) is a good option for identifying the cooling and melting points of the seeds to find the optimum storage temperature (Nadarajan et al., 2008).

Seed viability is a measure of whether the seed embryo is alive or not. For seed viability testing, chemical (tetrazolium test), physical (gently pushing or cutting the seed), and non-invasive (use of X-rays) methods can be used (Halbritter Rechsteiner et al., 2019; ISTA, 2022). In orchids, the tetrazolium test is a good option because the seeds are very tiny (dust-like) and difficult to handle. Seed germinability is usually measured using emergence tests to determine the germination potential of a seed lot. This enables the seedling that grows from the germinating seed embryo to be accessed to ensure that it has all the structures necessary to develop into a healthy plant *in situ* (ISTA, 2022). The seed germination percentage is essential to give an indication of the number of plants that can be provided for conservation, breeding, or reintroduction programmes in which large amounts of plants are usually required (Diantina, 2020). Germination can be assessed *in* or *ex vitro*; in orchids, *in vitro* germination is a good alternative as the media utilized can provide the nutrient requirements needed for asymbiotic germination (Dutra et al., 2009; Nadarajan et al., 2011). However, one of the constraints is that the media effectiveness can differ significantly between species (even within species sourced from different habitats within the same geographic regions, as these habitats may have resulted in locally adapted ecotypes) (Diantina, 2020; Hufford & Mazer, 2003).

In orchids, *ex situ* conservation through seed storage alone has not been recommended; the suggested conservation method is an integrated conservation strategy (*i.e.*, including a mixture of *ex situ* and *in situ* techniques) (Hawkes et al., 2012), as well as specific regulations in the countries of origin of the species. Seeds of most orchid species are considered orthodox (*i.e.*, desiccation-tolerant that can be stored relatively efficiently in conventional seed bank conditions). Still, they are relatively short-lived under storage (Yenson et al., 2021). Seed longevity depends on the seeds themselves, on the species characteristics (the seed of a species may be short-lived, but within those species constraints, the seed itself may be short-lived if of poor quality) but can also be affected by the storage conditions (Murdoch & Ellis, 2000). Longevity under storage can be extended by controlling the environmental factors, such as temperature, and relative humidity.

Guatemala is a biologically rich country located in the Mesoamerican region within one of the seven hotspots for conservation priorities in the Americas. It is also a very vulnerable region as many species within it (including orchids) are at risk of extinction (Myers et al., 2000). Guatemala has over 734 orchid species, all listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Some have been included in the List of Threatened Species of Guatemala (LEA) (Cameron, 2000; CONAP, 2009; López-Selva Quintana, 2016). *Lycaste* is a neotropical genus in which all its species are listed in CITES, and twelve in LEA (out of 46 species) (CONAP, 2009; WFO, 2021). Guatemalan orchid species have received little attention in terms *ex situ* conservation (although they are categorized as threatened) (CONAP, 2009; Species+/CITES, 2021). Therefore, this research aimed to evaluate some *ex situ* conservation strategies for three Guatemalan orchid species from the *Lycaste* genus (*L. cochleata*, *L. lasioglossa*, and *L. virginalis*) to contribute to local and global biodiversity conservation efforts. For this, the effect of different storage conditions (temperature, RH, and time) on seed viability and germinability was investigated, and DSC measurements were conducted to identify the lipid melting and crystallization point for each species.

4.2. Methods

4.2.1. Seed Material

Ten seed capsules of each species (*L. cochleata*, *L. lasioglossa*, and *L. virginalis*) were collected when ripe in the Experimental Orchid Station of the Archila's Family Farm in Cobán, Guatemala, and sent by courier to Massey University in Palmerston North, New Zealand (1-2 weeks). Upon arrival, seed capsules were allowed to dry in a room at 20°C and 55% RH (4-5 weeks) until release of the seeds (dehiscent capsules). The seeds of each capsule were placed in different glass vessels and stored under the same conditions for 1 to 3 weeks (20°C and 55% RH). After this, a tetrazolium test was performed for each seed capsule to assess the viability after the seeds had dehisced; and based on the results, seeds of five seed capsules per species were selected for this research (the ones with the highest viability rate). Then, to allow the handling of seeds for the different experiments, seeds of the different seed capsules and species were folded in 55 mm diameter filter paper envelopes (Appendix N).

4.2.2. Seed Desiccation

Seed desiccation or drying was conducted to equilibrate the seed samples to three relative humidity (RH) environments (15%, 25%, and 75%). The 15% and 25% RH environments were selected as following conventional seed banking equilibrium relative humidity conditions (Maschinski, 2022), and 75% to mimic the humidity conditions that seeds experience in their natural habitat (Weather Atlas, 2021) (Appendix H).

The target RH environments were generated using lithium chloride solutions (LiCl). LiCl solutions with different salt concentrations were prepared following the technical information sheet 09 defined by Kew Royal Botanic Gardens (2022) (Appendix O). The prepared solutions were monitored at regular intervals, using a hygrometer (Tiny tag data logger TV-4505), until they reached the equilibrated RH at 20 °C (4-5 weeks), (Appendix P). Upon reaching the equilibrium, the LiCl solutions were set in nine labelled air-tight sealed boxes filling about 20% of the total volume of each box (3 boxes per relative humidity) (IP67 waterproof junction box). After this, a metallic support mesh was placed within each box to provide a platform above the LiCl solution for the filter paper envelopes containing the seeds. The boxes containing the LiCl solutions and seed envelopes were placed at 20 °C until the seed reached the RH equilibrium (5-6 weeks) (Appendix Q). The seed's relative humidity status was monitored with a hygrometer (HygroPalm 23-AW).

4.2.3. Seed Storage Trials

For the evaluation of the seed longevity under storage, envelopes with seed samples were placed in sealed aluminium bags (-20°C, 5°C, and 20°C) and cryopreservation vials (for -196 °C) under the combinations of relative humidity and temperatures described in Figure 15.

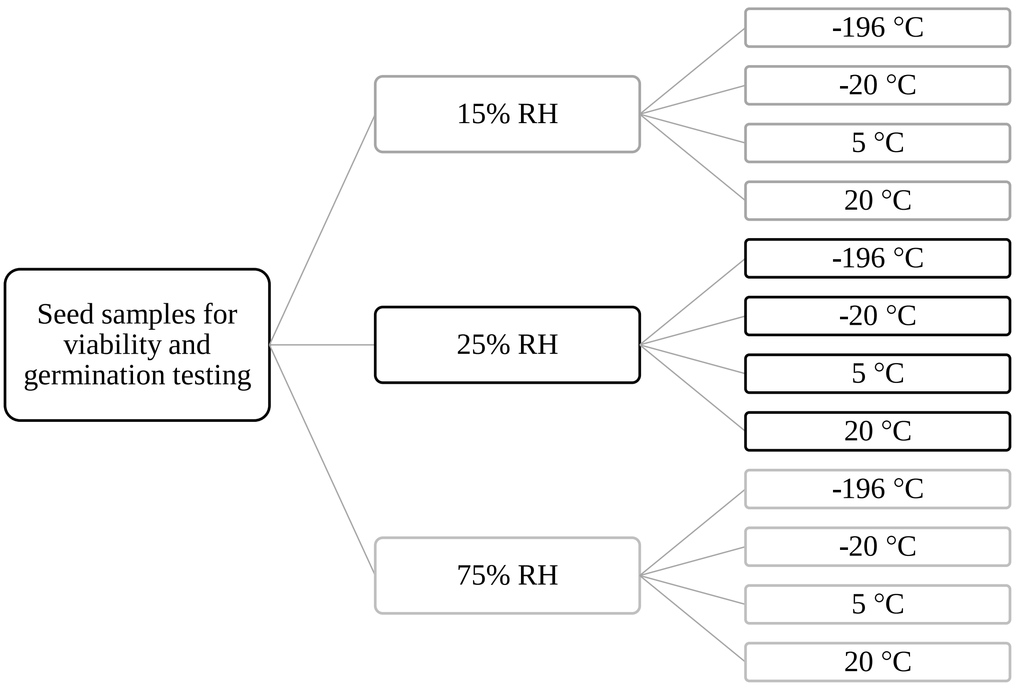

Figure 15. Different combinations of relative humidity and temperatures for the seed storage trials.

The viability and germination percentages of the seeds stored in each of the storage treatments were evaluated at 0 days, 10 days, 60 days, 100 days, 180 days, and 255 days after storage. Viability and germination were assessed following the methods previously described (section 3.2.2. and 3.2.3., respectively). For gemination, the Murashige and Skoog (MS) media with 2% of sucrose was used (Appendix M) (Murashige et. al., 1962).

4.2.4. Differential Scanning Calorimetry

Thermal behaviour in three *Lycaste* orchid seeds equilibrated to 15% relative humidity was determined using a Perkin-Elmer differential scanning calorimeter (DSC 8500) (Shelton, USA), calibrated for temperature and heat flow with zinc (melting point 419.5°C) and indium (melting point 156.6°C). Seeds weighing from 8-20 mg were hermetically sealed into a large volume (60 µL) stainless steel capsule (sample pan) using an O-ring with the aid of a Perkin-Elmer Universal Crimper (Shelton, USA). Sample weight was measured using a micro-balance (Model: XPR6UD5; Mettler-Toledo, Switzerland), and subjected to calorimetric assessment within 5 min of sample preparation. Samples were cooled from 25°C to -100°C and held for 1 min before rewarming to 25°C at a rate of 10°C per min. Exothermic- and endothermic-heat changes were derived from the lipid crystallization and lipid melt endotherm during the cooling and warming cycles. The onset temperature of the lipid thermal transition was determined as the temperature at which the tangent of the sharpest portion of the first peak intersected the baseline. The onset, peak and end temperatures were calculated using PYRIS software. The areas of melt and crystallization peaks were calculated from the area above the baseline and expressed as millijoule (mJ). The enthalpies for these transitions are presented as joule per gram of sample weight (J/g). Calorimetric data were collected from three replicates per treatment.

4.2.5. Data Analysis

For viability, the data analysis was conducted in three steps, firstly the initial viability of fresh seeds was compared between species using a two-way ANOVA. Secondly, viability data after desiccation (drying) was compared using a two-way ANOVA with species and RHs as independent variables. Lastly, viability data after storing the seeds under different storage environments was compared within each species using a Generalized Linear Model (GLM), with RH, temperature and storage time as independent variables. In every case, normality was assessed with the Kolmogorov-Smirnov test using 'R' (R Core Team, 2013); if the data did not follow normality, it was transformed with square root. Pairwise test comparisons were conducted when significant effects were found for individual variables or their interaction. Analyses were conducted using 'IBM SPSS Statistics' software version 28.0.1.1 (IBM SPSS Statistics, 2022).

For germination, the data analysis was conducted as described above but in two phases: germination data after seed desiccation and data after placing the seeds in storage. Graphs were drawn as described above.

4.3. Results

4.3.1. Variation of Seed Viability

4.3.1.1. Viability of Fresh Seeds

The Kolmogorov-Smirnov test confirmed that the initial viability data met the premises of normality ($p = 0.33$). Table 8 shows the seed viability of the fresh seeds of five capsules for each species revealing that there was no statistically significant difference in the initial means between the three species.

Table 8. Viability of fresh seeds of the Lycaste species.

Species	**Initial Viability (%)**
L. cochleata	85 ± 0.25
L. lasioglossa	90 ± 0.97
L. virginalis	75 ± 6.62

Percentages values (mean ± SE).

4.3.1.2. Seed Viability after Desiccation

The seed viability was assessed after desiccation; the data followed the premises of normality according to the Kolmogorov-Smirnov test (p=0.91). The seed desiccation process (but not RH) had a different effect on these three species; a two-way ANOVA revealed that the seed viability of *L. cochleata* was statistically significantly different from *L. lasioglossa* and *L. virginalis* after equilibration (Table 9).

Table 9. Seed viability after equilibrating seed of each species of the Lycaste genus to the required relative humidity (RH).

Species	Viability (%)		
	15 % RH	**25 % RH**	**75 % RH**
L. cochleata	41 ± 3.73 [a]	59 ± 5.15 [a]	55 ± 5.78 [a]
L. lasioglossa	42 ± 6.73 [b]	37 ± 8.65 [b]	27 ± 7.28 [b]
L. virginalis	44 ± 4.01 [b]	30 ± 10.1 [b]	29 ± 8.69 [b]

Percentage values (mean ± SE) are presented in the table. Different letters indicate significant differences across species at different relative humidity (Tukey test, $p < 0.05$).

4.3.1.3. Seed Viability after Storage

The Kolmogorov-Smirnov test showed that the data did not meet the premise of normality ($p < 0.05$), therefore, data were transformed using the square root to meet (or approximate) normality. A general linear model (GLM) revealed a significant effect of the relative humidity, temperature, and days after storage on seed viability in the different species, the graphs show the behaviour of seed viability in time the graphs show the trends of seed viability in time (Table 10; Appendix R; Appendix S; Appendix T). Significant interactions between RH and temperature ($p<0.001$), and RH and storage time ($p=0.01$) for *L. cochleata;* and between RH and temperature ($p<0.001$) for *L. lasioglossa* were also found. Pairwise comparisons between all possible treatment combinations for each species can be found in the Appendix DD and Appendix EE.

Table 10. Percentage of viability in different relative humidity, temperatures, and storage time of Lycaste cochleata, L. lasioglossa and L. virginalis.

	Lycaste cochleata	***Lycaste lasioglossa***	***Lycaste virginalis***
Relative humidity			
15%	44 ± 1.6 [b]	40 ± 1.65 [a]	39 ± 2.2 [a]
25%	46 ± 1.5 [a]	31 ± 1.59 [b]	27 ± 1.9 [b]
75%	39 ± 1.8 [c]	27 ± 1.63 [c]	25 ± 2.1 [b]
Temperature			
-196°C	27 ± 1.2 [c]	20 ± 2.0 [b]	22 ± 2.1 [b]
-20°C	48 ± 1.8 [a, b]	36 ± 1.7 [a]	33 ± 2.7 [a]
5°C	50 ± 1.4 [a]	38 ± 1.7 [a]	36 ± 2.4 [a]
20°C	45 ± 1.9 [b]	34 ± 1.9 [a]	30 ± 2.5 [a, b]
Storage time			
10 days	63 ± 1.2 [a]	50 ± 2.28 [a]	47 ± 3.2 [a]
60 days	53 ± 1.7 [b]	42 ± 1.71 [b]	40 ± 2.5 [a]
100 days	42 ± 1.6 [c]	34 ± 1.71 [c]	31 ± 2.4 [b]
180 days	34 ± 1.3 [d]	24 ± 1.56 [d]	23 ± 2.3 [b, c]
255 days	30 ± 1.5 [d]	20 ± 1.50 [d]	18 ± 2.2 [c]

Non-transformed percentage values (mean ± SE) are presented in the table. Statistical analyses were performed on square root transformed data. Different letters indicate significant differences in the transformed data (Tukey test, $p < 0.05$).

4.3.2. Variation of Seed Germination

4.3.2.1. Seed Germination after Desiccation

After seed desiccation, the germination percentages met the premises of normality (p=0.49). Table 11 shows the two-way ANOVA revealing that the seed germinability after desiccation was statistically significantly different among the three *Lycaste* species.

Table 11. Seed germination of each Lycaste species after equilibrating seed to the required relative humidity (RH).

Species	**Germination (%)**		
	15 % RH	**25 % RH**	**75 % RH**
L. cochleata	28 ± 3.47 [a]	36 ± 2.88 [a]	39 ± 3.24 [a]
L. lasioglossa	13 ± 3.19 [c]	21 ± 2.47 [c]	12 ± 1.11 [c]
L. virginalis	32 ± 5.64 [b]	26 ± 3.54 [b]	19 ± 3.20 [b]

Percentage values (mean ± SE) are presented in the table. Different letters indicate significant differences across species at different relative humidity (Tukey test, $p < 0.05$).

4.3.2.2. Seed Germination after Storage

Seed germinability data did not meet premises of normality ($p < 0.05$) after storage, data was transformed to approximately meet normality. A GLM revealed a significant effect on the seeds in all three species caused by the relative humidity, temperature, and days after storage (Table 12). The graphs show the behaviour of seed germinability in time (Appendix U; Appendix V; Appendix W). Significant interactions between RH and time ($p<0.001$), temperature and storage time ($p=0.003$), and RH, temperature, and storage time ($p=0.017$) for *L. cochleata*; and between RH and temperature ($p=0.04$), and temperature and storage time ($p=0.001$) for *L. lasioglossa*. Pairwise comparisons between all possible treatment combinations for each species can be found in the Appendix FF and Appendix GG.

Table 12. Percentage of seed asymbiotic germination on MS media with 2% of sucrose for different relative humidity, temperatures, and storage time of Lycaste cochleata, L. lasioglossa and L. virginalis.

	Lycaste cochleata	*Lycaste lasioglossa*	*Lycaste virginalis*
Relative humidity			
15%	36 ± 2.3 [a]	35 ± 1.1 [a]	34 ± 1.8 [a]
25%	28 ± 1.8 [b]	27 ± 1.4 [b]	29 ± 2.2 [a, b]
75%	29 ± 2.5 [b]	30 ± 1.3 [b]	25 ± 1.9 [b]
Temperature			
-196°C	17 ± 2.4 [b]	23 ± 1.3 [b]	16 ± 1.8 [b]
-20°C	33 ± 2.2 [a]	34 ± 1.1 [a]	37 ± 2.1 [a]
5°C	36 ± 2.6 [a]	34 ± 1.5 [a]	35 ± 2.2 [a]
20°C	37 ± 2.7 [a]	32 ± 1.7 [a]	33 ± 2.4 [a]
Storage time			
10 days	40 ± 2.3 [a]	38 ± 1.2 [a]	38 ± 2.2 [a]
60 days	26 ± 1.4 [b]	27 ± 0.8 [b]	26 ± 1.3 [b]

Non-transformed percentage values (mean ± SE) are presented in the table. Statistical analyses were performed on square root transformed data. Different letters indicate significant differences in the transformed data (Tukey test, $p < 0.05$).

4.3.3. Variation in Thermal Transitions in Seeds

DSC thermograms provided measurements for critical parameters, including enthalpy of lipid phase transitions between -90°C and 20°C. All three *Lycaste* species showed two lipid crystallization events for the cooling cycle, with the onset and end temperatures spread from -31.8°C to -43.2°C (Figure 16; Table 13). The onset temperature for lipid crystallization for *L. virginalis* is slightly lower, *i.e.*, -35.32°C compared with -31.83°C and -33.39°C in *L. cochleata* and *L. lasioglossa*, respectively.

Upon warming, all three species displayed three lipid melting events spread from -80 to -1.5°C. The lipid melting events for *L. cochleata* seeds ranged from -79 to -6°C, whereas for *L. virginalis* it ranged from -79 to -1.9°C and from -80 to -1.5°C for *L. lasioglossa*. The average of total melt enthalpy (cumulative value of all three melting events) was 16.5 J/g for *L. cochleata*, 13.8 J/g for *L. virginalis*, and 31.9 J/g for *L. lasioglossa* (Table 13). *L. lasioglossa* showed the highest melt area and enthalpy, whereas *L. virginalis* showed the smallest melt area and lowest melt enthalpy (Figure 17; Table 13).

Graphs of the calorimetric data collected from three replicates per species can be found in the appendix (*L. cochleata* Appendix X, Appendix Y; *L. lasioglossa* Appendix Z, Appendix AA; *L. virginalis* Appendix BB, Appendix CC).

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Table 13. Cooling and warming thermodynamic properties of three Lycaste species seeds following equilibration to 15% relative humidity.

Species	Sample Weight (mg)	Cooling: Onset temperature of lipid crystallisation (°C)	Cooling: End temperature of lipid crystallisation (°C)	Cooling: Area of lipid crystallisation (mJ)	Cooling: Enthalpy of lipid crystallization (J/g)	Warming: Onset temperature of lipid melt (°C)	Warming: End Temperature of lipid melt (°C)	Warming: Area of Melt (mJ)	Warming: Enthalpy of lipid melt (J/g)
L. cochleata	18.3 ± 2.39 [a]	-31.83 ± 5.76	-43.23 ± 10.0	-13.05 ± 5.20 [b]	-1.40 ± 0.27 [b]	-44.63 ± 27.89	-31.14 ± 26.26	98.92 ± 68.15	16.46 ± 4.198
L. lasioglossa	9.63 ± 0.98 [b]	-33.39 ± 5.34	-39.85 ± 5.53	-3.261 ± 0.82 [a]	-0.68 ± 0.07 [a]	-46.12 ± 27.21	-30.57 ± 27.91	104.5 ± 94.78	31.93 ± 14.95
L. virginalis	7.51 ± 0.56 [b]	-35.32 ± 4.09	-41.05 ± 7.26	-1.479 ± 0.94 [a]	-0.40 ± 0.05 [a]	-44.03 ± 28.79	-30.41 ± 27.58	34.07 ± 33.35	13.79 ± 11.37

Superscript of different letters within a column indicates significantly difference at p < 0.05 based on a one-way ANOVA analysis (Tukey HSD test). Data are presented as mean ± SD of the observed peaks in the figures 16 and 17 ($p < 0.05$) for three different treatments.

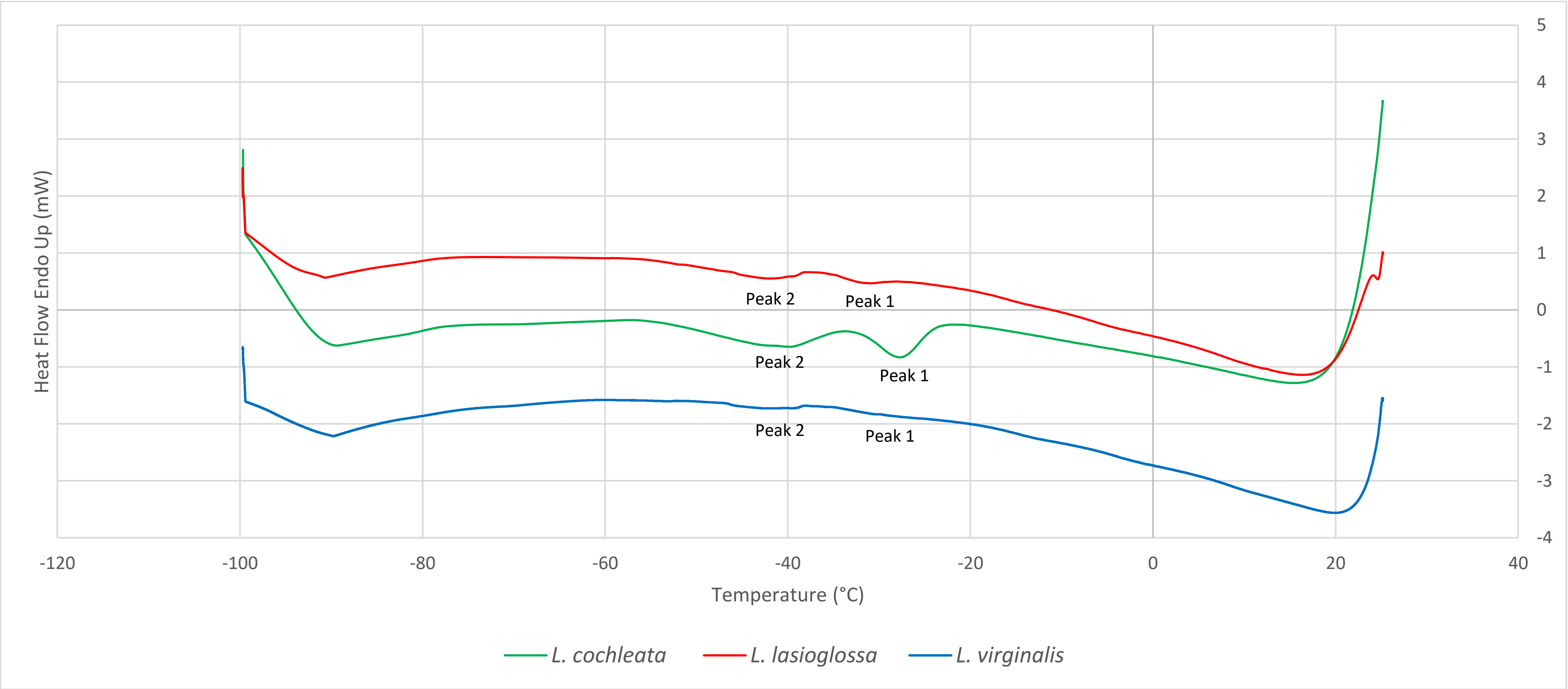

Figure 16. Cooling thermograms for the Lycaste species.

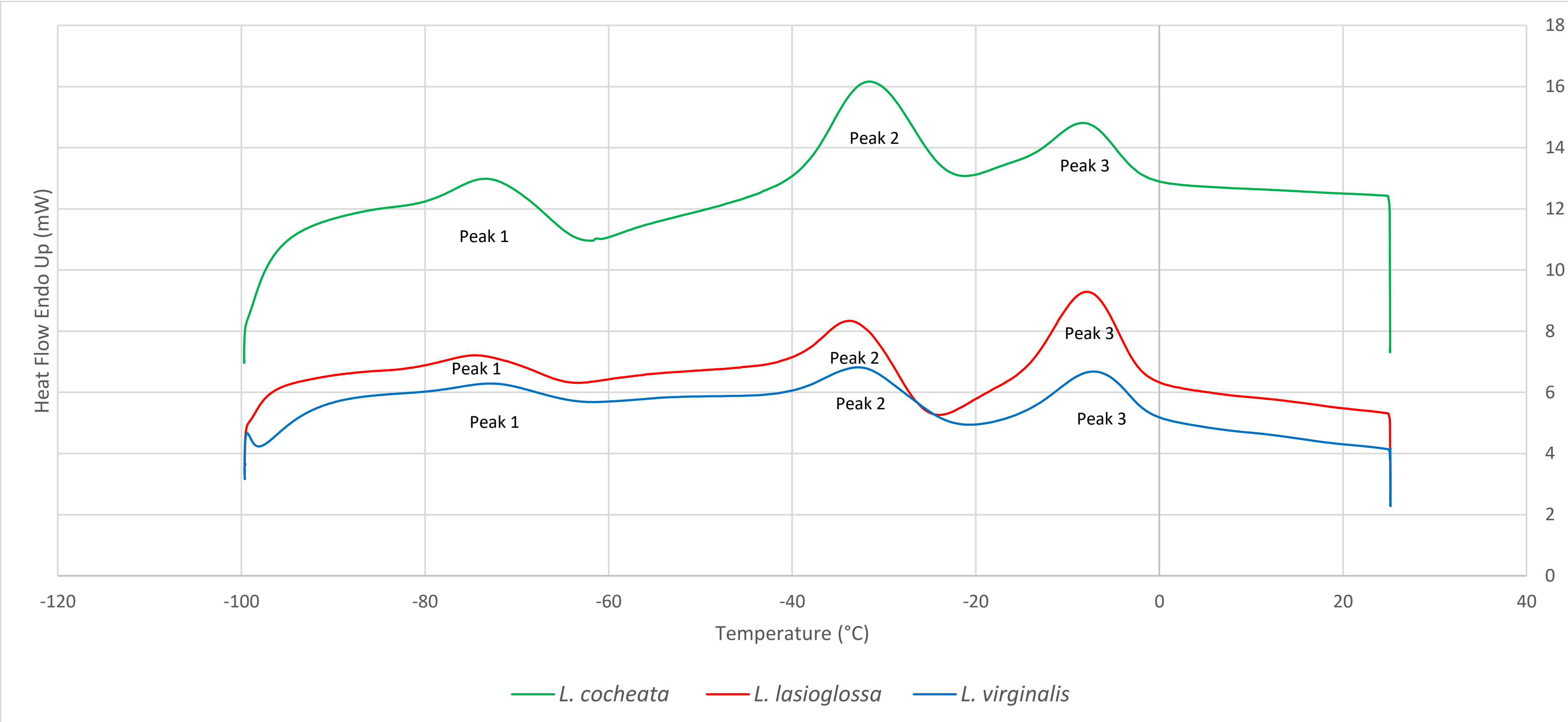

Figure 17. Warming thermograms for Lycaste species.

4.4. Discussion

Experiments on three Guatemalan *Lycaste* species were conducted to evaluate their suitability for *ex situ* conservation in a seed bank; for this, the seeds were desiccated in three different relative humidity environments (15%, 25%, and 75%). The viability of the seeds notably dropped during equilibration to all three storage RH (*L. cochleata* from 85% to 41% at 15RH, 59% at 25RH, 55% at 75RH; *L. lasioglossa* from 90% to 42% at 15RH, 37% at 25RH, 27% at 75RH; and *L. virginalis* from 75% to 44% at 15RH, 30% at 25RH, 29% at 75RH), which suggests that orchid seeds can be dried to the low seed moistures typical of seeds showing orthodox seed behaviour (Hong & Ellis, 1996), however the continued loss of viability during moisture equilibration without significant differences across the different relative humidity suggests the behaviour is not typically orthodox (Table 10). This pattern may be associated with the crystallization of storage lipids (Walters et al., 2021), which should not be problematic for orthodox seeds, however it could accelerate the seed deterioration.

There is little information on the seed desiccation response of *Lycaste* species, however, for *L. skinneri x Biifrenaria harrisoniana* the seed storage behaviour has been previously reported as orthodox (desiccation tolerant) (Royal Botanical Garden Kew, 2022). Although seed banking is recommended for the long-term conservation of orchid seeds, for these species and other species of the *Lycaste* genus, further investigation is needed to confirm the seeds' post-desiccation sensitivity and their aging kinetics (Nadarajan et al., 2021). For short-lived orthodox species other storage alternatives must be considered; yet, for short to medium-term storage, the longevity can be kept by reducing temperatures as low as possible (Berjak et al., 2004).

In this study, seed viability was assessed with the tetrazolium staining test, which provides information on the quality of the seeds, and has been previously utilized successfully in epiphytic orchids (Diantina, 2020). In all three species, the viability continually decreased in storage. After 255 days, the best combination of relative humidity and temperature for storage for *L. cochleata* was 25% RH at 5°C (20% viability) (Table 10; Appendix R); *L. virginalis* had similar viabilities when equilibrated at 15% RH and stored at 5°C, 20°C, and -20°C (15%, 11%, and 10%, respectively) (Table 10; Appendix T); and *L. lasioglossa* accomplished similar outcomes when dried at 15% and 25% RH at 5°C, 20°C, and -20°C (viabilities were between 10% to 7%) (Table 10; Appendix S).

To assess germination, MS media with 2% sucrose was chosen; the media was selected based on the results of Diantina (2020), but also correlates with our results on the effect of media on *in vitro* germination of three species of the *Lycaste* genus (Chapter 3), although the decision to use MS media was made prior to these results being available. Similar to the viability data, in all three species, the germination percentage in general continued to decreased under storage, excluding some treatments, where the germination after ten days of storage increased, but then after 60 days decreased. Germination after 100 days, 180 days, and 255 days of storage is still pending (germination scoring is not accomplished until six months after sowing to include plants in the final stage of development). However, the preliminary data shows that *L. cochleata* is germinating without significant differences in all combinations of RH and temperature (Table 12; Appendix U); *L. lasioglossa* is germinating better for seeds dried at 15% and 75% RH at -20°C and 20°C (Table 12; Appendix V); and *L. virginalis* seeds are germinating better when dried at 25% and stored at 20°C and 5°C (Table 12; Appendix W). So far, the germinability outcomes have shown favourable results compared with the viability when dried at 15% RH and stored at 20°C and -20°C for *L. lasioglossa*; and with seeds dried at 15% RH and 25% RH and stored at 5°C and 20°C for *L. virginalis*.

Both the viability and germination data varied across the *Lycaste* species evaluated; this is expected as in most plant species, the degree of viability and germinability of the seeds varies significantly between and within populations (Diantina, 2020). In the three species, the viability was higher than the germinability; the gap between them could be because the media used did not entirely fulfil the nutritional germination requirements of each species; however, to properly conclude this, further research on asymbiotic and symbiotic germination needs to be done. The variation in the viability and germination between the species could be due to several factors, such as the genetic basis of the species, the environmental conditions where the seeds developed (seed capsules for this research did not grow in a controlled environment), the position of the seed capsule on the plant and the age of the mother plant during seed setting and growing (McDonald, 1999; Seaton et al., 2010). All the seed capsules used in this research were pendulum suggesting capsule position may not have been a factor in the three *Lycaste* seed lots analysed.

In addition, the thermophysical properties of the lipids of the seeds were evaluated through differential scanning calorimetry (DSC). For this, exothermic and endothermic peaks and their areas in DSC thermograms were determined (Table 13). The transitions observed in the cooling and warming thermograms result from the lipids. In all three species, the thermogram presented in Figure 16 shows two exothermic events during cooling, a moderately sharp peak at approximately -24°C (Peak 1) and a broader, flatter peak at about -42°C (Peak 2). Whereas the thermogram in Figure 17 shows three endothermic events for each species during warming, the three peaks are relatively sharp at approximately -75°C, -35°C, -8°C (peaks 1, 2, and 3, respectively).

It has been reported that some dry seeds of oily-rich seed species can lose germinability when rapidly cooled to liquid nitrogen temperatures (-196°C) due to a glass transition event (*i.e.*, vitrification of the lipid compounds at around -90°C) (Pritchard & Seaton, 1993). Based on that premise, the damaging effects of storage of seeds equilibrated at 15% RH of *L. cochleata*, *L. lasioglossa*, and *L. virginalis* at -196°C could be due to lipid vitrification. In addition, the reduced longevity in the *Lycaste* seeds may be associated with the occurrence of the lipids with transitions taking place over the temperature range of -75 to -8 °C during warming; Figure 17, thus showing the lipids are in a metastable state, which could explain the rapid loss of viability at -20°C.

For the long-term storage of seeds, temperatures close to or within the lipid phase transitions should be avoided to avoid rapid seed deterioration due to unforeseen consequences to the seed cell structure (Hamilton et al., 2013; Van der Walt, 2022; Walters et al., 2001). This principle is associated with the recommendations from this research, where the storage of seeds at a temperature a few degrees above that at which the lipids have completed their thermophysical transitions appears to offer the best longevity in the *Lycaste* seeds (*i.e.*, 5°C followed by 20°C; Table 10; Table 12). All seeds showed significant loss of viability during equilibration to the storage moisture, irrespective of the relative humidity the seed was being equilibrated to. This viability decline slowed when seeds were placed in storage. Depending on the species, the best relative humidity for orchid seed storage was 15% and 25% relative humidity. It is likely that in the ten weeks taken for the seed to equilibrate to 15% or 25% relative humidity seeds were deteriorating. In future experiments, a control treatment of storing the seed at low temperature as soon as it had dehisced should be included so any effect of equilibration to lower moisture on seed viability can be determined.

4.5. Conclusions

Lycaste virginlis, L. cochleata, and *L. lasioglossa* are endangered species from Mesoamerica and should be considered a high conservation priority. In orchids, an integrated conservation approach is recommended (*i.e.*, including a mixture of *ex situ* and *in situ* techniques). In this regard, this research aimed to find the appropriate combination of relative humidity and temperature for seed storage to support *ex situ* conservation efforts of this species.

- Seeds of *L. virginalis* exhibit extended longevity at 5°C when stored at an equilibrium relative humidity of 15%.
- Seeds of *L. cochleata* have longer longevity at 5°C when stored at an equilibrium relative humidity of 25%.
- Seeds of *L. lasioglossa* exhibit extended longevity when stored at 5°C, 20°C, and -20°C when stored at equilibrium relative humidity both above and below 15% and 25%.

Differential scanning calorimetry (DSC) shows lipids' transitions over the temperature range -42 to -24°C during cooling and between -75 to -8°C during warming. Seed storage close to or within the lipid phase transitions should be avoided for long-term conservation of seeds. Based on our findings, storage temperatures below -75°C is recommended for the storage of three *Lycaste* species; however, further research needs to be done to validate these findings.

4.6. References

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Chapter 5.: General Discussion

5.1. Research Outcomes

This study was conceived with the overarching goal of contributing to the knowledge base on Guatemalan orchids. For this, three species were selected based on their cultural importance, availability of germplasm resources, their threat of extinction, and the absence of data required for their conservation:

- *Lycaste virginalis*, is considered as having a significant threat of extinction.
- *Lycaste lasioglossa*, is not considered at risk of extinction yet, but it could be if its use and trade are not regulated.
- *Selbyana cochleata* (synonym of *L. cochleata*), is an endemic species (*i.e.,* distribution limited to a specific habitat).

All these *Lycaste* species have an epiphyte growth form, are distributed in a tropical habitat and are from the same origin (Coban, Alta Verapaz, Guatemala). With the objective of generating information that could be useful for *ex situ* conservation through seed banking for this threatened species, this work was elaborated, and it is compiled in four chapters:

Chapter 1 presents a comprehensive review of the literature exploring global orchid biodiversity and conservation status, anthropogenic threats to orchids, orchid conservation status in Latin America, the *Lycaste* genus and its importance, and orchid conservation strategies. This review evidences the dire state of orchid conservation worldwide and in Latin America. In summary, orchids are the second-largest family of flowering plants, and most of its species are threatened due to over harvesting, habitat destruction, and climate change. They also have a complex biology, relying on symbionts for pollination, germination, and establishment (Chase et al., 2015; Fay, 2018). Therefore, it is imperative to develop conservation strategies to preserve orchid biodiversity. Guatemala is a biodiversity hotspot having over 700 orchid species (Cameron, 2000) *Lycaste* species have high cultural value in Guatemala, but little is known about their taxonomy, habitat distribution, genetics basis, population dynamics, dispersal system, reproductive behaviour, and germplasm physiology

(IUCN SSC, 2017), and no previous studies have explored their suitability for *ex situ* conservation. Therefore, this research aimed to fulfil knowledge gaps by characterizing seed morphology, investigating the effect of media on *in vitro* asymbiotic seed germination, and exploring seed viability and germination under different storage conditions.

Chapter 2 is a characterization of the seed capsules and seed morphology. The qualitative traits, such as physical appearance (*e.g.,* capsules with six ribs, elongated seeds, prolate-spheroid embryos), and colour were similar across the three species. Conversely, the quantitative traits of the seed capsules and seed varied significantly; the seed capsules' length varied within species, *L. cochleata* ranged from 7 to 9 cm, *L. lasioglossa* from 9 to 12 cm, and *L. virginalis* from 8 to 13 cm, the seed capsule dimensions may be species-specific traits.

The seed micro-morphological dimensions also vary among species. Based on the seed length, all three species are small-sized (*L. cochleata* 210 µm, *L. lasioglossa* 230 µm, and *L. virginalis* 260 µm) (Barthlott et al., 2014), with relative large-sized embryos (thus low SV/EV ratio; *L. cochleata* 1.22, *L. virginalis* 1.45, and *L. lasioglossa* 1.77) (Diantina, 2020); and air volume that widely ranges from 17% to 42% (*L. cochleata* 17%, *L. virginalis* 30%, and *L. lasioglossa* 42%,). *Lycaste* orchid seeds develop under dense canopies where they do not need to travel very long distances, and have developed to be dispersed by waterdrops falling onto the orchid plant from the canopy (hydrochore), (*i.e.,* because they are epiphytic species, with small seeds and relatively large embryos) (Arditti & Ghani, 2000). It is important to know the air volume of the seeds because it may contribute to seed deterioration due to the oxidation of the fatty acids (McDonald, 1999). Further research is needed to validate the findings of this study; more *Lycaste* species should be included to compare the seed morphology with the natural habitat distribution of the species, their dispersal mechanisms and germination success.

In Chapter 3, the effect of media on *in vitro* asymbiotic seed germination was evaluated for the three *Lycaste* species. The medias assessed were: Murashige and Skoog (MS), Knudson C, and terrestrial orchid medium BM-1. The three species germinated well and produced normal (healthy) plantlets on MS media. In Knudson C media, the germination rate was similar than on MS media for *L. cochleata* and *L. lasioglossa,* but very low for *L. virginalis;* still, healthy (high-quality) plantlets were grown. Conversely, in BM-1 media, the three species had very poor germination, and the quality of the plantlets was extremely poor (*i.e.*, decayed, necrotic, or discolored plantlets).

In the three *Lycaste* species, the germination percentage of the three media was lower than the viability percentage, indicating that none of the media assessed reached the potential germination suggested by the viability measured by the tetrazolium test. Further optimization of the media and methods is required to reach a higher germination on artificial media. Additionally, seed dormancy and aging should be studied to determine if they are a factor in low seed germination compared with the viability measured by the viability test.

Chapter 4 explored the effect of different storage conditions (RH, temperature, and time) on seed viability and germinability, aiming at *ex situ* conservation through seed banking. The seed viability was first tested when the seeds were fresh. After that, viability and germination were tested simultaneously after the seeds were equilibrated at different relative humidity, and at different times after placing the seeds in different storage conditions.

Initial seed viability was high but dropped significantly after desiccation across the three species. This could happen due to several reasons, primarily that it took around 8-10 weeks between the natural release and desiccation of the seeds (due to limited resources); in that period, the seeds were in a 55% relative humidity environment, which could accelerate the seed deterioration process. Alternatively, the desiccation process itself may impact the species' viability which may not appear until the seed has been in store for several months post-desiccation. Further research is needed to elucidate the cause of the viability decline during equilibration at 20°C.

In summary, the seed storage tests showed that the viability of *L. cochleata* and *L. virginalis* seeds is best maintained when seed is dried to 15% or 25% relative humidity and stored at 5 °C. For *L. lasioglossa*, the seed viability is also best maintained when dried to 15% or 25% of relative humidity, but it can be stored in a range from -20°C to 20°C.

Differential scanning calorimetry (DSC) was also conducted to identify each species' lipid melting and crystallization points. The lipids' transitions range from -42°C to -24°C during cooling and between -75°C to -8°C during warming. It has been recommended that seed storage should be avoided close to or within the lipid phase transitions; based on the DSC results, storage temperatures below -75°C is recommended for the storage of three *Lycaste* species. Still, more research is needed to validate these findings, as only 6.5% of the total species of the *Lycaste* genus (3 species out of 46) were studied.

All together this work provides the first in-depth study of the seed morphology, asymbiotic *in vitro* germination, and effect of storage conditions on viability and germination of *Lycaste* species, contributing to their *ex situ* conservation. Only a few studies have investigated similar aspects for other orchid species, for example, Diantina (2020) compared the seed morphological characteristics of six orchid species originating from different habitats and biogeographical regions (*Dendrobium strebloceras, D. lineale*, *D. cunninghamii* (epiphytic), *Gastrodia cunnninghamii*, *Pterostylis banksii and Thelymitra nervosa* (terrestrial)). Diantina (2020) also compared the asymbiotic *in vitro* germination and seedling development, and cryopreservation methods for those species. The findings of that study are similar to the ones in this research where a wide diversity in the quantitative morphological seed traits was found. MS media was also a good option for *in vitro* asymbiotic germination in epiphytic orchid species.

To date no one has reviewed the conservation status of orchids in Latin America. This study evidences the need of generating a more robust database for Guatemalan (and other Latin American) orchids and devise strategies for their conservation. While *ex situ* conservation works well for multiple species, this study reveals some challenges to applying the process to *Lycaste*, like rapid viability loss after moisture equilibration, possibly associated with transporting and manipulating seeds.

As part of global conservation efforts, a programme of the United Nations Convention on Biological Diversity denominated Global Strategy for Plant Conservation (GSPC) was established with the aim of holding at least 75% of threatened plant species in *ex situ* collections by 2020, preferably in the country of origin of the species. Currently, seed banks in 350 botanic gardens in 74 countries hold seeds from 57,051 species (Antonelli et al., 2020).

The Kew's Millennium Seed Bank (MSB) holds the world's most diverse store of seeds from wild species; seed collection has been undertaken in more than 95 countries (collectively recognized as the Millennium Seed Bank Partnership (MSBP)). The MSB holds 97,978 collections of 39,940 species from 190 countries and territories (updated on 01-July-2022). Del Valle University of Guatemala is listed as research collaborator of MSBP.

In Guatemala, the main seed banks according to CONAP (2012) are:

- Institute of Agricultural Science and Technology (ICTA): for maize, beans, wheat, rice, tomato, potato, and chili seeds.
- Agronomy Faculty of the University of San Carlos of Guatemala (FAUSAC): for peanut, chili, chilacayote, güicoy, miltomate, and blackberry seeds.
- Cunsuroc-USAC: for vara beans, passion fruit, nance, and native chili seeds.
- Conuroc-USAC: for forest species.
- Private sector, Agroselva, S.A., Agrokan, Pilones de Antigua, and P&C Maderas: together store more than 100 tree species, primarily coniferous and broadleaf.

5.2. Research Recommendations

Information on seed banking of orchids in Guatemala has not been found online; however, further enquiries to the local authorities, local stakeholders, private collectors, and conservationists could help determine if an institution is storing orchid seeds. Additionally, considering the implementation of stricter conservation policies can help to regulate the use of the most threatened species. As well as the establishment of an institution with seed banking and live collections of threatened orchid species, and sustainable farming practices (*i.e.*, seed preservation, limited harvests, contribution to wild populations), which will also return the cultivation of *ex situ* species to indigenous environments and locations.

Through this research project, we have identified areas where further research would be beneficial and supplement the results of this study. These are:

- The effect of the genetic basis of the species or intra-plant competition effects during seed setting and maturation on the seed's quantitative traits.
- Further germination studies are needed to find optimum media for the *Lycaste* species studied; for example, potential changes in the composition of the media could be

evaluated (*i.e.,* change the relative portions of compounds in the media to improve germination), or testing other media.

- To complement the results of the experimental storage tests, other factors that could affect the viability of the seeds after desiccation would need to be studied in detail. For example, seed maturation, collection frame time, time variations between the natural release of the seeds and desiccation, different desiccation methods, transportation effect, seed handling alternatives, and different storage conditions in terms of RH and temperature.
- Expansion of the study to include species beyond the 6.5% of the *Lycaste* genus studied here to determine if standardized protocols can be developed. This could be further expanded to other closely related taxa.

5.3. References

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Appendix Chapter 2

Forma LI

CONSEJO NACIONAL DE AREAS PROTEGIDAS
CONAP
PRESIDENCIA DE LA REPUBLICA
GUATEMALA, C. A.

Nº 00284 -B

LICENCIA DE INVESTIGACION

No. DRVE-001/2020

Nombre: Maria Alejandra Alfaro Pinto   No. Reg. I-DRVE-001-2020

Nacionalidad: GUATEMALTECA   Identificación: 2124 37739 0114

Institución:

Si existe contrato administrativo que ampara esta Licencia, especificar referencia:

Título de la Investigación: "Caracterización Morfológica y Química de las semillas del genero Lycaste y su comportamiento en diferentes condiciones de almacenamiento"

Institución nacional que avala la investigación: Universidad San Carlos de Guatemala

Nombre e identificación de otros investigadores participantes:

1.
2.
3.
4.
5.

Fecha de Emisión: Cobán, Alta Verapaz, 18 de Marzo 2020

Fecha de Vencimiento: Cobán, Alta Verapaz, 17 de Marzo 2021

Firma de Recibido

Appendix A. Research License issued by CONAP.

Forma LCA

CONSEJO NACIONAL DE AREAS PROTEGIDAS (CONAP)
GUATEMALA, C.A.

Serie A Nº 002143

LICENCIA DE COLECTA O APROVECHAMIENTO DE VIDA SILVESTRE

1. Nombre o razón social: María Alejandra Alaro Pinto
   Dirección: 2a. Calle 5-107 Colonia Lomas del Capitán, Amatitlán, Guatemala
   Teléfono: 4143-1949 Identificación: DPI: 2124 37739 0114

2. Tipo de colecta: comercial ______ científica XXX aficionada ______

3. No. de registro: I-DRVE-001-2020

4. Especies a colectar:

ESPECIES	CANTIDAD	FORMA
Lycate virginalis	10	Cápsulas con semilla
Lycaste cochleata	10	Cápsulas con semilla
Lycaste cruenta (Lndl.) Lndl.	10	Cápsulas con semilla
Lycaste lasioglossa Rchb.f.	10	Cápsulas con semilla
Lycaste guatemalensis Archila	10	Cápsulas con semilla

5. Ubicación de la colecta o aprovechamiento:
   Estación Experimental de Orquídeas de la Familia Archila

6. Número de registro de la propiedad: ______

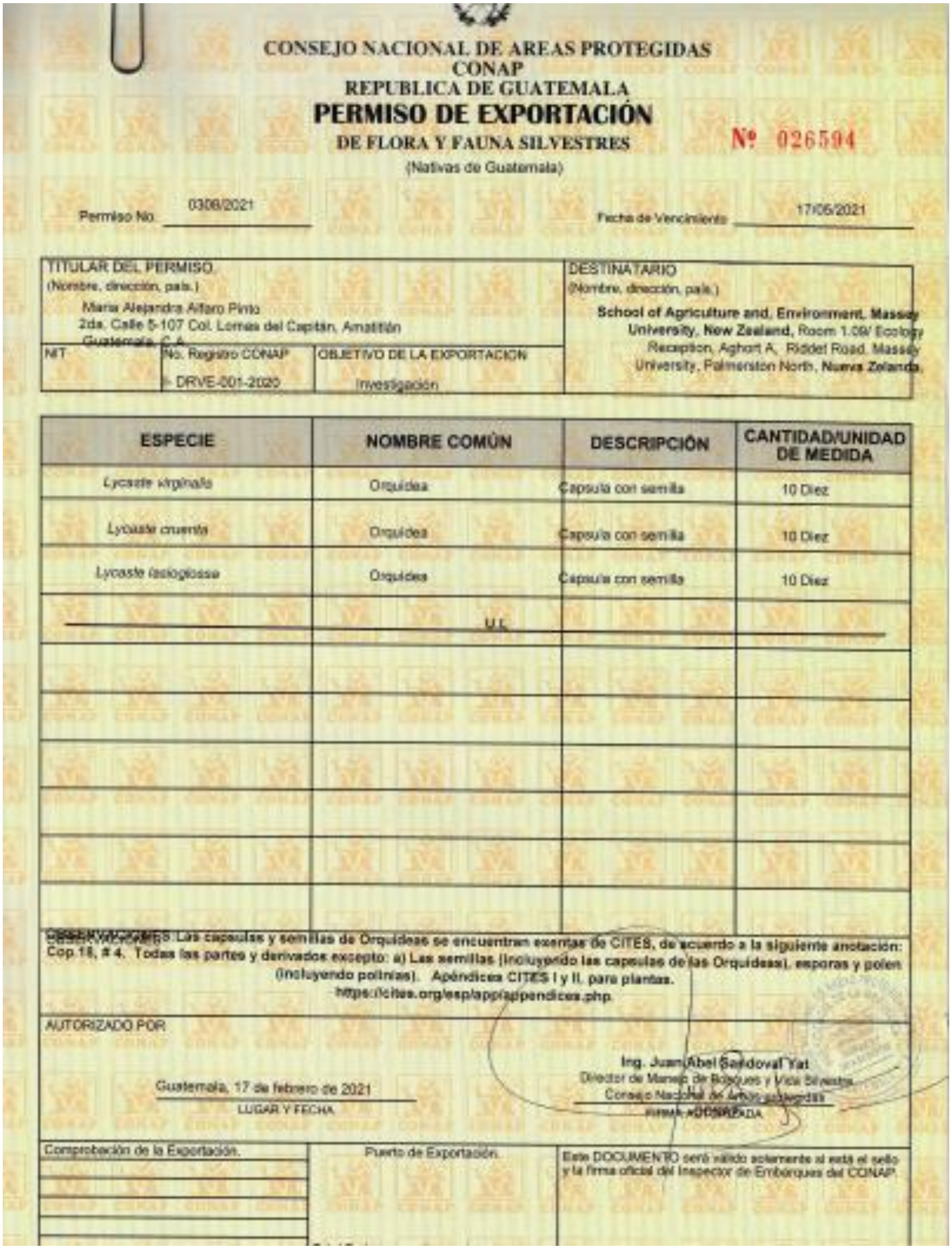
7. Técnicas de colecta autorizadas: ______

8. Nombre de colector(es) autorizado(s) e identificación: ______

9. Localidad de traspaso de material colectado:
   Escuela de Agricultura y Ambiente, Universidad Massey, Nueva Zelanda

Lugar y fecha de emisión: Cobán, Alta Verapaz, Marzo 18 de 2,020

Appendix B. Collection License for Use of Wildlife issued by CONAP.



CONSEJO NACIONAL DE AREAS PROTEGIDAS
CONAP
REPUBLICA DE GUATEMALA
PERMISO DE EXPORTACIÓN
DE FLORA Y FAUNA SILVESTRES
(Nativas de Guatemala)

Nº 026594

Permiso No. 0308/2021

Fecha de Vencimiento 17/06/2021

TITULAR DEL PERMISO
(Nombre, dirección, país.)
Maria Alejandra Alfaro Pinto
2da. Calle 5-107 Col. Lomas del Capitán, Amatitlán
Guatemala, C.A.

NIT

No. Registro CONAP: I- DRVE-001-2020

OBJETIVO DE LA EXPORTACION: Investigación

DESTINATARIO
(Nombre, dirección, país.)
School of Agriculture and Environment, Massey University, New Zealand, Room 1.09/ Ecology Reception, Aghort A, Riddet Road, Massey University, Palmerston North, Nueva Zelanda.

ESPECIE	NOMBRE COMÚN	DESCRIPCIÓN	CANTIDAD/UNIDAD DE MEDIDA
Lycaste virginalis	Orquidea	Capsula con semilla	10 Diez
Lycaste cruenta	Orquidea	Capsula con semilla	10 Diez
Lycaste lasioglossa	Orquidea	Capsula con semilla	10 Diez
	UL		

OBSERVACIONES: Las capsulas y semillas de Orquideas se encuentran exentas de CITES, de acuerdo a la siguiente anotación: Cop 18, # 4. Todas las partes y derivados excepto: a) Las semillas (incluyendo las capsulas de las Orquideas), esporas y polen (incluyendo polinias). Apéndices CITES I y II, para plantas.
https://cites.org/esp/app/appendices.php

AUTORIZADO POR

Guatemala, 17 de febrero de 2021
LUGAR Y FECHA

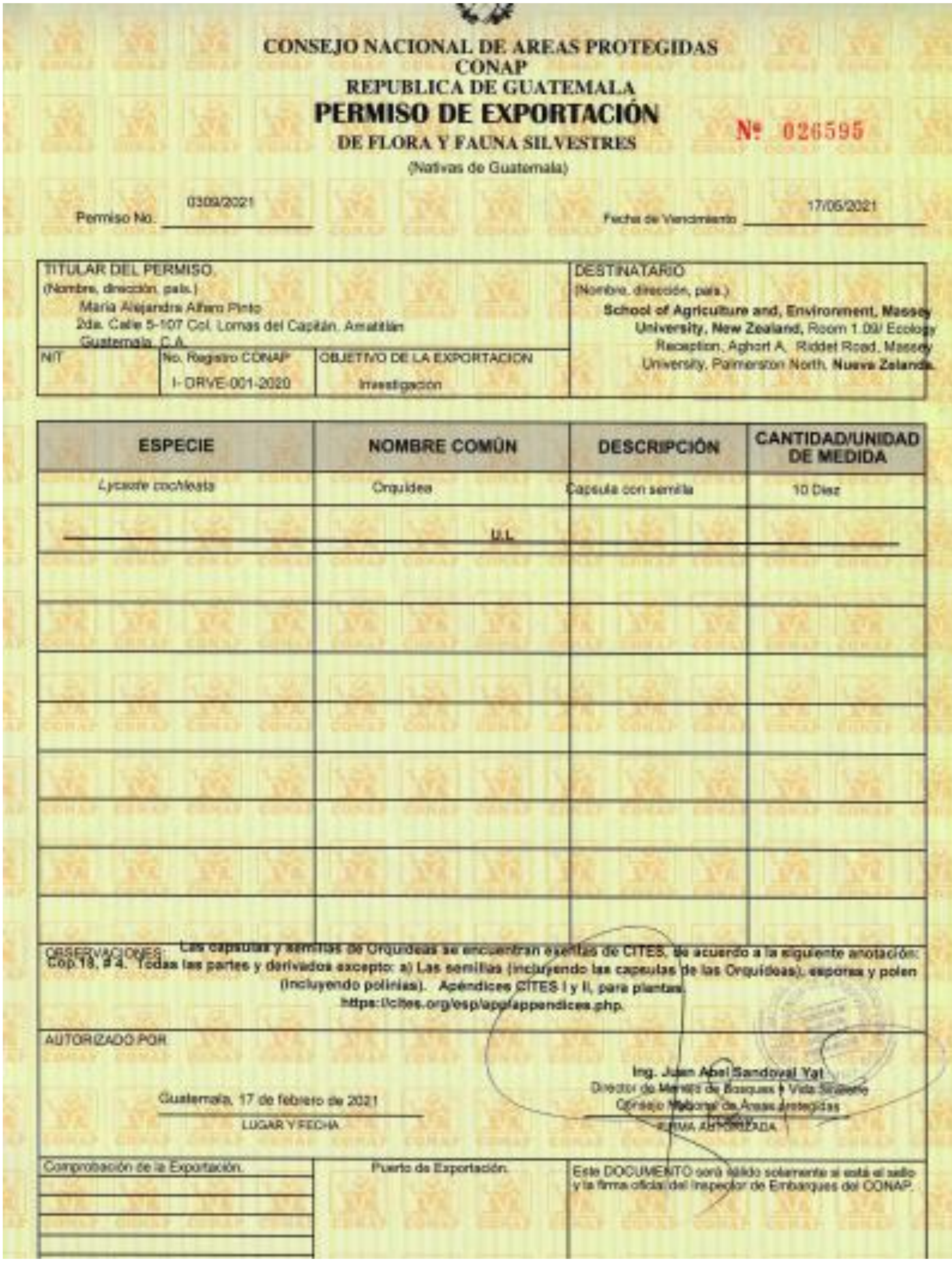
Ing. Juan Abel Sandoval Yat
Director de Manejo de Bosques y Vida Silvestre
Consejo Nacional de Áreas protegidas
FIRMA AUTORIZADA

Comprobación de la Exportación.

Puerto de Exportación.

Este DOCUMENTO será válido solamente si está el sello y la firma oficial del Inspector de Embarques del CONAP.

Appendix C. First Exportation Permit issued by CONAP.



CONSEJO NACIONAL DE AREAS PROTEGIDAS
CONAP
REPUBLICA DE GUATEMALA

PERMISO DE EXPORTACIÓN

DE FLORA Y FAUNA SILVESTRES

(Nativas de Guatemala)

Nº 026595

Permiso No. 0309/2021

Fecha de Vencimiento 17/06/2021

TITULAR DEL PERMISO.
(Nombre, dirección, país.)
María Alejandra Alfaro Pinto
2da. Calle 5-107 Col. Lomas del Capitán, Amatitlán
Guatemala, C.A.

NIT

No. Registro CONAP: I-DRVE-001-2020

OBJETIVO DE LA EXPORTACION: Investigación

DESTINATARIO
(Nombre, dirección, país.)
School of Agriculture and, Environment, Massey University, New Zealand, Room 1.09/ Ecology Reception, Aghort A, Riddet Road, Massey University, Palmerston North, **Nueva Zelanda.**

ESPECIE	NOMBRE COMÚN	DESCRIPCIÓN	CANTIDAD/UNIDAD DE MEDIDA
Lycaste cochleata	Orquidea	Capsula con semilla	10 Diez
	U.L.		

OBSERVACIONES: Las capsulas y semillas de Orquideas se encuentran exentas de CITES, de acuerdo a la siguiente anotación: Cop.18, # 4. Todas las partes y derivados excepto: a) Las semillas (incluyendo las capsulas de las Orquideas), esporas y polen (incluyendo polinias). Apéndices CITES I y II, para plantas. https://cites.org/esp/app/appendices.php.

AUTORIZADO POR

Guatemala, 17 de febrero de 2021
LUGAR Y FECHA

Ing. Juan Abel Sandoval Yat
Director de Manejo de Bosques y Vida Silvestre
Consejo Nacional de Areas Protegidas
FIRMA AUTORIZADA

Comprobación de la Exportación.

Puerto de Exportación.

Este DOCUMENTO será válido solamente si está el sello y la firma oficial del Inspector de Embarques del CONAP.

Appendix D. Second Exportation Permit issued by CONAP.

GOBIERNO DE LA REPÚBLICA DE GUATEMALA
MINISTERIO DE AGRICULTURA, GANADERÍA Y ALIMENTACIÓN -MAGA-
VICEMINISTERIO DE SANIDAD AGROPECUARIA Y REGULACIONES -VISAR-
DIRECCIÓN DE SANIDAD VEGETAL -DSV-

CERTIFICADO FITOSANITARIO DE EXPORTACIÓN
PHYTOSANITARY CERTIFICATE FOR EXPORT

No. DSV 1151104

NEW ZEALAND

Expreso Aéreo, Guatemala
febrero 17, 2021

CERTIFICACIÓN CERTIFICATION

DESCRIPCIÓN DEL ENVÍO DESCRIPTION OF THE CONSIGNMENT

MARIA ALEJANDRA ALFARO PINTO 2DA. CALLE 5-107 COLONIA LOMAS DEL CAPITAN, AMATITLAN, GUATEMALA

SCHOOL OF AGRICULTURA &ENVIRONMENT, MASSEY UNIVERSITY ROOM 1.09ECOLOGY RECEPTION, AGHORT A, RIDDET ROAD,MASSEY UNIVERSITY,PALMERSTON NORTH, NEW ZEALAND

N/A	1.00 BOX	0.7000 KGS SEMILLA DE ORQUIDEA	LYCASTE VIRGINALIS
N/A	1.00 BOX	0.7000 KGS SEMILLA DE ORQUIDEA	LYCASTE LASIOGLOSSA
N/A	1.00 BOX	0.7000 KGS SEMILLA DE ORQUIDEA	LYCASTE CRUENTA

GUATEMALA AEREO NEW ZEALAND CUSTOMS SERVICE

TRATAMIENTO DE DESINFESTACIÓN Y/O DESINFECCIÓN DESINFESTATION AND/OR DESINFECTION TREATEMENT

N/A N/A N/A N/A N/A

DECLARACIÓN ADICIONAL ADDITIONAL DECLARATION

N/A

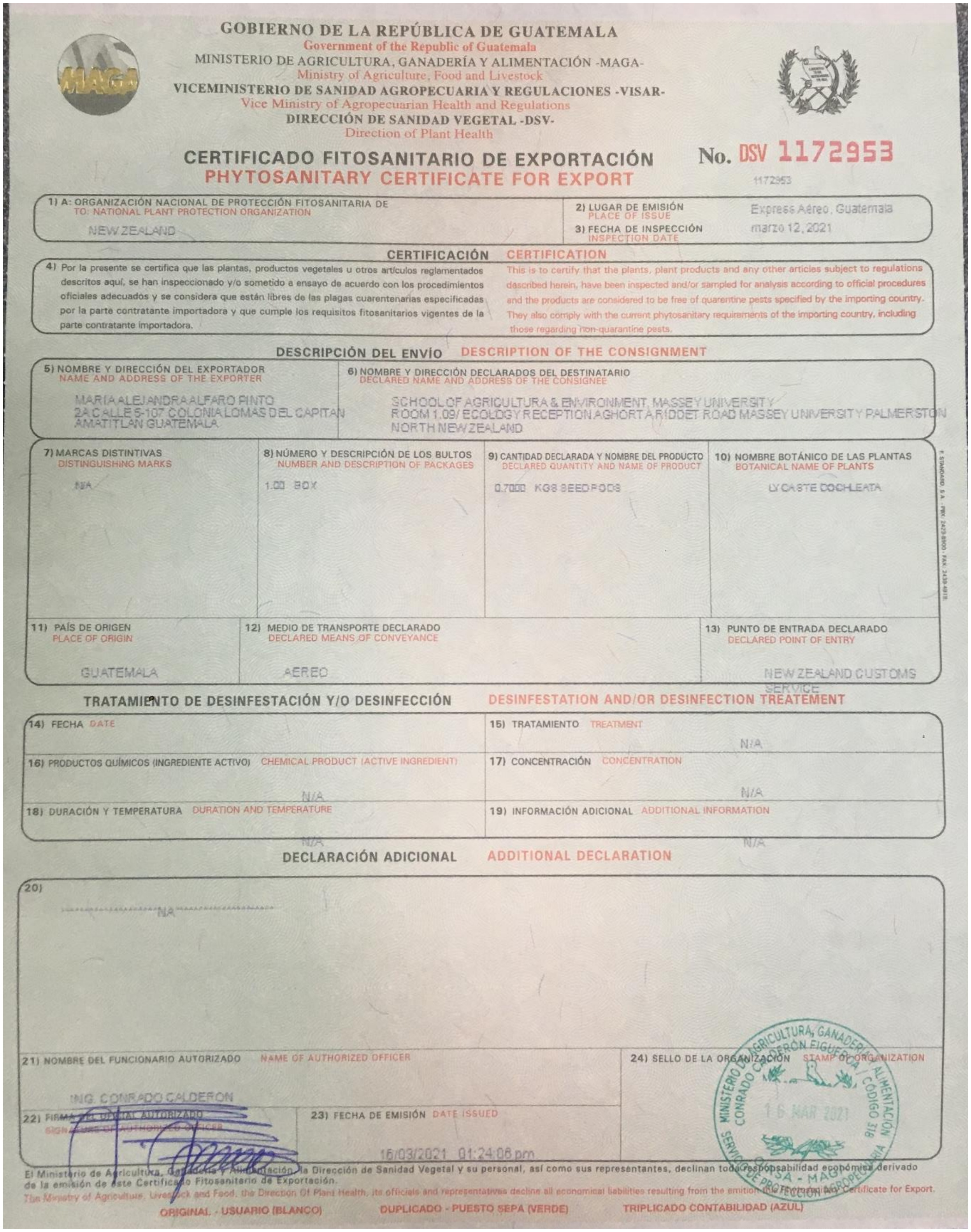
ING. MILTON ALEXANDER PEREZ SAMAYOA

17/02/2021 11:50:17 am

17 FEB 2021

ORIGINAL - USUARIO (BLANCO) DUPLICADO - PUESTO SIPA (VERDE) TRIPLICADO CONTABILIDAD (AZUL)

Appendix E. First Phytosanitary Certificate for Export issued by MAGA.



GOBIERNO DE LA REPÚBLICA DE GUATEMALA
Government of the Republic of Guatemala
MINISTERIO DE AGRICULTURA, GANADERÍA Y ALIMENTACIÓN -MAGA-
Ministry of Agriculture, Food and Livestock
VICEMINISTERIO DE SANIDAD AGROPECUARIA Y REGULACIONES -VISAR-
Vice Ministry of Agropecuarian Health and Regulations
DIRECCIÓN DE SANIDAD VEGETAL -DSV-
Direction of Plant Health

CERTIFICADO FITOSANITARIO DE EXPORTACIÓN
PHYTOSANITARY CERTIFICATE FOR EXPORT

No. DSV 1172953
1172953

1) A: ORGANIZACIÓN NACIONAL DE PROTECCIÓN FITOSANITARIA DE / TO: NATIONAL PLANT PROTECTION ORGANIZATION: NEW ZEALAND
2) LUGAR DE EMISIÓN / PLACE OF ISSUE: Express Aéreo, Guatemala
3) FECHA DE INSPECCIÓN / INSPECTION DATE: marzo 12, 2021

CERTIFICACIÓN CERTIFICATION

4) Por la presente se certifica que las plantas, productos vegetales u otros artículos reglamentados descritos aquí, se han inspeccionado y/o sometido a ensayo de acuerdo con los procedimientos oficiales adecuados y se considera que están libres de las plagas cuarentenarias especificadas por la parte contratante importadora y que cumple los requisitos fitosanitarios vigentes de la parte contratante importadora.

This is to certify that the plants, plant products and any other articles subject to regulations described herein, have been inspected and/or sampled for analysis according to official procedures and the products are considered to be free of quarantine pests specified by the importing country. They also comply with the current phytosanitary requirements of the importing country, including those regarding non-quarantine pests.

DESCRIPCIÓN DEL ENVÍO DESCRIPTION OF THE CONSIGNMENT

5) NOMBRE Y DIRECCIÓN DEL EXPORTADOR / NAME AND ADDRESS OF THE EXPORTER:
MARIA ALEJANDRA ALFARO PINTO
2A CALLE 5-107 COLONIA LOMAS DEL CAPITAN
AMATITLAN GUATEMALA

6) NOMBRE Y DIRECCIÓN DECLARADOS DEL DESTINATARIO / DECLARED NAME AND ADDRESS OF THE CONSIGNEE:
SCHOOL OF AGRICULTURA & ENVIRONMENT, MASSEY UNIVERSITY
ROOM 1.09/ ECOLOGY RECEPTION AGHORT A RIDDET ROAD MASSEY UNIVERSITY PALMERSTON NORTH NEW ZEALAND

7) MARCAS DISTINTIVAS / DISTINGUISHING MARKS	8) NÚMERO Y DESCRIPCIÓN DE LOS BULTOS / NUMBER AND DESCRIPTION OF PACKAGES	9) CANTIDAD DECLARADA Y NOMBRE DEL PRODUCTO / DECLARED QUANTITY AND NAME OF PRODUCT	10) NOMBRE BOTÁNICO DE LAS PLANTAS / BOTANICAL NAME OF PLANTS
N/A	1.00 BOX	0.7000 KGS SEEDPODS	LYCASTE COCHLEATA

11) PAÍS DE ORIGEN / PLACE OF ORIGIN	12) MEDIO DE TRANSPORTE DECLARADO / DECLARED MEANS OF CONVEYANCE	13) PUNTO DE ENTRADA DECLARADO / DECLARED POINT OF ENTRY
GUATEMALA	AEREO	NEW ZEALAND CUSTOMS SERVICE

TRATAMIENTO DE DESINFESTACIÓN Y/O DESINFECCIÓN DESINFESTATION AND/OR DESINFECTION TREATEMENT

14) FECHA DATE	15) TRATAMIENTO TREATMENT: N/A
16) PRODUCTOS QUÍMICOS (INGREDIENTE ACTIVO) CHEMICAL PRODUCT (ACTIVE INGREDIENT): N/A	17) CONCENTRACIÓN CONCENTRATION: N/A
18) DURACIÓN Y TEMPERATURA DURATION AND TEMPERATURE: N/A	19) INFORMACIÓN ADICIONAL ADDITIONAL INFORMATION: N/A

DECLARACIÓN ADICIONAL ADDITIONAL DECLARATION

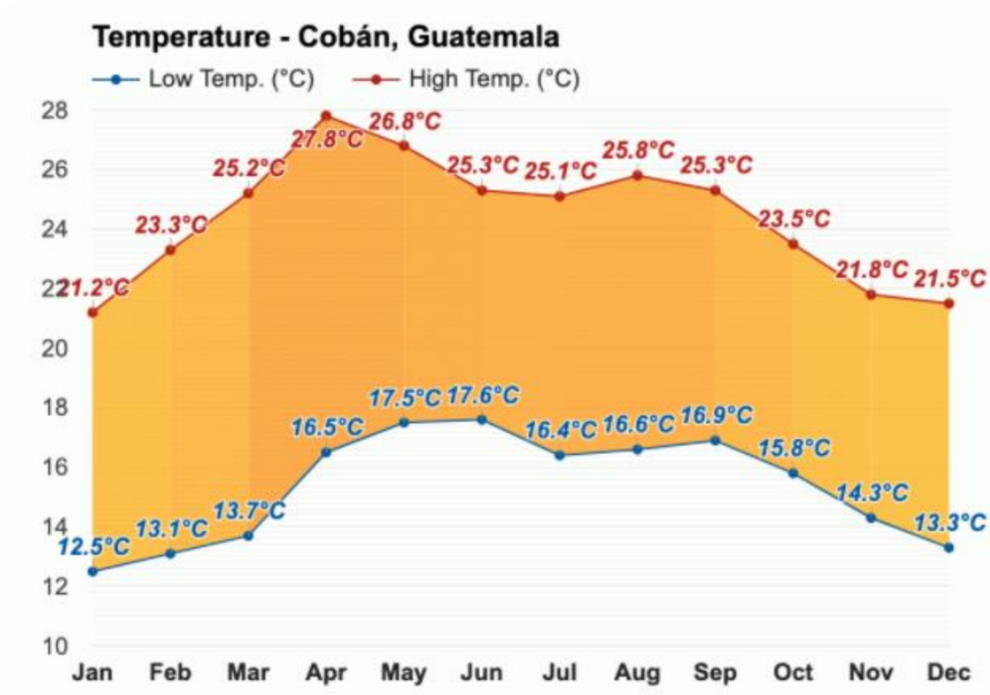
20) ----------NA----------

21) NOMBRE DEL FUNCIONARIO AUTORIZADO NAME OF AUTHORIZED OFFICER: ING. CONRADO CALDERON
22) FIRMA DEL OFICIAL AUTORIZADO SIGNATURE OF AUTHORIZED OFFICER
23) FECHA DE EMISIÓN DATE ISSUED: 16/03/2021 01:24:06 pm
24) SELLO DE LA ORGANIZACIÓN STAMP OF ORGANIZATION

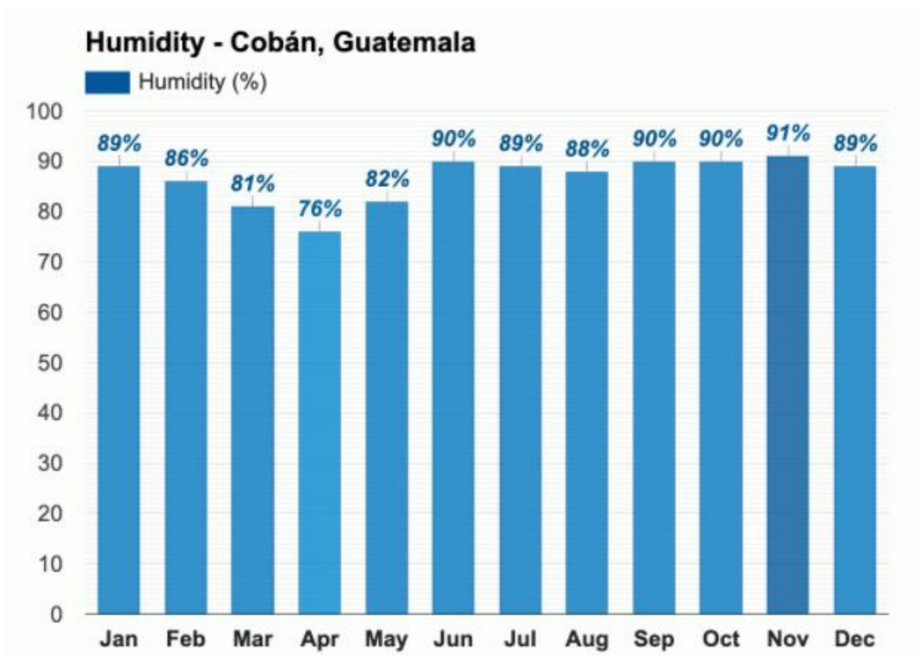
El Ministerio de Agricultura, Ganadería y Alimentación, la Dirección de Sanidad Vegetal y su personal, así como sus representantes, declinan toda responsabilidad económica derivado de la emisión de éste Certificado Fitosanitario de Exportación.
The Ministry of Agriculture, Livestock and Food, the Direction Of Plant Health, its officials and representatives decline all economical liabilities resulting from the emition of this Phytosanitary Certificate for Export.

ORIGINAL - USUARIO (BLANCO) DUPLICADO - PUESTO SEPA (VERDE) TRIPLICADO CONTABILIDAD (AZUL)

Appendix F. Second Phytosanitary Certificate for Export issued by MAGA.



Appendix G. Average temperature Cobán, Alta Verapaz, Guatemala. Taken from Weather Atlas (2021).



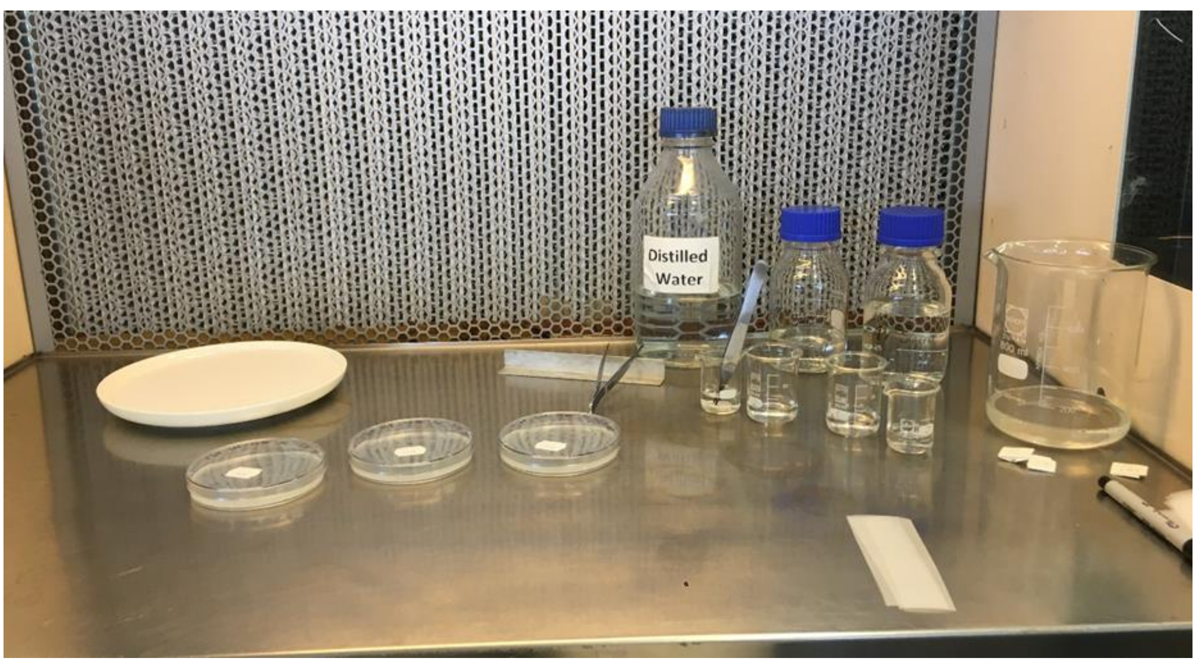
Appendix H. Average humidity at Cobán, Alta Verapaz, Guatemala. Taken from Weather Atlas (2021).

Appendix I. Plastic container with the seed capsules, silica gel and tissues.

Appendix J. Cooler used to ship the plastic containers with orchid seed capsules from Guatemala to New Zealand.

Appendix Chapter 3

Appendix K. Seed capsules placed in plastic containers to dry and release the seeds.



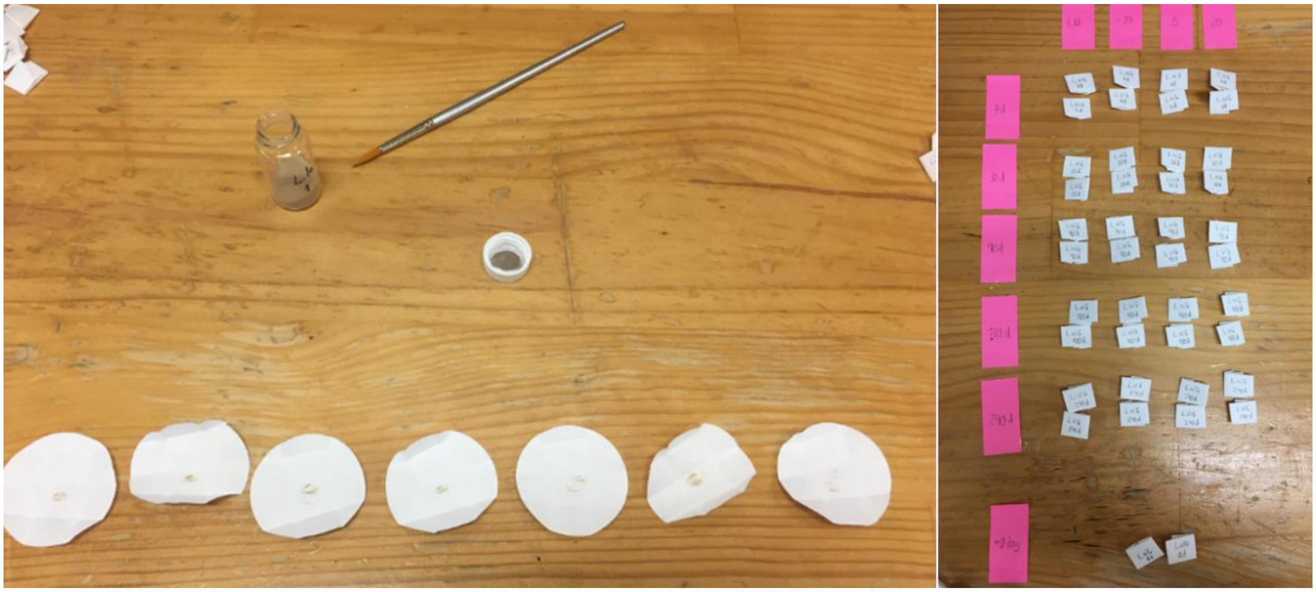
Appendix L. Seed disinfection chain under a laminar flow hood.

Appendix M. Comparative nutrient composition of the asymbiotic germination media: Murashige and Skoog (MS), Knudson C (KC), and terrestrial orchid medium BM-1.

	MS	KC	BM-1
Macronutrients (mM)			
Ammonium	20.61	13.82	-
Calcium	3	2.12	-
Chlorine	3	3.35	0.0021
Magnesium	1.5	1.01	0.83
Nitrate	39.4	10.49	-
Potassium	20.05	5.19	2.2
Phosphate	1.25	1.84	2.2
Sulfate	1.84	4.91	1.1
Sodium	0.1	-	0.2
Micronutrients (µM)			
Boron	100.26	-	161.7
Cobalt	0.11	-	0.105
Copper	0.1	-	0.1
Iron	183	90	100.2
Iodine	5	-	-
Manganese	89.3	30	147.9
Molybdenum	1.03	-	1.03
Zinc	29.9	-	34.8
Undefined organics (mg/l)			
Biotin	-	-	0.05
Casein hybrolysate	-	-	500
Folic acid	-	-	0.5
L-Glutamine	-	-	100
Glycine	-	-	2
Myo-inositol	100	-	100
Nicotonic acid	1	-	5
Pyridoxine. HCl (vit B6)	1	-	0.5
Thiamine. HCl (vit B1)	10	-	0.5
Total N (mM)	-	24.31	-
NH4:N03	-	1.32	-

Formulations taken from (Diantina, 2020; Kauth et al., 2008).

Appendix Chapter 4

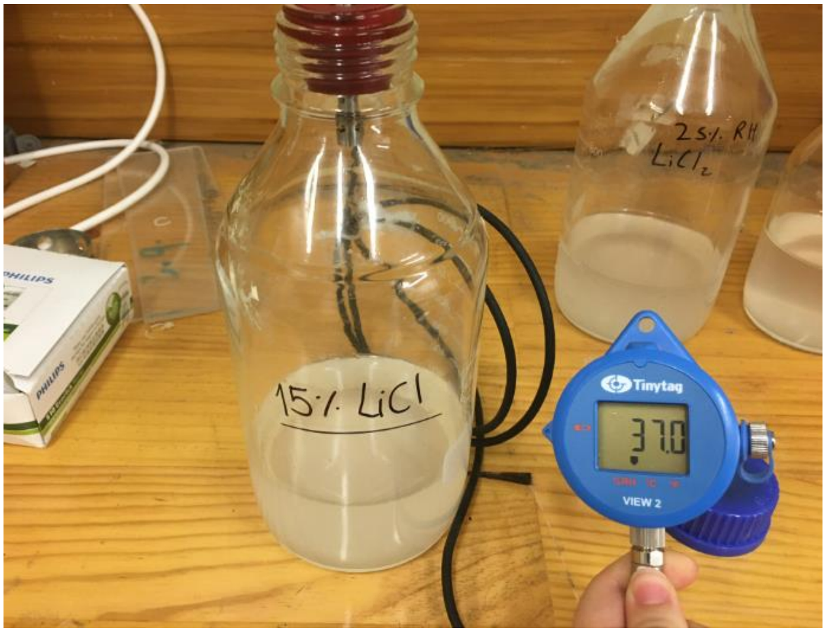


Appendix N. Lycaste seeds folded in filter paper envelopes (55 mm diameter).

Appendix O. LiCl weights needed to prepare the solutions at the different target relative humidity.

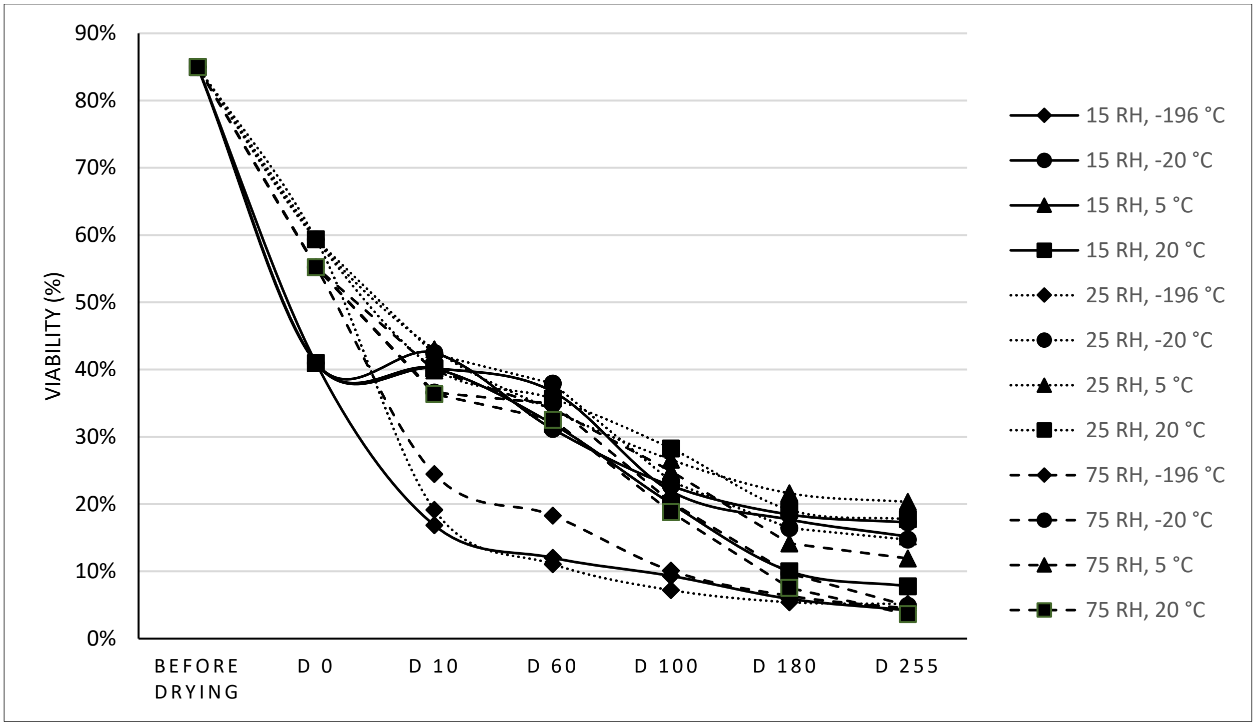
Weight of LiCl (g) dissolved in 200ml deionised water	Predicted RH (%) generated at 20ºC
147	15
116	25
42	75

Formulations modified from the Technical Information Sheet 09 of Kew Royal Botanic Gardens (2022).

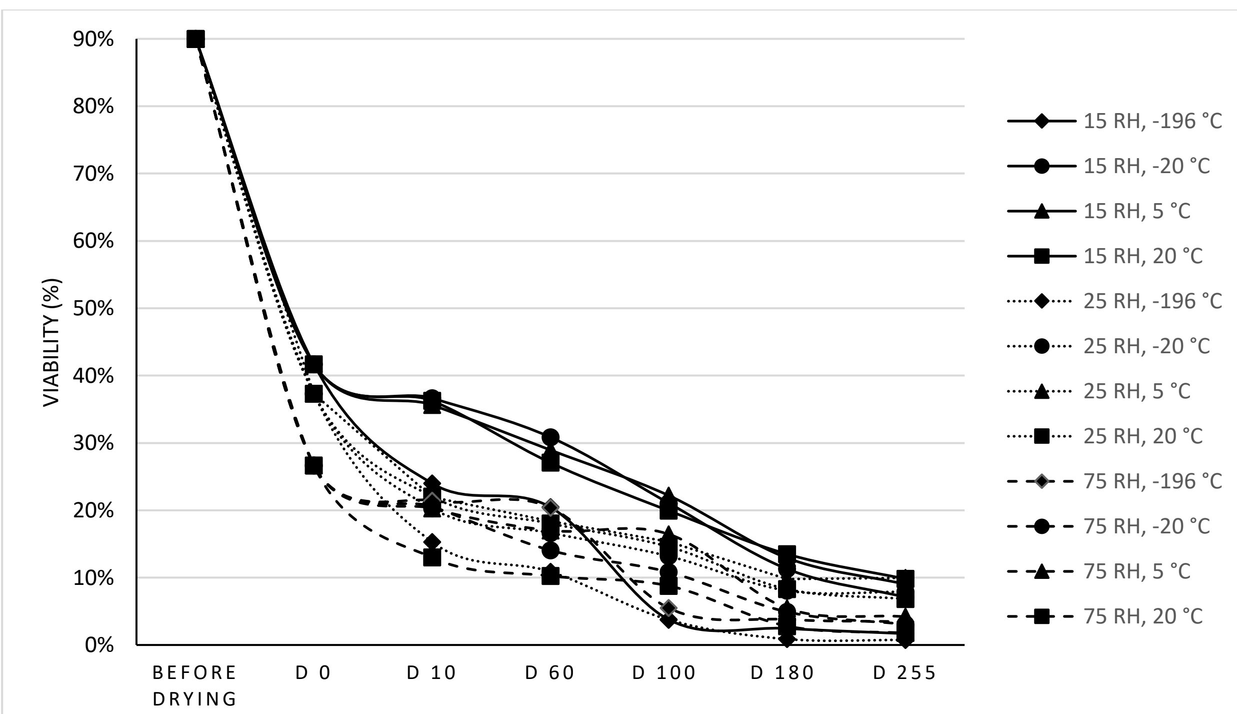


Appendix P. Checking of the relative humidity environments of the lithium chloride solutions (LiCl) with a tiny tag data logger TV-4505.

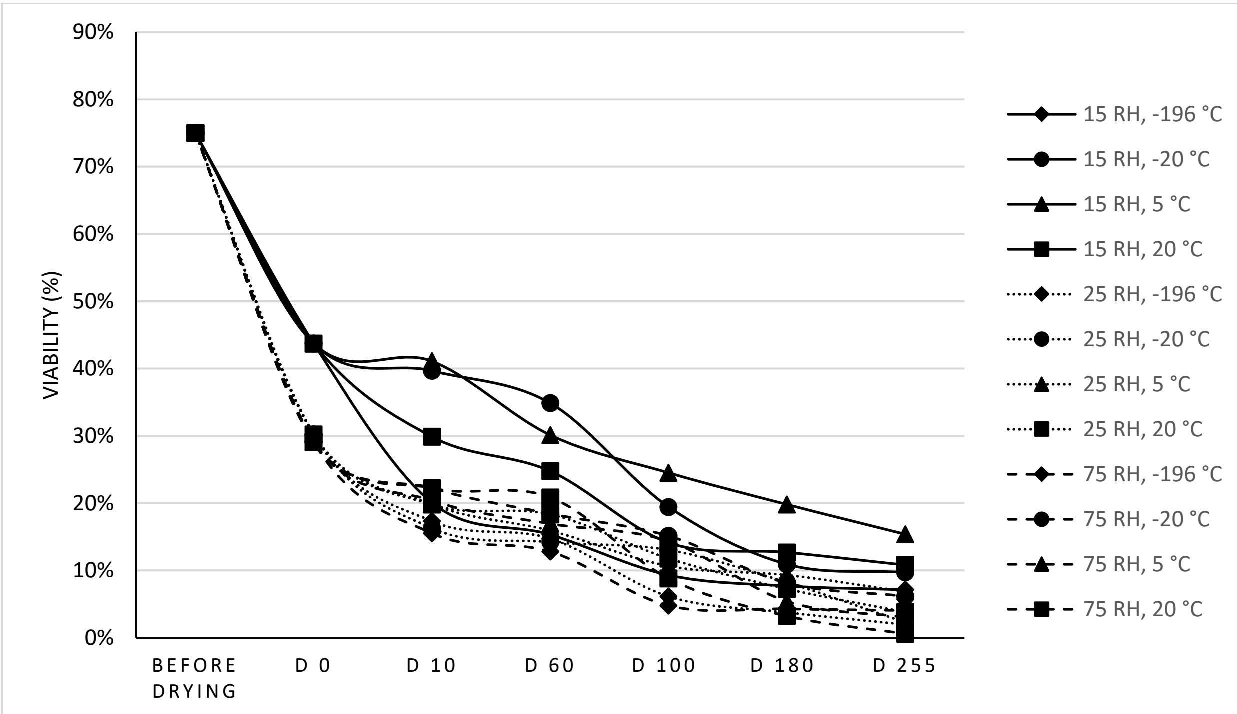
Appendix Q. Seeds envelopes reaching the RH equilibrium in boxes containing the LiCl solutions at 20 °C.



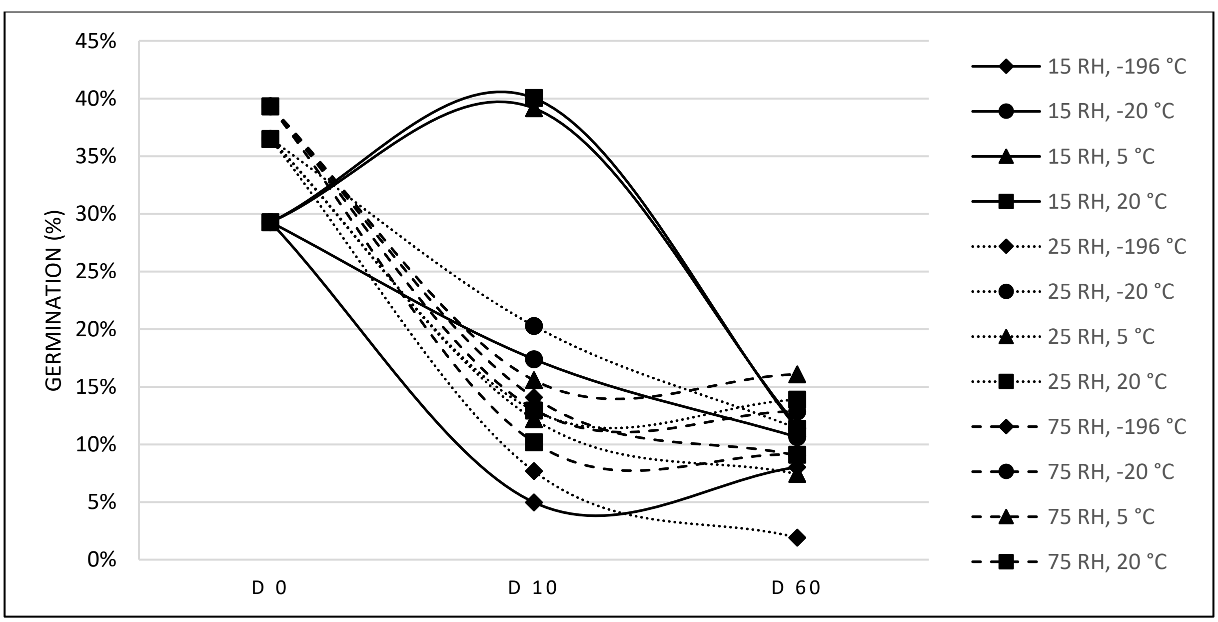
Appendix R. Variation in seed viability at different sampling times for Lycaste cochleata stored under different conditions.



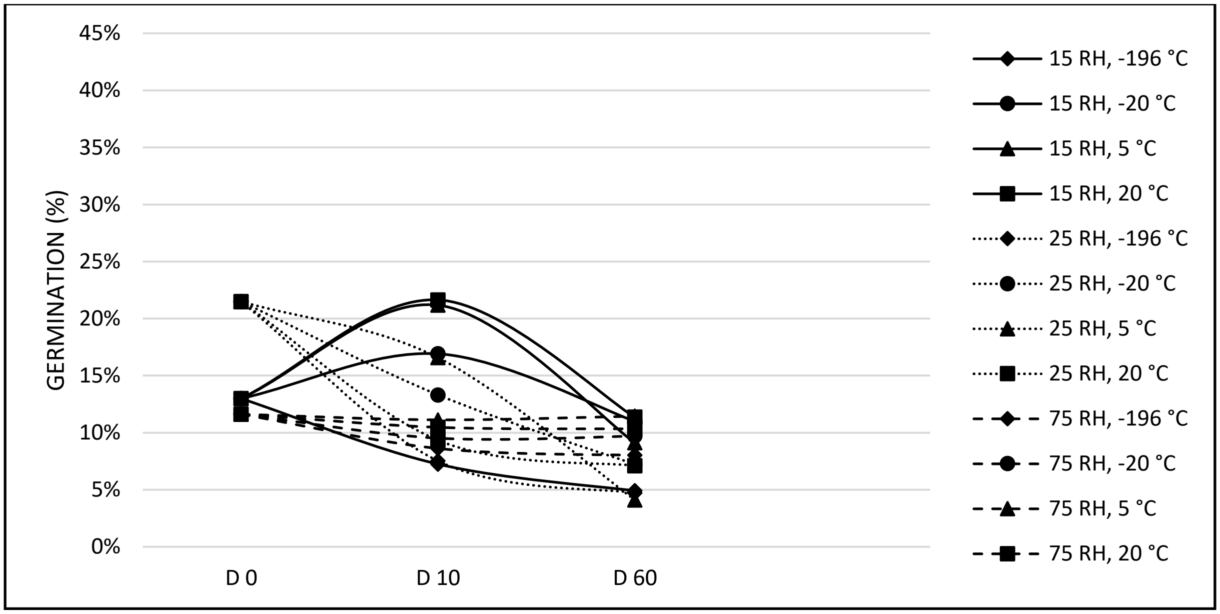
Appendix S. Variation seed viability at different sampling times for Lycaste lasioglossa stored under different conditions.



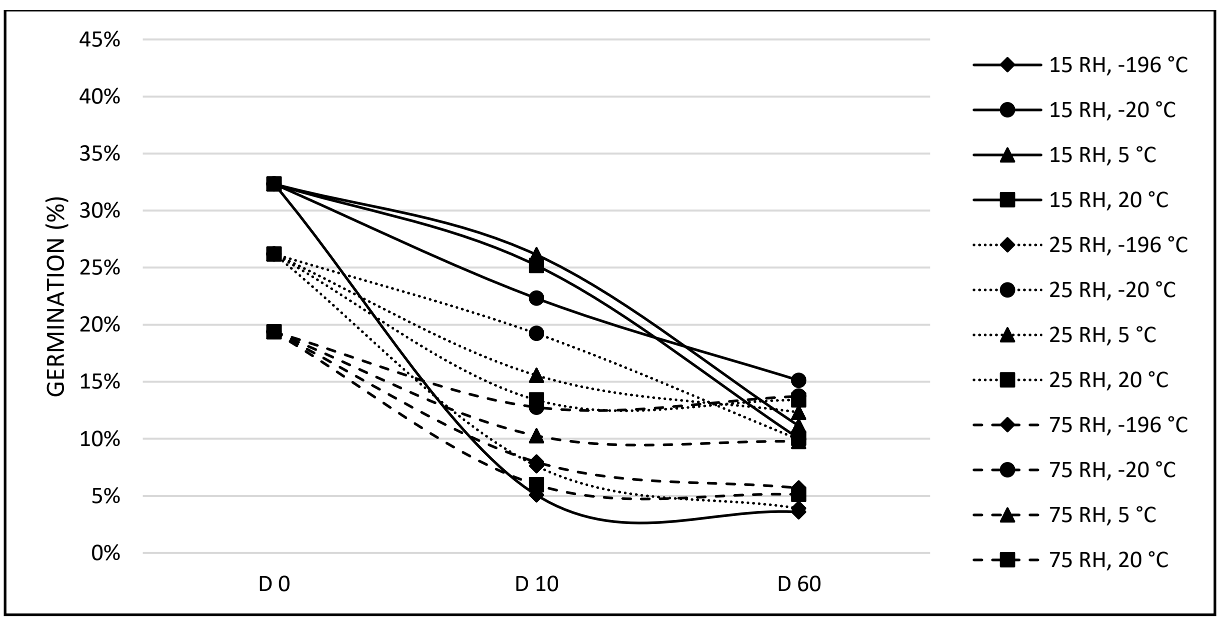
Appendix T. Variation of seed viability at different sampling times for Lycaste virginalis stored under different conditions.



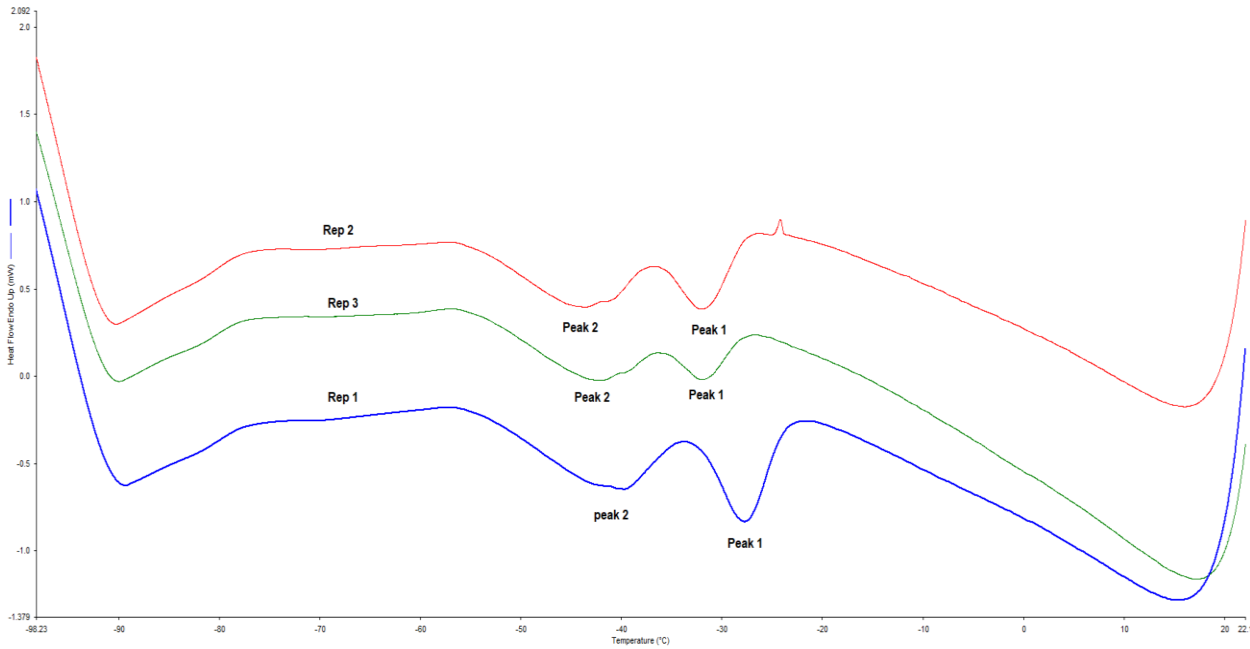
Appendix U. Variation of seed germination at different sampling times for Lycaste cochleata stored under different conditions.



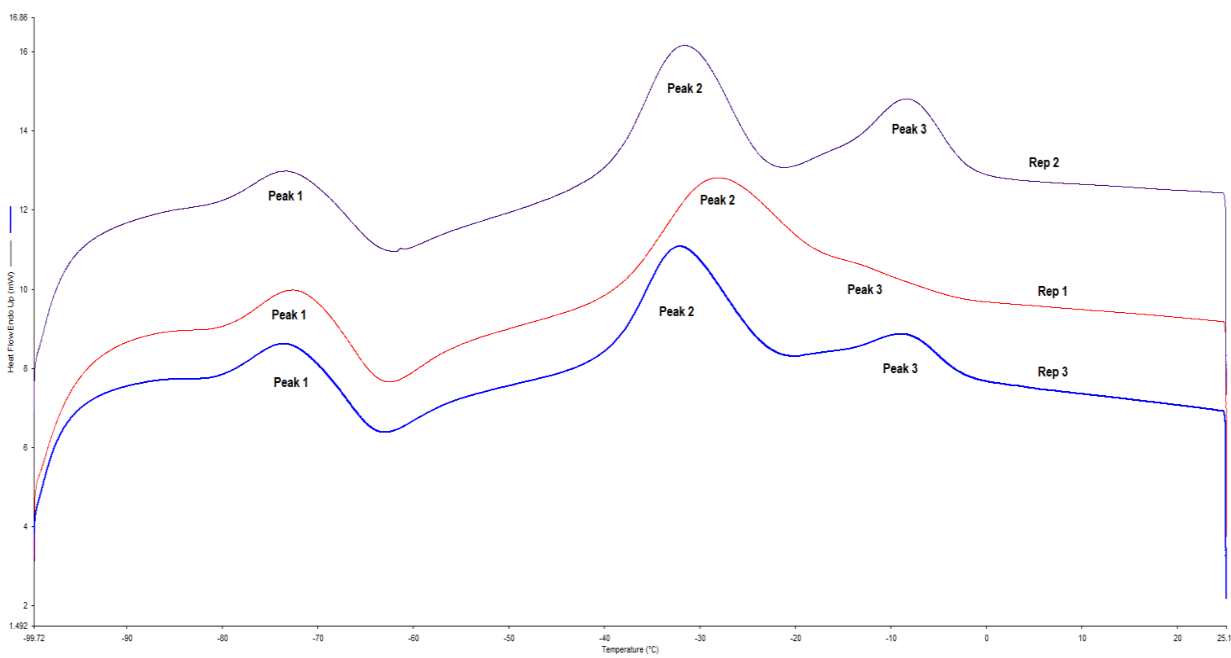
Appendix V. Variation of seed germination at different sampling times for Lycaste lasioglossa stored under different conditions.



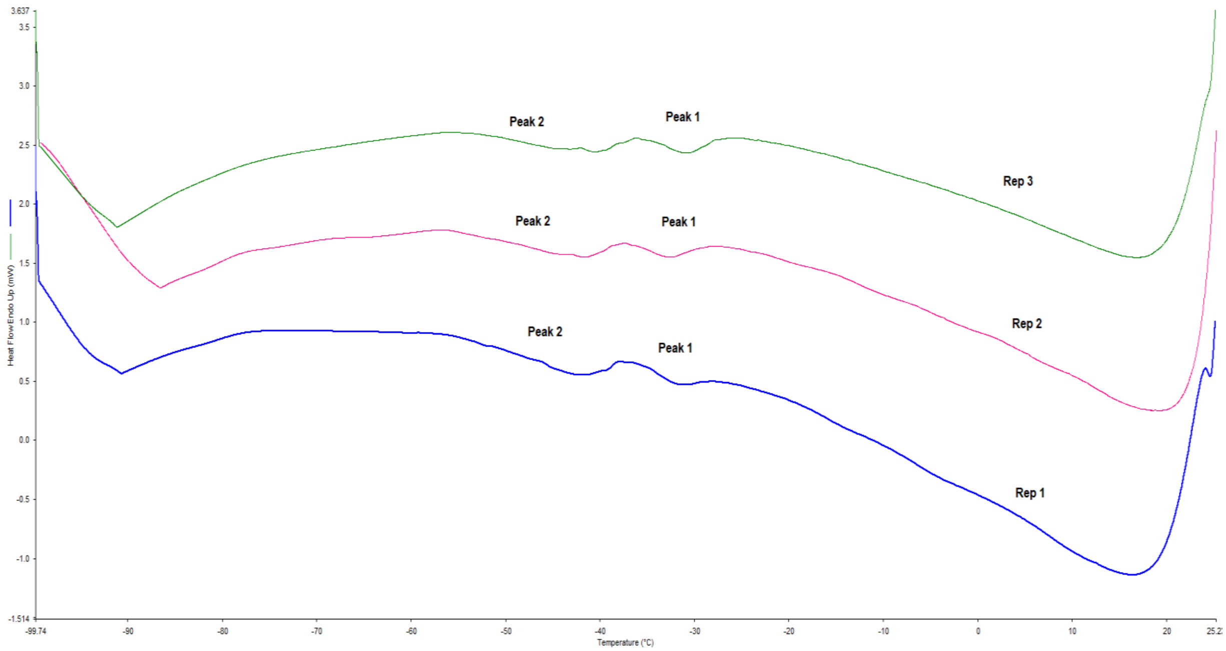
Appendix W. Variation of seed germination at different sampling times for Lycaste virginalis stored under different conditions.



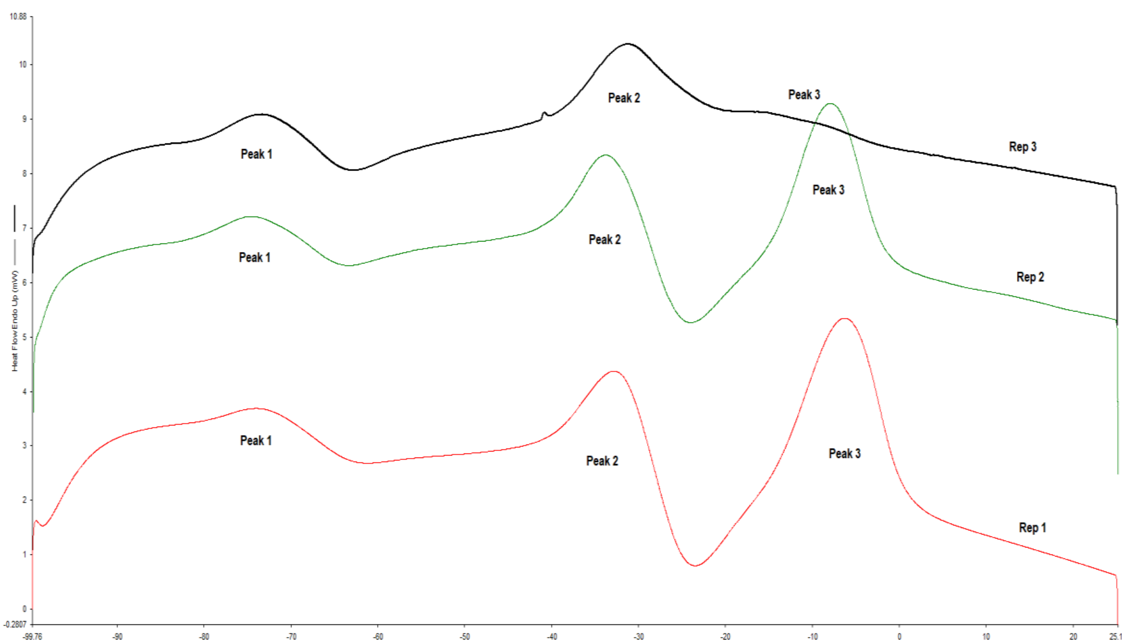
Appendix X. Cooling thermograms for Lycaste cochleata.



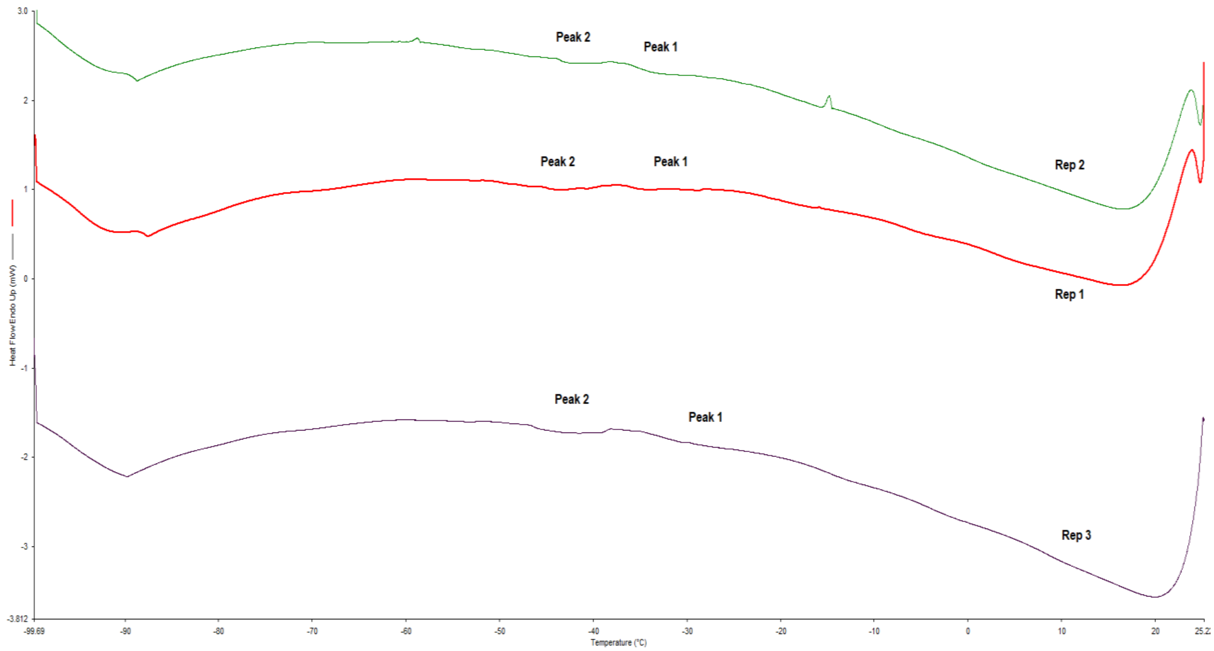
Appendix Y. Warming thermograms for Lycaste cochleata.



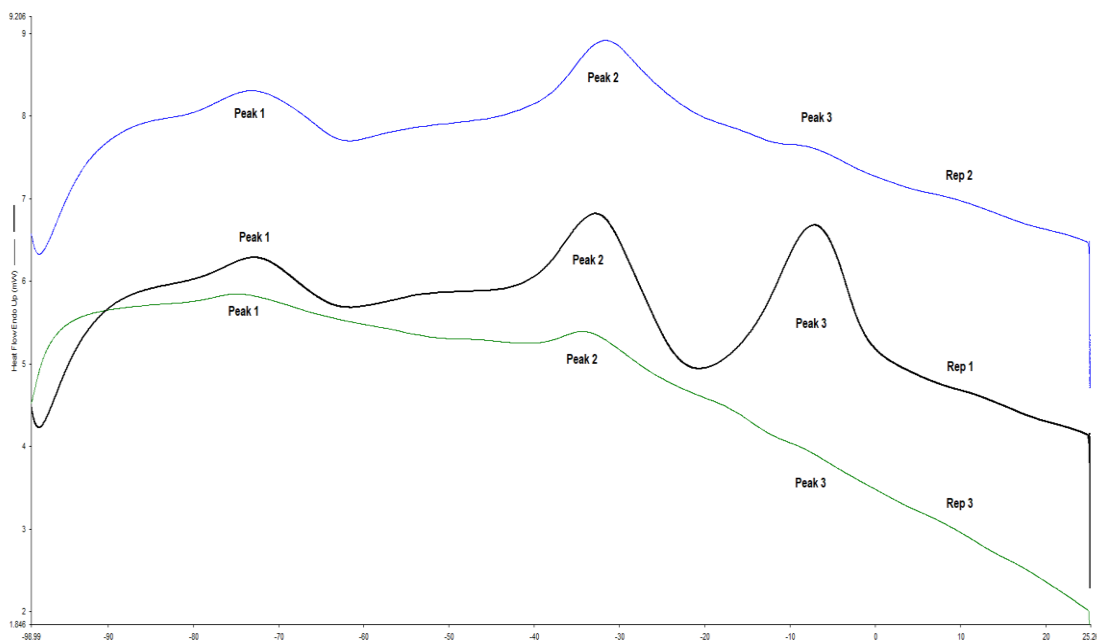
Appendix Z. Cooling thermograms for Lycaste lasioglossa.



Appendix AA. Warming thermograms for Lycaste lasioglossa.



Appendix BB. Cooling thermograms for Lycaste virginalis.



Appendix CC. Warming thermograms for Lycaste virginalis.

Appendix DD. Pairwise comparisons between all possible treatment combinations for viability of Lycaste cochleata.

	15RH, 5°C, 10days	25RH, 5°C, 10days	75RH, 5°C, 10days	15RH, 5°C, 60days	25RH, 5°C, 60days	75RH, 5°C, 60days	15RH, 5°C, 100days	25RH, 5°C, 100days	75RH, 5°C, 100 days	15RH, 5°C, 180days	25RH, 5°C, 180days	75RH, 5°C, 180days	15RH, 5°C, 255days
25RH, 5°C, 10days	0.723	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	0.776	0.508	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	0.667	0.417	0.893	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	0.416	0.219	0.614	0.722	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	0.325	0.161	0.508	0.608	0.887	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	0.003	0.00078	0.00861	0.01358	0.04038	0.06064	-	-	-	-	-	-	-
25RH, 5°C, 100days	0.040	0.013	0.086	0.120	0.258	0.337	0.419	-	-	-	-	-	-
75RH, 5°C, 100 days	0.014	0.004	0.035	0.051	0.127	0.176	0.654	0.738	-	-	-	-	-
15RH, 5°C, 180days	0.000	0.00003	0.00053	0.00094	0.00368	0.00621	0.444	0.095	0.201	-	-	-	-
25RH, 5°C, 180days	0.003	0.001	0.008	0.012	0.037	0.055	0.972	0.398	0.629	0.467	-	-	-
75RH, 5°C, 180days	0.000	6.8E-07	0.000021	0.00004	0.000	0.000	0.110	0.012	0.034	0.459	0.119	-	-
15RH, 5°C, 255days	0.000	2.4E-06	0.000062	0.000	0.001	0.001	0.185	0.025	0.064	0.621	0.197	0.821	-
25RH, 5°C, 255days	0.001	0.000	0.003	0.006	0.018	0.029	0.792	0.263	0.463	0.633	0.819	0.199	0.311
75RH, 5°C, 255days	0.000	5.2E-08	0.000002	4.3E-06	0.000026	0.000053	0.034	0.003	0.008	0.209	0.037	0.653	0.486
15RH, 20°C, 10days	0.981	0.707	0.792	0.683	0.429	0.337	0.003	0.042	0.015	0.000	0.003	5.3E-06	0.000017
25RH, 20°C, 10days	0.986	0.736	0.765	0.654	0.406	0.316	0.003	0.038	0.014	0.000	0.003	4.4E-06	0.000014
15RH, 20°C, 60days	0.194	0.086	0.337	0.423	0.677	0.792	0.118	0.508	0.299	0.015	0.109	0.001	0.003
25RH, 20°C, 60days	0.556	0.325	0.776	0.884	0.837	0.723	0.022	0.169	0.077	0.002	0.020	0.000082	0.000
75RH, 20°C, 60days	0.248	0.116	0.414	0.504	0.774	0.888	0.088	0.427	0.236	0.010	0.081	0.001	0.002
15RH, 20°C, 100days	0.000	0.000094	0.001	0.002	0.009	0.014	0.617	0.167	0.323	0.808	0.643	0.309	0.448
25RH, 20°C, 100days	0.073	0.027	0.146	0.197	0.383	0.478	0.287	0.824	0.569	0.053	0.268	0.006	0.012
75RH, 20°C, 100days	0.000	0.000024	0.000	0.001	0.003	0.005	0.419	0.086	0.186	0.968	0.440	0.486	0.653
15RH, 20°C, 180days	2E-08	2.7E-09	1.3E-07	2.8E-07	2.1E-06	4.7E-06	0.007	0.000	0.001	0.067	0.008	0.323	0.208
25RH, 20°C, 180days	5E-04	0.000	0.002	0.003	0.010	0.016	0.641	0.179	0.340	0.785	0.667	0.292	0.429
75RH, 20°C, 180days	5E-10	4.9E-11	2.9E-09	6.7E-09	5.9E-08	1.4E-07	0.00067	0.00002	0.000098	0.01032	0.00076	0.08423	0.04568
15RH, 20°C, 255days	8E-10	7.9E-11	4.6E-09	1.1E-08	9.1E-08	2.2E-07	0.0009	0.000029	0.00014	0.0132	0.00103	0.10145	0.05621
25RH, 20°C, 255days	2E-04	0.000034	0.0006	0.00104	0.00407	0.00688	0.46301	0.10145	0.21353	0.97665	0.48619	0.44001	0.60022
75RH, 20°C, 255days	2E-14	6.9E-15	1.1E-13	2.6E-13	2.2E-12	5.6E-12	2E-07	1.8E-09	1.4E-08	0.000011	2.4E-07	0.00032	0.00012
15RH, -20°C, 10days	0.794	0.92934	0.57256	0.47583	0.26168	0.19529	0.00112	0.01791	0.00588	0.000045	0.00098	1.1E-06	0.000004
25RH, -20°C, 10days	0.794	0.92934	0.57256	0.47583	0.26168	0.19529	0.00112	0.01791	0.00588	0.000045	0.00098	1.1E-06	0.000004

75RH, -20°C, 10days	0.621	0.379	0.844	0.951	0.769	0.653	0.016	0.138	0.060	0.001	0.015	0.000053	0.000
15RH, -20°C, 60days	0.182	0.080	0.320	0.404	0.653	0.769	0.127	0.529	0.316	0.017	0.117	0.001	0.003
25RH, -20°C, 60days	0.777	0.510	0.998	0.890	0.612	0.506	0.009	0.086	0.034	0.001	0.008	0.00002	0.000061
75RH, -20°C, 60days	0.471	0.261	0.681	0.791	0.931	0.817	0.031	0.216	0.103	0.003	0.028	0.000	0.000
15RH, -20°C, 100days	0.004	0.001	0.012	0.018	0.053	0.078	0.926	0.483	0.732	0.383	0.897	0.087	0.149
25RH, -20°C, 100days	0.004	0.001	0.011	0.017	0.049	0.073	0.942	0.465	0.712	0.400	0.917	0.092	0.158
75RH, -20°C, 100days	0.000	0.000041	0.001	0.001	0.005	0.008	0.493	0.112	0.234	0.942	0.517	0.414	0.569
15RH, -20°C, 180days	0.000	0.000055	0.001	0.002	0.006	0.010	0.534	0.129	0.262	0.897	0.560	0.376	0.524
25RH, -20°C, 180days	0.000	7.1E-06	0.000	0.000	0.001	0.002	0.276	0.045	0.108	0.779	0.295	0.662	0.838
75RH, -20°C, 180days	0.000	9.9E-10	5.1E-08	1.1E-07	8.8E-07	0.000002	0.004	0.000	0.001	0.044	0.005	0.240	0.149
15RH, -20°C, 255days	0.000	0.00002	0.000	0.001	0.003	0.005	0.396	0.078	0.172	0.939	0.417	0.512	0.681
25RH, -20°C, 255days	0.000	1.1E-06	0.000032	0.000061	0.000	0.001	0.135	0.016	0.044	0.518	0.146	0.931	0.890
75RH, -20°C, 255days	2E-13	3E-14	1.3E-12	3.2E-12	3.1E-11	7.7E-11	0.000002	2.2E-08	1.7E-07	0.000082	2.4E-06	0.002	0.001
15RH, -160°C, 60days	2E-07	2.9E-08	1.2E-06	2.6E-06	0.000017	0.000034	0.025	0.002	0.006	0.173	0.028	0.581	0.426
25RH, -160°C, 60days	7E-08	7.9E-09	3.6E-07	7.8E-07	5.5E-06	0.000012	0.013	0.001	0.003	0.105	0.014	0.433	0.295
75RH, -160°C, 60days	0.000	0.000022	0.000	0.001	0.003	0.005	0.410	0.082	0.180	0.954	0.430	0.498	0.665
15RH, -160°C, 100days	6E-09	7E-10	3E-08	8E-08	6E-07	1E-06	3E-03	1E-04	6E-04	4E-02	4E-03	2E-01	1E-01
25RH, -160°C, 100days	2E-10	2E-11	1E-09	3E-09	3E-08	7E-08	4E-04	1E-05	6E-05	7E-03	5E-04	6E-02	3E-02
75RH, -160°C, 100days	1E-08	2E-09	8E-08	2E-07	1E-06	3E-06	5E-03	3E-04	1E-03	5E-02	6E-03	3E-01	2E-01
15RH, -160°C, 180days	1E-11	1E-12	7E-11	2E-10	2E-09	4E-09	5E-05	9E-07	5E-06	1E-03	6E-05	2E-02	7E-03
25RH, -160°C, 180days	7E-12	9E-13	5E-11	1E-10	1E-09	3E-09	4E-05	6E-07	4E-06	9E-04	4E-05	1E-02	6E-03

75RH, -160°C, 180days	5E-11	5E-12	3E-10	7E-10	7E-09	2E-08	1E-04	3E-06	2E-05	3E-03	2E-04	3E-02	2E-02
15RH, -160°C, 255days	6E-14	1E-14	3E-13	9E-13	7E-12	2E-11	6E-07	6E-09	4E-08	3E-05	7E-07	7E-04	3E-04
25RH, -160°C, 255days	5E-12	6E-13	3E-11	7E-11	7E-10	2E-09	3E-05	4E-07	3E-06	7E-04	3E-05	1E-02	4E-03
75RH, -160°C, 255days	9E-13	1E-13	6E-12	1E-11	1E-10	3E-10	7E-06	9E-08	6E-07	2E-04	8E-06	4E-03	2E-03

	25RH, 5°C, 255days	75RH, 5°C, 255days	15RH, 20°C, 10days	25RH, 20°C, 10days	15RH, 20°C, 60days	25RH, 20°C, 60days	75RH, 20°C, 60days	15RH, 20°C, 100days	25RH, 20°C, 100days	75RH, 20°C, 100days	15RH, 20°C, 180days	25RH, 20°C, 180days	75RH, 20°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	0.071	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	0.001	4.6E-07	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	0.001	3.7E-07	0.970	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	0.061	0.000	0.202	0.188	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	0.009	9.4E-06	0.571	0.544	0.522	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	0.043	0.000	0.259	0.240	0.905	0.612	-	-	-	-	-	-	-
15RH, 20°C, 100days	0.822	0.123	0.000	0.000	0.032	0.004	0.022	-	-	-	-	-	-
25RH, 20°C, 100days	0.168	0.001	0.077	0.070	0.672	0.265	0.578	0.100	-	-	-	-	-
75RH, 20°C, 100days	0.603	0.228	0.000	0.000	0.013	0.001	0.009	0.777	0.047	-	-	-	-
15RH, 20°C, 180days	0.017	0.619	2.6E-08	2.1E-08	0.000018	6.7E-07	9.8E-06	0.034	0.000	0.075	-	-	-
25RH, 20°C, 180days	0.846	0.114	0.001	0.000	0.035	0.005	0.025	0.974	0.108	0.754	0.031	-	-
75RH, 20°C, 180days	0.002	0.229	5.5E-10	4.3E-10	6.4E-07	1.7E-08	3.3E-07	0.004	6.4E-06	0.012	0.518	0.004	-

15RH, 20°C, 255days	0.003	0.265	8.8E-10	6.9E-10	9.8E-07	2.6E-08	4.9E-07	0.006	9.4E-06	0.015	0.573	0.005	0.941
25RH, 20°C, 255days	0.654	0.197	0.000	0.000	0.017	0.002	0.011	0.829	0.057	0.944	0.062	0.806	0.009
75RH, 20°C, 255days	0.000	0.002	2.5E-14	2.3E-14	3.1E-11	6.9E-13	1.4E-11	3.1E-06	4.5E-10	0.000014	0.011	2.6E-06	0.071
15RH, -20°C, 10days	0.000	8.8E-08	0.777	0.806	0.108	0.379	0.143	0.000	0.035	0.000037	4.6E-09	0.000	8.7E-11
25RH, -20°C, 10days	0.000	8.8E-08	0.777	0.806	0.108	0.379	0.143	0.000	0.035	0.000037	4.6E-09	0.000	8.7E-11
75RH, -20°C, 10days	0.007	5.9E-06	0.638	0.610	0.461	0.929	0.546	0.003	0.222	0.001	4E-07	0.003	9.7E-09
15RH, -20°C, 60days	0.066	0.000	0.191	0.176	0.974	0.502	0.882	0.035	0.695	0.015	0.000021	0.039	7.7E-07
25RH, -20°C, 60days	0.003	0.000002	0.794	0.767	0.336	0.774	0.412	0.001	0.145	0.000	1.3E-07	0.002	2.8E-09
75RH, -20°C, 60days	0.014	0.000017	0.486	0.461	0.610	0.907	0.704	0.006	0.330	0.002	1.3E-06	0.007	3.4E-08
15RH, -20°C, 100days	0.717	0.025	0.005	0.004	0.148	0.029	0.112	0.546	0.340	0.358	0.005	0.569	0.000
25RH, -20°C, 100days	0.736	0.027	0.004	0.004	0.140	0.027	0.105	0.566	0.326	0.374	0.006	0.585	0.000
75RH, -20°C, 100days	0.688	0.181	0.000	0.000	0.019	0.002	0.013	0.865	0.064	0.912	0.055	0.838	0.008
15RH, -20°C, 180days	0.736	0.159	0.000	0.000	0.023	0.003	0.016	0.912	0.075	0.865	0.047	0.888	0.007
25RH, -20°C, 180days	0.435	0.356	0.000046	0.000038	0.006	0.001	0.004	0.590	0.023	0.811	0.134	0.569	0.025
75RH, -20°C, 180days	0.010	0.506	1E-08	8.1E-09	8.2E-06	2.7E-07	4.4E-06	0.021	0.000064	0.049	0.880	0.019	0.633
15RH, -20°C, 255days	0.574	0.245	0.000	0.000	0.012	0.001	0.008	0.749	0.043	0.970	0.082	0.723	0.013
25RH, -20°C, 255days	0.237	0.585	8.3E-06	6.9E-06	0.002	0.000	0.001	0.360	0.008	0.548	0.275	0.340	0.067
75RH, -20°C, 255days	0.000	0.009	2.6E-13	2.2E-13	4.3E-10	8E-12	1.9E-10	0.000026	5.8E-09	0.000099	0.039	0.000022	0.187
15RH, -160°C, 60days	0.055	0.928	2.7E-07	2.2E-07	0.000	5.8E-06	0.000067	0.099	0.001	0.188	0.690	0.091	0.275
25RH, -160°C, 60days	0.030	0.759	7.7E-08	6.1E-08	0.000043	1.8E-06	0.000024	0.056	0.000	0.116	0.859	0.052	0.402
75RH, -160°C, 60days	0.590	0.235	0.000	0.000	0.013	0.001	0.008	0.767	0.045	0.986	0.078	0.740	0.012
15RH, -160°C, 100days	0.008	0.459	6.6E-09	5.3E-09	5.8E-06	1.8E-07	0.000003	0.017	0.000046	0.040	0.822	0.015	0.686
25RH, -160°C, 100days	0.001	0.180	2.6E-10	2E-10	3.3E-07	8.2E-09	1.6E-07	0.003	3.4E-06	0.008	0.439	0.003	0.907

75RH, -160°C, 100days	0.013	0.560	1.6E-08	1.3E-08	0.000012	4.2E-07	6.5E-06	0.027	0.000092	0.060	0.936	0.024	0.576
15RH, -160°C, 180days	0.000	0.057	1.2E-11	9.6E-12	2E-08	4.3E-10	9.6E-09	0.000	2.4E-07	0.001	0.186	0.000	0.544
25RH, -160°C, 180days	0.000	0.048	8.3E-12	6.7E-12	1.4E-08	2.9E-10	6.5E-09	0.000	1.7E-07	0.001	0.162	0.000	0.500
75RH, -160°C, 180days	0.000	0.103	5.3E-11	4.3E-11	7.9E-08	1.8E-09	3.8E-08	0.001	8.8E-07	0.003	0.292	0.001	0.717
15RH, -160°C, 255days	0.000	0.004	6.8E-14	5.7E-14	9.9E-11	1.9E-12	4.6E-11	8.2E-06	1.4E-09	0.000034	0.020	0.000007	0.112
25RH, -160°C, 255days	0.000	0.039	5.5E-12	4.4E-12	8.8E-09	1.8E-10	4.2E-09	0.000	1.1E-07	0.001	0.138	0.000	0.450
75RH, -160°C, 255days	0.000	0.018	1.1E-12	9E-13	1.8E-09	3.6E-11	8.3E-10	0.000076	2.3E-08	0.000	0.073	0.000065	0.293

	15RH, 20°C, 255days	25RH, 20°C, 255days	75RH, 20°C, 255days	15RH, -20°C, 10days	25RH, -20°C, 10days	75RH, -20°C, 10days	15RH, -20°C, 60days	25RH, -20°C, 60days	75RH, -20°C, 60days	15RH, -20°C, 100days	25RH, -20°C, 100days	75RH, -20°C, 100days	15RH, -20°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-

15RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 255days	0.012	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 255days	0.058	9.7E-06	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 10days	1.4E-10	0.000052	6.9E-15	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	1.4E-10	0.000052	6.9E-15	1.000	-	-	-	-	-	-	-	-	-
75RH, -20°C, 10days	1.5E-08	0.001	3.6E-13	0.436	0.436	-	-	-	-	-	-	-	-
15RH, -20°C, 60days	0.0000	0.018	3.9E-11	0.100	0.100	0.440	-	-	-	-	-	-	-
25RH, -20°C, 60days	0.0000	0.001	1.1E-13	0.574	0.574	0.841	0.318	-	-	-	-	-	-
75RH, -20°C, 60days	0.0000	0.003	1.3E-12	0.309	0.309	0.834	0.585	0.679	-	-	-	-	-
15RH, -20°C, 100days	0.001	0.402	1.1E-07	0.002	0.002	0.022	0.159	0.012	0.042	-	-	-	-
25RH, -20°C, 100days	0.001	0.417	1.3E-07	0.001	0.001	0.021	0.150	0.011	0.039	0.979	-	-	-
75RH, -20°C, 100days	0.010	0.966	7.7E-06	0.000063	0.000063	0.002	0.021	0.001	0.003	0.429	0.444	-	-
15RH, -20°C, 180days	0.009	0.920	5.7E-06	0.000084	0.000084	0.002	0.025	0.001	0.004	0.467	0.485	0.951	-
25RH, -20°C, 180days	0.032	0.759	0.000045	0.000011	0.000011	0.000	0.007	0.000	0.001	0.229	0.240	0.723	0.677
75RH, -20°C, 180days	0.690	0.040	0.018	1.7E-09	1.7E-09	1.6E-07	9.7E-06	5E-08	5.4E-07	0.003	0.003	0.036	0.030
15RH, -20°C, 255days	0.017	0.917	0.000017	0.000031	0.000031	0.001	0.013	0.000	0.002	0.336	0.350	0.884	0.834
25RH, -20°C, 255days	0.082	0.500	0.000	1.8E-06	1.8E-06	0.000081	0.002	0.000031	0.000	0.108	0.114	0.469	0.430
75RH, -20°C, 255days	0.159	0.000071	0.679	5.2E-14	5.2E-14	4.8E-12	5.2E-10	1.3E-12	1.7E-11	1.2E-06	1.3E-06	0.000058	0.000044
15RH, -160°C, 60days	0.316	0.162	0.003	5.1E-08	5.1E-08	3.6E-06	0.000	1.2E-06	0.000011	0.019	0.020	0.147	0.128
25RH, -160°C, 60days	0.448	0.099	0.006	1.4E-08	1.4E-08	1.1E-06	0.00005	3.5E-07	3.5E-06	0.009	0.010	0.088	0.076

75RH, -160°C, 60days	0.016	0.934	0.000015	0.000035	0.000035	0.001	0.014	0.000	0.002	0.348	0.364	0.900	0.851
15RH, -160°C, 100days	0.746	0.033	0.023	1.1E-09	1.1E-09	1.1E-07	6.8E-06	3.3E-08	3.7E-07	0.002	0.002	0.029	0.024
25RH, -160°C, 100days	0.846	0.006	0.096	4.3E-11	4.3E-11	4.7E-09	3.9E-07	1.4E-09	1.7E-08	0.000	0.000	0.005	0.004
75RH, -160°C, 100days	0.633	0.050	0.014	2.8E-09	2.8E-09	2.5E-07	0.000014	7.8E-08	8.3E-07	0.004	0.004	0.044	0.037
15RH, -160°C, 180days	0.491	0.001	0.268	2E-12	2E-12	2.4E-10	2.4E-08	6.8E-11	9.1E-10	0.00003	0.000034	0.001	0.001
25RH, -160°C, 180days	0.448	0.001	0.302	1.3E-12	1.3E-12	1.6E-10	1.6E-08	4.6E-11	6.2E-10	0.000022	0.000025	0.001	0.001
75RH, -160°C, 180days	0.657	0.003	0.169	8.4E-12	8.4E-12	1E-09	9.5E-08	2.9E-10	3.9E-09	0.00009	0.000	0.002	0.002
15RH, -160°C, 255days	0.094	0.0000	0.854	1.6E-14	1.6E-14	1.1E-12	1.2E-10	3.4E-13	4.4E-12	3.3E-07	3.8E-07	0.00002	0.000015
25RH, -160°C, 255days	0.404	0.001	0.342	9.3E-13	9.3E-13	1E-10	1.1E-08	3E-11	4E-10	0.000016	0.000018	0.001	0.000
75RH, -160°C, 255days	0.254	0.000	0.510	2E-13	2E-13	2E-11	2.2E-09	5.5E-12	7.4E-11	4.1E-06	4.7E-06	0.000	0.000
	25RH, -20°C, 180days	75RH, -20°C, 180days	15RH, -20°C, 255days	25RH, -20°C, 255days	75RH, -20°C, 255days	15RH, -160°C, 60days	25RH, -160°C, 60days	75RH, -160°C, 60days	15RH, -160°C, 100days	25RH, -160°C, 100days	75RH, -160°C, 100days	15RH, -160°C, 180days	25RH, -160°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-

15RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 180days	0.092	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 255days	0.838	0.054	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 255days	0.732	0.201	0.574	-	-	-	-	-	-	-	-	-	-

75RH, -20°C, 255days	0.000	0.061	0.000	0.001	-	-	-	-	-	-	-	-	-
15RH, -160°C, 60days	0.302	0.573	0.202	0.518	0.012	-	-	-	-	-	-	-	-
25RH, -160°C, 60days	0.197	0.738	0.127	0.378	0.023	0.829	-	-	-	-	-	-	-
75RH, -160°C, 60days	0.822	0.051	0.981	0.560	0.000	0.194	0.120	-	-	-	-	-	-
15RH, -160°C, 100days	0.078	0.944	0.045	0.175	0.073	0.522	0.683	0.042	-	-	-	-	-
25RH, -160°C, 100days	0.017	0.546	0.009	0.049	0.237	0.218	0.328	0.008	0.595	-	-	-	-
75RH, -160°C, 100days	0.111	0.941	0.067	0.235	0.049	0.629	0.796	0.063	0.888	0.493	-	-	-
15RH, -160°C, 180days	0.003	0.254	0.002	0.012	0.520	0.073	0.125	0.001	0.290	0.631	0.219	-	-
25RH, -160°C, 180days	0.003	0.225	0.001	0.009	0.568	0.062	0.108	0.001	0.258	0.583	0.193	0.949	-
75RH, -160°C, 180days	0.008	0.383	0.004	0.024	0.372	0.128	0.205	0.004	0.427	0.808	0.337	0.821	0.774
15RH, -160°C, 255days	0.000	0.032	0.000041	0.000	0.822	0.005	0.011	0.000037	0.039	0.148	0.026	0.374	0.414
25RH, -160°C, 255days	0.002	0.194	0.001	0.007	0.619	0.051	0.090	0.001	0.223	0.532	0.166	0.895	0.942
75RH, -160°C, 255days	0.001	0.108	0.000	0.003	0.821	0.024	0.045	0.000	0.127	0.362	0.090	0.690	0.740

	75RH, -160°C, 180days	15RH, -160°C, 255days	25RH, -160°C, 255days
25RH, 5°C, 10days	-	-	-
75RH, 5°C, 10days	-	-	-
15RH, 5°C, 60days	-	-	-
25RH, 5°C, 60days	-	-	-
75RH, 5°C, 60days	-	-	-
15RH, 5°C, 100days	-	-	-
25RH, 5°C, 100days	-	-	-
75RH, 5°C, 100 days	-	-	-

15RH, 5°C, 180days	-	-	-
25RH, 5°C, 180days	-	-	-
75RH, 5°C, 180days	-	-	-
15RH, 5°C, 255days	-	-	-
25RH, 5°C, 255days	-	-	-
75RH, 5°C, 255days	-	-	-
15RH, 20°C, 10days	-	-	-
25RH, 20°C, 10days	-	-	-
15RH, 20°C, 60days	-	-	-
25RH, 20°C, 60days	-	-	-
75RH, 20°C, 60days	-	-	-
15RH, 20°C, 100days	-	-	-
25RH, 20°C, 100days	-	-	-
75RH, 20°C, 100days	-	-	-
15RH, 20°C, 180days	-	-	-
25RH, 20°C, 180days	-	-	-
75RH, 20°C, 180days	-	-	-
15RH, 20°C, 255days	-	-	-
25RH, 20°C, 255days	-	-	-
75RH, 20°C, 255days	-	-	-
15RH, -20°C, 10days	-	-	-
25RH, -20°C, 10days	-	-	-
75RH, -20°C, 10days	-	-	-
15RH, -20°C, 60days	-	-	-
25RH, -20°C, 60days	-	-	-
75RH, -20°C, 60days	-	-	-
15RH, -20°C, 100days	-	-	-
25RH, -20°C, 100days	-	-	-
75RH, -20°C, 100days	-	-	-
15RH, -20°C, 180days	-	-	-
25RH, -20°C, 180days	-	-	-

75RH, -20°C, 180days	-	-	-
15RH, -20°C, 255days	-	-	-
25RH, -20°C, 255days	-	-	-
75RH, -20°C, 255days	-	-	-
15RH, -160°C, 60days	-	-	-
25RH, -160°C, 60days	-	-	-
75RH, -160°C, 60days	-	-	-
15RH, -160°C, 100days	-	-	-
25RH, -160°C, 100days	-	-	-
75RH, -160°C, 100days	-	-	-
15RH, -160°C, 180days	-	-	-
25RH, -160°C, 180days	-	-	-
75RH, -160°C, 180days	-	-	-
15RH, -160°C, 255days	0.246	-	-
25RH, -160°C, 255days	0.717	0.459	-
75RH, -160°C, 255days	0.520	0.646	0.796

Appendix EE. Pairwise comparisons between all possible treatment combinations for viability Lycaste lasioglossa.

	15RH, 5°C, 10days	25RH, 5°C, 10days	75RH, 5°C, 10days	15RH, 5°C, 60days	25RH, 5°C, 60days	75RH, 5°C, 60days	15RH, 5°C, 100days	25RH, 5°C, 100days	75RH, 5°C, 100 days	15RH, 5°C, 180days	25RH, 5°C, 180days	75RH, 5°C, 180days	15RH, 5°C, 255days
25RH, 5°C, 10days	0.07544	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	0.02841	0.72651	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	0.49473	0.31225	0.15421	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	0.02113	0.65694	0.9301	0.12516	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	0.00772	0.43672	0.70408	0.05888	0.77296	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	0.10093	0.91586	0.64204	0.38206	0.56488	0.36133	-	-	-	-	-	-	-
25RH, 5°C, 100days	0.00596	0.3884	0.6486	0.04922	0.71659	0.94542	0.31728	-	-	-	-	-	-
75RH, 5°C, 100 days	0.00599	0.38991	0.65049	0.04953	0.71851	0.94663	0.31832	0.99758	-	-	-	-	-
15RH, 5°C, 180days	0.00178	0.22134	0.41898	0.01944	0.47884	0.70792	0.17285	0.76058	0.7592	-	-	-	-
25RH, 5°C, 180days	7.20E-05	0.03511	0.08932	0.00123	0.11208	0.21739	0.02442	0.24851	0.2481	0.43143	-	-	-
75RH, 5°C, 180days	3.00E-07	0.00085	0.00347	1.10E-05	0.00486	0.01405	0.00053	0.01772	0.01759	0.0452	0.2635	-	-
15RH, 5°C, 255days	9.70E-05	0.04261	0.10669	0.0016	0.13209	0.24851	0.03046	0.28253	0.28156	0.47884	0.94542	0.23178	-
25RH, 5°C, 255days	8.60E-05	0.03888	0.09878	0.00142	0.12298	0.23434	0.0276	0.26605	0.26511	0.4575	0.97026	0.24632	0.97567
75RH, 5°C, 255days	5.60E-08	0.00023	0.00107	2.20E-06	0.00152	0.00482	0.00014	0.00618	0.00615	0.01843	0.1413	0.77159	0.11989
15RH, 20°C, 10days	0.98335	0.07137	0.02667	0.48229	0.01986	0.00717	0.0961	0.00553	0.00557	0.00165	6.50E-05	2.90E-07	9.20E-05
25RH, 20°C, 10days	0.05415	0.91024	0.82281	0.24891	0.75205	0.52094	0.81706	0.47204	0.47332	0.27664	0.0489	0.00137	0.05859
15RH, 20°C, 60days	0.34813	0.4575	0.2481	0.82607	0.20742	0.10634	0.53809	0.08891	0.08932	0.03888	0.003	3.50E-05	0.00394
25RH, 20°C, 60days	0.01076	0.49644	0.77296	0.0758	0.84636	0.9301	0.42262	0.87934	0.88003	0.6359	0.17873	0.01015	0.20821
75RH, 20°C, 60days	0.00015	0.05388	0.13138	0.0023	0.16276	0.292	0.03977	0.33325	0.33218	0.5434	0.88003	0.19258	0.93133
15RH, 20°C, 100days	0.05003	0.88511	0.84959	0.2352	0.7757	0.54518	0.79076	0.49165	0.49254	0.29273	0.05294	0.00154	0.06391
25RH, 20°C, 100days	0.00168	0.21452	0.40696	0.01843	0.4691	0.69659	0.1668	0.74819	0.74681	0.98692	0.44118	0.0475	0.48987
75RH, 20°C, 100days	4.10E-05	0.02417	0.0651	0.00077	0.08326	0.16747	0.0167	0.19559	0.19504	0.35239	0.91212	0.32777	0.85531
15RH, 20°C, 180days	0.00257	0.2614	0.47795	0.02594	0.5434	0.77488	0.21022	0.82931	0.8274	0.93133	0.37154	0.03547	0.4202
25RH, 20°C, 180days	2.70E-05	0.01873	0.05294	0.00054	0.06771	0.13965	0.01284	0.16528	0.16444	0.30484	0.84959	0.37678	0.79076
75RH, 20°C, 180days	6.70E-09	4.60E-05	0.00023	3.00E-07	0.00034	0.00118	2.50E-05	0.00154	0.00154	0.00527	0.05766	0.49165	0.04811
15RH, 20°C, 255days	0.00014	0.05275	0.12916	0.00222	0.15909	0.28751	0.0388	0.32777	0.32671	0.53632	0.887	0.19559	0.93933
25RH, 20°C, 255days	5.00E-06	0.00587	0.01997	0.00012	0.02685	0.06104	0.00386	0.07454	0.07409	0.15762	0.58942	0.61815	0.53322
75RH, 20°C, 255days	2.30E-10	2.50E-06	1.60E-05	1.20E-08	2.60E-05	0.00011	1.30E-06	0.00015	0.00015	0.00059	0.01058	0.18379	0.00823
15RH, -20°C, 10days	0.9301	0.05831	0.02098	0.43388	0.01573	0.00551	0.0794	0.00422	0.00425	0.00125	4.70E-05	1.80E-07	6.40E-05
25RH, -20°C, 10days	0.0386	0.80556	0.92456	0.19353	0.85341	0.62465	0.71659	0.56488	0.56668	0.35129	0.06863	0.00228	0.08287

75RH, -20°C, 10days	0.02779	0.72023	0.99339	0.15182	0.93564	0.70931	0.6359	0.65177	0.65366	0.42262	0.09112	0.00355	0.1085
15RH, -20°C, 60days	0.6284	0.23093	0.10515	0.87866	0.08326	0.03815	0.2835	0.03067	0.03088	0.01115	0.00063	4.70E-06	0.00084
25RH, -20°C, 60days	0.00885	0.4608	0.72651	0.0647	0.79844	0.9745	0.38206	0.92208	0.9227	0.68024	0.20203	0.01242	0.23372
75RH, -20°C, 60days	0.00195	0.23178	0.43265	0.02083	0.49254	0.72023	0.18196	0.77651	0.7757	0.98285	0.41741	0.04261	0.46411
15RH, -20°C, 100days	0.07707	0.99226	0.72023	0.31728	0.65088	0.43143	0.92208	0.38206	0.38356	0.21739	0.03403	0.00083	0.04158
25RH, -20°C, 100days	0.00143	0.19559	0.37826	0.01616	0.43994	0.66072	0.15182	0.71521	0.71521	0.95512	0.47037	0.05275	0.51962
75RH, -20°C, 100days	0.00028	0.07856	0.18106	0.004	0.22051	0.37678	0.05766	0.42542	0.42421	0.65555	0.76773	0.13965	0.82281
15RH, -20°C, 180days	0.00057	0.11612	0.24891	0.00717	0.29399	0.48229	0.0881	0.53455	0.53322	0.77651	0.64671	0.09665	0.70269
25RH, -20°C, 180days	1.80E-05	0.01458	0.04311	0.00039	0.05491	0.11649	0.00975	0.13774	0.137	0.26418	0.78612	0.43057	0.72981
75RH, -20°C, 180days	5.70E-07	0.00132	0.00524	1.90E-05	0.00723	0.01997	0.00084	0.02515	0.02498	0.06036	0.32265	0.91959	0.28253
15RH, -20°C, 255days	1.20E-05	0.01054	0.0333	0.00026	0.04311	0.0938	0.00693	0.11159	0.11096	0.22344	0.71521	0.49116	0.66072
25RH, -20°C, 255days	1.60E-05	0.01337	0.0403	0.00034	0.05157	0.11034	0.00892	0.13012	0.12956	0.25259	0.76773	0.44605	0.71455
75RH, -20°C, 255days	8.30E-09	5.50E-05	0.00027	3.50E-07	0.0004	0.00137	3.10E-05	0.00179	0.00178	0.00599	0.06391	0.52094	0.05294
15RH, -160°C, 60days	0.05256	0.90082	0.83372	0.24456	0.76196	0.53189	0.80556	0.48052	0.4818	0.2835	0.05067	0.00144	0.06074
25RH, -160°C, 60days	0.00011	0.04511	0.11208	0.00174	0.13847	0.25838	0.03263	0.29273	0.292	0.49165	0.9301	0.22239	0.98335
75RH, -160°C, 60days	0.02608	0.70931	0.97926	0.144	0.94965	0.72023	0.62002	0.66831	0.6697	0.43549	0.0961	0.00383	0.1137
15RH, -160°C, 100days	1.20E-07	0.0004	0.00171	4.60E-06	0.00247	0.00755	0.00024	0.00983	0.00975	0.0275	0.18749	0.87295	0.15991
25RH, -160°C, 100days	4.30E-08	0.00019	0.00088	1.70E-06	0.00127	0.00403	0.00011	0.00531	0.00527	0.01596	0.12791	0.73365	0.1079
75RH, -160°C, 100days	3.10E-07	0.00088	0.00355	1.10E-05	0.00499	0.01439	0.00054	0.01807	0.01794	0.04622	0.26605	0.99339	0.23434
15RH, -160°C, 180days	3.90E-09	2.80E-05	0.00015	1.80E-07	0.00023	0.00083	1.60E-05	0.00109	0.00109	0.00373	0.0452	0.43143	0.03741
25RH, -160°C, 180days	8.60E-12	1.80E-07	1.30E-06	6.30E-10	2.20E-06	1.10E-05	1.00E-07	1.60E-05	1.60E-05	7.30E-05	0.00181	0.05888	0.00138

75RH, -160°C, 180days	3.70E-08	0.00017	0.0008	1.40E-06	0.00115	0.00366	9.70E-05	0.0048	0.00476	0.01458	0.11923	0.71521	0.10093
15RH, -160°C, 255days	6.30E-10	7.00E-06	4.20E-05	3.70E-08	6.40E-05	0.00025	3.80E-06	0.00034	0.00034	0.00125	0.01975	0.26418	0.01573
25RH, -160°C, 255days	8.60E-12	1.40E-07	1.00E-06	5.40E-10	1.70E-06	8.80E-06	7.30E-08	1.30E-05	1.20E-05	5.90E-05	0.00153	0.05256	0.00117
75RH, -160°C, 255days	1.80E-08	9.50E-05	0.00047	7.30E-07	0.00071	0.00224	5.70E-05	0.003	0.00298	0.00954	0.08891	0.6214	0.07409

	25RH, 5°C, 255days	75RH, 5°C, 255days	15RH, 20°C, 10days	25RH, 20°C, 10days	15RH, 20°C, 60days	25RH, 20°C, 60days	75RH, 20°C, 60days	15RH, 20°C, 100days	25RH, 20°C, 100days	75RH, 20°C, 100days	15RH, 20°C, 180days	25RH, 20°C, 180days	75RH, 20°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	0.12956	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	7.80E-05	5.00E-08	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	0.05388	0.0004	0.05157	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	0.00353	7.60E-06	0.33433	0.37597	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	0.19449	0.00344	0.01007	0.59356	0.13138	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	0.91024	0.09709	0.00014	0.07499	0.00551	0.2481	-	-	-	-	-	-	-
15RH, 20°C, 100days	0.05859	0.00047	0.0475	0.97567	0.35493	0.6214	0.08141	-	-	-	-	-	-
25RH, 20°C, 100days	0.4691	0.01944	0.00154	0.26843	0.03741	0.62278	0.55545	0.28474	-	-	-	-	-
75RH, 20°C, 100days	0.88132	0.18563	3.70E-05	0.03524	0.00182	0.13554	0.78227	0.0386	0.36245	-	-	-	-
15RH, 20°C, 180days	0.39668	0.01382	0.00236	0.32671	0.04978	0.706	0.47884	0.34672	0.92208	0.29782	-	-	-
25RH, 20°C, 180days	0.81706	0.21947	2.50E-05	0.0276	0.00136	0.11333	0.72023	0.03046	0.31519	0.94054	0.2593	-	-
75RH, 20°C, 180days	0.05231	0.72023	6.00E-09	8.00E-05	1.00E-06	0.00083	0.03765	9.30E-05	0.00553	0.0794	0.00376	0.09709	-

15RH, 20°C, 255days	0.91773	0.09934	0.00013	0.0733	0.00534	0.24456	0.99226	0.0794	0.5483	0.7894	0.47332	0.72789	0.0386
25RH, 20°C, 255days	0.5568	0.39974	4.60E-06	0.00922	0.00034	0.04835	0.47037	0.0103	0.1636	0.68701	0.12846	0.74489	0.20742
75RH, 20°C, 255days	0.00929	0.32565	2.20E-10	5.00E-06	4.50E-08	7.00E-05	0.00591	6.00E-06	0.00064	0.01605	0.0004	0.02054	0.57075
15RH, -20°C, 10days	5.60E-05	3.40E-08	0.94422	0.0424	0.292	0.00766	9.60E-05	0.0388	0.00118	2.50E-05	0.00175	1.60E-05	3.90E-09
25RH, -20°C, 10days	0.07625	0.00071	0.03643	0.90585	0.29654	0.69851	0.10384	0.92889	0.34018	0.05003	0.41043	0.04056	0.00014
75RH, -20°C, 10days	0.10048	0.00109	0.02608	0.81706	0.2452	0.77651	0.13322	0.84445	0.41199	0.06648	0.4818	0.05388	0.00023
15RH, -20°C, 60days	0.00075	8.70E-07	0.61257	0.17873	0.70182	0.0489	0.0012	0.16595	0.01054	0.00037	0.01525	0.00026	1.20E-07
25RH, -20°C, 60days	0.21947	0.00422	0.00817	0.54696	0.11484	0.95512	0.27473	0.57256	0.66692	0.15502	0.74819	0.12956	0.00104
75RH, -20°C, 60days	0.44118	0.01722	0.00179	0.28877	0.04158	0.65177	0.52662	0.30614	0.97026	0.3374	0.94783	0.292	0.00482
15RH, -20°C, 100days	0.03815	0.00023	0.0733	0.90458	0.46411	0.49165	0.05275	0.87934	0.21022	0.02342	0.25722	0.01817	4.40E-05
25RH, -20°C, 100days	0.49343	0.02212	0.00132	0.24891	0.03307	0.58852	0.58942	0.26418	0.96973	0.3884	0.89017	0.33878	0.00639
75RH, -20°C, 100days	0.79709	0.06739	0.00026	0.10669	0.00922	0.31966	0.89644	0.11484	0.66831	0.67592	0.58486	0.61629	0.02442
15RH, -20°C, 180days	0.67592	0.04411	0.00052	0.15421	0.01596	0.42262	0.76966	0.16595	0.7894	0.5483	0.71017	0.49116	0.01465
25RH, -20°C, 180days	0.75783	0.25349	1.60E-05	0.02147	0.00101	0.0938	0.66262	0.024	0.27204	0.88322	0.22427	0.94054	0.11649
75RH, -20°C, 180days	0.29883	0.68788	5.00E-07	0.00211	5.90E-05	0.01487	0.24144	0.00241	0.06312	0.39821	0.04811	0.45094	0.4202
15RH, -20°C, 255days	0.68788	0.29883	1.10E-05	0.01605	0.0007	0.07409	0.58942	0.01785	0.23093	0.80747	0.18471	0.87425	0.144
25RH, -20°C, 255days	0.73557	0.26511	1.50E-05	0.01986	0.00091	0.0881	0.64582	0.02196	0.26047	0.86413	0.21349	0.9227	0.12447
75RH, -20°C, 255days	0.05766	0.75342	7.40E-09	9.40E-05	1.30E-06	0.00096	0.04179	0.00011	0.00634	0.0881	0.00433	0.10669	0.97026
15RH, -160°C, 60days	0.05596	0.00043	0.04978	0.9887	0.36714	0.60604	0.07753	0.98514	0.27542	0.03661	0.33433	0.02871	8.60E-05
25RH, -160°C, 60days	0.95939	0.11433	9.70E-05	0.0618	0.00425	0.21739	0.94663	0.06771	0.50198	0.83813	0.43265	0.7757	0.0452
75RH, -160°C, 60days	0.10574	0.00118	0.02442	0.80036	0.2352	0.79268	0.13965	0.82607	0.42542	0.07051	0.49343	0.0571	0.00026
15RH, -160°C, 100days	0.17216	0.9096	1.10E-07	0.0007	1.50E-05	0.00542	0.13012	0.0008	0.02871	0.24032	0.02054	0.27542	0.6284
25RH, -160°C, 100days	0.11612	0.96547	3.80E-08	0.00033	6.00E-06	0.00284	0.08668	0.00038	0.01679	0.16747	0.0117	0.19869	0.7592

75RH, -160°C, 100days	0.24851	0.76636	2.90E-07	0.00141	3.60E-05	0.01038	0.19504	0.00158	0.04835	0.33218	0.03619	0.38158	0.48817
15RH, -160°C, 180days	0.04077	0.65177	3.70E-09	5.30E-05	6.10E-07	0.00055	0.02841	6.00E-05	0.00394	0.0618	0.00259	0.07661	0.9301
25RH, -160°C, 180days	0.00155	0.12555	8.60E-12	3.50E-07	3.10E-09	6.90E-06	0.00098	4.20E-07	7.90E-05	0.00298	4.90E-05	0.004	0.26724
75RH, -160°C, 180days	0.10912	0.94422	3.40E-08	0.00029	5.20E-06	0.00252	0.08035	0.00034	0.01537	0.15762	0.01058	0.18749	0.77843
15RH, -160°C, 255days	0.0175	0.44442	6.10E-10	1.30E-05	1.40E-07	0.00016	0.01153	1.50E-05	0.00132	0.02871	0.00088	0.03661	0.71521
25RH, -160°C, 255days	0.00132	0.11271	8.60E-12	2.90E-07	2.30E-09	5.30E-06	0.00083	3.30E-07	6.40E-05	0.00245	3.90E-05	0.00342	0.24746
75RH, -160°C, 255days	0.08035	0.8515	1.60E-08	0.00017	2.60E-06	0.00154	0.05794	0.00019	0.01007	0.11871	0.00682	0.14189	0.87934
	15RH, 20°C, 255days	25RH, 20°C, 255days	75RH, 20°C, 255days	15RH, -20°C, 10days	25RH, -20°C, 10days	75RH, -20°C, 10days	15RH, -20°C, 60days	25RH, -20°C, 60days	75RH, -20°C, 60days	15RH, -20°C, 100days	25RH, -20°C, 100days	75RH, -20°C, 100days	15RH, -20°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-

15RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 255days	0.47667	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 255days	0.0061	0.05415	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 10days	9.30E-05	2.90E-06	1.60E-10	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	0.10139	0.0145	1.00E-05	0.02891	-	-	-	-	-	-	-	-	-
75RH, -20°C, 10days	0.13082	0.02047	1.60E-05	0.02054	0.92146	-	-	-	-	-	-	-	-
15RH, -20°C, 60days	0.00117	5.80E-05	4.50E-09	0.54965	0.13266	0.10325	-	-	-	-	-	-	-
25RH, -20°C, 60days	0.26963	0.05596	9.20E-05	0.00618	0.65088	0.73173	0.04179	-	-	-	-	-	-
75RH, -20°C, 60days	0.51962	0.14929	0.00054	0.00136	0.36568	0.43672	0.0121	0.6999	-	-	-	-	-
15RH, -20°C, 100days	0.05157	0.00567	2.40E-06	0.05954	0.79844	0.71521	0.23434	0.45421	0.2277	-	-	-	-
25RH, -20°C, 100days	0.58303	0.17963	0.00076	0.001	0.31653	0.38206	0.00916	0.6359	0.93933	0.19258	-	-	-
75RH, -20°C, 100days	0.88828	0.37597	0.00355	0.00018	0.1434	0.18379	0.00207	0.35493	0.64016	0.07661	0.70269	-	-
15RH, -20°C, 180days	0.76196	0.28156	0.00184	0.00037	0.20542	0.25259	0.00391	0.4608	0.76058	0.1137	0.82281	0.88132	-
25RH, -20°C, 180days	0.6697	0.80421	0.02649	1.10E-05	0.03241	0.04389	0.00018	0.1079	0.25349	0.01416	0.29273	0.54965	0.43833
75RH, -20°C, 180days	0.2452	0.70408	0.14265	3.30E-07	0.00357	0.00534	8.30E-06	0.01794	0.0571	0.00127	0.07094	0.18034	0.12555
15RH, -20°C, 255days	0.59771	0.88003	0.03524	7.10E-06	0.02434	0.03403	0.00012	0.08617	0.21349	0.01023	0.24851	0.48229	0.37678
25RH, -20°C, 255days	0.65088	0.82473	0.02861	1.00E-05	0.0298	0.04104	0.00016	0.10139	0.24367	0.01294	0.28085	0.53189	0.42262
75RH, -20°C, 255days	0.04289	0.22427	0.53986	4.80E-09	0.00017	0.00028	1.40E-07	0.0012	0.00553	5.30E-05	0.00743	0.0276	0.0167
15RH, -160°C, 60days	0.0758	0.0097	5.30E-06	0.04077	0.91586	0.8274	0.17285	0.5568	0.29527	0.89206	0.25606	0.11034	0.15909
25RH, -160°C, 60days	0.95393	0.5183	0.0076	7.10E-05	0.08781	0.1137	0.00091	0.24367	0.47795	0.04389	0.53455	0.84004	0.71521

75RH, -160°C, 60days	0.137	0.02181	1.80E-05	0.01934	0.90585	0.98335	0.09821	0.75012	0.45094	0.70269	0.39668	0.19258	0.26232
15RH, -160°C, 100days	0.13266	0.48229	0.2593	6.80E-08	0.00117	0.00175	1.70E-06	0.00664	0.02555	0.00039	0.03285	0.09349	0.06104
25RH, -160°C, 100days	0.08851	0.3704	0.35382	2.50E-08	0.00058	0.00091	6.60E-07	0.00357	0.01476	0.00018	0.0192	0.05917	0.03888
75RH, -160°C, 100days	0.19869	0.62278	0.18106	1.80E-07	0.00234	0.00363	4.90E-06	0.01275	0.04332	0.00084	0.05369	0.14189	0.09821
15RH, -160°C, 180days	0.02911	0.16937	0.64671	2.30E-09	9.40E-05	0.00015	6.80E-08	0.00072	0.00344	2.70E-05	0.00463	0.01817	0.01058
25RH, -160°C, 180days	0.00102	0.01337	0.64393	8.60E-12	7.40E-07	1.40E-06	2.50E-10	9.30E-06	6.50E-05	1.80E-07	9.40E-05	0.00054	0.00027
75RH, -160°C, 180days	0.08249	0.35239	0.37154	2.10E-08	0.00052	0.00083	5.70E-07	0.00322	0.01347	0.00016	0.01759	0.05526	0.03619
15RH, -160°C, 255days	0.0119	0.08851	0.86222	4.00E-10	2.50E-05	4.40E-05	1.30E-08	0.00021	0.00115	6.70E-06	0.00154	0.00693	0.00389
25RH, -160°C, 255days	0.00084	0.01134	0.60604	8.60E-12	5.70E-07	1.10E-06	2.20E-10	7.20E-06	5.40E-05	1.40E-07	7.80E-05	0.00045	0.00022
75RH, -160°C, 255days	0.05917	0.28156	0.45257	1.00E-08	0.0003	0.00048	3.00E-07	0.00193	0.00879	9.30E-05	0.01162	0.04003	0.02481

	25RH, -20°C, 180days	75RH, -20°C, 180days	15RH, -20°C, 255days	25RH, -20°C, 255days	75RH, -20°C, 255days	15RH, -160°C, 60days	25RH, -160°C, 60days	75RH, -160°C, 60days	15RH, -160°C, 100days	25RH, -160°C, 100days	75RH, -160°C, 100days	15RH, -160°C, 180days	25RH, -160°C, 180days
25RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 100 days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 5°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-

15RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, 20°C, 255days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 10days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 60days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 100days	-	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 180days	-	-	-	-	-	-	-	-	-	-	-	-	-
75RH, -20°C, 180days	0.50198	-	-	-	-	-	-	-	-	-	-	-	-
15RH, -20°C, 255days	0.9301	0.57438	-	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 255days	0.98105	0.52269	0.94783	-	-	-	-	-	-	-	-	-	-

75RH, -20°C, 255days	0.12846	0.44442	0.15844	0.13554	-	-	-	-	-	-	-	-	-
15RH, -160°C, 60days	0.02247	0.00224	0.01679	0.02069	9.90E-05	-	-	-	-	-	-	-	-
25RH, -160°C, 60days	0.71521	0.27204	0.64671	0.6999	0.05035	0.0643	-	-	-	-	-	-	-
75RH, -160°C, 60days	0.04652	0.00572	0.03619	0.04332	0.00031	0.81131	0.11923	-	-	-	-	-	-
15RH, -160°C, 100days	0.31832	0.78035	0.37597	0.33325	0.65694	0.00074	0.15261	0.00189	-	-	-	-	-
25RH, -160°C, 100days	0.23372	0.65088	0.27542	0.2452	0.78804	0.00035	0.10267	0.00099	0.87425	-	-	-	-
75RH, -160°C, 100days	0.43388	0.9227	0.49343	0.45094	0.51656	0.00149	0.22533	0.00391	0.86603	0.72789	-	-	-
15RH, -160°C, 180days	0.0938	0.35812	0.11649	0.09991	0.90458	5.60E-05	0.03524	0.00017	0.55143	0.6898	0.42701	-	-
25RH, -160°C, 180days	0.00538	0.04411	0.00749	0.00587	0.24891	3.80E-07	0.00128	1.50E-06	0.09349	0.13921	0.05794	0.31728	-
75RH, -160°C, 180days	0.22134	0.63027	0.26232	0.23286	0.80939	0.00031	0.0961	0.00088	0.84959	0.97926	0.71017	0.71017	0.14851
15RH, -160°C, 255days	0.0452	0.21555	0.05766	0.04866	0.68788	1.40E-05	0.01465	4.80E-05	0.36245	0.47667	0.2614	0.78612	0.49473
25RH, -160°C, 255days	0.00454	0.03888	0.00629	0.00499	0.23008	3.00E-07	0.00108	1.20E-06	0.08287	0.12555	0.05157	0.29156	0.96547
75RH, -160°C, 255days	0.17024	0.53189	0.20821	0.18034	0.9096	0.00018	0.07008	0.00053	0.75342	0.88828	0.61629	0.80229	0.19504

	75RH, -160°C, 180days	15RH, -160°C, 255days	25RH, -160°C, 255days
25RH, 5°C, 10days	-	-	-
75RH, 5°C, 10days	-	-	-
15RH, 5°C, 60days	-	-	-
25RH, 5°C, 60days	-	-	-
75RH, 5°C, 60days	-	-	-
15RH, 5°C, 100days	-	-	-
25RH, 5°C, 100days	-	-	-
75RH, 5°C, 100 days	-	-	-

15RH, 5°C, 180days	-	-	-
25RH, 5°C, 180days	-	-	-
75RH, 5°C, 180days	-	-	-
15RH, 5°C, 255days	-	-	-
25RH, 5°C, 255days	-	-	-
75RH, 5°C, 255days	-	-	-
15RH, 20°C, 10days	-	-	-
25RH, 20°C, 10days	-	-	-
15RH, 20°C, 60days	-	-	-
25RH, 20°C, 60days	-	-	-
75RH, 20°C, 60days	-	-	-
15RH, 20°C, 100days	-	-	-
25RH, 20°C, 100days	-	-	-
75RH, 20°C, 100days	-	-	-
15RH, 20°C, 180days	-	-	-
25RH, 20°C, 180days	-	-	-
75RH, 20°C, 180days	-	-	-
15RH, 20°C, 255days	-	-	-
25RH, 20°C, 255days	-	-	-
75RH, 20°C, 255days	-	-	-
15RH, -20°C, 10days	-	-	-
25RH, -20°C, 10days	-	-	-
75RH, -20°C, 10days	-	-	-
15RH, -20°C, 60days	-	-	-
25RH, -20°C, 60days	-	-	-
75RH, -20°C, 60days	-	-	-
15RH, -20°C, 100days	-	-	-
25RH, -20°C, 100days	-	-	-
75RH, -20°C, 100days	-	-	-
15RH, -20°C, 180days	-	-	-
25RH, -20°C, 180days	-	-	-

75RH, -20°C, 180days	-	-	-
15RH, -20°C, 255days	-	-	-
25RH, -20°C, 255days	-	-	-
75RH, -20°C, 255days	-	-	-
15RH, -160°C, 60days	-	-	-
25RH, -160°C, 60days	-	-	-
75RH, -160°C, 60days	-	-	-
15RH, -160°C, 100days	-	-	-
25RH, -160°C, 100days	-	-	-
75RH, -160°C, 100days	-	-	-
15RH, -160°C, 180days	-	-	-
25RH, -160°C, 180days	-	-	-
75RH, -160°C, 180days	-	-	-
15RH, -160°C, 255days	0.49254	-	-
25RH, -160°C, 255days	0.13322	0.46782	-
75RH, -160°C, 255days	0.91024	0.58852	0.17694

Appendix FF. Pairwise comparisons between all possible treatment combinations for germination of Lycaste cochleata.

	15RH, -160°C, 10days	15RH, -20°C, 10days	15RH, 5°C, 10days	15RH, 20°C, 10days	25RH, -20°C, 10days	25RH, 5°C, 10days	25RH, 20°C, 10days	15RH, -160°C, 60days	15RH, -20°C, 60days	15RH, 5°C, 60days	15RH, 20°C, 60days	25RH, -160°C, 60days	25RH, -20°C, 60days
15RH, -20°C, 10days	0.00432	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 10days	1.70E-10	0.0002	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	5.90E-10	0.00062	0.85367	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	0.00087	0.73688	0.00432	0.01016	-	-	-	-	-	-	-	-	-
25RH, 5°C, 10days	0.09277	0.35971	9.60E-07	2.80E-06	0.14512	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	0.0337	0.73688	4.10E-05	0.00011	0.4136	0.73688	-	-	-	-	-	-	-
15RH, -160°C, 60days	0.77421	0.01801	1.40E-09	5.10E-09	0.00432	0.24076	0.09721	-	-	-	-	-	-
15RH, -20°C, 60days	0.07763	0.52052	9.30E-06	2.70E-05	0.24413	0.89086	0.84637	0.19383	-	-	-	-	-
15RH, 5°C, 60days	0.11691	0.47304	1.20E-05	3.30E-05	0.21716	0.95891	0.78754	0.26499	0.93879	-	-	-	-
15RH, 20°C, 60days	0.11691	0.5062	2.40E-05	5.80E-05	0.24416	0.93945	0.80927	0.26447	0.95446	0.97428	-	-	-
25RH, -160°C, 60days	0.85367	0.00091	1.80E-11	4.30E-11	0.00017	0.03663	0.01137	0.58324	0.0316	0.05404	0.05404	-	-
25RH, -20°C, 60days	0.06085	0.65154	4.10E-05	0.00011	0.34729	0.83396	0.90677	0.15753	0.90677	0.86423	0.87017	0.02548	-
25RH, 5°C, 60days	0.28889	0.16509	3.40E-07	9.60E-07	0.05853	0.74579	0.46906	0.54808	0.67244	0.73688	0.73688	0.15275	0.57592
25RH, 20°C, 60days	0.02565	0.77421	6.30E-05	0.00017	0.4758	0.68232	0.93945	0.07763	0.78754	0.73688	0.76175	0.00835	0.86423

75RH, -160°C, 60days	0.18696	0.28943	1.90E-06	6.00E-06	0.11691	0.87017	0.64382	0.40013	0.80927	0.86423	0.85841	0.09277	0.73688
75RH, -20°C, 60days	0.04568	0.67029	2.70E-05	6.90E-05	0.35141	0.78754	0.93879	0.12309	0.87017	0.84637	0.86008	0.01619	0.96186
75RH, 5°C, 60days	0.01619	0.85841	0.00012	0.00034	0.57411	0.57411	0.86423	0.05404	0.72388	0.67029	0.69326	0.00471	0.80558
75RH, 20°C, 60days	0.27942	0.17203	3.40E-07	1.00E-06	0.06085	0.75741	0.4758	0.53892	0.68232	0.74579	0.73688	0.14681	0.58468

	25RH, 5°C, 60days	25RH, 20°C, 60days	75RH, -160°C, 60days	75RH, -20°C, 60days	75RH, 5°C, 60days	75RH, 20°C, 60days
15RH, -20°C, 10days	-	-	-	-	-	
15RH, 5°C, 10days	-	-	-	-	-	
15RH, 20°C, 10days	-	-	-	-	-	
25RH, -20°C, 10days	-	-	-	-	-	
25RH, 5°C, 10days	-	-	-	-	-	
25RH, 20°C, 10days	-	-	-	-	-	
15RH, -160°C, 60days	-	-	-	-	-	
15RH, -20°C, 60days	-	-	-	-	-	
15RH, 5°C, 60days	-	-	-	-	-	
15RH, 20°C, 60days	-	-	-	-	-	
25RH, -160°C, 60days	-	-	-	-	-	
25RH, -20°C, 60days	-	-	-	-	-	
25RH, 5°C, 60days	-	-	-	-	-	

25RH, 20°C, 60days	0.40275	-	-	-	-
75RH, -160°C, 60days	0.86423	0.57411	-	-	-
75RH, -20°C, 60days	0.53172	0.88058	0.70693	-	-
75RH, 5°C, 60days	0.31389	0.90677	0.47908	0.82984	-
75RH, 20°C, 60days	0.98073	0.4136	0.86661	0.54081	0.32441

Appendix GG. Pairwise comparisons between all possible treatment combinations for germination of Lycaste lasioglossa.

	15RH, -160°C, 10days	15RH, -20°C, 10days	15RH, 5°C, 10days	15RH, 20°C, 10days	25RH, -20°C, 10days	25RH, 5°C, 10days	25RH, 20°C, 10days	15RH, -160°C, 60days	15RH, -20°C, 60days	15RH, 5°C, 60days	15RH, 20°C, 60days	25RH, -160°C, 60days	25RH, -20°C, 60days
15RH, -20°C, 10days	8.40E-05	-	-	-	-	-	-	-	-	-	-	-	-
15RH, 5°C, 10days	8.60E-08	0.15888	-	-	-	-	-	-	-	-	-	-	-
15RH, 20°C, 10days	2.30E-07	0.20345	0.91753	-	-	-	-	-	-	-	-	-	-
25RH, -20°C, 10days	0.00544	0.39433	0.02378	0.0346	-	-	-	-	-	-	-	-	-
25RH, 5°C, 10days	0.00015	0.92121	0.1345	0.17372	0.45344	-	-	-	-	-	-	-	-
25RH, 20°C, 10days	0.60043	0.00196	1.00E-05	1.90E-05	0.03973	0.00283	-	-	-	-	-	-	-
15RH, -160°C, 60days	0.45344	2.60E-06	1.70E-09	4.50E-09	0.00039	5.20E-06	0.20542	-	-	-	-	-	-
15RH, -20°C, 60days	0.06568	0.06692	0.00129	0.00204	0.42549	0.08909	0.23161	0.00784	-	-	-	-	-
15RH, 5°C, 60days	0.23161	0.01104	8.80E-05	0.00016	0.14662	0.01648	0.54782	0.04756	0.54782	-	-	-	-
15RH, 20°C, 60days	0.05759	0.11359	0.00304	0.0048	0.50795	0.13893	0.20345	0.00712	0.91	0.48761	-	-	-
25RH, -160°C, 60days	0.12542	3.10E-08	8.70E-12	2.50E-11	1.70E-05	7.00E-08	0.04354	0.47853	0.00059	0.00529	0.00057	-	-
25RH, -20°C, 60days	0.72458	0.00199	1.50E-05	2.50E-05	0.03594	0.00283	0.91	0.29199	0.20387	0.48761	0.17683	0.07509	-
25RH, 5°C, 60days	0.06169	7.60E-09	4.60E-12	8.70E-12	5.00E-06	1.80E-08	0.01968	0.29315	0.00018	0.00204	0.00018	0.74795	0.03786
25RH, 20°C, 60days	0.79609	3.00E-05	3.10E-08	7.30E-08	0.0026	5.70E-05	0.45344	0.62404	0.03594	0.14716	0.03159	0.21702	0.54782

75RH, -160°C, 60days	0.44896	0.00293	1.50E-05	2.80E-05	0.06199	0.00451	0.8228	0.11941	0.33134	0.7152	0.28809	0.01788	0.72537
75RH, -20°C, 60days	0.20528	0.01434	0.00012	0.00021	0.16977	0.02054	0.50978	0.0395	0.60043	0.93771	0.53018	0.00419	0.45344
75RH, 5°C, 60days	0.06131	0.07447	0.00151	0.00237	0.44865	0.09852	0.21702	0.00712	0.96357	0.53018	0.92368	0.00052	0.19302
75RH, 20°C, 60days	0.29199	0.00736	5.20E-05	9.40E-05	0.11656	0.01104	0.62404	0.0637	0.48675	0.91621	0.4357	0.00753	0.54782

	25RH, 5°C, 60days	25RH, 20°C, 60days	75RH, -160°C, 60days	75RH, -20°C, 60days	75RH, 5°C, 60days	75RH, 20°C, 60days
15RH, -20°C, 10days	-	-	-	-	-	
15RH, 5°C, 10days	-	-	-	-	-	
15RH, 20°C, 10days	-	-	-	-	-	
25RH, -20°C, 10days	-	-	-	-	-	
25RH, 5°C, 10days	-	-	-	-	-	
25RH, 20°C, 10days	-	-	-	-	-	
15RH, -160°C, 60days	-	-	-	-	-	
15RH, -20°C, 60days	-	-	-	-	-	
15RH, 5°C, 60days	-	-	-	-	-	
15RH, 20°C, 60days	-	-	-	-	-	
25RH, -160°C, 60days	-	-	-	-	-	
25RH, -20°C, 60days	-	-	-	-	-	
25RH, 5°C, 60days	-	-	-	-	-	

25RH, 20°C, 60days	0.12188	-	-	-	-
75RH, -160°C, 60days	0.00712	0.29315	-	-	-
75RH, -20°C, 60days	0.00164	0.12771	0.65358	-	-
75RH, 5°C, 60days	0.00016	0.03293	0.30948	0.57233	-
75RH, 20°C, 60days	0.00293	0.18629	0.80246	0.86308	0.46282