Fruit processing, seed viability and dormancy mechanisms of *Persoonia sericea* A. Cunn. ex R. Br. and *P. virgata* R.Br. (Proteaceae)

Lynda M. Bauer, Margaret E. Johnston and Richard R. Williams

School of Agronomy and Horticulture,

The University of Queensland Gatton,

Queensland, Australia 4343.

Corresponding author

Dr Margaret Johnston m.johnston@uq.edu.au

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Summary

The morphology of the fruit and difficulties with fruit processing impose major limitations to germination of *Persoonia sericea* and *P. virgata.* The mesocarp must be removed without harming the embryo. Fermentation of fruit or manual removal of the mesocarp was effective but digestion in 32% hydrochloric acid (HCl) completely inhibited germination. The endocarp is extremely hard and therefore very difficult and time consuming to remove without damaging the seeds. The most efficient method was cracking the endocarp with pliers, followed by manual removal of seeds. Germination was completely inhibited unless at least half of the endocarp was removed. Microbial contamination of the fruit and seeds was controlled by disinfestation and germination of the seed under aseptic conditions. The results suggest that dormancy in these species is primarily due to physical restriction of the embryo by the hard endocarp.

Introduction

Persoonia virgata R. Br. is an Australian native shrub with attractive, yellow, bell-shaped flowers. The stems, whether flowering or vegetative, are used as 'fillers' in floral bouquets. Even though markets exist for the foliage of *P. virgata*, it has not been introduced into commercial cultivation due to extreme propagation difficulties. Export and domestic markets therefore rely solely on a product harvested from the wild. *Persoonia sericea* A. Cunn. ex R. Br**.** is an ornamental shrub up to 1.5 m tall. It also has not been introduced in to the commercial trade, because of propagation difficulties.

P. virgata is an obligate seed regenerator (McFarland 1990). There are environmental concerns that continual harvesting of this species from wild populations over a number of years could deplete the seed bank and threaten the species. It has been demonstrated that any factor limiting seed production of *P. pinifolia* in consecutive fruiting seasons can significantly reduce seedling recruitment following recurring fires (Auld *et al.* 2000). These environmental concerns plus the horticultural potential of the genus, stimulated our study into the propagation of these species.

The fruits of *Persoonia* species are drupes, with a succulent exocarp and a thick, woody endocarp (Johnson and Briggs 1975). The skin (or epicarp) of the fruits is formed by the exocarp, which becomes leathery at maturity (Strohschen 1986). The next fleshy to "pulpy-sticky" layer is the mesocarp and "the inner hard stone is formed by the multi-layered endocarp which is derived from the inner epidermis of the ovary wall" (Strohschen 1986).

Regeneration of *Persoonia* species in the wild usually occurs after a disturbance such as bushfires (McIntyre 1969; Abbott and Van Heurck 1988). Germination after bushfires may occur because of the fracturing of the hard seed coverings due to the high, dry temperatures (Gill 1975), not withstanding the more recent discovery of the effect of smoke on breaking seed dormancy of some species (Brown 1993; Dixon *et al.* 1995). However there are no report of smoke promoting germination of *Persoonia* species.

McIntyre (1969) and Mullins *et al.* (2002) proposed that a combination of hard woody endocarp and a dormant embryo prevented the germination of *P. pinifolia and P.*

longifolia seed, as germination was only achieved with endocarp removal and gibberellic acid (GA_3) treatment. Both studies noted that germination could be affected by microbial contamination of the seeds, which interfered with the statistical analyses of results.

Our study commenced with a morphological examination of *P. virgata* fruit and seed. This was followed by a series of experiments aimed at determining the seed dormancy mechanism controlling germination.

Materials and Methods

Fruit and seed morphology

P. virgata fruits, seeds and early seedlings were dissected and examined under a stereomicroscope to determine the stages of seed development and the morphology of the mature seed.

General methods

Seed viability was determined using tetrazolium staining. At least half of the endocarp had to be manually removed and the surrounding testa disrupted to allow sufficient penetration of the solutions. The seeds were imbibed for 24 hours in distilled water (DW) at ambient temperatures. Imbibed seeds were placed into 1.0% 2,3,5-triphenyltetrazolium chloride (TZ) solution and held at 40°C in the dark for 24 hours. After rinsing with DW, seeds were cut longitudinally to expose the embryo. Deep-red to deep-pink embryos were recorded as viable, while white to pale-pink embryos were recorded as non-viable (Copeland and McDonald 1995).

To avoid contamination problems reported in previous germination studies of *Persoonia spp.* seeds (McIntyre 1969; Rintoul and McIntyre 1975; Mullins *at al.* 2002), seeds or endocarps plus seeds (E+S) were disinfested in sodium hypochlorite (2000 ppm chlorine plus Tween 20) for 2 h then rinsed with sterile DW before being placed into jars containing sterile agar medium. The medium contained the basal salts of de Fossard (1981) at half strength, without sucrose or vitamins. Groups of seeds were placed in 65mL glass jars containing 10mL of medium or single seeds in 30mL polycarbonate tubes with 8mL of medium. The cultures were incubated at 25°C ± 3°C under 16 hours light period of 2.7 to 5.4 µmol $m^{-2}s^{-1}$.

Experimental methods

Persoonia sericea

Fruits of *P. sericea* were collected from Murphy's Creek (latitude 27° 26´S, longitude 152° 06´E) in Queensland, Australia. Two mesocarp removal treatments, two chemical scarification treatments and six endocarp removal treatments were compared. The 24 treatments were replicated three times, with four fruits per replication.

The mesocarps were removed by either fermentation (placed in warm water and left to cool and soak for 3 days) or acid treatment (32% HCl for 3 h). The fruits were then washed through a sieve to completely remove the mesocarp. The E+S were left to dry at ambient temperatures. After mesocarp removal, E+S were soaked in either 98% sulphuric acid (H_2SO_4) or 5% caustic soda (NaOH) for 15 min. The E+S soaked in H_2SO_4 were rinsed in a solution of 5% Na_2CO_3 for 15 minutes to neutralise the acid followed by a rinse in tap water, while E+S exposed to NaOH were rinsed in tap water only. Portions of the endocarp were removed using a sharp scalpel to minimise

damage to the seed. The treatments were: a control with no endocarp removed; moist E+S with the endocarp pierced; the ends of the endocarp removed; half the endocarp removed longitudinally; the majority of the endocarp removed; or dry E+S with half of the endocarp removed longitudinally.

The E+S were cultured aseptically on basal medium. Four E+S were placed in each jar (replicate). Radicle or cotyledon emergence through the seed coverings was assessed twice weekly for 100 days. Contaminated cultures were removed from the experiment. Data collected were the number of contaminated replicates, number of days to germination, and number of germinated seeds. The percentages of non-contaminated seed germinated after 30, 60 and 100 days were calculated.

Persoonia virgata

Fruit of *P. virgata* were collected from Landsborough Road (latitude 26° 50´S, longitude 152° 57´E) Queensland, Australia. The mesocarps were removed using a sharp knife. The following endocarp removal treatments were applied to dry E+S: a control with no endocarp removed; the ends of the endocarp removed using a sharp scalpel; half or all of the endocarp removed by cracking the endocarp with pliers of a suitable size (Sidchrome® No. 200/28212). A mounted needle was used when necessary to extract the seeds from the endocarp and testa.

There were 20 replications of each treatment. The seeds and E+S were surface disinfested before being placed singularly in polycarbonate tubes containing basal medium. Radicle or cotyledon emergence was assessed twice weekly for 70 days. Contaminated cultures were recorded and removed from the experiment.

Data were collected on the numbers of contaminated and germinated seeds and the corresponding percentages calculated. An untreated sample of 40 seeds, and any seeds from the experiment that had not germinated or been contaminated, were subjected to a TZ test to assess their viability.

Experimental design and analysis

Both experiments were a completely randomised factorial design. There was a degree of non-orthogonality in these experiments due to the variation in the number of contaminated seeds removed.

For the *P. sericea* experiment with multiple seeds in each replication, a chi-square analysis was applied using the theoretical variance on arcsine transformed data (Steel and Torrie 1980). Least significant differences (Fisher's LSD [Ott 1993]) were calculated at 5 and 1% levels of significance, allowing for different replication numbers due to contaminated seeds.

For the *P. virgata* experiment with single seed replicates, a chi-square analysis was applied using *r x c* contingency tables (Steel and Torrie 1980). Mean separations were made using the normal approximation at a 95% confidence interval (Steel and Torrie 1980).

The mean number of days to germination was calculated only for treatments where seeds germinated. The data were log transformed to compensate for the exponential distribution of the number of days to germination. These data were then analysed

using analysis of variance with least significant differences (Fisher's LSD [Ott 1993]) calculated at 5 and 1% levels of significance.

Results

The fruits of both *P. virgata* and *P. sericea* contain an extremely hard endocarp 1 - 2 mm thick. The majority of the fruits were uni-locular and single-seeded. The embryos were enclosed by the remains of the endosperm (a thin transparent layer) surrounded by the testa (Figure 1). The five cotyledons, 6 - 7 mm long, recurved during germination.

Insert Figure 1

No germination occurred in *P. sericea* fruit treated with HCl to remove the mesocarp. Chemical scarification of the endocarp with NaOH resulted in a higher percentage germination ($P<0.01$) than H_2SO_4 (Figure 2), but this came at the expense of increased contamination. No E+S treated with H_2SO_4 became contaminated, whereas 33% of NaOH scarified E+S were contaminated ($P<0.05$).

Insert Figure 2

E+S seeds with either the endocarp pierced, or with none or only the ends of the endocarp removed, did not germinate. Moist E+S with half of the endocarp removed produced the highest mean germination percentage of 65% (Figure 3). The amount of endocarp removed did not significantly influence the contamination percentage, which was 16.7% across the three treatments that allowed germination.

Insert Figure 3

There was a significant interaction ($P<0.05$) between the effects of chemical scarification of the endocarp and the degree of endocarp removal on germination percentage at day 30 of the experiment, but not at day 60 or 100 (Figure 4). By day 30 E+S that had been treated with NaOH and then had half of the endocarp removed (CH) had a germination of 75% with a maximum germination of 87.5% by day 60. There was no significant germination in the other treatments until day 60 with the final germination less than half that of CH at day 100.

Insert Figure 4

Germination of *P. virgata* was totally inhibited when either no endocarp or just the ends of the endocarp were removed (Figure 5). However, when either half or all of the endocarp was removed, germination was 87.5% and 58.8%, respectively. The TZ test conducted on a sample of seed indicated that the sample was 87.5% viable, and hence all viable seed germinated when half of the endocarp was removed. With *P. virgata* seed, removal of half the endocarp resulted in the highest (60%) contamination ($P<0.05$). All other endocarp removal treatments resulted in 10–20% contamination.

Insert Figure 5

Discussion

The dormancy of *P. sericea* and *P. virgata* seeds must be due to the endocarp since removal of at least half of the endocarp allowed germination. Removal of just the ends or pricking of the endocarp, with minimal mechanical disruption, was not sufficient to allow germination. Either of these treatments should have been sufficient to enable the penetration of water and oxygen (Mayer and Poljakoff-Mayber 1989). Thus inhibition of germination by the endocarp must involve physical restriction of embryo development and expansion. A similar mechanism was postulated for *Eucalyptus pauciflora* where pricking seed gave only 28% germination but cutting either side on the seed, or isolation of the embryo, gave 87 to 91% germination (Bachelard 1967).

Seed may have been damaged during the endocarp removal process, as this was very difficult. It was easier to remove the endocarp from moist *P. sericea* E+S and these showed higher germination (65%) than with dry E+S (30%). The highest germination of *P. sericea* seed was 87.5%, obtained by fermentation followed by NaOH scarification and mechanical removal of half the endocarp from moist E+S. The remaining 12.5% of seed may not have been viable. Alternatively, the seed may have been excessively damaged by the treatments, most likely during removal of the endocarp, or they may have been dormant due to some other mechanism.

Eremophila maculata seed germination is also controlled primarily by physical dormancy due to the woody endocarp surrounding the seeds. However in this species chemical dormancy also played an important role; water-soluble germination inhibitors were found within the fruit wall (Richmond and Ghisalberti 1994). The

removal of the endocarp from *P. virgata* E+S by cracking with pliers was easier than cutting with a scalpel. Of the seeds that did not germinate with all or half of the endocarp removed, 75% were not viable. It is possible that the soft seeds were damaged whilst removing the endocarp, with germination only occurring when damage was minimised. Auld *et al.* (2000) found 85% of *P. pinifolia* seed were viable, which is similar to the results obtained *for P. virgata* and *P. sericea.*

The removal of the mesocarp using 32% HCl inhibited the germination of *P. sericea* seed. The concentration of HCl may have been too high, or the duration of its application too long, resulting in damage to the seed as has been reported for some tomato cultivars (Herrington 1981).

The seeds of both *P. sericea* and *P. virgata* showed similar responses to the endocarp removal. At least half of the endocarp must be removed for germination to occur, and care must be taken when removing the endocarp. It appears that the endocarp is mechanically restricting germination, but further experiments are needed to determine whether other dormancy mechanisms exist.

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Figure 1. A longitudinal section of a *Persoonia virgata* fruit.

Figure 2. The influence of chemical scarification of the endocarp on mean percentage germination of fermented, endocarp-enclosed seed of *Persoonia sericea.* Means separated using Fisher's LSD on arcsine transformed data. Different letters within a treatment time indicate significant differences at $P<0.01$.

Figure 3. The influence of endocarp removal on mean percentage germination of fermented, endocarp-enclosed seed of *Persoonia sericea*. Means separated using Fisher's LSD on arcsine transformed data. Different letters within a germination time indicate significant differences at $P<0.01$.

Figure 4. The interaction of chemical scarification and endocarp removal on mean percentage germination of fermented, endocarp-enclosed seed of *Persoonia sericea*.. S = H_2SO_4 treated, C = NaOH treated; H = moist removal of half the endocarp; DH = dry removal of half the endocarp; M = majority of endocarp removed. Means separated using Fisher's LSD on arcsine transformed data. Different letters within a germination time indicate significant differences at $P<0.05$.

Figure 5. Effect of endocarp removal on the percentage germination of endocarp-enclosed seed of *Persoonia virgata*. Treatment comparisons by chi-square *r x c* contingency table, with mean separation by 95% confidence intervals at the normal approximation.

Figure 1

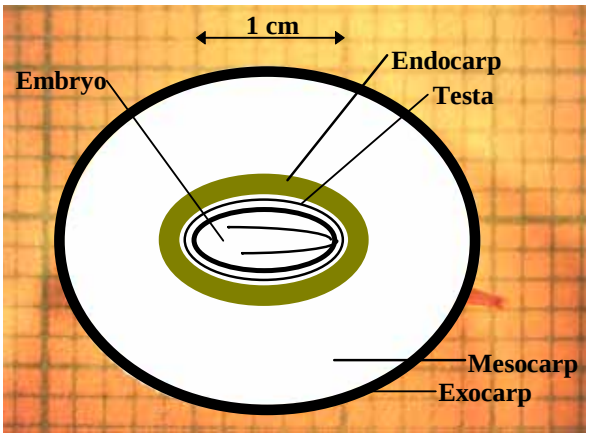


Figure 2

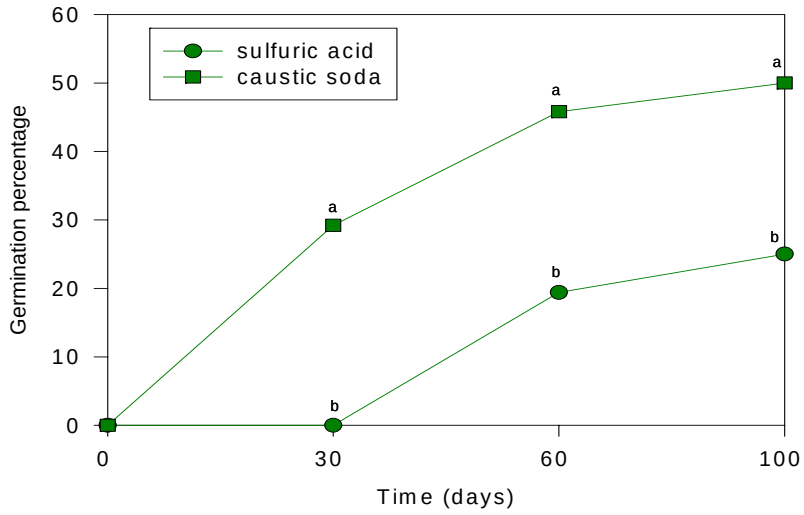

60
50
40
30
20
10
0
Germination percentage
sulfuric acid
caustic soda
a
a
b
b
a
b
0
30
60
100
Time (days)

Figure 3

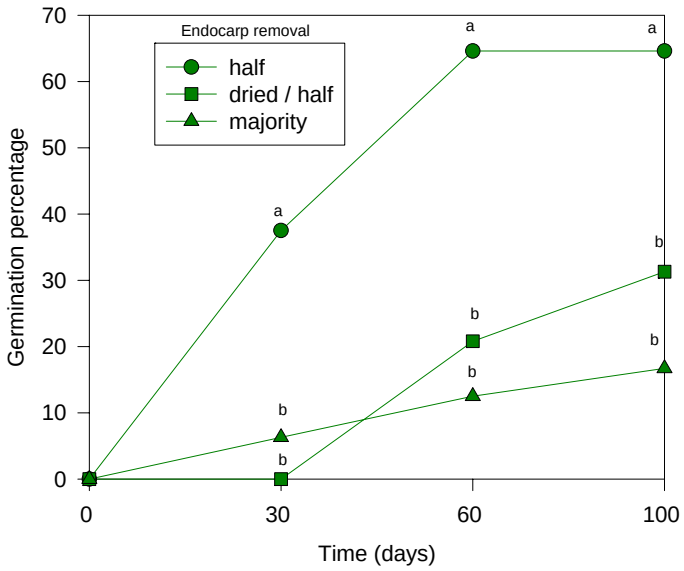

Endocarp removal
half
dried / half
majority
Germination percentage
Time (days)
0
10
20
30
40
50
60
70
0
30
60
100
a
a
a
b
b
b
b
b
b

Figure 4

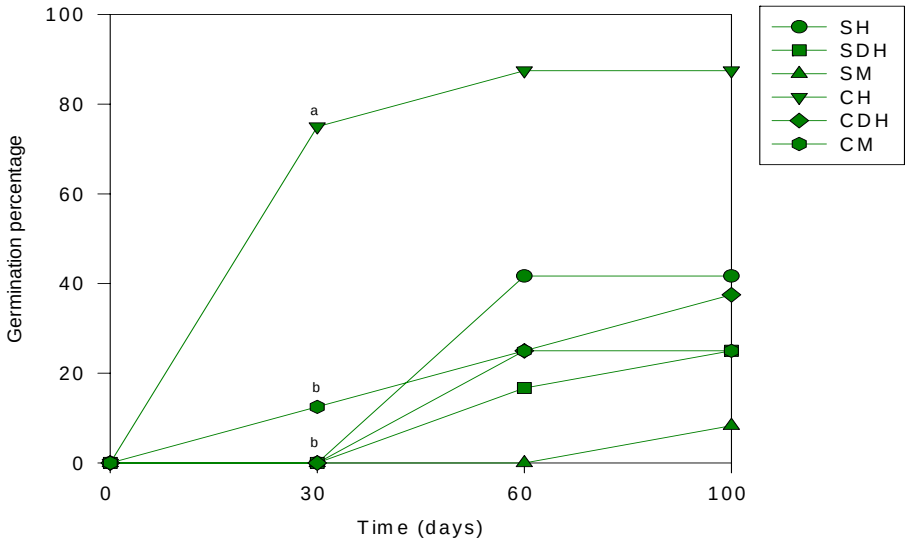
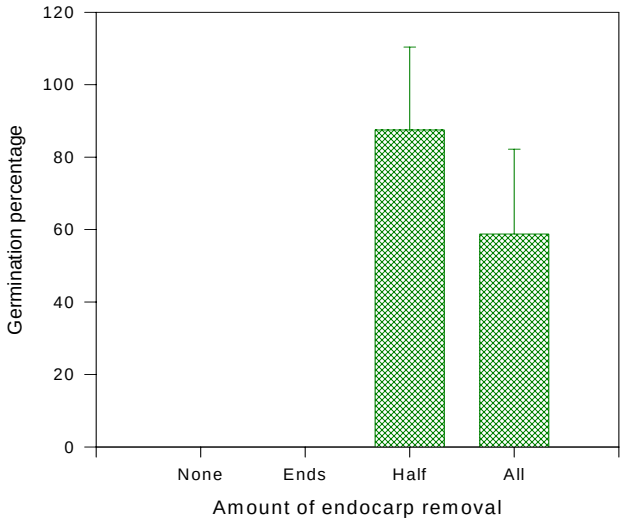

100
80
60
40
20
0
Germination percentage
0
30
60
100
Time (days)
a
b
b
SH
SDH
SM
CH
CDH
CM

Figure 5


120
100
80
60
40
20
0
Germination percentage
None
Ends
Half
All
Amount of endocarp removal