

Effect of temperature and scarification on seed germination of *Conanthera* spp. (Tecophilaeaceae)

Carlos De la Cuadra¹, Alexis K. Vidal^{1*}, Felipe Lagomarsino¹, Patricia Peñaloza¹, Leví M. Mansur¹, and Carlos Huenchuleo¹

¹Pontificia Universidad Católica de Valparaíso, Escuela de Agronomía, Casilla 4-D, Quillota, Chile.

*Corresponding author (kooichi.vidal@pucv.cl).

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ABSTRACT

The Chilean endemic genus *Conanthera* is comprised of five species of herbaceous cormous geophytes. They have ornamental value due to their bell-shaped flowers, which are blue, violet or white. Previous germination studies of *Conanthera campanulata* and *Conanthera trimaculata* were carried out at 22 °C, and manual mechanical scarification improved germination. Based on these results, it was suggested that physical dormancy is present in the non-scarified seeds. However, an improvement in germination after scarification is not enough evidence to conclude that the seed is non-permeable to water and thus has physical dormancy. The objective of the present study was to assess the testa water permeability via an imbibition test and to identify the optimum germination temperature in *C. campanulata* and *C. trimaculata*. Using 6-mo-old seeds, data from the imbibition tests showed that neither the seeds of *C. campanulata* nor *C. trimaculata* have physical dormancy. In the germination experiments, the temperature range for achieving high germination percentages was 10 to 15 °C, where germination reached 90% in less than 28 d. Temperature of 20 °C can be considered supra-optimal, while 5 and 25 °C inhibited germination. Manual mechanical scarification did not affect germination results at the range of 10 to 15 °C. However, at the supra-optimal temperature of 20 °C manual mechanical scarification increased germination, although levels were never higher than 25%.

Key words: *Conanthera campanulata*, *Conanthera trimaculata*, germination temperature, imbibition, scarification, seed dormancy.

INTRODUCTION

Conanthera (Ruiz & Pav.) is one of eight genera of Tecophilaeaceae (Simpson and Rudall, 1998), and it is comprised of five species: *C. bifolia*, *C. campanulata*, *C. parvula*, *C. trimaculata* and *C. urceolata*, which are endemic to Chile (Instituto de Botánica Darwinion, 2017). The genus has ornamental potential as a garden plant, pot plant and cut flower (Simpson and Rudall, 1998; Riedemann and Aldunate, 2001; Yáñez, 2001; Olate and Schiappacasse, 2013). The *Conanthera* species are perennial, herbaceous geophytes with tunicated corms; their leaves are basal, linear and glabrous; the inflorescence is a panicle with bell-shaped flowers with six straight or reflex blue, violet or white tepals, with or without spots; and the fruit is comprised of round tri-valve capsules, which produce black, opaque, ovoid seeds in variable numbers depending on the species (Muñoz, 2000). The conservation status is determined as of little concern for *C. campanulata*, while *C. urceolata* is at risk, and *C. bifolia*, *C. parvula* and *C. trimaculata* have not been evaluated (Ministerio del Medio Ambiente, 2017).

These species are common on sunny hillsides with well-drained soils (Riedemann and Aldunate, 2001), as well as in clearings and on slopes of sclerophyllous forests and shrublands (Teillier et al., 2005). According to Buerki et al. (2013), the habitats of *Conanthera* species are arid climates (*C. campanulata* and *C. urceolata*) or Mediterranean climates (*C. bifolia*, *C. campanulata*, *C. trimaculata* and *C. parvula*). In both of these climates, adult corms sprout and grow in

autumn and winter, flowering occurs in spring, and seeds are dispersed during summer. The summer in these regions is characterized by absence of rainfall. According to Baskin and Baskin (2014), seeds of herbaceous species in these climates can be categorized into predominantly having physiological dormancy (65%), followed by physical dormancy (20%) or other types of dormancy (12%), and a few having no dormancy (3%).

The germination requirement of some *Conanthera* species have been studied. Schiappacasse et al. (2005) found that *C. bifolia* requires stratification at 8 °C for 6 wk with subsequent germination at 15 and 20 °C, thus assuming the occurrence of physiological dormancy (Baskin and Baskin, 2014). Vogel et al. (1999) stated that for seeds of *C. campanulata* and *C. trimaculata* stored for 8- and 11-mo manual mechanical scarification improved germination at 22 °C. Based on this result, Vogel et al. (1999) suggested the presence of physical dormancy in seeds of these two species. However, Baskin and Baskin (2014) have explained that increased germination after scarification is not enough evidence to prove that seeds have physical dormancy, and thus an imbibition test is required to determine the presence or absence of physical dormancy.

The present study focuses on seed imbibition and determining the optimum temperature required for germination of *C. campanulata* and *C. trimaculata*. These results are important for the domestication, conservation and development of genetic improvement programs for these species.

MATERIALS AND METHODS

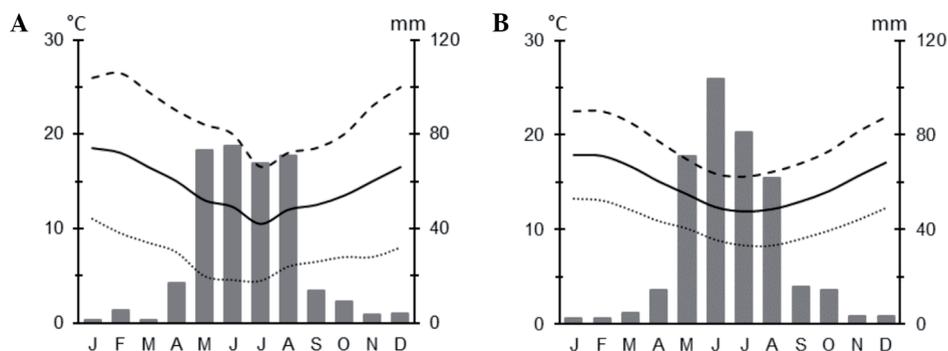
Seed origin

Seeds of *Conanthera campanulata* and *C. trimaculata* were collected at the end of February 2016 from dehiscent capsules from populations of plants growing in their natural habitats. Sampling of *C. campanulata* was done at Catapilco (32°31' S, 71°17' W), inland from Zapallar, Chile. This area is classified as semi-arid subtropical Mediterranean climate (Novoa and Villaseca, 1989) as shown in Figure 1A. Sampling of *C. trimaculata* was done at Laguna Verde (33°06' S, 71°40' W), a coastal area near Valparaíso, Chile. Its climate is classified as marine Mediterranean (Novoa and Villaseca, 1989), as shown in Figure 1B. Moreover, both areas, during the period from 1979 to 2015 have not shown significant warming trends due climate change (Burger et al., 2018). After seeds were collected, they were stored in paper envelopes at 20 ± 5 °C for 6-mo until imbibition (Experiment 1) and germination (Experiment 2) tests were carried out.

Experiment 1 - Imbibition tests

The imbibition tests of *C. campanulata* and *C. trimaculata* non-scarified and manual mechanically scarified seeds were carried out in a growth chamber at 15 °C in dark conditions. The manual mechanical scarification consisted in uniform abrasion around all seed surface with 80 grit wood sandpaper, removing the testa evenly until the endosperm was slightly

Figure 1. Climographs of subtropical semiarid Mediterranean climate (A) and marine Mediterranean climate (B) corresponding to the collection sites of Catapilco (32°31' S, 71°17' W) and Laguna Verde (33°06' S, 71°40' W) for the *Conanthera campanulata* and *C. trimaculata* seeds, respectively. Adapted from Novoa and Villaseca (1989).



Solid bars: Precipitation, solid line: average monthly temperature, dashed line: maximum monthly temperature, dotted line: minimum monthly temperature.

visible, avoiding seed damage (Mousavi et al., 2011). For each species, the experiment was arranged in a completely randomized design with three replicates of 25 seeds. The seeds in each replicate were weighed after 0, 6, 24, and 48 h of imbibition. Ten replicates of 100 seeds of each of the four cultivars were weighed using an electronic balance (model BP210S, Sartorius, Göttingen, Germany).

The relative seed mass increase by each replicate after each imbibition period was calculated using the equation: $\%Wi = ((Wi - Wf)/Wf) \times 100$, where Wi and Wf are the weight of imbibed and non-imbibed seeds, respectively (Shalimu et al., 2015). The relative seed mass increase was analyzed by ANOVA for each species. To compare the different treatments, the Tukey test was used with $P \leq 0.05$. Prior to the ANOVA test, the data were arcsine transformed to satisfy the assumptions of ANOVA (Walpole et al., 2012). The data were analyzed using Minitab 17 (Minitab Inc., State College, Pennsylvania, USA).

Experiment 2 - Germination tests

For each species, germination tests also were conducted for non-scarified and manual mechanically scarified seeds using a completely randomized experimental design. The manual mechanical scarification was carried out as described in Experiment 1. Before sowing, non-scarified and mechanically scarified seeds were disinfected for 3 min in a solution of 1% captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide; Captan 50WP, Arysta LifeScience North America, Cary, North Carolina, USA) and then rinsed three times.

Germination tests of non-scarified seeds were carried out in growth chambers at 5, 10, 15, 20 and 25 ± 2 °C, under dark conditions. For manual mechanically scarified seeds, germination tests were carried out only at 10, 15 and 20 ± 2 °C due to limitations of seeds. Each treatment had three replicates of 25 seeds each, which were placed in a 9 cm diameter Petri dish with absorbent paper soaked with 3 to 4 mL distilled water. The germination records were taken every 2 d for a maximum period of 4 wk. Seeds were exposed to room light while checking for germination. A seed was considered germinated when the radicle had emerged to a length of at least 2 mm. If seeds did not reach 85% germination in at least one of the temperature treatments at each storage months, a tetrazolium test was performed to control seed viability (International Seed Testing Association, 2003).

The germination data for each treatment was fit 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 have already germinated. The upper limit parameter d denotes the proportion of seeds that germinated during the experiment out 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} , this last parameter being the time elapsed in order to reach 50% germination of the maximum germination percentage.

For parameter estimation of the event-time model for each treatment, the extension package for the software environment R was used (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

Experiment 1 - Imbibition tests

The mass of seeds varies depending on the species. *Conanthera campanulata* seeds had an average mass of 1.67 ± 0.11 g per 1000 seeds, *C. trimaculata* seeds an average of 2.85 ± 0.14 g per 1000 seeds. Manual mechanical scarification reduced their average mass by 8% and 10%, respectively.

For both species, there is a significant interaction between scarification and imbibition time on the relative seed mass increase (Table 1). Such an interaction was only observed at 6 h of imbibition, where the increase in relative seed mass of non-scarified was always under 48%, while for manual mechanically scarified seeds it was higher than 58%. After 48 h of imbibition, the relative seed mass increase was greater than 80%, irrespective whether the seed was scarified or not. As imbibition time increased beyond 6 h, there was no difference in the relative seed mass between treatments (Figure 2).

Experiment 2 - Germination tests

Germination curves for the different treatments were fit to the model used by Ritz et al. (2013) (Figure 3). For non-scarified seeds stored for 6-mo, the highest values for the upper limit parameter d were obtained at 10 and 15 °C for both

species. The values of t_{50} at 10 and 15 °C showed differences, but not the values of b (slope at time t_{50}) for *C. campanulata* and *C. trimaculata*. At 5, 20, and 25 °C, after 28 d, the final germination percentage was zero or less than 5% (Table 2).

A similar result was observed for mechanically scarified seeds stored for 6-mo. The highest values of d were obtained at 10 and 15 °C, and the values of the other variables showed similar results. Germination was also zero at 5 and 25 °C after 28 d. For *C. trimaculata*, there were no differences in germination at 20 °C for scarified and non-scarified seeds. However, there was an effect of scarification on *C. campanulata* at 20 °C, although the final germination percentage (d) of mechanically scarified seeds was not higher than 25% (Table 2).

DISCUSSION

Indistinct if the seeds were non-scarified or mechanically scarified, germination was high ($d > 85\%$) for at least one germination temperature for each species. Based on this result, disinfection treatment before sowing was assumed harmless and as having no effect on viability of the seed. So a tetrazolium test was not required and although light requirement for germination is unknown, if there was any, it would have been fulfilled during seed germination evaluation, which was performed under room light conditions.

The testa of the seeds of *C. campanulata* and *C. trimaculata* stored for 6-mo did not confer physical resistance to water entry. This would mean that, unlike the findings of Vogel et al. (1999), the seeds of *C. campanulata* and *C. trimaculata* do not have physical dormancy. In addition, we can reconfirm the findings of Baskin and Baskin (2014) in that the effective way to determine physical dormancy in seeds is through imbibition testing. A similar characteristic of testa not conferring physical resistance to water entry can also be seen on *C. bifolia*, *Zephyra compacta*, *Z. elegans* and *Z. violiflora*, all of which are in the Tecophilaeaceae (Schiappacasse et al., 2005; Vidal et al., 2012; De la Cuadra et al., 2017).

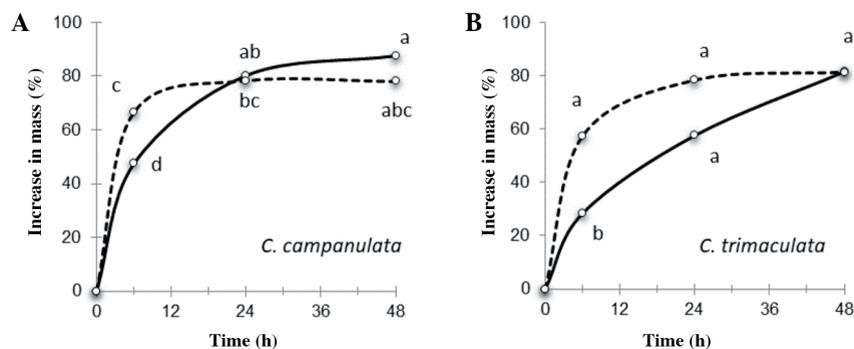
Table 1. Mean squares for relative seed mass increase of *Conanthera* spp. non-scarified and mechanically scarified seeds after 6, 24, and 48 h imbibition.

Source	DF	<i>Conanthera campanulata</i> MS	<i>Conanthera trimaculata</i> MS
Scarification (S)	1	0.0ns	485.8**
Imbibition (I)	2	455.4***	882.4***
S × I	2	142.4***	122.5*
Error	12	10.5	34.0

DF: Degrees of freedom; MS: mean squares.

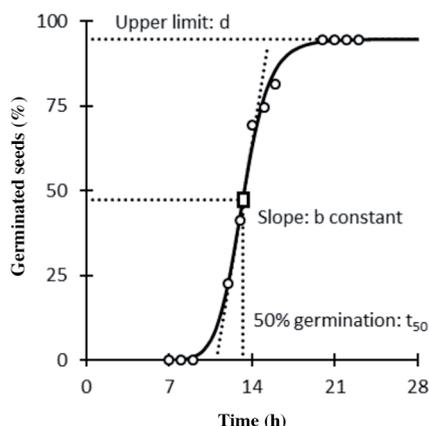
*, **, ***Significant at the 0.05, 0.01 and 0.001 probability levels, respectively, by the F test; ns: nonsignificant.

Figure 2. Imbibition curves for non-scarified (solid line) and mechanically scarified (dashed line) seeds of *Conanthera campanulata* (A) and *C. trimaculata* (B) at 15 °C.



Means followed by the same letter in each graph showed non-significant differences according to the Tukey test ($P \leq 0.05$).

Figure 3. Example of typical germination curves fit 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 elapsed 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.



Six-month-old non-scarified seeds of *Conanthera trimaculata* germinated at 10 °C.

Table 2. Parameter estimates (standard errors in brackets) of the log-logistic model $d/(1 + \exp[b\{\log(t)-\log(t_{50})\}])$ obtained by fitting the event-time model on *Conanthera* spp. seeds stored for 6-mo before sowing. The upper limit parameter d denotes the proportion of seeds that germinated during the experiment, t_{50} denotes the time by which 50% of the seeds that germinated during the experiment have germinated, and b denotes the slope of the germination curve at time t_{50} .

Germination temperature, °C	d	t_{50}	b
<i>Conanthera campanulata</i> , non-scarified seeds			
5	0		
10	97 (2)aA	11.4 (0.2)bB	-11.3 (1.3)aAB
15	96 (2)aA	8.0 (0.1)aA	-15.8 (1.8)aA
20	0		
25	0		
<i>Conanthera campanulata</i> , mechanically scarified seeds			
10	99 (1)aA	10.5 (0.3)bB	8.1 (0.8)aB
15	99 (1)aA	8.1 (0.1)aA	-11.6 (1.3)aAB
20	27 (6)bB	10.3 (3.2)abAB	-1.9 (0.5)bC
<i>Conanthera trimaculata</i> , non-scarified seeds			
5	0		
10	95 (3)aA	13.2 (0.2)bB	-11.8 (1.4)aA
15	94 (3)aA	11.0 (0.3)aA	-8.4 (0.9)abAB
20	4 (3)bB	10.7 (3.6)abAB	-3.8 (2.4)bB
25	0		
<i>Conanthera trimaculata</i> , mechanically scarified seeds			
10	97 (2)aA	13.1 (0.3)bB	-8.3 (0.9)aAB
15	94 (3)aA	11.5 (0.3)aA	-7.4 (0.8)aB
20	19 (11)bB	20.5 (13.2)abAB	-2.0 (0.2)bC

For each species and scarified treatment, mean values within a column followed by the same lower-case 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 of each species 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.

The temperature range for achieving high germination percentages for *C. campanulata* and *C. trimaculata* is between 10 and 15 °C. When analyzing variable t_{50} (time where 50% of the seeds that germinated during the experiment have germinated) for *C. campanulata* and *C. trimaculata*, the optimum temperature is 15 °C. For both species, 20 °C is considered supra-optimal. Five and 25 °C are outside the temperature range that allows seeds of the two study species to germinate. The seeds of *C. campanulata* and *C. trimaculata* have a behavior similar to that of a winter annual species, i.e. they germinate in winter and plants senesce in spring or summer, after seed set. Similar behavior is seen in other Mediterranean geophytes from Chile, such as *Leucocoryne* spp., *Trichopetalum plumosum*, *Pasithea coerulea* and *Schizanthus litoralis* (Jara et al., 2006; De la Cuadra et al., 2016) and others geophytes as *Crocus* spp., *Narcissus* spp., *Scilla autumnalis* and *Urginea maritima* (Marques and Draper, 2012; Skourti and Thanos, 2015).

According to Figueroa et al. (2004), the region of central Chile with a Mediterranean climate (from 30° to 37° S lat) is characterized by the presence of many species that respond to mechanical or chemical scarification of their seeds. However, manual mechanical scarification has no effect on the germination of *C. campanulata* and *C. trimaculata* in the temperature range of 10 to 15 °C. Only when germinating at the supra-optimal temperature of 20 °C does scarification increase germination, although the level achieved is rather low, not exceeding 25%. At the supra-optimal temperature of 22 °C, a similar effect has been reported by Vogel et al. (1999) for seeds of *C. campanulata* and *C. trimaculata*.

There is no evidence that 6-mo storage at 20 ± 5 °C has a detrimental effect on the viability of seeds of *C. campanulata* and *C. trimaculata*, as germination was over 93%. It is probable that the *Conanthera* seeds studied here are to be categorized as orthodox and show a high degree of longevity under storage. This has also been observed with others Mediterranean species in Chile such as *Alstroemeria* spp., *Calceolaria* spp., *Leucocoryne* spp. and *Nolana paradoxa* (Hong et al., 1998; De la Cuadra et al., 2002) and species from others Mediterranean regions (Juan-Vicedo et al., 2016).

The results reported in this manuscript are for 6-mo-old seeds, potentially after-ripened, and future studies using fresh seeds may not result in high germination percentages at 10 or 15 °C. It is not possible to discard that seeds were dormant at maturity and may have after-ripened during storage, it means that *C. campanulata* and *C. trimaculata* could have physiological dormancy.

CONCLUSIONS

The results of this study show that the seeds of *Conanthera campanulata* and *C. trimaculata* have no physical dormancy, and the temperature requirements for germination of 6-mo-old seeds, which are 10 to 15 °C, match those annual winter species that grow in the same Mediterranean habitats.

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