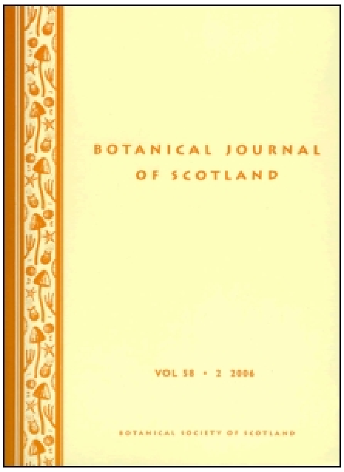

Botanical Journal of Scotland
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t909496046

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To cite this Article Ranwala, Sudheera M. W. and Naylor, Robert E. L.(2004) 'Production, survival and germination of bilberry (*Vaccinium myrtillus* L.) seeds', Botanical Journal of Scotland, 56: 1, 55 — 63
To link to this Article: DOI: 10.1080/03746600408685067
URL: http://dx.doi.org/10.1080/03746600408685067

Production, Survival and Germination of Bilberry (*Vaccinium myrtillus* L.) Seeds

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Summary

Bilberry (*Vaccinium myrtillus* L.) is a prominent species in moorland and woodland vegetation which spreads and survives mainly by vegetative growth from buds. Each berry contains many seeds but seedlings are rarely seen in Scotland. Thus, it is of interest to establish whether the lack of seedlings reflects a low production of viable seeds, their dormancy status, or germination requirements or seed longevity.

Samples of *V. myrtillus* berries were collected from moorland in the east Grampian mountains, Scotland. Each berry contained about 70 seeds. Small seeds did not germinate but large fresh seeds achieved over 80% germination in laboratory tests. Seeds stored dry for one year did not lose viability. Seeds stored in moist cold conditions (to mimic conditions in the soil over winter) lost viability progressively and none germinated after about 43 weeks. Germination occurred over the range 15-24 °C. These seed germination and longevity characteristics may currently severely limit the window of opportunity for germination of seeds of *V. myrtillus* in Scotland and have implications for the spread of *V. myrtillus* if average temperatures increase.

Key words: seed production, temperature, storage, dormancy, longevity.

Introduction

Vaccinium species generally produce many fruits (berries) and are thought to have a high fecundity. The berries are attractive to animals and so *Vaccinium* species are usually assumed to have good seed dispersal. Neverthless, they appear to lack large or persistent seed banks.

Bilberry (*Vaccinium myrtillus* L.) is a key species of Scottish moorland vegetation. It is a clonal plant and invades and regenerates from buds on the rootstock. Recruitment of bilberry seedlings at 'windows of opportunity' is suggested as one distinct pattern of recruitment among clonal plants (Eriksson & Froborg, 1996) but clearly must be dependent on a supply of fresh seeds for its success. Stands of *V. myrtillus* produce crops of globose berries 5-10 mm in diameter in most years. However, in Scotland and elsewhere, seedlings are rare (Miller & Cummins, 1987; Hester, Gimingham & Miles, 1991; Vander Kloet & Hill, 1994; Welch *et al.*, 1994; Welch, Scott & Doyle, 2000). There were no bilberry seedlings in over 1000 seedlings which emerged from soil samples taken in September 1998, April 1999, September 1999 and April 2000 from moorland vegetation containing bilberry in the eastern Grampian Mountains, Scotland

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(Ranwala, 2001). In studies on montane seed banks in various communities in the Cairngorm Mountains, Scotland Miller & Cummins, 2003 found that bilberry seeds were absent or scarce in the seed bank despite bilberry plants being prominent in the surface vegetation, with cover up to 60% at some sites.

Thus, it is of interest to establish the reasons for the scarcity of bilberry seedlings. Possible reasons are a low production of viable seeds, their dormancy status, their germination requirements and their longevity and survival in the soil.

In this paper we report the seed content of *V. myrtillus* berries, their morphometric characters, the temperature conditions conducive to their germination and conditions affecting seed longevity.

Materials and methods

The study site

All bilberry seeds were collected by hand from areas which had been ungrazed for two years, on Strathfinella Hill at the Macaulay Institute's Glensaugh Research Station in the east Grampian mountains (National Grid NO677782). The hillside faces north-west at an altitude of 200-250 m above sea level. Average annual rainfall is 1040 mm. The soil is a peaty podzol developed on drifts derived from Old Red Sandstone. Heather-dominated patches comprised about 85% of the area and grass-dominated patches (predominantly of *Deschampsia flexuosa*, *Agrostis capillaris* and *Festuca ovina*) occupied about 15% (Hester *et al.*, 1999). Bilberry was present as small patches of pure stands and patches mixed with heather, with mean overall cover of less than 5%.

Fruit and seed characteristics

Samples of mature ripe berries were hand picked in August 1998 and 1999.

In the laboratory, the fresh weights (to the nearest mg) and diameter (to nearest mm) of 50 berries were measured. The seeds were extracted from each berry separately, by crushing and mixing the berry with water and carefully separating the peel and pulp from seeds using forceps. All seeds were then washed with tap water several times and air-dried for 24 h. The total number of seeds in each berry was counted and weighed. Two types of seeds were distinguished. Large plump seeds (*i.e.* seeds about one mm in length) were separated manually under a magnifying glass from small flat (relatively lighter) seeds about 0.5 mm in length. The number and weight of each type of seed was recorded separately for each berry.

Bulk samples of seeds for germination experiments were obtained from samples of about 1000 berries. The crushed berries were introduced to a mechanical seed separator using sieves with aperture diameters 2000 μm, 560 μm and 315 μm. After two to three hours, the seeds on each sieve were picked from the remaining fruit parts using forceps. After extraction, seeds were used immediately in experiments.

Germination tests

Overall germination was assessed on a sample of 3554 seeds (all the seeds contained in 50 berries). Seeds, categorised as large or small, were placed in batches of 50, in Petri dishes lined with germination paper and moistened with 3 ml of deionised water. The Petri dishes were kept in a growth cabinet at 21 °C with a photoperiod of 16 h light and 8 h dark. The number of germinated seeds (defined as emergence of the radicle to at least 2 mm) was counted on alternate days until no further germination was observed for three consecutive recordings. The seeds left ungerminated at the end of the test were counted. In a separate experiment a batch of large seeds was divided into those which were pale or dark and the germination of 1000 of each was determined in a similar manner.

Temperature response

Germination was assessed on a temperature gradient plate (Grant Instruments, Cambridge, UK) at a range of constant temperatures from 9 to 28 °C (±0.5 °C) and prepared for use as described in Naylor, 1993. A piece of white plastic-backed filter paper ('Benchkote', Whatman, England) was placed on the base with its absorbent side up, and 250 ml deionised water added. A plastic grid, defining a matrix of 14 × 14 cells each 4 × 4 cm, was also cleaned with alcohol and placed on the paper. The outer cells were not used in the experiment. A folded germination paper (Whatman, 90 mm) was introduced into each cell and a further 3.5 ml of deionised water was added. The temperatures were checked in the front, middle and back rows until they equilibrated before commencing the experiment.

Bulk samples of seed were air-dried and stored in sealed foil packets for one week at either 20 °C (warm) or at 2 °C (cold). From each storage treatment, 20 seeds were placed in each of the 12 cells used in a row, each cell having a different temperature. There were four replicate rows for each storage treatment arranged in four randomised blocks. The top of the gradient plate was also covered with Benchkote with the absorbent side down and sealed with tape to reduce evaporation.

Germination was assessed every alternate day after setting seeds to germinate and continued until there was no germination for three consecutive days. The criterion for germination was emergence of the radical to greater than 2 mm. The number of seeds germinated was counted under a magnifying glass and the germinated seeds removed.

For each cell on the temperature gradient table, the final germination was expressed as a percentage. The median germination time ($t_{50,}$ the time to reach half of final germination) was estimated by linear interpolation. The rate of germination was calculated as $1/t_{50}$. The germination data were analysed using appropriate routines in GENSTAT.

Seed storage

One seed lot of over 1000 large seeds was air-dried, placed in a sealed foil packet and stored at 21 °C for one year. A further twelve seed lots of 1000 large seeds were stored in cold moist conditions by wrapping the seeds with a filter paper and moistening with 3 ml of deionised water before placing them in sealed foil seed packets and placing in a refrigerator at 2 °C. At four-week intervals a single packet was removed from storage the germination tested as described previously at 21 °C.

Results

Fruit size, seed size and colour

The average diameter of a *V. myrtillus* berry was 6.34 mm (Standard Error, SE = 0.22) and had an average fresh weight of 230 mg (SE = 16). Berries contained on average 71 (SE = 3) seeds. The total seed weight per berry was 0.106 mg (SE = 0.0021), contributing about 0.05 % of the fresh weight of a berry. Small, flat seeds of lighter weight comprised 72 % of seeds. There were 8 % large dark seeds and 20 % large pale seeds. There were positive relationships of berry fresh weight with both the total weight of seeds contained (total weight of seeds = 25 × berry weight (mg) + 1.8; n = 50; $R^2 = 0.57$) and the number of large seeds (number of large seeds = 0.2 × berry weight (mg) + 3.2; n = 50; $R^2 = 0.77$). Thus, heavier berries contained a greater weight of seeds and more large seeds than did lighter berries.

Germination

Germination of fresh seeds of *V. myrtillus* commenced nine days after setting to germinate and continued for 35 days. Final germination was 24 % overall (Table 1). However, small seeds did not germinate but germination of large seeds was 86 %. Large pale seeds germinated less well than large dark seeds. Median germination time did not differ with seed colour.

There was no germination of seeds below 15 °C. Germination occurred over the range 15 to 24 °C (Fig. 1). Most germination occurred at 21 °C. There was no difference in minimum temperature or optimum temperature for germination between seeds stored for one week in warm or in cold conditions. The germination rate ($1/t_{50}$) increased from 15 °C to 23 °C and decreased sharply above this range of temperatures (Fig. 1b).

Change in germination of bilberry seeds with storage

The germination of *V. myrtillus* seeds stored dry for one year was not appreciably different from that of fresh seeds (Table 2). The final number of seeds that germinated of seeds stored in moist cold conditions decreased linearly with time, by about 2.2 % seeds per week of storage, and was zero after 43 weeks. The period before germination commenced was shortest after 13 or 16 weeks storage. The median germination time was shortest after 21 weeks of cold wet storage but increased thereafter.

Discussion

Numbers of viable seeds produced

The fruits of *V. myrtillus* contain many seeds. Potential seed production is high: the number of seeds per berry was as high as 141 in this study and up to 95 in a study in Kola peninsula, Russia (I. Lyanguzova, pers. comm.). However, not all the seeds appear to be viable. Seeds which are small, flat and light do not germinate. This may account for some of the differences in reported germination of bilberry seeds if different workers selected only large seeds for their experiments. Many reports do seem only to have considered large seeds (Ritchie, 1956;

Table 1. Effect of seed size and colour on germination of fresh bilberry seeds.

		Seed size	Seed colour	
			Pale	Dark
		n = 3554	n = 1000	n = 1000
Final germination (%)	All seeds	24.2		
	Small, flat light seeds	0	nt	nt
	Large, plump heavy seeds	86.3	70.4	85.3
Median germination time (t_{50}; days)		17.5	13.5	14.0
Germination rate ($1/t_{50}$; seeds/day)		0.057	0.074	0.071

nt = not tested

Table 2. Germination of large bilberry seeds fresh and after dry or moist storage.

Storage period (weeks)	Storage conditions	Final germination (%)	Start of germination (day)	Median germination time (t_{50}; days)	Germination rate ($1/t_{50}$; seeds/day)
0 (fresh)	–	86	9	17.5	0.057
52	dry	83	12	16.5	0.061
4	moist	84	12	16.5	0.061
13	moist	75	9	14.0	0.071
16	moist	56	9	10.5	0.095
21	moist	53	10	10.0	0.100
24	moist	43	13	14.0	0.071
28	moist	32	10	14.0	0.071
33	moist	10	11	15.0	0.067
37	moist	7	10	16.0	0.063
41	moist	6	13	16.0	0.063
45	moist	3	14	16.0	0.063

Grime *et al.*, 1981; Vander Kloet, 1983; Jacquemart, 1997; Welch *et al.*, 2000). It is not clear whether the proportion of large seeds reflects pollen availability or assimilate supply.

Large seeds germinate to 70-80% even after dry storage for one year confirming the seeds behave in an orthodox fashion and are not recalcitrant, as are many seeds from fleshy fruits. It is possible that the darker large seeds represent more mature seeds.

At Glensaugh, local patches of *V. myrtillus* had a shoot density up to 400 reproductive shoots m^{-2} shoots which bore on average 2.5 fruits (Ranwala, 2001). The fruits contained on average 71 seeds of which 28% were large. This suggests a viable seed production equivalent to nearly 20 000 m^{-2}, albeit at a small patch scale. Thus, production of germinable seeds would appear not to limit the number of bilberry seedlings.

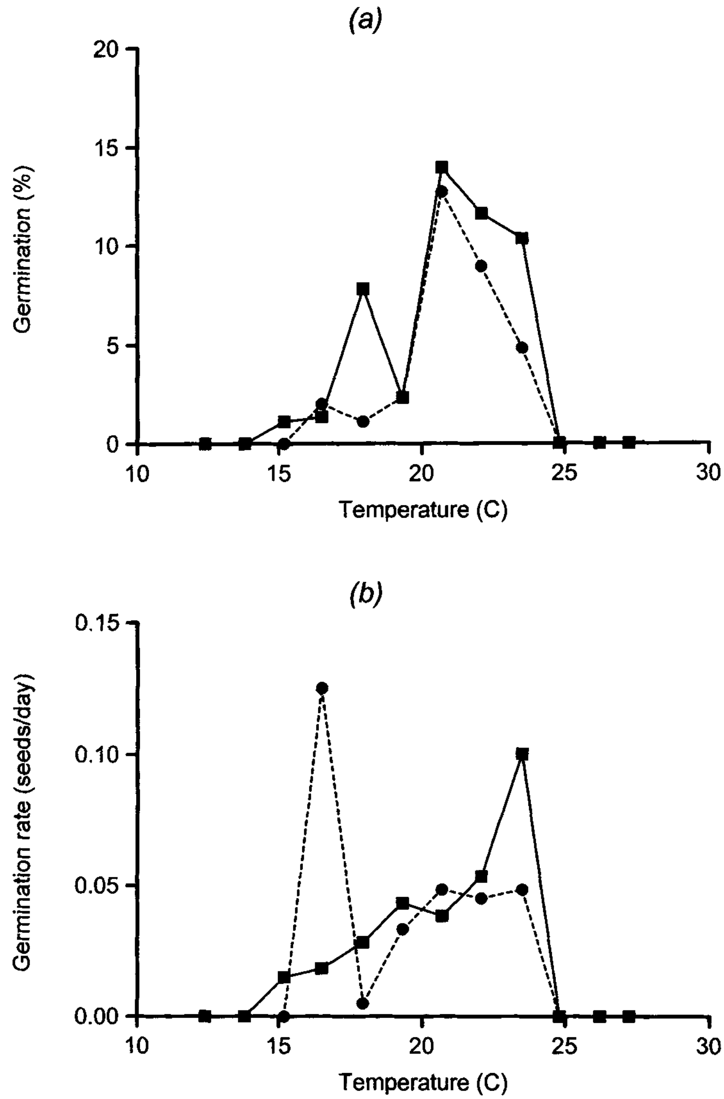

Fig. 1. Effect of temperature on (a) final germination (%) and (b) germination rate of bilberry seeds stored at 20 °C (—■—) or 2 °C (- - ●- -) for a week after collection.

Seed survival in the seed bank

If the lack of *V. myrtillus* seedlings is not due to low production of germinable seeds, then the incorporation of seeds into the seed bank and the survival of seeds in the soil may be critical. This paper gives no information on fruit consumption and dispersal of seeds. Welch *et al.*, 2000 list the animals which may be available to consume *V myrtillus* berries and suggest that the main dispersal agents may be small rodents feeding on fallen berries in autumn, a conclusion repeated by Miller & Cummins, 2003. *V. myrtillus* seeds are known to survive passage through the guts of animals (Reynolds, 1958). Thus they are likely to be introduced to the seed bank, albeit at a different location. Welch *et al.*, 2000 confirmed the germination of seeds from dung of frugivores, albeit survival of passage through the gut was estimated at 1%. The extent of seed predation in the soil is not known.

A cause of decline in seeds in the seed bank may be germination (Naylor, 2002) but the relatively high temperatures required for germination reported here, suggest that it would be unlikely to occur in Scotland except in the hottest months of the year. Only in July and August are soil temperatures at Glensaugh above 15°C for long enough. However, seed dispersal occurs in late August and September so most seeds must survive for almost a year in cool moist soil before experiencing temperatures that permit germination. The present results suggest this is unlikely.

The present results suggest that *V. myrtillus* seeds are unlikely to retain viability for more than one year in the soil. A survey of the seedbanks under a range of Scottish vegetation types showed *V. myrtillus* seeds to be absent or rare even under vegetation containing appreciable cover of *V. myrtillus* plants (Miller & Cummins, 2003). Thus, *V. myrtillus* appears not to have a large persistent seed bank but a transient seed bank.

The conclusions of the present work are that the ecological conditions in Scotland combined with the low persistence of seed before germination conditions may be experienced, account for the observed scarcity of *V. myrtillus* seedlings.

Implications for conservation

Vander Kloet & Hill, 1994 found that of six species of *Vaccinium* present in heathland vegetation in Newfoundland, only seeds of *V. angustifolium* and *V. boreale* were recovered from 120 soil cores. Seeds of *V. uliginosum* and *V. vitis-idaea*, two of the most common species, were not found in the seed bank although seeds that were experimentally buried germinated successfully when exhumed after six years. These authors suggested that the paucity of *Vaccinium* seed in the soil column may have been due in part to fungal rot and avian predation. In these present studies, the apparent scarcity of new seedlings of *V. myrtillus* despite the investment of biomass into fruit production is shown to be due to the failure of seeds to survive until appropriate temperatures permit germination.

Despite the success of vegetative reproduction, the lack of recruitment of new genetic individuals poses risks for the species. However, the poor seedling recruitment may not continue to be the case should average temperatures in Scotland rise thus broadening the 'window of opportunity' for germination. Intervention by collecting *V. myrtillus* seeds and storing dry is a possible management option to increase the number of seedlings of bilberry though soil surface modification may be needed to provide suitable microsites for germination.

Acknowledgements

Sudheera Ranwala thanks the Commonwealth Scholarship Commission in the

UK for the award of a Commonwealth Scholarship to pursue these studies in Aberdeen. Alison Hester of the Macaulay Institute, Aberdeen, Scotland is thanked for allowing access to her trial site and providing information. The Macualay Institute kindly supplied temperature data collected as part of the Environmental Change Network.

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(Accepted for publication 12 April 2004)