A determination of the horticultural potential of *Persoonia hirsuta* subsp. 'Yengo National Park' (Proteaceae)

Final Report for a project supported by the Australian Flora Foundation in 2018.

BOTANIC GARDENS OF SYDNEY

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Author declaration

This report is provided to the Australian Flora Foundation in fulfilment of the conditions of the grant awarded to the author in 2018. This report contains the experimental work conducted by Nathan Emery between January 2019 and January 2021 on the propagation of Persoonia hirsuta subsp. 'Yengo National Park'.

The work within this report is entirely that of the author's, except where indicated in the acknowledgements. This work is original.

This work would not have been possible without the support of the Australian Flora Foundation and the Australian Botanic Garden Mount Annan.

Dr Nathan Emery

November 2023

A determination of the horticultural potential of *Persoonia hirsuta* subsp. 'Yengo National Park' (Proteaceae)

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Summary

Persoonia hirsuta (Hairy Geebung) is an Endangered shrub that occurs in small, scattered populations across the Greater Sydney Region, New South Wales (NSW). Recently there has been uncertainty over the taxonomy of the *P. hirsuta* species complex, as the morphological variation has become more complex following the discovery of additional populations. Illustrating this, a formally unnamed sub-species, *P. hirsuta* subsp. 'Yengo National Park' occurs in the northern extent of the distribution in Yengo National Park, NSW. The nine plants in this population have a distinctive weeping form with longer and less hirsute leaves on average than the other populations. Recognising the uniquely attractive weeping vegetative form of *Persoonia hirsuta* subsp. 'Yengo National Park', the outcome of this project was to conserve the sub-species by investigating the potential for developing a successful horticulture protocol using seeds, vegetative cuttings and tissue culture techniques.

Vegetative cuttings were collected from all nine wild plants in May 2019 and transported to the Australian Botanic Garden Mount Annan (ABGMA) nursery for processing. Cuttings were separated into four treatment groups: 1) water soak (control); 2) EsiRoot; 3) EsiRoot + clonex green, and; 4) EsiRoot + clonex purple. Cuttings were monitored for root strike for 400 days. The growth of struck cuttings was recorded over nine months. Seed collection from extant plants was not possible due to the bushfires in NSW during summer 2019-20. Therefore, a stored seed collection from November 2017 was used for this study. Seeds were pre-treated with water (control) 1000ppm gibberellic acid (GA_3) or 2% smoke water and incubated at 25/15°C for ten weeks. Germinants were then transferred to potting mix and their growth was measured over nine months. Tip cuttings were taken from seedlings and initiated into tissue culture in August 2019. Plant segments were sub-cultured when suitable growth was observed.

Seed germination was the most successful propagation method with germination ranging from 70.9 ± 8.7% to 89.5 ± 1.6% among the treatments used. By comparison, vegetative cuttings required >70 days to strike roots, and strike rates were very low, ranging from 3% to 17% among treatments. The analysis showed higher concentrations of indole-butyric acid (IBA) significantly reduced the survival of cuttings. Survival was highest for cuttings soaked in an EsiRoot solution. Seedlings grew six times faster than struck cuttings following nine months of measurements in the glasshouse. Approximately 65% of plant tips were successfully initiated into tissue culture. However, there was a slow to response of the species to the culture medium, resulting in longer sub-culturing intervals of approximately 6-9 weeks.

Ex-flasking plantlets out of tissue culture was not possible in the project due to insufficient material available and plant mortality.

The results of this study demonstrate that seed germination provides the fastest and most successful method to produce *Persoonia hirsuta* subsp. 'Yengo National Park' plants. However, plant production using vegetative cuttings may be a viable option for selecting horticulturally desirable traits if juvenile plant material can be wild or *ex situ* sourced.

Introduction

Persoonia (Proteaceae) are an Australian endemic group of long-lived plants ranging from low prostrate or spreading shrubs to small trees. The genus is characterised by light green foliage with extensive morphological variation both within and among species, and inconspicuous yellow flowers (Emery and Offord 2018; Weston 2003). Following pollination, a mature ovule slowly develops into a large fleshy drupe, containing a woody endocarp with one or two seeds inside. Following the most recent taxonomic description of *Persoonia pauciflora* (Weston 1999), there are 99 species occurring over a wide range of ecosystems and regions, excluding central and arid regions. *Persoonia* species are either obligate-seeders or resprouters, capable of regenerating from branches and/or roots, and several species have long primary juvenile periods that may exceed beyond that of typical fire intervals in some dry sclerophyll ecosystems (Emery and Offord 2018).

Traditionally, *Persoonia* species have been desired by the horticulture industry for their attractive growth habits and foliage as feature plants, groundcovers, rockery plants or hanging plants (Offord *et al.* 2015; Wrigley and Fagg 1989). Stems of several species, such as *P. pinifolia* and *P. longifolia* have also occasionally been bush harvested for the attractive foliage and flowers as 'fillers' in floral arrangements. Despite the demand for *Persoonia* horticulture, many species are under-represented or not included due to difficulties surrounding seed germination (Ketelhohn *et al.* 1996; Mullins *et al.* 2002; Nancarrow 2001) and vegetative cutting propagation (Crowhurst 2006; Ellyard 1982; Perry 1997; Weston 2003). In addition, nine species are listed as threatened, endangered or critically endangered and are negatively impacted by factors such as habitat fragmentation and clearing, mining, inappropriate fire regimes, small population sizes and a lack of recruitment events. The restoration industry is also frequently unable to incorporate *Persoonia* species as part of replanting or translocation programs due to a lack of propagation material and knowledge of the species' ecological requirements.

Mass propagation is ideally achieved through seed germination where large plant numbers can be produced at minimal cost and effort. *Persoonia* seed germination is often unsuccessful due to complex dormancy mechanisms that require specific environmental conditions to overcome. Because many species occur in fire-prone ecosystems, germination may be triggered by fire cues. The first barrier to germination is the mechanical restriction of the woody endocarp that must weaken over time or be manually removed. Recent research demonstrated that the endocarp breaks down over several years *in situ* before germination occurs, but the required time can be shortened following favourable climate conditions (Chia *et al.* 2016; Emery and Offord 2019b; Norman and Koch 2008). Manual removal of at least half of the endocarp using a scalpel or bench-mounted vice enables germination to occur under controlled conditions in the laboratory; however, the process is labour-intensive and has a high risk of

damaging the seeds (Bauer *et al.* 2004; Emery and Offord 2019c). Furthermore, sown seeds are highly susceptible to microbial contamination. The application of a surface-steriliser or anti-fungal agent to the substrate reduces contamination rates but does not eliminate the problem.

Compared to seed germination, cutting propagation is an enticing method to mass produce plants with horticulturally desirable traits. Where seed germination requires an understanding of the ecological components that affect fruit and seed set in the field, vegetative propagation requires a problem-solving approach of identifying the conditions required for root strike (Malan 1990). To date, vegetative propagation of *Persoonia* has proven to be an arduous and long-term process as cuttings may require up to 12 months to strike roots. Common species such as *P. pinifolia, P. chamaepitys* and *P. longifolia* typically require 3-4 months for root strike to occur (Dewing 2000; Ellyard 1982), while rare species such as *P. hindii* and *P. hirsuta* often require 6-12 months for root strike (Catelotti and Offord 2017; Emery and Offord 2019c). Despite recent progress for improving strike rates of *Persoonia* species by collecting semi-hardwood cuttings in autumn (i.e. following the period of flowering and new growth), there is still significant differences in success among genotypes (Catelotti and Offord 2017). Individual genotypes are hypothesised to have varying levels of chemical inhibitors or promotors for rooting in shoots, but it may be possible to subsequently select genotypes that are more likely to strike roots (Bauer and Johnston 1999). Furthermore, root strike success might further increase following a secondary hormone treatment, but higher concentrations of indole-butyric acid (IBA) growth regulator ('hormone') can also negatively affect cuttings (Ellyard 1982; Emery and Offord 2019c). Therefore, further work is required to optimise protocols for processing *Persoonia* cuttings.

More recently, the difficulties surrounding seed germination and vegetative cuttings have led to trialling tissue culture as a potential propagation method. As part of a wider study, seven eastern Australian *Persoonia* species were successfully initiated into tissue culture, with most explants produced from embryos (Offord *et al.* 2015). This is because embryos are considerably easier to decontaminate than axillary shoots (Cambecedes and Balmer 1995; Ketelhohn *et al.* 1996) and possess juvenile characteristics that favour tissue culture establishment in many plants (C. Offord pers. obs.). It may be difficult to establish *Persoonia* explant cultures from embryos when selecting for specific horticultural traits, particularly as many species are partially self-incompatible (Emery and Offord 2018), but a genetically diverse explant collection from embryo tissue would be beneficial for restoration projects. Irrespective of the motivation, further experimentation is required to determine whether propagation *Persoonia* species through tissue culture is a viable method for achieving plant production targets.

The propagation issues discussed here are of concern for *Persoonia hirsuta* Pers. *P. hirsuta* (hairy geebung) is a spreading to decumbent shrub between 0.3 and 1.5m tall and has a disjunct distribution across the Greater Sydney region of New South Wales (NSW). The species is experiencing significant decline throughout much of its distribution, with many of the 21 historical populations now locally extinct or existing with fewer than ten individuals. Recent field surveys have failed to find plants in locations known from numerous historical records and several populations exist with a single isolated individual (Haynes 2015; Wilmott 2013). A total of 11 surviving populations are known and many of these under significant threat of habitat fragmentation and isolation from urban development, inbreeding and pollen limitation, poor recruitment and inappropriate fire regimes. Consequently, *P.*

hirsuta is listed as Endangered under the *Environment Protection and Biodiversity Conservation Act 1999* and the *NSW Biodiversity Conservation Act 2016*.

P. hirsuta was initially characterized into two sub-species: *P. hirsuta* subsp. *hirsuta* and *P. hirsuta* subsp. *evoluta* that occur on the eastern and western distribution extremes, respectively. Intergrades of the two sub-species were thought to occur clinally in an east-west direction (Weston and Johnson 1991). Taxonomic uncertainty of the *P. hirsuta* species complex has since arisen following the discovery of additional populations with varying morphological features. Illustrating this, a formally unnamed third putative sub-species, *P. hirsuta* subsp. 'Yengo National Park' occurs in the northern extent of the distribution in Yengo National Park, NSW (Holmes et al. 2018). There are 25 historical records of this subspecies, and survey work conducted in 2017 confirmed at least 9 plants persisting *in situ*. This subspecies is morphologically differentiated from southern populations of *P. hirsuta* by its weeping foliage that is typically less hirsute (Figure 1). Branches are often seen layering into the surrounding soil, but roots have not been observed on these branches (N. Emery, pers. obs. 2017). The uniquely attractive weeping vegetative form of *P. hirsuta* subsp. 'Yengo National Park' make it a desirable option for establishment in cultivation. Furthermore, establishing *P. hirsuta* subsp. 'Yengo National Park' in cultivation will help ensure this morphotype does not go extinct in the wild due to its small population size. Therefore, the outcome of this project was to conserve *P. hirsuta* subsp. 'Yengo National Park' by investigating the potential for developing a successful propagation protocol. Specifically, our aims were to determine whether plants can be successfully produced from seeds, vegetative cuttings or by tissue culture, and determine which method produced the fastest growth rate of young potted plants.

Materials and methods

Seed germination experiment

Seeds could not be collected in the field due to fruit dispersal coinciding with the unprecedented bushfire season that occurred throughout NSW in summer 2019-20. Fires burnt through the region where the known wild *P. hirsuta* subsp. 'Yengo National Park' plants occurred in November 2019. Consequently, the seed germination methodology was amended and used a recently stored seed collection at the Australian PlantBank.

The seeds used for this experiment were collected from nine wild plants in November 2017 and processed at the Australian PlantBank. In the laboratory, the fleshy exocarp and mesocarp layers were removed and the remaining pyrenes (i.e. endocarp and seed within) were cleaned using a mild bleach solution (2%) before being rinsed twice using reverse osmosis (RO) water. The pyrenes were transferred to a drying room set to 15°C and 15% relative humidity for two weeks and then placed in long-term storage at -20°C in a vacuumed-sealed foil packet.

Seeds were retrieved from storage in March 2020 and allowed to rehydrate and equilibrate at room temperature for 48 hours prior to experimentation. Pyrenes were soaked in RO water for two hours to soften slightly and then seeds were extracted by cracking the endocarp using a bench-mounted hand vice. Seeds with obvious physical damage to the embryo, cotyledons or testa were immediately discarded. The extracted seeds were agitated in a 2% bleach solution on an orbital shaker for 20 minutes and then rinsed twice in RO water. Seeds were sown in flat-bottom well-plates (Corning®

Figure 1. Typical spreading, weeping growth form of *Persoonia hirsuta* subsp. 'Yengo National Park'. Leaves are narrower and less hirsute than other populations of *P. hirsuta*.

Costar® TC-treated) with a 0.9% (w/v) water agar medium that were pre-applied with either 1ml of RO water (control), 1000 ppm GA_3 or 2% smoke water solution spread across the agar surface. One seed was sown per well, helping to prevent any microbial contamination from spreading to an entire plate of seeds (Figure 2). There were three replicates of 24 seeds per treatment, giving a total of 216 seeds. All plates were sealed using plastic and placed into an incubator set at a 25/15°C temperature regime with a 12-hour light/dark cycle. Germination was checked every 4-7 days for ten weeks, and a seed was scored as germinated when the radicle was at least 2mm in length. All non-germinated seeds were checked for viability using a cut-test and final germination results were adjusted accordingly.

All germinants were potted up into 90mm tubes containing a soil mix of crushed quartz and coir fibre with a low organic matter content. A total of 57 seedlings survived the transition from agar germination to soil mix. Seedlings were kept in a glasshouse at the Australian Botanic Garden Mount Annan (ABGMA) nursery. The above-ground height of each seedling was measured at five- and nine-months after potting up. Each seedling was subjectively assigned a health rate between 1 and 5 (1: brown, defoliated stems; 5: green, healthy) at each measurement.

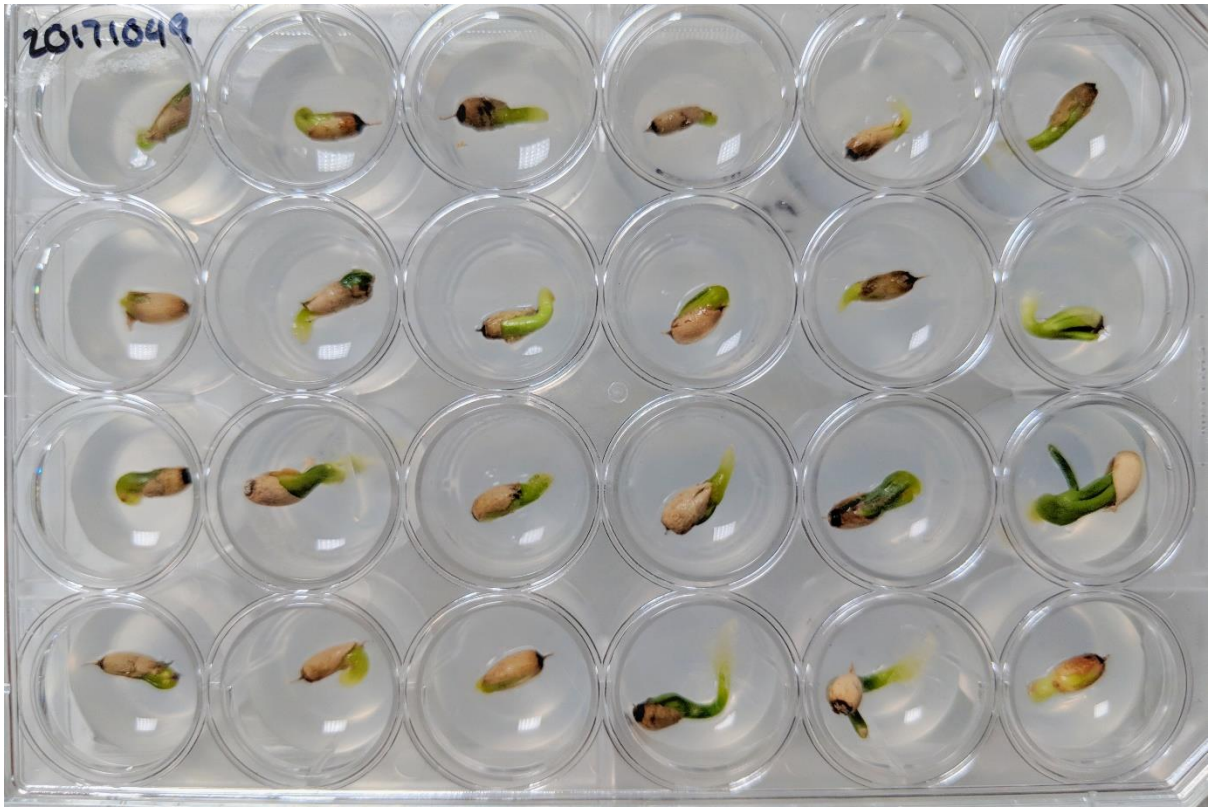

Figure 2. *Persoonia hirsuta* seeds sown in a 24-well plate to minimise and isolate microbial contamination during incubation.

Vegetative cuttings propagation

A composite collection of vegetative cuttings was collected from all nine known plants in May 2019. Cuttings consisted of semi-hardwood branchlets that were not flowering or fruiting. Although difficult to quantify in the field, enough plant material was taken for around 350-400 cuttings, and in accordance with national guidelines no more than 10% of any one plant was collected (Offord and Meagher 2009). Cuttings were transported from the field to the ABGMA nursery at 4°C in breathable plastic bags sprayed with sterilised water to prevent the cuttings from drying out.

In the nursery, the cuttings were initially sterilised by soaking in a 2% bleach solution for 15 minutes, and then rinsed under water. Stems were trimmed to around 7cm in length, and the linear foliage below the top 1cm was reduced to the petiole using sterilised secateurs. The cuttings were then assigned to one of four treatments:

(i) *Water control*: cuttings were soaked in water for approximately 30 minutes.
(ii) *EsiRoot soak*: cuttings were soaked in an EsiRoot proprietary growth solution for approximately 30 minutes.
(iii) *EsiRoot + Clonex green*: cuttings were soaked in an EsiRoot proprietary growth solution for approximately 30 minutes and then dipped in Clonex green rooting hormone gel.
(iv) *EsiRoot + Clonex purple*: cuttings were soaked in an EsiRoot proprietary growth solution for approximately 30 minutes and then dipped in Clonex purple rooting hormone gel.

The EsiRoot solution contained 4 ppm (25ml/4L) indole-butyric acid (IBA) and 1-Naphthylacetic acid (NAA). The Clonex rooting growth regulator gels provided an additional IBA treatment at 1.5g/L (green) or 3g/L (purple). The treated cuttings were then placed in 20mm coir plugs (Jiffy Preforma) that were held in polystyrene cells and placed in a glasshouse on controlled, heated benches (22 ± 2°C) and mist watered until root strike for up to 400 days.

The cuttings were periodically checked for roots and struck cuttings and the coir plug were potted in 90mm forestry tubes and re-potted later when necessary. The above-ground height and length of the longest branch of each struck cutting were quantified at five- and nine-months following root strike. Cutting-grown plants were also subjectively assigned a health rating as per the seedlings.

Tissue culture trial

A recent preliminary trial found that *P. hirsuta* axillary tip cuttings were more likely to be successfully initiated into tissue culture than seed embryos due to lower microbial contamination (Emery and Offord 2019c). Therefore, 34 tip cuttings were freshly harvested in August 2019 from eight two-month old seedings previously grown in the ABGMA nursery. Leaves were removed from plant growth tips leaving 2mm of the petiole to protect the bud during sterilisation. Tip cuttings were cut to 15mm segments, soaked in a 2% Alconox detergent and agitated on an orbital shaker for 30 minutes before being washed in RO water. The plant segments were then submerged in a 1% bleach solution with a drop of Tween surfactant and agitated on an orbital shaker for 20 minutes and re-rinsed in RO water.

Once cleaned, the plant segments were placed onto a Woody Plant Medium (WPM; McCown & Lloyd, 1981) containing 2 µmol/L benzyl adenine purine, 0.2 µmol/L IBA, 20 g/L sucrose and 9g/L agar (pH 6.0). Preliminary trials on *P. hirsuta* found that plantlets positively responded to WPM (A. Rollason, pers. comm. 2019).

Data analysis

All analyses were performed in the R environment (R Core Team 2020). A generalised linear model (GLM) weighted by the number of viable seeds with a binomial distribution was used to test the effects of chemical stimulants on final germination. The GLM assumptions were visually checked using the *gvlma* package (Pena and Slate 2019) and no data transformations were required. *P*-values were obtained using global analysis of variance (ANOVA) tests.

A time-to-event model was performed to analyse the time to 50% germination (t50), relative to the maximum germination of each treatment. This regression model takes into consideration that a seed may have germinated at any time point between survey intervals, and that non-germinated but viable seeds may have still germinated after the experiment was completed. The time-to-event model was performed using the *drc* package (Ritz *et al.* 2015).

A Cox regression model was used to test the effects of growth regulator treatment on the survival of cuttings in the nursery using the *survival* package (Therneau 2015). Cuttings that struck roots within the study period were excluded in the model. Hazard ratios and *P*-values were calculated for each hormone treatment relative to the control.

To examine the vigour of seedlings and cuttings propagated plants, growth was measured by plant height and calculated by the change in height between time-periods (net growth). The starting height,

that is, the initial height was also accounted for by calculating relative growth rates (RGR) of both propagation methods. RGR was expressed as the change in height between the first and final measurements. GLMs were used as outlined above to determine if there was a significant difference between propagation methods for net growth and RGR.

Results

Seed germination experiment

Persoonia hirsuta subsp. 'Yengo National Park' readily germinated in all three treatments, ranging from 70.9 ± 8.7% under the water control to 89.5 ± 1.6% when GA_3 was added (Figure 3). Although the effect of chemical stimulant on germination displayed some variation, there was no significant differences between the treatments ($P = 0.0515$). Treatment affected the rate of germination where t50 was lower for seeds treated with GA_3 (29.7 ± 1.6 days) than for seeds treated with smoke water or water (35.7 ± 1.6 days and 35.8 ± 1.3 days, respectively).

Vegetative cutting propagation

Vegetative cuttings required 2-3 months to strike roots (c. 71 days) and was very low among the treatments by 400 days. Final strike rate was highest for the water control treatment (17%). By comparison, cuttings that were given an additional IBA treatment (i.e. Clonex green or purple) had

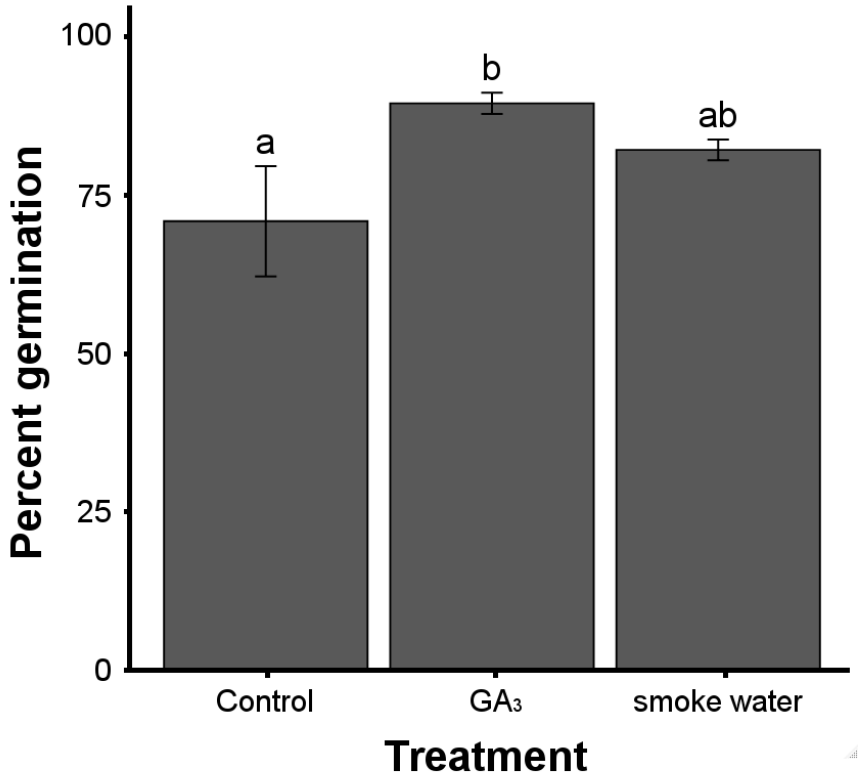

Figure 3. Final percent germination (mean ± s.e.) of *Persoonia hirsuta* subsp. 'Yengo National Park' seeds by treatment. Seeds were sown in 9g/L of water agar with 1ml of RO water (control), 1000 ppm GA_3 or 2% smoke water solution spread across the agar surface. Different lower-case letters indicate significant differences between treatments ($P < 0.05$).

<5% of cuttings strike roots (Figure 4a). The survival of cuttings in propagation significantly varied among hormone treatments (Figure 4b). Cuttings treated with EsiRoot + Clonex purple were more than three times less likely to survive compared to water control (Hazards ratio 3.33 ± 0.96; $P < 0.001$; Figure 5). In contrast, cuttings treated with EsiRoot had a reduced chance of dying relative to the water control treatment (Hazards ratio 0.59 ± 0.19; $P = 0.01$). The chance of survival was not statistically different between cuttings treated with EsiRoot + Clonex green and the water control (Figure 5).

Early plant growth

During the nine months of monitoring early plant growth in the nursery, three seedlings and one cutting died and were not included in the following results. Seedlings were significantly taller than cuttings after nine months ($P < 0.001$). On average, seedlings (27.4 ± 1.3 cm) were more than twice the height of cuttings (12.2 ± 1.5 cm) at nine months. Additionally, seedlings also grew around six times faster that cuttings (Table 1). Most of the growth of cuttings-propagated plants during the monitoring period occurred in lateral branches, but there was no significant difference in the length of the longest branch for plants from either propagation method ($P = 0.335$). Seedlings typically developed lateral branches after three months. All plants were healthy and showed minimal signs of yellowing foliage (health rating ≥4.8; $P = 0.173$; Figure 6).

Tissue culture trial

After four weeks, 35% of *P. hirsuta* subsp. 'Yengo National Park' explants were discarded due to microbial contamination. The remaining explants were successfully initiated into tissue culture (Figure 7). The species was slow to respond to the culture medium, resulting in long sub-culturing intervals of approximately 6-9 weeks. By November 2020 there were approximately 100 plantlets, which did not provide enough replication for trialling ex-flasking by the end of the study timeframe. Several mortality events associated with growth medium meant that sufficient plantlet numbers could not be achieved to continue this study.

Discussion

The results of this study demonstrate that *Persoonia hirsuta* subsp. 'Yengo National Park' can be successfully propagated *ex situ* from both seeds and vegetative cuttings and be initiated into tissue culture. Seed propagation is the fastest method to grow plants, with 50% germination being achieved in approximately 30 days. Seedlings typically grew slightly faster and more erect than cutting grown plants and 23% of seedlings did not produce any lateral branches by the end of the study period. Although not quantified in this study (due to its destructive requirement), seedlings are thought to produce more vigorous and dense root systems than cutting grown plants early on. These results reflect previous research on *P. hirsuta* which found greatest vigour and propagation success from seeds (Emery and Offord 2019c).

That germination was highest when GA_3 was added to the agar (89.5 ± 1.6%) might indicate some level of physiological dormancy. However, germination readily occurred in all treatments and was similar to other reported *Persoonia* species (Bauer *et al.* 2004; Chia *et al.* 2016; Emery and Offord 2019a; Emery and Offord 2019b; Nancarrow 2001). Sterilising seeds in a weak bleach solution did not prevent contamination as expected. Most of the microbial contamination observed in the experiment came

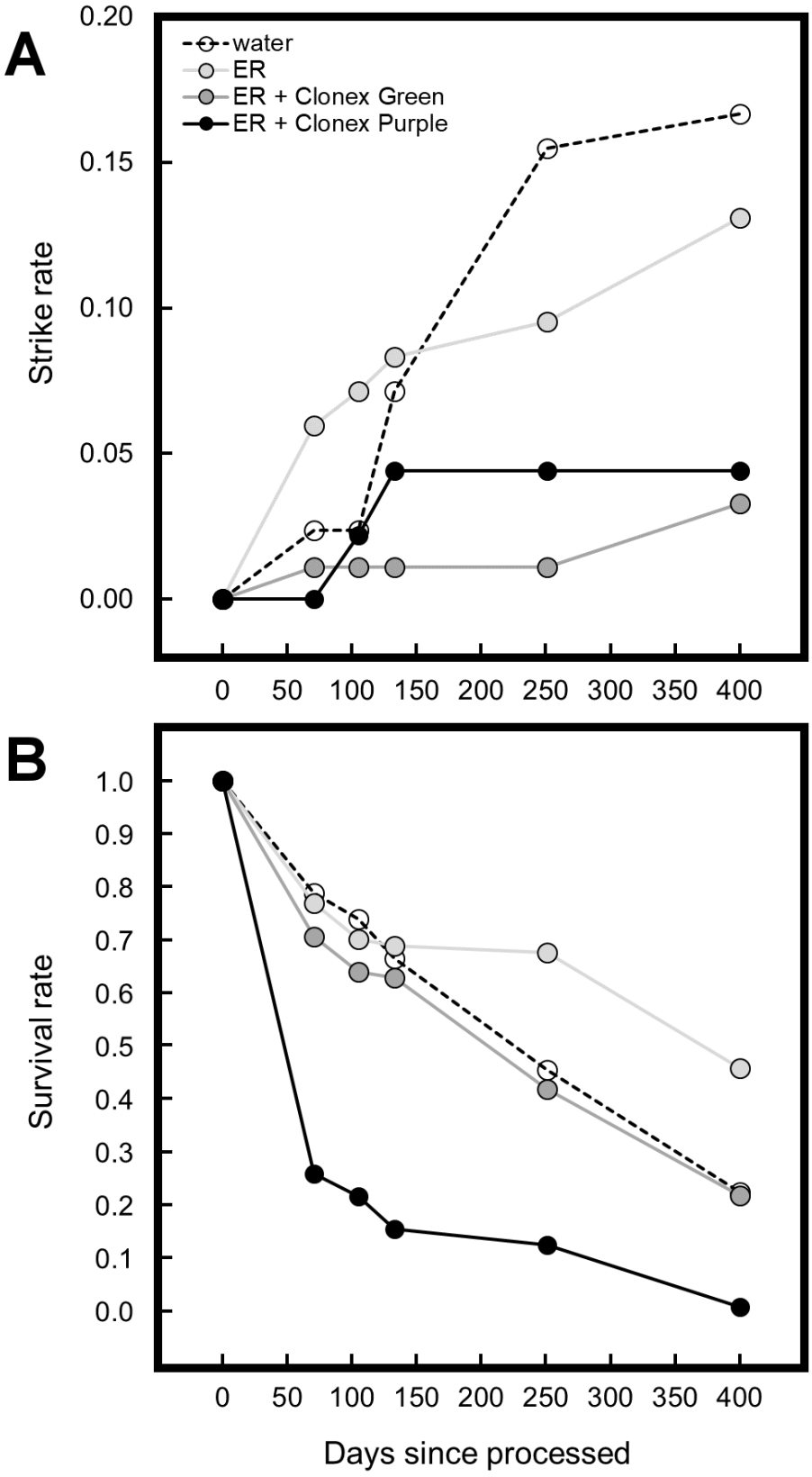

Figure 4. Graphs illustrating the strike rate (a) and survival rate (b) of *Persoonia hirsuta* subsp. 'Yengo National Park' cuttings over 400 days. Treatments were water soak (control; 'water'), EsiRook soak (ER), EsiRoot soak and Clonex green gel (ER + Clonex Green), and EsiRoot soak and Clonex purple gel (ER +Clonex Purple).

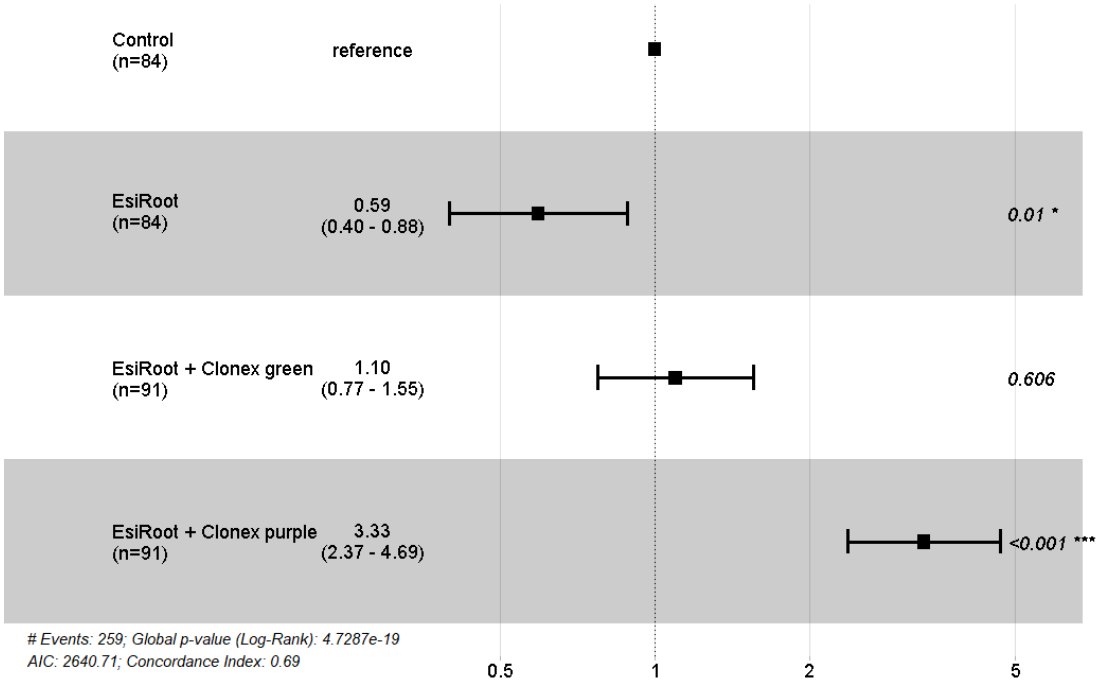

Figure 5. Hazards ratios of the root hormone treatments on the survival of *Persoonia hirsuta* subsp. 'Yengo National Park' cuttings compared to the water control treatment.

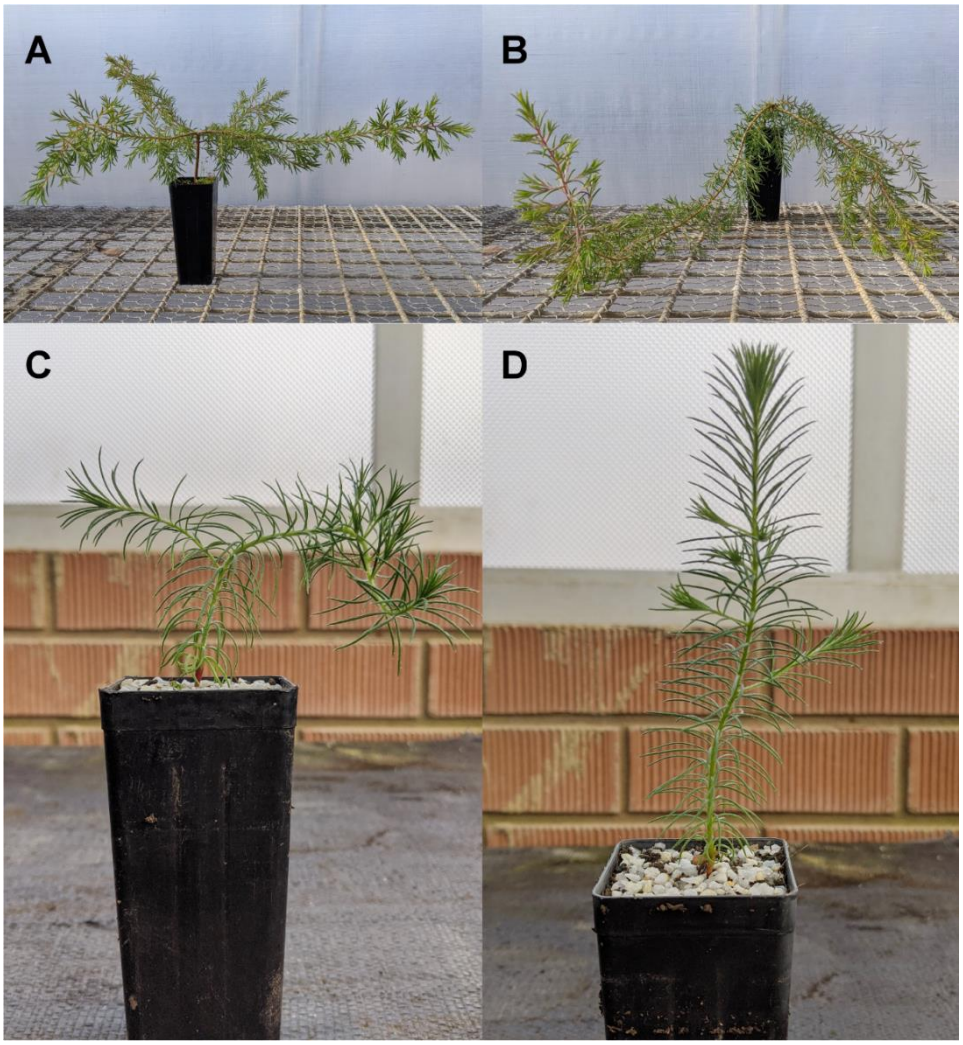

Figure 6. Examples of *Persoonia hirsuta* subsp. 'Yengo National Park' plants propagated from vegetative cuttings (A-B) or from seeds (C-D) in the Australian Botanic Garden Mount Annan nursery.

Table 1. Effect of propagation method on the net growth, relative growth rate (RGR) and health of *Persoonia hirsuta* subsp. 'Yengo National Park' plants following nine months of growth in the nursery. Data are means ± SE and different superscript letters between propagation methods for each factor are significantly different at $P = 0.05$.

Factor	Seedlings	Cuttings
Final height (cm)	27.4 ± 1.3 [a]	12.2 ± 1.5 [b]
Longest branch length (cm)	18.5 ± 2.5 [a]	15.2 ± 2.3 [a]
Net growth (cm)	16.9 ± 1.2 [a]	2.6 ± 1.9 [b]
RGR	0.12 ± 0.01 [a]	0.02 ± 0.01 [b]
Health (0-5)	4.8 ± 0.1 [a]	4.9 ± 0.0 [a]

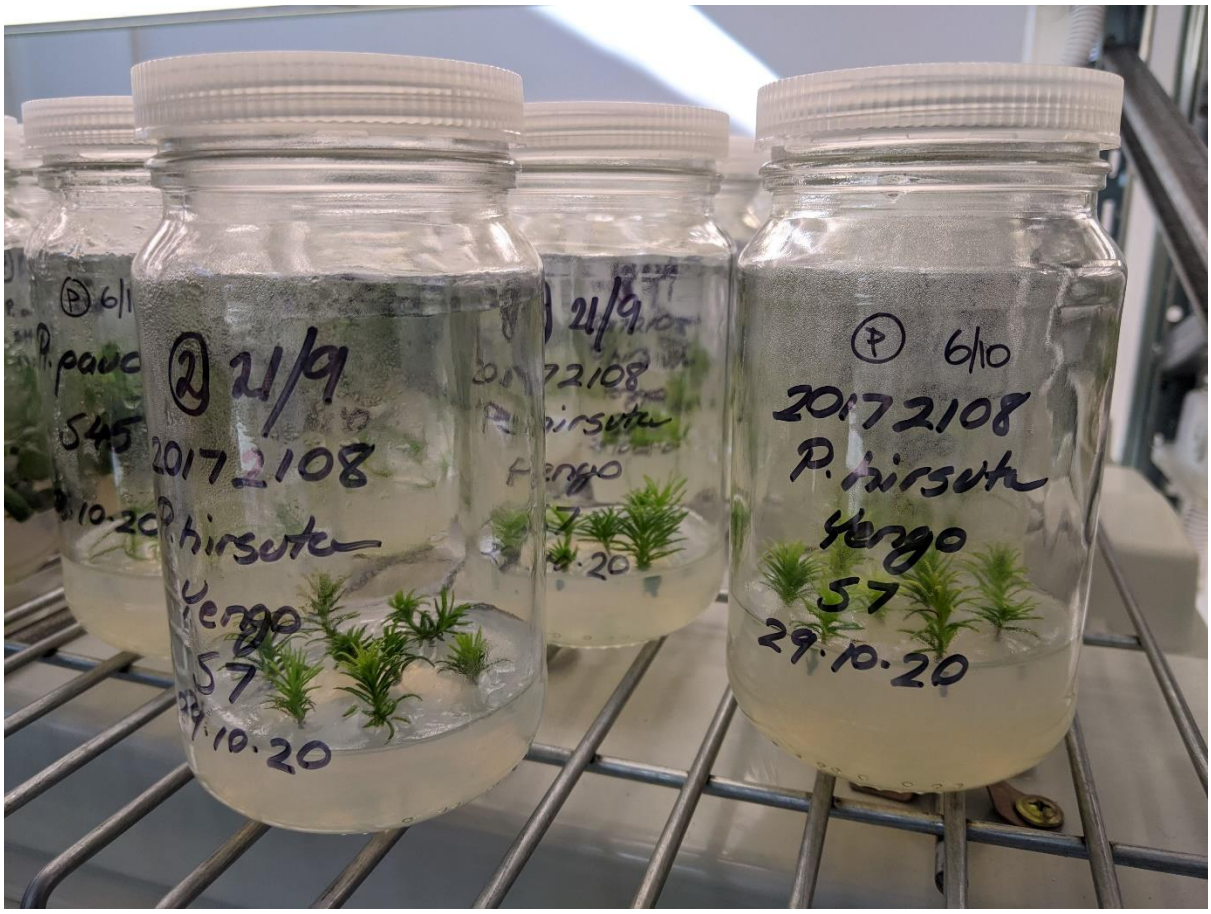

Figure 7. *Persoonia hirsuta* subsp. 'Yengo National Park' plant segments successfully initiated into tissue culture at the Australian PlantBank. The plant segments are growing on a Woody Plant Medium (WPM).

from within the seeds themselves, which suggests that surface sterilisation may be ineffective. However, microbial contamination outbreaks were contained by sowing individual seeds in well plates, negating the need to re-treat and sow seeds when contamination spreads across a Petri plate. For most *Persoonia* species, it may be preferable to germinate seeds without chemical stimulants as both GA_3 and smoke water may also promote microbial growth as similar chemical metabolites also occur in other microbes (Brian *et al.* 1954; Light *et al.* 2009).

Propagating *Persoonia* species from vegetative cuttings is a very slow and labour-intensive process that often results in mixed success. Strike rates of *P. hirsuta* subsp. 'Yengo National Park' were <20% among the four treatments. Similarly, strike rates of *P. pinifolia* and *P. chamaepitys* cuttings were comparatively low (20% and 40%, respectively) following a 1000 ppm IBA, 200 ppm NAA, and 200 ppm 2,4-dichlorophenoxy acetic acid treatment combination (Ellyard 1982). This author found that strike rates of *P. pinifolia* would almost triple when cuttings were treated a second time after two months. *P. hirsuta* subsp. 'Yengo National Park' showed a strong sensitivity to IBA which caused significant mortality of cuttings and the lowest strike rate was when Clonex Purple was used. The similarity in root strike rates among the other three treatments may reflect the variation in condition of the wild plants. Given the very low success of cuttings propagation reported here it should not be adopted for production without further experimentation or for a specific purpose such as an emergency plant rescue.

A key result from this study was that the highest root strike was for *P. hirsuta* subsp. 'Yengo National Park' cuttings soaked in water only. A similar result was reported for *Banksia dallanneyi* in Western Australia (Willyams 2015). Bauer *et al.* (1999) first reported root strike rates of *P. virgata* cuttings were significantly improved when juvenile plants were used and when cuttings were collected during seasons where rooting inhibitors were not present. Recent research on several east Australian *Persoonia* species found that strike rates improved four- to five-fold when cuttings were collected in autumn once the new growth had hardened, and when juvenile source plants were used (Catelotti and Offord 2017; Emery and Offord 2019c). In this study we collected cuttings from source plants in autumn 2019, but we were unable to examine any potential effects of plant age due to the very limited number of wild plants and the absence of juveniles.

Persoonia hirsuta subsp. 'Yengo National Park' can be successfully established in tissue culture using axillary tip cuttings. This method is more desirable than embryos for horticulture purposes as plants with specific traits can be targeted during initial sampling (Offord *et al.* 2015). Although the plantlets took to WPM, their growth was very slow, which meant that ex-flasking was not possible after 14 months in culture. Further experimentation and research are required to increase growth rates and determine whether plant production from tissue culture is possible.

Conclusion

The results of this study demonstrate that seed germination provides the fastest and most successful method to produce *Persoonia hirsuta* subsp. 'Yengo National Park' plants. Once established in potting mix, seedlings grew faster than plants propagated from vegetative cuttings during this study. However, access to *Persoonia* seeds remains a potential issue as it relies on several ecological factors, including climate, plant condition, population abundance and outcrossed pollination (Cadzow and

Carthew 2000; Emery and Offord 2019b; Field *et al.* 2005; Krauss 1994). Plant production using vegetative cuttings may be a viable option for selecting horticulturally desirable traits if juvenile plant material can be wild or *ex situ* sourced; but further experimentation is required to increase strike rate. From the results here, it is recommended to treat *P. hirsuta* subsp. 'Yengo National Park' with EsiRoot hormone and not Clonex gels.

Acknowledgements

This work was funded by the Australian Flora Foundation, with laboratory and nursery space and consumables provided by the Australian Botanic Garden Mount Annan. All plant material was collected according to NSW Scientific licence conditions (SL100569). We thank Veronica Viler for field and laboratory assistance, and Euan Mills for nursery propagation assistance.

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