The Australian
BOTANIC GARDEN
Mount Annan

# Western Sydney International Airport

## Threatened Flora Propagation Program Delivery Report

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# 1. Executive Summary

The Western Sydney International (WSI) Nancy Bird Walton Airport *Threatened Flora Propagation Program* (TFPP), was developed and commenced in August 2017. The TFPP has been delivered by the Australian Botanic Garden, Mount Annan (ABGMA) with the support of GHD ecologists between April 2017 and July 2019. The key objective of the TFPP was to develop the most effective approaches to the propagation and translocation of threatened native plant populations affected by the development of the airport, and to support the conservation of these species.

The program focused on three listed threatened species; *Pultenaea parviflora* (a shrub), *Marsdenia viridiflora* subsp. *viridiflora* (a climber) and *Pimelea spicata* (a low shrub) which are not commonly cultivated and have contrasting growth habits, ecological requirements and seed biology. Resulting from this program of seed/cutting collections, nursery propagation and ecological observations at the WSI site, the key results of the TFFP are:

- *Pultenaea parviflora* is a straightforward plant to propagate from seed and grow under nursery conditions. Cuttings had a low success rate and produced plants with low vigour. Seed can be collected easily using mesh bags, and the hard coated seed has a very long storage life under good storage conditions.

- *Marsdenia viridiflora* subsp. *viridiflora* produces small amounts of fruit/seed, making good quantity seed collections difficult. Germination and storage of seed is straightforward. Cuttings is the preferred method to produce plants, although they are slow to establish.

- *Pimelea spicata* is a difficult species to propagate and to collect seed from in quantity, particularly during drought conditions. Seed germination rates are low due to physiological dormancy, although treatment with gibberellic acid and smoke water improved germination. Cuttings are slow to establish, and even with well struck cuttings losses at the potting stage can be significant.

At the conclusion of Stage 1 of the TFPP, the following plant materials were held at the ABGMA, and provide a valuable resource for future conservation work at the WSI site:

- *Pimelea spicata* (190 plants in the nursery, 6,100 seeds held in PlantBank).

- *Marsdenia viridiflora* subsp. *viridiflora* (560 plants, 108 seeds).

- *Pultenaea parviflora* (500 plants, 50 seeds).

The combination of seed collections, germination/propagation experiments, nursery stock and documented propagation techniques generated by the TFPP are a significant advance on the existing propagation and conservation knowledge of these poorly known threatened species. As such, the TFPP comprises an 'other compensatory measure' component of the Biodiversity Offset Delivery Plan (BODP) for the WSI site and will help offset impacts to these threatened flora species.

# 2. Introduction

## Project background

The Western Sydney International (WSI) Nancy Bird Walton Airport is being developed on approximately 1780 hectares of land at Badgerys Creek acquired by the Australian Government in the 1980s and 1990s. Approval for the construction and operation of the airport is controlled by the *Airports Act 1996* (Cth) (Airports Act). The Airports Act provides for the preparation of an Airport Plan, which serves as the authorisation for the development of the airport. In addition, the Australian Government Minister for the Environment determined that the construction and operation of the airport would require assessment in accordance with the *Environment Protection and Biodiversity Conservation Act 1999* (Cth) (EPBC Act).

An Airport Plan was developed which provides details of the initial development being authorised, as well as a long-term vision of the airport's development over several stages. The Airport Plan contains several biodiversity conditions, which require mitigation and management measures to be implemented to reduce the potential impacts on biodiversity values and to offset unavoidable residual impacts.

## Project objective

Condition 33 of the Airport Plan requires the delivery of a *Threatened Flora Propagation Program* (TFPP), developed in consultation with the Federal Department of the Environment and Energy (DoEE), the NSW Office of Environment and Heritage and the Australian Botanic Garden, Mount Annan (ABGMA). The purpose of the TFPP is to help develop the most effective approaches to the propagation and translocation of threatened plant populations affected by the development of the airport and to support the conservation of these species. The threatened flora species that are the subject of the TFPP are:

- *Pultenaea parviflora* - a threatened shrub which is listed under NSW state and Commonwealth legislation as a threatened species.
- *Marsdenia viridiflora* subsp. *viridiflora* - a climber which is listed under NSW state legislation as an endangered population in the Liverpool Local Government Area.
- *Pimelea spicata* - a low spreading shrub which is listed as an endangered species under Commonwealth and NSW State legislation.

ABGMA has been engaged by GHD as a sub-consultant to undertake seed collections and propagation of these three species and to help deliver the TFPP. Located in Western Sydney, ABGMA specialises in native flora and is part of the Royal Botanic Gardens & Domain Trust (Department of Planning, Industry and Environment). ABGMA Science and Horticulture teams specialise in seed storage, propagation and the conservation of NSW threatened species. Operating out of PlantBank, a purpose-built seed storage and research centre, and the Horticultural Nursery, staff have extensive experience in collecting and conserving Western Sydney flora.

The Airport Plan conditions required the Department of Infrastructure, Regional Development and Cities (DIRDC) to prepare for approval a Biodiversity Offset Delivery Plan (BODP) to compensate for residual significant impacts associated with development of the airport. The BODP was prepared in accordance with condition 30 of the Airport Plan which, among other requirements, requires that the BODP takes into the *EPBC Act 1999 Environmental Offsets Policy October 2012* (EPBC Act Offsets Policy) (DSEWPaC 2012). Consultation with Environment has confirmed that the TFPP may be considered as a proportion of the 'other

compensatory measures' component of the BODP. To qualify for this approach, the program must be undertaken as part of a sound scientific framework, with adequate monitoring and reporting that genuinely increases the knowledge and understanding of the species (DSEWPaC 2012). The TFPP is a compensatory measure presented as an offset for impacts to *Pimelea spicata, Marsdenia viridiflora* subsp. *viridiflora* and *Pultenaea parviflora*.

The TFPP commenced in April 2017 with the collection of *Marsdenia viridiflora* subsp. *viridiflora* fruits and cuttings from the WSI site and the planning for collection of *Pimelea spicata* and *Pultenaea parviflora* material. The delivery of the TFPP continued throughout 2017 until July 2019 with successive rounds of collection of plant material, propagation trials and maintenance of nursery plants. This delivery report presents the results and conclusions of the implementation of the TFPP.

## Structure and purpose of report

The purpose of this TFPP Delivery Report is to:

- Provide an overview of the ecology and life history of the subject species *Pultenaea parviflora*, *Marsdenia viridiflora* subsp. *viridiflora*, and *Pimelea spicata*.
- Present the aims of the TFPP including propagation targets, research questions and the focus of various propagation trials undertaken to establish the best techniques to propagate the subject species.
- Outline the methodology for collection and propagation of plant material and the experimental design for propagation trials.
- Present the results of the collection program and propagation trials.
- Present key outcomes of the program and research findings as a guide to the successful propagation of the subject species.
- Demonstrate compliance with the Airport Plan conditions, BODP and EPBC Act offsets policy.
- Confirm how the delivery of the TFPP functions as an 'other compensatory measure' as part of the suite of biodiversity offsets implemented for the WSI site.

The TFPP has been prepared to help address the following research priorities for the threatened biodiversity of the Cumberland Plain identified in the *Cumberland Plain Recovery Plan* (DECCW 2010):

- undertaking trials to establish the propagation requirements for key Cumberland Plain species (including threatened species, populations and communities) to assist with restoration.
- researching the seed storage requirements of key Cumberland Plain species (including threatened species, populations and communities).

Sections 4 to 6 of this TFPP Delivery Report present the aims, methodology, results and key outcomes of the program separately for each of the three subject species. The report has been structured in this way so that the results could be readily accessed and summarised for each species individually to support future translocation or revegetation programs and to assist with their long-term conservation.

# 3. Scope of Works

The scope of works for the TFPP was to:

1. Make seed collections of *Pultenaea parviflora*, *Marsdenia viridiflora* subsp. *viridiflora* and *Pimelea spicata* from the WSI site, ensuring that adequate ex-situ seed collections are held at the Australian PlantBank at the ABGMA.
2. Make collections of cutting material from the WSI site and/or access other sources of seed to ensure that sufficient propagules are available to meet the program's targets.
3. Conduct seed germination and cutting trials to establish the best techniques to propagate and grow these three plant species.
4. Grow a minimum of 500 plants of each of *Pultenaea parviflora* and *Marsdenia viridiflora* subsp. *viridiflora*, to be available for use in revegetation programs or other conservation activities at program completion.
5. Grow up to a target of 1000 plants of *Pimelea spicata*, ensuring genetic sampling across the WSI site population to be available for an *ex-situ* potted collection, for use in revegetation programs or other conservation activities at program completion.
6. Report the key outcomes and research findings of the TFPP as a guide to the successful propagation of these species for future translocation or revegetation programs and to assist with their long-term conservation.

The approach and indicative program for the TFPP are outlined in the *Western Sydney Airport Biodiversity Activities Threatened Flora Collection and Propagation Plan for Pultenaea parviflora, Marsdenia viridiflora subsp. viridiflora and Pimelea spicata – August 2017* (ABGMA 2017).

The TFPP was undertaken by ABGMA with the support of GHD ecologists between April 2017 and July 2019.

The end use of the plants at the conclusion of the trial will be determined in conjunction with the implementation of the BODP. Most of the plants are likely to be used in revegetation programs to help maintain the population size and genetic viability of the regional populations of these species. A subset of the tube stock plants could be used to establish a longer-term potted ex situ collection at the ABGMA nursery. The end use of the propagated *Pultenaea parviflora, Marsdenia viridiflora* subsp. *viridiflora* and *Pimelea spicata* will be documented in the BODP Implementation Audit Report(s) and biodiversity monitoring plans for receiver sites as appropriate.

All seed and vegetative collecting activities are conducted under Royal Botanic Gardens & Domain Trust (RBG&DT), Section 132c Scientific licence No. 100569 issued by the NSW Office of Environment and Heritage (OEH, now DPIE) which includes seeds and cutting collection from NSW *Biodiversity Conservation 2016* (BC Act) listed species.

Seed and vegetative collection was performed in an ecologically sensitive manner, in accordance with the guidelines published in 'Plant Germplasm Conservation in Australia' (Offord and Meagher 2009) which are consistent with international standards. Seed collections were processed and stored at the Australian PlantBank at the ABGMA in accordance with Offord & Meagher (2009), i.e. dried at 15°C/15%RH, germination tested prior to banking and stored at -20°C in hermetically sealed laminated foil packets.

All seed and cutting collections were recorded on the Royal Botanic Gardens and Domain Trust (RBG & DT) living collections database with accurate GPS locations, and matching herbarium specimens lodged at Royal Botanic Gardens Sydney (RBG Sydney).

# 4. Pultenaea parviflora

## Species overview

*Pultenaea parviflora* is a small erect shrub from the Fabaceae family 1 m - 1.8 m high, producing yellow pea-like flowers between August and November. Seed is generally produced in early summer (December) which is egg shaped up to 5mm long. Seed has a hard coat with an aril, which can be readily dried and stored long term under seedbank conditions.

Distribution is restricted to the Cumberland Plain region in the western districts of Sydney, where it occurs on tertiary alluvium/lateritic clay in a number of woodland communities such as Castlereagh Ironbark Forest and Shale Gravel Transition Forest. *Pultenaea parviflora* is listed as an endangered species under the NSW BC Act and a vulnerable species under the EPBC Act.

**Figure 1** *Pultenaea parviflora*

Produces hard coated seeds which can persist in the soil seedbank and germinate following bushfire or disturbance.

## Program aims

The propagation of *Pultenaea parviflora* was the subject of a previous study at the ABGMA in 1990. This previous study was conducted in a relatively orthodox manner, whereas the TFPP assessed newer propagation technology. The aims of the TFPP for *Pultenaea parviflora* were to:

- collect seed from the WSI site and access the seed collection held at the Australian PlantBank at the ABGMA to grow a minimum of 500 plants of *Pultenaea parviflora* to be available for use in revegetation programs or other conservation activities at program completion.

- conduct seed germination and cutting trials to establish the best techniques to propagate this species including trial of germination of older seed collections (1991) held at PlantBank and cutting propagation using a combination of hormone soak solution, in combination with hormone gel application at base of cutting.

## Program methodology

### *Seed collection and propagation*

- November 2017 - Mesh bags were placed over fruiting branches of the remaining plants at Longley's Rd, (and tied off at the base) once a substantial amount of flower set (and pollination) were complete.

- February 2018 - Mesh bags were retrieved from the Longley's Rd site when plants were pulled out of the ground by vandals.

- Seed processing and cleaning at PlantBank laboratory - *50 viable seeds* obtained from this collection, which have been dried at 15%RH/15°C and packaged in foil packets, then stored in PlantBank seed vault at -20°C. Due to insufficient seed collected in 2017/18 collection from the WSI site, it was agreed that an existing *1992* WSI site collection held at PlantBank (3415 seeds) would be accessed to try and meet the propagation target.

- Seeds from the 1992 collection were hot water treated (~85°C) and soaked for a minimum two hours before sowing into punnets. Growing media consisted of 50% coarse sand and 50% perlite seed raising mix. Punnet was placed in a temperature controlled glasshouse (av temp 25°C) with heated benches operating at 25°C with no misting system. Watering was via capillary matting and hand watering when required).

- Potting media was a soil-less coir and quartz based mix, pH balanced to around 5.8 with a low phosphorus 8-9 month Controlled Release Fertiliser added, steam pasteurised at 60°C for 30 minutes.

- Germinated seedlings were potted into 50mm forestry tubes and grown on in a temperature controlled glasshouse until enough root mass was observed and suitable outdoor temperatures were achieved.

- Plants potted on from 50mm tube to 140mm pots. The average time taken to pot from 50mm to 140mm was four months.

**Figure 2** Seed grown *Pultenaea* in ABGMA Nursery

### *Cutting propagation*

- 77 cuttings were collected (02/11/17) from the Longley's Rd site and processed at ABGMA nursery.
- Prepared cuttings were soaked in 1% bleach solution and rinsed under running water. Whole cuttings were placed in a solution of Esi-Root auxin hormone treatment (1.6/L IBA + 1.6/L NAA) at a rate of 10ml/4ltr for 15 minutes followed by application of Clonex green hormone gel to the base of each prepared cutting.
- Cuttings were placed into 20mm preforma coir plugs and placed into an evaporative cooled and overhead mist system glasshouse on a heated bench operating at 25°C. Misting system controls are monitored daily to provide target humidification of 85% where possible.

## Program results

### *Seed propagation*

There were two rounds of propagation from seed, both from the same 1992 seed collection.

The first round in November 2017 resulted in the germination of 425 plants from 600 seed. There was a 77.5% germination rate achieved after using the standard hot water soaking treatment used at the ABG nursery for Acacias and other hard seeded Fabaceae species with physical dormancy.

The second round in February 2018 resulted in 75 plants from 100 seed.

In addition, a standard seedbank viability test was conducted, where 100 seeds were scarified with a scalpel, sown on 0.7gms/l water agar and incubated at 20°C (12 hrs light/12hrs dark). Seed germinated within 7 days resulting in 93% success (93 germinants). This result indicates a high level of viability has been maintained since the original collection in 1992, in line with expected viability under conservation storage conditions (15% RH at -20°C).

### *Cutting propagation*

Remaining *Pultenaea parviflora* plants at Longley's Road were in poor condition at the time cuttings were taken.

Poor condition of cutting material and historical low strike rates for cutting growing *Pultenaea* contributed to the 1% strike rate achieved. The lack of vigour and poor health of the plant propagated resulted in the decline and eventual death of cuttings.

Results were consistent with previous ABGMA work on this species (Chandler 1990) that found cutting propagation was characterised by low strike rate <50% and difficulty in growing on cutting-propagated plants.

At the conclusion of the TFPP in July 2019 a total of 500 *Pultenaea parviflora* had been propagated from seed.

## Key outcomes and ecological observations

Key outcomes and findings of the TFPP that may assist with the future propagation or translocation of *Pultenaea parviflora* include:

- Seed can be stored long term under basic seed storage conditions, i.e. the 1992 seed collection was packaged in heat sealed foil packets and stored at 5°C until 2006 before being moved to more optimal conditions in the freezer (-20°C) until present.

- Hard seeded species such as *Pultenaea parviflora* can be expected to maintain high levels of viability for several hundred years in current storage conditions.

- Seed can be readily propagated following a standard hot water treatment to assist in the breakdown of the hard seed coat and seed imbibition/germination.

- *Pultenaea parviflora* populations have been in consistent decline at the WSI site. ~75 plants were recorded at the WSI site in 1989 (Chandler 1990), in 2017 GHD recorded only 5 extant plants and following vandalism (removal of seed bagged plants) at the Longley's Rd site this species is represented by only one remaining plant.

- Nursery production of *Pultenaea parviflora* from seed was straightforward and the species would be a suitable candidate to establish as a potted/planted seed production collection, to enable seed to be harvested for future translocation/restoration of this species at the WSI Environmental Conservation Zone.

- Additional seed of *Pultenaea parviflora* is available from the 1989 and 1991 WSI site seed collections are held at PlantBank, if required for future translocation activities.

# 5. Marsdenia viridiflora subsp. viridiflora

## Species overview

*Marsdenia viridiflora* subsp. *viridiflora* is a twining climber from the Apocynaceae family with stems up to 4 m high and narrow leaves 2 - 12 cm long and 1 - 18 mm wide with a prominent midvein. Flowers are greenish yellow, bell-shaped in clusters of 3 – 10. It produces a milky latex when cut and has large underground tubers up to 16 cm diameter. It is able to regenerate from underground tubers. Fruit are large and pear-shaped, up to 80 mm long, producing seeds with a silky coma which assists with wind dispersal. Seed production in wild populations is limited and highly variable, presumably in response to seasonal conditions. Seed storage behaviour is considered to be orthodox, i.e. seed can be dried and stored, however longevity in storage is unknown. Cutting propagation methods had not been previously trialled at ABGMA, and it was expected that the irritant milky sap would make preparation and treatment of cuttings difficult. The slender, twining habit of this plant also limited the amount of suitable cutting material available from the small WSI site population.

*Marsdenia viridiflora* subsp. *viridiflora* is widely distributed throughout coastal and inland regions of NSW and Queensland. The range extends from the Sydney region through to west of Cairns, in Far North Queensland. The species is highly restricted in the Cumberland Plain region of Western Sydney, where populations occurring in the former Bankstown, Blacktown, Camden, Campbelltown, Fairfield, Holroyd, Liverpool and Penrith local government areas are listed as an endangered population under the NSW BC Act. This NSW listing is due to extinction risk associated with:

- reduction of population numbers to a critical level
- reduction of habitat
- a disjunct distribution at or near the limit of its geographic range

*Marsdenia viridiflora* subsp. *viridiflora* is known to occur in a range of Western Sydney vegetation communities, including Cumberland Plain Woodland which is listed as a critically endangered ecological community under the BC Act and the EPBC Act. At its southern limit, *Marsdenia viridiflora* subsp. *viridiflora* is known to occur in Western Sydney Dry Rainforest, which is listed as an endangered ecological community under the BC Act and a critically endangered ecological community under the EPBC Act.

**Figure 3** *Marsdenia viridiflora subsp.viridiflora* fruit forming.
Pods open to release black seeds with a silky coma

## Program aims

The propagation and seed biology of *Marsdenia viridiflora* subsp. *viridiflora* is relatively unknown, and so this study required an adaptive and flexible approach to assess current propagation techniques and seed characteristics. The aims of the TFPP for *Marsdenia viridiflora* subsp. *viridiflora* were to:

- collect seed from the WSI site.
- collect cuttings from the WSI site and/or access other sources of seed to ensure that sufficient propagules are available to grow a minimum of 500 plants of *Marsdenia viridiflora* subsp. *viridiflora* to be available for use in revegetation programs or other conservation activities at program completion.
  - conduct trials to establish the best techniques to propagate this species including trial of collection and extraction of seed from mature and unripe fruits.
  - Seed germination trials.
  - Cutting propagation trials.
- collecting seed from the WSI site to be held for long term storage at PlantBank.

## Program methodology

### *Seed collection and propagation*

There were two seed collections, one in 2017 and on in 2018.

- Seed collection of *Marsdenia viridiflora* subsp. *viridiflora* from the WSI site was very limited due to the small number of plants and extended dry conditions. Despite profuse flowering regularly observed throughout the year, fruit set was limited. ABGMA staff observed that the large fruit of *Marsdenia viridiflora* subsp. *viridiflora* ripens very slowly making it hard to judge seed maturity. Once the mature phase is reached seed dispersal via wind can occur quite quickly.

- September 2017 - Three (3) seed pods were collected from the *Marsdenia viridiflora subsp. viridiflora* and slowly ripened over 6 weeks in a container containing Lithium chloride solution (65% RH @ 20°C). Two of the pods were pest damaged and the remaining pod contained 82 seeds.

- 10 seeds were germination tested in an agar petri dish incubated at 20°C at our PlantBank facility, achieving an 80% germination rate, the resulting eight plants being passed on to the nursery for growing on. Two seeds were assessed as not being viable. Of the eight, seven still survive.

- The remaining 72 seeds are held in storage in PlantBank.

- September 2018 - A single mature pod was collected from Longley's Road and resulted in 36 mature seeds which are also held in storage in PlantBank.

### *Cutting propagation*

- Cuttings were prepared after soaking in 1% bleach solution and rinsed under running water. Whole cuttings were placed in a solution of Esi-Root 1.6/L IBA + 1.6g/L NAA at a rate of 10ml/4Ltr hormone treatment for 15 mins, followed by application of Clonex purple hormone gel to the base of each prepared cutting.
- Cuttings were placed into 20mm Preforma coir plugs and placed into an evaporative cooled and overhead mist system glasshouse on a heated bench operating at 25°C. Misting system controls were monitored daily to provide target humidification of 85% where possible.

## Program results

### *Seed Germination*

The seed germination test resulted in eight (8) plants being moved to the nursery of which seven (7) are currently held in 165mm pots.

### *Cutting propagation*

At the conclusion of the TFPP in July 2019 a total of 562 *Marsdenia viridiflora* subsp. *viridiflora* had been propagated and potted on to 50 mm forestry tubes comprising material collected in November 2017 and December 2018.

In total 1055 cuttings were taken with 765 successfully striking representing a 73% strike rate. Of those 765 successful cuttings potted 562 plants are being held by the Nursery, in addition to the seven seed grown plants.

## Key outcomes and ecological observations

Key outcomes and findings of the TFPP that may assist with the future propagation or translocation of *Marsdenia viridiflora* subsp. *viridiflora* include:

- *Marsdenia viridiflora* subsp. *viridiflora* was a difficult species to collect seed in quantity, due to sporadic and limited fruit set, possibly due to ongoing drought conditions. Propagation from cuttings is reliable.

- Collected unripe fruit could be successfully artificially ripened to maturity under conditions of controlled temperature and humidity. Seed from fruit treated in this way were successfully germinated. These results, and visible seed quality, are in line with expectations given previous studies completed on other wild sourced collections of *Marsdenia* species.

- Due to limited amount of seed available at the WSI site, the collections of 108 seeds (72 from 2017 and 36 from 2018) have been retained at PlantBank for future use if required. Cutting propagation was the main technique used to produce plants for this project.

- *Marsdenia viridiflora* subsp. *viridiflora* is a light twining plant which favours growing on thickets of *Bursaria spinosa*, and often difficult to detect. We did not investigate the presence or size of the underground lignotuber and recommend this be investigated if plant salvage is considered as an option prior to the commencement of WSI earthworks.

**Figure 4** Marsdenia fruit being bagged on Longley's Road

# 6. Pimelea spicata

## Species overview

*Pimelea spicata* is a low spreading shrub from the Thymelaceae family growing to 50 cm in height. Leaves are opposite and elliptical, to 20 mm long by 8 mm wide. Tubular flowers are white, pink-tinged up to 10 mm long, with four spreading petals and can appear at any time of the year. *Pimelea spicata* is not capable of vegetative propagation and is dependent upon seed production for recruitment of new individuals. The species flowers sporadically throughout the year, with good flowering often observed during summer, particularly after heavy rain and good soil moisture. The plant is able to quickly regenerate from underground rhizomes or tubers. Green fruits to 2.5 mm long are produced after flowering, which contain small brown seeds ~2 mm long. Seed production is sequential and usually sporadic. Longevity of seed in the soil seed bank is unknown, and long-term seed storage under controlled conditions has not been tested. Seed germination is known to be promoted by disturbance events such as fire, slashing/, grazing and soil disturbance (DEC 2005). Under laboratory conditions seed germination is known to be responsive to smoke, indicating that physiological seed dormancy is likely to be present.

The main distribution of *Pimelea spicata* is the Cumberland Plain region of Western Sydney, with some disjunct populations in the Illawarra region. On Cumberland Plain sites it is associated with Grey Box communities, particularly Cumberland Plain Woodland and Moist Shale Woodland where a diverse understorey of native grasses and herbs is still present. Key threats to the long-term survival of *Pimelea spicata* identified in the Recovery plan (DEC 2005) are:

- weed invasion and habitat decline.
- loss of habitat through land clearing and urban development.

**Figure 5** *Pimelea spicata* flowering.
Seed production is highly responsive to good soil moisture and summer rainfall

## Program aims

The physiology, seed biology and germination of *Pimelea spicata* is known to present challenges to propagation. Previous experience with this species at the ABGMA indicates a low strike rate for cutting propagation and physiological seed dormancy which must be overcome to achieve seed germination. In addition, Western Sydney often experiences hot dry summers, and drought conditions can persist for many months. Under drought conditions seed set can be extremely limited, and propagation material of poor quality. Hence an adaptive management approach to the TFPP was implemented in order to help provide the quantity and quality of material required to achieve propagation targets.

The aims of the TFPP for *Pimelea spicata* were to:

- collect seed from the WSI site.
- collect cuttings from the WSI site to ensure that sufficient propagules are available to grow up to a target of 1000 plants of *Pimelea spicata* to be available for use in revegetation programs or other conservation activities at program completion.
- Investigate field techniques to increase seed production and/or the health of plants for harvesting cuttings including supplementary watering and removal of competition from other plants.
- Conduct trials to establish the best techniques to propagate this species including trial of:
  - Application of germination treatments to overcome physiological dormancy of *Pimelea spicata* seed
  - cutting treatment trials
- maintain seed collections from the WSI site to be held at PlantBank.

ABGMA is currently implementing the Stage 2 TFPP to help establish a longer-term potted *ex-situ Pimelea spicata* collection at the ABGMA nursery. This potted *ex-situ* collection would provide a source of cutting material to support any future translocation or amenity planting of the *Pimelea spicata* population from the WSI site once the plants are removed during the Stage 1 construction works. This program would draw upon experience in propagation techniques gained throughout the TFPP. The proposal is for a potted collection of approximately 50 plants, comprising approximately 30 genetic individuals, informed by the results of a genetic study and selected to minimise kinship and maximise genetic diversity.

## Program methodology

### *Seed collection and propagation*

Persistent drought conditions throughout 2017-2018 and from late Summer 2019 until August 2019, were a significant limitation to the seed collecting and cutting propagation work on *Pimelea spicata* as part of the TFPP. The WSI site *Pimelea spicata* population, however responded to relatively short favourable summer 2018-19 rain events producing flowers and seed set.

Two significant seed collections were made in December 2018 (1900 seeds) and February 2019 (4200 seeds). Mesh bags were placed over fruiting branches (and tied off at the base) once a substantial amount of flower set (and pollination) were complete. Mesh bags were left on the plants for 4-6 weeks to allow seed to mature to dispersal point. Bags were retrieved by cutting of fruiting branch at the base of the plant and taken back to PlantBank for mesh bag removal and processing.

A composite seed sampling strategy was used across the population with an estimated ~50 plants bagged and sampled.

**Figure 6** Pimelea growth and flowering in response to rain

**Figure 7** Bagging of Pimelea seed

### *Cutting propagation*

Cutting propagation material was harvested as a *composite* collection (individuals were not tagged) from plants across the entire population along the Northern Road, with the aim of achieving the target 1000 plants produced at ABGMA nursery. A composite cutting collection approach was required to obtain enough cutting material during drought conditions. Some individual plants were later tagged as part of the genetic study.

Additionally, and as a separate component of the genetic study (*Conservation genomics of Pimelea spicata in support of management and translocation activities*), 100 plants at the WSI site were GPS mapped and sampled, to inform an *ex-situ* potted collection for the Stage Two TFPP and other conservation strategies. The genetic study includes samples from 15 other populations in addition to the WSI site.

The collection of cuttings from the WSI site was a result of many visits to site over the years, based on rain and temperature. Most visits were unproductive in terms of cuttings but allowed the regular assessment of plant condition. The limited amount of material and the target numbers needed did not allow for extensive propagation trials.

Treatment of cuttings was as follows:

- Prepared cuttings were soaked in 1 % bleach solution and rinsed under running water. Whole cuttings were placed in a container of fresh water followed by application of Clonex green hormone gel to the base of each prepared cutting.
- Cuttings were placed into punnets of 14% coir fibre, 28% crushed quartz and 58% perlite and placed into an evaporative cooled and overhead mist system glasshouse on a heated bench operating at 25°C. Misting system controls were monitored daily to provide target humidification of 85% where possible.
- Increased humidity conditions were provided for the cuttings in propagation by placing clear plastic hoods drained of excess moisture daily and cutting media was watered when required. The top vent was opened after one week and hoods removed on mild overcast days when light levels are low, otherwise hoods remained until root formation. Plants were checked daily for botrytis and other fungal pathogens and treated with Previcur fungicide if needed.
- Potting media is a soil-less coir and quartz based mix, pH balanced to around 5.8 with a low phosphorus 8-9 month Controlled Release Fertiliser added, steam pasteurised at 60°C for 30 minutes.

In response to drought induced moisture stress at the WSI site *Pimelea spicata* population since the commencement of the project (August 2017), Greening Australia bush regenerators were engaged in December 2017 to help investigate field techniques to increase seed production and/or the health of plants for harvesting cuttings by reducing competition from grass.

As this was an experimental approach, only a small number of 100 plants within the population at the WSI site were flagged and hand weeded. This was undertaken in a ~1 metre radius around individual *Pimelea* plants. Supplementary water was applied on two separate days to all 100 marked plants as well as opportunistically to other visible and accessible *Pimelea spicata* at the site.
Clearing around individual plants did not increase plant vigour and resulted in the death of most individuals. A combination of factors may have contributed to the plant loss:

- Mechanical damage/disturbance - potentially the result of the clearing techniques adopted, given *Pimelea* is not a robust plant.
- Grass appeared to provide some protection from heat and evaporation, which when removed made plants more susceptible to environmental factors.
- Follow up watering may not have been adequate once plants became more exposed.
- Ongoing drought.
- Increased predation - most plants targeted for clearing were along the “game trail”, thus increasing the likelihood of more targeted predation/browsing from goats, rabbits and others.

ABGMA followed up with the installation of some protective plant cages, which provided some protection from browsers.

The strategy in the form described above, did not increase the success of the propagation programme either in terms of plant health in the field, seed production or cutting strike rates.

**Figure 8** Pimelea in grassland before clearing

**Figure 9** Pimelea after clearing

## Program results

### *Seed collection and germination experiments*

**Standard germination experiments**

*November 2018 Collection* - An initial experiment was conducted on relatively immature but fully developed seed collected in November 2018 and resulted in a germination rate of 38%.

For all standard germination experiments, seed, with seed coat removed, was sown in 80mm petri dishes with 3-4mm depth of 0.7% water agar incorporated with Gibberellic acid (250ppm). Topically, 2ml of 2% smoke water was applied over the seeds on each plate and they were incubated at 10/20°C alternating temperature. Fifty seeds were used for each experiment, 10 seeds/5 agar plates.

*December 2018 Collection* - A second experiment was conducted on fully mature seed collected in December 2018. The treatment was as above except one sample was sown *without* the smoke water application. There was a higher rate of germination for the sample with both GA3 and smoke water (22%) compared to the GA3 only (16%) but the rate was lower than the fresh sample from the previous month (38%).

*February 2019 Collection* – Fully dispersed and bagged seed from the February 2019 collection was sown after drying and using the combined gibberellic acid and smoke water treatment resulted in a germination rate of 36%, comparable to the initial germination experiment.

Additionally, two further germination experiments were set up at two different alternating temperatures 20/10°C and 20/5°C (day/night), 12hr light/12hr dark. The seed coats *were not* removed from the seeds used in these experiments.

The difference between the two December samples, 16% and 22%, demonstrates smoke, from an appropriate fire regime, would promote germination. The germination rate of 22%, lower than the fresh material, could be due a negative response to the seed drying which may indicate that although the seed appeared fully mature, the full development of seed characteristics, necessary for long term storage, had not been achieved at the time of collection. The seed that was fully mature and post-dispersal achieved a similar rate of germination to the fresh collection in November 2018, likely indicating the completed maturation of the seed.

For the experiments with the seed coat not removed, results showed a delayed germination compared to seed coat removed but similar final germination rate, 36% for the 10/20°C temperature. The final germination rate at 5/20 was supressed at 30%.

**Figure 10** Pimelea seed for processing in the lab

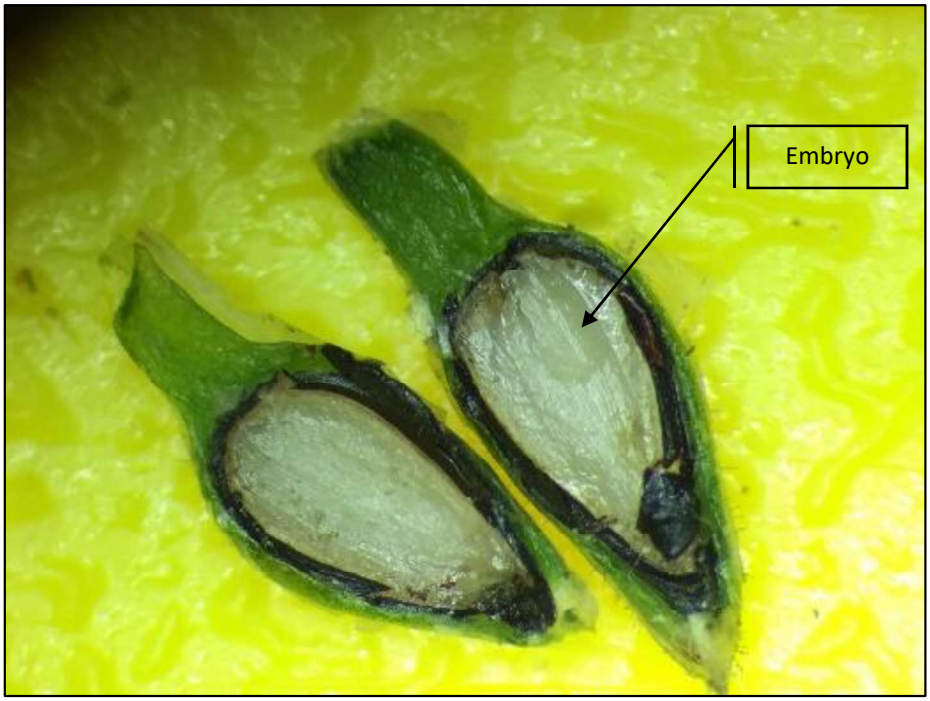


**Figure 11** Pimelea seed cross section under a microscope showing viable embryo

**Thermogradient Table Germination Experiment**

A thermogradient table allows simultaneous testing of multiple seed samples across a range of temperature combinations. Using a gradient from 5°C to 45°C and alternating the gradient every 12 hours enables the determination of optimum, upper and lower limits of germination across the range of night/day temperature combinations.

An experiment was set up in July 2019 to investigate optimal day/night germination temperatures for *Pimelea spicata.* A total of 450 seeds were used from the second collection event in February 2019. The thermogradient table (Grant Instruments Pty Ltd) provides the capacity to reproduce a range of programmed individual 'cells' set at a wide range of day/night temperatures simultaneously within the one unit.

The thermogradient table was set to alternate day and night temperatures both between a range of 5-45°C, with a grid formation of 9 x 9 squares containing germination plates. The temperature gradient and light conditions (light/dark) were alternated on a 12hr/12hr cycle. The temperature range was selected to reflect the typical maximum and minimum temperatures experienced in the collection location in the previous 12 months. The weather data was obtained from the Bureau of Meteorology website. The experiment was only conducted in the half of the table that had warmer day and cooler night to constant day/night temperatures, which best reflected the potential environmental temperatures of where this species grows.

Forty-five replicates of 10 seeds were used, and the seed coats were removed as a pre-treatment, which we believed would improve germination.

Each replicate of 10 seeds were placed in 45mm (diameter) plastic petri dishes, filled with 3-4mm depth of 0.7% water agar incorporated with Gibberellic acid (250ppm). Topically, 2ml of 2% smoke water was applied over the seeds on each plate. The experiment ran for 31 days in total, during which the plates where checked every 2-3 days in the first two weeks and 4-5 days in the following weeks. Each plate was sealed with cling film to reduce moisture loss.

**Figure 12** Thermogradient table
Showing the experimental setup and arrangement of individual day/night temperature treatment cells

Contour plots were generated to visually display the hotspots for cumulative germination and time till first germination for each replicate. These plots were generated in the statistical computer program R. They are displayed below.

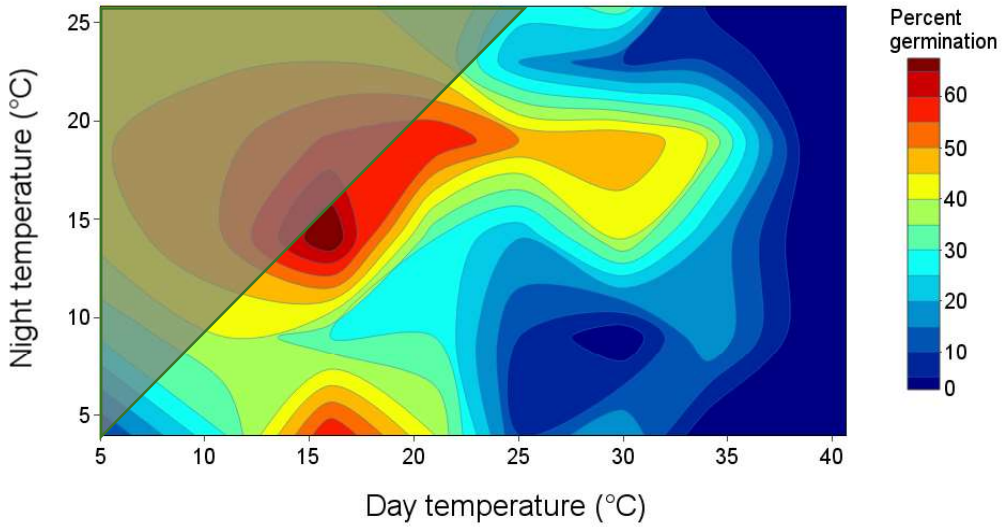


**Figure 13** Contour plot of *Pimelea spicata* cumulative germination % on a thermogradient table.

A 12hr/12hr alternating temperature gradient and light/dark schedule can be seen. Temperature range was 3-45°C (n=10). Note: no replicates were present in the temperatures greyed out.

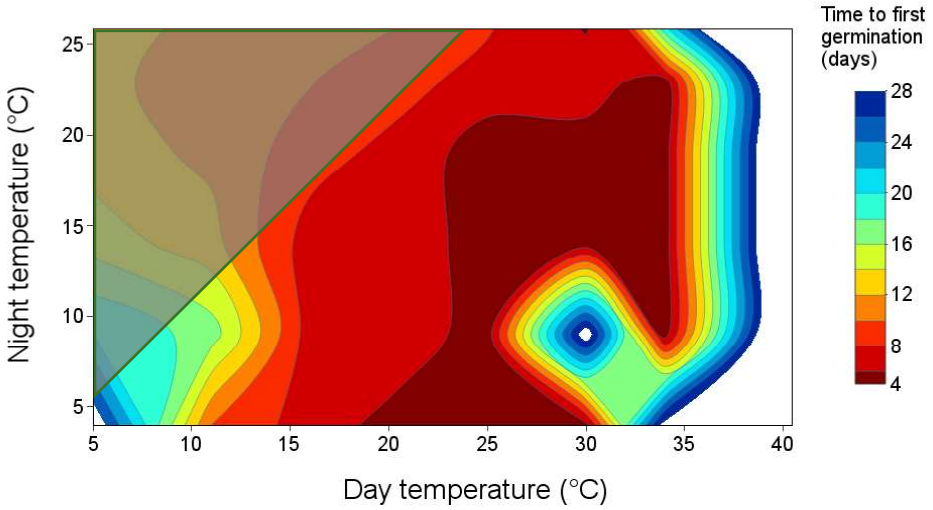


**Figure 14** Contour plot of *Pimelea spicata* time to first germination (days) on a thermogradient table.

Showing a 12hr/12hr alternating temperature gradient and light/dark schedule. Temperature range was 3-45°C (n=10). Note: no replicates were present in the temperatures greyed out and no seeds germinated at all in the area that is white

Contour plots indicate that at higher day temperatures there is a distinct suppression of germination (i.e. summer conditions with high temperature range). Highest germination was achieved at 15-20°C day/night treatment (cooler with less temperature range) which would equate with spring or autumn conditions when rainfall and soil moisture conditions are potentially more suitable. There was still some germination at lower than optimal temperature, but it was at a lower and slower rate.

*Summary Table of Germination Experiment (Thermogradient)*

| Collection date | Seed development phase | Seed state | Treatment | Germination temperature (alternating night/day) | Germination % |
|---|---|---|---|---|---|
| Nov 2018 | Slightly immature | Fresh (seed coat removed) | GA3 & SK[3] | 10/20 C | 38 |
| December 2018[1] | Mature but not fully dispersed | Dried (Seed coat removed) | GA3 | 10/20 C | 16 |
| December 2018[1] | Mature but not fully dispersed | Dried (seed coat removed) | GA3 & SK | 10/20 C | 22 |
| February 2019 | Fully mature and dispersed | Dried (seed coat intact) | GA3 & SK | 10/20 C | 36 |
| February 2019[2] | Fully mature and dispersed | Dried (seed coat intact) | GA3 & SK | 5/20 | 30 |
| February 2019[2] | Fully mature and dispersed | Dried (seed coat removed | GA3 & SK | 15/20[4] | 70[5] |

[1,2.] Seed from same collections. [3] GA 3 = gibberellic acid at 250ppm, SK = topical application of 2% smoke water. [4] Optimum temperature from thermogradient experiment. [5] single 10 seed replicate as opposed to 5 x 10 seed replicates for others.

Germinants were sent to the Nursery and grown on with the following results:

- 19/7/19 - 50 germinants received on agar and transferred into punnets containing seed mix. 13 survived and were potted up on 2/8/19, of those seven remain. Accession no. 2019-0626/1
- 22/7/19 - 23 germinants received on agar and transferred into punnets containing seed mix. 13 survived and were potted up on 2/8/19, of those eight remain. Accession no. 2019-0626/5
- 24/7/19 - 18 germinants received on agar and transferred into punnets containing seed mix. 15 13 survived and were potted up on 5/8/19, of those 13 remain. Accession no. 2019-0626/3
- 29/7/19 - 15 germinants received on agar and transferred into punnets containing seed mix. 8 13 survived and were potted up on 20/8/19, of which five remain. Accession no. 2019-0626/4
- 13/8/19 - 4 germinants received on agar and transferred into punnets containing seed mix. Three were potted up on 9/9/19 with all surviving. Accession no. 2019-0626/6

Most of the losses happened early in the process during the transfer from agar to potting media and can mainly be attributed to damping off. The losses that occurred after potting were of weak individuals that showed signs of damping off but were potted anyway in the hope that they might recover.

The tubestock is performing well and given low glasshouse temperature, is flowering and forming fruit, as experiments would indicate. It is too early to tell if any seed will be produced.

**Figure 15** Seed grown Pimelea spicata in flower and producing fruit in the glasshouse

**Figure 16** Close up of immature Pimelea spicata fruit

### *Cutting propagation*

Five propagation events were recorded between November 2017 and January 2019, with a total of 6182 cuttings taken. The results of the cutting propagation programs were as follows

- 1430 cuttings were successfully struck which equates to a 23% strike rate overall. The best results were achieved in 2018 where collections made in November and June achieved a 33% strike.
- The growing on of successful plants proved problematic with significant losses after potting. Of the total 1430 cuttings potted only 193 plants remain.
- The Greening Australia field intervention (targeted grass/weed removal) in December 2017 did not appear to notably increase the health or vigour of treated *Pimelea spicata* relative to other plants at the site. Installation of plant guards or herbivore exclusion fencing was trialled on select individuals but did not improve the health or vigour of the plants, possibly due to the overriding drought conditions.


**Figure 17** Cutting grown Pimelea in the glasshouse at ABGMA

At the conclusion of the TFPP in July 2019 a total of 230 *Pimelea spicata* had been propagated and potted on to 50 mm forestry tubes of which 193 remain.

## Key outcomes and ecological observations

Key outcomes and findings of the TFPP that may assist with the future propagation or translocation of *Pimelea spicata* include:

- Cutting propagation was highly dependent on plant vigour and restricted to Spring and cooler months when plant material was available. Even when good collections were made, strike rate remained low.
- The availability of material was also dependant on rainfall. Collections made during extreme summer weather were generally unsuccessful.
- Increased localised humidity around cuttings improved the strike rate.
- Maintaining cutting grown *Pimelea spicata* in pots long term is untested.
- Low seed germination rates in *Pimelea spicata* species are indicative of a physiological dormancy that limits germination to favourable environmental conditions. Seasonal temperature is often a key factor in maximising germination.
- The germination experiment on the thermogradient table shows a distinct suppression of germination at higher temperatures (summer).
- Although limited in replication due to seed quantity, the thermogradient experiment indicated that field germination of seedlings was more likely to occur in spring or autumn when soil temperatures were cooler and less variable than during the heat of summer.
- *Pimelea spicata* is highly responsive to favourable rainfall events during the warmer months and is able to recover, produce flowers and set seed within two months if soil moisture levels are adequate.
- Hand weeding around *Pimelea spicata* to reduce competition did not appear to increase the health or vigour of plants and did not notably aid cutting collection. There was some evidence of increased herbivory of treated plants.
- *Pimelea spicata* seed is a difficult species to collect in reasonable quantities at any one collection event, due to the sequential flowering/seed ripening. The use of fine mesh (organza) bags proved to be highly effective method to capture seed in quantity as it progressively matured. The collections made from the WSI site are some of the largest known collections for this species and will be retained at PlantBank for future conservation use and long term viability monitoring.

# 7. Conclusions

The TFPP has investigated a range of propagation techniques for all three species which can be successfully applied to further conservation work or translocations linked to the construction of the Western Sydney International (Nancy Bird Walton) Airport or the implementation of the Biodiversity Offset Delivery Plan (BODP).

Information on the species' ecology and propagation will be in the public domain, representing a significant indirect offset outcome for the BODP, and an improvement in our knowledge for these species, which are considered by restoration practitioners as difficult to propagate.

*Pultenaea parviflora* is a straightforward plant to propagate from seed and grow for future conservation projects and translocations. Seed can be collected easily using mesh bags, and seed has a very long storage life under good storage conditions.

*Marsdenia viridiflora* subsp. *viridiflora* seed germination was straightforward, although it was difficult to collect adequate seed. Cuttings remains the preferred method. Potted plants are slow to establish and challenging to maintain in large numbers in a nursery environment long term due to their need to twine and related space requirements.

*Pimelea spicata* is responsive to good rainfall events which provides a 'window' to collect seed and cutting material. *Pimelea spicata* can be a difficult species to collect seed in quantity due to this relationship, particularly during drought conditions. Seed germination rates are low, although improved with treatment with Gibberellic acid and smoke water. Cuttings are slow to establish and even on well struck cuttings losses at potting stage can be significant.

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