Germination Temperature and the Effect of Storage Time on the Seed Viability of *Zephyra elegans* (Tecophilaeaceae)

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Additional index words. after-ripening, Atacama Desert, geophyte, seed dormancy, seed germination, seed storage

Abstract. *Zephyra elegans* is a geophyte endemic to Chile that has horticultural value as a cut flower. Its natural habitat is a coastal desert climate with abundant cloud cover, little and variable precipitation, and mean monthly temperatures between 10 and 20 °C. It is hypothesized that the temperature requirements for germination of *Z. elegans* seeds are similar to those of *Zephyra compacta*, which shares the same habitat. As it is a species from a desert climate, it can be expected that its seeds remain viable during long periods of storage. The seeds used in this study were harvested in different years from plants grown from cultivated corms of *Z. elegans*. The germination test temperatures were 10, 15, 20, and 25 °C, and the dry storage times at room temperature (20 ± 5 °C) were 4, 16, 28, and 40 months. The temperature requirements for germination of *Z. elegans* seeds are similar to those of *Z. compacta*, which has been studied earlier, i.e., the temperature range for achieving high germination percentages is 10 to 20 °C, whereas 25 °C can be considered supraoptimal. After-ripening of the seeds occurred during dry storage, as shown by increased germination when tested at a supraoptimal temperature. The seeds had high viability after 40 months of dry storage at room temperature.

Zephyra elegans is a perennial herbaceous species of horticultural value. The genus *Zephyra*, belongs to the Tecophilaeaceae family, is endemic to Chile and comprises *Z. compacta*, *Zephyra cyanocrocus*, *Z. elegans*, and *Zephyra violiflora* (Buerki et al., 2013; Ravenna, 1988). *Zephyra elegans* has fibrous corms, narrow basal lanceolate leaves, panicle inflorescence, six-petaled actinomorphic flowers with straight or wavy edges, fruit in the shape of three-sided round capsules, and ellipsoid, flattened, rough, dark brown seeds (Muñoz and Moreira, 2000; Simpson and Rudall, 1998). Its conservation status is described as out of danger (Squeo et al., 2008). It has horticultural value as a cut flower because the plant is generally neat; with floral stems longer than 40 cm; comprising numerous flowers with a diameter of 2 cm; a white interior and pale blue exterior; and excellent flower lifespan (Bridgen et al., 2002; Kim and Ohkawa, 2001).

The natural habitat of this species is the desert coast of northern Chile between the region of Arica and Parinacota (18 °S) and the region of Coquimbo (29 °S) (Pinto and

Luebert, 2009; Ravenna et al., 1998). This habitat is mainly composed of sandy soils with a coastal desert climate and abundant cloud cover, which in accordance with the Köppen classification system would be classified as B_{Wn} (Juliá et al., 2008). As is also the case with *Z. compacta* (De la Cuadra et al., 2017), the climate in which *Z. elegans* grows experiences cloud cover and fog almost all year, little and highly variable rainfall from year to year, mean monthly temperatures from 10 to 20 °C, and a mean daily temperature oscillation of 7.5 °C. According to Antonioletti et al. (1972), the seasonality of this climate is defined by the frequency of the cloud cover and fogs, and by rainfall on 1–4 d in winter, rather than by annual changes in temperature. It is important to note that the El Niño–Southern Oscillation phenomenon occurs in this region, where unusually high rainfall (≥15 mm) leads to the emergence of more than 200 species of annual and geophyte plants, including *Z. elegans* (Gutiérrez, 2008; Vidiella et al., 1999).

Zephyra elegans may have survival strategies to take advantage of the sporadic years in which water from precipitation is available and to survive the dry years with little or no precipitation, as is common among desert species (Figueroa et al., 2004). According to Baskin and Baskin (2014), the main survival strategies of desert species include the formation of persistent seed banks and latency mechanisms that allow the seeds to remain

viable for long periods until conditions for germination and propagation of the plants become favorable.

Considering that *Z. compacta* lives in the same habitat as *Z. elegans* and that its germination temperature requirements are known (De la Cuadra et al., 2017), it can be hypothesized that the germination temperature requirements of *Z. elegans* seeds are similar to those of *Z. compacta*. In other words, high germination percentages can be obtained in the temperature range between 10 and 20 °C, whereas 15 °C can be considered optimum and 25 °C will inhibit germination. Because *Z. elegans* is a desert species, its seeds should also remain viable in dry storage for more than 1 year.

Based on the previous text, the objective of the present study is to determine the optimum temperature for germination, while also assessing the effect of storage time on the seed viability of *Z. elegans*.

Materials and Methods

The seeds came from 45 corms of *Z. elegans* collected in 2007 in the coastal desert region of the Atacama Desert, Chile, between Huasco (28°30'S, 71°15'W) and Bahía Sarco (28°51'S, 71°27'W). In 2009, the corms were grown in Viña del Mar, Chile (33°00'S, 71°30'W), and the seeds were then harvested and stored for 28 and 40 months until germination tests were carried out. In 2011, the same corms were grown in Quillota, Chile (32°54'S, 71°12'W), and the seeds were harvested and stored for 4 and 16 months until germination tests were carried out. All seeds were stored in paper envelopes at room temperature [20 ± 5 °C; 50% to 70% relative humidity (RH); darkness] at the Laboratory of Phytogenetics at the School of Agronomy of the Faculty of Agricultural and Food Sciences, at the Pontificia Universidad Católica de Valparaíso, located in Quillota, Chile.

The germination tests were carried out in chambers at temperatures of 10, 15, 20, and 25 ± 2 °C, in darkness, with seeds that had been stored for 4, 16, 28, and 40 months. After storage, the seeds were disinfected for 3 min in 1% Captan® solution [Captan® 50WP; N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide; Arysta LifeScience North America, Cary, NC] and then rinsed three times. Each treatment consisted of 200 seeds distributed on filter paper wetted with 4 mL of water in four petri dishes (9 cm in diameter) with 50 seeds in each dish. The germination results were recorded every 2 days for a maximum period of 6 weeks. Seeds were exposed to normal light while checking germination. A seed was considered germinated once the radicle had emerged 2 mm. If the seeds did not reach 85% germination for at least one of the test temperatures for each storage time, the viability of the nongerminated seeds was checked using the tetrazolium test (International Seed Testing Association, 2003).

Received for publication 29 Dec. 2017. Accepted for publication 23 Apr. 2018.

We gratefully acknowledge Luis Arriagada for collecting the corms of *Zephyra elegans* from which the seeds used in this study were obtained.

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The germination data for each treatment were fitted to the event-time model $F(t) = d/(1 + \exp[b\{\log(t) - \log(t_{50})\}])$ used by Ritz et al. (2013), where F defines a proper cumulative distribution function, which for each time point $t \geq 0$ returns the fraction of seeds that has already germinated. The upper limit parameter d denotes the proportion of seeds that germinated during the experiment of the total number of seeds present at the beginning of the experiment. The parameter b is proportional to the slope of F at time t equal to the parameter t_{50} , the time at which 50% of all seeds germinated during the experiment have germinated.

Parameter estimation of the event-time model for each treatment was carried out using the extension package for the software environment R (Ritz and Streibig, 2005). The statistical test used for comparisons was the test of equality of two means when population variances are not equal at $P \leq 0.05$ (Ritz and Streibig, 2005).

Results

As shown in Figure 1, germination curves for the different treatments were fitted to the model used by Ritz et al. (2013). The highest values of parameter d were obtained at 10, 15, and 20 °C. Irrespective of the storage time of 4, 16, or 28 months, in the range of 10 to 20 °C, values of $d \geq 93\%$ were obtained. Based on the values of the parameter t_{50} , within the germination range of 10 to 20 °C, the optimum temperature appears to be 15 °C. Regarding the nature of germination in relation to the value of parameter b , the uniformity tended to be higher, i.e., lower values of b , at lower temperatures (Table 1).

At 25 °C, storage time was seen to affect germination. Seeds stored for 4 months gave a maximum germination percentage (d) of 15%, and as storage time increased germination reached 90%. Following a similar pattern, the value of t_{50} decreased and the value of b increased from 16 months of storage, giving germination curves a more vertical shape as storage time increased (Fig. 2). Germinating at 25 °C, the seeds stored for 28 and 40 months gave similar values for d , t_{50} , and b . The value of b for seeds stored for 28 and 40 months increased, in some cases reaching the same level as seeds germinated in the range of 10 to 20 °C (Table 1).

Discussion

Seeds were incubated in darkness and checking for germination exposed them to room light. Independent of the germination temperature and storage time, with the exception of 25 °C and 4 months' storage, germination was high ($d > 85\%$). Though the light requirement for germination is unknown, any such requirement would have been fulfilled while germination was being checked. Although germination at 25 °C of seeds stored for 4 months reached only 18%, seeds from the same storage time incubated at temperatures from 10 to 20 °C saw germination

Table 1. Parameter estimates (SE in brackets) of the log-logistic model $d/(1 + \exp[b\{\log(t) - \log(t_{50})\}])$ obtained by fitting the event-time model. The upper limit parameter d denotes the proportion of *Zephyra elegans* seeds that germinated during the experiment, parameter t_{50} denotes the time at which 50% of the seeds that germinated during the experiment had germinated, and parameter b denotes the slope of the germination curve at time t_{50} .

Germ. ^z T°	d Upper limit	t_{50} 50% germ.	b Slope
Storage time at 20 °C: 4 mo.			
10 °C	98 (1) a A	14.5 (0.2) c I	-8.0 (0.5) a AB
15 °C	95 (2) a ABCD	6.8 (0.2) a A	-5.8 (0.4) b DE
20 °C	95 (2) a ABCD	11.8 (0.3) b G	-4.9 (0.3) b EF
25 °C	18 (3) b E	36.9 (2.9) d K	-5.5 (1.2) ab BCDEFG
Storage time at 20 °C: 16 mo.			
10 °C	96 (1) ab ABC	13.2 (0.2) c H	-7.7 (0.5) ab ABC
15 °C	98 (1) a A	8.9 (0.2) b DE	-6.8 (0.5) b BCD
20 °C	98 (1) a AB	8.3 (0.1) a BC	-8.2 (0.6) a A
25 °C	88 (3) b CD	18.8 (1.3) d J	-2.4 (0.2) c I
Storage time at 20 °C: 28 mo.			
10 °C	93 (2) ab BCD	17.8 (0.3) d J	-8.9 (0.6) a A
15 °C	95 (2) ab ABCD	7.9 (0.2) a B	-5.0 (0.3) c EF
20 °C	97 (1) a ABC	8.6 (0.2) b CD	-6.4 (0.4) b CD
25 °C	90 (2) B D	10.5 (0.3) c F	-4.1 (0.3) c FH
Storage time at 20 °C: 40 mo.			
25 °C	90 (2) D	9.5 (0.4) EF	-3.5 (0.2) GH

^zFor each storage time, mean values within a column followed by the same lowercase letter do not differ significantly ($P \leq 0.05$) according to a test of equality of two means when population variances are not equal. Mean values within a column followed by the same capital letter do not differ significantly ($P \leq 0.05$) according to a test of equality of two means when population variances are not equal.

higher than 95%, and therefore the viability test was not performed.

Germination of *Z. elegans* responded to temperature in a way that was consistent with the climate characteristics of its habitat. *Z. elegans* has a temperature range for high germination percentages of 10 to 20 °C, which coincides with temperatures that occur throughout the year in its natural habitat (Juliá et al., 2008). The lack of a single optimum temperature supports the idea that the seasonality of the coastal desert region of the Atacama is not defined by temperature variation but by the frequency of cloud cover, fog and rainfall (Antonioletti et al., 1972; Vidiella and Armesto, 1989). As with many desert species, germination is associated more with water availability than with temperature (Black et al., 2006).

At 25 °C, germination performance was found to depend on storage time. Germination of seeds stored for 4 months was 18%, after an incubation period of 8 weeks in darkness. A similar result, germination did not exceed 40%, was observed when germinating seeds in tissue culture medium under in vitro conditions, in darkness and at the same temperature of 25 °C (Vidal et al., 2012). For longer storage times of 16, 28, and 40 months, germination was over 88%, implying that 25 °C is a supra-optimal germination temperature.

Other species from the same habitat show similar responses. *Zephyra compacta*, for example, has a high germination (over 85%) in the temperature range from 10 to 20 °C, and an optimum germination temperature of 15 °C (De la Cuadra et al., 2017). Other geophyte herbaceous species native to Chile such as *Conanthera campanulata* (C. De la Cuadra, unpublished data), *Leucocoryne dimorphopetala* (De la Cuadra et al., 2016), and *Pasithea coerulea* (Jara et al., 2006; Schiappacasse et al., 2005) achieve

Fig. 1. Example of typical germination curves fitted to the event-time model (solid line). According to Ritz et al. (2013), the model equation is $d/(1 + \exp[b\{\log(t) - \log(t_{50})\}])$, where t_{50} is the time to reach 50% germination relative to the maximum germination percentage, which is the upper limit d of the germination curve. The slope of the germination curve at time t_{50} is proportional to b (with a proportionality constant that depends on d and t_{50}). The interpretations of the parameter estimates are shown with dotted lines in the figure. Seeds of *Zephyra elegans* stored for 4 months and germinated at 15 °C.

high germination at a narrower temperature range of 10 to 15 °C.

The viability of *Z. elegans* seeds was unaffected by storage at room temperature for up to 40 months. As with *Z. compacta* (De la Cuadra et al., 2017), germination of *Z. elegans* in the temperature range of 10 to 20 °C led to germination values of above 90% for seeds stored for 28 months at room temperature. Thus, the survival strategy of *Z. elegans* includes corm formation (Yañez

Fig. 2. Effect of storage time at $20 \pm 5^\circ\text{C}$ for 4 months (solid line, +), 16 months (dash line, ○), 28 months (dot line, □), and 40 months (dash and dot line, Δ) on the germination of *Zephyra elegans* seeds at temperatures of 10 (A), 15 (B), 20 (C), and 25 (D) in darkness. Lines represent germination curves fitted to the event-time model and markers represent observed data.

et al., 2005) and retention of seed viability for long periods of dry storage.

Complimentary to the above, *Z. elegans* appears to have a seed dormancy mechanism as a survival strategy. When incubated at the supraoptimal temperature of 25°C , germination increased with the increase in storage time, extending the germination range. This is evidence of after-ripening, which increases the temperature range for germination after seeds have been exposed to dry storage conditions at temperatures greater than 15°C (Finch-Savage and Leubner-Metzger, 2006). Additional evidence of after-ripening has been observed from seeds of *Z. elegans* harvested in 2015, stored at similar conditions as the present experiment ($20 \pm 5^\circ\text{C}$; 50% to 70% RH; darkness) and tested for germination at 15 and at supraoptimal temperature of 25°C . For seeds stored for 0, 2, and 4 months, germination at 15°C was 95%, 91%, and 94%, respectively; and for seeds germinated at 25°C , values were 1%, 3%, and 30%, respectively (C. De la Cuadra, unpublished data). Considering these data in view of the dormancy classification system (Baskin and Baskin, 2004), *Z. elegans* seeds have type 1 nondeep physiological dormancy.

In summary, seeds of *Z. elegans* stored for 4–40 months have a range for optimum

germination from 10 to 20°C and a supra-optimal temperature of 25°C . Seeds incubated at 25°C showed an increase in germination as the period of after-ripening increased.

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