NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
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Conservation of priority forests and forest openings in "Ethnikos Drymos Oitis" and "Oros Kallidromo" of Sterea Ellada
LIFE11 NAT/GR/1014 - "ForOpenForests"

ACTION C.7.
Ex situ conservation and propagation of keystone species of target habitats

DELIVERABLE C.7_1

Manual with protocols for seed collection, handling, storage and seed germination for the keystone species of all the target habitats

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ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ
ΤΟΜΕΑΣ ΒΟΤΑΝΙΚΗΣ

Διατήρηση δασών και ανοιγμάτων προτεραιότητας στον "Εθνικό Δρυμό Οίτης" και στο "Όρος Καλλίδρομο" της Στερεάς Ελλάδας
LIFE11 NAT/GR/1014 - "ForOpenForests"

ΔΡΑΣΗ C.7.
Εκτός τόπου διατήρηση και πολλαπλασιασμός ειδών-κλειδιών των οικοτόπων-στόχων

ΠΑΡΑΔΟΤΕΟ C.7_1.

Εγχειρίδιο με πρωτόκολλα για τη συλλογή, τον χειρισμό, την αποθήκευση και τη φύτρωση των σπερμάτων για τα «είδη-κλειδιά» όλων των οικοτόπων-στόχων

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SUMMARY

A seedbank of the orthodox keystone plant species of the target habitats of temporary ponds (3170*) and mountain grasslands (6210* and 6230*) has been created. Seeds of the 10 keystone or typical temporary pond species (3170*) and 28 mountain grassland species (6210*, 6230*) were collected. Peculiarities of seed collections as well as methodology for seed cleaning have been identified for all species.

Germination behavior has been studied in detail for the rare plants typical of the temporary ponds. Seeds of *Veronica oetaea** germinate at 5 °C in light, with extremely low rate, whereas seeds of *Verbena supina* germinate at 30 °C in light. Seeds of the other species are physiologically dormant, light requiring and germinate optimally at 15-20 °C; dormancy is broken either by afterripening (*Myosurus minimus* and *Ranunculus lateriflorus*), or by cold followed by warm stratification (*Juncus bufonius* and *Lythrum thymifolia*), or by warm stratification or short exposure at 40 °C (*Limosella aquatica*). Germination tests have also been performed for Seed Banking purposes, with afterripened seeds of 26 mountain grassland species (6210*, 6230*) and optimal germination (exceeding 80%) has been identified for 22 of them. For these 22 species, optimal germination is achieved at 15 or 20/10 °C and an additional to afterripening, pretreatment was not necessary. Light indifferent seeds have been produced by 7 species, while light inhibits seed germination only in *Allium achaium*. Light requiring seeds have been produced by 6 species, while other 8 species germinate optimally in light but the effect of darkness on seed germination has not been examined.

ΠΕΡΙΛΗΨΗ

Δημιουργήθηκε μία Τράπεζα σπερμάτων από ορθόδοξα είδη-κλειδιά των οικοτόπων-στόχων των εποχικών λιμνίων (3170*) και των ορεινών λειμώνων (6210* και 6230*). Σπέρματα από 10 είδη-κλειδιά των εποχικών λιμνίων (3170*) και 28 των ορεινών λειμώνων (6210*, 6230*) συλλέχθηκαν και προσδιορίστηκαν οι ιδιαιτερότητες ως προς τη συλλογή των σπερμάτων και τον χειρισμό των συλλογών για όλα τα είδη.

Η φυτρωτική συμπεριφορά των σπερμάτων μελετήθηκε διεξοδικά για όλα τα είδη των εποχικών λιμνίων. Τα σπέρματα της *Veronica oetaea** φυτρώνουν βέλτιστα στους 5 °C στο φως, αλλά με αρκετά χαμηλό τάχος φύτρωσης, ενώ τα σπέρματα της *Verbena supina* φυτρώνουν στους 30 °C στο φως. Τα σπέρματα των υπολοίπων ειδών έχουν φυσιολογικό λήθαργο, είναι φωτοαπαιτητικά και φυτρώνουν βέλτιστα στους 15-20 °C. Ο φυσιολογικός λήθαργος αίρεται είτε με μεθωρίμανση (*Myosurus minimus* και *Ranunculus lateriflorus*), είτε με ψυχρή ακολουθούμενη από θερμή στρωμάτωση (*Juncus bufonius* και *Lythrum thymifolia*), είτε από θερμή στρωμάτωση ή σύντομη έκθεση στους 40 °C (*Limosella aquatica*). Επίσης, πραγματοποιήθηκαν πειράματα φύτρωσης με στόχο την εύρυθμη λειτουργία της Τράπεζας Σπερμάτων, με μεθωριμασμένα σπέρματα από 26 είδη των ορεινών λειμώνων (6210*, 6230*) και επιτεύχθηκε βέλτιστη φύτρωση (πάνω από 80%) για 22 από αυτά, στους 15 ή 20/10 °C. Φωτοαδιάφορα σπέρματα παράγουν 7 είδη, φωτοαπαιτητικά σπέρματα παράγουν 6 είδη, ενώ το φως βρέθηκε να αναστέλλει τη φύτρωση των σπερμάτων μόνο στο *Allium achaium*. Τα υπόλοιπα 8 είδη φυτρώνουν βέλτιστα στο φως, αλλά η φύτρωση στο σκοτάδι δεν εξετάστηκε.

1. Introduction

Ex situ conservation of genetic material is a complement of in situ conservation, necessary in order to provide long-term insurance against catastrophic events and to facilitate plant re-introduction or population enhancement when necessary. Seed banking is based on protocols for seed collection, handling, storage and germination. The importance of seed collection and handling on seed banking is evident, however, seed germination tests are equally pivotal. While seeds are being stored, even under the most ideal conditions for long-term storage in seedbanks, they gradually lose their longevity. Therefore, seed collections must be checked for viability periodically and renewed whenever necessary. The most reliable method for testing seed viability is a germination test, since biochemical tests, such as tetrazolium test may be misleading (ENSCONET, 2009). Obviously the knowledge of how seeds germinate is also important so that seeds stored at a seedbank can be used for the production of new plants for in situ conservation. Moreover, germination tests provide information regarding the optimal germination conditions and timing of germination in the field. This information can be used especially for threatened and endemic species, for better understanding on the survival strategy of the species (Andreou et al. 2011).

This action includes the creation and function of a seedbank of the keystone plant species of the target habitats of temporary ponds (3170*) and mountain grasslands (6210* and 6230*) and *Juniperus foetidissima* (9560*) and *Pinus nigra* (9530*), as well as the propagation of selected species for planting for habitat restoration. The latter is included in the Deliverable C.7.2.

Regarding the typical species of *Juniperus foetidissima* forests, according to the results of action A.7, there are no keystone species of the habitat on Mt. Oiti, except from the tree itself. The other species occurring in the habitat are herbs and low scrub typical of other habitat types. The production of *Juniperus foetidissima* for restoration is one of the objects of action C.5. Thus, action C.7 did not include any species for the habitat 9560*.

The creation and function of a seedbank of the keystone plant species of the habitats 3170*, 6210*, 6230* and 9530* is fully described in the present deliverable and includes details regarding:

1. Seed collections that took place in the appropriate season for each plant species. Collections of the species of habitat 3170* were repeated whenever necessary, for the needs of plant replenishment at the project restoration sites (actions C.2 and C.4).
2. Seed cleaning, separation and sorting that were done manually at the Seedbank of UoA or at the establishments of IMFE, as necessary.
3. Seed germination tests for the species of habitats 3170*, 6210* and 6230* that have been administered at the establishments of UoA. It must be noted that the germination of *Pinus nigra* in Greece has been studied adequately for the needs of a seedbank (Skordilis & Thanos, 1997), so no experiments were performed.
4. Seed storage at the Seedbank of the UoA, according to International Standards.

Methodology

Seed collection and handling

Seeds of the 10 keystone or typical temporary pond species (3170*) and 28 mountain grassland species (6210*, 6230*) were collected from 2013 to 2019 (Tables 1 and 2, respectively). *Pinus nigra* cones were collected from Mt. Kallidromo in winter 2018.

Seed collections were cleaned manually, using steel sieves of various wire mesh sizes (125, 300, 400, 500, 630 and 900μm and 1.4mm) and, whenever necessary, seed aspirator (blower), and the surrounding tissues as well as any nonplant material were extracted. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Table 1: Seed collections from 10 keystone or typical temporary pond species (3170*).

Taxon	Family	Collection site [a]	Altitude, m	Collection date
Heliotropium supinum	Boraginaceae	Nevropoli – K	976-985	10.09.2016
				30.08.2016 [b]
				12.09.2019
Juncus bufonius	Juncaceae	Greveno – O	1896-1897	21.07.2014
Limosella aquatica	Scrophulariaceae	Livadies – O	1812-1821	23.06.2013
				20.07.2014
				14.07.2016
		Greveno – O	1896-1897	14.07.2016
Lythrum portula	Lythraceae	Livadies – O	1812-1821	03.08.2016
Lythrum thymifolia	Lythraceae	Alykaina – O	1917-1925	13.08.2013
		Livadies – O	1812-1821	13.08.2013
				20.07.2014
		Greveno – O	1896-1897	13.08.2014
				03.08.2016
Myosurus minimus	Ranunculaceae	Greveno – O	1896-1897	21.07.2014
				27.06.2016
Polygonum arenastrum	Polygonaceae	Livadies – O	1812-1821	23.11.2016
Ranunculus lateriflorus	Ranunculaceae	Livadies – O	1812-1821	23.06.2013
				30.07.2013
				20.07.2014
				14.07.2016
Verbena supina	Verbenaceae	Nevropoli – K	976-985	26.11.2015
				23.11.2016
				12.09.2019
*Veronica oetaea**		Alykaina – O	1917-1925	11.07.2014
		Livadies – O	1812-1821	23.06.2013
				11.07.2014
		Greveno – O	1896-1897	11.07.2014

a: Collection sites are located either in Mt. Kallidromo (K) or in Mt. Oiti (O).
b: Immature seeds were collected.

Table 2: Seed collections from 28 mountain grassland species (6210*, 6230*).

Taxon	Family	Collection site [a]	Altitude, m	Collection date
Achillea crithmifolia	Asteraceae	Gkioza – K	1288-1296	30.08.2016
Allium achaium	Alliaceae	Livadies – O	1812-1821	30.08.2016
		Greveno – O	1896-1897	30.08.2016
Alopecurus gerardii	Poaceae	Livadies – O	1812-1821	27.07.2016
Anthoxanthum odoratum	Poaceae	Livadies – O	1812-1821	26.06.2016
Bellardiochloa variegata	Poaceae	Livadies – O	1812-1821	27.07.2016
Brachypodium pinnatum	Poaceae	Gkioza – K	1288-1296	14.07.2016
Carex ovalis	Cyperaceae	Livadies – O	1812-1821	03.08.2016
Centaurea nervosa subsp. *promota*	Asteraceae	Livadies – O	1812-1821	30.08.2016
Chrysopogon gryllus	Poaceae	Isomata – K	994-1010	14.07.2016
Dianthus tymphresteus	Caryophyllaceae	Greveno – O	1896-1897	14.07.2016
Festuca sp.	Poaceae	Livadies – O	1812-1821	30.08.2016
Festuca polita	Poaceae	Isomata – K	994-1010	28.06.2016
Festuca cf. *valesiaca*	Poaceae	Gkioza – K	1288-1296	14.07.2016
Galium verum	Rubiaceae	Livadies – O	1812-1821	14.10.2016
Hieracium hoppeanum s.l.	Poaceae	Greveno – O	1896-1897	14.07.2016
Hypericum barbatum	Hypericaceae	Livadies – O	1812-1821	03.08.2016
Luzula multiflora	Juncaceae	Livadies – O	1812-1821	27.07.2016
Luzula spicata	Juncaceae	Alykaina – O	1917-1925	03.08.2016
		Livadies – O	1812-1821	27.07.2016
		Greveno – O	1896-1897	03.08.2016
Nardus stricta	Poaceae	Livadies – O	1812-1821	27.07.2016
Nepeta nuda	Lamiaceae	Gkioza – K	1288-1296	27.07.2016
Phleum alpinum	Poaceae	Livadies – O	1812-1821	27.07.2016
Potentilla recta subsp. *laciniosa*	Rosaceae	Gkioza – K	1288-1296	27.07.2016
Prunella laciniata	Lamiaceae	Gkioza – K	1288-1296	27.07.2016
Rhinanthus pubescens	Orobanchaceae	Greveno – O	1896-1897	14.07.2016
Rumex acetosella	Polygonaceae	Livadies to Greveno – O	c. 1850	27.07.2016
Silene roemeri subsp. *macrocarpa*	Caryophyllaceae	Livadies – O	1812-1821	03.08.2016
Stipa capillata	Poaceae	Gkioza – K	1288-1296	28.06.2016
Xeranthemum cylindraceum	Poaceae	Isomata – K	994-1010	14.07.2016

a: collection sites are located either in Mt. Kallidromo (K) or in Mt. Oiti (O).

Seed germination

Following an extended literature review of seed germination of all collected species and in general of species inhabiting temporary ponds (mainly from the Mediterranean region), germination experiments were performed initially with the most numerous seed collections. For each species, germination experiments were carried out using five samples of 20 seeds each, unless otherwise indicated, depending on seed availability. Seeds were sown on two layers of filter paper moistened with distilled water in Petri dishes. For the experiments in darkness, the dishes were subsequently placed inside lightproof, metal containers. Seeds were incubated at various constant (5, 10, 15, 20, 25, 30 °C) and alternating (20/10, 25/15, 30/20 °C) temperatures, in the light (light treatment) or in darkness (dark treatment), depending on seed availability. Germination experiments were conducted immediately after seed collection or after c. 4 months of afterripening (seeds were kept dry at c. 22°C). Measurements were made under white light (light treatment) or a under dim green safelight (dark treatment) and germinated seeds were removed from the dishes. The experiments were terminated when no additional seeds germinated for a period of 2 months; cut-tests were performed and germination percentages were corrected for viable seeds. The rate of germination was measured by the t_{50}, which is the time to 50% of final germination and was calculated according to the following formula:

$$t_{50} = t_1 + \{(N/2 - N_1) \times (t_2 - t_1) / (N_2 - N_1)\}$$

where N is final germination, N_1 and N_2 are germination percentages prior to and after N/2, respectively and t_1 and t_2 are the time taken to N_1 and N_2, respectively.

The effect of gibberellic acid (GA_3) was examined, by applying aqueous solutions of 1000ppm GA_3 either at the beginning of the experiment or after 2 months of imbibition in non-germinated seeds. For dormancy release purposes, warm stratification was also examined in various species, by imbibing seeds in water at 25°C for 2 months, unless indicated otherwise. In order to detect the induction of secondary dormancy (skotodormancy or thermodormancy, respectively), whenever necessary, the non-germinated seeds from the unfavorable conditions (darkness or unfavorable germination temperatures) were transferred to the optimal ones (light and 25 or 20 °C or 5 °C). For the release of secondary dormancy, GA_3 was added or imbibed dormant seeds were incubated at 25 °C (warm stratification) in light or darkness or seeds were left dry at 25 °C to afterripen.

An f-test followed by a t-test for each taxon separately was used to investigate the effect of light, GA_3 and cold/warm stratification on seed germination.

Seed storage

Seed collections were stored at the Seedbank of UoA, after seed germination was examined, according to ENSCONET Curation Protocols & Recommendations (2009). Dried seeds were stored in leak-proof glass containers of various sizes, depending on the size of each collection. Containers are air-tight, so that seeds will be prevented from absorbing moisture that will reduce their storage life. Above seeds, silica gel was added as a humidity indicator. Containers are also transparent, allowing seeds and humidity indicators to be seen (Fig. 1). Small seed containers were stored into a larger leak-proof glass container on top of silica gel (Fig. 2). All seed collections were stored at the seedbank at -20°C. The seeds of all species were presumed

as orthodox due to their small size, therefore storage at low temperatures is an appropriate method of preservation.

Figure 1: Dried seeds stored in leak-proof glass containers of various sizes (photo from S. Oikonomidis).

Figure 2: Leak-proof glass containers stored into larger leak-proof glass containers on top of silica gel (photo from S. Oikonomidis).

3. Results for temporary pond species (3170*)

Seeds of the 10 keystone or typical temporary pond species were collected from 2013 to 2016 from various populations on Mt. Oiti and Mt. Kallidromo. All seed collections were cleaned and stored at the Seedbank of the UoA, after seed germination was examined.

Heliotropium supinum

Both mature and immature seeds of *Heliotropium supinum* were collected from Nevropoli in 2016 (30.08.2016-immature seeds, 10.09.2016-mature seeds). Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 1.4mm and partially at 900μm. Afterwards, seed collections were homogenized and immature seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Bhatia (1985), *H. supinum* produces physiologically dormant seeds; dormancy can be released by afterripening or warm stratification and afterwards germination occur at alternative temperatures (with 10°C being the lower limit) in light. Thus, germination experiments were performed at 20/10°C, under both light and darkness, immediately after seed collection and after 1-3 months of warm stratification with the addition of GA_3. Moreover, the effect of boiling on seed germination was tested, in order to test the possibility of physical dormancy (dormancy due to impermeable seed coat). Germination was poor and never exceeded 30% (Table 3).

Table 3: Final germination of *Heliotropium supinim* seeds.

Temperature, °C	Light conditions	Pretreatment	Final germination, %	s.e.
20/10	Dark	-	0	-
	Light		0	-
	Dark	Warm stratification	1	1
	Light		8	2
	Dark	GA_3	2	1
	Light		30	9
20	Light	5'' boiling	0	-
	Light	30'' boiling	0	-
	Light	1' boiling	0	-

Juncus bufonius

Seeds of *Juncus bufonius* were collected in 2014 from Greveno, where the largest population of the species occur. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 125 and 300μm. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Due to high seed availability, germination experiments were performed at constant (5, 10, 15, 20, 25 °C) and alternating (20/10, 25/15 °C) temperatures, under both light and darkness. Final germination without any pretreatment does not exceed 27% and gibberellic acid failed to

promote germination at 20/10°C (Table 4). Therefore the effect of cold stratification, warm stratification, as well as their combination were examined (Tables 5 and 6).

Table 4: Final germination of afterripened *Juncus bufonius* seeds.

Temperature, °C	Pretreatment	Final germination (%)		s.e.		t_{50}
		light	darkness	light	darkness	light
5	AR	21	0	6,0	-	25
10	AR	16	0	2,4	-	14
15	AR	27	0	4,1	-	6
	AR + 6mo CS	0	0	-	-	-
20	AR	27	0	7,7	-	5
25	AR	12	0	4,1	-	6
20/10	AR	22	0	3,7	-	9
	AR + 6mo CS	0	0	-	-	-
	AR+GA_3*	12	-	-	-	-
25/15	AR	18	0	2,5	-	8

* germination experiment was carried out using one sample of 50 seeds

Table 5: Final germination of afterripened *Juncus bufonius* seeds, after cold stratification (CS), warm stratification at 20°C (WS 20) or 25°C (WS) or 30°C (WS 30) and the addition of GA_3, in light or in darkness (in D).

Initial temperature & light conditions	Final germination (%)	Treatment	Final germination (%) at 15°C in light, after treatment	s.e.
5°C, Light	21	-	21	6,0
5°C, Dark	0	WS (30) & CS	15	1,6
10°C, Light	16	GA_3	16*	2,4
10°C, Dark	0	WS (20 in D)	5*	3,2
15°C, Light	27	CS	48	9,0
15°C, Dark	0	CS (in D)	0	0,0
20°C, Light	27	GA_3	94*	1,9
20°C, Dark	0	CS	26	13,5
25°C, Light	12	CS & WS	34	9,7
25°C, Dark	0	CS & WS & CS (in D)	0*	0,0
20/10°C, Light	22	CS & WS & CS	32	5,1
20/10°C, Dark	0	CS & WS & CS (in D)	3	1,2
25/15°C, Light	18	-	18**	2,5
25/15°C, Dark	0	CS & WS & CS	29	8,1

* after treatment, seeds were moved at the initial temperature in light

** seeds were moved at 15°C in light

Table 6: Final germination of afterripened *Juncus bufonius* seeds, after 3 months of cold stratification (CS), followed by 1 month of warm stratification at 30°C in light (treatment 1 - TR1) and 1 month of cold stratification at 5°C in light (TR2).

Initial temperature & light conditions	Final germination (%) after 3 months of CS	Final germination (%) after TR1*	Final germination (%) after TR2 *	s.e.
10°C, Light	9	12	12	2,5
10°C, Dark	0	12	12	1,2
15°C, Light	34	57	57	3,4
15°C, Dark	0	1	1	1,0
20°C, Light	18	34	40	5,7
20°C, Dark	0	16	21	6,2
25°C, Light	0	-	17**	5,1
25°C, Dark	0	-	0**	-
30°C, Light	0	-	37**	4,6
30°C, Dark	0	-	5**	3,2
20/10°C, Light	0	16	-	-
20/10°C, Dark	0	12	-	-
30/20°C, Light	10	-	57**	9,0
30/20°C, Dark	0	-	0**	0,0

* after each treatment, seeds were moved at the initial temperature in light

** after treatment, seeds were moved at 15°C in light

Seeds of *Juncus bufonius* germinate optimally at 15 - 20 °C in light (57% and 40%, respectively), after a period of cold followed by warm stratification (Table 6). Final germination is improved with the addition of gibberellic acid (Table 5, 97% final germination at 20°C). Seedlings were checked for epicotyl dormancy and were found non-dormant. It is concluded that seeds of that species are able to germinate under natural conditions during autumn.

Limosella aquatica

Seeds of *Limosella aquatica* were collected in 2013, 2014 and 2016 from Livadies and also in 2016 from Greveno. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 125 and 300μm sieves. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Salisbury (1967), *L. aquatica* produces non-dormant seeds, which germinate in light within 5 days. Thus, germination experiments were performed at constant (5, 10, 15, 20, 25 °C) and alternating (20/10, 25/15 °C) temperatures, under both light and darkness, with afterripened and non-afterripened seeds from all collections. Final germination was null in all cases (results not shown). Therefore the effect of cold stratification, warm stratification, as well as their combination were examined with seeds from the most numerous collection (Tables 7 and 8).

Table 7: Final germination of afterripened *Limosella aquatica* seeds collected in 2013 from Livadies, after cold stratification (CS), warm stratification at 20°C (WS 20) or 25°C (WS) or 30°C (WS 30) and the addition of GA_3, in light or in darkness (in D).

Initial temperature & light conditions	Final germination (%)	Treatment	Final germination (%)	s.e.
			at 15°C in light, after treatment	
5°C, Light	0	-	0	-
5°C, Dark	0	WS (30) & CS	4	2,4
10°C, Light	0	GA_3	16	7,0
10°C, Dark	0	WS (20 in D) & GA_3	81	7,3
15°C, Light	0	CS	28	13,1
15°C, Dark	0	CS (in D)	13	4,6
20°C, Light	0	GA_3 & CS	25*	5,2
20°C, Dark	0	CS	0*	-
25°C, Light	0	CS & WS	17	6,4
25°C, Dark	0	CS & WS & CS (in D)	4*	1,9
20/10°C, Light	0	CS & WS & CS	18	14,4
20/10°C, Dark	0	CS & WS & CS (in D)	1	1,0
25/15°C, Light	0	-	64**	-
25/15°C, Dark	0	CS (in D)	52	13,1

* after treatment, seeds were moved at the initial temperature in light

** germination experiment was carried out using one sample of 50 seeds and seeds were moved at 20°C in light

Table 8: Final germination of afterripened *Limosella aquatica* seeds collected in 2013 from Livadies, after 3 months of cold stratification (CS), followed by 1 month of warm stratification at 30°C in light (TR1) and 1 month of cold stratification at 5°C in light (TR2).

Initial temperature & light conditions	Final germination (%) after 3 months of CS	Final germination (%) after TR1*	Final germination (%) after TR2 *	s.e.
10°C, Light	0	0	0	-
10°C, Dark	0	0	0	-
15°C, Light	2	2	2	2,0
15°C, Dark	0	2	2	2,0
20°C, Light	0	0	0	-
20°C, Dark	0	17	17	6,8
25°C, Light	1	-	2	1,2
25°C, Dark	0	-	6	1,9
30°C, Light	0	-	1	1,0
30°C, Dark	0	-	25	7,6
20/10°C, Light	0	0	-	-
20/10°C, Dark	0	0	-	-
30/20°C, Light	0	-	2	2,0
30/20°C, Dark	0	-	27	12,6

* after each treatment, seeds were moved at the initial temperature in light

** after treatment, seeds were moved at 15°C in light

Final germination was rather low and inconsistent in all treatments. A period of warm stratification followed by cold is necessary for optimal germination at 15 °C in light (Table 7; 52%). The addition of gibberellic acid after warm stratification increased final germination (Table 7; 81%).

Subsequently, the effect of warm followed by cold stratification as well as the effect of oxygen and exposure to higher temperatures (40 °C) for short periods were examined, in afterripened seeds collected in 2016 from Greveno (Tables 9 and 10). Afterripened seeds of *Limosella aquatica* from Greveno germinated optimally (57-66%) at 20 °C in light either after a short exposure at 40°C in darkness or after a period of cold followed by warm stratification (Table 9).

Table 9: Final germination of afterripened *Limosella aquatica* seeds collected in 2016 from Greveno, after various combinations of 1 month of cold stratification in darkness (CS in D) or in light (CS), 1 month of warm stratification at 25°C (WS) or 30°C (WS 30) in light and 2 days, 1 week or 2 weeks of exposure at 40°C.

Germination conditions	Pretreatments	Final germination (%)	s.e.	t_{50}
20°C, Dark	WS (30) & CS	0	-	-
20°C, Light	WS (30) & CS	9	1,9	5
20°C, Dark	CS & WS (30)	0	-	-
20°C, Light	CS & WS (30)	58	8,3	44
10°C, Light	40°C (in dark) for 2 days	0	-	-
15°C, Light		0	-	-
20°C, Light		57	6,0	4
25°C, Light		0	-	-
20/10°C, Light		0	-	-
20°C, Light	40°C (in dark) for 2 days & WS	37	15,8	2
10°C, Light	40°C (in dark) for 1 week	0	-	-
15°C, Light		15	5,2	11
20°C, Light		55	14,7	12
25°C, Light		1	1,0	-
20/10°C, Light		7	3,0	24
10°C, Light	40°C (in light) for 1 week	0	-	-
15°C, Light		0	-	-
20°C, Light		0	-	-
25°C, Light		0	-	-
20/10°C, Light		0	-	-
20°C, Light	40°C (in dark) for 1 week & WS	66	13,2	2
20°C, Light	40°C (in light) for 1 week & WS	2	2,0	-
20°C, Light	40°C (in dark) for 2 weeks	0	-	-
15°C, Light	CS (in D) & 40°C (in dark) for 1 week	0	-	-
20°C, Light		0	-	-

Table 10: Effect of oxygen on final germination at 20°C in light, after 1 month of warm stratification at 25°C in light followed by 1 month of cold stratification in light for afterripened *Limosella aquatica* seeds collected in 2016 from Greveno.

Seeds imbimbed:	Final germination (%)	s.e.	t_{50}
on top of 2 filter papers	9	1,9	5
between papers	21	5,4	6
no filter paper	14	4,3	4

In general, final germination was medium to low (never exceeding 66%) in all treatments unless gibberellic acid was added. Notably, final germination was not corrected for empty seeds since the extremely small seed size prohibited the performance of cut-tests. However, seedlings were checked for epicotyl dormancy and were found non-dormant. It is concluded that the vast majority of *L. aquatica* seeds will probably germinate under natural conditions during autumn, after short periods of exposure at extremely high temperatures during the summer months.

Lythrum portula

Seeds of *Lythrum portula* were collected in 2016 from Livadies. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 300 and 400μm and partially at 500μm. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Due to low seed availability and following an extended literature review of seed germination of other *Lythrum* species (Baskin & Baskin, 2014), germination experiments were performed with afterripened seeds at 20 °C in light and both afterripened and cold stratified seeds at 20 and 25°C in light. Final germination without any pretreatment reached c. 62% (Fig. 3). Cold stratification not only failed to promote final germination further but it reduced it significantly (data not shown). Seedlings were checked for epicotyl dormancy and were found non-dormant.

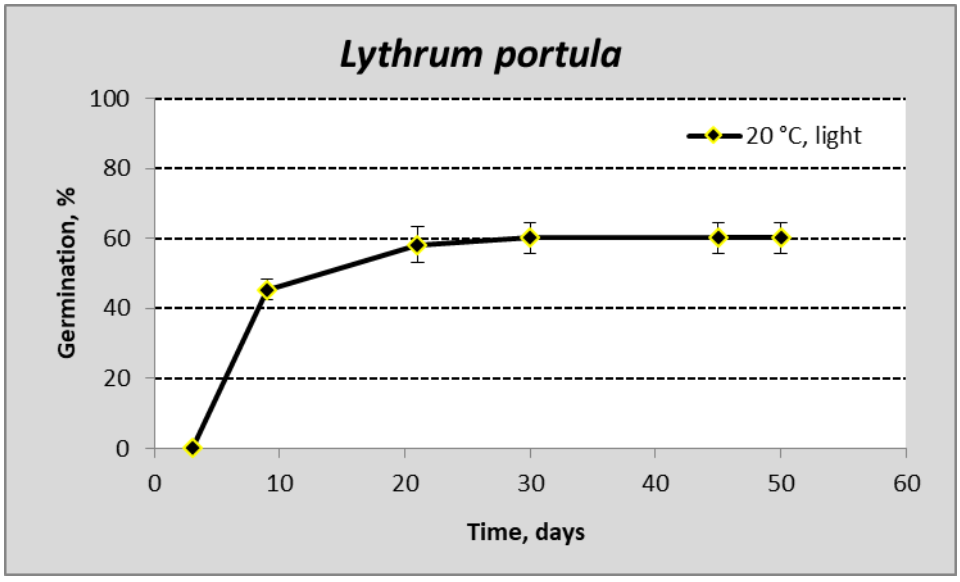

Figure 3: Light germination of *Lythrum portula* afterripened seeds, at 20°C.

Lythrum thymifolia

Seeds of *Lythrum thymifolia* were collected in 2013 from Livadies and Alykaina, in 2014 from Livadies and Greveno and in 2016 from Greveno. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 400μm and partially at 500 and 300μm. However, it must be noted that seeds retained at 300μm were

mainly empty. The seed collection from Livadies in 2014 with seeds mostly retained at 300µm, contained mainly empty seeds as proven by cut tests. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Seed germination of *L. thymifolia* has never been studied before, but according to literature (Baskin & Baskin, 2014), *Lythrum* species usually produce physiologically dormant seeds that need 3-4 months of cold stratification and germinate optimally at 20-35 °C in light. For *L. thymifolia* germination experiments were performed at constant (5, 10, 15, 20, 25, 30 °C) and alternative (20/10, 25/15 °C) temperatures, in continuous darkness and in light with afterripened seeds from the most numerous collection (Livadies-20.07.2014 and Greveno-13.08.2014). For seeds collected from Livadies, germination experiments were carried out using five samples of 10 seeds each, due to low seed availability.

Final germination without any pretreatment for seeds collected from Livadies in 2014 did not exceed 42% and reached 52% with the addition of gibberellic acid (Table 11). Subsequently, the effect of 3 months of cold stratification was examined, but final germination remained low (Table 11). Low final germination can be attributed to the high percentage of empty seeds in this seedlot.

Table 11: Final germination (before cut-tests) of afterripened *Lythrum thymifolia* seeds collected in 2014 from Livadies, after c. 4 months of afterripening (AR) and 3 months of cold stratification (CS) in darkness.

Germination conditions	Pretreatments	Final germination (%)	s.e.	t_{50}
5°C, Light	AR	6	2,4	-
10°C, Light	AR & CS	0	-	-
10°C, Dark	AR & CS	48	6,6	63
15°C, Light	AR & CS	0	-	-
15°C, Dark	AR & CS	0	-	-
20°C, Light	AR	42	5,8	10
20°C, Light	AR & CS	10	4,5	9
20°C, Dark	AR	0	-	-
20°C, Dark	AR & CS	0	-	-
25°C, Light	AR & CS	14	7,5	107
25°C, Dark	AR & CS	0	-	-
30°C, Light	AR & CS	8	5,8	105
30°C, Dark	AR & CS	0	-	-
20/10°C, Light	AR +GA3	52	8,6	6
20/10°C, Light	AR & CS	0	-	-
20/10°C, Dark	AR & CS	0	-	-

Germination was studied extensively with seeds collected from Greveno in 2014. Final germination without any pretreatment did not exceed 11%, unless gibberellic acid was added (Table 12). Subsequently, the effect of various durations of cold stratification followed by warm stratification were examined (Tables 12 and 13).

Table 12: Final germination of afterripened *Lythrum thymifolia* seeds collected in 2014 from Greveno, after cold stratification (CS), warm stratification at 20°C (WS 20) or 25°C (WS) or 30°C (WS 30) in light or in darkness (in D) and/or the addition of GA_3.

Initial temperature & light conditions	Final germination (%)	Treatment	Final germination (%)	s.e.
			at 15°C in light, after treatment	
5°C, Light	2	-	5	3,2
5°C, Dark	0	WS (30) & CS	29	12,4
10°C, Light	0	GA_3	78**	3,4
10°C, Dark	0	WS (20 in D)	26*	6,6
15°C, Light	9	CS & GA3	78	3,4
15°C, Dark	0	CS (in D)	17	4,4
20°C, Light	11	GA_3	75*	3,2
20°C, Dark	0	CS (in D)	12*	4,6
25°C, Light	1	CS & WS	95	3,2
25°C, Dark	0	CS & WS & CS (in D)	36*	8,0
20/10°C, Light	0	CS & WS & CS	47	16,4
20/10°C, Dark	0	CS & WS & CS (in D)	75	5,7
25/15°C, Light	0	GA_3	16***	4,0
25/15°C, Dark	0	CS (in D)	52	6,0

* after treatment, seeds were moved at the initial temperature in light

** after treatment, seeds were moved at 20°C in light

*** after treatment, seeds were moved at 5°C in light

Table 13: Final germination of afterripened *Lythrum thymifolia* seeds collected in 2014 from Greveno, after 3 months of cold stratification (CS – TR1), followed by 1 month of warm stratification (WS – TR2) at 30°C in light (30) and in darkness (30 D). After each treatment, seeds were moved at the initial temperature in light.

Initial temperature & light conditions	Final germination (%)	Final germination (%)	Treatment 2 (TR2)	Final germination (%)	s.e.
	after AR	after AR & CS (TR1)		after TR1 & TR2	
10°C, Light	0	10	1 mo WS (30)	28	4,4
10°C, Dark	0	0	1 mo WS (30 D)	58	6,4
15°C, Light	9	32	1 mo WS (30)	85	1,6
15°C, Dark	0	0	1 mo WS (30 D)	87	4,4
20°C, Light	11	10	1 mo WS (30)	75	8,2
20°C, Dark	0	0	1 mo WS (30 D)	75	4,5
25°C, Light	1	0	-	0*	0
25°C, Dark	0	0	-	7*	3,0
30°C, Light	0	8	-	21*	2,9
30°C, Dark	0	0	-	12*	3,4
20/10°C, Light	0	0	1 mo WS (30)	27	12,9
20/10°C, Dark	0	0	1 mo WS (30 D)	-	-
30/20°C, Light	-	0	-	3*	2,0
30/20°C, Dark	-	0	-	21*	5,1

* after treatment, seeds were moved at 15°C in light

The results show that afterripened seeds of *Lythrum thymifolia* germinate optimally at 15 °C (>80%) in light, after a period of cold followed by warm stratification. Seedlings were checked

for epicotyl dormancy and were found non-dormant. It is concluded that seeds of that species are able to germinate under natural conditions during autumn.

Myosurus minimus

Seeds of *Myosurus minimus* were collected in 2014 (MmG714) and 2016 (MmG616) from Greveno, where the largest population of the species occurs. Seed collections were cleaned manually, using steel sieves and viable seeds were retained at various wire mesh sizes (mainly at 400 – 630μm). Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Due to the high seed availability, germination experiments were performed at all constant and alternating temperatures available, under both light and darkness, with afterripened seeds from the most numerous collection (Greveno-21.07.2014). The effect of afterripening on seed germination was tested with seeds from 2016 collection (Fig. 4). The main germination characteristics were confirmed for both seedlots of *Myosurus minimus* (Table 14).

Table 14: Final germination of *Myosurus minimus* seeds collected in 2014 (MmG714) and 2016 (MmG616).

Temperature, °C	Seedlot	Pretreatment	Final germination (%)		s.e.		t_{50}
			light	darkness	light	darkness	light
5	MmG616	Afterripening	0	-	-	-	-
	MmG714	Afterripening	12	0	2,5	-	26
10	MmG616	-	64	-	6,0		14
	MmG616	Afterripening	94	-	2,9	-	12
	MmG714	Afterripening	85	0	3,1	-	14
15	MmG616	-	41	-	6,4	-	77
	MmG616	Afterripening	96	0	2,1	-	7
	MmG714	Afterripening	97	0	2,1	-	6
20	MmG616	-	7	-	3,4	-	79
	MmG616	Afterripening	74	-	4,9	-	6
	MmG714	Afterripening	97	1	3,0	1	6
25	MmG616	-	1	-	1,0	-	-
	MmG616	Afterripening	67	0	2,4	-	20
	MmG714	Afterripening	90	0	2,6	-	37
30	MmG616	Afterripening	3	-	2,0	-	3
	MmG714	Afterripening	0	0	-	-	-
20/10	MmG714	Afterripening	88	1	3,0	1	8
25/15	MmG714	Afterripening	82	0	7,3	-	7
30/20	MmG714	Afterripening	100	0	-	-	61

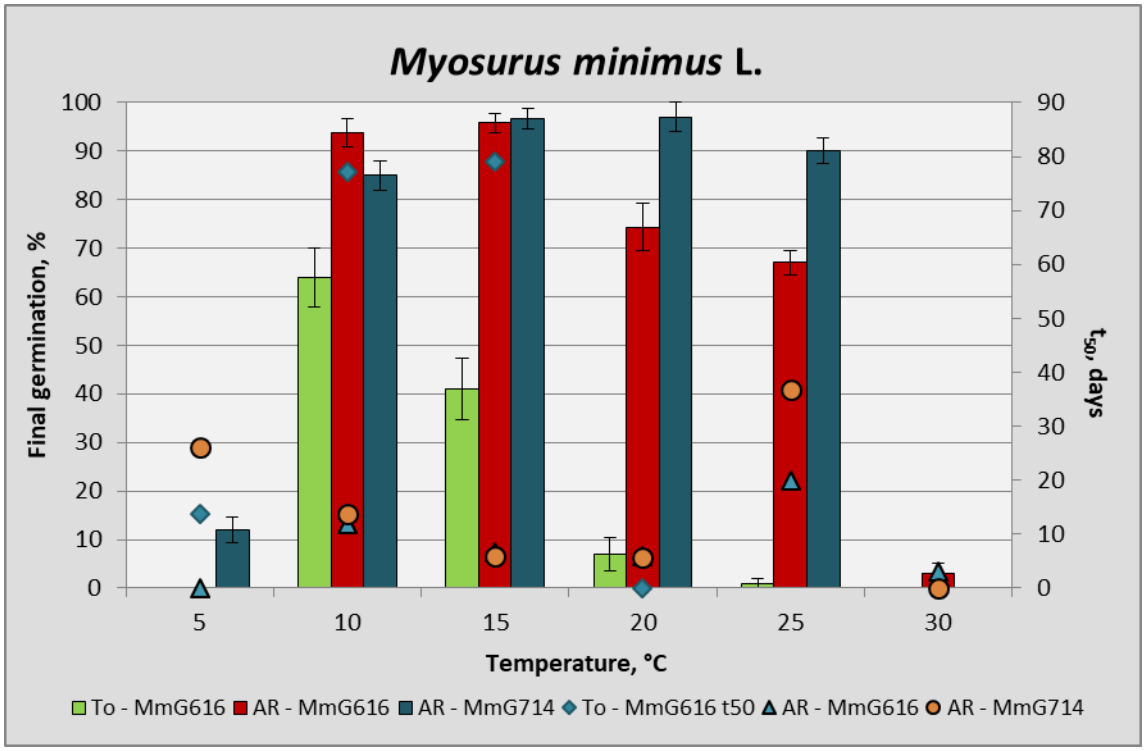

Figure 4: Final germination immediately after seed collection (T_0) and after 4 months of afterripening (AR) at constant temperatures in light for *Myosurus minimus* seeds collected in 2014 (MmG714) and 2016 (MmG616) from Greveno.

Germination was achieved in light, under a wide range of constant (10-25 °C) and all the alternating temperatures tested, for afterripened seeds. However, seeds germinate optimally, with final germination exceeding 95%, at typically Mediterranean temperatures (15-20 °C), in light. The rate of germination is also very high, since the t_{50} is approximately 6 days. Darkness completely prevents seed germination, but GA_3 can substitute for light (Table 13).

Secondary dormancy is imposed in darkness (skotodormancy) mainly in suboptimal temperatures (Table 16). Thermodormancy is imposed by unfavorable low temperatures (5°C) and not by 30°C (Table 15). Both skotodormancy and thermodormancy are released by GA_3. Seedlings were checked for epicotyl dormancy and were found non-dormant.

Table 15: Induction of secondary dormancy by suboptimal temperatures and its release by GA_3, afterripening (AR) and warm stratification in either light (WS) or darkness (WS in D) in *Myosurus minimus* seeds collected in 2014 (MmG714) from Greveno.

Temperature, °C	Final germination (%)		Treatment 2	Final germination (%)	s.e.
	in light	after TR1		after TR2	at optimal %
5	12	12*	GA3	93	1,2
5	7	7	WS	7	2,0
5	1	1	WS (in D)	1	1,0
5	1	1	AR	6	1,0
30	0	93	-	-	5,4

TR1: seeds were moved from 5 or 30°C to 15°C.

TR2: after treatment, seeds were moved at 15°C.

* seeds were moved at 25°C in light

Table 16: Induction of secondary dormancy by darkness and its release by GA_3, afterripening (AR) and warm stratification in either light (WS) or darkness (WS in D) in *Myosurus minimus* seeds collected in 2014 (MmG714) from Greveno.

Temperature, °C	Final germination (%)		Treatment 2	Final germination (%)	s.e.
	in darkness	after TR1		after TR2	at optimal %
5	0	98*	-	-	1,3
	0	0	WS	3	2,0
	0	0	WS (in D)	0	-
	0	0	AR	1	1,0
10	0	0	GA_3	100***	-
	0	0	WS	1	1,0
	0	0	WS (in D)	1	1,0
	0	0	AR	0	-
15	0	96	-	-	1,9
	0	98	-	-	2,5
	0	24	WS	77	8,3
	0	57	AR	78	6,4
	0	-	GA_3	100	-
20	1	93	-	-	1,9
25	0	5	GA_3	98	1,4
30	0	98**	-	-	1,4
20/10	1	52	GA_3	-	5,4
25/15	0	7	GA_3	97	2,0
30/20	0	100	-	-	-

TR1: seeds were moved from dark to light

* seeds were moved at 25°C in light

** seeds were moved at 15°C in light

TR2: after treatment, seeds were moved at the initial temperature in light

*** after treatment, seeds were moved at 20°C in light

It is concluded that seeds germinate under natural conditions during autumn, if they are on the soil surface. If buried, skotodormancy and thermodormancy are expected to be imposed during winter, creating a transient soil seed bank. These seeds are expected to germinate next autumn, after secondary dormancy is released by the higher summer temperatures or whenever they are brought to the surface. Although afterripening and warm stratification failed to break secondary dormancy, the abundance of the *M. minimus* seedlings at the temporary pond of Greveno in November 2015 confirmed this hypothesis.

Polygonum arenastrum

Seeds of *Polygonum arenastrum* were collected in 2016 from Livadies. However, no germination tests were performed. Germination tests were performed in a seedlot lot collected in 2013 and wrongly identified as *Polygonum arenastrum* while it was actually *Lythrum thymifolia*. So, the 2016 seed collection was cleaned and stored without germination tests. *Polygonum arenastrum* is a nitrophilous vegetation pioneer species common in the temporary ponds of the project sites but not a typical temporary pond vegetation species. Due to the limited time available after the

belated identification of the 2013 seedlot, priority had to be given to typical target habitat species.

Based on an extended literature review of seed germination of other *Polygonum* species as well as their seed size (Baskin & Baskin, 2014), *P. arenastrum* is expected to be a typical summer annual, producing physiologically dormant seeds, which need 3 to 5 months of cold stratification to overcome dormancy and germination is expected to take place at 35/20°C in light.

Ranunculus lateriflorus

Seeds of *Ranunculus lateriflorus* were collected twice during 2013 (RlL613) and also in 2014 (RlL714) and 2016 from Livadies (RlL716), where the largest population of the species occurs. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 630 and 900μm. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *Ranunculus lateriflorus*, but following an extended literature review of seed germination of other *Ranunculus* species (Baskin & Baskin, 2014), seeds were expected to be morphophysiologically dormant, requiring cold or warm stratification or their combination to break dormancy. Thus, germination experiments were performed at all constant and alternative temperatures available, under both light and darkness, with afterripened seeds from the most numerous collection (Livadies-23.06.2013). The effect of afterripening on seed germination was tested with seeds from the 2016 collection (Fig. 5). It was confirmed that the main germination characteristics were similar for all seedlots of *Ranunculus lateriflorus* (Table 17).

Germination was achieved in light, under a wide range of constant (10-20 °C) and at all the alternating temperatures tested, for afterripened seeds. Seeds germinate optimally in light, with final germination exceeding 95%, at the temperatures typical for Mediterranean climate species, i.e. 15-20 °C. The rate of germination is also very high, since the t_{50} is approximately 6 days. Darkness completely prevents seed germination, but GA_3 can substitute for light (data not shown).

Table 17: Final germination of *Ranunculus lateriflorus* seeds collected in 2013 (RlL613 or RlL713), 2014 (RlL714) and 2016 (RlL716).

Temperature, °C	Seedlot	Pretreatment	Final germination (%)		s.e.		t_{50}
			light	darkness	light	darkness	light
5	RlL613	Afterripening	19	0	4,0	-	28
	RlL713	Afterripening	28	0	4,6	-	41
	RlL714	Afterripening	55	0	2,5	-	41
	RlL716	Afterripening	0	-	-	-	-
10	RlL716	-	56	-	4,1	-	13
	RlL716	Afterripening	96	-	1,9	-	11
	RlL613	Afterripening	72	0	4,1	-	13
	RlL713	Afterripening	78	0	6,3	-	13
	RlL714	Afterripening	91	0	1,4	-	12

15	RIL716	-	56	-	6,6	-	22
	RIL716	Afterripening	100	-	-	-	6
	RIL613	Afterripening	96	0	2,0	-	6
	RIL713	Afterripening	93	6	2,9	1,9	6
	RIL714	Afterripening	94	0	2,5	-	3
20	RIL716	-	9	-	2,9	-	70
	RIL716	Afterripening	88	-	2,9	-	4
	RIL613	Afterripening	88	0	3,7	-	6
25	RIL716	-	0	-	-	-	-
	RIL716	Afterripening	81	-	3,8	-	5
	RIL613	Afterripening	84	0	2,7	-	5
30	RIL613	Afterripening	0	0	0,0	-	-
	RIL716	Afterripening	8	-	3,0	-	6
20/10	RIL613	Afterripening	93	0	2,7	-	8
25/15	RIL613	Afterripening	92	1	3,4	1,0	6
30/20	RIL613	Afterripening	89	0	4,3	-	5

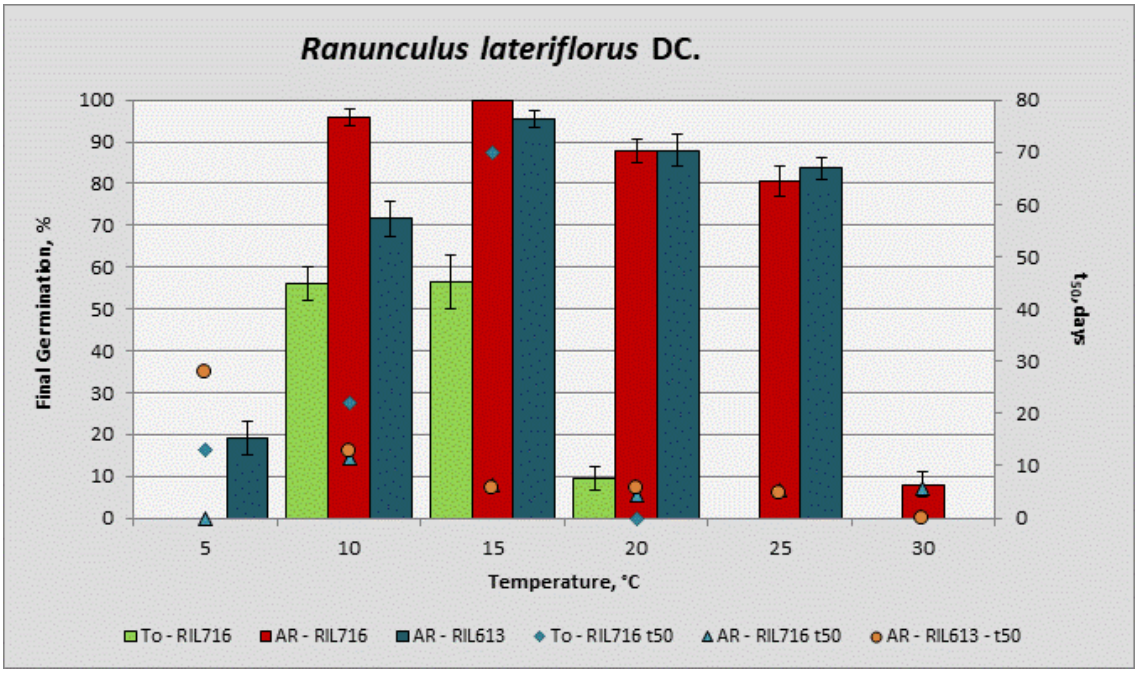

Figure 5: Final germination immediately after seed collection (T_0) and after 4 months of afterripening (AR) at constant temperatures in light for *Ranunculus lateriflorus* seeds collected in 2013 (RIL613) and 2016 (RIL716) from Livadies.

Secondary dormancy is imposed in darkness (skotodormancy) mainly in suboptimal temperatures (Table 18). Thermodormancy is imposed by suboptimal low temperatures (5°C) but not by by suboptimal high temperatures (30°C) (Table 19). Both skotodormancy and thermodormancy are released by GA_3 and warm stratification in light. Seedlings were checked for epicotyl dormancy and were found non-dormant.

Table 18: Induction of secondary dormancy by suboptimal temperatures and its release by GA_3, afterripening (AR) and warm stratification in either light (WS) or darkness (WS in D) in *Ranunculus lateriflorus* seeds collected in 2013 (RlL613).

Temperature, °C	Final germination (%)		Treatment 2	Final germination (%) after TR2	s.e.
	in light	after TR1			at optimal %
5	19	19	GA_3	96	2,0
5	18	18	WS	71	2,9
5	19	20	WS (in D)	38	4,4
5	21	21	AR	25	3,2
30	0	99	-	-	1,3

TR1: seeds were moved from 5 or 30°C to 15°C.

TR2: after treatment, seeds were moved at 15°C.

Table 19: Induction of secondary dormancy from darkness and its release by GA_3, afterripening (AR) and warm stratification in either light (WS) or darkness (WS in D) in *Ranunculus lateriflorus* seeds collected in 2013 (RlL613, RlL713) and 2014 (RlL714).

Temperature, °C	Seedlot	Final germination (%)		Treatment 2	Final germination (%) after TR2	s.e.
		darkness	after TR1			at optimal %
5	RlL613	0	-	WS	99**	1,1
	RlL613	0	1	WS	70	2,7
	RlL613	1	1	WS (in D)	33	8,9
	RlL613	0	0	AR	6	2,9
	RlL713	0	0	-	46*	6,2
	RlL714	0	6	-	69*	8,1
10	RlL613	0	0	GA_3	95	2,6
	RlL613	0	0	WS	85	5,3
	RlL613	1	1	WS (in D)	91	5,4
	RlL613	0	0	AR	38	5,7
	RlL713	0	2	WS	85	3,1
	RlL714	0	18	WS	90	4,2
15	RlL613	0	95	-	-	3,2
	RlL613	0	-	GA_3	96	1,7
	RlL713	0	64	WS	98	1,3
	RlL714	0	99	-	-	1,3
20	RlL613	0	92	-	-	4,0
25	RlL613	0	58	GA_3	92	2,9
30	RlL613	0	97*	-		1,3
20/10	RlL613	0	78	-	-	3,2
25/15	RlL613	1	84	-	-	3,3
30/20	RlL613	0	95	-	-	0,1

TR1: seeds were moved from dark to light

TR2: after treatment, seeds were moved at the initial temperature in light

* seeds were moved at 15°C in light

** after treatment, seeds were moved at 20°C in light

It is concluded that seeds germinate under natural conditions during autumn, if they are on the soil surface. If buried, skotodormancy and thermodormancy are expected to be imposed during winter and seeds will germinate next autumn, after secondary dormancy is released during summer (Skourti et al. 2016). The abundance of the *R*. *lateriflorus* seedlings at temporary ponds (Livadies) in November 2015 confirmed these hypotheses.

Verbena supina

Seeds of *Verbena supina* were collected from Nevropoli in 2015, 2016 and 2019. Germination experiments were performed with the 2015 and 2016 seedlots. Seed collection of this species was rather tricky due to the combination of asynchronous seed maturation/dispersal, severe grazing and low abundance in 2015, 2016 and 2017. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630μm and partially at 500μm. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C).

This is the first study of seed germination of *V. supina*. An extended literature review of seed germination of other *Verbena* species (Baskin & Baskin, 2014), showed that seeds are expected to germinate better in light than in darkness and be physiologically dormant with dormancy released by cold stratification. Thus, germination experiments were performed at constant (5, 10, 15, 20, 25, 30 °C) and alternating (20/10 °C) temperatures, in continuous darkness and in light, after seed collection and after 3 months of cold stratification.

Interestingly, final light germination of cold stratified or afterripened seeds reaches c. 60% at 30 °C, whereas no seeds germinated at 25 °C (Table 20). Darkness inhibits seed germination completely. Skotodormancy seems to be induced at the optimal temperature (30 °C). Gibberellic acid promotes final germination (88% at 20°C). However, the non-germinated seeds may be empty; cut-tests were not performed for the non-germinated seeds, but have been performed for a small portion of non-imbibed seeds of the seed collection and c. 60% of them found to be empty. Seedlings were checked for epicotyl dormancy and were found non-dormant. It is concluded that the vast majority of *V. supina* seeds will probably germinate under natural conditions if they are exposed at high spring or summer temperatures in light (soil surface).

Table 20: Final germination of mature *Verbena supina* seeds, immediately after seed collection, after 3 months of cold stratification (CS) or the addition of GA_3 and after seed transfer at 30°C, in light.

Germination conditions	Pretreatments	Final germination (%)	s.e.	t_{50}	Final germination (%) after transfer at 30°C, in light	s.e.
5°C, Light	-	0	-	-	-	-
10°C, Light	CS	0	-	-	58	10,7
10°C, Dark	CS	0	-	-	28	8,0
15°C, Light	CS	0	-	-	58	10,7
15°C, Dark	CS	0	-	-	56	7,5
20°C, Light	-	0	-	-	-	-
20°C, Light	GA_3	88	5,8	3	-	-

20°C, Light	CS	0	-	-	-	-
20°C, Dark	-	0	-	-	-	-
20°C, Dark	CS	0	-	-	-	-
25°C, Light	CS	0	-	-	-	-
25°C, Dark	CS	0	-	-	-	-
30°C, Light	AR	66	6,7	15	-	-
30°C, Light	CS	58	5,8	38	-	-
30°C, Dark	CS	0	-	-	0	-
20/10°C, Light	CS	0	-	-	-	-
20/10°C, Dark	CS	0	-	-	-	-

* germination experiments were carried out using 5 samples of 10 seeds each

Veronica oetaea*

Seeds of *Veronica oetaea* were collected in 2013 from Livadies and in 2014 from all three temporary ponds, Livadies, Greveno and Alykaina. Seed collections were cleaned manually, using steel sieves and seeds remained at 125 and 300μm sieves. Afterwards, seed collections were homogenized and seeds were maintained under laboratory conditions (c. 22 °C), until the onset of the germination experiments, several months following seed collections.

An initial study of the germination behavior of *Veronica oetaea* was conducted in the Mediterranean Forest Research Institute both at constant and alternating temperatures (> 15 °C) and following a chilling pretreatment in darkness; however germination was achieved only for seeds in which gibberellic acid was applied (Mantakas G. 2016, personal communication). From the literature, germination for weeds *Veronica arvensis* and *Veronica hederifolia* are known. *Veronica arvensis* germinates in a variety of constant and alternating temperatures while *Veronica hederifolia* germinates following a warm pretreatment.

Germination experiments in the current study were conducted with seed material collected in 2013, in a variety of constant and alternating temperatures (also lower than 15 °C), in light (12h photoperiod) and in complete darkness, both without any pretreatment and following a chilling pretreatment in light and in darkness at 5 °C. Regarding seed material collected in 2014, germination was tested at 5 ºC in light following the results of the previous experiments. In addition the effect of GA_3 was determined in all seed collections. All experiments are presented in Table 21 and figures 6-8.

Table 21: Final germination of *Veronica oetaea* seeds collected in 2013 (VoL613) and 2014 (VoL714, VoG714, VoA714). L: light, D: darkness.

Temperature, °C	Seedlot	Pretreatment	Final germination (%)		s.e.		t_{50}	transfer conditions	Final germination (%)		s.e.
			light	darkness	light	darkness	light		light	darkness	light
5	VoL613	-	35	3	5,2	-	133	-	-	-	-
	VoL714	-	28	0	2,0	-	98	-	-	-	-
	VoG714	-	16	0	1,9	-	100	-	-	-	-
	VoA714	-	1	0	1,0	-	138	-	-	-	-
10	VoL613	-	3	0	5,2	-	18	5 °C L/D	29	0	3,3
	VoL613	5°C L/D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	5°C D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-

15	VoL613	-	0	0	-	-	-	5 °C L/D	40	0	4,5
	VoL613	5°C L/D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	5°C D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
20	VoL613	-	0	0	-	-	-	5 °C L/D	44	0	1,9
	VoL613	5°C L/D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	5°C D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
25	VoL613	-	0	0	-	-	-	5 °C L/D	33	0	3,4
	VoL613	5°C L/D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	5°C D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
20/10	VoL613	-	0	0	-	-	-	5 °C L/D	31	0	6,2
	VoL613	5°C L/D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	5°C D - 3 mo (chilling)	0	-	-	-	-	-	-	-	-
	VoL613	GA_3 imbibition	84	-	1,9	-	-	-	-	-	-
	VoL714	GA_3 imbibition	81	-	2,9	-	-	-	-	-	-
	VoG714	GA_3 imbibition	80	-	2,2	-	-	-	-	-	-
	VoA714	GA_3 imbibition	71	-	2,9	-	-	-	-	-	-

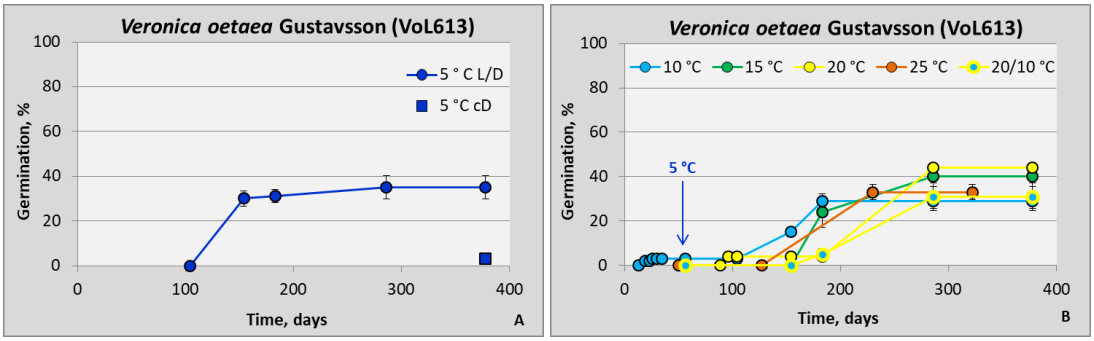

Figure 6: Germination of *Veronica oetaea* seeds collected from Livadies in 2013 (VoL613). A. Germination at 5 °C, in alternating light/darkness conditions (L/D) and continuous darkness (cD). B. Germination at 10, 15, 20 and 20/10 °C, in alternating L/D conditions. The arrow marks the time when the seeds were transferred from the initial temperature to the optimal 5 °C L/D conditions.

Germination was achieved only at the very low temperature of 5 °C, in light after c. 4 months (t50 c. 100 days or more), either initially placed in this temperature or transferred from another higher one. Germination percentage reached 16 to 44% in all seedlots and conditions with the exception of VoA713 seedlot, where germination did not exceed 1%. Seeds do not germinate in complete darkness. As mentioned above, secondary dormancy, i.e. thermodormancy is not imposed in *Veronica oetaea* seeds. High germination, above 70%, along with a high germination rate (t50 13-14 days) was succeeded only after seeds were imbibed in 1000 ppm gibberellic acid, in all seedlots used in the present study.

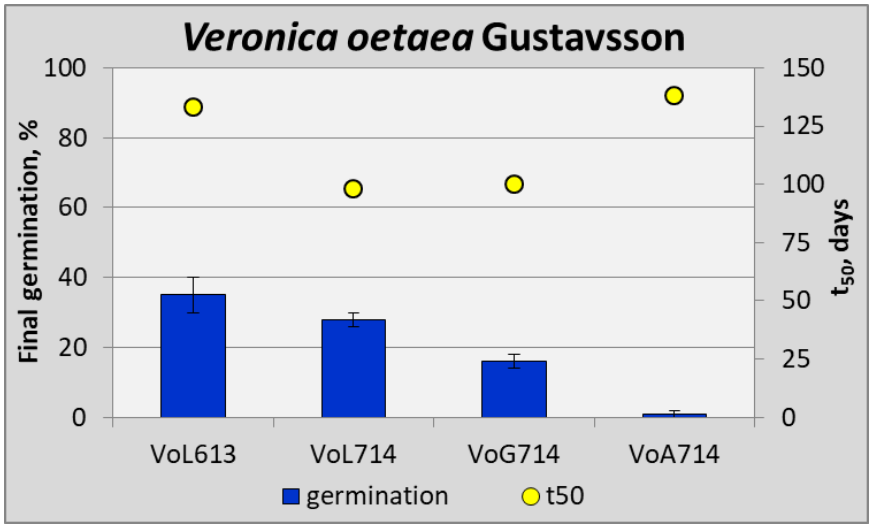

Figure 7: Germination of *Veronica oetaea* seeds at 5 °C (12h light/12h darkness). Seeds were collected from Livadies in 2013 (VoL613) and 2014 (Vo, Greveno in 2014 (VoG714) and Alykaina in 2014 (VoA714).

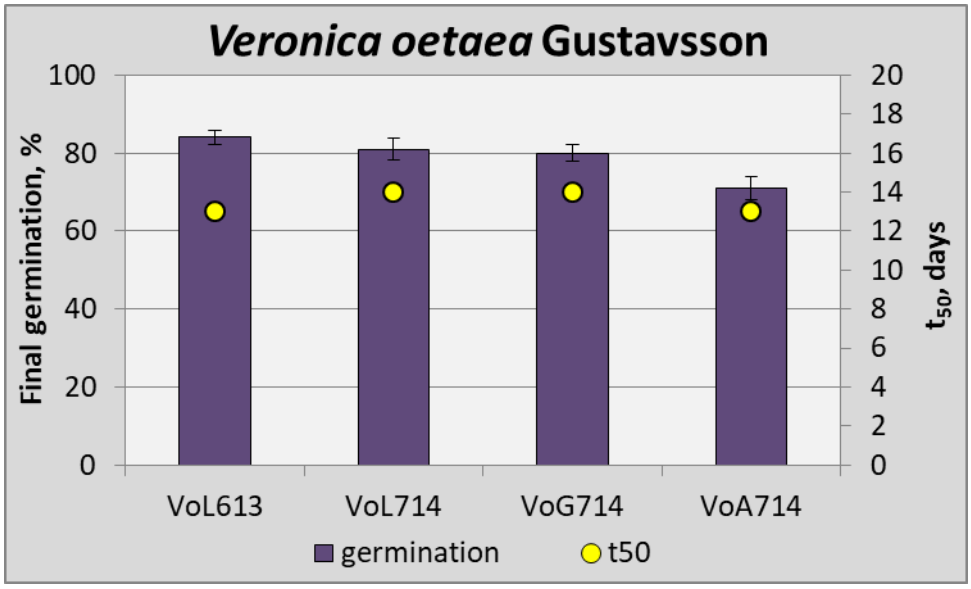

Figure 8: Germination of *Veronica oetaea* seeds at alternating temperatures 20/10 °C (12h light/12h darkness), imbibed with 1000 ppm GA_3. Seeds were collected from Livadies in 2013 (VoL613) and 2014 (Vo, Greveno in 2014 (VoG714) and Alykaina in 2014 (VoA714).

Further research is necessary to discover the favorable germination conditions for *Veronica oetaea*. However, the findings of this study have a significant value since this is the first time that seeds of *Veronica oetaea* have been reported to germinate without the use of the potent, but artificial, germination promoter gibberellic acid. Germination in such cold conditions and with such a slow rate leads us to conclude that germination takes place in late spring, following snow melt.

4. Results for mountain grassland species (6210*, 6230*)

Seeds of the 28 mountain grassland species (6210*, 6230*) were collected during 2016 from various populations on Mt. Oiti and Mt. Kallidromo. Seed collections were cleaned and stored at the Seedbank of the UoA, after seed germination was examined.

Achillea crithmifolia

Seeds of *Achillea crithmifolia* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 300, 400 and 500µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 0.2 and 0.3mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *A. crithmifolia*, but following an extended literature review of seed germination of other *Achillea* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released by cold stratification. Although the effect of light on seed germination was not always examined, the majority of *Achillea* species studied, seems to produce seeds that germinate better in light than in darkness. Thus, germination experiments were performed at 15 and 20/10°C, under both light and darkness, immediately after seed collection and after 1 month of cold stratification.

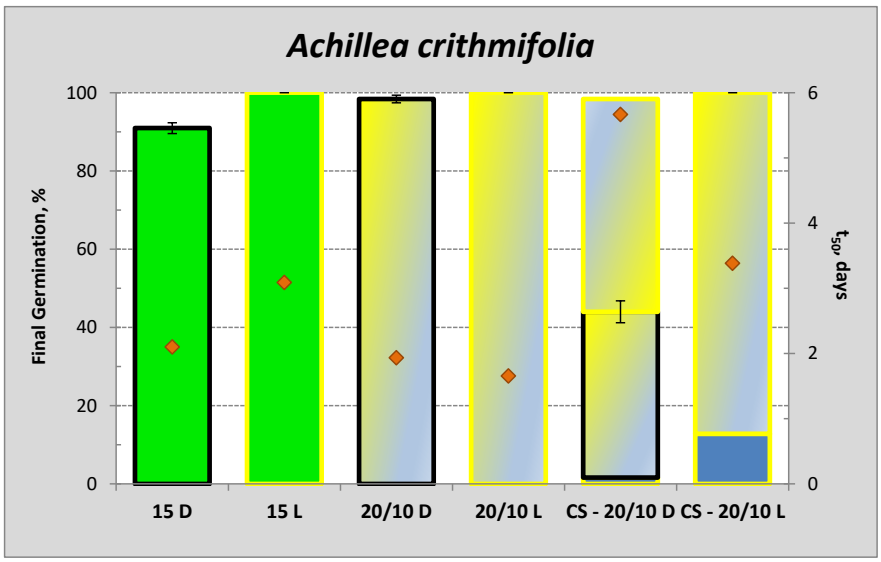

Figure 9: Final germination of *Achillea crithmifolia* seeds, immediately after seed collection and after 1 month of cold stratification (CS), at 15°C and 20/10°C in light (L-yellow border on bars) and in darkness (D-black border on bars).

Germination was achieved immediately after seed collection, at both temperatures tested under both light and darkness (Fig. 9). The rate of germination is also very high, since the t_{50} is approximately 2-3 days. A small portion of seeds germinated during cold stratification in light (blue part of the bar "CS-20/10 L") and the others germinated when transferred at 20/10°C in

light, but the rate of germination was lower compared to the rate of germination of non-treated seeds. Moreover, cold stratification prevented seed germination in darkness (black border of the bar "CS-20/10 D"), but secondary dormancy was not imposed, since all seeds germinated at 20/10°C when they were transferred from darkness to light (yellow border of the bar "CS-20/10 D"). It is concluded that seeds are able to germinate under natural conditions during autumn, if buried or on the soil surface.

Allium achaium

Seeds of *Allium achaium* were collected in 2016 from Livadies and Greveno. Seed collection was cleaned manually, without using steel sieves or a seed aspirator. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *A. achaium*, but following an extended literature review of seed germination of other *Allium* species (Baskin & Baskin, 2014), seeds were expected to be physiologically dormant, requiring cold or warm stratification to break dormancy. Although the effect of light on seed germination was not always examined, light inhibited germination of *Allium staticiforme* (Thanos et al. 1991). Thus, germination experiments were performed at all constant temperatures available, under both light and darkness, with afterripened seeds of both collections (Livadies and Greveno). Seeds from both collections showed the some germination characteristics. Final germination reached c. 90-100% at 5-20 °C in darkness, but the optimal temperature for germination was 15 °C at which the t_{50} was lower and around 4 days (Fig. 10). Light inhibited germination only at 20 °C, whereas no germination occur at 25 °C. It is concluded that seeds are able to germinate under natural conditions during autumn.

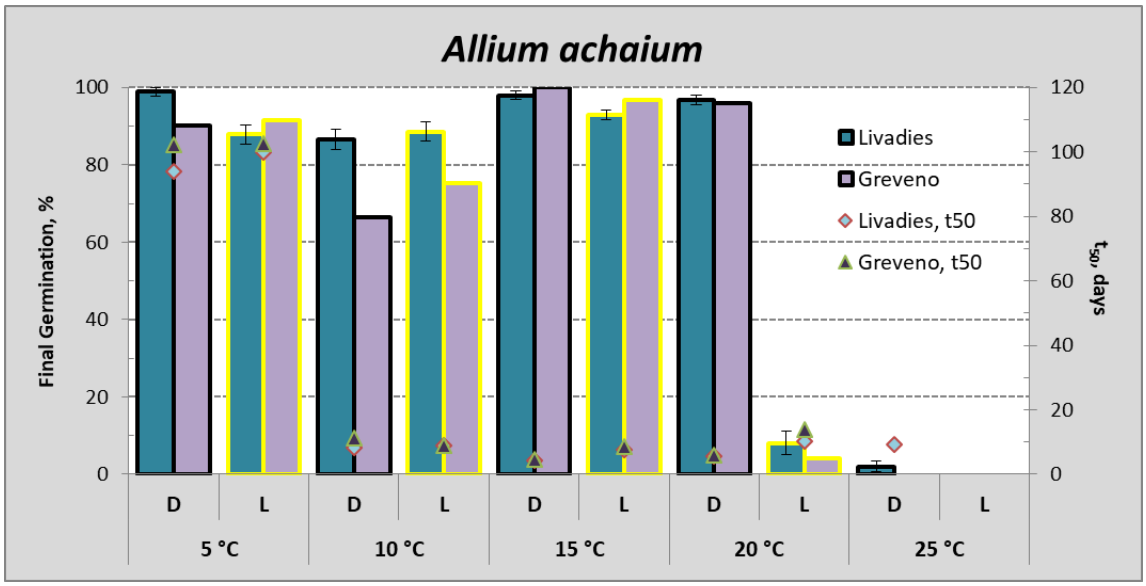

Figure 10: Final germination of afterripened seeds of *Allium achaium* from Greveno and Livadies, at various constant temperatures in light (L-yellow border on bars) and in darkness (D-black border on bars).

Alopecurus gerardii

Seeds of *Alopecurus gerardii* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 900μm and 1.4mm and partially at 630μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *A. gerardii*, but following an extended literature review of seed germination of other *Alopecurus* species (Baskin & Baskin, 2014), seeds were expected to be physiologically dormant and dormancy can be released by warm stratification. Moreover, the majority of *Alopecurus* species studied, seems to produce seeds that germinate better in light than in darkness. Thus, germination experiments were performed exclusively with afterripened seeds at 20/10°C in light, using five samples of 15 seeds each, due to low seed availability. Final germination reached 100% and the t_{50} was approximately 2 days (Fig. 11). It is concluded that seeds are able to germinate under natural conditions during autumn.

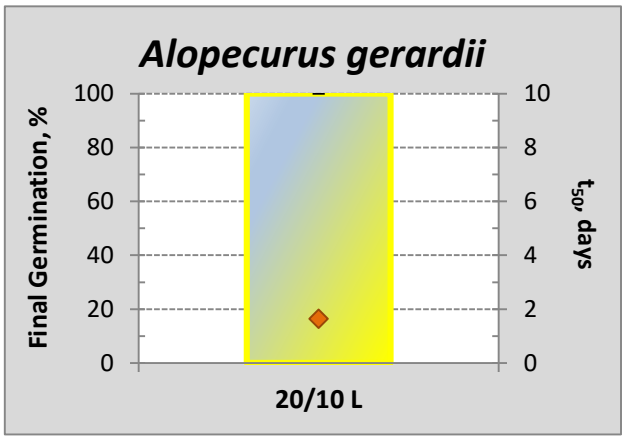

Figure 11: Final germination of *Alopecurus gerardii* afterripened seeds at 20/10°C in light.

Anthoxanthum odoratum

Seeds of *Anthoxanthum odoratum* were collected from Livadies in 2016. Seed collection was cleaned manually and seeds were separated from dead tissues using a seed aspirator at 1mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Platenkamp (1991), *A. odoratum* seeds germinate after the first autumn rains in October or November. Thus, germination experiments were performed with afterripened seeds at 15 and 20/10°C in both light and darkness. Germination was achieved immediately after seed collection, at both temperatures tested in light and the t_{50} was approximately 9-10 days (Fig. 12). Darkness partially inhibited germination at both temperatures tested, but secondary dormancy was imposed only at 20/10 °C, since seeds did not germinate adequately at 20/10°C when they were transferred from darkness to light (yellow border of the bar "CS-20/10 D"). It is concluded that the majority of *A. odoratum* seeds are able to germinate under natural conditions during autumn.

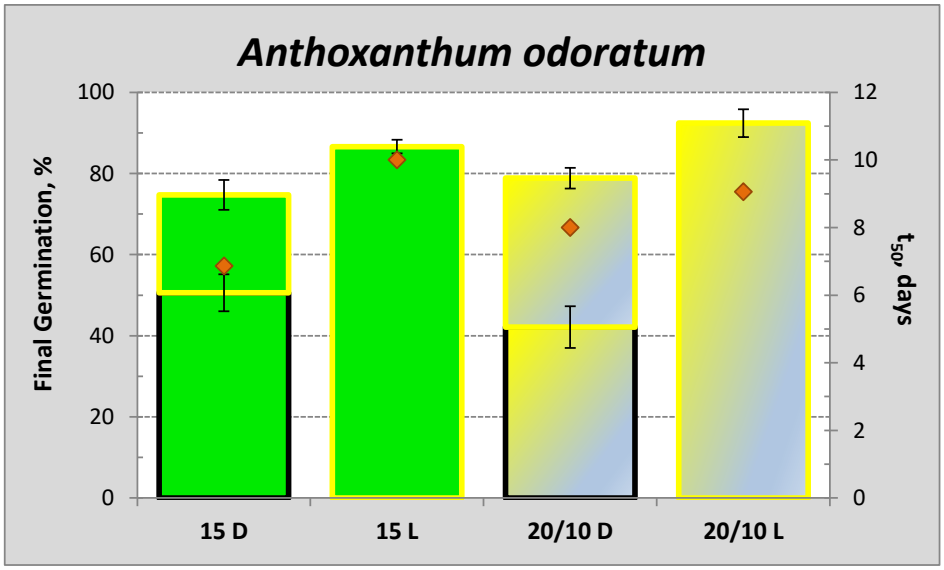

Figure 12: Final germination of *Anthoxanthum odoratum* afterripened seeds at 15°C and 20/10°C, in light (L-yellow border on bars) and in darkness (D-black border on bars).

Bellardiochloa variegata

Seeds of *Bellardiochloa variegata* were collected from Livadies in 2016. Seed collection was cleaned manually and seeds were separated from dead tissues initially, using a seed aspirator at 0.8mm air-flow. Afterwards, seed collection was cleaned using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900μm and 1.4mm and seeds were separated from dead tissues using a seed aspirator at 1.6 and 2mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Due to low seed availability and lack of information on *Bellardiochloa* seed germination, germination experiments were performed exclusively with afterripened seeds at 20/10°C in both light and darkness. Final germination reached 100%, the t_{50} was approximately 5-6 days and *B. variegata* produces light indifferent seeds (Fig. 13). It is concluded that seeds are able to germinate under natural conditions during autumn, if buried or on the soil surface.

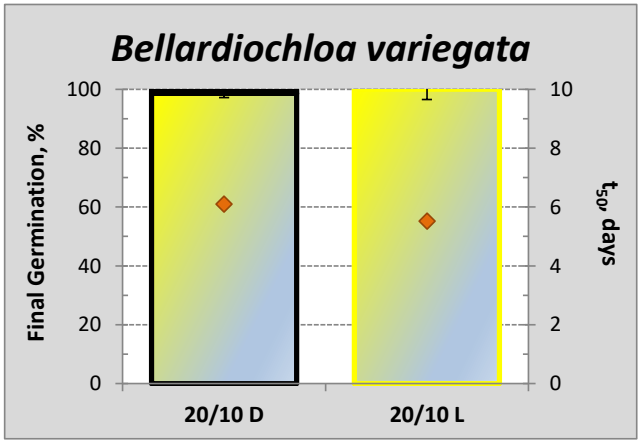

Figure 13: Final germination of *Bellardiochloa variegata* afterripened seeds at 20/10°C in light (L) and in darkness (D).

Brachypodium pinnatum

Seeds of *Brachypodium pinnatum* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 500 and 630μm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 0.5mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Czarnecka (2004), *B. pinnatum* seeds fail to germinate in autumn and seed germination occur partially during winter and mainly in spring. Germination experiments were performed exclusively with afterripened seeds at 20/10°C in light, due to low seed availability. Final germination reached 88% and the t_{50} was approximately 6 days (Fig. 14). It is concluded that *B. pinnatum* seeds are either non-dormant or require afterripening and not cold stratification in order to germinate. Thus, seed germination is expected to occur under natural conditions during autumn.

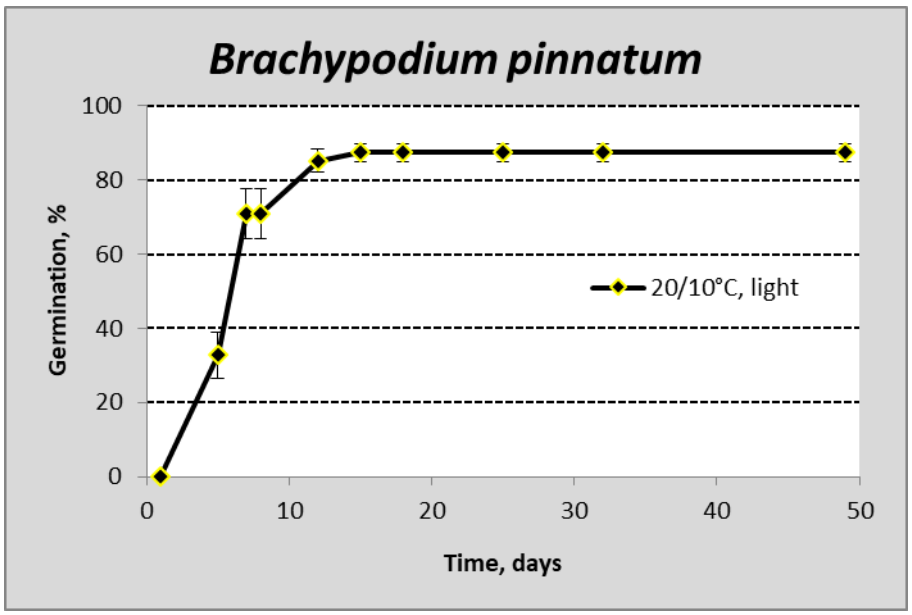

Figure 14: Light germination of *Brachypodium pinnatum* afterripened seeds, at 20/10°C.

Carex ovalis

Seeds of *Carex ovalis* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 900μm and partially at 630μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Schütz (1999), *C. ovalis* seeds germinate exclusively at alternative temperatures in light and cold stratification enhances light germination. Thus, germination experiments were performed with afterripened seeds at 20/10°C in light and in darkness. Moreover, the effect of cold stratification was examined. Final germination reached c. 100% and cold stratification only affected the rate of germination, by reducing the t_{50} from approximately 16 to 8 days (Fig. 15).

Darkness inhibited germination and secondary dormancy was imposed, since seeds did not germinate adequately at 20/10°C when they were transferred from darkness to light. The addition of GA_3 and warm stratification failed to release skotodormancy (data not shown), which was released after 1 month of cold stratification (blue border on the bar "20/10 D"). It is concluded that light requiring seeds are able to germinate under natural conditions during autumn, if they are on the soil surface. If buried, skotodormancy is expected to be imposed and seeds will germinate next spring, after secondary dormancy is released during winter.

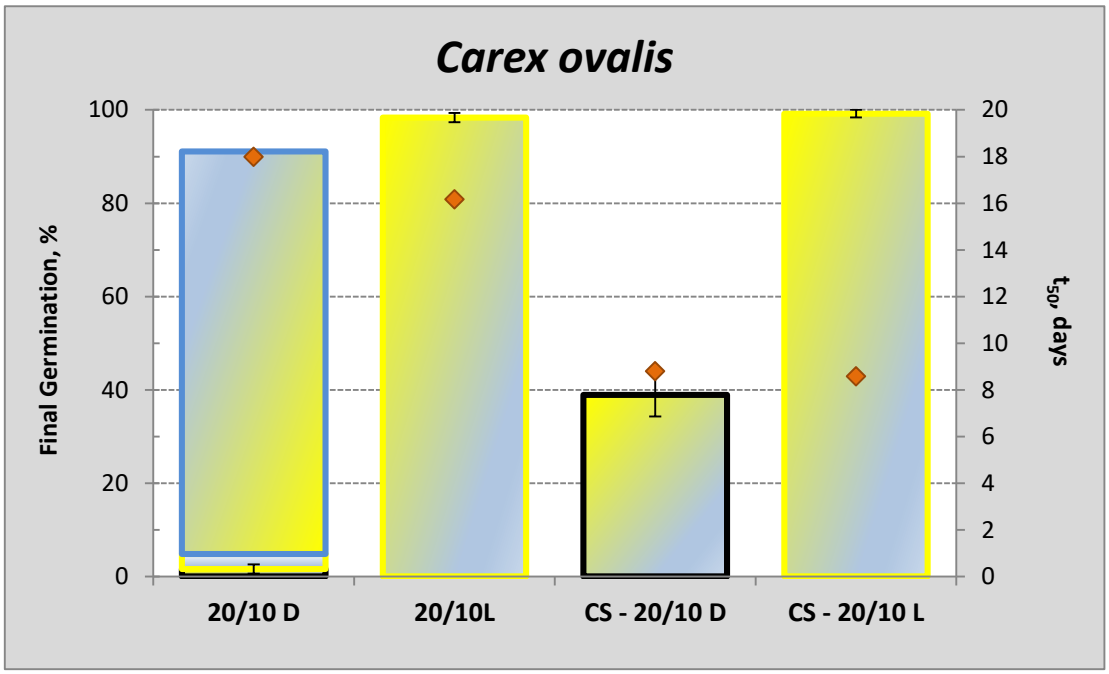

Figure 15: Final germination of *Carex ovalis* seeds, after dry storage and after dry storage and 2 months of cold stratification (CS), at 20/10°C in light (L-yellow border on bars) and in darkness (D-black border on bars) and the release of skotodormancy by 1 month of cold stratification (blue border on the bar "20/10 D").

Centaurea nervosa subsp. promota

Seeds of *Centaurea nervosa* subsp. *promota* were collected in 2016 from Livadies. Seed collection was cleaned manually, but all seeds were empty due to insect infection. Therefore, no seeds were stored in the Seed Bank and seed germination was not studied.

Chrysopogon gryllus

Seeds of *Chrysopogon gryllus* were collected from Isomata in 2016. Seed collection was cleaned manually without the usage of steel sieves or seed aspirator. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *C. gryllus*, but following an extended literature review of seed germination of other *Chrysopogon* species (Baskin & Baskin, 2014), seeds were expected to be physiologically dormant and dormancy can be released by afterripening. Moreover, the majority of *Chrysopogon* species studied, seems to produce seeds that germinate

better in light than in darkness. Thus, germination experiments were performed exclusively with afterripened seeds at 20/10°C in light and in darkness, using five samples of 15 seeds each, due to low seed availability. Seeds failed to germinate and they were left to warm stratified for 2 months. Seed germination never exceeded 18% and thus, the seeds were transferred at higher germination temperature (25 °C). Final germination remained low and even with the addition of gibberellic acid never exceeded 44% (data not shown). However, the non-germinated seeds may be empty; cut-tests were not performed for the non-germinated seeds, but were performed for a small portion of non-imbibed seeds of the seed collection and c. 70% of them found to be empty.

Dianthus tymphresteus

Seeds of *Dianthus tymphresteus* were collected from Greveno in 2016. Seed collection was cleaned manually, without using steel sieves or a seed aspirator. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *D. tymphresteus*, but following an extended literature review of seed germination of other *Dianthus* species (Baskin & Baskin, 2014), seeds were expected to be non-dormant or physiologically dormant, requiring cold or warm stratification to break dormancy. Thus, germination experiments were performed at all constant temperatures available, under both light and darkness, with afterripened seeds. Final germination reached c. 100% at 20 °C in light and the t_{50} was approximately 1 day (Fig. 16). Darkness inhibited germination in all temperatures tested. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

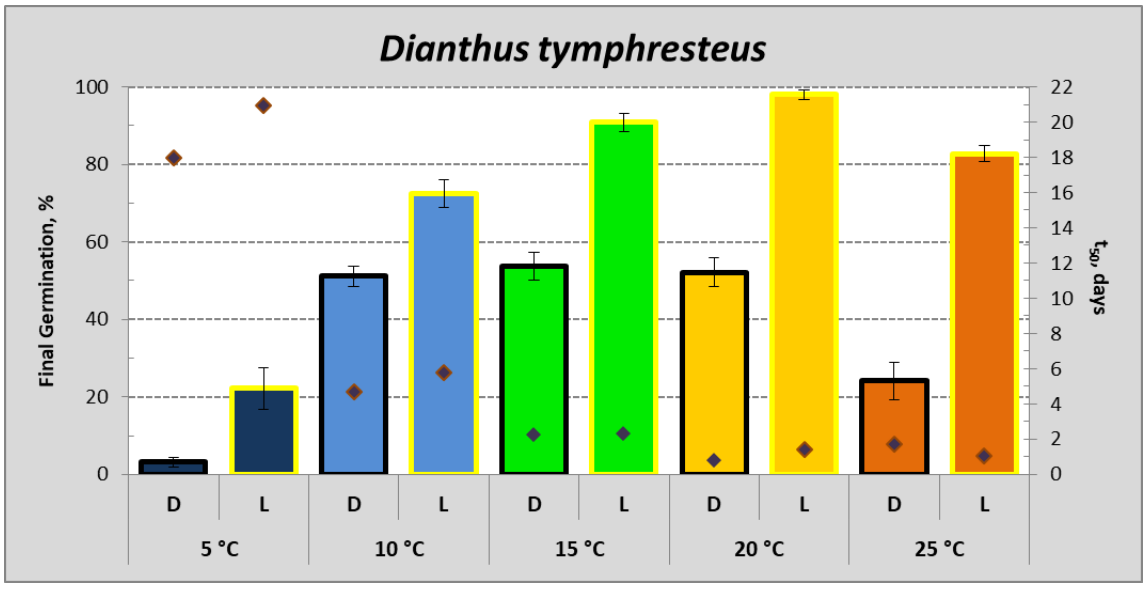

Figure 16: Final germination of afterripened seeds of *Dianthus tymphresteus* at various constant temperatures in light (L-yellow border on bars) and in darkness (D-black border on bars).

***Festuca* sp.**

Seeds of *Festuca* sp. were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 300μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Following an extended literature review of seed germination of *Festuca* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released mainly by cold stratification. Although the effect of light on seed germination was not always examined, *Festuca octoflora* seems to produce seeds that germinate better in light than in darkness (Hylton & Bass, 1961). Thus, germination experiments were performed at 15 and 20/10°C in light and in darkness. Moreover, the effect of cold stratification on seed germination was examined.

Final germination reached c. 97% at both 15 and 20/10°C in light (Fig. 17) and the t_{50} was approximately 4 days. Final germination in darkness was lower than light germination, but not in a statistically significant way. A small portion of seeds germinated during cold stratification (blue part of the bars "CS-20/10 L" and "CS-20/10 D"). Cold stratification not only failed to enhance the rate of germination, but also induced secondary dormancy, since stratified seeds failed to germinate adequately at 20/10°C both in light and in darkness (black and yellow borders of the bars "CS-20/10 L" and "CS-20/10 D"). Secondary dormancy was released by 1 month of warm stratification (orange border on the bar "20/10 D"). It is concluded that seeds are able to germinate under natural conditions during autumn, if they are buried or on the soil surface. If they failed to germinate, secondary dormancy is expected to be imposed during winter and seeds will germinate next autumn, after secondary dormancy is released during summer.

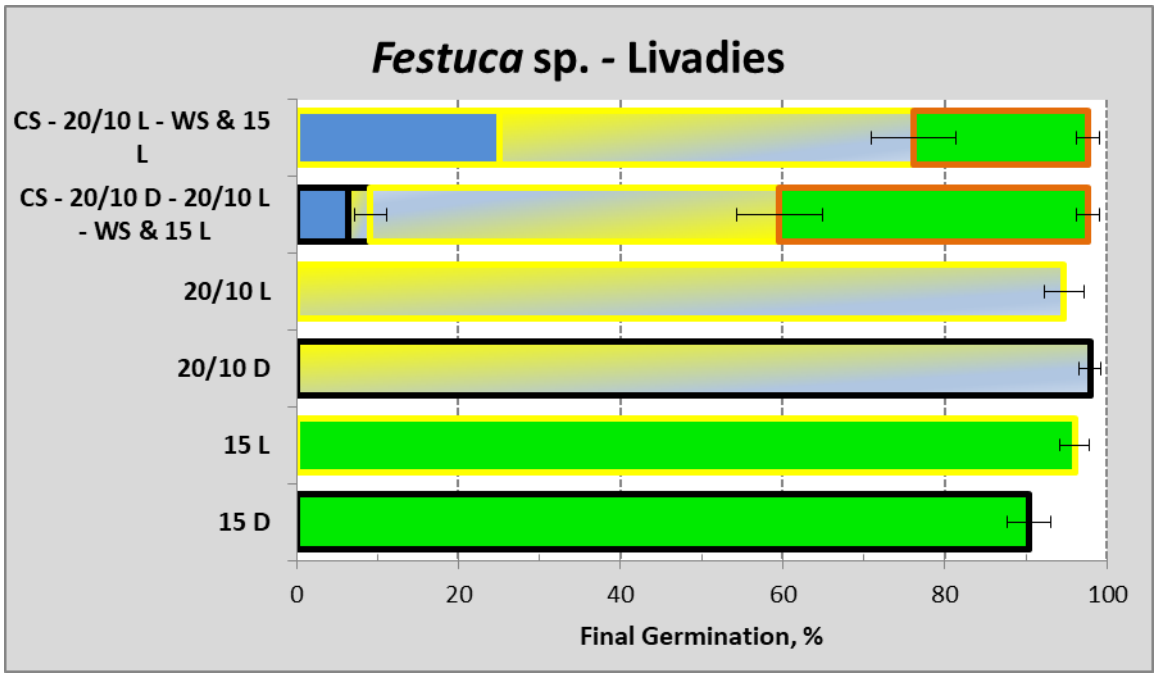

Figure 17: Final germination of *Festuca* sp. seeds collected from Livadies, at 15°C and 20/10°C in light (L-yellow border on bars) and in darkness (D-black border on bars), after seed collection and after 1 month of cold stratification (CS) and the release of secondary dormancy by 1 month of warm stratification (WS).

Festuca polita

Seeds of *Festuca polita* were collected from Isomata in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 500, 630 and 900µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 1mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *F. polita*, but based on the extended literature review of seed germination of *Festuca* species that has been described above and due to low seed availability, germination experiments were performed at 20/10°C in light and in darkness. Final germination reached 90-97%, the t_{50} was approximately 5 days and *F. polita* produces light indifferent seeds (Fig. 18). It is concluded that seeds are able to germinate under natural conditions during autumn, if buried or on the soil surface.

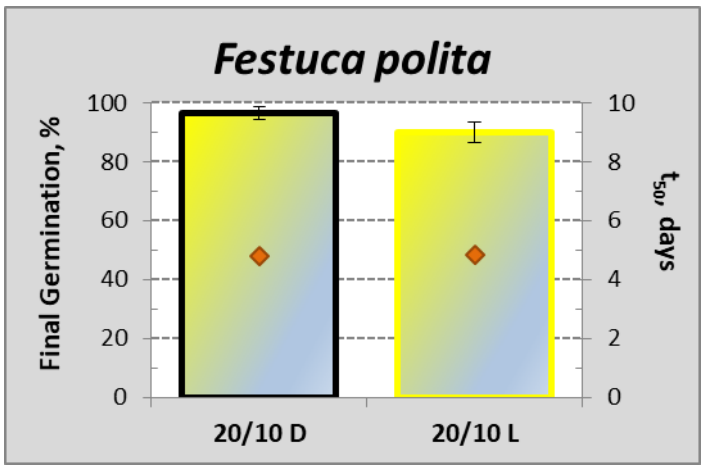

Figure 18: Final germination of *Festuca polita* seeds at 20/10°C in light (L) and in darkness (D).

Festuca* cf. *valesiaca

Seeds of *Festuca* cf. *valesiaca* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 1mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen. Germination experiments were not performed for this species, due to extremely low seed availability. However, based on the literature review of seed germination of other *Festuca* species, as well as the experiments performed from the other two *Festuca* species that have been collected from Mt. Oiti and Mt. Kallidromo, seeds of *Festuca* cf. *valesiaca* are expected to germinate adequately at 20/10°C in light and in darkness.

Galium verum

Seeds of *Galium verum* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained at 500, 630 and 900μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Liu et al. (2011), *G. verum* produces physiologically dormant seeds; dormancy can be released by 40 days of cold stratification or afterripening and afterwards germination occur at 20/5°C. Thus, germination experiments were performed at 20/10°C, under both light and darkness. Seeds failed to germinate and they were left to cold stratified for 4 months. Seed germination never exceeded 2% and the addition of gibberellic acid failed to enhance germination (data not shown). However, the non-germinated seeds may be empty; cut-tests were not performed for the non-germinated seeds, but were performed for a small portion of non-imbibed seeds of the seed collection and c. 95% of them were found to be empty.

***Hieracium hoppeanum* s.l.**

Seeds of *Hieracium hoppeanum* s.l. were collected from Greveno in 2016. Seed collection was cleaned manually and viable seeds were separated from dead tissues and empty seeds using a seed aspirator at 0.1mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *H. hoppeanum*, but following an extended literature review of seed germination of other *Hieracium* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released by cold stratification or afterripening. Although the effect of light on seed germination was not always examined, *H. pratense* seems to produce seeds that germinate better in light than in darkness (Panebianco & Willemsen, 1976). Thus, germination experiments were performed at 20/10°C in light and in darkness. Moreover, the effect of cold stratification on seed germination was examined.

Final germination reached 99% and 96% at 20/10°C in darkness and light, respectively (Fig. 19) and the t_{50} was approximately 5 days. A small portion of seeds germinated during cold stratification in darkness (blue part of the bar “CS-20/10 D”), but almost 84% of the seeds germinated during cold stratification in light (blue part of the bar “CS-20/10 L”). Cold stratification failed to enhance the rate of germination in darkness and was not found to be crucial for seed germination. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are buried or on the soil surface.

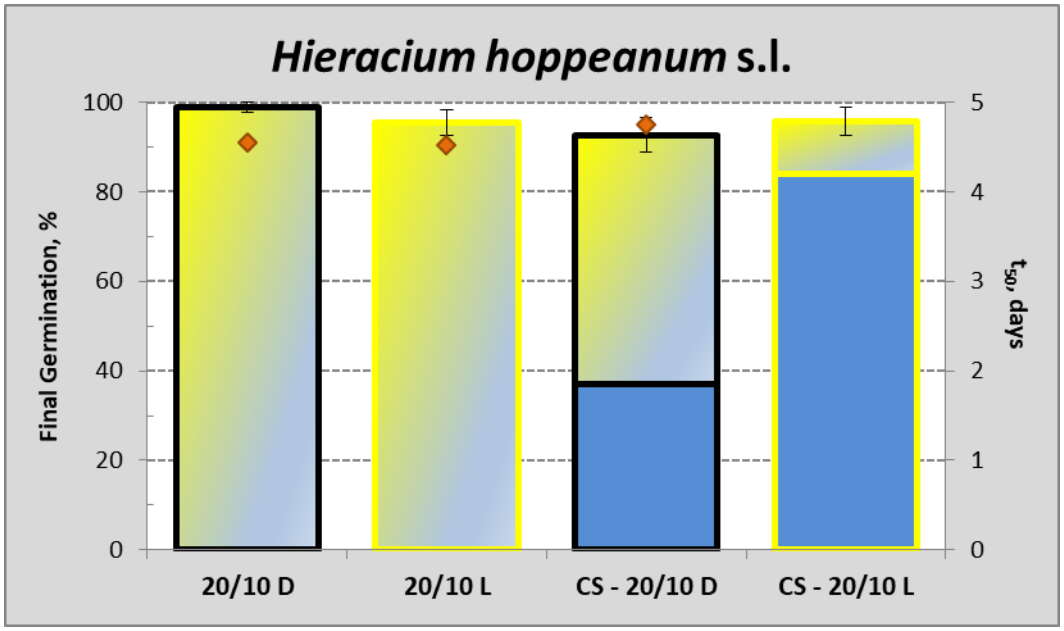

Figure 19: Final germination of *Hieracium hoppeanum* s.l. seeds at 20/10°C in light (L) and in darkness (D), after seed collection and after 1 month of cold stratification (CS).

Hypericum barbatum

Seeds of *Hypericum barbatum* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 400 and 500μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *H. barbatum*, but following an extended literature review of seed germination of other *Hypericum* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released by cold stratification or warm stratification. For the majority of *Hypericum* species, light seems to enhance seed germination. Thus, germination experiments were performed at 15 and 20/10°C, in light and in darkness. Moreover, the effect of cold and warm stratification on seed germination were examined.

Final germination reached c. 78% at both 15 and 20/10°C in light (Table 22) and the t_{50} was approximately 13 days. Darkness reduced final germination significantly, but secondary dormancy was not imposed. Both cold and warm stratification, as well as the addition of gibberellic acid failed to further enhance germination in a statistically significant way. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

Table 22: Final germination of *Hypericum barbatum* seeds at 15 and 20/10°C, in light and darkness, after various treatments.

Germination conditions	Pretreatment	Final germination (%)	Treatment	Germination after Treatment		
				Final germination (%)	s.e.	t_{50}
15°C, Light	AR	77	-	-	5,0	13
15°C, Dark	AR	23	Transfer to light	77	4,1	13
20/10°C, Light	AR	79	Addition of GA3	88	2,6	10
20/10°C, Dark	AR	8	Transfer to light	77	4,0	16
20/10°C, Light	AR+CS	68	-	-	3,8	12
20/10°C, Dark	AR+CS	0	Transfer to light	64	7,8	12
20/10°C, Light	AR+WS	27	Transfer at 15°C	52	5,5	4
20/10°C, Dark	AR+WS	3	Transfer at 15°C, in light	58	9,0	11

Luzula multiflora

Seeds of *Luzula multiflora* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and partially at 500μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Pegtel (1988), *L. multiflora* produces mainly non-dormant, light indifferent seeds, which germinate at 20 or 25/15°C. Due to high seed availability, germination experiments were performed at 20/10°C, under both light and darkness, with afterripened and both afterripened and cold stratified seeds. Final germination of afterripened seeds reached c. 100% in light and the t_{50} was approximately 13 days (Fig. 20). Cold stratification only affected the rate of germination, by reducing the t_{50} to approximately 5 days. Darkness reduced final germination significantly, but secondary dormancy was not imposed neither in afterripened nor in cold stratified and afterripened seeds. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

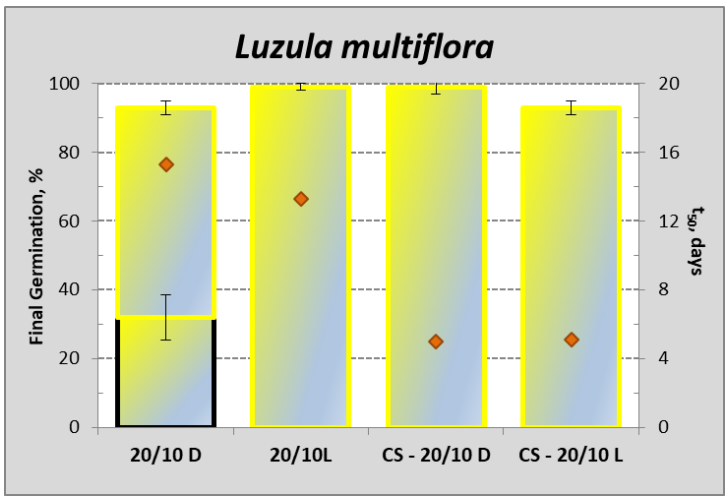

Figure 20: Final germination of *Luzula multiflora* afterripened and both cold stratified and afterripened (CS) seeds, at 20/10°C in light (L-yellow border on bars) and in darkness (D-black

border on bars). After the completion of germination in darkness, non-germinated seeds were moved from darkness to light (yellow border on D - bars).

Luzula spicata

Seeds of *Luzula spicata* were collected from Livadies, Alykaina and Greveno in 2016. Seed collections were cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and partially at 500μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Cummins & Miller (2000), *L. spicata* seeds did not germinate adequately at 20°C, not even after cold stratification, unless they are scarified. Germination experiments were performed with seeds of the most numerous collection (the one from Greveno) at 20/10°C in light, with afterripened and both afterripened and cold stratified (for 1-4 months) seeds. Final germination was null in all cases (data not shown). Therefore, afterripened seeds were left to germinate at 5°C in light (Fig. 21). Final germination reached 99% and the t_{50} was approximately 144 days. It is concluded that seeds germinate, with extremely low rate (within 4 months), under natural conditions by the end of winter.

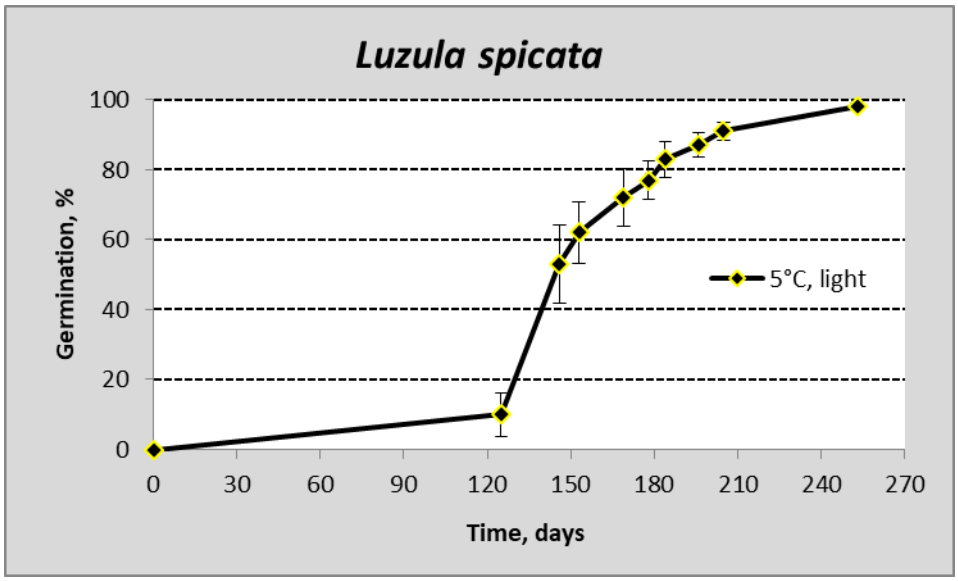

Figure 21: Light germination of *Luzula spicata* afterripened seeds, at 5°C.

Nardus stricta

Seeds of *Nardus stricta* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900μm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 1.7 and 2mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Grime et al. (1981), *N. stricta* produces physiologically dormant seeds; dormancy can be released by cold stratification and afterwards germination occur at 25/20°C in light.

Germination experiments were performed with afterripened seeds at 20/10°C in light and in darkness. Final germination reached 98% in light and the t_{50} was approximately 6 days (Fig. 22). Darkness did not affected seed germination in a statistically significant way. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are buried or on the soil surface.

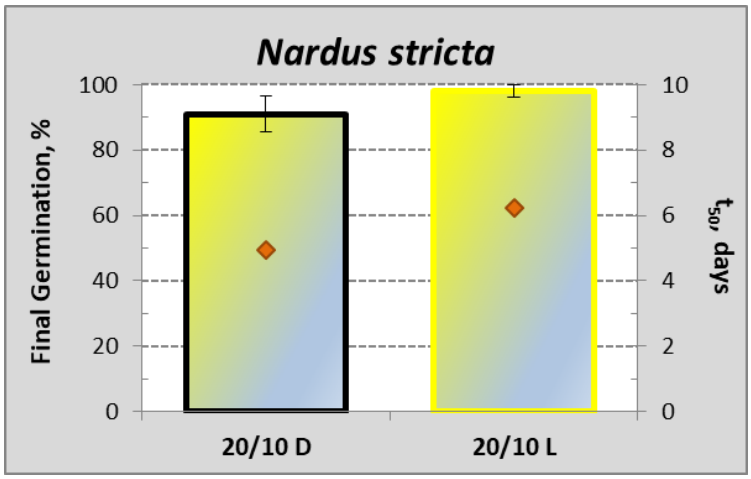

Figure 22: Final germination of *Nardus stricta* afterripened seeds, at 20/10°C in light (L) and in darkness (D).

Nepeta nuda

Seeds of *Nepeta nuda* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 1.7 and 2mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *N. nuda*, but following an extended literature review of seed germination of other *Nepeta* species (Baskin & Baskin, 2014), seeds were expected to be physiologically dormant and germinate in light after dormancy release by cold stratification. Thus, germination experiments were performed at 20/10°C in light. Final germination reached c. 45% and the addition of gibberellic acid failed to further enhance final germination (data not shown). However, the non-germinated seeds may be empty; cut-tests were not performed for the non-germinated seeds, but were performed for a small portion of non-imbibed seeds of the seed collection and c. 60% of them were found to be empty.

Phleum alpinum

Seeds of *Phleum alpinum* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 500 and 630µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 1.4mm air-flow. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Bliss (1958), *P. alpinum* produces non-dormant seeds that germinate better in light than in darkness. Thus, germination experiments were performed with afterripened seeds at 20/10°C in light. Final germination reached 96% in light and the t_{50} was approximately 3 days (Fig. 23). It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

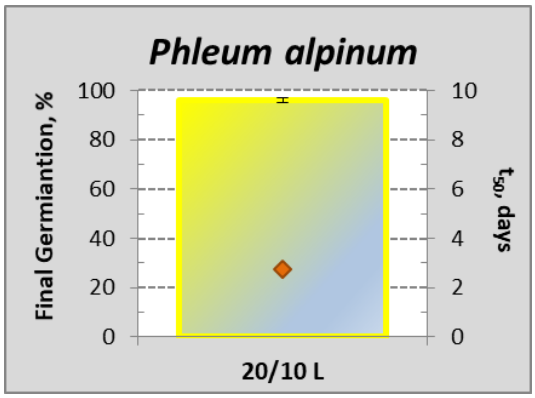

Figure 23: Final germination of *Phleum alpinum* afterripened seeds, at 20/10°C in light.

Potentilla recta* subsp. *laciniosa

Seeds of *Potentilla recta* subsp. *laciniosa* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 500, 630 and 900μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Baskin & Gaskin (1990), *P. recta* subsp. *laciniosa* produces non-dormant seeds that germinate better in light than in darkness. Thus, germination experiments were performed with afterripened seeds at 20/10°C in light, using five samples of 10 seeds each, due to low seed availability. Final germination reached 100% in light and the t_{50} was approximately 6 days (Fig. 24). It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

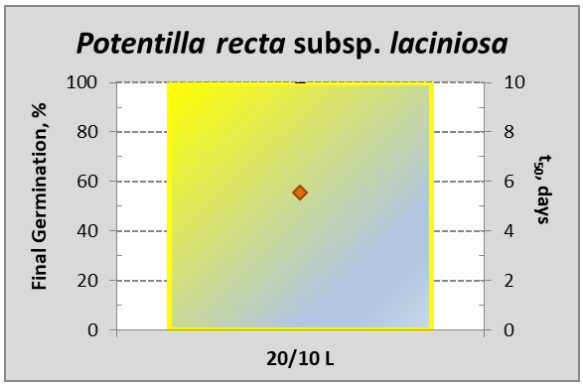

Figure 24: Final germination of *Potentilla recta* subsp. *laciniosa* afterripened seeds at 20/10°C in light.

Prunella laciniata

Seeds of *Prunella laciniata* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900µm. Afterwards, seeds were separated from dead tissues using a seed aspirator at 2.5 and 3.5mm air-flow, respectively. Finally, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Luna & Moreno (2009), seeds of *P. laciniata* are expected to be non-dormant and light requiring at 17.5 °C. Thus, germination experiments were performed with afterripened seeds at 15, 25 and 20/10°C in light and in darkness. Moreover, the effect of cold stratification was examined, since other *Prunella* species produce physiologically dormant seeds.

Final germination reached 100% in light at all temperatures tested and the t_{50} was approximately 3 days (Fig. 25). Darkness partially inhibited germination only at 15 and 20/10°C, but secondary dormancy was not imposed, since seeds germinated adequately when they were transferred from darkness to light (yellow border of the bars "15 D" and "20/10 D"). Cold stratification not only failed to enhance the rate of germination, but also negatively affected seed germination in darkness, since stratified seeds failed to germinate adequately at 20/10°C in darkness (black border of the bar "CS-20/10 D"). However, secondary dormancy was not imposed since seeds germinated adequately when they were transferred from darkness to light. It is concluded that seeds are able to germinate under natural conditions during autumn, if they are buried or on the soil surface.

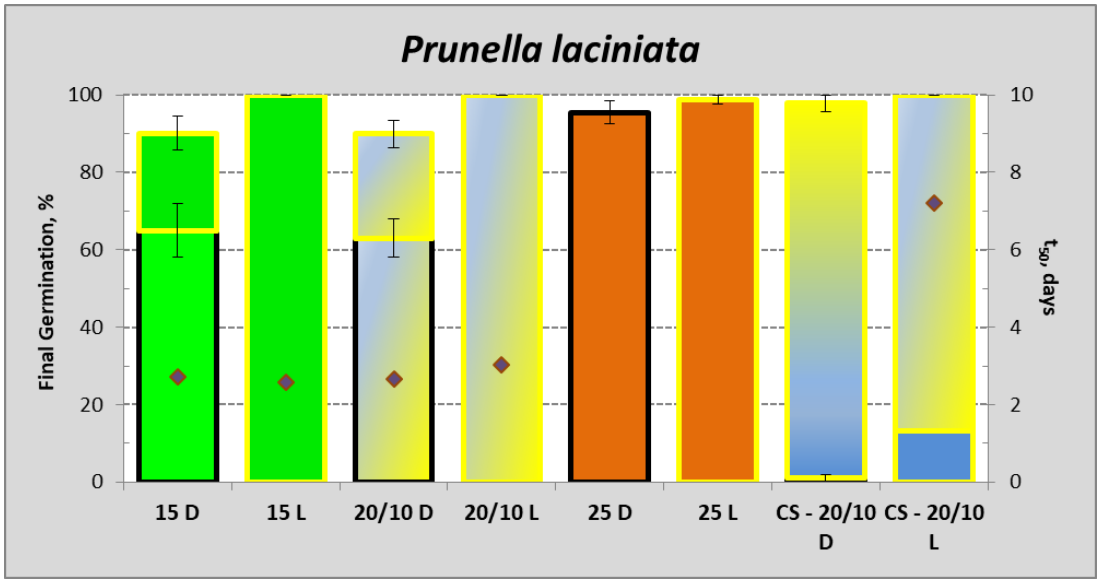

Figure 25: Final germination of *Prunella laciniata* afterripened and both afterripened and cold stratified (CS) seeds at 15, 20/10 and 25°C in light (L-yellow border on bars) and in darkness (D-black border on bars).

Rhinanthus pubescens

Seeds of *Rhinanthus pubescens* were collected from Greveno in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 1.4mm (sieves with larger wire mesh sizes will be helpful but were not available).

Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *R*. *pubescens*, but following an extended literature review of seed germination of other *Rhinanthus* species (Baskin & Baskin, 2014), seeds were expected to be physiologically dormant and dormancy can be released by cold stratification. Although the effect of light on seed germination was not always examined, *R*. *angustifolius* seems to produce seeds that germinate both in light and in darkness (Jensen, 2004). Thus, germination experiments were performed at 20/10°C in light and in darkness, using five samples of 15 seeds each, due to low seed availability. Final germination was null and the effect of cold and warm stratification as well as the addition of gibberellic acid on seed germination were examined. However, final germination was null in all cases (data not shown). Cut-tests were not performed for the non-germinated seeds, due to fungi infection, but cut-tests that have been performed for a small portion of non-imbibed seeds of the seed collection showed that c. 80% of them were dead and the remaining 20% of the seeds were empty.

Rumex acetosella

Seeds of *Rumex acetosella* were collected from the grasslands among Livadies and Greveno in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and partially at 500μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

According to Grime et al. (1981), *R*. *acetosella* produces physiologically dormant seeds that germinate after warm stratification at 20/15°C in light and in darkness. Thus, germination experiments were performed with afterripened seeds at 15 and 20/10°C in light and in darkness. Final germination was null and the effect of warm stratification as well as the addition of gibberellic acid on seed germination were examined. However, final germination was null in all cases (data not shown).

Silene roemeri* subsp. *macrocarpa

Seeds of *Silene roemeri* subsp. *macrocarpa* were collected from Livadies in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 630 and 900μm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *S*. *roemeri* subsp. *macrocarpa*, but following an extended literature review of seed germination of other *Silene* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released by cold or warm stratification. Although the effect of light on seed germination was not always examined, some species seems to produce seeds that germinate better in light than in darkness and others seems to produce seeds that germinate better in darkness than in light. Thus, germination experiments were performed with afterripened seeds

at 15, 25 and 20/10°C in light and in darkness. Moreover, the effect of cold stratification was examined.

Final germination reached c. 100% in light and in darkness at all temperatures tested and the t_{50} was approximately 4-5 days (Fig. 26). Cold stratification did not enhance the rate of germination, and a portion of seeds (c. 60%) germinated during stratification in light (blue part of the bar "CS-20/10 L"). It is concluded that seeds are able to germinate under natural conditions in autumn, if they are buried or on the soil surface.

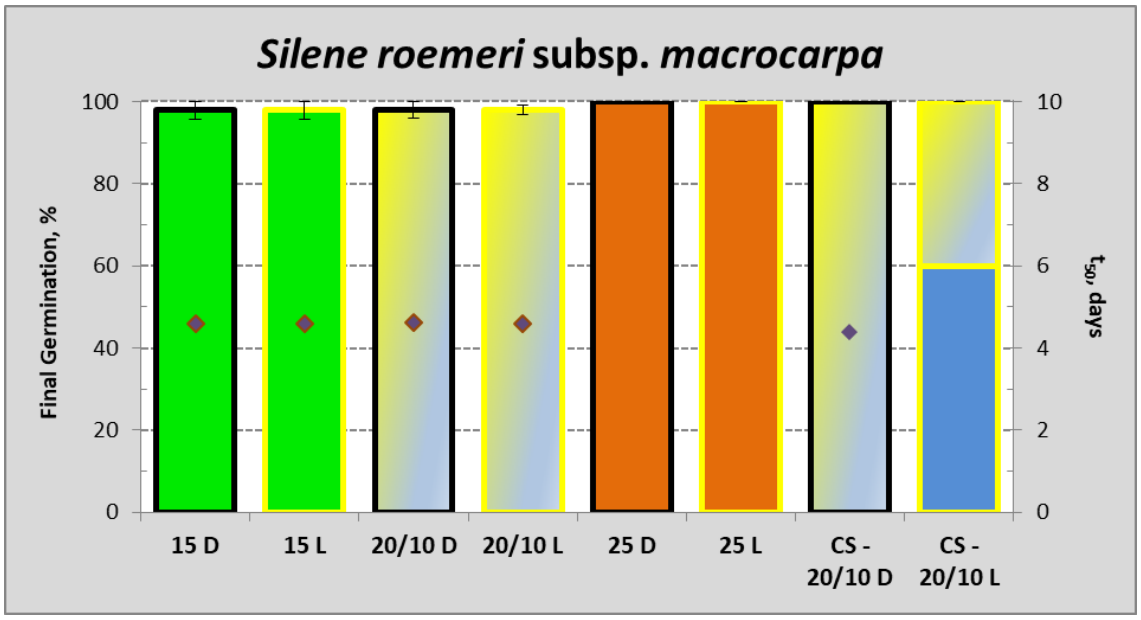

Figure 26: Final germination of *Silene roemeri* subsp. *macrocarpa* afterripened and both afterripened and cold stratified (CS) seeds at 15, 20/10 and 25°C in light (L) and in darkness (D).

Stipa capillata

Seeds of *Stipa capillata* were collected from Gkioza in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 900μm and 1.4mm. Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

Following an extended literature review of seed germination of other *Stipa* species (Baskin & Baskin, 2014), seeds were expected to be either non-dormant or physiologically dormant. If dormant, dormancy can be released by cold or warm stratification. Thus, germination experiments were performed with afterripened seeds at 20/10°C in light, using five samples of 12 seeds each, due to low seed availability. Final germination reached c. 91% and the t_{50} was approximately 7 days (Fig. 27). It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

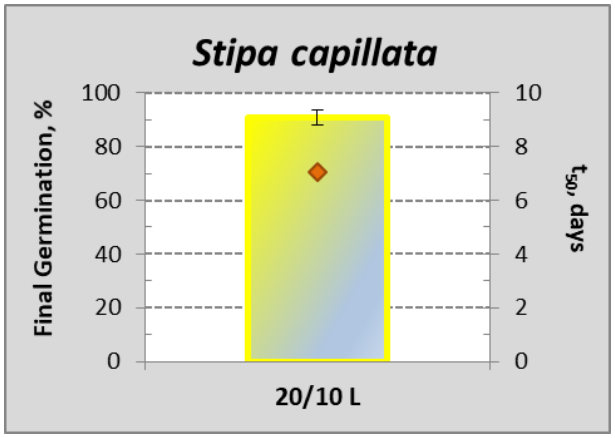

Figure 27: Final germination of *Stipa capillata* afterripened seeds at 20/10°C in light.

Xeranthemum cylindraceum

Seeds of *Xeranthemum cylindraceum* were collected from Isomata in 2016. Seed collection was cleaned manually, using steel sieves of various wire mesh sizes and viable seeds were retained mainly at 1.4mm (sieves with larger wire mesh sizes will be helpful but were not available). Afterwards, seed collection was homogenized and seeds were maintained under laboratory conditions (c. 22 °C), in order to air-dry and afterripen.

This is the first study of seed germination of *X. cylindraceum* and germination experiments were performed with afterripened seeds at 20/10°C in light, using five samples of 12 seeds each, due to low seed availability. Final germination reached 100% and the t_{50} was approximately 5 days (Fig. 28). It is concluded that seeds are able to germinate under natural conditions during autumn, if they are on the soil surface.

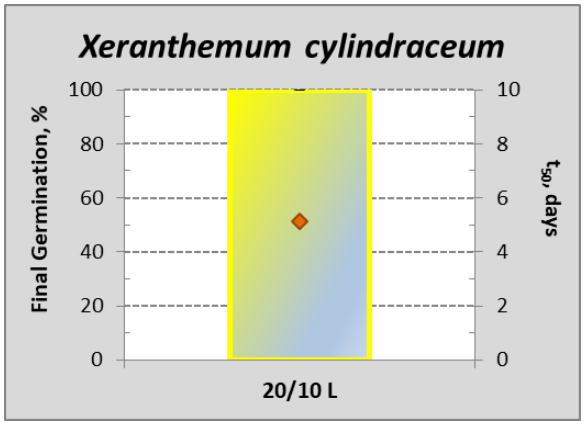

Figure 28: Final germination of *Xeranthemum cylindraceum* afterripened seeds at 20/10°C in light.

5. Instructions for seed collection, handling, storage and seed germination for the keystone species of all the target habitats

Instructions for seed collection, handling, storage and seed germination for 10 keystone or typical temporary pond species (3170*) and 28 mountain grassland species (6210*, 6230*) on Mt. Oiti and Mt. Kallidromo are given in Tables 23 and 24, respectively.

Table 23: Protocols for seed collection, handling, storage and seed germination for 10 keystone or typical temporary pond species (3170*) and for *Pinus nigra* (9530*).

Taxon	Proposed month for seed collection [a]	Seed handling (mesh size of sieves, airflow in mm)	Optimal germination conditions [b]	Seed storage [g]
Heliotropium supinum	September – October	900μm-1.4mm, -	GA_3 - 20/10 °C, L>D [c]	UoA
Juncus bufonius	July	125-300μm, -	CS&WS - 15 °C, L>D [d]	UoA
Limosella aquatica	June – July	125-300μm, -	WS - 15-20 °C, L>D [d]	UoA
Lythrum portula	June – July	300-500μm, -	20 °C, L [d]	UoA
Lythrum thymifolia	July – August	400-500μm, -	CS&WS - 15 °C, L=D	UoA
Myosurus minimus	June – July	400μm-630μm, -	AR - 15 °C, L>D	UoA
Polygonum arenastrum	November	-	- [e]	UoA
Ranunculus lateriflorus	June – July	630-900μm, -	AR - 15 °C, L>D	UoA
Verbena supina	September – November	500-630μm, -	(AR or CS) - 30 °C, L>D [f]	UoA
*Veronica oetaea**	July	125-300μm, -	5 °C, L>D	UoA
Pinus nigra	November – February	-	15-20 °C, L=D[h]	UoA

a: Proposed month is based on observations made during seed collections and apply only to the habitats at the specific conditions (altitude, climate) of Mt. Oiti and Mt. Kallidromo.

b: L>D: optimal germination in light; D>L: optimal germination in dark; L=D: light indifference; L: only germination in light tested; AR: afterripening necessary; CS: pretreatment with cold stratification necessary; WS: pretreatment with warm stratification necessary.

c: Optimal germination never exceeded 30%.

d: Optimal germination never exceeded 70%.

e: Germination experiments were not performed.

f: Seed germination immediately after seed collection (without any pretreatment) at 30 °C in light, was not examined and therefore the need of any pretreatment is uncertain.

g: Seeds were stored at -20°C, in the Seedbank of the University of Athens.

h: Based on literature (Skordilis & Thanos 1997). Light and cold stratification increase germination rate but not final germination.

Table 24: Seed collections from 28 mountain grassland species (6210*, 6230*).

Taxon	Proposed month for seed collection [a]	Seed handling (mesh size of sieves, airflow in mm)	Conditions for optimal seed germination [e]	Seed storage [k]
Achillea crithmifolia	August	300-500µm, 0.2-0.3mm	15 or 20/10 °C, L=D	UoA
Allium achaium	August	-, - [b]	15 °C, D>L	UoA
Alopecurus gerardii	July – August	900µm-1.4mm, -	20/10 °C, L	UoA
Anthoxanthum odoratum	June – July	-, 1mm	20/10 °C, L>D	UoA
Bellardiochloa variegata	July – August	-, 0.8mm	20/10 °C, L=D	UoA
Brachypodium pinnatum	July	500-630µm, 0.5mm	20/10 °C, L	UoA
Carex ovalis	July – August	630-900µm, -	(CS) - 20/10 °C, L>D [f]	UoA
Centaurea nervosa subsp. *promota*	July – August	Seeds were empty due to insect infection		
Chrysopogon gryllus	July	-, - [b,c]	WS - 20/10 °C, L=D [c,g]	UoA
Dianthus tymphresteus	July	-, - [b]	20 °C, L>D	UoA
Festuca sp.	August	300µm, -	15 or 20/10 °C, L=D	UoA
Festuca polita	June – July	500-900µm, 1mm	20/10 °C, L=D	UoA
Festuca cf. *valesiaca*	July	630-900µm, 1mm	- [h]	UoA
Galium verum	October	500-900µm, - [c]	- [c]	UoA
Hieracium hoppeanum s.l.	July	-, 0.1mm	20/10 °C, L=D	UoA
Hypericum barbatum	July – August	400-500µm, -	15 or 20/10 °C, L>D	UoA
Luzula multiflora	July – August	500-630µm, -	20/10 °C, L>D	UoA
Luzula spicata	July – August	500-630µm, -	5 °C, L	UoA
Nardus stricta	July – August	630-900µm, 1.7-2mm	20/10 °C, L=D	UoA
Nepeta nuda	July – August	-, 1.7-2mm [c]	20/10 °C, L [c]	UoA
Phleum alpinum	July – August	500-630µm, 1.4mm	20/10 °C, L	UoA
Potentilla recta subsp. *laciniosa*	July – August	500-900µm, -	20/10 °C, L	UoA
Prunella laciniata	July – August	630-900µm, 2.5-3.5mm	15 or 20/10 °C, L>D	UoA
Rhinanthus pubescens	July	1.4mm, - [d]	- [i]	UoA
Rumex acetosella	July – August	500-630µm, -	- [j]	UoA
Silene roemeri subsp. *macrocarpa*	July – August	630-900µm, -	15 or 20/10 °C, L=D	UoA
Stipa capillata	June – July	900µm-1.4mm, -	20/10 °C, L	UoA
Xeranthemum cylindraceum	July	1.4mm, - [d]	20/10 °C, L	UoA

a: Proposed month is based on observations made during seed collections and apply only to the habitats at the specific conditions (altitude, climate) of Mt. Oiti and Mt. Kallidromo.

b: Seed collection was cleaned manually without the usage of steel sieves or seed aspirator.

c: Seed collection contains mainly empty seeds. In the case of *Nepeta nuda* the percentage of empty seeds is high (c. 60%).

d: Sieves with larger mesh sizes would be helpful.

e: L>D: optimal germination in light; D>L: optimal germination in dark; L=D: light indifference; L: only germination in light tested; CS: pretreatment with cold stratification necessary; WS: pretreatment with warm stratification necessary. All germination tests were applied to afterripened seeds, therefore the need for afterripening is unknown.

f: Cold stratification only promotes the rate of seed germination.

g: Optimal germination never exceeded 30%.

h: Germination experiments were not performed, due to low seed availability.

i: Seed collection contains mainly dead and a few empty seeds.

j: Optimal germination conditions were not identified.

k: Seeds were stored at -20°C, in the Seedbank of the University of Athens (UoA).

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