SOUTH32 Illawarra Metallurgical Coal

THE *PERSOONIA* PUZZLE: RESEARCH REPORT ON THE CONSERVATION BIOLOGY OF *PERSOONIA HIRSUTA*

Acknowledgements

This report has been prepared on behalf of South32 by Alison Haynes, consultant in plant science and ecology, and David Gregory, Environmental Specialist at South32. Thanks to staff at Royal Botanic Gardens and Domain Trust (PlantBank at Mt Annan), in particular, Dr Nathan Emery, for their help in providing data and reviewing the document and giving permission to use photographs from numerous stages of the research.

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Cover images: Nathan Emery

Abbreviations

BC: Biodiversity Conservation Act 2016
EPBC: Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) (Federal)
SPRAT: Species Profile and Threats Database
TSC: Threatened Species Conservation Act 1995 (NSW)
UOW: University of Wollongong

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1. INTRODUCING *PERSOONIA HIRSUTA,* THE HAIRY GEEBUNG

Persoonia hirsuta, the Hairy Geebung, is one of several endangered plants found in fire-prone habitats of the Sydney Basin, NSW. Preventing its extinction demands site management, but also coordinated and carefully planned research that elucidates key biological and ecological knowledge to protect current populations, supplement these and establish new populations.

South32 involvement

South32 Illawarra Metallurgical Coal (referred to as South32) became involved in facilitating and supporting research into this rare and threatened plant species when a substantial population was found on one of its mine leases. The discovery was made during a flora survey and project impact assessment at Appin North, NSW (previously known as West Cliff Colliery) (Bower 2009). The research outlined in this report was undertaken as one of several conditions of an Environment Protection and Biodiversity Conservation Act 1999 (EPBC) approval for the Bulli Seams Operation Expansion at this site, notably expansion of underground mining.

Under these conditions the company engaged experts to undertake targeted research that would inform conservation knowledge of the species, in particular, of these aspects:

- Current understanding of *Persoonia hirsuta* ecology and genetics;
- Previously documented management and conservation actions;
- Pollination biology including the requirement of pollinators;
- Soil seedbank dynamics and the role of disturbances;
- Phenology and seasonal growth;
- Genetic structure, genetic diversity, minimum population size and management actions;
- Dieback disease and control techniques;
- Impact of fire on *Persoonia hirsuta* and its habitat;
- General analysis of threatening processes and available management/mitigation actions.

The targeted research, beginning 2013, took the form of three Honours projects at the University of Wollongong, NSW, parts of a PhD project (ongoing) supervised jointly by the University of Western Sydney and scientists at the Royal Botanic Gardens and Domain Trust

(PlantBank at Mt Annan) and several projects by PlantBank included in a wider ACARP study (Australian Coal Association Research Program).

Threats to *Persoonia hirsuta*

Persoonia hirsuta is a threatened plant, listed as endangered by both NSW and federal legislation. Key threatening processes listed by NSW and the Commonwealth relate to issues such as:

Loss of habitat. Clearing, urban development and habitat fragmentation.

Degradation of habitat/disturbance. Road, fire trail and power easement maintenance, bushrock removal, recreational use of habitat (especially trail bikes), stormwater run-off and nutrient enrichment at roadsides, coal mining activity.

Fire regime. Inappropriate, in particular, too frequent fire, by both hazard reduction and other unnatural ignitions that have increased fire frequency.

Biological. Low population numbers mean these threats are more acute. The European honey bee, *Apis mellifera* may contribute to the species' rarity since it is considered an ineffective pollinator of several *Persoonia* species. Competition and encroachment from dense vegetation that results in a more closed canopy could hinder germination and survival. Browsing rabbits may destroy seedlings. Cinnamon Fungus *Phytophthora cinnamomi* may be implicated in plant death (DECCW, 2005; Rymer et al 2005).

Species description

Persoonia hirsuta is a small shrub characterised by moderately hairy leaves and branchlets, hence its common name, the Hairy Geebung. Its growth habit is spreading to decumbent, reaching 0.3 to 1.5 m tall and up to 3 m wide in dry sclerophyll woodland largely in the Sydney Basin, NSW. It shares the typical yellow flowers of the *Persoonia* genus that have a tubular appearance, are approximately 1cm long, with petals that curve backwards once opened. Flowering peaks in summer while fruits drop in spring (Emery and Offord 2018).

Like other *Persoonia* it bears a fleshy fruit known as a drupe, which is green with red-purple striations when mature and approximately 1 cm long. Inside is a single smooth pyrene comprising a thick woody endocarp surrounding a single seed. Its convex reticulate leaves are 0.75 to 5 mm wide and 0.5 to 1.5 cm long (Figure 1).

Figure 1. Life history stages of *Persoonia hirsuta.* Top left: flower and leaf. Top right: shrub form. Bottom left: cotyledons emerging after germination. Bottom right: pyrenes. Images: Alison Haynes

Habitat and range

Persoonia hirsuta is endemic to NSW, Australia, and found in the Sydney Basin, in a restricted range of approximately 150 km around Sydney on sandy soils, especially on ridges in dry woodland and sclerophyll forest, (NSW Government 2013; Figure 2). Its range is from Yengo National Park near Gosford to the north, west to the Blue Mountains and south toward Wollongong and the Southern Highlands. The species is often found as an isolated individual, but historically, populations have also been noted of up to 156 plants (Bower 2009).

Figure 2. Known range (in pink) of *Persoonia hirsuta* in the Sydney Basin. Source: Office of Environment and Heritage.

Subspecies

Currently *P. hirsuta* is considered to be two subspecies: *P. hirsuta* subsp. *hirsuta* and *P. hirsuta* subsp. *evoluta.* The first has a range from Gosford south to the Royal National Park, south of Sydney, within 20 km of the coast and below 300 m altitude, while the second has been described as having a range from Putty District to Glen Davis to Hill Top at 350–600 m altitude (Weston 1995, Weston and Johnson 1991). PlantNET lists the difference between the two as being in the leaves, with subsp *hirsuta* having linear to narrow-oblong leaves that are 0.75 to 1.5 mm wide, with revolute margins; whereas subsp *evoluta* has spathulate to elliptic leaves, 1.5 - 5 mm wide and with revolute margins. Whether in fact these differences stand up genetically is the focus of one of the studies in the current research.

Biology and ecology

Previous to the work detailed in this report, almost nothing was known specifically about the biology of *Persoonia hirsuta* beyond its morphology and apparent range and habitat preferences and the fact it is an obligate seeder, i.e. fire sensitive, with adults killed by fire. This means new recruits are only by germination of seeds in the seedbank. If fires occur before new recruits have produced new seed, it can be detrimental to the plant's survival.

Persoonia genus: background and morphology

The *Persoonia* genus belongs to the Southern Hemisphere Proteaceae family, that includes amongst other genera, *Banksia*, *Grevillea* and *Telopea*. *Persoonia* consists of 100 species, all endemic to Australia. The genus includes both rare and common species, with several listed as threatened under state and/or federal environmental law. As well as *Persoonia hirsuta,* other *Persoonia* species considered endangered include *P. pauciflora, P. bargoensis, P. hindii* and *P. glaucescens. Persoonia* are shrubs and small trees with superficially similar floral and fruit traits, but which differ dramatically in distribution and abundance. Typically, flowers are yellow but occasionally they are red to white. The plants bear fruit in the form of a fleshy 'drupe' with a single smooth pyrene inside that has a thick woody endocarp surrounding one or two seeds and a variable number of cotyledons. These drupes are shed at maturity; seeds are held mechanically dormant by the woody endocarp and in some species the seeds are also physiologically dormant, and both are difficult to break artificially and not well understood (Myerscough et al 2001).

While the flowers tend towards a common form, the leaves in contrast are highly variable, with a length across the genus from 0.75 to 35 cm and a wide range of shapes: linear to orbicular, with cross sections that are both flat or terete (cylindrical) and leaf margins that are flat, recurved and revolute.

For most of the genus, the time to first flowering, also called the primary juvenile period, is unknown, but for species for which there is data, it is between three and 10 years (Auld and Ooi 2008). However, since initial flowering can be sparse, peak seed production may occur many years later, at twice the primary juvenile period.

Ecological significance

Persoonia are considered ecologically significant because of their fleshy fruit (drupe) that provides food for vertebrates (Figure 3), but they have to date been largely omitted from post mining restoration programmes because of difficulties in propagation, germination and translocation (Catelotti and Offord 2017). *Persoonia* grow in fire-prone habitats and hence have evolved a way to persist in the landscape by one of two mechanisms. In the first, obligate seeders are killed by fire, but the seeds in the soil survive to provide new recruits; the second, resprouting, is where an adult plant is not killed, and can regenerate from protected epicormic buds that sprout new growth after the fire. Within the genus, species fall into both these categories.

Figure 3. Picture shows fruit, of a type known as a drupe, and flower bud of *Persoonia hirsuta.* Image: Nathan Emery

Fire response, germination and dormancy

Knowledge about fire response of the *Persoonia* genus is patchy. While it has been accepted for some time that seedling recruitment is most likely after fire (Nield et al 2015), many of the finer aspects, especially those affecting restoration efforts such as *ex situ* production, remain elusive for many species. For instance, research into species such as *Persoonia longifolia*, a Western Australian Geebung used in mining rehabilitation projects, has highlighted the complexity of dormancy and germination mechanisms, with questions about seed burial time in the field, the role of smoke as a trigger to germination, as well as seasonal temperature cycles and endocarp weakening (see Emery & Offord 2018).

Disturbance of some kind is expected to be essential for germination and recruitment of all *Persoonia* - whether fire, or mechanical disturbance such as is involved when building roads or grading fire trails, but beyond that, each species is likely to have unique combinations of requirements for breaking dormancy and triggering germination. Barriers to germination may be both mechanical, in terms of the rigidity of the woody endocarp, and physiological, involving the endosperm (Emery and Offord 2018; Auld and Ooi 2008).

Pollination biology

Although numerous Proteaceae can be pollinated by animals, *Persoonia* flowers are too small, so they are insect pollinated. Many *Persoonia* species are pollinated by native bees, especially *Leioproctus* and *Exoneura* species (Bernhardt and Weston 1996). While the European Honey Bee, *Apis mellifera,* has also been observed on *Persoonia* species, its effectiveness as a pollinator is questioned since it appears to carry pollen in a manner that makes it difficult to transfer to the flower's stigma (the female, pollen accepting part of the flower) (Rymer et al 2005).

Persoonia show great variation in mating systems from self-compatibility to obligate-outcrossing (Rymer et al 2005). The trait of self-compatibility (that a plant can be fertilised by its own pollen) is thought to be an evolutionary response to rarity, but both common and rare *Persoonia* are found to be self-incompatible, i.e. obligate outcrossers, bringing that concept into question.

Challenges of conservation research of rare plants

Preventing the extinction of *Persoonia hirsuta* has two key aspects. Maintaining the quantity and quality of its habitat, including aspects such as fragmentation and connectivity, is one, and out of the scope of the current research. The other is advancing the understanding of its ecology and biology with a view to managing existing populations, supplementing these and establishing new ones.

Progressing on fronts such as understanding germination cues is challenging as these are complex and can include numerous factors, each with their own complexities such as timing or chronology. In simple terms - fire followed by rain would trigger very different responses by plants than rain followed by fire.

A further challenge for threatened species is that their status has practical implications for research - they can be hard to find; populations may be small and therefore sample sizes for experiments are often limited, which can present problems statistically.

Research approach

Since so little was known about *Persoonia hirsuta*, the research initiated here was designed to provide information on:

Baseline monitoring, demography and habitat. An Honours projects at the University of Wollongong revisited previously known populations, surveyed for current plants and analysed habitat and soil characteristics. It also quantified the seedbank at several sites. South32 continued to monitor the population at Appin North for growth, health and survival.

Conservation genetics of adult populations and the soil seedbank. Quantifying and analysing genetic diversity is the first step in assessing small populations which can be at risk of inbreeding depression. Conservation genetics can also give insight into mating systems and dispersal of genes between populations. In addition, a genetics approach can support or confirm the basis of the two subspecies.

Pollination biology. Identifying pollinators of a plant, how they forage within a plant and plant population and what pollen loads they carry aids understanding of a plant's interaction with its environment.

Seed biology. Many plants have complex germination requirements, so while seed storage is a protection against extinction, it can only assist site restoration or population expansion if *ex situ* germination is possible or *in situ* recruitment is well understood.

Restoration. Early experiments to propagate *Persoonia hirsuta* failed, therefore more research was needed into propagation and translocation methods, to determine, for instance, the best way to improve strike rate of vegetative propagation or to store seeds. The first translocation of the plant was attempted at Appin North.

Drivers of dieback. Understanding environmental factors driving dieback, especially with regard to soil properties and microbe/fungi associations will support management of this species.

Fire response. A wildfire in 2013 that killed an adult population provided the opportunity to observe the fire response, in terms of seedling emergence; an ecological burn experiment was also conducted at Appin North.

The remaining parts of the report are organised according to the specific conditions of South32's approval (Table A1, Appendix 1). Many of these aspects are interrelated: seed biology affects fire response, while dieback may influence restoration, and so on. Together they build significantly on the conservation biology of this rare and endangered plant.

2. PREVIOUS MANAGEMENT AND CONSERVATION ACTIONS

Persoonia hirsuta was listed as endangered in NSW in 1998 and federally in 2000, but, excluding later projects and research mentioned in the current report, was not subject to any management or conservation actions, nor has a recovery plan been prepared. In 2016 it was added to the NSW Saving Our Species programme, allocating it to site management to prevent extinction within the next 100 years. These milestones, in addition to South32's initial management actions, are detailed below.

1998: NSW listing

In 1998 *Persoonia hirsuta* was listed as endangered after a final determination of the NSW Scientific Committee, under the Threatened Species Conservation Act (TPC). This was due to evidence of a decline in the number of locations of populations, as well as a reduction in the number of individuals. Most populations were noted as being between 10 and 20 plants, making them vulnerable to local extinction, and some populations consisted of one to three plants. The endangered listing is now covered by the Biodiversity Conservation Act 2016.

The listing notes a range of threats, including increased fire frequency due to hazard reduction burns as well as other unnatural ignitions; loss of habitat due to development and degradation of habitat due to grazing, mountain biking and stormwater management.

2000: National listing

The plant was listed as endangered, nationally, in 2000 under the Environment Protection and Biodiversity Conservation Act 1999, referencing approved conservation advice. This advice noted that threats were inappropriate fire regimes, habitat fragmentation and destruction; possibly the European Honey Bee, *Apis mellifera* (as it was considered that it might be a poor pollinator of Geebungs) and contamination by Cinnamon Fungus, *Phytophthora cinnamomi,* which can kill Geebungs.

The advice lists a number of research priorities including implementing a monitoring programme for the species; a detailed population assessment; identifying additional populations; and germination and propagation trials to identify requirements to establish populations.

The conservation advice lists numerous recommendations for local and regional action, ranging from protecting existing populations and identifying the species' optimum fire regime, to collecting and storing seed and implementing a translocation protocol.

The listing notes that no recovery plan was made; nor was any Threat Abatement Plan identified as being relevant for the species.

2003: Fire Management Plan, Yengo National Park

Persoonia hirsuta ssp evoluta is mentioned in this plan simply as being located in the park - with a single population recorded in North West Yengo National Park. Track maintenance is listed as a significant threat. The fire management regime proposes an interval of 10 to 15 years to allow for seed storage; it suggests moderate intensity fires.

2009 onwards: site management by South32

Several vegetation surveys targeting or including *Persoonia hirsuta* were carried out by Biosis (2007, 2009) FloraSearch (2009) and by Niche Environment and Heritage (2012). These surveys document a core population of the species at Appin North, NSW (referred to in the report as West Cliff Colliery since it changed name in 2016), largely on Coastal Sandstone Ridgetop Woodland.

Guided by these assessments, South32's management of *Persoonia hirsuta* has included establishing a monitoring programme at Appin North. This involved a baseline assessment of the population in 2012, recording number and location of protected plants, plant condition and age class. The population has been monitored annually since then.

As works for the planned coal wash emplacement would remove several plants, a *Persoonia hirsuta* offset area of 18.4 ha was established and was considered to be sufficient to support a population of at least 150 plants, with a density of around eight plants per hectare.

Where land was cleared of native vegetation, existing land management practices at Appin North were followed, including pre-clearing surveys and inspections. Any *P. hirsuta* plants found in the area to be cleared were either translocated or used in research.

In planning the coal wash emplacement at Appin North actions were taken to minimise disturbance to the *Persoonia hirsuta* population, for instance by using dust control measures.

The mine site population is being enhanced by a series of propagation and translocation trials by PlantBank. Further detail is provided in section six of this report.

2016: NSW Saving our Species (SoS) programme

The Saving our Species programme, launched in 2016, listed *Persoonia hirsuta* with a recommendation for site management. This programme is a NSW 'movement involving volunteers, scientists, businesses, community groups and the NSW Government' (DPIE website) that aims to reduce extinction risk of species and ecosystems using a range of conservation projects and encouraging partnerships between, for instance, corporations and the community.

It includes nine kinds of management streams, from site managed species and threatened ecological communities to 'keep watch species'. Seven priority sites are proposed for *Persoonia hirsuta,* these being: Yengo; Parr; Maroota Ridge; Fred Caterson Reserve; Cromer; West Cliff mine (now called Appin North) and Bargo.

Summary

Persoonia hirsuta has been recognised as endangered in Australia for more than twenty years but up until the current focus has been subject to very little research or conservation action. Given that fact, the various research projects detailed in this report are all the more important and will not only aid to the understanding of the species, but will also enhance conservation and recovery of both the species and the broader category of threatened *Persoonia.* The research will also improve post-mining programmes that will benefit from including the genus in restoration of native vegetation.

3. DEMOGRAPHY AND HABITAT REQUIREMENTS

To support evidence-based management of threatened species, key information about their biology and ecology is needed, yet for many of Australia's 1342 threatened plants, little data exists (Threatened Species Recovery Hub 2020). Investigation of population locations, numbers, size and condition is a fundamental step in correcting this situation and critical to managing a species, especially when assessing the need for its reintroduction and translocation. Similarly, quantifying habitat requirements can guide reestablishment efforts and help set priorities for the protection and management of habitats.

An Honours project by UOW environmental science student, Stephanie Willmott, represents the first piece of research in a systematic attempt to build such knowledge about *Persoonia hirsuta* (Willmott, 2013). While the species had been listed for many years, little progress had been made on understanding its basic ecology. This Honours project sought to confirm known populations; assess the location and size of the soil seedbank and analyse vegetation communities to find indicator species. It laid the ground work for the next two Honours projects at the university, that investigated the conservation genetics of the adult populations (Haynes 2015) and the soil seedbank (Bunker 2016).

Rationale

Without assessing known populations, it's impossible to know the conservation status of a species. While the size of the above ground, adult population is important, quantifying the seedbank gives another indication of population size (Ayre et al 2021); it also gives an insight into dispersal patterns. Predictive habitat modelling combines variables that could include biotic as well as abiotic factors - from climate and altitude, to other vegetation. Identifying indicator (or characteristic) species, easily recognisable species associated with a particular habitat, is a common practice in ecology and land management. Such information can contribute to predictions of other sites where the species of interest could be found.

Assessment of previous populations

Willmott surveyed 20 sites in the Sydney Basin where *Persoonia hirsuta* had been previously recorded - based on sightings and herbarium specimens. The furthest north was Putty, between Wollemi and Yengo National Parks; the furthest south was Balmoral in the Southern

Highlands; the most eastern was Oxford Falls in northern Greater Sydney; and the most western, Nattai, in the Macarthur region.

Overview of methods. The individuals were located by ground field surveys and for each, the following variables recorded: elevation, height and condition. The latter was based on a seven point rating scale that considered percentage cover of leaves, fraction of the leaves that were brown or dull, and the presence of fruit and flowers.

Key findings. P. hirsuta plants were found at five of the 20 sites with varying size of population. These were: Appin North (35 individuals), Couridjah (30), Yanderra (17), Balmoral (1) and Castle Hill (1). The results conferred with conservation advice that the species was in decline and populations were decreasing and becoming locally extinct. It should be noted that not finding a population where previously reported could mean one of three outcomes: i) the population no longer exists; ii) the population still exists, but due to challenges of locating it, it was not found and iii) the initial sighting was not accurate.

Plant condition at the largest population (Appin North) was on average 'moderate', with 45 - 60% leaf cover, 50% of leaves brown or dull and some fruit or flowers. Other sites (Couridjah, Yanderra and the one plant at Balmoral) scored near to 'very good' with 75 - 90% leaf cover, less than 25% of leaves brown or dull and fruit and flowers present. The largest population, at Appin North, was believed to be at least 24 years old since the last reported fire on the site was in 1989.

Other comments. It was noted that due to the presence of a seedbank, the size of a population should be considered dynamic since it could change dramatically after disturbances such as fire. Population size was regarded as small compared to other *Persoonia* species, for instance *P. bargoensis,* another threatened *Persoonia,* tends to have populations of 50-300 individuals (Field et al 2005). Willmott also highlighted the fact that the population at Appin North could be senescing, and a prescribed burn might allow recruitment before the seedbank also lost viability. All sites showed high levels of disturbance, and many plants were observed at the side of fire trails, highlighting the potential role of mechanical disturbance in recruitment for this species.

Quantifying the soil-stored seedbank

Persoonia hirsuta is one of many fire-sensitive species in fire-prone habitat within the Sydney Basin to have a soil-stored seedbank. This is potentially a rich genetic reservoir since it may represent seed from both present and past individuals on a site, as well as that brought to the site by dispersers such as vertebrates. The seedbank can be accumulated over numerous seasons and therefore also be the result of a range of mating combinations amongst the plant population. Investigation of the seedbank can provide an indication of a population's resilience and ability to recruit after disturbance such as fire. The location of seed in relation to adult plants also gives insight into dispersal processes: is the seed primarily gravity dispersed, i.e. it falls off the adult plant and remains on the ground or is it removed by other processes including vertebrates eating the fruit and dropping the seed elsewhere?

Overview of methods. To quantify the soil-stored seedbank, the soil was investigated at the five sites where *P. hirsuta* had been found. Soil in a quadrat of 1 m^2 to a depth of 10 cm was sieved to isolate and count seeds. In the three sites with more than one individual, ten quadrats were placed directly below individual *P. hirsuta* plants and ten at random within the site but no further than 20 m from a *P. hirsuta* plant. In the other sites, with only one individual, one quadrat was deployed under a plant and 19 away from it.

Key findings. Seeds were found in the top 3 cm of the soil on all sites but one. In all cases they were from quadrats under the plant and none were found in the random quadrats. No seeds were found at the Castle Hill site. Seed density was described by site, with an average of between 0.75 seeds per m^2 at the Balmoral site; 1.3 for Couridjah and 5.3 and 5.6 for Yanderra and Appin North respectively. There was a trend for larger plants to have more seed beneath them, but it was not a statistically significant difference.

Seed predation was observed and thought to be by rodents, probably the bush rat, *Rattus fuscipes* and the black rat, *Rattus rattus* since other studies have shown these to be the main predators of *Persoonia* seeds in *P. mollis* subsp *maxima, P. lanceolata and P. glaucescens* (Rymer et al 2005).

Implications/discussion. As expected, like other *Persoonia* species, it appears that seeds are mostly dispersed by gravity since they were found directly in the shadow of an adult plant, and not in the spaces between them. The evidence of a seedbank was considered a sign that the population could regenerate if burnt - but testing seed viability, and therefore whether this might be true for any of the populations, was outside the scope of this study.

Habitat requirements

Habitat suitability modelling is used in conservation to identify a species' ecological niche; predict its presence in unsurveyed sites and prioritise conservation areas. Depending on the model being used, site variables can be a range of both biotic and abiotic. Abiotic variables could include climate (such as temperature and precipitation); topography (elevation, slope); soil type and land cover. Biotic variables could include vegetation structure (foliage cover and height) and dominant vegetation as well as measurements of fauna or, for instance, biotic interactions including herbivory.

Aim. This part of Willmott's thesis aimed to identify the most relevant environmental factors that might be used in a species distribution model for *Persoonia hirsuta.* The environmental variables measured at each site were:

- vegetation structure: canopy, mid storey and ground cover (including dominant structure);
- elevation;
- rock outcrop;
- disturbance;
- soil particle size;
- soil composition;
- ground cover - coarse fragments and leaf litter.

Overview of methods. Sampling involved 20 m quadrats and smaller quadrats inside these. Sites were the 20 locations surveyed for *Persoonia hirsuta.* At each study site a single 20 m x 20 m quadrat was chosen at random, but since the Appin North site was so variable, six quadrats were surveyed there.

Key findings

Canopy species. 18 canopy species were found across the sites, and in the sites where *P. hirsuta* persisted, there were nine canopy species: *Angophora costata, Banksia serrata, Eucalyptus gummifera, E. haemastoma, E. oblonga, E. piperata, E. punctata, E. sclerophylla* and *E. sieberi.* However, statistically there was no difference between sites.

Mid-storey species. Three mid-storey species were found to be associated with persistence of *P. hirsuta*: *Isopogon anemonifolius, Lambertia formosa* and *Leptospermum trinervium.*

Groundcover. In sites where *P. hirsuta* persisted, one species was found to be associated: *Bossiaea obcordata.*

Soil. On sites where *P. hirsuta* was found, percentage sand ranged from 56.74% in Yanderra to 64.53% in Appin North; silt from 31.27% in Couridjah to 38.51% in Balmoral and clay from 3.54% in Balmoral to 5.02% in Castle Hill. Willmott found that at the sites where *Persoonia hirsuta* still existed, the average soil particle size was smaller than for other sites: ranging from 116 µm at Castle Hill to 223 µm at the Appin site.

Elevation and site prediction. Sites with *Persoonia hirsuta* ranged from 106 m above mean sea level to 476 m. Other sites predicted by the habitat characteristics were Scheyville National Park, Oxford Falls and Thirlmere Lakes National Park.

Overall conclusion

For practical reasons, study sites for all aspects of this research are limited to locations which once supported, or were at least believed to have supported a *Persoonia hirsuta* plant, so while information on co-occurring vegetation and other habitat characteristics is useful, its value for habitat prediction is limited since all sites were potentially viable for the species. Nonetheless, this work confirmed the presence of five populations and supported the opinion that the species was in decline. It quantified the seedbank and indicated its location as mainly below adult plants as expected. All sites where *Persoonia hirsuta* had persisted had sand content of more than 50%. This is in line with reports that the plant favours sandy to stony soils (Bower 2009) and an association with habitats such as sandstone ridgetop woodland and sandstone plateau heath and woodland and other dry sclerophyll habitats. The research outlined in this section by Willmott laid the grounds for subsequent Honours projects at UOW, particularly with regard to population sites and seedbank location.

The study also highlighted the senescence occurring in the Appin North population and the concern over the absence of a germination cue (disturbance by heat or mechanical at this site). Willmott's thesis was a key driver of propagation and translocation trials at PlantBank as well as the burn trial at Appin North.

Other populations

Finding rare plants in small populations can be difficult, especially when not in flower. Further to the sites identified here, other locations of *Persoonia hirsuta* have since been located. (Willmott 2013, Emery and Offord 2019a, DPIE, 2021). The currently known sites are (Figure 4):

- Appin
- Avon Dam Catchment
- Bargo
- Castle Hill
- Couridjah
- Currarong (Blaxland Ridge)
- Glenorie
- Kenthurst
- Long Point
- Maroota Ridge
- Parr
- Wedderburn
- Yanderra
- Yengo

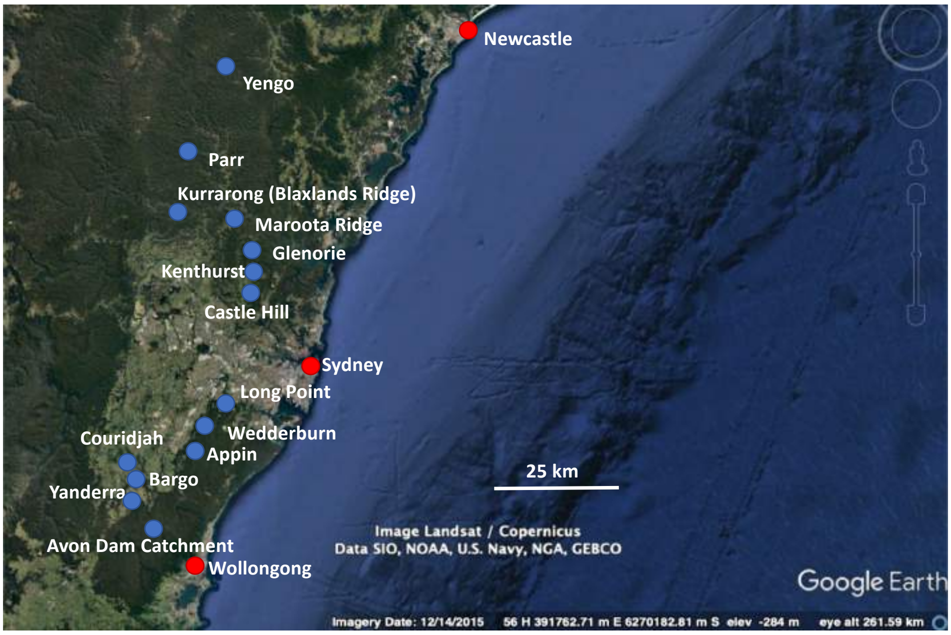

Figure 4. Map showing known populations (blue dots) of *Persoonia hirsuta* as of 2021. Sydney, Wollongong and Newcastle (red dots) are shown for context. Image: Google Earth

4. POLLINATION BIOLOGY OF *PERSOONIA HIRSUTA*

Pollination systems - interactions between breeding systems and pollination ecology - are key to species conservation since they may be disrupted by landscape processes like fragmentation and alterations of fire regimes, as well as by biotic factors such as introduced species (Rymer et al 2005). This is because of the central role that pollination has in a species' biology as the first in many steps to produce a fruit, with seed that has the potential to disperse and germinate, resulting in a new individual (recruitment). Clearly seed production is related to a plant's capacity to regenerate, whether as individuals senesce and die; after disturbances such as fire; or to disperse and establish new populations (Rymer et al 2005).

The resilience of a population relies in part on numerous characteristics of its pollinators including identity (assemblage), abundance and behaviour. For instance, changes in foraging behaviour of pollinators, including their patterns of movement between flowers, can lead to inbreeding depression and reproductive failure in small isolated populations; small populations may receive less visits from pollinators because, being small, their floral display size is smaller and less attractive. Such changes can alter mating systems - the degree to which individuals of a species or population can fertilise their own flowers ('selfing') or another plant's flower ('outcrossing') (Rymer et al 2005). And while selfing is sometimes considered to be an evolutionary response to rarity (with no breeding partners, seed production is still possible by self-fertilisation), the trade off may be a higher rate of inbreeding. If pollinator assemblage is less than optimal, a plant may receive visitors, but some of these species may be pollen 'thieves' rather than effective pollinators.

Pollination biology of the *Persoonia* genus

While vertebrates such as birds and small mammals are known to pollinate the large inflorescences of some Proteaceae, the *Persoonia* genus is instead pollinated primarily by native bees (examples: Figure 5). This was confirmed in a 1996 study of 20 *Persoonia* species (not including *P. hirsuta*) by Bernhadt and Weston, in which 22 different insect taxa were collected from flowers. In addition, pollen loads of the insects were analysed and pollinators observed in the field. The most 'efficient' pollinators were native bees of the genus, *Leioproctus* (within a family known as plaster bees), and also those of the genus, *Exoneura* (small social bees also known as reed bees).

Rymer et al (2005) confirmed that bees were the main pollinators of four *Persoonia* species in a study that compared common and rare *Persoonia* species. They found both the common and the rare species were obligate outcrossers but pollination was limited in the rare species due to fewer native bee visits, leading to reduced reproductive success: 20% of flowers matured fruits in rare species of *Persoonia* compared to 35% of flowers of common species. Both native bees of the *Leioproctus* genus and introduced European honeybees (*Apis mellifera)* were observed. The native bees visited less flowers on a plant but tended to travel further between plants for foraging. This pattern is expected to favour outcrossing. Honeybees made more visits to common than rare species and overall were the most frequent visitor to all species. The authors concluded that lower pollination effectiveness (by honeybees) contributed to the lower reproductive success (i.e. fruit set) in the rare *Persoonia.*

Figure 5. Pollinators of *Persoonia,* include left, green carpenter bee (*Xylocopa* sp.), and right, leafcutter bee (*Megachile* sp). Both genera have been observed on *Persoonia hirsuta* flowers. Images: Nathan Emery.

In Rymer's study, the authors concluded that for these species, plant breeding systems were not related to rarity. Instead, rare species were less fecund and less frequently visited by native bees. The authors argued that habitat fragmentation and altered fire regimes had changed the distribution and abundance of plants, with pollinator activity also possibly changing in response.

Overview of current research

Determining the pollination system of *Persoonia hirsuta* is important because some species in the genus are primarily outcrossing - i.e. involves pollinators, rather than selfing, which does not need an intermediary (Bernhadt and Weston 1996). Even where in theory, a plant can self fertilise, it may affect other stages of its life history. For instance, self pollination within the *P. mollis* complex does not generally result in a successful seed set (Krauss 1994).

Several aspects of the plant's pollination biology were investigated:

- Pollinator observations (leading to data on identification of pollinators, foraging time, number of flowers visited, pollinator assemblages);
- Pollination manipulations to determine the effects of outcrossing v selfing ;
- Pollination treatments (bagging) and fruit set.

This section draws on research by PlantBank and also a student project by Hannah Bunker at UOW.

Study one. Student report,* Pollinator Behaviour and Assemblages and Their Contribution to Rarity in *Persoonia hirsuta, Hannah Bunker

Bunker compared the rare *Persoonia hirsuta* with the common *Persoonia levis.* She observed pollination of *P hirsuta* at two sites: Appin North and Couridjah, and compared the two species at a single site in Couridjah. She studied pollinator assemblage, pollinator visitation and pollinator behaviour.

Overview of methods. Trial observations of 15 to 20 minutes on 11 plants in total were made using Go-Pro Hero 3 cameras and an observer on site. However, these did not result in useful video footage as either the frame was too large and pollinators were too small to identify; or the frame was too small and too few flowers could be observed. A larger sample of 40 plants in total were then observed for 15 minutes and for each pollinator observed behaviour was recorded: number of flowers visited and whether a nearby conspecific plant was visited. Insects were collected where possible and identified later. At Appin North, 14 *P. hirsuta* were observed while at Couridjah, 14 *P. hirsuta* and 12 *P. levis* were observed.

Key findings. Pollinators differed between sites for *P. hirsuta,* with the native bee genera *Camponotus, Exoneura* and *Leioproctus* being the most frequent at the Appin North site. At the Couridjah site, *Apis mellifera* was the most common pollinator. Visitors of *P. levis,* on the other hand, were *Leioproctus* species. *P. levis* received more visitors overall and more native bee visitations, but there was no difference between pollinator behaviour, whether between sites or between species.

Comments. The finding that *Apis mellifera* was the predominant pollinator at Couridjah is of note, given the concern that it may encourage selfing (Rymer et al 2005) and therefore contribute to rarity.

Study two. Pollination treatments, manipulations and observations at three sites, Glenorie, Parr and Appin North, by PlantBank scientists. (Emery and Offord, 2019a; Emery and Offord (in review)).

Background. Cross pollination by hand allows very specific control of which plant pollinates which, while 'bagging' prevents access by further pollinators. Open pollination allows for natural mechanisms to occur. Following flower maturation through to fruit set, gives insights into the plant's optimal mating system. These experiments are used to quantify self-compatibility, fruit termination and fruit set, or maturation. See Emery and Offord (2018) for a review of manipulative pollination experiments on *Persoonia.*

Overview of methods. Hand pollination of *Persoonia hirsuta* flowers was carried out at several sites to measure the effect on fruit set of selfing (using pollen from the same plant to fertilise a flower) and outcrossing (taking pollen from the flower of one plant and placing it on the stigma of a flower of a different plant), with four pollination treatments in all: autogamy (pollen from the same flower), selfing, crossed and open (Figure 6).

Standard pollinator observations were carried out at three sites, Glenorie, Parr and Appin North, to assess pollinator assemblages and behaviour. Field observations of pollinators were recorded by observing focal plants in a *P. hirsuta* population for one hour during the hours of 10am to 3pm, and repeated over three separate days. Records were made of: pollinator species observed; time foraging and in contact with the stigma.


Figure 6. Pollination treatments. Left, hand pollination; right, pollinator exclusion bags. Images: Nathan Emery

Key findings. More fruit developed from flowers left open to pollinators (between 22.8 and 44.8%) while both autogamy (0.6 to 1.6%) and selfing (2.6 to %) produced significantly less fruit. Across two sites, Glenorie and Parr pollinators were observed from six genera: *Amegilla*, *Hyalaeus*, *Leioproctus* (plaster bees), *Megachile* (leafcutter bees), *Tetragonula*, *Xylocopa* and *Zizina* (blue butterfly) . Taxa observed on six focal plants' flowers at Appin North were notably different to other sites and included beetles, flies and butterflies (Figure 7).

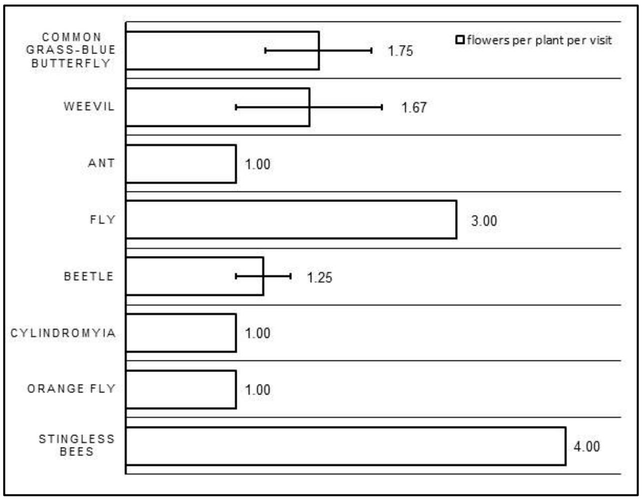

Figure 7. Pollinators observed at Appin North and number of flowers visited per plant. (pers comm, Emery, 2021)

Comments. Results for fruit set indicate that the species is capable of self fertilisation, although it appears to be less successful. The difference in pollinator assemblage at Appin North was interpreted as being a sub optimal assemblage, due to the population being small and isolated, which could result in reduced fruit set. In this situation the *P. hirsuta* flowers are thought to be outcompeted by other larger and more abundant species that are more attractive to local pollinators. It's also of interest since findings from other studies in this report indicate loss of a viable seedbank. The fact that statistically, day of survey was significant, reinforces the need to carry out pollinator observations over several days.

Overall summary

These studies build on knowledge about *Persoonia* pollination and confirm the importance of native bees in pollinator assemblages of the genus. Manipulations add weight to the suggestion that *Persoonia hirsuta* shows greater reproductive success with outcrossing. Where population size is reduced there may be no opportunities for outcrossing, which, as PlantBank's study shows, has a negative effect on fruit set and hence seed production. Another scenario is where the seedbank is not viable and a fire destroys an adult stand without possibility for regeneration. The research in this section will contribute to understanding how such situations influence the species' rarity, allowing for better management of the plant in terms of prioritising conservation projects such as *ex situ* breeding and *in situ* translocations to boost population growth and establish new locations.

5. SOIL SEEDBANK DYNAMICS

Given that 75% of plant species in the Sydney Basin have soil-stored seedbanks (Auld and Ooi 2008), understanding the range of responses and life-history traits, known as soil seedbank dynamics, will significantly contribute to their conservation. Such knowledge may, for instance, guide fire management to optimise population persistence in the field or improve *in situ* seed storage and propagation techniques that will enable new plantings.

These dynamics concern complex biological processes such as breaking of dormancy, germination cues, persistence and store of seed in the wild, as well as predation, viability and location of the seedbank. Simple questions regarding the abundance of seeds and how they move within and among populations also give insight to a species' vulnerability.

Whether in the canopy or in the soil, a viable seedbank ensures future recruitment, both in terms of sufficient numbers of new recruits, but also, potentially, in terms of genetic diversity. Soil seedbanks are considered a buffer against adversity since seeds can be stored for different lengths of time and potentially germinate at different times. For fire-sensitive plants like *Persoonia hirsuta* that are killed by fire, the soil-stored seedbank is the only source of new recruits. Fire frequency is also critical for these species, also referred to as obligate seeders. If the fire interval is too long, the adult population may senesce, and produce non-viable seed (Bradstock et al 1998, Whelan 2003; McKenna 2007). If the seedbank loses its viability, then the potential for the population to renew is lost and the risk of extinction heightened.

Soil seedbanks and conservation

Understanding these many aspects can improve conservation efforts at many points of a species' life history. At the landscape scale it can suggest how the species should be managed in terms of fire, for instance in the timing of prescribed fires. It can also suggest best practice for seed storage in an *ex situ* seedbank. In the field insight into how to trigger germination may boost local populations or help establish new ones.

Previous studies

Very little is known about the soil seedbank dynamics of *P. hirsuta*, hence research questions are wide, ranging from fire response, germination, both *in situ* and *ex situ*, recruitment and viability. Its seed is found within a drupe (a fleshy fruit) and is surrounded by a woody endocarp and a thin papery testa. The woody endocarp and seed are together known as a pyrene, which is expected to be at least partly transported by swamp wallabies, but mostly gravity dispersed.

Current studies

This section draws on numerous sources to begin to fill these research gaps: each of the three UOW Honours projects contribute to these questions, including a side project of the second Honours project. Together they address questions such as location, abundance and fire response of the seedbank. Two other studies also contribute: PlantBank investigated germination cues in laboratory experiments, including the effect of smokewater on germination rates, and of both heat shock and removal of the endocarp on germination success after storage; and the results of an ecological burn by South32 raised questions concerning fire response and seed viability.

Study one. Honours thesis, UOW.* The Demography and Habitat Characteristics of the Endangered *Persoonia hirsuta* (Hairy Geebung), *Stephanie Willmott, 2013

Willmott described the seedbank in terms of location and abundance at five sites. She found all seed was in the top 3 cm under the canopy of an adult plant, with an average seed density calculated by site: between 0.75 seeds per m^2 at the Balmoral site; 1.3 for Couridjah and 5.3 and 5.6 for Yanderra and Appin North respectively. Notably there was no seed found at one population. See section three for full details.

Study two. Honours thesis, UOW. Chapter Four -* Persoonia hirsuta *seedling emergence after spring fire: a spatial and temporal investigation in* Conservation Genetics of the rare and endangered plant, *Persoonia hirsuta* (Proteaceae), *Alison Haynes, 2015

This project, part of a thesis that focussed on conservation genetics, followed the emergence of seedlings for two years after a severe spring wildfire in October 2013 at Yanderra. The aims were to quantify emergence; assess location of seedlings in relation to adult plants burned in the fire, (now existing as skeletons); and to determine whether emergence was a 'first flush' after the first rainfall following the fire, or showed a delayed, seasonal emergence.

Overview of methods. The site at Yanderra of approximately 8000 m^2 was visited at regular intervals between April 2014 and October 2015. The study built on the data gathered by Willmott, which had recorded locations for 17 plants at the site.

Key findings. Over the study period a total of 68 seeds germinated to produce visible seedlings under the canopy of 11 plants. For the plants with germinations, numbers varied between one and 16, with an average of 4.25. For six plants, there were no germinations during the study period.

Depending on rates of mortality and germination, the population size fluctuated. It rose dramatically from seven to 33 in May, then grew steadily to a peak of 51 in November, one year and one month after fire. By the end of the study period, two years and two months after the fire, the population was 47.

In addition to the core area at the site, a further 28 seedlings were discovered in November 2015. Eighteen of these were grouped in clusters while the rest were more randomly distributed. The findings for *Persoonia hirsuta* at this site are similar to *Persoonia mollis* where two seedling cohorts after fire were found to be tightly clustered at small scales near dead adults (McKenna 2007).

Implications. The study confirmed the presence of a viable seedbank. It indicated a delayed germination and breaking of dormancy but given it is only one fire, this finding is not conclusive. The results aligned with the premise that seeds are primarily gravity dispersed, although some seeds appeared to have moved from an adult plant, a finding that is backed by genetic studies that showed some connection between populations.

Study three.* Conservation Genetics of the rare and endangered plant, *Persoonia hirsuta* (Proteaceae*), Alison Haynes, 2015

This microsatellite study assessed genetic diversity and connectivity of three populations of *Persoonia hirsuta:* Yanderra (seedlings), Couridjah and Appin North. It is described fully in section seven but a few details relevant to seedbanks are covered here. Protocols for DNA extraction and PCR (polymerase chain reaction, a method of DNA amplification) were optimised during the Honours research and built on subsequently, using additional microsatellites (Ayre et al 2021).

It was argued that, given the patchy nature of fire and the 'cryptic nature' of seedbanks, populations of plants with soil-stored seedbanks are often ephemeral, so that the true size is difficult to estimate accurately. The same can be said for diversity and population differentiation. It was expected that these populations had been small and isolated for several generations since much of their habitat was fragmented or disturbed (Bradstock and O'Connell

1988). The soil-stored seedbank was predicted to act as a buffer to these small, isolated populations, both against the loss of genetic diversity and population differentiation.

Key findings. The populations were more diverse than expected and this was explained by the persistence of the seedbank - with the diversity representing a historically more connected population, i.e. with more gene flow between populations than would appear to be currently possible. Genetic analysis revealed some gene flow between populations, indicating a degree of movement of genes - via seeds or pollen - between populations. The Yanderra population revealed a genetically diverse seedbank.

Study four.* Can the seedbank act as a reservoir of genetic diversity? A conservation genetic study of *Persoonia hirsuta, Hannah Bunker, 2016

This Honours thesis investigated the conservation genetics of *Persoonia hirsuta* in terms of the soil seedbank and is dealt with in more detail in section eight. Bunker sampled both canopy and soil seed to compare recent 'fresh' seeds with older ones at Appin North, Couridjah and Yanderra. Some of the insights into soil seedbank dynamics are outlined here, in particular with regard to how many seeds contained an embryo ('full') or were empty. For more details of methods and results of genetic diversity, see section eight.

Seed viability. At Appin North, the percentage of canopy seed across the population that was full ranged from 90.41% - 100%, with an average of 95.21% but the percentage of soil seed that was full ranged from 0% - 2.63%, with an average of 1.44%. At Couridjah, the percentage of canopy seed that was full ranged from 75% - 94.12%, with an average of 84.59%. The percentage of soil seed that was full ranged from 3.45% - 8.33%, with an average of 5.41%.

Implications. The low viability of seeds in the soil seedbank was a problem for the genetic research but at the same time gave an insight into possible limitations facing the species' survival. The research supports the hypothesis that the populations may have been historically connected since they are not genetically distinct. Bunker found greater variation in the seedbank than in adult leaf material and higher overall observed heterozygosity. This result confirms the importance of a seedbank in maintaining diversity.

Study five. Germination trials with removal of endocarp, short term storage and heat shock, PlantBank

Rare plants are often the subject of conservation plantings such as reintroductions and population supplementation. Propagating from seeds is one option and this requires specialist knowledge, not only about germination biology of a species, but also storage. Correct storage, which varies from species to species, is vital if global seed banking efforts are to have any impact on reducing rarity and preventing extinction.

Plants with woody endocarps, such as *Persoonia*, are often difficult to germinate *ex situ.* The endocarp is a mechanical dormancy mechanism: for dormancy to break and germination occur, the endocarp needs to weaken over time or, if part of *ex situ* production or research, be removed manually. However, while removing the endocarp may break mechanical dormancy, it may also expose the seed to contamination. In addition, the embryo is physiologically dormant and to induce germination requires specific temperatures and conditions, for instance, stimulants such as gibberellic acid or smoke (Emery and Offord 2019b). These species-specific cues are unknown for many Australian native plants. *Persoonia hirsuta* can be considered a 'model plant' in that many other high interest plants share similar issues.

The PlantBank researchers ran an experiment with *Persoonia hirsuta* seeds to determine the germination response to: heat shock; short-term *ex situ* storage at low and room temperature; and endocarp removal (Emery and Offord 2019b).

Heat shock is relevant for two reasons. Firstly, contamination from microbes has been a common problem for many laboratory germination studies of the *Persoonia* genus. While surface sterilisation with bleach surmounts the problem for some species, it has not helped in the case of *Persoonia*, and contamination has been observed originating from within the seed. The researchers asked whether heat shock could sterilise the seeds and enhance germination rates. Response to heat is also relevant since *Persoonia hirsuta* is found in fire-prone habitats. It is unknown whether heat enhances germination success by helping to break dormancy or triggering germination.

Overview of methods. The study used a total of 1000 fruit from 20 plants, believed to be an intergrade of the two *P. hirsuta* sub species, in Glenorie, in the Hills Shire district of Sydney. These were assigned a combination of treatments regarding temperature of storage, endocarp removal and heat shock. Storage treatments lasting six months were: low temperature (-20^{o}C), room temperature and no storage (control - i.e. they were incubated immediately after endocarp removal); heat shock consisted of placing seeds in a fan forced oven set at 80°C for 10 minutes. Seeds were then incubated for 12 weeks in a 15°/25°C alternating temperature regime following methods used successfully with other species of *Persoonia* (Catelotti and Offord 2017).

Key findings. Final germination of the control (no storage treatment) group ranged between 66% and 90%, depending on the other experimental treatments, compared to between 35% and 77% for the low temperature and between 6% and 84% for the room temperature storage. Removing the endocarp from the seeds reduced germination success, while statistically, the application of heat did not make a difference. Additionally, there were interactions between treatments. For instance, germination was significantly reduced when seeds were stored for six months without the endocarp and treated with heat. The effect on germination of removing the endocarp before storage is explained by the fact it exposed the seeds to contamination: seeds stored without an endocarp had contamination rates of 61-91%, compared to 16-26% for those that retained it.

Implications. The authors recommend that the endocarp is retained, whether for storage or for direct sowing, to increase the likelihood of germination and viable seedlings. They also emphasise the importance of optimising *in situ* conservation, given the difficulties of successful *ex situ* storage and germination of *Persoonia* species. Finally, experiments are needed to test germination rates after extended storage beyond six months.

This was the first documented successful germination trial of *P. hirsuta.* PlantBank have since successfully grown new plants from seed in the nursery and are gradually reintroducing individuals to the mine site (see section six for more detail). For a discussion of the next research steps to improve germination and translocation success, see section 11.

Study six. The effect of smokewater on germination success, PlantBank

Background. Many plants in fire-prone regions germinate in response to smoke, as the chemicals in it interact with numerous aspects of plant physiology including hormones such as gibberellins that regulate many development stages (Dixon et al 1995, Van Staden et al 2000). But by no means all Australian plants have been found to respond to smoke (41% of tested species showed smoke responsive germination in Carthey et al 2018) and the response of *Persoonia hirsuta* seeds is unknown.

Overview of methods. Seeds were collected from three populations: Glenorie, (batches from two years collection) Parr SCA and Yengo National Park. Seeds were extracted, treated by soaking in bleach then rinsed and sown on agar. For the treatment group, the agar included 2% smokewater pipetted across the agar one hour before sowing. Seeds were then incubated at 25/15^{o}C with a light regime of 12/12 hours. They were checked for germination weekly for 10 weeks.

Key findings. Smokewater did not increase germination and in one batch of seeds, it decreased it.

Implications. Although the seeds did not appear to respond to a smoke signal, it is possible that they require a more complex set of triggers for germination including, for example, temperature. Additionally, heat may be a more important fire cue for stimulating germination by weakening the endocarp, allowing the seed to push through.

Study seven. Ecological burn at Appin North by South32 and the Rural Fire Service, autumn 2016

A small scale prescribed burn was carried out in a 5 ha portion of the offset area at Appin North to measure germination from the soil-stored seedbank. While it was acknowledged that the *Persoonia hirsuta* population was likely senescing, and was estimated to be 24 years old, it was expected that enough viable seed had been produced in recent years for new recruits to germinate after the fire. The site was monitored for five years post fire and one seedling was observed in the last survey year (December 2020). Whether lack of recruits is due to characteristics of the prescribed burn such as the timing or temperature of the fire, or low viability of seedbank is discussed in more detail in section ten, which focusses on the fire response of *P. hirsuta,* and also provides more details on the ecological burn.

Overall conclusion and implications

Together these studies considerably advance the knowledge of soil-stored seedbanks in *Persoonia hirsuta.* Overall they suggest:

- Most seeds seem to be gravity dispersed, but some may be moved from the adult plant, possibly by birds or swamp wallabies (Willmott 2013, Haynes 2015);

- Seedbanks vary widely in viability, with one population recruiting well after a fire; yet other investigations revealing large numbers of empty seeds in the soil and though not conclusive, an ecological burn pointing to the possibility of a non-viable seedbank (Haynes 2015, Bunker 2016, South32 2016);
- Seedbanks appear to store genetic diversity that can act as a buffer against the effects of isolation and fragmentation (Haynes 2015, Ayre et al 2021);
- Seeds are best stored without removing the endocarp; heat alone does not appear to influence germination success (Emery and Offord 2019b);
- While smoke did not enhance germination, further research should investigate combinations of factors as, for example, seasonal temperatures combined with fire cues may be needed (Collette and Ooi 2021).

Numerous questions remain, such as how long and in what conditions seeds remain viable, but the work outlined in this chapter has laid a strong foundation for further conservation research by testing a number of theories regarding, for instance, germination success, and highlighting future directions.

6. IN THE FIELD: PHENOLOGY, SEASONAL GROWTH AND TRANSLOCATION

Ultimately the test of conservation programmes is the number of individuals surviving in the field - and that is the focus of the two main elements of research in this chapter. The first element is the annual monitoring of the core population at Appin North for South32 and the second is the first *Persoonia hirsuta* translocation experiment, by PlantBank, to boost this population.

Monitoring has three main objectives under NSW's ‘Saving our Species’ programme. These are to provide evidence on the status of threatened species and the effectiveness of conservation projects, to evaluate the effectiveness of conservation projects and to facilitate adaptive learning. In addition, monitoring data is critical to measure the success of management activities and to analyse potential reasons for decline (OEH 2018).

Translocation, defined as 'the deliberate transfer of plants or regenerative plant material from an *ex situ* or natural population to a new location, usually in the wild' (Commander et al 2018) covers a broad range of methods, from propagation to seeds, that can be used to supplement *in situ* conservation.

South32 annual monitoring

South32 has conducted annual population and condition surveys of the core population of *Persoonia hirsuta* at Appin North since the 2012 baseline survey by Niche where 44 individuals were found and each plant tagged with identification details. This monitoring has documented the survival of the individual plants as well as examined the growth and health of the plants. New recruits have also been recorded, tagged and monitored in subsequent years.

An annual report has been provided to the Department of Agriculture, Water and Environment (previously the Department of the Environment and Energy) in accordance with South32's EPBC approval conditions.

Baseline study 2012

At the baseline study, in 2012, 44 individuals were recorded in the core population and these were in good condition. The habitat was also considered to be in good condition with a 'good level of inherent resilience' i.e. capacity to regenerate; high level of native plant species richness; few exotic plants; and intact structural layers consisting of canopy, mid-storey, shrub and ground cover (Illawarra Metallurgical Coal 2020).

The last reported fire for the site was around 1989, and the site reflected a fire interval of that length, since *Banskia ericifolia* was senescing, and cover of annual herbs, grasses and obligate seeding short-lived shrubs was low.

Senescence and population reduction

The original population of 44 plants has been monitored annually for eight years during which time it gradually reduced in size until in 2017 none of the original baseline plants survived: 16 were burned in the ecological burn and the remaining 26 are believed to have died because of old age. Discounting the 10 plants that had been identified in the offset since baseline (2012), the offset has declined by 44 plants. The consultants noted that there were no visible impacts from dust nor signs of disease such as Cinnamon Fungus.

Recruitment

New plants were observed several times, with a total of nine *new* individuals recorded by 2016 (five in 2013, four in 2014). Many of these had germinated on disturbed parts of the offset area. In 2017, the population consisted of ten plants, all of which had emerged after the baseline study, including one new individual located that year. (By that time, all the original population had died, as detailed above). In 2019, 128 individuals were planted as part of a translocation study, outlined below.

2020 census

During the most recent monitoring, in spring 2020 the population was recorded as 10, with nine from the previous year, and a new plant found during monitoring after a prescribed burn. In addition, translocation survivors at the time of the census amounted to 28, bringing the total population in the offset area to 38 at the end of 2020.

Condition report, 2020

Persoonia hirsuta individuals were producing both flowers and fruit, in greater abundance on plants in dense bush compared to those in more open areas at roadsides or under powerlines. Vegetation in the offset area as well as surrounding area was recorded as good.

Translocation experiment

Translocations have a long history, with, for example more than 50 plant species known to have been deliberately moved at some time by Aboriginal Australians (Commander et al 2018). The use of translocation is increasing as more species are threatened by habitat loss, degradation and climate change, but only few translocations have resulted in self-sustaining populations because of low survival rates or inappropriate methods. An experimental approach, such as described in the current study, allows for testing of different scenarios and builds biological and ecological knowledge for future translocations, with the aim to gradually increase the chance of success.

Species vary in their preference for translocation technique. Some survive better when planted as seeds as it may reduce transplant shock following transfer from a nursery; but when seeds are difficult to obtain or to germinate, vegetative propagation is more practical.

Aims. This translocation experiment at Appin North analysed survival rates of juvenile plants, raised from seed and from cuttings. It also tested survival rates with different planting treatments (mulch and plant guards). Specifically, it compared growth, health and survival of plants under the treatments, and investigated root architecture as well as soil conditions. It also asked what characteristics need to be considered for site selection (Andres et al in review).

Overview of methods. Plants were raised from seeds and cuttings (64 in each group) and plants grown at the Australian Botanic Garden Mount Annan. The propagative material was from several sites: Appin North (fruit for 'local' seed); Glenorie (fruit for 'non-local' seed) and Yanderra (cuttings, Figure 8). Plants were raised in a glasshouse then shadehouse for several months before being acclimatised outdoors for four weeks before planting (Figure 9).

Figure 8. Staff from Australian Botanic Garden at Mt Annan collecting vegetative cuttings from *Persoonia hirsuta* plants. Image: Nathan Emery

In autumn 2019 the *Persoonia hirsuta* individuals were planted on South32's mining lease at Appin North at random in a block design, with four planting treatments in all: control; local mulch; plant guard; local mulch and plant guard (Figures 10 and 11). Mulch was litter collected from the surrounding area, while plant guards consisted of a hessian sleeve held in place by bamboo stakes. A soil sample was taken from each hole before planting.

Plants were watered weekly, with additional watering over summer, with approximately 0.5L per plant. Monitoring was approximately every 50 days for 20 months. The following variables were recorded each time: height, number of branches, reproductive state, herbivory and health.

Figure 9. Top: *Persoonia hirsuta* plants propagated from seeds under shadecloth at the PlantBank nursery. Below: a later growth stage in the nursery. Images: Nathan Emery.

Key results. After 20 months, 25 of the 128 translocated plants survived. All of these originated from seeds; and more than two thirds (72%) had been protected by plant guards.

Propagation type. The advantage of the seed-raised plants was observed in the summer of 2019/2020, approximately eight months after planting, during which rainfall was low and temperatures were high. All seedlings declined in health after translocation, irrespective of propagation type, and until October 2019, cuttings were in better health. During summer months, both plant types declined substantially, but as temperatures cooled in autumn 2020, plants from seeds picked up and put out new shoots.

Planting treatment. Plant guards are commonly used to deter herbivory (Figure 11). In this study, plants with guards were healthier on average and had a greater chance of survival, with 72% of survivors being protected by guards. Where guards were used, mulching increased health and height, particularly around a year after planting.

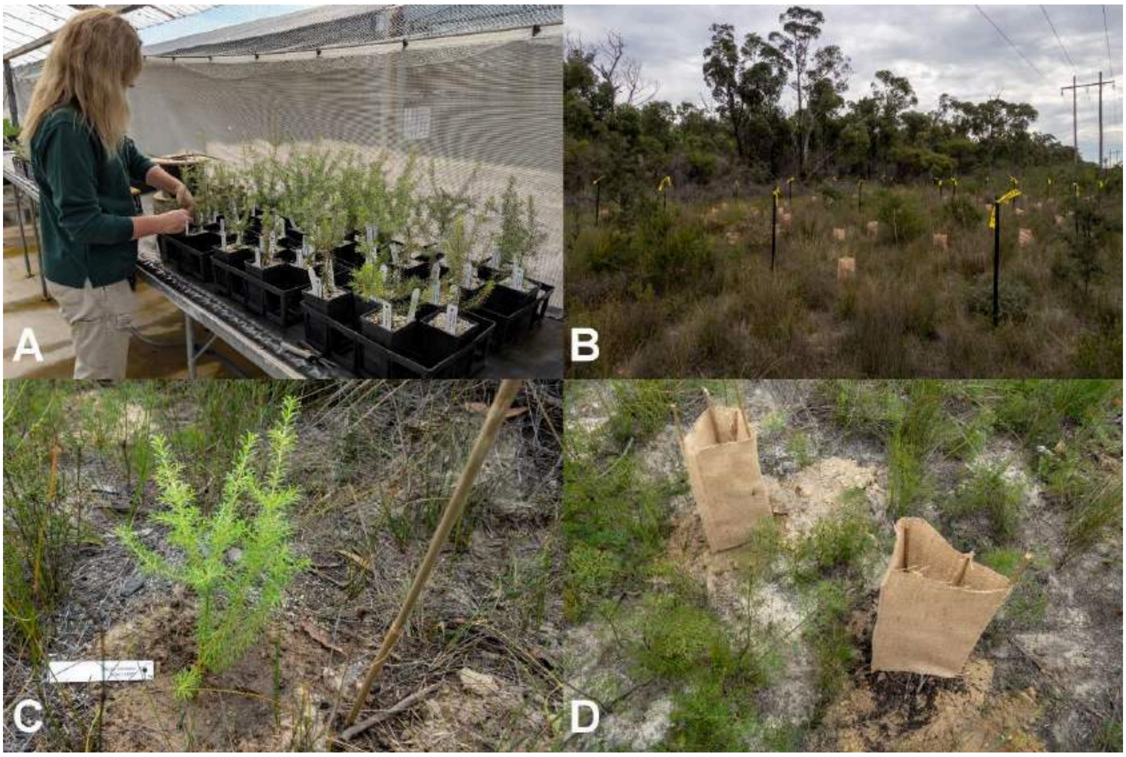

Figure 10. Preparation of *Persoonia hirsuta seedlings* for translocation (A) and translocation site (pilot study) at Appin North (B). The experiment investigated the effect of plant guards and mulch on plant survival (C and D). Image: Nathan Emery. Reproduced with authors' permission from Emery and Offord, 2019a.

Figure 11. Hessian guards used to deter herbivory of *Persoonia hirsuta* in the translocation experiment. Image: Nathan Emery

Root morphology. Root architecture could explain differences in survival between the two translocation types. Seedlings had deeper roots, with a finer root system, less horizontal branching and greater area; whereas cuttings' roots were clustered near the soil surface, with more lateral roots.

Seasonal growth

Two measures for translocant growth were taken both before translocation and on a monthly basis afterwards. The measures were plant height and number of branches, and were standardised to proportional growth rate. In the ten months of measurements before translocation plant height grew steadily until it averaged around 300% for all plants, while branching was greater between January and April 2020, than between June and December 2020, and pre-translocation there were on average around five times the branches than when measurements began.

During the translocation, results were focussed on differences between planting treatments as detailed above. Nonetheless, when temperatures cooled during late summer, 18 percent of plants that were assumed dead put on new shoots and leaves. Additionally of note: in the first four months post translocation, average height for all translocants decreased.

Second translocation

In May 2021, a second translocation was carried out with 90 *Persoonia hirsuta* at Appin North in the coal wash emplacement Stage 2 rehabilitation area, chosen because of soil properties, the fact there are two naturally occurring *P. hirsuta* plants there and that it has greater canopy cover than the first translocation site (Figure 12). The site consists of 50 m x 50 m within which plants are roughly 1 m apart, each with mammal plant guards to protect against predation. Plants are between two and four years old. Propagative material was collected from source plants over three years (2017-2019) from several wild populations throughout the species' distribution in an attempt to include a wide level of genetic diversity.

Figure 12. Site of the second translocation at Appin North planted in May 2021.

Plants from different source sites were separated wherever possible throughout the planting blocks within sites to increase the likelihood of out-crossing. It is assumed that some level of genetic variability exists among the seed-grown plants, and to a lesser degree the cuttings-grown plants. The translocation will be watered and monitored by employees of Mt Annan Australian Botanic Gardens as well as the Appin North Specialist Environment, with the aim of the translocation being to supplement the existing population at Appin North. An additional phase 3 translocation is planned at Appin North for 2022.

Comments and implications

Monitoring at Appin North confirmed the vulnerability of the population since despite some recruitment the population has reduced substantially. The data suggests the species' life span is around 30 years maximum, since the plants tracked in the baseline study are estimated to have been approximately 24 years old in 2012.

The translocation experiment confirmed strategies to proceed with in future translocations of *Persoonia hirsuta*: using plants raised by seed and using plant guards combined with mulching. Further consideration of planting location, in particular canopy cover and soil environment, may also improve success. The experiment represents a milestone for conservation of the species, especially since it combines other aspects of the research, such as successful germination *ex situ*.

7. ECOLOGY AND POPULATION GENETICS FOR CONSERVATION

Conservation genetics is a branch of population genetics and a standard tool used to investigate small populations. It involves analysing the genetic diversity of one or more populations to shed light on its potential vulnerability to inbreeding depression, to measure gene flow between populations (by pollen or by seed) and to estimate a species' or population's mating systems. This section draws on an Honours project at UOW which used microsatellites and leaf DNA extractions of *Persoonia hirsuta* subsp. *evoluta* and an ongoing genetics study at Mt Annan that is investigating the status of the subspecies and the diversity contained in *ex situ* compared to onsite populations. The UOW Honours project was extended with further DNA extractions using a wider range of microsatellites and additional analysis (Ayre et al 2021).

The problem of small populations

Low genetic diversity is a risk for small populations because it can lead to inbreeding depression, reduced fitness and enhanced risk of extinction (a scenario known as the 'extinction vortex'). Analysing diversity, therefore, is an important aspect of assessing the conservation status of a species. Conservation genetics also gives insight into processes such as mating systems and pollen and seed dispersal that are very difficult to quantify in other ways.

Wright's F statistics, used in the first study, are a collection of formulae relying on genotypes, in particular, heterozygosity scores based on the differences between what would be expected in an 'ideal' population (He - expected heterozygosity) and what in fact is measured in a population (Ho - observed heterozygosity). In simple terms, He is considered one of several measures of genetic diversity. A heterozygous individual has different genes for a particular locus in the DNA while a homozygous individual has two copies of the same gene. A key F statistic is F_{ST}. This is the fixation index, and is used to describe differentiation between populations.

The two studies outlined below represent the first steps in genetics research for *Persoonia hirsuta.*

Study one. (Conservation genetics). Microsatellite study involving identification of microsatellites, design of primers and polymerase chain reaction (PCR) protocols to analyse the conservation genetics of three populations. Honours thesis, Alison Haynes, 2015

Firstly, this study developed and optimised a set of species-specific microsatellite primers suitable for fine scale population analysis. This allowed investigation of: i) genetic diversity within and amongst the populations; ii) mating systems of the populations; iii) genetic connectedness of plants in the three sites (i.e. population differentiation of the populations); and iv) the conservation value of different populations.

Microsatellite primer design. Microsatellites are one of several molecular markers available to use in molecular population genetics. They are fragments of DNA, typically consisting of up to six nucleotides in a repeating motif. A significant benefit is that they are highly variable, so that individuals, and hence populations, may be differentiated at a fine scale. This is because microsatellites mutate at a high rate. Microsatellite primers, best designed for each species are used in the PCR stage of the process - where DNA is amplified to obtain a workable volume for sequencing. The gene technology company, ecogenics, based in Zurich, Switzerland, was commissioned to identify 10 polymorphic microsatellites in genomic DNA of *Persoonia hirsuta* leaf samples and develop 10 associated primers and PCR protocol.

Population sampling. Three populations known at the time were sampled for leaf tissue. These were adult populations at Appin North and Couridjah, and a third consisted of seedlings that had emerged after a 2013 fire that had consumed all adult plants at Yanderra.

DNA extractions, PCR, sequencing and genetic analysis. DNA was extracted from approximately 0.012g of silica gel dried leaf tissue using the cetyl trimethylammonium bromide (CTAB) method outlined by Doyle and Doyle (1990). PCR and sequencing protocols required a long optimisation period (Haynes 2015) due to the difficulties of working with this plant material, including interference of key reactions by plant secondary compounds. The final PCR protocol and sequencer information is detailed in Ayre et al (2021). GeneMapper v3.7 was used to score genotypes and data was further analysed using software including GenAlex and STRUCTURE.

Key results. Genotypes were obtained for: 32 adults at Appin North (core population in the offset area) and a further six in a cluster 1000 m away; 23 adults at Couridjah and 50 seedlings at Yanderra. All three populations showed similar genetic diversity in terms of He (expected heterozygosity) and allelic richness (number of alleles, or gene versions at a locus). Using several measures, including F_{ST}, the populations were found to be differentiated by a small but significant amount. Using an 'island model' (Wright 1931), showed that all locations either were or had been strongly interconnected. Migration rates were estimated to be 2.3 migrants per generation amongst all locations, and six migrants per generation between Couridjah and Yanderra. Analysis by both STRUCTURE and a PCoA (principal coordinate analysis) showed that the Appin North population was different to the others. It appears that Couridjah and Yanderra form one genetically similar group, while the Appin plants form another. Genetic statistics suggested that for all populations mating systems were mixed, but predominantly outcrossed.

Implications/interpretation. The populations showed greater genetic diversity than expected given their small size and isolation. Ayre et al (2021) interpreted this as evidence that there has not been enough time for habitat fragmentation and changes in the fire regime to impact genetic diversity or population differentiation. This scenario is considered to be largely due to the soil seedbank acting as a buffer, thereby reducing the rate of genetic loss. Other factors thought to have mitigated loss of diversity include a mating system that is predominantly outcrossing since this protects a population against genetic drift (random changes in the genetic composition). In addition it is argued that, like other fire sensitive species, *Persoonia hirsuta* populations are ephemeral and while they may be small immediately before a fire, post-fire stands can be much larger, as was the case for the population at Yanderra in this study.

It is particularly interesting that the three populations in this study showed similar allelic frequency and little geographic differentiation. This means that historically these populations appear to have been well connected by dispersal of pollen or seed. The F_{ST} value is the lowest found to date for the Proteaceae family, and appears to be at odds with the available evidence on seed and pollen dispersal, but again, it is argued that this reflects a relatively recent history of more extensive and contiguous distribution of the species than currently exists.

While genotyping seed as well as adults, and comparing adults and progeny was desirable, difficulties with PCR for seed meant this was not possible (Bunker 2016).

Apis mellifera has been observed in pollinator assemblages of *Persoonia hirsuta* (Rymer et al 2005) and is of concern since it is believed to promote inbreeding. However, the results of this study mean that either the honey bees are not in fact foraging in a manner that reduces outcrossing or that they are an ineffective pollinator (Bernhardt and Weston 1996) and not impacting mating choice.

Study two. (Taxonomy focus). Are the sub species a valid distinction? Genetics to improve conservation programmes for Persoonia hirsuta, such as translocation programmes.

This substantial investigation is well underway, with contributions by both ecologists and geneticists at PlantBank and Restore & Renew. Field work and sampling is complete and lab work, using SNPs (single nucleotide polymorphism, a genetic marker) has begun.

The study builds on the conservation genetics Honours project, investigating the population genetics of other *P. hirsuta* locations found since the initial study, and analysing genetic data for specific management applications such as translocation and seed collection. Questions include:

- Is separating the species into two sub species, *hirsuta* and *evoluta,* justified?
- Is there evidence of hybridisation?
- What are the relationships between populations?
- Is there gene flow between subspecies?
- What diversity is represented by *ex situ* collections and by the offset site capture?
- How should individuals be chosen for translocation?

Full results from sequencing are expected in the latter half of 2021, but preliminary results show similar clustering of genetic diversity as the microsatellite study: Yanderra, Couridjah and Balmoral populations showed similarity, and a separate cluster was identified that included Appin North. While there is distinct genetic differentiation and spatial clustering throughout the distribution of *P. hirsuta*, further work is underway to elucidate if this is typical for *Persoonia* in the Greater Sydney region. It is hoped that herbarium specimens can also shed light on historical patterns of diversity of the species.

Overall implications

The UOW study highlights the conservation value of individual *Persoonia hirsuta* populations since the Appin North population was found to be genetically distinct. In addition, the result from the seedlings at Yanderra confirmed the considerable genetic diversity that can be stored in the seedbank.

While the studies outlined here significantly advance knowledge of *Persoonia hirsuta,* they also act as invaluable case studies for the *Persoonia* genus, and for other obligate seeding plants, particularly of the Sydney Basin.

8. GENETIC DIVERSITY OF THE SEEDBANK

A long-lived soil seedbank may protect plants with small populations against local extinctions by providing a genetic reservoir. This acts as a buffer during unfavourable conditions, as even small contributions will add up over time to create diversity. In this way, when it germinates, a persistent seedbank can reintroduce genes that were lost in the above ground population, and in doing so, change the genetic make-up of the population. The effect of a soil seedbank means that for some species levels of diversity are similar to a common widespread species despite a patchy distribution (*Kennedia coccinea*: Bradbury et al 2016).

Genetic analysis of a population's seedbank complements investigation of its adult life stage and contributes to a better understanding of the species, especially the role of a soil seedbank in obligate seeding species such as *Persoonia hirsuta.*

UOW study: **Can the seedbank act as a reservoir of genetic diversity? A conservation genetic study of *Persoonia hirsuta, Hannah Bunker, 2016***

This chapter is based on a UOW Honours conservation genetics project by Hannah Bunker that used microsatellites and primers identified in a previous study (Bunker 2016, Haynes 2015). As the plant proved difficult to work with, a significant amount of research involved troubleshooting and optimisation of techniques such as DNA extraction, PCR protocols and sequencing procedures. Progress was also hampered by low seed viability, although valuable insights were gained through these trials.

Aims. The study's broad aims included to genotype seed from both canopy and soil, perform standard conservation genetic analysis on population differentiation and inbreeding and investigate patterns of mating such as outcrossing rates ad paternity analyses. It also aimed to compare genetic diversity and partitioning within adult and seedbank populations by comparing results with the previous UOW Honours thesis (Haynes 2015, Doyle and Doyle 1990).

Overview of methods. Both canopy and soil seed was sampled in 2014, 2015 and 2016 at Appin North and Couridjah populations. Canopy seed was collected when fruit was very rounded and appeared ripe, while soil seed was removed from the soil directly beneath an individual plant. In all, 329 seeds were collected, 111 from the canopy and 218 from the soil.

DNA extraction, PCR and sequencing protocols followed methods developed in the previous UOW Honours thesis (Haynes 2015, Doyle and Doyle 1990), based on cetyl trimethylammonium bromide (CTAB) extraction. See section seven for an overview of the microsatellite development. The protocol was modified after experimentation, including using less than 0.005 g of seed material, to improve DNA quality and amplification success rates. To counteract the inhibitory effects of secondary compounds such as polysaccharides in the leaf samples, PVP-40 was added to DNA samples. This modification has been found to reduce non-target binding and enzyme inactivity in downstream processes (Doyle 1991).

Key findings. Protocols were optimised sufficiently to obtain results from four of the ten microsatellites for 57 individuals. The study was hampered by small sample numbers because once opened many of the seeds were found to be empty, i.e. they did not contain an embryo, as required for DNA extraction. Of the 329 seeds collected, 223 were empty, with most of these being from the soil (211).

Genetic diversity: The four loci showed moderate variability, with between three and 13 alleles recorded at each of the loci across both sites as well as private alleles at both.

Population differentiation: This was estimated using two measures. F_{ST} was 0.050, which, being close to zero indicated low population differentiation. AMOVA (analysis of molecular variance) confirmed this, with a low between population differentiation of 5%.

Inbreeding: Several measures indicated a degree of inbreeding in the populations. The inbreeding coefficient, F_{IS}, revealed moderate inbreeding; while deficits in heterozygosity pointed to moderate to severe inbreeding.

Comparison of seed and adults. Numerous measures of diversity indicated greater genetic diversity in the seedbank compared to the adults. Both observed and expected heterozygosity was higher overall in seeds than adults; and the number of alleles also higher (on average 5.5 for seed; 4.3 for adults).

The fixation index (F_{ST}) values were different for seed compared to adult. The seeds' 0.050 (little differentiation) is in contrast to the adult population's value of 0.132, which indicated slightly restricted dispersal. Inbreeding was similar to the adult population.

Seed viability. As described in section five, the vast majority of soil seeds were empty. Only between 1 and 5% of seeds from the soil (across sites and collection year) contained an embryo, while canopy seeds were largely full, with site averages of between 84 and 95%.

Implication/limitations

Bunker emphasises that this data should be interpreted with caution since it is based on only four loci and a relatively small sample size. However the trends in the data are in line with the previous Honours research and further analysis of the adult population (Haynes 2015, Ayre et al 2021). In particular they support the hypothesis that the populations may have been historically connected. The F_{ST} for the seedbank is lower than expected, and indicates greater connectivity than might be expected given the landscape fragmentation experienced by this species. However, reviews show that species with seedbanks have lower F_{ST} values than those without them (Honnay et al 2008).

The low viability of seed was a problem for the genetic research but at the same time gave an insight into possible limitations facing the species' survival. Due to problems with the DNA protocol, a broader sweep of microsatellite results were not possible and sample sizes were too small to determine a number of the aims, such as outcrossing rates, outcrossing distances, or to perform paternity analysis.

Bunker found greater variation in the seedbank than in adult leaf material and higher overall observed heterozygosity. It is unclear how useful it is to compare seed and adult genetic parameters since these can fluctuate with life stage. For instance, diversity measured as heterozygosity may increase with age in some species as selection may favour heterozygosity when due to outcrossing after germination, i.e. a certain genetic combination may tend to survive longer than others, so that diversity gradually decreases as the population ages (Roberts et al 2014).

This result confirms the importance of a seedbank and supports a hypothesis that the soil seedbank is acting as a reservoir of diversity, potentially buffering the adult population from the effects of population isolation and fragmentation.

9. DIEBACK AND CONTROL TECHNIQUES

Dieback of *Persoonia hirsuta* has hampered restoration efforts of the species, and been a factor in the decline of existing populations (Catelotti and Offord 2017, Andres et al 2020). For some years, numerous researchers have noted a tendency for *Persoonia* species to die off suddenly in propagation trials, while populations in the field also seem to decline and disappear quickly. In both situations the cause is unknown. Possible reasons that the current study aims to assess are i) altered plant-soil interactions such as microbial or fungal associations hitherto not understood in this species; ii) disease caused by the Cinnamon Fungus, *Phytophthora cinnamomi*; iii) altered macronutrient availability, particularly reduced nutrients. These are explained below:

- Cinnamon Fungus

Many *Persoonia* species are killed following infection by Cinnamon Fungus (Environment NSW). This soil-borne, microscopic fungus, also known as root rot, can invade and destroy roots of susceptible plants, both native and introduced, and is spread by tools, footwear, vehicles and other disturbance. Where communities are infected with *Phytophthora* fire can increase the severity and the extent of disease.

- Plant-soil interactions

Many Proteaceae species have proteoid roots (Bernhadt and Weston 1996). These root systems, also known as cluster roots, consist of mats or clumps of very fine root hairs and enhance nutrient uptake by increasing the surface area of the root system as well as exudating compounds which increase the solubility of nutrient. However, the *Persoonia* genus is a member of Persoonioideae, a sub-family of Proteaceae, which is notable for not having these root systems (Watt and Evans 1999). It is hypothesised that the species may compensate for lack of proteoid roots by a symbiotic relationship with soil microbes, but this is untested.

- Nutrient availability

Wildfires have numerous complex effects on soil nutrient characteristics such as nutrient pool size, turnover rate and availability of nutrients (Fisher and Binkley 2000). For instance, fire tends to increase mineral nitrogen concentrations and availability, as well as soil carbon, which in turn increases microbial activity (Stirling 2019).

Current study

The section describes a study designed as part of a PhD by Sam Andres, supervised by the University of Western Sydney and scientists at PlantBank and which will continue for a further 12 months until the work is completed. The aim of the project is to assess environmental factors that may be linked to dieback, particularly those related to beneficial and detrimental microbes and to plant nutrition.

Numerous soil and site characteristics are being considered including: nitrogen and organic matter content and moisture of soil; fire history, other *Phytophthora* host species and light intensity of the sites. In addition, plant health and stress measurements will be taken.

Preliminary investigations indicate, for instance, that *P. hirsuta* plants are on average healthier on sites where fire has occurred within 20 years; that dieback occurs on sites with a greater number of species known to be *Phytophthora* hosts (in sites affected by dieback, 64% are known *Phytophthora* hosts, but in sites not affected by dieback, only 32% of species are known hosts); soil nitrogen levels are higher in populations affected by dieback, so too organic matter and soil moisture. Light intensity may be higher at affected sites.

Overview of methods. While exact procedures are to be finalised, it is expected that soil will be collected from underneath 10 plants at two sites, Parr and Yanderra, and used in a glasshouse study. This study will involve 60 replicates of *Persoonia pinifolia*, (a co-occurring obligate seeder) in addition to six plants for a fertilisation study, including one *P. hirsuta* plant from each of the two sites.

Plants will be monitored for growth, dying stems, and dieback to analyse in conjunction with soil and original site characteristics. For instance, soil will be analysed for the presence of Cinnamon Fungus and other pathogens, other microbes (that could be beneficial) as well as macronutrients such as nitrogen. Other experiments being considered are drought and/or light treatments.

Implications. This study will enhance understanding of the threats to *Persoonia hirsuta* in the field and significantly add to knowledge of its biology. Identifying soil organisms that associate with *Persoonia hirsuta* will represent a significant step in understanding the below ground ecology of this genus and contribute to improving survival of existing populations as well as newly translocated ones.

10. IMPACT OF FIRE

Plants that evolved in fire-prone landscapes such as the Sydney Basin are intricately dependent on fire in complex and numerous ways. For obligate seeders such as *Persoonia hirsuta*, fire is the main disturbance that triggers the processes leading to germination and since the adults are killed by fire, this interplay between fire and seed germination is a pivotal to its life history. Wild fire is described by its 'regime' - and it is the interaction between a plant and the fire regime that determines its persistence in the landscape (Whelan 1995). The fire regime consists of fire frequency, seasonality, severity and extent and may also be influenced by time since last fire and previous fire history of a site.

Fire frequency is particularly important for obligate seeders. Adults are killed by fire and a seedbank may germinate in place of a previous population. However, new plants will only produce seed when they reach maturity, in a time frame known as the primary juvenile period that varies according to species (Auld and Ooi 2008). This means that successive fires may cause local extinction if seedling that recruited in response to the first fire are killed by the second fire before they have produced new seed. The risk of extinction also depends on whether all viable seeds in the seedbank germinated after the first fire. But as well as being at risk of too frequent fire, obligate seeders may become locally extinct when fires are not frequent enough - as may be the case with fire suppression, particularly when habitat is near assets such as towns and settlements. In these cases, adult plants may senesce and a seedbank may become unviable.

Previous research

Previous to the research in this report, very little was known about the fire response of *Persoonia hirsuta,* except that it was fire sensitive, i.e. an obligate seeder and that, for those in the genus for which it was known, the primary juvenile period - from germination to producing seed as an adult, varied between three and ten years (Auld and Ooi 2008). For other plants that share its habitat within the Sydney basin, the average fire return interval is seven to 17 years (Bradstock and Kenny 2003). Current fire management for *Persoonia* species aimed at avoiding extinction or fire-driven population declines has been set at more than 10 years (Auld and Ooi 2008).

Current research

This section summarises what is known about the fire response of *Persoonia hirsuta* and traces the recent research to further knowledge about seedling emergence of *P. hirsuta* after fire; germination response to smoke and heat; the breaking of dormancy; and suggestions as to the optimal fire regime. The section is closely related to section five, which discussed soil seedbank dynamics. It draws on results of various projects. UOW Honours thesis two (Haynes, 2015) included a chapter on seedling emergence after a 2013 fire at Yanderra; South32 undertook an ecological burn at Appin North; and Mt Annan conducted germination trials, experimenting with smokewater, heat shock and endocarp removal.

Study one. Honours thesis, UOW. Chapter Four -* Persoonia hirsuta *seedling emergence after spring fire: a spatial and temporal investigation in* Conservation Genetics of the rare and endangered plant, *Persoonia hirsuta* (Proteaceae)*, Alison Haynes 2015

This study followed seedling emergence at Yanderra following a fire in October 2013, known as the Hall Road Fire, that originated in Balmoral, 5km west and burned 15, 726 ha (NSW Rural Fire Service 2018). The study site had been surveyed by Willmott and GPS locations recorded for 17 adult plants. The site was first visited in the following April to locate remains of these adults, and a number of small seedlings, under 1cm in height, were discovered. In all, 68 seeds were recorded to have germinated in the two years following the fire in the seed shadow of 11 burned adult plants (Figure 13). After two years the population consisted of 47 seedlings, plus an additional 28 that were found at the end of the study period. See section five for more details.

Figure 13. Left, burned skeleton of a *Persoonia hirsuta* plant. Right, tagged *Persoonia hirsuta* seedling. Yanderra, NSW, 2014. Images: Alison Haynes

Implications. The seedbank showed a strong and ostensibly, delayed, response to the fire. The population was greater post fire than beforehand, but only continued monitoring will determine whether these shrubs will survive to maturity; or whether competition, for example, will tend to reduce plant numbers in the seed clusters (Roberts et al 2013). Confirming fire history of the site previous to 2013 could help age the plants that were burned and give insights into the species' optimal fire frequency.

Study two. Ecological burn at Appin North by South32 and the Rural Fire Service, autumn 2016

A controlled experimental burn was carried out by the Rural Fire Service at the Appin North site, NSW, in a section of dry sclerophyll forest that was habitat for a population of *Persoonia hirsuta* and which had last burned more than 20 years previously (Figure 14).

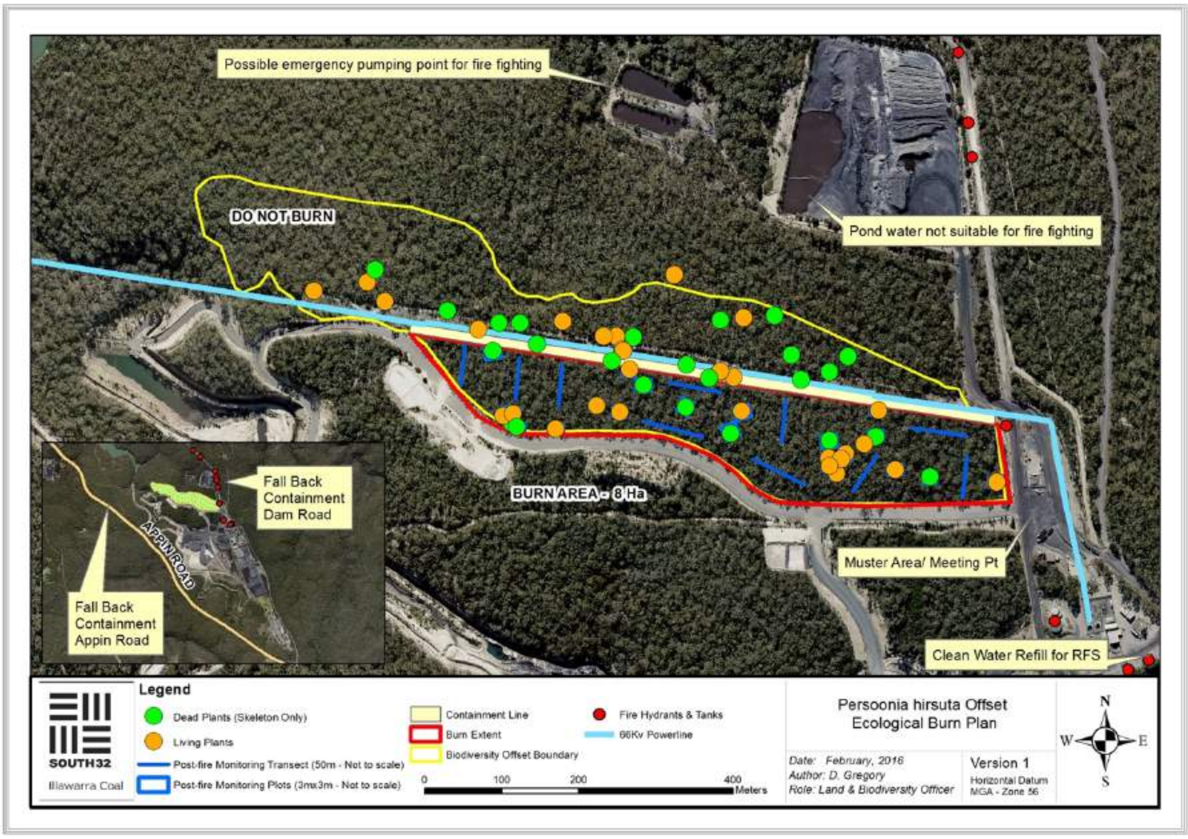

Figure 14. Map showing area burned in an ecological fire at Appin North in 2016.

Aims and hypothesis. The aim of the study was to promote germination of the seedbank and increase population numbers at the site. It was hypothesised there would be sufficient viable soil-stored seed to germinate, although it was noted that most *P. hirsuta* plants in the burn area were at least 24 years old and were likely to be senescing.

Methods. An area of 5 ha was burned on May 7 2016 (Figure 15). It consisted of dry sclerophyll forest with a shrubby understory, classified as Sandstone Ridge-top Woodland, with species including *Eucalyptus sclerophylla, Corymbia gummifera, Leptospermum trinervium* and *Banksia ericifolia*. Fuel was estimated to be 10 t/ha, consisting of medium density shrubby understory with moderate to high leaf loading and leaf litter depth of between 2 and 5 cm. Within the proposed burn area, 25 plants were tagged, nine of which were no longer living.

Figure 15. Ecological burn at Appin North, 2016.

Six temperature loggers were placed in the soil, three at depths of 1 cm and three at 3 cm throughout the burn site, to record temperatures during the fire.

The site was inspected three months after the fire, then at monthly intervals for one year. At each inspection, all burned skeletons were located and the area around the stem to 3 m^2 searched for germinations. In addition, 10 transects of 50 m each were also placed at random and the area each side searched each visit for seedlings.

Key results

Soil temperature. During the fire soil temperatures at a depth of 1 cm averaged 85.1°C while at 3 cm depth, soil temperature averaged 53.7°C.

Germinations. No germinations were recorded throughout the main monitoring season, nor during other visits up to October 2018. However, during annual monitoring of the area in December 2020, a single seedling was found.

Implications. Lack of recruits may be due to characteristics of the prescribed burn such as the timing of the fire, or soil temperature reached. It could also be due to low viability of the seedbank. Soil temperatures averaged 85°C at 1 cm depth and 54°C at 3 cm. For species in fire-prone habitats that rely on fire to break dormancy and trigger germination, optimum temperature varies both within and between species. For example, Liyanage and Ooi (2018) found optimum temperatures that broke physical dormancy fell between 65.4°C and 108.2°C in a range of species including both resprouters and obligate seeders; in addition, the duration at a particular temperature at, for instance, 2 cm depth, may not be long enough to be effective (Auld and O'Connell 1991). In this study, only one of the loggers out of six recorded a temperature above 80°C. Seed viability may also have been a serious obstacle to recruitment, considering that Bunker found less than one in 100 seeds contained an embryo. This alone would likely explain the result (Bunker 2016, see section five).

Study three. Germination trials with removal of endocarp, short term storage and heat shock, PlantBank

This has been described already in section five, and is summarised here. PlantBank researchers ran an experiment with *Persoonia hirsuta* seeds to determine the germination response to: heat shock; short-term *ex situ* storage at low and at room temperature; and endocarp removal (Emery and Offord 2019b). Heat may be important for sterilisation and protection against contamination, but response to heat is also of interest for plants in fire-prone habitats. It was unknown whether heat enhanced germination success of *P. hirsuta* by helping to break dormancy or triggering germination.

The study used a total of 1000 fruit from 20 plants; heat shock consisted of placing seeds in a fan forced oven set at 80°C for 10 minutes. The application of heat did not influence germination rates, but it is possible that the heat treatment, at 10 minutes, was not long enough. It is also possible that a combination of triggers is required to break dormancy and/or trigger germination.

An additional experiment, also described in more detail in chapter five, investigated the effect of smokewater on germination of extracted *P. hirsuta* seeds. Smoke did not increase germination.

Comments on fire response

Together these studies confirm basic information about the fire response of *Persoonia hirsuta*. In particular, adults are killed by fire and recruitment followed fire. In the case of the Yanderra fire, this recruitment appeared to be delayed and could possibly represent a seasonal delay in germination, but being only one fire it is not conclusive evidence. Failure to regenerate can be due to many reasons including low seed set, high seed predation, poor germination, high mortality from increased grazing pressure, or drought (Nield 2015). However, the lack of recruits one year after fire at Appin North is most likely explained by a non-viable seedbank. Comparing the two situations raises numerous research questions especially concerning soil temperature and timing of fire - the ecological burn being in autumn, while the Yanderra wildfire was in spring. Additionally, an endocarp weakened by a long series of wet and dry cycles (i.e. one that is several years old) may leave a seed vulnerable to damage by fire. Resolving such questions would greatly help fire management for this species. The germination studies suggest heat as an important aspect of fire, though not necessarily smoke, but further work is needed to understand how dormancy is broken and germination triggered in this species and could involve a suite of factors.

11. SUMMARY AND FUTURE DIRECTIONS IN RESEARCH ON *PERSOONIA HIRSUTA*

From almost no knowledge about the species' biology beyond its morphology, and the fact it is insect pollinated, the body of research outlined in this report has taken significant steps towards understanding the conservation needs of *Persoonia hirsuta*. The search effort resulted in 14 populations being recorded, many of which were sites for several studies. The key findings from this research can be summarised as follows:

Genetic diversity

Species-specific primers were developed for use in microsatellite studies that investigated both adult (leaf) and offspring (seed). Despite small isolated populations, diversity of adult stands were found to be higher than expected, and populations were differentiated but only to a small degree. Genetic analysis of adults revealed a mating system that is predominantly outcrossing (Ayre et al 2021, Haynes 2015).

Extracting DNA from seeds and running through microsatellite protocols was fraught with obstacles, from lack of seed embryo, to problems with DNA quality and downstream processes such as PCR and sequencing. This hampered analysis since sample size was small. Nonetheless results showed the seedbank was also diverse (and more diverse than adults) and in contrast to the adult population, showed moderate to severe inbreeding.

These studies have been interpreted as showing that the populations were historically more connected than they are currently; and also, that a seedbank harbours genetic diversity and can protect a population from loss of diversity. UOW studies using material from three sites showed all populations to be of conservation value since each contained private alleles.

Habitat

As predicted, sites containing *P. hirsuta* are sandy, with percentage sand ranging from 56.74% in Yanderra to 64.53% in Appin North; silt was 31.27% to 38.51%; clay content was low, from 3.54% to 5.02%. Soil particle size was smaller at sites where *P. hirsuta* persisted (and ranged from 116 µm to 223 µm) (Willmott 2013). Willmott found that *Persoonia hirsuta* sites ranged from 106 m above mean sea level to 476 m.

Seedbank

Most soil seeds are found in the top 3 cm of soil under the canopy of an adult plant. Recruitment after fire at one site also confirmed this pattern, supporting the suggestion that seeds are primarily gravity dispersed (Willmott 2013, Haynes 2015).

Seed viability

Opening seeds for one of the genetics studies revealed a high proportion of empty seed at two of the sites. One of these, Appin North, was the site of an ecological burn that resulted in no germinations (except for one several years after the fire) and it is hypothesised that low viability of the seedbank was a major reason.

Seed germination

Experiments showed removing the woody endocarp reduced germination success, while heat treatment did not effect germination. This means that the endocarp should be retained for when storing seeds in *ex situ* seed banks for conservation purposes, although longer term tests should be run as storage was limited to six months.

Pollination biology

Pollinator observations confirmed similar pollinators to that known for the genus overall, i.e. native bees of genera such as *Camponotus, Exoneura, Leioproctus* and *Megachile*. As well, the honeybee, *Apis mellifera* was a common pollinator, though it is unclear to what extent it is an effective pollinator (Bunker 2016). Across several sites a range of taxa was observed on flowers, including beetles, flies and butterflies, though again, their effectiveness is unknown, but suspected to be low. One of these sites, Appin North, was considered to have a less than optimal pollinator assemblage, which can result in reduced fruit set (Bunker 2015, Emery 2021 unpublished).

Fruit set

Manipulation studies support the concept that *P. hirsuta* is more successful reproductively when outcrossing. Flowers left open to pollinators develop more fruit, with much less being produced by autogamy and selfing (44.8%, 1.5% and 8.8% respectively) (Emery & Offord, in review).

Fire response

A wildfire at a known *Persoonia hirsuta* population site at Yanderra in spring 2013 afforded the opportunity to monitor seedling emergence. All 17 previously documented adults were killed by the fire while 68 germinations occurred in their place, during two years following the fire. In contrast an ecological burn in autumn at Appin North, on a section of the offset site with 16 living plants, resulted in one seedling, observed five years after the burn, and no recruitment before that.

Translocation

A pilot study found greater success with juvenile plants raised from seed compared to those raised from cuttings. In fact, after 20 months, only plants from seed survived. Survival was also enhanced by plant guards of hessian and by mulch consisting of local leaf litter. The survival rate overall was around 20% but the pilot study highlighted important methods that should increase future translocation success. A second translocation is now underway after planting in May 2021, using guards for all plants, in a site with greater canopy cover than the pilot study site.

Ongoing research

A number of studies are underway, with results yet to be documented. These include:

- Genetic studies to determine the species status regarding two sub species, *evoluta* and *hirsuta*. This study also examines parameters such as genetic diversity and differentiation of several populations.
- A field and glasshouse study of reasons for dieback of the species aims to determine plant-soil interactions involving both pathogens (such as Cinnamon Fungus) and beneficial microbes, as well as the effect of nutrient availability on plant health.

Research recommendations

Older *Persoonia hirsuta* populations are at the greatest risk of local extinction, either gradually due to plant senescence or quickly from a stochastic event, such as fire.This scenario points to three major research priorities: 1) fire response and optimal fire regime (following the determination of the species primary juvenile period), including the possibility of using effective prescribed burns to germinate the seedbank before it loses recruitment potential; 2) seedbank viability and longevity and 3) propagation efforts to boost, replace or reintroduce populations. These and other research questions are outlined in three broad categories below.

1. Seed biology

Seed germination

Related to fire response is the question of the role of fire in germination, in particular to what degree fire or heat can be used as a management tool that weakens the endocarp, whether in the field or *ex situ*. While preliminary investigations suggest heat is not involved in germination, it may be that a suite of factors is required, including smoke. Other valuable investigations include whether the endocarp provides chemical protection as well as physical protection.

Direct sowing

More research is needed on direct sowing methods, in particular regarding the endocarp as a limiting factor in germination. For instance, currently, there is no easy and quick way to extract the seed from the pyrene. Ideally, methods should be developed to enable batch processing of the seed for sowing, and to determine priming methods. If successful, this strategy could be utilised to more effectively include *Persoonia* species in restoration and rehabilitation projects.

Seedbank viability

How long do seeds remain viable in the soil; does this vary across sites and years? How do different treatments and extended storage effect germination success? These are critical questions for the persistence of a plant like *Persoonia hirsuta* that has experienced habitat fragmentation and a changing fire regime. Other avenues of research include:

- How the pollinator assemblage affects seed production;
- How does plant density and abundance influence reproductive success;
- How the seedbank accumulates on an annual basis;

- How the endocarp changes over time;
- How seed viability changes over time;
- How the fire regime and climate impact these processes.

2. Habitat

Propagation - site selection

Translocation shows promise as a conservation measure for this species but is a complex procedure with several stages, from collection of material (e.g. seed), through germination, growing of tube stock, site selection, preparation and planting. Following the first pilot study at Appin North, a number of avenues of research could be useful:

- Site selection including characteristics such as canopy cover and soil traits;
- Amelioration of extreme conditions (can seedlings be better protected through extreme weather days?)
- Does inoculation of roots with microbes improve survival?

Habitat/ range/ climate

Investigations have commenced which use species distribution models for *Persoonia hirsuta* that incorporate climate and soil data as well as fire history. This research is critical to predict suitable habitat for site selection and to identify at-risk populations in the light of climate change, coupled with translocation or other propagation trials.

The current study on dieback includes aspects of site characteristics which will be useful for management of the species. In addition, issues related to climate change need to be investigated:

- How adaptable is the species in terms of climate? (temperature and drought especially);
- Identification of climate refugia;
- Investigation of potential and methods for assisted migration;
- Identification the provenance of diversity, as a focus of seed collection for propagation and *ex situ* collections;
- Undertake a regional population census. Revisit known existing populations and undertake a detailed search for new plants. This is particularly important following the 2019 /2020 fire and for areas such as the Sydney Water Catchment area that have been little surveyed.

3. Ecology

Fire response

Many questions remain concerning the fire response of *Persoonia hirsuta* and its optimal fire regime. Optimal fire frequency appears to be less than 20 years, but analysing the fire history of known sites for the species could confirm this. Other questions concern the optimal soil temperature for regeneration, especially important if, on further analysis it is confirmed that fire suppression is threatening the persistence of the species. In this case, fire management that also includes prescribed fires, could prevent local extinction. Currently the the primary juvenile period is unknown.

Phytophthora risk

Further research is needed to determine the susceptibility of *P. hirsuta* to the soil pathogen *Phytophthora cinnamomi*. As the pathogen is known to occur in vicinity of the species habitat, it is possible that *Phyophthora* is contributing to the species decline.

Genetics

With preliminary data indicating significant spatial genetic differentiation, future research should determine if mixing plants from multiple genetic clusters for translocations risks population failure due to outbreeding depression.

Support of current projects and the questions that emerge

Much of the research outlined in this report is ongoing, so continuation of support to these is important, in particular to enable the completion of the wider genetic study, the current work on dieback, in particular soil interactions; and the monitoring of the translocation pilot and second translocation studies at Appin North.

Threatening processes, mitigation and management actions

Threatening processes listed in section one, such as habitat loss and disturbance, and inappropriate fire regimes, continue to be relevant, but research summarised in this document has provided new opportunities to specify potential mitigation and management actions (Table 1). For instance, once populations have been identified that are suffering from dieback, protocols can be introduced when visiting them such as cleaning all boots and equipment with ethanol to prevent contamination of other sites; while protecting current populations is crucial to mitigating the effects of further habitat loss, modeling habitat can also help - both to identify suitable current habitat and to identify future climate refugia. Management actions identified (Table 1) could be implemented on a site by site basis where feasible and practical.

Table 1. Threatening processes for *Persoonia hirsuta,* options for management response, and how research can mitigate the challenges.

Threat	Mitigation/management actions	Current/future research actions
Habitat loss	· Identify and protect areas where *P. hirsuta* occurs. · Ensure habitat connectivity between populations. · Identify climate refugia.	1. Survey areas where *P. hirsuta* historically occurs to accurately map its distribution. 2. Model potential areas of suitable habitat and conduct on-ground surveys to confirm.
Habitat disturbance	· Restrict road and trail maintenance around plants to limit disturbance. · Restrict slashing of *P. hirsuta* plants to 1m from ground level. · Minimise human activity around plants to reduce risk of disease. · Erect fencing around plants to restrict herbivores. · Conduct hand weeding to create a 20m buffer around populations.	1. Determine the level of above- and below-ground disturbance that negatively impacts plant survival.
Fire regime	· Maintain a fire regime at least 10-15 years where *P. hirsuta* occurs. · When conducting HR burns, ensure *P. hirsuta* plants are protected/buffered from fire. · Check for presence of a soil seedbank before burning.	1. Determine the longevity or half-life of seeds in the soil. 2. Quantify the primary juvenile period. 3. Determine impacts of fire seasonality and severity on recruitment. 4. Quantify population effects of post-fire recovery and recruitment. 5. Investigate the effects of fire on the endocarp.
Population size	· Monitor plant abundance, including adults and juveniles to capture long-term trends. · Conduct supplementary plantings at populations with <20 individuals to encourage cross-pollination and seed production. Plant genetically diverse translocants where appropriate. · Record local pollinators that visit *P. hirsuta* plants · Monitor fruit production rates on *P. hirsuta* plants.	1. Investigate the genetic diversity of populations across the distribution. 2. Establish whether there are outbreeding depression risks.
Dieback	· Boots and equipment should be cleaned with 80% ethanol prior to entering sites with *P. hirsuta* where there is risk of contamination. · Identify populations with signs of dieback and restrict access to these sites.	1. Determine if *P. hirsuta* is susceptible to *Phytophthora* or other soil pathogens. 2. Identify possible genotypes that are more resistant to soil pathogens. 3. Establish if there are nutrient thresholds that may trigger dieback.
Lack of recruitment	· Monitor the presence of seedlings and juveniles in populations. · Monitor soils adjacent to adult plants for presence of seedbank.	1. Quantify time required for endocarp to breakdown and trigger germination. 2. Determine if seeds are physiologically dormant.

Time frame of research

While short term studies can usefully guide conservation of a species, where possible long-term studies are highly recommended. Almost a decade of research was needed to understand enough of the ecology of *P. hirsuta* so that plants could be successfully propagated, and a translocation program could commence. Ideally, ongoing support is needed for translocation monitoring over several generations. Additionally, it may take up to a decade to monitor recruits to reproductive maturity. From these studies, it can be deduced whether the conservation measures are producing viable populations that will independently reproduce and continue to persist in the landscape.

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APPENDIX 1

Table A1. Table showing where condition requirements from EPBC 2010/5350 (Condition 3) have been addressed in this report.

Condition requirement	Details of condition	Section where addressed
a	Document current understanding of *Persoonia hirsuta* ecology and genetics;	3: Demography and habitat requirements
b	Outline previously documented management and conservation actions;	2: Previous management and conservation actions
c.i	Investigate pollination biology	4: Pollination biology of *Persoonia hirsuta*
c.ii	Requirements of its pollinators	4: as above
c.iii	Soil seed bank dynamics and the role of various disturbances (including fire) in germination and recruitment;	5: Soil seed bank dynamics
c.iv	Phenology and seasonal growth of *Persoonia hirsuta*	6: In the field: phenology, seasonal growth and translocation
c.v	Population genetic structure, levels of genetic diversity, minimum viable population size and management actions	7: Ecology and population genetics for conservation and 8: Genetic diversity of the seedbank
c.vi	Impact of dieback disease and control techniques on *Persoonia hirsuta* and its habitat; and	9: Dieback and control techniques
c.vii	Impact of fire on *Persoonia hirsuta* and its habitat	10: Impact of fire
d	Provide a general analysis of threatening processes and available management/mitigation actions.	11: Summary and future directions in research on *Persoonia hirsuta*

APPENDIX 2

Papers resulting directly from the research detailed in this report.

Andres SE, Powell J, Gregory D, Offord C & Emery N (in review) Assessing translocation management techniques through experimental reintroduction of the Endangered shrub *Persoonia hirsuta*

Andres, S, Emery, N, Gregory, D and Powell, J, 2020, Threatened plant translocation case study: 'Persoonia hirsuta' (Hairy Persoonia), Proteaceae, *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation, 28, 3, 14-17*

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