# Germination experiments with seeds from the native New Zealand woody plant flora

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## Abstract

This paper describes germination experiments intended to simulate conditions which native woody plant seeds would experience in nature. The results indicate that, when freshly collected seeds are tested: (a) The pattern of germination for each species is distinctive. (b) Germination percentages are very high for seeds in a well-lit, well-watered standard treatment maintained in Petri dishes. (c) Most of the seed species tested germinate in the dark. (d) Treatments maintained on soil give a lower percentage germination than those in the standard treatment. (e) Presence of the pericarp tissue inhibits germination for almost all species with fleshy fruit and for some species with dry fruit. (f) For most seed species, germination is completed within a year. A few species have the commencement of germination delayed for up to three years. (g) Few species have clear indications of requiring chilling treatment before the seeds will germinate, although seeds of many species germinate through the winter. (h) The triphenyl tetrazolium chloride test gives a lower estimate of potential germinability than the actual percentage of germination success in the standard test.

***Additional key words:*** *delayed germination, seed banks, seed ecology, triphenyl tetrazolium chloride, viability test.*

## Introduction

When, in the mid 1980's, I began to investigate the seed biology of plants of the native New Zealand flora (and specifically the germination requirements of seeds) the first thing which became clear was that published information on these subjects was sparse. Some unpublished data undoubtedly existed in the files of Botany Division, DSIR, Lincoln; Forest Research Institute, Rotorua; Seed Testing Laboratory, MAF-Tech, Palmerston North, as well as in some University theses and elsewhere. Various nurserymen and keen gardeners also know how to induce many kinds of seeds to germinate. However, detailed descriptions of controlled experiments are lacking for many species. Fountain and Outred (1991) have recently reviewed the published information about germination requirements of New Zealand seeds. There are still many gaps in knowledge for the flora overall.

The second problem for an ecologist beginning to investigate seed germination and dormancy was the incredibly confused and confusing state of the conceptual and terminological framework for these phenomena in the international seed physiological literature (cf., Baskin and Baskin, 1985; Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1982). There is no standardized set of concepts or terminology. This may reflect the complexity of the phenomena themselves but it also indicates an untidy and unresolved state of affairs among the physiologists who work in this field. For my own benefit and that of the undergraduates in the ecological classes I teach, I set out to clarify the concepts and terminology in a short paper (Burrows, 1989). The gist of this paper can be summed up by reference to a diagram from it (Fig. 1) which depicts the relevant points and explains the terminology that I will use here. The paper itself needs revision, in the light of some recent advances (some of which were discussed by Professor Black at this symposium).

Plant physiologists are usually interested in seeds in some rather different ways from ecologists, so I shall explain the seed biology phenomena which an ecologist would like to know about, with respect to each species being studied. These are:

1) The quantities of viable seeds being produced by the plant populations concerned, including seasonal patterns and year to year variations.
2) The modes and distances of dispersal of seeds.
3) The quantities of seeds that are being eaten and killed seasonally, and from year to year (as well as the identity of predators and factors of their ecology).

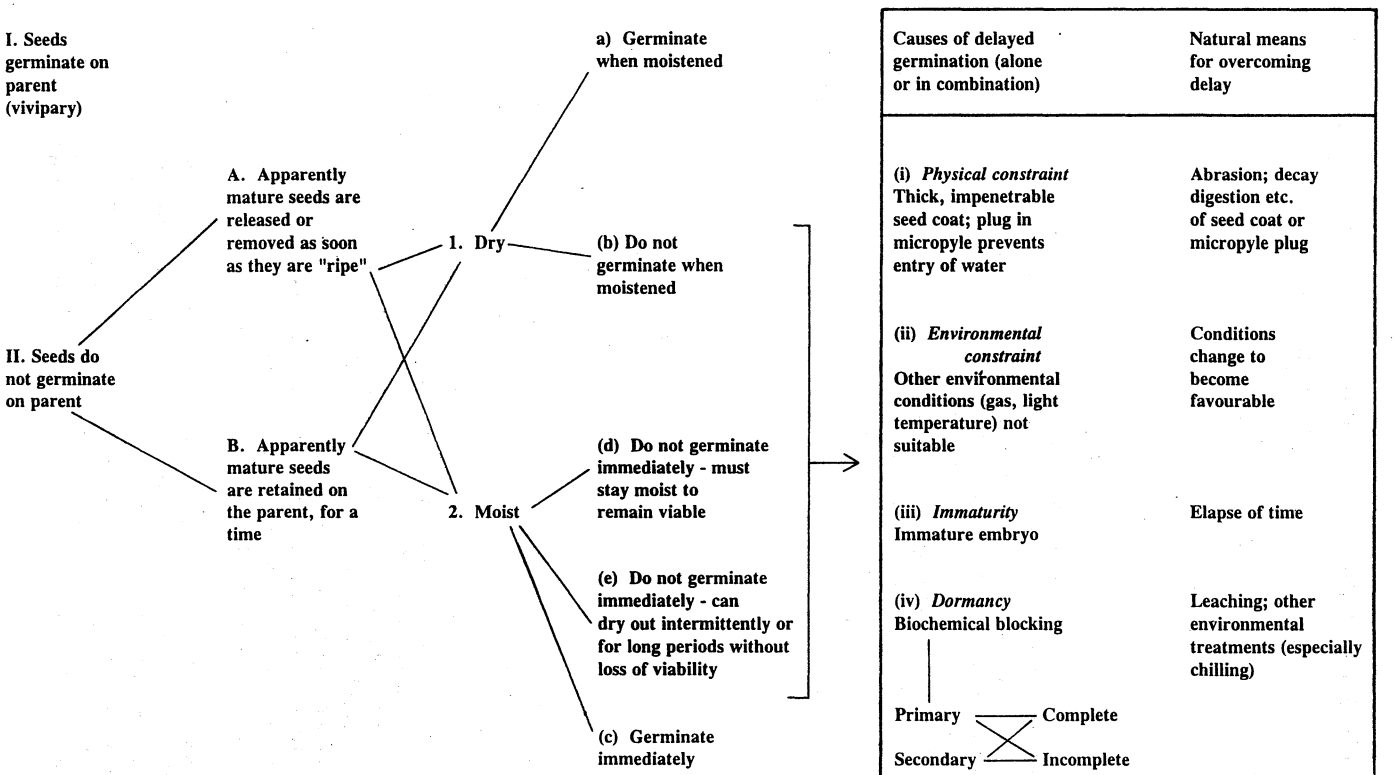


**Figure 1. Diagram indicating the kinds of behaviour exhibited by the seeds of wild plants. Germination of seeds is almost invariably delayed in some way, as the seeds of very few species are viviparous.**

4) The patterns and causes of germination delay and the nature of the resultant seedbanks.
5) The patterns of seed germination in relation to the seasonal or other environmental variations.
6) Factors affecting establishment of seedlings and the numbers of individuals in seedling populations.

Each of these items requires prolonged study.

I have been investigating the seed biology of plants in forest patches on Banks Peninsula for about eight years, with a view to elucidating as many of these matters for as many species as possible. I have been carrying out germination experiments since 1988. Here I will confine myself to discussing some results from simple experiments in conditions that simulate those that seeds would meet in nature. As any artificial storage is likely to affect the behaviour of the seeds subsequently, these experiments are always performed with freshly collected seeds. Some of the seed provenances tested have, in fact, come from Westland, Marlborough Sounds, or Riccarton Bush in central Christchurch. The prime aim of the experiments is to discover the conditions which the seeds require for germination in their native habitats. So far about 50 species have been tested, but only a few examples are considered here.

## Field and glasshouse procedures

Fresh fruit are collected from heavily-fruiting plants and treatments started within two to four days of collection. About 75% of the native woody species are fleshy-fruited and their seeds are dispersed after having been eaten by birds. Seeds of the remaining, dry-fruited, species are dispersed by wind, by gravity, or in streams. All but one set of seeds are removed from the pericarps and soaked for 24 h in tap water. This simulates passage through a bird (fleshy-fruit) or washing by rain (dry fruit). The modular sample for the treatments is 25 seeds. To maintain some control over external conditions the experiments are carried out in an unheated glasshouse on the University of Canterbury campus. The house is shaded by a row of trees partly to simulate forest conditions, partly to prevent overheating, which in hot, nor'west conditions, quickly dries out the experiments. Some direct sunlight is received in the house, especially in the early morning and late afternoon.

The temperature inside and outside the house is monitored. In general the weekly maxima and minima inside the house are 2-4°C higher than those outside. The conditions inside the house are probably close to those in the frost free, or nearly frost free, locations on Banks Peninsula and elsewhere, from which the seeds were obtained. The house is automatically spray-watered, weekly.

Experimental treatments are as indicated in Table 1. The triphenyl tetrazolium chloride (TTC) test, carried out on a seed sample immediately after the 24 h soaking, is an indicator of the potential germinability of the seed batch. The other tests, maintained in the glasshouse, are kept continually wet, on filter paper in petri dishes, with the exception of one set of two replicates which are placed, shallowly buried on a soil-pebble-vermiculite mix to simulate reasonably exactly the conditions on the ground in forest. The standard treatment, necessary for ensuring that seeds are always clearly visible, also simulates conditions on the ground and the dark treatment simulates conditions for seeds which are deeply buried.

Preliminary trials in 1988 showed that the germination of few seed species was influenced by the nicking of seed coats, even for hard-coated species such as *Pennantia corymbosa, Myoporum laetum* or *Rhopalostylis sapida*, so this treatment was not routinely used. However these same pilot studies showed that if seeds were left in the pericarps in most cases germination is inhibited. This applies to almost all fleshy-fruited species and some dry-fruited species. A treatment leaving seeds in the pericarp was carried out for each species. It simulates conditions if fruit are not eaten, or seeds are not dispersed from dry fruit.

The sets of treatments were maintained in the glasshouse until germination was completed. For most species this was within a year after the commencement of the trial. A few species for which experiments began in 1989, or 1990, have not yet finished germinating. As the experiments are begun for each species as soon as fruit is ripe, different temperature regimes are experienced by different species, or by different provenances of the same species. The detailed temperature records are not indicated here. Temperature extremes range from about +6 to +30°C in summer and from about -4 to +20°C in winter.

The experiments were checked at least weekly and often more frequently. The dark treatments were examined in a dark room illuminated only by a photographer's safe light. For most species, the criteria of germination is splitting of the outer seed coat, indicating that germination is well under way. For some species there is no coat splitting; the criterion here is usually the first sign of radicle emergence. In reality each species behaves differently in these respects. Some seed species (e.g., *Plagianthus regius*) change colour when about to germinate and in other species (e.g.,

**Table 1. Layout of germination experiments on seeds of New Zealand woody plants: modular experiment and treatments.**

| | | | | |
|---|---|---|---|---|
| **Module** | | | | |
| petri dish | 25 seeds | 1 circle filter paper | kept wet | kept in unheated glasshouse with automatic watering system |
| soil in plastic meat dish, perforated bottom | 25 seeds | potting mix + gravel, vermiculite 5:1:1 | well watered | |
| **Treatments** | | | | |
| Fruit straight off plants | | | | Replicates |
| Standard | flesh or capsules removed, soaked 24h in tap water, then placed in continually moist Petri dish | | | 4 |
| Dark | | ditto | wrapped in aluminium foil, examined under safelight in darkroom | 2 |
| Soil | | ditto | placed in rows in shallow grooves on soil surface | 2 |
| Tetrazolium for viability | | ditto | cut, soak overnight in tetrazolium chloride | 1 |
| Left in fruit | placed in petri dish as above | | | 2 |

*Dodonaea viscosa, Calystega tuguriorum*) the whole seed swells at this time.

## Results

Some examples of the patterns of germination which have been observed are shown in Figure 2. Summary germination data are shown in Tables 2 and 3. In each of these cases, except *Paratrophis*, at least two provenances of the species were tested and for each provenance the pattern was the same. It may be concluded from this that each species has a unique germination "signature". Other general points evident from the results are:

a) Percentage germination for the standard treatment is uniformly high (95-100%).

b) Most of these species germinate in the dark, but often more slowly than in light and with a lower percentage success. Only a few species, so far, have failed to germinate in the dark. Among them are *Alectryon excelsus, Tetrapathaea tetrandra* and *Carpodetus serratus*. The seeds of these species will germinate if, after a prolonged period in the dark, they are exposed to the light. Burial may be a means by which germination is delayed in field conditions.

c) The soil treatments almost invariably give lower percentage apparent germination success than the standard treatments. This may be because biological hazards such as fungi or collembola are relatively abundant in soil. The term 'apparent' germination signifies that actual germination percentages could be higher, but that some newborn seedlings are probably killed.

d) Almost all treatments where seeds are left in fruit result in very low germination (an exception is *Coriaria arborea*). This applies to fleshy-fruited species, but also to some dry-fruited species, e.g., *Pittosporum tenuifolium*. It is notable that the pericarps have an inhibitory effect both on seed germination and on their own decay. Some fleshy fruit retain their colour and integrity for months in the experimental conditions.

e) All seeds of most species tested so far germinate within a year. A small proportion of the seeds of some species (e.g., *Dodonaea viscosa, Myoporum laetum*) require more than a year to germinate. Some *Myoporum* seeds took three years. The first of a batch of *Melicope simplex* seeds germinated three years after the experiment was started and some of its seeds have not germinated after four years. Some other species have a trickle of seeds germinating each

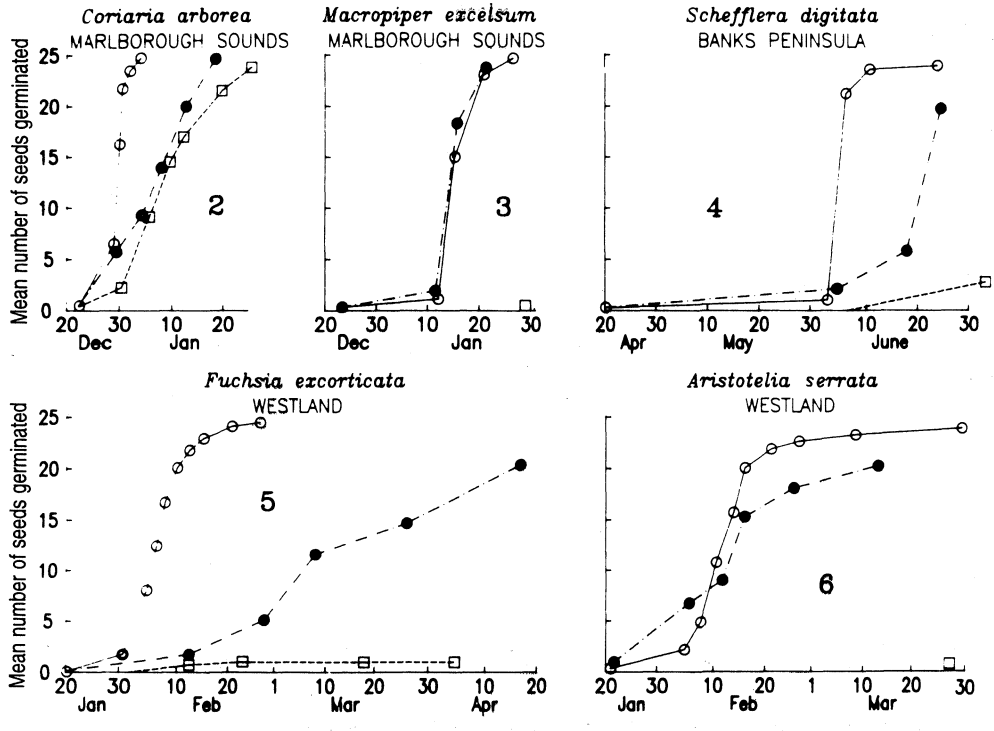


**Figure 2-6. Germination of five species (Fig. 2, 3, 5 and 6 in 1989; Fig. 4 in 1990) showing differences in rates (standard treatments) and differences for treatments where seeds were maintained in the dark and in fleshy pericarps. Treatments were: (O) standard, (●) dark, (□) in fruit.**

spring for at least four years (e.g., *Calystegia tuguriorum, Myrsine divaricata*).

f) As some seed species (*Macropiper excelsum, Coriaria arborea, Fuchsia excorticata*) germinate soon after the experiments are started in summer, they may require warm conditions to facilitate germination. Other species depicted in Figure 4, 7 & 8 (*Schefflera digitata, Pennantia corymbosa, Paratrophis microphylla*) germinate through the winter (with minimum temperatures as low as -4°C and maximum temperatures of 15-20°C). One of the species depicted, *Aristotelia serrata* (Fig. 6), had most of its seeds germinate in summer-autumn, but a small proportion of seeds overwintered and germinated in the following spring. A few other species (e.g., *Urtica ferox*) follow this pattern. *Pseudowintera colorata* seeds, placed in experimental conditions in autumn, germinate in the following spring. They may require cold treatment to spur them to germinate.

g) The TTC test almost invariably gives a lower estimation of potential seed germinability than the actual success rate in the standard germination test. Its use to gauge germinability accurately is, thus, suspect.

## Discussion and Conclusions

Without further, more refined, experiments in controlled temperature and light conditions both the identity of the cues for triggering germination and the causes for delayed germination in the seeds that have been tested are not very clear for many of the species. It seems likely, from the results so far, that selection of most seed species of the native woody plant flora has been affected neither by dark/light differences, nor by winter cold. The seeds of most species do not appear to require winter chilling to facilitate germination. The overall conclusion from this is that the climatic filter of

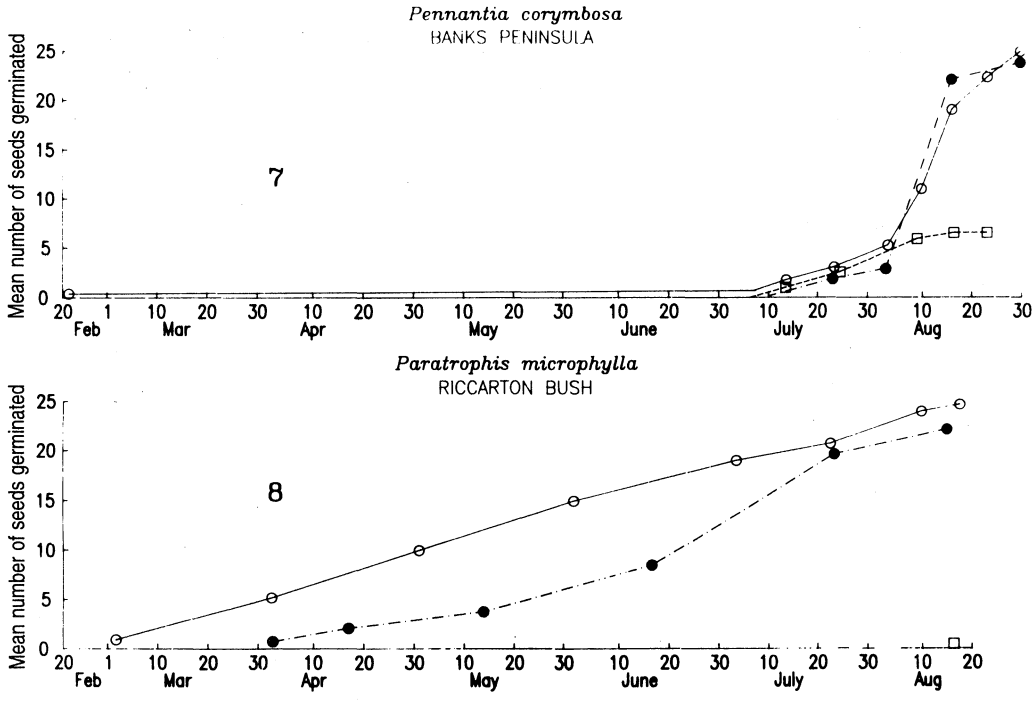


**Figure 7-8. Germination of two species in 1990. Other details as for Figs. 2-6.**

**Table 2. Final germination percentages for seeds of a range of New Zealand woody species sown under different conditions compared to their viability scores using the tetrazolium chloride test.**

| | Germination conditions | | | | % viability as indicated by TTC |
|---|---|---|---|---|---|
| | Light | Dark | In fruit | In soil | |
| *Coriaria arborea* | 100 ± 0[1] | 98 ± 2.4 | 92 ± 12.0 | 96 ± 5.6 | 76 |
| *Macropiper excelsium* | 100 ± 0 | 98 ± 2.4 | 0 | 84 ± 11.2 | 68 |
| *Schefflera digitata* | 98 ± 2.4 | 82 ± 14.0 | 30 ± 2.8 | 50 ± 8.4 | 84 |
| *Fuschia excorticata* | 98 ± 2.4 | 86 ± 2.8 | 6 ± 2.8 | 90 ± 2.8 | 80 |
| *Aristotelia serrata*[2] | 95 ± 2.0 | 86 ± 2.8 | 12 ± 5.6 | 54 ± 42.4 | 76 |
| *Pennantia corymbosa* | 100 ± 0 | 92 ± 5.6 | 26 ± 2.8 | 66 ± 14.0 | 88 |
| *Paratrophis microphylla* | 100 ± 0 | 90 ± 2.8 | 0 | 26 ± 2.8 | 88 |

[1] Standard errors of individual means
[2] Remaining seed germinated in light or dark in October-November (7-8 months later)

the Quaternary ice ages was not nearly as severe here as it was in the northern temperate zone, where most woody species have winter chilling requirements for germination (cf., Anon. 1948).

Some species (*Calystegia tuguriorum, Dodonaea viscosa*) have thick coated seeds and micropyle plugs. Presumably the plugs must decay before their seeds germinate. In a few other species slow germination

**Table 3. The timing of germination of seed of a range of NZ woody species.**

| Species | Days to start of germination | Timespread of germination (d) |
|---|---|---|
| *Coriaria arborea* | 7 | 6 |
| *Macropiper excelsum* | 20 | 13 |
| *Schefflera digitata* | 44 | 23 |
| *Fuschia excorticata* | 11 | 28 |
| *Aristotelia serrata* | 16 | 55 |
| *Pennantia corymbosa* | 139 | 50 |
| *Paratrophis microphylla* | 19 | 203 |

appears to be at least in part the result of immature embryos in otherwise apparently mature seeds (e.g., *Pittosporum tenuifolium, Pseudowintera colorata*). Biochemical blocking is probably also involved with these seeds and with others (such as *Pennantia* and *Paratrophis*, Figs. 7 and 8), where germination is slow. Much further experimentation is required to isolate the various causes of germination delay. At the other extreme are species such as *Griselinia littoralis* the seeds of which germinate rapidly but die if they dry out.

Seed banks for most seed species tested last from only a few weeks to a few months. Some of them are maintained on the parent plants in fruit. There is only one sure way of determining the longevity of seeds in soil seed banks and that is to put out trays of seed-free soil with which to trap falling seeds and subsequently examine the seed germination patterns.

The causes of germination inhibition by pericarps require careful experimentation. As a result of some bioassay experiments, I believe that there are chemical factors in the fruit tissue which are inhibitory. These studies will be described elsewhere.

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