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An experimental comparison of germination ecology and its implication for conservation of selected rare and endangered species of *Dianthus* (Caryophyllaceae)

Jeremi Kołodziejek, Jacek Patykowski, and Mateusz Wala

Abstract: The germination requirements of four taxonomically related taxa of *Dianthus* (*D. arenarius* L. subsp. *borussicus* Vierh., *D. carthusianorum* L., *D. gratianopolitanus* Vill., and *D. deltoides* L.) were studied under controlled conditions in a laboratory. A set of experiments were conducted to evaluate seed germination responses to storage period and cold stratification, different hydrogen ion concentrations (pH), potassium nitrate (KNO_3), temperatures, and light conditions. Experiments were also performed to study the effects of sowing depth and water supply on seedling emergence. There were no differences in germination between seeds incubated under light or dark conditions for any of the taxa we studied. Germination percentages increased significantly with increases in temperature. The taxa of *Dianthus* we studied showed similar responses to sand burial, i.e., seeds placed on or near the soil surface had maximum emergence, and emergence declined with increasing depth of seed burial. In the studied taxa, germination was reduced by declining osmotic potentials. Cold stratification increased the germination rate, but did not affect final germination percentage of *Dianthus* seeds. A higher concentration of nutritious solutions (KNO_3) negatively affected the germination percentage. The results show that seeds of the studied taxa are potentially germinable in the species' habitat at any time between April and October.

Key words: Dianthus, germination, nitrate, temperature, water stress.

Résumé : Les conditions de germination de quatre taxons de *Dianthus* apparentés d'un point de vue taxonomique (*D. arenarius* L. subsp. *borussicus* Vierh., *D. carthusianorum* L., *D. gratianopolitanus* Vill. et *D. deltoides* L.) ont été étudiées dans des conditions contrôlées en laboratoire. Une série d'expériences a été réalisée afin d'évaluer la germination des semences en fonction de la période de stockage et de la stratification froide, de différentes concentrations d'ion hydrogène (pH), de nitrate de potassium (KNO_3) et de différentes conditions de températures et de lumière. Des expériences ont aussi été réalisées pour étudier les effets de la profondeur d'ensemencement et de l'approvisionnement en eau sur l'émergence des semis. Chez tous les taxons étudiés, aucune différence de germination entre les semences incubées à la lumière ou à la noirceur n'a été enregistrée. Les pourcentages de germination augmentaient significativement en fonction de l'accroissement de la température. Les taxons de *Dianthus* étudiés présentaient des réponses similaires à l'enfouissement dans le sable, c.à.d que l'émergence était maximale chez les semences enfouies à la surface ou près de la surface du sol, cette émergence diminuant avec l'augmentation de la profondeur de l'enfouissement des semences. Chez les taxons étudiés, la germination était réduite par la diminution des potentiels osmotiques. La stratification froide augmentait le taux de germination, mais n'affectait pas le pourcentage final de germination des semences de *Dianthus*. Une concentration plus élevée en solutions nutritives (KNO_3) affectait négativement le pourcentage de germination. Les résultats montrent que les semences des taxons étudiés peuvent germer dans l'habitat naturel de ces espèces en tout temps entre avril et octobre. [Traduit par la Rédaction]

Mots-clés : Dianthus, germination, nitrate, température, stress hydrique.

Introduction

The genus *Dianthus* L. belongs to the dicotyledonous Caryophyllaceae family (Order: Caryophyllales). The family consists of 80 genera and 2000 species, which are either annual or perennial and occur mostly in the northern hemisphere, mainly in the Mediterranean and Irano-Turanian region. Traditionally, Caryophyllaceae are divided into three subfamilies: Alsinoideae,

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Table 1. Traits of the studied species of *Dianthus*.

Taxon	Life form	Seed length and width (mm)	Mean seed mass (mg)	Habitat preference
D. arenarius subsp. *borussicus*	Hemicryptophyte	1.9–2.3×1.3–1.9	0.324±0.005	Pine-dominated forests, dry grasslands on sandy soils, sand dunes
D. carthusianorum	Hemicryptophyte	2.0–2.4×1.5–1.8	0.330±0.004	Xerothermic grassland, xeric sand calcareous grasslands (*Koelerion glaucae*)
D. deltoides	Hemicryptophyte	1.1–1.6×0.7–1.0	0.094±0.002	Dry grasslands on sandy soils (*Diantho-Armerietum elongatae* Kraush 1959), xerothermic grassland
D. gratianopolitanus	Hemicryptophyte	2.7–3.1×1.8–2.2	0.726±0.008	Calcareous grasslands on shallow skeletal soils (*Seslerio–Festucion duriusculae*), pine-dominated forests (*Dicrano–Pinion*)

Note: Nomenclature corresponds to the following authors: life form (Ellenberg 1991); seed length and width (Bojñnanský and Fargašová 2007); seed mass (this study; the seed mass of each species was determined by weighing 1000 air-dried seeds, expressed in milligrams as the mean ± SD) and habitat preference (Hegi 1975). Plant nomenclature follows Tutin (1993).

Silenoideae, and Paronychioideae. *Dianthus* is the genus of about 300 species belonging to the subfamily Silenoideae (Rabeler and Bittrich 1993; Tutin 1993).

In our study, we focussed on the germination requirements of four closely related Caryophyllaceae taxa: *Dianthus arenarius* L. subsp. *borussicus* Vierh., *D. carthusianorum* L., *D. deltoides* L., and *D. gratianopolitanus* Vill. All four species have similar geographic distribution, occurring throughout Europe and Western Asia (Tutin 1993).

Dianthus arenarius subsp. *borussicus* is a caespitose perennial herb up to 45 cm tall. It spreads through both seeds and clonal growth. The species grows in pine-dominated forests on sand dunes and in dry grasslands on sandy soils in central and north-eastern Europe. The soil chemistry requirements for this species are unknown but in general, it appears to grow in soils that are acidic and deficient in macronutrients. *Dianthus carthusianorum* is a perennial hemicryptophyte, 15–45 cm high. It is distributed in southern, western, and central Europe (Meusel et al. 1965; Tutin 1993). It is more or less restricted to seminatural grasslands, which have been classified as priority habitats for conservation under the code number *6210 of the Council Directive 92/43/EEC (European Community 1992) and are important habitats for many red-listed light-loving and nitrophobic plant species (Hansson and Fogelfors 2000). *Dianthus carthusianorum* is species tolerant to high soil pH and low-nutrient levels (Ellenberg 1991). *Dianthus deltoides* is a loosely tufted perennial producing few-flowered (often solitary) inflorescences held on rounded, short, pubescent stems 15–30 cm tall. *Dianthus deltoides* needs nutrient poor, drought-prone soil in which vegetation cover develops slowly. This species exists in a variety of habitats, such as pastures, acid grasslands, open pine-dominated forests, off paths and roads on sandy soils. *Dianthus deltoides* is widespread throughout much of central, eastern, and northern Europe but becomes increasingly uncommon towards the south and west. *Dianthus gratianopolitanus* belongs to a suboceanic part of the European temperate zone, i.e., its range comprises western and central Europe (Meusel et al. 1965; Tutin 1993). It is found in calcareous and rocky grasslands of the alliance *Seslerio–Festucion duriusculate* Klika (1931) 1948, in well-lit coniferous forests belonging to the alliance *Dicrano–Pinion* Libb 1933, and also on acid soils in oak forests classified as *Calamagrostio arundinaceae – Quercetum petraeae* (Hartm 1934) Scam. et Pass 1959 community. In many European countries, *D. gratianopolitanus* is a rare and endangered species (Mirek et al. 2006).

Dianthus arenarius and *D. carthusianorum* are very similar with respect to seed size, whereas seeds of *D. gratianopolitanus* are two times larger. Seeds of *D. deltoides* are four to eight times smaller compared with the other examined species (Table 1).

Although these *Dianthus* species have received considerable attention and research (Partzsch 2011), there is a lack of information about their revegetation based on seed germination. Studying the germination requirements of closely related species with a similar geographic distribution allows one to attribute variation in germination requirements to differences in habitat preference (Table 2) between the species (Vandelook et al. 2008). Furthermore, it allows estimates of the critical factors for conservation of the studied taxa.

Therefore, laboratory studies were conducted to determine the effect of several environmental factors on seed germination and seedling emergence of *Dianthus* taxa. This study included examining (*i*) the effects of temperature, light, hydrogen ion concentration (pH), potassium nitrate (KNO_3), and water stress on seed germination, and (*ii*) the effect of burial depth in sand on seedling emergence.

Materials and methods

Seed collection

The seeds of four taxa included in this study (Table 1) were collected from wild plants of identical provenance growing in the experimental garden in the campus of University of Lodz, Faculty of Biology and Environmental Protection. During cultivation, the area was watered and

Table 2. Ellenberg index describing the effects of environmental conditions on vascular plants (*Dianthus* sp.).

Taxon	Light	Temperature	Continent	Moisture	pH[a]	Nitrogen[b]
D. arenarius subsp. *borussicus*	—	—	—	—	—	—
D. carthusianorum	8	5	4	3	7	2
D. deltoides	8	5	4	3	3	2
D. gratianopolitanus	9	7	4	2	7	1

Note: The scales range from 0 to 9, where 9 denotes the highest prevalence of plant (Ellenberg 1991).
[a]pH index: 3 indicates acidity; 7 indicates weakly acidic.
[b]N nitrogen index: 1 indicates nitrogen-poor soils; 2 indicates nitrogen-poor to moderately poor soils; 7 indicates nitrogen-rich soils; —, no data.

hand-weeded to maintain optimal growth conditions. Seeds were sampled between July and August 2014, depending on the time of ripening. Then, the seeds were air-dried at room temperature (21.5–22.0 °C) and stored in darkness at room temperature (21.5–22.0 °C). The seeds for the cold-stratified test group were stored in a refrigerator between two layers of nylon cloth, buried in a tray filled with moist sand at 4.5–5.1 °C.

General germination procedures

Three groups of the seeds were tested for germination: freshly matured (stored for 14 days) and after two different treatments: cold stratification for 120 days and dry storage for 270 days. The treatments were performed in continuous darkness. Average daily minimum–maximum temperatures were 4.5–5.1 °C in the cold environment, and 21.5–22.0 °C in the dry storage environment. The dry-stored seeds were subjected to a relative humidity of 30%–35%. All of the germination tests were conducted in 9 cm (diameter) glass Petri dishes on paper filters (Whatman No. 1) moistened with 3 mL of distilled water or an appropriate solution. To avoid the loss of water, the Petri dishes were sealed with parafilm. For each treatment, four replicates of 25 seeds were used; untreated seeds were the control for each experiment. The seeds were tested for germination under a 12 h photoperiod (Sylvania cool white fluorescent lamps, 25 μmol photons·m^{-2}·s^{-1} photosynthetically active radiation) or in darkness, which was achieved by wrapping the Petri dishes in a double layer of an aluminium foil. Every day, the percentage of germinated seeds was recorded. Newly emerged seedlings were removed from the Petri dishes. The seeds were regularly watered with distilled water to maintain stable and homogenous conditions. A dim green safe light was used for counts of the seeds tested in darkness. A seed was considered as germinated when radicle was visible. Germination percentages were determined on the basis of the number of viable seeds. Dead seeds were identified on the basis of their softness and brownish embryo colour, and these were not included in the calculations.

The rate of germination was estimated using a modified Timson's index of germination velocity: IGV = $(\Sigma G)/t$, where G is the cumulative seed germination percentage each day, and t is the total number of days germination percentage was tested (Khan and Ungar 1997). Therefore, if all of the seeds completed germination in first day, the Timson's index reaches a value of 100, which is the maximum possible value in this study (2000/20), whereas a lower value indicates less rapid germination.

Experiment 1: effect of temperature and light on germination

The germination tests were performed using fresh matured seeds (14 days after harvest) and those dry-stored at 21.5–22.0 °C for 270 days. The seeds were watered with distilled water. Germination was tested at 15/5 °C, 20/10 °C, and 25/15 °C, in light and darkness for 20 days.

Experiment 2: germination of cold-stratified seeds

To test whether *Dianthus* seeds require a cold-stratification period for germination, the germination tests were performed using cold-stratified seeds (120 days at 4.5–5.1 °C). The seeds were watered with distilled water. The germination conditions were the same as those described for Experiment 1 (15/5 °C, 20/10 °C, or 25/15 °C, light or darkness, 20 days of cultivation). The low temperature of stratification was chosen because it was used to simulate the coldest condition in winter in the morphophysiological dormancy (MPD) studies (Baskin and Baskin 2014).

Experiment 3: effect of water stress on seed germination

Osmotic solutions were prepared by adding polyethylene glycol 6000 (PEG 6000) to distilled water, as described by Michel and Kaufmann (1973). Freshly matured seeds were incubated in distilled water or the same volume of water solutions of PEG 6000 with osmotic potentials of −0.2 MPa, −0.5 MPa, −1.0 MPa, −1.2 MPa, and −1.5 MPa. Then, the seeds were incubated at 25/15 °C with light for 20 days.

Experiment 4: effect of pH on seed germination

Buffered pH solutions were prepared according to the method described by Lu et al. (2006). Freshly matured seeds were exposed to these buffer solutions of pH 4–9 in 1.0 pH unit increments. A 0.2 mol·L^{-1} sodium acetate buffer was used for pH 4.0. Buffered solutions of pH 5.0, 6.0, and 7.0 contained potassium hydrogen phosphate (0.2 mol·L^{-1}). For the buffered solutions of pH 8.0 and 9.0, a boric acid solution (0.2 mol·L^{-1}) and a borax solution (0.05 mol·L^{-1}) were used. Final pH adjustments of each buffer were made using 0.1 mol·L^{-1} HCl or 0.1 mol·L^{-1} NaOH. The seeds were incubated under the identical conditions to those described for Experiment 3 (25/15 °C with light, 20 days of cultivation).

Experiment 5: examination of seedling emergence

Freshly matured seeds were planted in sand–peat medium (90% sand and 10% peat moss, *v*/*v*) at nine depths (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 cm) in plastic pots (10 cm in diameter, 8 cm deep) and incubated in the growth chamber at 25/15 °C with light. The pots were watered throughout the study to maintain adequate moisture. Seedlings were considered to have emerged when cotyledons were visible. Emergence counts were recorded daily for a period of 20 days, during which the emerged seedlings were removed each day by cutting them at the soil surface.

Experiment 6: effect of KNO_3 on germination

Freshly matured seeds were watered with (*i*) distilled water (control), (*ii*) 0.5 mmol·L^{-1}, (*iii*) 10 mmol·L^{-1}, or (*iv*) 25 mmol·L^{-1} KNO_3 (specifically,NO_3^-) and incubated at 25/15 °C under light conditions for 20 days. All of the solutions were monitored and maintained at pH 6.1.

Data analysis

The germination percentage data were subjected to arcsine transformation and are reported in the tables as untransformed values. The significance of differences in germination among taxa and nitrate treatments was tested by two-way analysis of variance (ANOVA). Two-way ANOVA was also used to determine the effect of each taxon and sowing depth on seedling emergence. A four-way ANOVA was carried out to test the effects of main factors (taxon, treatment, light conditions, temperature) and of their interactions on the final germination percentage and rate of germination. Differences among means were considered to be significant at $P < 0.05$ using Tukey's multiple range test. Statistical analysis was carried out using Statistica version 10.

Results

Temperature and light requirements for germination

Germination percentages for all four studied taxa was generally very high in the groups incubated in all constant temperature regimes immediately after harvesting. The seeds of all four *Dianthus* species responded similarly to all temperatures tested (Tables 3 and 4). The temperature was an important factor because germination of these taxa was significantly ($P < 0.01$) greater at 25/15 °C than at lower temperatures in both light and darkness (Table 5). Germination in both darkness and light/dark was highest in *D. carthusianorum*, followed by *D. arenarius*, *D. gratianopolitanus*, and *D. deltoides* for all of the temperature regimes tested. However, for all four taxa, seed germination was higher under light than under dark conditions (Tables 3 and 4). Second-, third-, and fourth-order interactions that included temperature were of importance.

Table 3. Germination percentage (mean ± SD) of the untreated (fresh), dry-stored, and 4-month cold-stratified seeds of four *Dianthus* taxa at three temperatures (15/5 °C, 20/10 °C, and 25/15 °C) in light or in darkness.

Light condition	Temperature (°C)	Untreated	Dry stored	Cold stratified (4 months)
D. arenarius				
Light	15/5	57±3c	58±3c	51±4c
	20/10	68±5b	69±3b	67±3b
	25/15	86±2a	83±6a	85±5a
Dark	15/5	49±1c	54±1c	45±1c
	20/10	56±4b	57±2b	55±3b
	25/15	76±4a	80±3a	73±2a
D. carthusianorum				
Light	15/5	52±3c	56±3c	49±4c
	20/10	64±4b	62±3b	64±3b
	25/15	98±2a	97±5a	96±3a
Dark	15/5	26±2c	30±2c	25±1c
	20/10	68±3b	65±3b	65±2b
	25/15	91±3a	90±4a	89±4a
D. deltoides				
Light	15/5	40±4c	10±1c	34±1c
	20/10	59±3b	70±3b	58±4b
	25/15	96±3a	80±3a	93±3a
Dark	15/5	36±2c	37±2c	34±4c
	20/10	49±3b	46±3b	49±6b
	25/15	84±5a	87±4a	92±3a
D. gratianopolitanus				
Light	15/5	34±3c	36±1c	38±1c
	20/10	60±4b	56±3b	60±4b
	25/15	97±3a	99±6a	97±3a
Dark	15/5	7±2c	6±3c	6±4c
	20/10	56±2b	80±2b	55±6b
	25/15	88±3a	90±3a	87±3a

Note: Each value is the mean ± SD of four replicates of 25 seeds. Values followed by different lower-case letters within each column with the same light condition are significantly different ($P < 0.05$) in the germination percentage of the seeds among different temperatures, as analyzed using ANOVA followed by Tukey's multiple range test.

When all of the taxa were analysed together, the greatest rates of germination were found in the 25/15 °C temperature regime. A significant two-way interaction was found between each taxon and temperature treatment determining the rate of germination ($P < 0.01$). Germination preferences were similar for all taxa: after cold stratification and dry storage, germination occurred at all temperatures tested and in both light and darkness. However, cold stratification and dry storage had no significant ($P > 0.05$) effect on final germination percentage (Table 5).

Germination at different osmotic potentials

PEG solutions at osmotic potentials −0.2 to −1.0 MPa significantly ($P < 0.01$) inhibited the completion of seed germination, which was manifested by reduction of the

Table 4. Index of germination velocity of the untreated (fresh) dry-stored and 4-month cold-stratified seeds of four *Dianthus* species at three temperatures (15/5 °C, 20/10 °C, and 25/15 °C) under different light conditions for 20 days.

Light condition	Temperature (°C)	Untreated	Dry stored	Cold stratified (4 months)
D. arenarius				
Light	15/5	37±2c	39±2c	30±3c
	20/10	42±1b	44±2b	40±4b
	25/15	47±1a	48±1a	57±3a
Dark	15/5	4±1c	6±1c	1±1c
	20/10	38±2b	38±2b	33±1b
	25/15	49±1a	49±1a	59±2a
D. carthusianorum				
Light	15/5	23±4c	21±4c	27±2c
	20/10	46±5b	49±5b	52±4b
	25/15	57±6a	58±6a	64±3a
Dark	15/5	21±4c	22±3c	28±1c
	20/10	39±3b	36±3b	30±4b
	25/15	56±7a	57±4a	61±2a
D. deltoides				
Light	15/5	27±4c	28±2c	33±2c
	20/10	42±5b	42±5b	48±4b
	25/15	57±6a	58±5a	68±1a
Dark	15/5	22±4c	24±4c	34±1c
	20/10	31±3b	30±2b	43±4b
	25/15	53±7a	53±7a	58±2a
D. gratianopolitanus				
Light	15/5	37±5c	36±3c	43±2c
	20/10	42±1b	41±1b	51±1b
	25/15	59±1a	60±4a	67±2a
Dark	15/5	2±1c	2±1c	4±1c
	20/10	39±3b	40±3b	47±2b
	25/15	58±1a	57±1a	66±1a

Note: Each value is the mean ± SD of four replicates of 25 seeds. Values followed by different lower-case letters within each column with the same light condition are significantly different ($P < 0.05$) in the germination rate percentages of seeds among different temperatures, as analyzed using ANOVA followed by Tukey's multiple range test.

final germination percentage (Figs. 1A–1D). The seeds of the four species completed germination in 0 (water) and −0.2 MPa solutions; few seeds completed germination in −0.5 MPa solutions. The difference between the germination percentage in water (control) and in −0.2 and −0.5 MPa was significant ($P < 0.05$). No germination occurred in the PEG solutions at osmotic potential more negative than −0.5 MPa.

Effect of pH on seed germination

The pH value had a significant impact on germination of all studied taxa. *Dianthus arenarius* subsp. *borussicus*, *D. carthusianorum*, and *D. gratianopolitanus* seeds completed germination between pH 5 and 9, but the maxima were observed at pH ranging from 6 to 7 (Figs. 2A, 2B, and 2D). The seeds did not complete germination when the pH was 4. For *D. deltoides*, the final germination percentage was highest at pH 5. However, the seeds did not complete germination at pH 9 (Fig. 2C).

Table 5. Results of four-way ANOVA examining the effects of the studied factors on final germination percentage and rate of germination of four *Dianthus* taxa.

Factor	df	*F*	*P*
Percent germination			
(Tx) Taxon[a]	3	0.7	$P < 0.01$
(P) Pre-treatment[b]	1	0.9	ns
(T) Temperature[c]	2	22.4	$P < 0.001$
(L) Light[d]	1	0.7	$P < 0.01$
Tx × P	3	0.5	ns
Tx × T	6	18.5	$P < 0.01$
Tx × L	3	0.6	$P < 0.01$
P × T	2	19.5	ns
P × L	1	0.12	ns
T × L	2	14.6	$P < 0.01$
Tx × P × T	6	15.5	$P < 0.01$
Tx × P × L	3	0.11	$P < 0.01$
Tx × T × L	6	12.7	$P < 0.01$
P × T × L	2	15.3	$P < 0.01$
Tx × P × T × L	6	16.3	$P < 0.01$
Rate of germination			
(Tx) Taxon[a]	3	1.2	$P < 0.01$
(P) Pre-treatment[b]	1	25.9	ns
(T) Temperature[c]	2	21.2	$P < 0.001$
(L) Light[d]	1	0.5	$P < 0.01$
Tx × P	3	16.6	ns
Tx × T	6	22.1	$P < 0.01$
Tx × L	3	0.14	$P < 0.01$
P × T	2	38.9	ns
P × L	1	24.9	ns
T × L	2	14.6	$P < 0.01$
Tx × P × T	6	15.5	$P < 0.01$
Tx × P × L	3	0.11	$P < 0.01$
Tx × T × L	6	18.2	$P < 0.01$
P × T × L	2	24.4	$P < 0.01$
Tx × P × T × L	6	32.6	$P < 0.01$

Note: The seeds were subjected to germination tests in eight different environments when fresh and after two different treatments.

[a]*Dianthus arenarius* subsp. *borussicus*, *D. carthusianorum*, *D. deltoides*, *D. gratianopolitanus*.

[b]Cold stratification or dry storage.

[c]Germination test at 15/5 °C, 20/10 °C or 25/15 °C day/night.

[d]Germination test in light or in continuous darkness; ns, not significant ($P > 0.05$). Each treatment had four replicates.

Seedling emergence

No significant differences among species were observed with respect to seedling emergence. The highest seedling emergence of these taxa appeared on the soil surface, and the deeper the seeds were placed in sand, the lower the percentages of seedling emergence. No seedlings emerged from the seeds of any of the four taxa buried at a depth of 4 cm or greater. The two-way ANOVA indicated that sowing depth, taxon, and their combinations

Fig. 1. Effect of osmotic potential on the germination of the four tested species of *Dianthus*: (A) *D. arenarius* subsp. *borussicus*; (B) *D. carthusianorum*; (C) *D. deltoides*; (D) *D. gratianopolitanus*, at 25/15 °C (day/night), with a 12 h photoperiod, for 20 days. Means with the same letter do not differ (Tukey's test at $P < 0.05$). Vertical bars represent the standard deviation of the means.

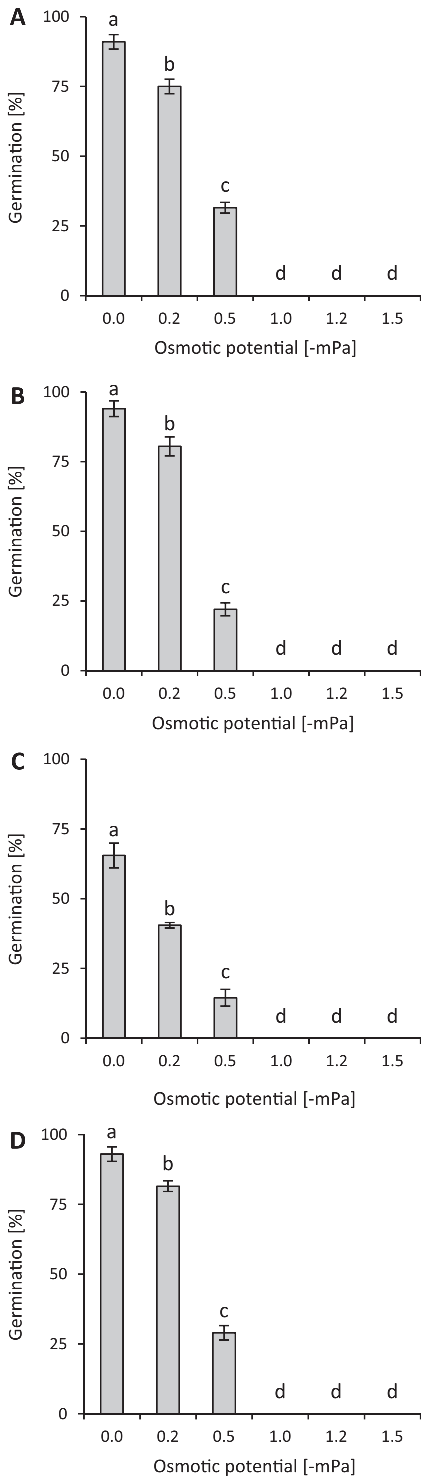

Fig. 2. Effect of pH on germination of four tested species of *Dianthus*: (A) *D. arenarius* subsp. *borussicus*; (B) *D. carthusianorum*; (C) *D. deltoides*; (D) *D. gratianopolitanus*, at 25/15 °C (day/night) with a 12 h photoperiod for 20 days. Means with the same letter are not significantly different ($P < 0.05$; Tukey's multiple range test). Vertical bars represent the standard deviation of the means.

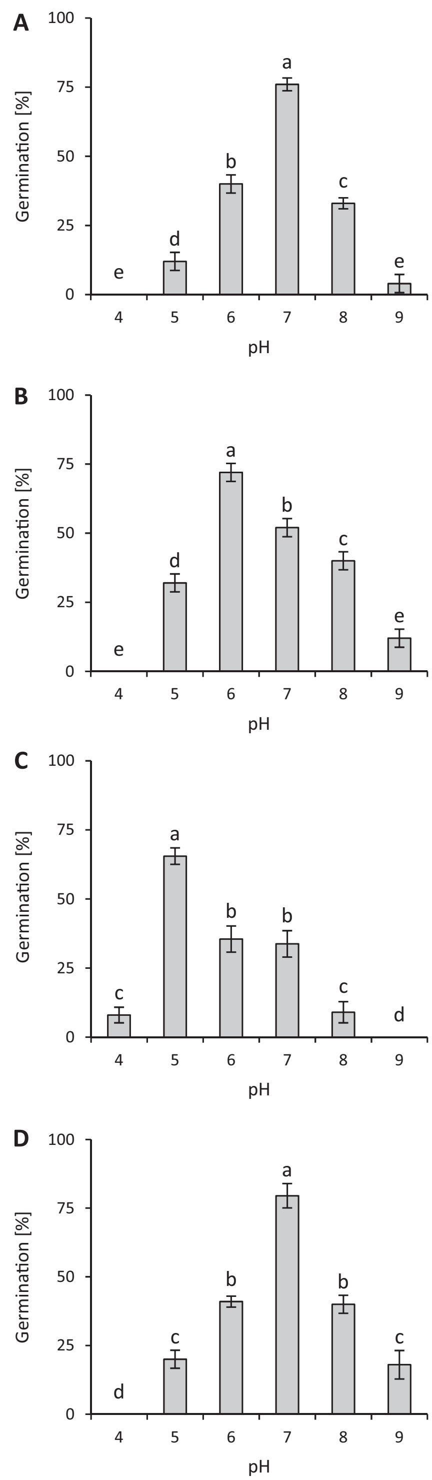

Table 6. Results of two-way ANOVA of characteristics of final seedling emergence percentage of four taxa in relation to sowing depth.

Factor	df	F	P
(Tx) Taxon[a]	3	225.0	ns
(B) Burial depth[b]	8	154.8	$P < 0.001$
Tx × B	24	24.8	$P < 0.01$

[a]*Dianthus arenarius* subsp. *borussicus*, *D. carthusianorum*, *D. deltoides*, *D. gratianopolitanus*.

[b]There were nine depths for sand burial (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3,5, and 4.0 cm). Each treatment had four replicates.

significantly affected final seedling emergence percentage (Table 6).

Effect of NO_3^- on seed germination

Regarding the effect of nitrate concentration on germination in the light, 0.5 mmol·L^{-1} KNO_3 was the most effective treatment in which germination of all four taxa was stimulated. Higher concentrations of potassium nitrate such as 10, and 25 mmol·L^{-1} were not found to be stimulatory (post-hoc comparison, $P > 0.05$). The highest concentration (25 mmol·L^{-1} KNO_3) decreased germination rate of all the studied taxa. When germination percentages and rate of germination were analysed, significant effects ($P < 0.01$) were found for nitrate concentration and interaction between taxon and nitrate concentration (Table 7).

Discussion

Seeds of all four studied taxa are nondormant (Baskin and Baskin 2014) and germinate very quickly after dispersal. It has been reported that seeds of other *Dianthus* species also complete germination both in the light and darkness just after collection and without any treatment (Partzsch 2011, 2013; Cogoni et al. 2012). Moreover, a high capacity for spontaneous germination directly after shedding is typical of many dominant Caryophyllaceae in open xerothermic vegetation units, such as *Gypsophilla fastigiata* L., *Viscaria vulgaris* Röhl., *Lychnis viscaria*, *Silene nutans* L., and *S. vulgaris* (Moench) Garcke (Partzsch 2011). Rapid germination has been associated with high-stress habitats, e.g., dry soil (Daws et al. 2002).

The *Dianthus* seeds examined in this study completed germination to high percentages in a wide range of temperatures. This is probably due to the fact that the seeds were completely nondormant when germination was tested. The temperature response of the studied *Dianthus* taxa is similar to that reported for other temperate Caryophyllaceae species, such as *Moehringia trinervia* (Clairv.) L., *Stellaria nemorum* subsp. *nemorum* L., *S. holostea* L., and *S. graminea* L. The optimum temperature reported for these species is 20 °C (Vandelook et al. 2008). Klavina et al. (2006) obtained similar results from experiments on seeds of *D. arenarius* subsp. *arenarius*. Similarly, Thompson (1970) showed in laboratory experiments that six species of the family Caryophyllaceae that shared common distributions had similar responses to temperature, except for variations in maxima and minima.

The majority of the species from the temperate regions require a cold winter period prior to germination or to increase their germination percentage in spring (Baskin and Baskin 2014). Nevertheless, our results showed that cold-stratification did not increase germination rate in any of the four taxa, suggesting that they are not specialized to complete germination in the spring. On the other hand, it was shown in this study that after four months of cold stratification, germination velocity (Timson's Index) of *Dianthus* seeds was significantly higher than those that had not been stratified ($P < 0.01$). Therefore, cold stratification increases growth potential of the embryo so that the radicle can break through the seed coat, resulting in completion of germination. Similar results were shown in nondormant seeds of *Picea mariana* (Mill.) B.S.P. (Wang and Berjak 2000) and *Pinus roxburghii* Sargent (Ghildiyal et al. 2009). The main mechanism responsible for this phenomenon might be degradation of endogenous abscisic acid (Li et al. 2011).

Nondormant seeds of many species complete germination well both in light and darkness (Baskin and Baskin 2014), among which the majority complete germination to higher percentages in light than in darkness (Vleeshouwers et al. 1995; Grime 2006; Baskin and Baskin 2014) compared with a relative few that complete germination to higher percentages in darkness than in light (Baskin and Baskin 2014). In this study, seed germination was higher in light than in darkness in all taxa. In general, small-seeded species are more likely to have a light requirement for germination than species with larger seeds (Milberg et al. 2000). Jankowska-Błaszczuk and Daws (1997) considered a seed mass of 1.5 mg as a threshold separating temperate forest herbs requiring light for germinating from those that do not. According to this criterion, all of the taxa we studied produce seeds below this threshold value and showed light requirement for the completion of germination. This suggests that successful germination and establishment of *Dianthus* requires a high-light environment. This also shows that the studied seeds are light sensitive, and our results may help explain why *Dianthus* usually colonize areas where soils are bare and exposed. *Dianthus* species produce a large amount of small seeds with no primary dormancy and the ability to complete germination over a wide range of temperatures, so they only have the potential to complete germination near the soil surface. However, because some *Dianthus* seeds also completed germination in complete darkness, this could explain why establishment occurs in dense, shaded areas such as pine forests (*D. arenarius* and *D. gratianopolitanus*).

Successful completion of germination by seeds may be directly related to the depth at which they are sown (Zhang and Maun 1990; Kołodziejek and Patykowski 2015). Seeds of all the taxa sown on the surface of sand

Table 7. Results of two-way ANOVA of characteristics of seed and rate of germination of four taxa in relation to nitrate concentration.

Dependent variable	Factor	df	F	P
Percent germination	(Tx) Taxon[a]	3	225.0	ns
	(B) Nitrate concentration[b]	8	154.8	$P < 0.001$
	Tx × B	24	24.8	$P < 0.01$
Rate of germination	(Tx) Taxon	3	156.8	ns
	(B) Nitrate concentration[b]	3	134.3	$P < 0.01$
	Tx × B	9	78.0	$P < 0.01$

[a]*Dianthus arenarius* subsp. *borussicus*, *D. carthusianorum*, *D. deltoides*, *D. gratianopolitanus*.
[b]There were four concentrations of nitrate (0, 0.5, 10, and 25 mmol·L^{-1}).

completed germination well, and the seedlings emerged well. But the deeper the seeds were placed in sand, the lower the rate of seedling emergence. This germination response to sand burial was similar to that observed in studies on the other species of Caryophyllaceae family (Whittington et al. 1988). It also contributes to a better understanding of the germination-based revegetation of studied taxa in natural as well as seminatural habitats and its implications for further protection plans.

Water deficit is usually the limiting factor for germination of nondormant seeds, affecting the percentage, speed, and uniformity of emergence (Gill et al. 2003; Kaydan and Yagmur 2008; Muscoloa et al. 2014). In this study we showed that germination of nondormant seeds was affected by increasing water stress: as osmotic stress increased, seed germination percentage decreased. Optimum germination occurred at osmotic potentials between 0 and −0.2 MPa. No germination occurred at an osmotic potential lower than −0.5 MPa. These findings indicated that the spread of *Dianthus* may be restricted to well-drained, relatively dry soils, owing to its ability to complete germination under conditions of low soil moisture. In *D. chinenesis* L. no germination was observed at an osmotic potential of −1.66 MPa (He et al. 2009). Poor seed germination under PEG-induced water deficit was also observed in different plants under both laboratory and field conditions (Gill et al. 2003). Slow and poor germination under water stress is obviously due to decreased osmotic potential of the germination medium, which restricts the water availability to the seeds (Wilson 1972; Takaki 1990; Hardegree and Emmerich 1994; Khera and Singh 2005).

The overall effect of exogenous application of KNO_3 was negative for germination in this study. With respect to seed germination in the natural environment, all of the studied species were found in very poor soil. The increased levels of NO_3^- in the medium could have caused a toxic effect that inhibited seed germination (Pons 1993). This is in agreement with the results obtained by Pérez-Fernández et al. (2006), who observed that maximum germination rates in eight species found in Mediterranean ecosystems were achieved with low concentrations of nitrate.

According to Ellenberg (1991), the abiotic conditions preferred by the four taxa of *Dianthus* taxa we studied are the same, except for pH. For these taxa, their microhabitats are sandy places with high temperature and intense light, which are favourable for germination and seedling survival. Based on the results, seeds of three *Dianthus* species (*D. carthusianorum*, *D. arenarius* subsp. *borussicus*, *D. gratianopolitanus*) germinated most readily at near-neutral pH, but could also germinate in both acidic and alkaline soils. High percentages of seed germination for these taxa over a broad pH range indicates that pH may not be a limiting factor for germination in moist soils. However, our data suggest that pH may be a limiting factor for the spread of *D. deltoides*, which preferentially germinated at pH 5.

The time for which the seed can persist is unknown, but such information is very important when considering the suitability of sites for restoration management. The seeds of the four *Dianthus* species we studied can retain high viability and germination capability after dry storage for up to one year. The ability to germinate in the dark suggests that *Dianthus* species does not form a long-lived seed bank. Partzsch (2011) indicated that Caryophyllaceae species have transient soil seed banks (i.e., *D. carthusianorum*, *G. fastigiata*, *S. nutans*) and long-lived seed banks (i.e., *Scleranthus perennis* L.). Therefore, for the species we studied, the establishment of stable populations seems to be dependent on continuous and uninterrupted revegetation of local individuals, both by seeds and clonal growth.

Conclusions

In conclusion, the seeds of all four of the studied taxa are nondormant and complete germination quickly after dispersal. Cold stratification increased their germination rate, but did not affect final germination percentage. *Dianthus* taxa require light for germination; thus germination is prevented when the seeds are buried deep in the soil. All of the seeds tested, except *D. deltoides*, completed germination most readily in near neutral pH or in both acidic and alkaline soils, indicating that the soil pH is not a limiting factor for their germination. These results indicate that the seeds of the studied taxa potentially germinate in the species' habitat at any time between April and October.

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References

Baskin, C.C., and Baskin, J.M. 2014. Seeds, ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, Calif.

Bojñnanský, V., and Fargašová, A. 2007. Atlas of seeds and fruits of central and east-European flora: the Carpathian Mountains region. Springer, London, UK.

Cogoni, D., Mattana, E.G., Fenu, G., and Bacchetta, G. 2012. From seed to seedling: a critical transitional stage for the Mediterranean psammophilous species *Dianthus morisianus* (Caryophyllaceae). Pl. Biosyst. **146**(4): 910–917. doi:10.1080/11263504.2011.647106.

Daws, M.I., Burslem, D.F.R.P., Crabtree, L.M., Kirkman, P., Mullins, C.E., and Dalling, J.W. 2002. Differences in seed germination responses may promote coexistence of four sympatric *Piper* species. Funct. Ecol. **16**(2): 258–267. doi:10.1046/j.1365-2435.2002.00615.x.

Ellenberg, H. 1991. Indicator values of vascular plants in central Europe 1. Indicator values of vascular plants not including *Rubus*. Scripta Geobot. **18**: 9–166.

European Community. 1992. European Community Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora (Habitat Directive), European Community, Brussels, Belgium.

Ghildiyal, S.K., Sharma, Ch.M., and Gairola, S. 2009. Effect of cold stratification on the germination of seeds of chirpine (*Pinus roxburghii* Sargent) from Indian Himalayan Region. Nat. Sci. **7**(8): 36–43.

Gill, P.K., Sharma, A.D., Singh, P., and Bhullar, S.S. 2003. Changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses. J. Plant Growth Regul. **40**(2): 157–162. doi:10.1023/A:1024252222376.

Grime, J.P. 2006. Plant strategies, vegetation processes, and ecosystem properties. Wiley, Chichester, UK.

Hansson, M., and Fogelfors, H. 2000. Management of a semi-natural grassland; results from a 15-year-old experiment in southern Sweden. J. Veg. Sci. **11**(1): 31–38. doi:10.2307/3236772.

Hardegree, S.P., and Emmerich, W.E. 1994. Seed germination response to polyethylene glycol solution depth. Seed Sci. Technol. **22**: 1–7.

He, X.Q., Du, C., Shao, Z., and Li, Q. 2009. Effect of salt and water stress on seed germination *Dianthus chinensis* L. *In* 2009 Academic Conference on Horticulture Science and Technology, Beijing, China. pp. 60–62.

Hegi, G. 1975. Illustrierte Flora von Mittel-Europa. Dicotyledons, J. F. Lehmanns, München, Germany.

Jankowska-Błaszczuk, M., and Daws, I. 2007. Impact of red : far red ratios on germination of temperate forest herbs in relation to shade tolerance, seed mass and persistence in the soil. Funct. Ecol. **21**(6): 1055–1062. doi:10.1111/j.1365-2435.2007.01328.x.

Kaydan, D., and Yagmur, M. 2008. Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl. Afr. J. Biotechnol. **7**(16): 2862–2868.

Khan, M.A., and Ungar, I.A. 1997. Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. Am. J. Bot. **84**(2): 279–283. doi:10.2307/2446089. PMID:21712207.

Khera, N., and Singh, R.P. 2005. Germination of some multipurpose tree species in five provenances in response to variation in light, temperature, substrate and water stress. J. Trop. Ecol. **46**(2): 203–217.

Klavina, D., Gailite, A., and Ievinsh, G. 2006. Initial responses of explants from rare and endangered coastal plant species during initiation of tissue culture. Acta Univ. Latv. Biol. **710**: 81–91.

Kołodziejek, J., and Patykowski, J. 2015. Effect of environmental factors on germination and emergence of invasive *Rumex confertus* in central Europe. Sci. World J. **15**: 170176, 1–10. doi:10.1155/2015/170176.

Li, W., Liu, X., Hanada, A., and Khan, M.A. 2011. Effect of cold stratification, scarification and hormones on germination of dimorphic seeds of *Atriplex centralasiatica* under saline conditions. Seed Sci. Technol. **39**: 82–92. doi:10.15258/sst.2011.39.1.08.

Lu, P., Sang, W., and Ma, K. 2006. Effects of environmental factors on germination and emergence of Crofton weed (*Eupatorium adenophorum*). Weed Sci. **54**(3): 452–457. doi:10.1614/WS-05-174R1.1.

Meusel, H., Jäger, E., and Weinert, E. 1965. Vergleichende Chorologie Der Zentraleuropäischen Flora. Gustav Fisher, Jena, Germany.

Michel, B.E., and Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. **51**(5): 914–916. doi:10.1104/pp.51.5.914.

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

Mirek, M., Zarzycki, K., Wojewoda, W., and Szeląg, Z. 2006. Red list of plants and fungi in Poland. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland.

Muscoloa, A., Sidaria, M., Anastasib, U., Santonocetoa, C., and Maggioc, A. 2014. Effect of PEG-induced drought stress on seed germination of four lentil genotypes. J. Plant Interact. **9**(1): 354–363. doi:10.1080/17429145.2013.835880.

Partzsch, M. 2011. Zur Keimungsbiologie acht ausgewählten Xerothermrasenarten – Teil 2: Caryophyllaceae. Hercynia, **44**: 127–144.

Partzsch, M. 2013. Growth performance and species interaction of *Festuca rupicola* Heuff. and *Dianthus carthusianorum* L. subjected to temperature increase and nitrogen addition. J. Plant Stud. **2**(2): 122–135. doi:10.5539/jps.v2n2p122.

Pérez-Fernández, M.A., Calvo-Magro, E., Montanero-Fernández, J., and Oyola-Velasco, J.A. 2006. Seed germination in response to chemicals: effect of nitrogen and pH in the media. J. Environ. Biol. **27**(1): 13–20. PMID:16850869.

Pons, T.L. 1993. Seed responses to light. *In* Seeds: the ecology of regeneration in plant communities. *Edited by* M. Fenner. CAB International, Wallingford. pp. 259–284.

Rabeler, K.R., and Bittrich, V. 1993. Suprageneric nomenclature in the Caryophyllaceae. Taxon, **42**(4): 857–863. doi:10.2307/1223270.

Takaki, M. 1990. Effect of water stress on seed germination and seedling growth in *Oryza sativa* L. Biol. Plant. **32**(3): 238–240. doi:10.1007/BF02890883.

Thompson, P.A. 1970. A comparison of the germination character of species of Caryophyllaceae collected in central Germany. J. Ecol. **58**(3): 699–711. doi:10.2307/2258530.

Tutin, T.G. 1993. *Dianthus* L. *In Flora Europaea. Edited by* T.G. Tutin, N.A. Burges, A.D. Chater, J.R. Edmondson, V.H. Heywood, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb. Cambridge University Press, Cambridge. pp. 188–204.

Vandelook, F., Van de Moer, D., and Van Assche, J.A. 2008. Environmental signals for seed germination reflect habitat adaptations in four temperate Caryophyllaceae. Funct. Ecol. **22**(3): 470–478. doi:10.1111/j.1365-2435.2008.01385.x.

Vleeshouwers, L.M., Bouwmeester, H.J., and Karssen, C.M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. J. Ecol. **83**(6): 1031–1037. doi:10.2307/2261184.

Wang, B.S.P., and Berjak, P. 2000. Beneficial effects of moist chilling on the seeds of black spruce (*Picea mariana* (Mill.) B.S.P.). Ann. Bot. **86**(1): 29–36. doi:10.1006/anbo.2000.1150.

Whittington, W.J., Wilson, G.B., and Humphries, R.N. 1988. The germination characteristics of seeds from *Lychnis viscaria* L. (*Viscaria vulgaris* Bernh.), *Potentilla rupestris* L. and *Veronica spicata* L. New Phytol. **109**(4): 505–514. doi:10.1111/j.1469-8137.1988.tb03727.x.

Wilson, A.M. 1972. Amylase synthesis and stability in crested wheatgrass seeds at low water potentials. Plant Physiol. **48**(5): 541–546. doi:10.1104/pp.48.5.541. PMID:16657835.

Zhang, J., and Maun, M.A. 1990. Sand burial effects on seed germination, seedling emergence and establishment of *Panicum virgatum*. J. Holarct. Ecol. **13**(1): 56–61. doi:10.1111/j.1600-0587.1990.tb00589.x.