



Three phylogenetically distant shade-tolerant temperate forest herbs have similar seed germination syndromes

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Abstract Plants growing in the same environment typically share ecophysiological characteristics enabling them to survive and reproduce when exposed to a common selection pressure. As such, seedlings establishing in temperate forest habitats cope with similar issues of low light availability and climate seasonality. We examined similarities in the germination syndromes in three shade-tolerant temperate forest herbs, *Allium ursinum* (Amaryllidaceae), *Mercurialis perennis* (Euphorbiaceae) and *Dioscorea communis* (Dioscoreaceae) with a distant phylogenetic affiliation. Seeds of *M. perennis* and *A. ursinum* are dispersed in spring and those of *D. communis* in autumn. Experiments on phenology of seed germination and seedling emergence in natural conditions revealed that seeds of all three species germinate in the following autumn. Seedlings do not emerge immediately but rather remain below the soil surface until late winter or early spring. Experiments in controlled laboratory conditions showed that seeds germinated best at intermediate autumn or spring temperatures (between 10 and 15°C) following a period at high summer temperatures (around 20°C). Seed germination of *Allium ursinum* is strongly photoinhibited, while moderate photoinhibition and no photoinhibition at all was observed in *Dioscorea communis* and *Mercurialis perennis*, respectively. Although seeds of all three species

are endospermic at dispersal, no embryo growth prior to germination was observed. The cotyledons functioned as a haustorium, recuperating the nutrient reserves in the endosperm post-germination. It can be concluded that although phylogenetically unrelated, the three species studied show a remarkable similarity in germination and seedling emergence strategy that is commonly observed in plants adapted to growing in shady conditions.

Keywords Dormancy · Embryo · Germination · Haustorium · Seeds · Woodlands

Introduction

Recent attention has been drawn to the importance of the belowground functional ecology of plants (Klimešová et al. 2018). The first stages in a plants life cycle usually take place below or near the soil surface as seeds germinate and start exploring the soil with their roots and, if buried, find their way to emerge above the soil surface. Below ground processes of seed germination and seedling emergence are relatively straightforward for many plant species. Once seeds are triggered to germinate, the embryo elongates until the radicle protrudes the seed coat and the shoot emerges shortly thereafter. In shade-tolerant species, however, more extensive belowground processes that precede seedling emergence, such as embryo growth prior to germination, root development and dormancy of the shoot, frequently occur (e.g. Barton and Schroeder 1942). Indeed, it has been demonstrated that seeds of shade-

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tolerant species share several ecophysiological characteristics, which are quite unique adaptations to the forest habitat (Bierzuchudek 1982; Whigham 2004).

Seeds of plants establishing in shade tend to have similar seed dimensions. Shade-tolerant plants in temperate forests typically have seeds that are larger as compared to congeneric open habitat species (Thompson and Hodkinson 1998) and tend to have small embryos and copious endosperm at dispersal, termed endospermic seeds (Hodgson and Mackey 1986; Vandeloos et al. 2012). Already early in the twentieth century, it was known that temperate forest species typically have seeds with a small embryo embedded in copious endosperm (Crocker 1916) and that very often the embryo grows within the seed after dispersal but before germination (Lakon 1911; Findeis 1917). The amount of embryo elongation prior to germination is species specific and is likely related to the initial size of the embryo and to the size of the seed (Vandeloos and Van Assche 2008a; Vandeloos et al. 2012). In some species it seems there is no exact correlation between the amount of embryo elongation and the onset of radicle protrusion, as embryo elongation and germination appear to be two different but interacting temperature dependent processes (Newton et al. 2013; Carta et al. 2014). Moreover, in certain shade-tolerant species with endospermic seeds, there is no embryo growth at all prior to germination and the embryo serves rather as a haustorium recuperating the reserves in the endosperm post germination (Barton and Schroeder 1942; Kondo et al. 2015).

Besides similarities in seed morphological characteristics, shade-tolerant plants also share similarities in the seed germination syndrome (Angevine and Chabot 1979; Baskin and Baskin 1988; Vandeloos and Van Assche 2008a; Baskin and Baskin 2014). In temperate and mountain climate forests, seedlings very often emerge in late winter and early spring thus avoiding frost damage, but profiting light availability before the canopy closes. In this context, two main strategies that enable seedling emergence in spring have been found. Firstly, seeds of some species require a period of low temperatures (< 10°C) to break dormancy, resulting in seed germination when temperatures rise in early spring and followed by seedling emergence shortly thereafter (Walck et al. 2000; Vandeloos and Van Assche 2008b). Secondly, many woodland species germinate at intermediate temperatures (about 10 to 15°C) in autumn, but seedlings remain belowground until temperatures rise in late winter and early spring (e.g. Baskin and Baskin 1985; Carta et al. 2014). A

strategy were seeds germinate in autumn and emerge in spring is often termed epicotyl dormancy in case an additional cold period is required to overcome shoot dormancy (Barton 1933). For species that germinate in autumn, the pre-treatment required to overcome dormancy can range from a 4 week period at 20°C in for example *Scilla bifolia* L. (Vandeloos and Van Assche 2008a), to more prolonged sequences of low winter and high summer temperatures in for example *Convallaria majalis* L. (Barton and Schroeder 1942). Given that seeds of shade-tolerant species are relatively large they usually do not require light or fluctuating temperatures to trigger germination (Jankowska-Blaszczuk and Daws 2007). Recent reviews have shown, however, that germination of some forest species is partially or completely inhibited by light, preventing germination at the soil surface (Carta et al. 2017; Vandeloos et al. 2018).

In this study, we aim to elucidate and compare embryo growth, seed germination in the soil and seedling emergence in natural conditions of *Allium ursinum* L. (Amaryllidaceae), *Dioscorea communis* (L.) Caddick and Wilkin (Dioscoreaceae) and *Mercurialis perennis* L. (Euphorbiaceae). These are three species that typically grow in the understory of temperate climate forests (Herny et al. 1999), but with closely related species that occupy very different habitat types. If seed characteristics of the species studied resemble more those of other less closely related shade-tolerant species, than those of close relatives occupying other habitats this may point to convergent evolution of seed characters in shade-tolerant forest species. Information on how dormancy break and germination is regulated in *A. ursinum*, *D. communis* and *M. perennis* is only fragmentary (Mukerji 1936; Burkill 1939; Ernst 1979). Therefore, experiments were performed in natural conditions to study phenology of seed germination and seedling emergence and in controlled conditions to test how temperature and light regulate seed germination. These results are discussed in relation to seed characteristics from phylogenetically and ecologically closely related species retrieved from literature.

Material and methods

Species description

Allium ursinum is a bulbous perennial growing in woodland and shady hedge banks (Tutin 1957). Reproduction occurs mainly through seeds, although vegetative

reproduction with bulbils can occur. Populations of *A. ursinum* are widely distributed in Europe, except in the evergreen regions of the Mediterranean, and the species' range extends eastwards all the way to Kamchatka, Russia. *Mercurialis perennis* is a dioecious, rhizomatous, perennial herb with predominant vegetative reproduction (Jefferson 2008). The species also grows in different types of woodland and more rarely in tall grasslands throughout Europe, except for the most northern region. Its distribution range extends to North Africa in the south and Iran in the east. *Dioscorea communis* is a climbing perennial herb with a large tuber (Burkill 1944), growing mostly in woodland margins, scrub and hedges. Reproduction only occurs through seeds. Similar to *M. perennis*, the distribution range extends to North Africa in the south and Iran in the East. The northern edge of the distribution range, however, is much smaller, extending only to Belgium, central Germany and Hungary. Seeds of *A. ursinum* and *D. communis* have embryos of the linear type whereas seeds of *M. perennis* have a spatulate embryo (Martin 1946).

Phenology of seed germination and seedling emergence

Allium ursinum seeds were collected in June 2004 from plants growing in a garden near Leuven, Belgium, where they had been introduced from wild plants growing about 5 km south from Leuven (50°49' N, 4°39' E). Seeds of *D. communis* were sampled in October 2003 in the same garden where they were introduced from wild material collected in Oudenaarde, Belgium (50°48' N, 3°35' E). The effect of cultivation on dormancy loss and germination strategy is expected to be minimal for perennials (Ensslin et al. 2018). Seeds of *M. perennis* were collected in May 2005 from a large wild population in a ravine forest in Anseremme, Belgium (50°14' N, 4°54' E). Seeds were always collected from multiple plants and pooled. After being collected, seeds were cleaned and stored at room temperature (about 20°C) and ambient relative humidity. To prevent seed mortality due to dry storage, experiments were started within two weeks after harvesting. Visibly nonviable seeds were excluded from the experiments. For each species, 50 seeds were sown at 1 cm depth in each of three separate plastic pots (9 × 9 × 9 cm) filled with potting soil and buried at soil level. Emerged seedlings were counted and removed every week for one year (*A. ursinum* and *M. perennis*) or two years (*D. communis*). Pots were covered with a net to prevent disturbance by birds and a

molluscicide was applied. Leaf litter was removed regularly from the pots to prevent covering by leaves.

To check germination phenology, 100 seeds of *D. communis* and 100 seeds of *A. ursinum* were buried in each of eight nylon bags and buried at 5 cm depth in October 2003 and June 2005, respectively, in a garden near Leuven. One bag was excavated every two months, and both germinated and non-germinated but intact seeds were counted. An insufficient number of seeds were available to perform this experiment for *M. perennis*.

Seed germination experiments

Seeds of *M. perennis* were collected in Anseremme in May 2006. Three replicates of 50 seeds were placed on filter paper moistened with distilled water in 9 cm diameter Petri-dishes. In control experiments, seeds were incubated at constant temperatures of 5°C, 10°C and 23°C, and at daily fluctuating temperatures of 15/6°C and 20/10°C (12h/12h). Seeds were incubated in light with a daily photoperiod of 12/12h, with the light period coinciding with the high-temperature part of the daily fluctuating temperature regime (PAR = 36 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with light provided by cool white fluorescent tubes, Philips TLD 80). In the second experiment, seeds were placed at 23°C for 4, 8, 12 and 16 weeks, and subsequently transferred to 5°C, 10°C, 15/6°C and 20/10°C for 16 weeks.

Laboratory germination experiments were performed with *A. ursinum* seeds collected from a natural population in the Botanic Garden Meise (50°55' N, 4°19' E) in June 2014. Three replicates of 40 seeds each were placed on agar in 9-cm diameter Petri-dishes and incubated for 36 weeks at constant 5°C, 11°C, 16°C and 20°C. Additional Petri-dishes were placed at a sequence of high to low temperatures, simulating the summer, autumn and winter sequence (Table 2). Seeds were incubated in light (8/16h at 11°C and 20°C; 12/12h at 5°C and 16°C) or in darkness by wrapping them in aluminium foil.

Seeds of *D. communis* were collected in October 2016 from plants growing in the Botanic Garden Meise but originating from Angers, France. Two replicates of 25 seeds were placed on agar in 9-cm diameter Petri-dishes and incubated for 64 weeks at constant temperatures of 5°C, 11°C, 16°C, 20°C and daily fluctuating temperatures of 23/9°C (12h/12h). Seed germination was also tested by moving Petri-dishes through a

sequence of winter-summer-autumn-winter temperatures or parts thereof for 64 weeks (Table 4).

In all experiments, germinated seeds were counted and removed from the dishes every week. Seeds incubated in darkness were exposed to light for a maximal one minute period during weekly germination counts. At the end of the experiment, viability was checked with a cut test. Only seeds that were white and firm were included in the results.

Embryo size

The size of the embryo as compared to the size of the seeds was determined by cutting seeds in half and measuring embryo length and seed length using a binocular microscope with a measuring ocular. In the case of *A. ursinum* and *D. communis* this was done for 20 freshly collected seeds and for 20 seeds randomly picked from the laboratory experiment immediately after germination. We also measured embryo length, seed length, embryo surface area and seed surface area of a single seed of *M. perennis* and, for comparative reasons, of eight additional *Allium* species available from the Botanic Garden Meise (Table 6).

Data analysis

The effects of light and temperature treatments on final germination in each species were analysed using a generalized linear model (GLM), with a logit link function and a binomial error structure, followed by a likelihood test. Significant differences in embryo size between freshly dispersed seeds and seeds at the moment of germination were tested for using a t-test. Assumptions for statistical analyses were checked prior to the analyses. All statistical analyses were performed in R Development Core Team (2013).

Results

Phenology of germination and seedling emergence

Seedlings of *M. perennis* and *A. ursinum* emerged after the first winter following sowing whereas seedlings of *D. communis* emerged after the second winter. Seeds of *M. perennis* buried in May 2005 started emerging in

January 2006 during frost free periods (Fig. 1a). By the end of March, almost 70% of the seeds had emerged as seedlings. Seedling of *A. ursinum* emerged in a very short time window from mid-March 2005 onwards when temperatures started rising (Fig. 1b). By the end of March, about 95% of the buried seeds had emerged as seedlings. Seeds of *A. ursinum* buried in nylon bags in June 2004 had germinated to 100% in the bag exhumed in October 2005 whereas none had germinated in the bags exhumed in August 2005. Seeds of *D. communis* buried in October 2003 emerged from March until mid-April 2005, by which time about 87% of the seeds buried had emerged as seedlings (Fig. 1c). In the nylon bag exhumed in October 2004, 53% of the seeds of *D. communis* had germinated whereas 62% of the seeds had germinated in February 2005. No additional germinated seeds were found thereafter.

Seed germination in controlled conditions

When incubated at one temperature condition without pretreatment, seeds of *Mercurialis perennis* germinated to 26.5% (binomial 95% CI: 18.7%, 35.7%) after 40 weeks at 15/6°C whereas none had germinated at any of the other temperature conditions tested. A pre-treatment at 23°C resulted in significantly higher germination at lower temperatures (Table 1). Seed germination percentage at 15/6°C increased up to 84.2%, following a four-week pretreatment at 23°C (Fig. 2). Following pre-treatment, seeds also germinated at 5°C and 10°C, but did not exceed 32% (Table 1).

Seeds of *A. ursinum* were strongly photoinhibited and did not germinate when exposed to light (Table 2). Without any pretreatment, seeds did not germinate within 36 weeks at any of the temperature conditions, except for a very low percentage at 16°C. Seeds that were incubated in a sequence of high to low temperatures germinated to significantly higher percentages when they were placed at 16°C for at least 10 weeks and subsequently transferred to 11°C (Tables 2, 3). Seeds that were placed at 20°C prior to transfer to 16°C already started germinating at 16°C and continued to germinate at 11°C (Fig. 3).

Seeds of *D. communis* germinated to > 90% when incubated at 16°C for 64 weeks both in light and in darkness (Table 4). Prolonged duration of incubation at 20°C, 16°C and 11°C significantly increased germination of *D. communis* (Table 5). Germination at 16°C was

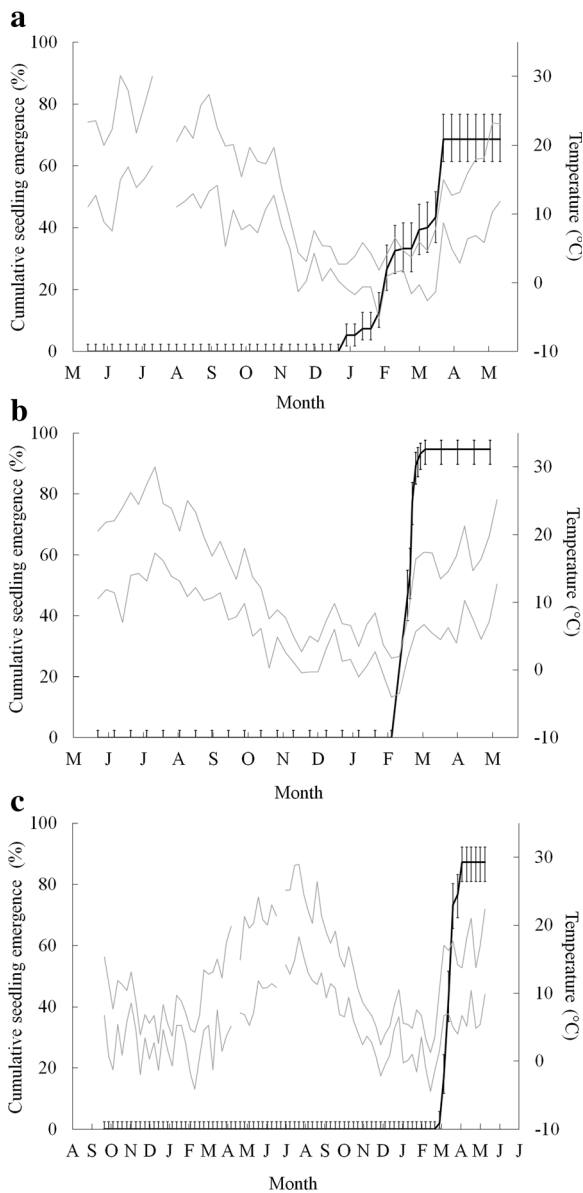


Fig. 1 Cumulative seedling emergence percentage (black line) of **a** – *Mercurialis perennis* seeds buried in May 2005, **b** – *Allium ursinum* seeds buried in June 2004 and **c** – *Dioscorea communis* seeds buried in October 2003. Error bars denote 95% binomial confidence intervals. Grey lines indicate daily maximum and minimum air temperatures averaged over a one week period. Measurements were not available for two weeks in July 2005 and for one week in May 2004 and in July 2004

faster and more synchronized when seeds were pre-treated first at 20°C. A high-temperature pre-treatment also resulted in higher germination percentages at low temperatures of 11°C and 5°C. A pre-treatment at 5°C significantly reduced final germination percentages

Table 1 Results of GLM on the effects of duration of pre-treatment at 23°C and of temperature during incubation on final seed germination of *Mercurialis perennis*

	Estimate	SE	z	P
(Intercept)	-0.69	0.11	-6.02	< 0.001
Duration	0.14	0.01	12.57	< 0.001
5°C	-3.78	0.20	-18.61	< 0.001
10°C	-2.01	0.13	-15.43	< 0.001

Incubation temperatures of 5°C and 10°C were tested against germination at daily fluctuating temperatures of 15/6°C (12h/12h). Seeds did not germinate at 20/10°C and as such were left out of the analysis

(Table 5). Although high germination percentages were reached in both light and darkness, seeds germinated significantly better in darkness.

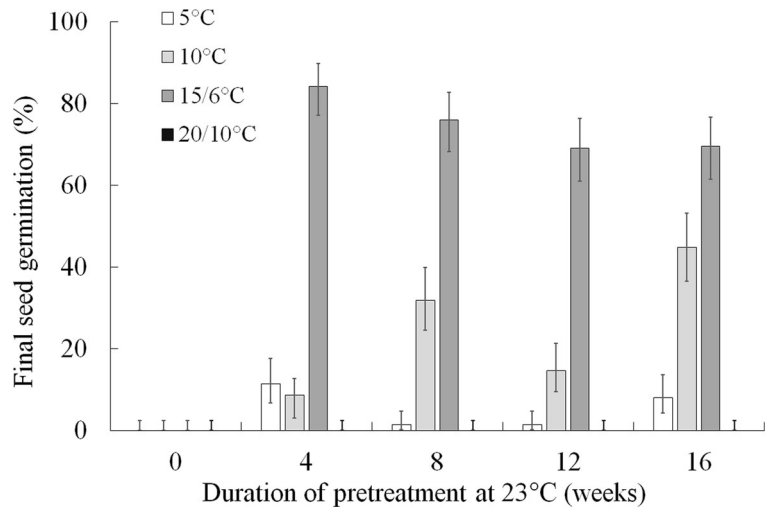
Embryo size

Seeds of *M. perennis* have spatulate embryos and occupy about 22% of the surface area in fresh seeds cut in half along the longest plane. Embryo growth prior to germination was not measured. In fresh *A. ursinum* seeds, the area occupied by the embryo is about 8% of the area of the seed, which is much lower as compared to that of other *Allium* species studied (Table 6). The embryo length in seeds of *A. ursinum* after dispersal (0.88 ± 0.03 mm, mean \pm SE) had not increased significantly ($P = 0.24$) at the moment of germination (0.93 ± 0.03 mm). The average seed length was 2.32 ± 0.04 mm. The embryo in seeds of *D. communis* occupies about 4.5% of the surface area in freshly dispersed seeds. The embryo length in freshly dispersed *D. communis* seeds was 1.60 ± 0.07 mm, which had increased significantly ($P = 0.03$) to 2.13 ± 0.06 mm in germinated seeds. This increase was, however, very small as compared to the mean seed length of 5.10 ± 0.07 mm and mainly caused by elongation at the radicular end when protruding the seed coat.

Discussion

Our experiments have shown that seeds of *A. ursinum* and *D. communis* germinate in autumn and seedlings emerge in late winter and spring. Seedlings of *M. perennis* also emerged in late winter whereas

Fig. 2 Final seed germination percentage of *Mercurialis perennis* after 16 weeks at 5°C, 10°C, 15/6°C and 20/10°C following pretreatment of 0, 4, 8, 12 and 16 weeks at 23°C. Error bars denote 95% binomial confidence intervals



laboratory experiments showed that seeds had probably already germinated in autumn. Since embryo growth prior to germination did not occur and seeds did not germinate immediately, all three species are thought to have physiological dormancy (Baskin and Baskin 2004). Dormancy is reduced during a warm summer period and seeds subsequently germinate when temperatures drop in autumn. The seedlings remain below the soil surface until they emerge in late winter and early spring when temperatures start to increase. This germination syndrome is

typically found in other shade-tolerant temperate and mountain forest herbs (see e.g. Baskin and Baskin 1985; Vandeloek and Van Assche 2008a; Copete et al. 2011).

Temperature and light requirement for dormancy break and germination

Seed germination percentages for *M. perennis* obtained in our experiments were much higher as compared to previous efforts (Jefferson 2008). This discrepancy can be explained

Table 2 Final seed germination percentage and 95% binomial confidence intervals of *Allium ursinum* incubated at different temperatures in darkness

20°C	16°C	11°C	5°C	36 weeks	Light			Darkness		
					Germ [%]	CI-	CI+	Germ [%]	CI-	CI+
0	→ 0	→ 0	→ 36 weeks	0	0	3	0	0	3	
0	→ 0	→ 36 weeks	→ 0	0	0	3	0	0	3	
0	→ 36 weeks	→ 0	→ 0	0	0	3	0.8	0.02	4.6	
36 weeks	→ 0	→ 0	→ 0	0	0	3	0	0	3	
10 weeks	→ 10 weeks	→ 10 weeks	→ 6 weeks	0	0	3	72.5	63.6	80.3	
4 weeks	→ 10 weeks	→ 10 weeks	→ 12 weeks	0	0	3	74.2	65.4	81.7	
10 weeks	→ 0	→ 16 weeks	→ 10 weeks	0	0	3	0.8	0.02	4.6	
0	→ 10 weeks	→ 16 weeks	→ 10 weeks	0	0	3	61.7	52.4	70.4	
0	→ 10 weeks	→ 10 weeks	→ 16 weeks	0	0	3	66.7	57.5	75.0	
0	→ 4 weeks	→ 10 weeks	→ 22 weeks	0	0	3	0	0	3	
0	→ 0	→ 16 weeks	→ 20 weeks	0	0	3	0	0	3	
0	→ 0	→ 10 weeks	→ 26 weeks	0	0	3	0	0	3	

No germination had occurred in light. Seeds were incubated at one single temperature for 36 weeks or at a sequence of high to low temperatures for a total of 36 weeks

Table 3 Results of GLM on the effects of temperature conditions during incubation in darkness on seed germination of *Allium ursinum*

	Estimate	SE	z	P
Intercept	-8.77	1.40	-6.25	< 0.001
20°C	0.05	0.04	1.54	0.12
16°C	1.20	0.18	6.73	< 0.001
11°C	-0.02	0.03	-0.59	0.55

Duration of incubation at 20°C, 16°C and 11°C was tested

by the fact that seeds were previously subjected to sub-optimal conditions for physiological dormancy break, such as exposure to frost (Mukerji 1936) or too high temperatures (Slade and Causton 1979). The requirement of passing through a sequence of relatively high summer temperatures, followed by low autumn temperatures for optimal germination in *A. ursinum*, was already observed (Ernst 1979). Seeds of *A. ursinum* are very strongly photoinhibited, which is also found in several other geophytic species (Carta et al. 2017). Photoinhibition is usually considered an adaptation

to avoid desiccation at the soil surface (Thanos et al. 1991), but this risk is very low in the deciduous forest habitat. Therefore, the adaptive advantage of photoinhibition is considered limited for plants growing in the mesic forest habitat and it has been argued that it could be a remnant of its evolutionary history (Vandelook et al. 2018). Photoinhibition also occurred in *D. communis*, but was much less strong. Such a moderate photoinhibition has also been observed in other *Dioscorea* species (Okagami and Kawai 1977).

Seeds of *D. communis* were known to be notoriously slow germinating (Burkill 1939), but virtually nothing was known about temperature requirements for dormancy break. Field germination of *D. communis* resembles that of forest herbs with a strategy sometimes referred to as ‘double dormancy’ in that nearly two years are required before seedlings emerge (Baskin and Baskin 2014). Seeds with double dormancy, however, require two periods of cold stratification, to break embryo dormancy and shoot dormancy, respectively, and germinate in the first spring after sowing (Barton and

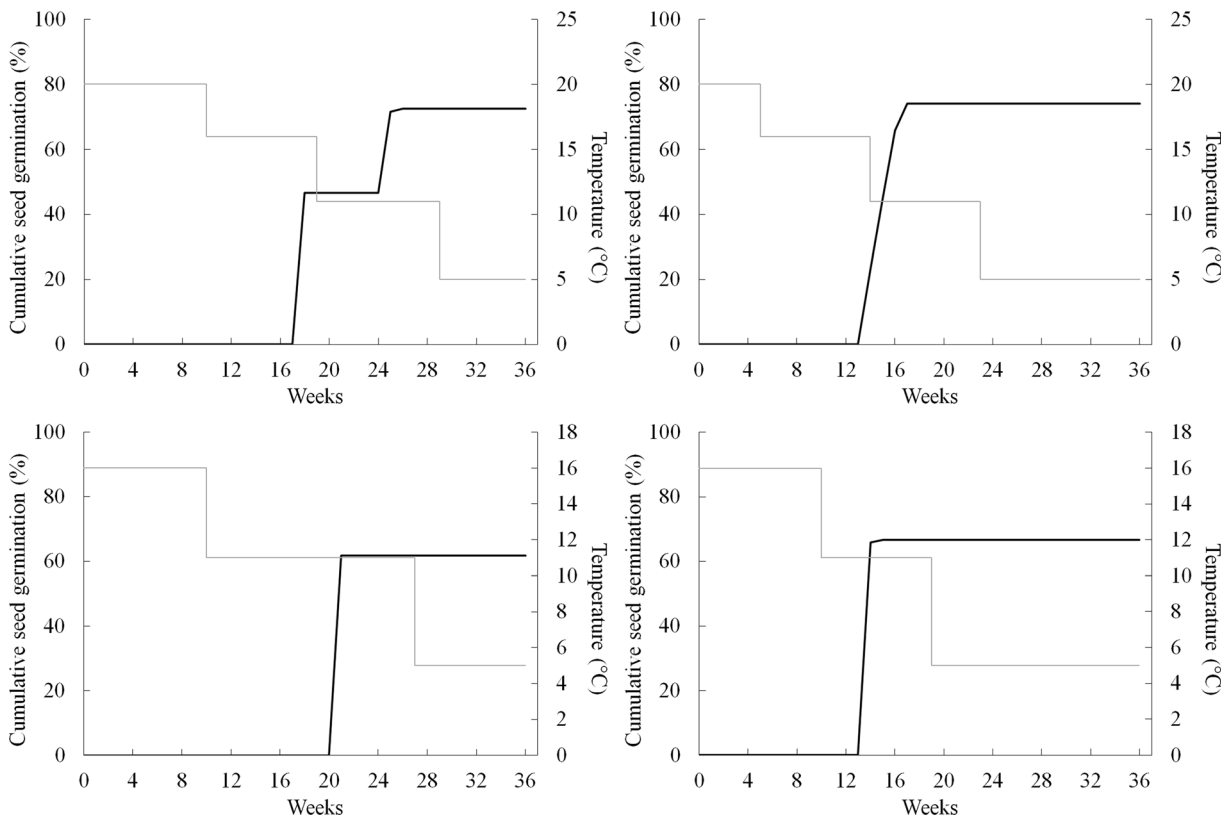
**Fig. 3** Cumulative seed germination percentage of *Allium ursinum* (black lines) during a sequence of high to low temperatures (grey lines)

Table 4 Final seed germination and 95% binomial confidence intervals of *Dioscorea communis* seeds incubated in light and darkness following pre-treatment at different temperatures

			Light			Darkness		
			Germination [%]	CI-	CI+	Germination [%]	CI-	CI+
		64 weeks 5°C	0	0	7.1	0	0	7.1
		64 weeks 11°C	0	0	7.1	48	33.7	62.6
		64 weeks 16°C	96	86.3	99.5	90	78.2	96.7
		64 weeks 20°C	6	1.3	16.5	18	8.6	31.4
	28 weeks 20°C →	36 weeks 5°C	14	5.8	26.7	92	80.8	97.8
	40 weeks 20°C →	24 weeks 5°C	88	75.7	95.5	100	92.9	100
12 weeks 5°C →	16 weeks 20°C →	36 weeks 5°C	0	0	7.1	26	14.6	40.3
12 weeks 5°C →	28 weeks 20°C →	24 weeks 5°C	30	17.9	44.6	42	28.2	56.8
	40 weeks 5°C →	24 weeks 11°C	0	0	7.1	0	0	7.1
	28 weeks 20°C →	36 weeks 11°C	100	92.9	100	100	92.9	100
	40 weeks 20°C →	24 weeks 11°C	100	92.9	100	100	92.9	100
12 weeks 5°C →	16 weeks 20°C →	36 weeks 11°C	58	43.2	71.8	100	92.9	100
12 weeks 5°C →	28 weeks 20°C →	24 weeks 11°C	88	75.7	95.5	92	80.8	97.8
	40 weeks 5°C →	24 weeks 16°C	24	13.1	38.2	64	49.2	77.1
	28 weeks 20°C →	36 weeks 16°C	90	78.2	96.7	94	83.5	98.7
	40 weeks 20°C →	24 weeks 16°C	100	92.9	100	96	86.3	99.5
12 weeks 5°C →	16 weeks 20°C →	36 weeks 16°C	82	68.6	91.4	96	86.3	99.5
12 weeks 5°C →	28 weeks 20°C →	24 weeks 16°C	94	83.5	98.7	96	63.3	99.5

Schroeder 1942; Kondo et al. 2015). Our experiments have shown that seeds of *D. communis* can germinate during prolonged exposure to intermediate temperatures (11°C and 16°C) and that germination proceeds faster and to higher percentages when seeds are exposed to higher summer temperature conditions first. In contrast to *D. communis*, dormancy in other *Dioscorea* species is broken by cold stratification (Okagami and Kawai 1982;

Terui and Okagami 1993), or by after-ripening at warm and dry conditions (Commander et al. 2009). Seeds of *D. communis* ripen in autumn but can remain attached to the mother plant until spring because birds are not keen on eating the red berries, as they contain irritating raphids (Burkill 1944).

Table 5 Results of GLM on the effects of light and temperature conditions during incubation on seed germination of *Dioscorea communis*

	Estimate	SE	z	P
Intercept	-1.65	0.19	-8.50	< 0.001
Light	-0.49	0.08	-5.97	< 0.001
5°C	-0.01	0.00	-2.73	0.01
20°C	0.03	0.00	8.36	< 0.001
16°C	0.04	0.00	12.57	< 0.001
11°C	0.02	0.00	7.12	< 0.001

Duration of incubation at 20°C, 16°C, 11°C and at 5°C prior to moving the seeds to higher temperature was tested. The effect of germination in light was tested against germination in darkness

Phenology of seed germination and seedling emergence

Seedlings of these forest herbs emerge above ground in spring. In the species we studied, a time-lag of several months exists between radicle protrusion and seedling emergence. Germination takes place in autumn and by the time seedlings emerge above ground an extensive root system has already developed. Such a strategy was first studied in detail in tree paeony, where it became clear that an additional stratification period at low temperature after germination was necessary to break dormancy of the epicotyl (Barton 1933). Later studies have shown that germination in autumn and seedling emergence in spring is fairly common in forest species and that it has evolved multiple times in very different genera such as *Convallaria*, *Quercus*

Table 6 Size and proportions of seed internal tissues of *Allium* species for which seeds were available at the Botanic Garden Meise

	Embryo length [mm]	Seed length [mm]	Embryo / Seed length	Embryo area [mm ²]	Seed area [mm ²]	Embryo / Seed area
<i>Allium cristophii</i> Trautv.	6.59	3.74	1.76	2.79	6.79	0.41
<i>Allium fistulosum</i> L.	4.68	3.63	1.29	2.24	7.71	0.29
<i>Allium flavum</i> L.	4.72	4.83	0.98	1.50	9.33	0.16
<i>Allium giganteum</i> Regel	11.15	3.48	3.20	5.05	6.31	0.80
<i>Allium karataviense</i> Regel	10.65	4.39	2.42	5.30	9.52	0.56
<i>Allium schubertii</i> Zucc.	10.58	5.08	2.08	6.23	15.64	0.40
<i>Allium sphaerocephalon</i> L.	3.99	3.87	1.03	1.36	5.80	0.23
<i>Allium tuberosum</i> Rottler ex Spreng.	4.21	3.88	1.09	1.77	9.71	0.18
<i>Allium ursinum</i> L.	0.88	2.32	0.57	0.27	3.23	0.08

and *Anemone* (Barton and Schroeder 1942; Fox 1982; Mondoni et al. 2008). Preliminary experiments have shown that cold stratification after seed germination is necessary for shoots of *A. ursinum* and *D. communis* to grow. Seedlings of *M. perennis*, on the other hand, are not dormant but just tend to develop slowly at low temperatures, as has been observed in some other temperate forest species (Vandelook and Van Assche 2008a). This also explains the more synchronized seedling emergence of *A. ursinum* and *D. communis* as compared to *M. perennis*. The adaptive significance of a time-lag between germination and seedling emergence has never been studied, but it may be a mechanism to avoid frost damage to the shoot, while an already developed root system gives the seedling a head start to optimally use the short period after the end of winter and before canopy closure.

Haustorial function of the embryo

In none of the species studied, the embryo grows substantially inside the seed before germination. The absence of growth of the embryo prior to germination has been observed in other temperate woodland herbs with endospermous seeds, such as *Polygonatum multiflorum*, *Convallaria majalis* (Vandelook pers. obs.), *Hyacinthoides non-scripta* (Vandelook and Van Assche 2008a) and *Convallaria keiskei* (Kondo et al. 2015), but minor morphological constraints on germination of endospermous seeds have been also reported for the Mediterranean genus *Romulea* (Carta et al. 2016). Kondo et al. (2015) proposed to expand the definition of morphological dormancy to seeds with: 'hypogeal germination and embryo growth after root emergence', but before shoot emergence as is

the case in the species studied here. We do, however, oppose such over-classification because it will lead to a loss of information on subtle differences in the continuum of dormancy mechanisms and germination strategies. Moreover, because there is no morphological constraint on seed germination in the species studied, we view any processes involved in regulating dormancy release and induction of germination as physiological. Studies on the growth of excised embryos in *Dioscorea* have shown that growth or development of the embryo is not a factor hindering germination of *Dioscorea*, but that it is rather physiological processes in the endosperm, very likely in interaction with the embryo, that determine dormancy characteristics (Terui and Okagami 1989, 1993). Given that many reserves are stored outside the embryo and that the seed remains attached to the developing seedling for a considerable period of time after germination (Burkill 1944; Tutin 1957; Jefferson 2008), it can be assumed that reserves are consumed by the seedling after germination and that the cotyledon(s) function as a haustorium (Lawton and Lawton 1967; Kondo et al. 2015).

Comparison of seed ecology and morphology

The embryo in ripe seeds of *A. ursinum* and *D. communis* are seemingly less developed as compared to that of congeneric species. The embryo in seeds of *Allium* species is often well developed and curved or spirally coiled (Hoffman 1933; Martin 1946). The genus *Allium* has an Holarctic distribution but the clade to which *A. ursinum* belongs to, consists of species with a Mediterranean distribution (Li et al. 2010). We can therefore assume that the seed germination strategy of *A. ursinum*, that is typical of shade-tolerant

forest herbs, is a derived strategy. Seeds of *D. communis*, like all other *Dioscorea* species studied so far, have a small embryo that is embedded in copious endosperm. The fan-like structure at the apical end of the embryo typically observed in several Asian and African *Dioscorea* species (Martin 1946; Lawton and Lawton 1967; Terui and Okagami 1989) is, however, not present in *D. communis*. The occurrence of the fan like structure in both early branching (*Dioscorea tokoro*) and advanced taxa (*Dioscorea bulbifera*) suggest that it has been lost in the evolution of *D. communis* (Wilkin et al. 2005). *Mercurialis* is a relatively small genus, composed of about a dozen species mostly Mediterranean and Central European (Krähenbühl et al. 2002). The evolutionary history of *Mercurialis* is uncertain, but it seems to be related to some tropical taxa (Wurdack et al. 2005). Within the genus *Mercurialis* there is a perennial clade, including *M. perennis*, and an annual clade (Krähenbühl et al. 2002). *Mercurialis annua* belongs to the latter clade and has a completely different germination strategy, as its seedlings are observed to germinate and to emerge throughout spring, summer and autumn (Magyar and Lukács 2002) and can build up a persistent seed bank (Jozef Van Assche, pers. comm.). In contrast to *M. perennis*, the time lag between germination and seedling emergence is much smaller, which may be related to the fact that *M. annua* seeds germinate at higher temperatures (unpublished results).

Conclusions

We can conclude that although phylogenetically unrelated, the three species studied show a remarkable similarity in germination and seedling emergence strategy that is typical for plants growing in shady conditions. Based on the fragmentary information we have on the germination syndrome of congeneric species, it seems that it is very different from that of the species studied. The woodland plant seed germination syndrome (i.e. germination in autumn after a warm period and seedling emergence in spring after a cold period) has evolved multiple times in different angiosperm clades, suggesting a clear evolutionary advantage in the woodland habitat and perhaps that this syndrome could evolve rapidly. The

absence of embryo growth prior to germination indicates that embryo development after dispersal does not hamper germination. Whether the haustorial function of the embryo as compared to embryo growth prior to germination provides a selective advantage in woodland species is still unclear and may very well be the result of differences in evolutionary history of woodland species.

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