Chapter 4 - Laboratory germination characteristics and seed mass of alpine species from the Victorian Alps

Summary

In this chapter, I explore the differences in seed mass and germination characteristics among ten alpine species from different elevations within the Victorian alpine zone. I used laboratory techniques to simulate germination during early spring (cool treatment) and summer (warm treatment) under standard moisture regimes and diurnal light fluctuations. No seeds were pre-treated in the experiments and all were tested for viability. I weighed all seeds before subjecting them to experimental conditions.

Across different altitudes, seed mass varied within each species, but only two of the ten species showed a significant relationship between seed mass and altitude of seed origin. Within each species, mean seed mass showed no significant correlations with final percent germination.

Final percent germination was high among seeds from all species with some species having >90% germination. Seeds of *Aciphylla glacialis* did not germinate under either treatment. Germination lag-times were short and germination speeds (T_{50}) were fast. Germination lag-times were significantly longer in the cool treatment compared to the warm treatment. Most species had reached their final percent germination after 20 days. Although the final percent germination values between seeds of different elevations were similar, the probability of germination was often higher in seed that originated from lower elevations.

I discuss my results in relation to the international literature on alpine and arctic seed germination characteristics. I discuss how various germination strategies affect alpine species which grow across altitudinal gradients and how subsequent seedling establishment among alpine plants may be influenced by seed mass and particular germination syndromes.

Introduction

Reproduction by seed in alpine areas has historically been considered a rare event (Billings and Mooney 1968). The short, cold growing seasons, large diurnal temperature fluctuations in summer and regular frosts with consequent soil heave are likely to hinder all phases of sexual reproduction (Bliss 1962; Brink *et al*. 1967; Billings and Mooney 1968; Bliss 1971; Bell and Bliss 1980; Körner 1999; Inouye 2000). Hence, the probability of successful sexual regeneration in alpine areas is thought to be low, and it has been widely accepted that plant dispersal and regeneration in alpine areas is largely through vegetative means such as rhizomes and re-sprouting (Bliss 1962; Billings and Mooney 1968; Bliss 1971). Despite this apparent lack of sexual regeneration under natural conditions, there is evidence that many alpine plants flower readily (Körner 1999) and produce hundreds of viable seeds (Sayers and Ward 1966; Chambers *et al*. 1987) that can contribute to extensive soil seed banks (Freedman *et al*. 1982; Cooper *et al*. 2004) and under laboratory conditions, germinate easily (Amen 1966; Sayers and Ward 1966; Bliss 1971; Kibe and Masuzawa 1994).

The stages of seedling emergence and subsequent establishment are the periods with the highest risk of mortality at all elevations (Urbanska and Schütz 1986; Körner 1999). Under the pressure of natural selection, therefore, patterns in seed germination are expected to vary with environmental factors in order to maximize seedling survival (Cavieres and Arroyo 2000). Environmental factors, such as soil moisture, the current and previous season's temperature, and the length of the snow season can act as selective pressures affecting the timing of alpine seed germination (Mooney and Billings 1961; Amen 1966; Chambers 1989; Cavieres and Arroyo 2000; Giménez-Benavides *et al*. 2005). These factors can vary predictably across an altitudinal gradient (see Chapter 2) and hence, germination patterns may be expected to vary accordingly and thus, explain the abundance and altitudinal distribution of alpine species (Chambers *et al*. 1990). However, studies comparing germination patterns across species within habitats have indicated that germination characteristics can partly be a function of life history traits (Baskin and Baskin 1971; Grime *et al*. 1981; Washitani and Masuda 1990). This therefore makes the relationship between germination and habitat type difficult to ascertain (Meyer *et al*. 1990). Within species and genera, however, differences in germination characteristics between habitats have been detected (Meyer *et al*. 1990; Schütz and Rave 1999; Giménez-Benavides *et al*. 2005), although results from different species are inconsistent. Hence, an overall 'alpine germination syndrome' is unlikely to exist (Körner 1999).

Very few studies have compared the effect of environmental factors on alpine seed germination within habitat type and species. Vera (1997) showed that seeds of *Calluna vulgaris* and *Erica cinerea* collected from the highest altitudes in the Cantabrian Mountains, North Spain, had the highest percent germination but it is unclear whether habitat type was consistent between seed collections.

In the current study, I examined the variation in seed germination between populations within species across an altitudinal gradient. I held habitat type constant by using seed from the peaks of mountains that vary in altitude, but where tall open herbfield is the habitat type across mountains. This approach also holds other aspects of species life-history relatively constant, making differences in germination characteristics in relation to the environmental factors across the gradient easier to detect (Cavieres and Arroyo 2000).

The main objectives of this study were to 1) characterise seed mass and germination responses within several alpine species across an altitudinal gradient, and 2) to compare seed mass and germination responses of each species between sites and experimental treatments.

Methods

Study sites

The study was conducted with seeds from ten alpine species from four families collected across eleven alpine sites. The study sites (Table 2.1 in Chapter 2) represent a gradient in environmental conditions from the 'warm and favourable conditions' at Mt Speculation (1668 m) where winter snow cover is usually marginal, to Mt Bogong (1986 m) where conditions are 'less favourable'; here, frosts are common all year round (Williams and Ashton 1987) and the growing season is four months at most. All sites are within the Alpine National Park, approximately 350 km from Melbourne and are within 40 km of each other, with most less than 10 km apart (Figure 2.1 in Chapter 2). In addition to the sites used in Chapter 2, I also used seed collected from Spion Kopje (1880 m, 36°50'00"S, 147°19'30"E), Mt McKay (1840 m, 36°52'35"S, 147°14'30"E) and Mt Howitt (1738 m, 37°10'30"S, 146°38'50"E). Mean annual rainfall of this region is relatively high compared to adjacent lowland areas, over 1800 mm (LCC 1982), with much of this precipitation falling as snow during winter. Average air temperatures follow seasonal variations with typical daily minima and maxima of between 2 and 27 °C in

summer and -6 and 12 °C in winter (Bureau of Meteorology *unpublished data*). Frosts are frequent and can occur at any time of year (Williams 1987). The growing season spans approximately seven months from snowmelt, usually the end of September, until late April, when the first substantial snowfalls of the winter begin. Key climatic parameters for the study sites, derived from long-term data by BIOCLIM are shown in Table 2.3 in Chapter 2. All sites are classed as 'Steep Alpine Mountains' after Costin (1957), are above the natural tree-line and are dominated by tall alpine herbfield vegetation. The lower peaks also contain species typical of adjoining sub-alpine woodland (Costin 1957). The underlying geology of the sites varies (Table 2.2 in Chapter 2). Despite such variation, the parent material in these regions rarely influences soil type and all soils are considered alpine humus, with a gravelly or skeletal structure (Costin 1962b; LCC 1982) and are acidic, ranging from pH 4 to 5.

Study species and seed collection

Ten study species were chosen on the basis of their conspicuous abundance in at least two of the study sites and whether ample seed was available for collection. From the Apiaceae, I chose *Aciphylla glacialis*. From the Asteraceae, I chose *Brachyscome rigidula*, *Brachyscome spathulata*, *Leucochrysum albicans* sub. sp. *albicans*, *Microseris* sp. 2, *Olearia phlogopappa* var. *subrepanda* and *Xerochrusym subundulatum*. From the Junceaceae, *Luzula acutifolia* was selected and from the Poaceae, *Trisetum spicatum* and *Rytidosperma nudiflora*. Seeds from these species were collected by hand over two months during the summer of 2003/2004. I took care that seed was mature and easily detached from the remaining stalks or inflorescences. The seed was placed in paper bags, transported to the laboratory and left to dry in a purpose built heated drying box, set at 17 °C, for two weeks. Seed was then stored in dry conditions with naphthalene crystals, to deter insects, at room temperature until the experiments began.

Germination trials

Each seed was assessed for viability prior to the germination experiments, by squeeze tests and inspection on a light box, to ascertain that each embryo was filled. Seed mass was determined by weighing to four decimal places. Twenty random seeds from each species were arranged on moist seed germination paper (A.S.C. seed filter paper, 1 mm thick, 84 mm diameter) in petri dishes, with eight replicates per species in each of the two temperature treatments. Petri dishes were sealed with laboratory Parafilm to prevent moisture loss. The germination trials were conducted in Thermoline Refrigerated

Incubators, with a bank of fluorescent tubes providing a light intensity of 48 microeinsteins m^{-2} s^{-1}. Eight replicates of each species were subjected to a 'cool' treatment (12/10°C with 14/10 hours day length, representing late winter/early spring conditions) and a 'warm' treatment (20/10°C with 12/12 hours day length, representing mid summer). Petri dishes were randomly placed in the incubators, checked every 2 to 4 days during the 'day' part of their cycle and re-randomised upon return to the incubator. Seeds were considered germinated when the radicle had emerged from the seed coat.

The germination trials were staggered due to space limitations and equipment failure. Seeds from all species were placed in the cool treatment on the 21st October 2004. *Brachyscome rigidula* seeds were placed in the warm treatment on the 29th October 2004. The remaining seeds did not start their warm treatment until the 5th July 2005 and hence, were stored for an extra nine months compared with their counterparts in the cool treatment. Too few seeds of *Rytidosperma nudiflora* were collected to allow this species to be included in the warm treatment. At the end of each test period, ungerminated seeds were scored for fungal attack and again for viability. Seeds deemed unviable or killed by fungus were removed from the analysis and scores of final percent germination in each petri dish were adjusted accordingly.

Data analysis

Germination characteristics were only compared within a species, rather than across species. I used multi-factorial ANOVA with Bonferroni post-hoc tests to compare seed mass across sites. I used linear regression to investigate the relationship between seed mass and altitude for species which were collected from three or more sites.

I describe the germination lag-time time, the number of days until seeds from each species first germinate, and the germination speed (T_{50}), the number of days until 50% of the final germination has occurred.

I used multi-factorial ANOVA with Bonferroni post-hoc tests to compare the final percent germination within and between the cool and warm treatments.

I used the Kaplan-Meier (K-M) product limit estimation function (Kaplan and Meier 1958; Lee 1992) to model the probability of germination during the experiment. Modelling the germination curves in this manner reveals the germination 'journey' that a group of seeds take on their way to reaching their final percent germination. This method also gives a mean probability of germination score at each sampling time which can be compared across sites within each species. The method uses germination data from every seed, in the form of elapsed time (days) to germination (right censored data) and also data

for seeds which did not germinate (non-censored data) (Giménez-Benavides *et al*. 2005). The values in the probability of germination curves are then constructed by subtracting the K-M values (0 to 0.9) from 1. Comparisons of paired germination probability curves across sites were then tested by non-parametric Log-rank tests, which provide a Mantel's χ^2 statistic and *P*-value. The shape of the probability of germination curves were remarkably similar to the percent germination curves and are therefore not presented (see Figure 5.3, in Chapter 5, for an example of germination probability curves). SYSTAT version 10 (Copyright SPSS Inc., 2000) was used for all statistical analyses.

Results

Seed mass

For some species, seed mass varied significantly across sites of different elevations (Figure 4.1). Overall, there were few trends between seed mass and altitude. However, *Olearia phlogopappa* var. *subrepanda* and *Leucochrysum albicans* sub sp. *albicans* both showed significant linear relationships between seed mass and altitude ($R^2 = 0.69$, $P < 0.001$; $R^2 = -0.17$, $P = 0.032$ respectively).

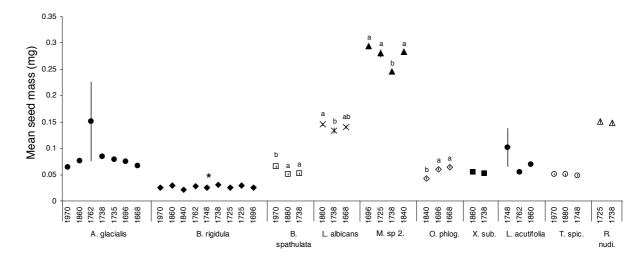Figure 4.1. Mean (± 1 standard error) seed mass and altitude for each species. Different labels (a, b or ab) above data points signify the Bonferroni significant differences within each species. Data points without labels showed no significant differences between sites. * Significant differences in seed mass between sites of *B. rigidula* in Appendix 4.1. For full species names see Appendix 4.2.

Germination characteristics

Germination lag-times, the time until the first germination of any seeds of each species was recorded, were short (2-19 days), especially for the forbs from the Asteraceae in the cool treatment (Table 4.1). The shrub *Olearia phlogopappa* var. *subrepanda* showed significantly longer lag times in both treatments than other species from the same family. The graminoid species showed consistently longer lag times than Asteraceae species in both treatments. Seeds of *Aciphylla glacialis* failed to germinate in both treatments and therefore germination speed and lag times are not available. All species showed significantly longer lag times (6 – 21 days) in the warm treatment than in the cool treatment (except *O*. *phlogopappa* var *subrepanda)*.

Germination speed (T_{50}) was particularly fast in the Asteraceae species in both treatments. Some species reached T_{50} at the first or second sample interval (Table 4.1).

Table 4.1. Germination speed (T_{50}) and germination lag-time (lag) for seeds in cool (12/10°C) and warm (20/10°C) treatments of ten species from various altitudes across the Victorian alpine zone. Dashes (-) indicate seed unavailability. No *Aciphylla glacialis* seeds germinated in either treatment, therefore germination lag and speed data is not available.

Species	Site and altitude (m) of seed origin	Cool treatment		Warm treatment	
		lag (days)	T_{50}	lag (days)	T_{50}
Aciphylla glacialis	Mt Bogong (1970)	N/A		N/A	
	Mt Hotham (1860)	N/A		N/A	
	Mt Buller (1762)	N/A		N/A	
	Mt Howitt (1738)	N/A		N/A	
	Mt Magdala (1725)	N/A		N/A	
	King Billy (1696)	N/A		N/A	
	Mt Speculation (1668)	N/A		N/A	
Brachyscome rigidula	Mt Bogong (1970)	2	2	6	8
	Mt Hotham (1860)	2	2	6	8
	Mt McKay (1840)	2	8	6	8
	Mt Buller (1762)	2	2	6	8
	Mt Stirling (1747)	2	8	6	8
	Mt Howitt (1738)	2	2	6	8
	Mt Magdala (1725)	2	2	6	8
	The Bluff (1725)	2	2	6	8
	King Billy (1696)	2	2	6	8
Brachyscome spathulata	Mt Bogong (1970)	2	8	11	14
	Spion Kopje (1880)	2	8	11	14
	Mt Howitt (1738)	2	8	11	14
Leucochrysum albicans subsp. *albicans*	Mt Hotham (1860)	2	2	8	6
	Mt Howitt (1738)	2	2	-	-
	Mt Speculation (1696)	2	2	6	6
Microseris sp. 2	Mt McKay (1840)	2	2	6	14
	Mt Howitt (1738)	2	2	6	11
	Mt Magdala (1725)	2	8	6	11
	King Billy (1696)	2	2	6	11
Olearia phlogopappa var. *subrepanda*	Mt McKay (1840)	19	28	17	39
	King Billy (1696)	19	35	21	37
	Mt Speculation (1668)	19	28	21	30
Xerochrysum subundulatum	Mt Hotham (1860)	2	8	6	11
	Mt Speculation (1668)	2	8	6	11
Luzula acutifolia	Mt Hotham (1860)	15	23	17	24
	Mt Buller (1762)	10	12	14	17
	Mt Stirling (174 7)	12	19	14	21
Trisetum spicatum	Mt Bogong (1970)	8	10	-	-
	Spion Kopje (1880)	8	10	11	11
	Mt Stirling (1747)	2	8	11	11
Rytidosperma nudiflora	Mt Magdala (1725)	8	10	-	-
	Mt Howitt (1738)	10	19	-	-

The germination curves for each species reflect the short germination lag times and rapid germination speeds (Figure 4.2). For most species, few seeds had germinated after 20 to 30 days, by which time the final percent germination values has mostly already been attained. Final germination was above 90% for many species, with little or no significant differences between sites within each species (Figure 4.3). There were no significant differences between final percent germination for any species at any site between the cool and warm treatments (paired t-tests, $P = >0.05$)

Within each species, the probability of seed germination between pairs of sites, over the course of the experiment, was often significantly different (Table 4.2 and Table 4.3), even though final mean percent germination was often the same among each pair. These pairwise comparisons between sites, within species and treatments, showed that seeds from lower elevations were more likely to germinate than those from higher elevations (Table 4.2 and 4.3). Of all the pairwise comparisons which showed significant differences in germination probabilities, 75% indicated that germination probability was higher in seed which originated from lower elevations. However, around half of all comparisons showed either no significant differences, or in some cases, that seed from a higher mountain peak had a significantly higher germination probability.

Within species, seed from the lower mountain peaks generally had a higher probability of germination in the cool treatment compared to the warm treatment (Table 4.4). However, seed from higher mountain peaks showed no trends in germination probability

Comparisons of seed properties

Within each species, mean seed mass showed no significant correlations with final percent germination. Neither lag-time nor germination speed were correlated with final percent germination using the Pearson/Spearman rank correlation co-efficient.
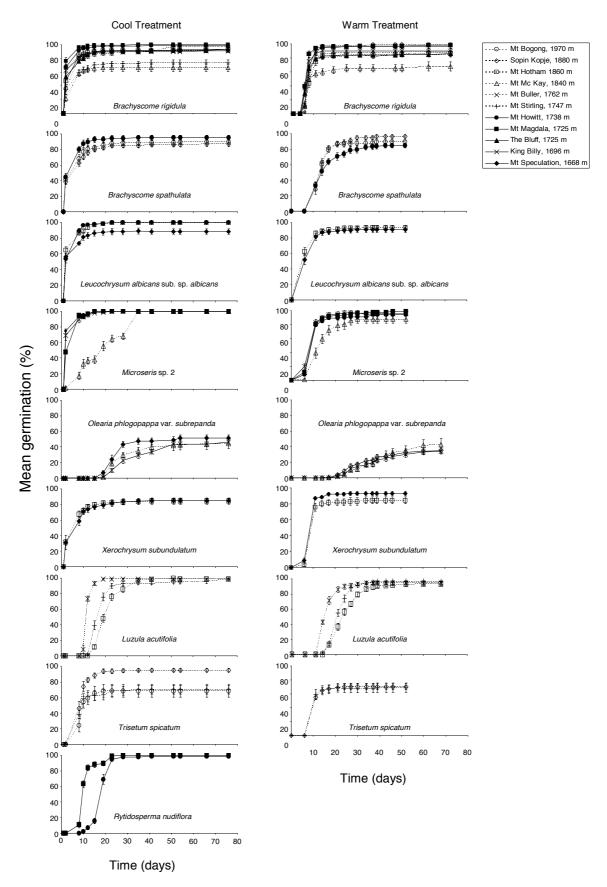
Cool Treatment

Warm Treatment

Mean germination (%)

Brachyscome rigidula

Brachyscome spathulata

Leucochrysum albicans sub. sp. *albicans*

Microseris sp. 2

Olearia phlogopappa var. *subrepanda*

Xerochrysum subundulatum

Luzula acutifolia

Trisetum spicatum

Rytidosperma nudiflora

Time (days)

Time (days)

- ---○--- Mt Bogong, 1970 m
- ···◇··· Sopin Kopje, 1880 m
- ···□··· Mt Hotham 1860 m
- ---△--- Mt Mc Kay, 1840 m
- ···×··· Mt Buller, 1762 m
- ···+··· Mt Stirling, 1747 m
- ——●—— Mt Howitt, 1738 m
- ——■—— Mt Magdala, 1725 m
- ——▲—— The Bluff, 1725 m
- ——×—— King Billy, 1696 m
- ——◆—— Mt Speculation, 1668 m

Figure 4.2. Mean (± 1 standard error) percent germination curves for the cool (12/10 °C) and warm (20/10 °C) treatments against time (days) for seeds from species from the eleven sites. No seeds from *Aciphylla glacialis* germinated in either treatment during the experiment and therefore no germination curves are available for this species.
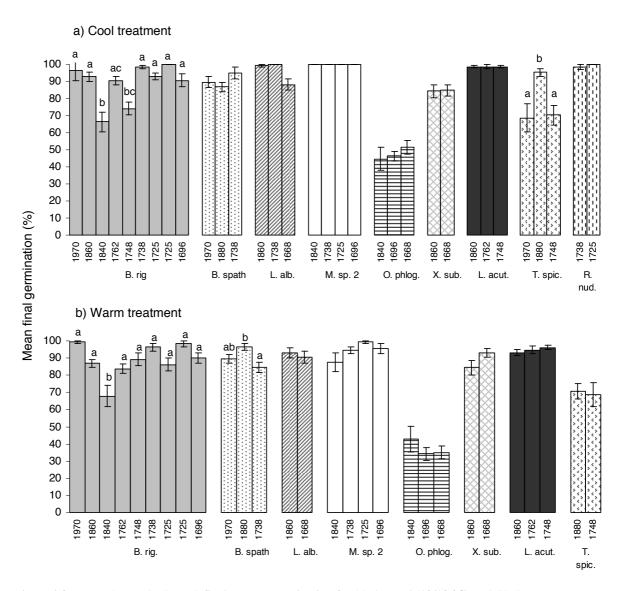
a) Cool treatment

b) Warm treatment

Figure 4.3. Mean (± standard error) final percent germination for (a) the cool (12/10 °C) and (b) the warm (20/10 °C) treatments. Different labels (a, b, or ab) above columns signify the Bonferroni significant differences within each species. Species columns without labels showed no significant differences between sites. No *Aciphylla glacialis* seed germinated under either treatment, zero mean final percent germination values are not presented. For full species names see Appendix 4.2.

Table 4.2. Pairwise comparisons of the probability of seed germination between sites, within each species, based on the probability of germination curves, explained by Mantel's χ^2 in the cool treatment. Significant differences are indicated by an asterisk(s). *** denotes $P \leq 0.001$, ** denotes $P \leq 0.01$, * denotes $P < 0.05$. Overall greater probability of germination between each pair is indicated in brackets with greater than (>) symbol between site names. Site name abbreviations and site altitudes are shown in Appendix 4.3. For full species names see Appendix 4.2.

Species	Pairwise comparisons by site (Mantel's χ^2).									
B. rigidula	**BO v HO** 0.054	**BO v MK** 25.098*** (BO>MK)	**BO v BU** 1.482	**BO v ST** 15.573*** (BO>ST)	**BO v HW** 27.857*** (HW>BO)	**BO v BL** 1.6881	**BO v MA** 2.762	**BO v KB** 0.946	**HO v MK** 26.993*** (HO>MK)	**HO v BU** 1.229
	HO v ST 16.816*** (HO>ST)	**HO v HW** 24.576*** (HW>HO)	**HO v BL** 1.397	**HO v MA** 4.592* (MA>HO)	**HO v KB** 0.755	**MK v BU** 19.355*** (BU>MK)	**MK v ST** 1.176	**MK v HW** 81.675*** (HW>MK)	**HO v BL** 1.397	**MK v MA** 16.293*** (MA>MK)
	MK v BU 19.355*** (BU>MK)	**MK v ST** 1.176	**BU v HW** 6.036* (HW>BU)	**BU v BL** 0.000	**BU v MA** 6.122* (MA>BU)	**BU v KB** 0.106	**ST v HW** 67.136*** (HW>ST)	**ST v BL** 25.438*** (BL>ST)	**ST v MA** 10.172*** (MA>ST)	**ST v KB** 14.709*** (KB>ST)
	HW v BL 14.505*** (HW>BL)	**BL v MA** 4.751* (BL>MA)	**BL v KB** 0.153	**MA v KB** 6.839** (MA>KB)						
B. spathulata	**BO v SP** 0.817	**BO v HW** 4.516* (HW>BO)	**SP v HW** 9.207** (HW>SP)							
L. albicans	**HO v HW** 0.239	**HO v SP** 14.667*** (HO>SP)	**HW v SP** 10.084*** (HW>SP)							
M. sp.2.	**MK v HW** 150.736*** (HW>MK)	**MK v MA** 132.109*** (MA>MK)	**MK v KB** 122.454*** (KB>MK)	**HW v MA** 10.519*** (MA>HW)	**HW v KB** 1.546	**MA v KB** 2.989				
O. phlog	**MK v KB** 0.482	**MK v SP** 1.540	**KB v SP** 3.959*** (SP>KB)							
X. sub.	**HO v SP** 0.409									
L. acutifolia	**HO v BU** 142.356*** (BU>HO)	**HO v ST** 14.235*** (HO>ST)	**BU v ST** 103.516*** (BU>ST)							
T. spicatum	**BO v SP** 42.294*** (SP>BO)	**BO v ST** 4.418* (ST>HO)	**SP v ST** 16.790*** (SP>ST)							
R. nudiflora	**MA v HW** 120.749*** (MA>HW)									

Table 4.3. Pairwise comparisons of the probability of seed germination between sites, within each species, based on the probability of germination curves, explained by Mantel's χ^2 in the warm treatment. Significant differences are indicated by an asterisk(s). *** denotes $P \leq 0.001$, ** denotes $P \leq 0.01$, * denotes $P < 0.05$. Overall greater probability of germination between each pair is indicated in brackets with greater than (>) symbol between site names. Site name abbreviations and site altitudes are shown in Appendix 4.3. For full species names see Appendix 4.2.

Species	Pairwise comparisons by site (Mantel's χ^2).									
B. rigidula	**BO v HO** 11.474*** (BO>HO)	**BO v MK** 35.964*** (BO>MK)	**BO v BU** 0.000	**BO v ST** 1.859	**BO v HW** 5.189	**BO v BL** 0.005	**BO v MA** 40.230*** (BO>MA)	**BO v KB** 8.704** (KB>BO)	**HO v MK** 8.244** (HO>MK)	**HO v BU** 11.834*** (HO>BU)
	HO v ST 2.152	**HO v HW** 18.112*** (HW>HO)	**HO v BL** 4.361	**HO v MA** 55.283*** (MA>HO)	**HO v KB** 17.400*** (KB>HO)	**MK v BU** 31.295*** (BU>MK)	**MK v ST** 15.394*** (ST>MK)	**MK v HW** 32.885*** (MK>HW)	**HO v BL** 4.361	**MK v MA** 68.968*** (MA>MK)
	MK v BU 31.295*** (BU>MK)	**MK v ST** 15.394*** (ST>MK)	**BU v HW** 0.299	**BU v BL** 2.691	**BU v MA** 13.773*** (MA>BU)	**BU v KB** 0.273	**ST v HW** 6.003	**ST v BL** 0.522	**ST v MA** 32.159*** (MA>ST)	**ST v KB** 7.667** (KB>ST)
	HW v BL 1.627	**BL v MA** 19.390*** (MA>BL)	**BL v KB** 3.337	**MA v KB** 4.101						
B. spathulata	**BO v SP** 0.793	**BO v HW** 3.241	**SP v HW** 10.046** (SP>HW)							
L. albicans	**HO v SP** 1.737									
M. sp 2.	**MK v HW** 22.017*** (HW>MK)	**MK v MA** 43.228*** (MA>MK)	**MK v KB** 29.767*** (KB>MK)	**HW v MA** 4.152* (MA>HW)	**HW v KB** 2.420	**MA v KB** 29.767*** (KB>MA)				
O. phlog.	**MK v KB** 1.516	**MK v SP** 0.537	**KB v SP** 0.288							
X. sub.	**HO v SP** 9.975** (SP>HO)									
L. acutifolia	**HO v BU** 53.221*** (BU>HO)	**HO v ST** 10.418*** (ST>HO)	**BU v ST** 38.660*** (ST>BU)							
T. spicatum	**SP v ST** 0.000									

Table 4.4. Pairwise comparisons of the probability of seed germination between the cool and warm treatments, within each species and site, based on the probability of germination curves, explained by Mantel's χ^2. Significant differences are indicated by an asterisk(s). *** denotes $P \leq 0.001$, ** denotes $P \leq 0.01$, * denotes $P < 0.05$. Overall greater probability of germination between each pair is indicated in brackets with greater than (>) symbol between treatments; C, cool; W, warm. Site name abbreviations and site altitudes are shown in Appendix 4.2. For species names abbreviations, see Appendix 4.3.

Species	Pairwise comparisons by treatment (Mantel's χ^2) within site										
	BO	SP	HO	MK	BU	ST	HW	BL	MA	KB	SP
B. rigidula	9.530** (W>C)	N/A	28.467*** (C>W)	1.123	12.642*** (C>W)	1.307	63.904*** (C>W)	19.470*** (C>W)	8.077** (C>W)	5.931	N/A
B. spathulata	37.019	14.496*** (W>C)	N/A	N/A	N/A	N/A	36.807*** (C>W)	N/A	N/A	N/A	N/A
L. albicans	N/A	N/A	50.367*** (W>C)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	12.439*** (W>C)
M. sp 2.	N/A	N/A	N/A	0.066	N/A	N/A	230.211*** (C>W)	N/A	156.251*** (C>W)	120.186*** (C>W)	N/A
O. phlogopappa	N/A	N/A	N/A	1.291	N/A	N/A	N/A	N/A	N/A	3.055,	10.739*** (C>W)
X. subundulatum	N/A	10.290*** (W>C)	30.974*** (C>W)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L. acutifolia	N/A	N/A	12.676*** (C>W)	N/A	68.135*** (C>W)	34.598*** (C>W)	N/A	N/A	N/A	N/A	N/A
T. spicatum	N/A	69.643*** (C>W)	N/A	N/A	N/A	8.666*** (C>W)	N/A	N/A	N/A	N/A	N/A

Discussion

General interpretation of results

Mean seed mass did not differ significantly across the altitudinal gradient for most species, nor were there significant trends between seed mass and final percent germination for any species at any site. In the germination experiments, where no seed was pre-treated, high mean percent germination was common at all sites across the altitudinal gradient, with seven of the nine species showing >90% germination in one or more sites. *Aciphylla glacialis* seeds did not germinate under either treatment. Apart from *A. glacialis*, the only species not to reach high final percent germination was *O. phlogapappa* var. *subrepanda* (shrub) which showed mean final percent germination of 40-55% in both treatments. Dormancy mechanisms were not apparent as seed from all species germinated within the time period of the experiment, except for *A. glacialis* seeds. Possible dormancy mechanisms of *A. glacialis* are explored in Chapter 5.

Germination lag times were short in both treatments for all species, between 2 and 11 days for most of the Asteraceae, and up to 21 days for grass, rush and shrub species. Short lag times indicate opportunistic germination strategies which enable germination to occur as soon as suitable conditions are present. This strategy is characteristic of alpine species (Bell and Bliss 1980) and may be indicative of species with a transient seed bank. However, my results suggested that persistent seed banks are present across the mountain-top sites (Chapter 3), as samples from spring and autumn were not consistently different within any species. The species used in the current study were rarely found in the seed bank samples and hence, there may be different strategies operating among different species at different life-history stages i.e. opportunists and those that "wait".

Neither germination lag-time, nor germination speed (T_{50}), were significantly correlated with final percent germination. However, these parameters can be important characteristics under natural conditions where seed germination may be limited to brief periods in the summer when sufficient soil water coincides with favourable ambient temperatures (Bliss 1971). I demonstrated that the overall germination 'journeys' encountered in this study were 1) early rapid germination and 2) late rapid germination. The germination journey that a group of seeds took was modelled using Kaplan-Meier probability curves. The comparisons between pairs of sites showed that the germination probability of seeds from lower altitudes, within species and treatments, was often higher than that of seeds from high altitudes. This result is not consistent among species, however, and was often caused by exceptionally low germination of seeds from

Mt McKay (1840 m), for reasons that remain unknown. However, roughly half the comparisons showed no difference in germination probability between high and low elevations.

When comparing the germination probability between the cool and warm treatments, seeds of five species had a higher germination probability in the cool treatment. The cool treatment simulated field conditions present at the start the growing season (early spring). Therefore, high germination probabilities in this treatment support the predominance of opportunistic germination strategies under natural conditions. This was more common for seeds from lower altitudes. The use of germination probability curves, therefore, can be a useful tool for comparing the germination probability of seeds over set time periods and across treatments (Giménez-Benavides *et al*. 2005).

Overall, I showed that the germination response of Victorian alpine seeds is fairly consistent across the two temperature regimes among naturally co-occurring species. Few differences in germination characteristics across the altitudinal gradient exist within species.

Seed mass and reproductive success

In my study, I found no trends relating seed mass to germination probability or success. My results are consistent with the findings of Molau (1993) who reported that relative reproduction success does not correlate well with resource allocation to reproduction, seed number, seed weight or germinability among seeds from arctic tundra species. Large seeds are more likely to have extra reserves available for germination and subsequent seedling emergence and survival (Holm 1994; Westoby *et al*. 1996; Vera 1997; Leishman *et al*. 2000; Pickering 2000; Pickering and Hill 2002). Large seeds, therefore, would be advantageous for plants growing at high altitudes in order to compensate for the various environmental stresses (Vera 1997; Pickering and Arthur 2003). However, large seeds require more maternal investment and therefore, at higher elevations or in more stressful environments, some species may adopt the conservative strategy of producing large but few seeds to compensate for the environmental stresses (Molau 1993). These species would be identified as *K*-strategists along the *r/K* continuum (MacArthur and Wilson 1967), which is the group most arctic and alpine flowering plants tend to fall into (Molau 1993).

However, within a species, examples of seed mass increasing with altitude are rare. Rather, there tends to be a high degree of variability in seed mass across an altitudinal range (Vera 1997; Moles *et al*. 2003; Pluess *et al*. 2005). Over a large

altitudinal gradient (1255 m, which is more than four times the size of the altitudinal gradient in this study), Lord (1994) found that mean seed mass of *Festuca novae-zelandiae* increased significantly with altitude. The size of flowers and other reproductive structures do not usually change with altitude (Fabbro and Körner 2004) and consequently, seed mass may also remain unchanged across altitudes. However, between congeneric, closely-related species the seed mass of alpine species may be higher than that of their lower dwelling temperate counterparts (Pluess *et al.* 2005). This could be investigated for *Brachyscome rigidula*, and *Microseris* sp. 2 in future studies as they occur at high altitudes above the treeline and in sub-alpine areas.

Germination characteristics

Final percent germination results in this experiment were within (or above) the ranges of other alpine plant germination studies using similar day/night temperature regimes and without seed pre-treatments (Bliss 1959; Amen 1966; Sayers and Ward 1966; Chabot and Billings 1972; Bell and Bliss 1980; Urbanska and Schütz 1986; Mariko *et al.* 1993; Kibe and Masuzawa 1994; Giménez-Benavides *et al.* 2005; Wesche *et al.* 2006). Optimum temperatures for alpine seed germination are notoriously variable but in many cases maximum germination occurs between 20 to 30 °C (Billings and Mooney 1968; Marchand and Roach 1980). Below these temperatures, percent germination can decline rapidly (Chabot and Billings 1972). A high temperature requirement for germination among alpine seeds may act as an important cue under natural conditions, ensuring seeds germinate during the warmer months following snowmelt (Billings and Mooney 1968; Chabot and Billings 1972). However, I found no significant difference in final percent germination between the two temperature treatments for any species. A likely explanation may be the small difference in temperature regimes between the cool and warm treatments.

In general, few alpine seeds show any forms of innate dormancy (only one species in my study, *Aciphylla glacialis*) and hence, dormancy may be controlled by the environment and low winter temperatures (Billings and Mooney 1968). This is exacerbated by the high temperature germination requirement (Chabot and Billings 1972), again ensuring developmental processes operate predominantly during favourable conditions (Billings and Mooney 1968). In the experiments, I simulated natural conditions by alternating the day and night temperatures, as diurnal temperature fluctuations have been found to significantly increase final percent germination rates compared with constant temperatures (Sayers and Ward 1966; McDonough 1969).

However, I did not simulate winter conditions prior to the germination trials which may account for the lack of germination in *Aciphylla glacialis*.

I found quick lag times and fast germination for seeds under both temperature regimes. These characteristics are indicative of seedling emergence at the very start of the growing season (see Chapter 6), corresponding with favourable environmental conditions immediately after snowmelt and limiting the risks associated with germination to a few weeks (Billings and Mooney 1968). Most seeds from all species in this experiment had reached 90% of their germination within 20 days, which may indicate that under natural conditions, the available 'window' for seed germination in the Victorian alpine zone is small and germination usually occurs quickly. I found no variations in lag-times and germination speeds from seeds collected across the altitudinal gradient. However, across regions with much stronger environmental gradients, seeds from high alpine areas or stressful habitats (i.e., snowpatch) can show significantly faster germination rates (Mariko *et al*. 1993; Lord 1994; Vera 1997; Shimono and Kudo 2005). This strategy takes advantage of every opportunity for germination, regardless of the probability of successful seedling establishment (Bell and Bliss 1980).

In contrast, species with intermittent or "cautious" germination syndromes may spread germination over extended periods, thereby restricting seedling establishment to rare intervals of favourable weather during the snow-free period (Bell and Bliss 1980; Schütz 2002) and protecting seedlings from the hazards of emerging too early during extended winters or in exposed locations (Meyer *et al*. 1990). Under natural conditions, seeds with this germination strategy may remain buried in the soil for many years before the right combination of external conditions coincide, prompting germination. Mark (1965) found *Chionochloa rigida* seeds germinated after four years in the laboratory, which may indicate such a strategy. In contrast, the species in this study, except perhaps *O*. *phlogopappa* var. *subrepanda* and *L*. *acutifolia*, tended to have an opportunistic germination strategy, with quick lag-times, fast germination and high final percent germination during the experimental period.

Conclusions

High rates of seed germination among co-existing species indicate many opportunities for seedling emergence. However, the strong risk of seedling mortality in alpine environments may enforce strategies that ensure germination is cued with favourable ambient conditions. In my experiments, I found few trends in germination characteristics between the cool and warm treatments, although germination probability was often higher among seeds in the cool treatment which originated from lower elevations. The seeds of the study species are likely to be opportunistic in their germination and therefore germinate immediately after snowmelt under natural conditions. I found few differences in final percent germination in seeds from different altitudes, possibly because the gradient used in this study was small. To fully understand the germination strategies of alpine plants, these laboratory germination trials are compared with patterns of seedling recruitment, emergence and establishment under natural conditions in Chapter 6.

Appendices

Appendix 4.1. Matrix of *P*-values which indicate any significant differences (*P* <0.05) in seed mass of *Brachyscome rigidula*, based on log-transformed data, after the Bonferroni adjustment, between sites of varying elevation (m, **bold** type).

	1668	**1696**	**1725**	**1738**	**1748**	**1762**	**1840**	**1970**
1668	1.000							
1696	0.000	1.000						
1725	0.000	0.022	1.000					
1738	0.000	0.000	0.550	1.000				
1748	0.000	1.000	0.108	0.001	1.000			
1762	0.000	0.024	1.000	1.000	0.088	1.000		
1840	0.000	0.221	0.000	0.000	0.066	0.000	1.000	
1970	0.000	1.000	0.358	0.003	1.000	0.241	0.022	1.000

Appendix 4.2. Abbreviations of species names used in Figures 4.1 and 4.3 and Tables 4.2, 4.3 and 4.4.

Abbreviation	**Species name**
B. rig	*Brachyscome rigidula*
B. spath	*Brachyscome spathulata*
L. alb	*Leucochrysum albicans* sub. sp. *albicans*
M. sp. 2	*Microseris* sp. 2
O. phlog	*Olearia phlogopappa* var. *subrepanda*
X. sub	*Xerochrysum subundulatum*
L. acut	*Luzula acutifolia*
T. spic	*Trisetum spicatum*
R. nudi	*Rytidosperma nudiflora*

Appendix 4.3. Abbreviations of site names used in Tables 4.2, 4.3 and 4.4.

Abbreviation	**Site name (m)**
BO	Mt Bogong (1970)
SK	Spion Kopje (1880)
HO	Mt Hotham (1860)
MK	Mt McKay (1840)
BU	Mt Buller (1762)
ST	Mt Stirling (1748)
HW	Mt Howitt (1738 m)
BL	The Bluff (1725)
MA	Mt Magdala (1725)
KB	King Billy (1696)
SP	Mt Speculation (1668)

Chapter 5 - Case study: seed germination of *Aciphylla glacialis* (Mountain Celery)

Summary

In this chapter, I investigate the germination characteristics of *Aciphylla glacialis* (Apiaceae). This species failed to germinate under the experimental conditions that nine other alpine species from the same region found favourable (see Chapter 4). I used seeds collected during the summer growing seasons of 2004 and 2005 from eight mountain tops in the Victorian alpine zone which form an altitudinal gradient of 302 m. I subjected the seeds to a cold-wet stratification treatment in an attempt to overcome what appeared to be seed dormancy in the experiments described in the previous chapter.

After approximately 15 weeks of the cold-wet stratification treatment, *A. glacialis* seeds began to germinate. Germination rates were fast among seeds from the mid to high altitude sites, as was the final percent germination. Final percent germination was lowest at Mt Speculation (1668 m) and Mt Bogong (1970 m) from seeds collected in 2004. In 2005, the lowest values were also from Mt Bogong. Between years, there was no significant difference between final percent germination for seed from any site. Across the altitudinal gradient, there were no significant relationships between altitude and any germination characteristics. Between sites, there were several significant differences in germination probability. However, there was no overall trend in germination probability between high and low sites.

I also discuss the role of seed dormancy mechanisms in relation to the alpine environment and speculate about the role of altitudinal gradients in relation to seed germination in this species.

Introduction

Seed germination, dormancy and environment

Seed germination and seedling emergence under natural conditions are often highly seasonal (Baskin and Baskin 1988). In alpine areas, where the changes in ground level temperatures are predictable due to the timing of snowmelt, temperature can provide a reliable seasonal germination cue for seeds located at or near the soil surface. In Chapter 4, I found that nine species from the Victorian alpine zone have high rates of germination, as do many species in alpine areas from the northern hemisphere (Amen 1966; Bliss 1971). Optimum temperatures for seed germination among alpine species worldwide are high, often between 10 and 30 °C (Amen 1966; Bell and Bliss 1980; Schütz 2002). Many seeds also germinate opportunistically whenever suitable ambient conditions are present (Bell and Bliss 1980; Körner 1999). Seed dormancy is therefore considered rare in these ecosystems (Amen 1966; Bliss 1971) and germination immediately following snowmelt, coinciding with abundant soil moisture and warmer ambient temperatures, is very common (Bliss 1962; Billings and Mooney 1968; Körner 1999).

Cold-wet stratification, as experienced over winter in an alpine landscape, may be required to break seed dormancy in the few alpine species that use such internal physiological mechanisms to restrain germination (Baskin and Baskin 1988; Probert 1992). This stratification may also be responsible for the timing of seedling emergence in these species. Photoperiodism also affects the seeds of many alpine species, ensuring that growth does not commence in periods which, by 'evolutionary experience', would be fatal for an emerging seedling (Körner 1999). Hence, there are various strategies to ensure that germination occurs in spring or early summer when conditions are most favourable for the survival of seedlings in the alpine zone.

Relationships between germination syndrome, habitat and geographical distribution have been difficult to ascertain (Baskin and Baskin 1988), even at the species level (Meyer *et al*. 1990), as germination characteristics can, in part, be a function of life history traits (Baskin and Baskin 1971; Grime *et al*. 1981; Washitani and Masuda 1990). However, if the aspects of life-history, habitat and distribution are relatively similar, different adaptations to environmental factors within species can be discerned more clearly (Meyer *et al*. 1990) as natural selection should favour seed germination patterns that promote seedling establishment (Cavieres and Arroyo 2000). Germination characteristics, therefore, are expected to vary predictably across an altitudinal and

environmental gradient. Evidence suggests that seed viability and seedling vigour may decrease within a species as altitude increases, due to environmental constraints such as short growing seasons, low temperatures and harsh conditions at high altitudes (Amen 1966; Billings and Mooney 1968). Therefore, germination strategies may be partly a function of life-history traits and partly due to habitat characteristics.

Characteristics of the Aciphylla genus

In this study, I aimed to investigate the germination behaviour of a locally common, but regionally rare, alpine forb which grows across an altitudinal gradient in Victoria. The focal species is the Mountain Celery, *Aciphylla glacialis* (F.Muell.) Benth. (Apiaceae). It is one of only three *Aciphylla* species found in Australia, all of which are restricted to alpine and sub-alpine regions. *A. simplicifolia* is uncommon in Victoria and mostly restricted to the high alpine areas of New South Wales (Costin *et al.* 2000), and the Tasmanian endemic, *A. procumbens* is restricted to bolster heath, heath and alpine sedge-land (Kirkpatrick 1997). The other forty species of this genus are found in New Zealand, where they occur over a wide range of habitats from lowland grasslands to alpine areas (Raven 1973).

As a dioecious, obligate out-crossing genus, *Aciphylla* species are dependent on pollinators for fertilization and are therefore likely to experience severe competition, both within and between species, for the few pollinators that are active at high altitudes (Inouye and Pyke 1988; Pickering 2000). The sex ratio of populations of *A. glacialis* are strongly male biased, with an overall ratio of female to male plants of 1:1.49, with males producing four times more flowers than females and therefore attracting and competing with females for pollinators with large floral displays (Pickering 2000). However, there is generally no change in the number of flowers with altitude for both male and female plants among the same sized populations (Pickering and Hill 2002). The proportion of biomass that females allocate to reproduction in *A. simplicifolia* can be 80% more than males based on the average weight of the inflorescences (Pickering and Arthur 2003) and nearly all female flowers produce fruit.

The great investment into reproduction by female *Aciphylla* plants is assumed to lower their survival and growth, especially at the higher alpine sites where the conditions for the accumulation of biomass are limited. The costs associated with female function at high altitudes could therefore result in females flowering less frequently (Pickering and Arthur 2003). At lower altitudes, resources are potentially more abundant (longer snow-free period, warmer temperatures), which could result in an equal flowering ratio between

males and females. Phenotypic differences in relation to environmental effects are also common in this genus, making *A. glacialis* a good model species for investigating the differences in germination across an altitudinal gradient. To my knowledge, no published germination studies exist for any *Aciphylla* species in Australia.

Chapter focus

Following the results in Chapter 4, when *A. glacialis* seeds did not germinate under laboratory conditions simulating early spring and summer, I ask: Can seed dormancy of *A. glacialis* be overcome by cold-wet stratification? Which dormancy syndrome does the behaviour of *A. glacialis* exhibit? Does the altitude of seed origin or the year of seed collection influence the germination characteristics?

Methods

Seed collection and study sites

Seed from *Aciphylla glacialis* (hereafter referred as *Aciphylla*) populations was collected during 2004, between January and March, from seven alpine peaks in Victoria, and again from three of the peaks in 2005. Seed was kept in dry, dark, stable conditions until the germination trials began in mid 2005. The peaks represent a gradient of 302 m in altitude from Mt Speculation (1668 m) to Mt Bogong (1986 m) (see Table 2.1 in Chapter 2). In addition to the sites used in Chapter 2, seed was also collected from Mt Howitt (1738 m, 37°10'30"S, 146°38'50"E), however, seed was not collected from Mt Stirling or The Bluff. All sites are within 40 km of each other, with most less than 10 km apart (Figure 2.1 in Chapter 2).

Mean annual rainfall of this region is relatively high compared to adjacent lowland areas, over 1800 mm (LCC 1982), with much of this precipitation falling as snow during winter. Average air temperatures follow seasonal variations with typical daily minimums and maximums of 2 and 27 °C in summer, and -6 and 12 °C in winter (Bureau of Meteorology *unpublished data*; Chapter 2). Frosts are frequent and can occur at any time of year (Williams 1987). The growing season spans approximately seven months from snowmelt, usually the end of September, until late April, when the first substantial snowfalls of the winter begin. All sites are classed as 'Steep Alpine Mountains' after Costin (1957), are above the natural tree-line and are dominated by tall alpine herbfield vegetation. The lower peaks also contain species typical of adjoining sub-alpine

woodland (Costin 1957). The underlying geology of the area varies (see Table 2.2 in Chapter 2). Despite this variation, the parent material in these regions rarely influences soil type and all soils are considered alpine humus, with a gravelly or skeletal structure (Costin 1962b; LCC 1982) and are acidic, ranging from pH 4 to 5.

Germination trial

Filled seeds were selected by visually assessing each seed using a light box and by gently squeezing each seed. Seeds from each site were then weighed in multiples of ten on an electronic balance, to three decimal places to determine seed mass. In a prior experiment, described in Chapter 4, *Aciphylla* seeds did not germinate under conditions that nine other alpine species found favourable (cool treatment: 12/10°C with 14/10 hours day length and warm treatment: 20/10°C with 12/12 hours day length). Thus, in this experiment, I sought to pre-treat the *Aciphylla* seeds with cold-wet stratification. I arranged 20 replicates of ten seeds from each site in petri dishes on a bed of moist cotton wool with an anti-fungal treatment (10 ml of Mancozeb solution). Petri dishes were then sealed with Parafilm to prevent moisture loss and kept at 2 °C in the dark. Seeds were exposed to daylight for a few minutes whilst they were being monitored for germination, every two to three weeks. Germination was considered to have occurred when the radical emerged from the seed coat, and petri dishes were monitored every one to two weeks after the first germination of seeds occurred. The experiment was terminated at 205 days when germination rates across sites had stabilised and incidences of fungal attack had become more frequent. The final values of germination were adjusted to represent percent germination of the viable seeds present, i.e. those that had not succumbed to fungus attack or were in fact unfilled at the start of the experiment.

Data analysis

Mean seed mass was compared across the sites of seed origin with a factorial ANOVA and Bonferroni post-hoc tests. Final percent germination was expressed as a percentage of viable seeds that germinated during the course of the trial. Germination lag-time was the time in weeks for germination to begin, and germination speed (T_{50}) was measured as the time in days taken to reach 50% of final germination. The final percent germination of seeds was compared between sites using ANOVA and Bonferroni post-hoc tests.

The shape of each percent germination curve, the germination 'journey' by which the group of seeds at every site take to reach the final germination score, were compared by modelling the germination curves as a probability of germination, using the Kaplan-

Meier (K-M) product limit estimation function (Kaplan and Meier 1958; Lee 1992; Giménez-Benavides *et al*. 2005), as in Chapter 4.

Linear regression, and in some cases Pearson/Spearman's rank correlations, were performed across populations between combinations of final percent germination, lag-time, germination speed, altitude of seed origin and seed mass.

SYSTAT version 10 (Copyright SPSS Inc., 2000) was used for all statistical analyses.

Results

Germination characteristics

Seeds from all elevations and years showed some germination under the experimental conditions (Figure 5.1). Across all sites, the mean final percent germination was 39 to 95% for seeds collected in 2004, and 30 to 95% for seeds collected in 2005 respectively. Seeds from Mt Hotham had the highest mean final percent germination in both years (95.5 ± 1.53). Comparisons across sites, of seeds collected in 2004, revealed that seeds from Mt Bogong and Mt Speculation (the highest and lowest sites respectively) had significantly lower mean final percent germination ($P < 0.05$) than seeds from all other sites (Figure 5.2). Of the seeds collected in 2005, those from Mt Bogong also had significantly lower mean final percent germination ($P < 0.05$) than the other sites (Figure 5.2). There were no significant differences between years in the final percent germination of seeds from Mt Bogong, Mt Hotham and Mt Buller ($P > 0.05$). Linear regression showed that there was no significant relationship between final mean percent germination and altitude.

The germination lag-time for all sites was approximately 15 weeks from the start of the experiment. As an exact date was not determined, lag-time is not included in the statistical analyses. Germination speed (T_{50}) varied across the gradient (Table 5.1). However, there was no significant relationship between T_{50} and the altitude of seed origin. T_{50} did, however, show a strong relationship with final percent germination ($R^2 = 0.81$, $P = 0.000$) indicating that fast germination speeds correlate with high final germination. Linear regression showed that there was no significant relationship between T_{50} and altitude of seed origin ($P > 0.05$).
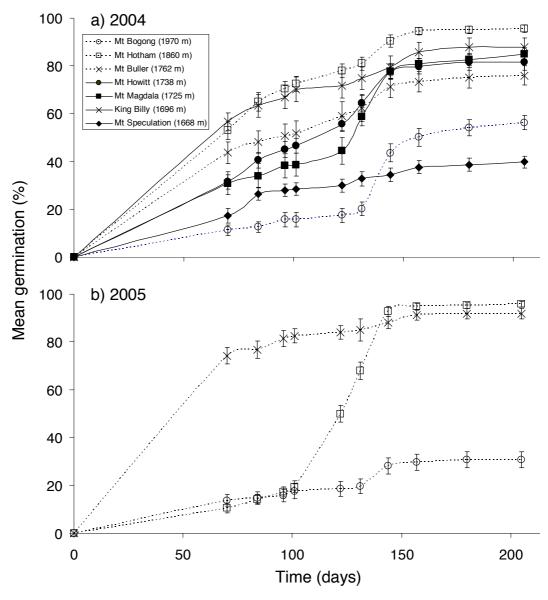
Figure 5.1. Mean percent germination (± 1standard error) of *Aciphylla glacialis* seed collected from the seven alpine sites in (a) 2004 and (b) 2005, over the duration of the experiment (days).
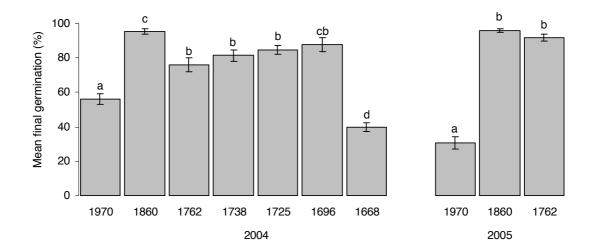
Figure 5.2. Mean final percent germination (±1standard error) of *Aciphylla glacialis* seed collected in 2004 and 2005 from different elevations (m). Different labels (a - d) above columns signify the Bonferroni significant differences within each year.

Table 5.1. Germination speed (T_{50}) of *Aciphylla glacialis* seeds from the seven sites, at various altitudes (m), collected in 2004 and 2005

Site of seed origin	Altitude (m)	Year of collection	T_{50} (days)
Mt Bogong	1970	2004	157
Mt Hotham	1860	2004	70
Mt Buller	1762	2004	96
Mt Howitt,	1738	2004	122
Mt Magdala	1725	2004	131
King Billy	1696	2004	70
Mt Speculation	1668	2004	205
Mt Bogong	1790	2005	205
Mt Hotham	1860	2005	122
Mt Buller	1762	2005	70

The probability of germination over time (Figure 5.3) indicates that there are few trends with altitude of seed origin; however, there are several significant differences between curves of different sites (Table 5.2). Of the 23 pairwise comparisons of germination probability by site over both years, nine of these showed that the higher altitude site of the pair had a greater overall probability of germination during the course of the experiment. Eleven showed that a lower site had a high germination probability. Hence, there were no overall trends in seed germination probability with altitude of seed origin.

The probability of germination over time between years was also significantly different for Mt Bogong, Mt Hotham and Mt Buller. Seeds collected in 2004 from Mt Bogong and Mt Hotham were more likely to germinate than seed collected in 2005 (Mantel's $\chi^2 =$ 16.264, $P = 0.000$; Mantel's $\chi^2 = 34.267$, $P = 0.000$ respectively). In contrast, seed from Mt Buller collected in 2005 had a higher probability of germination than that from 2004 (Mantel's χ^2, 34.025; $P = 0.000$).

Across seed from all sites, there were no significant relationships between seed mass and final percent germination, germination speed, germination probability, year or altitude of seed origin (see Chapter 4).
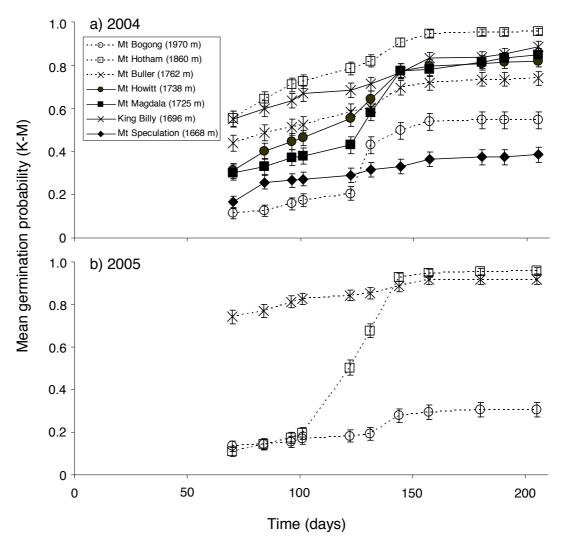
Figure 5.3. Mean probability of germination (± 1 standard error) of *Aciphylla glacialis* seed collected from the seven alpine sites in (a) 2004 and (b) 2005 based on Kaplan-Meier models over the duration of the experiment (days).

Table 5.2. Pairwise comparisons of the probability of *Aciphylla glacialis* seed germination between sites, based on the probability of germination curves, explained by Mantel's χ^2, from seed collected in 2004 and 2005. Significant differences are indicated by an asterisk(s). *** denotes $P \leq 0.001$, ** denotes $P \leq 0.01$, * denotes $P < 0.05$. Overall greater probability of germination between each pair is indicated in brackets with greater than (>) symbol between site names. Site name abbreviations are as follows: BO, Mt Bogong (1970 m); HO, Mt Hotham (1860 m); BU, Mt Buller (1762 m); HW, Mt Howitt (1738 m); MA, Mt Magdala (1725 m); KB, King Billy (1696 m).

Year / Site						
2004	**HO**	**BU**	**HW**	**MA**	**KB**	**SP**
BO	153.986*** (HO>BO)	30.323*** (BU>BO)	52.243*** (HW>BO)	53.297*** (MA>BO)	80.893*** (KB>BO)	4.540*** (BO>SP)
HO		33.978*** (HO>BU)	28.733*** (HO>HW)	35.362*** HO>MA)	7.264** (HO>KB)	164.768*** (HO>SP)
BU			0.838	0.685	10.988*** (KB>BU)	50.047*** (BU>SP)
HW				0.201	5.811* (KB>HW)	67.260*** (HW>SP)
MA					6.203* (KB>MA)	70.067*** (MA>SP)
2005	**HO**	**BU**				
BO	160.512*** (HO>BO)	195.719*** (BU>BO)				
HO		41.276*** (BU>HO)				

Discussion

General interpretation of results

Aciphylla seeds did not germinate under the laboratory conditions that seeds from nine co-occurring alpine species found favourable, as found in Chapter 4. This indicates that *Aciphylla* seeds may experience innate primary seed dormancy. In the current study, I showed that this dormancy may be broken by a low temperature stratification, which represents a natural dormancy breaking mechanism (Probert 1992) and implies that this species is a strict spring germinator. Consequently, the few observations of *Aciphylla* seedlings emerging in the field were restricted to spring and early summer (see Chapter 6).

The lag-time for *Aciphylla* seeds to germinate from all populations was around 15 weeks, during which conditions were kept at a constant dark 2 °C. Fifteen weeks roughly correlates with the length of a typical Victorian winter, taking into account inter-annual variability in snowfall (Hennessey *et al*. 2003). I therefore suggest that this germination lag-time is a direct result of evolutionary experience of these species growing in the Victorian alpine zone.

In this study, I observed considerable variation in germination characteristics between populations from different altitudes. However, final percent germination showed no significant trends with altitude, nor did seed germination probability. Therefore, germination in this species is likely to be a function of inherited seed traits (Grime *et al*. 1981; Washitani and Masuda 1990) and does not show strong adaptations to the changes in environmental factors found across sites. *Aciphylla* seeds also appear to act independently of ambient conditions and thus, the timing of germination may be reliant on seed dormancy alone. This restrictive germination pattern may protect *Aciphylla* seeds from the hazards of early germination (Meyer *et al*. 1990), especially if snow melts unusually early, leaving plants 'out in the cold' when overnight spring frosts are common (Bannister *et al*. 2005). The high rates of germination in this study, 70 - 90%, are within the range of alpine species from around the world (Sayers and Ward 1966; Bell and Bliss 1980; Mariko *et al*. 1993; Körner 1999).

High final percent germination was significantly related to fast germination speeds. Inherently fast rates of germination following snowmelt may be important during the early phases of seedling growth (Sayers and Ward 1966). This ensures that seedlings establish during periods of favourable weather and ample soil moisture (see Chapter 6)

and therefore, species capable of rapid germination have an early-season survival advantage.

The variation in germination characteristics between years from Mt Bogong, Mt Hotham and Mt Buller were statistically undetectable. Hence, the 2005 seeds followed the same patterns in percent germination as those from 2004. Seeds from Mt Hotham showed the highest final germination of all sites in both years. Such consistency between years is unusual for alpine species, as slight increases in temperatures during the seed maturation period can lead to improved seed germinability (Körner 1999). The timing of snowfall, snowmelt and ambient temperatures were not particularly unusual compared to the long-term trends in either 2004 or 2005 and therefore, the similarities in germination may be a result of the predictable environmental conditions that prevail at these sites annually.

Dormancy mechanisms

Dormancy makes intuitive sense in a landscape where winters are cold and severe. However, Billings and Mooney (1968) suggest that the protective layer of snow over winter may serve to insulate seeds sufficiently and hence, they need never fully evolve specific seed dormancy syndromes. Hence, most alpine species lack a seed dormancy mechanism (see Chapter 4) and the elapsed year or more between seed production and germination is an environmentally imposed cue (Amen 1966). However, non-dormant species may germinate in mid-winter if the snow melts unexpectantly, and therefore experience high mortality rates (Amen 1966).

Innate seed dormancy is most frequently imposed by seed coat inhibition among alpine species (Amen 1966; Urbanska and Schütz 1986). This mechanism can spread germination over an extended period and thereby encompass several periods of favourable growth. Thus, germination of some innately-dormant species may occur intermittently over many years, even if growing season conditions are favourable for seedling emergence (Mark 1965; Billings and Mooney 1968). Seed coat inhibition can be broken by scarification of seeds. Under natural conditions, abrasion of buried seeds by frost heave or strong winds at the soil surface, blowing seeds over rocky substrates, may induce the required scarification (Amen 1966; Billings and Mooney 1968). In future studies, deliberate scarification of *Aciphylla* seeds in combination with a chilling treatment, could confirm whether seed coat inhibition occurs in this species.

Cold stratification and cold-wet stratification are techniques used to break seed dormancy of many species from cold climates (Amen 1966; Sayers and Ward 1966;

Cavieres and Arroyo 2000). In my experiment, the length of the cold-wet stratification period required for germination (lag-time) was not precisely recorded. However, other studies clearly show that seeds originating from high elevations require longer stratification periods (Billings and Mooney 1968; Dorne 1981; Cavieres and Arroyo 2000). Cavieres and Arroyo (2000) showed that *Phacelia secunda* seed from 1600 m needed only one month cold stratification, whereas seed from 3400 m needed three months. This pattern may not be apparent in *Aciphylla* seeds as the altitudinal distribution of this species is relatively small and adaptations to differences in snow season length across the gradient may be minimal or undetectable.

Altitudinal gradients

I did not find a relationship between germination characteristics and the altitude of seed origin, possibly because the altitudinal gradient of sites which support *Aciphylla* populations was too small. Elsewhere, the variation in germination characteristics between populations across environmental gradients is common, especially amongst species that respond to variation in habitat. For example, the shrub *Artemisia tridentata*, which grows across a wide environmental range in North America from desert to montane sites, showed strong habitat related differences in seed germination patterns (Meyer *et al.* 1990). Thompson (1973) compared the germination requirements for *Silene vulgaris* across a wide geographical range and found that the temperature range for seed germination was broad across several habitats. However, within a habitat, germination characteristics between populations were remarkably similar (Thompson 1973). Some researchers have suggested that the highest percent germination and shortest germination speed may come from seeds which originate from the highest elevations, when compared to seed of the same species from lower elevations (Mariko *et al.* 1993; Holm 1994; Vera 1997). Vera (1997) showed *Calluna vulgaris* seeds that germinated in the high mountains in Spain also had the highest survival rate as seedlings at the highest altitudes. Large seeds at the highest altitudes may also have the highest survival rates as seedlings (Holm 1994). In Chapter 4, I found no significant relationship between *Aciphylla* seed mass and altitude, nor was there any significant difference between seed mass from high altitude or low altitude sites. Seed germination rates were significantly lower at Mt Bogong and Mt Speculation when compared to the other sites. Hence, *Aciphylla* may be near its upper and lower distributional limits at these sites. Pickering and Arthur (2003) speculated that the growth and fecundity of female *A. simplicifolia* plants may be limited at higher altitudes due to the high maternal investments made during seed production, in

combination with stressful environmental conditions. However, I found no general trends between altitude and final percent germination or germination probability for *Aciphylla* seeds. Therefore, any limitations that the female plants may be experiencing across the altitudinal gradient are not being translated into reductions in seed germinability.

Conclusions

In laboratory experiments, *Aciphylla glacialis* seeds showed germination responses typical of seeds with an innate primary dormancy syndrome. This is an effective way to ensure that spring germination is cued at the end of the winter snow season in an alpine landscape. Final percent germination was high, up to 95%, and no differences in germination were found in seeds collected in different years. Fast germination speeds were significantly related to high final percent germination. These germination characteristics of *Aciphylla* did not show significant trends across the altitudinal gradient of sites within the Victorian alpine zone.