# *SEED ECOLOGY III*

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***International Society***
***for Seed Science***
***Meeting on***
***Seeds and the Environment***

***"Seeds and Change"***

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## Preface

Extended abstracts included in this proceedings will be made available online.

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The extended abstracts in this proceedings were edited for clarity.

Seed Ecology III logo designed by Bitsy Schultz.

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# Germination Ecology of Dry Sandy Grassland Species Along a pH-Gradient Simulated by Different Aluminium Concentrations

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## Introduction

Species occurring along a soil pH gradient show different responses to acidic and calcareous conditions. Grassland ecosystems have developed under different soil conditions, and understanding mechanism of plant occurrence is essential for habitat interpretation. We studied Aluminium (Al) toxicity, because it is a possible major factor limiting plant growth in acidic soils (Tamás et al. 2006, Poschenrieder et al. 2008). Until now, only a few selected terrestrial plant species have been studied with respect to their Al-sensitivity (Rorison 1960, Grime and Hodgson 1969), and no larger set of species from one substrate type has been evaluated along a pH gradient. Furthermore, Ellenberg Indicator values (EIV) for soil Reaction were only validated for pH itself and for nitrogen nutrition (ammonium on acidic soils, nitrate on calcareous soils; Bogner 1968, Gigon 1971) but not for Al as a limiting factor. In this study, germination and root growth rate of dry sandy grassland species (from a gradient of very acidic to calcareous sandy soils and with different EIV) were examined along a simulated gradient of Al availability with the aim of showing that species exclusively occurring in calcareous (high pH) sandy grasslands are more sensitive to high Al concentrations than species from acidic sandy grasslands.

## Material and Methods

Sixteen species of calcareous, intermediate and acidic dry sandy grasslands and 4 species non-sensitive to pH were chosen representing Ellenberg indicator values (EIV) for Reaction from 1 (growing on very acidic soils) to 8 (growing on calcareous soils; Ellenberg et al. 1992). EIV's were additionally validated by analysing pH and Al-content in soil samples collected in the field. A filter-paper-based system was used to germinate and cultivate seeds in order to identify Al concentrations affecting different species. Al toxicity or Al tolerance was analysed by applying 10 Al concentrations from 1mM to 10 mM. In order to make comparisons with the natural situation, contents were measured after binding to the cellulose component of the filter papers. Germination and absolute root growth (ARG) as well as length of the root hair zone were studied and a critical concentration for each species was determined for ARG and length of the root hair zone.

## Result and Discussion

The results confirmed the hypothesis that acidic grassland species are more resistant to Al in comparison to intermediate and calcareous grassland species. With respect to germination, Al generally reduced germination rate, but no differences were found between acidic and calcareous species. As for root response, in high concentration of Al not only was root growth strongly reduced, but also typical symptoms like swollen and brown-coloured root tips or short stunted curved side roots were observed. Different critical concentrations were found for acidic, intermediate and calcareous species. These correlated significantly with the EIV's for Reaction, which can arguably be viewed as evidence for the importance of the factor Al for species distribution in sandy grasslands.

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# Induction and Release of Secondary Dormancy Under Field Conditions in *Bromus tectorum*

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## Introduction

*Bromus tectorum* L. is a facultative winter annual grass originally from Eurasia. During the past century, this species has become highly invasive in the western United States, where it has displaced millions of hectares of native vegetation. Seeds of *B. tectorum* lose primary dormancy through dry after-ripening, and nearly all seeds are capable of germinating in response to autumn precipitation. We have successfully characterized and modeled the loss of primary dormancy and germination in both laboratory and field studies (e.g., Christensen et al. 1996; Meyer and Allen 2009). However, a fraction of *B. tectorum* seeds may enter secondary dormancy in the soil and carry over across years as a component of the soil seed bank. Field studies were conducted to characterize the induction and release of this secondary dormancy.

## Methods

After-ripened samples of locally-collected *B. tectorum* seeds were placed in one of three contrasting microenvironments (buried under 0.5 cm of soil beneath 2 cm of previous-year *B. tectorum* litter, on the soil surface beneath 2 cm of litter, or on the surface of 2 cm of litter) at the xeric Whiterocks study site on two autumn dates (early September and mid-November). Field germination, post-retrieval laboratory germination, and seed viability were determined for replicated subsamples of seeds retrieved from the field at weather-dependent intervals over the course of one year.

## Results and Conclusions

Seeds placed on top of the litter failed to germinate in the field, became very dormant over the winter, and remained dormant during the following spring and summer (Figure 1, top panels). Over the course of one year, up to 90% of the seeds that became secondarily dormant were killed by the generalist seed pathogen *Pyrenophora semeniperda,* which is being examined in separate studies as a possible biocontrol for *B. tectorum.* In contrast with seeds on top of the litter, seeds that were buried in soil or placed beneath the litter in September had completely germinated by November. Seeds buried or placed on the soil surface in November did not experience early autumn rains, and these seeds became moderately dormant during the winter (Figure 1, middle and lower panels). A fraction of these late-planted seeds germinated in spring, particularly in the buried samples, while remaining fractions became largely non-dormant during the spring (buried seeds) and summer (seeds under litter). These data illustrate that variations in microenvironmental conditions largely determine secondary dormancy responses for this species, and help to explain the wide range of germination outcomes observed in the field. Depending on its position in the seed bank and on timing of the first effective precipitation in the fall, a seed may germinate, remain germinable, or enter secondary dormancy. Release from secondary dormancy is a gradual process, similar to release from primary dormancy; it is likely that these processes are governed by similar physiological mechanisms. In contrast, the mechanism of secondary dormancy induction in this species is not well understood.

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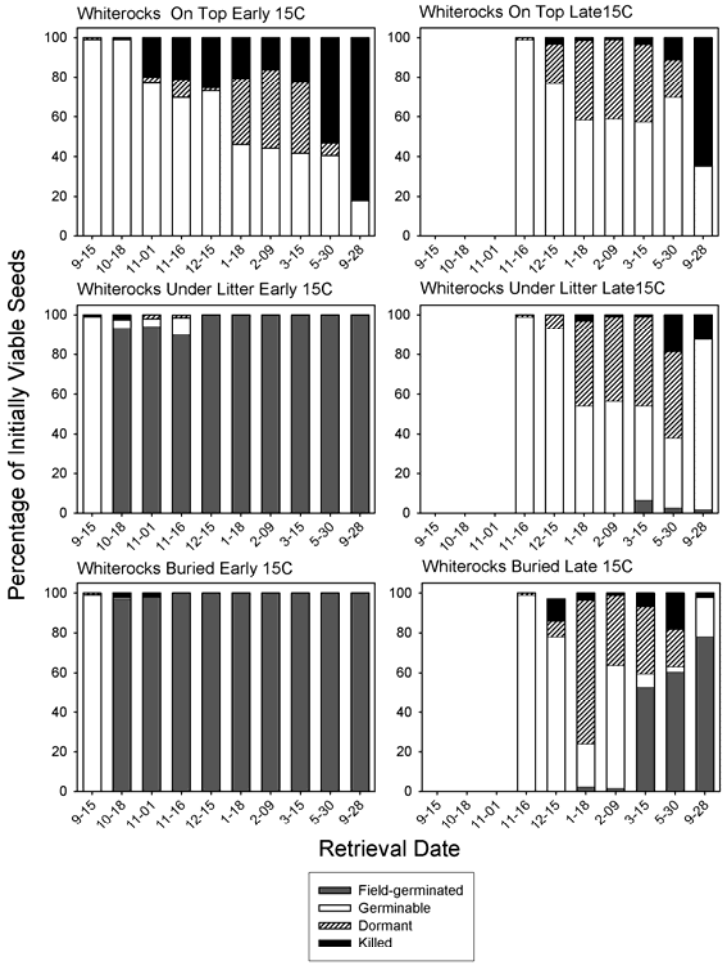
Figure 1. Germination or change in dormancy status in the field for initially nondormant seeds of *Bromus tectorum* placed on the surface of litter (top two panels), beneath litter (middle panels), or buried in soil beneath litter (bottom panels). Seeds were placed in the field in early September (left hand panels) or mid-November (right hand panels), retrieved on the dates indicated, then incubated at 15C. Viability loss (killed seeds) was almost entirely due to attack by the fungus *Pyrenophora semeniperda*.



# Seedling Production for Purposes of Biodiversity Restoration in the Brazilian Cerrado Region Can Be Greatly Enhanced by Seed Pretreatments Derived from Seed Technology

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## Introduction

The ***cerrado*** is a vast tropical savanna ecoregion of Brazil. It is characterized by an enormous range of plant and animal biodiversity. According to the WWF-Brazil, it is biologically the richest savanna in the world. However, the cerrado biome is under severe threat, due to needs of farming communities, mining, charcoal production and construction of hydro-electric dams. For durable long-term preservation and restoration of the cerrado biome, joint efforts are required, in which sociological, economic and biological interests are well integrated. However, at present, the greatest need to accommodate this purpose is a stable and uninterrupted availability of seedlings of a great variety of cerrado species.

Successful regeneration and storage of the seeds of these species are confronted with 2 major problems: 1) the occurrence of sometimes severe dormancy among seeds from orthodox species; 2) about 30% of the riparian woody species of the cerrado exhibit recalcitrant or intermediate behavior. The presence of dormancy severely hampers successful regeneration *via* seedlings after a short period of storage whereas recalcitrance poses a serious problem for longer-term storage. Dormancy and germination studies of cerrado natives allow the classification of new and existing species into classes and types of dormancy, as well as the identification of specific environmental requirements for germination and seedling establishment. With this knowledge we are now developing seed pretreatment protocols to eliminate often (very) slow and uneven germination, to guarantee continuous production of uniformly growing seedlings. Examples will be given of dormancy and germination mechanisms and successful priming or hormone treatments for *Solanum granulosum-leprosum, Solanum lycocarpum, Eremanthus erythropappus* and *Annona crassiflora* to enhance production of uniform and vigorous seedlings.

## Methods

Fruits of *Solanum lycocarpum*, *Solanum granulosum-leprosum* and *Annona crassiflora* were collected after natural dispersal in areas of natural occurrence of the species near the city of Lavras, Minas Gerais, Brazil (altitude 919 meters, latitude 21º14'S, longitude 45º00'W GRW). Fruit processing consisted of removal of the soft pulp, passage through a mesh or processing in a concrete mixer (for *A. crassiflora*), and washing under running water to separate the pulp from the seeds. Seeds from *Eremanthus erythropappus* were collected and subjected to an air blower to remove the empty seeds. All seeds were blotted dry with a paper towel if necessary and dried further in a climate-controlled room at 20°C and 60% relative humidity until equilibrium moisture content (8%) was reached. Seeds were stored at 5°C ± 2 in sealed plastic bags until the beginning of the experiments. Seeds of *S. lycocarpum*, *S. granulosum-leprosum and E. erythropappus* were surface-sterilized in 1% sodium hypochlorite and subjected to water potentials of 0.0 -0.2, -0.4 and -0.8 MPa (osmopriming) for different periods of time. After osmopriming seeds were rinsed in running water for approximately 2 min to remove any PEG residues. The seeds were dried over a saturated magnesium chloride solution (30% RH) for 17 h at 20º C until the seeds reached a moisture content of 8% (fresh weight basis). Physiologically dormant seeds of *A. crassiflora* from long-term burial experiments (da Silva *et al.,* 2007) were treated with different concentrations of gibberellins ($GA_{4+7}$).

Germination of all species was tested under optimal and/or stressed conditions (osmotic, salt) after which the germinated seeds were planted in small pots containing a substrate of ravine earth, organic soil and carbonized rice peel (5:2:1) in a semi-covered nursery, to evaluate seedling quality and emergence.

## Results and Conclusions

Both *Solanum* species are endosperm-retaining and display slow germination due to mechanical restraint of the micropylar endosperm. Complete germination of these species may take up to 6 weeks

under optimal conditions (Pinto *et al.* 2007). A hydropriming treatment of 15d at 15°C was most effective in improving germination. *Solanum lycocarpum* and *S. granulosum-leprosum* germination percentages increased from 70 to almost 100%, $t_{50}$ was reduced from 20 to 5d and, most importantly, uniformity and seedling emergence vigor were considerably improved (Table 1).
*Eremanthus erythropappus* seeds display slow and uneven germination, the completion of which may take up to 4 weeks under optimal conditions. These seeds also responded favorably to a pretreatment but in this case osmopriming in -0.4 MPa PEG at 15 °C for 5d proved to be most effective. Apart from a general improvement of germination performance, as seen in the other species, a very relevant feature of the pretreatment was the considerably increased tolerance of seed germination to osmotic, salt, and high-temperature stress (Fig. 1).
*Annona crassiflora* seed germination is extremely problematic. The species possesses morpho-physiolocal dormancy which takes 6-7 months to be released under field conditions (da Silva *et al.,* 2007). So far, no temperature regimes or priming- or other pretreatment protocols are known to speed up this process under laboratory conditions. However, a reasonably effective method to germinate these seeds is incubation in $GA_{4+7}$. This reduced germination time from 7 months to 4 weeks, albeit at total germination levels which hardly exceed 50% (Fig 2).
In conclusion, with relatively simple means it is possible to improve germination characteristics, uniformity of emergence, seedling properties and seed(ling) stress tolerance of cerrado species for large-scale regeneration and restoration purposes. Most pretreatments can easily be scaled up. Knowledge of germination and dormancy mechanisms and their regulation is particularly helpful in designing pretreatment protocols. Further seed enhancement procedures for cerrado species now also include seed coating and pelleting for direct seeding of larger areas.

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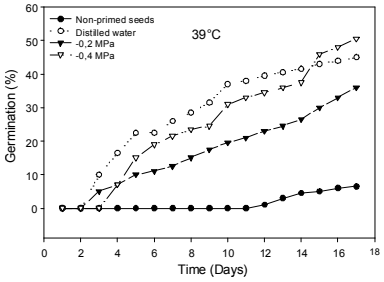


**Figure 1.** Effect of priming on tolerance to high temperature of *E. erythropappus* germination

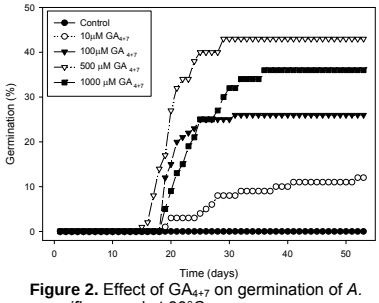


**Figure 2.** Effect of $GA_{4+7}$ on germination of *A. crassiflora* seed at 30°C.

**Table 1.** *S. lycocarpum* germination, and seedling properties 90 d after emergence.

| | Unprimed | Primed |
|---|---|---|
| Germination (%) | 70 | 95 |
| $T_{50}$ (days) | 20 | 5 |
| Shoot length RGR ($mm.d^{-1}$) | 0.093 ± 0.008 | 0.107 ± 0.014 |
| Shoot dry weight (g) | 0.81 ± 0.10 | 1.14 ± 0.17 |
| Root dry weight (g) | 1.18 ± 0.14 | 1.67 ± 0.25 |
| Dickson's Quality Index | 0.98 ± 0.17 | 1.24 ± 0.21 |

# Epicotyl Dormancy – Revisited

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## Historical

Lela Barton (1933) used "epicotyl dormancy" to describe the several-month delay between root and shoot emergence in seeds of *Paeonia*. This term has been used to describe the delay in shoot growth in seeds of white oaks and one black oak (*Quercus* spp., Fagaceae) with fully developed embryos and in those of a variety of species with underdeveloped embryos. Nikolaeva (1977) listed epicotyl dormancy as one of the kinds of morphophysiological dormancy (MPD), and she called it "deep simple epicotyl MPD." In temperate regions, seeds with deep simple MPD require the temperatures of summer → autumn → winter with both root and shoot emerging in spring, but in seeds with deep simple epicotyl MPD the root emerges in autumn and the shoot in spring.

## Recent Discoveries

Three lines of recent research have expanded our view of epicotyl dormancy. (1) Among species with underdeveloped embryos, additional levels of epicotyl MPD have been identified, *e.g.*, *Viburnum odoratissimum* has nondeep simple epicotyl MPD, and Nikolaeva *et al*. (1985) list *Lilium pensylvanicum*, *Ranunculus ficaris,* and *Silaum pratensis* as having nondeep complex epicotyl MPD. (2) The level of physiological dormancy (PD) in root and shoot may differ, *e.g.*, *Asarum canadense*, root (nondeep PD) and shoot (deep PD), or be the same, *e.g.*, *Daphniphyllum glaucescens*, root (nondeep PD) and shoot (nondeep PD). (3) In addition to Fagaceae, species with fully developed embryos and epicotyl dormancy have been found in the Oleaceae (temperate) and in the Clusiaceae, Fabaceae, Icacinaceae, and Rubiaceae (tropical). We still need a better understanding of (1) why the shoot in seeds of temperate species is insensitive to cold stratification until after root emergence, (2) effects of gibberellins on promotion (or not) of root and especially shoot growth, (3) how epicotyl dormancy is broken in tropical species, and (4) what are the ecological/evolutionary reasons for a species having epicotyl dormancy.

## Phylogeny

Epicotyl MPD has been reported in 10 families (Apicaceae, Aristolochiaceae, Boraginaceae, Caprifoliaceae, Daphniphyllaceae, Fumariaceae, Liliaceae, Paeoniaceae, Papaveraceae, and Ranunculaceae) and six orders (Piperales, Liliales, Ranunculales, Saxifragales, Apiales, and Dipsacales), with Boraginaceae being unplaced in the euasterids I clade (Fig. 1). The six families with epicotyl PD are Clusiaceae, Fabaceae, Fagaceae, Icacinaceae, Oleaceae, and Rubiaceae, and they occur in the Malpighiales, Fabales, Fagales, Garryales, Lamiales, and Gentianales, respectively. Thus, epicotyl MPD occurs in all the major groups of flowering plants except eurosids II and euasterids I, while epicotyl PD is restricted to eurosids I and euasterids I. Neither epicotyl MPD nor epicotyl PD is known to occur in the ANA-grade plants, but epicotyl MPD does occur in the Magnoliids.

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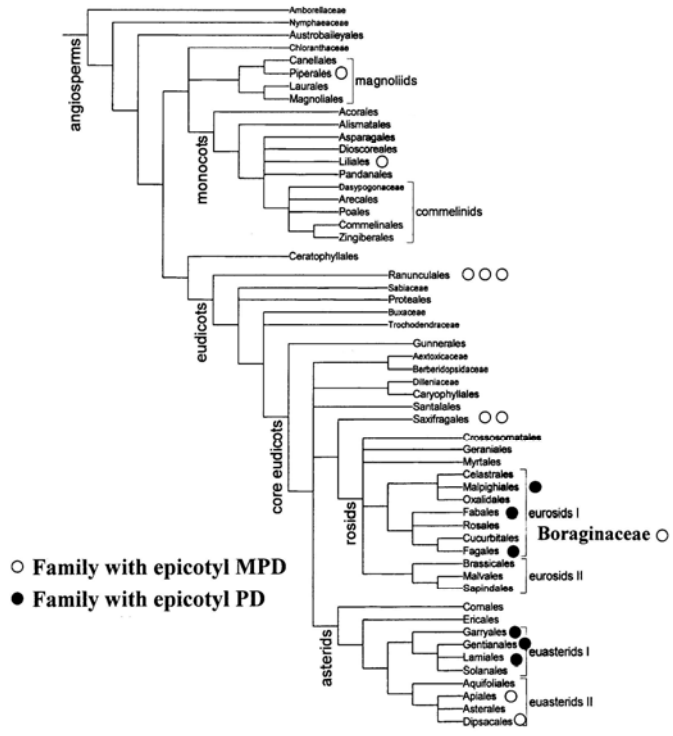


**Figure. 1.** Phylogenetic position of families with epicotyl morphophysiological dormancy (MPD) and with epicotyl physiological dormancy (PD). Phylogenetic diagram is from APG-II (2003).

**Effect of Inbreeding Depression and Population Size on Seed Germination: A Review**

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**Historical**

Darwin (1876) compared the effects of self- and cross-pollination on various aspects of plant growth and reproduction, including number and mass of seeds per fruit and per plant, seed size (mass), and relative time to germination. In 10 of 21 comparisons of relative time to germination, crossed seeds germinated faster than selfed seeds; in 10 selfed seeds germinated faster than crossed seeds; and in one there was no difference in timing. He considered earlier germination to be superior to later germination. His general conclusion from results of the many crosses reported in *Cross and Self-fertilisation* was that, "The first and most important of the conclusions which may be drawn from the observations given in this volume, is that cross-fertilisation is generally beneficial, and self-fertilisation injurious" [*i.e*., inbreeding depression]. (p. 436)

**What is inbreeding depression?**

Inbreeding depression (δ) is the reduction in mean fitness of selfed progeny ($W_s$) compared to that of outcrossed progeny ($W_o$), i.e. $W_s<W_o$.

$$\delta = 1 - (W_s/W_o) = (W_o - W_s)/W_o$$

Inbreeding depression also applies to reduction in mean fitness of offspring resulting from crosses between close relatives (biparental inbreeding depression). One also could have outbreeding depression, i.e. $W_s>W_o$. Thus, in the case of inbreeding depression, percentage/rate of germination of selfed seeds is lower than that of outcrossed seeds, whereas in outbreeding depression percentage/rate of selfed seeds is higher than that of outcrossed seeds. Some things to consider when evaluating inbreeding depression are summarized in Table 1.

**Results and Discussion**

Results of a survey of published papers that included 235 "case studies" of the effects of inbreeding vs. outbreeding on seed germination are: inbred < outbred, 117; inbred = outbred, 101; inbred > outbred, 17. Inbreeding depression also can result from small populations or from fragmentation of large populations, i.e. small fragments. For seed germination, we found published papers that included 43 comparisons of the effect of large continuous (X) vs. small fragment (Y) populations of a species: X<Y, 2; X>Y, 23; and X=Y, 18. Thus, percentage/rate of seed germination may or may not be lower in inbred- than in outbred seeds or in small- than in large populations. A summary of the effects of inbreeding on seed germination are presented in Table 2.

Lower percentage/rate of germination due to selfing or to crossing between close relatives may be due to the effects of inbreeding depression on seed size, seed viability, or dormancy-germination regulating processes *per se*. Differences in seed germination between inbred and outbred seeds is sometimes caused by smaller seeds of inbred plants that germinate to a lower percentage/rate than the larger seeds of outbred plants. Low seed viability (*i.e*. nonviable embryos) often is the cause of low germination percentage in small populations or fragments. Concerning the effect of inbreeding depression on dormancy-germination regulating processes, we suggest that in some cases the plant would gain more long-term fitness via fresh seeds being dormant rather than nondormant. This conclusion is the reverse of the assumptions in studies we reviewed that included information on the effect of inbreeding depression on seed germination: that it is more fit to germinate than not to germinate. In many of these studies, consideration was not given to how dormancy and germination fit into the life cycle of the plant in its habitat Further, in only a few of the 235 case studies did the authors pretreat seeds for dormancy break before incubating them for germination.

Table 1. Some things to consider when evaluating inbreeding depression.

- Competition may increase δ, *i.e*. density-dependent δ.
- Physical environment. δ generally (but not always) increases with stress; thus, ( $\delta_{field}$ / $\delta_{greenhouse}$) > 1.0 [but see Waller *et al*., 2008, who found that stress did not increase inbreeding depression and in fact sometimes decreased it].
- Breeding system and mating history. In general, outbreeders show greater inbreeding depression than selfers, and lower levels of δ are expected in populations with a long history of inbreeding –*i.e*. deleterious genes have been purged from populations of selfers (inbreeders).
- Life history stage. δ can vary among stages of the life cycle, but the majority of δ is expressed late in the life cycle of selfing species and early or late in the life cycle of outcrossing species. δ, especially in early stages of the life cycle, may be modified by maternal environmental effects.
- δ can vary considerably among families (family = offspring of a female) within a population, and among populations and habitats.
- δ may be affected by distances between crosses.
- δ can vary both geographically and from year-to-year at a single location for a species.

Table 2. Effect of inbreeding on percent and/or rate of seed germination.

- outcrossed plants ≥ or < selfed plants
- (selfed) selfing plants ≥ (selfed) outcrossing plants, *i.e*. strongly self compatible plants ≥ weakly self-compatible plants
- chasmogamous flowers (usually outcrossed) ≥ or < cleistogamous flowers (always selfed)
- female flowers ≥ hermaphroditic (selfed and outcrossed) flowers
- between population crosses ≥ or < within population crosses; both better than within population selfed
- populations with high genetic variation ≥ those with low genetic variation; however, germination generally (but not always) increases with heterozygosity
- large populations ≥ small populations
- continuous habitats ≥ fragmented habitats
- within populations, near- neighbor crosses ≥ or < far- crosses
- seeds of plants with low F (coefficient of inbreeding depression) ≥ than those of plants with high F, but generally germination decreases (which may be due to nonviable seeds) with increase in F
- distant populations (outbreeding depression) < populations in close proximity (hybrid vigor)
- between families crosses > within families crosses

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## Effect of Fire on a Seed Bank Pathogen and on Seeds of Its Host *Bromus tectorum*

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### Introduction

The generalist pathogen *Pyrenophora semeniperda* (Brittlebank and Adam) Shoemaker occurs primarily in cheatgrass (*Bromus tectorum* L.) seed banks, where it causes high seed mortality (Beckstead et al. 2007; Meyer et al. 2007). How does fire impact survival of a fungal seed pathogen, *P. semeniperda,* versus survival of the seeds of its cheatgrass host, the invasive *Bromus tectorum*? If fire completely destroys the pathogen, then cheatgrass seeds surviving the fire would be able to establish free of this natural enemy. Conversely, if the pathogen has high post-burn survival, then cheatgrass recovery from a post-fire seed bank could be negatively impacted.

### Methods

We investigated this question in a series of laboratory and seed bank studies. In the laboratory, we determined the 50% thermal death point ($TDP_{50}$, temperature causing 50% mortality) for cheatgrass seeds and for three life stages of the pathogen. Each were subjected to a range of temperatures in a muffle furnace (20 (control), 65, 100, 125, 150, 200 and 300°C) for 5 minutes and evaluated in subsequent viability assessment. Pathogen viability was assessed by placing the heat-treated life stage next to a bait seed during incubation. Seed viability was assessed via a germination test. In the field, we took advantage of two natural wildfires in cheatgrass monocultures in Utah and Washington, USA. Seed bank samples were taken from burned and adjacent unburned areas within a month of the fires. Samples were processed to remove all apparently viable and pathogen-killed cheatgrass seeds. Apparently viable seeds were incubated for 4 weeks at 20°C and scored as germinated, viable but dormant, killed in incubation by the pathogen, or nonviable/unfilled.

### Results and Conclusions

Results showed that the ability of the seed pathogen to survive high temperature and subsequently infect adjacent host seeds decreased with increasing temperature and varied among life stages. The $TDP_{50}$ for the pathogen across all life stages after a 5-minute heat treatment was 164°C (Fig. 1a). The conidial stage experienced 50% mortality at a lower temperature than the mycelial and stromatal stages, indication that conidia are more susceptible to fire. The $TDP_{50}$ for seeds (148°C) was 10% lower than the mean $TDP_{50}$ for the pathogen (Fig 1b), indicating that the seeds would be killed at a lower temperature than the pathogen. Wildfire dramatically reduced viable seed density at two sites; the Rattlesnake Mountain site had a 98% reduction, while the West Mountain site had an 85% reduction (Fig. 2a). The potential inoculum load for *P. semeniperda* measured as the density of field-killed cheatgrass seeds with stromata varied significantly between sites (Fig. 2b); however, fire only had a marginally significant ability to reduce the inoculum load. The ability of the pathogen to survive fire at the mycelial stage, internally in seeds, was indicated by the lack of any significant difference between burn treatments in density of incubation-killed seeds (Fig. 2c). Integrating our laboratory $TDP_{50}$ measurements with the seed bank data from wildfires, it is clear that annual grassland fires in our study were not hot enough to eliminate *P. semeniperda* from the seed zone. We conclude that *P. semeniperda* is as well-adapted to frequent, low-intensity fire as its cheatgrass host. Fires do not necessarily create an escape from this pathogen, thus allowing for post-firecheatgrass expansion.

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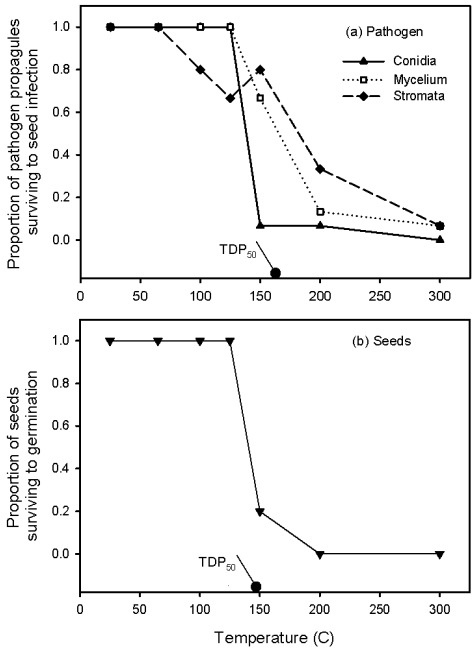


Figure 1. Thermal death curves showing the effect of radiant heat after 5-minute heat treatments on survival of *Pyrenophora semeniperda* life stages (a) and cheatgrass (*Bromus tectorum*) seeds (b). Thermal death points ($TDP_{50}$; temperature at which 50% of the individuals experience death) are indicated for the average of all pathogen life stages (a; $TDP_{50}$ = 164°C) and for cheatgrass seeds (b; $TDP_{50}$ = 148°C).

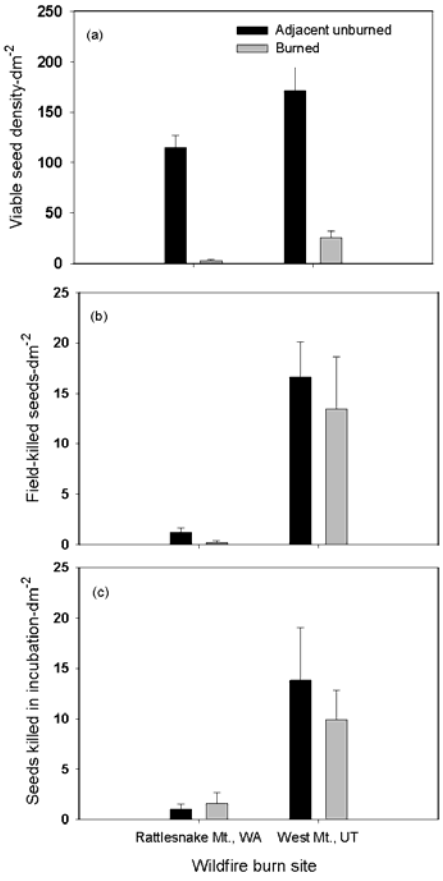


Figure 2. Effects of wildfires at Rattlesnake Mountain, Washington and West Mountain, Utah on cheatgrass (*Bromus tectorum*) seed bank viable seed density (a), field-killed seed density (b), and density of seeds killed in incubation (c); mean + 1 SE). Data represent ten (West Mountain) or twenty (Rattlesnake Mountain) soil samples collected from each treatment combination.

## Modeling and Predicting Changes in Dormancy in Soil Seed Banks

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### Introduction

Dormancy can be defined as an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal and gaseous conditions (Benech-Arnold et al. 2000). Dormancy can be classified as primary or secondary. Primary dormancy refers to the innate dormancy possessed by seeds when they are dispersed from the mother plant. Secondary dormancy refers to a dormant state that is induced in non-dormant seeds by unfavorable conditions for germination, or re-induced in once-dormant seeds after a sufficiently low dormancy had been attained. Predicting seed-bank dormancy level is important because timing and extent of seedling emergence in the field is strictly related to the dormancy state of the seed-bank. The possibility of predicting the dormancy state of the seed-bank, and consequently, timing and extent of seed emergence, has many practical applications. For example, in relation to increasing the efficacy of weed control methods, assessing both timing and extent of weed emergence through predictive models is of capital importance (Batlla and Benech-Arnold 2007). In addition, predictive models can help us to design practices for managing native or introduced plant populations (Allen et al. 2007). To accomplish this goal, the following steps need to be followed: first, the effect of the different environmental factors on the dormancy level of buried seeds must be comprehensively understood; second, the effect of those factors on the dormancy level of the seed-bank population must be quantified; third, the developed quantitative relationships must be included in a consistent modeling framework.In the present paper we show examples of practical approaches to accomplish these three steps.

### Environmental Factors Affecting Dormancy in Natural Soil Seed Banks--Conceptual Model

Environmental factors affecting dormancy level of buried seed-banks can be divided in two classes: dormancy level regulating factors and dormancy terminating factors, i.e., germination initiating factors (Benech-Arnold et al. 2000). The dormancy level regulating factors are related to seasonal synchronicity of seed germination in the field (Finch-Savage and Leubner-Metzger 2006). These factors alter the depth of dormancy, producing seasonal changes in the germination behavior of the seed-bank by altering the sensitivity of seeds to environmental signals. Soil temperature is one of the main factors governing seasonal changes in the seed-bank dormancy level in temperate environments (Benech-Arnold et al. 2000; Allen et al. 2007). On the other hand, for most seed populations, dormancy must be terminated by specific environmental signals (i.e. dormancy terminating factors) which, from an ecological point of view, are factors that indicate in a more immediately way that conditions are suitable for germination (Finch-Savage and Leubner-Metzger 2006). Under field conditions the most important factors that terminate dormancy of buried seeds are light and alternating temperatures. The degree or level of dormancy of a seed population establishes the width of the range of environmental conditions that allow germination. A low dormancy level is characterized by a wide range of environmental conditions permissive for seed germination, while seeds presenting a high dormancy level show a narrow range of environmental conditions permissive for seed germination. This relationship was first proposed by Vegis (1964) who introduced the concept of degrees of relative dormancy from the observation that as dormancy is released, the temperature range permissive for germination widens until it is maximal, while as dormancy is induced, the range of temperatures over which germination can proceed narrows, until germination is no longer possible at any temperature, and full dormancy is reached. For the summer annual *Polygonum aviculare* L., Batlla and Benech-Arnold (2003) demonstrated that during dormancy loss the range of temperatures permissive for germination widened as a consequence of a decrease in the lower limit temperature for seed germination ($T_l$). On the other hand, dormancy induction was characterized through a narrowing of this range due to an increase in $T_l$. In both cases, germination occurs in the field when soil temperature enters the permissive range.

### Temperature Effects on Seed Dormancy Level--Establishing Quantitative Relationships

Thermal-time based models have been used to establish quantitative relationships between temperature and dormancy changes in dry and moist stored seeds. For example, Batlla and Benech-

Arnold (2003) characterised *P. aviculare* dormancy loss through changes in the range of temperatures permissive for germination as a consequence of changes in the value of the mean lower limit temperature permissive for germination ($T_{l\ (50)}$). As a summer annual, *P. aviculare* seeds are released from dormancy by low winter temperatures (i.e., stratification) while high summer temperatures induce secondary dormancy. In order to quantify the effects of stratification time and temperature on seed population dormancy status (assessed through changes in $T_{l\ (50)}$) the authors used a thermal time index calculated as follows (Eqn. 1; Fig 1):

$$S_{tt} = \text{Days} \bullet (T_c - T_s) \qquad \text{Eqn. (1)}$$

where $S_{tt}$ is stratification thermal time units (°Cd), $T_c$ is the dormancy release "ceiling" temperature (°C) (the temperature at, or over, which dormancy release does not occur) and $T_s$ is the daily mean stratification temperature (°C). The optimal "ceiling" temperature for dormancy loss in *P. aviculare* seeds was 17 °C (Batlla and Benech-Arnold 2003).

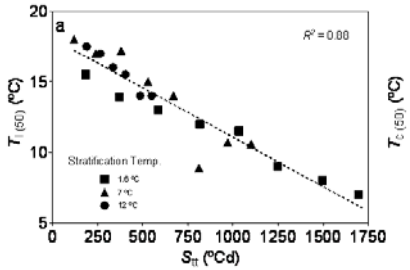


**Figure1.** Estimated values of the mean lower limit temperature ($T_{l\ (50)}$) for *P. aviculare* seeds stored moist at different temperatures plotted against stratification thermal time ($S_{tt}$)

The same index was proven to be effective to predict changes in the response of *P. aviculare* seeds to other environmental factors in relation to the stratification temperature experienced by the seeds, as for example, changes in sensitivity to light (Batlla and Benech-Arnold 2005), in the range of permissive water potential for seed germination (Batlla and Benech-Arnold 2004) and in seed sensitivity to alternating temperature cycles (Batlla et al. 2003).

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# Variation in Germination and Nursery Performance of *Prunus africana* and *Hagenia abyssinica* Populations in Ethiopia

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## Introduction

*Prunus africana* (Hook. F.) Kalm. and *Hagenia abyssinica* (Bruce) J.F. Gmel. are valuable timber and medicinal trees growing in montane habitats of Africa. Both species belong to the family Rosaceae. Recently, they have come under heavy pressure due to intense and selective harvest for their timber and medicine extraction. As a result, both are listed as endangered species in Ethiopia (EFAP, 1994; Negash, 1995). This implies the need for urgent conservation measures. Understanding the variation among populations of trees is essential to devise management strategies for their conservation and sustainable utilization. Re-establishing and expansion of indigenous species across a wider range through afforestation/ reforestation activities requires careful choice of populations and use of quality seeds. Therefore, *P. africana* and *H. abyssinica* seeds were studied under laboratory and nursery conditions, with the major objective of identifying and recommending superior populations for effective seed collection as well as seed source establishment.

## Methods

Mature seeds of *H. abyssinica* and *P. africana* were collected between May and July 2006 from phenotypically superior populations, each representing the potential remaining populations of the study species in the country. *Prunus africana* seeds were collected from potentially known seed production sites: Anferara, Menagesha, Bedele, Masha and Gambo areas. Likewise, *Hagenia abyssinica* seeds were collected from Adaba, Kore, Chilimo, Asella and Bekoji areas. The study sites were selected following the tree seed zone system developed for the country (Aalbæk, 1993). Seeds were transported to the seed laboratory of the Forestry Research Center, Addis Ababa. Subsequent to de-pulping, seeds were cleaned and sun-dried to 6–8% moisture content. Five replicates of 20 seeds were randomly sampled from each population and enclosed in 9.5mm Petri dishes following the procedures in Negash Mamo et al. (2006). Seeds were inspected daily and considered germinated when the radicle reached the size of the seed. Germination percentage was calculated as the cumulative percentage at 60 days after sowing (Demel Teketay,1996).

In order to asses growth performance at the nursery level an experimental plot with a completely randomized design was established. Fifty polythene plastic bags (20 × 20 cm when flat) were filled with soils. Five seeds were directly seeded per bags. No fertilizer or mycorrhizal inoculation was applied. The experiment was carried out for six months. Seedling height, collar diameter, and survival were recorded every month.

## Results and Conclusions

The study species showed significant variation among populations in both laboratory and nursery experiments. *Prunus africana* seeds collected from ‘Menagesha’, ‘Masha’, and ‘Bedele’ sites exhibited better performance, respectively. Likewise, *Hagenia abyssinica* seeds obtained from ‘Chilimo’, ‘Asela’ and ‘Bekoji’, respectively, were found to represent the best provenances. The study recommended these sites for bulk seed collection for future seed orchard establishment.

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**Stream Flow, Seed Suspension, and Seasonality: Hydrochory in a Semi-arid Stream, Verde River, Arizona, USA**

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**Abstract**
Riparian plant species have a unique opportunity for seed and vegetative propagule dispersal, namely dispersal through water—hydrochory. Hydrochory has been show to play an important role in both aquatic and terrestrial plant community composition in montane riparian areas of Scandinavia and the Western US, but has been little studied in semi-arid regions. In this study, we assessed hydrochory in the Verde River, a perennial river in semi-arid Arizona, USA. The Verde has two seasonal periods of peak discharge: one due to winter rains and spring snowmelt, and the other due to late summer monsoon rains. These high flows bring water, and presumably hydrochores, to the dry floodplains of the Verde River. In this study, we addressed three questions: How are hydrochore abundance, species richness, and composition influenced by (1) stream flow rate, (2) position within the water column, and (3) time of year. To address our questions, we collected hydrochores using seed seines suspended at two water depths, nine sites, and twelve times during 2009. Hydrochore samples were sown on potting soil in a greenhouse in which temperature and day length emulated seasonally changing field conditions. Two additional sets of samples were collected for a smaller scale study, where samples were placed in a growth chamber emulating spring field conditions. Current results indicate that numbers of germinants emerging from hydrochore samples varied as a function of all factors examined: stream flow and position within the water column. Of note, under conditions of high stream flows, significantly more germinants emerged from samples collected from the water column (vs. those collected from the surface.)

# Maturity, Germination and Longevity studies in a Narrow Endemic Columbine in the Italian Alps (*Aquilegia thalictrifolia* Scott et Kotschy).

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## Introduction

*Aquilegia thalictrifolia* Scott et Kotschy, or Lake Garda Columbine, is a critically endangered, narrow endemic species with a relict distribution around Lake Garda in the central Italian Alps. This species has attracted local research interest for many reasons: its peculiar distribution, following a relict pattern while this genus is usually considered in active migration; its singular ecology, restricted to the base of dripping wet cliffs; its complex germination requirements, having an underdeveloped embryo and a suspected morpho-physiological dormancy as inferred by preliminary investigations carried out in 2006, and last but not least its worrying conservation status. An integrated all-round ecological study was started in 2009, analyzing microclimate, population dynamics, fluctuating asymmetry in leaf and flower shape, and fruit set and seed set in all 17 fertile subpopulation of the species, complemented by germination studies and genetic analysis. A preliminary investigation on germination requirements carried out in 2006 suggested the presence of an underdeveloped embryo requiring warm stratification for its development, followed by cold stratification and alternating temperatures for final germination (Castellani, 2006).

## Methods

The germination trials presented here and still ongoing were started in autumn 2009 and were designed to test the initial hypothesis of a morpho-physiological dormancy requiring sequential warm and cold stratification for germination. The germination main cycle mimics the natural annual soil temperature experienced by the seeds in the wild, based on the temperatures measured by logger for 3 years in the first five cm of the soil of the investigated growing site. This main cycle includes 8 weeks of warm stratification at 20/10°C, 16 weeks of cold stratification at 1°C, and 2 constant (15°C and 20°C) and 3 alternating (15/5°C, 20/10°C and 25/15°C) final germinating temperatures. To serve as controls, this main cycle was carried out also in the absence of warm stratification or cold stratification or both. An additional trial was carried out with gibberellic acid (250mg/l) at 20°C. Each single test was performed in light (12h light and 12h dark) and dark. The germination medium selected was 1% agar-distilled water gel on Petri dishes, with 4 replications of 25 seeds each. Eight cooled incubators are in use with a 4000°K fluorescent light source. Dark conditions were simulated by sealing Petri dishes in foil bags and scoring was carried out in safe green light. All germination trials were carried out on freshly collected seeds from the same growing site. Seeds were considered ripe when their seed coat turned black and the fruit capsule was open. Comparative seed ageing studies were also carried out employing the Kew simplified protocol for comparative seed longevity testing (Davies *et al*. 2004), employing a single ageing conditions at 60%RH and 45°C.

## Results and Conclusions

Preliminary results are partially in contrast with the initial investigation carried out by Castellani (2006). Contrary to what was concluded in 2006, current experimental data show that dormancy can be removed without a warm stratification period and no alternating temperatures are necessary in the germination stage. As widely indicated for the *Ranunculaceae* (Martin, 1946; Baskin & Baskin, 1998) the current investigation confirms that the embryo is underdeveloped, and that it increases in length by approx. 30% in 8 weeks' time (fig. 1). A cold stratification period is necessary and sufficient to remove both the morphological and the physiological components of dormancy (fig. 2). Once dormancy is broken, seeds germinate similarly (>80%) in dark and light conditions, and/or under alternating and constant germination temperatures (Fig.3). Hormone treatments were capable of fully removing dormancy and promoting near-total germination within 2 to 3 weeks. The difference between the 2006 and 2009 investigations might be due to among-year differences or to different timing of seed collection. Careful field observations indicate that it is difficult to clearly identify the appropriate maturity stage: seeds turn black quite early while still in a partly open green follicle, but might remain for nearly a month in the same follicle as it turns brown and splits open on the upper side. The germination behaviour of seeds collected at different times might differ significantly, particularly if an underdeveloped embryo in involved. Therefore the apparent discrepancy between 2006 and 2009

data might also be explained by different times of seed collection. This might indicate that change in color is not a reliable indicator of seed maturity in *Aquilegia* and might explain the wide variation in response to the same germination conditions observed in different seed lots. Assuming that in 2006 seeds were collected at an earlier stage of development, fully ripe seed lots seems to require only a long cold stratification (at least 16 weeks), while black seeds from a green capsule might also require a warm period before the cold stratification and germinate better at final alternating temperature. To definitively demonstrate this hypothesis, a follow-up experiment is needed, testing seeds collected in the same year at different stages of fruit development. Comparative seed ageing studies confirmed this species is short-lived in storage, with a $P_{50}$ of 17 days and a MTD (mean time to death) of 24 days at 60%RH and 45°C, close to the lower benchmark represented by *Ranunculus sceleratus* L.

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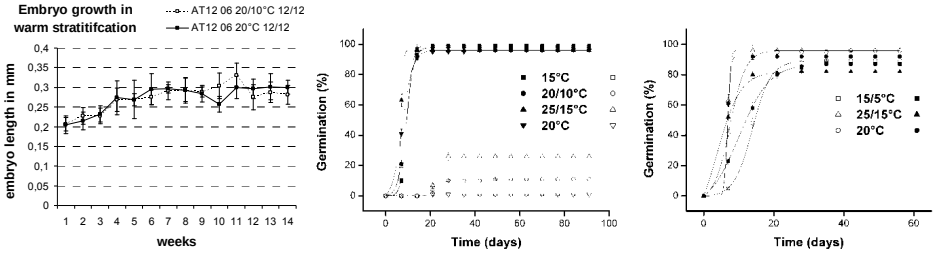


**Figure 1**. Embryo growth (mm) during warm stratification at constant (20°C) and alternating temperature (20/10°C)

**Figure 2**. Germination progress curves for seeds of *A. thalictrifolia* held at 4 different temperatures after 0 weeks (empty symbol) and 16 weeks of cold stratification at 1°C (closed symbol).

**Figure 3**. Germination progress curves for seeds of *A. thalictrifolia* held at 3 different temperatures in light (empty symbol) and dark (closed symbol) conditions, after 16 weeks of cold stratification at 1°C

# Seed Ecology of Australian Rutaceae

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## Introduction

In Australia large areas of natural vegetation have been removed and degraded resulting in high numbers of threatened species in urgent need of conservation. One family of noticeable importance is the Rutaceae, a taxonomically diverse and widespread group with many species currently classified as rare and threatened, and numerous others key components of different ecosystems. Species of Rutaceae are well recognized as producing seeds that are problematic to germinate as well as having highly variable seed quality. Conservation of Rutaceae is currently hampered by a lack of knowledge on their basic seed biology, seed dormancy and seed ecology. This purpose of this study was to investigate general seed biology, dormancy and ecology of selected Rutaceae, focusing on representative taxa from different ecosystems and biogeographical locations in both western and eastern Australia. The results from this study will aid in the conservation and utilization of native Rutaceae species and increase our overall understanding of seed dormancy mechanisms in Australian natives.

## Methods

Basic seed characteristics were investigated where possible on at least one species per genus in the Rutaceae family. These include seed weight, lengths, viability/seed fill (using x-ray), moisture content, embryo lengths, embryo type (Martin 1946) and some basic germination tests including the use of smoke component Karrikinolide (previously known as butenolide; Flematti *et al* 2004) and gibberellic acid ($GA_3$). Using a dichotomous key developed by Baskin and Baskin (2004), the results obtained for embryo type and germination, as well as imbibition tests, were used to determine the type of seed dormancy. To investigate how dormancy is moderated by environmental stimuli, a series of laboratory and field based studies were carried out on select species. These included after-ripening (dry, warm storage), cold and warm stratification, embryo extraction and nicking of seed coats. Burial studies were carried out to investigate changes in seed viability, dormancy state and germination in response to seasonal changes.

## Results and Conclusions

At least three different embryo types have been observed across the family; however the majority of species, particularly those found in the southern half of Australia, possess linear embryos running the full length of the seed. There are significant seed quality issues for many species of Rutaceae, with seed fill ranging from 0 to 98%. There were also marked differences in seed quality of separate populations of the same species suggesting quality may be provenance-driven (Figure 1). Only 1 species out of 19 tested so far was capable of germinating to 100% with no pretreatment and so was considered not to have seed dormancy. This species, *Micromelum minutum*, was also the only member of Rutaceae tested with a folded embryo and is only found in the northernmost parts of Australia.

A positive germination response to $GA_3$ was observed, but only in a few species and at very low percentages. Preliminary results indicate physiological dormancy is the dominant type in this family, as seeds are capable of imbibing water and have developed and differentiated embryos, but do not germinate under seemingly favourable conditions. After-ripening partially overcame dormancy in one species, with germination percentages steadily increasing from 0 to 50% after 12 months after-ripening (Figure 2). Current experiments using warm stratification are proving successful in the species *Diplolaena angustifolia* with germination percentages increasing to over 50%. Nicking and excision of embryos has also produced positive results; however this method of germination is not very efficient.

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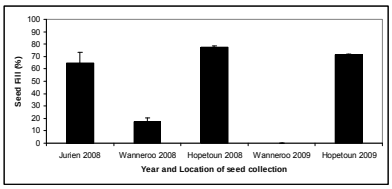


**Figure 1:** Average seed fill percentages of *Boronia ramosa* from various sites in Western Australia over two years, results obtained using x-ray analysis.

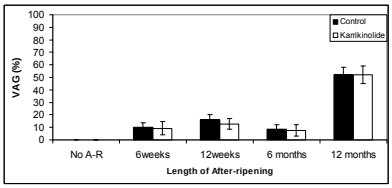


**Figure 2:** Mean viability adjusted germination (VAG) percentages of *Diplolaena angustifolia* when after-ripened (stored at 30ºC and 50% relative humidity) for 6 weeks, 3 months, 6 months or 12 months, followed by 8 weeks incubation at 15ºC.

# Does Cattle Dung Cause Differences in the Germination Response of Grazing Increasers vs Decreasers?

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## Introduction

Grazing is a fundamental factor in the shaping and maintenance of Mediterranean grasslands. A considerable proportion of herbaceous species in these systems are found exclusively in grazed or non-grazed sites, with relatively few species found in both zones (Peco *et al.* 2006). Depending on their response to grazing in terms of abundance or presence in a given zone, species are classified as increasers if they increase, or decreasers if they decline.To date, no experimental studies have taken into account the effect of dung on germination as a determinant of response to grazing. This paper strives to ascertain whether the rates of germination and root growth of species classified as increasers and decreasers are affected differently by cattle dung leachates.

## Methods

A phytotron germination experiment was conducted using seeds of 14 herbaceous species in five different families, seven increasers and seven decreasers (Table 1). A mix of air dried dung and distilled water in a 1:3 ratio was compacted to produce a highly concentrated extract (25% extract). This extract was diluted three times, and four different cattle dung leachate concentrations (1%, 5%, 10% and 25%) were generated. These four treatments, along with a pure distilled water control, were applied to the Petri dishes containing the seeds (25 seeds per dish; 6 replicates in each treatment). The replicates were subjected to 12 h of light at 20 ºC and 12 h of darkness at 10 ºC for 6 weeks. Each dish was examined to define the percentages of viable germinated seeds (GP), T50 (time lapsed until 50% of the seeds germinated) and root length after five days (L5) for at least four germinated seeds per species and treatment. Mixed effect linear models were used (Gelman *et al.* 2007), in which the response variables studied were GP, T50 and L5. The explanatory variables were leachate concentration, grazing response (increaser or decreaser), and interaction between the two. The family was used as grouping factor, allowing the intercept to vary between groups. Four different models were generated for each response variable: a linear response to treatment was assumed in the first, while the other three corresponded to piecewise regressions in which a response change threshold was assumed (at 1%, 5% and 10% concentrations). The models with the lowest score for the Akaike Information Criterion were selected (Sasaki et al. 2008).

## Results and Conclusions

The decreaser GPs clearly diminished between the control and the 1% concentration, while increasers were unaffected. At this concentration, the differences in germination between the two groups of species were maximized. Above the threshold, the increment in leachate concentration produced no significant changes in the increasers' GP, while the decreasers' GP increased slightly (Fig. 1a).

For all leachate concentrations, the increasers germinated faster than the decreasers. In the range between the control and the 5% concentration, the decreaser T50 rose proportionally to the leachate concentration, while at higher concentrations, no effect of higher concentrations was noted. The increaser T50 was not affected by the treatment throughout the concentration range (Fig. 1b).

The decreaser species L5 was affected negatively by the increments in leachate concentration above the 1% threshold. Increments in treatment concentration intensified the difference of L5 between the two species groups (Fig. 1c).

Increasers seemed to tolerate conditions generated by the presence of dung, while decreaser germination and root growth indices declined. This could have significant effects on the relationship between the colonising and survival capacity of seedlings in the two groups of species. There are also indications of a non-linear response of plant species to action by cattle, which might explain the existence of abrupt changes in floristic composition under certain levels of grazing pressure.

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**Table 1.** Species selected for the experiment, classified by family and response to grazing.

| Family | Increaser | Decreaser |
|---|---|---|
| ***Brassicaceae*** | *Brassica barrelieri* (L.) Janka | *Alyssum granatense* Boiss. & Reuter |
| ***Caryophyllaceae*** | *Spergularia purpurea* (Pers.) D. Don | *Silene scabriflora* Brot. |
| ***Leguminosae*** | *Astragalus pelecinus* (L.) Barneby | *Vicia lathyroides* L. |
| | *Trifolium glomeratum* L. | *Trifolium strictum* L. |
| ***Plantaginaceae*** | *Plantago coronopus* L. | *Plantago lanceolata* L. |
| ***Poaceae*** | *Poa annua* L. | *Micropyrum tenellum* (L.) Link |
| | *Vulpia muralis* (Kunth) Nees | *Vulpia ciliata* Dumort. |

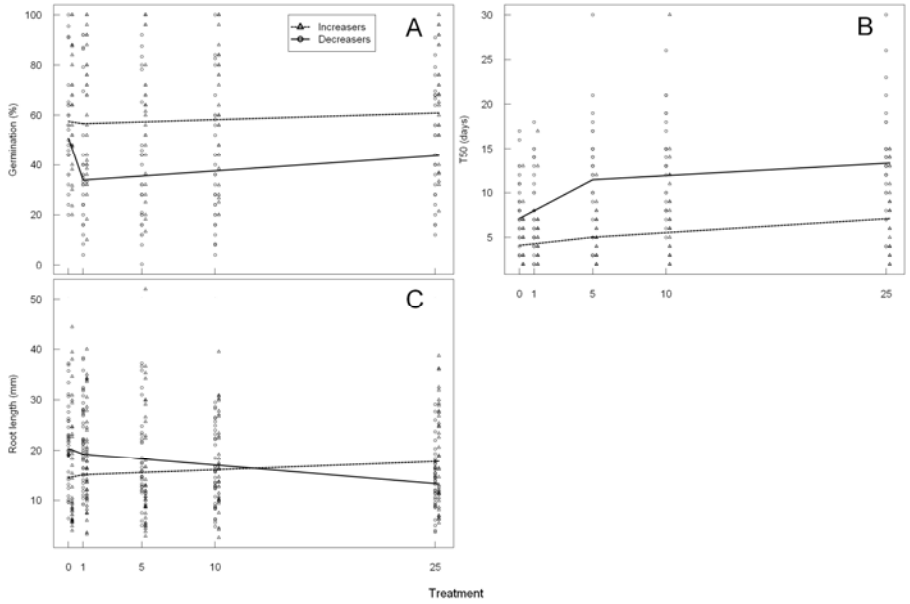


**Figure 1.** Effect of dung leachate concentration on GP (A), T50 (B) and L5 (C) of increasers and decreasers, analysed using piecewise regression.

# Effects of Light and Temperature on Seed Germination of *Ficus* spp. from Two Contrasting Tropical Microhabitats

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## Introduction

As members of a pan-tropical genus of trees, shrubs and vines occupying a wide variety of ecological niches, many *Ficus* species serve as keystone species in rainforest ecosystems in terms of providing a key food resource for some frugivores. Species-specific seed germination responding to the forest microenvironment may contribute to species coexistence in tropical forests (Vázquez-Yanes and Smith 1982, Daws et al. 2002). We investigated the effects of light and temperature gradient as affected by gap formation on seed germination of two pioneer tree species (*Ficus hispida* and *F. semicordata*) and two climax tree species (*F. altissima* and *F. auriculata*) to explain how the four *Ficus* species differed in their niches.

## Methods

Seeds of the four *Ficus* species were collected in Xishuangbanna Tropical Botanical Garden, air-dried after collection, and stored in darkness at room temperature (around 26C) until the start of the experiments. Tests of the effects of light and temperature on seed germination were set at 23/20 and 30C, and a R/FR (red/far-red light) ratio gradient (0.67, 0.52, 0.42 and 0.34) was created by filtering incandescent light through 0, 2, 4 and 6 layers of green plastic films, respectively. One layer of neutral-density black plastic film was used to determine the effect of light intensity on seed germination of the figs at 30C. All the experiments were carried out in incubators with 14h light/10h dark treatments. Final germination percentages were compared with analysis of variance (ANOVA).

## Results and Conclusions

The seeds of the four *Ficus* species were photoblastic and could not germinate in total darkness, but germination could be induced by very low PPFD (photosynthetic photon flux density), though it was delayed. Higher seed germination percentages for the pioneer species *F. hispida* and *F. semicordata* were obtained at higher R/FR ratios (0.67 and 0.52) than at lower R/FR ratios (0.42 and 0.34), and their seed germination was significantly inhibited by R/FR ratios of 0.52 and 0.42 at 23/20C as compared with 30C (Fig. 1a, b). In contrast, all R/FR ratios, both at 23/20 and 30C were effectively favourable for the seed germination of the climax species *F. altissima* and *F. auriculata* (Fig. 1c, d). Thus light response differences may help explain why these fig species occur in two contrasting microhabitats.

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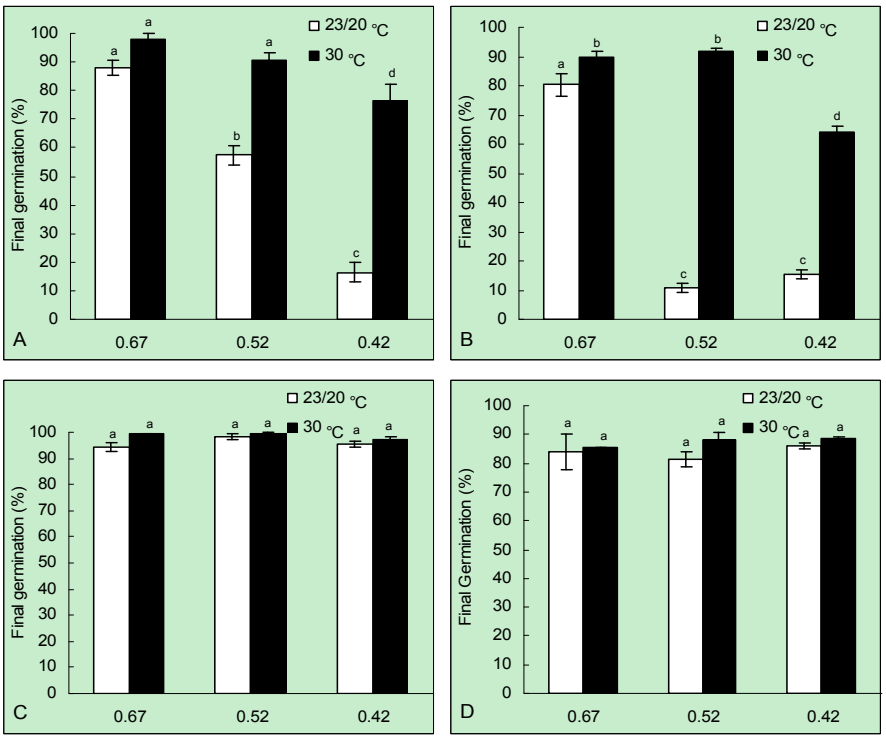


**Figure 1.** Effects of temperature on the response of *F. hispida* (A), *F. semicordata* (B), *F. altissima* (C) and *F. auriculata* (D) seeds to R/FR (mean ± *SE*). Treatments with different letters are significantly different at $P < 0.05$.

# Effect of the Acorn Pericarp on Germination of *Pasania konishii* (Fagaceae)

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## Introduction

Fagaceae is a major plant family in Taiwan and is represented by eight genera, one of which is *Pasania* with 13 species. *Pasania konishii* (Hayata) Schottky is endemic to Taiwan and is distributed in natural forests throughout the island at elevations from 500 m to 1,000 m (Liao 2000). The embryo contains high quality fatty acids that potentially can be used as cooking oil. The purpose of this study was to characterize seed germination by removing the acorn pericarp and by cold-stratifying acorns at 5$^{o}$C for different periods of time.

## Methods

Mature acorns were harvested from central Taiwan (23$^{o}$50'52"N, 120$^{o}$56'14"E; altitude 800 m asl) in September 2008; mean number of acorns per liter was 105, and mean thickness of pericarp 3.96 ± 1.91 mm. Acorns or pericarp-removed seeds of *P. konishii* were mixed with moist sphagnum moss (water content of the sphagnum moss was about 400% of dry mass), sealed inside polyethylene bags (0.04 mm in thickness) and incubated at 12 h/12 h alternating temperatures regimes of 30/20, 25/15 and 20/10$^{o}$C. They were exposed to 12 h of light (80-100 µmole m-2 sec-1, 400-700 nm) during the higher temperature and to 12 h of constant darkness at the low temperature. Each treatment consisted of three replications of 25 seeds each. Germination, i.e. radicle at least 2 mm long, was recorded weekly for 32 weeks. For cold stratification, fresh acorns were mixed with moist sphagnum moss (cut into small pieces and water content was about 400% of dry mass), sealed inside polyethylene bags and stored at 5$^{o}$C in darkness for 4, 16 and 32 months prior to testing for germination.

## Results and Conclusions

Fresh acorns incubated at 25/15$^{o}$C began to germinate after 14 weeks, and germination was 9% and 30% after 26 and 67 weeks, respectively (Fig. 1); further incubation resulted in acorn deterioration. Acorns incubated at 30/20 and 20/10$^{o}$C germinated to 7% and 21%, respectively, after 67 weeks. After 4, 16 and 32 months of cold stratification at 5$^{o}$C, seed germination rate and percentage increased, e.g., after 4, 16 and 32 weeks of cold stratification, germination at 25/15$^{o}$C began after weeks 3, 2 and 1, respectively, and 33%, 46% and 39% of seeds, respectively, germinated after 26 weeks. Removal of the pericarp from freshly harvested acorns promoted germination, and germination began in week 2 and increased to 59% in week10; final germination was 95% in week 18 (Fig. 1). Pericarps were observed before and after cold stratification by scanning electron microscopy, and evidence of softening and breakdown was found. Seedlings from cold-stratified acorns grew well in the greenhouse. Our results suggest that the pericarp influences germination of fresh acorns, and that cold stratification might increase growth potential of the embryo and help weaken the pericarp, thus shortening germination time.

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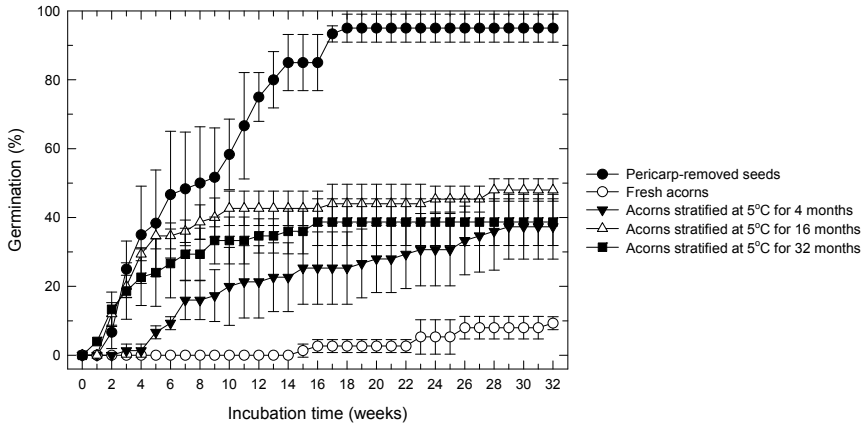


Figure 1. Effect of pericarp removal and cold stratification on germination of fresh acorns of *Pasania kinishii*.

# Seed Dormancy and Germination of *Cycas taitungensis* (Cycadaceae)

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## Introduction

*Cycas taitungensis*, previously misidentified as *C. taiwaniana*, is endemic to an area of about 290 ha in eastern Taiwan (22o51'30" - 22o52'30"N, 120o57'30" - 121o01'00"E) (Lin et al. 2000). The *C. taitungensis* Nature Reserve was established in 1986. However, this species has been attacked by the scale insect, *Aulacaspis yasumatsui,* and the larvae of the cycad blue butterfly (*Chilades pandara peripatria*), and many plants have died recently. To establish ex situ conservation of *C. taitungensis*, seeds were collected both from the Nature Reserve and a man-managed plantation for propagation. *Cycas taitungensis* is a dioecious species and seeds mature in October through December. The fresh *Cycas* seed has a small, linear embryo with a clear view of the coiled suspensor, and the embryo grows inside of the seed before the radicle emerges. This study had two primary objectives: (1) to characterize seed germination and to measure embryo length during incubation, and (2) to store seeds at 5oC for 6 and 12 months to test viability.

## Methods

Due to the presence of many scale insects, the sarcotesta was removed and seeds were soaked in Benomyl (fungicide and pesticide) for 1 hour before using them in experiments. Fresh seeds of *C. taitungensis* mixed with moist sphagnum moss were placed in sealable polyethylene (PE) bags (water content of the sphagnum moss inside PE bags was about 400% of dry mass) and incubated for 12 h/12 h at 25/15, 30/20 and 25oC. The daily photoperiod of 12 h in the incubators (80-100 µmole m-2 sec-1, 400-700 nm) was at the high temperature. The sphagnum moss used for seed germination was cut into small pieces. Each treatment consisted of three replications of 25 seeds. Germination, i.e., radicle at least 2 mm long, was recorded weekly for 32-60 weeks. Results were expressed as germination percentage.

## Results and Conclusions

Germination of seeds from 12 individual plants from the Nature Reserve at 30/20oC extended from weeks 3 to 18, and final germination ranged from 25% to 90% (Fig. 1). However, germination of seeds from the plantation began on week 17, and final germination was 58-60% (64% for seeds treated with GA3) (Fig. 2). These differences may be due to the wide range in time of pollination and thus to different degrees of seed maturity and embryo sizes (5-22 mm in length) in seeds from the Nature Reserve. For plantation seeds, there was a 6-week delay in germination of fresh seeds at 25/15oC compared to that at 30/20oC. Cold stratification of plantation seeds at 5oC for 12 months decreased time to beginning of germination by 5 weeks, but final germination decreased from 60% (fresh seeds) to 50%. Embryos in fresh plantation seeds were 7-9 mm long and increased in length by about 240% (in 17 weeks) before radicle emergence. Thus, seeds of *C. taitungensis* have morphophysiological dormancy, a primitive condition in seed plants.

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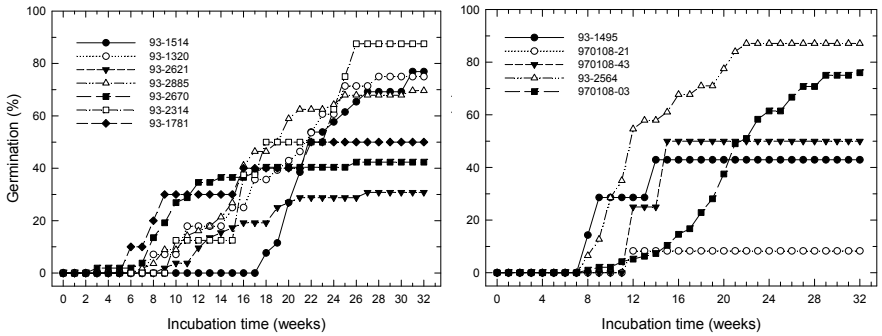


**Figure 1.** Germination of C. taitungensis seeds collected from 12 individual plants (7 left panel, 5 right panel) in the Nature Reserve

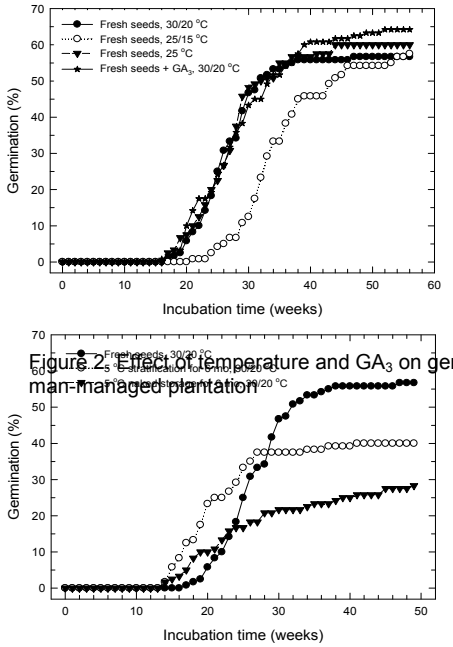


Figure 2. Effect of temperature and $GA_3$ on germination of *C. taitungensis* seeds collected in a man-managed plantation

## Restoring the Desert: Using Seed Ecological Information to Restore Arid-zone Mines

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### Introduction

Seeds are one of the main sources of plants for restoration. Limited research on restoration seed ecology has been undertaken in the arid-zone of Australia, even though the region covers 70% of the continent. We aimed to understand the ecophysiology of seed dormancy and germination of dominant species in the Western Australian arid-zone to maximise the efficiency of seed use and increase the species diversity in restored mining areas. Climate in the arid zone plays a major role in seed ecology, and we investigated how temperature and moisture may drive dormancy loss and cue germination. The Australian arid-zone also is fire-prone, so we tested the effect of an active compound in smoke, karrikinolide ($KAR_1$), on germination. The results of this study will help guide mine restoration efforts in arid Australia.

### Methods

Fresh seeds of 25 species were incubated at 25°C on water agar, or agar containing $KAR_1$. Physically dormant seeds (Fabaceae) were treated with hot (>90°C) water for 1 minute prior to incubation. Germination was scored regularly to investigate germination rate and final germination was determined after 28 days of incubation.

Seeds of *Grevillea* Knight (Proteaceae), *Goodenia* Sm. (Goodeniaceae) and *Triodia* R.Br. (Poaceae) species, which had low initial germination on water agar, were considered dormant. To alleviate dormancy we used warm (35°C) and dry (50% RH), warm and wet (water agar) or alternating wet and dry (2 days on water agar followed by 12 days at 50% RH) storage; conditions that mimic those experienced by seeds in the soil. After storage, seeds were incubated at 25°C on water agar, or agar containing $KAR_1$.

### Results and Conclusions

Research on 25 species revealed that relative proportions of dormancy types were similar to those described for hot deserts; i.e. there are few non-dormant species, and physical and physiological dormancy predominate. It is not surprising that we have not (yet) found morphological dormancy, as soil moisture is not generally available in the arid-zone for long periods of time, without which embryos would not be able to grow prior to germination (Baskin et al. 1998).

*Germination rate*

Some researchers hypothesise that seeds that germinate slowly are favoured in ecosystems with short rainfall events interspersed between drought periods (Fenner et al. 2005). This was not the case in a study on seeds from arid central Australia, as most germinated quickly (Jurado et al. 1992). We found that non-dormant seeds and seeds treated to alleviated dormancy from our arid ecosystem germinated quickly, with the time to 50% ($t_{50}$) germination of seeds of most species being just 1-4 days. We consider that although water may only be available in the soil for short periods, non-dormant seeds are able to take advantage of this soil moisture as they germinate quickly. However, Fenner et al. (2005) did not specify whether the seeds that germinated slowly were dormant or non-dormant. If the seeds with slow germination that were favoured in ecosystems with drought periods were in fact dormant, then their hypothesis correlates with this study, as we found that seeds of most species were dormant; once non-dormant, seeds germinated quickly.

The fast germination rates of non-dormant seeds have implications for restoration practices. Should seeds be pre-treated to break dormancy prior to seeding? If so, will they all germinate with the first large rainfall event? What does that mean for risk management – will seedlings die if they germinate after a rainfall event that isn't sufficient for establishment? Or will seeds only germinate when there is sufficient soil moisture for survival? Instead, should we broadcast dormant seeds, and let them come out of dormancy naturally? Or then do we run the risk that they may not germinate in the first season,

then lose viability, or be lost via predation or erosion before they are able to germinate? Further experiments are planned in an attempt to answer these questions.

*Dormancy and the smoke stimulant*

The Western Australian arid-zone is fire-prone and $KAR_1$ promoted germination of fresh seeds of two *Goodenia*, four *Grevillea* and two *Triodia* species. When we investigated dormancy loss of some of these species, we found that dormancy was (at least partly) alleviated by warm and dry storage (*Grevillea* spp.) or alternating warm wet, and warm dry storage (*Triodia* spp.). Germination of *Triodia* seeds increased on both water and $KAR_1$ agar as dormancy was alleviated over an 8-weeek storage period, with germination on $KAR_1$ consistently higher than that on water agar. For *Grevillea stenobotrya* F.Muell., 4 weeks of dry storage further increased germination of seeds treated with $KAR_1$. However, over 24 weeks of dry storage, germination of *G. stenobotrya* seeds incubated on water agar increased and almost reached the same level of germination as the $KAR_1$-treated seeds. Hence, the ability of $KAR_1$ to stimulate germination above control levels can change over time. Storing *Triodia* seeds for a longer period may reveal whether control germination eventually reaches that of $KAR_1$ seeds.

Whether or not the smoke-signal (in this case $KAR_1$) is a predominant germination cue of physiologically dormant species from the Western Australian arid-zone is an unresolved issue. In a previous study (Commander et al. 2009), one species (*Anthocercis littorea* Labill.) appeared to have an obligate requirement for $KAR_1$ as dry storage only increased germination of seeds when they were treated with $KAR_1$. Thus, seeds used for restoration purposes would require treatment with $KAR_1$. However, the present study shows that although germination of fresh seeds of several species is stimulated by $KAR_1$, dormancy was lost during dry storage and eventually seeds of some species were able to germinate without it. In their natural environment, seeds of those species may initially respond to a smoke cue when they are fresh, but after a period of dry storage they may lose dormancy and then not need a fire to stimulate germination. In a restoration context, seeds could either be treated with $KAR_1$ if practitioners aim to establish seedlings immediately, or seeds could be broadcast while they are still dormant and assume that soil storage will eventually alleviate dormancy without the need for fire, while risking seed predation and asynchronous germination.

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# Germination and Field Survival of *Sarracenia leucophylla* Seeds

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## Introduction

*Sarracenia leucophylla* Raf. (crimson pitcherplant) is native to the Gulf Coast region of the United States. The plants are found in Alabama, Florida, Georgia, Mississippi, and North Carolina, with populations in Florida and Georgia listed as endangered. *Sarracenia leucophylla* is a wetland indicator species found in bogs and wet pine savannahs. The plants are insectivorous, trapping and digesting insects to obtain nutrients lacking in the poor soils in which they grow. They are threatened by wetland draining for agriculture and development, invasive species, and also by illegal harvesting. They thrive under a naturally occurring fire regime and require plentiful sunlight.

## Methods

Thirty seed pods were harvested from a stand of *S. leucophylla*, located in Alabama 38 miles from the Gulf Coast, in August 2009. Pods were sealed in plastic bags and shipped overnight to Auburn, AL. Seeds were immediately harvested from brown pods. Green pods were allowed to mature, and the seeds were extracted when the pods showed signs of splitting open. All pods had opened and the seeds removed within eight days of harvest. The following tests were immediately conducted:

Laboratory Studies:

(1) One hundred seeds were scattered into a clear-lidded plastic box lined with moist paper. Seeds were sprayed with Spectracide® Immunox all-purpose fungicide (7 oz per gallon) and placed in a growth chamber set at a uniform 30°C. After 4 weeks, with no sign of germination, this was changed to 30°C with light for 16 hours and 25°C, no light, for 8 hours (hereafter referred to as the standard temperature regime).

(2) One hundred seeds were rolled into a moist paper towel, sealed inside a plastic bag, and placed in the cold storage room at 4°C. The bag was removed from the cold room after 60 days, and the seeds were scattered into a clear-lidded plastic box, sprayed with the fungicide, and placed in the growth chamber set at the standard temperature regime.

(3) One hundred seeds were soaked in 10% bleach for 1 minute, rinsed 3 times with distilled water, and divided into 2 lots of 50 seeds each. Two 1-liter plastic pots were lined in the bottom with pine needles and filled with a 50-50 mix of coconut husk and sand. Seeds were sprinkled on the top of these pots and then thoroughly sprayed with the fungicide. Nets were tented over the pots which were then placed in a tray filled with rain water.

(4) Seeds were placed in storage at 4°C. Every six months, a vial containing 400 seeds will be removed from storage and tested for viability.

Field Study:

One hundred seeds were placed in each of 56 5.1 by 7.6-cm screen-wire bags that were sewn with clear plastic thread. Three plastic tubs measuring 50.8 cms by 64.8 cms were filled with a 50/50 sand/ peat moss mixture. The screen-wire bags were divided into three lots and placed in a single layer on top of the sand/peat moss mixture, then covered with a layer of fine sand. The tubs were kept moist by a rainwater irrigation system. Bags were harvested and seed viability tested after 3 months in the growth chamber set at the standard temperature regime. Bags will be harvested at regular intervals for the next year.

## Results and Conclusions

*Sarracenia leucophylla* seeds harvested from pods and placed immediately in the growth chamber did not germinate, even when the temperature regime was altered from a constant 30°C to alternating 30°C/25°C. Conversely, 21 of the 100 seeds that were cold stratified for 60 days produced plantlets. After 3 months in the field, four bags of the seeds buried in the tubs were uncovered and brought into

the lab. Germinated as four replications of 100 seeds each, average percent germination of these seeds was 66%.

Only one seed of the 100 in the netted pots has germinated to date although the seeds are still clearly visible on the soil surface. The seed that germinated did so in October 2009; it has not visibly grown since germination.

After initial germination tests, a second seed sample was not taken for 7 months, instead of 6, because of mechanical difficulties with the growth chamber. Seeds left in the ground for 7 months showed no evidence of germination in the field. After 3 weeks in the germinator, they are averaging 18% germination. Dry seeds stored at 4$^{o}$C for 7 months and placed immediately in the growth chamber show no sign of germinating after 3 weeks, suggesting that seeds must be moist stratified in order to initiate germination.

It is evident from these studies that, despite the southern location of the seed source, some cold stratification is necessary to stimulate germination of *S. leucophylla* seeds and that seeds buried for at least 7 months retain viability. We will continue to harvest seeds from the field and will periodically test viability of seeds kept in cold storage.

# The Effect of Scarification and Stratification Treatments on the Germination of *Danthonia californica* Seed from Three Populations

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## Introduction

*Danthonia californica* Bolander (California oatgrass) is an important cool season perennial grass for prairie restoration as well as rangeland and wildlife habitat improvement in Pacific Coastal States USA. However, establishment is often confounded by delayed germination attributed to one or more types of seed dormancy. Dormancy varies from low to high among populations and among and within seed lots of the same population. Hulling/scarification (Trask 1996) or the use of concentrated sulfuric acid to erode the pericarp (Laude 1949) have been among the most successful methods promoting germination, but cold moist stratification or a combination of treatments have worked as well. This suggests the possibility of combined dormancy in some seed lots (Darris et al. 2008). In order to break dormancy, a series of four experiments were conducted to compare the effects of single and multiple treatments on the germination of *Danthonia californica*, including cold moist stratification (moist chilling), warm moist stratification, hulling, and acid or mechanical scarification of the seed coat.

## Methods

Seed from three natural populations of *Danthonia californica* [Polk Co, OR (747), Douglas Co., OR (415), and California (030)] were used in a series of four germination experiments. All experimental samples consisted of a random mix of terminal inflorescence seed and cleistogenes, typical of machine harvested *Danthonia* seed lots that have been aggressively threshed. Seed was stored under ambient air conditions in the office until used. All tests used standard germination boxes and paper placed in a growth room with a photoperiod of 16 hr light/8 hr dark at 20°C/15°C (Tables 1-2) or 25°C/20°C (Tables 3-4). Germination was recorded weekly for 21 days. Experimental design consisted of a completely randomized design with four replications and 50 seeds per replication. All data were transformed with an angular transformation and means compared using analysis of variance and LSD test at the alpha 0.05 level of significance.

Cold moist stratification occurred for 90 days at 3°C (Table 1) or 60 days at 3°C plus 30 days at 11°C (Table 3). Warm moist stratification was conducted in the dark by wrapping germination boxes in aluminum foil and placing them in a growth room at 25°C/20°C for 14 or 28 days prior to being uncovered (Table 4). Seeds were manually hulled by rubbing and peeling off the lemma and palea by hand to minimize any damage to the pericarp, and mechanically hulled by moving the seed between two rubber belts travelling at slightly different speeds (called a belt thresher). Scarification of the pericarp was accomplished with concentrated sulfuric acid (sp. gr. 1.84, as per Laude 1949), with a 50% dilute solution of sulfuric acid, or with a Forsberg seed scarifier using 320 grit sandpaper, all at varying time intervals (Tables 1-2). Seed was “nicked” by making a cut with a scalpel over the endosperm on the dorsal side of manually hulled seed (Laude 1949) (Tables 2-3).

## Results and Conclusions

Means with the same letter are not significantly different (P=.05) (Tables 1-4). Manually hulled then nicked seed germinated within 2% of total viability as determined by tetrazolium test (Tables 2-3), but manual hulling alone did not significantly improve germination over the controls for two of three populations. This suggests that for at least some populations, delayed germination is the result of a single, seed coat imposed dormancy. This result concurs with work by others (Laude 1949, Trask 1996), and does not indicate a combined dormancy mechanism. While the hull may still impose partial dormancy in some seed lots, manual removal is impractical and mechanical removal did not improve germination over no treatment. Mechanical hulling also increased the number of abnormal seedlings (Table 2). The fact that the embryo is in a vulnerable position and subject to physical injury (Laude 1949) probably explains the highly reduced germination from all sandpaper scarification treatments by a machine. In sharp contrast to Laude (1949), concentrated sulfuric acid treatments did not improve germination but were instead detrimental, if not lethal to the seed. In this study, 90 days of cold moist stratification alone or 28 days of warm moist stratification in the dark were virtually equivalent. They probably involve biochemical degradation or physical changes to the pericarp allowing for a higher

percent germination. A change in moisture imbibition is not likely the reason (Laude 1949). Cold moist or warm moist stratification are the simplest and most practical means to maximize germination of some populations of *Danthonia californica* without undue damage to the seed.

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Table 1. Effect of mechanical hulling, moist chilling, and scarification on germination of two populations of *Danthonia californica*

| Treatment | % germination by population | |
|---|---|---|
| | 415 | 747 |
| Total viability (TZ test) | 96 | 93 |
| 90 d | 88 a | 81 a |
| Hulled + 90 d | 53 b | 52 b |
| None (control) | 52 bc | 39 c |
| 90 d + 15 sec sand | 50 bc | 47 bc |
| Hulled | 44 c | 50 bc |
| 90 d + 5 min acid | 31 d | 18 e |
| Hulled + 15 sec sand | 17 e | 8 f |
| Hulled + 90 d + 5 sec sand | 8 f | 28 d |
| Hulled + 90 d + 10 sec sand | 8 fg | 7 f |
| Hulled + 90 d + 15 sec sand | 4 fgh | 1 g |
| Hulled + 90 d + 20 sec sand | 4 gh | 7 f |
| 20 min acid | 2 h | 20 de |
| Hulled + 90 d + 5 min acid | 0 i | 0 g |
| Hulled + 90 d + 10 min acid | 0 i | 0 g |
| Hulled + 90 d + 20 min acid | 0 i | 0 g |
| Hulled + 90 d + 30 min acid | 0 i | 0 g |
| Hulled + 90 d + 40 min acid | 0 i | 0 g |
| Hulled + 20 min acid | 0 i | 0 g |
| 90 d + 20 min acid | 0 i | 0 g |

Table 2. Effect of hulling and scarification treatments on germination of *Danthonia californica* (population 030)

| Treatment | % germination | |
|---|---|---|
| | abnorm | normal |
| Total viability (TZ test) | | 96 |
| Manually hulled + nicked | 2 | 97 a |
| Manually hulled | 0 | 82 b |
| 20 min 50% dilute acid | 0 | 80 bc |
| 5 min 50% dilute acid | 0 | 76 bcd |
| Machine hulled | 7 | 71 cd |
| None (control) | 0 | 65 de |
| Mach. hulled + 5 sec sand | 21 | 57 ef |
| 5 min acid | 5 | 48 f |
| Mach. hulled + 20 sec sand | 24 | 19 g |
| Mach. hulled + 5 min acid | 3 | 18 gh |
| 20 min acid | 8 | 10 h |
| Mach. hulled + 20 min acid | 2 | 0.5 i |

Table 3. Effect of manual hulling and moist chilling on germination of *Danthonia californica*

| Treatment | % germination by population | | |
|---|---|---|---|
| | 030 | 415 | 747 |
| Total viability (TZ test) | 96 | 96 | 93 |
| Man. hulled + nicked coat | --- | 95 a | 91 a |
| Man. hulled + 90 d cold/cool | 85 a | --- | --- |
| 90 d cold/cool | 70 b | 83 b | 59 b |
| Manually hulled | --- | 23 c | 20 c |
| None (control) | 53 c | 15 c | 14 c |

Table 4. Effect of warm moist stratification on germination of *Danthonia californica*

| Treatment | % germ. by population | | |
|---|---|---|---|
| | 030 | 415 | 747 |
| Total viability (TZ test) | 96 | 96 | 93 |
| 28 d warm | 72 a | 88 a | 81 a |
| None (control) | 68 a | 75 b | 47 b |
| 14 d warm | 44 b | 42 c | 46 b |

# Where Dispersal Meets Dormancy: "Hard" Seed Dispersal by Herbivorous Mammals

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## Introduction

Large herbivorous mammals (from *a*urochs to *z*ebra) are regarded as important vectors in seed dispersal, by ingesting/excreting large numbers of seeds from numerous grasses and herbs (endozoochory). In a seminal paper, Janzen (1984) hypothesized that these animals accordingly exert(ed) selection for seed attributes that favor this mode of dispersal. Yet, empirical support for this hypothesis is lacking. If a seed attribute is identified that unambiguously promotes endozoochory, Janzen's hypothesis on endozoochory-imposed selection could nonetheless be addressed directly; if this trait is amenable to micro-evolution (see below),this could even be done *in situ*.

To identify seed traits that are important in endozoochory, experiments have been performed in which known amounts of seeds were fed to ungulates, and mortality rates were related to seed attributes (e.g., Cosyns et al. 2005, Mouissie et al. 2005). As shown recently, however, suggestions on relevant attributes from these studies (e.g.,seed length, mass, shape) are often impaired due to an insufficient control of species interdependence in analyses (D'hondt et al. in press). In contrast, seeds that are water-impermeable at consumption consistently show high to complete survival (e.g., Gardener et al. 1993, Ramos et al. 2006). Water impermeability of seeds, or "hardseededness", is primarily a dormancy trait and occurs in several taxa (physical dormancy, e.g., in Fabaceae, Cistaceae). Due to its direct fitness consequences, it thus seems a suitable trait to study selection through endozoochorous dispersal. For micro-evolution to be possible, however, the trait furthermore needs (1) to exhibit sufficient intraspecific variation, and (2) to have a heritable basis. We explored these conditions for the degree of initial "hardseededness" in clover (*Trifolium* spp.); i.e., the proportion of seeds in mature infructescences (heads) that are impermeable to water and would thus survive herbivore consumption.

## Methods

Two field campaigns were performed to assess the level of intraspecific variation in initial hardseededness. In an elaborate campaign in summer 2008, we marked inflorescences of white clover (*Trifolium repens* L.) that had finished flowering at several natural sites, and collected them 26 days later (i.e., at seed maturity, but before dispersal). All seeds were dissected from these infructescences, and tested for water-impermeability (on moist blotting paper) and viability (tetrazolium staining). In a preliminary second campaign (summer 2009), we collected ripe infructescences from various other, mostly annual, clover species in the field, and tested their seeds similarly for impermeability and viability. To assess whether any variation in the proportion of impermeable seeds at maturity is due to genetic differences or environmental conditions, a greenhouse experiment was performed on the clonal *T. repens*. As humidity is considered the most influential environmental condition on the acquisition of physical dormancy in legumes (Hyde, 1954), we subjected two ramets of each of nine genetically distinct clones to either dry (minimum relative air humidity = 47%), mesic (61%), or wet (89%) conditions during seed maturation. The seeds were then dissected from infructescences and tested for impermeability and viability as before.

## Results and Conclusions

In *T. repens*, no less than 35% of the seeds proved permeable (i.e., non-dormant, or "soft") at maturity. The proportion of impermeable seeds within infructescences ranged from 0 to 100% (Fig. 1). For the other *Trifolium* species, this variation was less marked. Modally, infructescences contained high to full levels of dormant seeds in all species. Each of the nine clones showed the same response to humidity in the greenhouse experiment: put briefly, dry conditions yielded impermeable seeds, wet conditions yielded permeable seeds, and mesic conditions yielded combinations of both. The incidence of water-permeable seeds at maturity thus appears to stem from a plastic response within genotypes to humidity, not genetic variation (broad-sense heritability estimate ≈ .03). The phenotypic variation from the field (Fig. 1) probably likewise represents (micro)climatological variation between environments and seasons.

When consumed by herbivorous mammals, individual clover infructescences would substantially have differential fitness because of *in situ* variation in the degree of initial hardseededness. However, the suitability of this system to study endozoochory-imposed evolution seems limited due to the low heritability of this trait. Nonetheless, genes have been identified that affect the level of initial hardseededness in other legumes species (e.g. *Lupinus*, Boersma et al. 2007), but further elaboration is needed. In contrast to our understanding of seed dispersal by wind, ants or birds, the evolutionary ecology of seed dispersal by herbivorous mammals remains largely unexplored.

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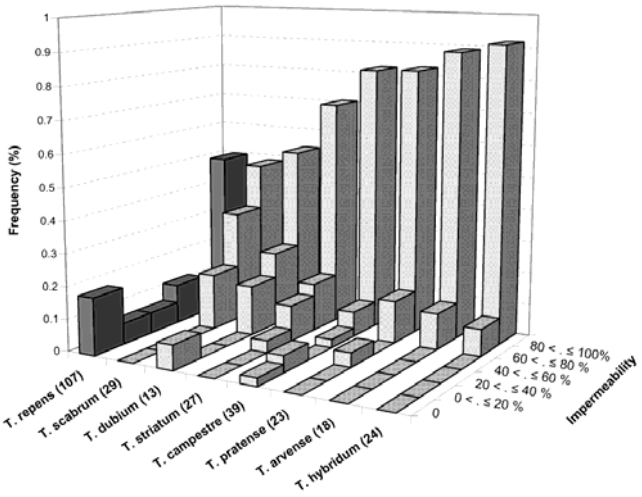


**Figure 1.** Frequency distribution for the proportion of water-impermeable to viable seeds held within mature infructescences of several clover species (*Trifolium* spp.). The number of investigated infructescences is given between brackets. The campaign for *T. repens* (2008) was performed apart from that for the other species (2009).

# Does Ethylene Play Some Role in the Germination Enhancement of Clustered *Reseda complicata* Seeds?

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## Introduction

*Reseda complicata* Bory (Fam. *Resedaceae*) is an endemic species of the Betic Mountains (South Spain). It grows between 2400 and 3050m and, like other of high mountain species, presents dormancy. Dormancy break can be induced, especially by temperature and light, and by some treatments such as physical and chemical scarification. Furthermore some phytohormones can also break dormancy. Previous studies indicated that Ethrel, a compound that hydrolyzes at pH>4 and produces ethylene, substantially increased the degree of germination in this species (Díaz-Miguel et al. 2010). In current literature only a few reports exist on density-dependent or clustering effects on seed germination. The objective of this study was to determine whether clustering of *R. complicata* seeds affected their ability to germinate and the possibility that ethylene is involved in this process.

## Methods

The *R. complicata* seeds were collected from natural populations from the Sierra Nevada of the Betic Mountains within the coordinates 37º05'44''N and 3º23´14´´W at 2500m altitude. The seeds were stored at 4ºC for three months before the study was initiated. For all experiments the seeds were surface-sterilized with sodium hypochlorite at 1% for 5 minutes and then washed with abundant, distilled, sterilized water. In the first experiment, the clustering effect on seed germination was tested. Four series of clusters of 10, 20 and 40 seeds were placed on two layers of filter paper resting on glass beads (6mm in diameter) in Petri dishes (9cm in diameter). These contained 20ml of distilled water. The same design wasused for seeds which were isolated instead of clustered. All germination experiments were carried out in a growth chamber at a temperature of 25/5$^{\circ}$C (light/dark conditions, 14/10h respectively). These were similar to summer conditions within the seed collection zone in the Sierra Nevada. In light, the seeds were exposed to cool-white fluorescent light (PAR, 20µmolm$^{-2}$s$^{-1}$at seed level). Seed germination was monitored every 24 hours for 31 days, and a seed was considered to be germinated when the radicle emerged.
To measure ethylene production, seeds are grouped or individually arranged on a filter paper strip 5x1cm with 20 seeds per strip. This, in turn, was placed on a disk of moistened filter paper in a Petri dish with four replicates in each case. They dishes were moved to a dark 20$^{\circ}$C chamber, and at several different times periods during germination, the strips were introduced into a 6x1cm vial with a screw lock where they remained for 4h of darkness. The ethylene given off was determined by gas chromatography.

Using the compiled data a further experiment was performed under the same conditions as the first experiment, but the seeds were sown in soil from the zone where the seeds were collected (4mm sifted and mixed with vermiculite 50%). The soil was placed into a 30x20cm tray that had a 2cm layer of quartz sand at the base. The tray was then divided into plots of 36cm$^2$ and 30 seeds were sown per plot, with 4 plots per treatment. The four types of treatment for the seeds were: a) Control (seeds imbibed in water for 12h and then sown isolated), b) Seeds pre-treated for 12h with 100ppm ethrel and then sown isolated, c) Seeds were once again imbibed in water for 12h and then placed within layers of soil in gelatine capsules, d) Seeds were pretreated in ethrel 100ppm for 12h and then encapsulated as in c). For thirty five days the time course of germination was followed and finally the seedlings were weighed from each plot and the data was statistically analyzed.

## Results and Conclusions

In all cases studied, the clustering of seeds positively affects the degree and rate of germination, although no differences between numbers of seeds per group were detected (Fig 1). Even though many species of seeds release substances that can inhibit the germination of other seeds, in some cases these effects can be stimulants (Murray, 1998). Based on the fact that ethylene is a significant stimulant for germination (Baskin et al. 2003), other authors associated the production of this substance with the increase in the germination of *Ottelia alismoides* seeds when they are grouped together (Yin et al. 2009). In the case of *R. complicata* seeds, the possibility that ethylene plays a significant role in this respect was examined in the second experiment. A higher level of ethylene was

given off when seeds were grouped together as opposed to when they were isolated (Fig.2). In the last experiment carried out (Fig. 3) the application of ethrel and the encapsulation of the seeds produced similar results, even though the germination rate (measured as T50) was higher in the first case: 4 and 12 respectively. The fact that the seeds that were pre-treated with ethrel and encapsulated didn't reach the values obtained in the other treatments could be due to a supraoptimal effect. In fact the application of ethylene concentrations greater than 100ppm led to similar results (data not shown). Finally one has to emphasize the fact that treatments b), c) and d) provoked a greater development of the seedlings compared with the control (1.6, 1.55, 1.25 and 1.1 mg/10 seedlings, respectively). With regard to the results obtained in these experiments, they suggest that there is a positive relationship between production of ethylene and the increase in germination observed as a result of clustering for *R. complicata* seeds.

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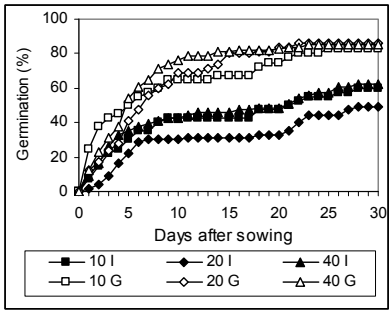


Fig. 1: Germination of *R complicata* seeds with relation to the number seeds per cluster. I: isolated, G: grouped

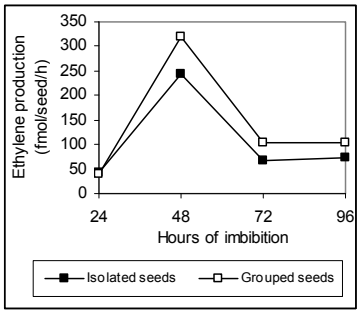


Fig. 2: Evolution of the ethylene released from *R. complicata* seeds

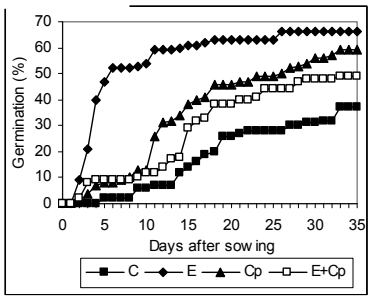


Fig.3: Germination of *R. complicata* seeds sown in soil. For more details see the text

**Predicting Optimum Germination Conditions for Wild Species Stored in Seed Banks.**

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**Introduction**

When conserving wild species *ex situ* in seed banks, it is important to be able to germinate all the viable seeds in the collections, at will. This is to ensure both the accurate monitoring of viability in storage, and that the full genetic variability and potential of a stored seed population is released for use, e.g., in species recovery and vegetation restoration projects.

Worldwide, around one third of all wild species studied are not exacting in their germination requirements (Baskin and Baskin, 1998). So long as they have sufficient moisture and the correct temperature they are relatively easy to germinate fully. The remainder possess varying degrees of several different kinds of seed dormancy, presumed to result from evolution to ensure that seedlings emerge when they are most likely to survive, and also to ensure that the emergence is spread over time ('bet hedging'). For seed banks holding very diverse seed collections of wild species, often the subjects of no previous germination research, seed dormancy (and choosing the best conditions to overcome it) can be a considerable problem, especially when rapid and synchronous germination is desired.

At the Millennium Seed Bank it has not been possible to conclude the initial germination test for over 20% of the collections, i.e., they are 'difficult', and optimal germination protocols are not yet established. This has significant practical consequences and resource implications, in a project that conducts around 14,000 germination tests every year, leading to a large and growing backlog of seed germination testing.

Here we review progress in optimising the use of the limited information available for most in-coming seed collections, to provide a decision support toolbox for germination testers, who usually seek the simplest conditions for full germination. In the past this has relied heavily on taxonomy, e.g. 'known' traits of families and closely related species, and a series of family 'algorithms' or protocols. However, the association of germination syndromes with climate has long been recognised (Baskin and Baskin, 1998); and the organised use climatic information in setting germination conditions for particular species and regional floras is increasing (e.g. Baskin and Baskin, 2003; Merritt et al., 2007). With the need to improve performance in the MSB, the focus is now on using available climate data to predict successful germination conditions on a global scale, alongside analysis of existing 'in-house' data and the published information of others.

**Methods**

Two approaches have been taken to providing seed testers with relevant data and information. First is an 'analytical' approach, where data-mining techniques, such as Bayesian Networks and Decision Trees have been used in attempting to discover predictive patterns in an existing large, but biased dataset – the Seed Bank Database (SBD). Results of preliminary collaboration with School of Information Systems, Computing and Mathematics, Brunel University are briefly described.

More attention is given here to a complementary, 'synthetic' approach. This uses basic collection data – species, and place and time of collection – to generate or 'predict' likely successful germination temperatures and dormancy-breaking pre-treatments. Collection geo-references are used in GIS with 'WorldClim' global interpolated climate surfaces (http://www.worldclim.org/ ; see also, Hijmans et al., 2005) to generate corresponding macroclimate data (microclimatic information being available only extremely rarely). These are then used together with dispersal time to estimate the time of germination in the field (mostly not recorded at the global scale), and thus both optimum germination temperature and temperature treatments likely to be successful in removing dormancy.

**Results and Conclusions**

Several examples of comparisons of successful germination conditions (and not so successful ones) will be compared with conditions predicted by the system, to demonstrate its efficacy. These include

diverse species from different habitats and closely-related species from different local habitats in the same region. They will show how the use of relatively coarse climate data (macroclimate modelled by interpolation) can provide valuable support in choosing successful germination test conditions for diverse species over a broad range of habitats.

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## Interactions across Seed and Adult Life Stages: Ecological and Evolutionary Consequences

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### Introduction

A life cycle is a sequence of developmental events that occurs within a sequence of seasonal change. In development, later life stages are contingent upon early life stages. The importance of early life stages in determining life cycles derives from the fact that they establish not only the physiological and genetic context for subsequent development, but also the seasonal context. As such, early life stages have compounding effects across the life cycle and are therefore likely to be subjected to intense natural selection, through direct effects on fitness as well as through their indirect effects on later life-history traits (Donohue et al. in press).

Germination behavior is one of the earliest phenotypes expressed by plants. It determines the seasonal environment experienced by new germinants and can influence the environment experienced by plants throughout their lives, thereby influencing the expression of and selection on post-germination traits. Germination, in turn, is strongly influenced by post-germination traits via maternal effects; the environmental conditions during seed maturation and immediately after dispersal strongly influence germination, and these conditions are determined by adult physiology and phenology. Thus early and late stages influence each other. Such interactions across life stages occur when one life stage determines the environment experienced by subsequent life stages. These interactions across life stages can influence life-history expression, genetic expression, and adaptive dynamics.

### Methods

Combining field and controlled-environment studies of various genetic materials, we measured natural selection on germination phenology, investigated the genetic basis of germination, characterized maternal effects on germination, and quantified the effect of germination and allelic variants of germination genes on adult life-history expression.

### Results and Conclusions

Germination phenology is under intense natural selection in natural and experimental populations, and loci associated with germination can change frequency extremely rapidly in response to such selection (Huang et al. 2010). Maternal effects on germination influence the strength and mode of natural selection on germination as well as the expression of genetic variation for germination (Donohue et al 2005, Chiang et al., unpublished results). Such maternal effects have the potential to contribute to variable natural selection on germination alleles and consequently contribute to the maintenance of genetic variation at germination loci. Germination, in turn influences the timing of reproduction and seed maturation. Under field conditions, the loci that had the largest effect on flowering phenology were actually loci that controlled germination timing. Such pleiotropic effects of germination, manifest under natural conditions due to phenotypic interactions across life stages, are expected to influence the evolution of germination, post-germination traits, and life-history. Because of interactions across life stages, whereby one life stage determines the environment experienced by the other stages, we cannot predict one life stage without knowledge of the others, nor can we elucidate the genetic basis of life traits without considering the effects of these interactions.

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# The Use of Agricultural Direct Drill Tynes to Improve Direct Seeding Results in Revegetation Using Australian Native Tree and Shrub Seeds.

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## Introduction

The regeneration and restoration of woodland areas in the Murray Catchment of NSW by the Murray Catchment Management Authority (CMA) is based on the principles of selection and protection of the best quality sites followed by appropriate management and restoration practices and appropriate incentives to achieve this. Unfortunately, as is the case of most of agricultural Australia, all available areas have been impacted upon to varying degrees. In most cases this has resulted in the complete loss of understorey shrubs across the landscape, with little or no regeneration potential (largely due to an exhausted soil seed bank) and complete lack of structural and species diversity.Direct seeding and the development of extensive areas of native Seed Production Areas (SPAs) has developed as the mainstay for broad scale native plant restoration programs in the Murray over the past two decades (with 2500 ha average area seeded and up to 1500kg of seed per annum used). There has been incremental adaptation and improvement in technology and great improvement in availability of seed supply (through SPAs) in this time under increasingly adverse climatic conditions. Agriculture has adapted to these changing conditions with the adoption of minimum tillage with deeper knife point tyne seedbed preparation to enhance germination, root penetration and seedling growth. The adoption and adaptation of these same tynes fitted to a towable direct seeding machine (towed by a modified utility vehicle), has enabled a direct replicated comparative trial against three differently adapted machines in 2009. Comparisons of multiple species, direct seeded across hundreds of sites all showed consistent improvement in results by the new machine over conventional machines (except where shallow rock is present). This was consistant over different ranges of soils and rainfall (total 3200 kilometres of sowing lines). To eliminate complexity of these multi species results on variable sites (and to get clear comparative results of the three machines) four cleared ex-agricultural sites (on different soil types) were established using a single species (*Atriplex nummularia* Lindl.) in replicated lines using three separate machines simultaneously. All sites confirmed the multi species observations of earlier germination, greater growth and greater survival using the agricultural tyne.

## Methods

Four separate trial sites were established on four different soil types (heavy clay, crusting clay, clay loam, and sandy loam) using three different direct seeding machines (unmodified Burford, modified Jenke parallelogram, and Primary Sales precision seeder bar). All sites were within 20km of Deniliquin in the southern Riverina of NSW, Australia. *Atriplex nummularia* seed was sown from each of the three machines in late June 2009 at a rate of c400g/km (c1200g/ha) in a replication of consecutive treatments in parallel lines across each site. Therehad been ca. 25mm of rainfall recorded in May and no more than 85mm recorded in Deniliquin over the growing season. Broad spectrum herbicide and insecticide was applied at all sites at sowing.
The sites were monitored for germination in July and for plant density, growth and survival in November 2009 and February 2010.

## Results and Conclusions

Despite the very dry winter/spring period there was good seedling emergence across all sites and soils. Seedling germination was consistantly higher on all soils using the Primary sales tyne than both other machines (Fig1). Average seedling height and growth was also greater for the Primary Sales tyne on all sites, but represented in Fig. 2 for the loamy clay site only. Monitoring for both subsequent dates showed that plant density of *Atriplex* seedlings (at all sites on all soils) was consistently higher and maintained with the use of the Primary Sales tyne represented for the loamy clay soil in Fig. 3. By February 2010, the average seedling density produced by the new machine was 12300 per hectare compared with an average of 2850 seedlings per hectare for the two older machines. Seedling growth was also greater, with an average height of 49cm compared to 33cm of the two older machines. This in turn resulted in a biomass of 1845kg dry matter per ha. for the new machine compared to an average of 156kg dry matter per ha. for the older machines, the difference being 1689 kg DM/Ha.

It is apparent from results to date that the adoption of the Primary Sales agricultural tyne has significant advantages for adoption in native plant regeneration and restoration projects. These advantages are primarily due to the deeper incised seedbed formation that assists native plant seed in promoting earlier germination, faster root penetration and greater growth and survival than the existing equipment. It also has the added advantage of enabling harvesting and deep penetration of rainfall in suboptimal conditions. This higher germination and survival advantage immediately translates into reduced seed use (a scarce and valuable resource), ability for more extensive coverage and more reliable results in harsh sites. The Primary Sales precision seeder bar is less invasive in sites with relatively intact ground layers and therefore creates less weed invasion and lower incidence of soil erosion resulting from works. It has the added advantage that it requires less experience and mechanical skill in setting up and operating. We consider that this is the greatest single improvement in direct seeding technology since the technique was adopted and anticipate that it will lead to the Murray Catchment Management Authority updating the majority of its machinery to the modified agricultural tynes. Current real and potential exotic weed competition still remains as a primary inhibiting factor in both seedling establishment and growth (depending on rainfall amount and timing). However these trials were not able to assess or progress any herbicide advances beyond the traditional broad spectrum glyphosate.

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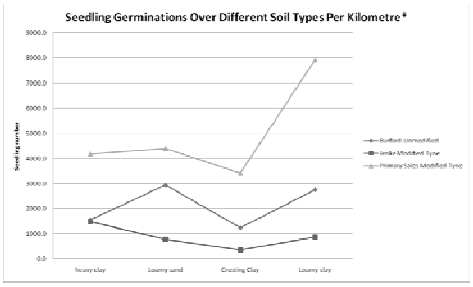


**Figure 1.** Seedling germination using three different drill tynes.

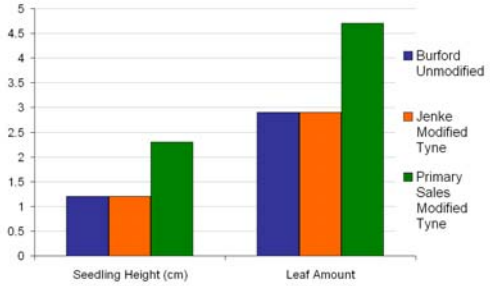


**Figure 2.** Average plant height and leaves per seedling of *Atriplex* grown on loamy clay soil.

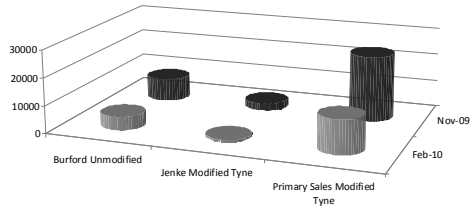


**Figure 3.** Plant density of Atriplex seedlings measured in Nov. 2009 (dark gray) and again in Feb. 2010 (light gray).

# Effects of Seed Storage on Germination of Two Succulent Desert Halophytes with Little Dormancy and Short-lived Seeds

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## Introduction

Dry storage at high temperatures increases germination percentage under optimum germination conditions for species with deep dormancy from the Saharo-Arabian phytogeographic deserts (Gutterman, 1994). However, few studies have assessed the effect of storage conditions and duration on species with transient seedbanks, in which seeds have no or low dormancy after ripening. In this study, the impact of storage period (up to 17 months) and storage conditions (natural habitats, warm, room temperature and cold) on germination level and speed were assessed in *Salsola imbricata* Forssk. and *Haloxylon salicornicum* (Moq.) Bunge. et Boiss. (Chenopodiaceae). The study also aimed at assessing the impact of the different storage conditions for a period of one year on temperature requirements of the two species. The two species are succulent shrubs dominant in different sandy habitats of the Arabian Gulf deserts. Fresh seeds have high germination level and speed immediately after dispersal and do not develop between-season seed banks under natural conditions (El-Keblawy et al. 2007; El-Keblawy and Al-Shamsi 2008).

## Methods

Fresh seeds of *H. salicornicum* and *S. imbricata* were collected during December 2004 from large populations around Al-Ain city, UAE. Seeds of each species were divided into five groups. Seeds of one group were incubated immediately after collection (within 5 days, fresh seeds). Seeds of the other four groups were put into 4cm x 6cm mesh bags. The bags were stored at room temperature, in a freezer at -4 °C (cold storage), in an oven adjusted at 40 °C ± 2 °C (warm storage) and on the soil surface in a desert habitat where the two species occur naturally. Seeds were tested for germination after 3, 9, 12 and 17 months of storage, in an incubator adjusted at 20 °C with continuous light. In order to assess the impact of different storage conditions on temperature requirement, seeds stored in room temperature, freezer and oven were tested for germination in four incubators adjusted at 15, 20, 25 and 30 °C in continuous light.

Germinated seedlings were counted and removed every other day for 14 days following sowing. The rate of germination was estimated using a modified Timson's index (germination velocity = $\Sigma G/t$), where G is the percentage of seed germination at 2d intervals and t is the total germination period. Two way-ANOVAs were used to assess the impact of storage condition and both seed storage period and temperature of incubation on final germination percentage and rate. A Tukey test was used to estimate least significant range between means.

## Results and Conclusions

Effect of storage period and storage condition

Storage for 3 months significantly increased germination of seeds stored under different conditions, compared to that of fresh seeds. In *H. salicornicum*, storage for 9 and 12 months completely inhibited germination of seeds stored in the field and significantly reduced germination of seeds stored in warm and room temperature conditions, but did not affect germination of cold stored seeds, compared to fresh seeds. Further storage for 17 months led for almost complete inhibition in germination of seeds stored in warm and room temperature conditions and to a significant reduction for the germination of seeds stored in the freezer (Table 1). For *S. imbricata*, 9-month storage inhibited germination of field seeds, but had no effect on seeds stored under the other conditions. Storage for 12 and 17 months significantly reduced final germination of cold, room and warm storage seeds, but did not completely eliminate it (Table 1). These results indicate that the two species have transient seedbanks. The high annual fruit production, even in dry years, coupled with high seed dispersal ability compensates for the high rate of mortality of seeds and adult plants of these species. Germination rate index increased with storage. This increase was insignificant for 3-month-stored seeds, but was significant for seeds stored 9 months in all storage conditions, except field seeds, which did not germinate. After 12 and 17 months, the increase in germination rate was significant for *S. imbricata* stored at the different conditions, but was significant only for seeds of *H. salicornicum* stored in the freezer. After 17 months, the germination rate of *H. salicornicum* seeds stored in room temperature and warm conditions was significantly lower than that of fresh seeds (Table 1).

Effect of storage condition and temperature of incubation

Fresh seeds of the two species germinated to significantly higher percentages at 20-25 °C compared to 30 ºC. In both species, cold storage had little effect on final germination and germination speed of seeds incubated at the different temperatures, compared to fresh seeds. However, room temperature and warm storages significantly reduced final germination and germination speed at the different temperatures, and the reduction was most pronounced at 30 °C, especially in *H. salicornicum* (Table 2). The significant reduction of germination speed in *H. salicornicum* at 30 °C is of ecological advantage in a desert that receives occasional rainfall in summer, such as that of the UAE. This would delay germination and consequently save germinated seedlings from the high temperatures and dry soils in summer.

To conclude, hydration-dehydration cycles at soil surface would have greater effect on seed physiological states and consequently would promote metabolic failure and viability loss of seeds with little dormancy. On the other hand, cold storage could reduce seed metabolism and consequently maintain seed viability and enhance germination speed.

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Table 1. Effects of storage period and storage condition on final germination percentage and germination rate index (mean ± SE) of *Haloxylon salicornicum* and *Salsola imbricata.*

| | | Haloxylon salicornicum | | Salsola imbricata | |
|---|---|---|---|---|---|
| Storage period | Storage condition | Ger. % | Ger. rate | Ger. % | Ger. rate |
| Fresh | Fresh | 76.0 ± 4.0 | 42.0 ± 0.1 | 65.0 ± 6.0 | 42.9 ± 0.0 |
| 3 months | Cold | 89.3 ± 3.5 | 46.9 ± 0.5 | 77.3 ± 7.4 | 41.1 ± 0.5 |
| | Field | 93.3 ± 3.5 | 42.5 ± 0.6 | 81.3 ± 3.5 | 43.7 ± 0.1 |
| | Room | 89.3 ± 7.1 | 45.0 ± 1.4 | 77.3 ± 9.3 | 44.6 ± 1.7 |
| | Warm | 84.0 ± 0.0 | 45.4 ± 0.5 | 89.3 ± 1.3 | 44.0 ± 0.5 |
| 9 months | Cold | 76.3 ± .9 | 48.8 ± 0.3 | 67.5 ± 4.3 | 49.5 ± 0.2 |
| | Field | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Room | 36.3 ± 3.1 | 46.4 ± 0.5 | 62.5 ± 3.2 | 49.7 ± 0.1 |
| | Warm | 56.3 ± 9.0 | 48.7 ± 0.6 | 60.0 ± 4.6 | 49.7 ± 0.1 |
| 12 months | Cold | 80.0 ± 2.0 | 48.9 ± 0.6 | 53.8 ± 7.5 | 47.1 ± 0.8 |
| | Field | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Room | 32.5 ± 6.0 | 42.0 ± 1.8 | 51.3 ± 6.9 | 46.6 ± 0.8 |
| | Warm | 33.8 ± 6.6 | 44.7 ± 0.8 | 43.8 ± 5.5 | 49.1 ± 0.2 |
| 17 months | Cold | 41.0 ± 3.4 | 49.3 ± 0.7 | 47.0 ± 3.0 | 49.4 ± 0.6 |
| | Field | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Room | 0.0 ± 0.0 | 0.0 ± 0.0 | 29.0 ± 1.9 | 47.7 ± 0.5 |
| | Warm | 2.0 ± 1.2 | 25.0 ± 5.0 | 26.0 ± 2.6 | 46.1 ± 1.3 |

Table 2. Effects of storage condition and temperature of incubation on final germination percentage and germination rate index (mean ± SE) of *Haloxylon salicornicum* and *Salsola imbricata* fresh seeds and seeds stored for 12 months .

| | | | Haloxylon salicornicum | | Salsola imbricata | |
|---|---|---|---|---|---|---|
| Storage period | Storage condition | Incub. temp. | Ger. % | Ger. rate | Ger. % | Ger. rate |
| Fresh | Fresh | 15 | 81.3 ± 2.7 | 41.6 ± 0.7 | 55.0 ± 2.9 | 41.6 ± 0.2 |
| | | 20 | 76.0 ± 4.0 | 42.0 ± 0.1 | 65.0 ± 2.9 | 42.9 ± 0.0 |
| | | 25 | 76.0 ± 4.6 | 41.3 ± 0.5 | 61.7 ± 6.7 | 42.6 ± 0.2 |
| | | 30 | 32.0 ± 2.3 | 39.8 ± 1.7 | 25.0 ± 2.9 | 42.9 ± 0.0 |
| 12 months | Cold | 15 | 85.0 ± 4.1 | 48.5 ± 0.1 | 66.3 ± 1.3 | 48.3 ± 0.4 |
| | | 20 | 80.0 ± 2.0 | 48.9 ± 0.6 | 53.8 ± 3.8 | 47.1 ± 0.8 |
| | | 25 | 77.5 ± 2.5 | 48.4 ± 0.5 | 42.5 ± 4.3 | 48.5 ± 0.4 |
| | | 30 | 28.8 ± 3.8 | 44.0 ± 1.8 | 31.3 ± 2.4 | 44.6 ± 0.7 |
| | Room | 15 | 27.5 ± 4.8 | 42.4 ± 1.6 | 55.0 ± 3.5 | 45.6 ± 0.5 |
| | | 20 | 32.5 ± 3.2 | 42.0 ± 1.8 | 51.3 ± 3.7 | 46.6 ± 0.8 |
| | | 25 | 28.8 ± 4.3 | 47.9 ± 0.9 | 50.0 ± 4.6 | 40.7 ± 2.2 |
| | | 30 | 3.8 ± 2.4 | 19.4 ± 11.2 | 11.3 ± 3.1 | 39.6 ± 0.7 |
| | Warm | 15 | 32.5 ± 4.8 | 43.1 ± 2.6 | 45.0 ± 2.9 | 46.1 ± 0.4 |
| | | 20 | 33.8 ± 3.1 | 44.7 ± 0.8 | 43.8 ± 2.4 | 49.1 ± 0.2 |
| | | 25 | 30.0 ± 2.0 | 46.2 ± 0.9 | 47.5 ± 4.8 | 48.9 ± 0.5 |
| | | 30 | 1.3 ± 1.3 | 9.7 ± 9.7 | 13.8 ± 4.3 | 27.2 ± 9.1 |

# Seed Dormancy, Persistence, and Germination Response Inform Restoration Capability in a Biodiverse Semi-arid Zone Ecoregion.

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## Introduction

Globally, the Pilbara region of Western Australia is one of the world's largest iron ore provinces. Predicted land disturbance for iron ore extraction, within this unique biogeographical region, is currently estimated at around 20 000ha, with regulatory requirements to restore the landscape to resemble its pre-disturbance environment after mine closure. A large deficit in fresh topsoil, which is the main source of reliable plant establishment in post-mining restoration (Bellairs and Bell 1993; Koch and Ward 1994; Rokich *et al.* 2000), necessitates seed-based research to supplement rehabilitation efforts. Research is currently focussed on typical Pilbara semi-arid zone species, comprising hard-seeded (e.g. *Acacia*, *Senna*, *Hibiscus* spp.) and dominant grass (*Triodia* spp.) species (Maslin and van Leeuwen 2008). The specific aims for the current study were (1) to examine what period of soil burial is required to alleviate primary dormancy, (2) to determine how long seeds of each species persist in the soil, (3) to test for fire responsiveness by germination in the presence of karrikinolide ($KAR_1$), (4) to compare whether burial site locations and soil composition (undisturbed soil versus re-profiled waste rock dumps) affect seed responses to aims 1-3, and (5) to utilise the knowledge of seed dormancy alleviation and soil persistence to develop effective seed broadcasting strategies.

## Methods

Seeds from three physically dormant (PY), *Acacia cowleana* Tate, *A. inaequilatera* Domin, and *Senna glutinosa* ssp. *pruinosa* (F.Muell.) Randell, and two physiologically dormant (PD), *Triodia* sp. Shovelanna Hill (S. van Leeuwen 3835) and *T. pungens* R. Br., species were buried in natural (undisturbed) vegetation and waste-rock dump sites. Seeds of *Triodia* species were buried both as the dispersal unit ('floret' - containing caryopses) and as pure caryopses (referred to 'seed' hereafter). Seeds/florets were contained within stainless steel mesh bags filled with sand and buried in four replicate sites at each location to a depth of 30-50mm. Samples were exhumed every two months, examined for field germination, decay in floret/seed fill, and the remaining filled floret/seed assessed for germination with or without $KAR_1$. Germination tests were conducted by incubating seeds for eight weeks at 30°C under a 12h/12h light/dark regime.

## Results and Conclusions

For seeds exhumed up to 12 months (PY species) or 8 months (PD species), no distinct differences in seed fill, germination, or $KAR_1$ stimulation is apparent between burial sites, except for seeds of *A. inaequilatera*. Thus, all data presented are for the undisturbed native vegetation site. Seeds of *Acacia cowleana* persisted in the soil with minimal viability loss (≤ 6%, Fig. 1A) and low proportions of seeds germinating (< 3%, Fig. 2A). In contrast, decline in seed fill has been observed for *Acacia inaequilatera* (43%) and *Senna glutinosa* ssp. *pruinosa* (89%) (Fig. 1A). Decline in seed fill can be partially attributed to PY alleviation with germination maximised after 2 months for *A. inaequilatera* (31%) and *S. glutinosa* ssp. *pruinosa* (73%) (Fig. 2A). Low levels in both field germination (< 20%) and $KAR_1$ stimulation is evident for PY species, except for an increase in $KAR_1$ germination of *S. glutinosa* ssp. *pruinosa* after 6 months (38% increase, Fig. 2C). For *Triodia* sp. Shovelanna Hill and *T. pungens*, florets and seeds persisted in the soil for at least 6 months (Fig. 1B) with germination exceeding 70%, except for *Triodia* sp. Shovelanna Hill florets (11%) (Fig. 2B). Seed fill of both species declined considerably after 8 months when compared to florets (Fig. 1B). Germination of the cleaned seeds in the field (< 5%) is unlikely to be a factor in this difference. Removing seeds from florets revealed considerable germination differences for control treatments (0 months) when compared to seeds remaining in florets (seeds > 41%, florets ≤ 1.1%, Fig. 2B). Germination in the florets appears to be delayed, with a period of burial required before germination levels approach the seed germination levels (e.g. *T. pungens* seeds versus florets, 0-6 months, Fig. 2B). Karrikinolide stimulated germination considerably in all germination units, except *T.* sp. Shovelanna Hill florets, whereby > 20% increase in germination was observed in the control treatments (0 month), and clearly removed the time delay for

germination that occurred under control conditions (e.g. *T. pungens* seeds, $KAR_1$ at 2 months versus $H_2O$ at 6 months, Fig. 2B & 2D).

Clear differences in dormancy type, field persistence, and germination have been observed for all species, indicating that species specific procedures need to be adopted. Tailoring seed mixes to account for species variation will maximise the chances of seed germination, successful establishment, and ensure that restored landscapes resemble the pre-disturbed environment.

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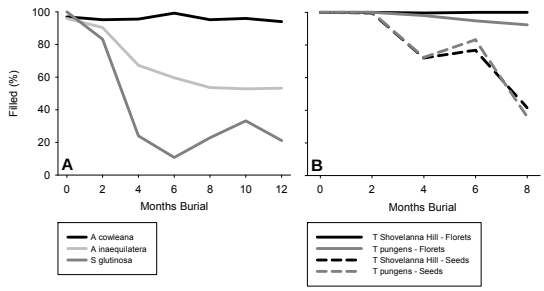


**Figure 1.** Seed fill percentages of PY (A) and PD (B) species buried in natural vegetation for up to 12 months.

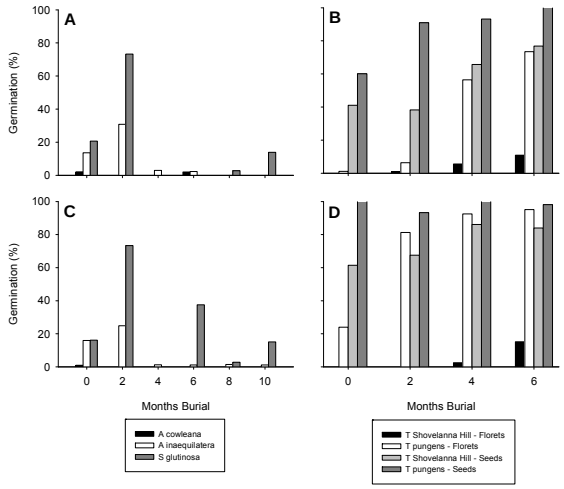


**Figure 2.** Germination of PY (left) and PD species (right) buried for up to 12 months in a natural vegetation site. Seeds/florets were incubated at 30°C under a 12h/12h light/dark regime on $H_2O$ (A-B) and $KAR_1$ (C-D) agar.

# Haplotype Variation for a Major *S*eed Dormancy Gene-Containing Region in the Lineage of Wild, Weedy, and Cultivated Rice

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## Introduction

Seed dormancy distributes germination in time and space to promote the survival of wild or weedy species in diverse ecological systems. Natural variation in seed dormancy has been associated with quantitative trait loci (QTL) in some model plant or crop species, including rice (*Oryza sativa* L.). We identified 10 QTLs for seed dormancy (*qSD*) from the weedy rice (*O. sativa* L. *f. spontanea*) line SS18-2. Weedy rice as a noxious weed accompanies cultivated rice worldwide, is morphologically intermediate between wild (*O. ssp*.) and cultivated rice, and is complex in origin (Oka 1988). We introduced individual seed dormancy QTLs into the background of the cultivated rice line EM93-1 to investigate genetic and evolutionary mechanisms of seed dormancy at a molecular level. *qSD12*, a major QTL accounting for >50% of the phenotypic variation, was narrowed to a genomic region containing three candidate genes (Gu et al. 2010, Fig. 1A). The objective of this research was to determine the origin, differentiation, and distribution of the *qSD12* seed dormancy gene based on haplotype variation across the candidate lines.

## Materials and Methods

A collection of 36 lines was selected from wild (4), weedy (10), and Asian (*O. sativa*, 19) and African (*O. glaberrima*, 3) cultivated rice (Fig. 1B). The wild rice lines represent the *O. rufipogon*, *O. officinalis*, and *O. alta* species. The lines of Asian cultivated rice belong to *indica* (13), *japonica* (4), and *javanica* (or tropical *japonica*, 2) subspecies. All these lines were genotyped with 13 markers distributed along the narrowed *qSD12* region of ~25 kb (Fig. 1A). These markers were designed to target only short (>3 nucleotides) insertions or deletions, which were identified by alignment of genomic DNA sequences from SS18-2 and EM93-1. All these markers are informative as they are co-dominant on a 6% non-denaturing polyacrylamide gel. Marker genotyping was conducted using the methods of Gu et al. (2010). Haplotypes were developed by sorting genotypes for the 13 marker loci. Phylogenetic analysis was conducted using ClustalV (http://www.sacs.ucsf.edu/Documentation/seqsoftware/ClustalW1.7/clustalv.html).

## Results and Discussion

A total of 16 haplotypes were detected from the 36 lines. Nine lines, including SS18-2 (the donor of the dormancy allele), the wild rice lines IRGA105400, IRGA 100161, IRGA 101077, and W0106, and the Asian cultivated rice lines LCBG, Kasalath, Moroberekan, and N22, represented 9 different haplotypes, respectively. The remaining 27 lines belong to the other 7 haplotypes. The weedy rice lines LD and S434 shared one haplotype, while the remaining 7 weedy lines(excluding SS18-2) belonged to the same 3 haplotypes as some *indica* or *japonica* lines. Intriguingly, the three African cultivated rice lines belonged to 2 haplotypes shared with some *indica* lines. The haplotype information demonstrates that *qSD12* is greatly diverse in the *Oryza* genus and this major seed dormancy locus has been subject to strong natural and artificial selections during evolution and domestication.
The 36 lines could be clustered into the wild (5), weedy (8), japonica (3), and indica (20) groups (Fig. 1B). Of the 36 lines, only SS18-2 has been detected to have a dormancy-enhancing allele at *qSD12*. SS18-2 is grouped with the 4 wild rice lines, suggesting that the dormancy allele may originate from wild rice (*O. ssp*). The weedy group consists of most (6/8) weedy rice lines and is phylogenetically closer to the wild than the *japonica* or *indica* group. It is possible that a functional *qSD12* dormancy allele may be also present in some weedy rice lines but rare in Asian and African cultivated rice. We are genotyping additional lines of wild, weedy, cultivated rice to test the above hypotheses.

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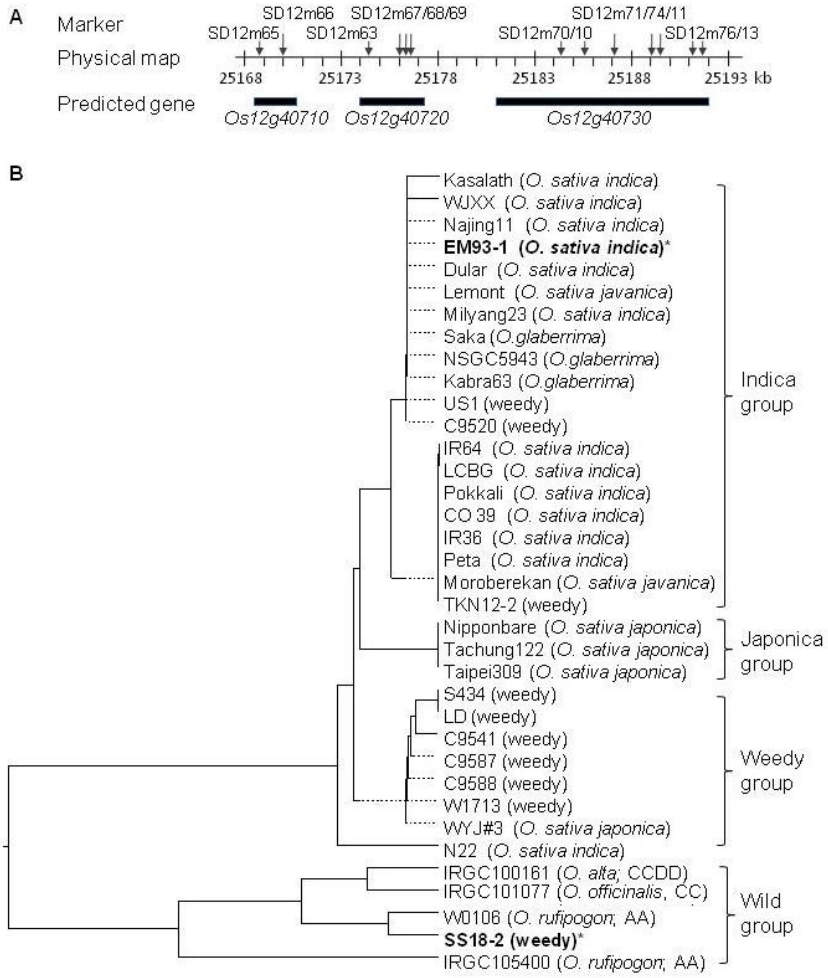


**Figure 1**. Phylogenetic relationships among 36 lines of wild, weedy, and cultivated rice. **A**. A partial physical map for the narrowed *qSD12* region. The three predicted genes are aligned against the physical map based on their lengths in the weedy rice line SS18-2. **B.** Dendrogram of the 36 lines developed based on genotypes of the 13 markers located within the *qSD12* candidate genes (**A**). EM93-1 and SS18-2 (*) are the parental lines used to map the *qSD12* seed dormancy QTL in the previous research.

## Relationship between Climate and Seed Germination at a Local Scale in a Narrow Endemic Species

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### Introduction

Variability in seed germination at the species level may be due to local genetic adaptation or to the environment during seed maturation. Experiments where the parent plants were grown under controlled conditions have found a correlation between germinability and higher temperature (T), reduced water availability and shorter days during seed maturation (Fenner & Thompson, 2005), although exceptions do exist. In the field, intraspecific variation in germination has usually been studied at broad geographical scales (Skordilis & Thanos, 1995). Besides latitude, elevation has been related to deeper dormancy and higher optimal T for germination (Cavieres & Arroyo, 2000), but less attention has been paid to climatic factors which are behind this relationship. In this work, we chose *Centaurium somedanum* M. Laínz, a narrow endemic species living in a restricted area in NW Spain, to test whether (a) differences in germinability exist at local geographical scale; (b) such differences may be explained by local climatic gradients; and (c) there is a differential response to incubation T depending on the elevation of the seed source.

### Methods

*Centaurium somedanum* is a perennial herb which lives in travertines and calcareous fens from 600 to 1700 m a.s.l. Sixteen populations are known in a 210 $km^2$ area. Seed collection took place in September-October 2009. We sampled all individuals bearing ripe fruits from 13 populations covering the entire distribution area of the species (table 1). After 3 wks in our laboratory (22ºC; 50%RH), we sowed seeds on 1% agar held in Petri dishes (4 dishes x 25 seeds per treatment). To assess the differences in germinability, we incubated fresh and cold-stratified seeds (12 wks, 5ºC, darkness) from each population at 22/12ºC. To test the response to incubation T, we also incubated fresh and cold-stratified seeds from CM, VA and MA (representing the elevation gradient) at constant T regimes of 15, 20, 25 and 30ºC. All incubations had a photoperiod of 12/12h (40 µmol $m^{-2}$ $s^{-1}$). We terminated the experiments after 4 wks. We obtained climatic data for the 13 populations from two sources: mean T and precipitation (P) values were extrapolated following Sánchez-Palomares et al. (1999); sun radiation (R) was obtained from the climatic model of Ninyerola et al. (2005). We used the mean values for the period from June (first flowering) to September (beginning of seed dispersal) as representative of the conditions during seed maturation. Statistical analyses were performed by fitting Generalized Linear Models (GLIM), with logit link function and binomial error distribution. To test the effect of the climatic variables on germinability, we fitted a main effects model with the mean values for *T*, *P* and *R* during seed maturation as explanatory variables (covariables), using a backwards model selection procedure. To test the effect of incubation T on germination, we fitted a factorial model with *incubation T* and *population* as fixed effects. We did the analyses separately for fresh and stratified seeds.

### Results and Conclusions

We found differences in germinability among populations, both in fresh and cold-stratified seeds (Table 1). Cold stratification improved germinability in all but one of the populations (BR), although the increase varied among them. Only one low elevation population (AG) achieved full germination, while in the others a percentage of the seeds were still dormant after cold stratification. *T*, *P* and *R* during seed maturation all had a significant effect on germinability, both in fresh and stratified seeds (Table 2). *T* had the greater effect. The effect was greater in the fresh seeds. The model selection procedure included the three variables in the final model. *Incubation T* had a significant effect on germination, both in fresh and cold-stratified seeds. No seeds germinated at 30ºC and only cold-stratified seeds germinated to a low % at 25ºC. These two *T* regimes were qualitatively different and were not included in the statistical analysis. The GLIM detected a significant difference between 20, 15 and 22/12ºC in stratified (Wald's $\chi^2 = 8.327$; $p = 0.016$) but not in fresh seeds. In fresh seeds an effect of *population* was detected (Wald's $\chi^2 = 23.107$; $p < 0.000$), but cold stratification eliminated the differences. Although the higher elevation population (MA) germinated slightly better at 20ºC while the lower elevation populations (CM and VA) had higher germination at 15ºC, no significant interaction *incubation T* x *population* was found. Our results confirm that seeds produced in colder places have a

deeper dormancy, with precipitation and sun radiation having a weaker, but significant effect. Different responses to incubation T, if they existed, were too weak to be detected. For seeds matured on the field, we have found a pattern of parental habitat effects similar to that found on controlled conditions studies. We conclude that slight differences in climate at a local scale may have a relevant effect in seed germinability.

**Acknowledgements**
We would like to thank S. del Río and Á. Penas for providing the T and P values for the populations; A. García-Torrico, R. Álvarez, J.I. Sanzo and E. Correia for their contribution to seed collection; and A. Colubi and G. González for their help with the statistics. This research was funded by the Spanish Ministry of the Environment (Fundación Biodiversidad) and the Government of Asturias (Plan de Ciencia, Tecnología e Innovación del Principado de Asturias).

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Table 2 Collection site data and % germination after 4 wks at 22/12ºC for the 13 populations involved in this study. Coordinates are given in UTM30, datum ED50. % germination is given as mean ± S.E. of 4 dishes.

| Population | Collection site | Coordinates (X/Y) | Elevation (m a.s.l.) | % germination | |
|---|---|---|---|---|---|
| | | | | Fresh | Cold strat. |
| AG | Aguinu | 234358/4778202 | 826 | 77 ± 6 | 98 ± 1 |
| AR | Foz de los Arroxos | 246321/4775163 | 1469 | 8 ± 3 | 64 ± 8 |
| BR | La Bruxa | 238425/4768456 | 1541 | 0 | 1 ± 1 |
| CM | La Malva (Carretera) | 235505/4779486 | 603 | 15 ± 3 | 37 ± 2 |
| CU | Cueiru | 239960/4785940 | 1346 | 6 ± 1 | 86 ± 5 |
| FM | La Malva (Área Forestal) | 235381/4779214 | 706 | 25 ± 4 | 56 ± 4 |
| FU | Furáu | 233611/4777589 | 870 | 38 ± 3 | 70 ± 2 |
| MA | Malconcecho | 246521/4766733 | 1672 | 1 ± 1 | 46 ± 1 |
| ML | Murias Llongas | 243164/4771164 | 1588 | 0 | 24 ± 3 |
| PU | El Puertu | 237229/4767118 | 1419 | 4 ± 3 | 15 ± 4 |
| TE | Veiga Teixera | 246479/4764980 | 1580 | 0 | 43 ± 4 |
| TR | Treméu | 245734/4766725 | 1625 | 2 ± 1 | 50 ± 5 |
| VA | El Valle | 239964/4773952 | 1283 | 11 ± 3 | 53 ± 8 |

Table 2 GLIM table for the effect of the climatic variables during seed maturation on seed germination.

| Model | Effects | Coefficient | S.E. | Wald's $\chi^2$ | p |
|---|---|---|---|---|---|
| Fresh seeds | Temperature | 2.153 | 0.164 | 171.865 | <0.000 |
| | Precipitation | 0.075 | 0.019 | 15.818 | <0.000 |
| | Sun radiation | 0.144 | 0.013 | 115.360 | <0.000 |
| Cold strat. seeds | Temperature | 0.714 | 0.071 | 100.078 | <0.000 |
| | Precipitation | 0.056 | 0.007 | 74.338 | <0.000 |
| | Sun radiation | 0.061 | 0.009 | 48.794 | <0.000 |

## Seed Biology and Germination of the Basal Angiosperm *Amborella trichopoda*

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### Introduction

The special phylogenetic position of *Amborella trichopoda* Baill., an endemic New Caledonian species, the only member of its order, as a sister to all the other angiosperms makes it a species of worldwide scientific interest, as it can help in understanding the evolution of flowering plants (Soltis et al. 2009). Only a few seed studies have revealed seed traits considered to be basal (Forbis et al. 2002; Baskin and Baskin 2005), with a rudimentary embryo located in a large endosperm surrounded by a thin integument (Bobrov et al. 2005). These kinds of seeds occur among members of the most primitive (basal) extant angiosperms, collectively called the ANITA grade. In order to better characterize seed/fruit structures, and to determine germination requirements and dormancy type, observations using binocular and scanning electron microscopy as well as germination experiments were conducted.

### Methods

*Fruit and seed characteristics:* Mature fruits of *A. trichopoda* were collected in July 2008 at the "Plateau de Dogny" (alt: 550-650m). Fruits were dissected in order to reveals their structure and to identify the seed position. After eliminating the fleshy part of the fruit, seeds were then dried under light (25°C) for one week before being used for different experiments. Before all dissections, seeds with their meso/endocarp were surface-disinfected for 5 min in a 4% solution of sodium hypochlorite, brushed during 30s with a toothbrush and then rinsed four times in sterilized distilled water to avoid contaminants. Seeds were observed entire, or transversally or longitudinally cut using binocular or scanning electron microscopy. In the latter case, methods described by Leon et al. (2005) were used to accomplish cryosectioning and scanning electron microscopy observation and mineral analysis. General characteristics of the seed (i.e., external and internal structure) were determined using the following methods: 1) for external structure, ten seeds were measured and mean values were determined, 2) for internal structure, three seeds in longitudinal section were observed and measured using SEM. and measurements were espressed as ranges. Seed viability was determined using the topographical tetrazolium test (ISTA 1999). Moisture content of four replicates of 25 seeds was determined gravimetrically (103 ± 2°C for 17 ± 1 h) following standard procedures (ISTA 1999). Chemical composition of seed storage reserves was determined using classical techniques for oil content (Griffiths et al. 2000), total proteins (Lowry et al. 1951) and sugars (Roe 1955).
*Seed germination trials:* Each germination assay was carried out with 100 pre-disinfected seeds placed in square pots (10 seeds per pots) on the surface of perlite/peat moss (1/1) and covered by a thin vermiculite layer. They were placed in a greenhouse under sprinkler watering on heated tables (25°C+/-3°C). A seed was regarded as germinated when the cotyledons emerged from the substrate. There were two pre-treatments consistting of: 1) scalpel scarification by slicing a piece of meso/endocarp at the end side of the hilum region, 2) soaking of seeds in concentrated sulphuric acid (95%) for 1, 5, 10, 15 or 20 min. after which seeds were thoroughly rinsed with distilled water before sowing.

### Results and Conclusions

Binocular observations confirmed the presence of a rudimentary embryo (Tobe et al. 2000), and its morphological development before the radicle emergence was monitored (Figs. 1; 2 B, F), thus characterizing morphological dormancy (Baskin and Baskin 2005). Scanning electron microscopy revealed for the first time the presence of small calcium and silicate crystals in the endosperm as well as large silicate crystals in the inner part of the mesocarp which constitute with the endocarp a hard layer surrounding the seed (Fig. 2 D-F). This layer seems to be responsible for mechanical inhibition. Such an inhibition is confirmed by the fact that a scarification of 20 minutes in pure sulfuric acid gives 80% germination (seed lot viability was estimated at 90%) within 90 days (compared to 0% without

scarification) with a latency time of 58 days (Fig. 3). This seemed to also indicate physiological dormancy; this may be confirmed by germination assays with $GA_3$. Indeed, a preliminary test with a 50 ppm dose seemed to promote germination but this still needs to be assessed with a large trial. *Amborella* seed dormancy might be defined as a "Nondeep simple morphophysiological dormancy" according to the Baskin and Baskin (2004) classification. Upcoming results will further test this hypothesis. We also determined moisture content and seed storage reserve composition (lipids, proteins and sugars). These elements and their role in seed conservation in the natural environment will be discussed.

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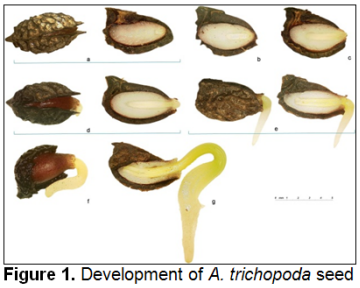

**Figure 1.** Development of *A. trichopoda* seed over time from sowing until cotyledon expulsion. a: whole and longitudinally cut seed with meso/endocarp just open; b, c: embryo development within the seed; d: germination *sensu stricto*; e-g: radical and cotyledon growth after radicle emergence.

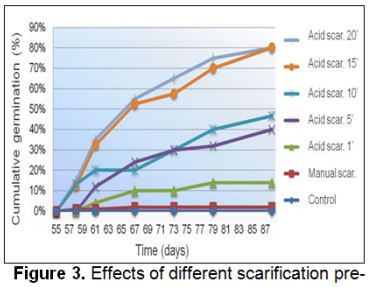


**Figure 3.** Effects of different scarification pre-treatments versus control on the germination of *A. trichopoda* at 25 +/- 3°C (Control: non-treated seeds; Manual scar.: scalpel scarification; Acid scar.: scarification with sulfuric acid during 1, 5, 10, 15 or 20 min.).

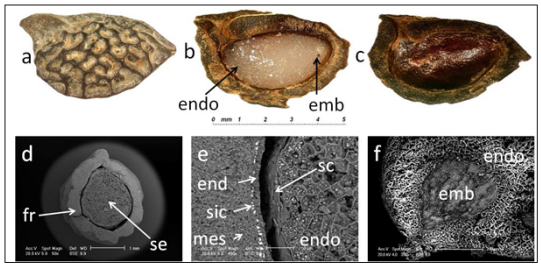


**Figure 2.** Photographic illustrations of *A. trichopoda* seed under binocular (a-c) and scanning electron microscope (d-f). a: whole seed; b, c, f: longitudinal sections (b and f showing the rudimentary embryo(emb) within the endosperm (endo)); d: transverse section showing internal fruit (fr) parts i.e. mesocarp (mes) and endocarp (end) separated by silicate crystals (sic) as well as seed (se) parts i.e. seed coat (sc) and endosperm (endo).

**Dormancy Cycling of Brassicaceae Species in the Field: Impact of Thermal Gradients and Nitrate on Seeds during Annual Cycles.**

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**Introduction**

Weeds remain a major constraint to cost effective crop production by interfering with yield. However, the way we manage weeds in the UK is increasingly constrained by the loss of chemical products available to control them following EU legislation coupled with environmental pressures to reduce herbicide inputs. A major challenge for farmers is therefore how to improve the reliability of non-chemical and integrated methods of weed control. Towards this end we aim to improve understanding of seed dormancy cycling in the soil, which is arguably the major obstacle facing this challenge, by evaluating contrasting cycling behaviour in three members of the *Brassicaceae, Alliaria petiolata, Sinapis arvensis* and *Thlaspi arvense*.

**Methods**

Seed samples from all three species were buried in a series of field trails and exhumed at monthly intervals for up to two years. Germination physiology of these seeds was studied under a wide range of conditions. These include light and dark, a range of temperatures, gibberellic acid (GA) and nitrate concentrations.

**Results and Discussion**

We illustrate below that these three species show contrasting dormancy behaviour in response to the same soil environment. We are currently developing models to describe these responses. The model outputs will be compared with historic weed emergence data, and used for investigating future climate change scenarios.

***Alliaria petiolata:*** Results show that this species is a summer annual that germinates and emerges exclusively in spring. The mechanism of dormancy relief appears simple and requires only an accumulation of time at low temperature to relieve dormancy and allow germination. Figure 1a shows that the timing of germination, and therefore seedling emergence, differs from year to year due to differences in the preceding winter weather. However, when these data are plotted on a scale of chilling degree days the germination curves converge (Figure 1b). Therefore it should be possible to develop a relatively simple model of accumulation of chilling degree days. Further analysis is underway to determine the most appropriate temperature for the upper and lower limits in which accumulation should be carried out to best predict germination time. *Alliaria Petiolata* seeds do not have a strong requirement for alternating temperatures, and do not have a light requirement and therefore germinate in the soil without disturbance. Indeed our results show that light inhibits germination and seeds that are held at the surface do not germinate despite exposure to a suitable low temperature environment. Buried seeds do not persist in the soil since all viable seeds appear to germinate; in contrast, seeds at the soil surface that are prevented from germinating by exposure to light persist beyond spring.

***Sinapis arvensis***: This is a persistent weed of arable crops. In the first year little germination was seen at constant temperature, but this increased in the second year (Figure 2). However, in the first year, sensitivity to nitrate increased dramatically from August to December, then declined in the spring before increasing again in the autumn. These changes in responsiveness seem related to changes in the soil environment such as increasing temperature and declining moisture content which appear to induce dormancy. The germination response to alternating temperature was analyzed on a thermogradient table (temperature range 0-30°C; maximum amplitude 23°C; 12 hour diurnal cycle in continuous light). Examples of the data we have collected for each sample are shown in Figure 3 for two contrasting retrievals (March and November). This showed that from autumn to mid winter the temperature response widened from small to larger amplitudes before diminishing in spring. The size of this response differed between years. These data indicate that the daily alternation of temperatures is the main determinant of seedling emergence patterns in this species. We therefore aim to develop a response-surface-based model from the large data set generated.

***Thlaspi arvense:*** Exhumed seeds indicate a preference for germination at higher temperatures, with germination potential increasing in late summer when these temperatures are likely to occur. Nitrate also stimulates germination of these seeds. In contrast to *Sinapis arvensis*, seed viability in this

species progressively declined throughout the field experiment (30% in the first 8 months) indicating that these seeds do not persist for long in the soil. Current analysis suggests a potential for seeds of this species to germinate from spring through to autumn in response to ambient conditions; remaining seeds are likely to germinate in the autumn, especially in agricultural soils disturbed for winter wheat/oil seed rape crops, so that persistence is not required.

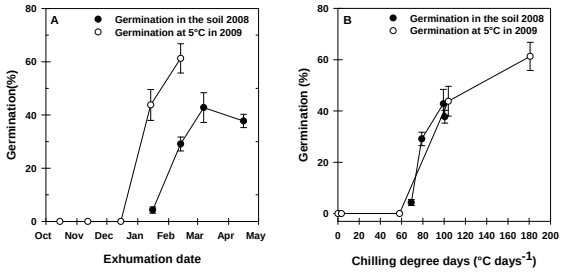


**Figure 1.** *Alliaria* germination (A) in the soil (2008) and at 5°C in the dark following exhumation (2009) and (B) in relation to the accumulation of thermal chilling time.

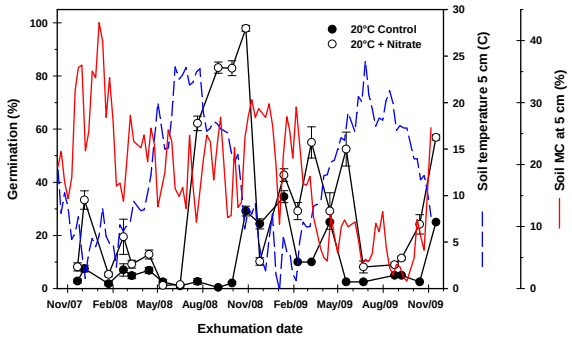


**Figure 2.** Sensitivity of *Sinapis* seeds exhumed from the field to 10 mM nitrate at 20°C

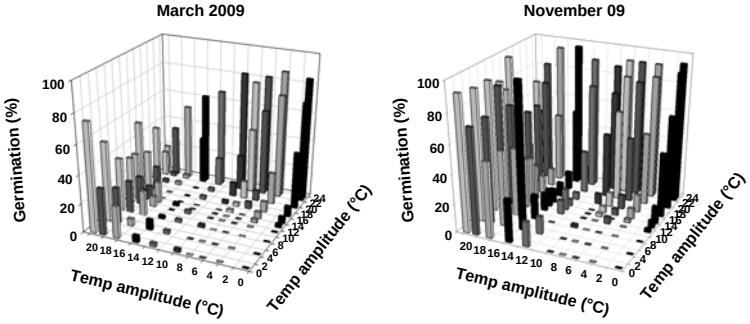


**Figure 3.** The response of *Sinapis* seeds to alternating temperatures folllowing exhumation after 4 (March) and 12 (November) months of burial in the field

## *Arabidopsis* Dormancy in the Field: a Molecular-Physiological Analysis Uncovers New Dormancy Cycling Behavior

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### Introduction

In recent years our understanding of seed dormancy has been increased by the molecular and physiological analysis of *Arabidopsis* wild types and mutants (Holdsworth et al. 2008a & b). In particular, transcriptome studies of dormancy cycling and germination mutants have identified distinct transcriptional profiles for the different stages of dormancy cycling and have identified dry after-ripening and dormancy as separate developmental processes (Cadman et al 2006, Carrera et al. 2007).

However, little is currently understood of the molecular/physiological events that underpin dormancy status in the soil seed bank where seeds are subjected to fluctuating environmental conditions that are known to influence dormancy status and thus the pattern of dormancy cycling in the field. In the absence of such data it is even unclear whether laboratory-based models of dormancy behaviour are relevant to the situation experienced by seeds in the natural environment. To address this we conducted a two-year field trial to evaluate changes in the physiological and molecular expression of dormancy cycling to evaluate how these changes compare to our continuing laboratory-based studies on dormancy cycling in *Arabidopsis* (Cadman et al. 2006; Finch-Savage et al. 2007).

### Methods

Four cohorts of *Arabidopsis* plants (ecotype Cvi) were raised in a glasshouse in spring 2007 to produce four biological replicates. The seeds from these were stored at -20 $^{o}$C until they were buried in October 2007 and May 2008 in field plots where soil temperature and moisture content (MC) were continually recorded. To distribute the seeds and aid their retrieval the seeds were mixed with 0.15-0.25 mm Ballotini balls contained in nylon mesh bags. At monthly intervals over two years seeds were exhumed and dormancy level evaluated by determining the after-ripening (AR) time required to enable 50% of the population to germinate (days AR50) (Footitt & Finch- Savage 2010). Changes in thermo-dormancy were evaluated by exposure to a range of constant temperatures, gibberellic acid (GA) and 10 mM nitrate. In the first year samples were collected for the molecular analysis of dormancy status; ongoing measurements include transcriptome (micro-array & QRT-PCR), hormone, and proteome analyses.

### Results and Conclusions

Upon burial, dormancy increased rapidly with the AR50 increasing from 50 days in the laboratory to >150 days after two months in the soil, peaking at 200d before declining back to 50d in one month during the spring (Fig. 1). Seeds were fully AR from June – August before dormancy increased again with AR50 reaching similar levels to those seen in the first winter.

In the first year as seed AR50 declined, thermo-dormancy declined and nitrate sensitivity increased and vice-versa as AR50 increased in late autumn. These changes were synchronous in the four biological replicates, but this synchrony was lost in the second year. Sensitivity to GA was also observed to change with the depth of dormancy. We will further discuss these changes alongside data for abscisic acid levels and available Omics data.

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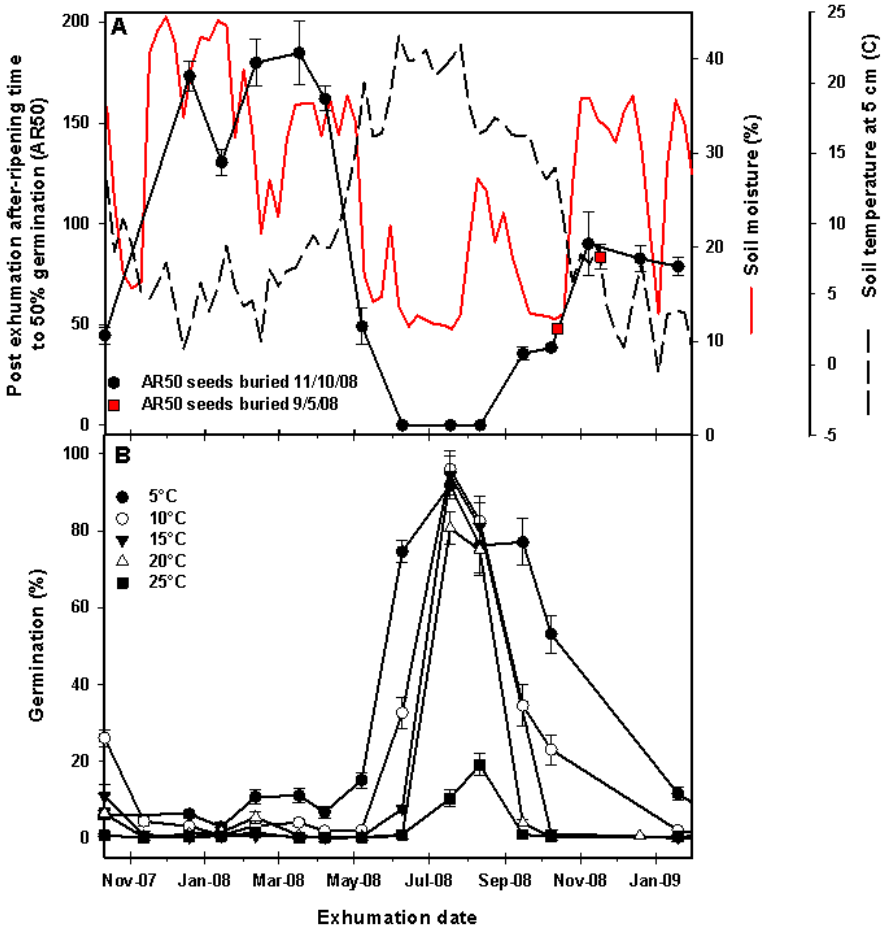


**Figure 1.** Dormancy cycling of *Arabidopsis* ecotype Cape Verdi Isle seeds buried in the field. A.) Changes in depth of dormancy measured as after-ripening (AR) time required to enable 50% of the population to germinate (AR50) following exhumation in relation to soil moisture and temperature at the burial depth. B.) Changes in thermodormancy of exhumed seeds over the range 5-25°C.

# Pigmentation and Symbiosis: Seed Properties and Their Implications for *Platanthera praeclara* (Orchidaceae)

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## Introduction

*Platanthera praeclara* is a terrestrial North American orchid protected under the Endangered Species Act of 1973 (US Fish and Wildlife Service). Dust-like orchid seeds require specific environmental conditions and microbial associations for successful germination in nature. The seeds for this threatened species are extremely slow to germinate and germination rates are very low (From 2002). Numerous non-extractable red bodies within *P. praeclara* seed testae that promoted germination *in vitro* were discovered in seeds collected from a Nebraska sandhills subpopulation on the Valentine National Wildlife Refuge. This is the first report of pigmented bodies within *P. praeclara* seeds.

## Methods

Seed collections were made in September 2005 from 2 different sub-populations. Seed sub-samples from each site were examined by high magnification on a compound microscope (Meiji Techno Co. Ltd). The seeds were later surface-disinfested and cultured aseptically on 50% MS medium (Murashige & Skoog, 1962) for germination tests. Cultures initiated with seeds from each accession were given identical treatments. Final germination counts were made by visual inspection at the end of 6 months.

## Results and Conclusions

Seeds in the *P. praeclara* sub-sample from South McKeel (SM) were found to contain a higher number of unidentifiable red bodies within the seed testae compared to those from West Sweetwater (WS). The presence of those red bodies is reported here for the first time. Seeds containing red pigments were photographed (Figure 1 A and B) on a compound microscope with camera attachment (MicroMetrics CMOS camera 3.2M). The presence of red bodies produced a higher rate of seed germination in this study. Final germination results for SM seeds versus those collected from WS are reported in Table 1. The microscopic size of the red bodies made them non-extractable and their properties cannot be identified with absolute certainty at this time. However, red bodies present in the seeds promoted a higher germination rate for *P. praeclara.* Orchid fusaria were first reported by Bernard (1903) as possible seed germination-promoting fungi. *Fusarium* fungi are generally pathogenic and may appear on seed surfaces as a result of wet environmental conditions during seed maturation. Carotenoids may be another possible explanation for the red pigmentation in or on *P. praeclara* seeds. Carotenoids can confer protection to seeds from excessive UV radiation. Red bodies observed were optically clear tissues entirely separate from other cells comprising the seedcoat or the embryos (Figure 1.). The red bodies did not contaminate *in vitro* cultures for the duration of the study.

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Table 1. Seed-borne red particulate bodies and germination percentages from two sites.

| Accession Site | % Seeds w/red bodies | % Germination In Vitro |
|---|---|---|
| West Sweetwater | 25.0 | 3.4 |
| South McKeel | 64.4 | 8.0 |

Random sub-samples were taken from the pooled seed accessions from each of two sites. Examination was carried out at high magnification with a compound microscope in phase mode.

Figure 1. A. Red body inside the *P. praeclara* seedcoat near the upper left side of the rudimentary embryo (arrow) 10x. B. Red bodies near the micropylar region (arrows) 20x.

# Comparative Genetics of Seed Dormancy between Tropical and Temperate Ecotypes of Weedy Rice

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## Introduction

Seed dormancy contributes to persistence of wild or weedy species in natural or agricultural ecosystems by distribution of germination of seeds in the soil seed bank over time. Natural variation in this adaptive trait is regulated by multiple genes and also modified by environmental factors. Weedy rice (*Oryza sativa* L. f. *spontanea*) accompanies cultivated rice (*O. sativa* L.), is distributed from tropical to middle temperate latitude areas (Oka 1988), and is greatly divergent from rice cultivars in dormancy traits (Gu et al. 2005). We developed weedy rice as a model system to elucidate genetic and evolutionary mechanisms of seed dormancy in the grass (Poaceae) family. Our previous research identified 10 quantitative trait loci (QTLs) associated with seed dormancy from a line of tropical weedy rice (Ye et al. 2010). In the present research, we introduced the genome from a line of temperate weedy rice into the same genetic background used for previous research to map seed dormancy QTLs. The two sets of QTLs were compared to provide a genome-wide pattern of differentiation in seed dormancy between tropical and temperate ecotypes of weedy rice.

## Materials and Methods

The highly dormant line LD, a temperate ecotype of weedy rice originating from East China (~36º N lat.), was crossed with the cultivated rice line EM93-1, and the hybrid $F_1$ was backcrossed (BC) with EM93-1 to generate the $BC_1F_1$ (EM93-1//EM93-1/LD) population. The $BC_1F_1$ plants were genotyped with rice microsatellite (RM) markers to develop a framework linkage map and evaluated for degree of dormancy by incubation of seeds partially after-ripened for a given number of days under controlled conditions (Gu et al. 2005). The mapping software WinQTLCart was used to scan for seed dormancy QTLs along the whole genome. Two LD-derived $BC_1F_1$ plants (#9 & #139) were backcrossed with EM93-1 to generate $BC_2F_1$ populations to confirm the QTLs detected from the $BC_1F_1$ and to identify additional seed dormancy loci from the advanced BC populations. QTLs detected from the LD-derived populations were compared with the 10 previously identified seed dormancy QTLs from the SS18-2-derived $BC_1F_1$ (EM93-1//EM93-1/SS18-2) or advanced populations (Ye et al. 2010). SS18-2 is a strongly dormant line of tropical weedy rice originating from Thailand (~15º N lat.).

## Results and Discussions

A total of 11 QTLs for seed dormancy (*qSD*) were identified from the LD-derived $BC_1F_1$ and $BC_2F_1$ populations (Table 1). These loci contributed a relatively major (>20%, e.g., *qSD12*), minor (<10%, e.g., *qSD1-2*, *9*, & *10*), or moderate (10-20%, e.g., *qSD1-1*, *4*, *6-2*, *6-3*, *7-1*, *7-2* & *8*) proportion to the phenotypic variances in percent germination of 1-, 11-, 21-, or 31-d after-ripened intact seeds or caryopses (hull-removed seeds). The parental lines LD and EM93-1 donate the dormancy-enhancing allele to 9 and 2 of the 11 loci, respectively. A similar observation was made from the SS18-2-derived populations, in which the parental line of weedy rice contributes the dormancy allele to 8 of the 10 QTLs (Ye et al. 2010). These results strongly indicate that weedy genotypes have retained a vast majority (~80%) of seed dormancy genes when they co-evolved with their co-specific crops in agro-ecosystems.

Eight of the 11 QTLs detected from the LD-derived populations co-locate with 8 of the 10 QTLs identified from the SS18-2-derived populations, which were tagged by the same panel of RM markers (Table 1). The co-location suggests that tropical and temperate ecotypes of weedy rice share most (~2/3) of the seed dormancy genes. It is possible that mutation at these conserved loci may have occured prior to the ecotypic differentiation or domestication of rice. Besides the major locus *qSD12*, the seven conserved QTLs also associate with other adaptive weedy traits, such as seed shattering, awn length, black hull color (*qSD4*, *7-2* & *8*), red pericarp color (*qSD7-1*), plant height (*qSD1-2*), and flowering time (*qSD1-1* & *10*). These associations suggest that the seed dormancy trait co-evolved with some other wild and domestication-related traits, which could be explained by several “adaptive haplotypes” at a QTL level.

Some seed dormancy QTLs were detected only in the LD-(*qSD6-2*, *6-2*, & *9*) or the SS18-2-(*qSD3* & *6-1*) derived populations. The detection of population-dependent QTLs suggests that tropical and temperate ecotypes of weedy rice may also have accumulated some specific genes for seed dormancy to enhance their adaption to different environmental conditions.

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**Table 1.** Summary of seed dormancy QTLs differentiated between the weedy rice line LD (temperate) or SS18-2 (tropical) and the cultivated rice line EM93-1.

| QTL [a] | Chromo-some | Nearest marker | Donor of the allele enhancing dormancy | Relative contribution to phenotypic variation [b] |
|---|---|---|---|---|
| *qSD1-1*$^{L,S}$ | 1 | RM220 | LD & SS18-2 | Moderate |
| *qSD1-2*$^{L,S}$ | 1 | RM315 | EM93-1 | Low |
| *qSD3* $^{S}$ | 3 | RM520 | SS18-2 | Low |
| *qSD4* $^{L,S}$ | 4 | RM252 | LD & SS18-2 | Moderate |
| *qSD6-1* $^{S}$ | 6 | RM314 | SS18-2 | Moderate |
| *qSD6-2*$^{L}$ | 6 | RM5745 | LD | Moderate |
| *qSD6-3*$^{L}$ | 6 | RM528 | LD | Moderate |
| *qSD7-1*$^{L,S}$ | 7 | RM21197 | LD & SS18-2 | Moderate |
| *qSD7-2*$^{L,S}$ | 7 | RM234 | LD & SS18-2 | Moderate |
| *qSD8*$^{L,S}$ | 8 | RM339 | LD & SS18-2 | Moderate |
| *qSD9*$^{L}$ | 9 | RM524 | LD | Low |
| *qSD10*$^{L,S}$ | 10 | RM271 | EM93-1 | Low |
| *qSD12*$^{L,S}$ | 12 | RM28607 | LD & SS18-2 | Major |

[a]: The superscripts indicate the QTLs detected in the SS18-2-($^{S}$) and/or LD-($^{L}$) derived populations.
[b]: Major (>20%), moderate (10-20%), or low (<10%) proportion of the phenotypic variance in percent germination explained by QTL in the $BC_1F_1$ or advanced populations segregating for more than one seed dormancy locus.

# Evaluation of Reproductive Success in *Senecio coincyi* Rouy, a Threatened Species from Spain

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## Introduction

*Senecio coincyi* Rouy (Asteraceae) is a threatened endemic species from the mountains of Sierra de Gredos, central western Spain. This species is protected by the Autonomous Community of Castile and León (Spain) and is included in the "In danger of extinction" category in the protected flora catalogue of that region. It was later catalogued as Vulnerable (VU) on the Red List of Threatened Spanish Vascular Flora (Moreno et al., 2008). In this paper, the results of a two-year investigation of *S. coincyi* population biology are shown. The aim of this work was to study several aspects of *S. coincyi* reproductive biology.

## Methods

Ripe cypselas of *S. coincyi* were taken from six populations located in Ávila province (Spain) in early summer 2007. Cypselas were stored dry under laboratory conditions at about 25ºC until the start of the germination trials in December 2007. Germination experiments were carried out in order to detect germination differences among: (1) populations, (2) individuals belonging to the same population, and (3) heads from the same individual plant. In all trials, four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 ml distilled water) in 7-cm diameter glass Petri dishes. Germination of cypselas was tested at different constant temperatures (10ºC, 15ºC, 20ºC, 25ºC and 30ºC) and the alternate temperatures of 25/15ºC under a 16/8h light/dark photoperiod. Cypselas belonging to the population SAN01 were tested for germination at alternate temperatures (25/15ºC) under a 16-h light photoperiod and additionally under constant darkness. At the end of the germination period, the final germination percentage (mean value ± standard error) and the mean germination time (MGT, mean value in days ± standard error) were calculated. The number of empty cypselas in each replicate was taken into account for calculating the final germination percentage.

## Results and Conclusions

The total number of *S coincyi* individuals is approximately 26,000, distributed in 13 populations over a 350 ha area. Its reproduction is basically sexual and characterized by a high plasticity regarding pollinators, as well as a remarkable facility to spread its seeds. A high production of flowers, fruits (cypselas) and seeds could be observed in the field. The mean number (± standard deviation) of heads per individual plant was 13 ± 6 (44 maximum-3 minimum), the mean number of flowers per head was 153 ± 34 (290-85), and the mean number of cypselas per head was 152 ± 38 (290-71). The effect of incubation temperature on the germination of cypselas from two populations is shown in Table 1. The final germination percentages (except at 10ºC) were very high (≥90%). Seed germination was not significantly affected by light conditions (16-h light photoperiod or constant darkness). Germination at 25/15ºC of cypselas belonging to six populations is shown in Table 2. Significant differences ($P$<0.05) were found among populations for final germination percentages and MGT values. Similarly, the number of empty cypselas varied significantly among populations (from 0 to 33%). However, no significant differences were found among the final germination percentages reached by cypselas belonging to different individuals of two populations (Table 3). In most cases, no significant differences ($P$>0.05) were found for the germination reached by cypselas of single heads from individual plants. In conclusion, obtained results seem to highlight the sexual reproductive ability of *S. coincyi*. This indicates that its conservation problems are not due to agents related to its reproductive biology, but mostly to other agents, such as the alteration of its habitat caused by the continuous and intense presence of livestock.

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**Table 1**. Effect of different temperature regimes on the final germination percentage and mean germination time (MGT) of *S. coincyi* cypselas belonging to two populations. Results after 20 days of incubation under a 16-h light photoperiod. For each population, mean values within a column followed by the same letters are not significantly different at the 5% level of probability.

| Population | {PRIVATE }Temperature | Germination (% ± SE) | MGT (days ± SE) |
|---|---|---|---|
| HER02 | 10ºC | 19 ± 3.28 a | 13.42 ± 0.22 d |
| | 15ºC | 98 ± 1.73 bc | 8.05 ± 0.06 c |
| | 20ºC | 98 ± 1.00 bc | 6.45 ± 0.19 ab |
| | 25ºC | 100 c | 5.87 ± 0.08 a |
| | 30ºC | 92 ± 3.34 b | 6.65 ± 0.38 b |
| | 25/15ºC | 98 ± 1.00 bc | 6.02 ± 0.11 ab |
| NAV02 | 20ºC | 98 ± 1.00 a | 7.77 ± 0.32 a |
| | 25ºC | 90 ± 3.75 a | 7.12 ± 0.44 a |
| | 25/15ºC | 91 ± 5.92 a | 7.55 ± 0.43 a |

**Table 2**. Final germination percentages and mean germination time (MGT) of *S. coincyi* cypselas belonging to 6 populations. Mean values followed by the same letters in a column are not significantly different ($P$>0.05). Results after 20 days of incubation under a 16-h light photoperiod at 25/15ºC.

| Population | Empty cypselas (% ± SE) | Germination (% ± SE) | MGT (days ± SE) |
|---|---|---|---|
| HER02 | 14 ± 7.00 b | 85 ± 4.39 abc | 7.50 ± 0.26 ab |
| PIE01 | 10 ± 2.24 ab | 92 ± 3.21 bcd | 7.52 ± 0.53 ab |
| SAN01 | 0 a | 99 ± 0.87 d | 8.90 ± 0.30 c |
| TIE01 | 33 ± 6.22 c | 74 ± 5.49 a | 8.82 ± 0.18 c |
| PEV01 | 0 a | 97 ± 1.66 cd | 8.07 ± 0.25 bc |
| NAV01 | 2 ± 1.00 ab | 84 ± 5.48 ab | 6.90 ± 0.20 a |

**Table 3**. Final germination percentages and mean germination time (MGT) of *S. coincyi* cypselas belonging to different individuals of two populations. Results after 20 days of incubation under a 16-h light photoperiod at 25/15ºC.

| Population | Individual plant Nº | Empty cypselas (% ± SE) | Germination (% ± SE) | MGT (days ± SE) |
|---|---|---|---|---|
| HER02 | 1 | 0 | 100 | 9.65 ± 0.25 |
| | 2 | 46 ± 1.41 | 96 ± 2.83 | 9.65 ± 0.25 |
| | 3 | 0 | 98 ± 1.41 | 6.40 ± 0.14 |
| | 4 | 8 ± 0.00 | 98 ± 1.41 | 9.55 ± 0.18 |
| | 5 | 0 | 100 | 10.25 ± 0.32 |
| | 6 | 4 ± 2.83 | 100 | 8.15 ± 0.32 |
| | 7 | 2 ± 1.41 | 100 | 7.70 ± 0.14 |
| | 8 | 20 ± 5.66 | 83 ± 7.42 | 9.70 ± 0.56 |
| | 9 | 0 | 100 | 7.25 ± 0.03 |
| | 10 | 14 ± 7.07 | 100 | 8.95 ± 0.03 |
| | 11 | 4 ± 2.83 | 93 ± 1.77 | 8.80 ± 0.42 |
| | 12 | 18 ± 1.41 | 95 ± 3.53 | 7.75 ± 0.11 |
| | 13 | 14 ± 1.41 | 90 ± 3.18 | 10.15 ± 0.25 |
| | *P* | 0.0001 | 0.0633 | 0.0001 |
| SAN01 | 1 | 44 ± 5.66 | 74 ± 4.95 | 8.85 ± 0.25 |
| | 2 | 8 ± 2.83 | 100 | 8.00 ± 0.35 |
| | 3 | 2 ± 1.41 | 91 ± 3.18 | 7.75 ± 0.25 |
| | 4 | 0 | 96 ± 0.00 | 10.15 ± 0.25 |
| | 5 | 0 | 98 ± 1.41 | 7.55 ± 0.11 |
| | 6 | 2 ± 1.41 | 98 ± 1.41 | 7.65 ± 0.25 |
| | 7 | 10 ± 4.24 | 96 ± 2.83 | 7.70 ± 0.07 |
| | 8 | 2 ± 1.41 | 94 ± 1.41 | 8.15 ± 0.18 |
| | 9 | 0 | 86 ± 4.24 | 6.50 ± 0.07 |
| | 10 | 10 ± 4.24 | 96 ± 2.83 | 8.90 ± 0.07 |
| | 11 | 2 ± 1.41 | 94 ± 1.41 | 7.90 ± 0.07 |
| | 12 | 8 ± 5.66 | 89 ± 3.89 | 9.00 ± 0.85 |
| | *P* | 0.0007 | 0.0589 | 0.0028 |

## Oceanic Dispersal: Can Seeds Really Float, Survive, and Germinate?

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### Introduction

Recent literature has suggested that long-distance seed dispersal via the ocean (oceanic hydrochory) is more prevalent than commonly thought (de Queiroz 2005; Nathan *et al.* 2008). In most cases, however, these findings are inferred from current plant distributions and phylogenetic data rather than from studies of seed dispersal *per se*. To assess the likelihood of oceanic hydrochory we investigated three factors we considered important for hydrochorous seed dispersal: buoyancy of seeds (Lopez 2001; van den Broek *et al.* 2005), survival of seeds in sea-water, and germination under saline conditions such as those that may be experienced when seeds are washed ashore (Woodell 1985; Martinez *et al.* 1992). We examined dispersal units (diaspores) of 13 angiosperms widespread in coastal regions of Western Australia (*Acacia cyclops* G (Fabaceae). Don, *A. rostellifera* Benth. (Fabaceae), *Cakile maritima* Scop. (Brassicaceae), *Clematis linearifolia* Steud. (Runnunculaceae), *Conostylis candicans* Endl. (Haemodoraceae), *Ficinia nodosa* (Rottb.) Goetgh., Muasya & D.A. Simpson (Cyperaceae), *Hardenbergia comptoniana* (Andrews) Benth. (Fabaceae), *Myoporum insulare* R. Br. (Scrophulariaceae), *Olearia axillaris* (DC.) Benth. (Asteraceae), *Rhagodia baccata* (Labill.) Moq. (Chenopodiaceae), *Scaevola crassifolia* Labill. (Goodeniaceae), *Spyridium globulosum* (Labill.) Benth. (Rhamnaceae) and *Tetragonia decumbens* Mill. (Aizoaceae)). We hypothesised that (1) diaspores would float in sea-water and remain viable during extended periods of exposure to sea-water; (2) diaspores would germinate under saline conditions, and; (3) at higher NaCl concentrations germination would be decreased or inhibited, though seeds may recover following transfer to fresh water.

### Methods

Buoyancy was assessed by placing diaspores of all species in containers of sea-water for up to 70 days. Where possible buoyancy of seeds (diaspores with appendages removed) was recorded for comparison with buoyancy of entire diaspores. To determine whether diaspores survived exposure to sea-water they were removed after 0, 7, 14, 21, 42, and 70 days, and germinated at 15ºC. To test germination under saline conditions, diaspores were incubated in each of seven concentrations of NaCl: 0 (fresh water), 50, 100, 200, 300, 400, and 500 mM (equivalent to sea-water). To determine whether germination inhibition was due to osmotic or toxic effects of NaCl, once diaspores incubated in NaCl reached maximum germination all diaspores were rinsed and transferred from NaCl to fresh water. Germination continued to be recorded to determine recovery.

### Results and Conclusions

Diaspores of most species floated, and for seven species >50% of diaspores initially floating remained buoyant after 14 days. A small percentage of diaspores of some species remained afloat for 70 days, for example, *R. baccata* (Fig. 1). In contrast, diaspores of *C. candicans* did not float well (Fig. 1). *C. candicans* was the only species where exposure time to sea-water related to a decrease in seed germination. Diaspores of other species survived in sea-water for up to 70 days and germination was not adversely affected by time in sea-water. Generally, seeds with physical or physiological dormancy survived. Predictably, germination decreased as salt concentration increased, however, most seeds recovered and germinated when transferred to fresh water (Fig. 2). The effect of salt on germination was categorised into four response types, three of which have been previously described in the literature (Woodell 1985). A novel germination response to salt was observed and will be described in this presentation. In general almost all species floated, survived and germinated in saline conditions, to varying extents, and for this reason we concluded that oceanic hydrochory is indeed a possible method of dispersal.

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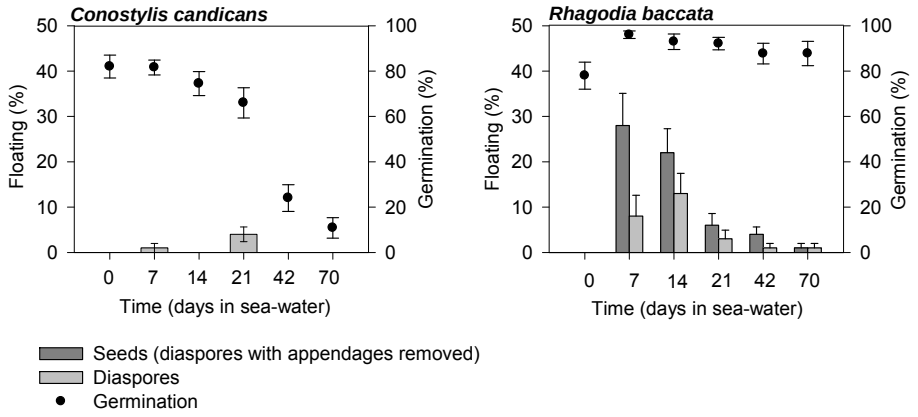


**Figure 1.** Buoyancy (floating %) and survival (germination %) of diaspores placed in sea-water for up to 70 days. Columns represent buoyancy of diaspores (mean% ± SE) between 0 & 70 days (n=100, 4 replicates of 25 per species). Germination (mean% ± SE) after exposure to sea-water for between 0 & 70 days is indicated on the secondary y-axis (n=100, 4 replicates of 25 per species).

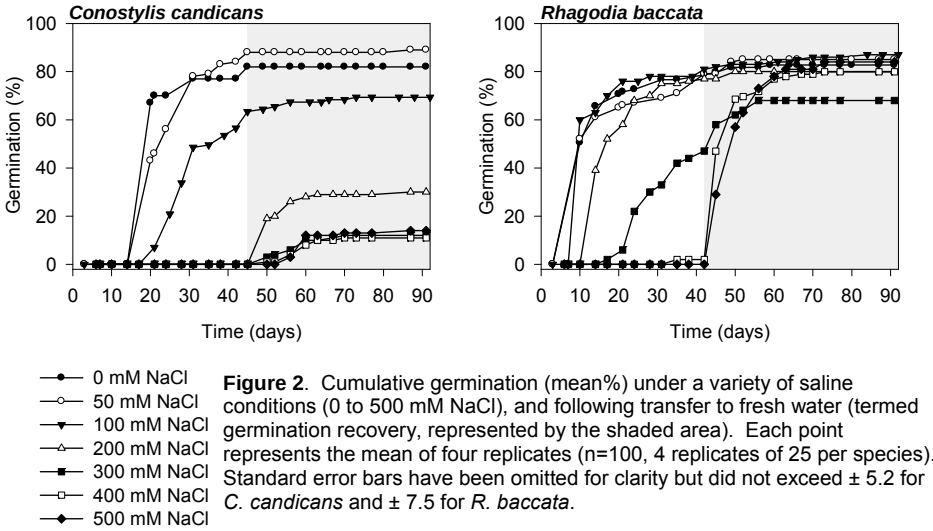


**Figure 2**. Cumulative germination (mean%) under a variety of saline conditions (0 to 500 mM NaCl), and following transfer to fresh water (termed germination recovery, represented by the shaded area). Each point represents the mean of four replicates (n=100, 4 replicates of 25 per species). Standard error bars have been omitted for clarity but did not exceed ± 5.2 for *C. candicans* and ± 7.5 for *R. baccata*.

## *Bromus tectorum*: Variation in Seed Dormancy Among Populations

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### Introduction

*Bromus tectorum* L. is an exotic annual grass that has invaded numerous plant communities and habitat types in the Intermountain Western United States. Laboratory germination tests were conducted on *Bromus tectorum* seeds collected from five populations (Table 1) near Reno, Nevada in order to answer three questions. 1) To what degree is *Bromus tectorum* seed dormant at seed dispersal time? Seed has been found to be dormant at dispersal (Meyer et al. 2009). 2) Do seeds from different populations respond to an after-ripening dormancy breaking treatment equally? Previous literature states a high degree of dormancy loss from after-ripening (Christensen et al. 1996). 3) Do seeds not germinate the first year after dispersal and carry over to the second-year growing season? Seed banks have been reported (Young et al. 1969, Young et al. 1975), however the time period of carryover can be highly variable (Smith et al. 2008).

### Methods

*Bromus tectorum* seeds were collected from each population every two weeks from May through July 2009. *Bromus tectorum* plant density, seed bank, and seed production was measured for each population. The populations were all in close proximity (<120 kilometers). Germination was tested within a week of seed dispersal at six temperature regimes (2C, 5C, 15C, 20C, 25C and 2/15C alternating). Germination tests were conducted for two weeks. Seeds were also tested at the six temperatures after four pre-treatments (6 weeks at 2C wet, 2C dry, or 40C dry, and 24 weeks dry at room temperature). Seeds from all populations were found to be at least 95% viable based on tetrazolium tests. Results are based on total germination after two weeks.

### Results and Conclusions

Unlike most previous findings, an identifiable amount of seed was nondormant at dispersal time with populations two and five often having the least dormancy (Figure 1). There was a great amount of variation in dormancy among the populations. It is possible that maternal environment at maturation is determining dormancy, as has been found previously in other species (Andersson et al. 1998). Similar to previous research, after-ripening strongly decreased the dormancy of two populations (Figure 2). However, three populations remained at least 89% dormant at 25C even after 24 weeks of after-ripening. Comparing June non-germinated seeds in the soil, the most dormant population (site 4) had the largest seed bank while the two least dormant populations (2 & 5) had the smallest (Table 1). In conclusion we find that *Bromus tectorum* seed dormancy can vary greatly among populations. Population five was 20% nondormant (25C) at seed dispersal time. This can account for 3500 nondormant seeds per $m^2$ being produced at this site, an amount not to be underappreciated. Our results stress the importance of acknowledging the variation of *Bromus tectorum* seed dormancy among populations and the need to further examine the environmental site conditions under which such diversity could be created.

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Table 1. Population sites, including dominant plant species, recent burn history, summer *Bromus tectorum* germination observations and *Bromus tectorum* characteristics.

*Bromus tectorum* population values are averages from random sampling of sites.

*seed banks determined by bioassay samples taken in June 2009

| Site | Dominant species | Elevation | Burn < 5 years | BRTE summer germination | soil | Brte Plants / $m^2$ | Tiller Length (mm) | Brte Seeds /plant | Seedbank* Seeds/$m^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **Wyoming sagebrush** *Artemesia tridentata wyomingensis*, (Beetle & A. L. Young) | 1365 | No | No | Loam/ Gravely | 291 | 106 | 54 | 109 |
| 2 | **Fremont's dalea** *Psorothamnus fremontii* (A.Gray) | 1329 | No | No | Sandy Loam | 17 | 190 | 599 | 6 |
| 3 | **Black greasewood** *Sacrobatus vermiculatus*, (Hook. Torr.) | 1280 | No | Yes Late June | Very Fine Loam | 104 | 87 | 115 | 38 |
| 4 | **Cheatgrass** *Bromus tectorum L.* | 1353 | Yes | No | Fine Loam | 411 | 85 | 99 | 301 |
| 5 | **Jeffery pine** *Pinus jefferyi (Grey balt.)* | 1758 | No | No | Sandy Loam | 512 | 248 | 64 | 12 |

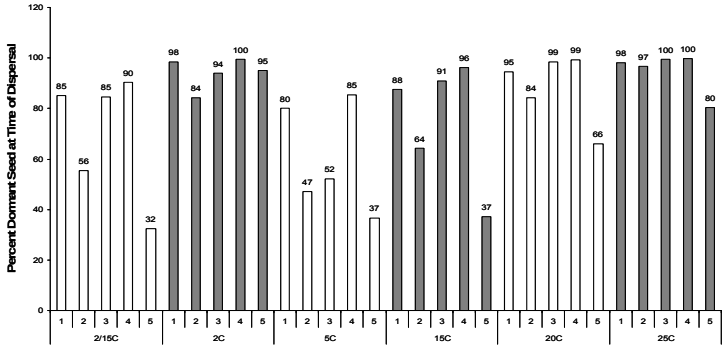


Figure 1. Percent of dormant seed based on lack of germination for all populations (1-5). Test conducted one week after seed collection. *seed collections for all population found to be at >95% viable based on Tetrazolium test.

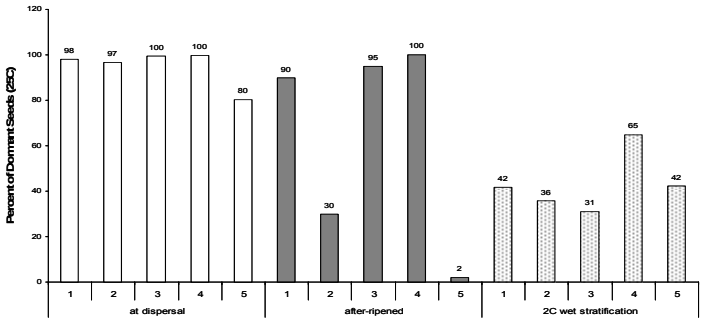


Figure 2. Percent of dormant seeds for each population (1-5) based on lack of germination at 25C tested at the time of seed dispersal, after 24 weeks room temperature storage and 6 weeks of cold moist prechill (2C) after time of dispersal. * seeds were all >95% viable for each population collection based on Tetrazolium test

# Does an Increase in the Duration of Cold Stratification Have an Effect on the Germination of *Eucalyptus nitens* H. Deane & Maiden and Three Other Victorian Eucalypt Species?

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## Introduction

Climate change may have serious implications for *Eucalyptus* species restricted to the montane forests of South Eastern Australia (Hughes *et al*. 1996a). Eucalypts in this region are expected to be very sensitive to even a low degree of climate change as they are known to have narrow ecological niches (Hughes *et al*. 1996b). *Eucalyptus nitens* H. Deane & Maiden is an endemic tree species occurring in cool mountain areas of central Victoria (Cook *et al.* 1991). It has a narrow distribution and typically occurs in pure stands between 800 and 1000m, at the ecotone between wet sclerophyll forest dominated by *Eucalyptus regnans* and montane wet forest dominated by *E. delegatensis* subsp. *delegatensis.* Cold moist seed stratification is not a requirement for germination of *Eucalyptus regnans*, but is a requirement to alleviate primary dormancy in *E. delegatensis* subsp. *delegatensis (*Close *et al*. 2002, Battaglia 1996*).* The requirements for *Eucalyptus nitens* are ambiguous, so we hypothesized that is would require a longer chilling requirement than *E. regnans* and a shorter period to alleviate primary dormancy than *E. delegatensis* subsp. *delegatensis* or *E. pauciflora.* We also tested the hypothesis that higher elevation populations both within and between species require longer chilling requirements to alleviate primary dormancy than populations at lower elevations.

## Methods

The effects of cold moist stratification on mean germination time (MGT) and germination percentage were tested under laboratory conditions for seven populations of *Eucalyptus nitens*, two populations of *E. regnans,* four populations of *E. delegatensis* subsp. *delegatensis,* and five populations of *E. pauciflora*. Treatment periods of 0, 1, 2, 4, and 6 weeks at 4°C were followed by 28 days at 20/15°C and 12/12 photoperiod. All remaining seed was assessed for viability at the conclusion. Species specific logistic regression models were used to assess the timing and percentage of germination under climate scenarios.

## Results and Conclusions

All populations germinated to some degree without any chilling, although germination percentage was lower in species known to exhibit primary dormancy. Significant differences were found within and between species in relation to MGT, germination % and length of chilling. The need for longer chilling requirements with increasing elevation was mixed. Modeling analysis identified species which require longer chilling periods to alleviate primary dormancy and stimulate germination are likely to be at a disadvantage during the regeneration phase compared to species which do not exhibit primary dormancy.

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## Seed Surface Structure in Central European Species

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### Introduction

Morphological dispersal syndromes are frequently used to assess the type of dispersal. In these approaches the type of dispersal is derived from morphological traits of the plant, especially its seeds (i.e., dispersules, which include all relevant appendages). For example, seeds with a pappus are categorized as being dispersed by wind, whereas seeds with hooks are assumed to be dispersed predominantly on the coats of animals. Here, we use an alternative method that allows quantification of gradual differences in seed morphology even within one morphological category. Based on digital image analysis, we calculate *seed surface structure* which describes the smoothness of a seed's surface on a continuous scale from 0 (rough surface) to 1 (smooth). In the following we will give a short overview of *seed surface structure* for a substantial proportion of the Central European flora and analyze differences in *seed surface structure* between species growing in different vegetation types. Additionally, we test whether *seed surface structure* can be used to predict terminal velocity of the seeds as an indicator for wind dispersal.

### Methods

The quantitative measure of *seed surface structure* (*SSS*) was already introduced and used to predict attachment and detachment of seeds to animal furs for small species sets (Römermann et al. 2005, Will et al. 2007). Here, we measured *SSS* for 999 species which are representative of the Central European flora by taking digital photographs of mostly 5 to 20 seeds per species under a binocular microscope (Nikon AZ 100). The photographs were then converted into binaries which were analyzed using NIS-Elements BR 3.0 (Nikon, Tokyo), a digital image analysis program.
*SSS* is calculated by dividing the perimeter of the smallest convex hull around the seed (calculated from the binary) by the actual seed surface (at the binary). *SSS* ranges from 0 to 1, with small values representing a rough, highly structured seed surface and large values a smooth surface.
*SSS* of a distinct species was calculated as the mean of all its measured seeds. The preferred vegetation type of the considered species was classified according to Korneck et al. (1998) and the life forms *sensu* Raunkiaer were taken from Ellenberg (1992). Finally, we collated data of the terminal velocity of these species based on our own measurements and from the LEDA-traitbase (Kleyer et al. 2008).

### Results and Conclusions

The general distribution of *SSS* within the Central European flora is negatively skewed, with 90% of the species having smooth seeds, here arbitrarily defined as seeds with SSS >= 0.4 (Fig. 1). The proportion of rough seeds (defined as seed with SSS<0.4) differs between vegetation types (Fig.2). In arable fields, for instance only 6% of the seeds are rough, whereas in alpine meadows more than 35% of the species have rough seeds. Rough seeds mostly have (many) hairs or awns and often have high wind dispersal potentials. Therefore, it is not surprising that seed surface structure is positively related to terminal velocity (Fig. 3). Significantly, the relationship is strongest for rough seeds, whereas it is hardly relevant for smooth seeds. Additionally, the large variation around the trend-lines indicates that SSS alone will not be sufficient to adequately predict terminal velocity. We therefore suggest considering additional functional traits, especially seed mass, in order to predict falling velocity of seeds from morphological traits with statistical models.

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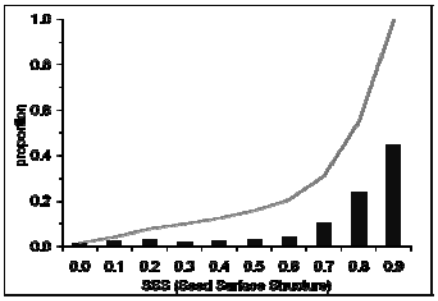


Fig. 1: Relative frequency (bars) and cumulative frequency (line) of seed surface structure for 999 species of the Central European flora.

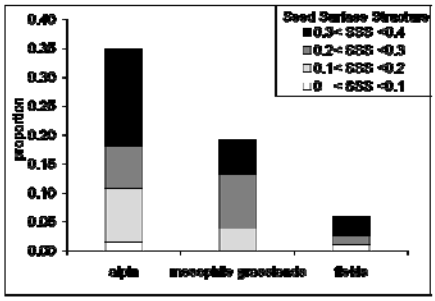


Fig. 2: Relative frequency of species with rough seeds, i.e., with seed surface structure (SSS) <0.4 for alpine meadows (N=140), mesophile grasslands (N=130) and arable fields (N=201).

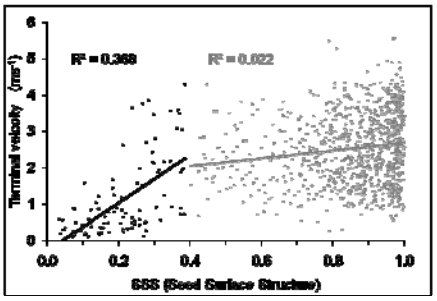


Fig. 3: Relationship between seed surface structure and terminal velocity. Data and trend-line of rough seeds (N=99) is plotted black, whereas grey colors are used for smooth seeds (N=900).

# Australian Alpine Seeds and Seedlings: Can They Cope with Change?

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## Introduction

Alpine environments occupy *ca*. 5200 km$^2$ of Australia's southeast mainland, the largest area being home to Australia's highest peak, Mt Kosciuszko (2228 m). Recently awarded inclusion on the National Heritage List, this alpine ecosystem is recognized as a center of plant diversity within Australia and one of the world's 187 biodiversity hotspots (IUCN / WWF, 1995). The Australian Alps have been identified as critically vulnerable by the International Panel on Climate Change. Already experiencing reduced snow cover / depth, increased summer temperatures and elevated $CO_2$, (Hughes, 2003), alpine species are being forced to move into cooler, wetter areas as their native environments become warmer and drier (Hughes *et al*. 1996; Fitzpatrick *et al*. 2008). Little is known about Australian alpine seed germination strategies, their ability to remain viable post-dispersal, or the resilience of alpine seeds and seedlings to future climate scenarios. However alpine altitudinal gradients offer unique insight since lower altitudes mimic future growing conditions when compared to current higher altitudes. Experiments are currently underway to investigate:

- Which alpine seeds germinate at dispersal and which postpone germination until the following growing season.
- Which alpine plants form persistent soil seed banks.
- How traits such as plant size, leaf area, seed production, seed viability and seedling establishment vary with altitude.

## Methods

*Germination experiment:* Mature seeds of 24 Australian alpine forbs were collected in Kosciuszko National Park (KNP) between Dec 2008 and April 2009. Seeds were cleaned and dried (15% RH) before being stored or 'banked' at -20°C prior to experimentation in Dec 2009. An additional 28 collections including 20 new species were made in KNP between Jan and March 2010. These 'fresh' seeds were stored for one week (15% RH, 15°C) prior to processing and inclusion in the experiment. Replicates of all collections (a total of 29 genera within 14 families) are currently being moved through germination conditions designed to mimic summer (8 wks at 25/15°C), early autumn (2 wks at 20/10°C), late autumn (2 wks at 10/5°C), winter (12 wks at constant 2°C), early spring (2 wks at 10/5°C), late spring (2 wks at 20/10°C) and summer conditions again. Light coincides with the warmest half of the regime and half of all replicates are wrapped in Al-foil to exclude light. Germination, defined as radicle emergence by >1 mm, is scored every 7 d. Seeds sown in the 'dark' are assessed under a green 'safelight'. All collections underwent viability testing before and at key intervals during the experiment using the TZ staining technique (ISTA, 2003).

*Soil seed bank experiment:* Soil samples to a depth of 10 cm were collected from quadrats at 50, 100, 150 and 200 m above the treeline (1940–1960m) along 3 transects in KNP in Jan 2010. Within each quadrat cover of all standing vascular plant species was recorded to the nearest 5%. Soil was stored at 15°C, 15% RH for 6 weeks before being spread in trays over sterilized sand and arranged randomly in temperature-controlled glasshouses. Temperature regimes were designed to mimic today's early growing season (20/10°C) and a warmer future scenario (30/20°C). Half of all soil samples were administered with 200mg/L $GA_3$ for the first 5 days in order to bypass any physiological seed dormancy (PD). All trays are currently watered twice daily with a fine spray-mist and are scored for germination (emergence) every 3-5 days, with each seedling remaining tagged and numbered until identification.

*Altitudinal gradient study:* Data and seeds from >30 individual plants spanning 8 species were collected in KNP along the maximum altitudinal gradient per species. Data included plant height, size and local density. Seeds from the reproductive module were collected as well as all available seeds on each plant. *Ex situ*, samples are currently being analyzed for vegetative parameters such as specific leaf area, vegetative mass and leaf mass area. In addition, seed mass, number and size, seed viability, number of seeds per head and total seed production per plant are being calculated. For 6 of

the species (for which seed numbers allow), we will carry out germination tests at optimal conditions per species and compare final percentage germination, time to first germinant and time to 50% germination across altitudinal gradients. Where possible, seedlings will be pricked out and planted in 'present' and 'future' controlled temperature glasshouse conditions (as described above) in order to investigate early seedling establishment under predicted climate change. Data will include seedling height and rosette diameter, above/below ground biomass and specific leaf area.

**Preliminary Results and Conclusions**

The 'move-along' seed germination experiment (Baskin and Baskin, 2003) was designed to mimic alpine soil temperature regimes in a shortened progression of seasons following summer dispersal. The experimental design makes the assumption that alpine seeds *in situ* are sufficiently hydrated throughout the year, i.e., with the opportunity to germinate at any stage. Preliminary data suggest that the majority of Asteraceae, Poaceae and Caryophyllaceae seeds appear likely to germinate immediately post-dispersal *in situ* (for example, *Leucochrysum albican* ssp *alpinum*, *Craspedia leucantha*, *Austrodanthonia alpicola* (Asteraceae) and *Colobanthus affinis* (Caryophyllaceae). Germination matched or exceeded estimated viability, with germination beginning within 7-14 days of sowing in 'summer' conditions, regardless of prior storage. In contrast, the majority of Apiaceae and Cyperaceae species tested did not germinate under 'summer' or autumn' conditions despite continued high viability. Exceptions were *Carex appressa* (Cyperaceae, 78% germination at 25/15°C, light/dark, post-banking), *Oreomyrhhis ciliata* (Apiaceae, 25% germination of fresh seeds at 25/15°C, light/dark) and *O. eriopoda* (Apiaceae, 82% germination at 25/15°C followed by 20/10°C, constant dark). *Oreomyrrhis eriopoda* is currently the only species tested for which germination was significantly greater in the dark compared to alternating light/dark, suggesting light-inhibited germination and a predisposition for persistent soil seed bank incorporation. In addition, 6% of *Aciphylla glacialis* (Apiaceae) seeds germinated at 2°C in the dark suggesting dormancy mechanisms to postpone germination until during or after the winter following dispersal. Such a germination strategy would enable a species to maximize the relatively short alpine growing season. Examination of *A. simplicifolia* and *A. glacialis* seed embryos, which appeared underdeveloped at dispersal, showed no sign of the elongation necessary for germination through summer and autumn temperature conditions. Therefore this development is assumed to have begun during 'winter'.

Alpine soil was collected after germination of transient seeds in spring 2009, and before the onset of seed dispersal of standing vegetation in summer 2010, in order to assess the *persistent* soil seed bank content. Following prolific seedling growth under glasshouse conditions, preliminary results suggest greater stimulation of germination in the 'cool' glasshouse compared to the 'warm'. This would suggest fewer seedlings emerging from the soil seed bank reserve under future climate scenarios. In addition, PD (overcome by $GA_3$) appears evident in the 'warm' glasshouse, indicating that some alpine species utilize seed dormancy mechanisms to postpone germination and perhaps take advantage of subsequent, more optimal growing seasons.

Protocols for reliable seed germination and plant propagation will contribute to effective management of alpine flora into the future. *Ex situ*, our work will improve conservation of Australian alpine flora at the Australian Alpine Seed Bank, while plans are underway for the creation of alpine beds and interpretive material in the Australian National Botanic Gardens to increase public awareness and appreciation of Australia's alpine biodiversity.

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# Cyclic Sensitivity Patterns in Seeds with Physical Dormancy

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## Introduction

Dormancy cycling is a phenomenon reported as early as the 1970's in seeds with non-deep-physiological dormancy (Baskin and Baskin 1985). Although there are claims for dormancy cycling in seeds with physical dormancy (PY) (Rolston 1978), there is no direct evidence for this. However, seasonal cyclic patterns of germination of seeds with PY have been observed in several Fabaceae (Van Assche et al. 2003; Taylor 2005) and Convolvulaceae (Jayasuriya et al. 2008a, 2009a) species. Taylor (2005) suggested a two-stage dormancy breaking model to explain this seasonal cyclic pattern. Jayasuriya *et al.* (2008a) further clarified this phenomenon with experimental evidence on species in Convolvulaceae. According to these models, there are two states of dormant seeds: the insensitive state, in which seeds are insensitive to the dormancy breaking treatment, and the sensitive state, in which seeds are sensitive to the dormancy breaking treatment (Jayasuriya et al. 2008a). These were termed as hard seeds and latent soft seeds, respectively, by Taylor (2005). Dormant seeds cycle between insensitivity ↔ sensitivity and there is a continuum between insensitive and sensitive states. Sensitivity cycling is driven by environmental stimuli such as temperature and soil moisture. Although direct evidence for sensitivity has been observed only for Fabaceae and Convolvulaceae, there is indirect evidence for sensitivity cycling in seeds with PY in Geraniaceae and Malvaceae as well (Jayasuriya et al. 2009b). Thus, sensitivity cycling may be a common phenomenon in seeds with PY.

## Sensitivity Cycling and PY Cycling

Although there are several claims that seeds with PY can cycle between dormancy and nondormancy (Rolston 1978, Norsworthy and Oliveira 2007), the evidence presented can also be explained by the sensitivity cycling phenomenon. In these claims there was no direct evidence to conclude that dormancy was broken prior to reentering into secondary dormancy. Dormancy break of seeds with PY occurs through formation of a physical break in a specialized morpho-anatomical area in the seed coat termed the water gap (Baskin et al. 2000). In most species, this physical break can not be resealed. However, in *Cuscuta* sp. (Jayasuriya et al. 2008b) and in some Fabaceae species (Hyde 1954) the hilar fissure, a preformed suture, acts as the water gap. Thus, there is a possibility that it can be resealed after a break. However, experimental evidence has shown that *Cuscuta* species cannot cycle between dormancy ↔ nondormancy (Jayasuriya et al. 2008b).

## Significance of Sensitivity Cycling

Physiological dormancy cycling has long been identified as a mechanism to ensure the timing of germination in favorable environmental conditions for seedling establishment. The sensitivity cycling mechanism serves the same ecological function in seeds with PY. Seeds with PY cannot cycle between dormancy ↔ nondormancy, and they germinate under a wide range of temperature conditions after the dormancy break. If there were no sensitivity cycling mechanism, seeds would be in the sensitive state throughout the year, would be sensitive to environmental cues for dormancy break, and would become nondormant. For most seeds with PY, the dormancy breaking cue is a short-term environmental change such as 35 $^{o}$C in a moist environment for 3 hr or 35 $^{o}$C in a dry environment for one week (Jayasuriya et al. 2009a). There is a possibility that these environmental cues could occur under unfavorable environmental conditions for seedling establishment. Sensitivity cycling ensures that the timing of dormancy release is synchronized with favorable environmental conditions for seedling establishment. Normally, seeds are dispersed in the insensitive state, and thus, they have to be exposed to certain environmental conditions for a longer period to become sensitive. Then sensitive seeds have to be exposed to a different environmental cue to become nondormant. The probability that a seed will be exposed to these two conditions sequentially is very low and hence, it reduces the danger of germination in unfavorable conditions. If sensitive seeds in the soil seed bank do not get exposed to dormancy breaking cues they can reenter insensitivity. Thus, sensitivity cycling also maintains the soil seed bank. Seed vigor is an important factor that determines the production of healthy seedlings. When sensitive seeds are kept for a long time, they lose vigor at a rapid rate, whereas insensitive seeds lose vigor slowly (Jayasuriya *et al.,* 2009c). Therefore, reentry of sensitive seeds to the insensitive state is important as a vigor-maintaining mechanism for seeds in the soil seed bank.

## Conclusion

Observed cyclic patterns in germination of seeds with PY can be explained well with the sensitivity cycling phenomenon. There is no experimental evidence for cycling of PY. Sensitivity cycling may be a common phenomenon in seeds with PY. Sensitivity cycling serves the same ecological function as physiological dormancy cycling, and both these phenomena ensure the timing of germination to coincide with favorable environmental conditions.

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# An Evaluation of Seed Scarification Methods of Four Native Lupinus Species

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## Introduction

Seed dormancy is a survival strategy that better ensures the persistence of a species. Dormancy is characterized as exogenous if caused by factors outside the embryo or endogenous if caused by factors within the embryo. Exogenous dormancy is further characterized as physical, mechanical or chemical, while endogenous dormancy may be physiological or morphological. Combinations of these factors may be responsible for dormancy in some species (Jones and Nielson, 1992).
Some lupines exhibit physical dormancy. The integrity of the physical barrier varies by species, accession and year. It has been demonstrated that dormancy in lupines is also affected by relative humidity at the time the seed reaches maturity (Quinlivan 1970). Highly dormant seed lots require scarification to assure acceptable germination rates when planted. We tested chemical, mechanical, and thermal scarification treatments to discover which was most predictable and yielded the highest germination.

## Methods

In this study we evaluated scarification treatments for four lupine species. *Lupinus argenteus* and *L. prunophilus* are physically dormant and were the primary focus of the scarification treatments. *Lupinus arbustus* and *L. sericeus* are not presumed to be physically dormant; however, these were treated to test the hypothesis that scarification would benefit germination. Seed was scarified chemically with a concentrated sulfuric acid or Clorox bath at incremental time increases; mechanically, using a Forsberg mechanical scarifier at incrementally increasing times; and thermally, using a hot water bath while varying exposure time to boiling water in one treatment and in a second treatment holding time constant at one minute and varying exposure temperature. Pilot studies were conducted in advance to determine a window of appropriate exposure intervals for each treatment method. For this study, ten treatments were conducted within the predetermined interval. Regression analysis was used to find optimal exposure for each species to each treatment type based on percent germination. Treated seed was place on moistened blotters in petri dishes at 20°C. Petri dishes were then placed in clear plastic bags to prevent blotters from drying out. Bags were left on a laboratory bench for the duration of the study. No Association Official Seed Analysts (AOSA) germination rules have been developed for the lupine species in this experiment. Generally AOSA rules indicate monitoring lupine germination for 18 days. In this study germination was recorded for 22 days. Germinated seeds were counted and removed 3 times during the 22 day period.

## Results and Conclusions

Every species except *L. arbustus* showed increased germination as a result of scarification. Optimal exposure times to each treatment differed between species. *Lupinus argenteus* responded best to acid and mechanical scarification. Germination peaked at 87% for acid scarification with five and a half minutes of treatment exposure. That is a 35% germination increase over the control (fig. 1a). Mechanical scarification of *L. argenteus* also peaked at 87% germination between 5.5 and 6.5 seconds exposure time. Unlike acid scarification for *L. argenteus,* mechanical scarification did not show the sharp decline after the optimal scarification point was reached but did become less predictable when exposure time was >7 seconds ( Fig 1b ). *Lupinus prunophilus* reacted best to the chemical scarification. Acid scarification of *L. prunophilus* did not cause a definite peak in germination. which increased for the duration of the treatment finishing at a 78% germination when exposed the full 10 minutes, which is a 46% increase over the control (fig, 1c). The Clorox bath treatment had the highest predicted germination at 79% when treated for 110 to 115 minutes. Mechanical scarification of *L.prunophilus* showed a lower peak at only 59% germination after two seconds andgermination declined by 30% after 10-second exposure. The 30% decline is possibly due to seed size; *L. prunophilus* is a much larger seed than *L. argenteus* and was visibly damaged to a much greater extent from the rotating action of the mechanical scarifier. *Lupinus. sericeus* is not hard-seeded; however it did show improvement in germination when scarified. The control for *L. sericeus* averaged

only 22% germination, while two seconds of exposure time in the mechanical scarifier increased germination by over 3.5 times, to 80%. This accession has demonstrated highemergence when used in greenhouse and field plantings without scarification; the low control germination percentage may mean that 22 days is not an adequate duration for germination without scarification. We demonstrated that some scarification on *L. sericeus* may help with increased uniformity of germination allowing 80% of the seed to germinate before 22 days. This could also lead to better establishment in a field setting by allowing scarified seed to put down a root more quickly than unscarified seed before soil moisture is lost. All scarification treatments on *L. arbustus* damaged the embryo, negatively impacting germination. The seed coat on *L. arbustus* is noticeably thinner than the other species in this study and this could explain the embryo damage. This study clarified which scarification methods yield best germination in these four lupine species and for these particular accessions. Continued research will focus on using this data as a baseline to test multiple accessions of these same species from Great Basin biotypes. This will allow us to better predict how each scarification method would act on a given species and thereby improve germination for cultivation of that species for use in restoration.

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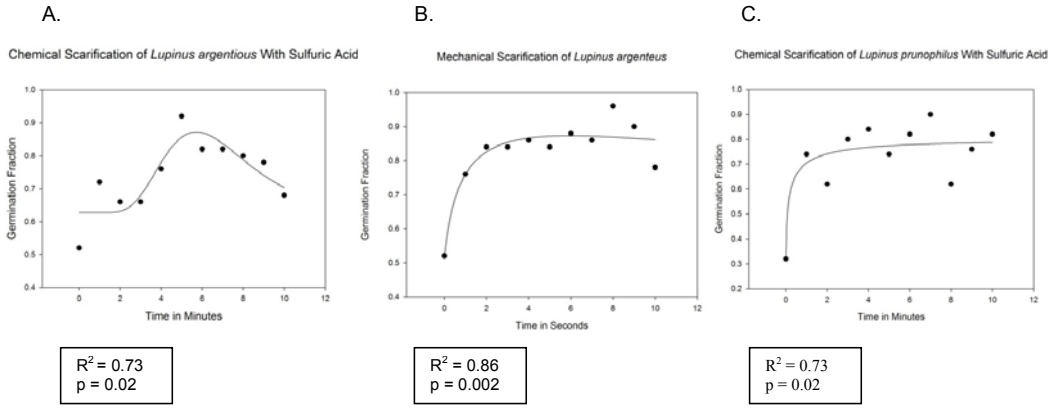


**Figure 1.** Fraction of germination vs. exposure time, non-linear regression equations used to predict germination response to a specific exposure treatment and time. A. Regression equation = Peak, Log Normal, 4 Parameter B. Regression equation = Log Normal, 4 Parameter C. Regression equation: Sigmoidal, Weibull, 4 Parameter

## Seasonal Sporulation-Revealed Complexity in Life Cycles of Norway Spruce Cone Rusts

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### Introduction

Seed crops of Norway spruce, *Picea abies* (L.) Karst., are significantly reduced by cone pathogens both in seed orchards and natural forests in Finland (Tillman-Sutela et al. 2004, Kaitera et al. 2007). Cone rusts cause malformation and premature opening of cones, prevent seed dissemination by cone resinosis, distort cone scales, increase the number of deformed seeds in cones, and reduce seed germination (Nelson and Krebill 1970, 1982, Ziller 1974, Sutherland 1981, Singh and Carew 1990). Seed crop losses have been reduced by spraying the trees using fungicides during pollination (Summers et al. 1986), but this treatment has not always resulted in an efficient control of rust diseases. The aim of this study was to increase accurate information about the sporulation and life cycle of Norway spruce cone rusts for improving disease control in seed orchards.

### Methods

In 2007-2008, sporulation of cone rusts was examined in cones and alternate hosts using scanning electron microscopy (FESEM). The frequencies of fruitbody stages of *Thekopsora* and *Chrysomyxa*, and their sporulation, were estimated at different times during the growing season. Life cycles of the observed cone rusts were presented, and the control of rust diseases was evaluated based on the observed life cycles.

### Results and Conclusion

Three rusts with different fruiting stages sporulated in cone scales. The most frequent and significant rust was *Thekopsora areolata* (Fr.) Magnus, which formed aecia in mid- and late summer in current-year cones and thus disrupted seed development. The majority of aecia sporulated a year later and no spermogonia were observed in cones. Uredinia developed on young *Prunus padus* L. leaves and telia developed in overwintered leaves. No basidia occurred on these leaves. For a detailed description of the results, see Kaitera et al. (2009a, b). The life cycle of *T. areolata* proved to be more complex than previously demonstrated, which complicates possible disease control. This is due to possible autoecism of the rust, which expands possibilities for rust infection. The rust may directly infect young developing cones with aeciospores from old cones. Consequently, cone infection may take place during the whole growing season, and the aeciospores may also spread long distances by air. The rust may be controlled by removing infected one-year-old and older cones from tree canopy or by removing infected leaves or entire trees of *P. padus*.

Another significant cone rust, *Chrysomyxa pirolata* Wint., formed undifferentiated fruitbodies that developed into either uredinia or telia on overwintered *Pyrola* spp. leaves in late spring and early summer depending on weather conditions. Telia were formed under wet conditions, whereas uredinia developed under dry weather. These fruitbodies sporulated in young cones. Spermogonia and aecia developed in current-year cones in early summer, and sporulated in the same year (Kaitera et al. 2009b). As a consequence seed development was disrupted. The life cycle of this rust, however, was typical for a heteroecious rust, which increases possibilities for disease control. The rust may be controlled by spraying the pistillate cones before and after telia formation on overwintered alternate hosts (*Pyrola* spp.). The alteration of undifferentiated fruitbodies into either uredinia or telia can be monitored.

A third rust, *Chrysomyxa ledi* (Alb. & Schw.) de Bary, produced spermogonia and aecia in single scales of current-year cones in the early and mid-summer. These fruitbodies sporulated during the same growing season (Kaitera et al. 2010). The life cycle of this rust was also typical for a heteroecious rust. Although this needle rust regularly causes severe rust epidemics in Norway spruce needles in Finland, most recently in 2009, the rust has probably only a small influence on seed development in Norway spruce cones. Therefore, there is no need to control this rust in cones.

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## Interaction between Ethylene and Reactive Oxygen Species in Regulation of Seed Germination

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### Introduction

Ethylene plays role in a wide spectrum of important physiological processes in plants including seed germination (Gianinetti et al. 2007). Recent studies have revealed a cross talk between ethylene and reactive oxygen species (ROS) in signaling for processes like programmed cell death and stomatal closure (de Jong et al. 2002, Desikan et al. 2005). Since ROS are also reported to be associated with the regulation of seed germination (Garnczarska et al. 2008), it is quite likely that an interaction between ethylene and ROS exists for regulation of germination. In the case of root growth and root hair tip growth, ROS produced by plasma-membrane located NADPH oxidase in the apoplast induces cell wall relaxation (Foreman et al. 2003). A similar role of apoplastic ROS in axis elongation during seed germination has been predicted. We have thus attempted to characterize ROS metabolism in germinating axes of *Vigna radiata* and to explore any possible cross talk with ethylene.

### Methods

Seeds of *Vigna radiata* (var B1) were incubated on filter paper moistened with distilled water or test solutions and incubated in darkness in a temperature-controlled germinator (30±2° C) and germination percentage was noted at intervals. Superoxide production by embryo axes after treatment was determined by NBT staining. In-gel assay for NADPH oxidase (NOX) and superoxide dismutase (SOD) was carried out in native PAGE preparations using NBT staining, while in-gel assay for peroxidase was performed using guiacol.

### Results and Conclusions

That ethylene has a role in seed germination was revealed from the effects of an ethylene synthesis inhibitor ($CoCl_2$) and an ethylene action inhibitor ($AgNO_3$) which retarded germination effectively. Similarly, the role of ROS was demonstrated by the inhibitory action of propyl gallate (a ROS scavenger) which was partially reversed by hydrogen peroxide. Involvement of other ROS, such as superoxide and hydroxyl radicals, was also proven by the action of methyl viologen (a superoxide producer) and a mixture of ascorbate and $Cu^{2+}$ to reverse $Ag^{+}$ inhibition. Apoplastic production of ROS may be initiated with superoxide production by NOX activity, which is controlled by ethylene as revealed from disappearance of NOX bands from $Ag^{+}$-treated axes in in-gel assay. However, such NOX bands intensified under simultaneous treatment with $Ca^{2+}$, indicating a possible involvement of $Ca^{2+}$ mediating the action of ethylene through activation of NOX. Once produced, superoxides are dismutated to $H_2O_2$ by SOD, particularly by Cu/Zn SOD, which is possibly located in the cell wall, and such activity was maximum during radicle emergence. Peroxidase activity, which may be involved in the production of hydroxyl radicals leading to cell wall relaxation, was studied in axes through in-gel assay. Ethylene has no direct influence on peroxidase as found from the effect of ethylene and $Ag^{+}$. However, the peroxidase enzyme is probably induced by ROS during germination since treatment with propyl gallate, β-mercaptoethanol and cycloheximide abolished activity in assay.

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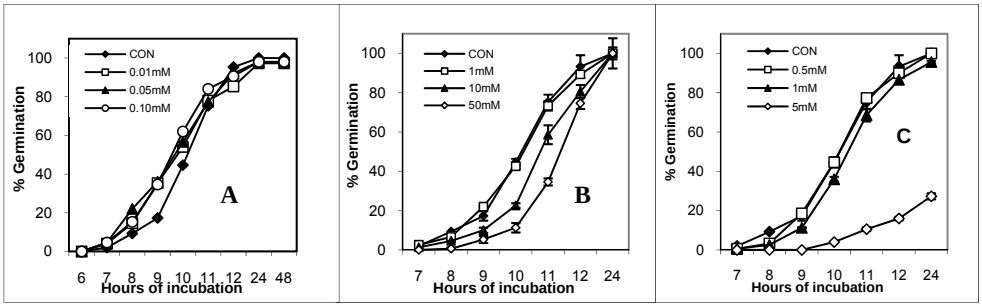


**Fig 1.** Effect of (A) ethylene (ethrel; 0.05 and 0.10mM), (B) cobalt chloride (1, 10 and 50mM) and (C) silver nitrate (0.5, 1 and 5mM) on germination percentage of *Vigna radiata* seeds during incubation in darkness at 30 ± 2 ºC.

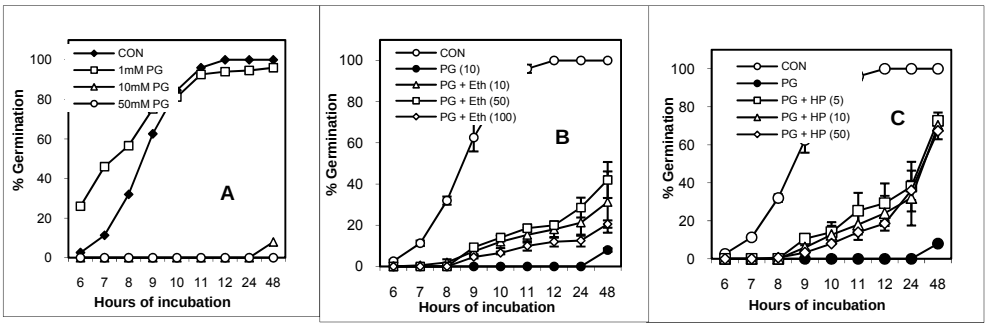


**Fig 2.** Effect of (A) propyl gallate (PG; 1, 10 and 50mM) on germination and recovery of germination by (B) hydrogen peroxide (HP; 5, 10 and 50mM) and (C) ethylene (ethrel; 10, 50 and 100 µM) from inhibition by propyl gallate (50mM) of *Vigna radiata* seeds during incubation in darkness at 30 ± 2 ºC.

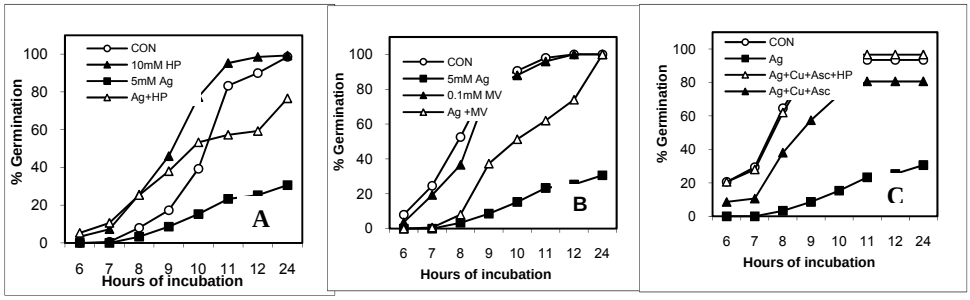


**Fig 3.** Recovery of germination by (A) hydrogen peroxide (HP; 50mM) and (B) methyl viologen (MV 0.1mM) and (C) mixture of $CuCl_2$ (1mM as pretreatment), hydrogen peroxide (10mM) and ascorbate (10mM) from inhibition by silver nitrate (Ag; 5mM) of *Vigna radiata* seeds during incubation in darkness at 30 ± 2 ºC.

# Use of the Angled Mirror Method to Estimate Three-Dimensional Seed Shape Parameters for *SeedR*

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## Introduction

Population-based hydrothermal time models (Bradford 1995, 2002; Gummerson 1986) are now omnipresent in seed science. By contrast, regression relationships cultivated at the individual-level have remained popular for decades in forestry. An advantage of individual-based models is that they facilitate comparison of the variability in underlying factors affecting larger processes of interest, as well as their relative importance. *SeedR* is a simple model intended to forecast seed hydraulic and thermal exchange based on seed morphological and physiological variables measured at the individual level. *SeedR* was developed to capture complex inter-relationships of seed with diurnal climate patterns, as well as atypical events (such as rainbursts or short dry spells). Internal parameters such as megagametophyte and embryo mass and shape are predicted from analysis of digital X-ray imagery. Three dimensional parameters necessary for the model include individual seed surface area and volume, under the shape assumption of $2^n$ partial ellipsoids fused at their equators (Keefe et al., in review). Individual seed surface area and volume, coupled with testa thickness and testa hydraulic conductivity, are used to estimate the exchange of energy between the seed and surrounding environment at the hourly time-step. Estimating partial axes for the major (a), intermediate (b) and minor (c) dimensions to parameterize the shape model requires a relatively quick and repeatable measurement method. We evaluated the use of an angled cosmetic mirror placed on a desktop scanner to obtain simultaneous, orthogonal measurements in three-dimensions at a fixed point in time.

## Methods

Simple random samples of fifteen seeds of a small (*Pinus contorta*), medium (*Pinus ponderosa*), and large-seeded (*Pinus edulis*) species in the *Pinaceae* were placed at each of six locations across a desktop scanner bed (Hewlett-Packard Co., Palo Alto CA). Seeds were positioned on a 3.175 mm thick glass section with a fixed, forty-five degree angle cosmetic mirror perpendicular to the upper edge of the scanner. In 2 cm increments, the intersection of the mirror plane with the glass was advanced from left to right, with new seeds drawn for measurement, without replacement, at each location. All partial axis pairs forming a $2^n$ fused ellipsoid shape were measured either directly, or in the mirror-reflected image. The ratio of the major axis, a, viewed directly and in the mirror, was to calibrate the measurement of c (reflected only): $c_{calibrated} = (a_{direct} / a_{mirror}) \times c_{reflected}$. Individual seed partial-axis pair lengths, surface area, volume, and surface area to volume ratios were derived for each seed, in order to determine whether bias resulted from seed and mirror position due to the angle formed by light source, reflected mirror image used to obtain the minor axis pair lengths, and photodetectors. All complete axes were measured with digital calipers for validation.

## Results and Discussion

Due to interaction of the light source, angled mirror, and photodetector position, images could not be obtained at 7, 9, or 11 cm from the left scanner bed edge. The reflected angle showing the minor axis profile of seeds distorted the image such that a portion of the profile was not visible. Based on linear regression fits of caliper and scan-measured primary axis lengths, three-dimensional profile images obtained with the mirror intersecting the scanner bed at 13 cm provided the most accurate of the 13, 15 and 17 cm positions. As the mirror was advanced to 15 cm and 17 cm, mirror-reflected images of the minor pairs, c1 and c2, and surface area to volume ratio, became negatively biased. This effect was greatest for the smallest seeds (*Pinus contorta*). Non-linear least squares regressions relating each full axis length (a,b,c) to the surface area to volume ratio were fitted (Figure 1) . As with earlier studies, the primary importance of the c dimension, which is not measured by most available seed imaging packages, was underscored. The minor axis, c, better predicted total seed surface area to volume ratio (RMSE=) than either the major (RMSE= ) or intermediate (RMSE=) axis lengths.

## Conclusions

The angled mirror method provides an accurate means for measuring the third dimension of conifer seeds across a range of sizes (a=1.4 mm to 5.4mm). The importance of measuring the third dimension in order to accurately calculate whole-seed physiological parameters for *seedR* is evident in

that this dimension is more closely related to surface area to volume ratio than either of the other two axes. Using the angled mirror method, it is possible to accurately estimate whole-seed shape parameters for conifer seeds exhibiting bilateral symmetry, and thus to use *seedR* to compare physiological interactions of individual seeds with ambient conditions for conifer species in a range of ecological settings.

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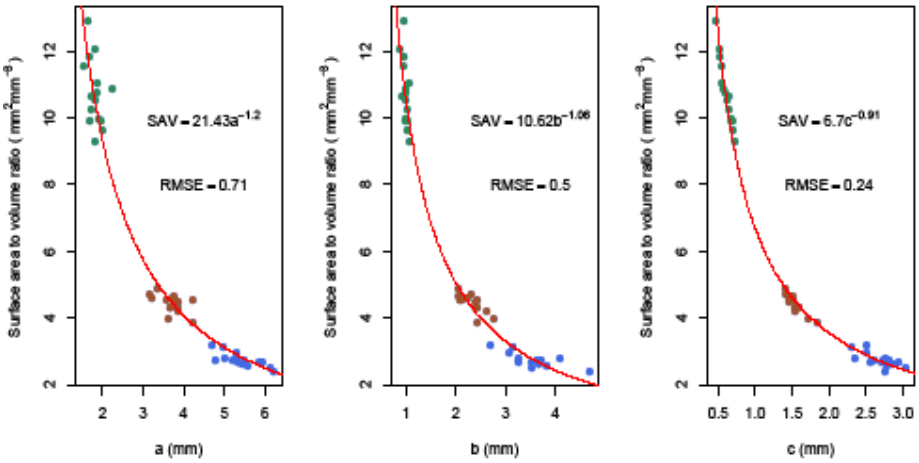


**Figure 1:** Non-linear least squares relationships of major (a), intermediate (b) and minor (c) axis lengths with surface area to volume ratio for *Pinus contorta* (green), Pinus ponderosa (brown) and *Pinus edulis* (blue) with mirror location at 13 cm.

# Ability of Methyl Jasmonate to Reduce Seed-borne *Alternaria porri* f. sp. *solani* Development and Induce Systemic Resistance in Tomato Plants

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## Introduction

*Alternaria porri* (Ell.) Neerg. f.sp. *solani* Ell. et. Mart. is one of the most damaging diseases of tomato, causing heavy economic losses on plants grown in the field and in greenhouses (Agrios 2005). Most attempts to control fungal pathogens involve the use of fungicides. Alternative methods include those that increase resistance of plants to plant pathogens. In the last decade, several new approaches to enhance the resistance of plants to diseases by using plant hormones as elicitors were developed (van Loon et al. 2006). Among plant hormones, jasmonates have been reported to play an important role. Treatment of plants with these compounds increases resistance of plants to necrotrophic fungal pathogens, a process called induced systemic resistance (ISR; Pozo et al. 2005). The present study was carried out to determine (1) whether methyl jasmonate (MeJA) has any activity against *Alternaria porri* f. sp. *solani in vitro,* (2) whether it safe for treated plants as a potential inducer of ISR, (3) whether it has an effect on enhanced tomato defense responses including antioxidant catalase, phenolic compounds and the related enzyme phenylalanine ammonia-lyase (PAL), directly involved in the biosynthetic pathway of phenol compounds, and (4) whether pretreatment of plants with MeJA provides significant protection against *A. porri.*

## Material and Methods

Seeds of *Lycopersicon esculentum* Mill. cv. Beta harvested in 2008 (germination > 80%) were obtained from the TORSEED Seed Company (Poland) and stored dry at -23 °C until the use. For preparation of stock cultures of fungus, PDA agar slants were inoculated with *Alternaria porri* f.sp. *solani* isolated from the above seeds. The method for conidial preparation, counting of germinated spores, and measurement of mycelial growth in presence of MeJA ( 0.01,01, 1mM) has been reported previously (Kępczyńska 1994). To determine the effect of MeJA on the germination process, the 50 seeds were soaked in MeJA water solutions at 0.01, 0.1, 1mM concentration (or a water control) for 60 min and then transferred to Petri dishes ( 5cm) on filter paper moistened with 1.5 ml of water. Germination of seeds was estimated every 24 h for 11 days. To test the effect of MeJA on seedling emergence, the seeds, after soaking in the different solutions of MeJA or water for 60 min, were sown in sterile soil in plastic pots and transferred to a growth chamber. Two-week-old tomato plants with fully expanded leaves placed in 2.7 L glass jars were used for 24 h treatment with gaseous MeJA. After 24h, seedlings were removed from the jars and inoculated with an *A. porri* f *sp. solani* spore suspension ($10^6$ spore's $ml^{-1}$). Four weeks after inoculation, to define disease severity, percentage of seedlings with necrotic spots and chlorosis was assessed. For biochemical analysis, 15-day-old tomato leaves from seedlings pre-treated 24 h with gaseous MeJA or from seedlings grown from 60 min soaked seeds were rapidly frozen in liquid nitrogen. The following methods for biochemical analysis were used: chlorophyll *a* and *b* - Lichthentaler and Welburn (1985), reducing sugars- Lever (1972), total phenols –Singleton and Rossi (1965), anthocyanins –Mancinelli (1984), PAL activity- Edwards and Kessman (1992) and catalase activity – Aebi (1964).

## Results and Conclusions

Application of MeJA to PDA medium at concentrations 0.01, 0.1 and 1.0 mM inhibited spore germination, mycelial growth expressed as mycelial diameter, and dry weight. Pretreatment of seeds with MeJA solution at 0.01 and 0.1 mM had no inhibitory effect on seed germination, seedling emergence, level of chlorophyll a+b or reducing sugars in seedling leaves, showing that at these levels it is not toxic for the plant. MeJA at 1mM did have an inhibitory effect on above physiological processes; therefore concentrations this high cannot be used to induce ISR. Pretreatment of seeds or seedlings with solutions or gaseous MeJA, respectively, at all tested concentrations, increased contents and activities of various defense biochemical markers: total phenols, anthocyanins, enzymes - PAL and catalase activities. Although levels or activity of all defense markers in tomato leaves were highest after pretreatment of seeds or seedlings with the highest (1 mM) concentration of MeJA, the protection against the fungus was not observed, probably because of phytotoxicity effects of the

MeJA. MeJA at all tested concentrations effectively inhibited development of fungus *in vitro*, however *in vivo* only at the 0.1 mM treatment significantly reduced disease development. Thus treatment of seeds or seedlings with MeJA at 0.1 mM provides efective, environmentally friendly, protection against the necrotroph *Alternaria porri* f. sp. *solani,* possibly through the involvement of an antifungal phenol compound, PAL and catalase activity.

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## Overcoming of primary dormancy in *Avena fatua* L. caryopses by smoke and smoke-derived butenolide - involvement of gibberellin and ethylene

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### Introduction

Smoke derived from burning plant material and smoke-water, produced by bubbling the smoke through water, stimulate seed germination of a range of plant species from fire-prone areas of South Africa, Australia, California and also species from fire-free environments including arable weeds and crop plants (Light et al. 2009). Although the role of smoke in seed germination has been known since 1990, it was only six years ago that a germination-active compound, a butenolide, from plant and cellulose-derived smoke was identified (Flematti et al. 2004, Van Staden et al. 2004). In comparison to smoke, butenolide was found to stimulate seed germination in a broader range of species. The discovery of butenolide created the possibility of studying physiological roles of new natural compounds and of a better understanding of the role of smoke in seed germination in soil. Previously it was found that caryopses and seeds of *Avena fatua* L., an important and widespread persistent weed, are sensitive to smoke-water and butenolide (Adkins and Peters 2001, Kępczyński et al. 2006, Daws et al. 2007, Stevens et al. 2007) The aim of this study was to elucidate the role of butenolide in relationship to ethylene and gibberellin in breaking dormancy in *Avena fatua* L. caryopses.

### Methods

*Avena fatua* seeds were collected in Poland near Szczecin in 2007 and stored at -20°C until used. Dormant seeds were then stored in dry conditions at 25°C. After various periods of storage, seeds were dehulled and incubated in 6-cm Petri dishes on filter paper moistened with distilled water or solutions of butenolide or gibberellin $A_3$ ($GA_3$). Caryopses from dormant seeds were incubated in the presence of smoke-water, butenolide, gibberellin $A_3$, ethephon, ACC (a precursor of ethylene biosynthesis), ancymidol (an inhibitor of gibberellin biosynthesis) or fluridone (an inhibitor of ABA biosynthesis). For 2,5-norbornadiene (NBD; an inhibitor of ethylene binding), ethylene (E) or NBD + E treatment, uncovered dishes with caryopses on filter paper with distilled water, butenolide or butenolide + ethephon or ACC solutions were incubated in tightly sealed glass containers. Most experiments were conducted at 20°C. For comparison of effects of smoke-water and butenolide, caryopses were incubated at 15,20, 25 and 30°C. In all experiments 3 replicates with 25 caryopses were used. Germination experiments were carried out in darkness and all manipulations were carried out in the dark under green safelight conditions. Caryopses were considered as germinated when the coleorhiza had protruded through the pericarp. After imbibition of caryopses for various periods in water, butenolide, or $GA_3$, α-amylase activity was measured. The effect of butenolide and $GA_3$ on the percentage of nuclei in various phases of the cell cycle in cells from tips of the radical coleorhiza, was determined by flow cytometry.

### Results and Conclusions

Dormant caryopses of *A. fatua* germinated partially at 15°C and did not germinate at 20 and 30°C. In the presence of smoke-water or butenolide, caryopses germinated almost completely at all temperatures. Dry storage of seeds at 25°C resulted in after-ripening. Germination of caryopses at 20°C gradually increased during prolonged dry storage up to 3 months. Caryopses from fully dormant seeds showed a higher requirement for exogenous butenolide or $GA_3$ than partly after-ripened or non-dormant ones. Ancymidol, an inhibitor of gibberellin biosynthesis, markedly reduced the stimulatory effect of butenolide. $GA_3$ completely antagonized the inhibitory effect of ancymidol in the absence or presence of butenolide. Ethephon, an ethylene-liberating compound, ethylene, and ACC, precursor of its biosynthesis, did not affect germination of dormant caryopses. 2,5-Norbornadiene and 1-methylocyclopropen, inhibitors of ethylene binding to its receptor, prevented the action of butenolide. The inhibition caused by norbornadiene was partially or completely relieved, depending on concentration, when these seeds were transferred to air. The inhibition due to the presence of norbornadiene can be antagonized by ethephon, ethylene and ACC. Both butenolide and gibberellin increased α-amylase activity before coleorhizal protrusion. Likewise, both compounds induced an increase in the proportion of cells with 4C amounts, DNA and an increased ratio of G2/G1. The results

indicate that butenolide is an active factor breaking dormancy in *Avena fatua* caryopses. Both ethylene and $GA_3$ are required for dormancy removal by butenolide. The effect of butenolide and $GA_3$ on germination of dormant caryopses is associated with increasing α-amylase activity and cell cycle activation.

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# Release of Dormancy in *Amaranthus retroflexus* L. Seeds by After-Ripening, Ethephon, Gibberellin $A_3$, Nitric Oxide and Hydrogen Cyanide

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## Introduction

Primary seed dormancy is common in wild plants and develops late in embryogenesis. This dormancy can be defined as lack of germination of intact viable seeds under conditions favorable for germination (Hilhorst 1995, Finch-Savage et al. 2006). Seed dormancy may permit survival after natural catastrophes, decrease competition between individuals of the same species, and prevent germination under conditions unsuitable for seedling growth. Primary dormancy in seeds of many plant species can be broken by different factors, i.e., dry storage, stratification, scarification or plant hormones. Likewise, nitric oxide and hydrogen cyanide may reduce seed dormancy. Chemical donors of nitric oxide lessen or remove dormancy in *Arabidopsis thaliana* and barley seeds (Bethke et al. 2004), as do donors of hydrogen cyanide in apple embryos and *Arabidopsis thaliana* seeds (Bethke et al.2006, Bogatek et al.1991). *Amaranthus retroflexus* L. seeds are dormant when freshly produced in the autumn. Primary dormancy in these seeds can be relieved by dry storage (after-ripening) at room temperature. Plant hormones such as gibberellins or ethylene may also release seed dormancy of *Amaranthus retroflexus* L. (Kępczyński et al. 1996, Kępczyński et al. 2003). The aim of the present study was to determine (1) sensitivity of *Amaranthus retroflexus* L. seeds in primary dormancy to ethephon, a compound liberating ethylene, and to $GA_3$ after different periods of dry storage at various temperatures, and (2) the role of nitric oxide (NO) and hydrogen cyanide (HCN) in releasing dormancy in these seeds.

## Methods

*Amaranthus retroflexus* L. seeds were collected in Poland in 2006. Seeds were air-dried and stored at $-20^0$ or at 5, 15, 25 and $35^o$ C for 8, 12 and 16 weeks in darkness. After various periods of storage, seeds were incubated in Petri dishes (6cm) on filter paper moistened with 1.5ml of distilled water, ethephon or $GA_3$ solutions. Germination assays were performed in darkness at $35^o$C. All manipulations were conducted under a safe green light. To determine effect of nitric oxide and hydrogen cyanide, donors for NO+HCN, HCN and NO, namely nitroprusside (SNP), S-nitroso-N-acetyl-DL-penicylamine (SNAP) and potassium ferrocyanide (Fe(II)CN), were used, respectively. Seeds were incubated in open 3.5 cm plastic Petri dishes on filter paper moistened with 0.8 ml of water or a solution of a nitric oxide scavenger (2-(4-carboxyphenyl)-4,4,5,5tetramethylimidazoline-1-oxyl-3-oxide; cPTIO). Petri dishes with seeds were sealed in 12cm glass Petri dishes together with one open 5 cm Petri dish containing 3.6 ml solution of the donor. The Petri dish containing the donor was replaced with a Petri dish containing water after 1, 3, 5 days, except in the experiment with cPTIO, where they were replaced after 7 days. Experiments involving the application of donors were conducted in white light (16/8h, 120 $\mu E\, m^{-2}\, s^{-1}$) at $24^o$ C. Each treatment included 5 replications of 50 seeds. Germination was defined as 2 mm radicle protrusion through the seed coat. Germinated seeds were counted after 7days.

## Results and Conclusions

Seeds of *Amaranthus retroflexus L.* were dormant at harvest; only 5% were able to germinate at $35^o$ C. After dry storage at 15-$35^o$ C for 12 weeks, 10 to 19% germination occurred. When seeds were dry -stored for 16 weeks, about 15 to 70% of seeds, depending on storage temperature, could germinate. Ethephon and gibberellin slightly stimulated germination of dormant seeds; 40 and 30% germinated, respectively. After dry storage at all temperaturesfor 12 weeks, 60-80% of seeds germinated in presence of ethephon or $GA_3$. All or almost all seeds could germinate in the presence of ethephon or gibberellin after storage at 5 to $35^o$ for 16 weeks. The greatest difference between germination in water, 15%, and in the presence of ethephon or gibberellin, 80-90%, was found after seeds were stored for 16 weeks at $5^o$ C

Seeds in primary dormancy germinated poorly in light (10%). Both NO+HCN and HCN alone stimulated germination of these seeds and 50- 60% were able to germinate. Nitric oxide alone did not affect germination. No stimulatory effect of NO+HCN or HCN was noted in the presence of the nitric

oxide scavenger cPTIO. These results indicate that after-ripening partially removed dormancy in *A. retroflexus* L. seeds, the effect increasing with increasing dry storage temperature. Dry storage of seeds increased sensitivity to both ethylene and gibberellin. Hydrogen cyanide may play a role in the control of primary dormancy in *A. retroflexus* seeds. Release of dormancy by hydrogen cyanide may require endogenous nitric oxide.

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## Chronosequence of Seed Bank and Vegetation in Abandoned Sheep Corrals

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**Abstract**
Seasonal grazing by sheep of the herbaceous understory vegetation in planted forests is a management tool to reduce risk of fire in semiarid Mediterranean regions (250-400 mm rainfall/year), while providing additional income to shepherds. Seasonal corrals are set in the forest, and after a few consecutive years of use are shifted to new sites. After abandonment, a nitrophilous vegetation composed of annual species with high dominance of thistles gradually develops in the corrals, concomitant with a reduction in soil salinity and dung decomposition, due to the seasonal rains. Abandoned corrals show high biomass production, but the ruderal vegetation is less palatable, leading to accumulation of flammable dry matter, and reducing the aesthetic value of the forest for public recreation. The annual vegetation in the abandoned corral emerges every year from its soil seed bank, composed of seeds produced *in situ* by the ruderal species in the corral, persistent seeds from the former seed-bank and seed-rain from the surrounding vegetation. We studied the dynamics of seed bank composition during vegetation recovery in the abandoned corrals. Age of corrals was determined from aerial photographs. Species composition and seed density were related to seed persistence and dispersal traits, and potential endozoochory, as well as to plant competitive ability and seed set in relation to gradual changes in soil resources in the abandoned corral. Vegetation recovery spans 15-20 years, and the pathway of recovery was found to be largely dependent on conditions in the abandoned corral.

## *Sphaeralcea munroana*: Strategies for Improving Seed Germination

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### Introduction

Munro's globemallow (*Sphaeralcea munroana*), endemic to the Great Basin, has suffered significant declines due to a changing climate and invasive species pressure. Despite strong efforts to restore native plants via seed propagation, few cases have been successful. Establishment failure can be attributed to seed germination requirements that are not met during planting. Restoration efforts hinge heavily on the ability to understand the germination strategy of the species. Munro's globemallow occurs primarily at the lower elevations on mountain slopes and in desert valleys. In these environments, seeds may be subject to fire or abrasion in flash floods. Poor *ex situ* germination in laboratory studies suggest that the seed requires scarification to break dormancy. This study evaluates methods for overcoming seed dormancy and improving germination success for *Sphaeralcea munroana* seeds.

### Methods

A study initiated to determine if seed coat impermeability is responsible for reduced imbibition and germination consisted of a 2 × 2 factorial block design with 4 replicates per treatment. Seeds were soaked in aerated, distilled water for 24 and 48 hr; 50% of the seeds were pierced prior to soaking. Germination took place on water-soaked filter paper inside Petri dishes. Seeds were subject to a 21 day alternating temperature cycle of 24°C (8 hr) and 17°C (16 hr). Germination was considered to be complete when the radicle had reached 5 mm in length (Page et al. 1966).

### Results and Conclusions

Initial research to determine if seed coat impermeability is responsible for poor germination rates focused on the effect of mechanical scarification and seed imbibition. The results show that physical dormancy plays an important role in germination. Seeds that were pierced exhibited higher germination than seeds in the non-pierced treatments. In addition, seed water absorption appeared to be slow in *S. munroana*, and pierced seeds may benefit from an extended submergence in water. Conversely, the prolonged water exposure of impermeable (non-pierced) seeds had deleterious effects, resulting in the lowest germination percentages. Scarification in the absence of water increased germination compared to the control; however, pierced, water-soaked seeds exhibited the highest germination capacity. This suggests that submergence in water following scarification may be necessary for successful germination. A subsequent study has been initiated to determine the effectiveness of various alternative scarification and stratification methods as possible dormancy-breaking techniques for *ex situ* germination.

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# Seed Dormancy of *Convallaria keiskei* and Effects of Light and Temperature on Radicle Emergence

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## Introduction

Seed dormancy of *Convallaria majalis* L. was investigated by Barton and Schroeder (1942) and it was classified as having double dormancy under morphophysiological dormancy (MPD; Baskin and Baskin 1998). Seeds need a first period of low temperature for radicle emergence and a second period of low temperature for shoot emergence. However, embryo growth was not investigated in this study, though an undeveloped embryo is one of the criteria for MPD. Information about the effects of light and temperature on germination is also insufficient. In this study, we focused on another species of the genus *Convallaria,* i.e., *Convallaria keiskei* Miq. (Liliaceae*,* Ruscaceae), which grows in the cool-temperate zone of Far East Asia. We monitored germination phenology and examined the temperature requirement for dormancy-break in seeds of this species.

## Methods

Seeds of *C. keiskei* were collected in October from a natural population in Sapporo, Japan.
The phenology of embryo growth and emergence of radicles and shoots were monitored out-of-doors. The requirements for radicle emergence were examined in the laboratory at various temperatures in light and darkness. For shoot emergence, seeds with an emerged radicle were buried at a depth of 1 cm in soil in polyethylene containers and were incubated at various temperatures.

## Results and Conclusions

Embryos in seeds of *C. keiskei* were oblong at maturity in autumn and did not grow until the initiation of radicle emergence in early summer following the first winter (Fig. 1). Shoot embryos of planted seeds grew after radicle emergence and kept growing simultaneously with root development and bud formation; however, the embryos disappeared belowground around December. Buds remained underground until the following spring. Shoots emerged from aboveground buds in late spring after the second winter. Low temperature was required for radicle emergence (Fig. 2). Seeds immediately after radicle emergence required warm → cold → warm temperature for shoot emergence. Therefore, seeds of *C. keiskei* have double dormancy as reported for seeds of *C. majalis*. Radicle emergence was strongly inhibited by light. Constant temperature was preferable to alternating temperature for radicle emergence (Fig. 2). Hence, radicle emergence would occur under the ground in the dark with less fluctuation of temperatures, and would never occur on the soil surface. We believe this is the first report describing the phenology of embryo growth in seeds having double dormancy. We believe also this is the first study to find that embryonic shoots in seeds with MPD do not grow until radicle emergence, as embryos needed to grow before radicle emergence in all previous studies on MPD.

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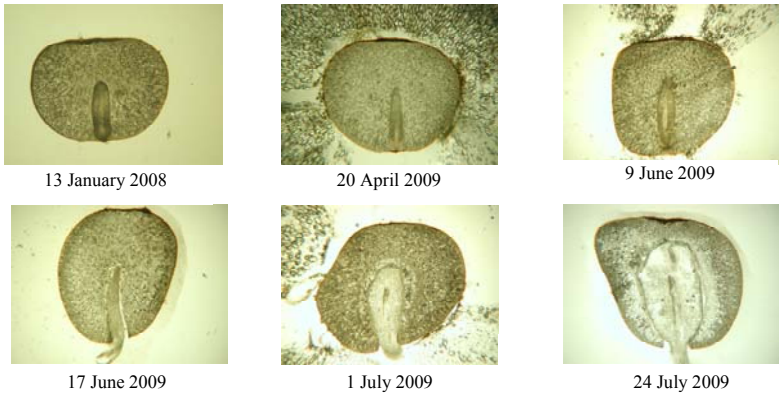


Figure 1. Phenology of embryo growth. Embryonic shoots grew after radicle emergence and kept growing together with root development and bud formation; however, embryonic shoots disappeared belowground around December

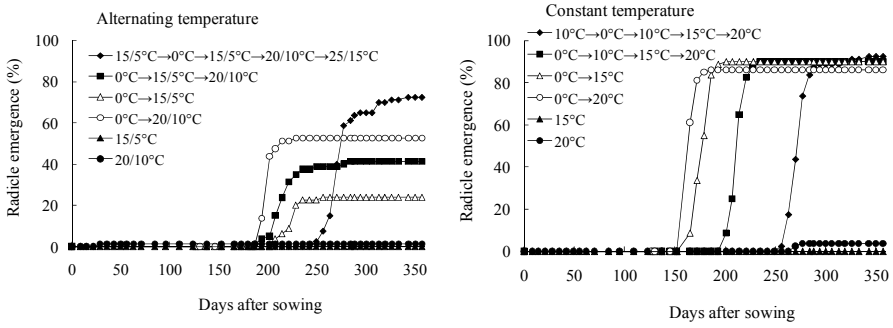


Figure 2. Effects of alternating temperatures and low temperatures on radicle emergence

## Plant Community Regeneration in Heterogeneous Habitats

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### Introduction

Spatial structure in plant communities arises as an outcome of intra- and interspecific competition, environmental heterogeneity and localized dispersal. Interplay between abiotic and biotic factors leads to habitats which are heterogeneous both in space and time. Although regeneration from seeds is frequently reported to be seed-limited (Myers & Harms 2009), local habitat conditions may play an important role as well. While several studies have addressed the impact of varying soil fertility and presence of arbuscular mycorrhizal fungi (AMF) on plant growth responses and plant-plant interactions, little is known about how these factors influence plant community regeneration in nature. We addressed the role of multiple interacting factors such as disturbance, soil fertility, seed limitation and presence of arbuscular mycorrhizal fungi (AMF) in determining plant community regeneration patterns.

### Methods

A field experiment in the herbaceous layer of a of boreonemoral spruce forest in Estonia was conducted. The experiment was set up as a 4 x 12 factorial design with disturbance, seed addition, soil fertility and presence of AMF as main factors. Every treatment combination had 10 replicates, resulting in a total of 480 patches, set out in a fully randomized arrangement. To test the influence of disturbance, soil from disturbed patches was removed; collected soil was pooled together, roots were removed in the laboratory and empty patches were filled with cleaned soil at the beginning of the experiment in May 2008. Vegetation remained undisturbed in intact patches. Seeds of *Viola mirabilis* L., *Prunella vulgaris* L. *Hypericum maculatum* Crantz. or no seeds were added to microsites to test seed limitation. Microsites were treated with fungicide throughout the season or left untreated to test for the influence of AMF; and soil fertility in microsites was raised with fertilizer, lowered with sugar addition or left untreated to test for the influence of soil fertility. Regeneration success from seed was estimated as the cumulative number of seedlings and as seedling biomass in a microsite 16 months after the beginning of the experiment.

### Results and Conclusions

Disturbance and seed addition were the main factors increasing cumulative number of seedlings in a microsite. When disturbed and intact microsites were analyzed separately, we found that seed addition increased the cumulative number of seedlings significantly in both. However, cumulative number of seedlings in disturbed microsites was additionally influenced by soil fertility level, being higher in untreated microsites than in those with fertilizer or sugar addition (Fig. 1 A). Cumulative number of seedlings in intact microsites was additionally influenced by fungicide addition (Fig. 1 B) and by interaction between these two factors, in that fungicide addition increased the cumulative number of seedlings in microsites even without seed addition (Fig. 1 C). Biomass of seedlings in a microsite was influenced by the interaction of disturbance and fungicide addition, indicating lower seedling biomass in disturbed microsites with fungicide addition compared to disturbed microsites with the natural fungal community.

Results of this study are in agreement with previous studies showing that seeds and microsite limitation are the main factors influencing plant community regeneration success by seeds (e.g., Zobel *et al*. 2000). However, these results clearly indicate the importance of considering multiple factors which simultaneously influence regeneration success, as the influence of one factor (e.g. ,soil fertility, presence of AMF) might depend on some other factor (e.g., disturbance).

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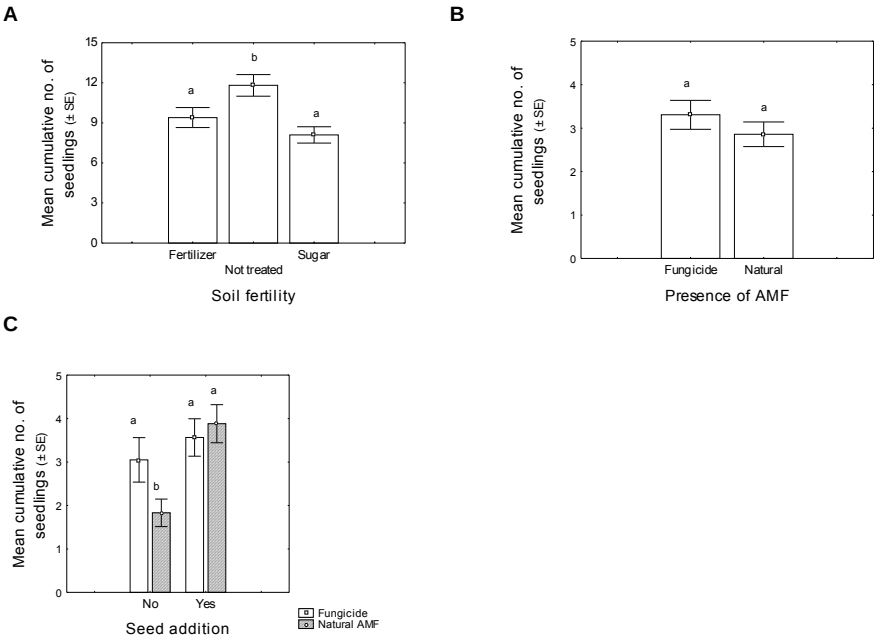


**Figure 1.** The influence of soil fertility (fertilizer, not treated, sugar addition) on mean cumulative number of seedlings (±SE) in disturbed microsites (A); The influence of presence of arbuscular mycorrhizal fungi (AMF) (fungicide treatment, natural AMF) (B), and interaction between seed addition (seeds added, not added) and presence of AMF (C) on mean cumulative number of seedlings (±SE) in intact microsites.

# Granivory in Terrestrial Isopods

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## Introduction

Granivory (seed feeding) has evolved in many animal groups. Seed predators contribute to seed losses and therefore they may be effective in the biological control of weeds or volunteer crops (Brust and House 1988, Marino et al. 1997, Westerman et al. 2003). Terrestrial isopods are generally considered detritovores (Sutton 1972, Zimmer 2002). Recent field observations indicated the existence of facultative granivory in terrestrial isopods, which was previously unknown (Saska 2008). Here we report on: (a) seed acceptance in the presence of alternative food, (b) size constraint for acceptance and consumption, (c) effect of temperature on seed consumption and (d) seasonal dynamic of seed consumption by terrestrial isopods.

## Material and Methods

We investigated weed seed consumption using terrestrial isopods common in agro-ecosystems in the Czech Republic in the laboratory from 2005-2009. In a choice experiment, *Armadillidium vulgare* Latreille consumed seeds of *Capsella bursa-pastoris* (L.) Med. and *Poa annua* L. when alternative food (plant litter) was present. In a no-choice experiment, seeds of seven plant species were offered to four isopod species (*A. vulgare*, *Oniscus asellus* Linnaeus, *Porcellio scaber* Latreille and *Porcellionides pruinosus* Brandt). The effect of increasing temperature on consumption by *A. vulgare* and *P. scaber* was established for *Stellaria media* (L.) Vill seeds. Seasonal dynamic of seed consumption by *A. vulgare* was recorded monthly in the laboratory (April-September 2007-2009) using seeds of *S. media*.

All experiments were conducted in glass Petri dishes (choice experiment – diameter 9 cm, height 1.5 cm; no-choice experiments - diameter 6 cm, height 1 cm). Seeds were exposed on seed cards (choice experiment) or on a moist filter paper and each treatment was replicated 10 (choice experiment) or 20 times. The dishes with seeds and isopods were kept in a climatic chamber (mean temperature: 20 °C, photoperiod: LD 17:7 h; except for temperature experiment in which the temperature regime varied from 5-30°C, respectively) for 3 days, after which the remaining seeds were counted. Seeds were considered eaten if less than half of the seed volume remained. If more then 50% of the seeds were missing, the missing seeds were replenished. In this way, seeds were always present in excess. After 3 days, the total seed consumption was summed over the experimental period.

## Results and Conclusions

*Amadillidium vulgare* consumed seeds even in presence of plant litter, suggesting that seeds are the natural food. In a no-choice experiment, the tested isopods differed in both acceptance (proportion of individuals that consumed seeds; Fig. 1) and consumption (both number and amount eaten) of seeds. The consumption increased linearly with isopod body mass in 6 out of 7 species tested. Size constraint was demonstrated for *A. vulgare* offered *Cirsium arvense* (L.) Scop. Seeds, as the probability that this large seed was eaten increased with isopod body size. The differences in the amount of seed consumption (converted to seed mass) relative to the body mass of isopods indicated that some seeds were eaten more intensively than others, suggesting the existence of preferences for different seed species.

We found that consumption (relative to isopod dry body mass) of *S. media* seeds by *A. vulgare* and *P. scaber* increased with increasing temperature (*A. vulgare*: $t_{118} = 9.25$, $p << 0.01$, $r = 0.65$; *P. scaber*: $t_{115} = 7.61$, $p << 0.01$, $r = 0.58$); consumption was highest at 25-30ºC. The lower temperature thresholds for consumption were established at 5 ºC for both species of terrestrial isopods. The overall consumption of *S. media* seeds relative to dry body mass did not differ between study years 2007-2009 (ANOVA, $F_{2,341} = 2.883$, $P = 0.057$); however, when monthly consumptions were separately compared between the years, we found significant differences in April, May and August (Fig. 2). Available data on granivory in terrestrial isopods originate from central Europe only. As terrestrial

isopods are more abundant and diverse in semi-arid regions, we encourage research in these zones where terrestrial isopods may be important granivores.

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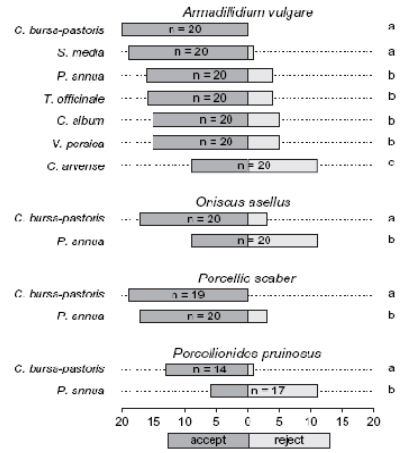
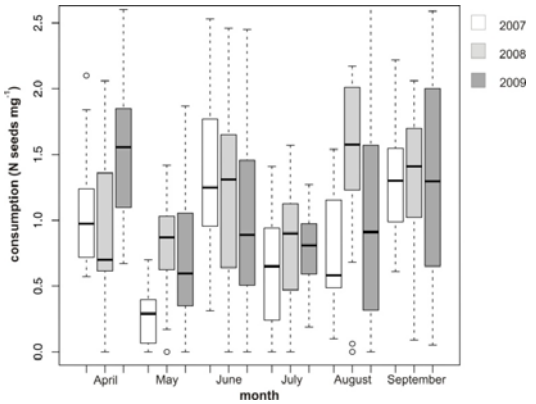



**Figure 1.** Acceptance of seeds by particular species of isopods. The same letters indicate seeds that did not differ in acceptance for particular isopod species (GLM-b, $P < 0.05$).




| Month | DF | deviance | *p-value* |
|---|---|---|---|
| April | 2,57 | 7.685 | *<<0.01* |
| May | 2,56 | 11.46 | *<<0.001* |
| June | 2,54 | 1.884 | *0.162* |
| July | 2,53 | 0.718 | *0.492* |
| August | 2,53 | 5.201 | *<<0.01* |
| *September* | *2,53* | *0.0521* | *0.949* |

**Figure 2.** Among-year (2007-2009) differences in consumption (relative to dry body mass) of *S. media* seeds by *A. vulgare*.

## Spatial Variation in the Effects of Pathogens on Seed Germination: How Habitat Affects Susceptibility to Soil Fungi

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For most plants, the majority of mortality occurs at the seed or seedling stage; soil fungi are believed to play a major role in this mortality. Populations of such fungi are expected to vary among habitats, in response to factors including soil moisture and the availability of hosts, potentially affecting recruitment by susceptible species.

My lab has investigated spatial variation in seed mortality for a wide range of woody and herbaceous temperate species. We have compared germination of buried seeds protected with fungicide (captan) *vs*. germination of seeds in water controls; this allows us to isolate the effects of fungal pathogens from other sources of mortality. Results indicate risks of attack often vary predictably among habitats.

For instance, the risk of attack may be increased by the proximity of conspecifics, as predicted by the Janzen-Connell Hypothesis. Losses of seeds of the forest tree *Tsuga canadensis* (L.) Carrière (eastern hemlock) to fungi were higher in conspecific-dominated sites than in sites dominated by the co-occurring tree *Acer saccharum* Marsh. (sugar maple; Kotanen 2007). *Tsuga canadensis* often recruits on fallen logs rather than the forest floor; comparing the effects of fungicide on seeds planted in logs *vs*. the forest floor suggested logs provide refuges from soil pathogens (O'Hanlon-Manners and Kotanen 2004b). In contrast, while germination of two herbaceous *Solidago* (goldenrod) species was lower in habitats dominated by conspecifics than in grass-dominated sites, germination was improved equally by fungicide in both (Figure 1). Finally, seeds of the weed *Ambrosia artemisiifolia* L. (common ragweed) buried in conspecific populations were less likely to germinate than seeds in ragweed-free sites (MacKay and Kotanen 2008), though this difference was not reduced by fungicide; consequently, the mechanism is unclear.

Attack also can be affected by the physical environment. For instance, canopy gaps are likely to be drier than the surrounding forest understory, and therefore less hospitable to fungal pathogens. Losses of seeds of the early successional tree *Betula papyrifera* Marsh. (paper birch) to soil fungi were greater in forest understory than in old fields or treefall gaps (O'Hanlon-Manners and Kotanen 2004a). This suggests *B. papyrifera* is prevented from establishing in moister understory environments in part by its susceptibility to pathogen attack. Similarly, many herbaceous species experienced higher seed mortality in wetter habitats, but fungicide reduced this difference, indicating that fungi represent a greater hazard in wetter soils (Blaney and Kotanen 2001).

We also have found many cases of both woody and herbaceous plants whose seed mortality does not follow such predictable patterns. Nonetheless, these examples suggest that for many ecologically important species, spatial patterns of recruitment are determined in part by seed pathogens. Population dynamics and even patterns of habitat occupancy may reflect spatial variation in the risk of fungal attack.

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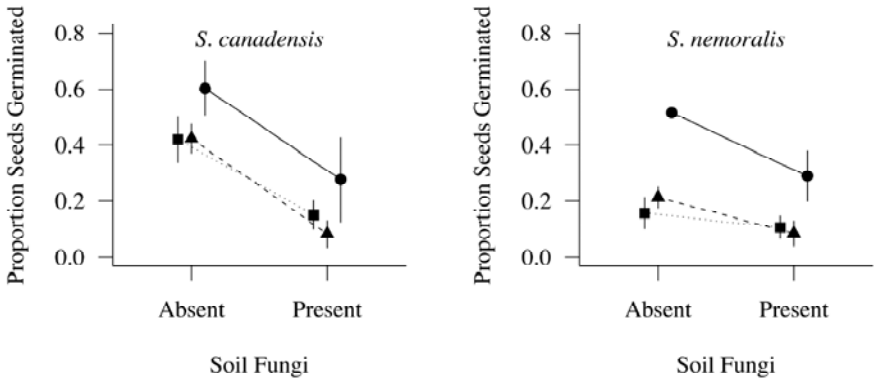


**Figure 1.** Germination success of *Solidago canadensis* L. and *Solidago nemoralis* Ait. when fungi were present *vs.* controlled by fungicide, in soils from habitats dominated by grass (circles), *Solidago canadensis* (triangles), and mixed *Solidago* spp. (squares). Symbols represent the mean ± SE proportion of seeds that germinated for each habitat and treatment combination. Germination differed significantly between habitats ($p < 0.1$) and fungicide treatments ($p < 0.01$), but there was no habitat x fungicide interaction ($p > 0.15$) (split-plot ANOVAs). Figure courtesy of Steven B. Hill.

## Light Requirement and Skotodormancy in Campanulaceae – an Ecophysiological Approach

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### Introduction

Campanulaceae is a cosmopolitan family of mostly herbs, comprising 84 genera and approximately 2300 species (Lammers 2007) and extending over six continents and a variety of habitats (except for major deserts). It is often divided into five subfamilies (Campanuloideae and Lobelioideae by far the larger and most widespread ones) with centers of speciation and distribution in the Mediterranean rim, Asia, S. Africa and N. America. Moreover, Campanulaceae are represented in the Greek flora by 119 taxa (8 genera) and shows the highest familial endemism (54%; Georghiou & Delipetrou 2010). Seed germination has previously been studied relatively poorly in regard to both geographical distribution and adequate numbers of representative Campanulaceae taxa. Thus a general overview of germination requirements for the entire family is currently attempted in the context of the first author's doctoral thesis. A comparative investigation of seed germination adaptations and relevant mechanisms is being carried out in association with the diverse ecological requirements and the evolutionary relationships within the family. Particular emphasis is placed on the effects of light and temperature on seed germination; data are presented for a considerable number of representative species.

### Methods

In total 134 seedlots from 113 taxa (111 species, 26 genera) have been studied; 43 seedlots are collections of our own and the remaining 91 were provided by several seed banks (Europe 82, Asia. 13, Africa 4, Americas 20 and Oceania 15). Species included in this study have been selected on the basis of taxonomic, evolutionary, species biology, ecological and conservation criteria. Germination experiments are conducted in a range of temperature and light conditions; light mediation is examined under diurnal white light / dark (L/D) conditions and in complete darkness (cD, without any intermediate measurements). In order to test skotodormancy induction, seeds that do not germinate in cD are subsequently transferred to L/D conditions.

### Results and Conclusions

Absolute light requirement for germination, observed in virtually all taxa examined (Fig. 1), is the major conclusion of this study. This property constitutes a collective characteristic of the family and, on the one hand, is in agreement with the documented association (Baskin & Baskin 1998) of small seed size with a positive germination photosensitivity (seed mass ranging from 5 to 1060 μg, less than 100 μg in 83% of the seedlots studied) and, on the other hand, conforms to the fact that most members of the family grow in open habitats. However exceptions to this 'rule' do exist and seem to be associated with large seed mass and certain habitat types. In some taxa, the absolute light requirement is partly substituted by alternating temperatures. Moreover, the induction of skotodormancy (in many species) is revealed for the first time in Campanulaceae. This secondary dormancy is imposed by the incubation of seeds in darkness while the extent of dormancy seems linked to temperature. The application of gibberellic acid both substitutes for light requirement and induces skotodormancy release (Fig. 2). At present, the ecological significance of skotodormancy remains unknown and various 'natural' means for its removal are currently under investigation.

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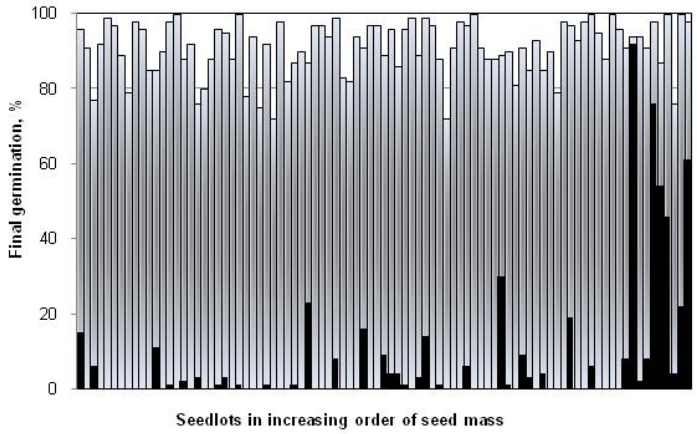


**Figure 1.** Final germination in L/D (grey bars) and cD (black bars) under the most favorable temperature regime, respectively, for 89 seedlots (that achieve a germination percentage >70% without any pre-treatment), representing 72 species (35 *Campanula*, 8 *Lobelia*) and 20 genera.

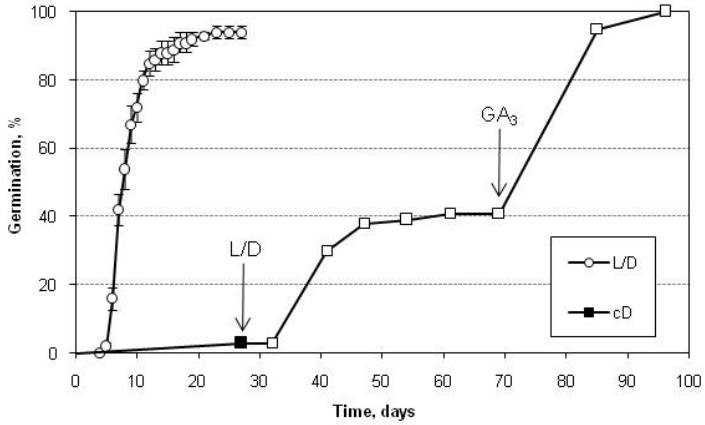


**Figure 2.** Time course of *Campanula sparsa* Friv. seed germination in 20/10 °C (12h/12h) under L/D and cD. At the time of germination completion in L/D, the ungerminated seeds from cD are transferred to L/D (arrow); 40 days after, gibberellic acid (1000 ppm) is also added (arrow).

# Improving Germination and Seedling Growth of *Festuca rubra by* Matriconditioning in the Presence of *Agrobacterium rhizogenes* Strains

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## Introduction

It is known that slow, asynchronous and unreliable germination and emergence due to low vigor of seeds leads to problems for successful crop production. A popular approach termed matriconditioning (MC) for improving seed performance has been to subject the seeds to controlled hydration, which can be achieved when seeds are held, for a defined period, in contact with a moist solid matrix with highly water-retentive capillary forces (Khan et al.1990). The purpose of this work was to integrate matriconditioning using solid carrier Micro-Cel E with strains of the bacterium *Agrobacterium rhizogenes,* to further improve seed performance of the common grass, *Festuca rubra* L.cv. Areta, frequently used as a turf grass and groundcover in lawn blends. *A. rhizogenes,* a soil gram-negative bacterium, induces adventious root formation at the site of infection in a large number of plants (Chilton et al. 1982). We were particularly interested to find out if *A. rhizogenes* strains LBA 1334 and 15834 have the ability to improve the root system, because deep and far-reaching grass root growth is responsible for nutrient uptake efficiency.

## Material and Methods

*Festuca rubra* L.cv. Areta seeds harvested in 2008 were obtained from TORSEED Seed Company (Poland) and stored dry at -23$^0$C until the use. The *A. rhizogenes* strains LBA 1334 and 15834 were maintained by regular subculture on solid LB medium. For matriconditioning experiments the bacteria were grown in liquid LBB medium for 24 h on a rotary shaker at 25$^0$C ±2 in the dark until an optical density of 0.6 at 600 nm was obtained. The seeds mixed with Micro-Cel E and water or bacterial suspension in ratio (by weight in grams) 1:0.4:0.8, respectively were placed in glass jars with caps loosely placed and transferred to 15$^0$C in light. After 5 days of MC the seeds were separated from Micro-Cel E and dried to their original weight. Dynamics of seed germination (50 seeds) on filter paper moistened with distilled water in Petri dishes ( 5cm) in darkness at 25$^0$C during 22 days was determined. Conditioned and non-conditioned seeds (25 seeds) wereplaced in Petri dishes (14 cm) and after 7-day incubation at 25$^0$C in a growth chamber at 16/8 (day/night) photoperiod the length of roots and blade were measured in mm. The fresh and dry weights were also determined.

## Results and Conclusions

Matriconditioning for 5 days at 15 $^0$C of *F. rubra* seeds improved speed of germination and increased the growth of seedlings (length of root and blade) at 25$^0$ C. *A. rhizogenes* strain LBA15834, present during matriconditioning increased the beneficial effect of MC on seed germination. After 6 days, when about 25% control and about 50 % matriconditioned seeds germinated, > 80% germination of matriconditioned seeds in presence of LBA15834 strain was observed. Both bacterial strains were very effective in stimulating seedling growth. The roots of seedlings from seeds matriconditioned in the presence of *A. rhizogenes* LBA 1334 and 15834 were 2- and 3-fold longer, respectively, as compared to the control seedlings, and the roots were more branched. Fresh and dry weights of seedlings obtained from seeds matriconditioned in presence of these strains was 2.5 and 3 time higher in comparison with control seedlings. These results clearly indicate that the matriconditioning of seeds in the presence of *A. rhizogenes* can be a successful approach to improving germination and growth of seedlings, especially the root system.

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## Seed Bank Patterns on Active and Stabilized Sand Dunes in Eastern Inner Mongolia, China

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### Introduction

Although many investigations on the soil seed banks of sand dunes have been performed (Yan et al., 2005; Liu et al., 2006; Liu et al., 2007), how sand burial and wind erosion affect seed bank patterns, and how the seed bank is linked to seedling emergence on the dune have not been clear. By means of deep sampling and long-term monitoring, this study compared seed bank pattern and its linkage to seedling emergence between the active and stabilized sand dune.

### Methods

This investigation was conducted in the Horqin Steppe (119°39′-120°02′E, 42°29′-43°06′N, 480m a.s.l.) of northeastern Inner Mongolia. Soil seed bank samples were taken on eight transects, 10m apart, from active (sampling in 0-100cm) and stabilized (sampling in 0-30cm) sand dunes during the period March 2007 to October 2008. Soil cores were collected at 15m intervals on each transect four times each year over the 2-year period to provide a qualitative and quantitative estimate of the viable seeds present. When the growing season began, emerging seedlings in each quadrat (1m × 1m) were identified, counted and removed at ten-day intervals to clarify the relationship between the seed bank and seedling emergence.

### Results and Conclusions

Seeds in the deep soil of the active dune and seeds in the hallow soil of the stabilized dune both played a major role in the soil seed bank. In the 0-100cm profile of the active sand dune there were two peaks of seed abundance; one peak was in 0-50cm depth increment, while the other was in 50-100cm depth increment. In the 0-30cm profile of the stabilized sand dune, seed number decreased from the shallow layer to the deeper layer. On the windward slope of the active sand dune, there were significantly more seeds on the upper section than in the middle section ($P<0.01$), but for the leeward slope, seed number was not significantly different among the lower, middle and upper sections. On the stabilized sand dune, seed number was not significantly different between the lower, middle and upper sections on either the windward and leeward slope. The correlations between seed bank and seedling emergence at different times, depths, and dune positions on the active sand dune were significantly higher than those on the stabilized sand dune, and the same pattern of higher correlation on the active dune was also observed between seedling emergence and rainfall.

The study indicated that: 1) the structure of seed bank is greatly regulated by sand burial and wind erosion on the active sand dune, and 2) the relationship between the seed bank and seedling emergence in the habitat with serious aeolian activity was closer than that in the habitat with slight aeolian activity.

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# Prior Imbibition of Seeds Reduces the Stimulatory Effect of the Smoke-derived Germination-Stimulant, Karrikinolide

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## Introduction

Seeds in unsaturated soils undergo wet-dry cycles in concert with rain events (Gonzalez-Zertuche *et al.* 2001). Hydration events can influence the dormancy state of seeds, although little is known about the physiology of why this occurs. The dormancy state of seeds in turn influences how sensitive seeds are to germination stimulants, ultimately affecting the efficiency of attempts to germinate seeds synchronously for the purposes of research, land restoration and weed management. In this study we investigated how the hydration state of seeds of *Brassica tournefortii*, a global agronomic weed, influenced their dormancy characteristics and response to the smoke-derived germination-stimulant karrikinolide. Specifically, we tested the hypotheses that dry seeds would be more sensitive to karrikinolide than fully imbibed seeds, and that germination responses would be linked to the internal concentrations of hormones involved in seed dormancy and germination, namely gibberellins and abscisic acid.

## Methods

Seeds were pre-equilibrated to one of three hydration states: dry (15% RH, 7% fresh-weight (f.w.) water content), fully-imbibed (100% RH, 86% f.w water content), or re-dried (fully-imbibed then dried to 15% RH, 7% f.w. water content), then tested for their germination response to two concentrations of karrikinolide ($KAR_1$) when exposed to it for periods of 3 minutes to 14 days. In addition, the endogenous concentrations and sensitivity to abscisic acid (in combination with an optimal concentration of $KAR_1$) and gibberellins were assessed for seeds of the different hydration states to give insight into the physiological factors underlying their response to karrikinolide. Further details of methods can be found in Long *et al.* (2010).

## Results and Conclusions

Dry seeds were most sensitive to karrikinolide, but only if they had never previously imbibed water. Seeds that were re-dried to 7% water content (equivalent to the 'dry seeds') were only as sensitive to karrikinolide as fully-imbibed seeds, indicating that a history of hydration has a greater influence on germination responses to karrikinolide than does seed water content. One possible explanation for the negative impact of hydration on the sensitivity of seeds to $KAR_1$ is that $KAR_1$ could not compete with an increased sensitivity to the dormancy-maintaining hormone, abscisic acid, which was also associated with prior imbibition. Although the effects of hydration state and history on gibberellins were also investigated, there was no significant impact on the endogenous levels of gibberellins or the sensitivity of seeds to exogenously-applied gibberellins. Thus, in contrast to the representations of Finch-Savage and Leubner-Metzger (2006), the relative concentrations of endogenous gibberellins and abscisic acid were not an indicator of the germinability of seeds in this study. We conclude that the hydration-history of seeds significantly influences their dormancy characteristics, and this relationship should be considered when applying germination stimulants in research and practice. In particular for $KAR_1$, it may be most effective for triggering germination of seeds in the field if applied just prior to the first rains that follow an extended dry period, such as at the start of autumn in Mediterranean-type climates.

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# Germination Strategies of Five Gypsum Annual Generalists in Contrasting Photoperiod, Temperature and Water Availability Conditions.

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## Introduction

In Mediterranean semi-arid gypsum steppes, a very diverse annual plant community occurs (Rivas-Martinez & Costa, 1970). Gypsum soils are known to have very stressful conditions, specifically toxic compounds such as magnesium and low water retention (Tadeo, 2000; Ferrandis et al., 2005). In this study we have tried to identify environmental cues that regulate germination response of five annual generalist species able to grow on gypsum soils (*Alyssum minus* Rudolphi, *Plantago afra* L., *Ziziphora hispanica* L., *Helianthemum salicifolium* (L.) Mill. Gard. and *Malva aegyptia* L.). Improving our knowledge about germination response of these gypsum annuals to different temperature, photoperiod and water availability scenarios may allow us to better understand trends of these species population dynamics under changing environmental conditions.

## Methods

We evaluated germination percentages and velocity of nearly 2000 seeds of each species in five different experimental scenarios simulating autumn, winter and spring photoperiod and temperature conditions in germination chambers. These scenarios cover a wide range of conditions of the life cycle and of habitats which these species inhabit: three different photoperiods (average night and day hours of October, January and April), two ranges of temperature (maximum and minimum temperature averages of each month), and two levels of water availability (continuous availability and an initial single irrigation). Germination was recorded every two to three days during 30 days of incubation. Total germination and velocity were analyzed with General Linear Models and germination curves were compared using the Kaplan- Meier procedure. SPSS v 15 was used.

## Results and Conclusions

Results showed that seeds were able to germinate in all scenarios but at different percentages and rates. Despite the low germination observed in April in the field under natural conditions (pers. observation), our experiment did not show great differences between April and October germination percentages, probably because the low germination observed in April in the field may be related to other complementary environmental cues not considered in this study, such as the withstanding of freezing conditions during winter (Baskin & Baskin, 1988). Results showed that the most important cue for germination was temperature (Escudero et al., 1997). In addition, water availability was very important: the higher the water availability, the larger the germination fraction (Freas & Kemp, 1983). The effect of the lack of water was more significant at high temperatures and long photoperiod scenarios than at low temperatures and short photoperiods. Species showed two contrasting germination strategies: i) fast germination (*Alyssum* and *Plantago*) and ii) slow germination (*Ziziphora*, *Helianthemum* and *Malva*). Differences in germination speed will presumably determine important differences in establishment hierarchies in the annual community (Keddy, 1990).

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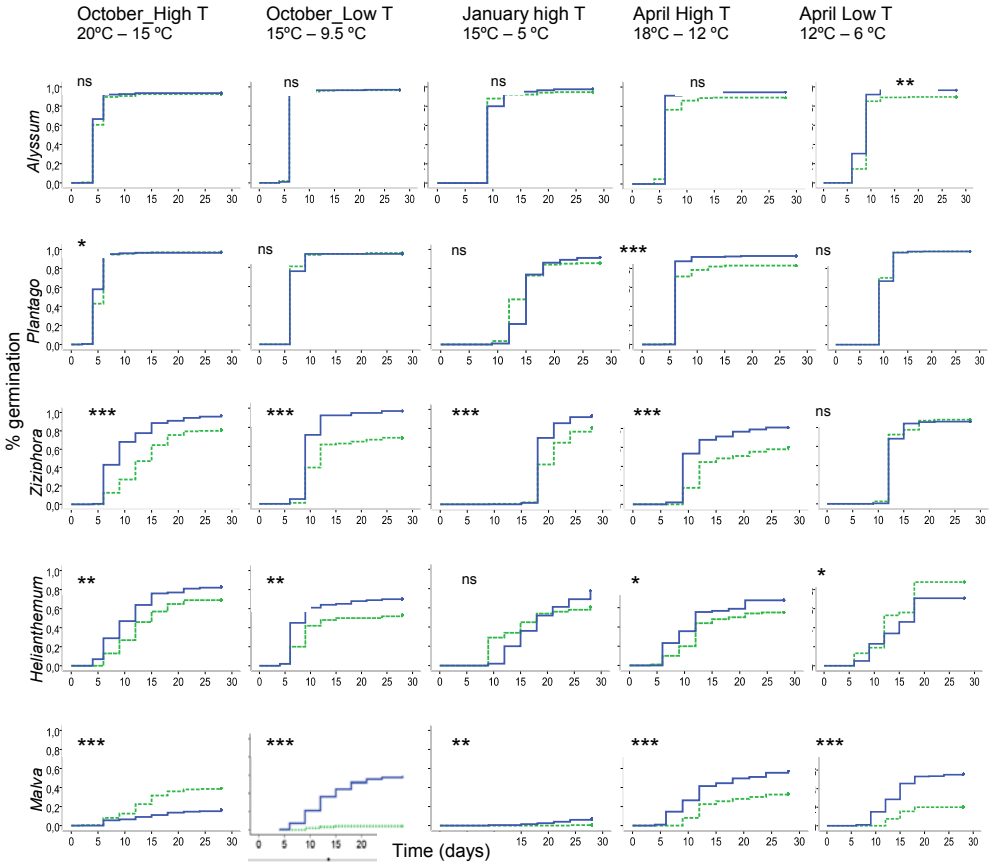


**Figure1**: Species germination proportion in each irrigation treatment. Solid line: continued irrigation and dashed line: single initial irrigation treatment. ***: p<0.001; **: p<0.01; *: p<0.05; ns: non-significant

**Abril- Extremas 18- 6ºC**

# Morphogenesis Responses of Embryos from Seedsof Five Soybean Varieties, Cultured In-Vitro, to Various Concentrations of NaCl.

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## Introduction

Soybean (*Glycine max,* L.) is an economically important crop of tropical and sub-tropical regions, since it is a source of protein. However its growth and development are generally limited by salinity (Papiernik *et al.,* 2005; Kao *et al.,* 2006;). In Indonesia, almost 24% of the mainland consists of beach swamp areas, suffering from intrusion of seawater; hence, high salinity is one of the most important problems in agricultural development on those regions. The increasing amounts of arable land undergoing salinization and increasing food demand from the growing population make it unavoidable to develop salt-tolerant crop varieties, for which it is necessary to identify the degree of salinity tolerance within varieties. Ultra-structural changes in cells and tissues due to high concentrations of salt have been reported for wheat (Kintzios *et al.,* 1997), while the root has been reported as the most sensitive part to NaCl applications for eggplant (Bintoro, 1985). The objective of this research was to examine differences in embryo morphogenesis and ultra-structure in the roots at the stage of initiation and regeneration of in-vitro cultured embryos from seeds of five local soybean varieties, in order to differentiate their tolerance to salinity.

## Methods

Mature seeds from five local soybean varieties, namely Wilis (V1), Kipas Putih (V2), Jaya Wijaya (V3), Tidar (V4) and Lokon (V5), were sterilized by soaking in detergent for 45 minutes, washed three times with sterile distilled water, soaked in Benlate for 20 minutes, then washed three times with distilled water. The embryos were then excised and used as explants, after sterilization by soaking for 5 minutes in 95% alcohol, 10% CaHOCl and a solution of 10% betadine and Tween-20. Each soaking was followed by washing three times with distilled water. Gamborg Medium (Gamborg, 1984 *in* Suryowinoto, 1996) was the medium used, with bacto-agar added at the concentration of 10g/l, and NaCl at 5 concentrations, namely 0 g/l (control) (G0), 2 g/l (G1), 4 g/l (G2), 6 g/l (G3) and 8 g/l (G4). The embryos were cultured in glass bottles. The research used a completely randomized design with three replicates. Observations were conducted on the number of shooted and rooted explants, number of shoots, dry weight of explants, root number, lengths and structures, radii of the cortex parenchyma and stele using micrograph (Kintzios *et al*., 1997), and chromosome number (Uitjewaal, 1987). All observations were carried out from the first week through three months after culture.

## Results and Conclusions

Increase in NaCl concentrations resulted in decrease of all parameters observed, although there were different responses for each variety. Embryo morphogenesis was mostly not affected for the Wilis variety (V1) even with increasing concentrations of NaCl up to 8 g/l; however there were significant decreases in dry weight of explants for all five varieties. The Wilis variety (V1) proved to be the most tolerant to salinity, followed by Jaya Wijaya (V3), Tidar (V4) and Kipas Putih (V2), while the least tolerant viariety was Lokon (V5). For root structures, 4 g/l NaCl in the medium produced bigger radii compared to the control (0 g/l NaCl), for the Wilis, Jaya Wijaya and Tidar varieties, but not for Kipas Putih and Lokon. The results suggest that for the more tolerant varieties, such as Wilis, Jaya Wijaya and Tidar, low concentrations of NaCl up to 4 g/l in the medium is not toxic to seedling growth, and might act to supplement potassium. Increased radicle elongation at low salinity of less than 0.2M NaCl has been reported for the salinity-tolerant species *Halocnemum strobilaceum* (Qu *et al.,*2008). Data for morphogenesis of embryos and root ultra-structures are shown in table 1. No difference in chromosome number was observed between Wilis as the most tolerant variety and Lokon as the least tolerant one. The number of chromosomes observed was 40 for both varieties, the commonly reported karyotype for soybean (either *Glycine max* or *Glycine soya*), which contains 2n = 40 chromosomes (Somaatmadja, *et al.,* 1985).

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Table 1. Embryo morphogenesis and root ultra-structures of five soybean varieties in various concentrations of NaCl

| Variety | NaCl Conc mg/l | No. of Shooted & rooted Embryos | No. of shoots | No. of Roots | Root Lengths (cm) | Seedling Dry wght. (g) | Radii of Cortex Parenchyma (µ) | Radii of stele (µ) |
|---|---|---|---|---|---|---|---|---|
| Wilis | 0 | 5.00a | 1.47ab | 8.22a | 13.57bc | 44.04a | 17.00 | 4.90 |
| | 2 | 5.00a | 1.47ab | 6.77ab | 13.23bcd | 34.83b | 21.96 | 12.00 |
| | 4 | 5.00a | 1.53a | 6.55bc | 14.04abc | 33.51bc | | |
| | 6 | 5.00a | 1.13cd | 4.66d | 8.37efg | 32.48bc | 29.92 | 19.07 |
| | 8 | 5.00a | 1.27bc | 3.66cd | 10.42c-f | 23.33ef | | |
| K.Putih | 0 | 4.33abc | 1.07cd | 9.11a | 15.10ab | 31.65bc | 23.02 | 10.33 |
| | 2 | 3.67cd | 0.80ef | 8.22a | 11.83b-e | 27.65c-e | | |
| | 4 | 3.33d | 0.67f | 6.33c | 11.74b-e | 24.59def | 18.01 | 9.61 |
| | 6 | 0.00f | 0.00g | 4.66d | 7.88fg | 20.15fgh | | |
| | 8 | 0.00f | 0.00g | 1.66g | 1.24hi | 21.00e-g | | |
| J.Wijaya | 0 | 5.00a | 1.27bc | 9.00a | 15.38ab | 30.96bcd | 10.13 | 5.06 |
| | 2 | 5.00a | 1.20cd | 8.33a | 14.63ab | 31.o6bcd | | |
| | 4 | 5.00a | 1.47ab | 6.33c | 14.50ab | 20.55fg | 21.73 | 10.27 |
| | 6 | 0.33f | 0.00g | 4.44d | 7.46fg | 13.86hi | | |
| | 8 | 0.00f | 0.00g | 1.88fg | 1.83h | 12.18i | | |
| Tidar | 0 | 5.00a | 1.07cd | 9.22a | 16.32ab | 34.19bc | 36.45 | 19.27 |
| | 2 | 4.00bcd | 1.00de | 8.55a | 19.38a | 35.55b | | |
| | 4 | 4.67ab | 1.13cd | 6.66bc | 13.80bc | 24.61def | 50.29 | 20.00 |
| | 6 | 0.00f | 0.00g | 2.77de | 6.07g | 14.35g-i | | |
| | 8 | 0.00f | 0.00g | 2.55ef | 2.56h | 11.29i | | |
| Lokon | 0 | 3.67d | 0.67f | 4.44d | 12.73bcd | 22.94ef | 23.61 | 11.57 |
| | 2 | 0.00f | 0.00g | 3.89d | 8.75efg | 23.24ef | | |
| | 4 | 0.00f | 0.00g | 4.11d | 9.24d-g | 19.6fgh | 20.60 | 9.56 |
| | 6 | 0.00f | 0.00g | 0.00i | 0.00i | 8.84i | | |
| | 8 | 0.00f | 0.00g | 0.00i | 0.00i | 7.95i | | |
| | | | | | | | | |

Note: Similar letters behind numbers in the same column indicate not significantly different at the 5% level of difference according to DMRT

# Predictors Controlling Seed Performance of a Strict Gypsophyte (*Centaurea hyssopifolia* Vahl.: Asteraceae) at Different Space and Time Scales and at Distinct Hierarchical Levels.

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## Introduction

Seed set and seed mass are known to be controlled by a complex set of variables acting at hierarchical levels and at contrasting spatial scales. This complexity makes prediction a difficult task. Seed set at the capitulum level and seed mass depend both on the quality and the amount of pollen load which in turn are affected by plant size, number of capitula produced by each individual, flower density in the neighbourhood, etc. as well as by the structural constraints limiting or favouring resource allocation to seeds depending on their spatial location in the plant. Increasing our knowledge about the role of these predictors on the reproductive output of plants at contrasting spatial and temporal scales in a hierarchical context could be of great relevance not only from an evolutionary point of view but also in order to improve management practices of species that are highly sensitive to the ongoing global change (Matesanz 2009).

## Methods

In our study, we conducted a modelling approach to identify the predictors that control seed performance at different space and time scales and at different hierarchical levels in *Centaurea hyssopifolia* Vahl., an endemic shrub of semi-arid gypsum steppes which is a strict gypsophyte. Seed set and seed mass variation were evaluated at different spatial and temporal scales. We considered four spatial scales: i) seed position within each capitulum (external or internal), ii) capitulum position within each individual (distance to the central axis of the plant) iii) neighbourhood of the target plant in the population (*Centaurea* and other perennial species cover at two distances from the target plant); iv) at four populations; and v) three contrasting phenology stages: early, peak and late fruiting. Four populations of *Centaurea hyssopifolia* were considered in our study. Twenty-five plants of each population and 10 capitula of each plant (when possible) were collected in early June, late June and mid July. More than 1,900 capitula were analyzed and nearly 11,000 seeds were weighed. The neighbourhood of each plant was characterized in two concentric circles of different radius (1.15 m and 2.8 m respectively), and the relative location of each capitulum from the central axis of its corresponding plant was measured. Seed set and seed mass were modelled by means of repeated measures GLM models using SPSS v.15.

## Results and Conclusions

Our results highlight the importance of the idiosyncratic effect of each population (Tables 1, 2 and 3), as has been detected in other studies (Luzuriaga et al 2006). Early-ripening capitula showed higher seed set as well as larger seeds than late ripening ones, probably because as summer approaches water availability is drastically reduced, which creates very stressful conditions for this species. Neither plant size nor total capitula exerted any significant effect on seed set but total capitula exerted a positive effect on seed mass (Table 3), probably because plant size is a surrogate for resource storage that will determine seed production (Colas et al 2001). Seed mass was affected by seed position in the capitulum (Wald´s Chi= 4.9; p=0.026); nevertheless capitulum position in the plant did not affect seed mass. We therefore detected small scale structural constraints for seed resource allocation at the capitulum level, but not at plant level due to branching architecture. Neighborhood characteristics exerted a contrasting effect on seed set. Total perennial cover in the nearest area of the target plant reduced seed set; conversely, *Centaurea* plant cover in the external ring area (1.15 m to 2.8 m apart from the target plant) caused an increase in seed set (Table 1). This effect was not detected for seed mass.

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| | Wald´s Chi | df | p |
|---|---|---|---|
| **Intercept** | 734.3 | 1 | 0.000 |
| | | | |
| **Fruiting season** | 184.1 | 2 | **0.000** |
| **Population** | 15.24 | 3 | **0.002** |
| **Fr. season * population** | 33.9 | 6 | **0.000** |
| **Capitulum position** | 0.63 | 1 | 0.43 |
| **Total number of capitula** | 0.30 | 1 | 0.58 |
| ***Centaurea* cover CENTER** | 1.4 | 1 | 0.24 |
| ***Centaurea* cover RING** | 5.1 | 1 | **0.024** |
| **Perennial cover_CENTER** | 8.68 | 1 | **0.003** |
| **Perennial cover_RING** | 1.847 | 1 | 0.17 |

**Table1.** Repeated measures GLM for the response variable SEED SET with Poisson distribution and link function= log. **Fruiting season**: early June, late June and mid July; **Capitulum position**: distance from the central axis of each plant; **total number of capitula** in each plant; ***Centaurea* cover_CENTER**: % of *Centaurea* plant cover in the circle with radius 1.15m around each target plant. ***Centaurea* cover RING:** % of *Centaurea* plant cover in the ring between 1.15 m and 2.8 m radius around each target plant. **Perennial cover_CENTER:** % of the rest of the perennial plant cover in the circle with radius 1.15m around each plant. **Perennial cover_RING:** % of the rest of the perennial plant cover in the ring between 1.15 m and 2.8 m radius around each target plant

| | Wald´s chi | df | p |
|---|---|---|---|
| **Intercept** | 18873.3 | 1 | 0.000 |
| | | | |
| **Plant(Population)** | 10235125.6 | 19 | **0.000** |
| **Capitulum (Plant(Population))** | 101713.5 | 19 | **0.000** |
| **Fruiting season** | 99.9 | 2 | **0.000** |
| **Population** | 144.15 | 3 | **0.000** |
| **Capitulum position** | 0.2 | 1 | 0.65 |
| **Fr. season * population** | 15.1 | 6 | **0.02** |

**Table2.** Repeated measures GLM for the response variable SEED MASS with Poisson distribution and link function= log. See abbreviations in Table 1.

| | Wald´s chi | df | p |
|---|---|---|---|
| **Intercept** | 2321 | 1 | 0.000 |
| | | | |
| **Fruiting season** | 89.2 | 2 | **0.000** |
| **Population** | 25.84 | 3 | **0.000** |
| **Fr. season * population** | 20.2 | 6 | **0.001** |
| **Capitulum position in the plant** | 2 | 1 | 0.08 |
| **Total number of capitula** | 4.5 | 1 | **0.03** |
| **Centaurea cover_CENTER** | 0.44 | 1 | 0.5 |
| **Centaurea cover RING** | 0.71 | 1 | 0.4 |
| **Perennial cover_ CENTER** | 2.9 | 1 | 0.09 |
| **Perennial cover_RING** | 0.007 | 1 | 0.93 |

**Table 3.** Repeated measures GLM for the response variable SEED MASS with Poisson distribution and link function= log. See abbreviations in Table 1.

## Mechanisms of the Glumes Affecting Seed Germination of *Leymus chinensis*

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### Introduction

*Leymus chinensis* (Poaceae) is a perennial, rhizomatous species of eastern region of the Eurasian steppes with great ecological and economic values. However, the seed dormancy mechanism is not well understood, especially how the glumes inhibit germination (Ma *et al*. 2008a). Tissues enclosing the embryo exert a constraint on its growth (coat-imposed dormancy), and the possible mechanisms of coats might be correlated with the water uptake, mechanical restraint, interference with gas exchange, and/or prevention of leaching inhibitors from the caryopsis and/or glumes (Bewley and Black 1994, Morris 2000). An interaction between the embryo and its covering structures may be the best explanation for the cause of nondeep physiological dormancy (Baskin and Baskin,1998). We tested the mechanism of inhibition of glumes on germination of *L. chinensis* seeds, considering their water absorption ability, endogenous hormone content, and microstructure.

### Methods

Water absorption ability of intact seeds (control) and naked seeds (with lemmas and paleas removed) were measured by the conventional weighing methods. The endogenous hormones of $GA_3$, Z, IAA, and ABA were measured using HPLC (Yi *et al*. 1997). The effects of $H_2SO_4$ on germination and on glume microstructure were measured as follows. *L. chinensis* seeds were soaked in 40%, 60%, 70%, 80% and 98% (chemically pure) $H_2SO_4$ for 10 min. Then, they were washed several times under tap water and incubated at 16/28°C to test for germination (Ma *et al*. 2008b). The microstructure of the external and internal surfaces of untreated glumes and the external surface of glumes treated with 40% $H_2SO_4$ were observed with SEM.

### Results and Conclusions

Water absorption for naked seeds was significantly lower than that of the intact seeds at each absorption time (Fig. 1). In the early stages of inbibition, both intact and naked seeds absorbed water rapidly and reached water contents of 68.1% and 44.4%, respectively, after 4 h, and then increased slowly. After 72 h, water content of naked seeds was 106.7% and that of intact seeds was 75.9%.

Concentrations of $GA_3$, Z, IAA and ABA were higher in intact than in naked seeds by 1.96, 2.58, 3.57 and 3.40 times, respectively (Fig. 2), indicating that these hormones primarily are present in the glumes.

Germination percentages of *L. chinensis* seeds treated with all concentrations of $H_2SO_4$ (40~98%) were significantly higher than that of the control ($p<0.05$; Fig. 3). There was no significant difference in germination percentages (77.8-83.3%; $p>0.05$) of seeds treated with 60-98% $H_2SO_4$. Germination of naked seeds (removal of glumes by hand) showed a similar germination percentage as those treated with 40% $H_2SO_4$, and these percentages were significantly higher than that of the control. SEM micrographs (Fig.4) showed that the external surface of glumes was compactly structured, and had a cuticle layer, and had many enclosed glume “lids” on the surface. The cuticle layer was eroded and most of the “lids” opened after treatment with 40% $H_2SO_4$, which also caused a significant increase in germination. The internal surface of the glumes contained many structures similar to needles, which might increase the mechanical resistance of the seed covering layers to embryo growth.

To summarize, our results show that the glumes were 1) water permeable, 2) contain germination promoters and inhibitors, and 3) contain compact structures that may play a role in mechanical resistance of the glumes. We suggest that the compact structures are at least in part responsible for the mechanical resistance of the glumes to embryo expansion, and as such they play a major role in seed dormancy of *L. chinensis*.

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We thank professors Jerry M. Baskin and Carol Baskin for revising this paper and giving many constructive suggestions. This work was supported by a grant from the Major State Basic Research Development Program of China (973 Program; No. 2007CB106800) and Action Plan of Chinese Academy of Sciences for West Development (KZCX2-XB2-13).

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**Comment [SE1]:** Axis labels of Figure 3 have germination misspelled.

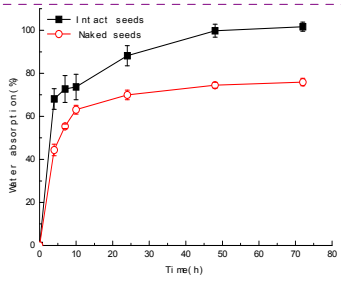


**Figure 1**. Water absorption curves of intact and naked *L. chinensis* seeds

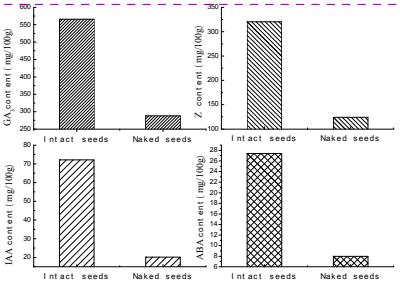


**Figure 2**. The content of endogenous hormones of intact and the naked seeds of *L. chinensis*.

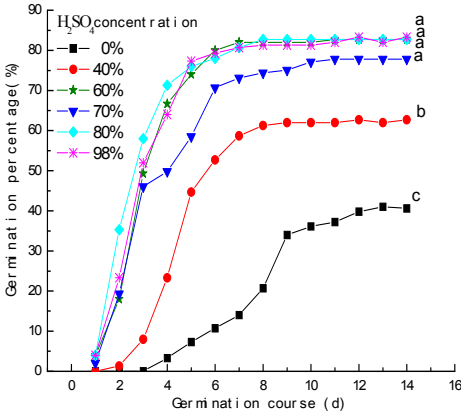


**Figure 3**. Effects of sulfuric acid on the germination course of *L. chinensis.*

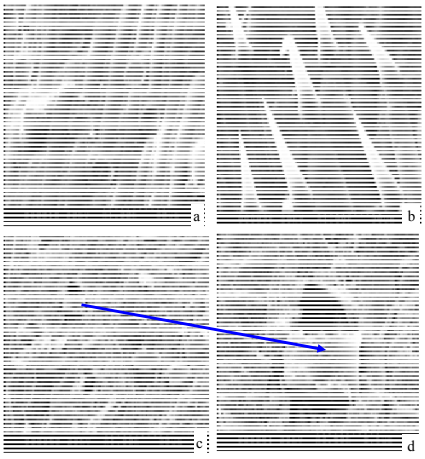


**Figure 4**. Surface structures of *L. chinensis* seeds glumes. a: External surface of glume of intact seeds; b: Internal surface of glume of intact seeds; c: External surface of glume treated with 40% $H_2SO_4$ for 10min; d: Partial amplification of picture c.

## Seed Production and Germination of the Endangered Species *Astragalus gines-lopezii*

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### Introduction

*Astragalus gines-lopezii* Talavera *et al.* (*Fabaceae*) is an endemic species from southwest Spain with a very limited area of distribution. There are only two known populations of this species, totaling less than 1000 individuals. This species was included on the Red List of Threatened Spanish Vascular Flora in 2008 in the category Endangered (EN) (Moreno 2008). The restricted area of distribution, limited number of individuals and their population structure could represent a high risk for survival of this species. Therefore, in order to detect potential threats, we have studied some key aspects of its reproductive biology. Fructification success and seed production were evaluated, and morphological and physiological seed variability were also studied.

### Methods

From each one of the two known populations of *A. gines-lopezii*, 50 individuals were randomly selected. For each plant, both the number of inflorescences and the number of flowers were recorded and then ripe fruits were collected and the number of ripe seeds per fruit was counted. For each population, the length, width and weight of seeds belonging to different individuals were determined. Seeds belonging to different individuals to be used for germination kept separately. To study the germination response of each population, seeds of several individuals per population were bulked. In all germination trials, seeds were incubated at alternating temperatures of 25/15ºC under a 16/8 h light/dark photoperiod. Mechanical scarification by sandpaper was used as presowing treatment applied for enhancing seed germination.

### Results and Conclusions

The average number of inflorescences and flowers per individual was 8.5 and 34, respectively. Plants converted the 42% of flowers into fruit and the mean number of ripe seeds per fruit was 12. For each population, great variability in seed weight, length and width were found among seeds belonging to different individuals (intrapopulation variability; Table 1). However, no significant differences were found between mean morphometric features of the two populations (interpopulation variability; Table 1). The germination percentages of control seeds (unscarified) ranged from 0% to 80% depending on the individual plant and the germination mean values of the two populations were 22% and 60% (Table 2). For each population, the scarified seeds reached higher final germination percentages (from 60 to 100%) and the mean values were very similar (97% and 99%) (Table 2). Therefore, as occurs in many *Fabaceae* taxa (see e.g. Baskin and Baskin 1998), the hard and impermeable seed coat seems to be the cause of the physical dormancy present in *A. gines-lopezii* seeds. Under natural conditions, the progressive erosion of the seed coat allows germination of the seeds of this species gradually. This species produces seeds with different degrees of physical dormancy and the degree of dormancy varies among individuals. Therefore, seeds are released from dormancy at different times and germinate intermittently over a determinate period. In conclusion, *A. gines-lopezii* seeds present a great intrapopulation morphological variablity as well as a high intra and interpopulation physiological variability. These results highlight that the source (origin) of seed samples should always be taken into account when defining models of germination behaviour, especially in wild species with a high degree of morphological and physiological variability. The results obtained in this study will be used in the development of a conservation programme for this species.

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**Table 1.** Length, width and weight of *A. gines-lopezii* seeds belonging to different individuals of the two populations (A and B). For each parameter, mean values followed by the same letter are not significantly different ($P$>0.05).

| Population and number of individual plant | Seed length ± SE (mm) (n = 10) | Seed width ± SE (mm) (n = 10) | Ratio of length to width ± SE | Seed weight ± SE (mg) (n = 2) |
|---|---|---|---|---|
| A1 | 2.88 ± 0.12 | 2,08 ± 0.07 | 1.40 ± 0.07 | 7.15 ± 0.00 |
| A2 | 2.67 ± 0.05 | 2.18 ± 0.11 | 1.29 ± 0.06 | 7.67 ± 0.19 |
| A3 | 2.76 ± 0.07 | 2.23 ± 0.05 | 1.22 ± 0.03 | 7.32 ± 0.00 |
| A4 | 3.27 ± 0.08 | 2.52 ± 0.05 | 1.31 ± 0.05 | 8.50 ± 0.52 |
| A5 | 3.25 ± 0.10 | 2.58 ± 0.08 | 1.27 ± 0.05 | 9.64 ± 0.00 |
| A6 | 3.19 ± 0.10 | 2.39 ± 0.06 | 1.34 ± 0.05 | 8.27 ± 0.05 |
| A7 | 2.95 ± 0.08 | 2.26 ± 0.08 | 1.31 ± 0.04 | --- |
| A8 | 2.80 ± 0.05 | 2.26 ± 0.08 | 1.26 ± 0.06 | --- |
| A9 | 2.91 ± 0.07 | 2.42 ± 0.05 | 1.21 ± 0.04 | 8.23 ± 0.24 |
| A10 | 3.00 ± 0.09 | 2.30 ± 0.09 | 1.32 ± 0.06 | 7.99 ± 0.04 |
| A11 | 3.15 ± 0.05 | 2.63 ± 0.07 | 1.21 ± 0.05 | 9.54 ± 0.36 |
| A12 | 3.26 ± 0.07 | 2.51 ± 0.04 | 1.30 ± 0.04 | 10.47 ± 0.18 |
| A13 | 3.16 ± 0.14 | 2.40 ± 0.09 | 1.32 ± 0.05 | --- |
| A14 | 3.12 ± 0.14 | 2.32 ± 0.08 | 1.42 ± 0.08 | 9.18 ± 0.00 |
| A15 | 3.22 ± 0.11 | 2.40 ± 0.04 | 1.35 ± 0.05 | 8.35 ± 0.16 |
| A16 | 2.93 ± 0.08 | 2.41 ± 0.06 | 1.22 ± 0.05 | 6.83 ± 0.00 |
| A17 | 3.05 ± 0.10 | 2.51 ± 0.07 | 1.23 ± 0.06 | 8.54 ± 0.00 |
| A18 | 2.95 ± 0.07 | 2.38 ± 0.08 | 1.25 ± 0.06 | 7.98 ± 0.04 |
| A19 | 2.95 ± 0.06 | 2.38 ± 0.06 | 1.25 ± 0.04 | 7.86 ± 0.07 |
| A20 | 3.28 ± 0.12 | 2.51 ± 0.09 | 1.32 ± 0.06 | 8.20 ± 0.02 |
| A21 | 3.11 ± 0.07 | 2.49 ± 0.08 | 1.26 ± 0.06 | 9.56 ± 0.10 |
| A22 | 2.90 ± 0.07 | 2.28 ± 0.06 | 1.28 ± 0.04 | 7.39 ± 0.17 |
| A23 | 3.07 ± 0.10 | 2.52 ± 0.09 | 1.23 ± 0.07 | 8.48 ± 0.26 |
| A24 | 3.04 ± 0.07 | 2.44 ± 0.06 | 1.26 ± 0.05 | 8.84 ± 0.30 |
| *P* | <0.000 | <0.000 | 0.456 | <0.000 |
| Mean values of the population A | 3.036 ± 0.003 a | 2.392 ± 0.027 a | 1.284 ± 0.011 a | 8.380 ± 0.195 a |
| B1 | 3.05 ± 0.07 | 2.46 ± 0.06 | 1.25 ± 0.06 | 8.78 ± 0.14 |
| B2 | 2.85 ± 0.06 | 2.52 ± 0.04 | 1.14 ± 0.03 | 7.38 ± 0.39 |
| B3 | 3.36 ± 0.17 | 2.62 ± 0.07 | 1.28 ± 0.06 | 9.79 ± 0.27 |
| B4 | 3.20 ± 0.07 | 2.30 ± 0.06 | 1.40 ± 0.04 | 9.15 ± 0.01 |
| B5 | 3.32 ± 0.15 | 2.33 ± 0.05 | 1.44 ± 0.10 | 7.53 ± 0.04 |
| B6 | 2.83 ± 0.08 | 2.24 ± 0.08 | 1.22 ± 0.07 | 7.08 ± 0.17 |
| B7 | 3.39 ± 0.09 | 2.51 ± 0.04 | 1.35 ± 0.04 | --- |
| B8 | 3.11 ± 0.09 | 2.39 ± 0.08 | 1.31 ± 0.04 | 7.32 ± 0.28 |
| B9 | 2.91 ± 0.09 | 2.39 ± 0.07 | 1.22 ± 0.05 | 8.20 ± 0.07 |
| B10 | 2.85 ± 0.08 | 2.36 ± 0.07 | 1.22 ± 0.05 | 7.80 ± 0.08 |
| *P* | <0.000 | 0.036 | 0.011 | <0.000 |
| Mean values of the population B | 3.089 ± 0.067 a | 2.412 ± 0.003 a | 1.283 ± 0.028 a | 8.114 ± 0.294 a |

**Table 2.** Final germination percentages of untreated seeds (control) and mechanically scarified seeds from the two *A. gines-lopezii* populations (A and B). Results after 35 days of incubation at 25/15ºC under a 16-h light photoperiod. Mean values followed by different letters within columns are significantly different ($P$<0.05).

| Population | Germination (% ± SE) | | |
|---|---|---|---|
| | Control | Mechanical scarification | S[1] |
| A | 60 ± 3.16 b | 97 ± 1.66 a | *** |
| B | 22 ± 2.24 a | 99 ± 0.87 a | *** |

| ANOVE table | df | MS | F | *P* |
|---|---|---|---|---|
| Treatment (T) | 1 | 8362.19 | 249.98 | <0.000 |
| Population (P) | 1 | 355.51 | 10.63 | 0.007 |
| T x P | 1 | 732.78 | 21.91 | 0.001 |
| Error | 12 | 33.45 | | |

[1] S, significance level: ***$P$<0.001

# Morphophysiological Seed Dormancy in *Ribes multiflorum* subsp. *sandalioticum* is Highly Sensitive to Warm Followed by Cold Temperatures.

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## Introduction

The seeds of most *Ribes* species (Grossulariaceae) have small embryos with a dormancy that can be broken by a long stratification period (Young and Young, 1992). However, seed coat dormancy is also suspected to occur (Pfister, 1974). On the basis of the seed dormancy classification system of Baskin and Baskin (2004), therefore, all the five classes of dormancy could occur in species of this genus. However, morphophysiological dormancy (MPD) has not been studied in detail in *Ribes* (Baskin and Baskin, 2007). In this study the effects of a move-along experiment (Baskin and Baskin, 2003) and $GA_3$ on seed germination of *R. multiflorum* Kit. *ex* Roem. *et* Schult. subsp. *sandalioticum* Arrigoni were investigated. The results provide some insight into the natural regeneration strategy of this rare mountain species, which is endemic to Sardinia (Italy) and grows up to 1000 m a.s.l. in only a few small populations.

## Methods

The rate of water uptake was assessed for scarified and non-scarified seeds by monitoring the change in weight during imbibition on the surface of 1% agar water, in order to assess any physical component in the dormancy. In addition, in an attempt to break morphophysiological dormancy, seeds were exposed to a prewarming (W: 25°C for 3 months) and prechilling (C: 5°C for 3 months) period as well as two combinations of warm and cold (W+C and C+W+C), before sowing on the surface of 1% agar water, at a range of constant temperatures (10-30°C) and one alternating temperature regime (25/10°C) in the light (12 h per day). Seeds were also sown on the surface of 1% agar water with 250 mg/L gibberellic acid ($GA_3$) and incubated in the light at the range of germination temperatures specified above.

## Results and Conclusions

*R. sandalioticum* seeds did not show any difference between scarified and untreated seeds in terms of imbibition rates, suggesting the lack of physical dormancy. Prewarming (W) and $GA_3$ treatments, followed by low germination temperatures (< 15°C), had a positive effect on the rate and final germination level (> 80%; (Fig. 1), while low germination (ca. 20%) was detected after prechilling alone (C) (Fig.1) and no germination occurred at warmer temperatures alone. At the end of the W+C and C+W+C treatments, ca. 70% and 35% respectively, of the initial sown seeds germinated during the cold treatment (C) after the warming (W), before moving to the germination conditions. Our data demonstrate the positive effect of warm stratification and $GA_3$ on breaking dormancy, suggesting the presence of MPD. The sensitivity of this species to low temperatures for seed germination highlight as well an increasing threat from global warming, as the level of natural emergence in the field may be reduced.

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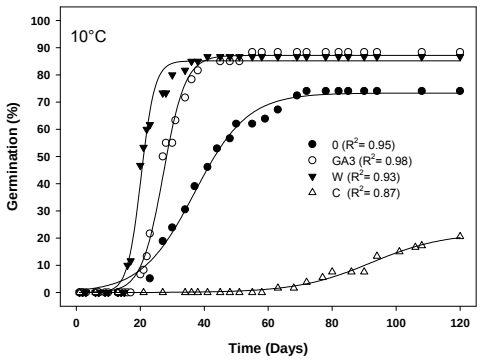


**Figure 1.** Germination trends at 10°C in the light (12 h per day), after each pretreatment: control (0), $GA_3$ 250 mg/L in the germination medium (GA3), prewarming (W) and prechilling (C). Points correspond to the actual data and solid lines indicate the fitted curves of the sigmoidal regressions. Data are the mean of three replicates of 20 seeds each.

# Seed Science at a Size That Matters – Managing Seed Resources to Deliver Large-scale, Biodiverse Restoration

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## Introduction

Key areas of seed biology and technology underpin management actions designed to initiate and/or accelerate the restoration of vegetation communities following disturbance. Where topsoil is limited or unavailable, seeds become a primary means of re-introducing biodiverse plant communities. But effectively using wild-sourced seeds for restoration is not without its challenges. Optimising each step in the chain of seed use in restoration, from collection, storage, propagation and delivery to site is necessary to ensure seeds are used to their full potential. Knowledge of these key areas is complex when dealing with biodiverse plant communities, as species-specific information must be tailored to site-specific needs.

To add to the complexities of working with wild seeds, in contemporary times the scale of restoration is vast, and increasing. It is now common for single restoration programs (often involving poorly-known ecosystems) to be tackling thousands, or even tens of thousands, of hectares. Community expectations for, and regulation of, environmental restoration standards are increasing to match. These factors are placing pressure on land managers to stipulate greater access to, knowledge of, and success in using diverse species for restoration.

## Methods and Results

When considering seeds as the primary source of plant re-introductions, commonly encountered shortfalls in seed knowledge and seed handling practices that hamper restoration outcomes include: (1) a limited understanding of the phenology of seed development and maturation for many species and the provenance variation for these factors that can lead to inappropriate timing of seed collection; (2) failure to document or understand the initial quality and viability of collected seeds; (3) unresolved seed dormancy alleviation and germination techniques for many dominant plant families; (4) the common application of poor seed banking procedures that do not consider the required storage duration or designated end use of seeds, and; (5) the typical low seedling establishment rates (< 10%) from broadcast seeds. Drawing on examples from the Australian flora, recognised as comprising some of the world's most diverse, endemic and ancient plants that pose some unique restoration challenges, I will highlight some advances as well as knowledge gaps in these key areas and lessons learned from experiences from intervention ecology.

## Conclusions

As seed ecologists we can contribute substantially to achieving biodiverse restoration. But how we actually use the seed ecological knowledge derived from our research to inform and improve restoration is key to meeting challenges of scale. Diverse fields of seed science require careful and deliberate integration to translate the science into restoration practice at an operational level. Effective working relationships with the restoration industry and the commercial seed industry are necessary. Targeted science demonstrating and achieving direct, on-ground environmental and economic outcomes will serve the dual purpose of increasing diversity in restoration and persuading the restoration industry of the benefits of investment in seed research.

# Ecological Genetics of Floret Mass Variation in *Bromus tectorum* (Poaceae)

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## Introduction

*Bromus tectorum* L. (cheatgrass, downy brome) is a highly invasive inbreeding annual grass that dominates millions of hectares of former shrubland in interior western North America. Factors contributing to its success include strong genetic regulation of key adaptive traits coupled with high phenotypic plasticity in response to resource availability (Meyer and Allen 1999a, b). Seed (floret) mass in this species has been shown to significantly impact biomass and reproductive output in the field, demonstrating its adaptive significance (Leger *et al*., 2009). In this investigation, the role of genetic variation versus seed maturation environment in regulating seed mass was examined in greenhouse studies with multiple populations and inbreeding lines.

## Methods

Seeds from individual plants were collected in the field from six *B. tectorum* populations representing a range of habitats. These seeds were weighed in four lots of 25 to obtain mean mass per 100 seeds. A subset of seeds from each plant was sown in container culture to produce a greenhouse-grown generation for further research. The progeny of this first greenhouse generation were then planted in a formal reaction norm experiment. Water stress during late seed maturation was manipulated at three levels to measure reaction norms for seed germination traits (Meyer and Allen 1999a, b), as well as seed mass. The mean seed mass for wild-collected seeds of each of the 18 inbreeding lines was compared with seed mass of two generations of greenhouse-grown seeds using linear regression. Seed mass reaction norms in response to maturation water stress were plotted for each inbreeding line.

## Results and Conclusions

There was a significant positive correlation between seed mass of greenhouse-grown progeny and their wild parents, indicating high heritability (Figure 1). Seed mass showed a plastic increase overall in the optimum growing conditions of the greenhouse. Mean seed mass in the wild generation varied among lines from 0.179 to 0.343 g/100seeds, while in the first greenhouse generation, mean seed mass ranged from from 0.271 to 0.409g/100 seeds. Lines with the smallest seeds showed the greatest seed mass increase in greenhouse cultivation (Figure 1A). This effect was even more pronounced in the second generation (Figure 1B). When plants of each line produced seeds under contrasting maturation water stress conditions, there was a general decrease in mass with increasing water stress. Mean seed mass at low, medium, and high water stress averaged 0.348, 0.319, and 0.281 g/100 seeds, respectively. Inbreeding lines varied considerably in the shape of the reaction norm response (Figure 2). For example, one Green River line, which had the lowest seed mass in the low stress treatment, showed no decrease with increasing water stress, while a Potosi Pass line, which had the highest seed mass under low stress, showed a dramatic linear decrease in mass with increasing water stress. These results indicate that inbreeding lines differ genetically in their plastic response to water stress as well as in mean seed mass. This combination of genetic control and phenotypic plasticity serves to ensure seed production under stress but also to maximize seed quality under optimal conditions.

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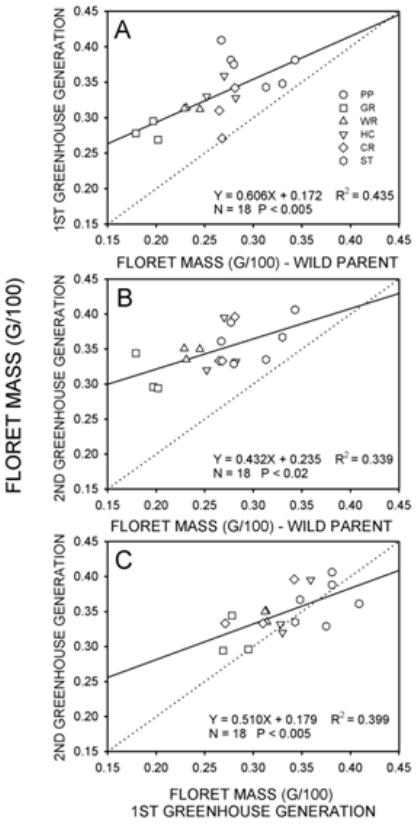


**Figure 1**. Changes in floret mass in greenhouse cultivation under optimal conditions for 18 inbreeding lines belonging to six populations of *B. tectorum*: (A) Change from wild-collected seeds to first-generation seeds produced in the greenhouse, (B) Change from wild-collected seeds to second-generation seeds produced in the greenhouse, and (C) Change from first-generation to second-generation-produced seeds in the greenhouse. Solid lines represent regression lines; dashed lines represent 1:1 relationship.

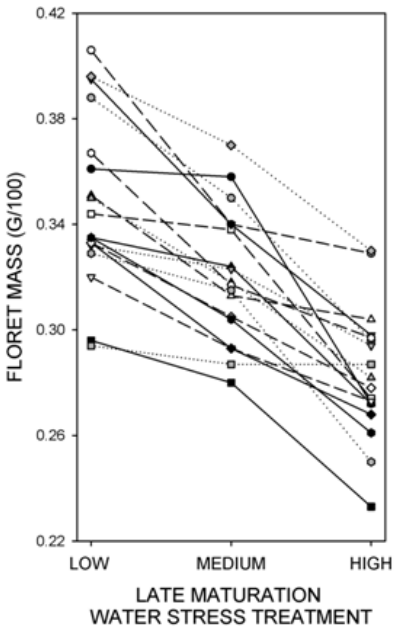


**Figure 2**. Floret mass reaction norms for 18 inbreeding lines belonging to six populations of *B. tectorum* in response to a gradient of water stress treatments applied during late seed maturation. Population symbols as in Figure 1: PP=Potosi Pass NV, GR=Green River UT. WR=Whiterocks UT, HC=Hobble Creek UT, CR=Castle Rock CO, ST= Strawberry Reservoir UT.

# Climate Change in Alpine Ecosystems: Will Seed Banking be an Effective Strategy to Halt Biodiversity Loss?

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## Introduction

Alpine ecosystems are considered to be particularly sensitive to global warming. According to recent predictions, if the climate warms by 4°C over the next century, species may have to move upwards *c.* 500m to find suitable climatic conditions (Thuiller 2007). In this scenario, many alpine species will have "nowhere to go", and of particular concern is their susceptibility to invasion by species from lower altitudes (Parolo and Rossi 2008). Collecting and banking seeds of plant species at risk of extinction in the face of rapid climate change is recognized as an effective tool for providing propagating material to re-establish wild plant populations (UNEP 1992). However, there are indications that seeds from plants growing in cold regions may be inherently short-lived in air-dry storage (Probert *et al.* 2009). The prospects of long term conservation for alpine species using conventional seed banking methods are therefore uncertain. Here, we present a case study of comparative seed longevity among 69 related species from European alpine and lowland locations. The reasons for low seed longevity are analyzed in terms of maternal environment and resistance to ageing.

## Methods

Seeds were collected in northern Italy, selecting 28 species from lowland sites (*c*. 0 to 400 m a.s.l.) and 41 species from alpine sites (*c*. 1800-2400 m a.s.l.). For each location the monthly mean temperature and total monthly rainfall values were obtained by querying WORLDCLIM data (http://www.worldclim.org/). Due to logistical and time constraints with comparing storage life span under gene bank conditions, seed longevity was determined using a standard rapid ageing protocol (Davies and Probert 2004). Seed lots were first rehydrated at 47% RH at 20°C for two weeks and then transferred to the ageing conditions at 60%RH at 45°C. At given intervals during the ageing period, samples of seeds were removed for germination testing.
Probit analysis was carried out on the data from the ageing experiments using GenStat (Version 11) in order to estimate the time for viability to fall to 50% ($p_{50}$). For each species and collection site, linear regression analyses were carried out to investigate the relationship between $p_{50}$ and mean temperature and daily rainfall during the flowering and seed setting period.

## Results and conclusions

Seed viability declined with increasing duration of the ageing treatments in all the species tested, with a wide variation across either species or sample provenance. The lowland seed lots were distinguished by a slower rate of deterioration or higher initial seed viability or both (e.g. Fig. 1). There was a significant correlation between seed longevity and either the temperature or the rainfall experienced by each species at the collection sites ($P < 0.001$). Species were progressively longer-lived with increased temperature and decreased rainfall of the collection sites, and accordingly, alpine species and populations were strikingly shorter-lived than their lowland counterparts.

The greater resistance to ageing in seeds of lowland plants may arise from selection pressure for seeds to survive warm, dry conditions following dispersal, whereas seeds of alpine plants are exposed to relatively cool, moist conditions which help to maintain viability. Reciprocal transplant experiments are planned to explore the extent to which longevity differences are controlled by genetic factors.

This study has highlighted a significant concern for the successful *ex-situ* conservation of alpine plants, which represent one of the groups most sensitive to the direct and indirect human impacts on plant diversity (Thuiller 2007). Conservation collections of alpine species held in seed banks will need to be re-tested more frequently to monitor their potential decline in viability during storage, and alternative storage techniques such as cryo-preservation should be considered for the shortest-lived taxa. However, under the current changing climate, short time-scale seed regeneration in storage should not be seen just as a remedy for maintaining collections that are declining in viability but also

as a strategy that would ensure both the conservation of high genetic diversity and novelty for replanting in future habitats.

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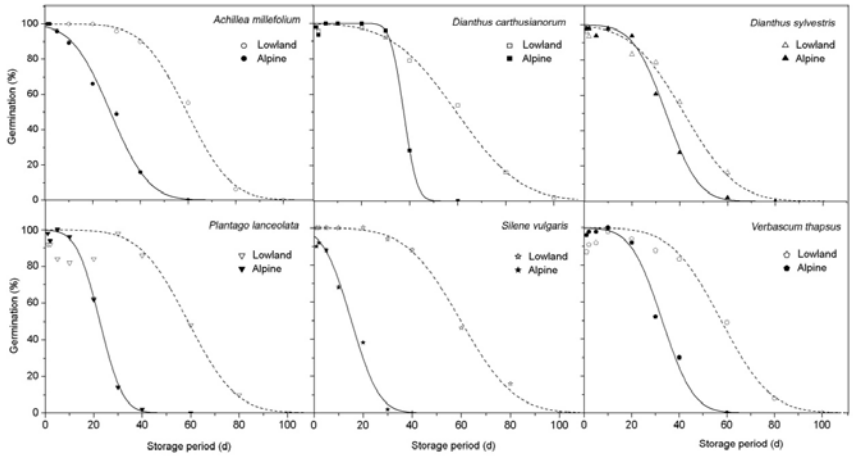


Fig. 1. Survival curves fitted by probit analysis for lowland and alpine populations of six species tested in this study, stored at 45ºC and 60% RH.

## Germination Characteristics of Czech Alien Neophyte Species

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### Introduction

Biological invasions by alien species represent a major threat to native biodiversity (e.g., Vitousek et al. 1996). Many studies exploring traits associated with invasiveness highlight the importance of reproductive characteristics (see references in Pyšek &. Richardson 2007). Seed germination is one of the key processes associated with the initial phase of plant establishment, and may influence the outcome of invasion in the new region. Here we assess the germination characteristics of Czech neophytes (alien species introduced after 1500 A.D.; see Pyšek et al. 2002, 2004) with regard to their invasion status, life history and residence time (time from which the transfer of the species to the Czech Republic is known to have occurred).

### Methods

The species set included 93 neophytes occurring in the flora of the Czech Republic (Central Europe), belonging to 70 genera and 32 families according to the Angiosperm Phylogeny Group classification (Stevens 2001). All species are either naturalized but not invasive, or invasive in the study region. To reveal germination requirements of individual species, seeds were collected from multiple localities in the field and germinated in the laboratory at four treatments: immediately after harvest (at 25/10, 20/15 and 15/5 $^{o}$C); after one month of dry storage (incubated at 25/10 $^{o}$C); and after 3 and 5 months of cold and wet stratification (incubated at 25/10 $^{o}$C) . Data were analyzed using logistic regression.

### Results and Conclusions

Proportion of germinated seed was significantly affected by germination treatment, life history, invasion status and resident time, with significant interactions of the effects. Invasive species germinated to higher post-treatment percentages but were more dormant than naturalized but non-invasive species. Annuals were more dormant than perennials, including monocarpic perennials. Immediately after harvest, the seed of annuals germinated to lower percentages than those of perennials (Table 1); germination of annuals was stimulated by higher temperatures. Species with long residence times germinated better than species with shorter residence times. There was a significant effect of phylogenetic position (family) and its interaction with other factors on the characteristics studied. The results indicate that germination characteristics differ among various stages of the invasion process and should be therefore taken into account when searching for determinants of plant species invasion success.

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Table 1: Germination (%) of neophytes in the Czech Republic with different invasion status and life history under four germination treatments (at harvest = germination immediately after the harvest; one month of dry storage = germination after one month of dry storage at room temperature; 3 month stratification = 3 months wet stratification at 1–5 °C; 5 month stratification = 5 months wet stratification at 1-5 °C. Germination was investigated under the temperature 25/10 °C.

| Invasion status | Life history | At harvest | One month of dry storage | 3 month stratification | 5 month stratification |
|---|---|---|---|---|---|
| Invasive | Annual | 38.4 | 48.3 | 73.8 | 81.6 |
| | Monocarpic | 37.7 | 37.7 | 70.1 | 74.7 |
| | Perennial | 58.9 | 60.6 | 77.5 | 79.9 |
| Naturalized | Annual | 30.3 | 47.1 | 69.3 | 65.0 |
| | Monocarpic | 62.3 | 69.1 | 75.3 | 76.4 |
| | Perennial | 42.2 | 53.6 | 70.7 | 76.7 |

# Seed Banks, Seed Dispersal, and Their Potential Implications for the Restoration of Degraded Land

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## Introduction

When plant species disappear from a landscape as a result of human interference (e.g., overgrazing or deforestation) restoration ecology attempts to re-establish such plant species within a scientific and ecological framework (Bakker et al., 1996). It is suggested that estimating the chance of plant re-establishment becomes easier with knowledge about the temporal and spatial dispersal of indicator species (Bakker et al., 1996). In this study differences in the number of seeds present in the seed bank of an overgrazed area are compared with the number of seeds present in the seed bank of a more lightly-grazed area on a stock and game farm in the Southern Kalahari Desert (Namibia). In addition seeds being transported within each of these areas were examined using two sampling techniques. The objective was to observe which elements of seed bank dynamics would be most crucial for future plant restoration ecology in a semi-arid environment.

## Methods

The spatial and temporal distribution of seed banks were quantified by taking core soil samples (n=6) from each site. The seed bank was sampled four times, directly after the 2008 seed release (July 2008), prior to the 2008/09 rainy season and seed germination (October 2008), after the 2008/09 rainy season and seed germination (February 2009) and after the 2009 seed release (July 2009). In addition, seeds dispersed by the wind were quantified using seed traps levelled with, and mounted above the ground surface. Seeds were extracted from samples using sieves and tweezers. After testing data for normality the appropriate analysis of variance test (ANOVA) was performed to test for differences in the data.

## Results and Conclusions

Seed densities were higher at the more lightly-grazed site than at the overgrazed site after seed release (July 2008 and 2009) as well as prior to germination (October 2008) (Fig. 1a). In fact seed densities were more than five times higher at the more lightly-grazed site during these periods. After germination (February 2009), however, there was no difference in seed densities between the two sites (Fig. 1a). In contrast our dispersal data revealed more seeds were in transport at the overgrazed site than at the more lightly-grazed site (Fig. 1b). The seeds do not however seem to have a high potential for deposition in the overgrazed area. The results show that manipulating the soil surface to allow seed deposition in an overgrazed arid area will be most crucial for the growth of seed banks and thus future restoration ecology. We therefore recommend that the effect of different topographic and surface cover manipulation techniques on seed deposition should be tested in similar overgrazed areas, where the topography is smooth and the soil particles are relatively small.

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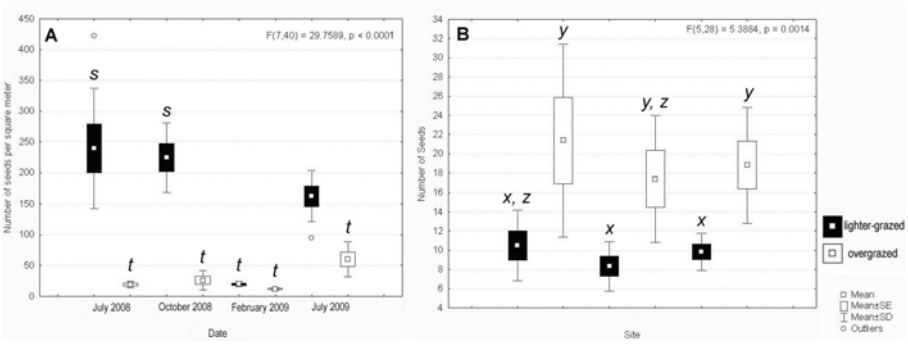


**Figure 1 A**) Number of seeds per square meter present in the seed bank at the more lightly-grazed and at the overgrazed sites over time. Sampling was done in Jul 08, Oct 08, Feb 09 and Jul 09. The One-Way ANOVA result is given in the top right hand corner. Periods that are statistically indistinguishable (LSD test) share a lower case letter (*s* or *t*). **B**) Number of seeds in transport captured at the more lightly-grazed and at the overgrazed sites during the 1 June – 15 July 2009 period. The One-Way ANOVA result is given in the top right corner. Sites that are statistically indistinguishable (LSD test) share a lower case letter (*x, y* or *z*).

# Factors Affecting the Germination and Seedling Emergence of a Floating-leaved Plant, *Trapa japonica*

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## Introduction

*Trapa japonica* Flerov. (=*T. bispinosa* Roxb. var. *iinumai* Nakano) is an annual floating-leaved plant; it is distributed throughout eastern Asia, including the Japanese archipelago. The species often forms dominant vegetation in shallow eutrophic lakes. Progress toward an ecological understanding of *Trapa* is important for lake management because dense stands lead to hypoxia (Caraco and Cole 2002) and hamper boat navigation. In Lake Mikata, Japan, the surface dominated by *T. japonica* has been increasing rapidly, and reached ~58% of lake area in 2009 (Fig. 1, Nishihiro et al. unpublished estimation). Information on germination phenology, soil seed bank dynamics, and factors affecting species abundance and distribution in the lake remains incomplete; however, such data are essential for appropriate lake management planning. We conducted laboratory experiments to determine the effects of temperature and light environment on dormancy break and germination, and the effects of water salinity on seed germination and seedling emergence. We postulated that saline water inhibits germination and/or emergence because *T. japonica* is absent from a section of Lake Mikata into which brackish water (<15 ‰ salinity) frequently enters.

## Methods

We sampled *T. japonica* seeds (Fig. 2a) from Lake Mikata on 3 October 2009. Seeds were stored in a 90 L tank for 40 days to facilitate after-ripening. To examine seed responses (the breaking or induction of dormancy) to low temperature, we compared germination in a test system either with or without chilling pretreatment (4 °C, 4 wk). Germination tests were carried out under constant temperature (8, 12, 16, 20, 24, 28, 32, and 36 °C). The light requirement for germination was assessed by comparing percentage germination in light (~200 µmol photons/$m^2$/s from white fluorescent lamps) and darkness under optimal thermal conditions after chilling pretreatment.

To examine water salinity effects on seed germination, we compared percentage germination after 10 days incubation at 24 °C (following 6 wk chilling pretreatment) among salinities of 0, 5, 10, 20, and 30 ‰. To examine effects on seedling mortality, seeds that had been incubated at 0 ‰ salinity for 1 wk after germination (Fig. 2b) were transferred to salinities of 0, 5, 10, 20, and 30 ‰. After 1 wk incubation, the survival of each seedling was assessed and recorded by observing the protruding primary stem (Fig. 2c). All seedlings without a primary stem were regarded as unable to grow (because roots had decayed).

ll tests were conducted with three replicates of 25 seeds. Seeds were submerged in a 1.8 L plastic container filled with de-ionized water.

## Results and Conclusions

The thermal germination test showed that low temperature was required to break primary seed dormancy: no seeds germinated without 4 °C pretreatment. Seeds out of dormancy germinated in a 16 - 36 °C temperature range. These observations suggest that *T. japonica* is adapted to germinate during the spring - summer period. There were no significant differences in germination percentages between light and darkness; thus, seeds likely germinate on the bottoms of turbid lakes.

Percent germination was significantly reduced by salinities >10 ‰. All seedlings died in salinities >5 ‰. Hence, the timing and spatial extent of brackish water entry probably affects the distribution and abundance of *T. japonica* in Lake Mikata.

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**Figure 1.** Lake Mikata. Rosettes of *Trapa japonica* are covering the water surface.

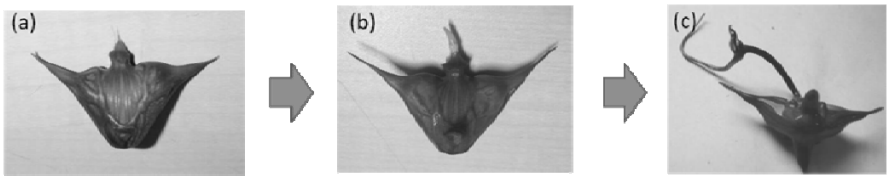


**Figure 2.** (a) Seed of *Trapa japonica*. (b) Germinated seed. (c) Seedling with primary stem.

## Water Relations in Embryo Axes of Germinating Recalcitrant and Orthodox Seeds

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### Introduction

The main difference between orthodox and recalcitrant seeds is the capacity of orthodox seeds for viability maintenance during water loss at the end of maturation and in the air-dry state. In contrast, recalcitrant seeds maintain high viability at high water content, which is preserved during maturation, then through the dormant state (if there is one), and up through germination. Physiological mechanisms providing desiccation tolerance innate to orthodox seeds and its absence in recalcitrant seeds have been clarified (Berjak, Pammenter 2008, Roach et al. 2010) and will not be considered here. The focus of this paper is on the distinctions and similarities in water status in orthodox and recalcitrant seeds prior to and during germination.

### Methods

Orthodox seeds were exemplified by *Vicia faba minor* L. (broad bean) and recalcitrant seeds by *Aesculus hippocastanum* L. (horse chestnut). Seeds were sampled in the Main Botanical Garden (Moscow). Horse chestnut seeds were stratified for 4 months in wet sand at 4°C and germinated in the dark at 26°C. Water content was measured by weighing. Longitudinal sections along the embryo axis were vitally stained with Neutral Red. Activity of acid vacuolar invertase was measured in dialyzed supernatant (Obroucheva and Lityagina 2009).

### Results and Conclusions

The difference in water status between orthodox and recalcitrant seeds is shown in Fig. 1. Orthodox seeds desiccate to an average water content of 8-9% fr.wt in embryo axes and can be stored without viability loss. Their transition to germination starts with seed imbibition from this low hydration level, which increases water content up to 72-73%, the water content at which growth in the axes begins. In the horse chestnut axes, water content decreases at maturation to 62-64% and is maintained without viability loss during dormancy and through to the transition to germination, culminating in the growth initiation at 74-75%. In these seeds, the water content of 62-64% corresponds to the level of $\Psi_{mat}$ saturation measured as water content in imbibed killed seeds. After dispersal, this level is maintained due to weak water evaporation through the wax-covered seed coat and high sucrose content (Obroucheva et al. 2006) providing a $\Psi_{osm}$ of –3Mpa, favoring water inflow. In imbibing broad bean seeds, the level of $\Psi_{mat}$ saturation is attained at 60% water content and results in activation of primary metabolism (Obroucheva 1999, 2010).

Another difference in water relations between orthodox and recalcitrant seeds manifests itself in the behavior of vacuoles, the main osmotic compartment and water reservoir. In maturing orthodox seeds, vacuoles are transformed to protein bodies, in which reserve proteins replace the vacuolar contents. In imbibing seeds, such protein bodies are restored to vacuoles prior to growth initiation. In horse chestnut seeds, the vacuoles present in freshly-fallen seeds are preserved during dormancy (Fig. 2) and become enlarged prior to growth initiation. The preserved vacuoles retain their tonoplast aquaporins (Obroucheva et al. 2010) and maintain the activity of vacuolar acid invertase (Fig. 3). The molecular mass of the enzyme and its subunits remained unchanged, as well as its substrate specificity (Obroucheva and Lityagina 2009). By the time of seed germination, its activity increases due to additional synthesis of enzyme molecules on long-lived DNA templates. To conclude, vacuoles in germinating orthodox seeds appear from protein bodies, whereas their enzyme machinery (vacuolar invertase in particular) is synthesized due to *de novo* gene expression after the growth commencement (Mutsuhashi et al. 2004). In recalcitrant seeds, the vacuoles formed at maturation are maintained through to germination, with their tonoplast proteins and enzymes preserved; this provides the more rapid and efficient germination typical of recalcitrant seeds.

**Acknowledgements**. The work was supported by RFBR (grant 08-04-00416) and RAS Program of cell and molecular biology.

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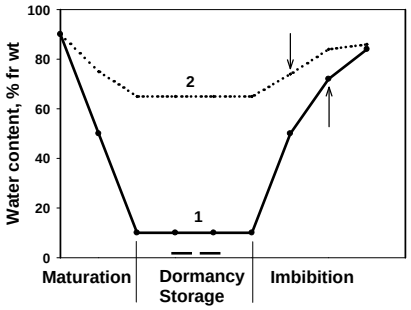


Figure 1. Water content in embryo axes of viable broad bean (1) and horse chestnut (2) seeds. Arrows indicate growth initiation (radicle emergence).

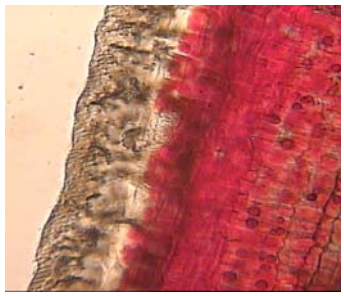

Figure 2. Production of crimson color by Neutral Red vitally staining acidic vacuolar contents in cortical meristematic cells of horse chestnut embryo axis.

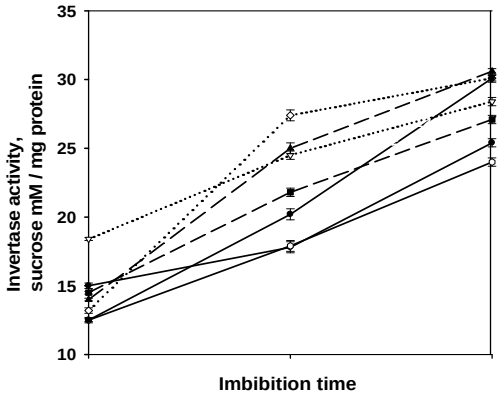


Figure 3. Activities of acid vacuolar invertase in embryo axes of horse chestnut seeds regularly sampled during deep dormancy (solid lines), dormancy release (dashed lines) and dormancy absence (dotted lines) and measured at the beginning of seed imbibition under optimum conditions, in the middle of imbibition time and at radicle emergence.

# Annual Cycles of Seed Dormancy: Evidence in Perennial Species of Tropical Monocotyledons

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## Introduction

*Syngonanthus* (Eriocaulaceae) is a predominantly herbaceous genus typical of the "campos rupestres" vegetation that occurs on open, rocky areas or on sandy high plains or swampy sites of the Espinhaço Mountain Range. The taxa of this genus have very small and light-sensitive seeds (Oliveira and Garcia 2005), characteristics that favor their longevity in the soil, although the persistence in the soil varies among the different species and according to their native environments (Thompson and Grime 1979). Unfavorable conditions in the local environment (such as soil temperature or humidity) can result in the development of secondary dormancy and extend germination over a considerably longer period of time (Baskin and Baskin 1988, Bewley and Black 1994). Annual cycles of dormancy have been described in species from temperate regions, but not yet in seeds from tropical regions where seasonal temperature variations are much milder. The present study evaluated the *in situ* longevity of the seeds of two species of *Syngonanthus* endemic to the mountainous regions of southeastern Brazil to determine if they formed seed banks and if their germination responses altered when buried under natural conditions.

## Methods

Seeds of *Syngonanthus bisulcatus* (Korn.) Ruhland and *S. verticilatus* (Korn.) Ruhland were collected in Serra do Cipó in the Cadeia do Espinhaço Mountain Range, Minas Gerais State, Brazil (19º12'-19º20'S and 43º30'-43º40'W). The average annual rainfall there is approximately 1600 mm (Marques *et al*., 2000), with the rainy season occurring from November to March (summer) and the dry season occurring from April to October (winter). The capitula were collected during the seed dispersal phase, removed to the laboratory, and subsequently shredded in a blender and sifted to separate out theseeds. These seeds were then placed in nylon bags and buried approximately 5 cm deep in the ground. Every two months, the seeds of both species were recovered and tested for their germinability, with a total of 34 months of experimental testing with *S. verticilatus* and 26 with *S. bisulcatus*. After removal from the soil, the seeds were incubated in Petri dishes on a double layer of filter paper moistened with distilled water and exposed to a 12 hr photoperiod at 20 ºC (the optimal germination temperature for both species). Testing was performed using four replicates of 50 seeds per treatment and germination was checked daily; germination was defined as the emergence of the embryonic axis (Scatena and Menezes 1993). As the germination data did not demonstrate normality or homogeneity using the Shapiro-Wilk and Brown-Forsythe tests respectively, the data was analyzed using the Kruskal-Wallis test, followed by comparisons using the Conover test at a 5% significance level (Conover 1999).

## Results and Conclusions

The seeds of both species are very small (< 0.02 mg) and will germinate almost immediately after sowing in the presence of light. Buried seeds of *S. verticilatus* remained viable even after 34 months in the ground, with germinability levels very similar to those of recently collected seeds (Fig. 1A). The seeds of *S. bisulcatus* demonstrated significant reductions in their germination percentages during the 26 months they were buried, with the oldest seeds demonstrating only ~50% of the germination seen in recently harvested seeds (Fig. 1B). The high germination percentages of the seeds of *S. verticilatus* seen after 34 months of storage in the soil indicate that this species fcan form a persistent seed bank (*sensu* Thompson, 1993). The maintenance of ~50% viability of the seeds of *S. bisulcatus* after 26 months demonstrated that this species can form a persistent seed bank in the soil (even though this bank would be relatively small; *sensu* Baskin and Baskin 1996). The stored seeds of *S. verticilatus* demonstrated significant reductions in their germination during the rainy season but a significant increase in germination during the dry season (August), with percentages very similar to those seen with recently collected seeds for three consecutive years (Fig. 1A); the seeds of *S. bisulcatus* showed similar responses, although their germination percentages were significantly lower than recently collected seeds ($P<0.05$; Fig. 1B). These results demonstrate that the seeds of both species develop secondary dormancy during the rainy season (summer) when temperatures and soil humidity are high. This acquired dormancy was lost during the dry season when the temperatures were lower and the

soils drier. The germination responses of the buried seeds of both species characterize an annual cycle of dormancy, which could be seen here to be related to the avoidance of germination during periods less favorable to seedling establishment.

**Acknowledgements**
Support for the research was received through the FBPN (Proc. 0662-20051) and FAPEMIG (Proc. CRAAPQ 4654-5.04/07). PGOliveira received a doctoral fellowship from FAPEMIG and QS Garcia a research productivity scholarship from CNPq.

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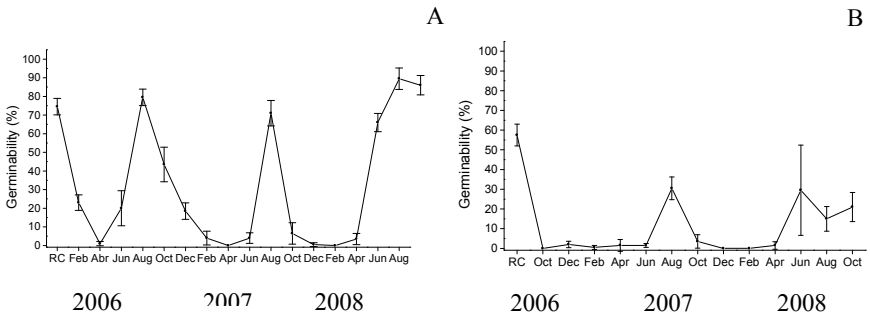


**Figure 1**. Germinability of the seeds of *S. verticilatus* (A) and *S. bisulcatus* (B) burried *in situ* (Serra do Cipó) for 34 and 26 months, respectively, and exposed to 12 hr photoperiod at 20 ºC in laboratory.

## Characterizing Dormancy in Seeds of Endangered Plant Species on Oahu, Hawaii

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### Introduction

The Oahu Army Natural Resources Program's Seed Laboratory (OANRP) studies the potential of seed storage as adequate genetic preservation for 51 federally listed endangered plant species on the island of Oahu, Hawaii. Additionally, OANRP propagates from seeds for the purpose of reintroduction for the majority of these species. Species-specific standardization of germination protocols allows for the adequate assessment of storage longevity. OANRP determines these protocols and applies the necessary conditions (e.g., media) to all viability assays, understanding that some conditions, such as chemical stimulants, may not be applicable to stored seeds. In this process, much has been documented on the germination behavior of fresh seeds, including how long seeds take to germinate and what conditions, including media, temperature, and scarification, contribute to the maximum germination for a particular species. Due to the severe rarity of most taxa investigated, our questions address storage potential only, but our data suggest possible dormancy classifications.

### Methods

Fresh seeds are sown on one or more different substrates and placed in Percival environmental controllers where temperatures mimic daily and nightly averages of the natural habitat. Seeds are checked every ten days and germination occurs when both the radicle and cotyledon(s) have emerged. After one year, if no germination occurs within a 30-day period, the remaining seeds are cut to determine viability, and the viability assessment is complete. Viable seeds with no germination 30 days after sowing are identified as dormant (Baskin & Baskin 2004). Viability of fresh seeds has been tested in 43 of the 51 species.

### Results and Conclusions

Sixty-three percent of the species tested have seeds that can be classified as non-dormant. Two species appear to have physical dormancy, and manual scarification of the seed coat is followed by increased germination (Fig. 1). *Hibiscus brackenridgei* subsp. *mokuleianus* (M. J. Roe) D. M. Bates (Malvaceae) is an example of a species that may have physical dormancy, as germination is consistently higher when scarification is applied. The remaining species (35%) have some type of physiological, morpho-physiological, or combinational dormancy (Fig. 2). *Neraudia angulata* R. S. Cowan (Urticaceae) exemplifies this category. Germination does not occur within the first month and seeds continually germinate for years after sowing. Future studies include measuring embryo lengths to differentiate between physiological and morpho-physiological dormancy and testing water impermeability in the species with potential physical dormancy. OANRP intends to use dormancy classification to aid in the stabilization of these rare species. Using dormancy-breaking methods for viability assays helps to determine whether seed storage is an acceptable long-term genetic storage method. Identifying dormancy classifications will allow for a better assessment of the viability of wild populations as well. Relating dormancy-breaking requirements to the occurrence of *in situ* seedling emergence, along with determining the type of soil seed banks these species create, will help in monitoring the stability of populations.

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Fig. 1: Scarification of seeds of *H. brackenridgei* subsp. *mokuleianus.*

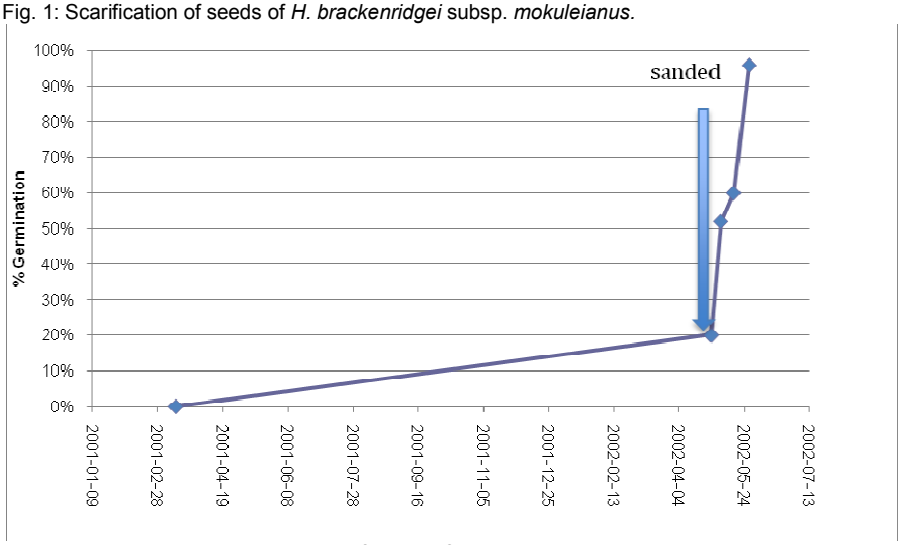
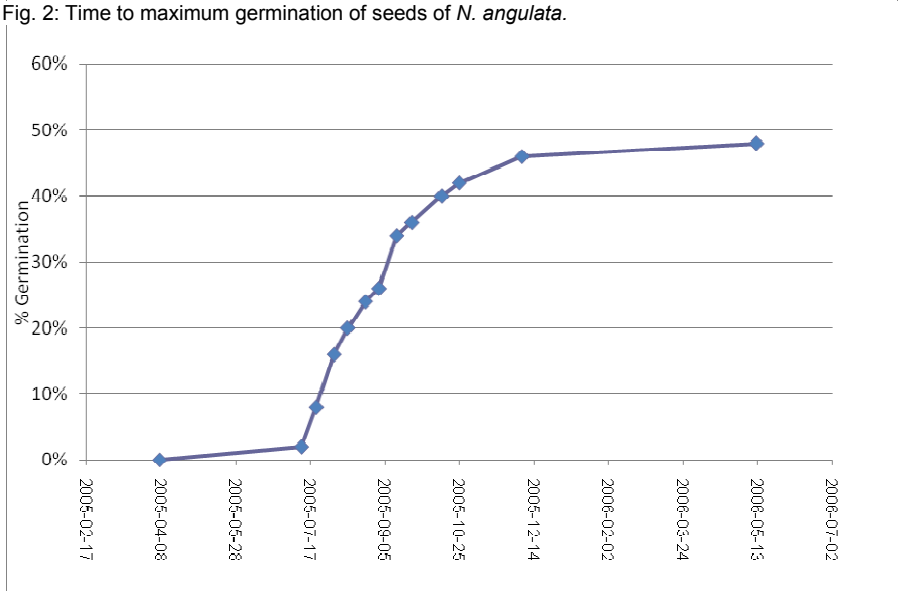



Fig. 2: Time to maximum germination of seeds of *N. angulata.*



# Individual Variation and Altitudinal Effects on Germination of *Miconia albicans* (Melastomataceae) From Rocky Grassland Savanna.

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## Introduction

In most plant species, there is a great variation in germination between and within populations and between and within individuals (Gutterman 2000). Previous studies have highlighted correlations between germination and altitude in temperate and Mediterranean ecosystems (Vera 1997, Giménez-Benavides et al. 2005, Wirth et al. 2010), but altitudinal effects on germination of tropical species remain largely unknown. In this study we investigate the effects of altitude on germination and seed mass of *Miconia albicans* at the Cerrado. *Miconia albicans* is a widespread shrub across vegetation types in the Cerrado vegetation (Brazilian savanna) with an obligatory apomictic mating system. We hypothesize that: 1) germinability and seed mass both increase with decreasing altitudes and 2) there is a high intra-specific variation in germinability caused by genetic differentiation among individuals.

## Methods

This study was conducted at Serra do Cipó, Southeastern Brazil, where the main vegetation is rupestrian fields (high altitude rocky grassland savanna). In this vegetation, plant communities colonize shallow, acidic, extremely nutrient-poor, excessively drained soils, and experience strong water shortage during the dry winters (Giulietti et al. 1997). In each location we collected seeds from 10 randomly chosen individuals along a 300-m transect. Locality 1 was located at 970m and Locality 2 was located at 1230m. At the study area, *M. albicans* fruits at mid-wet season but there is some phenological variation correlated with altitude. At higher elevation fruiting peaked in December while at lower altitude fruiting peaked in January. Seeds were collected around the canopy of fruiting individuals and 4 replicates of 25 seeds per individual were set to germinate in Petri dishes at 25ºC under a 12-hr photoperiod for 30 days. Four replicates of 25 seeds per individual were randomly chosen and weighed on a digital scale. Nested ANOVAs were used to determine the effects of individual and altitude on germinability (%, arcsine transformed), mean germination time (MGT, days) and seed mass (log transformed).

## Results and Conclusions

Both altitudinal and individual variation significantly affected germinability (Table 1). Germinability at higher elevation (64.2 ± 4.5%) was 3-fold higher than at lower elevation (20.8 ± 6.8%). Among-individual germinability ranged from 0 to 86%. We did not find any effect on MGT caused by altitude in spite of significant among-individual variation (Table 1). MGT was 10.56 vs 10.73 days in lower and higher altitude, respectively. Both altitudinal and individual variation significantly affected seed mass (Table 1). Mean seed mass at lower elevation (0.013g) was 2-fold higher than at higher elevation (0.007g).

Our findings are in accordance with previous studies that investigated individual variation and altitudinal effect on germination. In alpine environments, the few studies investigating the effect of elevation on seed weight within species have mainly detected a decrease in seed weight with increasing elevation (Wirth et al 2010). Seeds from heathlands in Spain collected at the highest sites reached higher and quicker germination than those from the lower ones. This pattern was mostly related to dormancy-breaking mechanisms (stratification), which is not the case with *M. albicans* (Vera 1997). On the other hand, Giménez-Benavides et al. 2005 found that germination was highly variable among altitudes, but results were inconsistent among species. Therefore, higher germinability at the higher elevation compared to the lower elevation, as found in this study has been previously described.

Variation in germinability among individuals at the same altitude is best explained by genetic differences, since *M. albicans* is an apomictic species. Plants at the same altitude are all under the same microhabitat conditions, but each individual has its own process of seed development and maturation. Nevertheless, variation in seed mass and germination among individuals at the same altitude can also be attributed to fruit position on the mother plant (Gutterman 2000). Our results show

that both altitudinal and individual variation have strong effects on intra-specific patterns of seed mass and germination, despite the lack of differences in germination timing. Plants at higher altitudes produced smaller seeds with higher germinability whereas plants at lower altitudes produced heavier seeds with low germinability. Further studies are needed in order to determine whether phenotypic plasticity or ecotype formation account for the described variations along an altitudinal gradient.

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**Table 1.** Results of the nested ANOVA for the effects of altitudinal and individual variation in germinability (%), mean germination time (MGT) and seed mass of *Miconia albicans*.

| Source of variation | Germinability | | MGT | | Seed mass | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | p |
| Altitudinal | 127.5 | < 0.000 | 0.71 | 0.791 | 744.5 | < 0.000 |
| Individual | 4.8 | < 0.000 | 2.88 | 0.007 | 26.8 | < 0.000 |

## Water-impermeable Seeds – What Are They For?

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### Introduction

Two strong selective forces shape the evolution of seed traits: seeds need to be able to select the right time and place to germinate, and they also need to avoid being eaten.

Water-impermeable seed coats [physical dormancy, hard seeds] occur in 15 plant families world wide, including economically important groups such as legumes. Hard-seededness has hitherto been interpreted in the light of germination regulation, i.e. as a dormancy-regulating trait.

Here, we test the hypothesis that water-impermeable seeds make more ecological sense as an anti-predator device, i.e. that dry seeds release few volatile chemicals and as a result seed predators that locate seeds primarily by olfaction find it difficult (or impossible) to find dry seeds.

### Methods

Hard and soft seeds of *Vicia sativa* L. and *Robinia pseudoacacia* L. were offered to a model seed predator, domesticated desert hamsters *Phodopus roborovskii* (Satunin), in caches of 20 hard or 20 soft seeds on top of or beneath either wet or dry gravel (Fig. 1). In follow-up experiments, seeds were also offered as circles of 20 seeds, 10 hard alternating with 10 soft seeds, on top of wet or dry gravel, and as mixed caches of 10 hard and 10 soft seeds beneath wet and dry gravel. We also used GC-MS to detect the volatile chemicals released by hard and soft seeds.

### Results and Conclusions

Water-impermeable seeds were up to 10 times less likely to be detected and removed by a rodent seed predator than naturally water-permeable seeds from the same species (Fig. 2). In contrast, soft seeds were not preferred to hard seeds when the latter were artificially imbibed (after scarification), nor when all seeds were presented dry. Our results show that water-impermeable seeds avoid detection and removal in moist environments. GC-MS analysis identified compounds that were released in much greater abundance by moist soft seeds compared to dry and hard seeds. The latter do not imbibe and therefore do not release metabolically-derived volatile substances that enable their predators to locate them.

Many mammalian granivores are scatter-hoarders that both re-cache their own and pilfer others' caches. In a second set of experiments, we found that the advantage of hard seeds is substantially increased for buried seeds, and persists even when the seeds are buried in mixed caches. Thus, in addition to predator protection, hard seeds may also confer dispersal advantages, as they will be "left behind" by granivores during finding and pillaging of caches.

Our findings imply that hard seeds are virtually invisible to their predators, even under moist conditions.

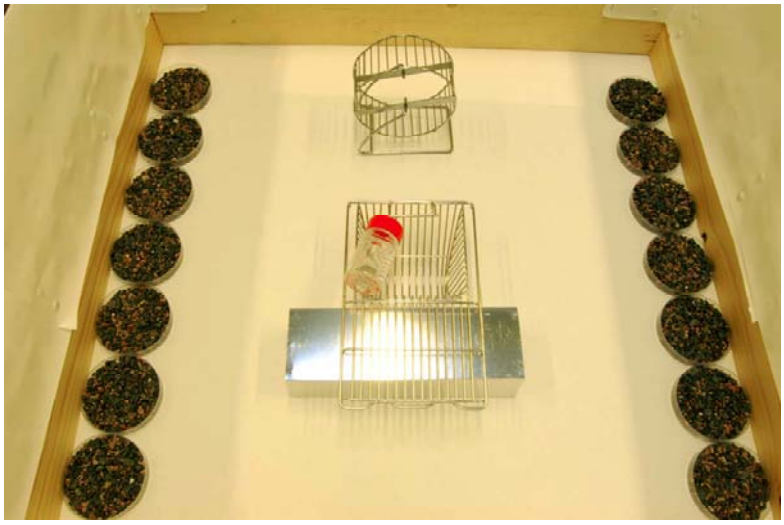

**Figure 1**. Experimental arena. Of the 14 dishes of gravel, six contain seeds.

a) Wet seeds on top of wet gravel

b) Wet seeds buried in wet gravel

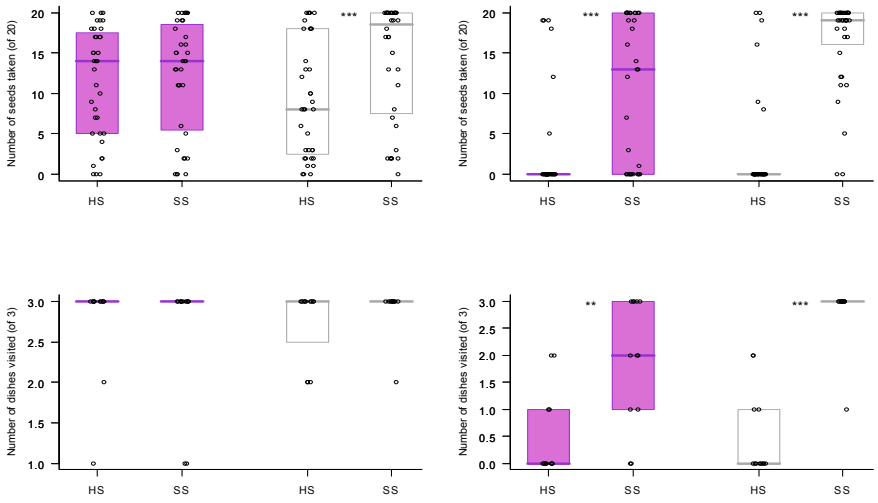


**Figure 2**. Results of first experiment, with wet (i.e. imbibed if soft) seeds (a) on top of or (b) beneath wet gravel (*Vicia* = pink, *Robinia* = clear). Hamsters readily located all caches when seeds were on top of gravel (bottom left) and removed most *Vicia* seeds (hard or soft), but preferred soft *Robinia* seeds. When seeds were buried, only caches of soft seeds were found (bottom right) and removed (top right).

## Dormancy Mechanisms and Germination Timing of *Dalea pinnata* (Fabaceae) Seeds

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### Introduction

*Dalea pinnata* (J.F. Gmel.) Barneby is a native forb present throughout the southeastern United States. This species is sometimes a component of seed mixes harvested for restoration and it presents an opportunity in terms of production and sales for the emerging native wildflower seed industry in the southeast. However, the germination ecology of *D. pinnata* has not been reported. Understanding dormancy mechanisms and germination timing may be important when determining success and monitoring criteria for restoration projects (Maschinski et al. 2005), considering germplasm conservation (Hong et al. 1998), and establishing propagation methods (Baskin and Baskin 2004). Our objective was to assess the germination timing and potential dormancy mechanisms in seeds of *D. pinnata*. Specifically, we address the following questions: 1) To what extent do seeds germinate after dispersal and burial? 2) Do seeds possess physical dormancy? 3) Can germination be enhanced using dormancy-breaking treatments?

### Methods

We harvested easily detachable legumes from a minimum of 50 randomly selected plants in central Florida during December 2006. Four 25-seed replicates were sown on quartz sand in 3.8 L pots, covered with a thin layer of sand and placed in a shadehouse (50% light transmittance). The sand was saturated once prior to seed sowing, and then once weekly if no rain occurred during the previous week. Emerged seedlings were counted weekly and removed. Data were collected for one year. For burial experiments, seeds were placed in four mesh bags with 320 seeds per bag and buried to a depth of 2.5 cm in each of four pots as described above. One bag was exhumed every three months. Rinsed seeds were then incubated at alternating temperatures that simulated conditions in Florida during the winter (22/11°C), spring (27/15°C), summer (33/24°C) and fall (29/19°C). Each treatment consisted of four 10-seed replicates. To determine the extent to which imbibition occurred, the increase in fresh mass over a 24 h period was recorded in four 25-seed replicates of scarified (i.e., nicked) and non-scarified seeds. Dormancy-breaking treatments consisted of soaking seeds in 18 M sulfuric acid for 5 or 10 min, nicking, or leaving the testa intact. Each treatment consisted of four 10-seed replicates. Germination tests in the dormancy experiment were conducted at 15, 25 or 35°C. with a 12-hr daily photoperiod (80 µmol/$m^2$/s, cool white fluorescent light). Viability was tested using tetrazolium staining techniques (Pérez et al. 2009).

### Results and Conclusions

Emergence gradually increased to 15% by week 44 after which no further emergence was observed. The majority of remaining seeds (64.0 ± 3.3%, mean ± SE) died, most likely due to seed pathogens or invertebrate activity. The intact seeds that remained (ca. 20%) were all viable based on TZ testing. Germination in bags did not exceed 8% regardless of burial duration; however, viability of residual seeds exceeded 90% (Fig. 1). Fresh mass increased about 12% for non-scarified seeds about 145% for scarified seeds (Fig. 2). After 24 hr, about 70% of scarified seeds had germinated, but no germination was observed for non-scarified seeds. Mechanically scarified seeds germinated to 100% regardless of incubation temperature, while germination of acid-scarified seeds ranged between 80 and 100%. Germination of control seeds did not exceed 45%. The differences in scarification treatments were significant (Table 1). Overall, seeds in these treatments completed germination in about 8 d, but seedlings incubated at 35°C succumbed quickly after emergence. The small initial germination and imbibition response in *D. pinnata* may be attributable to harvested seeds that have gained the ability to germinate but have not completed the maturation drying phase of seed development. The majority of seeds in the population, however, are physically dormant (i.e. water impermeable testa). Physical dormancy is most effectively overcome by mechanical scarification, but resultant seedlings should be grown out at 25°C. Finally, the ability of nearly all seeds to persist in the soil and to maintain viability for at least one year suggests formation of a soil seed bank.

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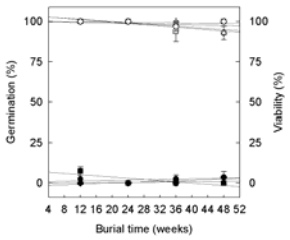


Figure 1. Mean (± SE) germination (closed symbols) and viability (open symbols) of buried *Dalea pinnata* seeds incubated at 33/24°C (circles), 29/19°C (triangles), 27/15°C (squares), or 22/11°C diamonds for 4 wk. Lines included as an aid to the eye. There were no significant effects of temperature ($F_{3,45}$ = 0.71, p = 0.55), burial duration ($F_{3,45}$ = 1.46, p = 0.24) or their interaction ($F_{9,45}$ = 1.81, p = 0.09) on germination. Viability was not significantly affected by temperature ($F_{3,45}$ = 0.31, p = 0.82) or temperature × burial duration ($F_{9,45}$ = 1.25, p = 0.29), but burial duration had a slight significant effect ($F_{3,45}$ = 2.49, p = 0.07).

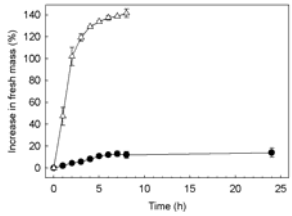


Figure 2. Mean (± SE) increase in fresh mass of *Dalea pinnata* seeds mechanically scarified by nicking the testa (open triangles) or with intact testa (closed circles).

Table 1. Analysis of variance for class comparisons between scarification treatments of *Dalea pinnata* seeds.

| | | | | Required *F* | |
|---|---|---|---|---|---|
| Source | df | MS | Observed *F* | 5% | 1% |
| Scarification | 3 | 2.65 | 126.40 | 2.89 | 4.44 |
| Ctrl v. Scarified | 1 | 7.27 | 264.32 | 4.14 | 7.47 |
| Acid v. Nicked | 1 | 0.40 | 19.10 | 4.14 | 7.47 |
| $Acid_{5min}$ v. $Acid_{10min}$ | 1 | 0.29 | 13.78 | 4.14 | 7.47 |
| Error | 33 | 0.02 | | | |

# Seed Dormancy and Germination Ecophysiology in *Viburnum furcatum* Blume ex Maxim. (Caprifoliaceae) with Underdeveloped Embryos

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## Introduction

The genus *Viburnum* (Caprifoliaceae) consists of 150 species of shrubs and small trees, geographically widespread in the temperate and warm areas of Asia and North America (Mabberley, 1997). *Viburnum furcatum,* a perennial deciduous shrub, is native to temperate Asia and is widely distributed in Japan, Korea and Russia (South Sakhalin and South Kurils). It naturally inhabits mesic woods and forest edges in the mountains. Based on previous studies on class of seed dormancy in the genus *Viburnum* (Baskin et al. 2009), we assumed that *V. furcatum* seeds too would have morphophysiological dormancy (MPD). However, to our knowledge, no information is available on seed dormancy and germination ecophysiology in *V. furcatum.* In the present study, we investigated germination phenology of *V. furcatum* seeds in outdoor field conditions and also investigated temperature requirements for embryo growth and for radicle and cotyledon emergences in controlled laboratory conditions to determine the class and level of seed dormancy (see Baskin and Baskin 2004) for *V. furcatum* seeds collected from a native population in Japan.

## Methods

Mature black fruits were collected on 1 and 6 October 2007 from plants growing in woods near Sapporo, Hokkaido, Japan. A week after collection, all seeds (true seed plus endocarp) were removed from pulp (exocarp and mesocarp) and dried under ambient room conditions (ca. 25°C) for 4-7 days before studies were initiated. On 10 October 2007, seeds were buried outdoors in soil on the campus of Hokkaido University, and embryo growth, radicle and cotyledon emergence were monitored at regular intervals for germination phenology. Daily temperatures at the soil surface were also measured using data loggers. Under controlled laboratory conditions, several experiments were conducted to determine the temperature requirement for embryo growth, and for radicle and cotyledon emergence. Additionally, to determine the specific level of dormancy, the effect of $GA_3$ on radicle emergence was tested. For all laboratory experiments, different constant, daily alternating and combinations of temperature sequence (i.e., move-along experiments) were used.

## Results and Conclusions

Embryos in mature seeds dispersed in October were underdeveloped and began to elongate in late June/early July of the following year; they were fully elongated by the end of August (Fig. 1). Radicle emergence began immediately after embryo growth and continued till mid October. Cotyledon emergence was observed during the second spring from mid-April to mid-May after snowmelt. In laboratory experiments, embryos hardly grew at 0°C during 90 days of incubation, and they fully grew at continuous temperature of 15°C and 25/15°C (60d) → 15/15°C (Fig. 2). Radicles began to emerge from seeds incubated at continuous temperature of 15°C and 25/15°C(60d) → 15/15°C. In seeds incubated in a simulated annual temperature sequence of summer (25/15°C) → autumn (15/5°C) → winter (0°C) → spring (15/5°C) → summer (25/15°C), embryo growth and radicle emergence were observed at summer/autumn temperature and cotyledon emergence occurred at spring temperature (Fig. 3). Percentage of cotyledon emergence increased when seeds with radicles were exposed to 0°C for 120 days and then incubated at 15/5°C. Consequently warm/intermediate temperatures are favourable for embryo growth and radicle emergence, and cool/intermediate temperatures are favourable for cotyledon emergence in *V. furcatum*. $GA_3$ did not substitute for the requirement for warm temperature for radicle emergence. Thus, based on germination phenology and temperature requirements for embryo growth, radicle and cotyledon emergence, *V. furcatum* seed has deep simple epicotyl MPD, as reported in a majority of other *Viburnum* species.

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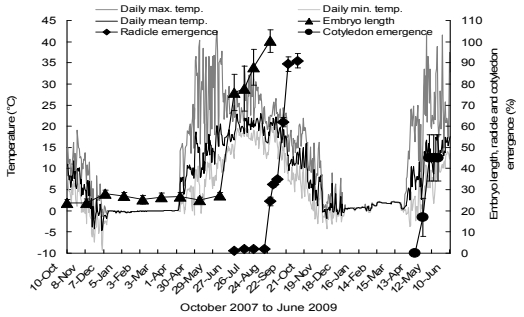


Fig. 1. Daily maximum, minimum, and mean temperatures and phenology of embryo growth, and radicle and cotyledon emergence in *V. furcatum* outdoors in Sapporo, Japan.

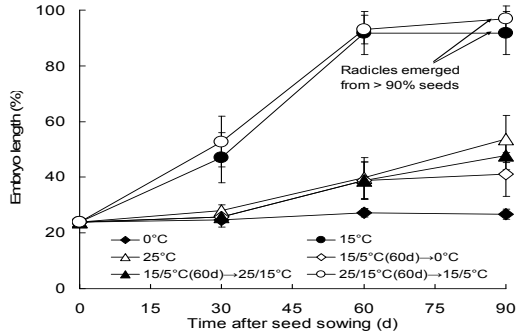


Fig. 2: Effect of constant and sequence of temperature on embryo growth of *V. furcatum*.

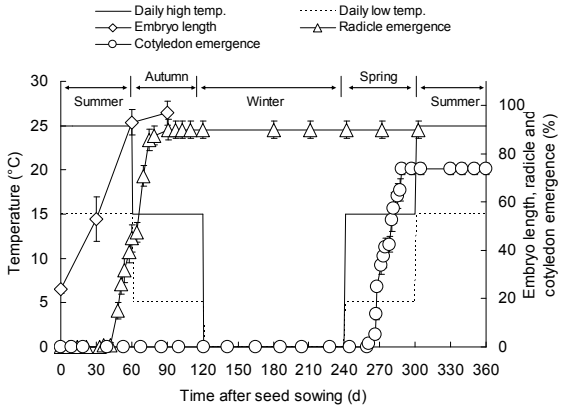


Fig. 3: Effect of simulated annual temperature sequence on embryo growth and on radicle and cotyledon emergence of *V. furcatum*.

# Soil Seed Banks of *Eupatorium adenophorum* (an Invasive Species) and Other Native Species under Different Tree canopies in a Central Himalayan Montane Subtropical Forest Ecosystem

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## Introduction

Understanding seed bank composition and density is especially important when communities have been invaded by exotic species (Cox and Allen, 2008). Effective management of invasive species requires detailed knowledge of their reproductive biology, including seed production, persistence in soil seed banks (Fenner and Thompson, 2005) and response to disturbance (Fisher et al. 2009). *Eupatorium adenophorum* has been categorized as one of the worst invasive alien weeds, naturalized in several parts of India and threatening native biodiversity by disruption of seed inputs of native species in the seed bank. The present work examined the composition of the soil seed banks under different forest canopies relative to abundance of *E. adenophorum*, as relatively very few data are available on soil seed banks from woodland communities (Thompson et al. 1997) considering different tree canopies (Godefroid et al. 2006). The objectives of the study were: (1) to investigate whether species composition and richness of soil seed banks are affected under different tree canopies; (2) to examine abundance of *E. adenophorum* in seed banks; and (3) to compare soil seed banks with above ground vegetation for restoration of native species.

## Methods

Soil samples were collected from sites where *E. adenophorum* was in abundance as ground vegetation, under two tree canopies, i.e., pure pine canopy and oak-dominated mixed canopy, and an open site. The study sites were located at 30°08'N latitude and 78°48E longitude and between 1118 – 1455 m in the central Himalayan Montane Subtropical Forest Ecosystem along the Srinagar-Pauri Garhwal National Highway in Uttarakhand in northern India. Within each selected site, four soil samples were collected horizontally and vertically in blocks of 16 x 16 cm in two layers (0-5 and 5-10 cm depth). Sampling was carried out during late spring (9. A pril 2009), before dispersal of fresh seeds and input in seed bank, and after emergence of autumn and spring germinating seeds. All samples (n = 24) were washed under tap water using a fine sieve (0.15 mm mesh width) to concentrate samples and remove fine soil particles from the sample (Ter Heerdt et al. 1996). The concentrated soil samples were immediately spread over sterilized sand in trays and incubated in a mist chamber for seedling emergence. The temperature inside the mist chamber was set at 25°C, but during mid-day during summer the temperature sometimes rose to 41°C. Emerged seedlings were counted and removed soon after their identification to species. Unidentified seedlings were transplanted into the pots and maintained until flowering for final identification.

## Results and Conclusions

The seed bank did show considerable differences under different tree canopies in terms of size, composition and depth of occurrence. All species and layers taken together, the species richness and soil seed bank size ranked as follows: open site > pure pine canopy > oak dominated mixed canopy (Fig. 1a,b). A total of 59.68% of seeds were found in the upper soil (0-5 cm) layer and altogether 43 species were present in soil seed bank. *Cyperus rotundifolia* showed exceptionally high seed density (12331 seedling/$m^2$) followed by *Mazus pumilus, Anagallis pumila* and *E. adenophorum* in the open site, whereas *Oplismenus burmannii (*2269 seedling/$m^2$) was highest in density under the pure pine canopy. Seed density of *E. adenophorum* (804 seedling/$m^2$) was maximum under the oak-dominated mixed canopy (Fig. 2). However, when all study sites are taken together for average ranking of seed density, *E. adenophorum* ranked first among all species. The aboveground vegetation represents only 67% of the species present in soil seed bank. *Mazus pumilus* was very rare in aboveground vegetation as compared to buried seeds in the open site. *Centella asiatica,* a very promising medicinal plant, was almost absent in aboveground vegetation but was present (68 seedling/$m^2$) in the seed bank at the open site. Thus, soil seed banks may play a crucial role, as a good source of germplasm, in the restoration of these ecologically and economically important native species.

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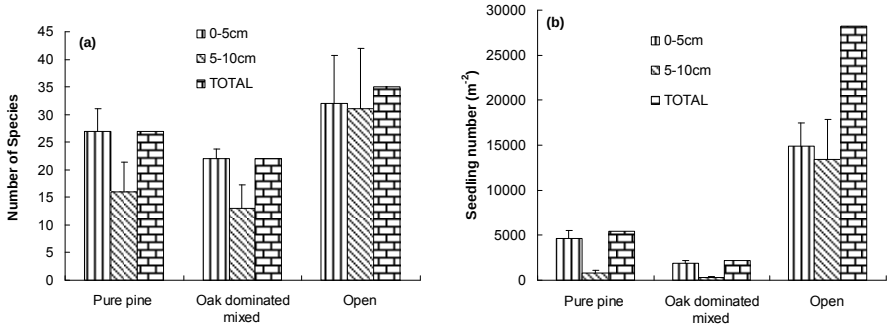


Fig. 1: Species richness and seed density in the seed bank of different soil layers under different overstory types.

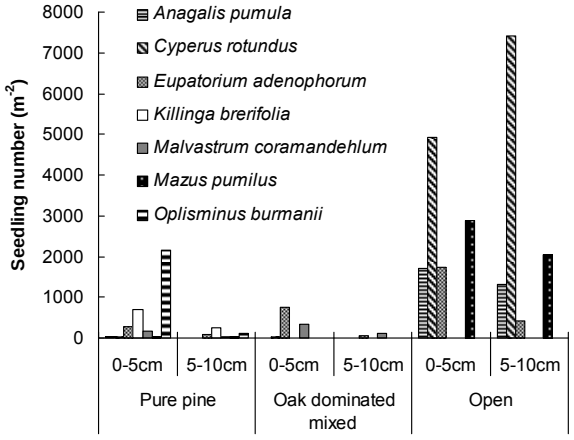


Fig. 2: Seed density of most prominent species in the seed bank.

## Why a Species Occurs Where it Occurs – The Role of the Germination Niche

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**Abstract**
The global and local distribution of a plant species is limited by climatic and edaphic filters. Despite these facts, it is not clear how these filters are acting on certain life history stages. Until now, climatic or edaphic envelopes are only restricted to the adult plant. However, germination requirements of a species may also affect its distribution and be responsible for its occurrence under certain habitat conditions. This is shown for species along hydrological gradients along river banks or lake and pond shores.

In a first experiment we tested the germination and establishment of a species set occurring along this gradient applying different water regimes from flooded to wet and moist/dry conditions. There were clear patterns which showed that germination and establishment occurred only under either moist/dry, wet or flooded conditions or at least mainly under one of the tested regimes.

In a second experiment we tried to find out which environmental factors are responsible that germination occurs only under certain hydrological conditions. Germination triggers were e.g. constant or fluctuating temperatures, aerobic or anaerobic conditions and light or darkness. Additionally, we found other seed ecological adaptations related to a species occurrence in a specific habitat along the studied gradient such as seed bank persistence and water dispersal potential. Germination but also seed ecological characteristics, therefore, allow to predict a species occurrence in the studied habitats along the hydrological gradients.

# Does Slow Germination Have to Mean the Presence of Dormancy?

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**Abstract**
Physiological dormancy in seeds of higher plants ensures that germination does not occur when conditions are unfavourable for seedling establishment. Until relatively recently, descriptions such as: 'inactivity of the meristems'; 'suspension of visible growth' and 'arrest in development of seed embryos' were commonly regarded as definitions of the dormant state in plant organs. However, many seed biologists now regard the time it takes seeds to visibly germinate as the main indicator of whether or not seeds are dormant. Yet a direct linear relationship between the rate of biological reactions and temperature has been known for over a hundred years and one consequence of this fact is that seeds germinate slowly at low temperatures. Indeed, for many cool temperate plants, seed germination only occurs at low temperatures, and as a result the process can take many weeks, particularly if embryo growth has to proceed inside the seed before germination is evident. But, are such seeds dormant? Unlike *Cardiocrinum cordatum*, and other species, where dormant embryos fail to develop under natural conditions for several seasons following dispersal, embryo development and growth in seeds of *Anemone nemorosa* and many other temperate species begins immediately after dispersal, and visible germination occurs under natural conditions without a period of developmental arrest. According to traditional definitions this cannot be regarded as dormancy. Such plants are able to achieve the same ends as those that have evolved true dormancy - seedlings are not produced when conditions are unfavourable - but, they achieve this by slowing development, rather than shutting it down completely, This paper will present evidence that questions whether 'morphological dormancy' is an appropriate term for describing slow germination in seeds with small embryos.

# Seed Bank Dynamics along an Experimentally Created Productivity Gradient in a Flood-plain Grassland

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## Introduction

The addition of limiting nutrients to herbaceous plant communities results in increased primary production and decreased plant species diversity (e.g., Lepš 1999, Sundig et al. 2005). Nitrogen addition intensifies competition for light and may thus restrict the density of local seed rain. High N levels directly affect soil seed bank, resulting in changes of species composition and diversity – high soil $NO^{2-}$ and $NO^{3-}$ concentrations may break physiological dormancy and stimulate seed germination (Baskin & Baskin 2001). This, in turn, results in a decline in density of viable seeds in the soil seed bank.

A handy method to decrease the plant-available N levels in soil is the addition of sucrose, sawdust etc. as C source to the community (e.g., Török et al. 2000, Corbin & D´Antonio 2004). Several studies have documented that C addition stimulates soil microbial activity that causes an increased immobilization of inorganic N. Also, there is evidence that addition of C has negative effect on aboveground vegetation biomass (e.g. Blumenthal et al. 2003). Changes in aboveground vegetation composition after C addition have been documented in several studies, but there is almost no experimental information about the soil seed bank dynamics in conditions where the productivity of a community decreases.

In order to investigate the dynamics of soil seed bank size and species richness in different productivity conditions we conducted a study on an experimentally created productivity gradient in a flood-plain grassland in south-eastern Estonia.

## Methods

The study site is a 2 ha patch of flood-plain grassland near Ahja river, south-eastern Estonia (58˚06´36˝N, 27˚4´14˝E), on sandy soil with pH (KCl) 4.6–5.2 and total nitrogen content 0.1–0.2%. Floods are rare, but may occur at an interval of ca. 10–12 years. The grassland is traditionally managed by annual mowing in early August. Productivity was increased by fertilization (long-lasting complex fertilizer 125 g $m^{-2}$ $yr^{-1}$; N – 20 g $m^{-2}$ $yr^{-1}$, P – 6 g $m^{-2}$ $yr^{-1}$ and K – 11 g $m^{-2}$ $yr^{-1}$) and decreased by carbon addition (1000 g of sucrose $m^{-2}$ $yr^{-1}$) during a seven-year experiment.
The soil seed bank was sampled at the end of June 2002 and 2009. The sampling time in June was scheduled to assess only the persistent part of seed bank. Eighteen permanent plots (8 × 8 m; 6 fertilized, 6 with sucrose and 6 control) were studied. Six soil cores (4 cm diameter) of 10 cm depth were collected at regular spacing. After removal of the litter, the samples were divided into subsamples of 0–5 cm and 5–10 cm depth. The 6 cores at each depth were pooled within treatment and plot. The soil samples were passed through a coarse sieve to remove roots and other dead plant material. The remaining material was spread in a thin layer over a sterilized potting soil in plastic trays. The emerged seedlings were identified as soon as possible, counted and removed during the duration of the growing period. No attempt was made to assess the number of ungerminated seeds remaining in the samples.

## Results and Conclusions

Twenty-seven vascular plant species were detected in the seed bank in 2002 and 38 species in 2009, of which 12 and 17 were not recorded in the vegetation, respectively. In 2002, 25 and in 2009 34 vascular species were recorded in the standing vegetation, 10 and 14 of which were not detected in the seed bank, respectively. The seed bank and standing vegetation shared 15 species in 2002 and 21 species in 2009, respectively.
The proportion of soil seed bank species which was represented also in aboveground vegetation was 47.1% for fertilized plots in 2003 and 26.7% in 2009; in plots with additional carbon the percentages were 45.5% and 59.1% and in control plots 52.9% and 51.9% accordingly. Thus, the similarity of the species composition between the soil seed bank and the aboveground vegetation decreased with N addition and increased with sucrose addition. The abundance of most species in the seed bank of

fertilized plots decreased during the experiment. *Gnaphalium uliginosum* L. and *Ranunculus acris* L. were species that disappeared entirely. *Campanula persicifolia* L. and *Agrostis capillaris* L. were species that established their seed bank with decreasing productivity.

The results of the 7-year field experiment showed that manipulation of productivity in two different directions – towards more fertile and less fertile conditions – did not cause marked changes in either species richness in the soil seed bank (Fig.1A) or in mean seed density (Fig. 1B), although there were significant changes in aboveground species richness and biomass (Zobel et al. 2010, manuscript). An interesting finding of our research is that increased productivity tends to lower and decreased productivity tends to enhance the variability of species composition in the soil seed bank (Fig. 1C).

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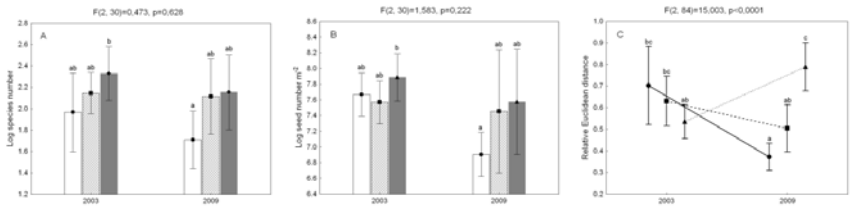


Figure 1. The dynamics of species richness (A), seed density (B) and variability of species composition (C) among experimental treatments. White bars denote fertilization; crosshatched and gray bars mark control and sucrose addition treatments (on A and B) respectively. The means marked with filled circles indicate fertilization; squares mark control and triangles mark sucrose addition treatments (on C) respectively.

# Active Dispersal of *Ambrosia trifida* Seeds by the Earthworm *Lumbricus terrestris*: Impact on Seed Survival and Plant Establishment

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## Introduction

Secondary seed dispersal of large-seeded weedy species is critical to seed survival and seedling establishment, yet these dispersal mechanisms are poorly understood. *Ambrosia trifida* L. is a native North American annual of increasing importance worldwide as an agricultural and allergenic weed. It produces large, short-lived seeds that are heavily consumed by rodents, birds, and insects. The naturalized European earthworm, *Lumbricus terrestris* L., removes giant ragweed seeds from the soil surface and stores large numbers of them in its burrows (Regnier et al. 2008). Seedlings subsequently emerge from the burrows, resulting in an association of the two species that is observed across the eastern U.S. Corn Belt (Schutte et al. 2010 *in review*). This association protects giant ragweed seeds from predation, but the benefits to *L. terrestris* are unclear since it cannot ingest the whole seeds. *Lumbricus terrestris* feeds on decaying organic debris collected from the soil surface and then buried inside its burrows and mounds (middens) over the burrow openings. It also collects inorganic materials such as pebbles, bits of brick, and plastic fragments, which physically resemble the hardened seeds of giant ragweed. Its reason for collecting inorganic debris and giant ragweed seeds is unknown. The objective of this research was to determine if *L. terrestris* forages actively rather than incidentally for giant ragweed seeds as a potential food source.

## Methods

*Lumbricus terrestris* individuals were raised singly in 15-cm-diam PVC pipes and given a choice of giant ragweed seeds, exact replicas of giant ragweed seeds made from plaster, and plaster "debris" resembling plant stems. Seeds and the plaster objects were arranged on the soil surface at two radii from the burrow: singly (inner radius), or in piles of six each (outer radius). The experiment was conducted twice, and each experiment included eight Lt individuals (replicates) monitored for 12 days. The PVC pipes were monitored nightly with time-lapse digital video, and data were collected on *L. terrestris* foraging behavior; and the number, type, and position of objects taken. Statistical analyses were performed with R using linear mixed models to test specific hypotheses on selectivity and foraging method.

## Results and Conclusions

The earthworm exhibited typical nocturnal foraging behavior by emerging from its burrow and collecting objects from the soil surface. It collected real seeds preferentially over artificial seeds and debris ($p < 0.01$), and it buried real seeds deeper than artificial seeds or debris ($p < 0.05$; Figure 1). We also observed the earthworm creating lateral tunnels and collecting surface seeds from below without emerging from the soil. Real seeds were more likely to be taken from below than above the soil surface compared to artificial seeds or debris ($p < 0.05$; Figure 2A), and also when the seeds were clustered in groups rather than arranged singly ($p < 0.01$; Figure 2B). Results suggest that *L. terrestris* is attracted strongly to seeds, possibly as a food source it detects by olfactory sensing from below or above the soil surface. Foraging from below the soil surface may be an adaptive behavior that reduces exposure to predators as the earthworm forages for seeds. These results provide evidence that *L. terrestris* collects seeds as a food source and that a mutually beneficial relationship has developed between *L. terrestris* and *A. trifida* that can increase *A. trifida* spread and persistence. Selective seed caching by this widely distributed earthworm species may alter plant community composition in agricultural and natural areas in North America and influence the evolution of seed traits.

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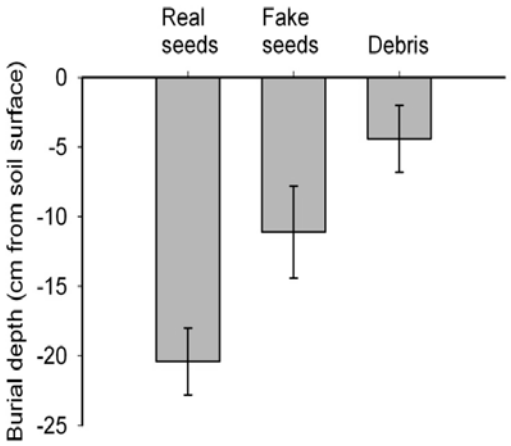


**Figure 1.** Burial depth of *A. trifida* seeds, plaster seed replicas ("fake seeds") and plaster debris collected by *L. terrestris*.

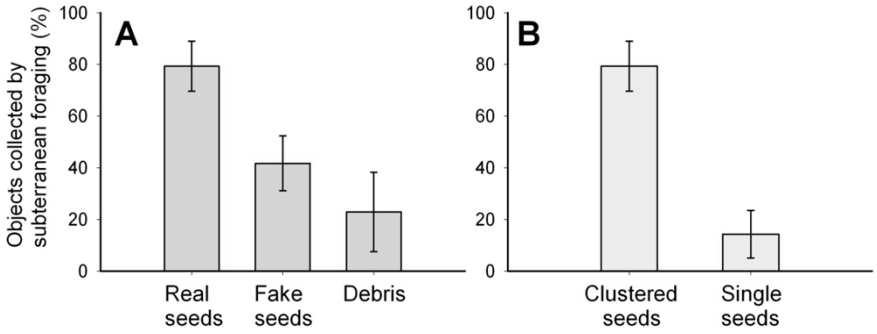


**Figure 2.** *Lumbricus terrestris* subterranean foraging of *A. trifida* seeds (A) in comparison with plaster seed replicas ("fake seeds"), and plaster debris; and,(B) when *A. trifida* seeds were clustered in groups rather than arranged singly.

# Seed Size and Photoblastism in Species Belonging to Tribe Cacteae (Cactaceae)

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## Introduction

Seeds have evolved mechanisms in order to detect environmental cues which enable them to restrict their germination to particular times and places to allow for a greater probability of seedling establishment (Khurana *et al.* 2001). One of these environmental signals is light.
The importance of light for seed germination and establishment has been well studied particularly in tropical forests, where light requirement for germination is tightly coupled to establishment. Germination behavior also depends on seed mass. Differences in seed mass have been related to the successional phase, as well as to the photoblastic response in tropical and temperate forests (Salisbury 1974, Milberg *et al*. 2000). This is understandable as small seeds lead to small seedlings that find it difficult to overcome the physical barrier imposed by soil and have less resources to survive and compete with established plants. Seed size is both genetically and environmentally determined, and it is also strongly correlated with species phylogeny, showing wide inter- and intraspecific variation (Khurana *et al.* 2001). Some seed traits such as small size, light requirement for germination and after-ripening period have been related to persistence in a soil seed bank. Within the Cactaceae we can find seed sizes than range broadly between less than 1 mm to above 5 mm and they show either a positive photoblastic response (*i.e.,* they require light for germination) or a non-photoblastic (*i.e.,* can germinate in light and in darkness) (Rojas-Aréchiga *et al.* 2000). Here, we determined the length and weight of seeds of each species studied. Then we determined the photoblastic response under controlled conditions of temperature (25 ˚C) and light (white light with a 12 h photoperiod and darkness) of several species belonging to tribe Cacteae (subfamily Cactoideae, family Cactaceae), in order to test if there is any relationship between photoblastic response and seed size in this tribe. For the Cactaceae inhabiting arid and semiarid environments of America, this relationship has not previously been studied.

## Methods

We collected mature fruits of 53 cacti species belonging to tribe Cacteae according to the phenology of each species and from different sites according to species distribution throughout Mexico. The life form for almost all species is globose, although some are globose-caespitose and barrel. The geographic distribution of the tribe Cacteae extends from Western Canada to Colombia, Venezuela and the Caribbean, and reaches a maximum diversity in Mexico, where it comprises 25 genera (Anderson 2001). We studied here species from 16 of these genera. We extracted the seeds from the fruits and left them to dry at room temperature (20 ± 2 ˚C). We randomly chose a sample of 50 seeds to determine length and mass. Each seed was individually weighed on an analytical balance (Sartorius CP225D). To obtain seed length we made five groups consisting of 10 seeds each. Each group was viewed and photographed under a microscope. Images were analysed with Vision Works Software (UVP Inc.), which allows the measurement of each seed within the sample. We correlated seed length and weight in order to find the relationship between variables with JMP software (v. 5.0, SAS).

To determine the photoblastic response, seeds were sown inside Petri dishes on 1% bacteriological agar. For all treatments four to seven replicates (depending on seed quantity for each species) with 25 seeds each were used. For light treatments, replicates were placed inside a transparent plastic bag in a germination chamber (Lab-Line model 844L). For darkness treatments, replicates were wrapped in two layers of aluminum foil and placed inside the germination chamber and checked one month later. For both treatments, temperature inside the germination chamber was 25˚ C with a 12 h photoperiod because these conditions are the optimum conditions for the germination of cactus seeds (Rojas-Aréchiga et al. 2000). Seeds were considered germinated once the radicle emerged from the testa.

**Results and Conclusions**

Seed length for the 57 studied species ranged from 0.545 mm (*Strombocactus disciformis* (DC.) Britton & Rose) to 3.02 mm (*Astrophytum capricorne* (A.Dietr.) Britton & Rose), with a qualitative range from the very small to the large seed category (Barthlott *et al.* 2000). More than half of the species studied (54.38%) may be classified as medium sized (*i.e.,* size ranged from 1.2 to 1.9 mm, Barthlott *et al*. 2000). Seed weights for 53 species studied ranged from 0.025 mg (*Strombocactus disciformis*) to 3.95 mg (*Echinocactus texensis* Hopffer). Most cacti seeds studied (71.6%) weighed less than 1 mg. We found that all studied species studied are positively photoblastic (*i.e*., they need light to germinate). Seed germination percentages among species varied greatly from less than 20% to 100%. There is a positive correlation ($R^2$ = 0.648, $P$ <0.0001) between seed length and seed weight for studied species, which supports findings for other species indicating that larger seeds are also heavier seeds. Results obtained suggest that light-dependent germination in members of this tribe is associated with phylogeny and/or life form rather than with seed mass. Information from available published literature showed that several Mexican species belonging to the same tribe are also positively photoblastic. A relationship between life form (barrel, globose and columnar) and light requirement among Cactaceae was first suggested by Rojas-Aréchiga *et al.* (1997), and has subsequently been supported by several papers. With respect to seed banks, a small size and a light requirement for germination are attributes that tend to favour the formation of a seed bank. Species studied may be classified as very small (8.77%) and small (17.54%), but there are no studies demonstrating the existence of seed banks for these species. However, the existence of aerial seed banks has been found for some positively photoblastic species belonging to tribe Cacteae. Also, rounded seeds, as the ones studied, are more prone to become part of a seed bank.This is the first paper that presents information on seed size in Cactaceae and its possible relationship with photoblastic response.

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# The Germination Niche as the Driving Force for the Occurrence of Aquatic Species – the Case Study of *Potamogeton* Species

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## Introduction

Species with extensive geographical and ecotopical distribution such as aquatic plants usually have a broad regeneration niche, which includes a germination strategy suited to avoiding the main risks of habitats they occupy (Grubb, 1977; Fenner et al., 2005). These risks are as follows: 1) inability of seeds and seedlings to withstand prolonged drought, because aquatic plants are fully adapted to living in a water environment; 2) emergence of seedlings in the season with low temperatures which can not only arrest subsequent growth, but also injure the seedlings; 3) germination of seeds into extremely variable environmental conditions that are changing both in space and time. To examine seed germination adaptations in aquatic habitats, two-phase germination experiments with five widely distributed species of *Potamogeton* (*P. compressus* L., *P.gramineus* L., *P.natans* L., *P.pectinatus* L., *P. vaginatus* Turcz.) were carried out.

## Methods

At the first stage of study experiments were conducted with intact seeds and seeds with broken seed coats (scarified seeds) to assess the ability of the seed coat to prevent germination under unfavourable conditions. In the second part of laboratory experiments, we analyzed the germination responses of seeds whose dormancy had been broken through scarification to a range of conditions reflecting aquatic habitats: constant and fluctuating temperatures, light and darkness, and different levels of oxygen supply (aerobic, flooded and hypoxic conditions). Germination experiments under hypoxic conditions were carried out only at the constant temperature because this combination of environmental factors is the natural situation.

## Results and Conclusions

In the first part of the experiment, we confirmed earlier reports that scarification significantly increases seed germination of *Potamogeton* species (Guppy 1897; Muenscher 1936; Teltscherová et al. 1973; Hay 2008). Presence of the hard seed coat can be interpreted as a protection against hazardous environmental conditions such as drought or freezing into ice. In the second part of the experiment, it was shown that scarified seeds germinate over a range of environmental conditions such as in light or darkness, under aerated, flooded or hypoxic conditions, and regardless of whether temperature is constant or fluctuating. This adaptation allows these species not only to germinate and establish under water but also to establish across a wide qualitative spectrum of aquatic habitats. This can serve as an explanation of the broad distribution of these aquatic plants.

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# Functional Ecology of Seed Persistence in the Soil – Insights from Germination in Cereal Weeds

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## Introduction

The persistence of seeds in the soil is crucial for the persistence of local populations and the reestablishment of plant communities after disturbances (Kalisz and McPeek 1992, Stöcklin and Fischer 1999). Seed persistence in the soil under field conditions is an important issue for restoration of plant communities and maintenance of local plant populations (Bossuyt and Honnay 2008). Primary and secondary dormancy as well as light requirements for germination are supposed to contribute to the formation of persistent seed banks (Baskin and Baskin 2006). Moreover, diurnally fluctuating temperatures can time germination and can therefore be linked to seed bank maintenance (Thompson *et al.* 1977). Whereas the importance of these factors has been often emphasized, and some of them studied for seed persistence, there is a lack of experimental data showing how these factors act through time. Here, we evaluate the relative importance of these factors using experimental data on soil seed mortality. Moreover, we want to test the hypothesis of an interaction between time after burial and germination ecological factors for the mortality of buried seeds.

## Materials and Methods

We studied annual plants from the agricultural landscape around the Luberon ridge in South Eastern France. In this work, we evaluated degree of dormancy (DD), light requirement for germination (RLG) and reaction to diurnally fluctuating temperatures (RFG) using germination experiments under controlled conditions. This was combined with an experimental comparative dataset on soil seed survival through time and germination parameters of 35 annual cereal weed species. This data set was gathered in a burial experiment under field conditions, described in more detail in Saatkamp et al. (2009), where seed samples were buried in 4 x 4 cm nylon mesh bags at 10 cm depth in a randomized block design. We estimated regression parameters using generalized linear mixed models (GLMM) including blocks, species and phylogenetic relationships as random effects and time after burial, DD, RLG and RFG as fixed effects using the MCMCGLMM routines in the R environment (Hadfield 2010).

## Results and Conclusions

Our data show that light requirements and dormancy are important features to maintain seeds ungerminated just after entering the soil, positively influencing survival of seeds in the soil. These effects diminish with increasing time after burial. Species with a mechanism to detect diurnally fluctuating temperatures have lower seed mortality in the soil. Unlike the other factors, this mechanism functions only after longer burial periods and especially in winter as compared to summer burial phases. These results confirm experimentally the functional role of dormancy and light for survival of seeds in the soil comparatively for a larger set of species. Our data highlight the detection of diurnally fluctuating temperatures as a third mechanism to achieve higher soil seed persistence for longer periods of burial. The functional analysis given in this article provides a basis for predictive studies on soil seed longevity. For future investigations, we thus recommend studying processes soon after burial and after longer periods of burial separately.

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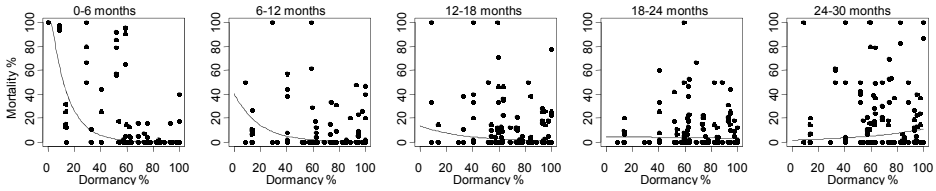


**Figure 1:** Interaction between time after burial and degree of dormancy: in early burial phases there is a clear declining mortality with increasing dormancy level; lines indicate parameter estimates from the generalized linear mixed model (GLMM) with Poisson error structure and log-link and blocks, species and phylogeny as random effects.

# Germination of *Miconia albicans* (Melastomataceae) along a Soil Fertility Gradient in the Brazilian Cerrado: a Three-year Study

Comment [SE2]:

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## Introduction

In most plant species, the seeds vary in their degree of germinability between and within populations and between and within individuals (Gutterman 2000). Habitat-mediated germination should be expected in habitats such as the Brazilian Cerrado *sensu lato* (Neotropical savanna), which is dominated by savanna, but at the same time with high landscape diversity. The dynamics of Cerrado vegetation are maintained by climate, fire regime, rainfall and soil properties (Oliveira-Filho et al. 2002). The vegetation physiognomies range from open environments with small shrubs such as *campo sujo* (CS; grasslands), through the *cerrado sensu stricto* (CE; savanna) to the *cerradão* (CD; woodland), a vegetation with a closed canopy and trees up to 15m high. Differences in soil fertility have been credited as the main factor controlling the variation among physiognomies. These vegetation types provide a natural gradient of structural complexity (CD > CE > CS) as well as nutritional environment stress (CS > CE > CD), thus offering an opportunity to test whether germination is affected by habitat. Here we evaluate for 3 years the differences in germination of *Miconia albicans* (SW.) Triana (Melastomataceae) seeds in individuals occurring along a soil fertility gradient. We test the hypothesis that: 1) germination is light-dependent; 2) germinability increases as nutritional stress decreases.

## Methods

*Miconia albicans* is 3-m tall shrub growing in aluminum-rich Cerrado soils across physiognomies. Ripe berries of 25 individuals per physiognomy were randomly collected in 2006, 2008 and 2009 from CS, CE and CD in Minas Gerais, southeastern Brazil. Eight replicates of 25 seeds per treatment were set to germinate in Petri dishes lined with moistened filter paper and incubated at 25ºC under 12-hr photoperiod or continuous darkness. Germination was checked by the emergence of the radicle and with daily observationns for 30 days, in order to calculate the mean germination time (MGT). A two-way ANOVA was performed in order to determine the effects of year of seed collection, physiognomy and year x physiognomy interaction on germinability and MGT.

## Results and Conclusions

Regardless of physiognomy, germinability was null or less than 3% under continuous darkness. Physiognomy ($F= 42.66$, $p<0.001$) and year of seed collection ($F= 23.33$, $p<0.001$) both affected germinability, but no significant interaction was detected ($F= 2.51$, $p<0.051$). For all years, germinability was statistically lower in CS but no significant difference was observed between CE and CD (Fig.1). We also found among-physiognomy ($F= 32.80$, $p<0.001$) and inter-annual variation in MGT ($F= 227.43$, $p<0.001$), but variation followed the soil fertility gradient only in 2006 and 2009, with lower values at cerradão, intermediate at cerrado and higher at campo sujo. Inter-annual variation in germinability and MGT was observed for all physiognomies (Fig. 2). Our results show that seeds of *M. albicans* are light sensitive, since its germination is light-induced and germination practically does not occur in darkness. As predicted, habitat-mediated germination followed the gradient, with lower germinability in the more stressed habitat and increasing germinability towards the cerradão (the upper extreme of the soil fertility gradient). Differences in environmental conditions such as temperature, mineral nutrition, water stress, light quality and day-length during plant development and seed maturation can account for differences in germinability of the seeds (Gutterman 2000). The described patterns were consistent through time and suggest that maternal effects play an important role in germination ecology in heterogeneous habitats.

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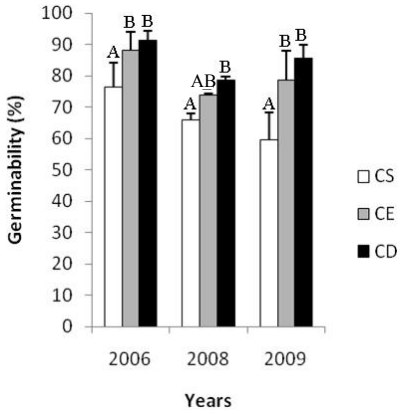


**Figure 1.** Germinability of *Miconia albicans* seeds collected from *campo sujo* (CS) *cerrado sensu stricto* (CE) and *cerradão* (CD) in the years 2006, 2008 and 2009. Different letters among physiognomies represent statistically different means by Tukey test (α =0.05).

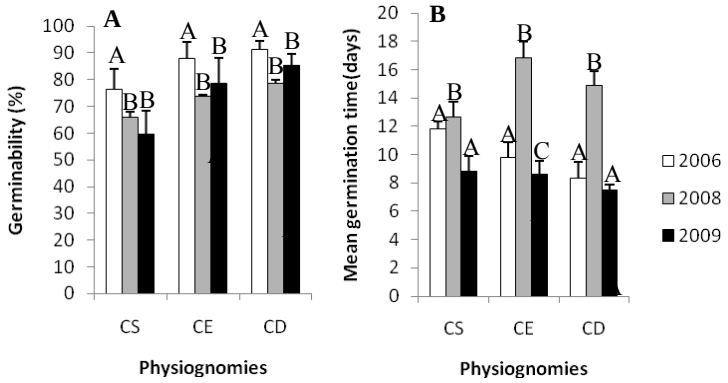


**Figure 2.** Inter-annual variation in germinability (A) and mean germination time (B) of *Miconia albicans* seeds collected from *campo sujo* (CS) *cerrado sensu stricto* (CE) and *cerradão* (CD). Different letters among physiognomies represent statistically different means by Tukey test (α =0.05).

# Seed Dormancy in *Hypericum philonotis,* a Weed Growing in Gaps of an Oak Forest

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## Introduction

*Hypericum* (L.) (Clusiaceae Lindey, Judd et al. 2002; Hypericaceae, Baskin and Baskin 2007) includes 360 herbaceous or shrubby species widely distributed in temperate and subtropical regions. Some of them, such as *H. perforatum* and *H. avicularиifolium,* have pharmacological interest (Çirak et al. 2007); other species, such as *H. gramineum* (Willis et al. 1997) and *H. perforatum*, have been studied from the ecological point of view (Pérez-García et al. 2006); the latter has potential to become a fast invader (Tisdale 1959). In contrast, *Hypericum philonotis* is an herbaceous species (Rzedowzki and Rzedowzki 2001) growing every 2-5 years in dense stands in forest gaps of the Parque Ecológico de la Ciudad de México (PECM). Plant population dynamics are initially determined by germination response and early seedling growth and recruitment (Zobel et al. 2000), in addition, it has been proposed that there is a relationship between seed size and species response to light and to fluctuating temperatures (Thompson et al. 1983). The seeds of *Hypericum philonotis* are very small, seed length is 0.5─0.8 mm and mean seed mass is 0.106 ± 0.005 mg. The purpose of this research was to identify the morphological and functional seed traits of *H. philonotis* and related them to the environmental factors prevailing in the microenvironment where they grow in the PECM.

## Methods

The seeds were collected during two production seasons, in June 2003 and in June 2005. Seeds collected in 2003 were scarified with concentrated hydrochloric acid (HCl 37.2%) for 0, 1, 2, 3, 4 or 5 min; germination of these seeds was tested at six constant temperatures (5, 15, 20, 25, 30 or 35 ºC), and at a fluctuating temperature (25─35 ºC) with or without the addition of 2000 ppm of gibberellins. Control seeds were not scarified and they were incubated without $GA_3$ at each temperature. Seeds collected in 2005 were pretreated with immersion in: a) acetone (3min), b) concentrated HCL (3min), c) hexane (1 min), d) hexane (3 min) or e) tap water (vigorous washing for 10 min), incubated with and without gibberellins (1000 ppm) and placed in growth chambers at 25 ºC in light or darkness. Another set of seeds pretreated with solvents or washed with tap water were also exposed to: 0, 1, 2, 3, or 4 weeks of stratification at 4 ºC, and then incubated at 25ºC in light. To test seed imbibition, the length and width of 30 seeds immersed in water (24 h) and 30 dry seeds were individually measured, micromorphology was observed with an optical microscope and the effect of the inhibitory activity of the acetone extract of a yellowish substance present in the *H. philonotis* seeds was tested on the germination and early growth of their own seedlings.

## Results and Conclusions

The treatments scarification, fluctuating temperature, acetone and gibberellins alone induced the highest probabilities of germination. Germination at constant temperature (20, 25 and 30 ºC) increased with the scarification time. Germination after any pretreatment was null at 5 ºC and low at 15ºC. In non-scarified seeds, the interaction between gibberellins and acetone or HCl promoted germination. In darkness, the probabilities of germination were near zero and gibberellins improved germination to very low percentages. Stratification times increased germination of seeds pretreated with hexane (1 min) or washed with tap water, while reducing germination in control seeds and those pretreated with acetone and HCl. The length and width of seeds incubated in water 24 h (0.502 ± 0.12 and 0.25 ± 0.05 mm respectively) were significantly higher than dry seeds (0.48 ± 0.13 and 0.23 ± 0.05 mm respectively) indicating water uptake by seeds. Observations in the photomicroscope showed the presence of a thick seed coat with lignified cell walls, the presence of lipids in the cuticle, and a lipid plug in the micropyle. With the immersion of seeds in acetone, an extract was obtained that inhibited the root and hypocotyl growth of the recently emerged seedlings. The restricted and sporadic presence of *H. philonotis* in gaps of the PECM seems be due to its complex dormancy. Morpho-functional seed characteristics combined with the biotic and abiotic environment regulate seed germination of the studied species. Due to the small seed size, seeds can be buried and be isolated from factors that trigger germination, as temperature fluctuation and light that may be acting as environmental cues for these seeds. Temperature fluctuation and acetone may melt or dissolve lipids

present in the seed covers, which apparently prevent root growth in the immature embryo. Gibberellins may remove the physiological dormancy in both sets of treatments. An afterripening period of 5 years did not alleviate dormancy. It is difficult to understand the inhibitory effect of the acetone extract on germination and on the root growth of *H. philonotis* in the field, because its non-polar nature and had no visible effect on seeds germinated with gibberellins. This suggests that increasing embryo vigor or removal of the seed covers is sufficient for *Hypericum* seed germination. It is necessary to evaluate how the inhibitor is liberated to the environment, its concentration in the soil and its removal under natural conditions. The fluctuation of temperature experienced by seeds in the upper layers of the soil may also eliminate the inhibitor and the restricting effect of all the components of the seed cover, including the thick layer of lipids. The observed emergence of pure *H*. *philonotis* stands may correspond to seed cohorts of different ages stored long-term in the soil, which have experienced the complete fulfillment of the environmental requirements that control the population dynamic of *H. philonotis*, unlike those seeds that have been recently dispersed.

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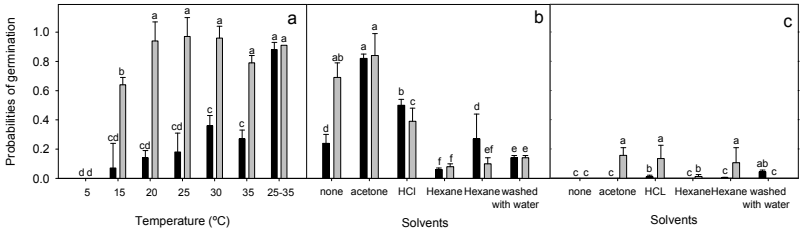


**Figure 1.** Effect of: a) temperature, b) immersion of seeds in solvents and incubation in light and c) immersion of seeds in solvents and incubation in darkness; with: 0 ppm (black bars) or 1000 ppm (gray bars) of gibberellins on the germination probabilities of *Hypericum philonotis* seeds. Letters indicate significant differences.

## Soil Seed Banks of *Eupatorium adenophora* and *E. odoratum* Along Road Sides in Yunnan

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### Introduction

Roads always provide good habitats for many invasive exotic species. *Eupatorium adenophora* (ED) and *E. odoratum* (EO) are common invasive species in subtropical and tropical regions. ED has a persistent soil seed bank and its seeds are dispersed before the rainy season (May). EO disperses its seeds in autumn (October). We attempted to determine the role of roads on the process of exotic species invasion.

### Methods

Ten roads were selected with wide range of construction history and construction designs in the middle and southern part of Yunnan. Twenty-six study points that had both invasive plants along the roadside and homogeneous vegetation cover were selected. At each point, 2-3 transects perpendicular to the road were established, and 10x10 x10 cm soil samples were taken along the transect at 0-1, 2-3, 4-5, 9-10, 14-15, 24-25, 29-30, 49-50, 99-100, and 199-200 m from the roadside. After quantification by germination in the greenhouse, soil seed density of each sample and relative density (percentage of the sample density relative to the sum of all sample densities in the same transect) were calculated.

### Result and Conclusions

In the subtropical region, seeds of *E. adenophora* were found in all samples (353) regardless of distance from the roadside or road type, with a density range from 100-107,000-m-2 and an average density of 9590±614-m-2. Soil seed density (one-way ANOVA, df=9,n=353, F=7.35, P<0.001) and relative density (one-way ANOVA, df=9,n=353, F=8.452, P<0.001) had a strong relationship with distance from the roadside. Soil seed density and relative density (Fig1.) increased with distance from the road to a distance of 4-5 m and then dropped down gradually. Seeds of *E. odoratum* were detected in the tropical region and its seeds were detected in 81.3% of soil samples with a highest density of 14,500- m-2, and average density of 2075±471- m-2. However, the relationship between distance and soil seed density (one-way ANOVA, df=5,n=48, F=0.98, P=0.44) and relative density (one-way ANOVA, df=5,n=48, F=2.46, P=0.05 was not significant (Fig. 2).

We concluded that: 1) seeds of *E. adenophora* and *E. odoratum* aggregate along the roadside in tropical and subtropical region of Yunnan, and that roads are important habitats for persistence of these two species, 2) soil seed density may influenced by the distance from the roadside, and roadside habitat may influenced by road maintenance, construction history and other vegetation factors.

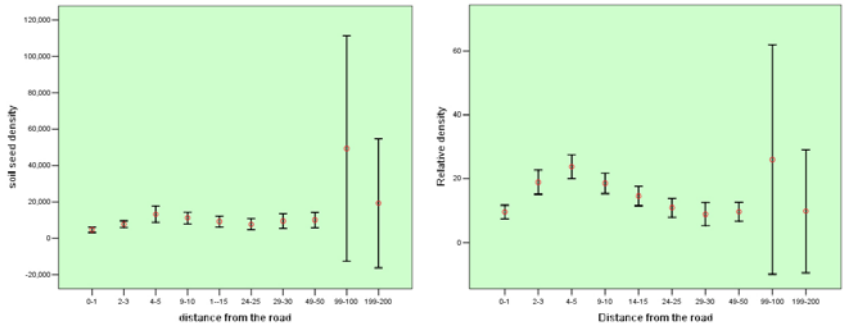


Fig.1 Soil seed density and relative density (Mean±2SE of ED and the distance from the road

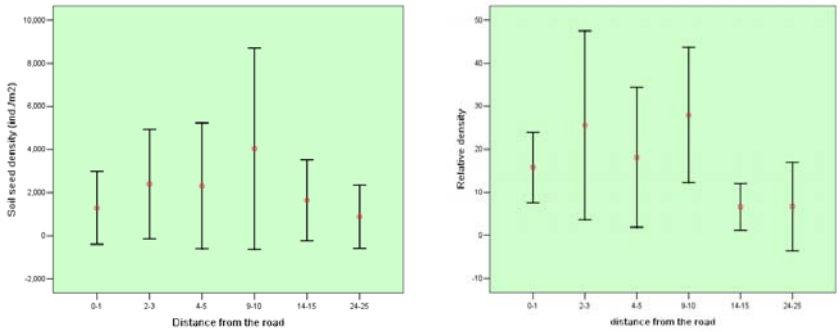


Fig 2. Soil seed density and relative density (mean±2SE ) of *EO* and the distance from the road

# Effects of Bird Frugivory on Germination of *Miconia* and *Clidemia* (Melastomataceae) Species from the Brazilian Cerrado

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## Introduction

The effects of bird frugivory on melastome berries are controversial, with reports on positive, null and negative effects of gut passage on seed germination (Elisson et al., 1993). In this study we investigate the effects of bird frugivory on germination of six *Miconia* species (*M. albicans*, *M. alborufescens*, *M. ferruginata*, *M. ibaguensis*, *M. irwinii* and *M. stenostachya*) and *Clidemia urceolata* from the Brazilian Cerrado (Neotropical savanna). We also compare the qualitative component of dispersal by comparing the effects of gut passage of seven bird species in four families.

## Methods

*Study site* - This study was conducted in "Estação Ecológica de Pirapitinga" (Três Marias, 18º21'34"S, 45º19'51"W, altitude 600 m) and Serra do Cipó (19º17'43"S, 43º35'39"W, altitude 1260 m), both in the state of Minas Gerais, southeastern Brazil. The climate of the region is characterized by a drier hot season between October and April and a wet/cold season between May and September. The vegetation is composed predominantly of cerrado (Neotropical savanna).

*Studied species* – All the species studied are trees and shrubs with heights from 1.5 to 5m, with contrasting phenology (Table 1). All species are widespread, occurring from Mexico to southern Brazil (Goldenberg, 2004), except *M. irwinii* and *M. alborufescens* which are endemic to the montane rocky savannas (Table 1). For the germination tests we selected seven birds species, distributed in four families: *Turdus leucomelas* and *Turdus amaurochalinus* (Turdidade), *Thraupis palmarum*, *Thraupis sayaca* and *Schistoclamys ruficapillus* (Thraupidae), *Mimus saturninus* (Mimidae) and *Zonotrichia capensis* (Emberezidae). These species have been frequently recorded eating fruits of Melastomataceae, although not all are primarily frugivorous (Sick, 1997).

*Germination tests* – Ripe berries were collected from at least 20 individuals per species and offered to captive birds. Gut-passed and hand-extracted seeds were set to germinate in Petri dishes incubated in BOD chambers under the constant temperature of 25ºC and a 12-hr photoperiod. Germination was checked daily for 30 days and % and mean germination time (MGT) were calculated. In order to determine the effects of seed cleaning, intact fruits were set to germinate under the same conditions (Samuels and Levey 2005).

## Results and Conclusions

Germinability from intact fruits was null (for *M. irwinii*, *C. urceolata*, *M. ferruginata* and *M. alborufescens*) or <3% (for *M. albicans*, *M. ibaguensis* and *M. stenostachya*; Table 2). Thus, fruit consumption removes the inhibiting effect of the fruit tissue, by allowing for the release from germination inhibitors and for removal of light-blocking pigmentation (Samuels & Levey 2005). Gut passage affected neither germinability nor MGT for *M. albicans*, *M. alborufescens*, *M. irwinii* and *M. stenostachya*, irrespective of bird species. Gut passage did not affect germinability but increased MGT of *C. urceolata* seeds (Table 2). However, seeds of *M. ibaguensis* and *M. ferruginata* ingested by birds presented significantly lower germinability compared to hand-extracted seeds, in spite of unaffected MGT (Table 2). We found differences in bird efficiency for *M. albicans*, *C. urceolata* and *M. alborufescens,* suggesting that birds differ qualitatively in their impacts on components of seed dispersal. This was expected since the loose melastome-frugivore interactions would result in diffuse co-evolution (Stiles & Rosseli, 1993). As gut-passage did not increase germination, the benefits provided by bird dispersal to Cerrado melastomes are restricted to seed cleaning and direct dispersal. Our results suggest that the variable outcomes resulting from complex fruit-frugivore interactions may affect recruitment of Cerrado melastomes.

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**Table 1.** Life-history and geographic distribution of the study species of *Clidemia* and *Miconia*.

| Species | Growth-form | Fruit color | Seeds/fruit | Fruiting phenology | Geographic range |
|---|---|---|---|---|---|
| *C. urceolata* | Shrub | Black | 168.8 ± 54.2 | Year-round | Widespread |
| *M. albicans* | Shrub | Green | 15.6 ± 3.4 | Mid-wet season | Widespread |
| *M. alborufescens* | Shrub | Black | 17.5 ± 2.4 | Early-wet season | Endemic |
| *M. ferruginata* | Tree | Black | 28 ± 5.1 | Dry-wet season transition | Widespread |
| *M. ibaguensis* | Shrub | Black | ± | Mid-wet season | Widespread |
| *M. irwinii* | Treelet | Black | 3.8 ± 2.3 | Dry-wet season transition | Endemic |
| *M. stenostachya* | Shrub | Black | 58.3 ± 8.2 | Mid-wet season | Widespread |

**Table 2.** Germinability (%) and mean germination time (MGT, days) of hand-extracted *Clidemia* and *Miconia* seeds (control) and seeds recovered from bird droppings.

| Species | | Cont | Ta | Tl | Ms | Zc | Tp | Ts | Sr | F |
|---|---|---|---|---|---|---|---|---|---|---|
| *C. urceolata* | % | 38ab | 5a | 30ab | - | - | - | 13a | 63b | 6.4* |
| | MGT | 12.6a | 27.5b | 26.9b | - | - | - | 26.5b | 26.8b | 10.01* |
| *M. albicans* | % | 52 | 52 | 71 | - | 67 | 63 | - | 57 | 1.92 |
| | MGT | 10.9a | 16.8b | 17.8b | - | 14.8b | 17.4b | - | 16.2b | 16.6** |
| *M. alborufescens* | % | 79 | 83 | 76.5 | - | 64 | 76 | 60 | 84 | 12.2 |
| | MGT | 9.1 | 9.3 | 8.4 | - | 11.4 | 9.6 | 9.8 | 8.6 | 3* |
| *M. ferruginata* | % | 36a | 11.25b | 9b | - | - | - | 17b | 14b | 7.5* |
| | MGT | 13.5ab | 15.76a | 11.1ab | - | - | - | 20.1b | 13.7ab | 3.8* |
| *M. ibaguensis* | % | 45a | 0 | 1b | 1b | 7b | 2b | - | - | 53.8** |
| | MGT | 20.3 | - | 13 | 27 | 15.3 | 26.5 | - | - | 1.74 |
| *M. irwinii* | % | 81 | - | | - | - | - | | 77.9 | 0.16 |
| | MGT | | - | 76 | - | - | - | 81.8 | | 0.26 |
| *M. stenostachya* | % | 41ab | 39ab | 37ab | 34ab | 17b | 40ab | - | 46a | 2.61* |
| | MGT | 16.6a | 17.8ab | 18.8ab | 22.5b | 17ab | 20.1ab | - | 16.3b | 3.48* |

Ta= *Turdus amaurochalinus*, Tl= *Turdus leucomelas*, Ms= *Mimus saturninus*, Zc= *Zonotrichia capensis*, Tp= *Thraupis palmarum*, Ts= *Thraupis sayaca*, Sr= *Schistoclamys ruficapillus*.
*p< 0.05; **p< 0.001. - interaction not studied.

We thank Instituto Chico Mendes (Três Marias/MG) for providing logistic support and CETAS/IBAMA (Belo Horizonte) for the permit to use bird species for the study.

# Do Seed Mass, Habitat and Geographic Distribution affect Light and Temperature Response of Melastomataceae Seeds from the Brazilian Cerrado?

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## Introduction

Vegetation referred to as rupestrian fields (high altitude rocky savanna) occurs in the Espinhaço Range of southeastern Brazil in areas above 900 m asl and is characterized by plant communities establishing in quartzite-sandstone substrate. The sclerophyllous plants often colonize shallow, acidic, extremely nutrient-poor, excessively drained soils, and experience strong winds, high sun exposure, frequent fires, high daily thermal amplitudes and water shortage during the dry winters (Giulietti et al. 1997). An outstanding feature of the rupestrian fields is habitat heterogeneity; local drainage systems dictated by topography diversify this environment by creating relatively humid or arid sites, often separated from each other by a few centimeters (Alves & Kolbek 2010).

Melastomataceae is a species-rich family in these rupestrian fields with a high diversity of life-forms, habitats, geographic distribution and seed size. Factors accounting for the structure and composition of plant communities in rocky grasslands have been investigated elsewhere, but seed-mediated mechanisms have been neglected thus far (Brandle et al. 2003). The aims of this study were to determine the germination requirements for 34 species and to test the following hypotheses: 1) light requirement decreases with increasing seed mass; 2) germination requirements are related to microhabitat conditions; and 3) widespread species have wide temperature ranges (eurythermic) compared to endemic species which have narrow temperature ranges (stenothermic).

## Methods

The effects of light and temperature on germination were determined under laboratory conditions for 34 species of melastomes distributed in 13 genera. Seeds (4 replicates of 50 seeds, 25 for *Miconia* and *Leandra*) were collected at Espinhaço Range, southeastern Brazil and set to germinate in Petri dishes incubated at the constant temperatures of 15, 20, 25, 30 and 35ºC under a 12:12h light: dark cycle (PPFD = 25.86 µmol $m^{-2}$ $s^{-1}$) or in continuous darkness for 30 days. For each replicate we calculated mean germination time (MGT) and for each species we calculated the light requirement index (LRI) at optimum temperature. Dry seed mass was determined by weighing four replicates of 100 seeds on a digital scale after drying at 70ºC for 5 days. Seed mass and LRI were determined for an additional 4 species, totaling 38 species.

Data in percentage were arcsine transformed and significant differences in germinability and MGT among temperatures within each species were determined through ANOVA followed by Tukey test ($\alpha$= 0.05). Optimum temperature was defined as the temperature(s) which allowed maximum germinability in least amount of time. A linear regression was used to determine relationships between seed mass and LRI. We ran chi-square tests to determine differences in frequency data. After establishing temperature ranges for germination, we used a T-test for independent samples to explore differences between widespread vs. endemic species and between mesophytes vs. xerophytes.

## Results and Conclusions

Both light and temperature significantly affected the germination of all studied species. All species were photoblastic with null or <5% dark germination. Thus, for all species LRI was one or nearly one ( $\overline{x}$ = 0.991) and there was no significant relationship between seed mass and LRI ($r^2$=36.5; p=0.085), in spite of the enormous variation in seed mass (155-fold). Only 44% of species germinated at 15ºC, and 37% germinated at 35ºC. As a consequence, the optimum temperature range was 20-25ºC for most species (Fig. 1). Germination was skewed towards low values of germinability, and optimum temperatures could not be determined for 10 species (29.4%) due to low or null germinability regardless of temperature (Fig. 1). Low or null germinability in the 20-30ºC regime may be the result of seed dormancy, which may be particularly important in the tribe Microlicieae, but dormancy may also have evolved in a few species of Miconieae and Melastomeae. Half of the studied species were

stenothermic ($\leq$ 10ºC temperature range) and half were eurythermic ($\geq$ 15ºC temperature range), with no significant difference in their frequency between widespread and endemic species ($\chi^2$ =0.29; p= 0.58). Accordingly, temperature range for germination did not differ (t= 0.589; p= 0.562) between widespread (14.17 ± 5.97ºC; SD) and endemic species (12.96 ± 5.97ºC). During the wet season, mesic soils experienced a 6.6ºC daily temperature variation whereas in xeric soils daily variation was 11.4ºC. Unexpectedly, mesophytes had wider temperature range than xerophytes. The proportion of xerophytes in stenothermics was greater than expected by chance ($\chi^2$=5.85; p= 0.015), but no significant differences were found for eurythermics ($\chi^2$= 0.06; p= 0.81).

Our partial results show that: 1) all studied melastomes are photoblastic and expected to have soil persistence. The photoblastic response is probably a phylogenetic constraint resulting from the production of small-sized seeds, rather than an adaptation for open environments; 2) soil temperatures do not match germination requirements, suggesting that germination-mediated habitat selection is not strong enough to structure plant communities in rupestrian fields. This is sustained by the lack of support to the niche breadth hypothesis. We put forward the idea that factors controlling seedling establishment, rather than seed germination, determine habitat selection and constrain the geographic expansion of endemic species.

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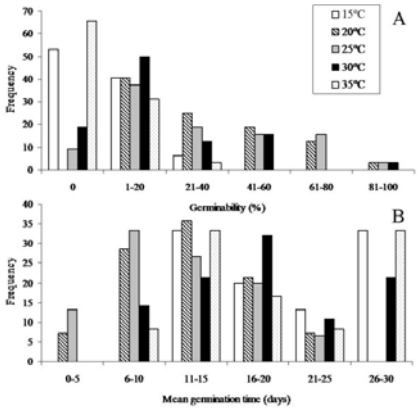


Figure 1. Distribution pattern of germinability (A) and mean germination time (B) of the study species of Melastomataceae.

## Germination Ecology of Melastomataceae from Neotropical Montane Savannas: Effects of Phylogeny, Habitat, Growth-form, Dispersal Mode and Geographic Distribution.

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### Introduction

Melastomataceae is a pantropical family, with high diversity and endemism in the Brazilian Cerrado. In the rupestrian fields (montane rocky grassland savannas) this species-rich family exhibits a high diversity of habitats (mesic and xeric), growth-forms, dispersal mode (wind- and bird-dispersed) and geographic distribution (endemic and widespread). Thus, it is an interesting taxon for studying the factors that control dormancy and germination as well as testing hypotheses on the ecological and historical correlates of seed germination (Brändle et al. 2003, Luna and Moreno 2009). Here, we present the preliminary analyses of the effects of phylogeny, habitat, growth-form, dispersal mode and geographic distribution range on germination of 41 species of melastomes distributed in three tribes (13 Miconieae, 17 Microlicieae and 11 Melastomeae) at Serra do Espinhaço, southeastern Brazil.

### Methods

In 2008 and 2009, seeds were extracted from mature fruits ($n \geq 20$ individuals) at Serra do Espinhaço, southeastern Brazil, an important center of plant endemism and diversity as well as human impact (Giulietti et al. 1997). The climate is mesothermic with rainy summers and dry winters (Giulietti et al. 1997) and the dominant vegetation is called rupestrian fields. Plant communities establish on shallow, acidic, extremely nutrient-poor and excessively drained soils. The soil mosaics in the rupestrian fields include xeric habitats such as rocky outcrops and mesic sites such as the gallery forests, peaty bogs and moist grasslands (Alves and Kolbek 2010).

Species selection was conducted in order to maximize variation in life-history. Our species subset included 26 shrubs, 4 trees, 11 sub-shrubs, 22 mesophytes and 19 xerophytes, 13 bird-dispersed (all in Miconieae) and 28 wind-dispersed (in Microlicieae and Melastomeae), 20 endemics of rupestrian fields, and 21 widespread species. Four replicates of 50 (25 for *Miconia* and *Leandra*) seeds per species were set to germinate in Petri dishes incubated in growth chambers under optimum conditions (between 20 and 30ºC under 12:12h light:dark cycle; PPFD = 25.86 µmol $m^{-2}$ $s^{-1}$) for 30 days. One-way and two-way ANOVAs were run to determine the effects of phylogeny, life-history and habitat on germinability (percentage data were arcsine transformed) and on mean germination time (MGT). Statistics followed Wang et al. (2009).

### Results and Conclusions

There was a huge among-species variation in germinability, with mean values ranging from 0.5% to 86%. We also found a 5-fold variation in MGT, with mean values ranging from 3.58 to 17.98 days. Phylogeny, life-history and habitat were significantly correlated with both germinability and MGT, but there was no correlation between germination parameters and geographic distribution range (Table 1). We found higher germinability and lower MGT in Miconieae (48.9 ± 4.3%) compared to Melastomeae (34.5 ± 3.8%) and Microlicieae (25.5 ± 2.8%). Germinability of trees (67.8 ± 5.19%) was higher than that of shrubs and sub-shrubs, and seeds of sub-shrubs took more time to germinate compared to the other life-forms (14.53 days vs. 9.89 for trees and 11.26 for shrubs). Mesophytes had higher germinability and a 1.7-fold reduction in MGT relative to xerophytes. Two-way ANOVAs detected significant interactions between tribe x distribution, tribe x growth-form, habitat x distribution and distribution x dispersal mode (Table 2).

Our results corroborate recent studies showing ecological and phylogenetic correlates with germination (Luna and Moreno 2009, Wang et al. 2009). Phylogeny-dependent variation in germinability can be explained by higher seed mass in Miconieae compared to the other tribes (data not shown). Higher seed mass in Miconieae may also account for longer MGT in this tribe and for the higher germinability of bird-dispersed (48.9 ± 4.3%) as opposed to wind-dispersed species (29.1 ± 2.28%).

Faster germination of mesophytes compared to xerophytes could prevent seed runoff during the wet season, and higher germinability in mesophytes points to the role of environment as an important selective pressure. Absence or $\leq 5\%$ germinability was related to seed dormancy or embryo-lacking (nonviable) seeds. The present data suggests a disproportionate occurrence of dormancy and/or seed nonviability in Microlicieae, with dormancy evolving in species of Miconieae and Melastomeae that disperse seeds at the transition between wet and dry seasons. As dormancy could have strongly affected our results (Wang et al. 2009), seed anatomy and physiology studies are being conducted in order to determine the evolution of dormancy in the family.

Significant interactions among functional groups suggest that the patterns of germination and the evolution of seed dormancy are more complex than previously thought. Our data do not support the niche breadth hypothesis (Brändle et al. 2003) that predicts broad germination niches for widespread species compared to endemic ones but instead show that regeneration strategies are dependent upon life-history, habitat, and evolutionary history. We argue that seedling establishment is the main ecological filter driving habitat selection in heterogeneous habitats and that ultimately dictates constraints on the geographic expansion of endemic species. Our ongoing studies include analysis of seven additional species and also aim to determine the role of seed mass and phenology on germination. Phylogenetically controlled analyses are needed to clarify the role of phylogeny in dormancy and germination.

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**Table 1.** Results of one-way ANOVAs for the effects of phylogeny, life-history and habitat on germinability (%) and mean germination time (days) of 41 Melastomataceae species from Serra do Espinhaço, Brazil.

| Source of variation | df | Germinability | | Mean germination time | |
|---|---|---|---|---|---|
| | | F | p | F | p |
| Tribe | 2 | 12.516 | 0.000 | 6.016 | 0.003 |
| Growth-form | 2 | 18.355 | 0.000 | 5.750 | 0.004 |
| Habitat | 1 | 4.231 | 0.041 | 13.976 | 0.000 |
| Distribution | 1 | 2.211 | 0.139 | 3.128 | 0.079 |
| Dispersal | 1 | 21.915 | 0.000 | 10.133 | 0.002 |

**Table 2**. Results of two-way ANOVAs showing the independent effects of one of two main factors and interaction effects on germinability between the two variables: Tribe (T), Growth-form (GF), Habitat (H), Geographic distribution (GD) and Dispersal mode (DM).

| Source of variation | Effect of A | | Effect of B | | A x B | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | p |
| T/GF | NC | | NC | | 4.218 | 0.018 |
| T/H | 13.385 | 0.000 | 6.673 | 0.011 | 2.665 | 0.073 |
| T/GD | 23.218 | 0.000 | 1.804 | 0.181 | 18.980 | 0.000 |
| GF/H | 18.563 | 0.000 | 3.728 | 0.055 | 0.681 | 0.508 |
| GF/GD | 18.360 | 0.000 | 3.838 | 0.052 | 2.192 | 0.115 |
| GF/DM | NC | | NC | | 1.74 | 0.189 |
| H/GD | 2.481 | 0.117 | 1.304 | 0.255 | 16.666 | 0.000 |
| H/DM | 1.868 | 0.174 | 21.381 | 0.000 | 3.638 | 0.058 |
| GD/DM | 10.207 | 0.002 | 44.259 | 0.000 | 39.364 | 0.000 |

NC – not calculated due to lost df.

# Germination Dynamics and Seedling Frost Resistance of Invasive and Native *Impatiens* Species Indicate Adaptation to Local Climatic Conditions

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## Introduction

Traits associated with early ontogenetic stages are among the key factors determining the spread of alien annual plants in their secondary distribution areas (Pyšek & Richardson 2007; Grotkopp & Rejmánek 2007). Invasion success of some alien plants is considered to be associated with inter-population differentiation and adaptations to local conditions (Dietz and Edwards 2006, Pyšek *et al*. 2009; Kollmann and Bañuelos 2004; Allan and Pannell 2009). To obtain an insight into these processes we investigated intra-specific differentiation in four species of *Impatiens* (Balsaminaceae) with different origins and invasion status.

## Methods

The experiment included *I. noli-tangere*, which is native in central Europe, and three alien species differing in their invasion status in the region (highly invasive *I. glandulifera*, less invasive *I. parviflora* and potentially invasive *I. capensis*). Germination, seedling recruitment, and frost resistance of seedlings were measured in the laboratory and in an experimental garden using seed collected from five natural populations of each species. Germination was tested under a cold, wet stratification regime. Seedlings were cultivated under temperatures simulating the gradually changing daily temperature recorded in the field in spring. Seedlings with the first pair of stem leaves were exposed to conditions simulating morning frost, i.e., at
-9 °C for one hour. Except for *I. capensis*, the results were related to climatic conditions in the original localities (average and minimum temperature measured at the locality, and average temperature and date of the last frost in the region based on long-term meteorological data).

## Results and Conclusions

Alien species were characterized by earlier germination and seedling appearance, and by lower frost resistance, differing thus from the native *I. noli-tangere*. Differences in the performance of populations were found for all the species studied, and related to climate in early spring at the seed-source localities, which indicates that individuals are adapted to local conditions. In the native *I. noli-tangere*, the adaptations were in terms of the time of germination and seedling appearance, and the differences among populations were explained by temperature at the locality from which the seed was collected (Fig 1). Adaptations to frost occurred in both invasive species. In *I. glandulifera* the differences among populations in resistance to frost were explained by field temperature at the seed-source locality (Fig 2) and corresponded to the pattern of appearance of seedlings in the garden. *Impatiens parviflora* was the most sensitive of all the species to frost and the inter-population differences were explained by the date of the last frost in the seed-source region (Tolasz et. al. 2007). In addition, the date of the last frost, together with temperature at the seed-source locality, explained the pattern of germination in this species.

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Fig 1. Relationships between the time to germination of 50% of the seeds and average temperature at the source localities in the second half of March for *Impatiens noli-tangere.*

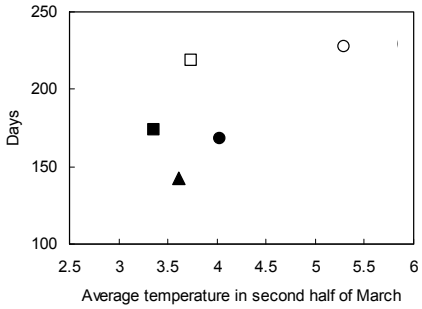
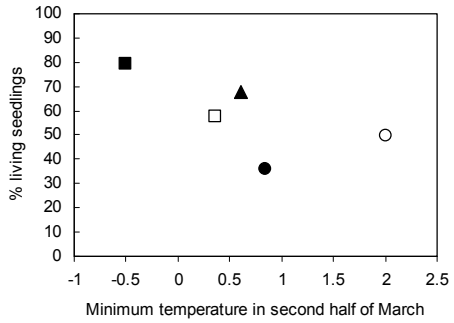



Fig 2. Relationship between percentage of seedlings that survived exposure to frost and minimum temperature at the source localities in the second half of March for *Impatiens glandulifera*.



## *Seed Reserves in Nineteen Tree Species in a Tropical Deciduous Forest*

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### Introduction

Functional diversity in seeds is expressed in several traits, such as the chemical composition of the reserves stored (Orozco Segovia and Sanchez Coronado in press). Seed reserves are key to understanding early plant growth (Kitajima 2002, Hanley *et al*. 2004). Lipids, carbohydrates and proteins are the main seed reserves. Proteins are the major N source for seeds, but other nitrogen compounds such as amino acids may be found (Ching 1972, Fenner 1985). To test if seeds reserves partitioning, seed dry mass and germination parameters may be good indicators of seedling performance, we studied the composition of seed reserves (non-structural carbohydrates, lipids, nitrogen content, proteins and total amino acid content) and germination parameters (lag time and germination rate) of 19 coexisting tree species from a highly seasonal tropical deciduous forest in NW Mexico.

### Methods

The seeds of the 19 studied species (Table 1) were collected in 2006 at the Biological Station of Chamela (19°30'N, 105°03'W) on the Pacific coast of Mexico. We determined seed dry mass and seed reserve concentration (lipids, nitrogen and non structural carbohydrates, proteins and amino acids) of the studied species. Germination tests for these species were also conducted. The relationships among seed reserves and seed dry mass, germination parameters and seedling relative growth rate (RGR, previously reported) were examined. We also evaluated the relationship between RGR and seed biomass taking into account seed reserve composition.

### Results and Conclusions

Reserve partitioning differed widely among the 19 studied species (Fig. 1). The species could be separated into two groups independently of their taxonomic identity: high-lipid seeds (HLS) and high-non-structural-carbohydrate seeds (HNSCS) species. In all 19 species and in the HNSCS group, seed biomass was negatively related to RGR of seedlings. Once physical dormancy was removed by scarification, in 18 of the 19 species studied, seeds began germinating within the first 14 d. Short germination lag times have been considered an evolutionary response to a short growing season and to facilitate efficient competition for resources (i.e., light and water), which may be limiting in the tropical deciduous forest (Garwood 1983, Khurana & Singh 2001). Nitrogen was positively related to germination rate in all the studied species. This could be related positively to water uptake and reserve mobilization (Lieffering *et al*. 1996, Hara and Toriyama 1998). Allocation of N suggested different strategies in N partitioning patterns, e.g., *Enterolobium cyclocarpum* had a high N concentration but its amino acid concentration was higher than protein concentration, whereas in *Caesalpinia eriostachys* protein concentration was higher than amino acid concentration (Fig. 2). HLS, facultative sun-requirement and slow growth rate as opposed to HNSCS, obligate sun-requirement and fast RGR may represent two strategies to survive and establish during the short growing season, characterised by water stress produced by rainfall seasonality and erratic rains, characteristic of tropical deciduous forest. The variability of reserve partitioning represents part of the functional diversity that is necessary to establish in the heterogeneous and changing environment of Chamela deciduous forest.

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**Table 1.** List of studied species; the nomenclature is in accordance with Lott (2002).

| | Species | Family |
|---|---|---|
| 1 | *Crescentia alata* H.B.K. | Bignoniaceae |
| 2 | *Tabebuia rosea* DC. | Bignoniaceae |
| 3 | *Ceiba pentandra* Gaertn. | Bombacaceae |
| 4 | *Cochlospermum vitifolium* Spreng. | Cochlospermaceae |
| 5 | *Ipomoea wolcottiana* Rose. | Convolvulaceae |
| 6 | *Acacia farnesiana* Willd | Fabaceae |
| 7 | *Acacia* sp. | Fabaceae |
| 8 | *Apoplanesia paniculata* Presl. | Fabaceae |
| 9 | *Caesalpinia coriaria* Willd. | Fabaceae |
| 10 | *Caesalpinia eriostachys* Benth. | Fabaceae |
| 11 | *Caesalpinia platyloba* S. Wats. | Fabaceae |
| 12 | *Enterolobium cyclocarpum* Griseb. | Fabaceae |
| 13 | *Lonchocarpus eriocarinalis* Micheli. | Fabaceae |
| 14 | *Pithecellobium dulce* Benth. | Fabaceae |
| 15 | *Gyrocarpus jatrophifolius* Domin. | Hernandiaceae |
| 16 | *Swietenia humilis* Zucc. | Meliaceae |
| 17 | *Coccoloba barbadensis* Jacq. | Polygonaceae |
| 18 | *Ruprechtia fusca* Fern. | Polygonaceae |
| 19 | *Hintonia latiflora* Bullock. | Rubiaceae |

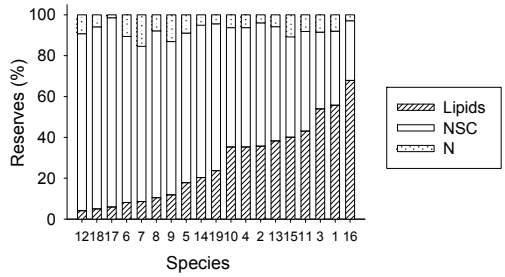


**Figure 1.** Seed reserve partitioning standardized to 100%. Numbers correspond to species of Table1.

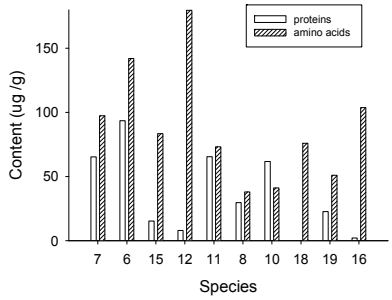


**Figure 2.** Thermostable proteins and amino acids stored in seeds of ten species studied. Species are ordered left to right according their N concentration. Numbers correspond to species of Table1.

# Herbicide-resistant *Lolium rigidum* have Greater Seed Dormancy: the Australian Cropping System Leads to Coexistence of Mechanisms to Evade Weed Control

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## Introduction

*Lolium rigidum* Gaud. (annual or rigid ryegrass) is the most economically significant weed in southern Australian cropping systems. In order to improve management of *L. rigidum*, knowledge of the variability in, and factors affecting, dormancy release and emergence within populations is essential. The primary aim of this study was to investigate the possibility to predict seed dormancy of *L. rigidum* over a large scale.

Temperature and rainfall during plant growth, seed development, and seed after-ripening have been shown to modulate dormancy status (Steadman et al. 2003a,b, 2004a,b) so climatic variables may be expected to be predictive for dormancy. Herbicide resistance status has also been linked with dormancy in *L. rigidum*, with a greater percentage of individuals with resistance due to a target-site ACCase mutation being grown from seeds exhibiting deeper dormancy than individuals exhibiting either herbicide susceptibility, or resistance due to a non-target site cytochrome P450-based enhanced metabolism mechanism (Vila-Aiub *et al.*, 2005). Therefore herbicide resistance status was also considered as a potential predictor for dormancy.

## Methods

Dormancy at harvest of mature seed and monthly during after-ripening over summer was measured for 406 populations collected from across the 14 million hectare grain-growing region of southern Western Australia. Logistic growth curve models were fitted to the change in germination with dormancy release for each population, with the resulting equation used to estimate four parameters describing dormancy status of the population. For determination of relationships with these parameters, long-term and current-year temperature and rainfall parameters were obtained for each population (Australian Bureau of Meterology, 2003), and resistance of seedlings to four commonly-used in-crop herbicides (diclofop-methyl, sethoxydim, clethodim and sulfometuron) was measured (Owen et al. 2007).

## Results and Conclusions

Following extensive statistical analysis, it was difficult to find consistent relationships with climate that could be used to predict the dormancy release pattern at this large scale. There were distinct spatial clusters of populations exhibiting strong dormancy clearly evident, however these clusters occurred at a variety of locations across the grain belt leading to very weak associations with the climatic parameters. Indeed, groups of populations located close together (and therefore experiencing a very similar climate) had contrasting dormancy characteristics, suggesting a role for differences in farming practices at the local level.

Higher levels of herbicide resistance to all four herbicides correlated with greater dormancy (Fig. 1). As this represents two modes of action (ACCase- and ALS-inhibitors) and a contrast of generalist (metabolic) and target-site mutation mechanisms, it is unlikely that a direct pleiotropic effect of resistant alleles, or even linkage of germination-influencing alleles to resistant alleles is the reason for this correlation. We explain this coexistence of deeper dormancy and herbicide resistance in terms of the selection pressures exerted by the cropping system used for decades. In the southern Australian winter cropping system it is common practice for both pre-seeding and in-crop weed control strategies to be applied within any year that a field is cropped. Under this system it is only the more dormant portion of the population (which avoids the pre-seeding control event by germinating afterwards) that then receives the selection pressure posed by the in-crop herbicide application. Consequently, it is the plants that combine dormancy and herbicide resistance that successfully reproduce and contribute to the seedbank. This strong link between herbicide resistance and seed dormancy suggests a potential

value in predictive modelling, and provides evidence for the need to consider the effect that crop management practices can have on weed population dynamics.

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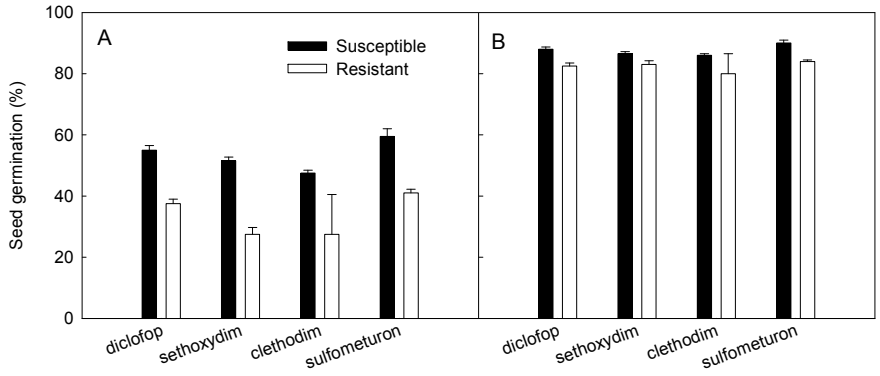


**Figure 1.** Germination of mature seeds soon after collection (A) or following after-ripening over the summer for 4000°Cd (B). Germination was measured under conditions that are ideal for this species; 25/15°C with a 12 hour photoperiod. Bars indicate the mean and se as estimated by a generalized linear model fitted to the dormancy release data. Seeds were produced by plants grown from populations that exhibited resistance (20% or more of individuals survived herbicide application) or susceptibility (less than 1% survival) to one of four herbicides applied at field rates.

# Climate Change in Unpredictable Ecosystems: A Soil Seed Bank Perspective

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## Introduction

Soil seed banks play an important role in annual plant populations by buffering populations from temporal variation (Claus and Venable 2000) and by avoiding the demographic effects of reproductive failure (Evans and Cabin 1995). Such seed banks contain dormant seeds that refrain from germination even when placed under conditions that are generally suitable for germination. Within this framework we asked: Do plant populations from climatic unpredictable environments such as an arid and semiarid ecosystems have a higher proportion of dormant and larger seeds than more predictable mesic ecosystems? This question is answered by considering soil seed bank dynamics as they are of crucial importance in highly variable and unpredictable environments. Soil seed banks play a determinant role in plant community dynamics, as differential plant germination strategies may buffer against inter-annual variability in growing conditions.

## Methods

We selected the strong climatic gradient in Israel, for testing predictions regarding the effects of global climate change on plant community dynamics. Soil seed banks from four field sites following a North to South aridity gradient were studied. Furthermore, climate change scenarios were experimentally tested with rainfall manipulations using rainout shelters and irrigation systems under field conditions. Environmental conditions at these sites respectively represent mesic Mediterranean, Mediterranean, semiarid, and arid conditions. Soil seed bank samples were collected at the end of the summer drought and before the onset of fall rains (late September). Each sample measured 5 × 5 cm to a depth of 5 cm, and included seeds on the surface and plant litter. These samples were later spread in plastic trays (12 × 14 cm, 6.5 cm depth, with drainage holes) on a gauze sheet placed on top of a 3 cm thick layer of perlite. The trays were irrigated during winter in a nethouse at the Botanical Gardens of Tel Aviv University, Israel. Emerging seedlings were identified, counted and continuously removed until no further emergence was observed (mid March). The overall seed germinability in each soil sample was determined by replicating the described procedure for each tray for three consecutive germination seasons (3 winters).

## Results and Conclusions

Following eight years of monitoring, the results indicated significant differences in seedling densities among the study sites along the aridity gradient (Fig. 1a). Seedling density increased with increasing rainfall conditions. Significant differences were also noted when comparing seed density between habitats (open gaps vs. shrub understory). Higher seedling densities were observed at the shrub understory at the arid part of the gradient while the opposite was noted at the mesic part (i.e. higher seedling density at the open gaps between shrubs). This phenomenon indicated shrub facilitation effects at the arid part of the gradient and competitive exclusion at the more humid part of the gradient. Moreover, important differences among years were clearly noted as response of rainfall conditions of the year when seeds were produced. The results collected showed no clear response of the soil seed bank to the rainfall manipulations (Fig. 1b). According to our expectations we should have observed a decreased in seed density under droughting conditions while the opposite by increasing rainfall through irrigation. The results were not consistent and in some cases opposite to expected. The short-term resistance of the soil seed bank to the rainfall manipulations is discussed within the framework of evolutionary adaptation of these plant communities to high temporal and spatial variability.

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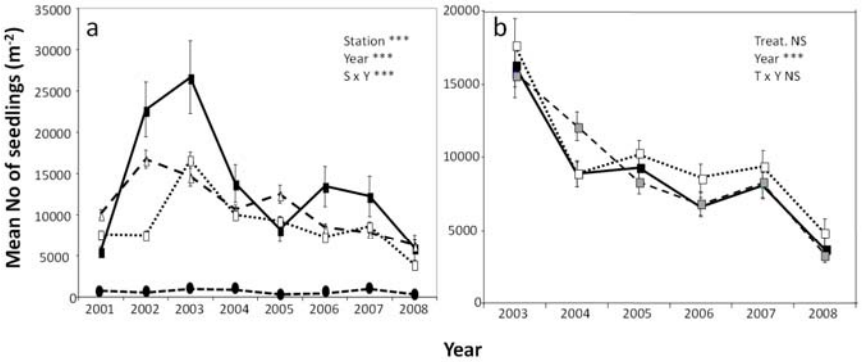


**Figure 1.** (a) Changes in seedling densities among study sites along the aridity gradient. Black square: mesic Mediterranean; white triangle: Mediterranean; white square: semiarid; black circle: arid. (b) Changes in seedling densities at the semiarid site under different rainfall manipulation treatments. Black square: control; white square: drought; grey square: watering.

## Seed Bank Dynamics of Arid Region Streams and Implications for Restoration

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**Abstract**
Riparian zones of dryland streams support a diverse assemblage of species representing a wide array of life history strategies. Given the highly variable flow regime and episodic occurrence of extreme disturbance, many of the plants are ruderals that remain quiescent until suitable conditions for establishment occur. Here we summarize community patterns of seed traits and soil seed banks for two desert rivers in Arizona. Along the Hassayampa River, herbaceous plant species sort along lateral hydrogradients based in part on seed size (diaspore mass). Obligate wetland plants, with the smallest seed mass, occur along wet stream banks while the mesic and xeric (and larger seeded) species grow on the higher and drier floodplains and terraces. Patterns in the soil and litter seed banks differ among zones. Seed traits of the litter reflect that of extant vegetation, but the deeper soils in all zones have small viable seeds of wetland affinity. Such a pattern increases likelihood that wetland ruderals will germinate widely after intense scouring floods mobilize sediments, restructure stream geomorphology, and reshuffle microsites. Surveys of the below-dam Agua Fria revealed persistence of viable wetland seeds in the soils of the xeric shrublands, which are the predominant post-dam vegetation type. The buried wetland seeds provide a potential source for revegetation should stream flows be restored. These and other results have implications for restoration of dammed and dewatered rivers. Information is needed, however, on the length and persistence of this riparian-wetland legacy.

# Identification and Characterization of the Water Gap in Physically Dormant Seeds of Geraniaceae, with Special Reference to *Geranium carolinianum* L.

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## Introduction

The water impermeable cell layer in the seed coat of Geraniacae is a continuous layer of palisade cells (except in the chalazal region) that is located below the outer polygonal and middle parenchyma layer(s) of the outer integument (Boesewinkel and Been, 1979; Schulz *et al*., 1991; Meisert *et al*., 1999). The gap between palisade cells of the chalaza is filled with a chalazal plug (=suberized stopper, *sensu* Boesewinkel *et al*., 1979) that maintains seed coat impermeability. The chalazal cleft previously has been reported to be the water gap (i.e., location of initial water entry) in innately permeable seeds of Geraniaceae (Meisert *et al*., 1999). The primary aim of this study was to re-evaluate the location of the water gap and to characterize its morphology and anatomy in physically dormant seeds of Geraniaceae, with particular reference to *G. carolinianum*.

## Methods

Length, width, mass, anatomy and germination of two seed types (light brown and dark brown) of *G. carolinianum* were compared. Location, anatomy and morphology of the water gap was characterized using free-hand and microtome tissue sectioning, light microscopy, scanning electron microscopy, dye tracking, blocking and seed burial experiments.

## Results and Conclusions

The two seed types do not differ significantly in length, mass, seed coat anatomy or germination. Intact seeds did not imbibe water while heat treated seeds and scarified seeds did imbibe. Treatment with dry heat at 80 $^{o}$C for 7 days caused a colour change in the palisade cells adjacent to the micropyle. After 4 months of burial in soil, seeds exhibited a similar colour change in the micropylar region. Germination of buried seeds with colour change was significantly higher (100 %) than those without visible colour change (20 ± 3.2 %). These results are similar to those of heat-treated and nontreated seeds. When placed in water, the 'hinged valve' (blister) erupted at the site of the colour change, exposing the water gap. Dye tracking showed that water enters through the water gap and not through the chalaza. This was also supported by a blocking experiment. Blocking of the micropylar region of heat-treated seeds significantly reduced imbibition of water compared to seeds with chalaza blocked (Fig. 1). Morphology and anatomy in the water gap region differs from that in the rest of the seed coat (Fig. 2 A, B). Morphology of the seed coat in the water gap region is similar in *G. carolinianum*, *G. columbinum*, *G. molle* and *G. pusillum* and differs from that of the closely related species *Erodium cicutarium*. Dislodgment of swollen 'hinged valve' palisade cells adjacent to the micropyle caused the water gap to open in physically dormant seeds of *G. carolinianum,* and it is clear that initial water uptake takes place through this gap and not via the chalazal opening as previously reported. This water gap ('hinged valve gap') differs from water gaps that previously have been described for other families in morphology, anatomy and location in the seed coat.

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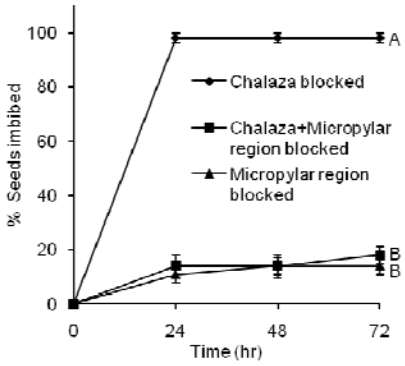


**Figure 1.** Percentage of *G. carolinianum* seeds that imbibed during 72 hr of incubation at 20/10 °C with chalaza blocked, chalaza+micropylar region blocked and micropylar region blocked. Different letters indicate significant differences between treatments ($p<0.05$).

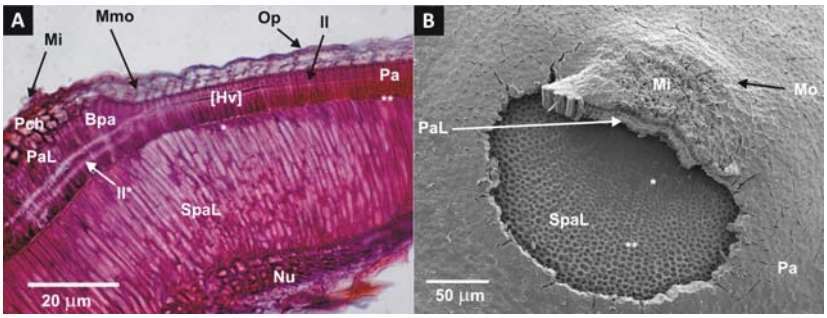


**Figure 2.** Light and electron micrographs of micropylar region of *G. carolinianum*: (A) longitudinal section through micropylar region of a dormant seed (water gap closed); (B) water gap of a nondormant seed without outer permeable cell layers and hinged valve dislodged (imbibed in water for 20 min). Bpa, bent palisade cells; Hv, hinged valve; ll, light line; ll*, widened light line in the micropylar region; Mi, micropyle; Mmo, multi-layered middle parenchyma cells; Mo, single layer of middle parenchyma cells; Nu, nucellus; Op, outermost polygonal parenchyma cell layer; Pa, palisade cells; PaL, elongated palisade cells of micropylar region; Spa, subpalisade cells; SpaL, elongated subpalisade cells of micropylar region; *, subpalisade cells with smooth outer periclinal cell wall; **, subpalisade cells with concave outer periclinal cell wall.

# Global Warming and Seed Germination – the Case of *Nepeta sphaciotica*, an Alpine Cretan Endemic

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## Introduction

Recent predictions of the effects of climate change on geographical distribution and conservation status of plants have shown significant and increasing impacts, particularly (among others) for narrow endemic species of Mediterranean mountains (Thuiller et al. 2005). However, there is a relative scarcity of relevant studies concerning the specific effects of global warming on plant reproductive biology and seed germination, in particular. *Nepeta sphaciotica* is a critically endangered species of European Community priority (Fournaraki et al 2009); it is a perennial sub-shrub with a single population on a bare, scree slope of Lefka Ori Mountains (Crete), at ca. 2300 m asl (Thanos 2008). The plant blossoms in August, its seeds mature during September and for a period of 5-6.5 months (usually from November to May) the plant is covered by snow (Fig. 1).

## Methods

Germination experiments were carried out with seeds sown on agar gel or water and incubated in growth chambers with temperature and light control, at various constant temperatures, under a daily photoperiod (12/12 h, light/dark) or in complete darkness. Additional experiments were performed under light and temperature conditions closely simulating the actual and projected climates.

## Results and Conclusions

Laboratory studies of *Nepeta sphaciotica* seed germination show an absolute, phytochrome-mediated, requirementfor light (as in *Nepeta rtanjensis*; Todorovic et al. 2007) and, similar to several alpine plants, a thermometer-type response to temperature. Thus, germination is fully manifested at 15 °C or above and fully inhibited at 10 °C or below, while chilling temperatures, in contrast to most alpine species (e.g., *Nepeta troodi*; Kadis et al. 2010), are completely ineffective in promoting or enhancing germination. On the basis of actual observations and temperature data, recorded for several years in the plant habitat, field germination takes place in June, immediately after snow melt, when average temperatures exceed 15°C (while those prevailing after seed maturation and dispersal, between September and November, never exceed 10 °C). Lab experiments closely simulating both current and future conditions (the latter from several versions according to the projections of various climatic scenarios) have confirmed that, on the one hand, no germination takes place under the actual autumn conditions and, on the other, that most seeds would germinate in autumn under the projected, warmer climate. It is postulated (Fig. 2) that the anticipated, warmer autumn temperatures (ca +5 °C as projected by the rather moderate climatic scenario B2a) will induce untimely seed germination that will undoubtedly result in seedling demise during the prolonged period of snow cover (or by freezing temperatures in case of no snow) thus jeopardizing population regeneration and, in the long run, the species survival itself.

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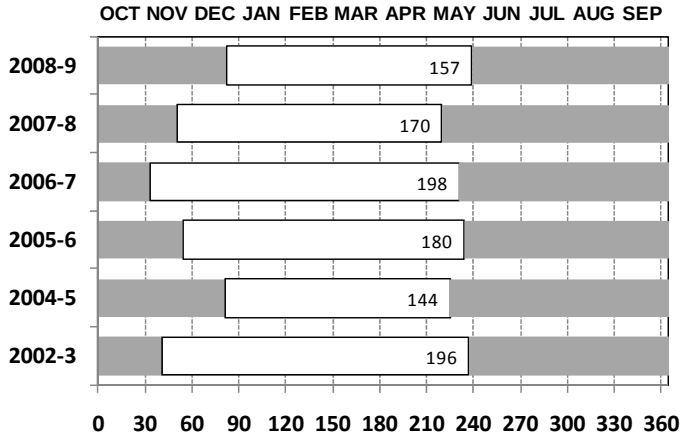


**Figure 1.** Timing and duration of snow cover (white bars) at Svourichti summit (2300 m asl), Lefka Ori (Crete); numbers in bars indicate snow cover duration in days.

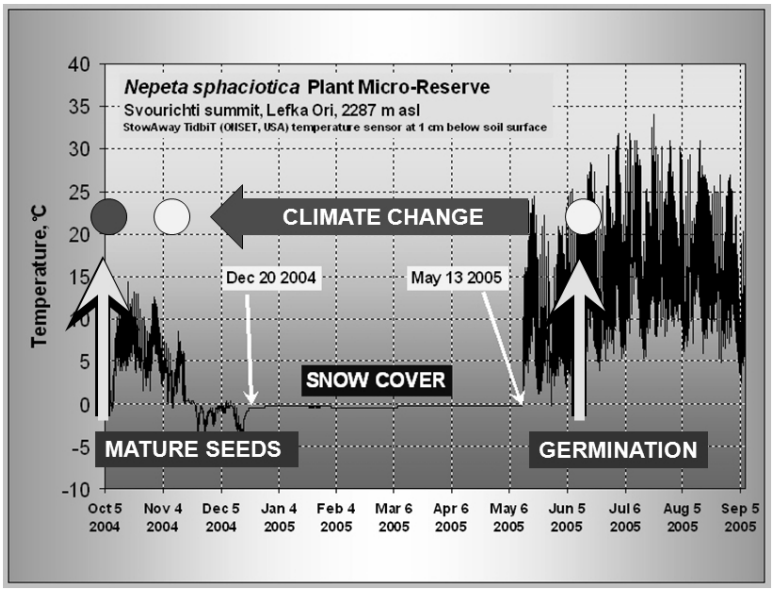


**Figure 2.** The postulated, climate-change-driven shift of *Nepeta sphaciotica* seed germination (white circles) from the current, post-snow, 'vernal' occurrence to a future, autumnal one.

# Advanced Structural Adaptation of Seeds to Bird-dispersal – Profit with Risk for Survival of Tree-line *Cembrae*

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## Introduction

Five alpine and boreal tree-line *Cembrae* pines, i.e. *Pinus cembra*, *P. sibirica*, *P.koraiensis* and *P. pumila* in Eurasia, and *P. albicaulis* in western North America, have a close and successful interaction with two nutcrackers, *Nucifraga caryocatactes* and *N. columbiana* (Lanner 1982, Tomback 1982, Kremenetski et al. 1998). The interaction is considered to have evolved initially in Eurasia to 0,6 – 1,3 million years ago (Lanner 1996, Krutovskii et al. 1994). This coevolved relationship ensures an important food source for nutcrackers and dispersal of seeds for *Cembrae* pines (Tomback & Linhart 1990). Nutcrackers have special traits: a long and sturdy bill for harvesting and planting of seeds, a sack-like sublingual pouch for transporting, and excellent spatial memory for retrieving seed caches. The *Cembrae* pines in turn have features adapted to bird-dispersal: indehiscent cones, large wingless seeds with solid seed coats, and survival in the soil seed bank for several years (op.cit). Highly developed interaction has been up to now beneficial to both a tree and a bird, even if cone and seed structures make the *Cembrae* obligate mutualists to nutcrackers to an extent that dispersal by wind is impossible. Currently seed crops and natural regeneration of whitebark pine (*P. albicaulis*) are, however, endangered due to altered fire regimes, introduced disease (*Cronartium ribicola*), native insect infestations (*Dendroctonus ponderosae*) and warming climate. Consequently, there is a pressing need to produce seedlings for restoration of this tree-line species. Delayed germination and the rarity of soil seed banks among conifers led us to study basic structures of *Cembrae* seeds and their differences from wind-dispersed conifer seeds.

## Methods

We used scanning and transmission electron (FESEM, STEM) and fluorescence microscopy, resin embedded sections and histochemical staining to examine the basic structures of untreated seeds and structural changes of *P. albicaulis* and *P. sibirica* seeds taken at different stages of the 90-day-long pre-treatment simulating conditions in the soil seed bank.

## Results and Conclusions

In addition to external traits of *Cembrae* cones and seeds, even the maturation of the surface and inner structures advanced in contrast to the development patterns seen in wind-dispersed conifers (Tillman-Sutela and Kauppi 1995). The hard seed coat and the depleted nucellar layers were the first to attain structural and chemical maturity as indicated by brown colour due to phenolic compounds (Tillman-Sutela et al. 2008), which protect seeds against fungi and predators in the soil seed bank. The brown colour of the seed coat also appears to be the stimulus for caching seeds (Tomback and Linhart 1990). In contrast to the surface structures, the megagametophyte tissue still had an abundance of starch and soluble lipids as a sign of chemical immaturity at the harvest. Both structural and chemical maturity of seeds prior to initiation of the cold period is indispensable for the wind-dispersed tree-line conifers, e.g., *Pinus sylvestris*, because seeds stay in cones during the winter. *Cembrae* seeds are, however, planted in soil and covered by snow and do not encounter very low temperatures.

Rigorous climatic conditions and short growing season are considered the main reasons for irregular seed crops and poor regeneration success (Sarvas 1962). Warming of the climate may increase seed production in boreal and alpine tree-line trees due to improved embryo growth and maturation, but it also results in smaller amounts of snow and in deeper freezing of the soil in these extreme conditions. Freezing of the soil to a great depth can injure tree roots and impair stress-tolerance of trees against fungi and predators (Solantie 2000, Tierney et al 2001), which would diminish cone and seed production. This may lead to less frequent visits by nutcrackers and decreased dispersal of seeds, because nutcrackers can also use the seeds of other conifers or even hazelnuts (*Corylus avellana*) beyond *Cembrae* stands as in southern Finland and Sweden. Shallow snow cover would also facilitate tracking of caches by nutcrackers and thus reduce the number of potential seedlings.

Early structural differentiation of the embryo, including well-cutinized epidermis, numerous stomata, glandular trichomes and resin ducts, was observed in *Cembrae* seeds during the pre-treatment that simulated conditions in the soil seed bank. When buried in the soil for one or more years embryos continue structural maturation and differentiation to attain readiness to germinate early, at the time of snowmelt and spring rains under the conditions in exposed sites. Shorter periods and smaller amounts of snowmelt can cause increased drought particularly in early summer. It would impair germination success, even if the embryo cavity is filled with malleable pectinous tissue that regulates moisture balance and metabolism of the seed, and also survival of young seedlings. The diminishing distribution of these tree stands would disturb the biodiversity of both the flora and fauna, because conifer seeds are rich in lipids and have great food values for many birds and mammals. Based on advanced adaptation of both the surface and inner structures of seeds specifically for bird-dispersal and for later maturation in the soil seed bank, the interaction with nutcrackers is changing from a profit to a major risk for survival of alpine tree-line *Cembrae* in a warming climate.

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## Evolution of Embryo Size in Apiaceae

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**Abstract**
A comparative study on the internal morphology of angiosperm species has shown that an enormous variation in relative embryo size exists amongst seeds of different species. While early angiosperm seeds are characterized by a small embryo and copious reserve tissues, an evolutionary trend is present in which the relative embryo size increases in more derived angiosperm lineages. In order to obtain a clearer picture of the evolutionary forces that are behind the enlargement of relative embryo size in several angiosperm lineages, a thorough study at low taxonomic level was needed. Therefore we performed a detailed investigation of the embryo size in seeds of Apiaceae species. The Apiaceae family contains about 3000 species that are distributed throughout the whole world, except for the tropics, and their seeds are known to exhibit considerable inter-specific variation in relative embryo size. Seeds of more than 200 Apiaceae species were obtained mostly from seed banks around the world. The mean and variation of the relative embryo size of these species was determined by cutting 20 seeds in half and measuring embryo and seed length. A phylogenetic hypothesis was constructed using ITS-sequence data. By using a molecular-seed morphological approach we aimed to determine: (i) the ancestral character state, (ii) whether there is an evolutionary trend towards increasing or decreasing embryo size within the Apiaceae family, (iii) to what extent the relative embryo size is a conserved trait. Additionally we attempted to infer the evolutionary forces which might be responsible for the modifications of the relative embryo size. We did this by taking phylogeny into account when correlating embryo size with, for example, habitat preference, life-cycle and geographic distribution.

# The Seed Stage and Its Importance for Desert Annuals

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## Introduction

Desert annual plants are frequently used to illustrate principles of adaptation to variable environments, population dynamic functions of dispersal and dormancy, and how temporal variation may promote species coexistence. All of these topics involve ecological and evolutionary responses to environmental variability. All of these topics end up focusing on the ecology of seeds, though most of the discussion is theoretical. I will illustrate these issues with data collected on Sonoran Desert annuals at the Desert Laboratory in Tucson, AZ. It has been suggested that combining the collection of long-term ecological data with a diversity of more short term focused approaches is a particularly powerful way to gain synthetic insights in ecology. We are using a 28-year data set on variation in demographic vital rates along with more short term investigations to determine the role of seed ecology in adaptation to variable environments, species coexistence, and community dynamics in response to climate change.

## Adaptation to Variable Environments

Organisms in variable, unpredictable environments are expected to evolve adaptations to reduce risk. Evolutionary bet hedging is a reduction in short-term fitness in favor of long-term reduction of risk. It is an adaptive strategy used to buffer organisms from temporal variation. For annual plants, delayed germination is considered to be a bet hedging mechanism in the face of variable and unpredictable success following germination. Theory predicts that the amount of bet hedging, measured as the proportion of seeds that forgo germination, should be related to the year-to-year variation in fitness. To test bet hedging theory we used our long-term demographic data on natural germination fractions and the 28-year sequence of data on temporal variation in per germinant fecundity. As predicted, species that experienced greater variation in per germinant fecundity had lower average historic field germination fractions. In other words, species with reproductive success that varies greatly from year to year have a higher proportion of dormant seeds on average. This analysis has quantified the actual, natural long-term fitness variance for a set of coexisting species and related it to their long-term average dormancy in that same undisturbed field setting.

## Species Coexistence and Diversity

Seed ecology plays a central role in species coexistence and species diversity. Ecologists have explored and cataloged the potential mechanisms by which competing species stably coexist. Three coexistence-promoting mechanisms are likely to be of potential importance to the structure of this winter annual community. First, resource partitioning is certainly involved and is reflected in habitat and range differences for coexisting plants. Another mechanism of potential importance is frequency-dependent seed predation. In this scenario, high density makes the seeds of a particular species more attractive to seed predators, which reduces the growth rate of common species. In seed tray feeding trials which systematically varied seed densities of different winter annual species, we have documented that the preference of rodents for each species is highest when seeds of that species are most abundant.

We have also quantified the magnitude of the storage effect for our system. The storage effect is a coexistence promoting mechanism that requires environmental variation that is experienced somewhat differently by different species and also a buffering life stage such as persistent seed banks. Our long-term data set demonstrates that species have differences in their germination responses to environmental variation and in their survival and fecundity responses to environmental variation. For our system, the low-density advantage due to germination variation alone increases population growth rate by ≈ 0.052. The low-density advantage due to per germinant fecundity variation alone adds 0.025 to the low density growth rate. An additional increase of 0.027 comes from the covariance of germination fraction and reproductive variation, giving a total low density growth rate advantage due to the storage effect ≈ 0.103. At our field site there are millions of individuals of most species. Thus any positive recovery rate will be adequate to result in indefinite coexistence. The observed storage effect is substantial, equivalent to a doubling time of 7 years for a species recovering from low density. Most of this storage effect is due to the ecology of seeds.

Species diversity of germinated plants has changed from year to year as population sizes vary and all species do not emerge in great numbers in every year. Species richness and Shannon diversity index for seedlings were both positively related to precipitation. Species richness and diversity taking into account seed banks is much more constant. Despite long-term trends in population and climate (discussed below) there has not been any sustained tendency for diversity to increase or decrease. This long-term stability in species diversity despite highly variable population dynamics confirms that strong coexistence-promoting mechanisms are at work in this community.

**Climate Change and Community Dynamics**

Long-term ecological data sets have recently been used to investigate the response of biotic communities to global climate change. Over the last 25 years, the Sonoran Desert has been getting warmer and drier. Climate models predict that this trend will continue and that the Southwestern United States will be impacted more by climate change than other parts of the US. The heating and drying has resulted in pushing germination of winter annuals later and later in the year, to December rather than October. This means that germination has been occurring under colder temperatures. We used our long-term data set to determine how the winter annual community has responded to these changes in weather and germination conditions, and found that the abundance of all winter annuals has decreased over the last 25 years. Also, some species were impacted more than others, leading to a change in community composition. Species composition has shifted in favor of demographically buffered, slower growing, water-use-efficient, cold adapted species.

In order to determine what stages in the lifecycle of our plants was responsible for inter-annual changes in abundance, we performed a series of regressions of different stage transitions against total annual change in population size. Transition from seeds produced in the previous year to seeds germinating in the current year, was the life history stage primarily responsible for changes in the abundance for seven of our nine focal species. Thus, germination ecology seems to be the primary driver of change in community composition. These results demonstrate the importance of germination ecology for biotic response to climate change. Also, responses to climate change can be unexpected: heating and drying have caused delays in germination that have led to lowered temperatures during germination, favoring an increase in cold adapted species.

**Conclusions**

We have shown that seed ecology plays the central role in adaptation to variable environments, species coexistence, and community dynamics in response to climate change for desert annuals. One might go as far as to say that desert annuals are persistent communities of seeds that occasionally put forth green plants that function as “fruiting bodies” much like fungal mycelia that occasionally produce mushrooms. While human observers of deserts usually only see these growing plants, only a superficial understanding of desert annuals is possible from observation of these “ephemeral fruiting bodies”. The real action is in the ecology of the seeds.

# Germination Responses Among Sympatric Facultative Fire Ephemerals Differing in Dormancy Classes

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## Introduction

Among Mediterranean regions of the world, fire-stimulated germination is particularly prevalent in Australia, California, and South Africa (Baker et al. 2005). A functional group of species found in these regions is fire ephemerals. Species in this group predominantly germinate after fire, are short-lived, and persist between fires as seeds in a soil seed bank. In South-west Australia, *Podotheca angustifolia* (Labill.) Less. (Asteraceae), *Austrostipa macalpinei* (Reader) S.W.L. Jacobs & J. Everett (Poaceae), *Trachymene pilosa* Sm. (Apiaceae), and *Levenhookia pusilla* R.Br. (Stylidiaceae) are monocarpic ephemerals that establish primarily after fire, but are not obligate fire followers (Western Australian Herbarium 2010). Although embryos in seeds of *Podotheca* and of *Austrostipa* are fully developed at maturity, those in seeds of *Trachymene* and of *Levenhookia* are underdeveloped. The purpose of our study was to compare the germination strategies among these four species in relation to their dormancy and their fire ecology.

## Methods

Freshly ripened seeds of the four species were collected from plants growing in *Banksia* woodlands near Perth, Western Australia or in *Eucalyptus marginata* (jarrah) forest near Pinjarra, Western Australia in November 2008. During December 2008, seeds were placed in nylon bags in a *Banksia* woodlands and in sealed polyethylene boxes (50% relative humidity) at 30°C in an incubator. Between January and April 2009, seeds were retrieved from the field and removed from the boxes and incubated in light and in darkness at 18/7°C (simulated winter), 26/13°C (spring and autumn), and 33/18°C (summer) on substrates moistened with distilled water, smoke water (SW), and the smoke compound karrikinolide ($KAR_1$). Embryo growth was monitored for *Levenhookia* and for *Trachymene* from January to April. To monitor germination phenology, seeds of the four species were sown on top of soil in pots in December 2009 and placed in a non-temperature-controlled tunnel-house. The soil was watered once a week until mid-April 2009 when watering commenced daily. In late April 2009, treatment pots were exposed to aerosol smoke for 1 h whereas control pots were not. Emergence of seedlings was monitored daily for the duration of the study.

## Results and Conclusions

No (or very few) freshly matured seeds of *Podotheca*, *Austrostipa*, and *Levenhookia* germinated in light or in darkness over a range of temperatures during 6 weeks of incubation. Although a few fresh seeds of *Trachymene* germinated in light, moderate to high percentages of seeds germinated in darkness with SW or with $KAR_1$ and low percentages with water. Dry after-ripening under field and laboratory conditions increased germination in light and in darkness for all species, particularly at low temperatures (Fig. 1). However, as seeds came out of dormancy, germination was stimulated to a greater degree with SW and with $KAR_1$ than with water. Once dormancy loss was complete, germination was about equal in all treatments. During after-ripening in the field and laboratory, no embryo growth occurred between January and April in seeds of *Levenhookia* and of *Trachymene*. Seedling emergence of all species from soil occurred from late April to May with the commencement of low habitat temperatures and regular rainfall. Sequence for the start of emergence was: *Podotheca* > *Austrostipa* = *Trachymene* > *Levenhookia*. The delay in emergence of *Trachymene* and *Levenhookia* was due to elongation of their embryos before radicle protrusion as compared to *Podotheca* (Fig. 2). Moreover, higher emergence took place from smoked soils than from non-smoked soils for *Levenhookia*. Germination of these species occurs regardless of smoke-related cues when they are non-dormant, but smoke-stimulated germination is highly dependent on the seed's degree of dormancy.

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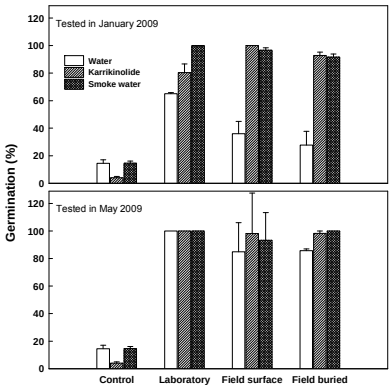


**Figure 1.** Mean (± SE) germination of *Trachymene pilosa* seeds after-ripened under laboratory conditions (50% relative humidity, 30°C), sown on the soil surface in the field, or buried in soil in the field. Seeds were tested for germination in January 2009 and in May 2009 at 18/7°C for 6 weeks in light on a substrate moistened with three solutions. Control seeds were not exposed to after-ripening conditions but incubated under the same conditions as after-ripened seeds.

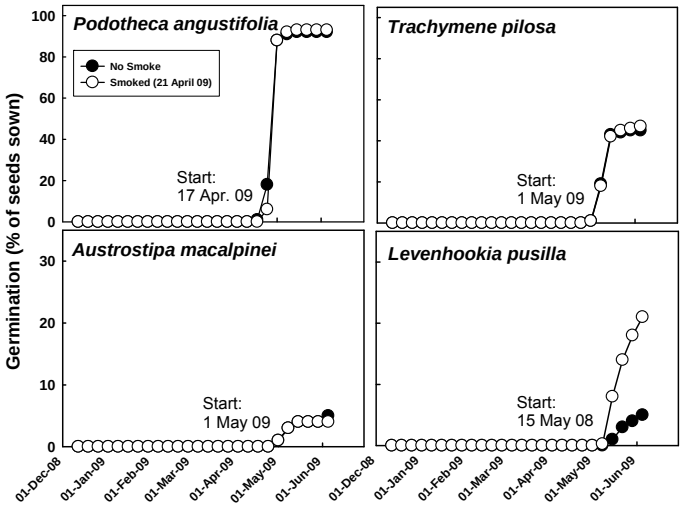


**Figure 2.** Mean (± SE; all SEs ≤ 7%) germination of seeds from four species of fire ephemerals. Seeds were sown on soil in a non-temperature controlled shed-house on 9 December 2008 with pots exposed to smoke on 21 April 2009. Soil was watered weekly from the day of sowing until 17 April 2009, when daily watering commenced. The date when the first seedlings emerged is indicated in each panel. The bottom two panels are scaled differently from the two top ones.

# Effect of Phylogeny, Life History and Habitat on Seed Germination of 69 Arid and Semi-arid Zone Species from Northwest China

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## Introduction

Life history traits of plants such as seed size and germination are not only inherited but also impacted by the external environment; thus, they are subject to natural selection. Life history attributes of plants may be determined by genetic and/or multiple environmental factors, such as a dry desert environment, acting simultaneously. Therefore, to assess the role of natural selection on seed germination at the community level, we should take into account as many variables as possible, including various life history traits and environmental conditions during seedling establishment.

The objectives of our study were to assess (a) whether differences in seed germination among species from the same community are related to phylogeny, life history traits, and/or seedling establishment conditions; and (b) what proportion of germination variation among species could be attributed to phylogenetic background, life history attributes, and environmental conditions. Through this community-level study, it is expected that the primary and secondary factors controlling seed germination of species in the arid /semi-arid zone can be identified.

## Materials and Methods

Mature seeds of 69 species from the arid/semi-arid zone of the Hexi Corridor in northwest China (Gansu province) were incubated in light and in darkness at an alternating temperature regime of 20 / 5°C (12:12 h). Final germination percentage, days to first germination and light requirement for germination of the 69 species were determined under laboratory conditions. One-way, two-way and multi-way ANOVAs were used to determine the effects of phylogeny and various life history and ecological attributes on final germination percentage and days to first germination.

## Results

*Germination characteristics*--Final germination percentages (GP) of the 69 species in both light and darkness had a bimodal distribution, whereas, days to first germination (DG) in both light and darkness was skewed toward short periods of time.

*Correlation between germination and phylogeny, life-history traits, and habitat*--In light, variance in final germination percentage was accounted for by phylogeny (13.6 %), dispersal mode (11.9 %), seed mass (3.6 %), and habitat (0.2 %); while in darkness it was accounted for by dispersal mode (17.5 %), phylogeny (12.7 %), seed mass (5.7 %), and habitat (0 %). In addition, there was a significant difference in mean germination percentage in light between phylogenetic groups ($F = 2.825$, $p < 0.01$) and an insignificant difference in mean germination percentage in darkness ($F = 1.807$, $p > 0.05$). There was a significant difference in mean germination percentage between dispersal categories (in light: $F = 8.966$, $p < 0.001$; in darkness: $F = 6.647$, $p < 0.05$) (Fig.1). In particular, seeds of wind-dispersed species have higher germination percentages than those of unassisted and vertebrate-dispersed species.

*Seed mass and germination of species in the two habitats*--Seed mass of the xerophytes (27 species) ranged from 0.145 mg to 63.50 mg, with a mean and median of 14.73mg and 6.356 mg, respectively. Seed mass of the mesophytes (42 species) ranged from 0.057 mg to 20.26 mg, with a mean and median of 3.13 mg and 1.33 mg, respectively. There was a significant difference in seed mass between the two plant groups ($F = 20.067$, $p < 0.001$); mean seed size of the xerophytes was significantly larger than that of mesophytes. Xerophytes tended to have a lower mean germination percentage (37 % and 39 % in light and darkness, respectively) than mesophytes (55 % and 49 % in light and darkness, respectively). Correlation analyses showed a significant negative correlation between germination percentage and seed mass of the 69 species. For the 27 xerophytes, there was an insignificant negative correlation between germination percentage and seed mass. However, for

the 42 mesophytes, there was a significant negative correlation between germination percentage and seed mass (Fig.2).

**Conclusions**

1) The variance in final germination percentages among species is largely dependent upon phylogeny and dispersal mode but that it is also influenced by seed mass and habitat.

2) The effects of dispersal mode and seed mass on final germination percentage among species were phylogenetic group- and habitat-specific.

3) Wind-dispersed seeds had higher germination percentages than unassisted and vertebrate-dispersed seeds.

4) By comparison with xerophytes, mesophytes tended to have smaller seed mass, higher mean germination percentages and a greater effect of seed mass on final germination fractions.

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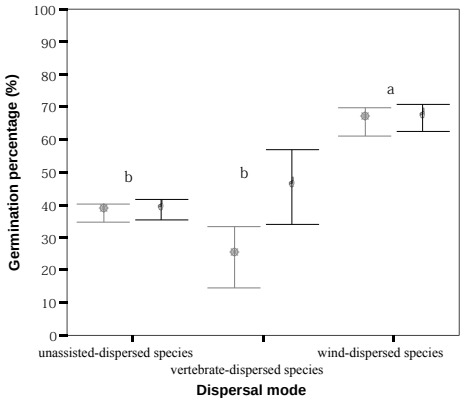


**Figure 1.** Difference in mean germination percentage between dispersal categories in light (star) and in darkness (hand).

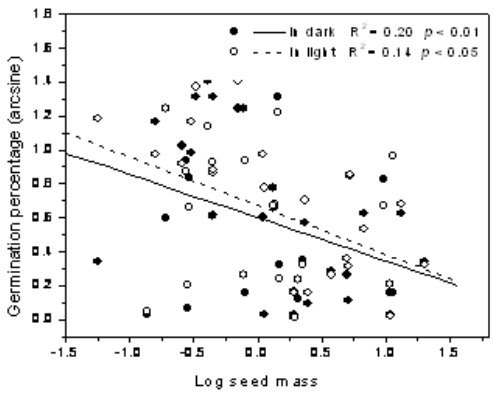


**Figure 2.** Relationship between germination percentage (arcsine) and seed mass (log) in 42 mesophytic species.

## Germination of Dimorphic Seeds of the Desert Annual Halophyte *Suaeda aralocaspica* (Chenopodiaceae), a C-4 Plant without Kranz Anatomy

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### Introduction

*Suaeda aralocaspica* is a summer monoecious annual and is commonly found only in the inland cold desert of the Junggar Basin, Xinjiang, China. It grows in saline-alkaline sandy soils. Plants bloom in August and produce dimorphic fruits and seeds on the same plant in September. We hypothesized that *S. aralocaspica* has developed a special strategy in the seed stage that is part of its suite of adaptations to the harsh cold salty desert habitats. Thus, we asked: what are the differences, if any, in (1) dormancy-breaking and germination requirements of the dimorphic seeds of this species, and (2) ability of the two seed morphs to recover from salt stress. We then used the results of our experiments to construct a conceptual model of its germination ecology.

### Methods

Fresh seeds of *S. aralocaspica* were collected from dry inflorescences in natural populations growing in desert saline soils (44º19′N, 86º57′E; 429 m a.s.l.). To investigate their germination behavior, fresh seeds of each morph were incubated at daily (12/12 h) temperature regimes in continuous light and in dark (seeds in black bags) for 20 d. Germination of fresh black seeds of *S. aralocaspica* in the light or dark in all temperature regimes was less than 40%, indicating a proportion of black seeds are dormant. Therefore, effects of cold stratification, seed coat scarification, seed coat removal, gibberellic acid and dry storage on dormancy-break were tested to explore the kind of dormancy in black seeds. The effects of different concentration gradients of NaCl on germination of seeds (brown seeds, black seeds and black seeds after 8-week cold stratification) were tested at a daily temperature regime of 10:25ºC in light. Ungerminated seeds from 20 d NaCl pre-treatments were rinsed three times with distilled water and then incubated for 10 d in Petri dishes that contained 2.5 mL distilled water.

### Results and Conclusions

Fresh brown seeds of *S. aralocaspica* are highly permeable to water, have a fully developed embryo, and are non-dormant. Thus, they can germinate rapidly to high percentages in water over a wide range of alternating temperature regimes in light and in dark. Fresh black seeds exhibit nondeep physiological dormancy (*sensu* Baskin & Baskin 2004) that can be broken by a few weeks of cold stratification. Furthermore, promotion of germination by GA3, afterripening in dry storage and disruption or removal of the testa also indicates that these seeds have non-deep PD. Germination tests of black seeds of *S. aralocaspica* after various periods of cold stratification indicated that during dormancy break the minimum temperature at which seeds could germinate to a moderate to high percentage decreased. Thus, it is concluded that black seeds of *S. aralocaspica* have Type 2 nondeep PD, the type expected for a summer annual such as *S. aralocaspica* (Baskin and Baskin, 2004). Data from this investigation with *S. aralocaspica* indicated differences in the responses of brown and black seeds to salinity. Compared to fresh black seeds, brown seeds are much more salt tolerant. When nondeep PD was broken by 8 weeks cold stratification, germination percentages and germination velocity of black *S. aralocaspica* seeds in distilled water and in saline solutions were significantly higher than for those that had not been stratified. A model summarizing the dynamics of seed dormancy, germination and potential to form a seed bank for dimorphic seeds of *S. aralocaspica* is shown. This study demonstrated that both morphological and physiological polymorphism exist in seeds of this species. These differences presumably represent the combination of different complementary adaptative strategies in one plant and have ecological significance for its successful survival in inland cold salt deserts.

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## Post-Dispersal Seed Removal by Rodents and Blue Fan Palm Distributions: An Approach to Start Understanding Desert Oasis Dynamics

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Information on the current processes controlling the distribution patterns of relict palm populations at the limit of their distribution in America are poorly known. We explored the importance of post-dispersal seed removal by vertebrates, recruitment and distribution patterns of the blue fan palm, *Brahea armata*, in Baja California peninsula, by evaluating (i) the levels of blue fan palm seed removal by vertebrates at two spatial scales and the initial fate of dispersed seeds, (ii) the spatial distribution and association of seedlings and adults at two spatial scales, (iii), and seed removal levels and seedling densities based on density and distance to adult palm trees.

Overall, seed removal levels were low at all sites (in 78% of cases, seeds were found intact, N = 256), and varied at regional but not at local scales (Fig. 1a). Small rodents were apparently responsible for most removal. The fate of removed seeds was significantly different among the three sites ($\chi^2$ = 21.7, df = 2, P = 0.0002; Fig. 1b). Seeds were removed from 0.5 up to 20 m from the stations in LL and from 0.5 up to 13 m in CAT (Fig. 1b). Adult density (AD) and nearest-neighbor distance (NND) differed among oases ($\chi^2$= 26.8, df = 2, P<0.00001; $\chi^2$ = 52.6, df = 2, P<0.00001, AD and NND, respectively; N = 128 data points in both cases); but seedling densities did differ locally ($\chi^2$ = 24.8, df = 4, P = 0.0001). In general, ADs, measured at random points, increased as NND decreased (Fig. 2a, b). CAT had the lowest AD and the highest NNDs, LL represented the intermediate case, and SPM showed the highest ADs and the most aggregated adult palm distribution (Fig. 2a, b). Whereas seed removal did not vary with distance from seed sources or parent trees (survival of seeds was neither significantly associated with AD: $\chi^2$ = 7.2, df = 8, P = 0.5, nor with NND: $\chi^2$ = 10.3, df = 8, P = 0.3); a weak positive association between seedlings and adults at the whole patch level indicated that establishment tended to occur near those grid cells where adults had established successfully (maximum seedling densities occurred at an AD category of more than one adult within an area of 10-m radius ($\chi^2$ = 12.6, df = 4, P = 0.01; Fig. 3a), and at a NND category of 0–5 m ($\chi^2$ = 10, df = 4, P = 0.04; Fig. 3b)). However, the spatial analysis of distance index (SADIE) showed a negative association between seedling and adult densities within the patches, indicating that within cells where growth was most successful, seedlings established preferentially in relatively open spaces (Table 1).

The exploration and scaling of seed removal and blue fan palm distribution patterns was important to help identify potential factors that might be significant in their establishment. We did not find evidence that post-dispersal seed removal by rodents is a main factor defining the recruitment patterns in these oases. In contrast, the effect of flood pulses seems to be significant and may have strong and conclusive effects on palm seedling distributions. We suggest that local factors (canyon physiography, nurse plants and/or objects) at each particular canyon are affecting post-dispersal seed removal activity patterns by rodents, as well as providing vital protection for palm seedling establishment from the extreme floods.

**Figure. 1** Patterns of seed removal: (**a**) percentages of cases with zero, one, two, three, and four seeds left at each site and plots within sites. San Pedro Mártir (SPM) included beci2 and bepal2 plots, Cataviña (CAT) included San Antonio (sa) and Santa Inez (si) plots, and La Libertad (LL) included ta2, tna1, and tna2 plots; (**b**) range of distances (meters) of blue fan palm seed removal by vertebrates (using the method of colored threads) and percentage of cases in which removed seeds were missing, dead or alive in each site.
**Figure. 2** Distribution patterns of adult palms in San Pedro Mártir (SPM), Cataviña (CAT), and La Libertad (LL): (**a**) Percentages of each adult density category (AD: 0, 1, and >1 adults in a 0.03ha area); and (**b**) Percentages of each nearest-neighbour distance category (NND: 0-5 m, 5-10 m, >10 m), measured at random points in all sites.

**Figure. 3** (**a**) Percentages of random points with seedlings (area: 0.0007ha), for each adult density category (AD: 0, 1, and >1 adults); and (**b**) at each nearest-neighbour distance category (NND: 0-5 m, 5-10 m, >10 m), in San Pedro Mártir, Cataviña, and La Libertad.

**Figure 1** **Figure 2** **Figure 3**

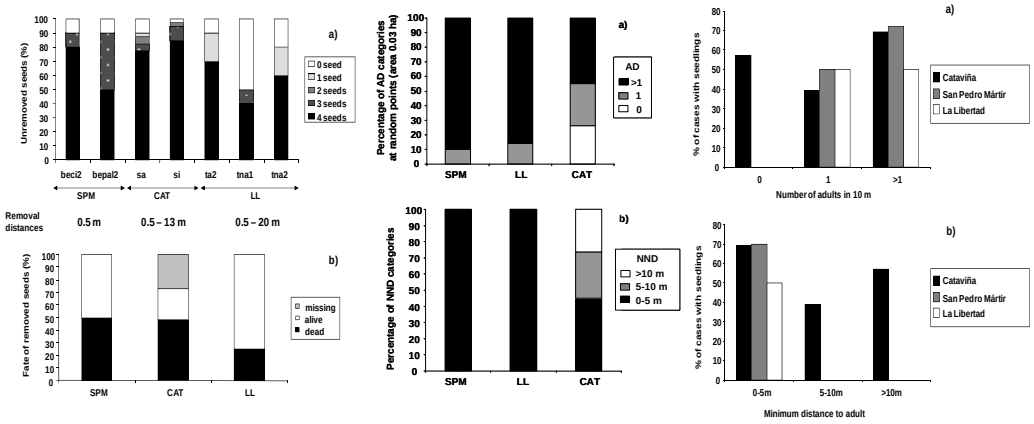


**Table 1** SADIE index and probabilities (P) of blue fan palm adults and seedlings, at two spatial scales (''patch'' and ''within-patch''), for the three study sites, Cataviña, La Libertad and San Pedro Mártir

| Scales | Sites | Study plot (size) | | *Ia* (P) | n | *X*(P) |
|---|---|---|---|---|---|---|
| **Patch** | CATAVIÑ A | SI 2 (0.65 ha) | Adults | **1.29 (0.05)** | | 0.15 (0.05) |
| | | | Seedlings | **1.72 (0.0005)** | 263 | |
| | | SA T (1.5 ha) | Adults | 1.47 (0.07) | | 0.18 (0.02) |
| | | | Seedlings | 1.26 (0.15) | 605 | |
| **Within-patch** | | SI 2 (0.2 ha) | Adults | **1.56 (0.008)** | | **-0.44 (>0.99)** |
| | | | Seedlings | **1.44 (0.02)** | 63 | |
| | | SA T (0.2 ha) | Adults | **2.1 (0.009)** | | **-0.58 (>0.99)** |
| | | | Seedlings | 1.2 (0.21) | 81 | |
| **Patch** | LIBERTAD | TNA1 (1 ha) | Adults | 0.9 (0.7) | | 0.3 (<0.01) |
| | | | Seedlings | **2.2 (0.0003)** | 275 | |
| | | TNA2 (1 ha) | Adults | 0.74 (0.96) | | 0.1 (0.06) |
| | | | Seedlings | **1.5 (0.03)** | 556 | |
| **Within-patch** | | TNA1 (0.2 ha) | Adults | **3.02 (0.0003)** | | **-0.9 (> 0.99)** |
| | | | Seedlings | **3.1 (0.0003)** | 49 | |
| | | TNA2 (0.2 ha) | Adults | 1.02 (0.4) | | **-0.7 (>0.99)** |
| | | | Seedlings | 0.8 (0.7) | 74 | |
| **Patch** | SP MARTIR | Bepal 2 (1 ha) | Adults | 1.4 (0.07) | | 0.15 (0.02) |
| | | | Seedlings | **4.3 (0.0003)** | 444 | |
| | | Beci 2 (0.5 ha) | Adults | 0.99 (0.4) | | 0.09 (0.07) |
| | | | Seedlings | **4.6 (0.0003)** | 488 | |
| **Within-patch** | | Bepal 2 (0.2 ha) | Adults | **3.5 (0.0003)** | | **-0.5 (>0.99)** |
| | | | Seedlings | **3.4 (0.0003)** | 134 | |
| | | Beci 2 (0.2 ha) | Adults | **3.5 (0.0003)** | | **-0.9 (>0.99)** |
| | | | Seedlings | **4.1 (0.0003)** | 97 | |

*Ia* spatial aggregation index (in bold significant aggregation patterns), n total number of counts, *X* overall spatial association index between adults and seedlings (in bold significant dissociation patterns)

# The Role of Ex Situ Seed Conservation in Rare Plant Reintroductions

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## Introduction

The Oahu Army Natural Resources Program (OANRP) implements conservation actions for the stabilization of 51 federally listed endangered plant species on the island of Oahu, Hawaii. To achieve stabilization, each species is required to have 3 populations of at least 50 reproducing individuals. Thirty-eight of these species require one or more reintroductions to meet this stabilization criterion. Reintroductions have already been initiated for 28 of these species. Another stabilization requirement is that every species must have adequate ex situ genetic representation. Seed storage is the preferred method of ex situ representation due to the relative low cost of maintaining collections and its superior capability in representing the genetic variability of an individual or taxon. With wild populations dwindling at an alarming rate, resource managers need to complete collections for ex situ genetic storage, find and fence suitable habitat for outplanting, and determine if there are threats that need to be mitigated. Storing seeds gives managers the time they need to complete all of these management actions so that reintroductions can be accomplished.

## Methods

Seeds are the propagule source for almost all OANRP reintroductions. Since many plants are only collected once before they die, and 15 of the 51 species have less than 50 individuals remaining, it is critical to understand the storage potential of each species. To both temporarily store seeds for reintroductions and create genetic storage collections, storage longevity is tested. Testing has been initiated for 80% of the 51 total species. Tests are ideally initiated from bulk seed collections (from more than one individual plant), and stored at several different relative humidities and temperatures. Viability is tested immediately after collecting, and then at 1, 2, and 5 years, followed by every five years until collections are exhausted. From these tests, a decline in viability can be tracked within the different treatments (Fig. 1a & b).

## Results and Conclusions

Of the species tested, only 2 have been determined to be desiccation sensitive, while the majority appears to be traditionally orthodox or fall into a more intermediate classification of long-term storage. From the ex situ collections, over 100 reintroductions have been initiated, and over 1500 reintroduced plants have originated from stored seed. More recently, the storage longevity data has allowed OANRP to set recollection intervals for either the extant wild plants (founders) or the stock at the reintroductions that represent extant or extirpated plants. The recollection interval is the length of time a seed collection adequately represents a plant or population in the seed bank before another collection must be made. Additional collections are necessary to maintain viable and genetically diverse storage collections. Once a 30-40% decline in viability is detected, the recollection interval is set for that length of storage time (Fig. 1a). If no decline has been detected, the recollection interval is set 5 years greater than the length of time tested and adjusted as needed. An example of this type of interval is for the species *Schiedea trinervis* (H. Mann) Pax & K. Hoffm.(Caryophyllaceae) (Fig. 1b). Since no decline in viability has been detected after ten years of storage, the recollection interval is set at 15+ years. Recollection intervals have been determined for 20 taxa to date (Table 1). Some recollections may happen early, particularly when the original collection was obtained from an isolated wild plant (selfed) of a known or assumed outcrossing species. As reintroductions age and new generations become reproductive, new genetic combinations can enhance the quality of the ex situ collections.

Table1.

| Species | Recollection Interval |
|---|---|
| *Chamaesyce celastroides* var. *kaenana* | 5+ |
| *Cyanea crispa* | 10+ |
| *Cyanea grimesiana* subsp. *obatae* | 10+ |
| *Cyanea superb* subsp. *superba* | 10+ |
| *Cyrtandra dentata* | 5 to 10 |
| *Delissea subcordata* | 10+ |
| *Dubautia herbstobatae* | 15+ |
| *Flueggea neowawraea* | 10 |
| *Hedyotis parvula* | 10+ |
| *Hibiscus brackenridgei* subsp. *mokuleianus* | 10+ |
| *Lobelia gaudichaudii* subsp. *koolauensis* | 5 to 10 |
| *Melanthera tenuifolia* | 10+ |
| *Neraudia angulata* | 10+ |
| *Sanicula mariversa* | 5 to 10 |
| *Schiedea kaalae* | 10+ |
| *Schiedea nuttallii* | 10+ |
| *Schiedea obovata* | 10+ |
| *Schiedea trinervis* | 15+ |
| *Tetramolopium filiforme* | 15+ |
| *Viola chamissoniana* subsp. *chamissoniana* | 10 |

Fig. 1a: Germination of Fresh & Stored Seeds of *Viola chamissoniana* Ging. subsp. *chamissoniana* (Violaceae)

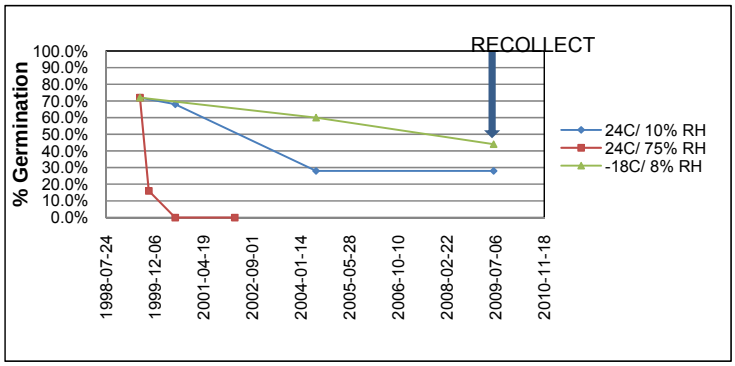
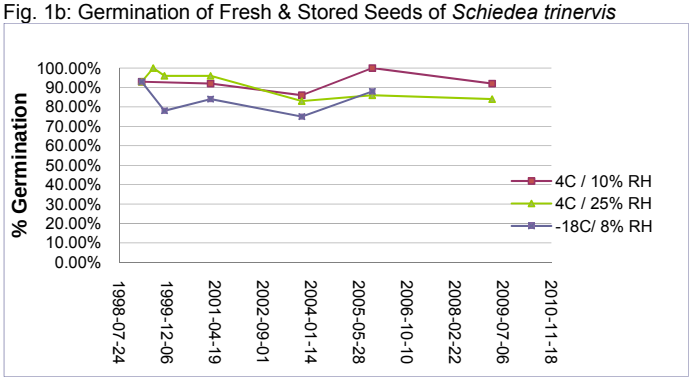



Fig. 1b: Germination of Fresh & Stored Seeds of *Schiedea trinervis*



# Light Requirements for Germination and Imbibed and Dry Longevity of Hawaiian Seeds

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## Introduction

Most studies of seed longevity are done on desiccated seeds under laboratory conditions. Fewer studies are done under imbibed conditions. Very few studies compare longevity under desiccated and imbibed conditions using seeds from the same collections. Many seeds are imbibed under field conditions, but the lack of studies comparing imbibed with desiccated longevity make it difficult to apply results of studies of longevity of dried seeds to seeds in nature. This study compares longevity of seeds of Hawaiian plants from the same seed lots stored under both imbibed and desiccated conditions. It shows that, for many species that require light for germination, there is a positive correlation of longevity in wet and dry storage over a two year period.

## Methods

Seeds were collected from 37 species of native Hawaiian plants and weighed. Fresh seeds were tested for germination. Seeds to be kept imbibed in the dark were plated onto 1% water agar in petri dishes which were then wrapped in aluminum foil (Baskin and Baskin 1998) and stored at 23°C. Other seeds from the same collections were stored under standard institutional seed banking conditions, i.e. dried to equilibrium with 20% or <10% relative humidity at 23°C, 5C°, or -18°C. After two years, the foil was removed from the petri dishes, and the dishes were placed in a growth chamber where they were exposed to light. At the same time, samples of seeds from the same seed lots stored under seed banking conditions were also sown onto petri dishes and placed in the growth chamber. Every 10 days, germinating seeds were counted and removed.

## Results and Conclusions

After 2 years in dark storage, 24 species had not germinated in the dark, but did after exposure to light ("Light"). Ten species did not germinate after exposure to light ("Dark"). For 8 of these species, all viable seeds germinated in the dark. In 3 species, some seeds germinated in the dark, others after exposure to light ("Both"). Most Light species were herbs and shrubs. Most Dark species were trees (Table 1). Median seed weight was 0.21 mg for Light seeds (n = 21), 38 mg for Dark seeds (n = 10) ($p = 0.0003$, Mann-Whitney test; Figure 1). There were both imbibed and dry storage data for 19 Light species. Of these, 12 germinated better after dry storage, 5 germinated better after imbibed storage, and there was no difference for 2. ($p = 0.08$, cumul. binom. distrib. $p = 0.5$, $n = 19$; Figure 2.) Percent germination under dry and imbibed storage were positively correlated (Pearson's correlation coefficient = 0.483), with notable outliers.

The results demonstrate that many small-seeded species of Hawaiian plants are capable of surviving for 2 or more years in an imbibed state in the dark. As many seeds in the soil are permanently imbibed (Wuest 2007), this shows that they are physiologically capable of forming persistent seed banks. For most species, there is little direct experimental data for longevity of seeds in soil seed banks, but the results demonstrate that data for longevity under dry storage can give insights into potential longevity in the soil. These findings further extend the results of Long et al. (2008) in applying laboratory studies of seed longevity to longevity under field conditions.

**References**

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**Table 1.** Growth forms of plants with seeds germinating after exposure to light following two years of dark imbibed storage ("Light") compared to those that all germinated in the dark or failed to survive dark storage ("Dark"). Growth forms from Wagner et al. (1999).

| | Sedge/grass | Herb | Shrub | Shrub/tree | Shrub/tree/liana | Tree |
|---|---|---|---|---|---|---|
| Light | 4 | 2 | 12 | 4 | 1 | 1 |
| Dark | - | 1 | 1 | 2 | - | 6 |

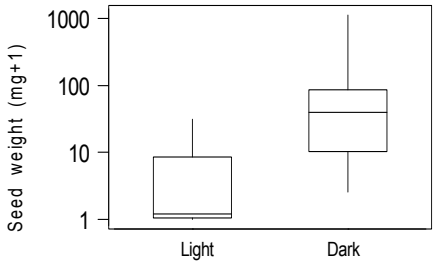


**Figure 1.** Box plots of seed weights for seeds germinating after exposure to light following two years of dark imbibed storage ("Light") compared to those that all germinated in the dark or failed to survive dark storage ("Dark"). Weights in $Log_{10}$ milligrams, with 1 mg added to make values positive.

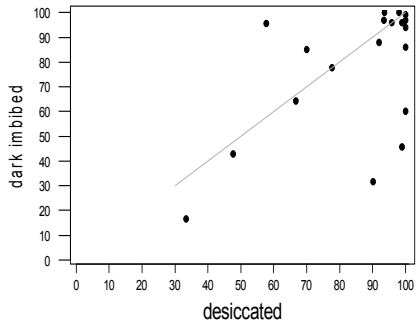


**Figure 2.** Percent germination after two years storage for seeds surviving imbibed storage. The horizontal axis shows germination after desiccated storage under seed banking conditions. The vertical axis shows germination after imbibed storage in the dark. Seeds along the diagonal line have the same longevity in both desiccated and imbibed storage. Percentages for each species were normalized by dividing by the highest observed percent germination for that species.

# Dormancy Breaking and Germination Requirements of Seeds in Five Native Shrubs in Urumqi, China

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## Introduction

It is urgent to rehabilitate degraded ecosystems in the arid regions of China. *Caragana acanthophylla, Astragalus arbuscula, Atraphaxis spinosa, A. virgata* and *Convolvulus tragacanthoides* are component shrub species of natural vegetation in the arid Urumqi region in NW China (Ye et al. 2005). They are tolerant of drought and soil infertility (Zhao et al, 2001). To understand their role in the revegetation of the area, it is necessary to know their regenerative processes. Because some or all seeds of these species are dormant, in this study we explored dormancy characteristics and dormancy-breaking requirements.

## Methods

Ripe seeds were collected from natural populations of each species. For each treatment, three replications of fifty seeds were incubated in Petri dishes with two sheets of filter paper moistened with de-ionized water. Seeds were stratified at a constant temperature of 5C for 2, 4, 6, 8 weeks and tested for germination in light and darkness at 12/12 h daily alternating temperature regimes of 15/6, 20/10, 25/15 and 30/20C for 2 or 4 weeks. In *Caragana acanthophylla, Convolvulus tragacanthoides* and *Astragalus arbuscula,* seeds were scarified before germination testing. Four replications of 200 seeds each were sown on the surface of soil collected from the habitat in pots (25 cm diameter, 20 cm height) and placed outdoors. The pots were inspected for seedlings weekly or biweekly. The experiment was terminated after 14 months.

## Results and Conclusions

Dormancy break in seeds of the two *Atraphaxis* taxa occurred during cold stratification at 5C for 6 or 8 weeks; seeds stratified at 5C and incubated at 12/12h daily temperature regimes of 15/6, 20/10, 25/15 and 30/20C germinated faster and to a significantly higher percentage at each temperature regime than non-stratified seeds (Table 1). Their seeds exhibited shallow physiological dormancy. Dormant seeds of *Caragana acanthphylla, Convolvulus tragacanthoides* and *Convolvulus tragacanthoides* immediately germinated after seed coat scarification; germination percentages averaged 99.32%, 100% and 97.31% respectively. Their seeds exhibited physical dormancy. In the field, seedling emergence occurred in the spring of the following year for *Atraphaxis taxa,* in the autumn of the current year for *Convolvulus tragacanthoides,* and in the autumn of the current year and the following spring for *Caragana acanthophylla* (Table 2). Based on dormancy characteristics and requirements for release of seed dormancy, we can sow seeds of these shrubs in the autumn to promote germination, especially at early spring habitat temperatures. Alternatively, we can crack the seed coats for *Caragana acanthphylla, Astragalus arbuscula* and *Convolvulus tragacanthoides* before sowing in spring.

**References**

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Table 1 Germination percentages (Mean ± SE) for seeds of two *Atraphaxis* taxa incubated at four alternating temperature regimes following stratification for 0 to 8 weeks

| Stratification time (weeks) | Incubation time (weeks) | Germination percentages (%) | | | |
|---|---|---|---|---|---|
| | | 15/6C | 20/10C | 25/15C | 30/20C |
| *Atraphaxis spinosa* | | | | | |
| 0 | 2 | 2.09±0.05 | 4.10±0.70 | 6.21±0.06 | 8.75±1.06 |
| 0 | 4 | 2.76±0.36 dC | 8.86±0.45 dB | 11.07±1.10 dB | 23.50±1.06 dA |
| 2 | 2 | 14.14±2.46 | 34.59±1.92 | 35.33±2.70 | 37.94±1.44 |
| 2 | 4 | 19.52±3.14 cB | 38.79±2.03 cA | 40.67±5.04 cA | 54.29±0.09 cA |
| 4 | 2 | 10.31±0.58 | 37.59±0.51 | 45.22±3.35 | 40.06±2.56 |
| 4 | 4 | 18.76±1.11 cdC | 47.65±0.79 cB | 54.12±1.57 cB | 64.59±2.35 cA |
| 6 | 2 | 17.46±3.71 | 48.59±3.04 | 56.70±0.69 | 56.71±1.60 |
| 6 | 4 | 53.70±3.55 bB | 80.62±1.88 bA | 80.02±2.73 bA | 88.08±5.42 bA |
| 8 | 2 | 59.07±3.52 | 89.86±4.83 | 91.28±10.27 | 99.33±3.83 |
| 8 | 4 | 68.77±4.40 aC | 91.25±4.99 aB | 94.63±7.84 aAB | 100.00±0.00 aA |
| *Atraphaxis virgata* | | | | | |
| 0 | 2 | 12.23±0.65 | 12.23±0.65 | 12.24±1.56 | 13.19±1.43 |
| 0 | 4 | 17.03±0.50 cB | 31.33±1.01 cA | 22.20±1.21dAB | 31.20±3.12 dA |
| 2 | 2 | 50.06±0.80 | 66.62±2.79 | 48.50±3.16 | 77.66±3.22 |
| 2 | 4 | 58.29±1.00 bB | 77.64±5.46 bA | 63.64±1.70 cB | 86.04±2.03 cA |
| 4 | 2 | 51.25±5.71 | 70.82±5.16 | 70.63±1.64 | 77.50±2.04 |
| 4 | 4 | 63.44±4.53 bB | 86.39±3.31 bA | 80.12±1.74 bA | 88.39±2.43 cA |
| 6 | 2 | 58.72±3.90 | 77.89±1.92 | 81.16±6.00 | 81.45±1.51 |
| 6 | 4 | 91.18±4.26 aA | 95.96±6.69 aA | 97.97±4.87 aA | 95.87±2.52 bA |
| 8 | 2 | 89.17±3.16 | 96.53±1.63 | 92.43±2.84 | 96.57±2.81 |
| 8 | 4 | 93.24±4.38 aA | 97.21±1.71 aA | 95.88±6.75 aA | 98.64±5.48 aA |

Table 2. Germination percentage in incubation and in the field (mean±SE)

| Speceis | Incubation (25/15°C) | Field (first year) | Field (next year) |
|---|---|---|---|
| *Caragana acanthophylla* | 62.19±4.69 a | 17.33±1.55 | 73.33±3.28 b |
| *Atraphaxis spinosa* | 11.07±1.10 c | 0 | 16.00±2.35 d |
| *Atraphaxis virgata* | 22.20±1.21 bc | 0 | 100 a |
| *Convolvulus tragacanthoides* | 33.22±0.77 b | 58.67±2.48 | 58.67±2.48 c |
| *Astragalus arbuscula* | 23.46±1.65 bc | - | — |