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Germination of *Persoonia myrtilloides* and *Persoonia levis*©

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Seeds of *Persoonia levis*, in which half the endocarp was removed and soaked in 350 ppm gibberellic acid (GA_3) at 20°C and 16-h photoperiod, resulted in significant levels of germination (SNK: $p<0.01$). However, such seeds, which did not have GA_3 applied, resulted in marginally insignificant levels of germination (SNK: $0.05<p<0.10$). There was also a high variation in percent germination between individual trees. Fresh seeds of *P. myrtilloides* did not germinate under the same conditions and treatments that *P. levis* was exposed to. However, 4-month-old seed that had their endocarps half removed did germinate at 15°C in the dark, but GA_3 concentration had no effect on germination percentages (ANOVA: $F=0.484$, $p>0.5$). Either after-ripening occurred in this species or the 5°C drop in temperature, allowed germination to occur. Furthermore, chilling fruits at 4°C and leaching endocarp treated seeds in 7.5% ethanol solution significantly reduced germinability in *P. myrtilloides* ($t=3.6$, $p<0.005$ and $t=3.35$, $p<0.005$, respectively).

INTRODUCTION

Persoonia species produce drupes, which comprise a fleshy mesocarp, a hard woody covering (endocarp) and a thin papery seed coat housing the embryo (Stroschen, 1986). This genera of plants has proven difficult to germinate in the past, however, recent work on *P. virgata* and *P. sericea* under aseptic conditions has shown that removal of at least half the endocarp was significant in overcoming dormancy (Ketelhohn et al., 1994, 1998).

The first aim of this study was to determine if endocarp removal could be successfully employed to overcome dormancy in two east-Australian *Persoonia* spp., *P. levis* and *P. myrtilloides*. The second aim was to determine an incubation temperature and gibberellic acid concentration that would yield the maximum germination rate.

MATERIALS AND METHODS

Germination Trial for *Persoonia levis*. Thirty-six ripe fruit from each of ten replicate *Persoonia levis* trees were collected from Crown Land near Nowra on 19 Nov.1998 and fermented in a container of water for 25 days. All fruits were chemically scarified in concentrated H_2SO_4 for 15 min. Mesocarps were then treated mechanically by removing the fibrous pulp with a scourer, washed in tap water, and sterilised by shaking in 1% hypochlorite for 20 min before rinsing in distilled water (dH_2O). Four fruits from each tree were then randomly allocated to a specific germination treatment. Seven germination treatments were conducted: four endocarp treatments in which the hard covering was split longitudinally in half using a vice, leaving half the endocarp intact, whilst the remainder had no endocarp treatment (controls).

Treatments for Endocarp Treated and Control Fruits.

Control: Fruits untreated.

Chilled: a) Fruits placed on moist filter paper in petri dishes at 4°C for 2 weeks.
b) Fruits maintained in a freezer at –20°C for 2 weeks.

Treatments for Endocarp Treated Fruits Only.

GA_3 Soak: Fruits with endocarps treated were soaked in 350 ppm GA_3 for 22 h.

All treated fruits were incubated on 15 Dec. 1998 at 20°C with a 16-h photoperiod for 7 weeks, and a successful germination was scored when either the cotyledons or radicles first appeared. Seeds were watered weekly with 0.1g 100 ml^{-1} Thiram in dH_2O to inhibit fungal growth. Germination was quantified by the percentage of successful germinants within a single petri dish over the 7-week period.

Data was analysed by a Model 1 univariate ANOVA with data arcsine transformed; variance homogenous at alpha = 0.01 and normality assumed. F test was conducted with alpha = 0.01. A Student-Newman-Keuls (SNK) test was later performed.

Germination Trial for *Persoonia myrtilloides*. Several ripe fruits from ten *P. myrtilloides* trees were collected from the Newnes State Forest north of Lithgow on 20 Dec.1998 and exposed to the same experimental protocol as *P. levis*. There was one exception. All fruits were mixed together and shaken so that tree identity was unknown. Forty fruits were then randomly allocated to a germination treatment, four fruits were randomly selected from each treatment batch and introduced to ten replicate petri dishes for incubation on 22 Dec. 1998.

The remaining fruits from the above trial were stored at room temperature for 4 months and incorporated into a new trial on 20 April 1999. The exceptions to the above methodology were that all fruits had their endocarps treated and there was no fermentation step. Five randomly chosen batches of 40 fruits were then soaked in one of five concentrations of GA_3 for 22 h: 0, 150, 350, 450, and 750 ppm and incubated at 15°C for 16 weeks in the dark. In addition, two other germination treatments were included in the design:

- Forty fruits with endocarps split in half were leached in 7.5% ethanol solution for 2 weeks before being soaked for 22 h in 450 ppm GA_3.

- Forty fruits were kept at 4°C for 2 weeks on filter paper moistened with 0.1 g 100 ml^{-1} Thiram in dH_2O before having their endocarps half removed.

Statistical analysis for comparing the percentage of germinants within each dish amongst the five different GA_3 concentrations was conducted with a Model 1 univariate ANOVA with variance homogenous at alpha=0.05. F tests were conducted at alpha=0.05. For comparing percent germination of 450 ppm / 450 ppm + leached, and 0 ppm / 0 ppm + fridge, t-tests were conducted with alpha = 0.05; variance homogenous at alpha = 0.05 for both sets of comparisons.

RESULTS

Germination Trial of *Persoonia levis*. For all treatments where chilling was involved, no seeds germinated within the 7-week period. Germination only occurred when seeds had their endocarps split in half and were soaked with GA_3 (mean = 50%) or dH_2O (mean = 17.5%). Compared to the control (mean = 0%), the germination of *P. levis* exposed to the endocarp and GA_3 treatment was highly significant (SNK: $p < 0.01$). However, when compared to endocarp treated seeds soaked in dH_2O, the

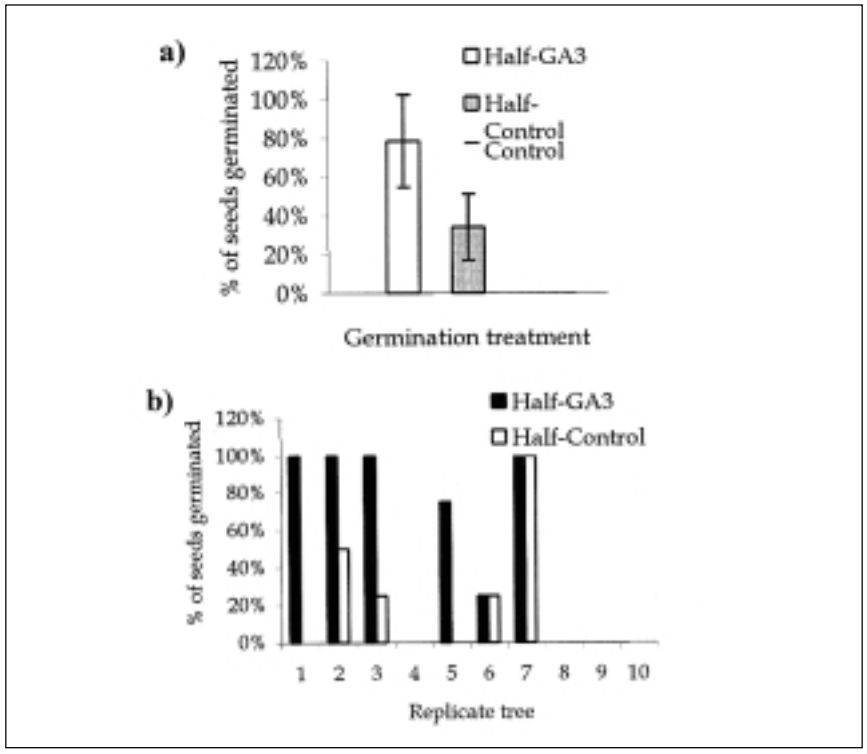

Figure 1. (a) Mean percentage germination (arcsine transformed) of *Persoonia levis* seeds (n = 10 trees) from three treatments indicated in legend box (ANOVA: $F_{2,27} = 5.49$, $P = 0.0096$). Error bars are standard error. (b) Percentage of *P. levis* seeds (four seeds per dish) germinated from fruits of ten trees exposed to two germination treatments indicated in legend box.

application of GA_3 proved to be marginally insignificant (SNK: 0.05<p<010) in enhancing germination rates (Fig. 1a.). The endocarp treated seeds soaked in dH_2O did not significantly enhance germination rates relative to control seeds (SNK: p>0.05; Fig. 1a). There was high variation in germination rates amongst the two treatments producing germinants, and this was particularly evident from trees that either had germinable fruits (six trees) or none at all (Fig. 1b.).

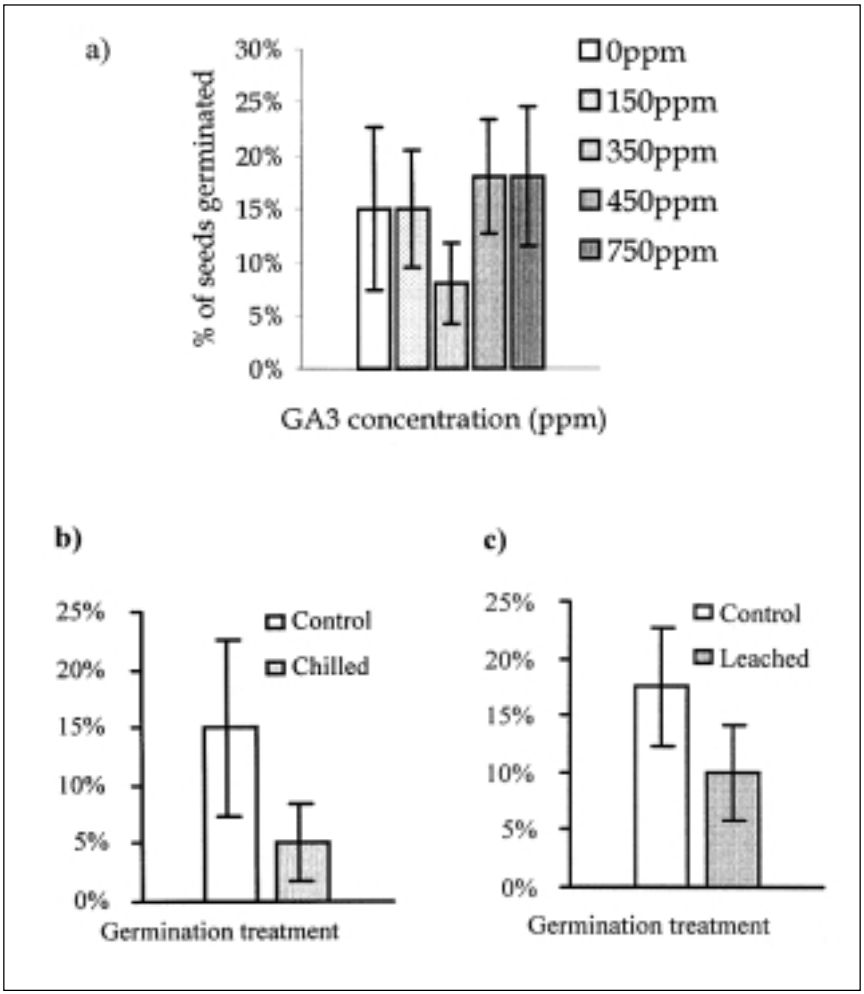

Figure 2. (a) Mean percentage of *Persoonia myrtilloides* seeds (n = 10 dishes: 4 fruits/dish) successfully germinated from five GA3 concentrations on seeds with endocarps split in half (ANOVA: $F_{4,45} = 0.484$, $P = 0.75$). Error bars are standard error. (b) Mean percentage of *P. myrtilloides* seeds (n = 10 dishes: 4 seeds/dish) successfully germinated from two treatments (chilled and not chilled) on seeds with endocarps split in half (t-test: $t_{2,18} = 3.6$, $P<0.005$). Error bars are standard error. (c) Mean percentage of *P. myrtilloides* seeds (n = 10 dishes: 4 seeds/dish) successfully germinated from two treatments (leached and not leached) on seeds with endocarps split in half (t-test: $t_{2,18} = 3.35$, $P<0.005$). Error bars are standard error.

Germination Trial of *Persoonia myrtilloides*. No successful germinants resulted from the first germination trial. However, seed that had been stored for 4 months did produce successful germinants (Fig. 2a, b, c). It was found that GA_3 concentration had no significant effect on germination rate ($F = 0.484$, $p>0.5$; Fig. 2a), whilst the chilled treatments significantly reduced germination success relative to endocarp treated seeds soaked in dH_2O ($p<0.005$; Fig. 2b). Likewise, leaching endocarp treated fruits in 7.5% ethanol solution prior to soaking in 450 ppm GA_3 significantly reduced germinability ($p<0.005$; Fig. 2c).

DISCUSSION

Given the other studies on *P. virgata* and *P. sericea*, removal of at least half the endocarp was also important in overcoming dormancy in *P. myrtillodies* and *P. levis*, although the reason for this requirement is still uncertain. It is most likely to allow embryo expansion or oxygen diffusion, possibly both. Gibberellic acid, which is believed to mobilise the endosperm, was important in *P. levis* for achieving significant germination (Jhuree et al., 1998). In contrast, this hormone had no significant effect on the germination of *P. myrtilloides*, which suggests it has an intrinsic mechanism of endosperm mobilisation. The chilling requirement is also believed to mobilise seed reserves, however significant reductions in the germination of both plant species occurred (Jhuree et al., 1998). This treatment was applied before the endocarps were split. Maybe oxygen diffusion is important in the seed mobilisation process, therefore, the chilling requirement should be repeated on endocarp treated seeds to test this possibility.

Unusually, fresh *P. myrtilloides* seeds did not germinate which hints at an after-ripening effect since 4-month-old seed germinated readily. However, this could have been attributed to reducing the incubation temperature to 15°C. Further work needs to be conducted on fresh and old seeds at 15°C to test for after-ripening.

There was also a high variation in germination percentages amongst individual trees of *P. levis*, which partly explains the low sensitivity in detecting differences amongst treatment means. To improve the experimental design for future work, "tree" should be treated as a factor and the number of fruits per tree increased to minimise the chance of partitioning a good or bad batch of fruits to each germination treatment.

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