THE EFFECTS OF SHORT-TERM STORAGE ON GERMINATION IN *MECONOPSIS* VIG. (PAPAVERACEAE)

Alan Elliott[1] *& Gregory J. Kenicer*[2]

ABSTRACT

The viability and germination rate of seed of five species and four cultivars of *Meconopsis* was tested. The seed was stored in both refrigerated (*c.*4°C) conditions and at ambient temperature (8–17°C) to reflect typical conditions for seed storage in the smaller botanical garden and amateur enthusiast's collection. Seed that had been stored in each of these conditions was sown sequentially 2, 3, 4 and 5 months after harvest. Seed batches were also treated with 0.25mg/l gibberellic acid (GA) or left untreated. Each of the four combination treatments was associated with an overall reduction in viability and germination rate as time after harvest increased (to a minimum of 66.7% viability and 33.7% germination, except for two species with high viability but no germination in the study period). These reductions were not significantly different between treatments, and use of GA did not significantly increase germination rate. We studied the role of underlying genetic relations on germination by relating our results to the most recent comprehensive phylogeny of the genus, and we suggest that ecology plays a more important role than phylogeny in germination.

INTRODUCTION

The genus *Meconopsis* was established by Viguier (1814) in *Histoire Naturelle Pavots Argémones*. The decision to establish the genus was based on *Papaver cambricum* (*M. cambrica*) having a short style and no sessile stigmatic disc. The history of the genus is described in Taylor's (1934) monograph, which remains the definitive taxonomic account of *Meconopsis*. However a revised monograph of the genus is being written by Dr Christopher Grey-Wilson to include new species discovered in the past 70 years and to resolve current taxonomic issues. Current estimates suggest *Meconopsis* comprises 40–50 species (Taylor, 1934; Cobb, 1989; Grey-Wilson, 2000; 2006).

Meconopsis species are threatened by habitat loss and their overuse in traditional Chinese medicine. Another threat is climate change; the genus is found only in alpine and montane zones and does not compete well against other plants that are encroaching on these habitats because of global warming.

About 25 of the 40–50 species of *Meconopsis* are perennial monocarpic: the plants die once their seed has been set (pers. comm. Dr Christopher Grey-Wilson, 8 March 2008,). For these monocarpic species especially, it is important to maintain a healthy, viable collection of seed for conservation and research (Sulaiman, 1993; Grey-Wilson & Mitchell, 2007).

[1]Alan Elliott is a graduate in Horticulture with Plantsmanship BSc (Hons) at Royal Botanic Garden Edinburgh and the corresponding author for this paper.
Address: 20A Inverleith Row, Edinburgh EH3 5LR
Email: a.elliott@rbge.org.uk
[2]Gregory J. Kenicer is a Lecturer in the Education Department at Royal Botanic Garden Edinburgh.
Address: As above.
Email: g.kenicer@rbge.org.uk

Meconopsis species are distributed from Pakistan in the west; along the Himalayan chain through Kashmir, Himachal Pradesh (India), Nepal, Tibet/Xizang Autonomous Region (China), Sikkim, Bhutan, Arunachal Pradesh (India), and Burma/Myanmar; and north east into the Chinese provinces of Yunnan, Sichuan, Gansu and Qinghai (Fig. 1). The type for the genus, *M. cambrica*, is the only representative outside the Himalayan chain and is found in western Europe (Taylor, 1934; Cobb, 1989; Kundu, 2008).

Kundu's (2008) *Compendium of Papaveraceae s.l. in the Indian subcontinent* lists the countries where *Meconopsis* is found. Kundu listed two species, *M. horridula* and *M. prattii*, as occurring in Japan. However, Akiyama (2006) in *Flora of Japan* makes no mention of *Meconopsis*, and the native members of Papaveraceae could not be mistaken for *M. horridula* and *M. prattii*. Therefore Kundu is probably mistaken in listing *Meconopsis* as occurring in Japan.

Meconopsis taxa have long been desirable plants in horticulture, with the first record of a species, *M. simplicifolia*, flowering in the UK in 1848 from seed sent by J.D. Hooker from Sikkim (Taylor, 1934). As the exploration of the Himalaya continued, new species were introduced and the genus increased in popularity. Taylor's monograph has a detailed account of the history of the genus in cultivation. The introduction of fresh material continued until the late 1940s, with collections by plant hunters such as Ludlow and Sherriff introducing many plants, not just *Meconopsis*, into cultivation. *A Quest for Flowers* (Fletcher, 1975) recounts the hunting exploits of Ludlow and Sherriff and gives a historical insight into the lengths to which they were prepared to go to bring back what Sherriff described as 'alpine aristocrats'.

In the UK, much of the recent horticultural work on *Meconopsis* has been done under the auspices of the *Meconopsis* Group (Meconopsis Group, 1999) since its foundation in 1999, with support from the Royal Botanic Garden Edinburgh (RBGE). Dr Evelyn Stevens, the group's coordinator, summarised their work on the naming of the big perennial blue poppy hybrids and cultivars in *Sibbaldia* 4 (Stevens, 2006).

A student project carried out as part of the first author's second year of the Horticulture with Plantsmanship degree at RBGE showed that *Meconopsis* does not germinate well under horticultural conditions after being stored in the seed bank at RBGE (Elliott, 2006). The genus is often described as difficult to propagate and cultivate, a statement supported by Dr Stevens (pers. comm.12 March 2008). Encouraged by the *Meconopsis* Group, the first author developed his research project and further investigated germination techniques and seed storage for the genus. Part of his BSc Honours research project focused on this topic, and the results are presented here.

Seed dormancy in Papaveraceae

Seed dormancy in Papaveraceae has been well researched because of the family's importance as an economically important medicinal group (Bernath, 1998) and to understand its longevity in soil seed banks because various taxa are regarded as agricultural weeds (Cirujeda *et al.*, 2006; Karlsson & Milberg, 2007).

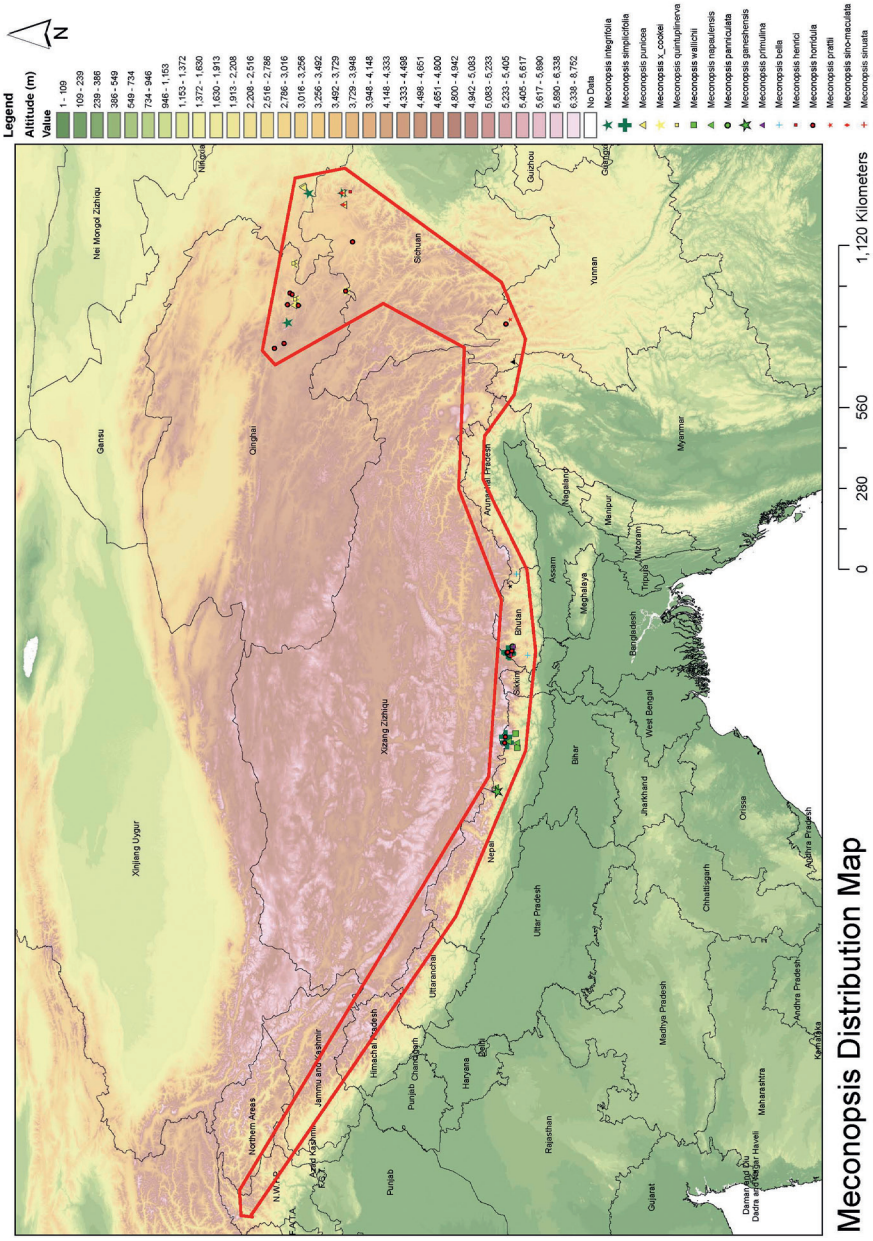

Fig. 1 Distribution map of *Meconopsis* created in ArcMap9.2 using information from satellite data GTOPO30 (2009) and locality information from Taylor (1934) and Kundu (2006).

Dormancy delays germination until more suitable conditions arise, thus improving the survival rate. *Dormancy type* is a classification of the mechanisms that govern the initiation, maintenance and breaking of dormancy, with the exact conditions needed to break dormancy differing both interspecifically and intraspecifically depending on the evolutionary path taken and on ecological pressures (Baskin & Baskin, 1998) Members of Papaveraceae are mainly from arctic, cold temperate and warm temperate climatic zones, and the seed show morphophysiological dormancy according to Baskin and Baskin (1998). Temperate and arctic plants have evolved with a range of dormancy types to prevent germination before favourable growing conditions arise (Baskin & Baskin, 1998).

The morphophysiological mechanism

Morphophysiological dormancy includes two distinct dormancy mechanisms. The morphological component is the degree of embryo development at the time of dispersal; the cotyledons and hypocotyl–radicle are present, but the embryo is underdeveloped in size and needs further time to grow before it can germinate. The critical size at which germination occurs is species-specific (Baskin & Baskin, 1998; Finch-Savage & Leubner-Metzger, 2006). The physiological component is the hormonal balance within the seed. The breaking of this dormancy type needs warm or cold stratification or a hormonal application to promote germination by shifting the balance of hormones to favour germination rather than maintenance of dormancy (Baskin & Baskin 1998). This part of the mechanism enables seed to effectively delay germination until favourable climatic conditions prevail and allow post-germination seedling survival. This is an important survival mechanism in temperate regions where plants must survive the extreme aspects of seasonality.

Previous work on Meconopsis seed germination

Horticultural literature on methods of germination for *Meconopsis* is long established (Cox, 1934; Cross, 1940; Amsler, 1945; Thompson, 1967). Early debate, which continues today, focused on the choice of spring or autumn sowing (Cox, 1934; Cross, 1940; Heath, 1981; Cobb, 1989; Beckett, 1994; Grey-Wilson, 2000).

Cross (1940) advised autumn sowing to enable a longer growing season than if seed were sown in spring. However, he conceded that spring-sown seed "showed no evidence of spring germination being inferior". Spring sowing is advised for most *Meconopsis* species, except *M. punicea*, which needs to be exposed to cold (Cobb, 1989; Grey-Wilson, 2000). Cox (1934) pragmatically advised dividing seed and sowing half in autumn and half in spring to double the chances of successfully raising *Meconopsis*, although the literature contains no evidence that it does.

Amsler (1945) described freezing seed in a block of ice to promote germination of *M. betonicifolia*. First, desiccated stored seed are placed in a container with a small amount of water in warm conditions (20–30ºC) for 24h. The rehydrated seed in the container of water are then placed in a freezer for 24h. The resulting block of ice is

removed from the freezer and allowed to melt before the seeds are sown in a seed pan. Amsler reported that germination of the treated seed "was so profuse that I pulled up many", whereas for the untreated seed, "not a single one came up".

More sophisticated testing by Thompson (1967), using controlled conditions, showed that *M. horridula*, *M. latifolia*, *M. regia*, *M.* × *sarsonsii* and *M. napaulensis* fail or have poor germination rates when sown immediately after harvesting. The results showed that some *Meconopsis* species need a period of post-harvest ripening of approximately 6 weeks, which is an example of morphological dormancy. Thompson's testing did not rely on any pretreatment or stratification and also showed that *Meconopsis* species can have modest germination rates without pretreatment before sowing.

The use of hormones to break seed dormancy and promote germination in *Meconopsis* is well known (Deno, 1993; Sulaiman, 1993). The use of gibberellic acid (GA) for this purpose has been described by Deno (1993) and Sulaiman (1993) and shows that *Meconopsis* species also have a physiological component to their dormancy strategy.

Deno (1993) pretreated seed from several *Meconopsis* taxa and found that GA and a prior cold stratification for 3 months has little effect on the percentage of germinating *M. betonicifolia* seed, and in one case GA treatment damaged the seed of *M. betonicifolia* (using the invalid var. *pratensis* name). This result suggests that there is no dormancy-breaking requirement for *M. betonicifolia*. Deno found that alternating temperatures and the light and dark period had little effect on germination in the *Meconopsis* species tested.

Sulaiman (1993) carried out germination and dormancy-breaking tests on *M. simplicifolia*, *M. paniculata* and *M. villosa*. He showed that in these three species, GA and darkness decrease and GA and light increase germination rates. These results show that GA promotes germination only in combination with light. Therefore the dormancy strategy of *Meconopsis* uses light as an additional component to physiological dormancy.

Roberts *et al.* (1997) found that prechilling and a GA presoak increases the germination rate of *M. betonicifolia*. The results of Deno (1993), Sulaiman (1993) and Roberts *et al.* (1997) show slightly differing responses to GA, suggesting that GA is useful but not essential for the germination of *Meconopsis* seed.

The results in Table 1 from the Millennium Seed Bank (Liu *et al.*, 2008) show that *Meconopsis* species can germinate well after prolonged storage in a deep freeze.

The first author's research project investigated the germination of *Meconopsis* seed in response to short-term ambient and cool storage to reflect the typical conditions used on a small scale by botanical gardens and amateur horticulturists.

Relations in Meconopsis

Several phylogenetic studies have investigated relations between *Papaver* and *Meconopsis* (Jork & Kadereit, 1995; Kadereit *et al.*, 1997; Yuan *et al.*, 2003; Carolan *et al.*, 2006). With the exception of Yuan *et al.* (2003), these studies focus on *Papaver*, using *Meconopsis* and other genera within Papaveraceae as outgroups. Yuan *et al.* (2003) investigated relations within *Meconopsis* as part of PhD research.

Species	Age of oldest collection (years)	Change in germination
M. betonicifolia	19	100% to 86.2% over 19 years
M. dhwojii	2	100% at collection
M. discgera	1	83% at collection
M. gracilipies	23	95% to 67% over 23 years
M. horridula	19	96% to 90% over 16 years
M. latifolia	14	100% to 100% over 13 years
M. napaulensis	25	95% to 86% over 25 years
M. paniculata	2	89% to 89% over 2 years
M. villosa	2	100% at collection

Table 1 Results of germination testing for *Meconopsis* species at the Millennium Seed Bank (Liu *et al.*, 2008)

The studies by Jork and Kadereit (1995), Kadereit *et al.* (1997) and Carolan *et al.* (2006) all highlighted problems with the current classification of *Meconopsis*. These three studies show that the various species of *Meconopsis* are split between three disparate groups in Papaveraceae. Only 14 of the *c.*40–50 species of *Meconopsis* were sampled, but they include subgenus *Discogyne* and six of eight series in subgenus *Eumeconopsis* (Taylor, 1934; Grey-Wilson, 2000); the sample therefore covers the genus reasonably well. *Meconopsis cambrica* (the type species) appears within *Papaver*, while *M. chelidonifolia* and *M. villosa* (series Chelidonifoliae) sit completely outside even *Papaver*. All remaining species sampled form a single (monophyletic) group within *Papaver*. Final taxonomic decisions are pending.

Genetic relations within *Meconopsis* have been further clarified by PhD research carried out in China by C. Yuan (Yuan *et al.*, 2003). This study, along with those mentioned earlier, used DNA sequence data from the nucleus (internal transcribed spacer) and chloroplast (*trn*L-F) to reconstruct relations.

To determine whether seed germination patterns reflect relations in *Meconopsis*, the work of these previous authors was synthesised, producing the most up-to-date analysis of the group. This analysis provided evidence to interpret whether germination is linked to underlying genetic relations or is based on other factors, such as the ecology of the species or individual treatments used. The appendix shows the data and methodology used to produce the phylogenetic tree.

MATERIALS AND METHODS

Harvest and processing

Seed from six species and four hybrids or cultivated forms (Table 2) were harvested at RBGE on 18 August 2008. *Meconopsis* ‘Lingholm’ was harvested on 17 August 2008

Sample code	Name	Donor
AE1	*M.* 'Lingholm'	
ES02A	*M. grandis*	E. Stevens
ES16	*M. betonicifolia*	E. Stevens
ES17	*M. betonicfolia* 'Alba'	E. Stevens
ES18	*M. betonicifolia* 'Hensol Violet'	E. Stevens
ES26	*M. quintuplinerva*	E. Stevens
ES27	*M.* 'Clint Callens'	E. Stevens
ES32	*M. punicea*	E. Stevens
ES33	*M.* 'Lingholm'	E. Stevens
RBGE1	*M. betonicifolia*	RBGE
RBGE2	*M. napaulensis* (hort.)	RBGE
RBGE3	*M. superba*	RBGE

Table 2 *Meconopsis* seed samples used for analysis and discussion in germination tests

from the first author's garden. Seed came from plants of the same accession at the same location. The seed capsules were placed facing downwards in white paper bags to enable the seed to air dry. This was done at ambient temperature. After 7 days, seed easily fell from the capsule to the bottom of the bag when shaken.

Blotting paper was used to remove the chaff from the seed; the paper enabled seed to roll off but held most of the chaff in place. Dissecting forceps were used to pick any remaining chaff from the seed. The seed generally came away cleanly from the capsules and little cleaning was needed, except for *M.* 'Lingholm', which has hirsute capsules.

Once the seed had been cleaned, each batch of seed from a single accession was divided into two roughly equal quantities for storage under different conditions. Each sample was placed in a cellulose seed packet with the identifying information written on the front: accession number (if applicable), date harvested and testing code (e.g. AE01 and RBGE01).

Seed from the national scientific collection of the UK National Council for the Conservation of Plants and Gardens (NCCPG) was harvested and processed by Dr Evelyn Stevens and stored in a cool room before being forwarded. Seed of *Meconopsis punicea* were provided by Dr James Cobb. Once received, samples with sufficient numbers of seed were divided roughly into two equal quantities, for different storage conditions, and placed in cellulose seed packets as described above.

Seed packets were stored in Kilner jars, with one half stored in a domestic fridge at *c.*4ºC and the other stored at ambient temperature (8–17ºC). The jars were opened only during seed sowing.

Sowing and incubation

The first test used 60 seeds from each sampling: three replicates, each with 20 seeds. Subsequent tests used 30 seeds: three replicates of each individual, with 10 seeds for each sample stored under the different storage conditions. This reduction was to enable an extended period of testing of as many samples as possible with the limited numbers of seed available.

Seeds were placed on a single sheet of 9cm Whitman's no. 1 filter paper in a Petri dish. Depending on the treatment, either 5ml of distilled water or 5ml of an aqueous solution of GA (BHD Laboratory Supplies, Poole, UK) at a concentration of 0.25g/l were added to the dishes. Each Petri dish had the sample identification number written on it, as well as the replicate number (e.g. RBGE1.1, RBGE1.2 and RBGE1.3). The Petri dishes were then placed in batches of six in a large sealable sandwich bag to maintain constant humidity. Seeds were sown in clean but not sterile conditions.

Fig. 2 Growing cabinet used in the germination and viability testing at RBGE. Photo: Alan Elliott.

Seeds were incubated in a growth chamber (Fig. 2) at 20ºC (±5ºC) with a 12h on/12h off light regimen. The seeds were examined by eye every 2–3 days to check for fungal contamination and to top up with an additional 2ml of distilled water if needed. If fungal growth was seen on the filter paper or the seed, then affected seeds were removed and recorded as dead. The remaining seeds were rinsed and placed on fresh filter paper in a new Petri dish.

Checking and germination recording

Normal germinations were recorded and the seedlings removed to prevent recounting. Seeds were classified into four categories defined by the International Seed Testing Association (2008) (Table 3).

Results for the storage conditions seed trial

Viability

Tables 4 and 5 show the percentages of viable seed for ambient and chilled stored seed, respectively, for each test sample at the end of the four tests. Germination rates ranged from 66.67% to 100% for ambient stored seed and 50% to 100% for chilled stored seed.

Viability or germination classification	Definition
Normal	Seedling has two intact green cotyledons, free from disease and free from the testa; a primary root with root hairs. The seed is considered to have germinated.
Abnormal	Seedlings have damaged, incomplete cotyledons; primary root damaged or incomplete; root hairs absent. The seed is considered to have germinated.
Fresh	Inspection shows seed to be ungerminated but firm and free from fungal infection. The seed is assumed to be viable.
Dead	Seed neither hard nor fresh and have not produced any part of a seedling structure. The seed is considered to be non-viable.

Table 3 Classification of seed type in viability tests (International Seed Testing Association, 2008)

Taxon	Code	Viability (%)			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	19 Dec – 16 Jan 2009 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. 'Lingholm'	AE01	93.33	83.33	73.33	73.33
M. grandis	ES02A	86.67	96.67	70.00	100.00
M. betonicifolia	ES16	86.67	73.33	70.00	83.33
M. betonicifolia 'Alba'	ES17	96.67	96.67	80.00	76.67
M. betonicifolia 'Hensol Violet'	ES18	66.67	76.67	80.00	73.33
M. 'Lingholm'	ES33	100.00	96.67	86.67	86.67
M. betonicifolia	RBGE01	93.33	96.67	80.00	80.00
M. napaulensis (hort.)	RBGE02	73.33	100.00	76.67	93.33
M. superba	RBGE03	86.67	93.33	66.67	90.00

Table 4 Percentage of viable *Meconopsis* seed stored at ambient temperature (8–17ºC) for each of the four tests

Table 6 shows the total viability of all *Meconopsis* taxa in the trial at the end of each of the four test periods.

Chi-square tests (Fowler & Cohen, 1990) were carried out using Minitab15. The graph in Fig. 3 shows an increase in viability with time for seed stored chilled. The graph for the ambient-stored seed (Fig. 4) shows a fluctuation in viability with time over the four test periods.

Germination

Tables 7 and 8 show the percentages of germination for ambient and chilled stored seed, respectively, for each test sample at the end of the four tests. Germination rates ranged

Taxon	Code	Viability			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	19 Dec – 16 Jan 2009 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. 'Lingholm'	AE01	50.00	93.33	86.67	100.00
M. grandis	ES02A	90.00	96.67	100.00	100.00
M. betonicifolia	ES16	76.67	90.00	90.00	93.33
M. betonicifolia 'Alba'	ES17	83.33	100.00	100.00	76.67
M. betonicifolia 'Hensol Violet'	ES18	96.67	90.00	90.00	100.00
M. 'Lingholm'	ES33	100.00	100.00	96.67	100.00
M. betonicifolia	RBGE01	66.67	90.00	96.67	96.67
M. napaulensis (hort.)	RBGE02	80.00	100.00	90.00	96.67
M. superba	RBGE03	70.00	86.67	100.00	96.67

Table 5 Percentage of viable *Meconopsis* seed stored under chilled conditions (*c.*4ºC) for each of the four tests

Storage	+56 days	+91 days	+126 days	+164 days	Total	Mean	SD
Ambient storage	235 (87.04%)	244 (90.37%)	205 (75.93%)	227 (84.07%)	911 (84%)	227.75	19.84943
Chilled storage	214 (79.26%)	254 (94.07%)	255 (94.44%)	258 (95.56%)	981 (91%)	245.25	20.90255

$\chi^2_3 = 6.018$, $P = 0.111$.

Table 6 Total viable Meconopsis seed at the end of each test under two different storage conditions

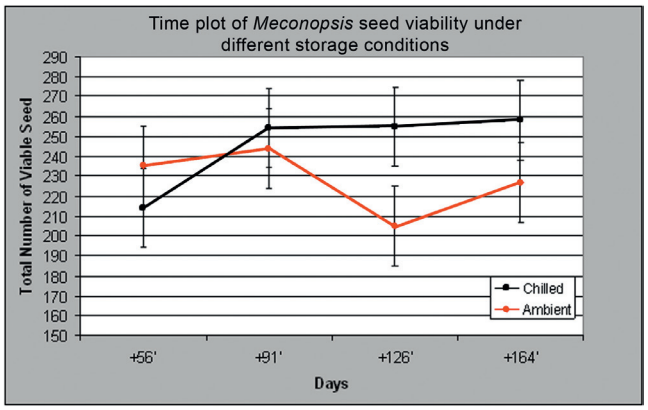

Fig. 3 Graph showing time plot of *Meconopsis* seed viability, comparing storage conditions. Chilled storage = black line, Ambient storage = red line. Vertical bars indicate standard deviations. Note that fungal contamination in the first sample-set was a factor in the low initial values.

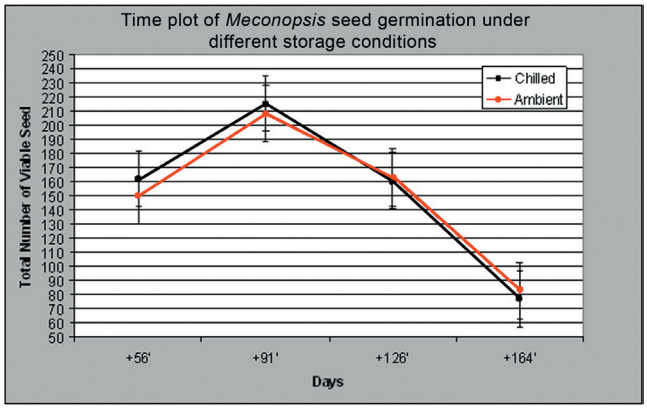

Fig. 4 Time plot of *Meconopsis* seed germination, comparing storage conditions. Chilled storage = black line, Ambient storage = red line. Vertical bars indicate standard deviations.

Species	Code	Germination			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	14 Nov – 12 Dec 2008 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. 'Lingholm'	AE01	46.67	76.67	43.33	43.33
M. grandis	ES02A	50.00	90.00	63.33	40.00
M. betonicifolia	ES16	60.00	73.33	70.00	30.00
M. betonicifolia 'Alba'	ES17	56.67	66.67	63.33	56.67
M. betonicifolia 'Hensol Violet'	ES18	60.00	60.00	50.00	26.67
M. 'Lingholm'	ES33	93.33	90.00	76.67	40.00
M. betonicifolia	RBGE1	36.67	83.33	53.33	16.67
M. napaulensis (hort.)	RBGE2	43.33	86.67	73.33	10.00
M. superba	RBGE3	53.33	66.67	50.00	13.33

Table 7 Percentage of germinated seed stored at ambient temperature (8–17ºC) for each of the four tests

from 13.33% to 93.33% for ambient stored seed and 13.33% to 100% for chilled stored seed.

Tables 9 and 10 show the total germination rates of all *Meconopsis* taxa in the trial at the end of each test period. For the first two test periods, chilled stored seed had higher rates of germination than ambient stored seed. For the last two test periods, ambient stored seed had higher germination rates than the chilled stored seed. Overall, the total germination rate was marginally higher when the seed was chilled. There was a 1.01% percentage difference in the total germination rate between ambient stored (55.93%) and chilled stored seed (56.94%). Fig. 4 shows a slight rise and then fall in the germination rate over the four test periods.

Species	Code	Germination			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	19 Nov – 16 Jan 2009 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. 'Lingholm'	AE01	46.67	60.00	33.33	13.33
M. grandis	ES02A	13.33	60.00	66.67	46.67
M. betonicifolia	ES16	63.33	76.67	86.67	23.33
M. betonicifolia 'Alba'	ES17	33.33	83.33	36.67	60.00
M. betonicifolia 'Hensol Violet'	ES18	96.67	86.67	50.00	20.00
M. 'Lingholm'	ES33	100.00	100.00	96.67	30.00
M. betonicifolia	RBGE01	66.67	73.33	73.33	20.00
M. napaulensis (hort.)	RBGE02	80.00	96.67	40.00	16.67
M. superba	RBGE03	40.00	80.00	53.33	26.67

Table 8 Percentage of germinated seed stored under chilled conditions (*c.*4ºC) for each of the four tests

Storage	+56 days	+91 days	+126 days	+164 days	Total	Mean	SD
Ambient storage	150	208	163	83	604	151	50.42091
Chilled storage	162	215	161	77	615	153.75	57.04603

$\chi^2_3 = 0.716$, $P = 0.870$.

Table 9 Total germination of *Meconopsis* at the end of each test under two different storage conditions

Storage	+56 days	+91 days	+126 days	+164 days	Total
Ambient	55.56	77.04	60.37	30.74	55.93
Chilled	95.29	79.63	59.63	28.52	56.94

Table 10 Total percentage germination of Meconopsis seed at the end of each test under two different storage conditions

Gibberellic acid test

Table 11 shows that monthly germination rates decreased with time. Seeds sown with GA solution had higher total germination rates in the first two test periods than those sown with distilled water. However, in the final test period, seeds sown with GA solution had lower total germination rates than those sown without it. Fig. 5 shows a declining germination rate over the three test periods.

Day	*M.* 'Lingholm'	*M. betonicifolia*	*M. superba*	*M. napaulensis*	Total	Mean
91	15	17	24	16	72	18
126	10	11	17	11	49	12.25
164	9	9	7	5	30	7.5
						SD = 5.33357
Day	*M.* 'Lingholm' + GA	*M. betonicifolia* + GA	*M. superba* + GA	*M. napaulensis* + GA	Total	Mean
91	18	22	24	29	93	23.25
126	10	22	16	13	61	15.25
164	4	6	8	5	23	5.75
						SD = 8.335303

$\chi^2_2 = 2.863$, $P = 0.239$.

Table 11 Germination test, showing comparison of *Meconopsis* taxa with distilled water only and those with distilled water and gibberellic acid (+GA).

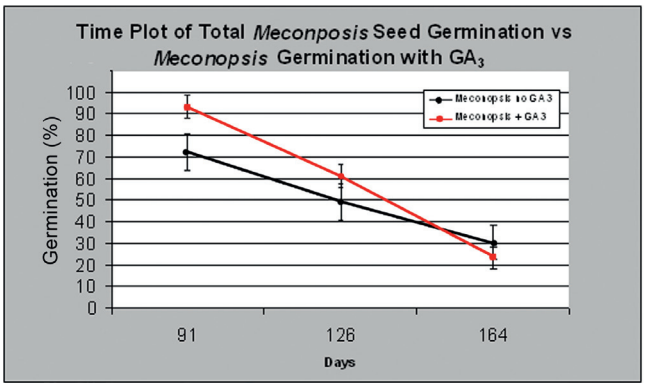

Fig. 5 Time plot of total *Meconopsis* seed germination, comparing hormone treatments. Without addition of GA_3 = black line, with GA_3 = red line. Vertical bars indicate standard deviations

DISCUSSION

Germination responses to different treatments

The viability results do not show a traditional pattern of viability dropping off with time. We attribute the initial loss of viability to seed being recorded as dead as a result of fungal contamination. It was observed that instances of fungal contamination decreased with time. The viability rate fluctuated from month to month, as shown in Table 4, but the mean rate remained high (84% for ambient storage and 91% for chilled storage). This result suggests that over this period there is little advantage to chilled storage of *Meconopsis* seed over ambient storage in the short term. Our study did not use seed that had been subject to deep freezing, although this is the generally accepted method of long-term storage for *Meconopsis* seed for both amateur and professional horticulturists.

Germination rates were low for the numbers of viable seed, with both ambient and chilled stored seed achieving only 56% germination within the test period. Deno's (1993) study showed similar germination rates, ranging from 48 to 70%, over the same test period. The germination rate in our study is lower than results for the Millennium Seed Bank at Kew (Liu *et al.*, 2008), which ranged from 90 to 100% when completely sterile conditions were used. However, low germination rates may not be problematic for horticulturists, because a single capsule contains many seed.

Our results for GA treatment are similar to those of Deno (1993; 1996), in that they showed both positive and negative results. Compared with untreated seeds, seeds treated with GA had increased germination rates in the first two tests and decreased rates in the final test; however, these differences were statistically non-significant. We used GA at a concentration of 0.25mg/l, slightly higher than that used by the Millennium Seed Bank (0.2mg/l) but lower than that used by Roberts *et al.* (1997) (10 mM: 3.48mg/l). Gibberellic acid may have first promoted germination, but as the internal hormonal balance shifted to favour dormancy, the GA concentration was insufficient to achieve the high GA:abscisic acid ratio needed to promote germination, as detailed by Finch-Savage and Leubner-Metzger (2006).

In our study, GA produced a small but statistically non-significant increase in germination rate. This agrees with the results of previous studies (Deno, 1993; 1996; Sulaiman, 1993; Roberts *et al.*, 1997), which show slightly differing responses to GA, suggesting that GA may be useful but not essential in the germination of *Meconopsis* seed. Further research using different GA concentrations and across species would clarify the effects of GA.

The tailing off of germination rate, despite the constant high level of viability, suggests that the seed's dormancy strategy began to take effect after day 91. Again, this result agrees with previous studies describing good germination in autumn (after harvest) and again in late spring (after cold stratification) (Cox, 1934; Cross, 1940; Cobb, 1989; Grey-Wilson, 2000).

Horticulturists should note that *Meconopsis* does not need special storage conditions in the short term. Ambient storage of harvested seed is as effective as chilled storage to achieve reasonable levels of viable seed to sow in the next season.

Germination responses in different taxa

The conditions used in our study favoured some taxa. The mean total germination rate of 56% was not representative of all taxa. Three of the series used – *Grandes*, *Robustae* and *Superbae* (Fig. 6; Taylor, 1934) – germinated. Members of *Grandes* and *Robustae* (including *M. grandis*, *M. napaulensis* and *M. betonicifolia*), as well as the series *Grandes*-like hybrid *M.* 'Lingholm', achieved high germination rates (over 75%; Tables 7 and 8).

In contrast, *M. punicea*, *M. quintuplinervia* and *M.* 'Clint Callens' (*M. quintuplinervia* × *M. betonicifolia*) failed to germinate over the different tests (Tables 12 and 13), despite the seed remaining viable – plump, firm and generally uninfected by fungal pathogens. This result suggests that the dormancy mechanism of these taxa differs

Species	Code	Viability			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	19 Dec – 16 Jan 2009 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. quintuplinerva	ES26	100.00	100.00	100.00	100.00
M. 'Clint Callens'	ES27	100.00	100.00	100.00	100.00
M. punicea	ES32	50.00	90.00	90.00	100.00

Table 12 Percentage of viable seed of *Meconopsis quintuplinerva*, *M.* 'Clint Callens' and *M. punicea* stored chilled at 4ºC

Species	Code	Germination			
		14 Oct – 11 Nov 2008 (+56 days)	14 Nov – 12 Dec 2008 (+91 days)	19 Dec – 16 Jan 2009 (+126 days)	26 Jan – 23 Feb 2009 (+164 days)
M. quintuplinerva	ES26	0.00	0.00	0.00	0.00
M. 'Clint Callens'	ES27	0.00	0.00	0.00	0.00
M. punicea	ES32	0.00	0.00	0.00	0.00

Table 13 Percentage of germinated seed of *Meconopsis quintuplinerva*, *M.* 'Clint Callens' and *M. punicea* stored chilled at 4ºC

substantially from that of the other species tested; both species are closely related (Fig. 6) and in series *Simplicifoliae*. The remaining member of this series, according to Taylor (1934), is *M. simplicifolia*, which is not in cultivation.

The failure to germinate seed of *M.* 'Clint Callens' suggests that the dormancy mechanism of *M. quintuplinerva* rather than that of *M. betonicifolia* is present in this interseries hybrid.

For *Meconopsis punicea* and *M. quintuplinerva* Cobb (1989) and Grey-Wilson (2000) recommend exclusive autumn sowing to enable sufficient cold stratification over winter to achieve germination. The seed tested in our study, although stored in refrigerated conditions, was dry, which does not reflect the moist and ambient chemical conditions of seed sown for winter stratification. Our results and those of previous studies suggest that these two species probably have a specific dormancy-breaking requirement unlike that of the other taxa tested. Series *Simplicifoliae* has a sister grouping, series *Primulinae*, none of whose members were tested in our study. It would be interesting to study germinability in series *Primulinae* and determine if its dormancy mechanism is similar to that of series *Simplicifoliae*.

Our results show that some taxa are inherently more germinable under the 20ºC (±5ºC) with a 12h on/12h off light regimen than others. It is probably incorrect to assume a 'one size fits all' germination behaviour for *Meconopsis*, because of the wide range of habitats in which its species are found. Evolutionary responses to the ecology of habitats probably gave rise to different requirements for germination among

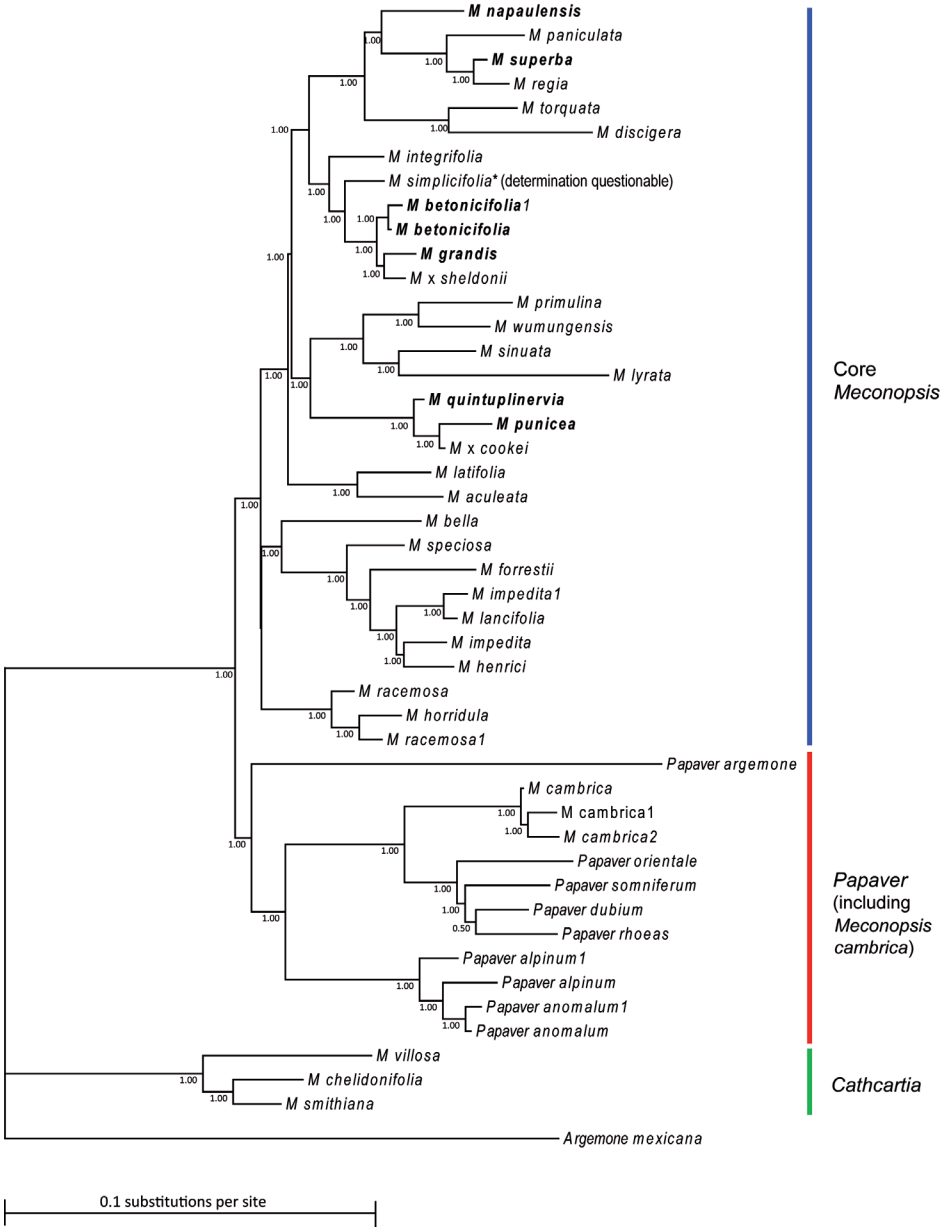

Fig. 6 New phylogeny of *Meconopsis* produced for this BSc honours research project, based on nuclear (ITS) and chloroplast (trnLF) DNA sequence data. The diagram shows the relationships among accessions of *Meconopsis* available on Genbank to date. For details of the Bayesian analysis used to construct this tree please see appendix. Numbers below and to the left of nodes indicate posterior probability support values for those nodes. Coloured bars show genera into which *Meconopsis* sensu Taylor (1934) species fall. Species names in bold correspond to those sampled for seed germination behaviour in this study. All species labels are presented as per determinations on Genbank, but the accession of *M. simplicifolia* marked with an asterisk (*) is presumed to be a misidentification or laboratory error.

Meconopsis species. *Meconopsis quintuplinerva* and *M. punicea* are found on open shrubland and open pasture and are subject to different local climatic conditions to those of *M. betonicifolia* and *M. grandis*, which are found in the herb layer of forest understory. These ecological differences undoubtedly determine the development of different dormancy strategies, with responses to light and temperature potentially having different degrees of importance in the behaviours of the species.

Continual conscious and arbitrary selection of plants and seed by horticulturists will also have played a selective role in the lineages of *Meconopsis* that persist in cultivation.

CONCLUSIONS

Horticulturists should note that storage of seed in cool or ambient conditions makes little difference in the short term. Also, there is only a limited decrease in viability and germination rate with time, so sowing most species fresh or stored makes little difference to germination success. Treatment with GA might slightly increase germination rates, but not significantly.

Readers should note that the experimental conditions in our study were clean (although not sterile) and so did not replicate the real life conditions of germinating seed in compost. Given the large numbers of seed present in a capsule, even very low germination rates can provide a good return of individual plants.

For species from series *Simplicifoliae*, longer germination periods, potentially with stratification to break dormancy, are needed (Cobb, 1989; Grey-Wilson, 2000).

Further studies of germination in *Meconopsis* should aim to consider at least the following in looking for patterns of germination behaviour: ecology, underlying phylogeny and the role of human selection on germination.

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APPENDIX

The phylogeny presented here is based on nuclear internal transcribed spacer and chloroplast (*trn*L-F) results taken from existing data on Genbank (Table A1). Following Carolan *et al.* (2006), *Argemone mexicana* was used as an outgroup. Bayesian analysis used MrBayes version 3.1 (Huelsenbeck & Ronquist, 2001) and was run on a Samsung X60 portable computer with an Intel Centrino Duo core mobile processor (1.83 GHz, 1 Gb RAM) mobile processor. By default, the personal computer version of MrBayes

version 3.1 runs two independent analyses in parallel, enabling cross-correlation at the expense of longer computing time. We ran two separate kinds of search: one with the model determined by Modeltest input (i.e. a user-defined model) and a second search with MrBayes left to estimate its own models of evolution.

We used the default settings for searching: four Markov chain Monte Carlo chains, with chain temperatures set as default with one million repetitions, sampling every 1000 replicates. Burnin was first set at 10% (100,000) trees. Likelihood stationarity was then checked by plotting likelihood values against sample number (in Microsoft Excel). The burnin could then be adjusted upwards as needed.

Parameters in MrBayes were set to enable the program to estimate its own model of evolution, and the analysis was run twice to ensure congruent topologies and comparable branch lengths with each run.

Sample	GenBank no. ITS	GenBank no. *trn*L-F	Reference
Meconopsis delavayi	AY328285	AY328211	Yuan *et al.* (2003)
M. aculeata	AY328263	AY328227	Yuan *et al.* (2003)
M. bella	AY328279	AY328218	Yuan *et al.* (2003)
M. betonicifolia	AY328292	AY328236	Yuan *et al.* (2003)
M. betonicifolia 1	DQ250323	DQ251174	Carolan *et al.* (2006)
M. cambrica	AY328299	AY328243	Yuan *et al.* (2003)
M. cambrica 1	DQ250277	DQ251128	Carolan *et al.* (2006)
M. cambrica 2	DQ250278	DQ251129	Carolan *et al.* (2006)
M. chelidonifolia	AY328300	AY328246	Yuan *et al.* (2003)
M. discigera	AY328277	AY328221	Yuan *et al.* (2003)
M. forrestii	AY328287	AY328219	Yuan *et al.* (2003)
M. grandis	AY328237	AY328235	Yuan *et al.* (2003)
M. henrici	AY328281	AY328209	Yuan *et al.* (2003)
M. horridula	AY328261	AY328208	Yuan *et al.* (2003)
M. impedita	AY328280	AY328210	Yuan *et al.* (2003)
M. impedita 1	AY328284	AY328213	Yuan *et al.* (2003)
M. integrifolia	AY328288	AY328229	Yuan *et al.* (2003)
M. lancifolia	AY328282	AY328212	Yuan *et al.* (2003)
M. latifolia	AY328264	AY328226	Yuan *et al.* (2003)
M. lyrata	AY328267	AY328215	Yuan *et al.* (2003)
M. napaulensis	AY328269	AY328228	Yuan *et al.* (2003)
M. paniculata	AY328272	AY328223	Yuan *et al.* (2003)
M. primulina	AY328266	AY328217	Yuan *et al.* (2003)

M. punicea	AY328293	AY328238	Yuan *et al.* (2003)
M. quintuplinerva	AY328295	AY328239	Yuan *et al.* (2003)
M. racemosa	AY328262	AY328207	Yuan *et al.* (2003)
M. racemosa 1	AY328257	AY328206	Yuan *et al.* (2003)
M. racemosa 2	AY328259	AY328205	Yuan *et al.* (2003)
M. regia	AY328273	AY328224	Yuan *et al.* (2003)
M. simplicifolia	AY328289	AY328230	Yuan *et al.* (2003)
M. sinuata	AY328268	AY328216	Yuan *et al.* (2003)
M. smithiana	AY328301	AY328247	Yuan *et al.* (2003)
M. speciosa	AY328286	AY328220	Yuan *et al.* (2003)
M. superba	AY328274	AY328225	Yuan *et al.* (2003)
M. torquata	AY328278	AY328222	Yuan *et al.* (2003)
M. villosa	AY328302	AY328245	Yuan *et al.* (2003)
M. wumungensis	AY328265	AY328214	Yuan *et al.* (2003)
M. × *cookei*	AY328294	AY328231	Yuan *et al.* (2003)
M. × *sheldonii*	AY328291	AY328234	Yuan *et al.* (2003)
Argemone mexicana	AY328303	AY328248	Yuan *et al.* (2003)
Papaver anomalum	DQ250264	DQ251115	Carolan *et al.* (2006)
P. anomalum 1	DQ250263	DQ251114	Carolan *et al.* (2006)
P. alpinum	DQ250268	DQ251119	Carolan *et al.* (2006)
P. alpinum 1	DQ250261	DQ251112	Carolan *et al.* (2006)
P. dubium	DQ250322	DQ251173.1	Carolan *et al.* (2006)
P. rhoeas	DQ250272.1	DQ251123	Carolan *et al.* (2006)
P. somniferum	DQ250306	DQ251157	Carolan *et al.* (2006)
P. argemone	DQ250298	DQ251149	Carolan *et al.* (2006)
P. orientale	DQ250291	DQ251142	Carolan *et al.* (2006)

Table A1 Samples used in the phylogenetic study: GenBank sample numbers and references (Benson *et al.*, 2008)