Perth Airport

Propagation, Research and Monitoring Plan for Wavy-leaved Smokebush and **Keighery's Macarthuria.**

Report to Perth Airport PTY LTD.

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Revision Schedule

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Boorloo worlak kornt kaadatj Wadjak moort Noongar boodja-k wer baalabang kalyakoorl noyinang Noongar boodja-k. Ngalak kaadatj Noongar Birdiya koora-koora yeyi wer boordakan.

Perth Airport acknowledges the Whadjuk Noongar people as the Traditional Custodians of this region and respects their ongoing connection to this land. We pay our respects to Elders past, present and emerging.

1 Executive Summary

A condition of the (Commonwealth) Ministerial approval MS20-000014 for the construction of a new runway at Perth Airport is to deliver and obtain approval for a Propagation, Research and Monitoring Plan (this plan) to mitigate the loss of genetic diversity and to reduce residual significant impacts to two threatened plant species, **Wavy-leaved Smokebush** (*Conospermum undulatum*) and **Keighery's Macarthuria** (*Macarthuria keigheryi*) which will be disturbed by the development.

Specifically, the goal of this plan is to successfully propagate, establish, secure and maintain new, viable population(s) of Wavy-leaved Smokebush and Keighery's Macarthuria within five years of undertaking any activity which results in an impact to the two plant species. The total number of individuals requiring establishment for each species were originally presented as conditions of the project's Ministerial Approval. These preliminary propagation targets, however, were based on historic field survey data compiled over a larger project area than will be impacted. Therefore, these target numbers required updating when actual impact to each species was determined). Following approval of the New Runway Project (via MS20-000014) in November 2020, much work undertaken to clarify numbers of surviving plants via resurveying of populations of the two species and to redesign aspects of the Project to reduce the clearing footprint and, subsequently, impact on the two species. This resulted in updated numbers of individual plants impacted by the development (for both species), as presented in the Offset Strategy for the New Runway Project (NRP) which was approved by the Commonwealth Minister for the Environment on the 15th May 2024. It is these updated numbers that informs the target (minimum) numbers of plants to be established via translocation program outlined in this Plan. Specifically, the numbers of plants predicted to be impacted by the NRP are now 18 individuals of *Macarthuria keigheryi* (translating to minimum of 29 plants to be established via translocation) and 307 individuals of *Conospermum undulatum* (translating to minimum of 466 plants to be established via translocation). It is emphasised that these numbers represent the minimum that need to be successfully established to compensate for their loss, however, for the purposes of contingency and achieving population viability, a much greater number of successfully translocated plants will be aimed for. Achieving this goal will require: 1) new ecological and genetic research to inform establishment strategies; 2) development of propagation techniques and seed orchard establishment to produce plants for the new populations; 3) evidence of successful establishment of viable populations; and 4) contingency plans in the event that new population(s) have not successfully been established after the five-year period.

The project proposal is guided by:

- the most recent Recovery Plans for the two species;
- government legislation and policy around translocation and use of threatened species;
- the ANPC Translocation Guidelines (Commander et al. 2018); and
- scientific literature from research into the ecology and genetics of the two species.

This plan consists of four main components that cover the propagation, translocation, monitoring, and research activities, respectively. Although these are presented as separate sections, they represent an integrated approach to establish and maintain viable populations of the Wavy-leaved Smokebush and Keighery's Macarthuria (Figure 1).

The propagation program covers all aspects of seed collection and germination and is integrated with the genetic research. Manual cross-pollination between carefully selected genotypes can greatly improve seed viability and seedling fitness, as well as resulting in greater and faster germination. This program also includes protocols for the vegetative reproduction of the species via cuttings to be propagated if insufficient seeds are attainable in the wild population. Lastly, it includes the development of tissue culture techniques for maintaining the genotypes of the plants threatened by the proposed land clearing, and to ensure mass propagation in the event of future declines of the two species.

The translocation program includes a selection process for suitable translocation site(s) if maintaining populations *in situ* is not achievable, and outlines the techniques and protocols to establish a viable population of the target species in the required timeframe.

The monitoring program is designed to assess the achievement of germination and translocation goals and to establish triggers and procedures for remedial actions if goals are either not achieved or are assessed as unlikely to be achieved within the desired timeframe.

The monitoring program is divided into two stages, the first (covering the first five years) which focusses on ensuring adequate numbers of viable plants are established and they are healthy/growing, in order to meet the conditions of the projects' Ministerial Approval. The second stage (years 6 to 10) monitors, evaluates and provides additional research opportunity around the assessment of more long-term population viability.

The monitoring program is complemented by the research program and will also include modelling and genetic characterisation of established plants and newly generated seedlings in the translocated population to ensure that heterozygosity is maintained through generations and to ensure long-term population viability.

Lastly, the research program has been designed as an adaptive management approach to the above three components, where learning and improvement is achieved by judiciously monitoring on-ground works, each being effectively established as experiments where effects, either positive or negative, can be unambiguously attributed to certain actions or treatments.

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2 Introduction

2.1 Project Background

Recent and forecasted increases in flight traffic at Perth Airport have necessitated the construction of a new runway which is proposed to be located on the eastern side of the Perth Airport Estate (hereafter referred to as the 'Estate'), and currently planned for construction between 2024-28. The construction of this new runway will require clearing of up to 129 hectares of native vegetation (in good or better condition) which will subsequently impact populations of two threatened flora species: *Conospermum undulatum* and *Macarthuria keigheryi*.

2.2 Project Approvals

Approvals to construct the new runway have been obtained via submissions of a Major Development Plan (MDP) (Department of Infrastructure, Transport and Regional Development [DITRD], now the Department of Infrastructure, Transport, Regional Development, Communications and the Arts [DITRDCA]). A condition of both approvals is the development (and approval) of a plan to establish new viable populations of the two species (this plan). Specifically, the wording of the MDP Ministerial condition MS20-000014 relating to the two species is:

"To mitigate the loss and reduce residual impact on genetic diversity of Wavy-leaved Smokebush (*Conospermum undulatum*) and Keighery's Macarthuria (*Macarthuria keigheryi*) on the Swan Coastal Plain, the approval holder must prepare a detailed Propagation, Research and Monitoring Plan(s) for approval by the Minister for the Environment. This plan must be submitted and approved by the Minister for the Environment prior to undertaking any activity which results in an impact to Wavy-leaved Smokebush or Keighery's Macarthuria. The Propagation, Research and Monitoring Plan(s) must include but not be limited to:

1. a commitment to propagate, establish, secure and maintain new, viable population(s) of at least 250 individuals of Wavy-leaved Smokebush and at least 1160 individuals of Keighery's Macarthuria within five years of undertaking any activity which results in an impact to Wavy-leaved Smokebush or Keighery's Macarthuria;
2. details of the methodology, sequencing and timing for propagation of individuals, rootstock and/or seedbank and establishment of the new population(s);
3. details of how the plan will increase scientific knowledge of the genetics and ecology of the taxa;
4. evidence and criteria that will be used to demonstrate successful propagation of individuals such that viable population(s) of Wavy-leaved Smokebush and Keighery's Macarthuria are established; and
5. details of contingency measures that will be implemented in the event that new population(s) have not successfully been established after the five-year period".

Following approval of the New Runway Project (via MS20-000014) in November 2020, much work undertaken to clarify numbers of surviving plants via resurveying of populations of the two species and to redesign aspects of the Project to reduce the clearing footprint and, subsequently, impact on the two species. This resulted in updated numbers of individual plants impacted by the development (for both species) as presented in the Offset Strategy for the New Runway Project (NRP) dated 5th April 2024. It is these updated numbers that informs the target (minimum) numbers of plants to be established via translocation program outlined in this Plan. (Note: These target numbers are derived using the Offset Calculator as specified in the aforementioned Offset Strategy). **Specifically, the numbers of plants predicted to be impacted by the NRP are now 18 individuals of *Macarthuria keigheryi* (translating to minimum of 29 plants to be established via translocation) and 307 individuals of *Conospermum undulatum* (translating to minimum of 466 plants to be established via translocation). It is emphasised that these establishment numbers represent the minimum which need to be successfully established to compensate for their loss, but in reality, for the purposes of contingency and achieving population viability, much greater number of successfully translocated plants will be aimed for.**

In order to meet these commitments, PAPL has engaged with suitable qualified ecologists (research scientists) from Edith Cowan University (ECU) who have developed this Plan in line with project approvals as well as relevant legislation, guidelines and policy.

2.3 Relevant Legislation, Guidelines and Policy

This plan has been directed by a number of existing plans, policies and guidelines on translocations of threatened plant species, including the most recent (Interim) Recovery Plans for the two species (DEC 2009a, DEC 2009b) and relevant government legislation and policy concerning the translocation, collection and use of threatened species (see list below). Additionally, current guidelines for plant species translocations, including the *Australian Network for Plant Conservation* (ANPC) *Translocation Guidelines* (Commander et al. 2018) and the *IUCN Guidelines for Reintroductions and other Conservation Translocations* (IUCN 2013), as well relevant scientific literature from research into the ecology and genetics of the two species, have been used to guide the direction and construction of this plan.

It is important that the actions and recommendations outlined in this plan are aligned with, and contribute positively to, the existing interim recovery plans for the two species – that is, this plan and its proposed works should aim to improve conservation outcomes and management of the two species more broadly. Alignment and consistency of this plan with interim recovery plans for the two species and other relevant policies and procedures are outlined in Table 1.

Legislation and government policy relevant to this plan include:

1. DBCA's Corporate Guideline No. 36 on "Recovery of Threatened Species through Translocation and Captive Breeding or Propagation", which states that all translocation plans must be approved by The Director of Science and Conservation, and only after review of a written Translocation Proposal.
2. Commonwealth Government's EPBC Act (specifically Chapter 4) and associated policy statement 'Translocation of Listed Threatened Species – Assessment under Chapter 4 of the EPBC Act' outlines procedures, guidance and circumstances for approval of conservation translocations under this Act.
3. EPBC Act 1999 Environmental Offsets Policy (DSEWPaC 2012) and other Offset Guidance documents (Table 1 addresses how this plan is aligned to Appendix A: Criteria for research and education programs of this Commonwealth Offset Policy).
4. Airports Act 1996 outlining requirements associated with major developments and relevant approvals on Commonwealth land.
5. Legislation and permits governing collection and use of threatened flora, at both Commonwealth and State (WA) levels.
6. Relevant (interim) recovery plans for the two species relevant to both State and Commonwealth jurisdictions, specifically "Keighery's Macarthuria (*Macarthuria keigheryi*) Recovery Plan" (DEC 2009a) and "Wavy-leaved smokebush (*Conospermum undulatum*) Recovery Plan (DEC 2009b).
7. Land-owner and/or relevant management authority approval and support for chosen translocation site(s).

2.4 Consistency with Offsets Policy: Criteria for Research Programs

Consistency between the research program for the two threatened flora species and the requirements for research programs, as per Appendix A of the EPBC Act 1999 Environmental Offsets Policy (DSEWPaC 2012), is outlined below in Table 1.

Table 1: Consistency with offset policy requirements for research programs (DSEWPaC 2012).

Offsets Policy Requirement	Proposed offset
Research or Educational Program Requirements:	
1. endeavour to improve the viability of the impacted protected matter	The proposed research program primarily aims to enhance conservation outcomes of the two species by improving our understanding of their ecology and genetics, focusing on identified gaps in our knowledge. Much of the proposed research is devoted to improving the design and delivery of the propagation/translocation works so that new viable population(s) are more likely to be established. For instance, genetic profiling of individuals and modelling will enable selection of the most appropriate parent plants to ensure optimal genetic diversity for translocation. Ecological research to identify and overcome reproduction barriers (pollination, germination, early survival) will lead to higher rates of plant establishment in the field. Successful establishment of viable populations is not only an important conservation outcome for these species, but will contribute to better approaches to translocation of rare plant species more broadly.
2. be targeted toward key research/ education activities as identified in the relevant Commonwealth approved recovery plan, threat abatement plan, conservation advice, ecological character description, management plan or listing document. Where Commonwealth approved guidance documents are not available or are insufficient in detail, the department will consider additional information sources such as state and territory management plans or peer reviewed scientific literature to inform priority offset activities	The proposed research explicitly addresses research as recommended in the interim recovery plans for the two species. Specifically, it addresses the following recommendations for research as listed in the *C. undulatum* recovery plan (DEC 2009b): • Optimal fire and mechanical disturbance regime to maximise population size and health. • Seed and germination biology, such as rate of seed set and size of soil seed banks. • Age structure of *Conospermum undulatum* populations. • Hybridisation between *Conospermum undulatum* and *Conospermum stoechadis* and *Conospermum triplinervium*. • Genetic differentiation between *Conospermum undulatum, Conospermum stoechadis* and *Conospermum triplinervium* using a higher number of genetic markers and with sampling from a broader range of populations of *C. undulatum* than in previous investigations by Close et al. (2005) • Genetic characterization of *C. undulatum* populations using a higher number of genetic markers to improve estimates of original genetic diversity and monitor and manage diversity over time following translocation. Although no specific research recommendations are made in the (interim) recovery plan for *Macarthuria keigheryi* (DEC 2009a), it does recommend translocation as "desirable…. if attempts to stimulate regeneration are not successful". Much of the planned research for this species is designed to assist and improve translocation outcomes. DBCA supports research designed to provide information and actions relevant to in situ management of existing population, which for *M. keigheryi* includes include management and monitoring of populations of disturbance ephemeral species (such as methods to survey or search for populations, including searching for "dormant" populations, as well as methods to monitor number or health of soil-stored seed).
3. be undertaken in a transparent, scientifically robust and timely manner	This plan, including the research program, will be made publicly available. The research program has been devised by a team of experts from Edith Cowan University in plant ecology and genetics (all having much published research in these fields), and with specific research experience on at least one of the species. They have considerable experience in writing and delivering research plans.

Offsets Policy Requirement	Proposed offset
	Research project timelines and deadlines are outlined in Section 4.
4. be undertaken by a suitably qualified individual or organisation in a manner approved by the department	The research team from ECU all have PhD qualifications and considerable relevant research experience in plant genetics, ecology and propagation. Project leads, Drs Eddie van Etten and David Field, have current H-indices of 24 and 17, respectively (Google Scholar). Dr Nicola Delnevo, who recently completed his PhD examining the ecology and genetics of *C. undulatum* and has 9 relevant peer-reviewed publications to date, will be part of the project team. This project has the support of ECU School of Science and fits within the scope and activities at the Conservation and Biodiversity Research Centre.
5. consider best practice research approaches	Being highly experienced researchers at a national and international level in these fields mean that research will be based on current best practice and cutting-edge research outputs expected. This includes the use of whole genome sequencing to clarify genetic patterns and potential hybridization and genetic models to test translocation strategies. State-of-the-art techniques, equipment, procedures, and analyses will be applied.
Research Program Requirements:	
1. will be tailored to at least a postgraduate education level; however, there will be scope to engage other educational levels in educational programs (see below)	The research program features a post-doctoral fellow and up to two PhD students to support the research.
2. will present findings that can be peer-reviewed	Publication in high-impact peer-reviewed journals is standard practice for the Research Team. The planned research is designed to be scientifically innovative and of broad scientific and conservation interest; therefore, it is likely to be suitable for publications in reputable international journals.
3. will publish findings in an internationally recognised peer-reviewed scientific journal or be of a standard that would be acceptable for publication in such a journal. Publications should be submitted to free open access journals. Data and information collected should have creative commons licensing and be free and accessible	As outlined above, research is designed to be publishable, both in terms of the specific scientific outcomes and the more general findings relating to best translocation practice. The budget includes provision for publication fees and other costs associated with open access journals.
4. research outputs should inform future management decisions on the protected matter and, where possible, be readily applicable to other similar matters (species groupings etc.).	The proposed research is explicitly designed to lead to improved conservation outcomes and management practices for the two threatened species. Additionally, lessons learnt are likely to assist plant translocation programs more generally, as well as advising management approaches for similar species, e.g. other species of *Conospermum* and *Macarthuria*, including appropriate fire management.
Proponent Requirements:	
1. select an institutional or individual host (for the purpose of executing the program)	An open tender process for the Propagation, Monitoring and Research Plan was initiated and followed by PAPL, with ECU being the successfully tenderer.

Offsets Policy Requirement	Proposed offset
through an internationally available open tender process or provide evidence that the program can be successfully undertaken in-house. The department will not be responsible for processing tenders. Where appropriate, the tender should complement an existing research institution's (e.g. National Environmental Research Program Hub) work program as it relates to the matter of national environmental significance. This will be the responsibility of the proponent; however, the department will require that proponents follow the department's guidelines	The proposed research is strongly aligned to one of ECU's four main research themes and priority areas, the 'Natural and Built Environment'. The research team members belong the Conservation and Biodiversity Research Centre of the university, which focuses on improved understanding of biodiversity and its management and conservation in urban areas.
2. provide updates on progress and key findings to the department through periodic reporting	PAPL will provide an annual report to the Department of progress and key findings for their information.
3. ensure that funds are managed appropriately and that auditable financial records are kept and maintained	As is standard practice for all research projects at ECU, budgets, including all financial transactions, will be regularly maintained and reported, and are audited on an annual basis.
4. apply a 'no-surprises' policy to the publication, whereby research publications and outputs are provided to the department at least 5 working days before release.	ECU will provide to PAPL any outputs intended to be published prior to submission. PAPL will provide DCCEEW with any publications and outputs at least 5 working days before release.

2.5 Species Background

The southwest region of Western Australia represents a global biodiversity hotspot due to its exceptional floristic diversity, especially among medium-sized shrubs of the Proteaceae and Myrtaceae (Hopper and Gioia 2004; Mittermeier et al. 2004). The ecological integrity of this region has been compromised by land clearing and fragmentation, over-exploitation, and introduction of alien species, particularly in urban and agricultural areas. Southwest Australia represents a priority area to preserve our natural heritage and its evolutionary potential. Some 325 threatened plant taxa are known from the region, two of which are the subject of this plan. Key information relating to the two species are outlined below, with more complete and specific detail available in Section 7.2.

2.5.1 Conospermum undulatum

2.5.1.1 Distribution

Thirty-five populations of *Conospermum undulatum* are currently recorded within a restricted area in the eastern Perth Metropolitan area between the suburbs of Maddington and Maida Vale within the Swan Coastal Plain bioregion (DBCA Threatened Flora databases; accessed 13/9/2021). Survey results of thirty-two of the DBCA-listed populations in 2018-2019 (Delnevo 2020) showed that 11 populations consisted of between 0 and 4 individual remaining plants and the existence of 17 functional populations, varying in size from a few individuals to several hundred. More detail is presented in Section 7.2.1.

Listed status: *Conospermum undulatum* was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 (now Biodiversity Conservation Act 2016) in 1997 and is currently listed in the threatened flora of Western Australia (W.A Government Gazette, 2018). It has been assessed as "Vulnerable" using IUCN red list criteria under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

2.5.1.2 Ecology

This species generally flowers in September to October with mature fruit ready for collecting from early November. The actual dispersal unit is an indehiscent fruit (with the seed remaining inside the fruit after dispersal); for the purposes of this plan the term 'seed' will refer this dispersed fruit. Pollination is a critical element of *C. undulatum* reproduction. This species relies on native insect pollinators for fertilisation and the production of viable seeds. The native bee *Leioproctus conospermi* is the most important pollinator, followed by native ants (Delnevo et al. 2020a). The composition of the pollinator assemblage and the flower visitation rate have been shown to be negatively impacted by habitat fragmentation and there is evidence of pollen quantity and quality limitation in fragmented populations (Delnevo et al. 2020a). A modest relationship has been shown to exist between *C. undulatum* and an endangered Threatened Ecological Community, Banksia Woodlands of the Swan Coastal Plain, SCP20a. *Banksia* woodlands may provide important habitat for the poorly known native pollinators of *C. undulatum*.

2.5.1.3 Seed germination

The germination rate for seeds treated with gibberellic acid and smoke water has been estimated to be 17.3% (Delnevo et al. 2019) and 11% (Closer et al. 2006, but based on surrogate species *C. stoechadis*), which suggest that *C. undulatum* seed has physiological-imposed dormancy (Close et al. 2006). Higher rates of germination have been achieved by DBCA. Seed germination is known to be significantly lower in small and isolated populations, again stressing the significant impacts of land use change on the reproduction of the species (Delnevo et al. 2019).

2.5.1.4 Hybridisation

C. undulatum is purported to hybridise with other species of its genus. A high degree of variability in the leaf morphology of *C. undulatum* is present in the populations located at the northern and eastern ends of the species distribution range, suggesting the presence of putative hybrid plants within these areas. As the Perth Airport Estate populations are on the eastern edge of the species distribution, possible hybrid individuals have been identified within the populations at a proportion of 28.4% (Delnevo et al. 2021). Genetic testing is required to determine accurately whether individuals within the NRP area are *C. undulatum* or a hybrid of two or more species. Hybrid individuals are not able to be used for population establishment (propagation and translocation) of true *C. undulatum*, and do not have the same offset requirements, therefore, hybrids identified via genetic testing will need to be excluded from the impact area population for the purposes of this offset project.

Since early drafts of this plan, subsequent and superior genetic analyses have been performed on the Perth Airport populations of *C. undulatum,* as well as on nearby population of *C. undulatum* and the three putative hybrid parent species (Delnevo et al. 2022). The main findings of this study differed substantially from the previous ones as no hybrids plants were detected in the Perth Airport populations. This new study did however find two distinct genetic populations in northern part of the species Perth Airport distribution which constituted by far the largest populations of plants by number (almost all of which will be cleared due to the New Runway Project). The smaller sub-populations in the very south-east corner of the Perth Airport Estate were similar genetically to other populations of *C. undulatum* collected outside the airport. The main implication of this new genetic study is that the two large populations at Perth Airport are potentially unique genetic entities which should be preserved where possible during the translocation process.

2.5.2 Macarthuria keigheryi

2.5.2.1 Distribution

Macarthuria keigheryi (Molluginaceae) was first described in 1989, with the type specimen collected from the Cataby area. The species was known from six populations, but recent surveys have found two additional populations in Moore River National Park and in the vicinity of Beermullah (some 20 km north of Gingin). Five of the known populations occur within a 5 km radius of Welshpool and Kewdale, south-east of Perth, in an area that is experiencing rapid development for housing and industry. *Macarthuria keigheryi* senesces in summer, resprouting from the rootstock and stem bases in winter, following rainfall. Large fluctuations have been recorded in some populations; for example, the Cataby population was recorded as having one extant plant in 1991 and none in 1996, but was then resurveyed in 2006 with over 10,000 plants estimated in the population (DEC 2009a). An increase of plant numbers has been observed following firebreak maintenance and fire (DEC 2009a), suggesting that the species may be fire/disturbance responsive. However more research is urgently required on its disturbance and seed ecology to determine the nature and strength of such responses as it is also possible that fluctuations may reflect seasonal variation in detectability due to plant senescence over summer/autumn months (as observed at Perth Airport by authors of this plan). There may also be variation in response across the range of the species. Studies on the demography and seed ecology of the species, including soil seed bank dynamics, is therefore highly desirable.

2.5.2.2 Listed status

The species was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 in 1997 due to clearing of its winter wet swamp habitat for urban development and agricultural use. It is currently listed in the threatened flora of Western Australia (W.A Government Gazette 2018) and has been ranked as "Endangered" using IUCN red list criteria under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

2.5.2.3 Ecology and genetics

The species generally flowers in spring with mature fruit available on plants between December and February (DEC 2009a). Pollination of the sweet-smelling flowers appears to be performed by small insects including native bees, flies and wasps. However, no work has been done to clearly define the pollinator assemblage of the species. Seed falls from the mother plant and are readily gathered and dispersed by ants. Genetic characterisation of the Beermullah and Estate populations using eight microsatellite loci (Neville, 2016) showed a strong genetic structure between the two investigated populations and a high genetic variation among the subpopulations at the Estate location. Moreover, although not extensive, results from this work showed the likely presence of clonal growth in *M. keigheryi*. The high variation among the Estate subpopulations suggests limited gene flow even across short distances and may indicate that pollination is performed by small native insects rather than the highly mobile introduced honeybees.

Deeper ecological investigations are needed for *M. keigheryi* to define the range of effective pollinators and the mating system of the species. Moreover, as for *C. undulatum*, investigations across more populations using more powerful genetic markers will provide a deeper understanding of the genetic diversity of remnant populations.

2.5.2.4 Seed germination

Seed germination has been demonstrated to be very low in the laboratory environment (5% using gibberellic acid; Close et al. 2006); nonetheless seed appears to persist for long periods in the soil seedbank and germinates in response to disturbance.

More detailed reviews of the literature pertaining to the two species are available in the Appendix (Section 7.2).

3 Propagation, Translocation and Research Methods

The overall aim of this plan is to achieve successful establishment of new viable populations of *Conospermum undulatum* and *Macarthuria keigheryi*, with target population numbers determined by final project approval conditions (i.e., MDP Approval) once informed by current number of individuals residing *in situ* that will be impacted. Current prescribed (minimum) numbers within the MDP approval are 1160 *Macarthuria keigheryi* individuals and for *Conospermum undulatum,* 250 individuals. However. the target (minimum) numbers of translocation plants adopted in this Plan, reflects revised population targets presented within the approved Offset Strategy, being at least **29 *M. keigheryi* and 466 *C. undulatum* plants** (based on the most recent disturbance footprint to estimate the numbers of plants to be impacted). It is important to emphasise that these 'targets' represent minimum numbers of plants to be fully established in the field to ensure that approval conditions are met. In reality, the strategies and works outlined in this Plan aim to achieve much higher numbers of established plants for reasons of contingency and to achieve the underpinning goal of population viability.

Potential risks associated with the implementation of the propagation and translocation activities of this plan are captured within the Risk Register, presented in the Appendices (Section 7.1)

An overview of the methods to achieve this aim of new viable populations are summarised below with their inter-relationships shown in Figure 1:

1. **Propagation Preliminary Assessment**: An estimate of the quantity of seed and cuttings required for collection, as well as a description of methods employed and a justification of assumptions and rationale for estimates, based on the target minimum population size and identified risk factors, are given. Also includes development of targets, success criteria etc. (Section 3.1.1).
2. **Collection of Propagation Material**: Seed, cuttings and tissue will be collected from the Estate populations, primarily focussed on plants to be cleared for the New Runway Development (although as a contingency, this may need to be supplemented with collection from outside the Estate, and/or via living collections, such as seed orchards, if insufficient quality seed/tissue is obtainable) in order for the new population(s) to retain the genetic makeup of the impacted population (Sections 3.1.2 and 3.1.3).
3. **Plant Propagation:** Collected seed will be germinated in laboratory and transferred to suitable pots/tubes in nursery (Section 3.1.2); stem cuttings will be established in nursery (Section 3.1.3). Plant propagation will be informed (and improved) by the following research components:
    a. Vegetative Propagation Research (Section 3.4.1.1)
    b. Seed Research (Section 3.4.1.2)
    c. Experimental Crossing Research (Section 3.4.1.3)
4. **Translocation Preliminary Assessment**: determination of habitat requirements (e.g. species range, soil, pollinator presence), development of targets, monitoring protocols and completion criteria that inform monitoring (Section 3.2.1)
5. **Review of Previous Translocations** (Section 3.2.3): This evaluates available information on two known previous attempts to translocate the species, and includes lesson learnt from these attempts.
6. **Process for Selecting Translocation Sites and Source Plants:** Here the selection criteria for choosing appropriate translocation site(s) are described (Section 3.2.3) and the process for selecting source populations and plants (Section 3.2.4) as informed by the genetic research and modelling.
7. **Establishment of population at translocation sites:** This includes site preparation techniques and planting approaches to maximise survival of tubestock planted (Section 3.2.5)
8. **Site Management and fencing:** On-going site management and procedures to ensure maximum survival of transplanted plants at the translocation site(s) are outlined in Section 3.2.6.
9. **Monitoring (Section 3.3):** designed to track progress against success criteria – this includes development and rationale for success criteria based on project goals/targets (Section 3.3.1), monitoring

protocols (Sections 3.3.2) and procedures (3.3.3 for stage 1 and 3.3.4 for stage 2), and contingency plans and associated corrective actions in the case of goals/targets not being met (Section 3.3.5).

10. **Research:** A comprehensive research program designed to inform and improve overall translocation success, as well as contribute to improved scientific understanding and knowledge to better manage and conserve these two threatened plant species. Specifically, the proposed research aims to inform the following components of the plan:

    a. **Population and individual plant selection,** including clarifying the status of *C. undulatum* hybrids (Section 3.4.2.3), clonal status of *M. keigheryi* (Section 3.4.2.4), and genetic diversity and structure of both species across its broader distribution (Section 3.4.2.5);

    b. **Translocation methods,** including modelling (and testing) of various translocation scenarios based on genetic research (Section 3.4.2.2), as well as specific field-based research on factors, such as fire, influencing pollination, recruitment and survivorship in wild populations (Sections 3.4.3.3 and 3.4.3.4).

    c. **Propagation methods,** including specific research exploring the effect of different seed treatments (Section 3.4.1.2) and cutting techniques (Section 3.4.1.1), as well as the effectiveness of purposeful cross-pollination by hand (Section 3.4.1.3).

As there is considerable scope to improve propagation, translocation and site management techniques for both species, an experimental approach will be adopted for propagation and translocation activities so that techniques and outcomes can be refined and improved over time, as well as increasing numbers of plants available for transplanting in the field and optimising the use of available seed and tissue. The propagation and translocation components of this plan are thus integrated with research component to provide adaptive management process (see Figure 1), ensuring the best possible success rate.

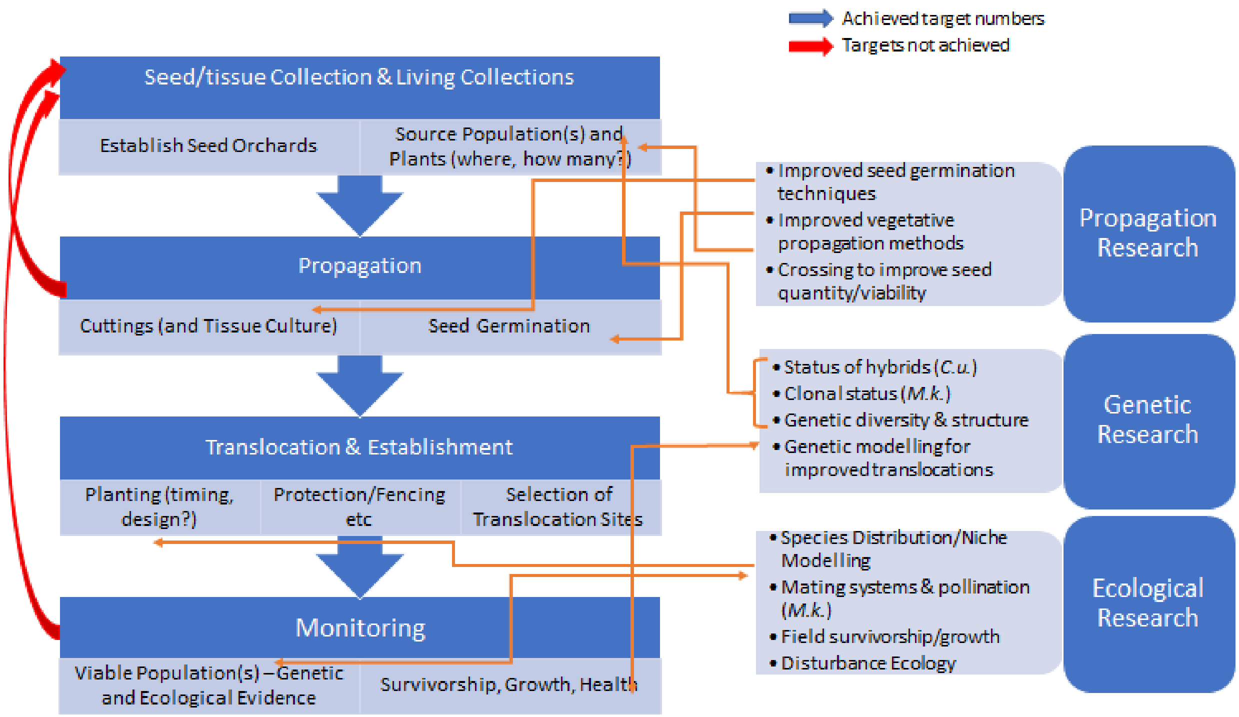

Figure 1. Elements of the Propagation & Monitoring Plan (left) showing sequence of activities and inter-relationships with the Research Plan components (right)

3.1 Propagation

3.1.1 Preliminary Assessment

The aim of the preliminary assessment is to develop an initial strategy for the successful establishment of <u>at least</u> the target population numbers while taking into consideration the species' ecology and risk factors. For the propagation phase of this project, this includes determining for each species: the location of the source of material used for propagation, the type of material to be collected from the source population(s), the amount of material to be collected, and the timing around material collections.

3.1.1.1 Source Material Location

Perth Airport populations of both species will be the source for seeds, stem cuttings and other propagules given these plants, or at least many of them, will be destroyed, and given preliminary studies suggesting unique genetic forms exist at Perth Airport (at least for the two largest populations of *C. undulatum*). Material may be sourced from populations external to Perth Airport if deemed necessary and beneficial to propagation success, required genetic diversity and/or target numbers, but only on the proviso that there will be no genetic dilution or potential to impact the genetic integrity of the existing or established populations. Collection of plant tissue outside Perth Airport Estate is also dependent on all additional required approvals (e.g. DBCA collection permits) being obtained.

Selection of localities, populations and specific plants for seed collection and cuttings will be informed from an initial genetic modelling exercise designed to optimise genetic diversity and adaptability in the translocated population(s) and in any living collections established – see section 3.4.2.2 for details of this genetic modelling.

3.1.1.2 Type of Material and Method

Plants will need be propagated via a variety of methods (seed germination, cuttings and, possibly, tissue culture – although this latter technique is a contingency action, see Section 3.3.5) to ensure adequate numbers of new plants are propagated in the desired timeframe. Living collections, such as seed orchards and nursery collections, will need to be established in the early stages to provide a more reliable source of seeds and plant tissue. Each plant will be given a unique number and labelled with 'permanent' label and marker, with any seed lot or plant tissue collected, or indeed any plant propagated, also labelled with this source plant number. This will be critical in the appropriate selection of individuals for translocation, as well as tracking plants in the monitoring phase (see Sections 3.2.4 and 3.3, respectively)

3.1.1.3 Timing

Collection of plant material from the Perth Estate for propagation is required before clearing of vegetation for the new runway (i.e., before approx. late 2024, based on current project timeline). As *C. undulatum* flowers in spring with seed fall occurring in early to mid-November, flowers will need to be bagged each spring from October 2021 (using organza bags) and these collected mid-November to early December each year until the vegetation is clear or access is no longer available (Note: two seasons of collections have already been achieved). *M. keigheryi* may flower progressively over several months from spring to summer; consequently, seed will be collected across several field trips from late spring to mid-summer. Cuttings of both species will be taken in autumn (generally best done at end of growing season), although this is dependent on rainfall and growing conditions. Continued collection of seed/cuttings is possible from plants on the Estate not cleared as part of the runway development (the numbers of these are yet to be established), as well as from populations outside the Perth Airport Estate (dependent on outcomes of genetic studies).

3.1.1.4 Survivorship Rates and Risk Factors

The minimum amount of material requiring collection and propagation, as well as the methods employed, will be informed by known and predicted success/risk factors, such as rates of seed viability, germination rate, root strike rate, translocation mortality/ survivorship etc. In general, much larger numbers, than the target population numbers, will need to be first propagated in the nursery to allow for a certain amount of seedling deaths in both the nursery and field. These rates enable an initial estimation of the quantities of propagules and plants required (refer to Table 2 and Table 3).

Seed viability is defined as the proportion of a seed stock with intact embryos (indicating the potential to germinate) and are known to be high (>90%) for both species (Table 2). Although the number of viable seeds produced per plant is typically low and highly variable (from plant to plant), for *C. undulatum* (Delnevo et al. 2020), it is possible to visually recognise and separate viable seed from unviable seed (based on size of fruit and orientation of hairs on fruit); hence it will be possible to ensure collected and sorted seed will have high rates of viability (Delnevo 2021).

Initial estimates of germination rates of viable seed in a laboratory environment are 20% for *C. undulatum* and 5% for *M. keigheryi*, based on previous studies (Table 2). There is considerable scope to improve these germination rates through the planned research (see Section 3.4.1.2). Cuttings strike rates are relatively high based on previous research (Table 2; Close et al 2006), indicating this to be a reliable propagation method.

Survivorship of propagated plants in the nursery is not specifically known for the two species, so an estimate based on general nursery survivorship of native plants has been used (of 95%). This does assume correct procedures, proper care and optimal conditions for growth and development are applied, which are all standard nursery procedures.

Survival rates of tubestock planted in the field are poorly known for these two species, but are likely to vary widely from year to year, as well as specific site factors and prevailing weather. Field survivorship of seedlings planted in translocation sites in southwest Australia varies widely, depending on species and conditions, typically ranging from 20-50%, but sometimes exceeding 80% (Commander et al. 2018). Based on these ranges, a 25% survival rate over a 5-year period has been used, which is relatively conservative, but is reasonable and achievable based on previous translocation attempts of *C. undulatum* seedlings (see Section 3.2.2). One known previous translocation attempt of *M. keigheryi* reported very low field survivorship (see Section 3.2.2), but there were several extenuating circumstances, particularly relating to the extreme site conditions, which are likely to explain the large amount of tubestock death over the first few years. It is accepted, however, that the translocation procedures for *M. keigheryi* are poorly understood, from seed germination to field planting techniques. In this plan, a research-informed and experimental approach will be implemented, specifically designed to improve propagation and translocation outcomes over time.

Risk factors and predicted success rates used to aid in quantification of material collection are summarised in Table 2.

Table 2: Informed and Predicted Risk Factors

Predicted Success Rate (Risk Factor)	C. undulatum	M. keigheryi
Seed Viability %	90%	90%
Germination Rate %	20%	5%
Cutting Strike Rate %	33%	75%
Nursery Survivorship Rate %	95%	95%
Translocation Survivorship Rate %	25%	25%

N.B.: Predicted seed viability %, germination % and cutting strike % based on Close et al. 2006 (both species) and Delnevo 2021 (for *C. undulatum*). For *C. undulatum*, a method has been developed to recognise viable seed based on appearance and size of the fruit which will ensure very rates of seed viability in the seed stock (Delnevo 2021). Translocated plant survivorship rates are unknown, therefore a generalised conservative estimate has been applied.

In the Perth region, most seedling deaths occur over the first summer after planting, when hot, dry conditions prevail (Commander et al. 2018; Brundrett et al. 2020). Data collected from the monitoring and field research programs from the first year of plantings will inform general levels of field establishment and survivorship, which can then be applied to set the appropriate numbers of propagated plants in subsequent years.

3.1.1.5 Initial Propagation Strategy

Table 3 presents the preliminary estimates of required number of plants, seeds and cuttings to at least reach the target numbers of plants to be established, based on risk factors and estimated survivorship rates indicated in Table 2. An annual breakdown of these estimated numbers is presented in Table 4. These numbers should be regarded as interim values to guide planning and will be reviewed and adjusted annually in an iterative fashion based on results of monitoring and research programs.

Using the predicted field survivorship factor (% surviving in field over 5 years) of 25%, in the order of four times that of the target numbers of individuals should be planted at the translocated site to achieve the minimum target numbers of individuals at the 5-year mark (Table 3). The number of propagated plants will need to be adjusted up or down depending on the field survival rates actually achieved (as reported from the monitoring program), especially those recorded in the first-year plantings over their first summer period which will be particularly informative. Given that collected seeds alone are not likely to achieve these plant numbers, a certain number of plants will also need to propagated using cuttings. The ratio of seedlings to cuttings to be propagated (Table 3) has been estimated from the predicted amount of seed available for collection, as well as what is logistically achievable in terms of propagation in the nursery. Additionally, seed orchards and nursery collections will need to be established in the early stages to provide a more reliable source of seeds and plant tissue (see below).

Using the predicted germination, viability and nursery-survival rates (see Table 2), it is estimated that in the order of 5500 viable seed will be required for *C. undulatum*, and some 1,500 for *M. keigheryi*. These predicted high numbers of seed required presents a considerable challenge for seed collection, but is achievable based on known and observed seed production and plant numbers. Also, for M. keigheryi, this assumes 5% germination (based on the only study known by Close et al. 2006) which in itself suggests the true seed dormancy mechanism and effective treatment(s) have yet to be discovered for this species. Given the relatively high seed viability in this species and the intensive research program proposed to discover the prevailing germination cues and best treatments, it is likely that higher rates of germination will be obtained, and hence far fewer seed will be required than anticipated.

Using the predicted strike rates (combined with nursery survival estimates), it is estimated that approximately 3000 cuttings will need to be taken for *C. undulatum*, but only around 90 for *M. keigheryi*.

As much seed as possible will be collected before clearing (and kept separate by plant), with genetic analyses then informing which plants are best used for seeds and cuttings (Section 3.4.2).

Table 3: Preliminary estimates of minimum required number of plants, seeds and cuttings to at least reach target numbers of plant establishment at translocation site(s).

Factor	***C. undulatum***	***M. keigheryi***
Target population established at 5 years (# of plants)	>466	>29
Field factor (predicted % surviving in field over 5 years)	25%	25%
Minimum tubestock predicted to be required for translocation (# of plants)	At least 2068 sourced from: - Seedlings: 932; - Cuttings: 932	At least 120 sourced from: - Seedlings: 60; - Cuttings: 60
Viable seed required (# of seed)	At least 5450 (based on 20% germination, 90% viability & 95% nursery survival)	At least 1,400 (based on 5% germination, 90% viability & 95% nursery survival)

Factor	*C. undulatum*	*M. keigheryi*
Cuttings required (# of stems to be collected and prepared for cuttings)	At least 2943 (based on 33% strike rate & 95% nursery survival)	At least 90 (based on 75% strike rate & 95% nursery survival)

Table 4: Preliminary timeline estimates of numbers of plants, seeds to be collected, seedlings raised, and cuttings successfully propagated. Note: these are minimum targets of seeds and plants per year. Note: Year 0 refers to seed collection required before commencement of the project and which has been largely achieved.

Species	**Material**	**Year**					**Totals**
		0	**1**	**2**	**3**	**4**	
C. undulatum	Seeds	2,100	2,000	800	400	150	5450
	Seedlings	-	332	200	200	200	932
	Cuttings	-	432	200	150	150	932
	Plants (tubestock)	-	764	400	350	350	1864
M. keigheryi	Seeds	600	300	300	100	100	1,400
	Seedlings	-	40	10	5	5	60
	Cuttings	-	40	10	5	5	60
	Plants (tubestock)	-	80	20	10	10	120

N.B. Seed collection will be mostly from wild (Perth Airport Estate) in year 0 (and hence is relatively large), but in other years will be mostly from seed orchards and nursery collections (although can be augmented by extra collection of wild seed). Some seed collected in early years will be kept in storage for germination in later years. Plants propagated in year 0 will be planted at translocation site(s) in year 1.

3.1.1.6 Alternative Seed & Cutting Sources including Seed Orchards and other Living Collections

3.1.1.6.1 Conospermum undulatum

Collecting such large quantities of *C. undulatum* seed from wild populations is unlikely to be possible (due to modest seed set combined with limitations on collecting seed from threatened plants), and so some additional and alternatives sources of seed will be required. Seeds of this species may be available in seed collections, but we note limited availability at the DCBA Threatened Flora Seed Centre (TFSC). Therefore, establishment of cultivated plants in the nursery (using potted plants) and/or at a field site for purposes of seed production (i.e., a seed orchard), as well as provision of cutting material, will be required. However, the selection of source plants and populations for establishing seed orchards and the arrangement of plants must be carefully planned to avoid deleterious genetic impacts in the offspring. Cuttings can flower and set seed within 7-10 months and hence it will be most expedient to establish a seed orchard in the nursery using cuttings (as done by Close et al. 2006), again as long as a representative sample of genetic diversity is captured in the source plants (see section 3.4.2.5). Also, it may be possible to rescue mature plants earmarked to be cleared at the Estate (by transferring to large pots) or existing plants in cultivation may be able to be sourced (e.g., from previous research programs) to more rapidly establish a mature seed orchard in the nursery and/or field. A seed orchard established in the nursery is advantageous as appropriate care and maintenance of plants is more achievable. However, a seed orchard established at a field site (e.g., protected remnant bushland on the Perth Airport Estate or at/near chosen translocation site) is more likely to attract the required pollinators. It is for this reason that both field- and nursery-based seed orchards will likely need to be established. In addition to the establishment of seed orchards, vegetative propagation will augment numbers (refer Section 3.1.3).

3.1.1.6.2 Macarthuria keigheryi

Collecting the estimated quantities of *Macarthuria keigheryi* seed (at least 1,400 seeds; Table 3 and Table 4) from wild populations at Perth Airport Estate alone will be challenging; therefore some of the seed collected from the Estate will be used for the establishment of cultivated plants in nursery (using potted plants) and/or at a field site for purposes of additional seed production (i.e., a seed orchard). The seed collection procedure for *Macarthuria keigheryi* will be as follows:

1. Mobilisation to Perth Airport Estate wild populations during early to late summer, between December 2021 and February 2022 (note: somewhat achieved already);
2. Collection of dried flower remains (sepals) containing mature fruit collected in the field (seed stored separately in paper envelopes for each plant and labelled with plant number);
3. Dried flowers will be taken to ECU laboratory or nursery for seed removal and cleaning (removal of visually unviable or damaged seed);
4. Germination (Section 3.1.2.1) for seed production in laboratory or nursery until target numbers of seed is reached.

Contingency and supplementary options (see Section 3.3.5 for more details):

- Seed sourcing from plants outside of clearing area within the Perth Airport Estate (although actual numbers are not yet known, it is likely that some plants will remain for ongoing seed collection).
- Seed sourced from nursery-raised plants and field seed orchard.
- Seed sourcing from populations outside of Perth Airport Estate (as guided by genetic analyses, availability and permits).

The species generally flowers in spring with mature fruit available on plants between December and February (DEC, 2009a). Seed will be obtained from wild populations in early to mid-summer. As seed is typically not immediately visible on the plant, dried flower remains (sepals) containing mature fruit will need to be collected in the field, followed by seed removal in the laboratory or nursery. It is proposed that prioritisation will be given to collections from wild populations within the Perth Airport Estate, particularly from plants to be cleared; however, the earliest that mature seed is likely to be available is December 2021. Further, any decent-sized populations retained within the Estate should be prioritised for ongoing seed collection in subsequent years. Based on our seed collection attempts in 2021-2023, we will explore the potential to either bag, net or trap fallen seed during the 2024/25 seed collection period.

3.1.1.7 Conospermum undulatum

In summary, the seed collection procedure for *C. undulatum* is:

1. Bagging of flowers for all mature plants in Perth Airport wild populations at end of flowering season (note: achieved in late October 2021 to 2023, with ongoing annuals seed collections until population cleared);
2. Collection of bags containing fallen fruits (stored separately in paper envelopes for each plant; achieved);
3. Fruit to be taken to ECU laboratory or nursery and sorted to remove fruit with visually unviable seed;
4. Germination (Section 3.1.1.7) for seed production in laboratory or nursery until target numbers of seed is reached.

Contingency and supplementary options (see Section 3.3.5 for more details):

- Seed sourcing from plants outside of clearing area within the Perth Airport Estate (although actual numbers are not yet known, it is likely that a small number of plants will remain for ongoing seed collection).
- Seed sourced from established seed orchards.
- Seed sourcing from populations outside of Perth Airport Estate (as guided by genetic analyses, availability and permits).

This species generally flowers in September to October with mature fruit ready for collecting from early November. As fruits tend to readily fall off plants when mature, fabric bags (typically made of muslin or organza) will be placed around inflorescences at the end of the flowering season to capture seeds (typically from mid-October). For this species, each fruit can have a maximum of only one seed, and the seed is not removed from the hard fruit for germination; rather it is the fruit which is collected and sown. Seed collection is initially required from the Estate population (before clearing of site), and then from known larger populations of species elsewhere as these will have higher levels of seed set and viability (as well as genetic diversity). The Research Plan (Section 3.4) includes crossing experiments aimed to increase seed viability and quantity.

It is anticipated that approximately 5500 seed will need to be collected to achieve the target number of transplants in the field (to produce the initial target of 932 seedlings for planting; Table 3). Some 3000 seed is likely to be available from Perth Airport populations (based on known seed set rates from Delnevo (2020), and confirmed during collection in 2021-2022), with at least the same number available from other large populations nearby. However, it is accepted that collecting such large amounts of seed from wild populations may be difficult (due to low seed set combined with restrictions on collecting seed from threatened plants), and so some additional and alternatives sources of seed may be required. However, selection of the locations of source population of such seeds must be known to avoid deleterious genetic impacts in the newly established population. For this reason, seeds will be only sourced from the Perth Airport Estate populations, unless this is strictly required for contingency purposes and genetic analyses have indicated alternate sources would result in positive outcome (and all additional approvals have been granted). Another strategy to improve seed availability is based on purposeful crossing and is incorporated in the Research Plan (see section 3.4.1.3). However, it is likely that these wild sources of seed will be inadequate to provide the anticipated numbers of required seed, and so the early establishment of a seed orchard, in the nursery and/or field will be undertaken (if possible) in order to provide a more reliable and consistent supply of seed. Given plants grown from seed of this species can take several years to reach reproductive maturity and set reasonable numbers of viable seed, plants in the seed orchard will be established where possible via cuttings (or perhaps rescuing of mature plants from populations to be cleared). Again, selection of plants for the seed orchard needs to be informed by genetic analyses of wild populations.

3.1.2 Seed Germination & Production of Seedlings

3.1.2.1 Macarthuria keigheryi

Despite high levels of seed viability reported in wild populations, *M. keigheryi* seed do not germinate in the laboratory environment without pre-treatment, even with several months of after-ripening (Close et al. 2016). Seed pre-treatment with the plant hormone gibberellic acid enables some seed germination but at low levels (~5% for both fresh and 3-month-old seed; Close et al. 2016). In the absence of other information, this germination treatment should be used for collected seed, at least initially. The seed germination procedure for *Macarthuria keigheryi* will be as follows:

1. After-ripening of seed via storage in protected and cool conditions for at least three months;
2. Seed pre-treatment by soaking seed in solution containing 1% gibberellic acid for 24 h (or other improved techniques);
3. Germination on filter paper or sterile agar-plates;
4. Transfer of germinants into suitable pots with native plant soil mix;
5. Grow for at least several months in nursery and then adequately hardened-off (gradual transition to an outdoor environment without supplemental irrigation);
6. Planting in the field (Section 3.2.4) at around 5-8 months of age, OR
7. Plant retained in nursery for continuation of seed production.

The improvement of germination rates by experimenting with various seed germination trials is included under the Research section (3.4.1.2), and new treatments and refinements should be adopted where improved germination is demonstrated. Improved germination will lead to less seed needing to be collected from wild populations.

3.1.2.2 Conospermum undulatum

Seed germination (which occurs from within fruits) of *C. undulatum* will follow protocols established by Close et al. (2006); Cochrane (2007) and Delnevo et al. (2019a) who all have successfully germinated the species:

1. nicking the hard fruit wall to reveal the seed;
2. soaking fruit in smoke water solution for 24 h;
3. soaking in Plant Preservative Mixture for 1 h;
4. plating onto sterile agar plates containing 1% gibberellic acid solution;
5. incubating at 15 °C with 12 h photoperiod until seeds germinate (can take up to 3 months);
6. Transfer of germinant into suitable pots with native plant soil mix;
7. Grown for at least several months in nursery and then adequately hardened-off (gradual transition to outdoor environment without supplemental irrigation);
8. Planting in the field (Section 3.2.4) at around 5-8 months of age; OR
9. Plant retained in nursery for continuation of seed production.

This can result in 20-40% germination but can be very slow with some seed taking several months to germinate. So, there is some scope to improve germination rates (both % and speed), as well as establishing the optimum combination of gibberellic acid and smoke water, which are addressed in the research plan below (see section 3.4.1.2).

3.1.3 Tubestock via Cuttings

3.1.3.1 Macarthuria keigheryi

The species can be successfully propagated by stem cuttings with previous research at Kings Park achieving a strike rate of ~75% (Close et al. 2006). Based on this research, the technique for cuttings of this species is:

1. collection of plant material (stems with leaves) in early autumn or the end of growing period (when most semi-hardwood is available on plants);
2. selection and preparation of semi-hardwood stem cuttings about 5-10 cm in size;
3. dipping cuttings in commercial hormone powder or gel (IBA at ~3g/L);
4. placement in regular soil mix for cuttings on below-heated misting bed;
5. transfer to standard propagation mix for native plants after root strike;
6. adequate hardening-off before transplanting in the field at between 4 to 8 months, OR
7. retained in nursery for seed production.

It is anticipated that at least 90 cuttings (Table 3) will need to be taken to meet target number of plants, which should be achievable given the reported high/fast strike rate (75% of cuttings strike within 5 weeks; Close et al. 2006). However, given the challenges faced in collecting and germinating seed (based on current knowledge), larger number of cuttings can be taken if required to reach final overall numbers of tubestock for translocation. Cuttings prepared in summer/autumn should be available for planting in the field in late winter/early spring.

3.1.3.2 Conospermum undulatum

Conospermum undulatum has also been successfully propagated by cuttings, but with lower root strike rate than *M. keigheryi*, with best results (33% strike rate) with semi-hardwood stem cuttings dipped in hormone (IBA) gel (Close et al. 2006). Cuttings will be propagated following same technique as described above for *M. keigheryi*. Cuttings reportedly strike roots within a few weeks (Close et al. 2006), therefore cuttings propagated in summer or autumn would likely be ready for planting in the field in late winter to early spring of the same year, or if root strike is slow, then the following winter. However, cuttings taken in winter-spring period, will only be suitable for planting in the following winter.

3.1.4 Tissue Culture

Tissue culture has considerable potential for mass propagation of plants using limited vegetative material, and so is often an ideal propagation method for highly threatened plant species. Tissue culture will only be required as a remedial action if seed germination and cuttings do not yield adequate numbers of plants for translocation within the first three years of the plan. The tissue culture protocols have not yet been developed for the two species, but the method is potentially suitable given the reported success and relative ease of stem cuttings. Although tissue culture will only be deployed if required, development of successful tissue culture protocols is still seen as considerable benefit to the conservation of the two species as it effectively ensures mass propagation in the event of future declines in the species – that is, it is an important contingency measure for the conservation of the two species. Additionally, tissue culture can be a means of preserving tissue from specific genotypes, such as those of plants to be destroyed at the Estate. For these reasons, development of tissue culture protocols for the two species is included under the research plan (see Section 3.4.1.1).

3.2 Translocation

3.2.1 Preliminary Assessment

The aim of the translocation preliminary assessment is to develop an initial strategy for the successful establishment and viability of target population numbers while taking into consideration species ecology and risk factors. For the translocation phase this includes determining for each species: the suitable characteristics of the translocation site(s), the timing around planting, the method of planting (including spacing), and the monitoring and management required.

Factors which influence the survival of nursery-raised tubestock planted at the chosen translocation sites have already been covered in Section 3.1.1, with an initial field factor of 25% used to predict numbers of plants required (Table 2). These risk factors include plants dying due to drought (hot, dry conditions over summer-autumn, as well as years with below-average rainfall), herbivory (for example by rabbits and kangaroos, depending on site chosen), and lack of suitable substrate (with compacted, water-repellent and nutrient-depleted soils disfavouring plant survival). Site treatments and on-going site management strategies to reduce these risks are described in the sections below.

To reduce risk of plant death after translocation, the timing of planting will be prioritised when soil moisture levels are at or near their yearly peak, which tends to be June to August. Therefore, it is proposed that seed and cuttings be propagated as early as possible in the year (or late in previous year) so seedlings in tubestock are at least 6 months of age and up to 8 months old when planted. Seedlings generally need at least two months of hardening-off before planting (see Section 3.1.2).

Preliminary estimates of the numbers of plants to be propagated each year, and therefore available for planting at translocation sites each year, are outlined in Table 4. These are calculated based on estimated risk/success factors justified in Table 2. If these plants are successfully propagated and the predicted field survival rates achieved, then overall targets should be met at year 5. Regular monitoring of plant survival and condition in the field will enable testing of these assumptions, and any adjustments required to the number of plants to be propagated.

3.2.2 Review of Previous Translocation Efforts

As part of their environmental offset requirements associated with the Gateway WA development (a major road construction venture), Main Roads (WA) were required to establish new populations of *Conospermum undulatum* and *Macarthuria keigheryi*. The *Conospermum* plants were translocated to a site at Pioneer Park, whereas the *Macarthuria* was translocated to a site within Dundas Road Reserve. Main Roads, via their contractors, used a combination of directly transplanting mature plants and planting tubestock propagated in a nursery using both cuttings and collected seed. Transplanting mature plants involved digging up mature plants within development area, transferring to pots, maintenance in a nursery for some period, and then planting in the field during winter. These translocation projects did not have any success translocating mature plants for either species. Both species had some deaths of mature plants in the nursery and all translocated mature plants died at the translocation receiver site.

Based on the most recent monitoring of the planted *C. undulatum* tubestock (Main Roads 2019), only 2.9% of tubestock propagated via cuttings survived at 4 years, and 25.7% of tubestock propagated using collected seed survived at 2 years. The vast majority of tubestock deaths occurred over the dry and hots summers in the first two years after planting. The marked difference in survivorship between the two propagation techniques could be due to different planting years, with the cuttings being planted in a drier winter some two years earlier than the seedlings. Another monitoring study (Gateway WA 2016a) reported 39% survival of *C. undulatum* seedlings some 16 months after planting.

For *M. keigheryi*, some 1595 tubestock were propagated from stem cuttings and planted over three years (2014-2016). As of late 2017, only 6 plants were surviving in the field, which represents a survival rate of 0.4% (Emerge Associates 2018). No clear reason for this very low survivorship rate was given, but potential contributing factors included harsh (exposed) conditions of the offset site, unfavourable substrate (it being a waste disposal site previously), below-average winter-spring rainfall, inappropriate planting methods and maintenance, and specific ecological attributes of the species which may disfavour cultivation (Emerge Associates 2018). At another translocation site, 6% survival was reported two years after planting seedlings (tubestock) (Gateway WA 2016b).

It is important that lessons learnt from these previous translocations are heeded to avoid repeating mistakes. The key learnings emerging from this initial review of the previous translocations are: 1) adequate irrigation and mulching are important during summer dry periods to maintain soil moisture and reduce drought deaths; 2) protection by overstorey seems to increase survival of tubestock; 3) open, very disturbed sites are extremely harsh, and so translocation sites within existing bushland may provide more favourable conditions. Direct transplanting of mature plants from one site to another is appealing but was not successful; this approach is not a formal part of this plan (although it will be attempted to establish mature seed orchards more quickly; see Section 3.3.5). The review here demonstrates ~25% field survivorship is feasible for *C. undulatum* seedlings, but for *M. keigheryi*, much improvement in propagation and field planting techniques and management is required. Overall, the previous translocation program designed and implemented for *M. keigheryi* demonstrates the dangers of an uninformed approach to translocation; that is, attempting revegetation of species where almost nothing is known about the reproductive ecology and propagation of the species. In this plan, we adopt a research-led and adaptive management approach which is more likely to achieve improved success over time. Further, through our monitoring program, we aim to detect low field survival rates early in the program, and implement remedial actions (such as extra seed collection, improved habitat management etc).

Before commencing the translocation programs for the two species, it will be prudent to further review and evaluate these and any other translocation attempts of these two species (e.g., through new site visitations) to obtain the most up-to-date outcomes, lessons-learned and recommendations for future strategies.

3.2.3 Selection of Translocation Sites

3.2.3.1 Site Selection Process

At present, *Conospermum undulatum* and *Macarthuria keigheryi* are restricted to a few fragmented populations on the Swan Coastal Plain (SCP). As the species is seemingly restricted to old, highly leached sand dunes on the eastern side of the SCP, it is likely that *C. undulatum* and *M. keigheryi* were both rare species with a naturally narrow distribution range before European settlement. The identification of potentially suitable site(s) for establishment of new populations is therefore vital to achieve effective translocations as not all remnant patches of bushland can be compatible with the survival of the species. For this reason, all options to keep the populations of *C. undulatum* and *M. keigheryi in situ* within Perth Airport tenure needs to be explored as this would maximise the conservation outcome for the species, while minimising the risk of deleterious genetic impacts on other populations and/or other closely related species.

A detailed species distribution model (SDM) will be used to improve translocation site selection. This approach is crucial due to the complex mosaic of aeolian and alluvial soils that characterises the ranges of these two species, resulting in a well-defined environmental envelope. A similar approach has been used in a spatial analysis of genetic differentiation of *C. undulatum* (Delnevo et al., 2021.) and will be implemented for the identification of suitable sites for both *C. undulatum* and *M. keigheryi*. Detailed description of the statistical machine-learning model and variable selection is given in Section 3.4.3.2 below.

Once potential translocation sites have been broadly identified by means of the SDM, their suitability will be carefully assessed on site to determine the degree of vegetation degradation. It has been shown that habitat degradation has a negative impact on the presence of the most important groups of pollinators of *C. undulatum*, and this may be true also for *M. keigheryi*. Moreover, the presence of weeds has been identified as a threat for the establishment of seedlings in both *C. undulatum* and *M. keigheryi*. Indeed, seedlings would have to compete with weeds and existing native plants for nutrient, space, and light resources. For this reason, the selected site will require pre-treatments before transplanting of new material as outlined is section 3.2.5 below. The most suitable translocation sites will then be selected based on land vesting and purpose, where possible, and acquisition of new sites will also be considered. Based on detailed preliminary assessment, several large patches of retained and secure bushland on the Perth Airport Estate have been identified as being suitable habitat for both species (in terms of matching soil, geomorphology and vegetation) and will comprise the sites for the planned translocation, pending required approvals.

Although the Ministerial conditions state new population(s) need to be established, it is possible that sites where extant populations were previously recorded (but now likely extinct), or where there are very low population numbers, and hence the population are not functional or viable in the long term, may be suitable candidate sites. Such sites have the benefit of supporting populations in the past, and hence were (at least historically) suitable habitat for the species.

Criteria for the final selection of suitable sites will then follow the conceptual framework reported in Figure 2 on the next page; actions to mitigate/fix problems encountered following the conceptual framework are addressed in sections below.

Site selection flow chart:

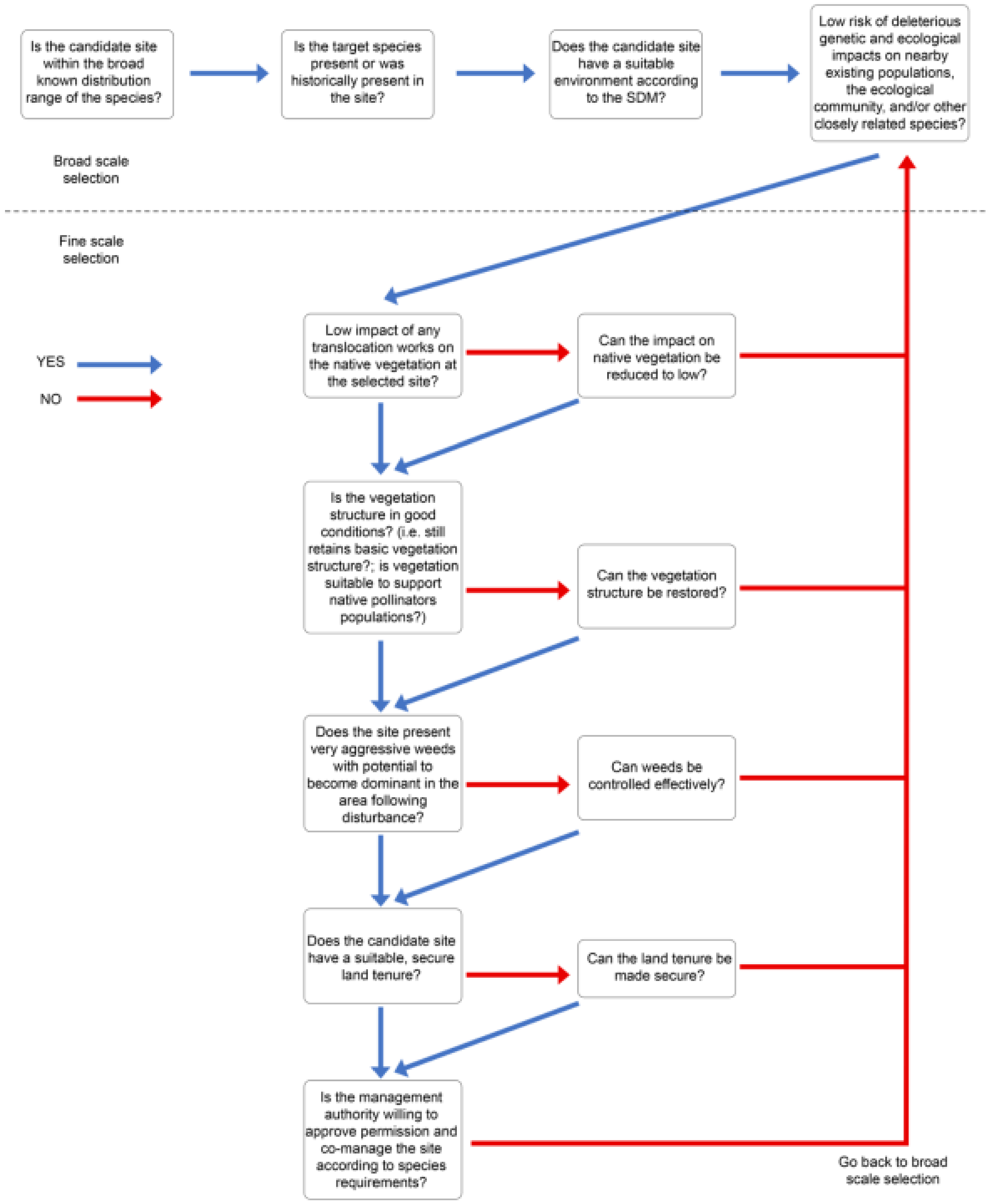

Figure 2. Conceptual framework reporting criteria for the final selection of suitable translocation sites.

3.2.4 Selection of source sites and individual plant selection

In general, plants grown for the translocation sites should encompass a diverse range of the gene pool sourced from a range of natural populations collected from the wild or established in the seed orchard. This is especially important for these species as they have few remaining populations, many of which contain lower levels of genetic diversity. However, given our understanding of the genetic distinctiveness of the major Perth Airport population of *C. undulatum* (Delnevo et al. 2022), the focus should be to preserve the genetic diversity of this population.

Plants originating from each source population will be genotyped to ascertain their genetic ancestry (hybrid or purebred), level of inbreeding, and estimate of the level of population structure and demographic history of each population (see section 3.4.2). These genetic measures will enable the characterisation of the starting population used in the translocation, which enables improved planning translocation of individuals based on their genetic makeup and provides a reference point to compare with ongoing monitoring of the genetic composition following translocation establishment (see section 3.4.2.2). Genome-wide genetic markers will enable accurate estimate of these individual and population characteristics. For example, heterozygosity fitness correlations can be examined in the seed orchard and enable a way to select plants that are likely to be more vigorous at the translocated sites. To maintain high levels of genetic variability at the translocated sites, the genetic information will also be used to maximise diversity within and between individuals. The amount of mixing of more differentiated source populations will be investigated (see section 3.4.2.5) to ensure risks of potential outbreeding depression are minimised. Any population pairs which show evidence of outbreeding depression will not be planted in close proximity to each other in the translocated sites. Similarly, the genetic information on individuals in the seed orchards will be used to avoid planting close relatives in close proximity at the translocated sites.

Threatened species characterised by just a few remaining populations may not respond to translocation to secure sites. Such species may require active management to ensure establishment and ongoing recruitment of new individuals. Is important to remember that the manipulative techniques implemented during active management can directly influence the survival of individuals. While this may be desirable in the short term, it can also affect the genetic characteristics and spatial patterns of such population and thus its evolutionary trajectory in the long term (Frankham et al. 2017). Therefore, it is important to genetically characterise the founder population to keep track of its genetic layout and to trigger remedial procedures (see sections 3.4.2.2 and 3.4.2.3)

3.2.5 Establishment Techniques & Protocols at Translocated Site(s)

Planting of tubestock (nursery-raised plants raised from either seed or cuttings) at the translocation site(s) shall follow standard tree-planting procedures following appropriate site treatments, namely:

1. Weed control achieved via selective herbicide or mechanical means (depending on site) several weeks before planting;
2. Shallow ripping or roughening of surface soil in areas to be planted (depending on site);
3. Dig holes bigger than root mass and carefully plant tubestock and return soil around root mass creating small furrows;
4. Water seedlings initially, and add soil wetting agent and small amount of slow-release fertiliser designed for native plants;
5. Add mulch around, but not touching, tubestock (best sourced from shredded native plants without seed or pathogens).

All individuals will continue to be tagged with a unique identifier to allow all field data to be linked back to source population and genetic information together with future monitoring of quantitative traits and survival. Site treatments will ultimately depend on the condition of soils and vegetation at the specific translocation site(s) (which are yet to selected), but would likely encompass some degree of weed control, some manual surface roughing (to reduce surface crusting/compaction), and application of soil wetter/conditioner (to improve water infiltration and wettability).

Spacing of individuals from each other and from other similar-sized plants will be based on typical spatial patterns observed from other populations of the two species.

3.2.6 Fencing and Site Management

Juvenile individuals of both *C. undulatum* and *M. keigheryi* are reported to be highly susceptible to rabbit browsing and will require exclusion fencing to protect the establishing plants from this exotic herbivore. Therefore, fencing (combined with other forms of rabbit control such as baiting) is required as a means to help establish individuals that will be transplanted at a juvenile stage. Fencing will also be required to reduce the impact of grazing on the survival of recruits produced *in situ* once the translocated plants reach maturity. In the instance of the selected translocation site falling within a Threatened Ecological Community (TEC), such as *Banksia attenuata* woodland over species-rich dense shrublands (floristic community type 20a), care will be taken to ensure that the condition of the TEC is not adversely affected by any translocation activities. In particular, clearing for fence lines can be highly disturbing to sensitive vegetation communities, which often do not grow back as before, and can facilitate weed invasion. Therefore, if the site falls within a TEC, fence lines will need follow existing tracks or breaks even if this means a larger area needs to be fenced.

If the translocation site(s) are already cleared or partially cleared, ongoing weed and ground-cover management will need to be implemented, most likely through regular application of herbicides directly to such exotic plants. Further, even if the site is little disturbed and relatively intact with few to no weeds, it is possible that ground disturbance from planting (which should be kept to minimum) will encourage some weeds. It is for this reason that weed presence and cover is an important measure in the monitoring plan (see Section 3.3 below). Pest animal and plant control is planned for the life of the project, although the specific control prescriptions will be informed from monitoring results in an adaptive management framework.

Although both species seem to be relatively fire-tolerant (being resprouting species, see Research Section 3.4.3.4), they are likely to be sensitive to fire as juveniles given their rootstock and lignotubers are yet to fully develop, which covers the first five-year period of this plan. Therefore, it will be important to avoid fires at the site(s) at least until further investigations indicate otherwise. Such fire avoidance will likely require fire breaks around the larger property and access limitation. Moreover, hazard reduction burning may be considered on site (and in the vicinity) prior to planting. Such treatment not only reduces fire hazard for several years, but it is also likely to encourage plant establishment by increasing resource availability and lowering competition (provided ongoing weed control).

Lastly, to minimise the impact of drought on newly established plants and mitigate expected losses over the warmest months, watering will be undertaken for at least the first few summers. This site management technique has been found to have positive impacts on newly translocated plant species (see Section 3.2.2).

3.3 Monitoring

3.3.1 Monitoring Goals and Success Criteria

Monitoring is an integral component of all translocations, primarily to confirm whether goals of the translocation have been met, and to establish triggers and procedures for remedial action if goals are not met or assessed as unlikely to be achieved within the desired timeframe. In this plan, monitoring is also integrated with various research questions, with monitoring data used to inform various ecological and genetic studies (Figure 1).

The broad goals of this Propagation, Research and Monitoring Plan are (as outlined in the scope – see Section 2.2) to:

- reach or exceed target plant (minimum) establishment numbers in the field at translocation site(s) (i.e. at least 466 individuals of *C. undulatum*, and 29 individuals of *M. keigheryi*);
- ensure the translocated population(s) are viable.

The Ministerial conditions state that these goals need to be achieved within 5 years of initial clearing or disturbance of Estate plants as part of the runway expansion project, but also that remedial action be included in the plan in the case goals are not met. For the second goal, that of viable populations, given the relative slow growth rates of the species, the time they take reach to reproductive maturity (and hence seed set), the episodic nature of natural recruitment (possibly linked to fire events), and the challenges of propagating and successfully transplanting such large numbers of plants in the field, it is possible that long-term viability will not be able to be fully assessed within 5 years, however indicators of viability (on trajectory) can be assessed. Therefore, the monitoring will be divided into two distinct five-year stages, each with a set of goals as outlined below:

Stage 1, Years 0 to 5 to fulfill condition of approval:

- Target numbers of plants (see Section 2) are successfully established in the field;
- Ensure that such plants are healthy and growing;
- Early genetic and ecological indicators of viability (on trajectory).
- Contingency plans where viability is not likely or achieved.

Stage 2, Years 6 to 10 for ongoing research requirement:

- Ongoing monitoring of population viability in the translocated populations of the two species,

Given different numbers of plants will be propagated and planted in the field each year (based on seed availability and logistic constraints; see Table 2 to Table 4), targets for each year have been developed to facilitate monitoring of translocated populations (Table 5; Table 6).

Table 5: Annual (minimum) targets* of seed collection, nursery propagated plants, tubestock planted at translocation site(s), and plants established for *Conospermum undulatum.*

Year	Seed Collected	Seedlings Propagated	Cuttings Propagated	Tubestock Planted	Plants Surviving**
0 (source)	2100	-	-	-	-
1	2000	332	432	764	764
2	800	200	200	400	782
3	400	200	150	350	741
4	150	200	150	350	816
5	C	C	C	C	554
6	C	C	C	C	466
7	C	C	C	C	466
8	C	C	C	C	466
9	C	C	C	C	466
10	C	C	C	C	466
Total	5450	932	932	1864	466

* Targets calculated using assumptions and estimates as per Tables 2 to 4. Contingency ('C') actions (i.e. extra collection, propagation and planting) to be determined/undertaken only if targets are not met.

**An estimation based on 25% survival of tubestock planted (see Section 3.4.1), but as most deaths are likely over the first two summers following planting, a 50% survival rate is applied for both the second and third year of tubestock plantings (which represents a 25% survival overall); this is also based on late spring monitoring so assumes all tubestock planted that year are still surviving.

Table 6: Annual (minimum) targets* in terms seed collection, plants successfully propagated in the nursery, tubestock planted, and plants established for *Macarthuria keigheryi*

Year	Seed Collected	Seedlings Propagated	Cuttings Propagated	Tubestock Planted	Plants Surviving**
0 (source)	600	-		-	-
1	300	40	40	80	80
2	300	10	10	20	60
3	100	5	5	10	40
4	100	5	5	10	40
5	C	C	C	C	32
6	C	C	C	C	29
7	C	C	C	C	29
8	C	C	C	C	29
9	C	C	C	C	29
10	C	C	C	C	29
Total	1400	60	60	128	29

* Targets calculated using assumptions and estimates as per Tables 2 to 4. Contingency ('C') actions (i.e. extra collection, propagation and planting) to be determined/undertaken only if targets are not met.

** An estimation based on 25% survival of tubestock planted (see Section 3.1.1), but as most deaths are likely over the first two summers, a 50% survival rate is applied both for the second and third year of tubestock plantings (which represents a 25% survival overall); this is also based on spring monitoring so assumes all tubestock planted that year are still surviving.

3.3.2 Preliminary Assessment of Monitoring Protocols and Processes

In addition to regularly monitoring the numbers of surviving plants (to gauge whether or not targets are being met or exceeded, or at least are on track to reach targets), the monitoring program will also need to measure the health and size of plants to assess how well plants are performing and developing. It is expected plants will grow and develop over time and reach expected life history stages, such as reproductive maturity, at about the same time as in wild populations. Monitoring of plant growth and condition can also serve as an early warning system to flag potential adverse conditions and stresses on the plant (including herbivory, plant disease, nutrient deficiencies etc), and enable remedial actions to be implemented which may avert plant deaths in future months/years. However, the size/health measurements used will need to be cognisant of the particular growth habits and form of the two species. *C. undulatum* develops as a multi-stemmed shrub which resprouts from a below-ground lignotuber, and although some stems can die (e.g., due to drought or physical damage), the plant can still survive by developing new shoots. Therefore, for this species, the number and growth (length and diameter) of stems, should be monitored. For *M. keigheryi*, more needs to be known about its annual growth patterns and behaviour (which will be addressed by the Research Program). This species potentially dies back over summer, but typically survives as rootstock underground. Therefore, change in size/condition from one spring to the next (when plants are expected to be at their maximum development above ground) is likely to be most informative. Again, measurement of stem numbers and length of selected stems is likely to be best indicator or plant health and growth. Regular and aligned monitoring of selected site conditions (ecological indicators), such as moisture and nutrient levels, will assist in identifying potential reasons for any substantial decline in plant size and condition recorded.

A viable population as defined by the Ministerial Statement is a self-sustaining population of mature individuals capable of undergoing natural population processes, such as producing viable seed and recruitment of subsequent generations, and containing sufficient genetic diversity to represent the genetic composition of the individuals removed from the project area.

To be assessed as viable, populations therefore need to satisfy three attributes - demographic, ecological and genetic. Demographically, enough plants need to be established to ensure ongoing persistence at the site – this number is not known but at least initially will be taken as the target numbers (as a minimum). Ultimately, a Population Viability Analysis (PVA) will be conducted for both translocated populations, as well as for the species overall, however key species attributes to conduct PVAs are currently unknown. As this information becomes available through the Research Plan, then PVAs are to be completed and will contribute important evidence on the viability of the final translocated populations after 5 years. Ecological measures which indicate viability include having sufficient numbers of plants that are healthy, growing and completing critical life-history stages (e.g. flowering, pollination, viable seed set, field germination) to predict the likelihood of ongoing recruitment of new plants. Genetically, populations need to have sufficient genetic diversity and heterozygosity to produce adequate viable seed (i.e. avoidance of inbreeding effects). Moreover, the genetic makeup of the population must be of similar genetic diversity to the original populations (no less than 5% drop in observed heterozygosity and 90% of genets retained). The time required to reach some of these milestones may be greater than the initial 5 years of the plan. At the completion of the Stage 1 period an assessment will be undertaken of the success in achieving the two monitoring goals. Where viability cannot conclusively be ascertained an assessment will be conducted to ensure the population is on the right trajectory to viability. This will trigger the requirement to commence Stage 2 monitoring for an additional five-year period, or until viability is able to be verified within the five-year period.

3.3.3 Monitoring Actions – Stage 1 (0-5 years)

The specific actions of the first stage of the monitoring program are:

1. Tag all planted tubestock at translocation sites (of the both species) as they are planted (this will include cross-ref to original source plants and plants will also be georeferenced and mapped using high-accuracy GPS or GNSS);
2. Biannual plant counts, and assessment of the size and health of each plant (see Section 3.3.2) in: i) mid-spring (primarily to assess plant establishment of newly transplanted plants of current year) and; ii) mid-autumn (to assess survivorship over summer drought);
3. Biannual measurement of key ecological indicators within permanent quadrats set up in the translocation site in the vicinity of transplants, including: i) weed cover and composition; ii) soil pH and major macronutrients; iii) soil moisture; iv) herbivore presence (e.g. via grazing activity and scat counts). Timing will be same as plant counts above;
4. Annual (spring) measurement of flowering, pollinator activity, seed set and other reproductive indicators;
5. Annual (spring) counts of recruitment of new individuals in the population (by scouting field site and surrounding area) – these plants will also be tagged as discovered;
6. Collection of plant tissue (typically one to few leaves) of certain individuals (for genetic analysis of transplants) – in years 3 and 4.

Monitoring in stage 1 will proceed for 5 years from the start of the project as per timeline above.

3.3.4 Monitoring Actions - Stage 2 (6 -10 years)

1. Biannual plant assessments at end of each spring and autumn, including numbers and size/health status of all surviving plants. Any new recruits to be tagged and geo-referenced as above;
2. Annual measurement of key reproductive indicators, including floral display (spring), number and proportion of viable seed set (summer), density of soil seed bank, and level of pollinator activity (spring-summer);
3. Annual measurement of key ecological indicators (as per Stage 1 above);

4. Annual assessment of population viability using above parameters, including PVAs and analysis of genetic diversity (relative to natural populations)
5. Testing response of selected plants (or section of translocation site) to disturbance such as fire (Optional)

We will consider the translocation successful if the monitoring targets for viability and minimum plant numbers in Stage 2 are met for two successive years. However, to ensure our commitments and agreements are met, the monitoring program will continue to the end of Stage 2.

3.3.5 Contingency Plans and Corrective Actions

3.3.5.1 Plan for not achieving target numbers

Propagation and planting will aim to exceed target numbers in the first three planting seasons to compensate for unexpected losses due to unforeseen events that cannot be effectively pre-emptively managed. An initial estimate of four times the number of required plants will be aimed for in terms of planting, although this will be reassessed in year 2 based on initial monitoring of year 1 plants, and then reassessed annually thereafter based on regular monitoring of translocation site(s). If numbers in first (autumn) monitoring of year 3 are below target then infill planting in winter of year 3 (and year 4 if necessary) will be required, again in exceedance of targets to compensate for likely seedling death in the field. Yearly targets are outlined in Table 5 and Table 6.

In the event that the number of plants able to be propagated, via both cuttings and seedlings, are below that required to successfully establish plants in the field at translocation site(s), despite research trials to improve propagation success (see Section 3.1), then tissue culture will be considered as an alternative propagation method. For this reason, establishment of tissue culture protocols (methodological standards) will be developed for the two species as part of the research program (Section 3.4.1.1). This knowledge is also important conservation outcome for these species. Further, it will enable the preservation of key genotypes from populations to be cleared at Perth Airport (although cryogenic storage or very low temperature refrigeration of plant tissue are also options here).

Ensuring that native pollinators are present at the new site or that the occurring natural vegetation can support them is part of the site selection process. However, if seed set is low at the translocation site due to unforeseen lack of natural pollination, hand pollination may be required to increase the reproductive output of the newly established population while ways of increasing pollinator abundance will be examined. Hand pollination should be based on the genetic compatibility of individuals, which will be known, to avoid early abortion of embryos and the propagation of only a few genotypes.

3.3.5.2 Plan for not achieving viability within 5 years

If PVAs or the other measures of viability indicate that population viability is not achieved, or the trajectory suggests it is unlikely to be achieved within 5 years then Stage 2 monitoring will be undertaken. If the trajectory suggests it is unlikely to be achieved within 10 years then contingencies will be required to be implemented depending on the reason(s) for the lack of viability. If, for instance, simply low plant numbers are the reason, then it will be a matter of propagating and transplanting more individuals (in numbers suggested by the PVA as likely to lead to viability). If it is lack of sufficient genetic diversity, then targeted propagation and selection of plants of particular genotypes may be undertaken. If lack of recruitment is the underlying cause, then prescribed burning, application of smoke water, or other ways to stimulate germination in the field (as informed by the research plan) can be implemented.

Table 7: Summary of Monitoring and Contingency/Corrective Actions

Performance targets/ Indicators	Monitoring	Timing (Year) or Frequency	Triggers	Corrective actions
Germination Target of 20% for *C. undulatum* & 5% for *M. keigheryi* at year 1 to 4	Seed germination rates after 3 months	Weekly monitoring of germination	Less than 10% for *C. undulatum* & 3% for *M. keigheryi* in any year 1 to 4	- Investigate Cause /Risk Factors - Review seed germination procedures and equipment, and remedy as required. - Procure additional seed to compensate lower than expected germination - Incorporate additional experiments in research program to improve overall seed germination %
Seed production rate below 10 viable seed per plant (on average) for both species	Seed production rates per plant from annual collection in spring/summer each year 1-4	Annually, following seed collection and cleaning	Less than 5 viable seed per plant on average in any year 1-4	- Investigate Cause /Risk Factors - Initiate cross-pollination by hand in field and nursery - Increase collections from outside Airport estate - Increase numbers of plants established in seed orchards - Increase germination attempts/efforts to counteract lower seed production %
Cuttings success (root strike) rate of 33% for *C. undulatum* & 75% for *M. keigheryi*	% cuttings that strike roots and begin active growth	Weekly monitoring of cuttings in nursery	Root strike rate falls below 20% for *C. undulatum* & 50% for *M. keigheryi*	- Investigate Cause /Risk Factors - Review methodology, equipment and structures, and remedy as required - Procure and perform additional cuttings to compensate - Conduct further experiments to improve success rate of cuttings - Use tissue culture as an alternative propagation approach if propagation rates cannot be improved
Survivorship of 95% in nursery in any year 1-4 (both species)	% of propagated plants surviving in nursery	Weekly monitoring of nursery plants for growth, health and survivorship. Hourly monitoring of	Nursery Population less than 80% in any year 1-4	- Investigate Cause /Risk Factors - Review nursery conditions (temperatures, misting, light, irrigation) and improve as required - Increase germination and cutting attempts/efforts to counteract lower nursery survivability %

Performance targets/ Indicators	Monitoring	Timing (Year) or Frequency	Triggers	Corrective actions
		nursery environment (temperature etc)		- Source additional/external seed if required
Near 100% survivorship of planted tubestock at translocation site(s) within 3 months of planting (years 1-4)	Monitoring of plant health and survivorship	Annual Spring monitoring (following winter planting)	Less than 80% tubestock surviving within 3 months of planting	- Investigate Cause /Risk Factors - Further investigation of causes of plant death, e.g., herbivory, physical damage, drought, nutrient deficiency? - Improvement of translocation site conditions, via watering, fertilisation, fencing, as suggested by investigations - Increase translocation attempts/efforts (in-fill planting) to counteract lower survivability % - Source additional/external seeds and cuttings
Survivorship of planted tubestock at least 25% at year 5 at translocation site(s), for both species	Monitoring of plant health and survivorship	Biannual monitoring (Autumn and Spring)	Survivorship of planted tubestock following either 1st or 2nd summer falls below 30% of planted numbers (both species)	- Investigate Cause /Risk Factors - Assess if any ecological indicators have changed over time, e.g. weed cover, pH, soil nutrients - Further investigation of possible causes of plant death, e.g. herbivory, drought, nutrient deficiency? - Improvement of translocation site conditions, via watering, fertilisation, fencing, as suggested by investigations - Increase translocation attempts/efforts (in fill planting) to counteract lower survivability % - Obtain additional tubestock via seed germination and cuttings
Majority of surviving tubestock are deemed healthy and growing	Monitoring of plant health	Biannual monitoring (Autumn and Spring)	Greater than 50% of surviving plants demonstrate a decline in health score and/or a reduction in plant size over one calendar year	- Investigate Cause /Risk Factors - Assess if any ecological indicators have changed over time - Further investigation of possible causes of poor plant health, e.g. herbivory, drought, nutrient deficiency? - Improvement of translocation site conditions, via watering, fertilisation, fencing, as suggested by investigations

Performance targets/ Indicators	Monitoring	Timing (Year) or Frequency	Triggers	Corrective actions
Flowering and seed production rate at translocation site similar to wild populations for plants of similar age (in years 6-10)	Floral display (flowers per inflorescence and plant) and viable seed per plant	Annual (spring-summer) monitoring of translocated plants for flowering and seed set rates	Population flowering/seeding at rates half or less than that known for plants from wild populations of similar age	- Investigate Cause /Risk Factors - Improvement of translocation site conditions - introduction of pollinators, improving areas to attract pollinators - Manual pollination
Recruitment rate (number of new individuals naturally regenerating) of translocated populations similar to rate of wild populations (years 6-10)	Number of new individuals in populations arising from natural recruitment per year	Bi-annual (autumn and spring) monitoring of plant numbers and condition	Recruitment rate (number of new individuals naturally regenerating) of translocated populations less than half that known for wild populations	- Investigate Cause /Risk Factors - Improvement of translocation site conditions - introduction of pollinators, improving areas to attract pollinators - Improved site treatments to enhance recruitment success, e.g. weeding, summer irrigation, improved fencing, small-scale burning - Manual pollination

3.4 Research

The research component of this project has been designed to be integrated with the plant propagation, translocation and monitoring programs, rather than be undertaken in isolation (Figure 1). The research components allow for an adaptive management approach to the propagation and translocation program, where learning and improvement can be achieved by judiciously monitoring on-ground works, each being effectively set up as experiments where effects, either positive or negative, can be unambiguously attributed to certain actions or treatments. Given the limited seed and tissue availability, which is typical of threatened plant species, it is anticipated that such integration will be of great benefit project. For example, plants produced during propagation research will planted at translocation sites (provided seed/tissue is sourced from plants growing within the Perth Airport Estate) and hence will not be wasted. The conceptual plan above (Figure 1) illustrates how each of the below research programs are to be integrated with the propagation, translocation and monitoring components of this project.

Further, this research is designed to provide broader ecological and genetic knowledge to assist in the management of other populations of the species, including any retained populations on Perth Airport lands (which is the case for both species). Where the research contributes to this broader understanding of the species and their conservation management, is indicated in the plan.

3.4.1 Propagation research

3.4.1.1 Vegetative Propagation

Objectives:

1. Improve success (root-strike) rates in stem cuttings to >50% for *C. undulatum.*
2. Develop tissue culture protocols for the two species.

Methods:

1. Establish two-factor experiment on stem cuttings of *C. undulatum* to compare effects of different hormone concentrations (0, 0.2%, 0.5% and 1% IBA) and type of wood (softwood vs. semi-hardwood).
2. Monitor cuttings for root strike and growth rates.
3. Analyse data to determine effects of treatments, if any, on cuttings. Deploy any improved techniques in cuttings program (Section 3.1.3).
4. For tissue culture, standard experiments in terms of explant selection & disinfestation, media composition (particularly hormone concentrations) and incubation conditions will be required to establish successful tissue culture protocols (Smith 2012).

Timeline:

Year 1 (cuttings) & Year 2 (tissue culture)

Equipment/consumables required (if relevant):

- Glasshouse with misting bed;
- Potting shed for preparation of cuttings, media, pots etc;
- Laminar flow cabinets and sterilisation facilities (e.g. autoclaves) for tissue culture; research (or similar elsewhere).

Location:

ECU Plant Nursery Complex and labs or similar facility elsewhere.

Outcomes:

- Improved cutting success rate will mean more efficient and effective use of plant stems, which are limited in availability for rare species, to propagate new plants. Fewer cuttings will need to be done in nursery, thereby saving time and resources (Section 3.1.3).
- Development of successfully tissue culture protocol will enable tissue culture to be deployed as a contingency measure (see Section 3.3.5).

3.4.1.2 Seed Research – further trials to improve germination success

Objectives:

1. Determine effect of standard germination treatments (scarification, stratification, seed coat manipulation, hormones, smoke) to improve germination of *M. keigheryi*.
2. Further refine existing treatments to optimise germination for *C. undulatum*.

Methods:

1. For *M. keigheryi*, treat replicated batches of seed, each with single seed treatment which covers range of standard seed treatments (including hot water, nicking of seed coat, extended incubation in either warm or cool conditions, and application of gibberellic acid or smoke water at various concentrations), as well as control seed batch (no treatment);
2. For *C. undulatum*, treat seed batches with various concentrations of smoke water or specific smoke compounds such as karrikinolide (Chiwocha et al. 2009), including 'zero' controls;
3. Sterilise surface of seed using plant preservation mixture;
4. Germinate seed in sterile water agar within petri dishes placed in seed germination cabinets set for winter soils temperature of ~15^{o}C;
5. Measure seed imbibition via regularly weighing sample of seeds;
6. Monitoring of seeds for germination;
7. Analyse data to determine effects of various treatments;
8. Repeat analyses exploring combined effects of any successful treatments (for *M. keigheryi*).

Timeline:

Years 1 (initial trials) and Year 2 (main trials)

Equipment/consumables required (if relevant):

Seed germination cabinet(s); sterilisation equipment; seed germination media.

Location:

ECU research labs and plant nursery complex.

Outcomes:

- Establish technique(s) to successfully overcome seed dormancy in *M. keigheryi* (as currently not known).
- Improved germination rates (%) which will mean less seed needs to be collected (Section 3.1.2).
- More efficient use of seed with limited availability.
- Provide extra seedlings for translocations (successful germinants will be deployed to the propagation program, i.e. not wasted).

Note: Given likely seed limitations for both species, larger scale and 'blue-sky' experiments (e.g., involving novel treatments) may be conducted on closely related, but more common species (such as *Macarthuria australis* and *Conospermum stoechadis*), where possible. Successful techniques discovered could then be applied to the two threatened species, thereby minimising seed wastage.

3.4.1.3 Experimental crossing to improve seed quantity, viability, and seedling fitness

Objectives:

1. Increase seed quantity, viability, and seedling fitness through controlled cross-pollination.
2. Identify which genetic attributes of populations and individuals best predict increased seed quantity, viability, and seedling fitness.

Methods:

1. Pollen donor (paternal) and maternal plants will have pollination bags placed around inflorescences to exclude pollinators.
2. Mature pollen collected from 50-80 plants of *C. undulatum* and *M. keigheryi* from within the airport site and four populations nearby the airport population.
3. On recipient plants (maternal), manual hand pollination from pollen parents both within and between populations of *C. undulatum* and *M. keigheryi* to be performed. Pollination technique will follow the approaches developed by Delnevo et al. (2020).
4. Pollination bags placed over inflorescences until fruit set. Fruit collected when mature to count total fruit set and total seed counts.
5. Seeds germinated in the glasshouse to assess germination rate and early seedling viability. These controlled crosses (obtained in section 3.1) will be used in translocations where plant survivorship and fitness will be monitored.
6. Statistical analyses to test for correlations between genetic characteristics of individuals (obtained in section 3.4.2.5) and the seed quantity, germination rate, and seedling fitness of resultant offspring.
7. In the event that plants at the target site will not be available for cross pollination, crosses will be performed once cuttings have reached flowering stage.

Timeline:

Genetic diversity and heterozygosity data available in first half of year 1 (see section 3.4.2.5) to guide controlled crossing experiments in second half of year 1.

Equipment/consumables required (if relevant):

Pollination bags, plant tags, paintbrushes, seed envelopes, pots, soil, fertilizer.

Location:

Crossing experiments performed in the field, germination experiments in the labs and glasshouse at ECU. Translocation of offspring from crosses performed at the identified site for translocations for both species.

Outcomes:

- This research will increase seed production and seedling germination, providing a larger seed stock and seedling number to commence translocations.
- Improve translocation success through use of genetically diverse seeds generated from cross pollination between populations, which is crucial for long-term viability of the population to adapt to changing environments.
- Improve general propagation guidelines for threatened species by testing the value of cross pollination that maximises genetic variability for seed production, germination and offspring viability.

3.4.2 Genetic research

3.4.2.1 Reference Genomes

Objectives:

1. Assemble a reference genome for *C. undulatum* and *M. keigheryi*.
2. Assemble an annotated genome for *C. undulatum* and *M. keigheryi*.

Methods:

1. High Molecular Weight (HMW) DNA will be extracted from leaf tissue from a single representative individual of each species using specialised kits designed for HMW plant DNA (Circulomics Nanobind).
2. DNA extractions tested for quantity and quality using Nanodrop and Qubit. The presence of long reads ~50KB required for HMW DNA will be tested using Agilent Tapestation at ECU.
3. Half of the DNA sample for both species will be sent to Australian Genome Research Facilities (AGRF) for sequencing on Pacific Bioscience (PacBio) HiFi single-molecule real-time (SMRT) platform.
4. In parallel, the other half of the HMW DNA samples for both species sequenced using Oxford Nanopore sequencer based at ECU.
5. Analysis protocols will use genome assemblers designed specifically for HiFi reads (hifiasm) and Oxford Nanopore (Pore-C). A hybrid assembly combining both data built using Hybrid de novo genome assembly pipeline (Unicycler).*
6. mRNA will be isolated from four tissue types (leaves, flowers, buds, roots) with MagMax RNA extraction kits (Thermo Fischer) following standard protocols. Samples taken from the same reference individuals used above, plus additional biological replicates.
7. mRNA libraries constructed using poly A pulldown approach and RNA sequenced at AGRF facilities.
8. The RNA sequence data will be used to build an annotated genome using the program FINDER.*

* - Bioinformatic analyses will use High Performance Computing (HPC) system at ECU and when required for large computational tasks this will be run using a paid service to access fast clusters computing remotely (DUG computing services).

Timeline:

The reference genome and annotation will be built during the first two years.

Equipment/consumables required (if relevant):

- PippenPulse electrophoresis system and MinilON sequencing system, pipettes (multi-channel steppers) and measurement scales;
- DNA and RNA extraction kits and general lab consumables (pipette tips, chemicals);
- DNA and RNA sequencing services (AGRF);
- HPC computer for bioinformatics and data storage, software licensing, external HPC usage (DUG).

Location:

Molecular lab work conducted at ECU laboratories. DNA sequencing outsourced externally to AGRF.

Outcomes:

- Obtaining a reference genome for both species will improve the success of re-sequencing individuals throughout the genetic components of the translocation and monitoring of the project.
- This information will provide more precision in accurately aligning DNA sequences to the common reference and enable improved estimates of population genetic structure, relatedness, hybrid identification and broader demographic and evolutionary insights into the history of populations and species.
- Obtaining an annotated genome will allow for the detection of which DNA sequences encode functional genes.
- The annotated genome will allow for the detection of deleterious mutations associated with inbreeding in small populations, allowing for better detection of poor fitness individuals in translocation design.

3.4.2.2 Modelling of translocation scenarios

Objectives:

1. Determine the optimal strategy for establishment of a genetical diverse crossing designs given genetic composition of individuals.
2. Determine the optimal composition in the translocated population that best represents available genetic variability of the source populations.

Methods:

1. Individual genotypes will be simulated using the program SLIM and a spatially-explicit forward simulation model written by DField (EvolRes) as an alternative more detailed model.
2. The simulation will be setup with one set of genetic markers (n = 1000) that will relate to individual fitness variation due to adaptation to the local conditions and other genetic markers (n = 1000) representing genetic load due to deleterious alleles (cause inbreeding depression).
3. Individuals will randomly or selectively interbreed (depending on scenario, see next point). Offspring dispersal will follow estimates of dispersal curves for plants with similar pollination and seed dispersal characteristics, and sites for new individuals will be subject to density dependent carrying capacity representative of local plant densities.
4. Modelling scenarios tested will include: (a) different founder numbers randomly drawn from source populations at a seed orchard, (b) selective translocations choosing individuals with maximum heterozygosity individuals, (c) selective mixing of different nearby source populations (both small and large populations), (d) selective mixing of closely related vs. distantly related populations.

5. Simulation parameters will be calibrated by real genome-wide data gathered from the source populations (see section 3.4.2.5).
6. Simulation outputs will be run for a few generations and over longer time scales, with replicates of each scenario providing confidence intervals of translocation outcomes in terms of mean fitness, mean heterozygosity and inbreeding coefficients, and the skewness of genetic composition in translocated populations.

Timeline:

- Simulations will be conducted in year 1 and 2.
- Equipment/consumables required (if relevant):
- The HPC computer listed above will be used to run the simulations (see section 3.4.2.1).

Location:

- General ECU laboratories.

Outcomes:

- The simulations will enable explicit testing of the consequences of alternative strategies for crossing designs (see section 3.4.1.3).
- The simulations will identify the optimum strategy to maximise genetic variability in the crossing and translocation plan, thus ensuring the newly established populations are not inflicted by inbreeding depression and are better equipped to cope with long-term environmental change.

3.4.2.3 Status of Conospermum undulatum hybrids

Objectives:

1. Determine the status of hybrids and clarify putative parent species of the *C. undulatum* airport population.
2. Determine whether this population contains a divergent lineage that needs to be considered as a different entity in the translocation plan.

Methods:

1. Plants will be tagged and georeferenced using GNSS.
2. Leaf material will be collected from all the individuals of *C. undulatum* in the Estate population, which comprises pure *C. undulatum* plants and putative hybrids.
3. Pure individuals from three different *Conospermum* species that are (or were) sympatric with *C. undulatum* will be sampled from reference populations to facilitate hybrids detection.
4. Leaf samples will be freeze dried and stored in silica gel until DNA will be extracted using a modified CTAB extraction method (Byrne et al., 2001).
5. High quality DNA will then be sent to the Australian Genome Research Facility (AGRF) for building short read DNA libraries and next generation sequencing.
6. Such sequences will be used to obtain tens of thousands of genome-wide single nucleotide polymorphism (SNP) markers for all the investigated species, enabling comprehensive genetic analyses and sufficient power to identify the genetic ancestry of individual plants.

Timeline:

Data will be available at the beginning of year 1 of the project (2024) and will also guide the controlled crossing experiments in second half of year 1 (see timeline in Section 4).

Equipment/consumables required (if relevant):

- PippenPulse electrophoresis system, pipettes (multi-channel steppers) and measurement scales.
- DNA extraction kits and general lab consumables (pipette tips, chemicals). DNA sequencing services (AGRF).
- HPC computer for bioinformatics and data storage, software licensing, external HPC usage (DUG).

Location:

Molecular lab work conducted at ECU laboratories.

Outcomes:

- Obtaining a clear understanding of the status of putatively hybrid individuals will inform accurate selection of propagation material, ensuring hybrid genotypes will be kept separated from the pure *C. undulatum* plants that will be used for propagation and establishment of the new population.
- This is needed to avoid the deleterious effects of genetic and demographic swamping in the newly established population. While possible hybridisation can occur for *C. undulatum*, currently it is not an issue for Perth Airport population of *M. keigheryi*.

3.4.2.4 Clonal status of Macarthuria keigheryi

Objectives:

1. Determine and quantify clonality in *Macarthuria keigheryi*.
2. Investigate clonal architecture of the species to maximise sampling effort.

Methods:

1. Plant tissue will be sampled from plants within twenty 5 x 5 m plots with similar abundance of the target species in the Estate population.
2. Plant tissue will be collected following the same protocol in large wild populations north of Perth to provide reference data, as well as from other known populations.
3. Each *M. keigheryi* plant will be tagged and mapped within each plot prior to tissue sampling.
4. Preliminary testing aimed at determining the amount of tissue required for DNA extraction and the best extraction method will be performed for this species to minimise the impact of tissue sampling on the threatened *M. keigheryi*.
5. SNP markers will be used to explore the number of different multilocus genotypes (MLGs), as well as clonal diversity measures (i.e. clonal richness, evenness, diversity).
6. Analyse data through dedicated software such as GENODIVE 3.0 (Meirmans, 2020) or R packages (e.g. RClone; Bailleul et al., 2016).

Timeline:

Field sampling to commence from Jul/Aug of year 1 (likely 2024) when plants emerge from rootstock.

Genetic data available early year 2 (likely 2025) (refer to timeline in Section 4).

Equipment/consumables required (if relevant):

- General field equipment and consumables (e.g. labels, tags, GPS,).
- PippenPulse electrophoresis system, pipettes (multi-channel steppers) and measurement scales.
- DNA extraction kits and general lab consumables (pipette tips, chemicals). DNA sequencing services (AGRF).
- HPC computer for bioinformatics and data storage, software licensing, external HPC usage (DUG).

Location:

- Sampling will be performed both on the Estate and in wild large populations north of Perth, as well as other known populations.
- DNA extractions will be conducted at ECU laboratories.

Outcomes:

- Obtaining a clear indication of the spatial arrangement of *M. keigheryi* clones.
- This will provide important information in relation to seed/cuttings collection to prevent oversampling of dominant MLGs and, consequently, to increase genetic diversity in the newly established population.

3.4.2.5 Genetic diversity and structure of Conospermum undulatum and Macarthuria keigheryi

Objectives:

1. Characterise the level of genetic diversity within each of the *C. undulatum* and each of the *M. keigheryi* populations.
2. Characterise the level of genetic diversity between each of the *C. undulatum* and *M. keigheryi* populations to identify unique population structure contained across populations of each species.

Methods:

1. Leaf tissue will be sampled from all *M. keigheryi* individuals from the Estate population and from 40 plants in each known populations of the species and stored in silica gel.
2. Leaf tissue will be sampled from *C. undulatum* collected from 20 plants from each of the 17 populations.
3. DNA extractions performed using modified CTAB (see 3.4.2.3) and MagMax (ThermoFischer) kits when DNA is degraded.
4. The same method described above (see 3.4.2.3) will be used to obtain SNP markers using ddRAD-seq to investigate the extent of the genetic diversity within populations.
5. Programs ADEGENET (Jombart, 2008) and HIERFSTAT (Goudet 2005) will be used to produce genetic diversity measures such as expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (AR) and inbreeding coefficients (F_{IS}).
6. Genome wide average levels of relative differentiation (F_{ST}) and absolute differentiation (D_{xy}) between all population pairs will be estimated using the software GENODIVE 3.0 (Meirmans, 2020).
7. The same software will be used to calculate the genetic distance matrix to be used in a Principal Coordinate Analysis (PCoA). To visualise population structure and similarity between samples, a PCoA ordination plot
8. The Bayesian clustering algorithm implemented in STRUCTURE v. 2.3 (Pritchard et al., 2000) will be used to assess the putative number of differentiated genetic clusters.

9. To account for extended genetic structure along a cline, spatially explicit landscape genetic methods will be used, including spatial principal component analysis (sPCA) and Moran's eigenvector map (MEM; Legendre et al 2015) analysis based on genotypes and spatial

Timeline:

Leaf sampling, DNA sequencing and analyses commenced in year 1.

Equipment/consumables required (if relevant):

- General field equipment and consumables (e.g. labels, tags, GPS,).
- PippenPulse electrophoresis system, pipettes (multi-channel steppers) and measurement scales.
- DNA extraction kits and general lab consumables (pipette tips, chemicals). DNA sequencing services (AGRF).
- HPC computer for bioinformatics and data storage, software licensing, external HPC usage (DUG) (same requirements as above section 3.4.2.1).

Location:

Molecular work conducted at ECU laboratories.

Outcomes:

- Obtaining genetic diversity within populations will enable selective crossing designs and optimal translocation designs to maximise genetic variability.
- Maximising genetic diversity in crossing designs and translocations will allow for designing an optimal individual design than minimises the risk of inbreeding and maximises the potential adaptive variability available in the establishing translocated population.
- Identifying population pairs with unique population structure in the species will assist in translocation designs to avoid mixing of potentially differentially adapted populations or hybrids.

3.4.3 Ecological research

3.4.3.1 Plant Establishment and Survival Research

The monitoring program outlined in Section 3.3 will likely generate important data on establishment and survival rates of seedlings and juveniles of the two species, as well as environmental factors (both physical and biological) influencing establishment/survival of translocated plants. To assign causality of the various site treatments imposed would however require control sites and plants to be established, such as unfenced/unprotected seedlings, non-weeded sites, and sites with no soil treatments. The number of plants used as controls however should be minimised given the importance of reaching target numbers, but should be sufficient to provide enough statistical power to make valid comparisons. Note: other proposed ecological studies, such as described below in section 3.4.3.4 on 'Disturbance Ecology', will shed light on levels of seedling survivorship in extant populations (and factors controlling this) which will provide important context for results found in the monitored translocated populations, as well being broadly applicable to the management of all populations of these two rare species.

3.4.3.2 Species Distribution & Niche/Microhabitat Studies and Modelling

Objective:

1. Improve the basis for site selection by defining and mapping the environmental envelope of the two target species.

Methods:

1. Environmental and climatic measurements for the area of interest will be gathered and mapped through geographic information system (GIS).
2. Such measurements will be rasterised to obtain spatially explicit data sets for relevant environmental, topographic, soil, and climatic variables.
3. The identification of suitable sites for translocation of *C. undulatum* and *M. keigheryi* will then be performed with statistical machine learning modelling techniques, such as random forest or maximum entropy, to model habitat suitability based on the GIS data sets as predictor variables, and presence and pseudo-absence coordinate data.
4. The model will generate habitat suitability scores for all the cells of the map, allowing the identification of translocation sites with suitability scores above a defined threshold. This modelling approach forms an integral part of the broader site selection process as outlined in section 3.2.3 above.

Timeline:

Modelling will be conducted at the beginning of year 1, with preliminary investigations to occur before year 1 (See timeline in section 4).

Equipment:

- GIS software and relevant maps and shapefiles to be run on the HPC computer listed in section 3.4.2.1 above.

Location:

ECU laboratories.

Outcomes:

- Identifying the most suitable location where to establish new populations of the threatened *C. undulatum* and *M. keigheryi*.
- Identifying possible locations to survey in order to find unknown populations of the two species.

3.4.3.3 Mating system and pollination of *Macarthuria keigheryi*

Objective:

1. Understand the basic mating and pollination requirement of M. keigheryi.
2. Define the composition of the pollination assemblage of M. keigheryi.
3. Determine the relative contribution of each pollinator group to the reproduction of the species.

Methods:

1. Field observations (in person and with cameras) will be required to identify the main flower visitors of *M. keigheryi*. Such surveys will be carried both in the Perth Airport population and in the larger northern populations, the latter being used as reference intact populations. Surveys will be conducted during the

entire flowering season of the target species to determine the exact composition of the pollinator assemblage.

2. To understand the relative importance of each group of pollinators, insects will be collected from flowers of *M. keigheryi* using clear bags after recording whether there was stigmatic contact by the insect. To avoid contamination of pollen load a new bag will be used for every insect.
3. Cold anaesthesia will be induced by placing the bag containing the insect on ice, and remove pollen non-destructively by dabbing in a standardised manner pollinator with fuchsin-stained gel to produce slides. The captured insect will be released as soon as the pollen has been sampled.
4. The microscope slides produced with the gel cubes will be analysed and pollen will be sorted by species. This will provides a good guide to major patterns of flower usage.

Timeline:

Ecological investigations will commence during the entire year 1 flowering season (2024), depending on approvals).

Equipment/consumables required (if relevant):

- General field equipment and consumables (e.g. camera, notebooks).

Location:

- Recording and sampling will be performed both on the Estate populations and in wild large populations north of Perth.

Outcomes:

- Obtaining a clear understanding of the mating system of *M. keigheryi* to guide the establishment of a new population.
- Define the pollinator assemblage required by the species in order to achieve self-sustainability in the new population.
- This will also add valuable information to understand vegetation requirements of pollinators and to ensure their presence in the newly established population.

3.4.3.4 Disturbance Ecology

Objectives:

1. Clarify the role of fire in the regeneration of *M. keigheryi* and *C. undulatum* by seed.
2. Quantify the soil seed bank for the two species.
3. Determine the effect of grazing by rabbits and other large herbivores on *M. keigheryi* and *C. undulatum.*

Methods:

Note: As prescribed fires are typically not permitted within the Perth Airport Estate (due to potential impact of smoke on visibility), response of the two species to fire using experimental burns cannot be planned with any confidence. However, there is potential to utilise prescribed burns conducted in other reserves by DBCA or local councils; as these cannot be guaranteed, they are included under 'Optional Additional Research' below. Further, under seed germination research (Section 3.4.1.2), responses of seed to heat and smoke will be studied, which will complement the research described below to determine the effect of fire on regeneration by seed.

1. Establish permanent 1m^2 quadrats within populations of the two species remaining at Perth Airport Estate and other areas where the species occur ;
2. Immediately adjacent to quadrats, collect topsoil samples (surface to 5 cm depth) within small sub-quadrats of 0.25 m^2.
3. Spread topsoil from each sub-quadrat into three seedling trays in glasshouse with each tray either: a) treated with boiling water (to simulate heat effect of fire); b) treated with smoke water (to simulate smoke effect of fire); and c) just water (control). Keep trays moist and at suitable temperatures in glasshouse and monitor trays weekly for germination over several months.
4. Tag all existing plants of the threatened species occurring in quadrats and any new seedling recruits (for monitoring).
5. Establish herbivore-exclusion mesh around half the quadrats (leaving the other half accessible to herbivores);
6. Monitor quarterly all tagged plants in quadrats (as well as any new emerging plants) for plant size (e.g., stem number, diameter and length), reproductive status and condition for at least 3 years;
7. Repeat seed bank assessment (step 3) within quadrats at 3 years;
8. Analyse results to determine the effect of heat/smoke on seed germination from soil seed bank, and the effect of herbivory on plant survival, condition, recruitment, and seed bank density.

Timeline:

These will be a multi-year trials set up in years 1-2 and monitored until year 3-4.

Equipment/consumables required (if relevant):

Pegs, tape measures, GPS, fire-resilient plant tags.

Location:

Perth airport and/or other protected field site(s) containing natural populations of the species.

Outcomes:

- Clarification of the germination response to fire of the two species.
- Evaluation of the importance of the soil seed bank to recruitment and overall population resilience.
- Clarification of population dynamics including degree to which species die back over summer drought (especially important for *M. keigheryi*).
- The potential damage wrought by herbivores, particularly rabbits, will be assessed, including interactions with fire (e.g., is post-fire herbivory greater and are young seedlings or resprouts more susceptible to grazing).
- Improved understanding of factors influencing growth and recruitment of the two species, which will better inform the propagation (Section 3.1.2)and establishment of the two species at translocations sites (Section 3.2.5).

3.4.4 Optional Additional Research

If prescribed burning is planned in other reserves containing either of the two species, then it may be possible to use these as experimental burns to directly study the effects of fire, both in terms of survivorship of adult plants and their potential for vegetative resprouting, and in terms of the fire-stimulation of germination in the field. This will require close co-operation with relevant land managers (e.g. LGAs, DBCA), but it is quite possible that at least one reserve for each of the species will need hazard-reduction burning over the initial 5 years of the research program (based on fire management regulations and requirements in peri-urban zones). Such research will require pre-fire survey and establishment of permanent monitoring quadrats within populations of the species, as well as measurement of quadrats within populations immediately outside the burn area (as non-burnt controls). Monitoring of quadrats should start before burning and then for at least 3 years post fire.

4 Project Implementation Schedule

As per the MDP Approval condition, the implementation of this Propagation, Research and Monitoring Plan must commence (latest) within 18 months of approval of the PAPL NRP Offset Strategy (approved 15/5/2024). Components of this plan (i.e., seed collection, genetic testing, translocation site assessment) have already commenced in advance of this requirement.

The proposed project implementation schedule is split into two stages. Stage 1 includes the 5-year population establishment phase as per the project conditions of approval (Table 8). This stage also includes initial monitoring and research implementation which are integrated with the propagation and translocation efforts. Stage 2 represents the following 5-years phase (years 6 to 10) and includes ongoing monitoring and assessment of population viability over the longer term, and contingency actions (as required) (Table 9).

It may be necessary to amend and update the implementation schedule from initially proposed due to plan review and approvals, and in keeping with adaptive management processes over the substantial duration and complexity of this project.

On completion of the required population establishment, the translocation areas (sites) will be transferred to the relevant land manager for management and protection for conservation purposes. Given the priority is to establish the new translocated populations within retained remnant bushland solely within the Perth Airport Estate, the relevant manager will be Perth Airport Pty Ltd.

Table 8: Stage 1: First 5 years: establishment phase, initial monitoring and research implementation. N.B. PVA = Population Viability Analysis. Monitoring of plant establishment and survival also includes other measures as described in s3.3.3.

Stage 1	Task	2023 - Year 0				2024 - Year 1												2025 - Year 2												2026 - Year 3					
		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Procedural & Reporting	1. Draft for comments																																		
	2. Plan approvals																																		
	3. Organise contracts, arrange permits																																		
	4. Update number of C.u. (airport) accounting for hybrids																																		
	5. Progress report																																		
	7. Draft final report																																		
	8. Final 5 year Reporting																																		
Propagation & Translocation	1. Bag flowers C.u. (airport)																																		
	2. Collect tissue C.u. (airport)																																		
	3. Collect seed C.u. (airport)																																		
	4. Collect seed M.k. (airport)																																		
	5. Review of previous translocation attempts																																		
	6. Seed germination																																		
	7. Stem cuttings																																		
	8. Establishment of seed orchards in nursery																																		
	9. Collect tissue C.u. & M.k. (all nearby populations)																																		
	10. Fencing																																		
	11. Site Preparation																																		
	12. Rescue & pot airport plants (if and when required)																																		
	13. Trial initial field plantings																																		
	14. Field plantings – Year 2 (aim for large initial establishment)																																		
	15. Bag flowers C.u. in the nursery to control cross-pollination																																		
	16. Collect pollen C.u. from nursery plants and wild populations																																		
	17. Collect pollen M.k from nursery plants and wild populations																																		
	18. Collect seed C.u.																																		
	19. Collect seed M.k.																																		
	20. Collect stems/tissue C.u. & M.k.																																		
Monitoring	1. Establishment																																		
	2. Survival																																		
	3. Final survival – all years																																		
	4. Evaluation of population viability (using early indicators)																																		
Research	1. Preliminary habitat/niche modelling to guide site selection																																		
	2. Lab work for genetic analysis of Status of C.u. hybrids																																		
	3. Preliminary analysis of C.u. (airport) for hybrid identification																																		
	4. Clonal status of M.k. - field sampling																																		
	5. Mating systems/pollination of M.k. – field sampling																																		
	6. Clonal status and genetic analyses of M.k																																		
	7. Initial genetic diversity and structure analyses for C.u. & M.k.																																		
	8. Experimental crossing for seed improvement – set up																																		
	9. Experimental crossings – viability tests																																		
	10. Propagation research – seed germination & cutting trials																																		
	11. Genetic modelling for population/plant selection																																		
	12. Fire trials – autumn burn																																		
	13. Tissue culture protocols																																		
	14. Reference genome – draft assembled																																		
	15. Fire trials – spring burn; autumn burn monitoring																																		
	16. Final genetic diversity and structure analyses for C.u. & M.k.																																		
	17. Genetic analyses of surviving plants																																		
	18. Fire trials – monitoring Autumn																																		
	19. Fire trials – monitoring Spring																																		
	20. Propagation research – data analyses and write up																																		
	21. Assembly and analyses of reference genomes (both species)																																		
	22. Genetic & ecological analyses of surviving plants & write-ups																																		
	23. Fire/disturbance papers – write up																																		
	24. Final genetic analyses of surviving plants																																		
	25. Genetics of translocation success – analysis and write-up																																		

Table 8 (Ctd.): Stage 1: First 5 years: establishment phase, initial monitoring and research implementation. N.B. PVA = Population Viability Analysis

Stage 1	Task	2026 - Year 3						2027 - Year 4												2028 - Year 5											
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Procedural & Reporting	1. Draft for comments																														
	2. Plan approvals																														
	3. Organise contracts, arrange permits																														
	4. Update number of C.u. (airport) accounting for hybrids																														
	5. Progress report																														
	7. Draft final report																														
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Propagation & Translocation	1. Bag flowers C.u. (airport)																														
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	4. Collect seed M.k. (airport)																														
	5. Review of previous translocation attempts																														
	6. Seed germination																														
	7. Stem cuttings																														
	8. Establishment of seed orchards in nursery																														
	9. Collect tissue C.u. & M.k. (all nearby populations)																														
	10. Fencing																														
	11. Site Preparation																														
	12. Rescue & pot airport plants (if and when required)																														
	13. Trial initial field plantings																														
	14. Field plantings – Year 2 (aim for large initial establishment)																														
	15. Bag flowers C.u. in the nursery to control cross-pollination																														
	16. Collect pollen C.u. from nursery plants and wild populations																														
	17. Collect pollen M.k from nursery plants and wild populations																														
	18. Collect seed C.u.																														
	19. Collect seed M.k.																														
	20. Collect stems/tissue C.u. & M.k.																														
Monitoring	1. Establishment																														
	2. Survival																														
	3. Final survival – all years																														
	4. Evaluation of population viability (using early indicators)																														
Research	1. Preliminary habitat/niche modelling to guide site selection																														
	2. Lab work for genetic analysis of Status of C.u. hybrids																														
	3. Preliminary analysis of C.u. (airport) for hybrid identification																														
	4. Clonal status of M.k. - field sampling																														
	5. Mating systems/pollination of M.k. – field sampling																														
	6. Clonal status and genetic analyses of M.k																														
	7. Initial genetic diversity and structure analyses for C.u. & M.k.																														
	8. Experimental crossing for seed improvement – set up																														
	9. Experimental crossings – viability tests																														
	10. Propagation research – seed germination & cutting trials																														
	11. Genetic modelling for population/plant selection																														
	12. Fire trials – autumn burn																														
	13. Tissue culture protocols																														
	14. Reference genome – draft assembled																														
	15. Fire trials – spring burn; autumn burn monitoring																														
	16. Final genetic diversity and structure analyses for C.u. & M.k.																														
	17. Genetic analyses of surviving plants																														
	18. Fire trials – monitoring Autumn																														
	19. Fire trials – monitoring Spring																														
	20. Propagation research – data analyses and write up																														
	21. Assembly and analyses of reference genomes (both species)																														
	22. Genetic & ecological analyses of surviving plants & write-ups																														
	23. Fire/disturbance papers – write up																														
	24. Final genetic analyses of surviving plants																														
	25. Genetics of translocation success – analysis and write-up																														

Table 9: Stage 2: Years 6 to 10: ongoing monitoring and assessment of population viability, and contingency actions (as required). N.B. PVA = Population Viability Analysis

Stage 2	**Task**	**2029 - Year 6**				**2030 - Year 7**				**2031 - Year 8**				**2032 - Year 9**				**2033 - Year 10**			
		Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4
Procedural & Reporting	1. Contingency plan (based on outcomes of Stage 1)																				
	2. Revision of contingency plans (as required)																				
	3. Draft final report																				
	4. Final report (all years)																				
Monitoring (biannual)	1. Plant establishment/health																				
	2. Site condition assessment																				
	3. Population viability analysis (PVA)																				
	4. Final PVA																				
Contingency Actions	1. Cuttings and seed germination (as required)																				
	2. In-fill planting (as required)																				
	3. Site treatments (as required)																				
	4. Population/genetic analyses and modelling (to inform PVA)																				
	5. Papers & presentations																				

5 Reporting

5.1 Progress & Scientific Reporting

The following reporting is planned from the ECU research team for PAPL:

- monthly project update reports, including forward plans, completed and new actions, new findings and conclusions, and any schedule updates or modifications.
- annual reports detailing propagation and translocation progress, including plant numbers against targets, as well as specific research results and outcomes.

For broader scientific communication of research findings, ECU aim to write and publish at least six peer-reviewed paper (all will be open-access as per offset requirements) and undertake three presentations at relevant international or national conferences across the project timeline.

ECU will provide to PAPL any outputs intended to be published prior to submission. PAPL will provide DCCEEW with any publications and outputs at least 5 working days before release.

5.2 Annual Progress Report

PAPL will provide an annual progress report to the Department of progress and key findings. This will be provided to DCCEEW for their information.

5.3 Compliance Reporting

As per MDP Approval Condition 7, PAPL will publish The Propagation, Research and Monitoring Plan (this plan) on the website no later than 7 business days after approval of the plan by the Minister for the Environment, and will keep this plan published on the website for the period of its operation. Any subsequent major revisions of this plan due to changes of circumstances will be submitted for review and approval by DCCEEW, and will be published on the website for the period of its operation.

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7 Appendices

7.1 Risk Assessment and Mitigation and Corrective Actions

Risk	Outcome	Mitigation	Corrective actions
Insufficient seed/cuttings available at Airport Estate during pre-clearing timeframe	- Lack of tubestock for planting and seed orchard establishment, fall short of plant targets	- Early determination of requirements (e.g. seed set rates) via survey of Estate populations	- Seek alternate propagule sources, e.g. other populations if appropriate and supported by genetic analyses - Salvage whole plants from Estate into large pots for future seed/cuttings
Delay in acquiring a translocation site or sites	- Timeframe for population establishment no longer achievable, condition not met	- Early site selection, assessment of several options, back-up sites - Extension of nursery phase (i.e. plants alive in nursery for longer period)	- Maintain seedbank/nursery at ECU/elsewhere to ensure no loss of genetic materials
Translocation site becomes unsuitable or is determined to be unsuitable based on new evidence after propagation/translocation efforts have commenced or failed	- Loss of genetic material, budget and time - Project failure	- Thorough site selection process taking into account all known and predicted success factors	- Contingency site selection
Drought deaths greater than predicted	- Death/Impact of population at translocation site; shortfall in plant targets - Increased propagation/translocation effort required in subsequent years	- Set up reticulation, - Check long-term weather forecast - Obtain source of supplementary watering	- Increase watering, improve infrastructure - Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials
Storm damage (or other inclement weather)	- Death/Impact of population at translocation site - Increased propagation/translocation effort in subsequent years	- Plant guards installed - Check weather forecast	- Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials - Infill planting of lost individuals

Risk	Outcome	Mitigation	Corrective actions
Wildfire at translocation site	- Death/Impact of population at translocation site (young plants being potentially more susceptible to fire) - Increased propagation/translocation effort in subsequent years	- Consider fire breaks &/or prescribed burning before 1st planting - Indicative weather forecast and fire weather warnings - Liaise with local fire teams re: access and fire plan	- Infill planting of lost individuals - Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials
Herbivory at translocation site	- Death/Impact of population at translocation site - Increased propagation/translocation effort in subsequent years	- Establish appropriate fencing - Pre-planting rabbit control and monitoring - Plant guards installed	- Improve fencing and other exclusion actions
Damage from unauthorised site access, vandalization	- Death/Impact of population at translocation site - Increased propagation/ translocation effort in subsequent years	- Secure and low-visibility site, signage (if required), establish fencing - Frequent monitoring for unauthorised access impacts - Consider CCTV or similar remote surveillance	- Implement additional site security measures - Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials - Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials
Poor root strike on cuttings	- Nursery strike rate lower than predicted; more plants need propagating	- Use best practice techniques and skilled staff, modified as per research findings. - Trials to explore better techniques	- Seek alternate methods - Propagate more cuttings to compensate
Ineffective or low nursery germination	- Nursery germination rates lower than predicted; more plants need propagating	- Use best practise techniques and modified as research findings. - Trials	- Improvement of Germination methodology - More targeted experiments
Ineffective nursery pollination	- Nursery flowering/ Seeding Rate lower than predicted	- Use best practise techniques as per research?...	- Improvement of nursery pollination methodology or nursery conditions

Risk	Outcome	Mitigation	Corrective actions
		- Trials	-
Unsuitable conditions for nursery survivorship	- Nursery survivorship lower than predicted	- Use best practise nursery procedures and structures Nursery hygiene at industry standard	- Increase germination attempts/efforts to counteract lower seed production % - Improve nursery hygiene and procedures
Best practice translocations methods unknown for species	- Translocation survivorship lower than predicted - More plants need propagation and planting	- Survivorship trials prior to mass planting - Appropriate site selection and treatments - Monitoring program designed to detect declining plant health early	- Procure additional seed/Propagates for translocation efforts - Continual population germination efforts in nursery as contingency for translocation failure - Maintain seedbank/nursery at ECU/elsewhere with contingency genetic materials - Improve site treatments and management, e.g. weed control, fertiliser etc
Lack of pollinators at translocation site	- Translocation population viability rate lower than predicted	- Site selection to consider all possible habitat requirements and connectivity for pollinators of both species, - implement sufficient protections (fencing) and management infrastructure (water) prior to planting	- Hand Pollinate - Attraction of pollinators via bee 'hotels' and other methods - Addition of mature potted plants to attract pollinators
Lack of germination and recruitment at translocation sites	- Translocation natural recruitment (germination in situ) rate lower than predicted - Population viability below predicted	- Use best practise techniques as per research - Field trials of fire responses - Ecological research	- Seek alternate methods - Consider prescribed burning - Site treatments to enhance chances of seedling recruitment

Risk	Outcome	Mitigation	Corrective actions
Translocation site soil incompatibility with species	- Translocation population Viability rate lower than predicted	- Pre-determine soil suitability prior to site selection (as part of site selection process)	- Soil type modification, application - Fertilisation - Alternative site ready as contingency

7.2 Species Background

Fragmentation of natural habitats is a widespread phenomenon that leads to a change in the sizes of remnant plant populations and their spatial arrangement (i.e. increased isolation) (Young et al., 1996; Aguilar et al., 2008). The resulting small and isolated plant populations often present reduced fitness compared to larger populations, and this could lead to reduced reproductive output and increased seedling mortality (Holmes et al., 2008). Several ecological and genetic aspects may be involved. These include altered pollinator presence and behaviour, pollen limitation, especially in self-incompatible species, and expression of inbreeding depression because of reduced gene flow between unrelated individuals. The southwest region of Western Australia represents a global biodiversity hotspot due to its exceptional floristic diversity, especially among medium-sized shrub of the Proteaceae and Myrtaceae (Hopper and Gioia 2004; Mittermeier et al. 2004). The ecological integrity of this region has been compromised by land clearing and fragmentation, over-exploitation, and introduction of alien species. Southwest Australia represents a priority area to preserve our natural heritage and its evolutionary potential.

7.2.1 *Conospermum undulatum*

Conospermum (Proteaceae) is a genus endemic to Australia with its centre of distribution being the southwest corner of Western Australia. In particular, *Conospermum undulatum*, commonly known as wavy-leaved smokebush, was first collected by James Drummond in 1839 and described by John Lindley the same year. The species is currently known from approximately 17 mostly small populations between Maddington and Maida Vale, in the Swan Coastal Plain biorion. Prior to European settlement it is likely to have had a fairly continuous distribution within its current range, with most populations able to exchange fertile propagules. It is however located in an area of Perth that has been, and it is, rapidly being developed for housing and industry. Drastic land use change in the area started in the 1950s and urbanisation has more than doubled since the 1970s, resulting in the fragmentation of much of the remnant bushland in which *C. undulatum* occurs. The species was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 (now Biodiversity Conservation Act 2016) in 1997 and is currently listed in the threatened flora of Western Australia (W.A Government Gazette, 2018). It has been assessed as "Vulnerable" using IUCN red list criteria under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

7.2.1.1 Description

Conospermum undulatum is a lignotuberous plant that grows as an erect, compact shrub up to 1.5 m tall with distinctive fibrous, longitudinally fissured stems. The glabrous leaves are to 12 cm long and 3.8 cm wide with a characteristic undulating margin. This species relies on massive population-level floral display for attracting pollinators (i.e. mass-flowering species; Heinrich & Raven, 1972). The flowering period usually ranges from late August to late October and during this reproductive season its white inflorescences dominate the (non-fragmented) landscape. *Conospermum* flowers have a sessile ovary with a single pendulous ovule (Douglas 1997) and, after pollination has occurred, they develop cone-shaped fruits, covered with tan-orange hairs, containing only one seed (i.e. achenes). While there is no conclusive evidence in the field, where a remarkable low number of seedlings and juvenile plants was observed, *C. undulatum* germination response in the lab is known to be slow and variable and can take several months.

7.2.1.2 Distribution and habitat

Conospermum undulatum is a geographically restricted species known from 25 historical populations between the Swan and Canning Rivers. However, only 17 populations currently contain extant plants. Of the known populations of *C. undulatum*, 47% are in areas currently identified as the endangered TEC (Threatened Ecological Community) *Banksia attenuata* woodlands over species rich dense shrublands, also known as Swan Coastal Plain community type 20a (SCP20a; *sensu* Gibson et al. 1994). The relationship between *C. undulatum* and the endangered community appears modest but not unimportant, especially considering that *Banksia* woodlands may provide important habitat for the poorly known native pollinators of *C. undulatum*.

Thirty-five populations of this species are listed on DBCA Threatened Flora databases within a restricted area between Maddington and Maida Vale on the eastern side of the Swan Coastal Plain bioregion. Nicola Delnevo, as part of his PhD, visited 32 of these populations in 2018-19 (all those listed at the time) and found 11 populations either consisted of less than four individual plants, or no plants could be found at all. Excluding these populations and a few (4) small populations within or contiguous with large populations, he reported 17 functional populations, varying in size from a few individuals to several hundred (Delnevo 2020). Since this study, some three new small populations have been reported by DBCA staff. Prior to European settlement, the species is likely to have had a fairly continuous distribution within its current range, with most populations able to exchange fertile propagules. Therefore, according to IUCN criteria, the species was likely to comprise a single population before urbanisation.

The Swan Coastal Plain comprises four broad dune successions running roughly parallel to the existing coastline and their geological ages increase moving away from the coast. The distribution range of *C. undulatum* is found on the eastern part of the plain, towards the Darling Scarp, on the oldest succession, namely the older Bassendean dune system. The dunes are made up of bleached white-grey sands and, because of their age, they present a low carbonate status and are now mostly pure silica sand (McArthur & Bettenay, 1974). *Conospermum undulatum* appears to grow on rapidly drained soils at the interface between the plain and the scarp. The entire distribution range of the species is characterised by sandy soils, including deep sands of the Bassendean system, as well as areas of the Pinjarra and Forrestfield (foothills of Darling Scarp with raised very old dunes from when sea levels were at the base of scarp) systems where sand deposits occur, sometimes over heavier soils, such as clays.

7.2.1.3 Biology and Ecology

Conospermum undulatum is a long-lived resprouter species, which means that individual shrubs can resprout from semi-subterranean woody lignotubers after disturbance such as fire or mechanical damage. Although younger plants are single stemmed, they start to form multiple stems when regenerating after disturbance or responding to stress (e.g., drought); thus, the number of stems could be a useful proxy for plant age. As a resprouting species (readily producing new shoots from underground lignotubers after disturbance or stress), it is assumed to be tolerant of a wide range of fire regimes. However, observations and measurements conducted during a pilot study at two sites experiencing prescribed burns by the City of Kalamunda (for hazard reduction purposes) showed many individual *C. undulatum* plants not resprouting after a relatively hot fire in long-unburnt vegetation where large amounts of fuel (mostly leaf litter) had accumulated around the stems of plants (van Etten & Delnevo, unpubl.). It is quite possible that these plants were killed by such hot fires, but this needs further study to confirm.

Pollination is a critical element of *C. undulatum* reproduction. This species relies on native insect pollinators for fertilisation and the production of viable seeds. The native bee *Leioproctus conospermi* is the most important pollinator, followed by native ants and argid sawflies, while dipterans appeared to be poorly effective pollinators (Delnevo et al. 2020a). Despite being generally excluded from pollinator assemblages, ants were found to contribute significantly to the seed set of *C. undulatum* (Delnevo et al. 2020b). Honeybees were frequently recorded visiting flowers of *C. undulatum*, but they are unable to effectively pollinate the small flower of this species. Honeybees robbing behaviour and the consequent depletion of floral resources for more effective pollinators is likely to limit the reproductive output of *C. undulatum*. The composition of the pollinator assemblage and the flower visitation rate were negatively impacted by habitat fragmentation and there is evidence of pollen quantity and quality limitation in fragmented populations (Delnevo et al. 2020a).

The species has a poor seed dispersal, mainly limited to the first 20 m from the mother plant. Limited seed dispersal was hypothesised initially from the frequent clumping of plants in undisturbed populations (Close et al. 2006), and then precisely quantified through genetic analyses (Delnevo et al. 2021). The overall seed viability ratio for *C. undulatum* indicates significant level of aborted infertile seeds with the ratio of non-viable to viable seeds being 4.71. Such low levels of fertile seeds are consistent with the resprouting ability of the species (Lamont et al. 2011). However, there is a significant drop in seed viability in small and isolated populations compare to large and connected ones, highlighting the negative effects of habitat fragmentation on *C. undulatum* (Delnevo et al. 2019).

The germination rate for seeds treated with gibberellic acid and smoke water was 17.33%. The response to gibberellic acid indicates that *C. undulatum* seed has physiological-imposed dormancy (Close et al. 2006). Seed germination was significantly lower in small and isolated populations, again stressing the significant impacts of land use change on the reproduction of the species (Delnevo et al. 2019).

Recent genetic investigations of *C. undulatum* showed fixation index close to 0, moderate levels of genetic differentiation, and weak genetic structuring across the entire distribution range of *C. undulatum* (Delnevo et al. 2021). This is compatible with an outcrossing system and the apparent lack of response to the fragmentation effects on the genetic layout of the species can be attributed to the combined effect of pervasive gene flow through the pre-fragmented landscape and the relatively long lifespan of the resprouter *C. undulatum*.

A high degree of variability in the leaf morphology of *C. undulatum* is present in the populations located at the northern end of the species distribution range and at the Perth Airport Estate (the Estate) site (on the eastern edge of its distribution), suggesting the presence of putative hybrid plants. The possibility of hybrids between *C. undulatum* and *C. triplinervium* plants from a nearby population was excluded in a previous study (Close et al. 2006) and recent molecular investigations found only eight plants assigned as likely *C. undulatum* x *C. triplinervium* hybrids, likely representing pre-fragmentation admixture events (Delnevo et al. 2021). On the other hand, the latest genetic investigation showed that possible historical hybridisation occurred between *C. undulatum* and *C. canaliculatum* in the Estate population, where 28.4% of the plants were assigned with high probability to the F1 *undulatuḿ canaliculatum* group. As of today, there are no large populations of *C. canaliculatum* present in the area, but one plant resulted assigned as pure *C. canaliculatum* within the Estate population, suggesting a possible high historical proximity of the two species in the area. This was also supported by the presence of backcrosses between hybrids and pure *C. canaliculatum* (Delnevo et al. 2021).

The presence of hybrids between *C. undulatum* and closely related species has been preliminary investigated by Delnevo et al (2021) but remains an important knowledge gap for the conservation of the species. This preliminary investigation was performed at the Estate using 14 microsatellite markers and suggests possible historical hybridisation between *C. undulatum* and *C. canaliculatum*. However, due to the close phylogenetic proximity of the species involved in the possible hybridisation, microsatellite markers have little discriminatory power. Therefore, more powerful genome-wide genetic markers are needed to clearly define the presence of hybrids within this threatened population.

7.2.1.4 Threats

Conospermum undulatum was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 (now *Biodiversity Conservation Act 2016*) in 1997. The main threats are continuing land clearing, degraded natural habitat, road and firebreak maintenance, lack of fire, weeds, recreational activities and rabbit grazing. The species is listed as Vulnerable (VU) under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

- **Land clearing (direct).** *Conospermum undulatum* distribution range can be characterised as an intermediate-age stable landscape (*sensu* Mucina & Wardell-Johnson, 2011; Gosper et al., 2020). It is therefore likely that *C. undulatum* was a naturally rare species with a naturally narrow distribution range before European settlement. Nonetheless, land clearing and fragmentation of the habitat are now exerting unprecedented pressure on this system. The area where *C. undulatum* occurs is undergoing rapid urbanization with a quarter of all known plants being located on subdivided blocks and many other populations affected by clearing for urban development. Several fragments have gone extinct in recent years due to land clearance.
- **Land clearing (indirect)** and degradation of natural habitat. Anthropogenic fragmentation of natural habitat has been found to have negative effects on the essential interactions between C. undulatum and its pollinators. Such effects are not limited only to reduced pollination events and, therefore, reduced pollen quantity, but extended to negative consequences both in the short/medium term (i.e. pollen quality and reproduction) and in the long term (i.e. reduced pollen flow leading to genetic erosion and reduced fitness due to expression of inbreeding depression). Most plants of C. undulatum are located in degraded natural vegetation remnants and/or are surrounded by degraded habitat. Such degradation has a negative impact on the presence of the most important groups of pollinators. Built-up residential areas have been shown to be effective barriers to pollen flow between populations.
- **Road and firebreak maintenance**. Numerous Conospermum undulatum plants are located on road reserves and some others are located on or border firebreaks. These plants are threatened by road and firebreak maintenance and spraying of verge vegetation with herbicide.

- **Lack of fire.** The vegetation remnants in which some populations of Conospermum undulatum occur have not been burnt for a long period of time. Existing plants are senescing, causing a decline in reproductive output.
- **Weeds are a threat to most populations of Conospermum undulatum**. Weed species compete for resources and weed competition reduces seedling survival.
- **Recreational activities. Horse riding, motor biking and four-wheel driving threaten several populations**. These activities directly damage Conospermum undulatum plants and also cause soil disturbance which encourages weed invasion.
- **Rabbit grazing.** Conospermum undulatum is susceptible to grazing by rabbits and, while mature plants have some capacity to resprout, seedlings are particularly vulnerable.

7.2.1.5 Habitat critical to the survival of the species, and important populations

Habitat critical to the survival of *Conospermum undulatum* includes the area of occupancy of remnant populations; areas of similar habitat surrounding important populations (i.e. sand and rapidly drained sandy clay soils, on flat or gently sloping sites), as these areas provide potential habitat for natural range extension and/or for providing habitat for native pollinators or biota essential to the continued existence of the species; and additional occurrences of similar habitat that may contain important populations of the species or be suitable sites for future translocations or other recovery actions intended to create important populations; and the local catchment for the surface and/or groundwater that maintains the habitat of the species.

A significant aspect that emerged from recent work on *C. undulatum* is the importance of maintaining high population floral displays to avoid creating small patches not attractive to pollinators due to a lack of resources. Moreover, small remnant populations are more likely to have a restricted availability of genetically unrelated mates, leading to a further reduction of the reproductive output. Maintaining a consistent inter-population pollen flow, therefore, appeared crucial for small populations where natural seed set is too low to ensure long-term population persistence.

All known plants of *Conospermum undulatum* are found in fragmented remnant bushland in an area that is around 55 km^2. The populations referred to in this summary are a reflection of this fragmentation, and often reflect changes in ownership or tenure of land across otherwise contiguous groups of plants, and are not analogous to a 'population of a species' as defined under the EPBC Act. The continuity of *C. undulatum* across its pre-fragmentation distribution range has been confirmed through genetic analysis showing high level of gene flow in the pre-fragmented landscape and low genetic structure among current fragments (Delnevo et al. 2021).

Early signals of the negative impact of habitat fragmentation on *C. undulatum* populations emerged from recent paternity investigations, and in particular, from the lack of pollen immigration detected in most of the populations. Results from this analysis suggested that although gametes of *C. undulatum* can flow unimpeded through large expanses of unfragmented bushland, inter-population gene flow is non-existent between urban fragments.

No clear source or sink populations to be prioritised for conservation based on their contribution to within- and between-populations allelic diversity were identified in recent investigations. Therefore, given the low number of *C. undulatum* individual all populations are important for the conservation of the species. Since demographic stochasticity is likely to play an important role in the survival or the extinction of extremely fragmented populations, the short-term goal of conservation planning should focus on avoiding further decline in the size of medium and large populations of *C. undulatum* and to increase the number of individuals in small populations.

7.2.2 Macarthuria keigheryi

Little is known about the biology and ecology of *M. keigheryi* (Molluginaceae). The species was first described 1989, with the type specimen collected from the Cataby area. The species was known from six populations, but a survey conducted in 2008 found only four of the initial six populations containing extant plants. As *Conospermum undulatum*, it occurs in an area that has been, and it is rapidly being developed for housing and industry.

The species was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 in 1997 due to clearing of its winter wet swamp habitat for urban development and agricultural use. It is currently listed in the threatened flora of Western Australia (W.A Government Gazette, 2018) and has been ranked as "Endangered" using IUCN red list criteria under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

7.2.2.1 Description

Macarthuria keigheryi is a small erect subshrub up to 40 cm tall with hairy, bright yellow to green stems. Two other species of *Macarthuria* (*M. australis* and *M. apetala*) co-occur with *M. keigheryi* but neither have hairy stems (Lepschi, 1996). The leaves are present mainly at the base of the stems and on young growth. They are narrowly obovate to elliptic in shape, 2.7 - 11.5 mm long and 0.7 - 3.5 mm broad. Flowering occurs from September to December and from February to March. The five petals are narrow, falling early. There are eight stamens joined at the base. The style is small, divided into three. *Macarthuria keigheryi* is distinguished from other members of the genus by a dense covering of golden hairs on the stems and leaves and by the large number of flowers (up to 25) in the dense headed inflorescence. Seeds are dark brown to almost black faintly reticulate-areolate so as to appear almost smooth, shining, broadly comma-shaped, 1.3-1.4 mm long, and it presents an elaiosome (a white fatty appendage that is attractive to ants for its nutritional value and enables ants to easily carry the seed).

7.2.2.2 Distribution and habitat

Five of the six known populations occur within a 5 km radius at Welshpool and Kewdale, south-east of Perth. The other population is isolated from the other five and occurs west of Dandaragan. This population was recorded as having extant plants in 1991 and then rediscovered in 2006 with over 10,000 plants.

Macarthuria keigheryi prefers low-lying, winter wet, damp grey/white sands, and at Welshpool and Kewdale it grows in open patches with low tree canopy cover among heathland and jarrah-Banksia woodland. It grows with *Kingia australis*, *Banksia attenuata*, *B. menziesii*, *Eremophila pauciflora*, *Nuytsia floribunda*, *Melaleuca seriata*, *Patersonia occidentalis* and *Alexgeorgea nitens*. At the Dandaragan population, it grows in *Banksia* woodland with *Banksia menziesii*, *B. attenuata*, *Eucalyptus todtiana*, *Nuytsia floribunda* and other wetland species (Brown et al 1998; Keighery 2001; Atkins 2004).

7.2.2.3 Biology and ecology

Macarthuria keigheryi senesces in summer, resprouting from the rootstock and stem bases in autumn, following rainfall. An increase of plant numbers was observed following firebreak maintenance (Population 1) and Departmental file records show that the largest *M. keigheryi* populations were recorded following fire, suggesting that the species may be fire/disturbance responsive.

Field counts have recorded dramatic changes in the size of some *Macarthuria keigheryi* populations over time, with highest numbers of plants being recorded following fire or soil disturbance (such as grading of firebreaks), which suggest the species is responsive to disturbance (DEC 2009a). This could be due to either a substantial seed germination response to fire/disturbance and/or mass resprouting from underground rootstock following disturbance. The proposed study of clonality (see Section 3.4.2.4) will shed light on the on the degree to which emerging stems in the same patch or sub-population are from same plant. Seeds are apparently not responsive to heat shock (at least in terms of heat treatment imposed by Close et al. 2005), and the proposed seed research should clarify the role of smoke water as a stimulant for germination. Plants of this species tend to die back to rootstock during summer and then re-emerge from soil when rains arrive in autumn-winter (Close et al. 2005), but the degree to which this occurs is worthy of further investigation. It is possible that the large variation in observed plant numbers in some populations reflects the seasonal variation in presence and number of above-ground stems, or may be due to uncertainty in differentiating between individual plants where multiple stems emerge from the soil. The complementary proposed research into clonality should again result in greater certainty in monitoring population sizes and dynamics.

Pollination of the sweet-smelling flowers seems to be performed by small insects including native bees, flies and wasps. Fruiting occurs between December and February. Research conducted by Botanical Gardens and Parks Authority in 2005 revealed that stems of older *Macarthuria keigheryi* plants grow horizontally, become buried in the surface of the soil and give rise to new shoots from buds on the upper surface of the stem. This suggest the possible presence of clones within populations and must be taken into account when sampling material for translocations or reintroductions.

Although seed germination was low in the lab (5% using gibberellic acid; Close et al. 2006), seed appears to persist for long periods in the soil seedbank and germinates in response to disturbance. Seed falls from the mother plant and are readily gathered and dispersed by ants.

7.2.2.4 Threats

The species was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 in 1997 due to clearing of its winter wet swamp habitat for urban development and agricultural use. The main current threats are land clearing, inappropriate fire regimes, road and firebreak maintenance and construction, weeds, grazing, and other impacts of development.

- **Land clearing.** *Macarthuria keigheryi* has a distribution range that spans over 160 km. However, all but one population are located within the Swan Coastal Plain and occur on a number of different of land tenures with two populations on land managed by the Department for Planning and Infrastructure (DPI). Long term plans for a road reserve are a threat to both of these populations. The clearing of native bushland is continuing in the Perth metropolitan area, especially in commercial areas around Kewdale and Welshpool where most populations occur. This threatens the long-term survival of the species and reduces the habitat suitable for translocation. Details on the exact composition of the pollinator guilt of *M. keigheryi* are missing, but native bees, flies, and wasps were observed visiting its flowers. Therefore, clearing of supporting habitat can negatively impact the presence of native pollinators.
- **Mining.** The largest population of *M. keigheryi*, located in Dandaragan ~100 km from the Perth populations, is likely to be impacted by mining exploration and development, with two mining companies having exploration leases over the area in which the population occurs.
- **Inappropriate fire regime.** As seed germinates in response to fire disturbance (Close et al. 2006), the soil seed bank would rapidly be depleted if fire is too frequent and recur before seedlings can reach maturity or before adult plant can complete their reproductive cycle after regenerating from rootstock. Fire is also known to facilitate weed invasion. Weeds are currently restricted to the edges of *M. keigheryi* habitat but can quickly invade the post-fire habitat, causing additional problems to seedling establishment (see below).
- **Road and firebreak maintenance** threatens all road reserve populations and populations on Commonwealth lands. Threats include grading, chemical spraying, and the slashing/mowing of roadside vegetation. These actions also encourage weed invasion.
- **Weeds.** Adult plants but especially seedlings have to compete with weeds for nutrient, space, and light resources. Weeds can dramatically increase in density and biomass following fire or other disturbances. The increase in weed biomass can start a positive feedback cycle where the initial increase of weeds increases

the fire hazard due to the easy ignition of high fuel loads that are produced annually by many weed species, increasing the frequency of controlled burns, which facilitates the growth of more weeds and the loss of native vegetation. Weeds can also exacerbate grazing pressure.

- **Grazing.** Rabbits and possibly kangaroos are thought to be major problems in at least three of the five Perth populations. In addition to direct browsing, the grazing of associated species can have other detrimental effects in areas inhabited by *M. keigheryi*, such as soil disturbance and addition of nutrients.

7.2.2.5 Habitat critical to the survival of the species, and important populations

The habitat comprising all existing known populations, as well as areas of surrounding areas of natural vegetation that provide potential habitat for natural range expansion and suitable habitat for pollinators is considered critical for the survival of the species. The local catchment area and ground water systems that maintain the damp-land habitat of the species is to be preserved.

All areas of similar habitat (i.e. low-lying winter wet damp sands with *Kingia australis*, *Banksia attenuata*, *B. menziesii*, heathland or *Banksia* woodland) that may contain the species or is suitable for future translocation is habitat critical to survival.

Given that *M. keigheryi* is listed as 'Endangered' under the EPBC Act, and is only known from six populations, it is considered that all wild and future translocated populations are important populations.

7.2.2.6 Benefits to other species or ecological communities

Many plants of *M. keigheryi* occur in bushlands where *C. undulatum* also occurs and actions implemented to improve the security of habitat of one species may also benefit the other. Many bushland areas that contain the smokebush have occurrences of four Threatened Ecological Communities (TECs). In most cases it is TEC 'SCP 20a', however occurrences of 'SCP 3c', 'SCP 20b' and 'SCP 8' have also been recorded. Moreover, *Banksia* woodlands (SCP 20) provide vital habitat for over 20 nationally threatened species such as Carnaby's and forest red-tailed black cockatoos, the western quoll and western ringtail possum; and are particularly important considering that *Banksia* woodlands may provide important habitat for the poorly known native pollinators of *C. undulatum* and likely *M. keigheryi*.

7.3 MDP Approval Conditions

The table below presents the MDP approval conditions relevant to the two species, as well as the section of this Plan where these conditions are addressed (where relevant). It should be noted that the MDP Approval conditions relating to population establishment quantum are considered superseded, due to the conditioned pre-clearance survey for both species, and the reduced construction footprint, both resulting in changes to the offset requirement that has been approved within the NRP Offset Strategy.

Aspect	MDP Ministerial (MS2-000014) Conditions	Plan Section
Clearing (All)	1. - To manage and minimise the impacts of the development upon protected matters (…) the proponent must not, and must ensure that all persons involved in the development do not: 1.d. - clear more Wavy-leaved Smokebush individuals than the greater of 206, or the number of individuals demonstrated by the pre-clearance surveys required under Condition 6 to be growing in the project area; 1.e. - clear more Keighery's Macarthuria individuals than the greater of 855, or the number of individuals demonstrated by the pre-clearance surveys required under Condition 6 to be growing in the project area;	N/A
Wavy-leaved Smokebush and Keighery's Macarthuria	2. - To offset the loss of protected matters as specified in Condition 1, the proponent must submit an Offset Strategy consistent with the Environmental Offsets Policy for approval by the Minister for the Environment.	NRP Offset Strategy
	5. - In addition to the Offset Management Plan or Plans, to mitigate the loss of genetic diversity of Wavy-leaved Smokebush and Keighery's Macarthuria on the Swan Coastal Plain and to reduce residual significant impacts resulting from the development, the proponent must submit a Propagation, Research and Monitoring Plan in sufficient time to obtain approval by the Minister for the Environment within eighteen months of the date of approval of the Offset Strategy. The proponent must implement the approved Propagation, Research and Monitoring Plan. The Propagation, Research and Monitoring Plan must include (but need not be limited to): 5.a. - a commitment to propagate, establish, secure and maintain new viable population(s) of at least 250 individuals of Wavy-leaved Smokebush and at least 1160 individuals of Keighery's Macarthuria for a specified period. <u>These numbers will be amended based on actual impacts determined following detailed design and the most recent surveys;</u> 5.b. - a forecast of propagation progress within five years of undertaking the first activity (including clearing) which is likely to result in an impact to Wavy-leaved Smokebush or Keighery's Macarthuria;	This Plan, Section 2
	5.c. - details of the proposed methodology, sequencing and timing for propagation of individuals, rootstock and/or seedbank and establishment of the new population(s);	Section 2

Aspect	MDP Ministerial (MS2-000014) Conditions	Plan Section
	5.d. - details of how the plan will increase scientific knowledge of the genetics and ecology of Wavy-leaved Smokebush and Keighery's Macarthuria; 5.e. - evidence and criteria that will be used to demonstrate successful propagation of individuals such that viable population(s) of Wavy-leaved Smokebush and Keighery's Macarthuria are established; and 5.f. - details of contingency measures that will be implemented in the event that new population(s) have not been successfully established after the five-year period.	Section 3.1.1.5 Sections 3 & 4 Section 3.4 Section 3.3 Section 3.3.5 Section 5

7.4 Glossary of key terms

Adaptive management – improved management of natural assets via a process of learning by monitoring and targeted research

Annotated genome – the complete list of DNA sequences that encode for expressed genes of a species.

Cross pollination – pollination of a flower or plant with pollen from another plant.

Fitness – the reproductive output of an individual plant (e.g., absolute number of seeds produced).

Genetic diversity – the amount of genetic variability found within a specified population of individuals. Most commonly measured as the average heterozygosity in a population (see below).

Heterozygosity - the presence of two different alleles (versions of a gene) at a position in the genome. Can me averaged across many positions in the genome to asses overall heterozygosity of individuals. Can be measured at population level, whereby high heterozygosity in a population means lots of genetic variability, and low heterozygosity in a population means little genetic variability.

Propagation – refers in this plan to propagation of plants, the purposeful multiplication of plants for a desired end use. Specifically utilises plant propagation techniques, such as cuttings, seed germination and tissue culture.

Propagule – the basic plant organ, tissue or structure used to propagate plants; typically seed, spores, or stem tissue.

Reference genome – the complete DNA sequence of a representative individual from a species. This will include coding DNA (see annotated genome) and non-coding DNA sequences.

Seed dormancy – the imposed barriers preventing seed germination under favourable conditions; these barriers typically need to be overcome via one or several dormancy-breaking treatments.

Seed orchard – a collection of plants established in either the field or nursery for the main purpose of supplying seed for propagation activities. Flowering and seed set can be encouraged through applying appropriate growing conditions, fertilisers, extra water etc.

Seed viability – the % of a seed stock or collection with healthy, intact embryos, typically viewed following a cut test or via seed x-ray machine.

Seedling viability – the % of seedlings that survive to adult phase.

Translocation - the purposeful and planned transfer of a species (or regenerative material) from an *ex situ* collection or natural population to a new location, usually in the wild.

Tubestock – young plants raised in small pots either from seed or cuttings

Viable population – a population which is self-sustaining in terms of reproduction, recruitment and survival over the long-term time scales, and one containing sufficient genetic diversity to represent the genetic composition of the broader species distribution.