An evaluation of the desiccation tolerance and seed germination requirements of Chatham Island Forget-me-not (*Myosotidium hortensia*)

C.R. McGill[1], H.A. Outred[2], M.J. Hill[3], K.A. Hill[3] and D.W. Fountain.[2]

[1]Seed Technology Centre, Institute of Natural Resources, College of Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand.
[2]Institute of Molecular Biosciences, College of Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand.
[3]New Zealand Seed Technology Institute, P O Box 84, Lincoln University, Canterbury, New Zealand.

Abstract

Chatham Island forget-me-not (*Myosotidium hortensia* (Decne.) Ballon) is endemic to the Chatham Islands, New Zealand, where it is now restricted to the outer islands. The germination behaviour of seed of *Myosotidium hortensia* is poorly understood with some reports suggesting the seed will germinate readily if fresh but others describing the germination as erratic. There is also speculation in the literature that the seed may be recalcitrant. Seed of *Myosotidium hortensia* was evaluated for recalcitrant behaviour by determining if desiccation to low seed moisture content (SMC) caused a loss of viability. Seed was harvested at two moisture contents, 47 % (green seed) and 36 % (dark brown or black seed), and air dried to a final moisture content of 7.5 %. As moisture content declined seed viability and germination were monitored. At 7.5 % SMC, viability was 82 % and germination 78 % for seed harvested at 36 % SMC, and 89 % and 92 %, respectively, for seed harvested at 47 % SMC. Seed placed on a temperature gradient plate at a range of temperatures showed less than 5 % germination after 11 weeks. However removal of the seed coat allowed germination to reach 88 % within two weeks. To determine how the coat imposes dormancy a series of dormancy-breaking treatments were applied to seed. Treatments that weakened the coat were most effective in allowing germination to proceed. The likely dormancy mechanisms in *Myosotidium hortensia* are discussed.

Additional key words: germination inhibitor, imposed dormancy, oxygen uptake, recalcitrance, seed coat

Introduction

The flora of New Zealand represents a unique genetic resource (Fountain and Outred, 1991) with approximately 75 % of the indigenous flowering plants found nowhere else (Laing and Blackwell, 1949). However, Given (1990) reports that 10-15 % of the New Zealand flora is either threatened or restricted to localised areas. To conserve and regenerate this flora an ability to store and germinate the seed of native flora is essential, but our knowledge of the germination and storage requirements of many of these species is at best limited and often non-existent (Fountain and Outred, 1991; Bannister and Jameson 1994). Information that is available is frequently anecdotal and found in the non-scientific literature; for example, home gardening guides. This information is often conflicting or inconclusive. There is therefore an urgent need not only to increase our knowledge of the germination and storage requirements of our native flora but also to place much of the existing information in a scientific context.

Chatham Island forget-me-not (*Myosotidium hortensia* (Decne.) Ballon) is a member of the Boraginaceae family. It is the only species in the genus and is found naturally only in the Chatham Islands (44°S, 176°W) a group of islands approximately 750 km east of mainland New Zealand. *Myosotidium hortensia* is susceptible to trampling damage from stock and is palatable to both sheep and pigs. It is now largely confined to the outer islands of Pitt, South East, Mangere and Little Mangere (Wilson and Given, 1989). The plant is characterised by large (up to 30 cm across) deeply grooved, glossy, green leaves. In late spring dense pyramidal flower clusters appear. These can range in colour from pale to dark blue. There is also a white form in cultivation which is sometimes referred to as "Alba" (Metcalf, 1993). Each

flower contains a four-lobed ovary. This develops into a fruit containing four nutlets, each nutlet containing a single seed (Moore and Irwin, 1978).

Maloy (1992) reports that seed will germinate readily if fresh but Metcalf (1995) comments that the germination is erratic and seedlings may not emerge until the following spring. Metcalf (1995) also suggests that seed will store for 12 months or more but some commercial growers have difficulty in maintaining seed viability for that length of time. Fountain and Outred (1991) also note that *Myosotidium hortensia* produces seedlings shortly after shedding which may indicate recalcitrant behaviour but that without a knowledge of the moisture status of seed this is speculative.

This work therefore aimed to determine whether seed of *M. hortensia* is recalcitrant and to determine its germination requirements, specifically the temperature or temperatures at which germination is both rapid and maximal.

Materials and Methods

Seed material

Seed used in these experiments was obtained from *Myosotidium hortensia* "Alba" plants planted at the rear of the Seed Technology Centre, Massey University, Palmerston North. The plants were approximately 16 months old when transplanted into the site in July 1996. The plants were grown in a bark-based (700 kg/m^3) medium. Flowering began in late September 1996 and seed was ready for harvest in January 1997.

Seed moisture content, viability and germination capacity

In all experimental work the following protocols were used to determine SMC, seed viability and germination capacity.

Seed moisture content (SMC) was determined using the forced air oven method (ISTA, 1993). Fifteen intact seeds per replicate were dried at 103°C for 17 h and the seed weight before and after drying used to calculate the SMC content on a fresh weight basis.

Seed viability was determined using the topographical tetrazolium test. Twenty-five seeds per replicate were preconditioned by rolling them in moistened 38 lb regular weight seed germination paper (Anchor Paper Company). The roll was placed in a jar with approximately 2 cm of water in the bottom. The jar and roll were placed in a plastic bag at 20°C for 24 h. After 24 h a scalpel was used to make a 5-10 mm incision in the cotyledonary area of softened seed coat and the coat peeled off. The de-coated embryo was placed in a solution of 1 % phosphate-buffered 2,3,5-triphenyl tetrazolium chloride solution (Enescu, 1991) at 20°C for a further 24 h. Seed was classified as viable if both the radicle and plumule area and greater than 50 % of both cotyledons, but including that part of the cotyledons near the radicle, showed uniform red staining (adapted from Enescu, 1991).

Seed germination capacity was determined by germinating 25 seeds per replicate. Seeds were dusted with thiram (using the ratio of 1 seed volume of thiram for every 50 seeds) and germinated in plastic boxes on steel blue seed germination blotters (Anchor Paper Company) at alternating temperatures of 10°C (± 2°C) for 16 hours and 15°C (± 2°C) for 8 hours in the dark (except for setting up and counting germination). This choice of temperature was based on the range of temperatures experienced at the Waitangi Meteorological Office in the Chatham Islands (1956-1980) in January. At this station the mean daily maximum was 17.6°C and the mean daily minimum 11.4°C (Thompson, 1983).

In all germination trials each replicate was treated as a block and treatments were randomised within that block. Seed germination was monitored weekly. Germination was scored when the radicle or cotyledon had emerged from the seed coat. Median time to 50 % germination (T_{50}) was calculated using the formula of Coolbear *et al.* (1984).

Desiccation experiment

Seed heads were harvested on 21 January 1997. Nutlets were separated from the stem material and classified according to colour as either green or black. Within each colour classification nutlets were mixed through a riffle divider three times and then, using the same divider, split into four replicates. Nutlets in each replicate were placed as a single layer in an aluminum pan (internal diameter 370 mm) and placed at 20°C and a relative humidity of 75-85 %. An indication of moisture loss from the nutlets was obtained by monitoring weight loss from the pan over time. At moisture content intervals of approximately 10-15 %, nutlets were sub-sampled and the seed removed from the nutlet. SMC, viability and germination rate and percentage were determined immediately. Limited amounts of green seed meant only 10 - 15 seeds per replicate were used for SMC, viability and germination assessment.

Germination experiments

Seed storage regime: Two further lots of seed were harvested on 23 January and 31 January 1997. Each lot was classified according to colour, split into four replicates per colour and dried at 20°C and 75-85 %

relative humidity using the same procedures as in the desiccation experiment. When seed had desiccated to its equilibrium moisture content (approximately 7.5 % SMC), both lots were bulked, seed was extracted from the nutlets and placed in sealed storage (12/20/50 micron laminated polyester/aluminium foil/polythene packets) at 5°C until required for the temperature gradient plate and dormancy-breaking experiments.

Temperature gradient plate experiment: A Grant Temperature Gradient Plate (Grant Instruments Limited, Cambridge, United Kingdom) was used to determine the optimum germination temperature(s). The temperature gradient plate was set to run a one-way gradient, using a temperature range of 5°C to 24°C. The plate was located in a controlled environment room operating at 20°C and with continuous lighting. The plate was positioned so that lighting was directly overhead, to reduce the effect of shading. Limited space on the plate meant only one replicate could be evaluated at each run. Seed was washed for 2.5 hours in running water at 17°C, then air-dried at 20°C and a relative humidity of 75-85 % to the original SMC. This seed was dusted with thiram and set to germinate on the plate on steel blue seed germination blotters on top of a single layer of K-22 Kimpak® (Anchor Paper Company). Seed was placed on one half of the plate at 1°C increments (37 mm) up the plate. Unwashed seed, dusted with thiram, was set to germinate as above on the other half of the plate.

After 11 weeks the majority of the seed remained ungerminated. All ungerminated seed was removed from the plate and the seed coat removed from half of it. This and unpeeled seed was set to germinate. Germination was scored as either radicle or cotyledon emergence in unpeeled seed and radicle growth in peeled seed. Radicle growth was defined as occurring when the radicle was longer than 2 mm. No further replicates were set up on the plate. The remaining seed was used for the dormancy breaking experiments.

Dormancy-breaking experiments: A dormancy-breaking experiment was set up in January 1998. Seed was pre-conditioned as detailed for the topographical tetrazolium test and then treated in the following ways: seed coat removed (peeled); seed coat cut completely around the seed just above the radicle; seed coat chipped at the distal end; seed washed for 24 hours in running water at 20-22°C.

Treated seed and control seed was set to germinate as described early. Peeled seed was also set to germinate using the standard methodology except that seed leachate was used as the imbibition medium rather than water.

The leachate was obtained by placing 80 seeds and 25 ml of distilled water in a 125 ml conical flask. The flask was wrapped in aluminium foil and agitated on an orbital shaker at 20°C for 24 h.

This experiment was repeated in March 1998 using seed remaining from the 23 January 1997 harvest and 7 January 1998 harvest. The peeling (germinated on water only), cutting and chipping treatments described above were repeated. An additional treatment of piercing the seed coat in the cotyledonary area with a dissecting needle (diameter 0.6 - 0.8 mm) was included as a less severe manipulation of the coat. Since supply of 1998 harvest seed was limited, the piercing treatment was applied to 1997 harvest seed only.

Data analysis

Data were analysed using the analysis of variance (ANOVA) procedure in SAS for Windows (Release 6.12, SAS Institute, Cary, NC, USA). The T_{50} was log transformed to normalise it. T_{50} means presented in this paper are for untransformed data.

Results

Desiccation experiment

The moisture content at harvest was 35.5 % for black seed and 47.4 % for green seed (Table 1). At 20°C and 75-85 % relative humidity both lots reached a final (equilibrium) moisture content of approximately 7.5 %. As SMC declined seed harvested green became black. Within each colour classification desiccation did not cause a loss in germination percentage at any moisture content. Therefore only data at harvest and equilibrium moisture contents are presented. After desiccation there was a significant difference (P < 0.05) in the normal germination percentage of black seed compared to green.

There was a significant decline in the viability of seed harvested at 47.4 % (P < 0.05), as determined by the tetrazolium test, but not in seed harvested at 35.5 %.

After desiccation seed harvested at 35.5 % moisture content (dried to 7.6 % moisture content) had a significantly higher percentage (P < 0.05) of dead seeds in both viability and germination tests than seed harvested at 47.4 % and dried to a 7.4 % moisture content. There was no significant difference in the rate of germination at any moisture content (Table 1).

Germination experiments

Temperature gradient plate: After 11 weeks on the temperature gradient plate little (< 5 %) or no germination was observed at any temperature, in either washed or unwashed seed (Fig. 1). However, removal of the

Table 1. Moisture content, viability, germination percentage and germination rate of Chatham Island forget-me-not *Myosotidium hortensia* seed harvested at 47.4 % (green) and 35.5 % (black) seed moisture content and desiccated at 20°C and 75-85 % relative humidity to an equilibrium moisture content of 7.4 %.

Colour	Seed moisture content (%)	Viability (Tz)[1] (%)	Dead (Tz)[1] (%)	Normal germination (%)	Dead (%)	T_{50}[2] (days)
Green	47.4	98	0	90	2	145
Green	7.4	89	3	92	2	136
Black	35.5	84	10	79	17	129
Black	7.6	82	12	78	20	135
LSD (P<0.05)	4.23	7.1	5.7	12.1	8.6	19

[1] Tetrazolium test; [2] Time to 50 % germination.

seed coat allowed germination, as measured by radicle growth, to proceed rapidly. In peeled seed 88 % radicle growth was reached within two weeks compared to only 1 % in unpeeled seed. In unpeeled seed, it took five weeks to reach 91 % radicle emergence after removal from the plate (Fig. 1).

Dormancy-breaking experiment: In both trials germination of untreated seed was erratic and slow (Fig. 2). Washing seed for 24 hours did not improve the rate of germination compared to untreated seed, but

weakening or removing the seed coat allowed a dramatic improvement in the germination rate (Table 2.) Germination was most rapid when the seed coat was completely removed or the seed coat was cut above the radicle. When the coat was chipped at the distal end of the seed the rate of germination was significantly slower (P < 0.05) than the other cutting or peeling treatments. Despite the fact that this may be an experimental artifact resulting from early radicle growth being masked by the
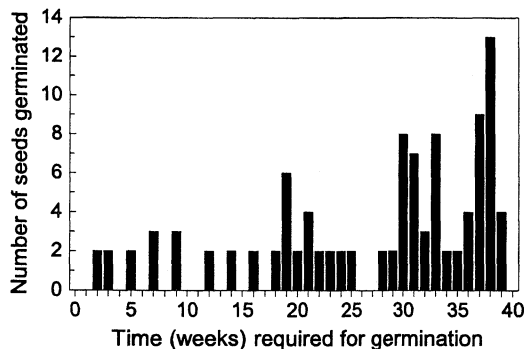

Figure 1. Comparison between the rate of radicle growth in peeled seed and radicle emergence in unpeeled seed. Percentages are for the pooled emergence of seed removed from different temperatures on the temperature gradient plate.
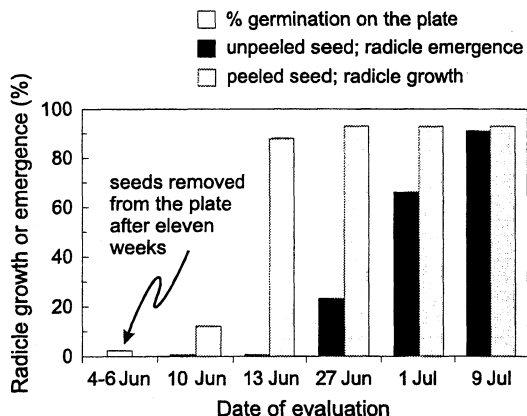

Figure 2. Germination distribution of untreated *Myostidium hortensia* seed over time. Germination counts are the pooled weekly emergence for all replicates. Total number of seeds germinated was 104.

Table 2. Germination rate, germination percentage and radicle emergence of Chatham Island Forget-me-not (*Myosotidium hortensia*) after dormancy-breaking treatment.

Treatment \ Trial	Rate of germination (T-50, days)		Final radicle emergence (%)		Normal germination (%)	
	1997	1997a[1]	1997	1997a[1]	1997	1997a[1]
untreated control	176.4[a]	216.8[a]	96[a]	83[a]	80[a]	81[a]
24 hour washing	167.3[a]	-	92[a]	-	85[a]	-
seed coat chipped at the distal end	16.9[b]	18.1[b]	91[a]	86[a]	59[b]	61[b]
seed coat cut above the radicle	13.4[c]	13.1[c]	88[a]	81[a]	55[b]	52[b]
peeled seed germinated on water	12.4[c]	12.3[c]	89[a]	79[a]	46[b]	48[b]
peeled seed germinated on seed leachate	12.3[c]	-	89[a]	-	22[c]	-
seed coat pierced in the cotyledonary area with a dissecting needle	-	30.8[d]	-	82[a]	-	82[a]

Treatment means with the same letter are not significantly different at P < 0.05
[1] data from the second (March 1998) dormancy experiment.

surrounding seed coat, chipping did allow the cotyledonary tissue to emerge from the coat at the position of the chip. Whether this occurs as a direct result of expansion of the cotyledons or radicle growth or a combination of both is unclear.

Piercing the seed coat also allowed germination to proceed at a significantly faster rate (P < 0.05) than in the untreated seed, but at a significantly slower rate than for the other dormancy breaking treatments. In 84 % of the seed that germinated it was observed that the coat ruptured at the entry point of the desiccating needle. There was no significant difference in the germination rates between the two trials for respective dormancy breaking treatments.

Final radicle emergence percentage did not differ among treatments within each trial but the normal germination percentage for peeled, cut or chipped seed was significantly lower (P < 0.05) than untreated seed. The difference in the normal germination percentage between untreated seed and the peeling, cutting and chipping treatments was largely due to an increase in the number of abnormal seedlings, in particular seedlings with abnormal, glassy, opaque root development (data not shown). Although the imbibition medium (water or seed leachate) used did not affect the germination rate, the normal germination percentage for peeled seed germinated on seed leachate was significantly lower (P < 0.05) than all other treatments. In this treatment, as with peeled seed germinated on water, there was an increase in the number of abnormal seedlings but in addition the radicle and/or the cotyledons of both normal and abnormal seedlings rotted (data not shown).

There was no difference in the normal germination percentage between seed whose coat had been pierced and control seed (Table 2).

Discussion

The data presented in Table 1 demonstrate that seed of *M. hortensia* "Alba" can be desiccated to a low SMC without loss of viability. It is, therefore, unlikely that *M. hortensia* is recalcitrant as speculated by Fountain and Outred (1991). The difference in the viability percentage of seed harvested at 47.4 % is difficult to explain, particularly given that there was no decline in the germination percentage of the same seed associated with desiccation. The explanation may simply be that because evaluation of the staining in the tetrazolium test contains a strong subjective element (Perry, 1987) some viable seed may have been classified as doubtful. Importantly, although the data suggest the seed viability declined, the viability still remained high. Several authors (Maloy, 1992; Matthews, 1993) observe that *M. hortensia* will germinate readily if fresh. A comparison of our T_{50} data in Table 1 provides no evidence to suggest that fresh seed germinated any more readily than seed that had been desiccated to low moisture content. Maloy (1992) and Matthews (1993) may be drawing their conclusions on the observation of early emergence of a small percentage of the seed population but as neither provide germination percentages this is speculative. Our germination data confirm the observations of Metcalf (1995) that germination is both erratic and slow (Fig. 2). Although there are reports in the literature (Baskin *et al.*,

1998; Drennan *et al.*, 1993) of a correlation between seed colour and dormancy status, this is not the case here where both green and black seed exhibited dormancy. There is, however, no evidence from this work to conclude that the dormancy mechanism, or mechanisms, in both are the same. Dormancy, in black seed at least, is likely to be a function of the seed coat (Fig. 1).

The reason for the higher percentage of dead seed in seed harvested at a 35.5 % SMC is unclear, although some of the dead seeds were surrounded by a slime characteristic of bacterial growth. The delay in harvesting this naturally drier seed may have resulted in a concomitant increase in bacterial loading on the seed. This is speculative and will require further investigation, but it may be an important factor in defining the best timing of harvest in this species.

The seed coat may impose dormancy in one or more ways; by restricting water uptake (Tomer and Singh, 1993); preventing loss of inhibitors from the seed; the presence of inhibitors within the coat; restricting gas exchange with the embryo (Qi *et al.*, 1993; Edelstein *et al.*, 1995), or physically constraining embryo growth (Stabell *et al.*, 1998).

The SMC rose during pre-conditioning (data not presented) suggesting that restriction of water uptake by the seed coat is not a dormancy factor in *Myosotidium hortensia*. Washing the seed did not improve the rate of germination, nor did germinating seed on seed leachate impede the rate of germination. These two findings discount the possibility of water-soluble inhibitors in the seed coat delaying germination.

The peeling, cutting and chipping treatments, which were most effective in allowing germination to proceed, both weaken the seed coat, alleviating any physical constraint by the coat on embryo growth, and improve gas exchange to and from the embryo. Piercing the coat will improve gas exchange (Bewley and Black, 1994) but in *Myosotidium hortensia* this treatment was also sufficient to weaken the coat so that it ruptured at the prick point when germination began. It is unclear whether germination was allowed to proceed because of weakening of the coat or because of increased oxygen uptake. If the latter is correct the coat may have ruptured at the prick point simply because it was a weakness in the coat. Equally possible is that the dormancy may be imposed by a combination of both. Stabell *et al.* (1998) suggest that restriction of both oxygen uptake and embryo growth by the seed coat is responsible for the dormancy in the Boraginaceae houndstongue (*Cynoglossum officinale*).

The peeling, cutting and chipping treatments all involved major surgical manipulation of the seed. The damaging effect of this surgery was seen in an increase in the number of seedlings that developed roots with a glassy, opaque appearance. The data presented in this paper are from seed that had been in store for at least twelve months. The development of glassy, opaque roots is often observed in aged seeds, but, control seed here did not show abnormal root development. It is, therefore, unlikely that ageing is the cause of the damage observed. It is possible that ageing may have made the seed more susceptible to damage but this is also unlikely given that freshly harvested (1998) seed treated in the same way also showed damage (data not shown).

In peeled seed germinated on seed leachate the damage was compounded by rotting of the seed and seedlings. This suggests that while seed leachate does not prevent germination (radicle growth) *per se* it may nonetheless contain compounds toxic to the seed and developing seedling.

The rate of germination in control seed is slow compared to those reported in nurseries (A Butler, *pers. comm.*). The may be partly because germination in the nursery is reported when first observed rather than at the median germination time reported here. Nonetheless the germination conditions used in this study eliminate any wetting and drying cycles that may promote germination in the soil or potting mix. Baskin and Baskin (1982) found that seeds of *Cyperus inflexus* germinated faster when exposed to wetting and drying cycles. Interactions between the seed and soil microbial activity are also removed. These may help weaken the coat, increasing the germination rate in the nursery situation. Regardless, the slow and erratic germination behaviour of *Myosotidium hortensia* remains a problem in the production system.

Acknowledgements

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