Forest Systems
24(1), e013, 13 pages (2015)
eISSN: 2171-9845
http://dx.doi.org/10.5424/fs/2015241-06197
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Germination ecology of the endemic Iberian daffodil *Narcissus radinganorum* (Amaryllidaceae). Dormancy induction by cold stratification or desiccation in late stages of embryo growth

José María Herranz, Elena Copete, Miguel Ángel Copete* and Pablo Ferrandis

E.T.S.I.A.M., Department of Plant Production and Agricultural Technology, Botanical Institute of the University of Castilla-La Mancha, University Campus s/n, Albacete 02071, Spain

Abstract

Aim of study: We studied the germination ecology of a threatened daffodil in order to develop a protocol to produce plants *ex-situ* from seeds, a key tool for population reinforcement.

Area of study: Experiments were carried out both outdoors and in the laboratory in Albacete (Spain).

Material and methods: Embryo length, radicle and shoot emergence were analyzed to determine the level of morphophysiological dormancy (MPD). Effects on germination of cold stratification or desiccation in late stages of embryo growth were also studied.

Main results: Mean embryo length in fresh seeds was 1.36 mm, needing to grow up to 2.20 mm to be able to germinate. In the laboratory, embryo growth occurred during warm stratification (28/14, 25/10 ºC), and then radicle emerged when temperatures went down (15/4 ºC in darkness). Phenology study in outdoors conditions revealed that embryo grew during summer-early autumn, short time after seed dispersal in nature (i.e., May); radicle emerged in autumn. The shoot however did not emerge until late winter-early spring, because it was physiologically dormant and required a cold (5 ºC) period of 30 days to break dormancy. Early cold temperatures interrupted the embryo growth and induced dormancy in seeds whose embryo had grown 30% with respect to the initial length. Desiccation in seeds whose embryo had grown 30% did not induce dormancy, but did it when the embryo growth reached 70%.

Research highlights: Seeds of *Narcissus radinganorum* have deep simple epicotyl MPD.

Key words: dormancy break; radicle emergence; shoot emergence; epicotyl MPD; phenology; secondary dormancy.

Citation: Herranz, J.M., Copete, E., Copete, M.A., Ferrandis, P. (2015). Germination ecology of the endemic Iberian daffodil *Narcissus radinganorum* (Amaryllidaceae). Dormancy induction by cold stratification or desiccation in late stages of embryo growth. Forest Systems, Volume 24, Issue 1, e-013, 13 pages. http://dx.doi.org/10.5424/fs/2015241-06197.

Received: 02 May 2014. **Accepted:** 15 Dec 2014

http://dx.doi.org/10.5424/fs/2015241-06197

Funding: Financial support from the Regional Government of Castilla-La Mancha (Plan Regional I+D+i, Consejería de Educación y Ciencia): "Creation of a germplasm bank of threatened wild flora in the Botanical Garden of Castilla-La Mancha" (PAI07-0088-0300) and "Germination ecology of twelve singular or threatened plant species with morphophysiological dormancy" (PEII10-0170-1830).

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Miguel Ángel Copete: miguel.copete@uclm.es

Introduction

The genus *Narcissus* L. belongs to the monocotyledon family Amaryllidaceae, which includes about 900 species in 60 genera (Mabberley, 2008). Many species of this family at seed-dispersal time have seeds with underdeveloped, although fully differentiated, linear embryos, which need to grow before the seed germinates (Baskin & Baskin, 1998). Seeds with underdeveloped embryos can have morphological dormancy (MD), if embryo growth and radicle emergence are completed in about 30 days under suitable conditions, or morphophysiological dormancy (MPD) when an additional physiological mechanism preventing embryo growth and germination occurs (Nikolaeva, 1977; Baskin & Baskin, 2004). Requirements for embryo growth are little known in Amaryllidaceae (Vandelook & Van Assche, 2008; Copete *et al*., 2011). Thus, in this study special attention was given to the analysis of environmental conditions that promote embryo growth and subsequent germination in *Narcissus radinganorum* Fdez. Casas, an endemism of Western Mediterranean

region with a geographical distribution restricted to the southern foothills of the Iberian System, in Eastern Spain (Fabregat & López, 2003).

At seed-dispersal time, mean embryo length (E) in seeds of *N. radinganorum* is 1.36 mm (s.e.=0.03, n=20), while that of the endosperm (S) is 3.38 mm (s.e.=0.04, n=20). Hence, the E:S ratio is 0.40, suggesting that the embryo is underdeveloped. As a part of a preliminary study, seeds of *N. radinganorum* were incubated for one month in light/dark and in permanent darkness at temperatures occurring in its natural habitat during the course of the year (5, 15/4, 20/7, 25/10, 28/14 and 32/18 ºC), none germinating. However, seeds germinated after two months of warm stratification followed by incubation at cool (15/4 ºC) temperatures. Taken together, the small embryo and the lack of germination during one month of incubation strongly suggest that seeds of *N. radinganorum* have MPD.

Narcissus radinganorum is an endangered species (Moreno, 2008) which belongs to *Pseudonarcissi* DC. section, with origin centre in the main southern mountain ranges of the Iberian Peninsula, and with non-pronounced morphological differences respect to other *Narcissus* species, because of an active, current speciation process (Ríos-Ruiz *et al*., 1999). Indeed, it is morphologically close to *Narcissus eugeniae* Fdez. Casas from which differentiates by a longer floral pedicel (5-10 mm). Although all species in the section have a high vegetative propagation ability by bulbs, seed germination ecology is not known in detail (Bañares *et al*., 2003). Such information should be essential for *ex-situ* plant production, both for ornamental aims (plants in this section are known as trumpet daffodils and are commonly cultivated in horticulture) and for the reinforcement of wild populations, since propagation from seeds is the unique via for maintaining genetic variability, crucial aspect when it comes to endangered species (Cerabolini *et al*., 2004).

Up to now, germination ecology in the *Pseudonarcissi* section has been studied in few species. In *N. pseudonarcissus* (Vandelook & Van Assche, 2008), *N. eugeniae* (Copete *et al.*, 2014) and *N. hispanicus* (Copete *et al.*, 2011), growth of the embryo is continuous throughout summer, radicle emergence occurs in early autumn and shoot emergence is delayed up to late winter-early spring, so corresponding to deep simple epicotyl MPD pattern. In *N. alcaracensis* (Herranz *et al.*, 2013b), dormancy break and embryo growth require only cold stratification and seeds have intermediate complex MPD, while in *N. longispathus* (Herranz *et al.*, 2013a) dormancy break, embryo growth and radicle emergence require warm stratification followed by cold one, corresponding to non-deep complex MPD pattern (Baskin & Baskin, 1998). The existence of three levels of MPD remarks the importance of this section to understand the evolutionary relationships between the different levels of MPD in *Narcissus* (Herranz *et al.*, 2013a).

A previous work (Herranz *et al.*, 2013a) suggested that in *Pseudonarcissi* section species colonizing habitats with a seasonally summer water stress, the presence of deep simple epicotyl MPD constitutes an adaptation for seedling survival during the dry season. In this work, we aimed to confirm this hypothesis by analysing several germination traits of *N. radinganorum,* such as the effect of environmental conditions on embryo growth, phenology of embryo growth and germination, dormancy break in buried seeds and influence of warm or cold stratification on germination, whose study is essential in species with MPD (Baskin & Baskin, 1998; 2004; 2005; Herranz *et al.*, 2010; Kondo *et al.*, 2004; Vandelook *et al.*, 2007). On the other hand, it is known that in imbibed non-dormant seeds of some species, unfavourable environmental conditions for germination, such as extreme temperatures, hypoxia, and water stress, can induce secondary dormancy (Hilhorst, 1998; Baker *et al.*, 2005). In this sense, we also analyzed the next hypotheses: i) exposure to low temperatures induces dormancy, as in *N. hispanicus* (Copete *et al.*, 2011) and *N. eugeniae* (Copete *et al.*, 2014); ii) desiccation after embryo growth induces secondary dormancy, as in *Lomatium dissectum* (Scholten *et al.*, 2009) and *Anemone nemorosa* (Ali *et al.*, 2007).

Therefore, the main goals of the present study were to analyze:

a) The phenology of embryo growth, radicle and seedling emergence.
b) Germination responses of seeds buried in soil and exhumed periodically.
c) The effects of temperatures and GA_3 on dormancy break and embryo growth.
d) The influence of warm and cold stratification, as well as temperature and light/dark conditions during incubation on germination.
e) The effect of low temperature on shoot emergence from seeds with an emerged radicle.
f) To determine whether low temperatures can induce dormancy in non-dormant seeds.
g) The effect of desiccation of seeds in different stages of embryo growth on germination.

Material and methods

Plant material and seed source

Narcissus radinganorum Fdez. Casas is a bulbous geophyte, 20-40 cm height when flowering with one

flower in the apex. It is endemic to mountains of Central-Eastern Spain (Sierras de Palomera, Ayora y Caroch, in Southern Iberian System, province of Valencia), where it lives between 600-1,000 m.a.s.l. on limestone substrates. The natural habitat of the species are typically grasslands with seasonal moisture, belonging to the association *Molinio arundinaceae-Ericetum erigenae* (Fabregat & López, 2003), where *N. radinganorum* coexists with *Molinia caerulea*, *Schoenus nigricans*, *Scirpus holoschoenus*, *Dorycnium hirsutum*, *Tetragonolobus maritimus*, etc. Plants flower in early April and seeds ripen in late May within capsules containing 35-40 seeds each one. Because of its small geographical distribution area, the low number and disruption of its populations, and the vulnerability to disturbances of its natural habitat, *N. radinganorum* was included with the category of "EN" (endangered) in the Atlas and Red Book of Threatened Vascular Flora in Spain (Bañares *et al.*, 2003) and in the Red List of the Spanish Vascular Flora (Moreno, 2008).

The seeds for this study were collected in La Hunde (Ayora, Valencia, Eastern Spain), 980 m.a.s.l., 30SXJ5427, where the most important population of the specie lives, exceeding 16,000 individuals (Fabregat & López, 2003). Fruits with the same level of ripeness, when the capsules change from green to yellow and begin to open for seed dispersal, were collected on 22 May 2006 from about 50 plants to do preliminary works and on 25 May 2007 to do the experiments described here. Ripe capsules were spread out in the laboratory to allow them to open and the seeds to fall out. Seeds were dried in the laboratory (22 ºC, 60% r.h.) until analysis beginning, on 1 June 2007.

Outdoor experiments

The aim of these studies was to determine the timing of the main events in the seed/seedling stage of the life cycle of *N. radinganorum*, in relation to the seasonal temperature cycle, from seeds kept under near natural temperature conditions in a non-heated metal frame shadehouse, located in the experimental field of the Technological School of Agronomy in Albacete (60 km from La Hunde). The temperature was recorded throughout the study. The growing medium in pots and trays containing the seeds was a mixture of sterilized peat and sand (2:1 v/v), which was watered to field capacity once a week throughout the year, with two exceptions. First, pots and trays were watered only twice a month in July and August, to simulate summer moisture stress that is common in the Mediterranean area. Secondly, water was withheld when the substratum was frozen in winter. Thus, temperature and soil moisture conditions were similar to those in the natural habitat of *N. radinganorum*.

Phenology of embryo growth and of radicle and shoot emergence

On 1 June 2007, seven groups of 50 *N. radinganorum* seeds each were mixed with fine-grained sterilized sand. Each group was placed in a fine-mesh polyester cloth bag and buried 5-cm deep in a pot. Each bag was labelled and the labels were not buried, which made it easy to recover each bag individually. One bag was removed monthly from July 2007 to January 2008, and the seeds were separated from the sand using a 1-mm sieve. The seeds with emerged radicles were used to record the percentage of radicle emergence throughout the phenology study. Embryos were excised from 20 healthy-looking seeds and their lengths measured using an ocular micrometer. These values were used in two ways: (1) monthly mean embryo length was calculated to analyze embryo growth throughout the experiment; and (2) each month these 20 values were grouped into size-classes to study temporal changes in the distribution of embryo size structure from July to January.

Embryos from seeds whose radicle had emerged during burial were recorded as having a critical embryo length. The critical embryo length for radicle emergence is the length of the embryo at the time the seed coat splits but before the radicle emerges (Vandelook & Van Assche, 2008). In *N. radinganorum*, the critical embryo length for radicle emergence was 2.62 mm (s.e.=0.04 mm, n=20, range=2.20-3 mm). The 20 seeds used for this calculation had received warm (28/14 ºC) plus cool (15/4 ºC) stratification treatments. The critical "embryo length : seed length" (E:S) ratio, calculated by averaging the E:S ratios of those 20 seeds, was 0.78 (s.e.=0.01, n=20, range=0.69-0.90). The minimum individual E:S ratio (i.e., 0.69 in our study), was considered the "threshold E:S ratio", a reliable indicator that dormancy is being overcome (Copete *et al.*, 2011).

On 1 June 2007 as well, three trays with drainage holes were filled with the growing medium, and 200 *N. radinganorum* seeds were sown 5-mm deep, equidistant from each other in each tray to avoid contact between them. The three replicates were placed in the shadehouse. From June 2007 to May 2009, seed trays were examined once a week and emergent shoots were counted and removed.

Radicle-dormancy break in buried seeds

On 1 June 2007, seven groups of 200 *N. radinganorum* seeds each were mixed with fine sterilized sand,

and then placed in a fine-mesh polyester cloth bag, buried 5-cm deep in a pot with the mixture of peat and sand, and watered as described above. One bag was exhumed on the first day of each month for next 7 months. All healthy, ungerminated seeds were incubated in darkness at 15/4 ºC (one of the most favourable thermoperiods for radicle emergence) for 30 days. Recovered seeds were classified into four categories: (1) seeds germinated (radicle emerged) within the bag during the buried period; (2) viable non-dormant seeds whose radicles emerged at 15/4 ºC; (3) viable dormant seeds that did not germinate at 15/4 ºC and had healthy embryos; and (4) non-viable seeds that were dead (they were soft and did not contain a firm, white embryo).

Laboratory experiments

Experiments were conducted in temperature and light-controlled conditions. Germination chambers (Ibercex model F-4, Madrid, Spain) were equipped with a digital temperature and light-control system [± 0.1 ºC, cold white fluorescent light, 25 $\mu molm^{-2}s^{-1}$ (1350 lux)]. Seeds were tested for radicle emergence in a 12 hour daily photoperiod (hereafter light) and in continuous darkness (hereafter darkness), which was achieved by wrapping Petri dishes in a double layer of aluminium foil, at constant temperature of 5 ºC and at 12/12 hour daily temperature regimes of 15/4, 20/7, 25/10, 28/14, and 32/18 ºC. In the 12/12 hour alternating temperatures treatments, the high temperature coincided with the light phase and the low temperature with darkness, to simulate day/night conditions.

The fluctuating temperatures used in the germination tests simulated mean maximum and mean minimum monthly temperature that characterize the annual climate cycle in the seed-source region (Sierra de Ayora, Valencia): 15/4 ºC, November and March; 20/7 ºC, October and April; 25/10 ºC, September and May; 28/14 ºC, August and June; and 32/18, July. The 5 ºC treatment simulated the mean temperature recorded during winter months: December, January and February.

Germination percentages were computed based on the number of apparently viable seeds, i.e. seeds whose embryos were white and firm. More than 95% seeds were viable.

Effect of temperature on embryo growth

The goal of this experiment was to determine the effect of (1) a range of temperatures of cold (5 ºC) or warm (25/10 and 28/14 ºC) stratification on embryo growth and (2) a cold or a warm stratification pre-treatment on embryo growth at cool (15/4 ºC) temperatures for 45 days.

First, we measured the mean length of embryos in freshly matured seeds. Then, 20 seeds were placed on two sheets of filter paper moistened with distilled water in a 9-cm Petri dish at room temperature for 24 hours. Embryos were excised from imbibed seeds with a razor blade and their lengths measured using a dissecting microscope equipped with a micrometer.

Two hundred seeds were placed in each of three 16-cm Petri dishes on two sheets of filter paper moistened with distilled water and sealed with parafilm. Each dish was placed in light at 5, 25/10, and 28/14 ºC. After 30, 60, 90, and 120 days, 20 healthy seeds were randomly extracted from each temperature treatment, and their embryos were excised and measured, calculating the E:S ratio too. Mean length and standard error were calculated for each sample of 20 embryos.

In addition, after 90 days at 5, 25/10, and 28/14 ºC, 20 seeds from each temperature were transferred to 15/4 ºC in light for 45 days (treatment A), and other 20 seeds at 15/4 ºC in darkness for 45 days (treatment B). After incubation, embryos were excised and measured; mean length and standard error were calculated for each sample. Embryos from seeds that had germinated were recorded as fully elongated, i.e. the critical length for germination.

Effect of GA_3 on embryo growth

The purpose of this experiment was to determine if GA_3 promotes embryo growth and radicle emergence. Thus, 50 seeds were placed in each of two 9-cm Petri dishes on filter paper moistened with a GA_3 solution (1,000 ppm) or with distilled water (0 ppm), respectively. The dishes were sealed with parafilm and placed at 25/10 ºC in light for 90 days. At the end of the experiment, embryos were excised from 20 seeds from each dish to determine mean embryo length and to compare the values obtained for each GA_3 concentration (1,000 ppm *vs*. 0 ppm).

Effect of temperatures of stratification and incubation on radicle emergence

The aim of this experiment was to determine the optimal pre-treatment of stratification and temperature of incubation for radicle emergence. Seeds were stratified 90 days in light and exposed to the following

treatments: (1) cold (5 ºC), (2) warm (28/14 ºC) and (3) warm at a combination of high temperatures (treatment C; 28/14 ºC for 30 days + 25/10 ºC for 30 days + 20/7 ºC for 30 days). In June 2007, 1,300 seeds were placed on each of three 16-cm Petri dishes on moist filter paper, assigning one dish to each of the treatments described above. In each treatment, after 90 days, four replicates of 25 seeds each were transferred to 5, 15/4, 20/7, 25/10, 28/14, and 32/18 ºC, both in light and in darkness, for 45 days. Seeds incubated in light were examined for radicle emergence at intervals of 4 days, and seeds incubated in darkness, at the end of the test.

Temperature requirement for shoot emergence

The goal of this study was to determine whether cold stratification is required for shoot emergence in radicle-emerged seeds and, if so, how long stratification should be. The experiment may also show the most favourable temperature for shoot growth.

Seeds with emerged radicles were placed in 9-cm Petri dishes on two sheets of filter paper moistened with distilled water and incubated in light. Three groups of 100 seeds each (four replicates of 25 seeds) with roots 2-3 mm in length were moist cold (5 ºC) stratified for 0, 30 or 60 days, respectively. After each cold stratification treatment, seeds were transferred to 20/7 ºC for 60 days. In addition, three groups of 100 radicle-emerged seeds each (four replicates of 25 seeds) were stratified at 5 ºC for 60 days and then incubated at 15/4, 20/7 and 25/10 ºC, respectively for 60 days, to determine the best temperature for shoot growth.

Induction of dormancy by low temperatures

The purpose of this experiment was to determine if low temperatures that occur in late autumn in nature may induce secondary physiological dormancy (PD) in seeds whose primary PD had been overcome and embryos had begun to grow but were not fully elongated.

In June 2007, three lots of 200 seeds each were placed in 9-cm Petri dishes in light to test the effect of two different sequences of stratification (warm plus cold, and warm plus warm) on germination. First, the three lots were placed at 28/14 ºC for 90 days. Secondly, one of the seedlot was transferred to 5 ºC for 45 days, other one to 20/7 ºC for 45 days, and the third one to 25/10 ºC for 45 days. Finally, four 25-seed replicates from each lot were used to test germination at 15/4 ºC in light for 45 days.

Induction of dormancy by desiccation in different stages of embryo growth

Some studies indicate that desiccation of seeds can induce secondary dormancy (Wood *et al.*, 2000; Shen *et al.*, 2001). On the other hand, in *Anemone nemorosa* (Ali *et al.*, 2007) and in *Lomatium dissectum* (Scholten *et al.*, 2009), loss of desiccation tolerance occurs at late stages of embryo growth inside the seed. So, we tested the effect of dehydration on dormancy status in two different stages of embryo growth.

Firstly (treatment 1), in June 2007, one lot of 150 seeds placed in a 9-cm Petri dish on two moist sheets of filter paper was stratified at 28/14 ºC in light for 60 days (embryo length = 1.78 ± 0.04 mm, percentage of embryo growth = 30.9%). Then, seeds were dried (seed moisture content was reduced from 47 to 8%) and stored at room temperature (22 ºC) for 60 days, which approximates to the period that seeds may remain dry during the summer in areas of the Mediterranean region. Subsequently, four 25-seed replicates were rehydrated and transferred to 15/4 ºC in darkness for 30 days, recording the germination percentage and the embryo length at the end of this period.

Secondly (treatment 2), another lot of 150 seeds was stratified at 28/14 ºC in light for 60 days, and then incubated at 15/4 ºC in darkness for 15 days. In this period, 20% seeds germinated. Then, ungerminated seeds (embryo length = 2.33 ± 0.05 mm; percentage of embryo growth = 71.3%) were dried and stored at room temperature as described above. The seeds (four replicates of 25 seeds) were then rehydrated and transferred to 15/4 ºC in darkness for 30 days.

As control, four 25-seed replicates was stratified at 28/14 ºC in light for 60 days and then incubated at 15/4 ºC in darkness for 30 days, with no dry storage between stratification and incubation.

Statistical analysis

The effect of duration of incubation at different temperatures and the effect of incubation temperatures on the length of embryos were analyzed by a two-way ANOVA. Seed germinability was evaluated by the final cumulative germination percentage, which was compared among treatments by ANOVAs. When significant main effects existed, differences were detected by a multiple comparison Tukey test. Prior to analyses, normality (Cochran test) and homoscedasticity (David test) of data were checked. Values of the final cumulative germination percentage were squared-root arcsine transformed.

Results

Outdoor experiments

Phenology of embryo growth and of radicle and shoot emergence

At the beginning of the burial experiment on 1 June 2007, mean embryo length was 1.36±0.03 mm. Embryos grew slowly between this date and 1 September, when the mean length of embryos was 1.71±0.05 mm (Figure 1). During this period, mean maximum and minimum daily temperatures were 31 and 14 ºC, respectively. However, between 1 September and 1 November, when mean maximum and minimum temperatures were 23 and 9 ºC, respectively, embryos grew rapidly. Thus, on 1 November embryo length was 2.43±0.04 mm, and 35% buried seeds had germinated (radicle emergence). On 1 December, embryo had grown to the critical embryo length of 2.62 mm, and 96% buried seeds had germinated. The remaining seeds were unviable.

All embryos from seeds buried on 1 June 2007 were in the two smallest size classes (Figure 2). Embryos grew during the summer months, and in August, September, and October, a broad range of size classes was represented. By November, all embryos were in the three largest size classes. On 1 December, 100% embryos were in the largest (>2.50 mm) size class.

Although on 1 December 2007, 96% seeds buried on 1 June had emerged radicles, shoots did not emerge until 1 March 2008 (18%; Figure 1). During this 3-month period (December-March), mean daily maximum and minimum temperatures in the shadehouse were 12 and 0 ºC, respectively. Accumulative shoot emergence reached 93% on 1 April 2008, and 99% on 1 May 2008.

Radicle-dormancy break in buried seeds

Almost half of the seeds buried in June and retrieved in July (Figure 3) had become non-dormant after 1 month, since 48% radicles emerged when incubated 30 days at 15/4 ºC in darkness. During this time, mean daily maximum and minimum temperatures were 29 and 12 ºC, respectively. In August, after two warm months of burial, the percentage of non-dormant seeds was 89% (s.e.=3.84). All apparently viable seeds retrieved on 1 October were non-dormant, but no seed germinated in the bag until 1 November.

Laboratory experiments

Effect of temperature on embryo growth

The mean length of embryos in seeds cold-stratified (5 ºC) in light for 90 days was 1.40±0.03 mm (Table 1),

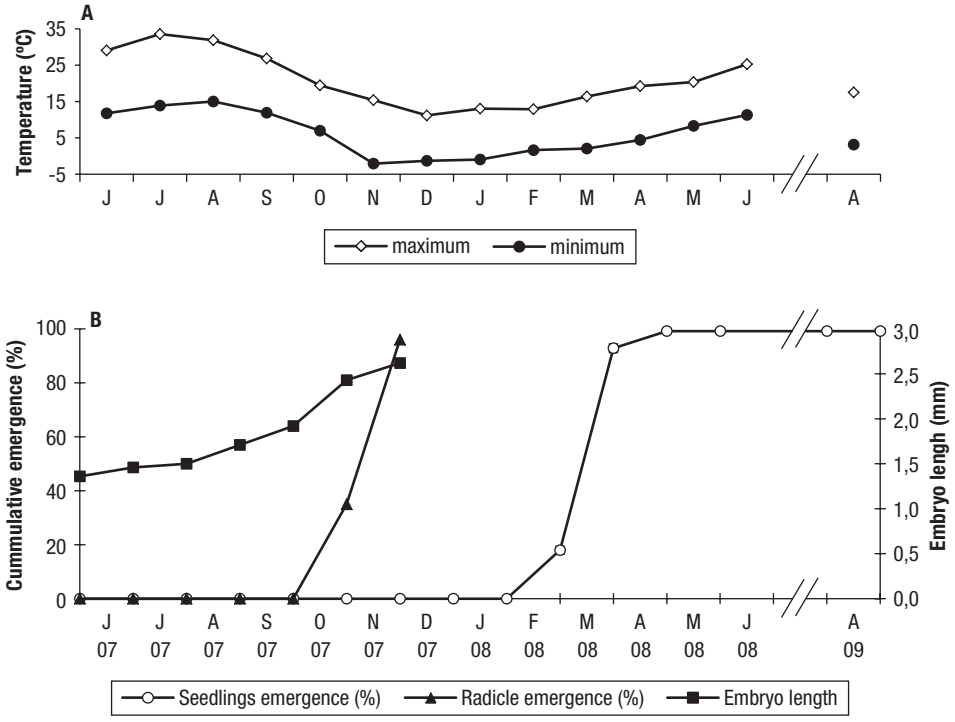

Figure 1. Mean daily minimum and maximum air temperatures (A) and phenology of embryo growth and of radicle and shoot emergence (mean±s.e.) from seeds sown on soil in a non-heated shade-house in June 2007 (B).

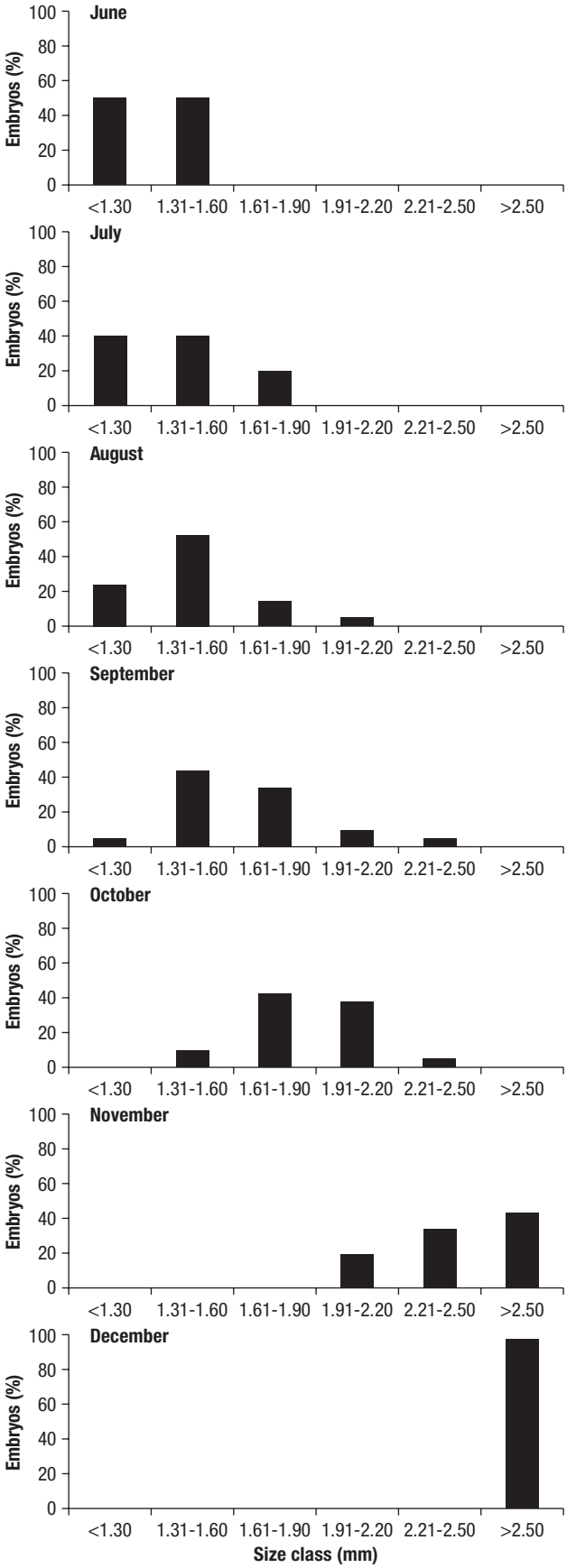

Figure 2. Changes in size-class distribution of embryos in seeds sown in June 2007 and recovered/measured monthly from July to December.

being slightly higher than the mean in freshly matured seeds (1.36±0.3 mm), and no embryo achieved the threshold E:S ratio (0.69) for germination. The embryo growth was also very small and no embryo achieved the threshold E:S ratio when seeds cold-stratified were transferred to 15/4 ºC in light or in darkness for 45 days (treatments A and B, respectively).

Embryos of seeds warm-stratified for 90 days reached a mean length of 1.92±0.05 mm at 25/10 ºC, and 1.83±0.04 mm at 28/14 ºC. At 25/10 ºC, 10% embryos reached the threshold E:S ratio. When these seeds were transferred to 15/4 ºC in darkness for 45 days (treatment B), the mean embryo length was ≥2.54 mm, and germination percentages were ≥90%.

Effect of GA_3 on embryo growth

GA_3 did not stimulate the embryo growth or promote the germination. Even, after 90 days at 25/10 ºC embryo length in seeds imbibed in a GA_3 solution of 1,000 ppm (1.66±0.06 mm) was slightly lower than in seeds imbibed in distilled water (1.92±0.05 mm). (Data not shown otherwise).

Effect of temperatures of stratification and incubation on radicle emergence

Seeds cold (5 ºC)-stratified for 90 days did not germinate when they were transferred to the different incubation temperatures (Figure 4). In contrast, warm-stratification treatments during 90 days at 28/14 ºC or at a combination of high temperatures (i.e., treatment C: 28/14 ºC for 30 days + 25/10 ºC for 30 days + 20/7 ºC for

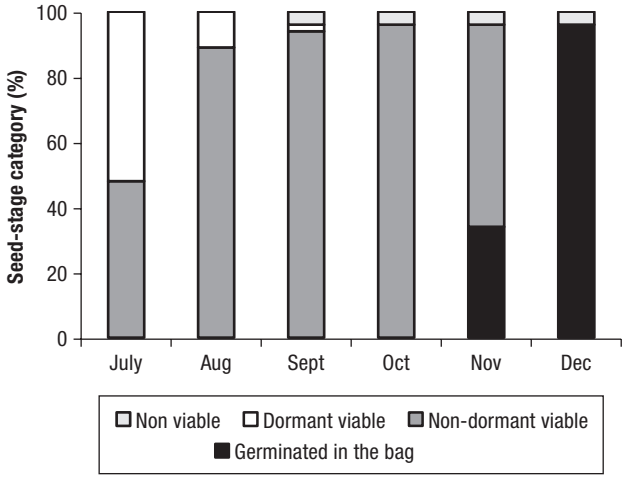

Figure 3. Rate of dormancy break of radicles in seeds of *Narcissus radinganorum* buried on 1 June 2007 and exhumed monthly from July to December.

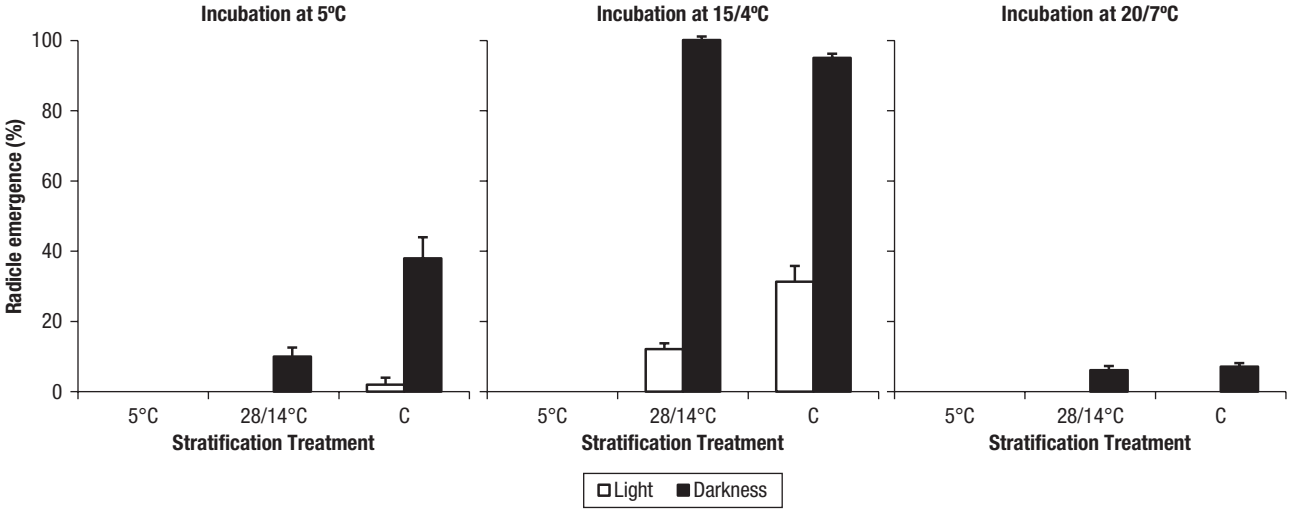

Figure 4. Effect on radicle emergence of different stratification treatments for 90 days in light (5 ºC, 28/14 ºC, and treatment C: 28/14+25/10+20/7 ºC) followed all by incubations for 30 days at 5, 15/4, and 20/7 ºC, in both light and darkness. Error bars indicate s.e. For each temperature x light incubation condition we used four 25-seed replicates.

Table 1. Influence of incubation temperature and duration on embryo growth (mm, mean±s.e.) in *Narcissus radinganorum* seeds.

		Incubation temperatures in light		
		5 ºC	**25/10 ºC**	**28/14 ºC**
Time of incubation (months)	**1**	1.37 ± 0.03^{Aa} (0 , 0)	1.61 ± 0.03^{Ab} (0 , 0)	1.68 ± 0.04^{Ab} (0 , 0)
	2	1.39 ± 0.02^{ABa} (0 , 0)	1.82 ± 0.06^{ABb} (0 , 0)	1.78 ± 0.04^{Ab} (0 , 0)
	3	1.40 ± 0.03^{ABa} (0 , 0)	1.92 ± 0.05^{BCb} (0 , 10)	1.83 ± 0.04^{Ab} (0 , 0)
	4	1.44 ± 0.02^{ABa} (0 , 0)	1.93 ± 0.04^{BCb} (0 , 5)	1.85 ± 0.04^{Ab} (4 , 6)
Treatment A		1.46 ± 0.02^{ABa} (0 , 0)	2.08 ± 0.07^{Cb} (20 , 30)	2.24 ± 0.07^{Bb} (10 , 30)
Treatment B		1.49 ± 0.04^{Ba} (0 , 0)	2.54 ± 0.04^{Db} (90 , 95)	2.60 ± 0.00^{Cb} (100 , 100)

Values followed by different uppercase letters within a column or different lowercase letters within a row are significantly different ($p<0.05$). The first number in parentheses is the percentage of radicle emergence, and the second one is the percentage of seeds whose E:S ratio is > threshold E:S ratio (=0.69). Treatment A: Incubation at 15/4 ºC in light following stratifications of 3 months at 5, 25/10, or 28/14 ºC. Treatment B: Incubation at 15/4 ºC in darkness following stratifications of 3 months at 5, 25/10 and 28/14 ºC. n= 20 for each embryo measurement.

30 days), were highly effective in breaking dormancy, especially when the seeds were incubated at 15/4 ºC in darkness, reaching percentages of radicle emergence ≥94%. Treatment C was more effective in breaking dormancy than that of 28/14 ºC followed by 5 ºC.

The optimum incubation temperature was 15/4 ºC, followed by 5 ºC, and 20/7 ºC. Above these temperatures, no seed germinated (data not shown otherwise). The germination percentages reached were significantly higher in darkness than in light.

Temperature requirement for shoot emergence

Cold temperature (5 ºC) promoted shoot emergence from seeds with emerged radicles. Consequently, prolonging the cold pre-treatment time shortened the incubation time for shoot development. Thus, cold-stratified seeds reached 72% shoot emergence in only 30 days after they were transferred from 5 ºC during 30 days to 20/7 ºC (total time = 60 days). In seeds not cold-stratified, shoot emergence at 20/7 ºC after 60 days reached only 18% (Figure 5).

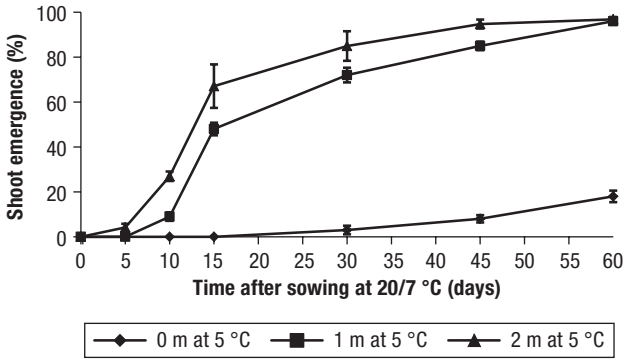

Figure 5. Shoot emergence in germinated seeds of *Narcissus radinganorum* at 20/7 ºC after 0, 1, or 2 months of cold treatment at 5 ºC. Error bars indicate s.e. For each cold-stratification duration we used four 25-seed replicates.

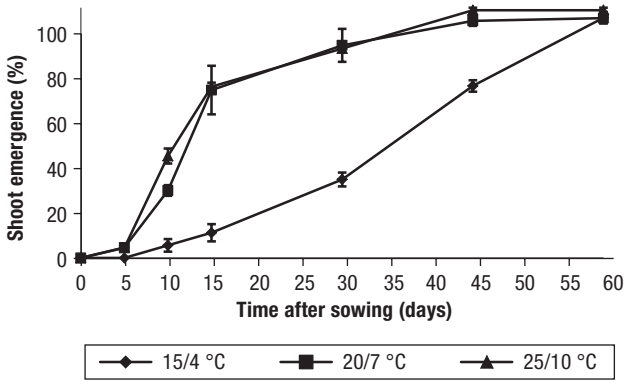

Figure 6. Shoot emergence in germinated seeds of *Narcissus radinganorum* incubated at different temperatures following 60 days of cold treatment at 5 ºC. Error bars indicate s.e. For each incubation temperature we used four 25-seed replicates.

Following 60 days of stratification at 5 ºC, nearly 85% shoots had emerged after 30 days of incubation at 20/7 ºC and 25/10 ºC, contrasting with the low shoot emergence at 15/4 ºC (31%; Figure 6). At this temperature, shoot emergence was slower than at 20/7 ºC and at 25/10 ºC, but after 60 days the final percentages were similar.

Induction of dormancy by low temperatures

Seeds placed at 28/14 ºC for 90 days, transferred subsequently to 25/10 ºC or 20/7 ºC for 45 days, and then incubated at 15/4 ºC for 45 days germinated ≥76% (Table 2). In contrast, seeds transferred from 28/14 ºC to 5 ºC and then incubated at 15/4 ºC germinated to only 1%. Thus, in almost all seeds the exposition at 5 ºC induced secondary dormancy.

Induction of dormancy by desiccation in different stages of embryo growth

When seeds stratified at 28/14 ºC in light for 60 days were incubated to 15/4 ºC in darkness for 30 days, without previous desiccation (control), the germination percentage reached 99% and embryos grew up to the critical length (i.e., 2.60 mm; Table 3). If seeds stratified as described above (embryo length = 1.78 ± 0.04 mm, percentage of embryo growth =30.9%) were submitted to desiccation for 60 days and then incubated at 15/4 ºC in darkness, the germination percentage reached 98% and the embryo length 2.58±0.02 mm. In seeds desiccated for 60 days when the embryo length was 2.33 mm (percentage of embryo growth = 71.3%) and then incubated at 15/4 ºC in darkness, the germination percentage was 85%. Thus, a little portion of the seeds were induced to secondary dormancy.

Discussion

Fresh seeds of *N. radinganorum* have an underdeveloped embryo at the time of dispersal in late spring (embryo length=1.36±0.03 mm). Embryos had to grow until they reached at least 2.20 mm to germinate (critical embryo length=2.60±0.04 mm, n=20, range=2.20-3.00 mm). Therefore, seeds have MD. On the other hand, since embryo growth and radicle emergence were not completed at suitable temperature, light, and moisture conditions in about 30 days, seeds also had PD and required a dormancy-breaking treatment. Thus, *N. radinganorum* seeds have MPD (Baskin & Baskin, 1998; 2005).

Under warm stratification (25/10 or 28/14 ºC) for 90 days, followed by cool temperatures (15/4 ºC in dark-

Table 2. Induction of dormancy by low temperatures (5 ºC) in *Narcissus radinganorum* seeds.

Stratification in light	Incubation in light	Germination (%) (mean±s.e.)
28/14 ºC (90 d)	5 ºC (45 d) + 15/4 ºC (45 d)	1 ± 0.87^{A}
	25/10 ºC (45 d) + 15/4 ºC (45 d)	76 ± 3.16^{B}
	20/7 ºC (45 d) + 15/4 ºC (45 d)	81 ± 2.60^{B}

Seeds were submitted to a warm stratification at 28/14 ºC for 90 days, followed by 45 days at 5 ºC, or at 25/10 ºC, or 20/7 ºC (control temperatures), and then incubated at 15/4 ºC for 45 days in light conditions (four 25-seed replicates). The year of seed collection was 2007. Values followed by different uppercase letters within columns are significantly different (p<0.05).

Table 3. Induction of dormancy by desiccation (60 days of dry storage at 22 ºC) in *Narcissus radinganorum* seeds.

Previous stratification in light	Subsequent treatment	Incubation at 15/4 ºC in darkness (30 d)	
		Germination (%)	Embryo length (mm)
28/14 ºC (60 d) (embryo length = 1.78 ± 0.04; percentage of embryo growth = 30.9%)	CONTROL 0 d of dry storage	99 ± 0.87^{A}	2.60 ± 0.00^{A}
	TREATMENT 1 60 d of dry storage	98 ± 1.00^{A}	2.58 ± 0.02^{A}
	TREATMENT 2 20 d at 15/4 ºC in darkness (ungerminated seeds: embryo length = 2.33 ± 0.05; percentage of embryo growth = 71.3%) + 60 d of dry storage	85 ± 1.66^{B}	2.54 ± 0.03^{A}

Seeds were submitted to three different treatments following a warm stratification for 60 days at 28/14 ºC in light: Control (0 days of dry storage), treatment 1 (60 days of dry storage), and treatment 2 (20 days of incubation at 15/4 ºC in darkness + 60 days of dry storage). Finally, seeds were incubated at 15/4 ºC in darkness for 30 days (four 25-seed replicates). Values followed by different uppercase letters within columns are significantly different (p<0.05).

ness) for 45 days, the embryos grew and then the radicles emerged from seeds. However, embryos grew only a little and no seed germinated if seeds were first cold stratified (5 ºC) for 90 days and then incubated at 15/4 ºC (Table 1). Hence, embryo growth (i.e., loss of morphological dormancy) occurs during exposition to warm and cool temperatures. This response indicates that seeds of *N. radinganorum* have some level of simple MPD (Baskin & Baskin, 1998). Since the embryo growth starts when seeds are exposed to favourable temperatures, it means that MD and PD are overcome simultaneously. That fact allows us to discard the presence of non-deep simple MPD, as in this level first PD is broken and then the embryo grows rapidly (MD) (Baskin & Baskin, 1994). The lack of GA_3 stimulation on embryo growth and germination also contributes to discard this level. Intermediate and deep simple MPD are discarded because once the embryo growth occurs, seeds do not need cold exposition to emerge the radicle (Figure 1). Deep simple double MPD can be discarded too, because in this level shoot emergence only occurs after the second winter (Kondo *et al.*, 2011).

The embryo growth patterns observed in laboratory trials are corroborated by the phenological tests under closed natural conditions in the non-heated frame shadehouse (Figures 1 and 2). So, the high summer temperatures provide the appropriate conditions that result in embryo growth and radicle emergence as temperatures decline in autumn. However, shoot emergence only occurs three months later, once seeds with emerged radicles are subjected to low winter temperatures. The delay between the radicle and shoot emergences is known as epicotyl dormancy (Barton, 1936). Both in non-deep and in deep simple epicotyl MPD, the break of PD in root occurs in response to a sequence of warm and cool temperatures, indicating that this is non-deep. The difference between both levels of MPD is that of non-deep simple epicotyl MPD does not require cold stratification to break PD of the shoot, as in *Viburnum odoratissimum* (Baskin *et al.*, 2008). Nevertheless, the opposite occurs in seeds with deep simple epicotyl MPD. In *N. radinganorum*, cold stratification of seeds with emerged radicles increased the rate at which shoot emerged when these seeds were exposed to spring (20/7 ºC) temperatures (Figure 5). So, in non-cold stratified seeds shoot emergence at 20/7 ºC after 45 days only reached 8%, but it came up to 48% when radicle-emerged seeds were cold stratified for 30 days and then incubated at 20/7 ºC during 15 days (total time 30+15 = 45 days). Therefore, we conclude that *N. radinganorum* seeds have deep simple epicotyl MPD, confirming the initial hypothesis.

From an ecological point of view, deep simple epicotyl MPD involves an excellent adaptation to temperate regions with marked seasonal changes during the year. At seed dispersal in late spring-early summer, the presence of an undeveloped and dormant embryo prevents germination after occasional summer storms and the mortality of seedlings which could emerge. Thus, radicle emergence is delayed until autumn, season with more appropriate temperature and humidity conditions to offspring survival. On the other hand, the presence

of a shoot with PD prevents shoot emergence during winter months, avoiding the risk of frost for young seedlings. However, under soil the winter temperatures are damped and the root can continue growing, and so the seedlings have a well-developed root system when the shoot expands in spring (Kondo *et al.*, 2004; Copete *et al.*, 2011).

The presence of a well-formed root system in spring allows seedlings to exploit more efficiently the available water resources until summer drought (Kondo *et al.*, 2004). In addition, when the spring comes, the shoot can develop rapidly by the growth stimulation from previous cold winter temperatures, what facilitates the accumulation of underground reserves and prepare small plants to face summer water stress. All the above reasons may show this MPD level as a good adaptation to environments with summer water stress, as the rushes and grasslands with seasonal moisture which constitute the habitat of *N. radinganorum*.

Narcissus radinganorum seeds are dispersed in late spring-early summer, a time similar to that of other temperate deciduous forest herbs with epicotyl dormancy, whose main period of seed dispersal is between May and July, e.g. *Hydrophyllum appendiculatum* (Baskin & Baskin, 1985), *Erythronium japonicum* (Kondo *et al.*, 2002) and *Hexastylis heterophylla* (Adams *et al.*, 2003). Such a dispersal phenology allows seeds to be warm-stratified during summer, which is essential for subsequent radicle emergence at slightly decreased temperatures in autumn (≤20/7 ºC, Figure 4). This radicle emergence pattern under controlled conditions in the laboratory is in agreement with that obtained in outdoor experiment of dormancy break of radicles in buried seeds (Figure 3). Although in this experiment 89% seeds had broken dormancy on 1 August, emerged radicles did not appear in the exhumed bags until 1 November, probably because of the absence of radicle emergence at September temperature (27/12 ºC) prevents their presence in the bags exhumed on 1 October. Under controlled conditions of laboratory, the warm stratification sequence at decreasing fluctuating temperatures (30 days at 28/14 ºC + 30 days at 25/10 ºC + 30 days at 20/7 ºC), followed by incubation at 15/4 ºC, was the most effective in promoting radicle emergence. This temperature sequence is that with the highest ecological meaning since it closely recreates the temperature conditions in the natural habitat of the plant during August, September, October, and November.

The stimulation of germination in darkness incubation conditions found in this study also occurs in *Narcissus hispanicus* (Copete *et al.*, 2011), *N. eugeniae* (Copete *et al.*, 2014), *N. alcaracensis* (Herranz *et al.*, 2013b), and *Delphinium fissum* subsp. *sordidum* (Herranz *et al.*, 2010), although this is not the general trend in seeds with MPD (Baskin & Baskin, 1994; Hidayati *et al.*, 2000). The ability to germinate in darkness, i.e. while seeds remain buried, hinders the formation of permanent soil seed banks (Milberg *et al.*, 2000) and explains the results obtained in the phenological experiments performed in this study.

The deep simple epicotyl MPD pattern found in *N. radinganorum* is very similar to that in the close species *N. eugeniae* (Copete *et al.*, 2014), which inhabits the most Northern, mountainous and cold areas of the Iberian System (Mayoral & Gómez-Serrano, 2004). The main difference is that in *N. radinganorum*, the rapid shoot emergence only needs one month of previous cold (5 ºC) stratification in seeds with emerged radicles, *versus* the eight weeks required by *N. eugeniae*. Such a difference seems to be related to colder winters in the habitat of *N. eugeniae* (mean temperature of December, January and February=3.1 ºC *versus* 4.9 ºC; Elías & Ruiz, 1981).

Ninety nine percent seeds kept at 28/14 ºC for 90 days to initiate dormancy break and embryo growth, transferred to 5 ºC for 45 days (winter temperature) and then incubated at 15/4 ºC did not germinate, while those that were not cold stratified germinated ≥ 76% (Table 2). These data indicate that low temperatures induced non-dormant seeds into secondary dormancy. That fact has been detected only in seeds of *N. hispanicus* with deep simple epicotyl MPD (Copete *et al.*, 2011). On the other hand, cold stratification also induced secondary dormancy in seeds of the winter annuals *Papaver rhoeas* (Baskin *et al.*, 2002) and *Chaerophyllum taintinturieri* (Baskin & Baskin, 1990), which have non-deep simple MPD. Although in the phenological test of shoot emergence 99% seeds became emergent seedlings in the next spring (Figure 1), the process was facilitated by sowing seeds to 0.5-1 cm depth in darkness conditions, what stimulated germination. Seeds which in the natural habitat stayed in the topsoil exposed to light, and whose radicles did not emerge in autumn could be induced to secondary dormancy by the effect of low winter temperatures, avoiding the germination in the first spring after dispersal. Moreover, these seeds would be more susceptible to suffer desiccation, what can induce secondary dormancy in 14% seeds (i.e.=99-85), whose embryos have increased their lengths in a 71.3% with respect to the initial size (Table 3). The same occurs in *Anemone nemorosa* (Ali *et al.*, 2007) and *Lomatium dissectum* (Scholten *et al.*, 2009).

This study also shows that if the embryo growth increase is lower than 30% with respect to that at the time of dispersal, the desiccation does not induce dormancy (Table 3), in accordance with the record that many species with seeds dispersed with underdeveloped embryos

are fully desiccation tolerant (i.e., orthodox seeds *versus* recalcitrant) (Tweddle *et al.*, 2003; Ali *et al.*, 2007).

In the natural habitat of *N. radinganorum*, the induction of secondary dormancy by low temperatures and/or dehydration is a factor which can delay the germination of a seed cohort beyond the first vegetative period after dispersal, contributing to the formation of persistent seed banks (Baskin & Baskin, 1998). Thus, *N. radinganorum* appears to have several mechanisms that contribute to population persistence, including both vigorous vegetative reproduction from bulbs and germination from seeds of various ages.

Narcissus radinganorum is a threatened species highly sensitive to habitat disturbance, which could make necessary population reinforcements with plants originated from seeds. In such a case, this study provides a successful protocol promoting radicle and shoot emergence and thus an accurate guideline on how to grow plants from seeds.

Acknowledgments

The authors thank Raquel Herranz Ferrer for their assistance with laboratory work.

References

Adams CA, Baskin JM, Baskin CC, 2003. Epicotyl dormancy in the mesic woodland herb *Hexastylis heterophylla* (Aristolochiaceae). J Torrey Bot Soc 130: 11-15. http://dx.doi.org/10.2307/3557520

Ali N, Probert R, Hay F, Davies H, Stuppy W, 2007. Post-dispersal embryo growth and acquisition of desiccation tolerance in *Anemone nemorosa* L. seeds. Seed Sci Res 17: 155-163. http://dx.doi.org/10.1017/S0960258507783149

Baker KS, Steadman KJ, Plummer JA, Merritt DJ, Dixon KW, 2005. The changing window of conditions that promotes germination of two fire ephemerals, *Actinotus leucocephalus* (Apiaceae) and *Tersonia cyathiflora* (Gyrostemonaceae). Ann Bot 96: 1225-1236. http://dx.doi.org/10.1093/aob/mci274

Bañares A, Blanca G, Güemes J, Moreno JC, Ortiz S (eds), 2003. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid. 1072 pp.

Barton LU, 1936. Germination and seedling production in *Lilium* sp. Contrib. Boyce Thompson Inst 8: 297-309.

Baskin CC, Baskin JM, 1994. Deep complex morphophysiological dormancy in seeds of the mesic woodland herb *Delphinium tricorne* (Ranunculaceae). Int J Plant Sci 155: 738-743. http://dx.doi.org/10.1086/297212

Baskin CC, Baskin JM, 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. CA: Academic Press, San Diego, USA. 666 pp.

Baskin CC, Baskin JM, 2005. Seed dormancy in wild flowers. In: Flower seeds: biology and technology (Mc Donald MB, Kwong FY, eds). CAB International. Wallingford, U.K. pp: 163-185. http://dx.doi.org/10.1079/9780851999067.0163

Baskin CC, Milberg P, Andersson L, Baskin JM, 2002. Non-deep simple morphophysiological dormancy in seeds of the weedy facultative winter annual Papaver rhoeas. Weed Res 42: 194-202. http://dx.doi.org/10.1046/j.1365-3180.2002.00275.x

Baskin CC, Chien CT, Chen SY, Baskin JM, 2008. Germination of *Viburnum odoratissimum* seeds: a new level of morphophysiological dormancy. Seed Sci Res 18: 179-184. http://dx.doi.org/10.1017/S0960258508042177

Baskin JM, Baskin CC, 1985. Germination ecophysiology of *Hydrophyllum appendiculatum*, a mesic forest biennial. Am J Bot 72: 185-190. http://dx.doi.org/10.2307/2443546

Baskin JM, Baskin CC, 1990. Germination ecophysiology of seeds of the winter annual *Chaerophyllum tainturieri*: a new type of morphophysiological dormancy. J Ecol 78: 993-1004. http://dx.doi.org/10.2307/2260948

Baskin JM, Baskin CC, 2004. A classification system for seed dormancy. Seed Sci Res 14: 1-16. http://dx.doi.org/10.1079/SSR2003150

Cerabolini B, De Andreis R, Ceriani RM, Pierce S, Raimondi B, 2004. Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens*. Biol Conserv 117: 351-356. http://dx.doi.org/10.1016/j.biocon.2003.12.011

Copete E, Herranz JM, Copete MA, Ferrandis P, 2014. Inter-population variability on embryo growth, seed dormancy break, and germination in the endangered Iberian daffodil *Narcissus eugeniae* (Amaryllidaceae). Plant Species Biol 29, e72–e84. http://dx.doi.org/10.1111/1442-1984.12032

Copete E, Herranz JM, Ferrandis P, Baskin CC, Baskin JM, 2011. Physiology, morphology and phenology of seed dormancy-break and germination in the endemic Iberian species *Narcissus hispanicus* (Amaryllidaceae). Ann Bot 107: 1003-1016. http://dx.doi.org/10.1093/aob/mcr030

Elías F, Ruiz L (eds), 1981. Estudio agroclimático de la región de Castilla-La Mancha. Consejería de Agricultura. Junta de Comunidades de Castilla-La Mancha, Toledo (Spain). 247 pp.

Fabregat C, López S, 2003. *Narcissus radinganorum* Fern. Casas. In: Atlas y Libro Rojo de la Flora Vascular Amenazada de España. (Bañares A, Blanca G, Güemes J, Moreno JC, Ortiz S, eds). Dirección General de Conservación de la Naturaleza, Madrid (Spain). pp: 798-799.

Herranz JM, Ferrandis P, Martínez-Duro E, 2010. Seed germination ecology of the threatened endemic Iberian *Delphinium fissum* subsp. *sordidum* (Ranunculaceae). Plant Ecol 211: 89-106. http://dx.doi.org/10.1007/s11258-010-9775-0

Herranz JM, Copete E, Ferrandis P, 2013a. Nondeep complex morphophysiological dormancy in *Narcissus longispathus* (Amaryllidaceae): Implications for evolution of dormancy levels within section *Pseudonarcissi*. Seed Sci Res 23 (2): 141-155. http://dx.doi.org/10.1017/S0960258513000056

Herranz JM, Copete MA, Ferrandis P, 2013b. Environmental regulation of embryo growth, dormancy break and germination in *Narcissus alcaracensis* (Amaryllidaceae), a

threatened endemic Iberian daffodil. Am Midl Nat 169: 147-167. http://dx.doi.org/10.1674/0003-0031-169.1.147

Hidayati SN, Baskin JM, Baskin CC, 2000. Dormancy-breaking and germination requirements of seeds of four *Lonicera* species (Caprifoliaceae) with underdeveloped embryos. Seed Sci Res 10: 459-469.

Hilhorst HWM, 1998. The regulation of secondary dormancy: the membrane hypothesis revisited. Seed Sci Res 8: 77-90. http://dx.doi.org/10.1017/S0960258500003974

Kondo T, Mikubo M, Yamada K, Walck J, Hidayati SN, 2011. Seed dormancy in *Trillium camschatcense* (Melanthiaceae) and the possible roles of light and temperature requirements for seed germination in forests. Am J Bot 98: 215-226. http://dx.doi.org/10.3732/ajb.1000151

Kondo T, Miura T, Okubo N, Shimada M, Baskin CC, Baskin JM, 2004. Ecophysiology of deep simple epicotyl morphophysiological dormancy in seeds of *Gagea lutea* (Liliaceae). Seed Sci Res 14: 371-378. http://dx.doi.org/10.1079/SSR2004182

Kondo T, Okubo N, Miura T, Honda K, Ishiwaki Y, 2002. Ecophysiology of seed germination in *Erythronium japonicum* (Liliaceae) with underdeveloped embryos. Am J Bot 89: 1779-1784. http://dx.doi.org/10.3732/ajb.89.11.1779

Mabberley DJ, 2008. Mabberley's plant-book. A portable dictionary of plants, their classifications and uses. 3rd ed. Cambridge University Press. New York. 1040 pp.

Mayoral O, Gómez-Serrano MA, 2004. Sobre la distribución de *Narcissus eugeniane* Fern. Casas (Amaryllidaceae) en Castilla-La Mancha. Flora Montiberica 28: 83-89.

Milberg P, Andersson L, Thompson K, 2000. Large-seeded species are less dependent on light for germination than small-seeded. Seed Sci Res 10: 99-104. http://dx.doi.org/10.1017/S0960258500000118

Moreno JC (coord), 2008. Lista Roja 2008 de la Flora Vascular Española. Dirección General de Medio Natural y Política Forestal. Ministerio de Medio Ambiente y Medio Rural y Marino. Madrid (Spain). 86 pp.

Nikolaeva MG, 1977. Factors controlling the seed dormancy pattern. In: The physiology and biochemistry of seed dormancy and germination (Khan AA, ed). Amsterdam: North-Holland. pp: 51-74.

Ríos-Ruiz S, Rivera-Núñez D, Alcaraz-Ariza F, Obon De Castro C, 1999. Three new species of *Narcissus* L. subgenus *Ajax* Spach (Amaryllidaceae), restricted to the meadows and forests of south-eastern Spain. Bot J Linn Soc 131: 153-165. http://dx.doi.org/10.1111/j.1095-8339.1999.tb01846.x

Scholten M, Donahue J, Shaw NL, Serpe M, 2009. Environmental regulation of dormancy loss in seeds of *Lomatium dissectum* (Apiaceae). Ann Bot 103: 1091-1101. http://dx.doi.org/10.1093/aob/mcp038

Shen ZX, Parrish DJ, Wolf DD, Welbaum GE, 2001. Stratification in switchgrass seeds is reversed and hastened by drying. Crop Sci 41: 1546-1551. http://dx.doi.org/10.2135/cropsci2001.4151546x

Tweddle JC, Dickie JB, Baskin CC, Baskin JM, 2003. Ecological aspects of seed desiccation sensitivity. J Ecol 91: 294-304. http://dx.doi.org/10.1046/j.1365-2745.2003.00760.x

Vandelook F, Bolle N, Van Assche JA, 2007. Seed dormancy and germination of the European *Chaerophyllum temulum* (Apiaceae), a member of trans-atlantic genus. Ann Bot 100: 233-239. http://dx.doi.org/10.1093/aob/mcm090

Vandelook F, Van Assche JA, 2008. Temperature requirements for seed germination and seedling development determine timing of seedling emergence of three monocotyledonous temperate forest spring geophytes. Ann Bot 102: 865-875. http://dx.doi.org/10.1093/aob/mcn165

Wood CB, Pritchard JW, Amritphale D, 2000. Desiccation-induced dormancy in papaya (*Carica papaya* L.) seeds is alleviated by heat shock. Seed Sci Res 10: 135-145.