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Regeneration from seed in herbaceous understory of ancient woodlands of temperate Europe

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- **Background and Aims** European ancient woodlands are subject to land use change and the distribution of herbaceous understory species can be threatened because of their poor ability to colonize isolated forest patches. The regeneration niche can determine the species assembly of a community and seed germination traits may be important descriptors of it.

- **Methods** We analysed ecological records for 208 herbaceous species regarded as indicators of ancient woodlands in Europe and, where possible, collated data on seed germination traits, reviewed plant regeneration strategies and measured seed internal morphology traits. The relationship between plant regeneration strategies and ecological requirements was explored for 57 species using ordination and classification analysis.

- **Key Results** Three regeneration strategies were identified. Species growing in closed canopy areas tend to have morphological seed dormancy, often requiring darkness and low temperatures for germination, and their shoots emerge in early spring, thus avoiding the competition for light from canopy species. These species are separated into two groups: autumn and late winter germinators. The third strategy is defined by open-forest plants with a preference for gaps, forest edges and riparian forests. They tend to have physiological seed dormancy and germinate in light and at warmer temperatures, so their seedlings emerge in spring or summer.

- **Conclusion** Seed germination traits are fundamental to which species are good or poor colonizers of the temperate forest understory and could provide a finer explanation than adult plant traits of species distribution patterns. Seed dormancy

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type, temperature stratification and light requirements for seed germination are important drivers of forest floor colonization patterns and should be taken in account when planning successful ecological recovery of temperate woodland understories.

Key words: ancient woodland indicator species, herbaceous forest understory, regeneration niche, seed dormancy, seed ecology, seed germination traits, seed internal morphology, temperate forest.

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INTRODUCTION

Ancient woodlands are forests that have not been cleared for a certain period of time, the duration of which differs throughout Europe, ranging among 150 and 400 years before present depending on the landscape history of each region, (Hermy et al. 1999). Ancient Woodland Indicators (AWIs) are plants associated with ancient woodlands, whose presence could be taken as an indicator of a long history of forest cover (Peterken, 1974). AWIs are generally considered to be stress-tolerant species with poor colonizing capacity due to their heavy seeds, low seed production, limited long-distance dispersal mechanisms (Hermy *et al.*, 1999; Verheyen *et al.*, 2003) and short-lived soil seed bank (Bossuyt et al.2002; Bossuyt and Honnay, 2008). Because of these traits, AWIs are vulnerable to human-induced environmental changes, and require active restoration efforts (Verheyen *et al.*, 2003). While current reforestation interventions focus mainly on the tree layer (McClain *et al.*, 2011; Francis and Morton, 2001), the natural recolonization of the forest understory may be very slow (Bossuyt and Hermy, 2000; Brunet *et al.*, 2011; Peterken and Game, 1984). Nevertheless, the herbaceous understory contributes the highest species richness and has the highest rates of extinction amongst forest layers (Gilliam, 2007). It also plays a functional role in the ecosystem, influencing the composition of the canopy by competitive interaction with the juvenile stages of overstory species (Gilliam, 2007). Thus, it is important that understanding the ecology of understory species is incorporated into good forest restoration practices (Blakesley *et al.*, 2013). However, basic research on seed functional traits is still needed to enable AWIs to be included in the restoration species pool and to encourage their supply through the native seed industry (Ladouceur *et al.*, 2018).

A key requirement, so far unmet, towards a better inclusion of AWIs in reforestation efforts is to define their regeneration strategies in terms of reproductive traits and ecological requirements. Reproductive traits determine the colonization capacity of understory herbs and

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the ecological niche in which they can successfully regenerate (Grubb, 1977). In trait-based studies, the most widely used reproductive trait is seed mass (Jiménez-Alfaro *et al.*, 2016). Although some correlations of seed mass with seed production or seedling growth have been proposed, seed mass says little about when and where a seed will germinate (Larson and Funk, 2016). For instance, the temperature requirements for dormancy loss and germination allow seeds to track the cycle of the seasons (Probert, 2000), while the germination requirements for light and temperature fluctuations serve as a gap detection mechanism (Pearson *et al.*, 2002). However, germination traits are rarely measured in wide-scale ecological studies (Jiménez-Alfaro *et al.*, 2016) and poorly represented in trait databases (Dürr *et al.*, 2015; Kattge *et al.*, 2011). Nonetheless, their importance is being increasingly recognized (Saatkamp *et al.*, 2018; Fernádez-Pascual *et al.*, 2019).

The aim of this study is to analyse the current data and information, available from literature and databases, on plant reproduction by seed in ancient woodland understories, focusing on species described in the literature as AWIs for European temperate broadleaf forests. We hypothesize a relationship among the environmental preferences of AWIs, defined by the Ellenberg Indicator Values (EIVs, Ellenberg and Leuschner, 2010) and the expression of their functional regeneration and seed germination traits. From the analysis of our dataset we aim to recognize groups of species with specific regeneration strategies and to discuss them as potential adaptations to variable forest habitats. In particular, we expect that shade tolerant herbs possess regeneration strategies that allow to reduce the competition for light at the time of seed germination or seedling emergence. The role of seed dormancy, and the environmental cues required to break it, may be pivotal to this regard.The implications of our findings will be discussed in relation to ancient forest restoration.

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MATERIALS AND METHODS

AWI species list

A list of 208 herbaceous species regarded as AWIs in temperate Europe was compiled from published data from eight North Western and Central European countries (Crawford, 2009; Hermy *et al.*, 1999; Kimberley *et al.*, 2013; Kirby, 2006; Perrin and Daly, 2010; Schmidt *et al.*, 2014; Verheyen *et al.*, 2003; Wulf, 2003). Latin names were standardized according to "Plants of the World Online" (http://www.plantsoftheworldonline.org/, accessed 16th February 2019).

Ecological requirements and reproductive traits

As a proxy of the ecological requirements of each species, EIVs for temperature, continentality, light, soil moisture, nutrients and pH were obtained from Ellenberg and Leuschner (2010).

The TRY database provided several traits related to plant reproduction by seed: number of seeds produced per plant, seed dry mass, seed width and length, seed terminal velocity, and plant height (Campetella *et al.*, 2011; Ciocarlan, 2000; Dainese and Bragazza, 2012; Everwand *et al.*, 2014; Fitter and Peat, 1994; Fry *et al.*, 2014; Gachet *et al.*, 2005; Garnier *et al.*, 2007; Green, 2009; Hickler, 1999; Hill *et al.*, 2004; Kleyer *et al.*, 2008; Kühn *et al.*, 2004; Milla and Reich, 2011; Moretti and Legg, 2009; Ordoñez *et al.*, 2010; Paula *et al.*, 2009; Peco *et al.*, 2005; Prentice *et al.*, 2011; Royal Botanic Gardens, Kew, 2011; Sandel *et al.*, 2011; Spasojevic and Suding, 2012; Vile, 2005; Wirth and Lichstein, 2009; Wright *et al.*, 2004). Seed terminal velocity, measured in ms^{-1}, expresses the maximum rate of fall of a seed after dispersal. When more than one record of a species trait was available in TRY, the average value was used. Duplicated records and outliers were removed before averaging.

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Embryo measurements

Embryo morphology is the main determinant of morphological seed dormancy (Baskin and Baskin, 2014). For 106 of the 127 endospermic AWI species listed, seeds were obtained from the Millennium Seed Bank, Royal Botanic Gardens, Kew, or collected in the wild. Twenty seeds of each species were imbibed on 1% agar-water for 24 hours. Thereafter, seeds were cut longitudinally, and photographs taken of the internal seed structure using a camera (AxioCam HRc, Carl Zeiss MicroImaging, Jena, Germany) mounted on a Stemi SV 11 Microscope (Carl Zeiss, Welwin Garden City, Herts, UK). Ten photographs of viable and normally developed seeds were selected per species. Embryo and internal seed areas were measured using the software Axiovision 3.1.2.1 (Carl Zeiss MicroImaging GmbH, Jena, Germany) and the ratio between them calculated. This parameter was defined as the "embryo:endosperm ratio", as the internal area not occupied by the embryo was always filled with endosperm. Low embryo:endosperm ratios may signify that the embryo needs to grow and develop before germination can occur. All Orchidaceae (16 species), Orobanchaceae (three species) and three parasitic Ericaceae (*Monotropa hypopitys, Orthilia secunda* and *Pyrola minor*) that possess "micro" seeds with undifferentiated embryos (Martin, 1946) were not dissected. Fifty-nine species from genera reported to have no endosperm were also not dissected. For non-endospermic seeds, the embryo:endosperm ratio was considered to equal 1. Embryo types were classified following Martin (1946). The categories of "micro" seed (< 0.2 mm long) and "dwarf" seed (0.3 to 2 mm long) referred to seed size rather than embryo morphology. Following the revision of Martin's classification in Baskin and Baskin (2007), species with "micro" seeds were assigned to the "undifferentiated" category while species with "dwarf" seeds were classified according to their embryo morphology.

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Germination traits

Because reliable germination traits were, for the most part, unavailable in databases, a systematic search strategy was devised to obtain these traits from published journal articles. A Boolean search string was built including: (1) all species names connected with an "OR" operator; and (2) the following string: "AND (seed AND (germination OR dormancy))". To also include those papers on woodland understory plants where no species name appeared in the title or abstract, a second string was used. In this case, instead of the species list the following terms were included: "ancient woodland indicators", "woodland understory species", "woodland herbs" and "forest understory". The strings were used to run searches in the Web of Science, accessed in June 2016. Initially, 924 papers were found. These were filtered by title and abstract, keeping only papers that clearly referred to genera and species in the list and included germination experiments on them. Studies in which seeds were exposed to only one germination temperature were excluded unless other parameters, such as the effect of light, fluctuating temperature or a dormancy breaking treatment were also investigated. The screening reduced the papers to 55 relevant references. The bibliography cited by these 55 works provided another 26 relevant papers, totalling 81 papers (marked with a "*" in the references section). Further information was extracted from the germination compendium of Baskin and Baskin (2014). Specific germination data were produced by the authors for *Hypericum androsaemum* and *Stachys sylvatica*.

The following traits were extracted from the available sources: dormancy type; stratification requirements; minimum, maximum and effective germination temperatures; germination response to light and fluctuating temperatures. Physiological dormancy refers to seeds that are water permeable but where a physiological inhibition prevents radicle emergence (Baskin and Baskin, 2014). Morphological dormancy is present in seeds with small embryos that need to elongate before germination can occur (Baskin and Baskin, 2004).

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If an additional physiological block to germination is present, this dormancy type is defined as morphophysiological (Baskin and Baskin, 2004). Physical dormancy relates to a physical barrier to germination, e.g., seed coat impermeability to water. Stratification requirements were defined when a positive response to a cold (“C”, 0-10°C) or warm (“W”,10-25°C) stratification treatment was reported in the literature. The effective germination temperature was considered the condition, in each study, resulting in the highest seed germination percentage. Maximum and minimum temperatures were defined as the temperature above and below which seed germination was not observed. When the effective germination temperature of a species differed across different studies, the temperature at which final germination was greatest was selected. When alternating temperatures were used, the mean constant temperature was calculated, considering the time spent at the warmer and cooler phases.

Three phenological traits were also collected from the literature: month of flowering peak, dispersal season (when seeds were collected) and germination season (when radicle emergence was observed in simulated natural conditions). When such data were not available, the ECOFLORA database, accessed in May 2017, was consulted (Fitter and Peat, 1994).

Statistical analysis

Quantitative data were checked for normality, using the Shapiro-Wilk test, and for autocorrelation, using the Pearson correlation coefficient. A Principal Component Analysis (PCA) was applied to the EIVs to assess the variability in the ecological requirements of AWIs. This analysis was performed only for the 191 species that had information on EIV (Supplementary data Table SI). With the goal of identifying general regeneration strategies in ancient woodland understories, an ordination and classification analysis was performed on the reproductive traits. Because not all traits were available for all species, before performing

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an ordination analysis of the regeneration traits the first step was to select a group of traits that: (1) provided a full data coverage for as many species as possible; (2) represented the full spectrum of reproductive processes; and (3) did not supply redundant information. According to data availability, 57 species were found to comply with the criterion of complete information on the following nine traits: flowering phenology;germination phenology; plant height; seed dry mass; embryo:endosperm ratio; seed dormancy type; stratification requirement; effective germination temperature and germination response to light. To remove outliers, this dataset did not include species representing the rarest categories of seed dormancy (*Geranium robertianum*, *Geranium sanguineum, Lathyrus linifolius* and *Vicia sepium* which possess combinational or physical dormancy) and stratification requirement (*Paris quadrifolia*, which requires cold + warm stratification). Data were ordered using a Factorial Analysis for Mixed Data (FAMD, Pages, 2004). The resulting two principal axes of the FAMD were tested for correlation with the EIVs for light, moisture, nutrients and pH. Then, the two FAMD axes were classified with a Hierarchical Clustering on Principal Components (HCPC, Husson *et al.*, 2010) to define regeneration strategies. Euclidean distance was calculated between all the species in the maps of individuals, taking into account only the first two dimensions of the FAMD. Clusters were aggregated using the Ward method and 40 iterations were performed. All data analyses were performed with the statistical software R (R Core Team, 2017), using the package FactoMineR (Lê *etal.*, 2008).

RESULTS

The final species list included 208 AWI species distributed across 45 families and 124 genera (Supplementary data Table S1). Table 1 reports the number of species for which information on each trait was available.

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Ecological requirements of European AWIs

The indicators for temperature, light, nutrients and moisture all have a median value of five. Continentality have a median of three, indicating low seasonal and daily thermal variation (Fig. 1a). The pH indicator ranges from two to nine, indicating a broad spectrum of pH preferences amongst woodland species (Fig. 1a). Finally, the light EIV varies between one and nine, describing species ranging from forest edges and gaps to closed canopy areas (Fig. 1a). The first two components of the PCA explain 57% of the variance (Fig. 2, Supplementary data Table S2). The first component represented mainly variation in nutrients, pH and light requirements (Pearson's r = 0.86, 0.71 and -0.48, respectively, Supplementary data Table S2). It orders species along a degree of decreased adaptation to shade-tolerance and increased adaptation for poor and acidic soils. The second component represents variation in moisture and light (Pearson's r = 0.72, 0.64, Supplementary data Table S2), grouping together species from open and moist sites. Temperature and continentality contribute little to the main axes, so these two EIVs were not included in subsequent analyses.

Reproductive traits of ancient woodland indicators

Flowering occurs from February to September, with a peak in June, indicating that average understory AWIs tend to flower in late spring/early summer (Fig. 1b). Sixty-three species possess seeds which are dispersed in summer (June to August); thirty-five are disperse seeds in autumn (September to November) and 20 species in spring (March and April). None disperses seeds in winter (Fig. 1b). Two-thirds of autumn-dispersed species require cold stratification. Ninety-four species are reported to germinate in spring, 11 in summer, 29 in autumn and one, *Vicia sepium*, either autumn or spring (F. Vandelook, unpublished data). Finally, nine species are known to germinate in late winter when the temperature is still cold and the forest canopy open.

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Seed production per plant is skewed towards low seed numbers (Fig. 1c), with 75% of the species producing less than 1,400 seeds per plant. Only 10 species produce more than 30,000 seeds per plant. Seed production per plant and seed dry mass were negatively correlated (Pearson's r = -0.65).

Seed dry mass is skewed towards lighter seeds (Fig. 1c), with 75% having a value < 4.28 mg. The maximum value, 197 mg, was reported for *Ruscus aculeatus* (Asparagaceae). Seed length ranged between 0.37 and 14.5 mm and seed width between 0.1 and 10 mm; both traits are skewed towards smaller values (Fig. 1c), are positively correlated with seed dry mass (Pearson's r = 0.74 and 0.89, respectively) and negatively with seed number per plant (Pearson's r = -0.50 and -0.67, respectively).

Seed terminal velocity has a mean value of 3.03 ms^{-1} (Fig. 1c). Plant height in AWI has average and median values of 0.4 m, and >75% of the species have an adult plant height < 0.6 m.

Seed internal morphology

Eleven categories of embryos were identified in the AWI list, following Martin (1946) and Baskin and Baskin (2007). Species with bent, folded or investing embryos have negligible endosperm, while it was sometimes present in species with linear and spatulate embryos. All other categories identified included only endospermic seeds. Table 2 reports the number of species assigned to each embryo type category. The ratio varied between 0.01 and 1 and the data have a bimodal distribution, with most species divided between those two extremes. The average embryo:endosperm value is 0.48 and the median is 0.25 (Fig. 1d). For all species with rudimentary and 17 out of 52 species with linear embryos a morphological component to seed dormancy is reported.

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Seed germination traits

Physiological dormancy is the most prevalent dormancy class (71 species),, 38 species are classified as morphophysiologically dormant and none as just morphologically dormant. Only four species possess physical dormancy and one, *Geranium robertianum* (Vandelook and Van Assche, 2010), has a combination of physical and physiological dormancy (Fig. 3a).

Seeds with physiological dormancy lose dormancy during a defined period of imbibition at a specific temperature, generally referred to as a stratification treatment. The imbibition temperature may be cold or warm to reflect the winter or summer average temperatures. Some seeds may require a combination of warm followed by cold stratification (W+C) or the opposite (C+W). Seventy-six species in our list required cold stratification, 13 warm, 10 W+C and one species, *Paris quadrifolia* (F. Vandelook, unpublished data), requires C+W. Ten species had no need for stratification (Fig. 3a). In species with epicotyl dormancy, an additional cold stratification period is required after radicle emergence to break shoot dormancy (Eriksson, 1994; Kondo *et al.*, 2004; Mondoni *et al.*, 2008; Mondoni *et al.*, 2009; Mondoni *et al.*, 2013; Takagi, 2001).

Effective temperature for germination ranges from 2°C (*Hordelymus europaeus*, Ten Brink et al. 2013) to 33.5°C (*Scirpus sylvaticus*, Grime *et al.*, 1981) with a mean of 16.6°C (SD 5.5, SE 0.5) and a median of 17.5°C (Fig. 3b). Temperature extremes for germination vary considerably amongst species. The minimum germination temperature, defined as the lowest temperature at which germination occurred, ranges between 0°C and 20°C. The maximum germination temperature, above which no germination occurred, is between 10°C and 38°C. Minimum and effective germination temperatures present a skewed distribution towards low values while the distribution of the maximum germination temperature is evenly distributed. However, the effective temperature has significant and positive correlation only with the maximum germination temperature (Pearson's r = 0.66).

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Alternating temperatures increase germination for 27 species, while the rest are reparted among species indifferent to fluctuating temperatures (19 species) or requiring constant temperature to germinate (14 species) (Fig. 3a). The latter group is dominated by species with morphophysiological dormancy .

The response of germination to light, expressed as photosynthetic active radiation (PAR),is positive for 68 species, while 26 germinate equally in light or in complete darkness (Fig. 3a). Eleven germinate better in darkness than in the light, all of which are monocotyledons that can be grouped in three categories: a grass (*Bromus ramosus*) and two groups of species in the order Asparagales: orchids (*Dactylorhiza maculata*, *Epipactis helleborine*, *Neottia ovata*, *Platanthera clorantha)* and large, endospermic geophytes *(Allium ursinum, Colchicum autumnale, Convallaria majalis, Galanthus nivalis, Maianthemum bifolium, Narcissus pseudonarcissus*).

No information is readily available on the germination of 76 of the AWIs, often species belonging to genera with a difficult taxonomy (e.g. *Hieracium*, *Pulmonaria*) or to groups for which germination in the laboratory is known to be challenging (e.g. orchids or parasitic plants). Many of the studies on the germination of 22 AWIs with “micro” seeds were not included in our analysis because seeds were germinated only in buried mesh nets and so no data on germination temperature or other germination traits were available.

Regeneration strategies in ancient woodland understories

The ordination of reproductive traits with a FAMD suggest certain general regeneration patterns in the European AWIs (Fig. 4a). The first component (Dim 1) explains 29% of the variance. All the continuous variables contribute positively and significantly to it, excluding seed mass which has a negative correlation coefficient (Supplementary data Table S3). All the qualitative variables are highly significant ($p < 0.01$). Stratification and light requirements for

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germination are the qualitative variables that contribute most to explain the first axis (R^2 = 0.66 and 0.54, respectively, $p < 0.01$, Supplementary data Table S3). The distribution of the categories along the first component separates the species into two regeneration strategies:

> 1) Taller plants that flower relatively late with seed germination in spring. Their seeds are smaller and have more developed embryos. Seeds have physiological dormancy and require cold stratification for dormancy loss. Their germination requires warmer temperatures and light.
>
> 2) Shorter plants that flower earlier with seed germination in autumn. They have larger seeds with smaller embryos, morphological or morphophysiological dormancy requiring warm temperatures or a combination of warm and cold stratification for dormancy loss. Germination occurs at cooler temperatures and is photoinhibited or indifferent to light.

The second group includes species more associated with closed forests (*sensu* Hermy *et al.*, 1999). The position of each species on the first axis thus indicates its affinity with ancient closed woodland as expressed by its regeneration strategy. The ecological requirement for moisture is marginally correlated with the first axis of the FAMD (Spearman rho = 0.253, p = 0.056), where species from the first group require wetter habitats. (Fig. 4c). The EIV for light is not significantly correlated with the first axis but presents a correlation coefficient higher than the other EIVs (Spearman rho = 0.128, p =0.341, Fig. 4b) and follows similar pattern to the moisture EIV.

The second axis of the FAMD (Dim 2) explains 13% of the variance (Fig. 4a). Effective germination temperature and relative embryo size are the quantitative variables significantly correlated to this dimension (Pearson's r = 0.31, $p < 0.01$ and 0.30, p = 0.02, respectively, Supplementary data Table S3) while the contribution of all the qualitative variables is

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significant. The second axis orders species primarily associated with mature forest. Germination season is the categorical trait with the strongest association with the second axis ($R^2 = 0.65$, $p < 0.01$), followed by dormancy type ($R^2 = 0.33$, $p < 0.01$), light requirement ($R^2 = 0.22$, $p < 0.01$) and stratification requirements ($R^2 = 0.15$, $p < 0.01$). Therefore, this component separates species with bigger embryos and autumn germination, in the upper part of Fig. 4a, from species with smaller embryos and winter germination.

The HCPC clustering confirms the patterns shown by the FAMD and enables the identification of three groups. For each one, the species closest to its centre is indicated in brackets:

1) Group 1 *(Carex brizoides)* (41 species): The majority of species in this group flower late, and germination occurs mostly in spring. The group includes tall species with small seeds. Embryos are relatively big and belong to the capitate, peripheral or investing categories. Seeds of all species possess physiological dormancy and 95% of them require cold stratification. Germination occurs at warmer temperatures and 82% of seeds require light for germination.

2) Group 2 *(Narcissus pseudonarcissus)* (6 species): These species flower the earliest and all seeds germinate in autumn. Epicotyl dormancy or a slow development of the shoot delays emergence until late winter or spring. Plants are short, seeds of all species are endospermic, and the embryos are smaller and linear or spatulate. Seeds of half of the species possess morphophysiological dormancy and the other half physiological dormancy, requiring warm or warm plus cold stratification. Germination occurs at lower temperatures and seeds from 80% of the species only germinate in darkness.

3) Group 3 *(Polygonatum odoratum)* (10 species): Seeds of these species germinate in late winter or spring and possess large seeds with small, rudimentary embryos. Seeds

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of eight of the species have morphophysiological dormancy and require cold stratification. Germination occurs at low temperatures and seeds of seven species germinate equally well in light or darkness.

The affinity of each species tested with the clusters is reported in the dataset and the association of each trait and supplementary variable with the HCPC clusters was statistically tested (Supplementary data Table S4).

DISCUSSION

In woodland understory communities, the ecological requirements for the adult plants are not always matched by similar preferences in the early life stages. For example, species that have high values of the EIV for light, such as *N. pseudonarcissus* or *Conopodium majus*, possess seeds that prefer germination in the dark. Moreover, species with broad ecological requirements, such as *C. majus*, can be both indicators of meadow communities (Rodwell, 1998) and of ancient forests (Blandino *et al.*, 2018) in different parts of their distribution range.

Seed dormancy is a mechanism that prevents seeds from germinating during suitable environmental conditions of temperature and water availability (Baskin and Baskin, 2004). The ecological advantage conferred by the presence of dormancy is the avoidance of the development of seedlings in adverse seasons, and/or the spread of seed germination over several years which ensures cohort survival in case a generation is lost during a particular year. In temperate forest understories, the main factors that can limit seedling survival and development are light deficiency during the summer months and extremely cold temperatures during winter. Therefore, it is not surprising that our findings on European species do not

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differ from the global assessment of dormancy types and average germination temperature for nemoral understory species described by Baskin and Baskin (2014).

Adaptation to forest clearings and hedges

Species of group 1 have the highest ecological requirements for light and moisture (Fig. 4b-c). Thus, these plants are associated with open situations, including clearings, forest edges and riparian forests. In these environments, more light is available and temperature fluctuations are less buffered than under the canopy or leaf litter. The need for temperature fluctuation is a requirement typical of small-seeded species that tend to germinate close to the soil surface (Probert, 2000). Germination of small seeds too deep in the soil may result in seedling death as there are insufficient reserves in the seed to support growth up to the surface. Bigger seeds, with more stored reserves, can germinate at greater depths in the soil, where daily temperature fluctuations are less detectable. Alternating temperatures can also be a cue to detect gaps in the canopy, where larger temperature fluctuations are expected. It has been demonstrated that strong temperature fluctuations can trigger germination in tropical, forest edge species (Wood *et al*., 2000).

The requirement of light for seed germination is a known gap-detection mechanism (Pearson *et al.*, 2002). As noted by Ten Brink *et al.* (2013), forest gap herbs have a positive germination response to light, and require cold stratification and relatively warm temperatures to germinate. Indeed, species in group 1 have physiological dormancy and lack traits associated with morphophysiological dormancy, such as linear or rudimentary embryos. All these traits point to dormancy being removed during winter and emergence in late spring or summer. Because they are not limited by canopy shading, species adapted to forest openings can afford to germinate relatively late, when the risk of frost has passed, and the thermal environment is optimal for seedling growth. Furthermore, the production of many small seeds and the greater plant height make the species of group 1 more efficient for longer

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distance seed dispersal than species in the other groups. Species from this cluster also have slower seed terminal velocity. This trait is directly proportional to seed mass, and it can also be influenced by seed shape. A slow terminal velocity allows wind-dispersed seeds to travel greater distances and, together with release height, and relatively open spaces, increases dispersal distance (Tackenberg *et al.*, 2003). In fact, adult plant height has been demonstrated to be a major driver of dispersal distance, more important than seed mass (Thomson *et al.*, 2011). Overall, species from this cluster can be regarded as good colonizers (Verheyen *et al.*, 2003) adapted to germinate in forest edges and canopy gaps.

Adaptation to closed canopy forests

Conversely, species from groups 2 and 3 have traits that characterize them as poor colonizers: short plants with big, short-lived seeds (Verheyen *et al.*, 2003). These are species more associated with closed canopies and lower values of the EIVs for light and moisture. Their germination indifference to light, or the requirement of darkness for germination (photoinhibition), can indicate preference for shady habitats or the necessity to germinate under leaf litter. While germination in group 3 was indifferent to light, photoinhibition was prevalent in group 2, mainly composed of lilioid monocots. In these taxa, inhibition of seed germination by light has been observed frequently (Carta *et al.*, 2017). This phenomenon is common in species from warm and dry climates, where absolute dark can be a cue of deep burial in the soil, away from the danger of desiccation. However, photoinhibition occurs also in phylogenetically related species from other habitats where desiccation is not a feature, and its retention can be explained by phylogenetic inertia (Vandelook *et al.*, 2017). In any case, forest species that germinate in autumn and emerge in spring can benefit from germinating underground; this ensures that the radicle and developing shoot will be buried and protected throughout winter. Moreover, all the species of group 2, with the exception of *Mercurialis*

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perennis, are bulbous geophytes that, being able to germinate in darkness, can ensure the development of bulb at an adequate depth in the soil.

Overall, this study confirms the ability of understory species of temperate forests with large seed mass to germinate in the dark (Jankowska‐Błaszczuk and Daws, 2007, Carta *et al.*, 2017). An important exception to this rule is represented by orchids (Carta *et al.*, 2017, Waes and Debergh, 1986). Large endospermic seeds have enough reserves for the embryo to grow and germinate even from far below the soil surface. In fact, orchids often need to establish a symbiotic relationship with fungi to germinate (Baskin and Baskin, 2014), and the absence of light may be indicative of incorporation in the soil, where the symbiotic fungi can be found.

The two closed canopy groups also include all the AWIs with a morphological component to their dormancy. Morphological dormancy has been reported as an important feature of herbaceous species from temperate forests of the Northern hemisphere (Baskin and Baskin, 2014). Its frequency in this type of habitat may be explained by the predictable moist conditions that characterize forest understories, allowing seeds with underdeveloped embryos to remain imbibed long enough to complete embryo growth. In the Apiaceae, Vandelook, Janssens and Probert (2012) found a negative correlation between relative embryo size and seed mass, plant longevity, shade requirement and precipitation: species with lower relative embryo size were more common in stable and moist environments, such as closed canopy temperate forests. A convergent evolution in regeneration strategies between core forest species derived from different evolutionary lineages can be observed in the clusters identified and has been described by Vandelook (2009).

The regeneration strategies of the two closed canopy groups differ mainly in their reproductive phenology. Thus, their regeneration niches are separated in time but not necessarily in space. Plants in group 2 are the earliest to flower: they usually disappear in late

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spring, after seed dispersal, and are replaced by other species (Newton *et al.*, 2013, 2015). Early flowering woodland plants take advantage of a season in which the tree canopy is still open and there is plenty of light available for flower and fruit development. Species of this group also tend to germinate in autumn, after a warm period. Autumn can be a favourable season for germination in broadleaved temperate forests, because temperatures are still relatively high in the day, while the tree canopy begins to open. However, emergence of the seedling above the litter layer can expose it to winter frosts. A possible solution is to decouple the timing of radicle and shoot emergence. While the radicle emerges and establishes itself in the autumn and during winter, securing a place for the seedling in the forest floor, the shoot will start to grow only after a cold period (Mondoni *et al.*, 2008, Takagi, 2001), or will keep growing but at a very slow pace during the coldest months (Blandino *et al.*, 2018, Newton *et al.*, 2013, 2015; Vandelook and Van Assche, 2008a). In the latter case, seedlings are frost-resistant (e.g. *Galanthus nivalis*). Nonetheless, even though the relative embryo size is smaller than the overall mean, only half of the species in group 2 possess morphological dormancy. In fact, one species, *Mercurialis perennis*, possesses physiological dormancy and two have been reported to have linear embryos that do not grow prior to germination (*Allium ursinum* and *Convallaria majalis*; Vandelook, 2009). In the latter cases, the embryo act as a haustorium by absorbing nutrients from the endosperm after germination. This type of germination was described for the genus *Yucca* (Horner and Arnott, 1966) and can be a further adaptation to germination in the dark because it allows the shoot to start developing before emerging from the soil or leaf litter.

Species of group 3 flower later than species of group 2. Most of them possess seeds with underdeveloped embryos that are dispersed in summer. The embryos grow during autumn and winter and most of the species in the group germinate after a cold stratification in late winter or early spring, when the tree canopy is still open. Seedling emergence occurs soon

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after germination. Therefore, the two clusters of closed canopy species share a common timing of seedling emergence in early spring, although this result is achieved by different regeneration strategies. Early emergence represents a competitive advantage in seasonal temperate forests and is an indication of adaptation to this habitat. Group 3 is characterized by the prevalence of morphophysiological dormancy and rudimentary embryos. There is agreement among different authors that a morphological component of dormancy is an ancestral character of angiosperms (Baskin and Baskin, 2004; Forbis *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006; Willis *et al.*, 2014) associated with a low relative embryo size (Forbis *et al.*, 2002; Martin, 1946). Willis *et al.* (2014) stated that some dormancy classes occur more in certain lineages but observed also a high degree of convergent evolution. In group 3, 80% of the species possess seeds with morphophysiological dormancy; these plants belong to the Apiaceae, Asparagaceae, Papaveraceae and Ranunculaceae families which are reported to show this type of dormancy in 63, 86, 94 and 96% of the studied species, respectively (Baskin and Baskin, 2014; Willis *et al.*, 2014).

Phylogeny was not included in our analysis but it is evident how it plays an important role in describing the HCPC grouping. In fact, with the exception of *Hordelymus europaeus* (Poaceae), no species from the families included in group 2 and 3 are found in group 1 (Supplementary data Table S1). Even though some families show a greater affinity for regeneration in a closed canopy environment, they are not necessarily phylogenetically related but could have independently evolved a similar expression of the germination traits.

Implications for forest recovery

Within the 57 species analysed with FAMD, a clear distinction can be made between: (1) good colonizer species that benefit from forest gaps and edges (group 1); and (2) core forest species, well adapted to survive in a stable environment but with limited ability to establish in new habitats. Our findings broadly concur with the study by Verheyen *et al.* (2003) that

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compared understory species from temperate Europe and North America. Based on life history and reproductive traits, dispersability was found to be a limiting factor for colonization and poor colonizers usually have complex seed dormancy breaking requirements. Another limitation to recruitment for core forest species with big seeds is that they do not form a persistent soil seed bank (Bekker *et al.*, 1998, Thompson *et al.*, 1997). Thus, after deforestation their seeds are soon depleted from the soil (Honnay *et al.*, 2002). A subsequent enrichment by ruderal species that form a long-lived soil seed bank means that recent forests on former arable land can take up to 100 years before the ruderal seed bank is depleted (Bossuyt and Hermy, 2002). Therefore, an efficient conservation measure to assist natural recolonization by core forest species in recent forest patches could be the control of the ruderals e.g., by minimising gaps in the canopy and disturbance in the herb layer (Honnay *et al.*, 2002). Moreover, it has been demonstrated that new forests, established as close as possible to ancient ones, can receive significantly more propagules of core woodland species than isolated forest patches (Honnay *et al.*, 2002), where reintroductions may be necessary.

When using seeds for ecological recovery of temperate woodland understories, the type of dormancy and the need for stratification are major constraints that can influence the success of an intervention. It is important to assess, for each species, the best vector for reintroduction (i.e. plants vs. seeds) according to its reproductive traits and regeneration biology. It has been demonstrated that big-seeded species are often more limited by seed availability than small-seeded ones (Clark *et al.*, 2007) and that seedling emergence and development is a more critical stage of plant development than seed germination (Turnbull *et al.*, 2000). Therefore, more research is necessary on the seedling survival strategies of temperate forest herbs and the main constraints to species continuity after *in situ* reintroduction by seed.

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LITERATURE CITED

Asterisks (*) preceding a reference indicate that it provided data for the review.

*Abdalla ST, McKelvie AD. 1980. The interaction of chilling and gibberellic acid on the germination of seeds of ornamental plants. *Seed Science and Technology* 8:139-144.

*Adams AW. 1955. *Succisa pratensis* Moench (*Scabiosa succisa* L.). *Journal of Ecology* 43: 709-718. doi:10.2307/2257031

*Ahmad H, Hitchmough JD. 2007. Germination and emergence of understorey and tall canopy forbs used in naturalistic sowing mixes. A comparison of performance in vitro v the field. *Seed Science and Technology* 35: 624-637. doi:10.15258/sst.2007.35.3.10

*Barton LV, Schroeder EM. 1942. Dormancy in seeds of *Convallaria majalis* L. and *Smilacina racemosa*. *Contribution from Boyce Thompson Institute* 12: 277-300

*Baskin CC, Milberg P, Andersson L, Baskin JM. 2000. Germination studies of three dwarf shrubs (*Vaccinium*, Ericaceae) of Northern Hemisphere coniferous forests. *Canadian Journal of Botany* 78: 1552-1560. doi:10.1139/cjb-78-12-1552

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* 14: 1-116. doi:10.1079/SSR2003150

Baskin CC, Baskin JM. 2007. A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. *Seed Science Research* 17: 11-20. doi:10.1017/S0960258507383189

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Baskin CC, Baskin JM. 2014. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination, 2nd edn.* San Diego: Academic Press.

*Beckmann M, Bruelheide H, Erfmeier A. 2011. Germination responses of three grassland species differ between native and invasive origins. *Ecological Research* 26: 763-771. doi:10.1007/s11284-011-0834-3

Bekker RM, Bakker JP, Grandin U et al. 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology* 12: 834-842. doi:10.1046/j.1365-2435.1998.00252.x

*Berg H, Redbo-Torstensson P. 1999. Offspring performance in three cleistogamous *Viola* species. *Plant Ecology* 145: 49-58. doi:10.1023/A:1009848318794

Blakesley D, Buckley P, Fitzgerald J. 2013. *Realising the wildlife potential of new native woodland*. East Malling: East Malling Research.

*Blandino C, Fernández- Pascual E, Marin M, Vernet A, Pritchard HW. 2018. Seed ecology of the geophyte *Conopodium majus* (Apiaceae), indicator species of ancient woodland understories and oligotrophic meadows. *Plant Biology* 21: 487-497 doi: 10.1111/plb.12872

Bossuyt B, Hermy M. 2000. Restoration of the understorey layer of recent forest bordering ancient forest. *Applied Vegetation Science* 3: 43-50. doi:10.2307/1478917

Bossuyt B, Heyn M, Hermy M. 2002. Seed bank and vegetation composition of forest stands of varying age in central Belgium: consequences for regeneration of ancient forest vegetation. *Plant Ecology* 162: 33-48. doi:10.1023/A:1020391430072

Bossuyt B, Honnay O 2008. Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. *Journal of Vegetation Science* 19: 875-884. doi:10.3170/2008-8-18462

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Brunet J, Valtinat K, Mayr ML, Felton A, Lindbladh M, Bruun HH. 2011. Understory succession in post-agricultural oak forests: Habitat fragmentation affects forest specialists and generalists differently. *Forest Ecology and Management* 262: 1863-1871. doi:10.1016/j.foreco.2011.08.007

*Brändel M, Schütz W. 2005. Temperature effects on dormancy levels and germination in temperate forest sedges (*Carex*). *Plant Ecology* 176: 245-261. doi:10.1007/s11258-004-0117-y

*Browne K. 1995. *A study of the Oxlips of Shadewell Wood*. BSc dissertation. Anglia Polytechnic University (now Anglia Ruskin University), Cambridge, UK.

*Campbell MH. 1985. Germination, emergence and seedling growth of *Hypericum perforatum* L. *Weed Research* 25: 259-266. doi:10.1111/j.1365-3180.1985.tb00643.x

Campetella G, Botta-Dukát Z, Wellstein C, et al. 2011. Patterns of plant trait-environment relationships along a forest succession chronosequence. *Agriculture, Ecosystems & Environment* 145: 38-48. doi:10.1016/j.agee.2011.06.025

Carta A, Skourti E, Mattana E, Vandelook F, Thanos CA. 2017. Photoinhibition of seed germination: occurrence, ecology and phylogeny. *Seed Science Research* 27: 131-153. doi:10.1017/S0960258517000137

Ciocarlan V. 2000. *Illustrated Flora of Romania. Pteridophyta et Spermatopyta,* 2nd edn. Bucarest: Ceres.

Clark CJ, Poulsen JR, Levey DJ, Osenberg CW. 2007. Are plant populations seed limited? A critique and meta- analysis of seed addition experiments. *American Naturalist* 170: 128-142. doi:10.1086/518565

*Coombe DE. 1956. *Impatiens parviflora* DC. *Journal of Ecology* 44: 701-712. doi:10.2307/2256857

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Crawford C. 2009. Ancient woodland indicator plants in Scotland. *Scottish Forestry* 63: 6-19.

*D'Antuono LF, Lovato A. 2004. Germination trials and domestication potential of three native species with edible sprouts: *Ruscus aculeatus* L., *Tamus communis* L. and *Smilax aspera* L. *Acta Horticulturae* 598: 211-218. doi:10.17660/ActaHortic.2003.598.31

Dainese M, Bragazza L. 2012. Plant traits across different habitats of the Italian Alps: a comparative analysis between native and alien species. *Alpine Botany* 122: 11-21. doi:10.1007/s00035-012-0101-4

*Davy AJ. 1980. *Deschampsia caespitosa* (L.) Beauv. *Journal of Ecology* 68: 1075-1096. doi:10.1038/147742b0

*Dillon K, Reichard SH. 2014. Effect of temperature on the seed germination of garden loosestrife (*Lysimachia vulgaris* L.). *Natural Areas Journal* 34: 212-215. doi:10.3375/043.034.0210

Dürr C, Dickie JB, Yang XY, Pritchard HW. 2015. Ranges of critical temperature and water potential values for the germination of species worldwide: contribution to a seed trait database. *Agricultural and Forest Meteorology* 200: 222–232. doi: 10.1016/j.agrformet.2014.09.024

*Doucet C, Cavers PB. 1997. Induced dormancy and colour polymorphism in seeds of the bull thistle *Cirsium vulgare* (Savi) Ten. *Seed Science Research* 7: 399-407. doi:10.1017/S0960258500003810

Ellenberg HH, Leuschner C. 2010. *Vegetation Mitteleuropas mit den Alpen, 6th edn.* Stuttgart: Eugene Ulman KG.

Eriksson O. 1994. Seedling recruitment in the perennial herb *Actaea spicata* L. *Flora* 189: 187-191. doi:10.1016/S0367-2530(17)30585-6

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Ernst WHO. 1979. Population biology of *Allium ursinum* in Northern Germany. *Journal of Ecology* 67: 347-362. doi:10.2307/2259355

Everwand G, Fry EL, Eggers T, Manning P. 2014. Seasonal variation in the capacity for plant trait measures to predict grassland carbon and water fluxes. *Ecosystems* 17: 1095-1108. doi:10.1007/s10021-014-9779-z

Fernández-Pascual E, Mattana E, Pritchard HW. 2019. Seeds of future past: climate change and the thermal memory of plant reproductive traits. *Biological Reviews* 94: 439-456. doi.org/10.1111/brv.12461

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* 171: 501-523. doi:10.1111/j.1469-8137.2006.01787.x

Fitter AH, Peat HJ. 1994. The Ecological Flora Database. *Journal of Ecology* 82: 415. doi:10.2307/2261309 URL http://ecoflora.org.uk/ [accessed 11 May 2017].

Forbis TA, Floyd SK, de Queiroz A. 2002. The evolution of embryo size in angiosperms and other seed plants: implications for the evolution of seed dormancy. *Evolution* 56: 2112-2125. doi:10.1111/j.0014-3820.2002.tb00137.x

Francis J, Morton A. 2001. Enhancement of amenity woodland field layers in Milton Keynes. *British Wildlife* 12: 244-251.

Fry EL, Power SA, Manning P. 2014. Trait-based classification and manipulation of plant functional groups for biodiversity-ecosystem function experiments. *Journal of Vegetation Science* 25: 248-261. doi:10.1111/jvs.12068

*Gillot P. 1925. *Recherches chimiques et biologiques sur le genre Mercurialis.* PhD Thesis. University of Nancy, France.

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Gachet S, Véla E, Tatoni T. 2005. BASECO: a floristic and ecological database of Mediterranean French flora. *Biodiversity Conservation* 14: 1023-1034. doi:10.1007/s10531-004-8411-5

Garnier E, Lavorel S, Ansquer P, et al. 2007. Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Annals of Botany* 99: 967-985. doi:10.1093/aob/mcl215

Gilliam FS. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *Bioscience* 57: 845-858. doi:10.1641/B571007

*Graae BJ, Verheyen K, Kolb A, et al. 2009. Germination requirements and seed mass of slow-and fast-colonizing temperate forest herbs along a latitudinal gradient. *Ecoscience* 16: 248-257. doi:10.2980/16-2-3234

*Graves JD, Taylor K. 1988. A comparative study of *Geum rivale* L. and *G. urbanum* L. to determine those factors controlling their altitudinal distribution. III. The response of germination to temperature. *New Phytologist* 110: 391-397. doi:10.1111/j.1469-8137.1988.tb00277.x

Green W. 2009. *USDA PLANTS Compilation.* URL http://bricol.net/downloads/data/PLANTSdatabase/. [accessed 2 February 2016].

*Grime JP, Mason G, Curtis AV, Rodman J, Band SR. 1981. A comparative study of germination characteristics in a local flora. *Journal of Ecology* 69: 1017-1059. doi:10.2307/2259651

Grubb PJ. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews* 52: 107-145. doi:10.1111/j.1469-185X.1977.tb01347.x

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Harris SM, Doohan DJ, Gordon RJ, Jensen KIN. 1998. The effect of thermal time and soil water on emergence of *Ranunculus repens*. *Weed Research* 38: 405-412. doi:10.1046/j.1365-3180.1998.00117.x

*Hassell RL, Dufault RJ, Phillips T, Hale TA. 2004. Influence of temperature gradients on pale and purple coneflower, feverfew and valerian germination. *HortTechnology* 14: 368-371.

Hermy M, Honnay O, Firbank L, Grashof-Bokdam C, Lawesson JE. 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation* 91: 9-22. doi:10.1016/S0006-3207(99)00045-2

*Herranz JM, Copete MÁ, Ferrandis P, Copete E. 2010. Intermediate complex morphophysiological dormancy in the endemic Iberian *Aconitum napellus* subsp. *castellanum* (Ranunculaceae). *Seed Science Research* 20: 109-121. doi:10.1017/S0960258510000048

Hickler T. 1999. *Plant functional types and community characteristics along environmental gradients on Öland's Great Alvar (Sweden)*. MSc thesis. University of Lund, Sweden.

*Hiirsalmi H. 1969. *Trientalis europaea* L. A study of the reproductive biology, ecology and variation in Finland. *Annales Botanici Fennici* 6: 119-173.

Hill MO, Preston CD, Roy DB. 2004. *PLANTATT - attributes of British and Irish Plants: status, size, life history, geography and habitats.* Huntingdon: Abbotts Ripton, Centre for Ecology & Hydrology.

*Hitchmough JD, Gough J, Corr B. 2000. Dormancy and germination in a wild collected ecotype of *Trollius europaeus*. *Seed Science Technology* 28: 549-558.

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Honnay O, Bossuyt B, Verheyen K, Butaye J, Jacquemyn H, Hermy M. 2002. Ecological perspectives for the restoration of plant communities in European temperate forests. *Biodiversity Conservation* 11: 213-242. doi:10.1023/A:1014531011060

Horner HT, Arnott HJ. 1966. A Histochemical and ultrastructural study of pre- and post-germinated *Yucca* seeds. *Botanical Gazette* 127: 48-64.

Husson F, Josse J, Pagès J. 2010. *Principal component methods-hierarchical clustering - partitional clustering: why would we need to choose for visualizing data?* Technical Report 1-17. Rennes, France: Agrocampus, Applied Mathematics Department.

*Hutchinson TC. 1968. *Teucrium Scorodonia* L. *Journal of Ecology* 56: 901-911. doi:10.2307/2258113

*Jankowska-Błaszczuk M, Daws MI. 2007. Impact of red: Far red ratios on germination of temperate forest herbs in relation to shade tolerance, seed mass and persistence in the soil. *Functional Ecology* 21: 1055-1062. doi:10.1111/j.1365-2435.2007.01328.x

*Jauzein P, Mansour A. 1992. Principaux facteurs de la germination de *Heracleum sphondylium* L : importance de l'oxygène. *Agronomie* 12: 85-96. doi:10.1051/agro:19920108

*Jensen K. 2004. Dormancy patterns, germination ecology, and seed-bank types of twenty temperate fen grassland species. *Wetlands* 24: 152-166. doi:10.1672/0277-5212(2004)024[0152:DPGEAS]2.0.CO;2

Jiménez-Alfaro B, Silveira FAO, Fidelis A, Poschlod P, Commander LE. 2016. Seed germination traits can contribute better to plant community ecology. *Journal of Vegetation Science* 27: 637-645. doi:10.1111/jvs.12375

*Jung LS, Winter S, Eckstein RL, et al. 2011. *Colchicum autumnale* L. *Perspectives in Plant Ecology, Evolution and Systematics* 13: 227-244. doi:10.1016/j.ppees.2011.04.001

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Kattge J, Díaz S, Lavorel S, et al. 2011. TRY - a global database of plant traits. *Global Change Biology* 17: 2905-2935. doi:10.1111/j.1365-2486.2011.02451.x

Kimberley A, Blackburn GA, Whyatt JD, Kirby K, Smart SM. 2013. Identifying the trait syndromes of conservation indicator species: how distinct are British ancient woodland indicator plants from other woodland species? *Applied Vegetation Science* 16: 667-675. doi:10.1111/avsc.12047

Kirby K. 2006. Ancient Woodland Indicator (AWI) plants. In: Rose F, eds. *The wildflower key*. London: Penguin Group, 558-575.

Kleyer M, Bekker RM, Knevel IC, et al. 2008. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology* 96: 1266-1274. doi:10.1111/j.1365-2745.2008.01430.x

*Kondo T, Miura T, Okubo N, Shimada M, Baskin C, Baskin J. 2004. Ecophysiology of deep simple epicotyl morphophysiological dormancy in seeds of *Gagea lutea* (Liliaceae). *Seed Science Research* 14: 371-378. doi:10.1079/SSR2004182

*Kosiński I. 2008. Long-term variability in seed size and seedling establishment of *Maianthemum bifolium*. *Plant Ecology* 194: 149-156. doi:10.1007/s11258-007-9281-1

Kühn I, Durka W, Klotz S. 2004. BiolFlor - a new plant-trait database as a tool fo plant invasion ecology. *Diversity and Distributions* 10: 363-365.

Ladouceur E, Jiménez- Alfaro B, Marin M, et al. 2018. Native seed supply and the restoration species pool. *Conservation Letters* 11: 1-9.

Larson JE, Funk JL. 2016. Regeneration: an overlooked aspect of trait-based plant community assembly models. *Journal of Ecology* 104: 1284-1298. doi:10.1111/1365-2745.12613

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Lincoln WC. 1981. Laboratory germination of *Cirsium vulgare* - bull of spear thistle. *Newsletter of the Association of Official Seed Analysts* 55: 67-68. doi:10.15258/sst.2008.36.3.29

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* 25: 1-18. doi:10.18637/jss.v025.i01

*Maas D. 1989. Germination characteristics of some plant species from calcareous fens in southern Germany and their implications for the seed bank. *Holarctic Ecology* 12: 337-344.

Martin AC. 1946. The comparative internal morphology of seeds. *The American Midland Naturalist* 36: 513-660. doi:10.2307/2421457

*Masselink A. 1980. Germination and seed population dynamics in *Melampyrum pratense* L. *Acta Botanica Neerlandica* 29: 451-468.

McClain CD, Holl KD, Wood DM. 2011. Successional models as guides for restoration of riparian forest understory. *Restoration Ecology* 19: 280-289. doi:10.1111/j.1526-100X.2009.00616.x

*McLean A. 1967. Germination of forest range species from Southern British Columbia. *Rangeland Ecology & Management* 20: 321-322.

*Meisert A. 2002. Physical dormancy in Geraniaceae seeds. *Seed Science Research* 12: 121-128. doi:10.1079/SSR2002104

*Michaux B. 1989. Reproductive and vegetative biology of *Cirsium vulgare* (Savi) Ten. (Compositae: Cynareae). *New Zealand Journal of Botany* 27: 401-414. doi:10.1080/0028825X.1989.10414121

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Milberg P. 1994. Germination ecology of the polycarpic grassland perennials *Primula veris* and *Trollius europaeus*. *Ecography* 1: 3-8. doi:10.1111/j.1600-0587.1994.tb00071.x

Milla R, Reich PB. 2011. Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. *Annals of Botany* 107: 455-465. doi:10.1093/aob/mcq261

*Mondoni A, Probert R, Rossi G, Hay F, Bonomi C. 2008. Habitat-correlated seed germination behaviour in populations of wood anemone (*Anemone nemorosa* L.) from northern Italy. *Seed Science Research* 18: 213-222. doi:10.1017/S0960258508084997

*Mondoni A, Probert R, Rossi G, Hay F. 2009. Habitat-related germination behaviour and emergence phenology in the woodland geophyte *Anemone ranunculoides* L. (Ranunculaceae) from northern Italy. *Seed Science Research* 19: 137-144. doi:10.1017/S0960258509990067

*Mondoni A, Orsenigo S, Rossi G. 2013. Ecophysiology of embryo development and seed germination of the European woodland herbaceous perennial *Corydalis cava* (L.) Schweigg. & Körte subsp. *cava* (Fumariaceae). *Plant Species Biology* 28: 215-223. doi:10.1111/j.1442-1984.2012.00380.x

Moretti M, Legg C. 2009. Combining plant and animal traits to assess community functional responses to disturbance. *Ecography* 32: 299-309. doi:10.1111/j.1600-0587..05524.x

*Myerscough PJ. 1980. *Epilobium angustifolium* L. *Journal of Ecology* 68: 1047-1074. doi:10.2307/2259474

*Newton RJ, Hay FR, Ellis RH. 2013. Seed development and maturation in early spring-flowering *Galanthus nivalis* and *Narcissus pseudonarcissus* continues post-shedding with little evidence of maturation in planta. *Annals of Botany* 111: 945-955. doi:10.1093/aob/mct051

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Newton RJ, Hay FR, Ellis RH. 2015. Ecophysiology of seed dormancy and the control of germination in early spring-flowering *Galanthus nivalis* and *Narcissus pseudonarcissus* (Amaryllidaceae). *Botanical Journal of the Linnean Society* 177: 246-262. doi:10.1111/boj.12240

*Nichols GE. 1934. The influence of exposure to winter temperatures upon seed germination in various native American plants. *Ecology* 15: 364-373. doi:10.2307/1932351

*Nomizu T, Niimi Y, Watanabe E. 2004. Embryo development and seed germination of *Hepatica nobilis* Schreber var. *japonica* as affected by temperature after sowing. *Scientia Horticulturae* 99: 345-352. doi:10.1016/S0304-4238(03)00115-8

Ordoñez JC, Van Bodegom PM, Witte JM, Bartholomeus RP, van Hal JR, Aerts R. 2010. Plant Strategies in Relation to Resource Supply in Mesic to Wet Environments: Does Theory Mirror Nature? *The American Naturalist* 175: 225-239. doi:10.1086/649582

*Packham JR. 1978. Biological Flora of the British Isles: *Oxalis acetosella* L. *Journal of Ecology* 66: 669-693. doi:10.2307/2259158

*Packham JR. 1983. *Lamiastrum galeobdolon* (L.) Ehrend. & Polatschek (*Galeobdolon luteum* Hudson; *Lamium galeobdolon* (L.) Nath.). *Journal of Ecology* 71: 975-997. doi:10.2307/2259606

Pagès J. 2004. Analyse Factorielle de Donnees Mixtes. *Revue Statistique Appliquee* 4: 93–111.

*Parić A, Hindija J, Muratović E, Pojskić N, Bajrović K. 2008. Breaking dormancy of two endemic *Lilium* species: *Lilium bosniacum* (G. Beck) Beck ex Fritsch and *Lilium :martagon* L. var. *cattaniae* Vis. *Seed Science and Technology* 36, 788-791. doi:10.15258/sst.2008.36.3.29

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Paula S, Arianoutsou M, Kazanis D, et al. 2009. Fire-related traits for plant species of the Mediterranean Basin. *Ecology* 90: 1420-1420. doi:10.1890/08-1309.1

Pearson TRH, Burslem DFRP, Mullins CE, Dalling JW. 2002. Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. *Ecology* 83: 2798-2807.

Peco B, De Pablos I, Traba J, Levassor C. 2005. The effect of grazing abandonment on species composition and functional traits: the case of dehesa grasslands. *Basic and Applied Ecology* 6: 175-183. doi:10.1016/j.baae.2005.01.002

*Pérez-García F, Huertas M, Mora E, Peña B, Varela F, González-Benito ME. 2006. *Hypericum perforatum* L. seed germination: interpopulation variation and effect of light, temperature, presowing treatments and seed desiccation. *Genetic Resources and Crop Evolution* 53: 1187-1198. doi:10.1007/s10722-005-2012-3

*Perglová I, Pergl J, Skálová H, Moravcová L, Jarošík V, Pyšek P. 2009. Differences in germination and seedling establishment of alien and native *Impatiens* species. *Preslia* 81: 357-375.

Perrin PM, Daly OH. 2010. *A provisional inventory of ancient and long- established woodland in Ireland. Irish Wildlife Manuals, 46.* Dublin: National Parks and Wildlife Service, Department of the Environment, Heritage and Local Government.

Peterken GF. 1974. A method for assessing woodland flora for conservation using indicator species. *Biological Conservation* 6: 239-245. doi:10.1016/0006-3207(74)90001-9

Peterken GF, Game M. 1984. Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. *Journal of Ecology* 72: 155-182. doi:10.2307/2260011

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Pietikäinen A, Kytöviita M-M, Vuoti U. 2005. Mycorrhiza and seedling establishment in a subarctic meadow: effects of fertilization and defoliation. *Journal of Vegetation Science* 16: 175-182. doi:10.1658/1100-9233(2005)016[0175:MASEIA]2.0.CO;2

*Piotto B, De Noi A. 2003. *Seed propagation of Mediterranean trees and shrubs*. Rome:Agency for the Protection of the Environment and for Technical Services (APAT). doi:10.1079/SSR2003158

Prentice IC, Meng T, Wang H, Harrison SP, Ni J, Wang G. 2011. Evidence of a universal scaling relationship for leaf CO2 drawdown along an aridity gradient. *New Phytologist* 190: 169-180. doi:10.1111/j.1469-8137.2010.03579.x

*Pritchard WH, Wood JA, Manger KR. 1993. Influence of temperature on seed germination and the nutritional requirements for embryo growth in *Arum maculatum* L. *New Phytologist* 123: 801-809. doi:10.1111/j.1469-8137.1993.tb03791.x

Probert RJ. 2000. The role of temperature in the regulation of seed dormancy and germination. In: Fenner M, eds. *Seeds: the ecology of regeneration in plant communities*. Wallingford: CABI Publishing, 261-292.

*Probert RJ, Smith RD, Birch P. 1986. Germination responses to light and alternating temperatures in European populations of *Dactylis glomerata* L. V. The principle components of the alternating temperature requirement. *New Phytologist* 102: 133-142. doi:10.1111/j.1469-8137.1985.tb03658.x.

*Qiu J, Bai Y, Coulman B, Romo JT. 2008. Mechanisms regulating seedling emergence of orchardgrass (*Dactylis glomerata* L.) and western wheatgrass (*Pascopyrum smithii* [Rydb.] L.): Dormancy change, seed fate and seeding date. *Environmental and Experimental Botany* 62: 185-194. doi:10.1016/j.envexpbot.2007.08.003

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

R Core Team. 2017. *R: A Language and Environment for Statistical Computing.* URL https://cran.r-project.org [accessed 9 June 2017].

Rodwell JS. 1998. *British plant communities. Vol. 3, Grasslands and montane communities*. Cambridge: University Press.

Royal Botanic Gardens Kew. 2008. *Seed Information Database (SID).* URL http://data.kew.org/sid/. [accessed May 2011].

Saatkamp A, Cochrane A, Commander L, et al. 2018. A research agenda for seed- trait functional ecology. *New Phytologist* 221: 1764-1775. doi: 10.1111/nph.15502

*Salisbury EJ. 1969. The reproductive biology and occasional seasonal dimorphism of *Anagallis minima* and *Lythrum hyssopifolia*. *Watsonia* 7: 25-39.

Sandel B, Corbin JD, Krupa M 2011. Using plant functional traits to guide restoration: A case study in California coastal grassland. *Ecosphere* 2: 1-16. doi:10.1890/ES10-00175.1

Schmidt M, Mölder A, Schönfelder E, Engel F, Schmiedel I, Culmsee H. 2014. Determining ancient woodland indicator plants for practical use: A new approach developed in northwest Germany. *Forest Ecology and Management* 330: 228-239. doi:10.1016/j.foreco.2014.06.043

*Schütz W. 1997a. Are germination strategies important for the ability of cespitose wetland sedges (*Carex*) to grow in forests. *Canadian Journal of Botany* 75: 1692-1699.

*Schütz W. 1997b. Primary dormancy and annual dormancy cycles in seeds of six temperate wetland sedges. *Aquatic Botany* 59: 75-85. doi:10.1016/S0304-3770(97)00028-4

*Schütz W, Rave G. 1999. The effect of cold stratification and light on seed germination of temperate sedges (*Carex*) from various habitats and implications for regenerative strategies. *Plant Ecology* 144: 215-230. doi:10.1023/A:1009892004730

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Scurfield G. 1954. *Deschampsia flexuosa* (L.) Trin. *Journal of Ecology* 42: 225-233. doi:10.2307/2256995

*Slade EA, Causton DR. 1979. The germination of some woodland herbaceous species under laboratory conditions: a multifactorial study. *New Phytologist* 83: 549-557.

Spasojevic MJ, Suding KN. 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. *Journal of Ecology* 100: 652-661. doi:10.1111/j.1365-2745.2011.01945.x

*Stanisavljević R, Djokić D, Milenković J, et al. 2011. Germinação de sementes e o vigor de plantas jovens de azevem italiano, dactilis e timóteo após a colheita e o armazenamento. *Ciência e Agrotecnologia* 35: 1141-1148. doi:10.1590/S1413-70542011000600014

Tackenberg O, Poschlod P, Bonn S. 2003. Assessment of wind dispersal potential in plant species. *Ecological Monographs* 73: 191-205. doi:10.1890/00125

*Takagi H. 2001. Breaking two types of dormancy in *Polygonatum odoratum*. *Journal of the Japanese Society for Horticultural Science* 70: 416-423.

*Taylor K, Markham B. 1978. *Ranunculus ficaria* L. (*Ficaria verna* Huds.; F. *ranunculoides* Moench. *Journal of Ecology* 66: 1011-1031. doi:10.2307/2259310

*Taylor K, Woodell SRJ. 2008. Biological Flora of the British Isles: *Primula elatior* (L.) Hill. *Journal of Ecology* 96: 1098-1116. doi:10.1111/j.1365-2745.2008.01418.x

*Ten Brink DJ, Hendriksma HP, Bruun HH. 2013. Habitat specialization through germination cueing: a comparative study of herbs from forests and open habitats. *Annals of Botany* 111: 283-292. doi:10.1093/aob/mcs253

*Thompson K. 1989. A comparative study of germination responses to high irradiance light. *Annals of Botany* 63: 159-162. doi:10.2307/2403382

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Thompson K, Bakker J, Bekker R. 1997. *The soil seed banks of northwest Europe: methodology, density and longevity*. Cambridge: University Press.

*Thompson PA. 1968. The effect of some promoters and inhibitors on the light controlled germination of strawberry seeds; *Fragaria vesca semperflorens* Ehr. *Physiologia Plantarum* 21: 833-841. doi:10.1111/j.1399-3054.1968.tb07308.x

Thomson FJ, Moles AT, Auld TD, Kingsford RT. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology* 99: 1299-1307. doi:10.1111/j.1365-2745.2011.01867.x

Turnbull LA, Crawley MJ, Rees M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88: 225-238. doi:10.1034/j.1600-0706.2000.880201.x

*Valletta A, Attorre F, Bruno F, Pasqua G. 2008. In vitro asymbiotic germination of *Orchis mascula* L. *Plant Biosystems* 142: 653-655. doi:10.1080/11263500802411205

*Van Assche J, Van Nerum D, Darius P. 2002. The comparative germination ecology of nine *Rumex* species. *Plant Ecology* 159: 131-142. doi:10.1023/A:1015553905110

*Van Assche JA, Vandelook F. 2006. Germination ecology of eleven species of Geraniaceae and Malvaceae, with special reference to the effects of drying seeds. *Seed Science Research* 16: 283-290. doi:http://dx.doi.org/10.1017/SSR2006255

*Van Waes JM, Debergh PC. 1986. In vitro germination of some Western European orchids. *Physiologia Plantarum* 67: 253-261. doi:10.1111/j.1399-3054.1986.tb02452.x

Vandelook F. 2009. *Seed germination ecology of temperate woodland herbs*. PhD Thesis. Katholieke Universiteit Leuven, Belgium.

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

*Vandelook F, Van Assche JA. 2009. Temperature conditions control embryo growth and seed germination of *Corydalis solida* (L.) Clairv., a temperate forest spring geophyte. *Plant Biology* 11: 899-906. doi:10.1111/j.1438-8677.2009.00194.x

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

*Vandelook F, Van Assche JA. 2008b. Deep complex morphophysiological dormancy in *Sanicula europaea* (Apiaceae) fits a recurring pattern of dormancy types in genera with an Arcto-Tertiary distribution. *Botany* 86: 1370-1377. doi:10.1139/B08-103

*Vandelook F, Van De Moer D, Van Assche JA. 2008. Environmental signals for seed germination reflect habitat adaptations in four temperate Caryophyllaceae. *Functional Ecology* 22: 470-478. doi:10.1111/j.1365-2435.2008.01385.x

*Vandelook F, Van Assche JA. 2010. A combined physical and physiological dormancy controls seasonal seedling emergence of *Geranium robertianum*. *Plant Biology* 12: 765-771. doi:10.1111/j.1438-8677.2009.00290.x

Vandelook F, Janssens SB, Probert RJ. 2012. Relative embryo length as an adaptation to habitat and life cycle in Apiaceae. *New Phytologist* 195: 479-487. doi:10.1111/j.1469-8137.2012.04172.x

Vandelook F, Newton RJ, Carta A. 2017. Photophobia in Lilioid monocots: photoinhibition of seed germination explained by seed traits, habitat adaptation and phylogenetic inertia. *Annals of Botany* 121: 405-413. doi: 10.1093/aob/mcx147

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Verheyen K, Honnay O, Motzkin G, Hermy M, Foster DR. 2003. Response of forest plant species to land-use changes: a life-history trait-based approach. *Journal of Ecology* 91: 563-577.

Vile D. 2005. *Significations fonctionnelle et écologique des traits des espèces végétales exemple dans une succession post-culturale méditerranéenne et généralisations.* PhD Thesis. University of Montpellier, France.

*Voss N, Welk E, Durka W, Eckstein RL. 2012. Biological flora of Central Europe: *Ceratocapnos claviculata* (L.) Lidén. *Perspectives in Plant Ecology, Evolution and Systematic* 14: 61-77. doi:10.1016/j.ppees.2011.09.004

*Vranckx G, Vandelook F. 2012. A season- and gap-detection mechanism regulates seed germination of two temperate forest pioneers. *Plant Biology* 14: 481-490. doi:10.1111/j.1438-8677.2011.00515.x

*Wheeler B, Hutchings MJ. 2002. Biological Flora of the British Isles: *Phyteuma spicatum*. *Journal of Ecology* 90: 581-591.

Willis CG, Baskin CC0, Baskin JM, et al. 2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist*, 203, 300-309. doi:10.1111/nph.12782

Wirth C, Lichstein JW. 2009. The imprint of species turnover on old-growth forest carbon balances - insights from a trait-based model of forest dynamics. In: Wirth C, Gleixner G, Heimann M, eds. *Old-growth forests: function, fate and value*. Heidelberg: Springer Berlin, 81-113.

Wood CB, Pritchard HW, Amritphalea D. 2000. Desiccation-induced dormancy in papaya (*Carica papaya* L.) seeds is alleviated by heat shock. *Seed Science Research* 10: 135-145. doi:10.1017/S0960258500000143

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

Wright IJ, Reich PB, Westoby M, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827. doi:10.1038/nature02403

Wulf M. 2003. Preference of plant species for woodlands with differing habitat continuities. *Flora-Morphology, Distribution, Functional Ecology of Plants* 198: 444-460. doi:10.1078/0367-2530-00118

Accepted Manuscript

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcac003/6502456 by guest on 11 January 2022

FIGURES

Fig. 1: Distribution of ecological and regeneration traits values of herbaceous European Ancient Woodland Indicators. In all boxplots the data represented are not transformed. a) Distribution of Ellenberg Indicator Values. C, continentality; L, light; M, moisture; N, nutrients; T, temperature. b) Distribution of reproductive phenology data. Categorical traits are represented by stacked columns. AUT =autumn; SPR = spring; SUM = summer; WIN = winter; DD = data deficient. c) Distribution of seed yield and dispersal traits. TV, terminal velocity. d) Distribution of relative embryo size values E:E, Embryo to Endosperm ratio.

Fig. 2: Principal Component Analysis of Ellenberg Indicator Values for 191 European Ancient Woodland Indicators. The percentages express the proportion of variance explained by each component. C, continentality; L, light; M, moisture; N, nutrients; T, temperature.

Fig. 3: Germination traits of European Ancient Woodland Indicator species. a) Stacked columns with the proportion of each level of the categorical dormancy types and germination requirements. “DD” (“data deficient”) indicates data not available. PYPD, physiological + physical; PY, physical; PD, physiological; MPD, morphophysiological; C, cold; W, warm; W+C, warm + cold; C+W, cold + warm; NS, no need for stratification; A>C, species requiring alternate temperatures; A=C, species indifferent to temperature fluctuation; C>A, species requiring constant temperature; L>D, species requiring light; D>L, photoinhibited species; L=D, species indifferent to light. b) Boxplots showing minimum (Min), effective and maximum (Max) germination temperatures.

Fig 4: Factorial Analysis of Mixed Data (FAMD) of regeneration traits and hierarchical clustering on its principal components (HCPC). a) Distribution of the numerical variables (left) and of the categorical traits (right). From left to right a gradient of growing capacity to colonize new habitats was described. From bottom to top germination occurs progressively

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later in the year. E:E, Embryo to Endosperm ratio, PD, physiological dormancy; MPD, morphophysiological dormancy; C, cold stratification; W, warm stratification; W+C, combination of warm + cold stratification; L>D, species requiring light; D>L, photoinhibited species; L=D, species indifferent to light; AUT, autumn; SPR, spring; SUM, summer; WIN, winter. Graphs b) and c) indicate the variation of Ellenberg Indicator Values (EIV) for light and moisture. The dark red dots represent the species with highest values for the variable (> than the 4th quartile of its distribution) while the yellow dots are the species with the lowest values (< than the 1st quartile). d) The three clusters of species identified by HCPC are group 1 (*Carex brizoides,* in red), group 2 (*Narcissus pseudonarcissus*, in blue) and group 3 (*Polygonatum odoratum*, in green).

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TABLES

Table 1 Number of species and percentages, from the original list of 208 AWI, for whom each trait was available. EIV = Ellenberg Indicator Value; E:E = embryo to endosperm ratio

Trait name	**N. of species**	**%**
Ecological preferences		
EIV for Temperature	191	92
EIV for Continentality	191	92
EIV for Light	191	92
EIV for Moisture	191	92
EIV for Nutrients	191	92
EIV for pH	191	92
Reproductiove phenology		
Flowering month	204	98
Dispersal season	118	57
Germination season	144	69
Regeneration and seed traits		
Plant heigth	207	99
Seed dry mass	182	87
Seed length	167	80
Seed width	130	62
Seed terminal velocity	99	48
Embryo type	208	100
E:E ratio	165	79
Germination traits		
Dormancy type	114	54
Stratification requirements	110	53
Effective germination temperature	108	52
Minimum germination temperature	79	38
Maximum germination temperature	78	37
Temperature fluctuation	60	29
Light requirement	105	50

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Table 2 Embryo type and embryo:endosperm ratios of European Ancient Woodland indicators.

Embryo type	Number of species	Number of endospermic species	Mean ratio	SD ratio
Rudimentary	19	19 (13)	0.03	0.01
Capitate	14	14 (13)	0.09	0.02
Broad	4	4 (4)	0.11	0.05
Lateral	19	19 (16)	0.12	0.03
Linear	52	45 (38)	0.28	0.33
Peripheral	6	6 (6)	0.59	0.08
Spatulate	52	20 (16)	0.82	0.27
Bent	12	0	1	0
Folded	3	0	1	0
Investing	5	0	1	0
Undifferentiated	22	ND	ND	ND

Numbers in parentheses indicate the number of species measured to calculate the mean ratio for each embryo type category. SD indicates the standard deviation from the mean embryo:endosperm ratio. Species with undifferentiated embryos that were not dissected (ND) for image analysis are indicated.

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Figure 1

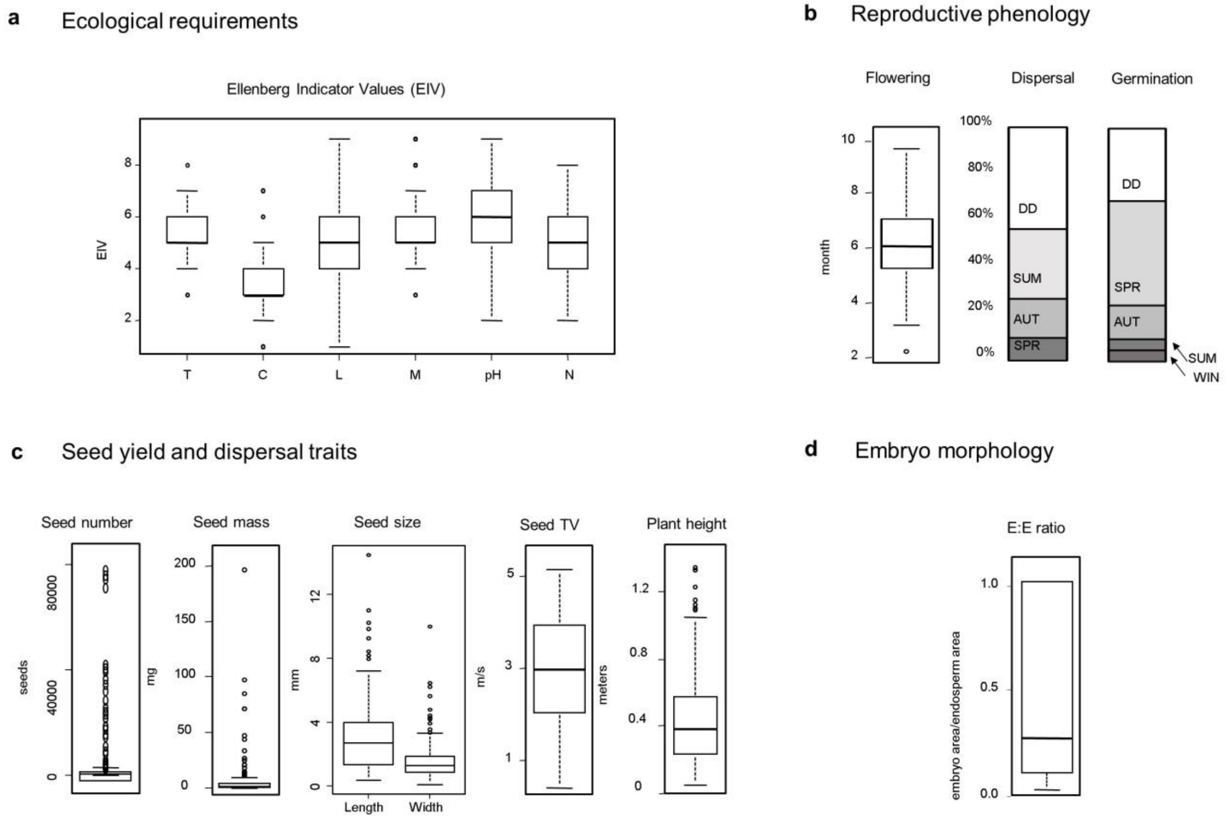

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Figure 2

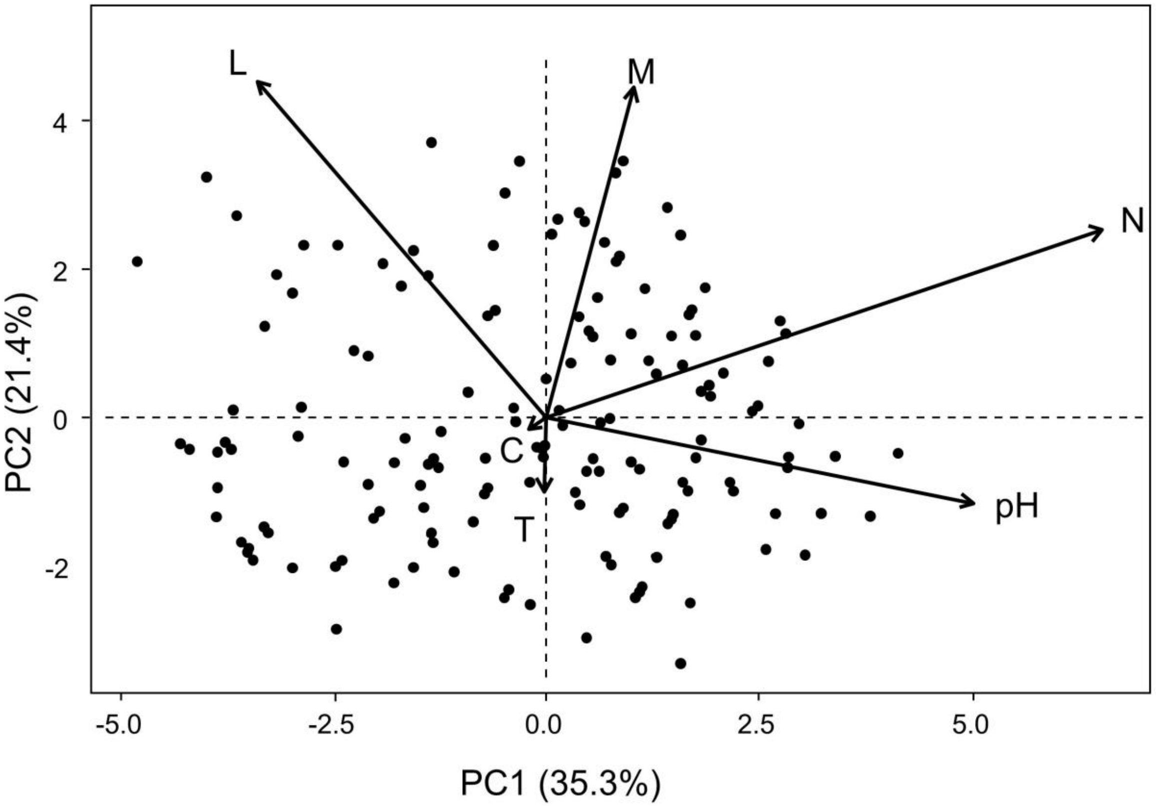

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Figure 3

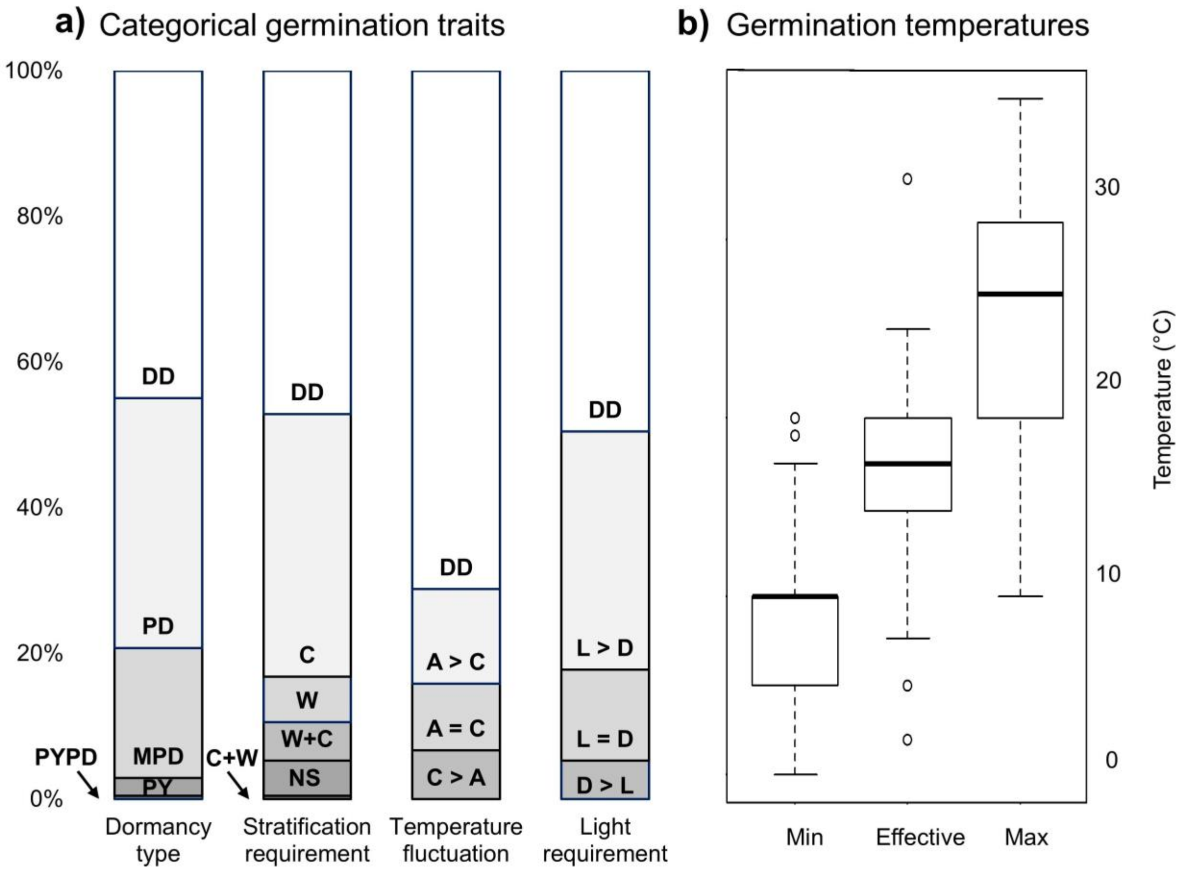

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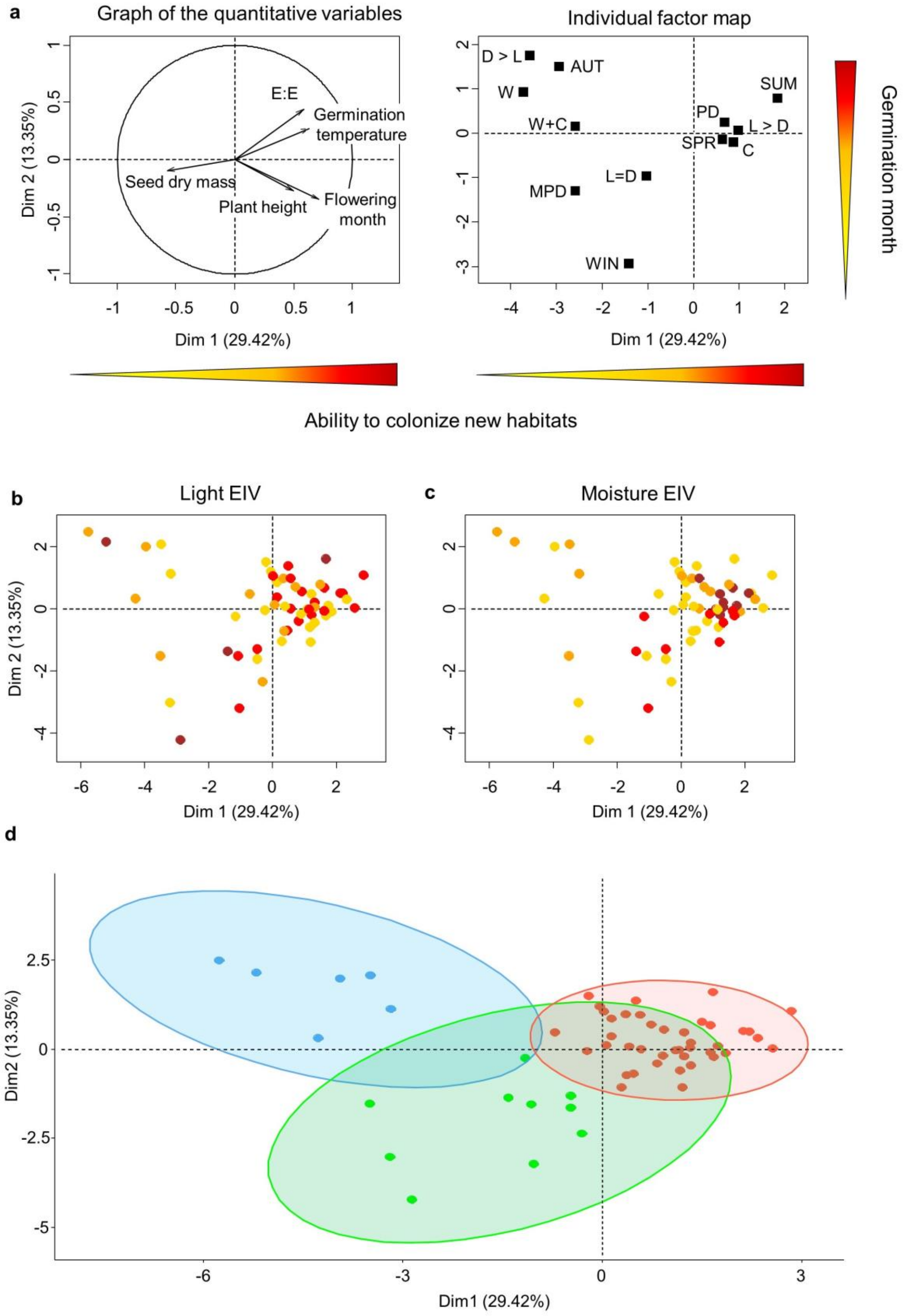
Figure 4



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