Final report on the Australian Flora Foundation funded project

Propagation of *Persoonia virgarta* for the development of a new floricultural export crop.

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ABSTRACT

Propagation of *Persoonia virgarta* for the development of a new floricultural export crop.

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A study of the propagation, growth and development of *Persoonia virgata* was conducted to form a basis for the domestication of this species. *P. virgata* is an attractive, yellow-flowered shrub endemic to Australia. Stems are harvested commercially from naturally-occurring populations all year round, and sold on both the export and domestic flower markets. The cultivation of *P. virgata* as a floricultural crop is currently restricted by its propagation difficulties.

Consistent results from the seed germination experiments showed that at least half removal of the endocarp from the *P. virgata* fruit is essential for germination. This was attributed to the endocarp restricting embryo expansion and development. The morphology of *Persoonia* fruit makes the endocarp removal process extremely difficult. The endocarp is extremely hard and 1 - 2 mm thick, and is surrounded by 6 - 8 mm of mesocarp. Nonetheless, it was shown that by first removing the mesocarp, and then using pliers of correct size (by a skilled operator), the endocarp could be cracked open and the seed released. The success of germination also relied upon the reduction of microbial contamination of the seeds. This can be aided by removing the mesocarp using hydrochloric acid, and disinfesting the seed and the endocarp (E+S) with a 2 hour soak in bleach before culturing aseptically.

Rooting success of *P. virgata* cuttings was highly dependent on the use of juvenile cutting material, which was supported by the success of rooting seedling explants and the complete failure of rooting mature explants *in vitro*. Cuttings from the different seedling genotypes showed a high variation in rooting success.

This study showed that the propagation of *P. virgata*, both sexually and asexually, is possible. Further research is required to make the propagation methods outlined more commercially-viable.

1. INTRODUCTION

The genus *Persoonia* belongs to the Proteaceae family, and was named in 1798 by Edward Smith in honour of Christiaan Hendrick Persoon (1755 - 1836), a South African-born mycologist (Baines 1981; Closs and Orchard 1985). The genus has been taxonomically reviewed, with the latest review stating that there are 98 *Persoonia* species (Weston 1995). Most species are commonly known as geebungs, which was derived from the Aboriginal term jibbong, meaning 'the fruits of certain species' (Baines 1981; Closs and Orchard 1985). *P. sericea* A. Cunn. ex R. Br. has been given the common name of Silky Geebung (Baines 1981; Neldner 1992) but no record has been found of a common name for *P. virgata* R. Br.

Persoonia virgata is an attractive, yellow-flowered shrub (Plates 1.1 and 1.2) endemic to coastal areas of south-east Queensland and northern New South Wales. Stems of *P. virgata* are harvested commercially from naturally-occurring populations all year round, and sold on both the export and domestic flower markets. The most accurate data on the number of stems of *P. virgata* and other *Persoonia* species exported has been obtained from Environment Australia, who issue export licences for Australian native species (Table 1.1). This table shows that stems of *P. virgata* have been exported since 1990, with small quantities exported until 1995. However, in 1996 almost one million stems were exported, and for the first two months of 1997, 491 000 stems had been exported to the Netherlands, Germany and Switzerland. *P. longifolia* R. Br., a Western Australian species, is also bush harvested and exported in large quantities. This species has been exported since at least 1987, with half a million stems exported in 1996 and 460 000 stems exported in the first two months of 1997.

Plate 1.1: A flowering *Persoonia virgata* plant growing on a roadside at Tuan, near Maryborough.

Plate 1.2: A close-up of the flowering stems of *Persoonia virgata*.

Table 1.1: The number of stems of *Persoonia* species exported from Australia between 1987 and February 1997 (Source : Environment Australia????).

Year	***Persoonia virgata***	***Persoonia longifolia***	***Persoonia saccata***	**Other *Persoonia* spp.**
1987	0	13 791	1	1 152
1988	0	7 360	6	25
1989	0	6 818	0	28
1990	4 400	6 503	0	11
1991	770	9 070	651	1
1992	3 100	37 224	5 915	36
1993	0	84 186	2 500	18 193
1994	4 610	193 708	14 630	1 502
1995	9 500	156 180	29 300	1 350
1996	981 900	578 970	71 705	500
to Feb 1997	491 000	460 000	0	0

The selling of *P. virgata* from the Brisbane flower market (Christensen Flower Market, Rocklea Markets, 1998 pers. comm.) occurs all year round, whether the stems are in flower, bud, fruit or a vegetative state. About 150 bunches are sold per week, with 20 stems per bunch, at A$3.95 per bunch. However, during busy periods such as Christmas and Valentine's Day, the number of bunches sold is increased to 150 per day. The constant demand for this foliage would make it attractive to a flower grower to provide a constant cash flow during periods when seasonal foliage / flower crops are not available.

The Australian wildflower industry was reviewed by Karingal Consultants (1994, 1997). They presented indicative data of heavily bush harvested native species from Western Australia, with *P. longifolia* being sixth in 1993 with 798 558 stems bush harvested, and then fourth in 1995 with 1.36 million stems. The changing levels of exploitation of different genera in Western Australia has been related to factors such as:

(i) changes in conservation practices, (ii) seasonal factors, (iii) fashion changes, and (iv) urban development. Urban development was proposed as being a major factor to the loss of access to wildflowers for commercial harvesting (Karingal Consultants 1997). Therefore, to maintain the quantity and continuity of supply required for the export markets, which pay premium prices, a change is needed to move from reliance on bush harvesting to the cultivation of commercially-valuable native species.

Even though markets exist for the foliage of *P. virgata,* it has not been introduced into commercial cultivation due to extreme propagation difficulties. Markets therefore rely solely on a bush-harvested product, and the natural habitats of *P. virgata* will suffer if the level of harvesting or exploitation is continued at its current rate for many more years. Another problem of bush harvesting is that the supply and quality of the product is controlled by drought, bushfires, grazing, land development, pest and disease outbreaks and nutritional deficiencies. Watkins (1982) believes that cultivation of commercially valuable plants is essential to provide the quality and continuity of supply required by the export market. Other important environmental aspects of bush harvesting to consider are: damage to perceived low value plants which hamper the picker; the repeated removal of superior stems and flowers may have a long term effect of changing the natural genetic diversity of the population; reduction of the seed bank decreasing regeneration after fire; and the possible spread of plant pathogens, weed seeds and insects through ecosystems (Webber *et al.* 1996).

Many of the problems of bush harvesting can be overcome by the domestication of these commercially-viable plants. The domestication of a native plant species has been described as involving three processes (Webber *et al.* 1996). Firstly, a study of the plant in its natural environment, including areas such as taxonomy and distribution. The second and often most difficult process is to investigate possible propagation methods. The final process is the selection of elite types, which may be an ongoing process throughout domestication.

The domestication of plants in the Old World was reviewed by Zohary and Hopf

(1994). It was stated that genetically, domestication of fruit trees meant changing the reproductive biology of the plants, "by shifting from sexual reproduction (in the wild) to vegetative propagation (under cultivation)". Clonal propagation assured the dependable supply of desired genotypes, with fruits that were readily propagated by vegetative methods becoming the first domesticated fruits in the Near East.

Frith (1990) believes that the future development of Australian species on the export market will depend on the maintenance of breeding programmes. This allows for the development of new cultivars to meet the changing tastes of a fashion industry. However, prior research on the desired crop, such as pollination biology and seed germination protocols, need to be developed before a breeding programme can begin. The selection of superior forms of flannel flowers *(Actinotus helianthi* Labill.) (von Richter and Offord 1996), *Conospermum* species (Seaton and Webb 1996) and *Baeckea behrii* (Schlechtendal) F. Muell. (Slater 1996) are currently being investigated, which has only been possible due to the prior development of successful seed and vegetative propagation techniques.

The growing demand for cut stems of *Persoonia virgata* on the export and domestic markets suggests that domestication is essential. This species and many others from the genus *Persoonia* also have potential as ornamental crops. One Melbourne wholesale native plant producer has reported that *"Persoonia pinifolia* is also increasing in popularity, and despite increasing production numbers every year, the nursery still cannot produce enough to satisfy the demand" (Saunders 1997).

The development of propagation protocols for *P. virgata* could be used to aid the conservation of endangered species. In the 1996 edition of Rare or Threatened Australian Plants, there are 44 Persoonias listed, of which two are presumed extinct (Briggs and Leigh 1996). A Conservation Research Statement and Species Recovery Plan for *P. nutans* R. Br. [classified as being endangered by Briggs and Leigh (1988, 1996)] has been prepared by the New South Wales National Parks and Wildlife Service (G. Robertson 1996 pers. comm.). Robertson stated that *P. nutans* is "restricted to a tertiary alluvium that only occurs on the Cumberland Plain (the plain

bounded by the Blue Mountains, the Hawkesbury River, and higher sandstone areas to the south and east)". Urban development of Western Sydney is severely reducing the area of suitable habitat. The development of propagation protocols is essential for recovery programmes of endangered species in this genus.

The work reported here is to form a basis for the domestication of *P. virgata,* with the long term aim of introducing this species into commercial cultivation. This will lead to: a reduction in the reliance of bush harvesting for commercial quantities; helping conserve the communities exploited; producing a more reliable source of harvested material of better quality; and providing a new crop for commercial flower growers that can be harvested all year round for continual cash flow. The specific objectives of this study were to:

1 Determine the major factors affecting seed dormancy and develop a method for germinating seed of *P. virgata*.

2. Determine the factors influencing the rooting ability of *P. virgata* cuttings and develop a method of vegetative propagation.

2. Germination of *Persoonia* species

2.1 Introduction

McIntyre (1969) and Abbott and Van Heurck (1988) reported that regeneration of many *Persoonia* species in the wild usually occurs after a disturbance, such as bushfires. Germination after bushfires may occur because of the fracturing of the hard seed coverings due to the high, dry temperatures (Gill 1975), or due to the smoke produced from the combustion of vegetation (de Lange and Boucher 1990; Brown 1993; Dixon *et al.* 1995).

It was believed that the hard woody endocarp and a dormant embryo prevented the germination of *P. pinifolia* R. Br. seed, as germination was only achieved with extracted seed when gibberellic acid (GA_3) was applied (McIntyre 1969). Seed extracted by pliers

did not germinate when stratified for 3, 6 and 12 weeks at 1 to 2°C (34 to 36°F). A hot water treatment was applied to fruit that had the mesocarp removed by using a scalpel. The fruits were then cracked open and the seeds removed, and this also gave no germination. GA_3 was applied at 400, 266, 66.5, 13.3 and 0 ppm to extracted old (>1 year old) and young (current season's) seeds in petri dishes kept in the dark at 21°C (70°F). No untreated seed germinated but old and young seed germinated when treated with GA_3. The best results achieved were approximately 30% germination of old seeds with 400 ppm GA_3, and approximately 50% germination of young seeds with 266 ppm GA_3. However, these experiments were severely contaminated and no statistical analyses were undertaken.

It was then suggested that an endogenous inhibitor may have been responsible for limiting the germination of *P. pimifolia* seed (Rintoul and McIntyre 1975). Extracted seeds treated with GA_3 at 350 and 500 ppm gave 40 and 30% germination respectively (10 seeds per treatment). Once again, 60 - 70% contamination occurred, which limited total germination.

Emergence, early survival and growth of seedlings of six tree species in Western Australia has been investigated, including *P. longifolia* and *P. elliptica* R. Br. (Abbott 1984). Three random sites on undisturbed litter were caged to prevent fruit theft, and 30 fruit of each species placed in each site. Germination of the other species commenced within 1 month of starting the experiment, and this was when soil moisture exceeded 15%. The *Persoonia* species did not germinate. Further experiments investigated the effect of shading with no shade cloth, 50 and 80% shade; and the effect of the type and quantity of litter, comparing leaf litter removal by light raking or low intensity fire, and the addition of *Eucalyptus marginata* Donn ex Sm. litter. Five fruits of both *Persoonia* species were distributed within each of three sites for both experiments. The *Persoonia* species did not germinate under any of these treatments, which the author suggested may explain the low abundance of these species in the jarrah forest.

Abbott and Van Heurck (1988) applied numerous treatments to *P. elliptica* seed, though precise details of the treatment were not given. Treatments included: 12 hour photoperiod

versus continuous light; soaking seeds in distilled water; cool storage of seed at 4°C for several weeks; filing the ends of the seeds; hot water submersion for 15 minutes; acid scarification for 3 hours; GA_3; burning; and varying the litter type and depth as a growing medium. None of the treatments induced seed germination, even though it was found that the seed viability (tested using tetrazolium chloride) of this species was high, ranging from 48% to 100% viability depending on the population. However, a limitation of the study was that no treatments were applied in combination, preventing any interactions.

Seeds of 94 species native of Western Australia which were known to be difficult to germinate, including *P. longifolia*, have been fumigated with smoke to enhance germination (Dixon et al. 1995). Sown punnets received cool smoke in a sealed tent for 90 minutes, after which they were transferred to a glasshouse. It was not stated if the fruits had been scarified or not. *P. longifolia* did not germinate in either the control or smoke treatments. If the concentration of the smoke was too high germination may have been inhibited (Brown 1993). Of five species of Proteaceae tested by Brown (1993), only *Protea compact*a and *Serruria phylicoides* R. Br. showed improved germination with smoke extract treatments. It was stated that this was the first report of a positive response to smoke application of any species in the South African Proteaceae.

Thus previous attempts to germinate *Persoonia* seed have been unsuccessful. Controlled germination experiments have been limited by contamination problems. A variety of treatments have been applied to overcome of dormancy of *Persoonia* seed but none have given better than 50% germination. This suggests that several interacting dormancy mechanisms may be involved, namely;

(i) The mesocarp surrounding the seeds may contain germination inhibitors. Leaching the fruit or removal of the mesocarp by either fermentation or acid treatment may overcome this problem.

(ii) The hard, woody endocarp surrounding the seeds could inhibit germination by either limiting embryo development or expansion, or limiting oxygen and/or water permeability to the embryo. An increase in oxygen availability or the scarification of the endocarp (mechanical or acid) could relieve this inhibition.

(iii) *P. pinifolia* seeds may have dormant embryos. Embryo dormancy has been released in some species by stratification, leaching or the application of chemicals.

The aim of this study was to determine whether these dormancy mechanisms regulate the germination of *P. virgata* seed.

2.2 General materials and methods

Fruit collection

Fruits of *P. sericea* were collected from Murphy's Creek on 2 and 19 June and 14 July 1992. Fruits of *P. virgata* were collected from Landsborough Road on 6 September 1993 and 11 November 1997, Tuan on 13 September 1994 and Toolara State Forest 1004 on 22 July and 9 September 1995. Unless otherwise specified, the fruits were collected into paper bags and stored at ambient temperatures.

Mesocarp removal

Three mesocarp removal techniques were used throughout the germination studies. The acid treatment involved soaking the fruits in 32% hydrochloric acid (HCl) for 3 hours, with occasional stirring of the fruits. The fruits were then washed through a sieve with water to completely remove the mesocarp, and the endocarp enclosed seeds (referred to as E+S) were left to dry at ambient temperatures. Alternatively, fruits were fermented by placing them in warm water and leaving them to cool and soak for 3 days. The fruits were stirred periodically during this time. These fruits were also washed through a sieve to completely remove the mesocarp, and left to dry at ambient temperatures. This process was similar to that outlined by Macdonald (1986). A control treatment involved drying the fruits intact. The fruits were placed on germination trays lined with germination paper, and placed in a drying incubator at 20°C ± 2°C until the mesocarps were dried. The time for this to occur varied with the fruits, and generally took from 1 - 3 weeks. The dried fruits from all treatments were stored in plastic bags at ambient temperatures.

Chemical scarification of the endocarp

Fruits (or E+S) were soaked in either 98% sulphuric acid (H_2SO_4) or 5% caustic soda

(NaOH) for varying lengths of time. The fruits (or E+S) exposed to H_2SO_4 were rinsed in a solution of 5% sodium carbonate (Na_2CO_3) for 15 minutes prior to the tap water, to neutralise the acid (Macdonald 1986), while fruits (or E+S) exposed to NaOH were rinsed in tap water only.

Chemical application

After the fruits (or E+S or seeds) had been surface disinfested, the chemicals were applied by soaking the fruits (or E+S or seeds) in the chemicals for 22 hours in a laminar flow unit. The chemicals applied were either a control treatment of sterile distilled water, hydrogen peroxide (H_2O_2), or filter-sterilised gibberellic acid (GA_3), at varying concentrations.

Aseptic culturing

For each experiment, fruits (or E+S or seeds) were cultured aseptically following surface disinfestation, due to contamination problems reported in germination studies of *P. pinifolia* seeds (McIntyre 1969; Rintoul and McIntyre 1975). The fruits (or E+S or seeds) were disinfested in a solution of sodium hypochlorite (NaOCl) containing 2000 ppm chlorine, with the addition of a wetting agent (Tween 20® at a rate of 2 drops per litre). The container with fruits (or E+S or seeds), NaOCl and the wetting agent was shaken periodically for 30 minutes, during which a vacuum was applied to remove air bubbles from the surface of the fruits (or E+S or seeds) and improve contact with the disinfecting solution. Fruits (or E+S or seeds) were rinsed with sterile distilled water in a laminar flow cabinet until cultured.

The basal medium used is listed in Table 2.1. It contained the basal salts of de Fossard (1981) at half strength plus sucrose and Difco-Bacto-agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 25 minutes. The medium was placed into either 65 mL glass jars (containing 10 mL of medium when more than one seed was used per replication) or 30 mL polycarbonate tubes (containing 8 mL for single seeds).

Table 2.1: The culture medium used for germinating seeds of *Persoonia* species in an aseptic environment.

Ingredient	Concentration
Minerals	
Macronutrients	
KNO_3	10 $mmolL^{-1}$
NH_4NO_3	10 $mmolL^{-1}$
$MgSO_4.7H_2O$	1.5 $mmolL^{-1}$
$NaH_2PO_4.2H_2O$	1.0 $mmolL^{-1}$
$CaCl_2$	1.5 $mmolL^{-1}$
Micronutrients	
$CuSO_4$	0.05 $\mu molL^{-1}$
Na2MoO4	0.50 $\mu molL^{-1}$
$CoCl_2$	0.05 $\mu molL^{-1}$
H_3BO_3	75.0 $\mu molL^{-1}$
$MnSO_4.4H_2O$	50.0 $\mu molL^{-1}$
$ZnSO_4.7H_2O$	20.0 $\mu molL^{-1}$
KI	2.50 $\mu molL^{-1}$
FeNaEDTA	50.0 $\mu molL^{-1}$
Growth Factors and Amino Acids	
Inositol	0.28 $\mu molL^{-1}$
Thiamine.HCl	0.74 $\mu molL^{-1}$
Nicotinic Acid	0.02 $\mu molL^{-1}$
Pyridoxine.HCl	1.20 $\mu molL^{-1}$
Sucrose	30.0 gL^{-1}
Agar	7.5 gL^{-1}

The cultures were kept under a 16 hour daylength in a culture room maintained at a constant temperature of 25°C ± 3°C. The intensity of the lighting ranged from 5.4 to 2.7 $\mu mol\ m^{-2}s^{-1}$ (400 to 200 lux) from the inner to the outer side of the shelf respectively. The jars or tubes were re-randomised twice weekly to minimise the influence of this variation in light intensity.

Seed viability testing

Potential seed viability can be tested using the standard tetrazolium staining technique as outlined by Moore (1973). The biochemical staining using salts of tetrazolium is accepted as a primary method of germination assessment for some species such as tree species where dormancy breaking is particularly difficult (Coster 1988). In a tetrazolium test, hydrogen from the respiration processes of living tissues combines with the absorbed

tetrazolium solution to form a red, stable, non-diffusible dye (formazan). This red staining makes it possible to distinguish the living (respiring) parts of the seed from the colourless, dead parts (Moore 1973).

The viability of *Persoonia elliptica* seed has been checked with tetrazolium chloride (Abbott and Van Heurck 1988), though no details of the procedure undertaken were specified. It has been stated that specific procedures such as soaking times are often not critical to successful staining (Weber and Wiesner 1980**)**. The procedure used in this study involved removing at least half of the endocarp from the seeds. The testa surrounding the seed also had to be broken to allow sufficient penetration of the solutions.

The seeds were imbibed for 24 hours in distilled water, at ambient temperatures. The seeds were then placed in a small glass jar, and a 1.0% (w/v) solution of 2,3,5-triphenyltetrazolium chloride (TTC) was added to cover the seeds. The enclosed jar was placed in an incubator, in the dark at 40°C, for 24 hours. The TTC solution was then discarded and the seeds rinsed in distilled water. The seeds were cut longitudinally to expose the embryo, with viability determined from the degree of staining of the embryo. When the whole of the embryo stained a deep red to deep pink, the seed was recorded as being viable; a white to pale pink colouration was recorded as being non-viable (Plate 2.1). A viability percentage was calculated.

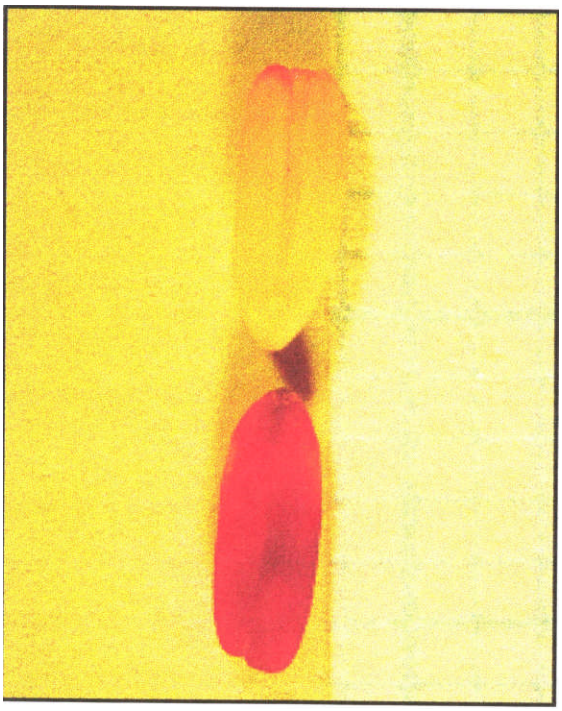

Plate 2.1: The viability of *Persoonia virgata* seeds was determined from the degree of staining of the embryo. Viable (bottom) - embryo stained a deep red to deep pink; Non-viable (top) - embryo stained pale pink or remained white.

Statistical methods

All seed germination 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 experiments with multiple seeds in each replication, a chi-square analysis was applied using the theoretical variance (Steel and Torrie 1980). The experiments were analysed using a general linear model with the statistical package SYSTAT (SYSTAT 1992). Data enumerating the proportion of seeds that germinated were transformed to angles using an arcsine transformation, with the angles expressed in radians. The arcsine transformation was used as the percentages covered a wide range. Before transformation, zero and 100 percent germination values were increased or decreased by l/4*n* percent respectively, where *n* was the number of uncontaminated seeds remaining per replicate (Steel and Torrie 1980; Gomez and Gomez 1984). Analyses of variance were applied to

the germination data, to obtain the treatment and interaction sums of squares required for the chi-square analysis. 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 experiments with single seed replicates, a chi-square analysis was applied using *r x c* contingency tables (Steel and Torrie 1980; Zar 1984). Mean separations were made using the normal approximation at a 95% confidence interval (Steel and Torrie 1980).

Any contaminated seeds within a treatment were disregarded in calculating germination percentages, to minimise any effect contamination may have had on germination percentages. Data enumerating the proportion of seeds that were contaminated, for treatments in which germination occurred, were analysed using the chi-square statistic for an *r x c* contingency table (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 was subjected to a log transformation due to the exponential distribution of the number of days to germination. This data was then analysed using analysis of variance with least significant differences (Fisher's LSD [Ott 1993]) calculated at 5 and 1% levels of significance.

2.3 Fruit morphology of *Persoonia virgata* and limitations to the study.

The fruits of *Persoonia* species are drupes (Plate 2.2), with a succulent exocarp and a thick, woody endocarp (Johnson and Briggs 1975).

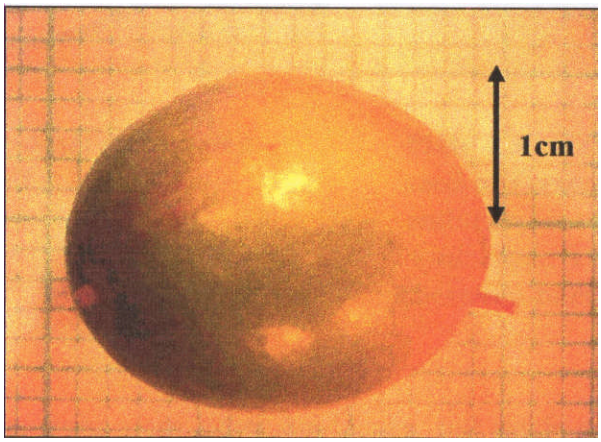

Plate 2.2: The fruits of *Persoonia virgata* are drupes.

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) (Figure 2.2). The endocarp is extremely hard and can be 1 - 2 mm thick (Plate 2.3). Krauss and Johnson (1991) describe the endocarp as "a solid, continuous woody structure composed of stone cells with very small lumina".

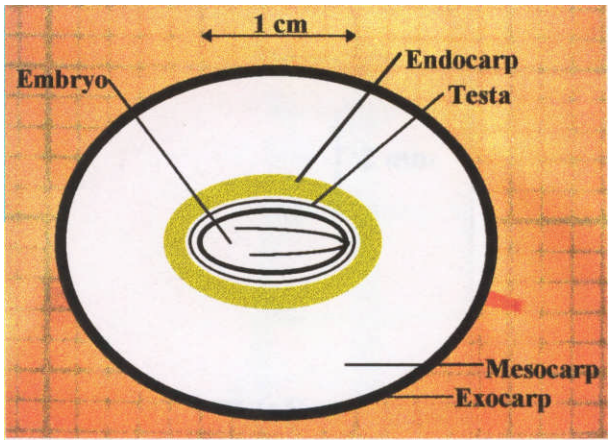

Figure 2.2: A longitudinal section of a *Persoonia virgata* fruit.

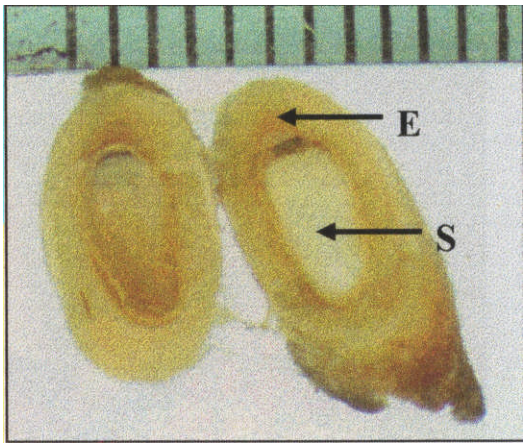

Plate 2.3: An endocarp enclosed seed of *Persoonia virgata*, with the endocarp cut longitudinally. (E = endocarp; S = seed). Space between bars equals 1mm.

In previous reports the fruits were either uni-locular and single-seeded, or obliquely bi-locular with one seed per loculus (Bentham 1870; Stanley and Ross 1986). The embryos usually contained more than two (Weston 1991), and up to six cotyledons (Strohschen 1986). There have been no reports on the development of the embryos into seedlings.

In this study 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, which was surrounded by the testa. The embryos generally developed five cotyledons. Longitudinal sections of the embryos showed that the cotyledons were 6 - 7 mm long, and recurved with germination (Plates 2.4 and 2.5). At the central base of the cotyledons is the plumule, which develops above the recurved cotyledons, and the radicle develops below the cotyledons.

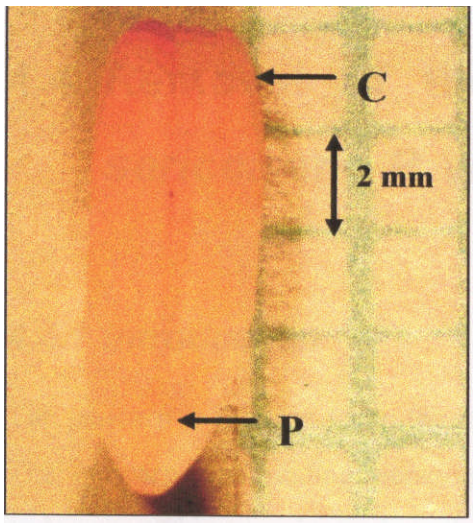

Plate 2.4: A longitudinal section of a *Persoonia virgata* embryo showing the cotyledons (C) and the plumule (P)

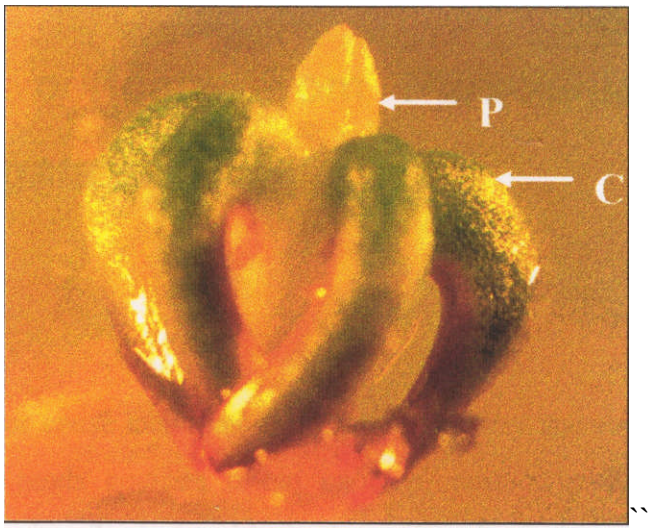

Plate 2.5: As *Persoonia virgata* embryos develop into seedlings, the cotyledons (C) recurve and the plumule (P) grows upward. (Magnification x3)

The morphology of the fruits was a major limitation in the following studies. Firstly, a method of mesocarp removal that did not harm the embryo had to be developed. Secondly, the endocarps were extremely hard and therefore very difficult to remove without damaging the seeds. A large number of seeds were wasted during this removal

process, and the process was extremely time-consuming. In some experiments the number of replications was limited due to these factors. Finally, the fruits and seeds were easily contaminated, and reducing the presence of fungi and bacteria during the germination studies was difficult. Contamination completely destroyed some experiments.

2.4 Removal of the pericarp from *Persoonia sericea* and *P. virgata* seed.

The pericarp is thought to be the major cause inhibiting germination of many species. The pericarp surrounding *Persoonia* seeds consists of an exocarp, mesocarp and endocarp. This experimental section primarily deals with the issue that the endocarp may provide a mechanical constraint to embryo development and expansion. However, other factors involved with pericarp removal will be discussed.

Complete germination of *Zamia integrifolia* seeds was achieved when the pulp was removed and the seed scarified at both ends (Smith 1978). No germination occurred without seed scarification. With all scarification treatments, germination was significantly lowered when the pulp was not removed.

The primary inhibition of seed germination in *Eucalyptus pauciflora* and *E. delegatensis* has been attributed to the mechanical resistance of the seed coat (Bachelard 1967 a,b). Extracted embryos or seeds with the coverings cut longitudinally on both sides gave 87.5 and 91.5% germination respectively, compared to 25% germination for seeds with the coverings pricked. Therefore facilitating gas and water exchange through pricking the seed coverings was not as effective in promoting germination as removing the physical resistance applied by the seed coverings.

Mechanical inhibition of the endocarp on the germination of *Persoonia sericea* and *P. virgata* seed.

The aim of these experiments was to determine whether the endocarp is a mechanical

constraint to the germination of *P. sericea* and *P. virgata* seed.

Materials and methods

Experiment I - *Persoonia sericea*

Fruits of *P. sericea* collected from Murphy's Creek on 2 June 1992 were used. Two mesocarp removal treatments (Fermented or Acid-treated), two chemical scarification treatments (1. 98% H_2SO_4, 15 min or 2. 5% NaOH, 15 min) and six endocarp removal treatments

1. Moist E+S, pierced
2. Moist E+S, ends removed
3. Moist E+S, half removed longitudinally
4. Moist E+S, majority removed
5. Dry E+S, half removed longitudinally
6. Control (none removed)

were compared. The 24 treatments were replicated three times, with four fruits per replication.

The mesocarps were removed by either fermentation or acid treatment (HCl) (Section 4.2.2). The cleaned endocarp enclosed seeds (E+S) were stored in plastic bags at ambient temperatures for 9 months, before the following treatments were applied (22 February 1993). The woody endocarp enclosing the seed was scarified with either 98% H_2SO_4 (which was neutralised with 5% Na_2CO_3) or 5% NaOH for 15 minutes. The endocarp was then removed using a sharp scalpel to minimise damage to the seed. The treatments were: a control with no endocarp removed; moist E+S that were pierced, had the ends removed, had half removed longitudinally, or 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 recorded and removed from the experiment.

Data were collected on the number of contaminated replicates, number of days to

germination, and number of germinated seeds. The germination percentages after 30, 60 and 100 days were calculated.

Experiment II - *Persoonia virgata*

Fruits of *P. virgata* were collected from Landsborough Road on 11 November 1997, and stored in a domestic refrigerator for 2 days, before the mesocarps were removed by hand using a sharp knife.

The endocarp removal treatments were : 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 correct size (Sidchrome® No. 200/28212). A mounted needle was used when necessary to extract the seeds from the endocarp and testa. Twenty replications were made of each treatment. Some E+S that were cracked open contained larvae or seed wasps, that were identified as possibly belonging to the Chalcidoidea superfamily. These seeds were not used in the experiment. Following the endocarp treatments, the seeds and E+S were surface disinfested in NaOCl solution) for 2 hours 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 replicates and germinated seeds. Total germination and contamination percentages were calculated.

An untreated sample of 40 seeds, and any seeds from the experiment that had not germinated or been contaminated, were subjected to a tetrazolium test to assess their viability.

Results

Experiment I - *P. sericea*

Mesocarp removal

There was no germination from fruits treated with HCl to remove the mesocarp, though 19.4% of fermented E+S germinated. The HCl treatment was therefore disregarded and

all the following results pertain to the fermented P. sericea E+S.

Chemical scarification of the endocarp

The germination percentages at 30, 60 and 100 days were higher in E+S scarified with NaOH rather than with H_2SO_4 (Figure 2.4). However, this positive effect of NaOH on germination percentages came at the expense of increased contamination. No E+S treated with H_2SO_4 became contaminated, whereas NaOH scarified E+S showed a significantly higher ($P<0.05$) level of contamination at 33.3%.

•

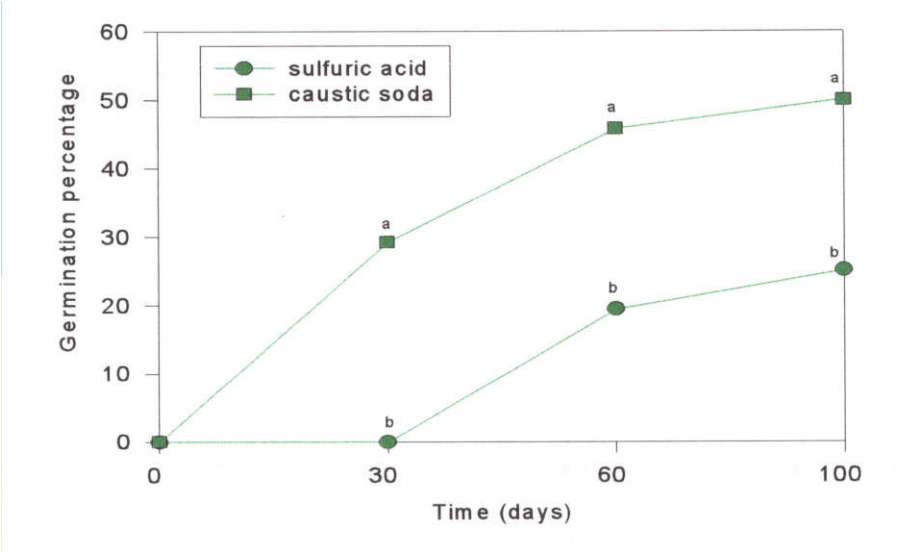

Figure 2.4: The influence of chemical scarification of the endocarp on the germination of fermented endocarp enclosed seed of *Persoonia sericea*. Mean percentage germination after 30, 60 and 100 days. Means separated using Fisher's LSD on arcsine transformed data. Different letters within a treatment time indicate significant differences at $P<0.01$.

Endocarp removal

Endocarp enclosed seeds that were either pierced, or had none or the ends of the endocarp removed, did not germinate. Moist E+S with half of the endocarp removed produced the highest mean germination percentage at 30, 60 and 100 days of the experiment, of 37.5, 64.6 and 64.6% respectively, when compared with all other endocarp removal treatments (Figure 2.5).

The amount of endocarp removed did not significantly influence the mean total contamination percentage, which was 16.7% for the three endocarp removal treatments that germinated.

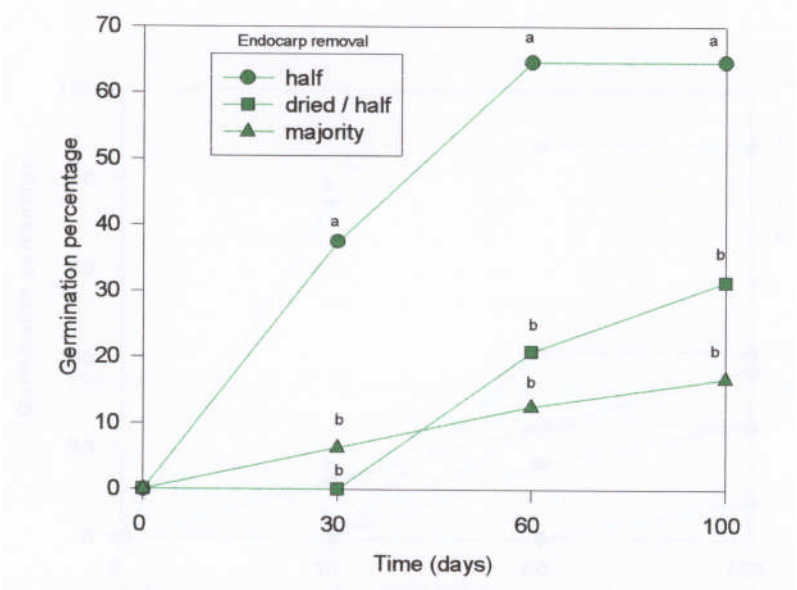

Figure 2.5: The influence of endocarp removal on the germination of fermented endocarp enclosed seed of *Persoonia sericea*. Mean percentage germination after 30, 60 and 100 days. Means separated using Fisher's LSD on arcsine transformed data. Different letters within a germination time indicate significant differences at $P<0.01$.

Chemical scarification and endocarp removal

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 no differences at day 60 or 100 (Figure 2.6). By day 30 of the experiment, E+S that had been treated with NaOH and then had half of the endocarp removed had produced 75.0% germination, with a maximum germination of 87.5% being reached by day 60. Endocarp enclosed seeds that had been treated with NaOH and then had the majority of the endocarp removed, showed 12.5% germination by day 30, though this was not significantly different to the treatment combinations that had not germinated. Treatment of E+S with NaOH followed by removal of half the endocarp reduced the mean number of days to germination (25 days) compared to all other treatment

combinations (Figure 2.7). In comparison, E+S treated with H_2SO_4 and followed by the removal of the majority of the endocarp germinated in 84 days.

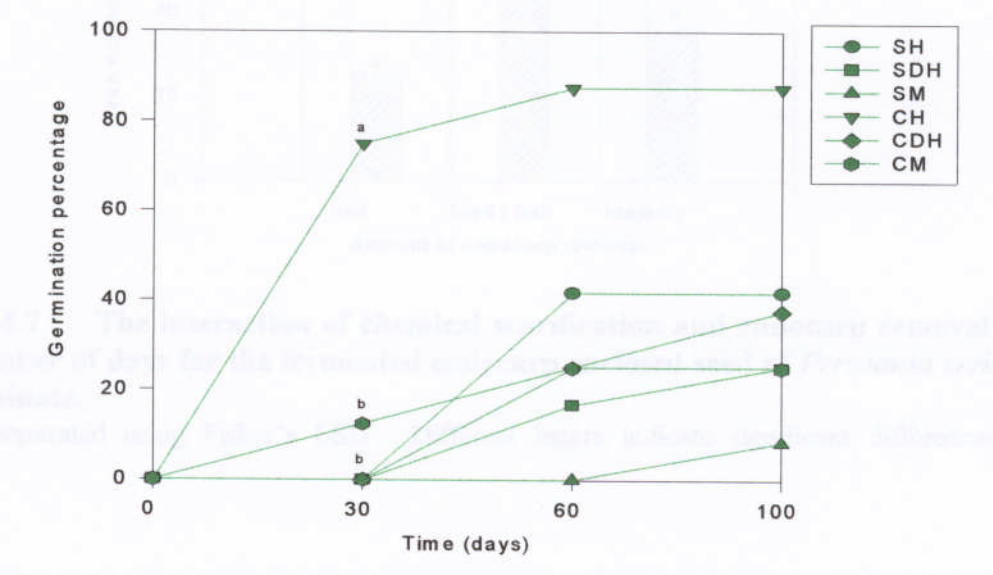

Figure 2.6: The interaction of chemical scarification and endocarp removal on germination of fermented endocarp enclosed seed of *Persoonia sericea*.

Mean percentage germination after 30, 60 and 100 days. Means separated using Fisher's LSD on arcsine transformed data. Different letters within a germination time indicate significant differences at $P<0.05$.

Legend : CDH : caustic soda and dried before half of the endocarp removed
CM : caustic soda and the majority of the endocarp removed
CH : caustic soda and half of the endocarp removed
SDH: sulfuric acid and dried before half of the endocarp removed
SM: sulfuric acid and the majority of the endocarp removed
SH : sulfuric acid and half of the endocarp removed

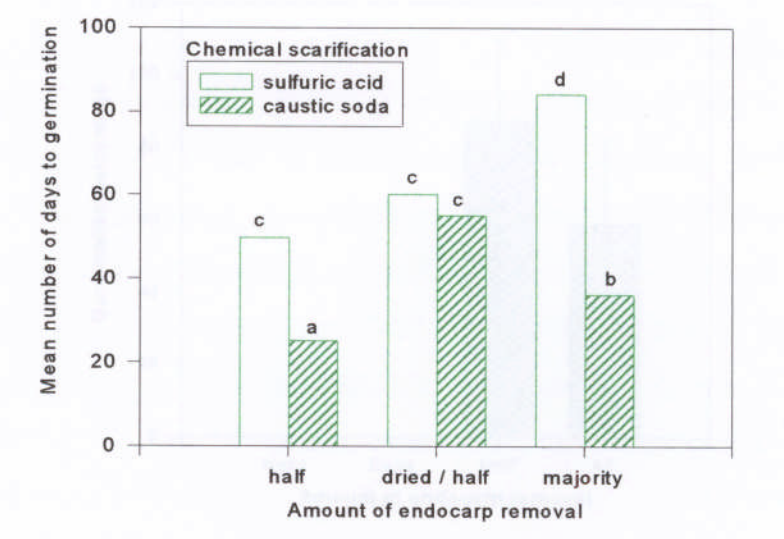

Figure 2.7: The interaction of chemical scarification and endocarp removal on the number of days for the fermented endocarp enclosed seed of *Persoonia sericea* to germinate. Means separated using Fisher's LSD. Different letters indicate significant differences at $p<0.05$.

Experiment II - *Persoonia virgata*

Germination percentages

When either no endocarp or just the ends of the endocarp were removed from *P. virgata* E+S, germination was totally inhibited (Figure 2.8). However, when either half or all of the endocarp was removed, germination percentages were 87.5 and 58.8, respectively. The tetrazolium test conducted on a sample of seed indicated that the sample was 87.5% viable, and therefore 100% germination of viable seed was obtained when half of the endocarp was removed.

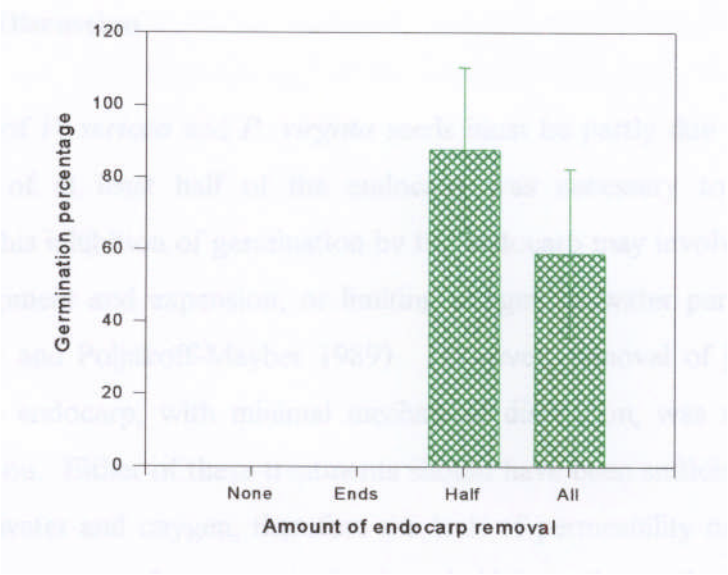

Figure 2.8: Effect of endocarp removal on the germination of endocarp enclosed seed of *Persoonia virgata*. Treatment comparisons by chi-square *r x c* contingency table, with mean separation by 95% confidence intervals at the normal approximation (indicatetd by the vertical lines).

Contamination percentages

Contamination levels were significantly influenced ($P<0.05$) by the degree of endocarp removed from the E+S, with half removal of the endocarp resulting in the highest contamination (Figure 2.9).

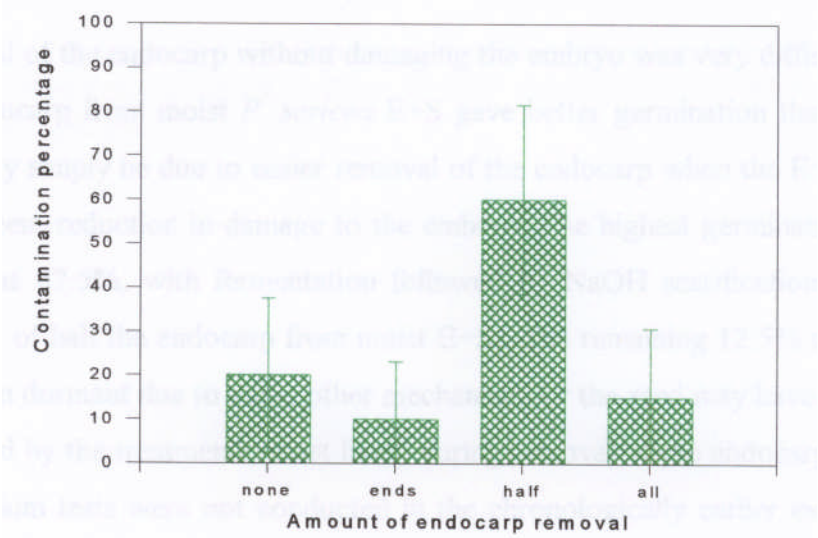

Figure 2.9: Effect of endocarp removal on the contamination of endocarp enclosed seed of *Persoonia virgata*. Treatment comparisons by chi-square *r x c* contingency table, with mean separation by 95% confidence intervals at the normal approximation (indicated by the vertical lines).

The dormancy of *P. sericea* and *P. virgata* seeds must be partly due to the endocarp since removal of at least half of the endocarp was necessary to get significant germination. This inhibition of germination by the endocarp may involve either limiting embryo development and expansion, or limiting oxygen or water permeability to the embryo (Mayer and Poljakoff-Mayber 1989). However, removal of just the ends or pricking of the endocarp, with minimal mechanical disruption, was not sufficient to cause germination. Either of these treatments should have been sufficient to enable the penetration of water and oxygen, therefore the lack of permeability mechanism seems unlikely. The requirement for removal of at least half the endocarp is consistent with a mechanical barrier to embryo growth. 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 - 91% germination (Bachelard 1967b).

Removal of the endocarp without damaging the embryo was very difficult. Removal of the endocarp from moist *P. sericea* E+S gave better germination than with dry E+S. This

may simply be due to easier removal of the endocarp when the E+S is moist and a consequent reduction in damage to the embryo. The highest germination of *P. sericea* seed was 87.5%, with fermentation followed by NaOH scarification and mechanical removal of half the endocarp from moist E+S. The remaining 12.5% of seed may have still been dormant due to some other mechanism, or the seed may have been excessively damaged by the treatments, most likely during removal of the endocarp. Unfortunately tetrazolium tests were not conducted in the chronologically earlier experiments of the study with *P. sericea* and *P. virgata* seed, as there was a lack of seed due to the wastage from inexperience in successfully removing the endocarps. It may be that only 87.5% of *P. sericea* seeds were viable, as found for *P. virgata* seeds.

Endocarp was removed from *P. virgata* E+S with a pair of pliers, which was an easier method than cutting with a scalpel. Of the seeds that did not germinate, the one remaining with half removal of the endocarp was not viable, and two of the three remaining from the complete removal of the endocarp were not viable. It is thus possible that the soft seed is being damaged whilst removing the endocarp, with germination only occurring when damage is minimised. By removing half of the endocarp it appears that total germination of viable seeds may have occurred. However, this treatment also produced the highest contamination level, which is difficult to explain.

The seeds of both *P. sericea* and *P. virgat*a 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 need to determine whether other dormancy mechanisms exist.

Endocarp removal on *Persoonia virgata* seed.

Experiments with *P. sericea* and *P. virgata* fruits have used chemical scarification in an attempt to aid the removal of the endocarps. However, the endocarp was still extremely hard to remove after 30 minutes of exposure to 98% H_2SO_4. The aim of this experiment

was to investigate the effect of longer durations of H_2SO_4 scarification on the ease of removing the endocarp from *P. virgata* seed, and on subsequent germination percentages.

Materials and methods

Fruits of *P. virgata* collected near Tuan on 13 September 1994, and stored in paper bags in a cool room maintained at 5°C and 24% relative humidity for about 9 months (9 June 1995), were used in this experiment. A comparison was made of four chemical scarification treatments combined with three endocarp removal treatments. The 12 treatments were replicated 20 times, with one seed per replicate.

Mesocarp Removal		Chemical Scarification		Endocarp Removal
Fermented		98% H_2SO_4 for 1. 1 hour 2. 3 hours 3. 6 hours 4. Control (distilled water)		1. Half removed longitudinally 2. Majority removed 3. Control (none removed)

The mesocarp was removed by fermentation. Five replications of each treatment were then prepared each day for four days, beginning on 20 June 1995. The endocarps were scarified using 98% H_2SO_4 for either 1, 3 or 6 hours, with a control of soaking the E+S for 6 hours in distilled water. The chemically scarified E+S were then cracked in a pair of pliers (Sidchrome® No. 200/28212) before having either half or the majority of the endocarp removed using a sharp scalpel. Endocarp enclosed seeds with no endocarp

removed were used as controls.

The E+S were cultured aseptically on basal medium. Radicle or cotyledon emergence through the seed coverings was assessed twice weekly for 100 days. Contaminated cultures were recorded and removed from the experiment.

Data were collected on the number of contaminated seeds, number of days to germination, and the number of germinated seeds. The germination percentages after 30, 60 and 100 days were calculated.

Results

Germination was completely inhibited when no endocarp was removed, and so this treatment was not included in the analyses of the other treatments. The duration of the chemical scarification did not influence germination percentages, time to germination, or total contamination percentage, when averaged over the endocarp removal treatments (data not shown).

Endocarp removal

Endocarp enclosed seed that had the majority of the endocarp removed gave the highest germination percentage (47.5) and germinated faster (39.0 days) than E+S that had half of the endocarp removed (26.2%, 53.7 days). The same levels of contamination were recorded for E+S that had either half or the majority of the endocarp removed (Table 2.2).

There was no significant interaction between the chemical used to scarify the endocarp and the degree of endocarp removal for percentage or time to germination, or the level of contamination (data not shown).

Table 2.2: **Effect of endocarp removal on germination, time to germination and percentage of contamination of endocarp enclosed seeds of *Persoonia virgata.***

Endocarp removal	Mean germination percentage at			Mean number of days to germination #	Mean contamination percentage *
	30 days *	60 days *	100 days *		
Half removed	0.0 b	13.8 b	26.2 b	53.7 b	23.7
Majority removed	12.1 a	43.7 a	47.5 a	39.0 a	23.7
Significance level	P<0.01	P<0.01	P.05	P<0.05	NS

Treatment comparisons by chi-square *r x c* contingency table, with mean separation by 95% confidence interval at the normal approximation. Different letters indicate significant differences. Treatment comparisons by ANOVA on log-transformed data. Mean separation by Fisher's LSD, with different letters indicating significant differences.

The chemical scarification of the endocarp did not promote germination, and the longer durations of acid treatment did not inhibit germination. The longer durations did not make the endocarp any easier to remove, or reduce any contamination problems in culture. It would therefore seem that even longer durations are required if chemical scarification is to be of any benefit in removing the endocarp for the germination of this species. Longer durations, however, increase the risk of damage to the seed as noted for *Zamia furfuracea* seed (Dehgan and Schutzman 1983). Moore *et al*. (1974) reported that longer durations of reduced acid activity were more beneficial to the germination of blackberry seeds.

Preliminary experiments with *P. sericea* E+S (L. Ketelhohn, unpubl data 1993) showed that soaking the E+S for 10 or 20 hours in 50% H_2SO_4 weakened the endocarp at a point near one end of the E+S. These endocarps were easier to remove, and always split open along the same region when using the pliers. However, no germination occurred in these

preliminary experiments. The normally white seeds were partially black after removal from the endocarp. These seeds were possibly damaged by acid penetrating through the weak point of the endocarp during the scarification treatment.

Cracking the endocarp using pliers appears to be the safest technique for removing the seed from the endocarp with minimal damage. In earlier experiments the endocarp was removed using a sharp scalpel following acid scarification. This process was extremely time-consuming and dangerous for the operator. McIntyre (1969) also used a pair of "small long nose pliers" to crack the woody endocarp of *P. pinifolia* seed, with no mention of seed damage from this process. In a later report on germinating *P. pinifolia* seed, Rintoul and McIntyre (1975) stated that "many more embryos were damaged in the method used to free the seed (cracking with a large pair of pliers)". The authors suggested that future work should possibly aim at overcoming the dormancy problem without the need of removing the seed. However, with *P. virgata* seed at least, the endocarp is very thick and hard (Plate 2.3), and germination does not occur without at least partial removal of the endocarp. Once the operator has gained some experience, cracking E+S with pliers is faster and with less wastage of seeds than endocarp removal. with just a sharp scalpel. The pliers must be the correct size for the E+S and the operator must be skilled.

2.5 Conclusions

From these seed germination studies, the following can be said about the dormancy factors which may regulate the dormancy of *Persoonia virgata* seeds :

(i) Mechanical dormancy

Removal of at least half of the endocarp longitudinally from the seed is required to relieve the mechanical constraint placed on the embryo. Care must be taken not to damage the embryo whilst removing the extremely hard endocarp. Mechanically removing the endocarp with the aid of a pair of pliers of the correct size is the easiest and fastest method developed from this study. Longer durations (up to 6 hours) of chemical scarification of the endocarp have indicated that it does not make the endocarp any easier

to remove or reduce contamination problems (data not shown).

(ii) Chemical inhibitors

While the presence or absence of chemical inhibitors in the mesocarp has not been conclusively proven, the exudate collected from *P. virgata* fruit did not inhibit the germination of radish or cucumber seeds (data not shown).

Germination experiments using *P. virgata* fruit that were fermented or dried intact resulted in the fermented fruit giving higher germination percentages. It is possible that inhibitors have been leached from the fruits during the fermentation process. A leaching and germination assay study using *P. virgata* seed is required to conclusively ascertain if water-soluble inhibitors are present in the mesocarp.

(iii) Water uptake

There was no significant water uptake by *P. virgata* seeds under imbibing conditions with or without the endocarp (data not shown). In earlier experiments, germination was still inhibited when the ends of the endocarp were removed from *P. sericea* and *P. virgata* E+S, allowing for increased water and oxygen penetration to the embryo. This shows that the overriding effect of the endocarp is its physical constraint on the embryo.

(iv) Embryo dormancy

The application of GA to fresh or cool-stored *P. virgata* seed did not promote germination, indicating that the embryos are either not dormant or that GA is not a critical factor in overcoming the dormancy (data not shown). It would appear that the embryos of *P. virgata* seeds are not dormant, as earlier findings have shown that once the endocarp is at least half removed no other treatment is required to obtain 100% germination of viable seeds.

This study has conclusively shown that contamination and the physical restriction of the endocarp surrounding the seeds of *P. virgata* are the major factors limiting germination. The endocarp must be at least half removed to relieve the mechanical dormancy that it imposes. The morphology of *Persoonia* fruit makes this process extremely difficult, as the endocarp is extremely hard and 1 - 2 mm thick, and is surrounded by 6 - 8 mm of

mesocarp. Nonetheless, it has been shown that by first removing the mesocarp, and then using pliers of correct size (by a skilled operator), the endocarp may be cracked open and the seed released without much difficulty.

3. CUTTING PROPAGATION OF *PERSOONIA VIRGATA.*

3.1 Introduction

There are few published studies on the rooting of *Persoonia* cuttings (Ellyard 1981; 1982). After 14 weeks, rooting of tip cuttings from cultivated plants of *P. pinifolia* R. Br. and *P. chamaepitys* A. Cunn. increased from 5 to 20% and from 1 to 40% respectively, when treated with a combination of 1000 ppm IBA, 200 ppm naphthalene acetic acid (NAA), and 200 ppm 2,4-dichlorophenoxy acetic acid (2,4-D) (Ellyard 1981), although only 15 cuttings were tested in each treatment. Moreover, recutting the base of any unrooted cutting and retreating it with auxins further improved rooting percentages to over 80% for both species after another 11 weeks. The timing of the plant growth regulator retreatment was further investigated (Ellyard 1982). A greater percentage of *P. pinifolia* cuttings rooted when the retreatment was applied at 8 weeks (57.5%), rather than at 12 weeks (25.0%), with rooting percentages calculated 12 weeks after the time of retreatment. Ellyard suggested from both studies that the long time the cuttings spent in the propagation environment may have resulted in either the leaching of inhibitors or the slow accumulation of phenolic compounds which, together with the auxins, may have stimulated rooting.

Several factors, which can be interrelated, are involved in limiting the rooting ability of certain woody species that are considered difficult to propagate by cuttings. The aim of this study was to determine whether the following factors are important for the rooting of *P. virgata* cuttings:

(i) Juvenile cuttings are often easier to strike roots than mature cuttings (Gardner 1929).

(ii) Differences in rooting capability may be related to anatomical factors, with the abundance of phloem fibre cells (Kachecheba 1975) or suberised cortical tissues (Williams *et al.* 1984) limiting rooting either physiologically or mechanically.

(iii) Different plant genotypes may produce varying rooting responses (Sedgley *et al.* 1991).

(iv) The time of year that the cuttings are collected may influence the rooting response. This factor may be related to physiological processes resulting from shoot growth activity (Lanphear and Meahl 1963), or rooting cofactor or inhibitor levels (Curir *et al*. 1992).

3.2 General materials and methods

Collection and handling of plant material

Plant material was collected on 2 November 1994, 7 and 14 February 1995 from container-grown stock plants of *P. virgata*, maintained at the UQG Plant Nursery. The plant material was placed into moist polyethylene bags.

The plant material was trimmed so that the cuttings were approximately 10 cm in length. The cuttings were then soaked for 10 minutes in an aqueous solution of NaOCl (600 ppm chlorine) and rinsed thoroughly in tap water.

Plant growth regulators

The base of the cutting (1 cm) was trimmed and the lower third of the leaves were removed before treating with the plant growth regulators. The plant growth regulators were applied either as a talcum powder (Stimroot®) or as a solution. The solutions were applied by dipping the basal 2cm for 3 to 5 seconds, allowing the solvent to evaporate from the cuttings before planting.

Stock solutions of the auxins IBA and NAA, and the cytokinin BAP, were prepared one or two days before the experiments. The powders (SIGMAThi, plant cell culture tested) were dissolved in 80% ethanol, with appropriate dilutions also being made with 80% ethanol. Precipitation problems were encountered in preliminary experiments with the dilution of IBA using 50% ethanol. The stock solutions were stored in glass bottles in a

refrigerator, at approximately 5°C. The BAP/IBA ratios were similar to those used for *Eucalyptus laevopinea* R. Bak. micropropagation (de Fossard 1981).

Propagation environment

The trays of cuttings were placed on benches in a propagation house. Bench heating at 25°C was controlled by a temperature sensor embedded in a bench. Hot water, at approximately 40°C, was circulated through a network of polyethylene pipes embedded in the benches covered with gravel, whenever the temperature fluctuated below 25°C.

The relative humidity of the propagation house was calculated from the wet and dry bulb temperatures (Anon. 1964) recorded hourly by a datalogger, from December 1993 until November 1995. It was maintained between 64 - 100% by a fogging system controlled by a thermostat. The trays of cuttings were manually irrigated on a daily basis.

The light intensity in the propagation house varied with the season, and also with the use of shadecloth on the roof during the summer period. The light intensities on the benches ranged from 200 microeinsteins m^{-2} sec^{-1} in September, to 65 microeinsteins m^{-2} sec^{-1} in March, with the use of Marix® tents on the benches reducing these intensities to 100 and 40 microeinsteins m^{-2} sec^{-1}, respectively (Table 3.1).

Table 3.1:The light intensity (microeinsteins m^{-2} sec^{-1}) in the propagation house at Gatton College throughout the year, measured at 12 noon on a clear day.

Environment	March	June	September	December
Outside (full sunlight)	1 800	1 200	1 550	1 900
Propagation house :				
open benches	65*	160	200	120*
tent covered benches	40*	80	100	70*

*Propagation house had outer shadecloth covering the roof during these times.

Anatomical studies

The following histological procedures were used to study the stem anatomy of *P. virgata*. The stems were fixed in formaldehyde acetic alcohol (90 : 5 : 5 of 70% ethanol : formaldehyde : acetic acid, by volume) for varying lengths of time according to the experiment. The fixed tissues were dehydrated in 70, 85, 95 and 100% tertiary butyl alcohol solutions. After dehydration, the tissues were maintained in liquid paraffin : tertiary butyl alcohol (1 : 1, v/v) for 1 hour at 40°C. The tissue was then transferred to paraffin wax for 1 hour at 60°C. The wax was decanted and replaced with more paraffin wax for 24 hours at 60°C. The tissues were then embedded with melted 58°C wax and left to solidify. Sequential sections of the stem (approximately 7 µm thick) were cut on a microtome. The sections were dewaxed by 3 x 10 minutes changes of Xylol and through a series of ethanol solutions, before being transferred to a 1% aqueous solution of Safranin O for 15 minutes staining. The sections were then dehydrated and counterstained in a 1% ethanol / clove oil based Fast Green for 15 seconds. The sections were passed through two changes of Xylol before being permanently mounted in Xam neutral mounting medium (BDH, U.K.) (Johnston 1994).

3.3 Preliminary studies

Various preliminary cutting experiments were conducted with little or no rooting success. Due to the small number of cultivated *P. virgata* stock plants, preliminary cutting experiments relied on cutting material collected from plants growing in natural populations. These plants therefore may have been water stressed and / or nutrient deficient.

One study was conducted with bush-harvested cutting material of *P. virgata* collected from State Forest 959, Gympie District in south-east Queensland in June 1993 (Ketelhohn *et al.* 1994). Less than 1% rooting was achieved. However, the results indicated that a toxicity effect was occurring when cuttings were treated with 8 000 ppm IBA as a solution. A comparison of propagation media showed that the Growool® was superior to Oasis® blocks or peat : perlite : vermiculite (1:1:1, by volume) mixture in Jiffy® peat pots, based on survival of cuttings and callus production. Oasis® blocks are

rigid, open-celled, water-absorbing foams that are recommended to be always kept moist (Smithers-Oasis, sales brochure). The use of a fogging propagation system with the manual irrigation allowed for the Growool® blocks to be not as moist as the peat mixture or as dry as the Oasis® blocks. These different water holding capacities of the media used would have a direct effect on the callus production and death of cuttings, and with the use of a different propagation environment, the results may have been different.

The next preliminary experiment was a comparison of the rooting of cuttings from bush-harvested plants and a limited number of cultivated stock plants, in March 1994. The cuttings were pulsed with sucrose (0 - 20%), and dipped in a solution of 4000 ppm IBA combined with BAP at either 0, 4, 8 or 12 ppm. The cuttings were placed into Growool® blocks, in a fogging propagation system, on benches with or without Marix ® cloth tents. The Marix® tent reduced the light intensities from 65 microeinsteins m^{-2} sec^{-1} on open benches to 40 microeinsteins m^{-2} sec^{-1} under the tent, and minimum air temperatures were increased by up to 5°C under the tent. Minimal rooting of cuttings (0.7%) resulted from this experiment. However, all of the cuttings that did produce roots originated from the cultivated stock plants and had been placed in the propagation environment without the Marix® tent. Of these cuttings, 12.5% produced roots from a range of plant growth regulator and sucrose treatments (Table 3.2). Even though there was a limited number of cuttings available from the stock plants, the results did indicate that future experiments should use cultivated stock plants, with the cuttings placed in the fogging propagation environment on open beds.

Table 3.2: The number of cuttings rooted from cultivated *Persoonia virgata* stock plants, with the listed sucrose pulsing and plant growth regulator treatments, placed in the propagation environment without the Maria® tents, ten cuttings per treatment..

Number of cuttings rooted	Plant growth regulator treatment	Sucrose pulse (%)	Number of weeks to rooting
1	4000 ppm IBA	10	25 weeks
1	4000 ppm IBA & 4 ppm BAP	0	24 weeks
1	4000 ppm IBA & 8 ppm BAP	10	20 weeks
2	4000 ppm IBA & 12 ppm BAP	10	14 and 25 weeks

3.4 Physiological age of *Persoonia virgata* cutting material *in vitro*.

Introduction

In cutting propagation of difficult-to-root woody plant species, ease of adventitious root formation often declines with the age of the parent plant. Tip cuttings from cultivated plants of *P. pinifolia* and *P. chamaepitys* have produced roots, though it took 25 weeks (Ellyard 1981). Therefore, to quickly determine if the physiological age of the stock plant is affecting the rooting of *P. virgata* cuttings, a comparison was made of the rooting ability of juvenile and mature shoots *in vitro*.

Ex vitro cuttings taken from the tip, median and basal sections of *Eucalyptus camaldulensis* Dehnh. seedlings have shown differences in rooting ability (Bachelard and Stowe 1963). Tip cuttings formed fewer roots and at a slower rate than the basal cuttings. It was suggested that substances important for root initiation, including auxins, may be more concentrated in the basal than the tip segments, or there may be gradients of hormones and / or inhibitors. The aim of this experiment was to determine if the physiological age of the explant, explant type and plant growth regulators influence the

rooting of *P. virgata* shoots *in vitro*.

Materials and methods

The experiment consisted of two physiological ages of explant material, two explant types and three agar media. The agar media contained either no auxins, 2 µM IBA + 2 µM NAA, or 4 µM IBA + 4 µM NAA). These treatments were applied factorially in a completely randomised design. There was a total of 12 treatments, with one explant age and type combination (seedling nodal explants) replicated 10 times with each medium due to the limited amount of explant material available. The other three explant age and type combinations were replicated 13 times with each media.

Vegetative shoots of *P. virgata* were collected from mature stock plants growing in the nursery and from seedlings that were growing aseptically. The shoots were trimmed to tip explants approximately 1 cm long, and the next 1 cm of the shoots were used as nodal explants.

The trimmed mature explants were rinsed in tap water for 30 minutes before surface disinfestation on 14 February 1995. The base of the explants were recut before being placed in polycarbonate tubes containing basal medium, for an initiation period in an aseptic environment. The cultures were placed in the culture room for two weeks, with only non-contaminated explants used in the experiment.

The tip and nodal cuttings of both the seedling and mature explants were transferred to polycarbonate tubes of basal medium containing either 0 µM IBA and 0 µM NAA, 2µM IBA and 2 µM NAA, or 4 µM IBA and 4 µM NAA. The cultures were placed into the culture room. The cultures were first assessed after 4 weeks for any change in appearance (29 March 1995). The explants were rated from 1 to 4, with 1 - no change, 2 - the base swollen, 3 -callus development, or 4 - root development. These ratings have been referred to as "stem ratings". The stem ratings were analysed using a generalised linear model (McCullagh and Nelder 1989) with a Poisson error and logarithmic link function. An "analysis of deviance" table was produced for the explant type and plant growth regulator applications. The deviance was tested against a chi-square distribution for significant levels. Treatments with significant differences were compared with approximate 95%

confidence intervals for the proportions. The confidence intervals were calculated using the method of Blyth and Still (1983).

Any explant unrooted at the time of this first assessment was recut at the base and transferred to new media. The cultures were finally assessed for stem ratings after another 4 weeks (8 May 1995).

Rooting percentages of the seedling explants were analysed at the time of the final assessment. A chi-square analysis was applied using an *r x c* contingency table, with significant differences compared with 95% confidence intervals, using the normal approximation (Steel and Torrie 1980).

Results

The physiological age of the explant influenced the stem ratings, with no roots produced on the mature tip or nodal explants. These explants were unchanged at both times of assessment. This treatment was therefore disregarded in the statistical analyses, and the following results pertain to the seedling *P. virgata* explants only.

Plant growth regulator applications

The auxins incorporated into the agar media significantly affected ($P<0.01$) the stem ratings of the explants, at both times of assessment, and when averaged over explant types (Figures 3.6 and 3.7). When 2 or 4 µM of IBA and NAA were added to the media, a greater proportion of explants showed root development at both times of assessment. After 4 weeks, 43 and 64% of the explants had produced roots on the media containing 2 and 4 µM of IBA and NAA, respectively (Figure 3.6). By 8 weeks, these values had increased to 65 and 82%, respectively. However, when no auxins were added to the medium, a greater proportion of explants showed no change throughout the experiment.

After 8 weeks few explants showed callus development, regardless of the level of auxins applied to the media (Figure 3.7). An analysis of total rooting percentages indicated that the media containing 2 or 4µM of IBA and NAA produced 65.2 and 81.8% rooting respectively, both of which were significantly higher ($P<0.01$) than the 13.0% rooting achieved when no auxins were present (Figure 3.8).

Explant type

The explant type (tip versus nodal) did not significantly influence the stem ratings or total rooting percentages when averaged over the plant growth regulator applications throughout the experiment (data not shown). There was no significant interaction between the plant growth regulator applications and the explant type on the stem ratings or total rooting percentages throughout the experiment (data not shown).

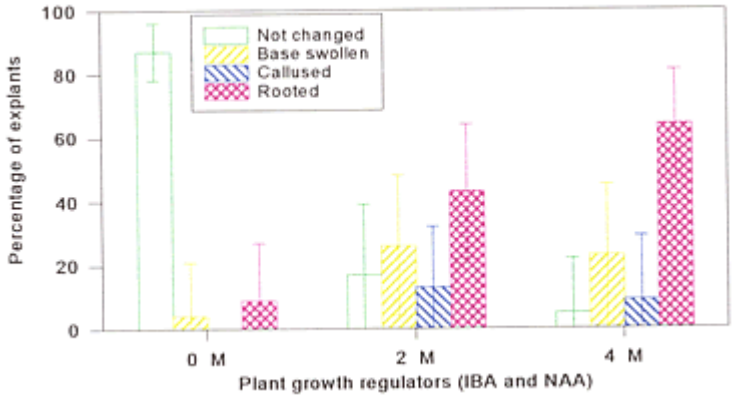

Figure 3.6: The stem ratings of *Persoonia virgata* seedling explants *in vitro*, when placed on agar media containing different levels of auxins, after 4 weeks. Vertical lines indicate mean separation by 95% confidence intervals using the normal approximation.

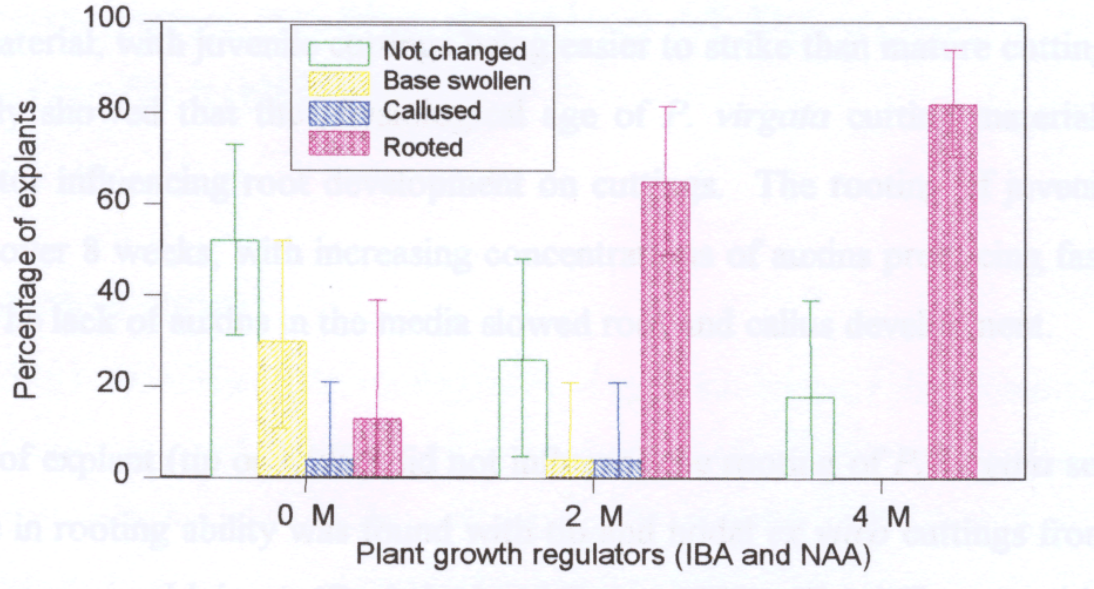

Figure 3.7: The stem ratings of seedling *Persoonia virgata* explants *in vitro*, when placed

on agar media containing different levels of auxins, after 8 weeks. Vertical lines indicate mean separation by 95% confidence intervals using the normal approximation.

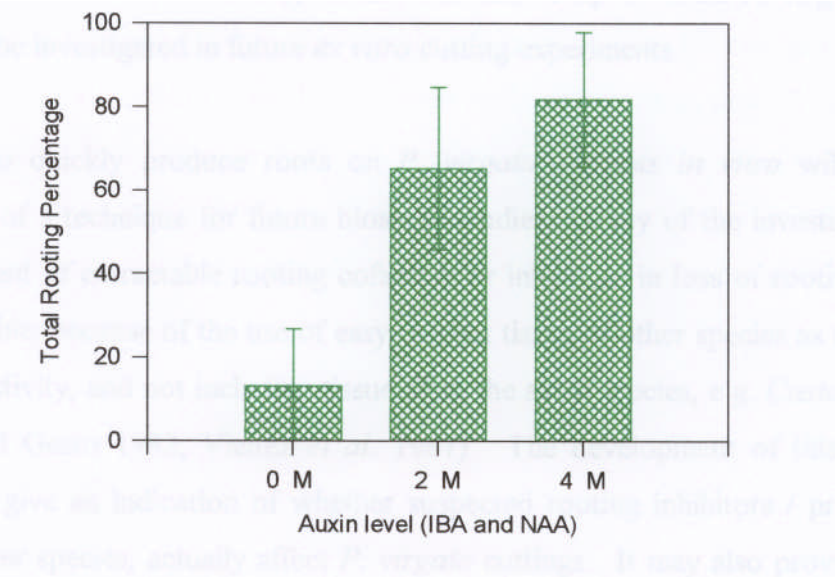

Figure 3.8: The effect of auxins on the rooting percentage of *Persoonia virgata* explants in vitro, when averaged over cutting type, at 8 weeks. Vertical lines indicate mean separation by 95% confidence intervals using the normal approximation.

Discussion

The rooting ability of many woody species is influenced by the physiological age of the cutting material, with juvenile cuttings from seedlings being easier to strike than cuttings from mature plants. This *in vitro* study showed that the physiological age of *P. virgata* cutting material may be a major factor influencing root development on cuttings. The rooting of seedling explants occurred over 8 weeks, with increasing concentrations of auxins producing faster rooting results. The lack of auxins in the media slowed root and callus development.

The type of explant (tip or nodal) did not influence the rooting of *P. virgata* seedlings. A difference in rooting ability was found with tip and nodal ex vitro cuttings from seedlings of *Eucalyptus camaldulensis* (Bachelard and Stowe 1963). The difference with *P. virgata* seedlings may be due to the smaller segments required for *in vitro* rooting, as compared

with *ex vitro*, diminishing any possible gradients of auxins or rooting inhibitors / promoters. The different environmental conditions of the *in vitro* and *ex vitro* systems, as well as the longer availability of nutrients and auxins in the agar medium for *in vitro* explants, may also influence rooting results. The use of tip or nodal *P. virgata* cuttings still needs to be investigated in future *ex vitro* cutting experiments.

3.5 Stock plant maturity, cutting type and genotype on the rooting of *Persoonia virgata* cuttings.

Introduction

Three factors may influence the rooting of *P. virgata* cuttings: (i) plant genotype, (ii) plant maturity / juvenility, and (iii) seasonal or environmental conditions. Plant genotype has affected the rooting of cuttings from other difficult-to-root Proteaceae species. When 10 cuttings were taken from eight plants each of *Banksia hookeriana* and *B. prionotes* at two-month intervals for one year, variations in the rooting ability of individual plants ranged from 0 to 80% during the winter period of high rooting (Sedgley *et al*. 1991). Similarly, genotype has influenced the rooting of *Leucospermum* cuttings, with rooting success varying from 5 to 93% (Brits 1986).

The rooting of *P. virgata* explants *in vitro* was influenced by the physiological age of the explant. No rooting of the mature explants occurred, regardless of whether it was tip or nodal, or whether auxins were supplied in the media. Both the tip and nodal explants of seedlings showed similar rooting responses to each other. This may have been due to the small segments required for in vitro rooting preventing possible gradients of rooting cofactors or auxins.

The aim of this experiment was to determine if cutting type, maturity of the stock plant and plant genotype influence the rooting of *P. virgata* cuttings.

Materials and methods

Cutting material of *P. virgata* was collected from stock plants at the UQG Plant Nursery

on 7 February (summer) 1995. Seedlings, mature plants, and plants grown from rooted cuttings that had not yet flowered (hereafter called juvenile plants), were the sources of tip and nodal cuttings. Hence, the juvenile plants may not necessarily represent six different genotypes. The seedlings were originally transplanted from either the Landsborough Road or Tuan sites, or were germinated in previous seed experiments. The mature plants had been transplanted as seedlings from the Landsborough Road site in June 1992 and had subsequently flowered.

The experiment was a completely randomised factorial design with three sources of plant material (seedlings, juvenile and mature plants) and two cutting types (tip and nodal cuttings). Superimposed on this was a comparison of different genotypes. The treatments from juvenile plants were replicated nine times (due to the lower number and size of these stock plants), and the other treatments were replicated 26 times. Each replication contained five cuttings. A reference number was allocated to each stock plant and each cutting was labeled accordingly, to allow the effect of individual plants on rooting to be measured. There were 15 seedling, six juvenile and three mature stock plants used in the experiment, and were distributed evenly between the cutting types.

Each stem was soaked in the NaOCl solution (600 ppm chlorine). Tip cuttings were 6 cm long and nodal cuttings the next 6 cm of stem. Each cutting was dipped into a solution of 1000 ppm IBA and 1000 ppm NAA, then inserted into Growool® blocks placed in wire trays and placed in the propagation house on open benches. The trays of cuttings were rotated weekly to minimise any environmental variation.

Anatomical observations were made on the segment of cuttings inserted in the Growool® blocks. Cuttings were randomly sampled from each treatment combination at the start of the experiment and 5, 10 and 15 weeks later. The segments were placed in tubes containing formalin acetic acid (FAA) until the sections were prepared and mounted on slides (July 1995). Observations were made of the continuity of phloem fibre cells forming a band.

During the fifteenth week of the experiment (23 May 1995) the cuttings were assessed for death of cutting, length of cutting, and callus or root development. Cutting length was

measured from the tip of the shoot to the top of the Growool® block. At the same time, the basal ends of non-rooted cuttings were recut and retreated with the solution of IBA and NAA and placed in new Growool® blocks. Root development was assessed again 11 months from the start of the experiment (3 January 1996) when the experiment was terminated. Cuttings were recorded and removed from the experiment at the time of rooting, and therefore a final assessment of callus was not done due to the small number of cuttings remaining.

Comparisons of rooting percentages were made with a chi-square analysis using *r x c* contingency tables. Mean separations were made using the normal approximation at 95% confidence intervals (Steel and Torrie 1980). Comparisons of the percentage of cuttings producing any callus were made with a chi-square analysis. Mean separations were made using a pairwise chi-square test. An association between callus production and rooting success was assessed with a chi-square analysis.

An analysis of variance was applied to the length of cuttings data using a general linear model with the statistical package SYSTAT (SYSTAT 1992). Least significant differences (Fisher's LSD [Ott 1993]) were calculated at 5 and 1% levels of significance, allowing for different replication numbers of some treatments.

Results

Survival of cuttings

All cuttings survived for the 11 month duration of the experiment.

Rooting percentages

Few *P. virgata* cuttings had rooted 15 weeks after insertion (May 1995), with no differences among the stock plant maturities or between cutting types. However, after eleven months (January 1996), cuttings from seedlings and juvenile plants had produced rooting percentages (32.4% for seedlings, 36.7% for juvenile plants) that were 10 times higher than mature cuttings (3.2%) ($P<0.01$), when averaged across cutting types. The cutting types did not influence the rooting percentages.

Due to the low rooting percentages obtained from the cuttings of mature plants,

comparisons of rooting percentages of cuttings from individual stock plants were made only for the seedling and juvenile plants. Five of the seedling genotypes produced 80% or greater rooting, while the remaining 10 seedling genotypes produced less than 40% rooting (Figure 3.11). Cuttings from three of the latter genotypes did not root at all.

The six juvenile plants used for cuttings produced rooting percentages between 20 and 61% which were not statistically different (Figure 3.12).

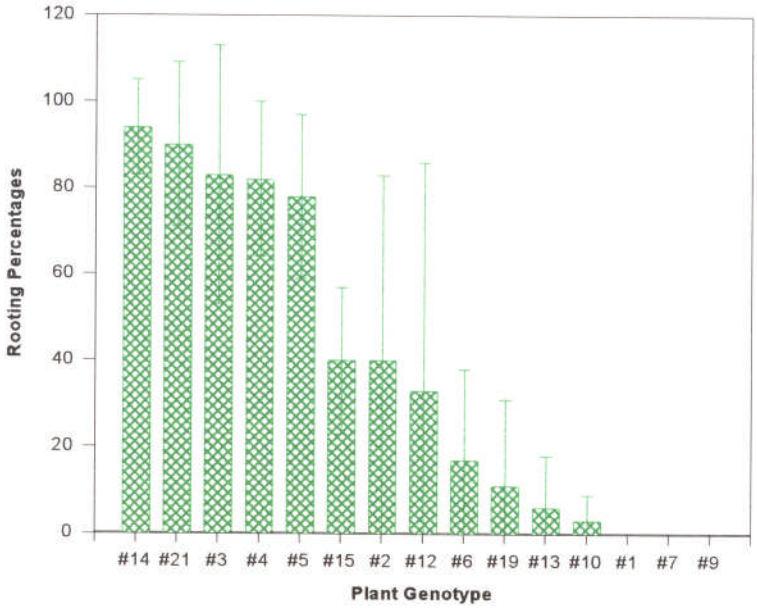

Figure 3.11: Effect of seedling plant genotypes on the rooting percentages of *Persoonia virgata* cuttings. Vertical lines indicate mean separation by 95% confidence intervals using the normal approximation. Calculated on different sample sizes for each genotype : #1 - 6; #2 - 5; #3 - 6; #4 - 17; #5 - 18; #6 - 12; #7 - 20; #9 - 48; #10 - 30; #12 - 3; #13 - 16; #14 - 17; #15 - 30; #19 - 9; #21 - 10.

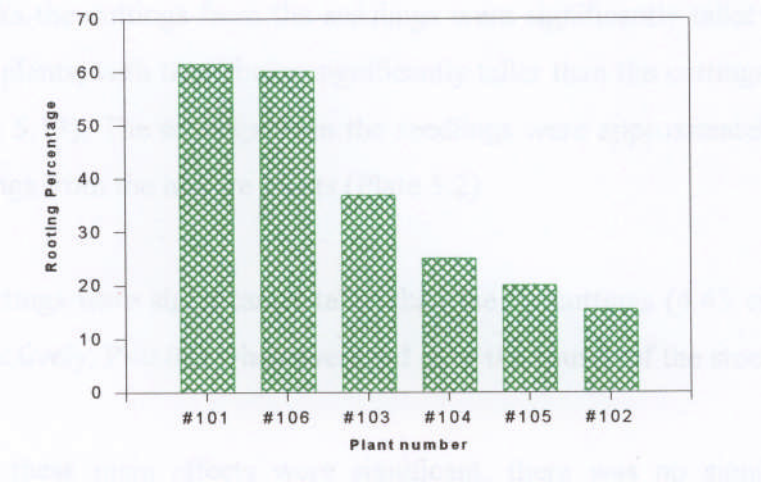

Figure 3.12: Effect of individual juvenile plants on the rooting percentages of *Persoonia virgata* cuttings. Sample sizes for each plant : #101 - 13; #102 - 13; #103 - 19; #104 - 12; #105 - 10; #106 - 10.

Length of cuttings

After 15 weeks the cuttings from the seedlings were significantly taller than the cuttings from juvenile plants, with these being significantly taller than the cuttings from the mature plants. The cuttings from the seedlings were approximately 1.5 times taller than the cuttings from the mature plants.

The nodal cuttings were significantly taller than the tip cuttings (4.43 cm compared with 3.48 cm respectively; $P<0.01$) when averaged over the source of the stock plants.

Even though these main effects were significant, there was no significant interaction between the effects of the source of the stock plant and the cutting type on the mean length of cuttings at 15 weeks of the experiment (data not shown).

Anatomical assessments

At the start of the experiment, juvenile plant nodal cuttings had limited phloem fibre bands, and tip cuttings had no bands. On the other hand, the phloem fibre cells formed nearly continuous bands in both tip and nodal cuttings from mature plants and the seedlings. After 5 weeks callus production, parenchymatous cells had disrupted the phloem fibre band so that groups of these cells were completely isolated in cuttings from

seedling, mature and juvenile plants.

Discussion

These results show that juvenility and genotype are critical factors in the rooting of *P. virgata* cuttings. Rooting percentages of seedling genotypes in this experiment ranged from zero to 94%. Furthermore, the juvenile plants that were originally propagated by cuttings must be from a line of plants with some rooting ability. Therefore the rooting ability was apparently inherited by the daughter cuttings, which would thus make it possible to select for genotypes which will root.

The differences in the rooting of genotypes may be due to the presence of a chemical inhibitor or promoter of rooting in the shoots. A component inhibitory to rooting has been found in mature cuttings of *Eucalyptus grandis* (**P**aton et al. 1970). It is possible that mature cuttings and the genotypes of *P. virgata* that are difficult to root may also contain rooting inhibitors.

Less than 4% of cuttings from mature *P. virgata* plants produced roots. The greater rootability of juvenile versus mature cuttings, particularly in woody species, has been long recognised. Gardner (1929) found that in 18 out of 21 tree species, cuttings from young seedlings rooted more readily than cuttings from older trees. The decline in rooting ability of mature cuttings has been correlated with anatomical changes in the phloem, particularly the pattern of lignification (Beakbane 1961). The anatomical examination of *P. virgata* cuttings showed greater continuity of the phloem fibre bands in both the seedling and mature stem sections, suggesting there was no causal role of lignification as a barrier to rooting ability in the cuttings from the seedlings. Furthermore, the sclerenchyma ring is always disrupted at the point of insertion of leaves and axillary buds on the stem, thus providing an opening for the growth of root primordia (Sachs et al. 1964). The close arrangement of leaves on *P. virgata* stems could result in frequent disruption of the phloem bands. Also, 5 weeks into the experiment parenchymous tissues (callus) was seen to be displacing the phloem fibre cells in *P. virgata*, and may have created larger openings for root primordia.

Callus itself has been proposed as an inhibitor of, or barrier to, rooting in balsam poplar (Cormack 1965), Douglas fir (Bhella and Roberts 1975) and red raspberry (Wu and Overcash 1971). There was no correlation between callusing and rooting success of *P. virgata* cuttings, suggesting there is no positive or negative influence of callusing. Similar percentages of both seedling and mature cuttings produced callus, whereas few mature cuttings produced roots. Furthermore, emergence of roots through callus was observed in some *P. virgata* cuttings (Plate 3.6). It would therefore appear that the callus produced is not necessarily a barrier to rooting.

It has been suggested that environmental or seasonal factors which stimulate growth inhibit root initiation and development of *Juniperus horizontalis* "Plumosa" cuttings (Lanphear and Meahl 1963). The authors postulated that rooting cofactors may not be mobilised to the site of root initiation during this period of shoot growth. This effect was not observed during the propagation of *P. virgata* cuttings, with maximum shoot growth resulting from the seedling cuttings, which subsequently rooted, and minimum shoot growth from the mature cuttings, where few produced roots. Further research is needed to determine if the time of year when *P. virgata* cuttings are collected influences rooting.

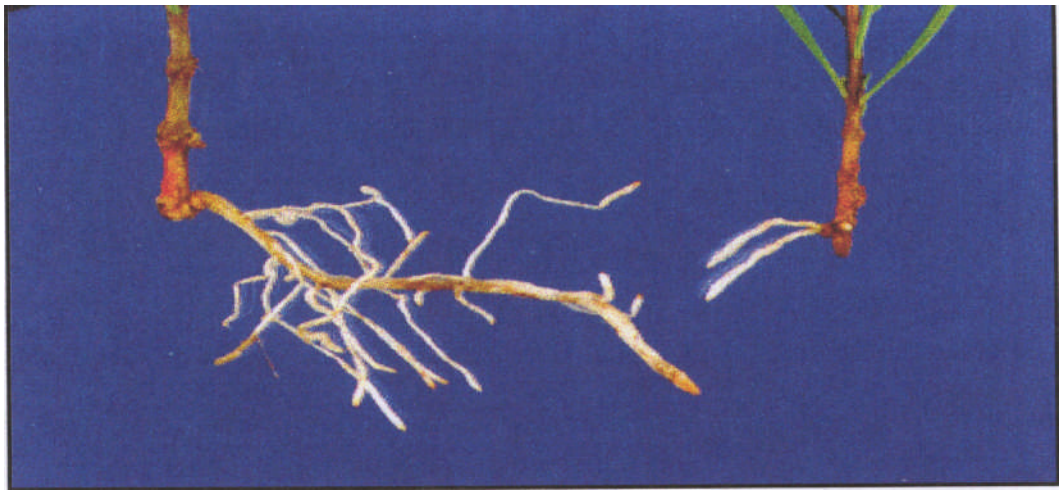

Plate 3.6: Cuttings of *Persoonia virgata* that have produced roots, with roots developing from the callus.

It is still unclear what the underlying factors are that control the rooting of *P. virgata* cuttings. As expected, seedling and juvenile plant material are superior to mature plant

material for rooting success, but the reasons for this need to be determined. Differences between genotypes in the rooting ability of cuttings from seedlings were observed.

3.6 Conclusions

From this study, the following are required for the successful rooting of *Persoonia virgata* cuttings:

(i) Juvenile cutting material roots much more readily than mature cutting material, and so it is essential that *P. virgata* stock plants are maintained in juvenile condition.

(ii) There are clear differences in rooting ability between genotypes. Therefore, selection of easy rooting genotypes is essential, as this can severely affect the overall rooting percentages obtained in a commercial operation.

4. RECOMMENDED PROPAGATION METHODS

While many questions still remain to be answered, this thesis has advanced our knowledge of the constraints on propagation of *Persoonia*. As a consequence, a more reliable method can be recommended (**Table 4.1**). For the initiation of the greatest number of new plants, a combination of seed germination and rooting of explants in vitro should be used.

Table 4.1: Recommended techniques for the propagation of *Persoonia virgata*.

Step	Technique	Reason for technique
1	HCl removal of the mesocarp then 15 min soaking in 5% Na2CO3)	May reduce microbial contamination Reduce embryo damage from HCl
2	Half to majority removal of endocarp	Relieve mechanical constraint of endocarp
3	Disinfestation of seed (2hrs NaOCl)	Reduce contamination
4	Aseptic germination of seed	Reduce contamination
5	*In vitro* culture of seedling shoot tip and nodal explants on agar medium with 4uM of IBA and NAA	Provides more than one plant from each seed.

5. REFERENCES

Abbott, I. (1984). Emergence, early survival, and growth of seedlings of six tree species in Mediterranean forest of Western Australia. For. Ecol. Manage. 9:51-66.

Abbott, I. and Van Heurck, P. (1988). Widespread regeneration failure of *Persoonia elliptica* (Proteaceae) in the northern Jarrah forest of Western Australia. J. Roy. Soc. West. Aust. 71:15-22.

Bachelard, E.P. (1967a). Effects of gibberellic acid, kinetin, and light on the germination of dormant seeds of some Eucalypt species. Aust. J. Bot. 15:393401.

Bachelard, E.P. (1967b). Role of the seed coat in dormancy of *Eucalyptus pauciflora* and *E. elagatensis* seeds. Aust. J. Biol. Sci. 20:1237-1240.

Bachelard, E.P. and Stowe, B.B. (1963). Rooting of cuttings of *Acer rubrum* L. and *Eucalyptus camaldulensis* Dehn. Aust. J. Biol. Sci. 16:751-767.

Baines, J.A. (1981). "Australian Plant Genera : An Etymological Dictionary of Australian Plant Genera". (Society for Growing Australian Plants : Chipping Norton).

Beakbane, A.B. (1961). Structure of the plant stem in relation to adventitious rooting. Nature. 192:954-955.

Bentham, G. (1870). "Flora Australiensis : A description of the plants of the Australian territory". Vol. V. (Reeve and Co. : London).

Bhella, H.S. and Roberts, A.N. (1975). Changes in origin and rate of development of root initials in Douglas-fir stem cuttings. J. Amer. Soc. Hort. Sci. 100(6):643646.

Blyth, C.R. and Still, H.A. (1983). Binomial confidence intervals. J. Amer. Statist. Assoc. 78:108-116.

Briggs, J.D. and Leigh, J.H. (1988). "Rare or Threatened Australian Plants". (Aust. National Parks and Wildlife Service : Canberra).

Briggs, J.D. and Leigh, J.H. (1996). "Rare or Threatened Australian Plants". (CSIRO : Canberra).

Brits, G.J. (1986). The influence of genotype, terminality and auxin formulation on the rooting of *Leucospermum* cuttings. In "Growing and Marketing of Proteas". Vol. 3. pp. 80-87. (Ed. P. Mathews). (Proteaflora Enterprises : Melbourne).

Brown, N.A.C. (1993). Promotion of germination of fynbos seeds by plant-derived smoke. New Phytol. 123:575-583.

Coster, R.M. (1988). The use of tetrazolium and sequential sampling as an alternative to germination testing for cereal seed. Plant Varieties and Seeds. 1:75-84.

Closs, J. and Orchard, A.E. (1985). *Persoonia* in Tasmania. Aust. Plants. 104(13):180-85.

Cormack, R.G.H. (1965). The effects of calcium ions and pH on the development of callus tissue on stem cuttings of balsam poplar. Can. J. Bot. 43:75-83.

Curir, P., Sulis, S., Bianchini, P., Marchesini, A., Guglieri, L. and Dolci, M. (1992). Rooting herbaceous cuttings of *Genista monosperma* Lam. : Seasonal fluctuations in phenols affecting rooting ability. J. Hort. Sci. 67(3):301-306.

Dehgan, B. and Schutzman, B. (1983). Effect of H_2SO_4 and GA_3 on seed germination of *Zamia furfuracea*. HortScience. 18(3):371-372.

Dixon, K.W., Roche, S. and Pate, J.S. (1995). The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. Oecologia. 101:185-192.

Ellyard, R.K. (1981). Effect of auxin combinations on rooting *Persoonia chamaepitys*

and *P. pinifolia* cuttings. Proc. Int. Plant Prop. Soc. 31:251-255.
Ellyard, R.K. (1982). The rooting of *Persoonia chamaepity*s and *P. pinifolia*. Canberra Botanic Gardens report.
De Fossard, R.A. (1981). "Plant Tissue Culture Propagation". (Filmfiche : Sydney).
Frith, G. (1990). Breeding and selection of Australian plants for homes and offices in the Northern Hemisphere. Agric. Sci. 3(5):17-20.
Gardner, F.E. (1929). The relationship between the age and the rooting of cuttings. Proc. Amer. Soc. Hort. Sci. 26:101-104.
Gill, A.M. (1975). Fire and the Australian flora : A review. Aust. For. 38:4-25.
Gomez, K.A. and Gomez, A.A. (1984). "Statistical Procedures for Agricultural Research". 2nd Ed. (John Wiley and Sons : New York).
Johnson, L.A.S. and Briggs, B.G. (1975). On the Proteaceae – the evolution and classification of a southern family. Bot. J. Linn. Soc. 70:83-182.
Johnston, M.E. (1994). The biology, harvesting and propagation of *Caustis blake*i. PhD Thesis, University of Queensland Gatton College.
Kachecheba, J.L. (1975). Anatomical aspects of the formation and growth of roots in stem cuttings of some species of *Hibiscus*. I. Stem anatomy and its relation to the formation and growth of roots. Hort. Res. 14:57-67.
Karingal Consultants. (1994). "The Australian Wildflower Industry. A Review". Rural Industries Research and Development Corporation, Research Paper No. 94/9.
Karingal Consultants. (1997). "The Australian Wildflower Industry. A Review –Second Edition". Rural Industries Research and Development Corporation, Research Paper No. 97/64.
Ketelhohn, L.M., Johnston, M.E. and Gage, J. (1994). Propagation of *Persoonia* species by seeds and cuttings. Proc. Int. Plant Prop. Soc. 44:36-40.
Krauss, S.L. and Johnson, L.A.S. (1991). A revision of the complex species *Persoonia mollis* (Proteaceae). Telopea. 4(2): 185-199.
De Lange, J.H. and Boucher, C. (1990). Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. S. Afr. J. Bot. 56(6):700-703.
Lanphear, F.O. and Meahl, R.P. (1963). Influence of endogenous rooting cofactors and environment on the seasonal fluctuation in root initiation of selected evergreen cuttings. Proc. Amer. Soc. Hort. Sci. 83:811-818.
Macdonald, B. (1986). "Practical Woody Plant Propagation for Nursery Growers". Vol. 1. (Timber Press : Portland).
McCullagh, P. and Nelder, J.A. (1989). "Generalised Linear Model". 2nd Ed. (Chapman and Hall : London).
McIntyre, D.K. (1969). The germination of dormant *Persoonia pinifolia* R.Br. seeds by the use of gibberellic acid. Canberra Botanic Gardens Report 69/6.
Mayer, A.M. and Poljakoff-Mayber, A. (1989). "The Germination of Seeds". (Pergamon Press : Oxford).
Moore, J.N., Brown, G.R. and Lundergan, C. (1974). Effect of duration of acid scarification on endocarp thickness and seedling emergence of blackberries. HortScience. 9(3):204-205.
Moore, R.P. (1973). Tetrazolium staining for assessing seed quality. In "Seed Ecology". Ch. 20. pp. 347-366. (Ed. W. Heydecker). (Butterworths : London).

Neldner, V.J. (1992). "Vascular Plants of Western Queensland". Queensland Botany Bulletin No. 11. (Qld Dept of Environ. And Heritage : Brisbane).
Ott, L. (1993). "An Introduction to Statistical Methods and Data Analysis". 3rd Ed. (PWS-Kent : Boston).
Paton, D.M., Willing, R.R., Nicholls, W. and Pryor, L.D. (1970). Rooting of stem cuttings of *Eucalyptus*: A rooting inhibitor in adult tissue. Aust. J. Bot. 18:175-183.
Rintoul, I. and McIntyre, D.K.(1975). Investigations into seed dormancy in Persoonia pinifolia. Canberra Botanic Gardens Report 75/14.
Saunders, F. (1997). Nursery in-tune with the bushland. Aust. Hort. 95(1):11-12.
Seaton, K.A. and Webb, M.G. (1996). Development of *Conospermum* for cut flower markets. Proceedings for the Fourth National Workshop for Australian Native Flowers. The University of Western Australia. Pp. 191-196.
Sedgley, M., Wirthensohn, M. and Fuss, A.M. (1991). Selection and breeding of banksias. International Protea Association Conference Proceedings. Pp. 192200.
Slater, A.T. (1996). Selection and assessment of improved cut flower forms of *Baeckea behrii.* Proceedings for the Fourth National Workshop for Australian Native Flowers. The University of Western Australia. Pp. 178-184.
Smith, G.S. (1978). Seed scarification to speed germination of ornamental cycads (*Zamia* spp.). HortScience. 13(4):436-438.
Stanley, T.D. and Ross, E.M. (1986). "Flora of south-eastern Queensland". Vol. II. (QDPI : Brisbane).
Steel, R.G.D. and Torrie, J.H. (1980). "Principles and Procedures of Statistics : A Biometrical Approach". 2nd Ed. (McGraw-Hill : Auckland).
Strohschen, B. (1986). Contributions to the biology of useful plants. 6. Anatomical studies of fruit development and fruit classification of *Persoonia pinifolia* R.Br. Angewandte Botanik. 60:257-265.
SYSTAT. (1992). "SYSTAT for Windows : Statistics". Version 5 Ed. (SYSTAT Inc. : Evanston, Illinois).
Von Richter, L. and Offord, C. (1996). Flannel flowers as cut flowers. Proceedings for the Fourth National Workshop for Australian Native Flowers. The University of Western Australia. Pp. 253-258.
Watkins, P.A. (1982). Overview of the wildflower industry in Western Australia. Proceedings of the National Technical Workshop on Production and Marketing of Wildflowers for Export. The University of Western Australia. Pp. 9-15.
Webber, J., Johnston, M., Ketelhohn, L. and O'Brien, S. (1996). Domestication of potential new floricultural crops. Proceedings of the First Australian New Crops Conference. The University of Queensland Gatton College. Vol. 2. pp. 127-134.
Weber, G.P. and Wiesner, L.E. (1980). Tetrazolium testing procedures for native shrubs and forbs. J. Seed Technol. 5(2):23-34.
Weston, P.H. (1991). *Persoonia.* In "Flora of New South Wales". Vol. 2. pp. 4-19. (Ed. G.J. Harden) (N.S.W. Univ. Press : Kensington).
Weston, P.H. (1995). *Persoonia.* Flora of Australia. 16:50-125.
Williams, R.R., Taji, A.M. and Bolton, J.A. (1984). Suberisation and adventitious rooting in Australian plants. Aust. J. Bot. 32:363-366.
Wu, L.L. and Overcash, J.P. (1971). Anatomical structure of red raspberry hybrid cuttings rooted under mist. J. Amer. Soc. Hort. Sci. 96(4):437-440.

Zar, J.H. (1984). "Biostatistical Analysis". (Prentice-Hall : London)
Zohary, D. and Hopf, M. (1994). “Domestication of Plants in the Old World – The origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley”. 2nd Ed. (Clarendon Press : Oxford).

6. A LIST OF PUBLICATIONS FROM THIS RESEARCH.

(Please note that the author's maiden name was Ketelhohn).

Book Chapters

Ketelhohn, L.M., Johnston, M.E. and Gage, J. (1996). In "Native Australian Plants, Horticulture and Uses". Ch. 5. pp. 56-67. (Eds K.A. Johnson and M. Burchett). (UNSW Press : NSW).

Refereed Journals

Bauer, L.M., Johnston, M.E. and Williams, R.R. (1999). Plant genotype, juvenility and mechanisms of inhibition of rooting *Persoonia virgata* R.Br. cuttings. Australian Journal of Experimental Agriculture. 39:1029-34.

International Edited Journals

Ketelhohn, L.M., Johnston, M.E. and Williams, R.R. (1996). Propagation of *Persoonia virgata* for commercial development. Acta Horticulturae. 454:157164.

Ketelhohn, L.M. and Johnston, M.E. (1995). The propagation of *Persoonia virgata*. Proceedings of the International Plant Propagators' Society. 45:118-121. (Paper presented as the winner of the Rod Tallis Youth Award).

Ketelhohn, L.M., Johnston, M.E. and Gage, J. (1994). Propagation of *Persoonia* species by seeds and cuttings. Proceedings of the International Plant Propagators' Society. 44:36-40. (Paper presented as the runner-up of the Rod Tallis Youth Award).

Conference Proceedings

Bauer, L., Johnston, M. and Williams, R. (1999). *Persoonias* - Potential for domestication. Proceedings of 5th Australian Wildflower Conference - "New Flowers, Products and Technologies". pp. 119-121.

Ketelhohn, L.M., Johnston, M.E. and Williams, R.R. (1996). Plant genotype and juvenility influences on the rooting of *Persoonia virgata* cuttings. Proceedings of the Fourth National Workshop of Australian Native Flowers. The University of Western Australia. pp. 90-96.

Webber, J., Johnston, M., Ketelhohn, L. and O'Brien, S. (1996). Domestication of potential new floricultural crops. Proceedings of the First Australian New Crops Conference. The University of Queensland Gatton College. Vol.2. pp. 127-134.

Ketelhohn, L.M., Johnston, M.E. and Gage, J. (1994). The propagation of *Persoonia* species. Proceedings of the Third National Workshop of Australian Native Flowers. The University of Queensland Gatton College. pp. 4.18-4.22.

Technical Papers
Johnston, M., Webber, J., Ketelhohn, L., and O'Brien, S. (1996). The domestication of potential new floricultural crops for the export market. Native Link. Winter Edition, p.4.

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