Factors affecting germination of great bindweed (*Calystegia silvatica*) seeds

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Abstract Great bindweed (*Calystegia silvatica*) invades riparian plantings in New Zealand but little is known about the factors influencing seed germination of this species, the number of seeds produced per flower or whether seed banks build up in the soil below infested sites. Dormancy-breaking treatments involving scarification and/or pre-chilling of seeds were evaluated. The effect of temperature on germination was also studied. The presence of viable seeds in capsules on vines and in the soil beneath established stands was quantified. Great bindweed seeds needed scarification but not a period of cold temperature to germinate. Germination occurred from 5˚C to 25˚C but germination was greater and more rapid at higher temperatures. Seed capsules contained an average of only 2.3 seeds, and the soil beneath plants had, on average, only 21.9 seeds/m^2. Seeds were large with one thousand seeds weighing 43.4 g. Once the hard seed coat is broken, seeds will germinate readily at warmer times of the year, but seed production is not prolific so seeds might not be that important for spread of the species.

Keywords dormancy, germination, great bindweed, riparian zones, temperature

INTRODUCTION

Riparian zones are the areas between waterways and farmland, and they are now often planted with native shrubs, flaxes and sedges in New Zealand to help reduce the impact of farming on waterways (Maseyk et al. 2018). Great bindweed (*Calystegia silvatica*) is a deciduous, perennial weed with rhizomes and trailing aerial stems that often establishes in these riparian zones, growing over the planted species and frequently covering them completely (Gawn 2013). This aggressive growth of great bindweed can harm the establishment and survival of new plantings (Wilson-Davey et al. 2009). Although great bindweed dies back each winter, it reinvades infested areas in spring because of the survival capacity of its rhizomes (Gawn et al. 2013).

The spread and distribution of great bindweed occurs mostly via rhizomes so seeds are not considered essential for reproduction. Popay et al. (2010) reported that few seeds are produced. However, seeds could, neverthless, play a role in colonising new sites along waterways in a similar way to rhizomes, especially after floods (Williams 2009). Related species such as field bindweed *(Convolvulus arvensis*) and pink bindweed (*Calystegia sepium*) are known to produce greater amounts of seed (Williams 2009; Popay et al. 2010). These species have seeds with a hard coat that needs to be broken before they can germinate (Parsons & Cuthbertson 2001). Seeds of the native New Zealand bindweed (*Calystegia tuguriorum*) also have an impermeable coat that needs to be broken for germination to occur (Burrows 1996).

Field bindweed seeds are known to germinate at a wide range of temperatures from 5˚C to 40˚C, with the highest reported germination rate

occurring when temperatures alternated between 20˚C and 35˚C (Brown et al. 2009). However, limited information is available about great bindweed seeds and the conditions under which they can germinate. This study sought to address this knowledge gap by assessing the effect of scarification and temperature on dormancy and germination of great bindweed seeds, and also to provide information on the number of seeds produced per flower and the size of soil seed banks for this species.

MATERIALS AND METHODS

Experiment 1 (Seeds per flower)

To get some indication of whether great bindweed flowers do produce seeds or not, seed capsules were collected in June 2012 from great bindweed plants growing over trees and shrubs at a waste area next to the Turitea Stream on Poultry Farm Road, 2 km south of Palmerston North. Gawn (2013) determined this population to be *Calystegia silvatica* ssp. *disjuncta*. A total of 550 full, unopened seed capsules were collected from these plants. Seeds per capsule were counted, and categorised following visual inspection as either healthy, shrivelled, under developed, insect damaged or affected by fungi. The healthy seeds were weighed to determine the thousand-seed weight.

Healthy seeds were used to determine ways to overcome dormancy (Experiment 2) and to test the effect of temperature on germination (Experiment 3).

Experiment 2 (Breaking dormancy)

Three dormancy-breaking treatments (scarified only; scarified and pre-chilled; and pre-chilled only) were compared in a randomised complete block design with four replicates, each replicate with 25 seeds. Two sets of seeds were scarified by piercing the edge of each seed, away from the embryo, using a scalpel. Half the scarified seeds and half the unscarified seeds were then subjected to the pre-chilling treatment, which involved keeping moistened seeds for 7 days at 5°C rolled within Anchor germination paper. Seeds for all three treatments were then kept within rolls of Anchor germination paper moistened to run-off with distilled water at 25°C in darkness for 27 days. Due to limited seed supplies, an untreated control was not included but rather the three treatments were compared with each other for maximum effectiveness.

Germination was assessed after 7, 15, 22, and 27 days, and the number of normal germinated seeds, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds were recorded. Seeds were categorised as having germinated normally when the emerged seedling (hypocotyl + primary root) was greater than 0.5 cm in length and healthy, which was a similar classification to the minimum standard used by Steinbauer and Grigsby (1959). Abnormal seeds were those that had emerged but were deformed or unhealthy. Dead seeds were those in which water entered the seed (causing seeds to enlarge) but nothing germinated and the seed case was empty and/or soft. Fresh ungerminated seeds were those in which water had entered the seed and the seed remained healthy, but no germination occurred. Hard seeds were those that were impermeable to water and no germination occurred.

Experiment 3 (Temperature effects on germination)

The third experiment was conducted to determine the optimal temperature range for the germination of great bindweed seeds. The seeds were all scarified then germinated within rolls of Anchor seed-germination papers as described above. There were three replicates of 25 seeds for each temperature (5°C, 10°C, 15°C, 20°C and 25°C) in a completely randomised design. The seeds were then assessed 7, 14, 21 and 28 days after incubation in the dark and the numbers of normal germinated seedlings, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds were counted.

Experiment 4 (Seeds in soil)

Soil samples were taken from two sites near Palmerston North where great bindweed had grown for several years to determine the concentration of seeds under stands of great

bindweed. One site was described above, i.e. a waste area near the Turitea Stream. The other site was a waste area behind the Plant Growth Unit of Massey University. In September 2012, a total of 600 soil cores (5.3 cm diameter to a depth of 3.2 cm) were taken – 500 from the Turitea Stream site and 100 from the site behind the Plant Growth Unit. Seeds were separated from the soil by wet sieving and counted. All seeds were then scarified and germinated at 25°C within Anchor germination paper.

Statistical analysis

To comply with assumptions of ANOVA (i.e. normality of distribution and homogeneity of variances), all seed categories were arcsine (y+1) transformed before ANOVA and means were separated by Fisher's protected tests at 5% probability. Each seed category was analysed separately using a one-way ANOVA. The original untransformed data were used to construct tables and figures.

RESULTS

Experiment 1 (Seeds per flower)

There was an average of 2.3 seeds per unopened capsule sampled. Of these, 64.4% were considered to be healthy, 6.2% were underdeveloped, 15.7% were shrivelled, 6.1% had been damaged by insects and 7.6% had fungal growth on them. The thousand-seed weight for healthy seeds was 43.4 g.

Experiment 2 (Breaking dormancy)

Most of the scarified seeds germinated within 7 days (regardless of pre-chilling), and the remainder of the scarified seeds that germinated had done so by 15 days after the start of incubation. In contrast, only low numbers of unscarified seeds germinated and these continued to germinate throughout the 27 days (Fig. 1). Significantly more seeds germinated in the two scarification treatments than in the pre-chilling-only treatment (Table 1). Only 14% of the seeds that were unscarified and pre-chilled had germinated within the 27 days of

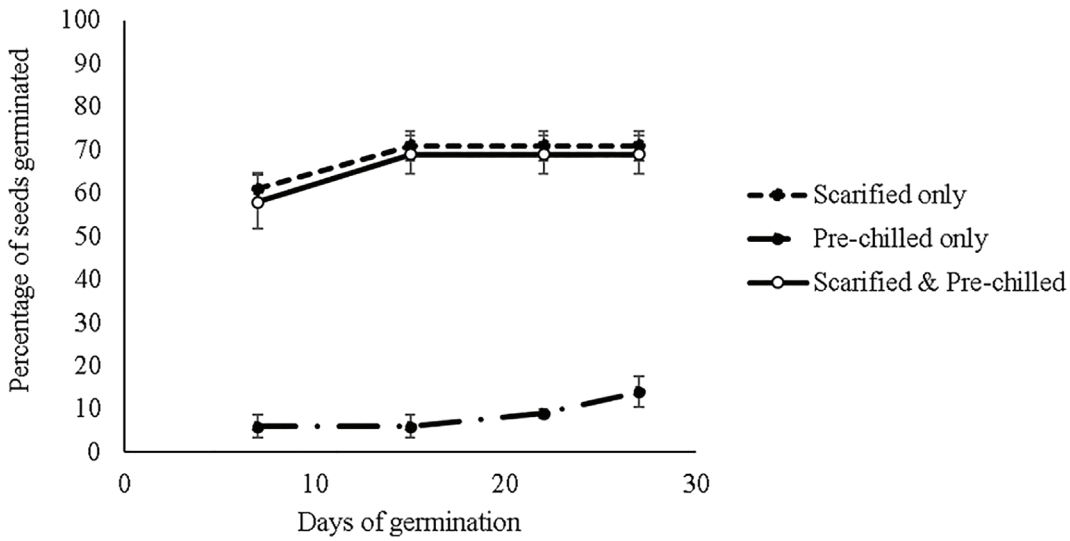

Figure 1 Average cumulative percentage of normal great bindweed seedlings which germinated at 25°C in Experiment 2 for treatments involving only scarification, only prechilling (i.e. non-scarified) and scarified & prechilled. Error bars indicate standard error of the means.

Table 1 Percentage of normal and abnormal seedlings and dead, fresh ungerminated or hard great bindweed seeds for scarified only, scarified plus pre-chilled or pre-chilled only treatments in Experiment 2.

Treatment	Normal	Abnormal	Dead	Fresh ungerminated	Hard
Scarified only	69 a	7	24 a	0	0 b
Scarified & Pre-chilled	71 a	1	28 a	0	0 b
Pre-chilled only	14 b	0	10 b	4	72 a
P value	0.0001	0.265	0.021	0.081	0.0001

Different letters indicate significant differences between mean values within columns according to Fisher's protected tests (α=0.05).

the experiment, and most of the ungerminated seeds were considered to be hard seeds, so would probably germinate once the hard seed coat was broken. The number of dead seeds was higher for scarified seeds (with or without pre-chilling) than unscarified seeds (i.e. pre-chilled only). However, it was difficult to differentiate between hard seeds that were alive and hard seeds that were dead, so this difference in dead seeds may not have been significant had this been easier to ascertain.

Experiment 3 (Temperature effects on germination)

At 15, 20 and 25°C, most seeds had germinated by 7 days after the start of incubation. In contrast, most of the seeds kept at 10˚C did not germinate until ≥14 days (Fig. 2). By 14 days, similar numbers of seeds had germinated for these four temperatures and no more germinated later. However, no seeds kept at 5°C germinated within the first 14 days and germination by 28 days was low (Table 2; Fig. 2).

Experiment 4 (Seeds in soil)

At the site beside the Turitea Stream, there was estimated to be 22.7 seeds/m^2, whereas at the waste area behind the Plant Growth Unit, the soil seed bank for great bindweed was estimated to be 18.1 seeds/m^2. All seeds recovered from the cores germinated within 7 days of incubation after being scarified.

DISCUSSION

The results from Experiment 2 showed that the majority of great bindweed seeds do need to have their seed coat broken before germination can occur. These results corroborate findings from a British PhD study from 50 years ago on what was probably the same species (Brummitt 1963). A number of species closely related to great bindweed (i.e. pink bindweed, New Zealand bindweed and field bindweed) each require seed scarification in order to germinate (Burrows 1996; Parsons & Cuthbertson 2001). In the field, this scarification could be caused by insects, microbial action or abrasion from movement down riverbeds (Burrows 1996). Some unscarified but pre-chilled great bindweed seeds did germinate normally but the remainder did not germinate and were recorded as either hard seeds or fresh ungerminated. This may have occurred because those seeds had been scarified by other means in the field prior to testing, such as exposure to temperature fluctuations within the capsules.

There were no differences for the scarified treatments between those that were pre-chilled and those that were not pre-chilled (Experiment 2), which indicates that a period of cold is not needed as part of the dormancy breaking process prior to great bindweed seed germination. Thus, great bindweed seeds do not appear to require overwintering before germination can

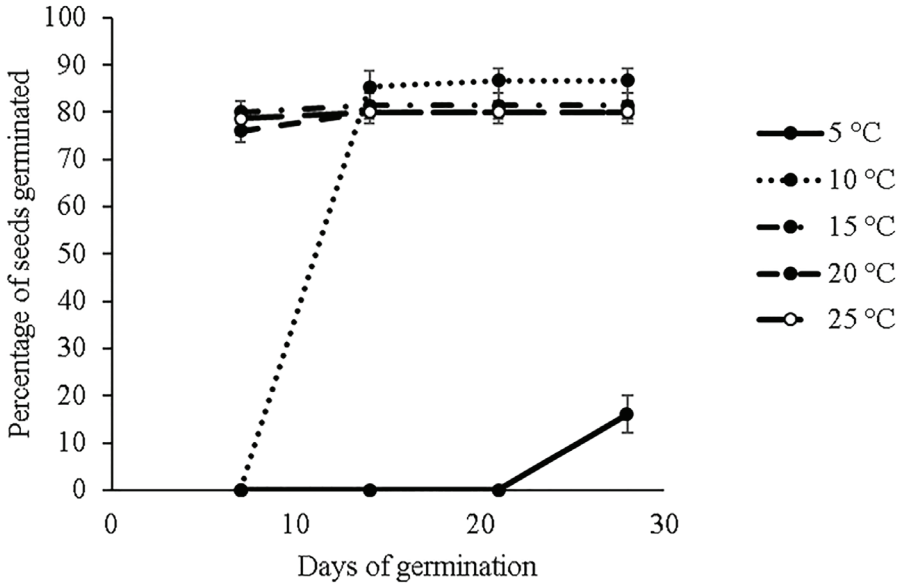

Figure 2 Average cumulative percentage of normal great bindweed seedlings which germinated at each temperature in Experiment 3.

Table 2 Percentage of normal seedlings, and dead or hard great bindweed seeds across five different temperature regimes after 28 days in Experiment 3. The experiment was conducted with scarified seeds.

Temperature (°C)	Normal	Dead	Hard ungerminated
5	16 b	11 c	73 a
10	87 a	13 bc	0 b
15	83 a	17 ab	0 b
20	80 a	20 a	0 b
25	80 a	20 a	0 b
P values	0.0001	0.033	0.0001

Different letters indicate significant differences between mean values within columns according to Fisher's protected tests (α=0.05).

occur. However, temperature is important for germination. Seedling germination occurred most rapidly at temperatures between 15 and 25°C. Germination did occur down to temperatures of 5°C, a temperature at which seed germination of field bindweed is also known to occur (Brown et al. 2009). However, germination was slower for temperatures lower than 15°C. Germination was slow at 5°C with germination occurring more than 21 days after the start of incubation. Also, there were significantly fewer germinated seeds than for warmer temperatures when the experiment ended at 28 days. More seed may have germinated after a longer period at 5°C but is possible that these would have been out-competed by neighbouring plants in the field such as grasses at this temperature.

Popay et al. (2010) claimed that most flowers of great bindweed do not form seeds. However, at the Palmerston North site that was selected for

this study, the results of the current study show that many of the flowers did appear to produce seeds, but only a very small number per flower. The seeds were found in air-filled capsules in June when counts were made, which could easily have been washed down-stream if the site was flooded, allowing them to reach new riparian zones. Likewise, soil washed down-stream could contain great bindweed seeds. However, in both cases, the number of seeds involved would probably be much lower than for seeds of most other weed species as, in general, seeds are produced more prolifically by other species.

At a thousand-seed weight of 43.4 g, the seeds of great bindweed are larger than most other weed seeds (Stevens 1957). For example, gorse has a thousand seed-weight of 6.2 g and variegated thistle is 19.4 g (Harrington et al. 2011), and these are both weed species that are considered to have quite large seeds.

Great bindweed seeds could be attractive to seed-feeding organisms such as birds and mice. However, the movement of seeds within flood waters could allow scarification to occur so great bindweed would be expected to establish well if deposited down-stream following a flood. Thus, seeds could possibly help with dispersal to new sites following floods, though if rhizome fragments were also washed down-stream, these would presumably establish new infestations more readily, as suggested by Williams (2009).

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