NATIONAL RECOVERY PLAN FOR THE SUNSHINE DIURIS Diuris fragrantissima

Anna H. Murphy, Alan Webster, Colin Knight and Karen Lester





Department of Sustainability and Environment Prepared by Anna H. Murphy, Alan Webster, Karen Lester (Department of Sustainability and Environment, Victoria) and Colin Knight (Melbourne Zoo).

Published by the Victorian Government Department of Sustainability and Environment (DSE) Melbourne, 2008.

© State of Victoria Department of Sustainability and Environment 2008

This publication is copyright. No part may be reproduced by any process except in accordance with the provisions of the *Copyright Act* 1968.

Authorised by the Victorian Government, 8 Nicholson Street, East Melbourne.

ISBN 1 74152 482 2

This is a Recovery Plan prepared under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999, with the assistance of funding provided by the Australian Government.

This Recovery Plan has been developed with the involvement and cooperation of a range of stakeholders, but individual stakeholders have not necessarily committed to undertaking specific actions. The attainment of objectives and the provision of funds may be subject to budgetary and other constraints affecting the parties involved. Proposed actions may be subject to modification over the life of the plan due to changes in knowledge.

Disclaimer

This publication may be of assistance to you but the State of Victoria and its employees do not guarantee that the publication is without flaw of any kind or is wholly appropriate for your particular purposes and therefore disclaims all liability for any error, loss or other consequence that may arise from you relying on any information in this publication.

An electronic version of this document is available on the Department of the Environment, Water, Heritage and the Arts website www.environment.gov.au

For more information contact the DSE Customer Service Centre 136 186

Citation: Murphy, A.H., Webster, A., Knight, C and Lester, K. 2008 National Recovery Plan for the Sunshine Diuris *Diuris fragrantissima*. Department of Sustainability and Environment, Melbourne.

Cover photograph: Sunshine Diuris *Diuris fragrantissima* by Gary Backhouse.

Contents

Summary3	
Species Information	
Description	3
Taxonomy	3
Distribution	4
Population Information	4
Habitat	4
Decline and Threats5	
Recovery Information6	
Existing conservation measures	6
Strategy for recovery	8
Recovery Objectives	8
Program Implementation	8
Program evaluation	8
Recovery Objectives	9
Management practices	10
Affected interests	11
Role and interests of indigenous people	11
Benefits to other species	11
Social and economic impacts	11
Acknowledgments12	
Bibliography	
Duration of Recovery Plan and Estimated Costs14	

Figures

|--|

Summary

The Sunshine Diuris *Diuris fragrantissima* D.L. Jones & M.A. Clem. is one of Australia's most threatened orchids. Endemic to Victoria, the species was once abundant on the grassy plains to the west of Melbourne. It has suffered a catastrophic decline in range and abundance since European settlement, and is now confined to a single location at Sunshine in the western suburbs of Melbourne, where about 30 plants remain, and a second site at Altona, where reintroductions are occurring. Approximately 200 mature plants survive in cultivation. Major threats include weed invasion, predation, illegal collection, altered fire regimes and changes to land use in rail reserves. The Sunshine Diuris is listed as <u>Endangered</u> under the Australian Government *Environment Protection and Biodiversity Conservation Act* 1999 (EPBC Act), and <u>Threatened</u> under the Victorian Government *Flora and Fauna Guarantee Act* 1988. Fewer than 30 plants survive in the wild, at a single site that is suffering severe threats, and the species is very close to extinction in the wild. An intensive recovery program is required to prevent the extinction of the Sunshine Diuris. The first Recovery Plan for the species (Backhouse *et al.* 2000) was adopted in 2000. This Recovery Plan is a revision and update of the earlier plan, and details the species' distribution and biology, conservation status, threats, and recovery objectives and actions necessary to ensure its long-term survival.

Species Information

Description

The Sunshine Diuris is a terrestrial herb, emerging annually from a lobed, subterranean tuber. It has 2–3 slender, channelled, grass-like green leaves up to 30 cm long. A slender green stem to 35 cm tall bears 1– 10 scented flowers coloured white with variable purplish hues and streaks, while the lateral sepals are green. The dorsal sepal is triangular and erect, while the lateral sepals are long and slender (to about 60 mm), inrolled, drooping and parallel. The ovate petals project obliquely upwards. The labellum is strongly trilobed, with the lateral lobes curving upward while the fan-shaped mid-lobe projects forward. Flowers open sequentially up the raceme, the lowest flowers often having shrivelled before the top flower buds have opened (description from Backhouse & Jeanes 1995; Jones 1988). In dry years, plants tend to be small, with few flowers. In wet years, plants can be relatively large, with high flower counts, and cultivated plants can be larger than those found in the wild.

Leaves emerge in late autumn, following the onset of seasonal rains. Flowering commences in late October, through November and is completed by early December. By late December the leaf has shrivelled, and if pollination has occurred, the seed capsule is ripening. Seed set occurs about 4–6 weeks after individual flowers open, with early flowers taking longer to ripen seed than flowers opening later (A. Garnham, Deakin University, pers. obs.). The species survives the late summer and early autumn as a dormant tuber. Reproduction is almost entirely from seed, although vegetative reproduction is observed occasionally (A. Garnham pers. obs; Cropper 1993). The irregularly shaped, lobed tuber is replaced annually.

Very little is known of the biology or ecology of Sunshine Diuris. It grows in a complex relationship with a mycorrhizal fungus *Tulasnella calospora* Boudier (Warcup 1971), that initiates seed germination, and assimilates nutrients for the orchid. The degree of dependence upon the fungus, particularly of mature plants, is not known. Some individuals of Sunshine Diuris have survived for over nine years in the wild (Cropper 1993) and over 20 years in cultivation (D. Tonkinson, La Trobe University and C. Knight, Melbourne Zoo, unpubl. data). The Sunshine Diuris is believed to be pollinated by small native bees (Tonkinson 1985), the purplish colour of the flowers mimicking the colour of native lilies that often grow with *Diuris* species. The bees may also be attracted by the strong fragrance of the orchid. Very low natural rates of pollination have been reported, with a maximum of only 7% of flowers producing seed pods (Cropper 1993). Hot summer fires are likely to enhance flowering the following spring(s), and most recruitment has been observed 1–2 years after a summer fire. Fires may indirectly promote seed germination and seedling establishment by altering soil nutrient levels and by reducing competition from associated grasses. This effect may act directly upon the orchid, or indirectly through the fungal symbiont.

Taxonomy

The Sunshine Diuris was originally called the White Diuris *Diuris alba* R. Br., a species now known to be confined to the central coast of New South Wales (Bishop 1996). It was later known as the white form of the Purple Diuris *Diuris punctata* var. *albo-violacea* Rupp *ex* Dockr., until it was recognised and described as a

separate species, *D. fragrantissima* D.L. Jones & M.A. Clem. (Clements 1989). The species is most similar to the Wedge Diuris *Diuris dendrobioides* Fitzg., a threatened orchid from grasslands in southern New South Wales and northern Victoria. The Wedge Diuris was included in *D. fragrantissima* by Walsh & Entwistle (1994), although most other authors (eg. Jones 1988; Clements 1989; Gullan *et al.* 1990; Backhouse & Jeanes 1995; Bishop 1996) have retained *D. dendrobioides* as a separate species.

Distribution

The Sunshine Diuris is endemic to the basalt plains immediately to the west of Melbourne (Fig. 1), in the Victorian Volcanic Plain IBRA Bioregion (*sensu* DEH 2000). The species has undergone a catastrophic decline in range and abundance, and only a single wild population, comprising about 30 plants, remains, at Sunshine, in Melbourne's west. Cultivated plants have been introduced to a small reserve at Altona,. While general locations have been indicated, exact locations and land management details have not been provided in order to deter disturbance to the sites or illegal collection of plants.



Figure 1. Distribution of Diuris fragrantissima in Victoria

Population Information

In situ

The sole remaining natural population of Sunshine Diuris occurs at Sunshine, where about 30 plants remain. An introduced population was first established at the Altona reserve in 1982, and 89 plants were planted out over the next few years, but these were believed to have died out by 2001. In 2004 and 2005, 700 seedlings were planted out, and about 120 of these flowered in the spring of 2005.

Ex situ

About 200 plants survive in cultivation, the bulk at Melbourne Zoo and the remainder at the Royal Botanic Gardens (Melbourne) and in private collections. These plants originated from some plants taken from the wild population at Sunshine about 30 years ago, and have provided seed for *ex-situ* cultivation and research. Many hundreds of seedlings are now being produced to increase the population in cultivation, and for reintroductions to the wild. The population in cultivation is vital to the future of the Sunshine Diuris.

Habitat

The Sunshine Diuris was confined to the grassland plains immediately to the west of Melbourne, particularly between Werribee and Sydenham (Cropper 1993; Jones 1988; Parsons 1981), where it grew in native grasslands dominated by Kangaroo Grass *Themeda triandra*, on heavy basalt soils, often with embedded basalt boulders. The sole remaining natural population at Sunshine occurs in a small (0.1 ha) remnant of Western (Basalt) Plains Grassland. *Themeda triandra* dominates this site, with *Austrodanthonia* species, *Dianella longifolia, Dianella revoluta, Tricoryne elatior, Pimelea humilis* and *Dichanthium sericeum* subsp. *sericeum* also present. The soil is shallow heavy clay with a friable dark surface layer, and has scattered

exposed basalt boulders. This site, as well as the introduction site at Altona (which has similar habitat to that at Sunshine, although the soil is a deeper cracking clay) should be considered as habitat that is critical to the survival of the species.

Decline and Threats

Although with a limited natural distribution, the Sunshine Diuris was once common in the native grasslands to the west of Melbourne around the time of European settlement. Since then the species has suffered a catastrophic decline in range and abundance. As early as 1900, remaining populations appear to have been confined to railway reservations (A. Garnham pers. comm.). Concern at the plight of the Sunshine Diuris was expressed over 70 years ago. In 1934, noted orchidologist W.H. Nicholls (cited in Willis 1951) wrote that 'the species was at one time exceedingly plentiful, but is now becoming scarce'. Willis (1951) believed that 'Victoria's most beautiful orchid' was in dire peril of extinction. Its decline has been attributed to widespread habitat destruction and degradation. By 1970, only seven populations remained, and by 1980 the species was restricted to the single remaining site at Sunshine rail reserve, where about 100 plants survived. This population continued to decline through the 1980s, with 67 flowering plants recorded in 1982, 11 in 1989, and only one in 1992. No flowering plants were seen for five years, until a single flowering plant was found at the site in October 1997, and three flowering plants were recorded in October 1998. Close monitoring of the site commenced in 2000, with an attempt to estimate total population size, not just flowering plants. At least 24 wild plants (of which 12 flowered) plus three introduced plants were known to be alive in spring 2007.

Habitat destruction and degradation has undoubtedly been the prime reason for the decline to virtual extinction in the wild of this beautiful orchid, and almost all sites where the Sunshine Diuris once occurred have now been destroyed. Much of the original native grasslands to the west of Melbourne have been destroyed for agricultural, industrial and urban development, and the remaining areas are mostly small, highly fragmented and usually substantially degraded. These habitats once covered about 10% of Victoria, but have now been reduced to less than 1% of their original extent (Barlow & Ross 2001; Stuwe 1986). During the 1970s, four of the five last known stands of Sunshine Diuris, all growing along railway reserves, were destroyed by soil and ballast dumping, cultivation or herbicide use (Parsons 1981).

The Sunshine site and the translocation site at Altona are under severe threat. The sites are tiny and surrounded by industrial and residential development. It is highly likely that, with the habitat substantially reduced and so few plants remaining, ecological processes such as natural pollination have also been disrupted. Very low natural rates of pollination have been reported, with a maximum of only 7% of flowers producing seed pods (Cropper 1993), which is possibly a function of the rarity of the orchid and hence few opportunities for pollinators to find flowers and effect pollination. Current major threats include weed invasion, predation from introduced herbivores, altered fire regimes and changes to land use in rail reserves.

Weed invasion

Many weed species threaten both the Sunshine and Altona sites. Invasion by the exotic grass *Nassella neesiana* is extensive, and now poses a very serious threat in spite of ongoing efforts to control weeds. Other serious weed species include annual grasses such as *Briza maxima*, *Vulpia bromoides* and *Aira* spp., *Foeniculum vulgare*, *Plantago lanceolata*, *Romulea rosea* and *Medicago* species. While weed control is essential for continued survival of the Sunshine Diuris, this needs to be carefully implemented, as indiscriminate herbicide use has itself been a problem in past years (Tonkinson 1985).

Predation

Predation of tubers by the introduced House Mouse *Mus musculus*, was believed responsible for a mortality of perhaps 70% of plants during the mid-1980s (Cropper 1993). Damage of plants by introduced species of slugs and snails is an ongoing problem.

Altered fire regimes

Periodic summer fires are probably beneficial to the Sunshine Diuris, by reducing competition and promoting suitable conditions for seedling recruitment. However, too frequent or ill timed fire may be a threat, causing damage to plants, increasing seedling mortality and destroying immature seedpods. Long fire-free intervals within grasslands may also be a threat, by allowing the development of dense swards of Kangaroo Grass that inhibits regeneration of smaller native herbs including orchids (McDougall 1989). Ironically, fencing of some of the population at Sunshine in the 1950s, and subsequent exclusion of fire, led to the disappearance

of plants within the fenced area over the next 25 years. Plants survived outside the fenced area in habitat that was apparently subjected to periodic burning.

Human interference

Human interference at the Sunshine site is an ongoing problem, especially as it is such a tiny area within the metropolitan zone of the city. Arsonists have set fire to the reserve several times. Trampling, particularly by visiting naturalists, has been a major problem, with up to 15% of plants broken or damaged in some years (Cropper 1993). Some plants have also been illegally removed, and two stems with maturing seed capsules were suspected to have been removed in 2005. Future industrial and transport development may also pose a threat.

Recovery Information

Existing conservation measures

The plight of the Sunshine Diuris has received considerable attention for several decades now. The former Native Plants Preservation Society (NPPS) undertook some recovery actions in the 1950s, most notably fencing a small (~80m²) area at Sunshine. The School of Botany at La Trobe University (SB-LTU) has had a substantial input into the conservation of the species and was responsible for many of the early conservation management actions, and initiated the Sunshine Diuris Research Project in 1982. In recent years other organisations, including the Department of Sustainability and Environment, University of Melbourne, Victoria University, Melbourne Zoo, Society for Growing Australian Plants (Basalt Plains Group), Australasian Native Orchid Society Victorian Group (ANOS VicGroup), Royal Botanic Gardens Melbourne, Australian National Botanic Gardens and local field naturalists have participated in actions to aid the recovery of Sunshine Diuris. An Action Statement for the Sunshine Diuris (Webster & McKay 1993) was published and a Recovery Plan (Backhouse *et al.* 2000) prepared and implemented. Following is a summary of major recovery actions undertaken to date.

Sunshine site - Management

Considerable liaison took place with the Sunshine site land managers by the Native Plants Preservation Society and SB-LTU in the early 1980s. The Native Plants Preservation Society even leased the Sunshine site for some years to protect the plants there. A Vegetation Management Agreement with the site manager was approved in 1990, and the Sunshine site was listed as Category one site (highest priority for protection). In 1982 the Department of Crown Lands and Survey, in conjunction with SB-LTU, fenced and signposted the Sunshine site in an effort to reduce damage. The site was even supervised during flowering periods in the 1980s to prevent disturbance to and theft of plants. The Sunshine site has been burnt regularly since 1982 to control competition from native grasses and weeds, and to promote flowering and seedling establishment. Baits have been laid for mice, snails and slugs to control predation, and weed control undertaken by volunteers and contractors with a particular focus on the removal of *N. neesiana*. Fencing surrounding the site was upgraded in 1999 and the site has been periodically burnt (every 3–5 years) to enhance flowering and recruitment, and control competition.

Cross-pollination between wild and cultivated plants has taken place in order to maximise the development of viable seed and to maintain genetic diversity. Seed collection has also continued, and *in situ* seed sowing trials are in progress, although to date no natural germination has occurred. Trials to detect the mycorrhizal fungus *in situ* using buried seed ('seed baiting') have been undertaken since 2001, so far without success.

Research on biology and ecology

The School of Botany at La Trobe University has undertaken a considerable amount of autecological research on the species, as part of the Sunshine Diuris Research Project. Research by SB-LTU, the University of Melbourne, Royal Melbourne Institute of Technology University, Royal Botanic Gardens, Victoria University and the Melbourne Zoo has addressed a number of factors relating to the survival of the species. These have included the investigation of the plant's molecular biology, genetic variation, habitat characteristics, life history, population structure, seasonal variation, hand pollination, seed collection, propagation and translocation as well as the effects of weed invasion and of burning regimes. A mycorrihzal fungus recently isolated from wild Sunshine plants is closely related to *T. calospora* and to one isolated from cultivated plants (Z. Smith, University of Melbourne, pers. comm.).

Survey

When in flower, the Sunshine Diuris is a showy, conspicuous species and as such, is easily detected in the field. Searches for the species at historical sites were initiated by SB-LTU in 1980, but the orchid was only found at one site (the current Sunshine site). Since then, many remnant native grasslands in Melbourne's west have been searched by botanists and orchid enthusiasts, so far to no avail.

Propagation

In 1978, J.H. Warcup of the Waite Agricultural Research Institute in South Australia developed a technique to propagate plants from seed, using a symbiotic method of seed germination. The National Botanic Gardens in Canberra also successfully propagated plants from seed in 1981, using the Warcup method. Seedlings were then sent to the RBG and La Trobe University to establish an *ex situ* collection. The mycorrhizal fungus was again isolated and cultured in 2002 (T. Huynh, University of Melbourne, unpubl. data) and 2006 (Smith 2006).

Researchers at La Trobe University were also successful in developing *in vitro* germination of seedlings (using the fungal symbiont obtained from the Australian National Botanic Gardens, Canberra) in 1985. The population in cultivation at La Trobe was maintained until 1991, and was then transferred to Melbourne Zoo, with some plants subsequently distributed to the RBG and specialist growers in ANOS to spread the risk. Improved propagation techniques have been developed by horticulturists at Melbourne Zoo, the RBG and ANOS VicGroup members, and the number of plants in cultivation has increased substantially, with over 200 mature plants now held in cultivation. Plants have been successfully propagated at Melbourne Zoo by tuber division and scattering seed around potted parent plants. A commercial growing facility, Western Laboratories (South Australia), was contracted as part of Recovery Plan implementation to propagate plants using asymbiotic techniques. The lab has produced over 700 plants for reintroductions and is continuing to produce several hundred seedlings per year for future reintroductions. The RBG has recently commenced trials growing plants symbiotically, and currently has several hundred plants in flask. In 1999, seed was lodged with Kings Park and Botanic Garden (Perth) and the RBG for long-term storage, to safeguard the species from extinction.

Ex situ conservation and translocation

Translocations to conserve the Sunshine Diuris were attempted as far back as 1950, when the NPPS translocated some plants (and other orchids) from the remaining population at Sunshine into a fenced area within a wireless mast site at Sydenham (Willis 1951). Few plants were seen after 1952, and the translocation was considered to have failed, although at least one plant survived until 1967, the last year a flowering plant was seen (D. Jones, CSIRO, pers. comm.). Six cultivated plants were reintroduced to the Sunshine site in 2000; four plants emerged and flowered during 2004, while three emerged and two flowered in 2005.

In 1982, SB-LTU established an orchid reintroduction site within the Altona reserve. Twelve seedlings propagated at the Australian National Botanic Gardens and 33 tubers from the Waite Institute were initially introduced, followed by another 36 seedlings planted in July 1985. There was a high mortality of seedlings, but several plants matured and flowered for some years after the reintroduction. Natural pollination and seed set were observed, but there was apparently no seedling establishment at the site (R. Parsons, La Trobe Uni, unpubl.). The last flowering plant was seen in 2000, 18 years after the original reintroduction, but it is not known if any of the original introduced plants still survive.

With the propagation of large numbers of plants by Western Laboratories, reintroductions to the Altona site recommenced in spring 2004, when 300 plants were planted out. This was followed by two subsequent plantings of 100 then 300 plants during summer and autumn 2005. This reintroduction used a multifactorial trial design to test a number of variables in reintroduction methodology, including different planting times (spring, summer and autumn), adding additional fungi previously isolated from *D. fragrantissima* to the soil, and tilling the soil prior to planting (Smith 2006, 2007b). Follow-up management included supplementary watering when rainfall was below average.

Genetic analysis of D. *fragrantissima* by the RBG has guided the breeding program to ensure that the diversity present in the *ex situ* population is maintained (E. James, RBG unpub. data), and further genetic studies were undertaken from 2003–2005 (Smith *et. al.* 2005a, 2005b, 2007a). The Victorian Threatened Orchid Recovery Team Cultivation Committee has also produced cultivation guidelines to maximise consistency and effectiveness of cultivation. A reintroduction strategy and plan for Sunshine Diuris has also been produced (TORT 2002).

Information and awareness

A considerable amount has been written on the Sunshine Diuris, both to highlight its plight and guide recovery management. Its history and conservation status were documented by SB-LTU for inclusion in the Register of Rare and Endangered Native Plant species in Victoria (Stuwe 1980). Cropper (1993) prepared a detailed historical account of its conservation and provided management recommendations. An education kit entitled 'Native Grasslands in the Melbourne Area' was produced (DCE 1992), with funding from the Australian Heritage Commission, and circulated to school groups and interested organisations. There has been considerable liaison with land managers, government agencies, local residents, native orchid societies, naturalists and other community groups, and a media campaign highlighting the plight of the orchid. An information brochure was produced for the broader community that outlined the plight of the species, and a number of papers have been presented at conferences to the scientific community and orchid enthusiasts (eg. James *et al.* 2001; Knight 2002; Knight *et al.* 2001; Richards 2002; Thompson 2002; Smith *et. al.* 2005a, 2000b). Information has also been disseminated via the Internet outlining current conservation objectives and actions, and via talks to state-wide orchid recovery forums.

Strategy for recovery

The strategy for recovery of the Sunshine Diuris will be to focus on protection and management of the two locations where the species grows (one natural and one reintroduced population), maintenance of the population in cultivation, and increasing reintroductions to the wild.

Recovery Objectives

The **overall objective** of recovery is to minimise the probability of extinction of the Sunshine Diuris in the wild, and to increase the probability of important populations becoming self-sustaining in the long term.

Within the 5-year life span of this Recovery Plan, the **Specific Objectives** for recovery of the Sunshine Diuris are to:

- Maintain and enhance the original wild population at Sunshine.
- Maintain and enhance the introduced population at Altona.
- Maintain a population in cultivation.
- Reintroduce cultivated plants to the wild.

Program Implementation

The Recovery Plan will run for five years from the time of adoption of a Final Plan, and will be managed by the Sunshine Diuris Recovery Team, consisting of scientists, land managers, horticulturists and community members, which has been established to oversee the recovery of the species. Implementation of individual actions will remain the responsibility of the relevant agencies and organisations identified in the Recovery Plan (subject to available resources), who will be responsible for preparing work plans and monitoring progress toward recovery within their own jurisdiction. Lead agencies will maintain liaison with each other through the Recovery Team, to exchange information on the species, monitor progress towards recovery, and facilitate the end of program review.

Program evaluation

The Recovery Team will be responsible for informal evaluation of their progress. Near the termination of the Recovery Plan, an external reviewer will be appointed to undertake a formal review and evaluation of the recovery program. This Recovery Plan will be reviewed within five years of the date of its adoption.

Recovery Objectives

Objective 1. Maintain and enhance the population at Sunshine.

Performance criteria: The population at Sunshine is maintained at a minimum of 30 plants.

1.1 Monitor plants and habitat

Each plant at the Sunshine site is tagged and monitored individually, and monitoring will occur at at plant emergence, during peak flowering and seed-set. Data collected will include a count of the number of Sunshine Diuris individuals, flowering and seed-set, plant dimensions (leaf and stem measurements) and condition (including predation) and associated native and exotic species. Pollinator baiting trials will also be conducted to aid identification of the pollinator and its habitat requirements.

1.2 Undertake pest control

Integrity of the Sunshine site and survival of the plants are seriously threatened by weeds and pest animals. Ecologically sensitive weed control will continue and will be undertaken by a contractor. A number of pests and predators are present, including snails, slugs and the introduced House Mouse. Control techniques will include fencing and caging of plants as well as baiting for slugs and snails. A larger area of VicTrack land is proposed to be fenced and intensively managed.

1.3 Undertake ecological burning

Although little data is available on the impact of altered fire regimes on Sunshine Diuris, the absence of fire is likely to be a significant threatening process. Data collected by SB-LTU for Sunshine Diuris shows a significant increase in individuals at the Sunshine site after a fire in 1982. However, no significant increases were recorded after other burn events (Cropper 1993). This may be due to numerous additional threats being active at the site during this period as well as a very diminished population. Despite this, the absence of appropriate fire regimes should be considered a serious threatening process (Backhouse *et al.* 2000). Ecological burns will continue to be undertaken at Sunshine by DSE.

Objective 2. Maintain and enhance the introduced population at Altona.

Performance criteria: The introduced population at Altona is maintained at a minimum of 1000 plants.

2.1 Monitor plants and habitat

Each plant at the Altona site is tagged and monitored individually, and monitoring Altona will occur at plant emergence, during peak flowering and seed-set. Data collected will include a count of the number of Sunshine Diuris individuals, flowering and seed-set, plant dimensions (leaf and stem measurements) and condition (including predation) and associated native and exotic species. Pollinator baiting trials will also be conducted to aid identification of the pollinator and its habitat requirements.

2.2 Undertake pest control

Integrity of the Altona site and survival of translocated plants are threatened by weeds and pest animals. Ecologically sensitive weed control will continue and be undertaken by Parks Victoria. A number of predators are present, including snails, slugs and the introduced House Mouse. Techniques will include fencing and caging of plants and control of slugs and snails.

2.3 Undertake ecological burning

The planning and implementation of ecological burns following guidelines established by Parks Victoria will continue, in order to provide conditions for the orchid's flowering, pollination and seedling recruitment.

Objective 3. Maintain a population in cultivation.

Performance criteria: Establish at least 3,000 plants in cultivation, with 50% of the population growing with their associated fungal symbiont.

3.1 Produce seedlings using *in vitro* propagation techniques.

Large numbers of seedlings are now being produced by a commercial laboratory using sterile *in vitro* propagation techniques. This method is an effective way of boosting the number of plants held in cultivation

and is essential for reintroduction efforts, and for conserving and maintaining genetic diversity. *In vitro* propagation will continue over the next five years and will aim to produce 500 seedlings annually for cultivation and reintroduction.

3.2 Develop techniques for symbiotic inoculation

Currently, laboratory-produced seedlings are grown asymbiotically, without the mycorrhizal fungus. Techniques to isolate and culture mycorrhizal fungus are now well understood for *D. fragrantissima*, but further improvement of techniques to inoculate asymbiotically grown seedlings is required. Plants that are cultivated with their fungal symbiont may be more vigorous and have a higher survival rate when planted out, but this needs to be tested. Seedlings have been produced using symbiotic techniques at the RBG in 2004–2005, using a fungus isolated by Tien Huynh from cultivated plants maintained at Melbourne Zoo (Rob Cross RBG pers. comm.). These seedlings have been deflasked, and are currently growing in the RBG nursery. A second symbiotic germination of *D. fragrantissima* seed has been undertaken in 2005–2006, using two isolates from wild plants growing at Sunshine. Trials are continuing to determine seedling survival after deflasking and dormancy, and to understand how asymbiotically produced seedlings subsequently become infected by mycorrhizal fungi (Z. Smith, pers. comm.).

3.3 Increase plant numbers in cultivation

The Melbourne Zoo and private orchid growers (ANOS VicGroup) have an excellent history of maintaining the Sunshine Diuris in cultivation. Their role in the *ex situ* conservation of the species also ensures that plants in cultivation are spread across a wide range of growers. The recovery effort will continue to support and encourage both these groups to maintain and increase plant numbers in cultivation, as well as supporting the symbiotic propagation program at RBG. Melbourne Zoo will continue to produce seedlings for reintroduction and *ex situ* conservation using tuber division and scattering seed around potted parent plants. The cultivation program will produce seedlings from a large number of crosses to maximise genetic diversity in accordance with recent research (E. James, RBG, unpub. data). The RBG is producing symbiotically germinated plants, which will also increase the population in cultivation and produce more plants for reintroductions.

Objective 4. Reintroduce cultivated plants to the wild.

Performance criteria: Maintain the reintroduction trials effort at Altona and establish at least one additional reintroduction site.

4.1 Reintroduce plants to Altona reserve

The Altona reserve is currently the principal reintroduction site, and reintroductions into this site will continue, in tandem with site management, intensive after-care and monitoring. A translocation brief has been prepared (Sunshine Diuris Recovery Team 2003) that includes guidelines for pre- and post-translocation site management to minimise loss of plants. To date, 700 seedlings have been planted out at the site in 2004 and 2005, and at least another 500 plants will be planted out at the site. Plants of at least two years of age will be used for reintroductions, as larger tubers appear to enhance subsequent growth and survival (Smith 2006).

4.2 Establish an additional reintroduction site

Ultimate recovery of the Sunshine Diuris will necessitate having several secure populations containing large numbers of plants, to reduce the risk of extinction. While the reintroduction will continue at Altona, at least one more site suitable for reintroduction will be identified and prepared during the life of this recovery plan. Another grassland reserve, recently transferred to public ownership for management by Parks Victoria, as well as several grassland reserves managed by Brimbank Citv Council and Hume City Council, have been identified for further investigation. At least one additional site will be selected and prepared as a future reintroduction site.

Management practices

Implementation of this Recovery Plan will have two main streams; *in situ and ex situ* conservation. *In situ* conservation will be implemented through habitat protection at Sunshine and Altona Reserve and reintroductions. On-ground site management will aim to mitigate threatening processes to prevent declines and create conditions for maintenance or increase of population size. Major threats requiring management include competition from pest plants, inappropriate fire regimes and human interference. A range of strategies will be necessary to alleviate these threats including weed control, fire management, fencing, and

control of pest animals. Providing information to land owners, managers and the broader community in the region will increase awareness of the species, provide for increased protection of existing populations, an increased likelihood of new populations being found, and a reduced risk of inadvertent damage occurring. *Ex situ* conservation measures will be essential, including maintenance of a population of mature plants as a seed bank and *in-vitro* propagation to produce large numbers of plants for reintroduction.

Affected interests

Only two, very small populations (one wild, one introduced) of Sunshine Diuris exist, so few parties are affected by the implementation of this Recovery Plan (Table 1). The land managers of the Sunshine and Altona sites are aware of the presence of the species and the conservation significance of these sites. All other stakeholders involved in this Recovery Plan have been consulted and approve of the responsibilities outlined in this document. Affected interests include:

Table 1. Affected interests

Organisation	Interest
Department of Sustainability and Environment	Recovery program manager, coordinating on-ground works.
Parks Victoria	Land manager, <i>in situ</i> management, participant in recovery program
Royal Botanic Gardens Melbourne	Symbiotic germination, cultivation, seed and fungal storage and research, participant in recovery program
Melbourne Zoo	Cultivation, participant in recovery program
Australasian Native Orchid Society	Community group, cultivation, <i>in situ</i> management, participant in recovery program
Brimbank City Council	Land manager of potential habitat, participant in recovery program
public corporation	Land manager, Sunshine site
Western Laboratories	Contract grower of D. fragrantissima seedlings
Port Phillip & Western Port Catchment Management Authority	Regional Authority

Role and interests of indigenous people

Indigenous communities on whose traditional lands the Sunshine Diuris occurs will be advised, through the relevant DSE Regional Indigenous Facilitator, of this Recovery Plan. Opportunities to involve indigenous communities in the implementation of the Recovery Plan will be explored once it is finalised.

Benefits to other species

By maintaining the wild site at Sunshine and the introduction site at Altona, a number of grassland species will also be protected. Species associated with the threatened Western (Basalt) Plains Grassland Community are well represented at this site. Maintenance of this site also protects a threatened and undescribed *Dianella* species that also occupies the site. The Sunshine Diuris is a highly attractive plant and as a result, has acted as a flagship species for the Western (Basalt) Plains Grassland community. No adverse impacts are expected to any other indigenous species.

Social and economic impacts

No negative social and economic impacts are expected as a result of the implementation of this Recovery Plan. The Sunshine Diuris is now restricted to just two sites, both on public land; one on a rail reserve at Sunshine and the second on reserved Crown land at Altona, and agreement has been reached with managers of both sites for continued conservation of the orchid there.

Acknowledgments

Our thanks to Cam Beardsell (Parks Victoria), Fiona Coates (DSE), Simon Cropper (Botanicus Australia), Rob Cross (Royal Botanic Gardens, Melbourne), Andrew Garnham (Deakin University), Sue Hadden (DSE), Tien Huynh (University of Melbourne), Adrian Moorrees (DSE), Gary Slater (formerly Zoos Victoria), Zoe Smith (University of Melbourne), James Todd (DSE), Dick Thomson (ANOS) and Dale Tonkinson (DSE) for information and comments on the Recovery Plan.

Bibliography

Backhouse, G.N. and Jeanes, J.A. 1995. The Orchids of Victoria. The Miegunyah Press, Carlton.

- Backhouse, G.N., Webster, A. and Arnott, J. 2000. Sunshine Diuris (*Diuris fragrantissima*) (Orchidaceae: Diuridinae) Recovery Plan 1998 2002. Department of the Environment and Heritage, Canberra.
- Barlow, T.J. and Ross, J.R. 2001. Vegetation of the Victorian Volcanic Plain. *Proceedings of the Royal Society of Victoria* 113: xxv–xxviii.
- Bishop, T. 1996. *Field Guide to the Orchids of New South Wales and Victoria*. University of New South Wales Press, Sydney.
- Clements, M.A. 1989. Checklist of Australian Orchidaceae. Australian Orchid Research vol 1.
- Cropper, S.C. 1993. Management of Endangered Plants. CSIRO Publications, Melbourne.
- DCE 1992. Native Grassland Management in the Melbourne Area Grassland Guidelines Kit. Department of Conservation and Environment, Melbourne and Victorian National Parks Association.
- DEH 2000. Revision of the Interim Biogeographic Regionalisation of Australia (IBRA) and the Development of Version 5.1. Summary Report. Department of the Environment and Heritage, Canberra.
- Gullan, P.K., Cheal, D.C. and Walsh, N.G. 1990. *Rare or Threatened Plants in Victoria*. Department of Conservation and Environment, Melbourne.
- James, E., Akiyama, S. and Knight, C. 2001. Building a Life Raft for *Diuris fragrantissima*. In 'Abstracts, Annual Meeting of the Society for Conservation Biology'. University of Hawai'i, Hilo.
- Jones, D.L. 1988. Native Orchids of Australia. Reed, Frenchs Forest.
- Knight, C. 2002. Sunshine Diuris: A cultivation-led recovery. In 'Abstracts, Mutual Gains, Co-operative Orchid Conservation in South-eastern Australia'. Royal Botanic Gardens, Melbourne.
- Knight, C., James, E. and Akiyama, S. 2001. Reversing the decline of *Diuris fragrantissima*. In 'Abstracts, Proceedings of the First International Orchid Conservation Congress'. Perth.
- McDougall, K. 1989. 'The Re-establishment of *Themeda triandra* (Kangaroo Grass): Implications for the Restoration of Grassland. Department of Conservation, Forests and Lands, ARI Technical Report Series No. 89, Melbourne.
- PV 2000. State of the Parks 2000. Parks Victoria, Melbourne.
- Parsons, R.F. 1981. The Conservation of *Diuris punctata* var. *albo-violacea* (Sunshine Diuris). La Trobe University Botany Department (Unpublished), Bundoora.
- Richards, H. 2002. *Diuris fragrantissima*, its demise and rescue. In 'Mutual Gains, Co-operation Orchid Conservation in South-eastern Australia'. Royal Botanic Gardens, Melbourne.
- Smith, Z. F. 2006. Developing a reintroduction plan for the threatened terrestrial orchid *Diuris* fragrantissima. PhD Thesis, The University of Melbourne, School of Resource Management, Faculty of Land and Food Resources, Melbourne, Australia
- Smith, Z.F., Murphy, D.J., James, E.A. and McLean, C.B. 2005a. Molecular investigation of the *Diuris* '*punctata*' group in south-eastern Australia. *Selbyana* 26(1) 217–228.
- Smith, Z.F., James, E.A. and McLean, C.B. 2005b. Investigation of the phylogenetic relationships between *Diuris fragrantissima* and its closest relatives using AFLPs. *The Environmentalist* in press.
- Smith, Z.F., James, E.A. and McLean, C.B. 2007a. Investigation of the phylogenetic relationships between Diuris fragrantissima and its closest relatives using AFLPs. The Environmentalist 27(1): 217–226.

- Smith, Z.F., James, E.A. and McLean, C.B. 2007b. Experimental reintroduction of the threatened terrestrial orchid *Diuris fragrantissima*. Lankesteriana 7(1–2): 377–380.
- Stuwe, J. 1980. *Diuris punctata* var. *albo-violacea*. In 'A Register of Rare and Endangered Native Plant Species in Victoria'. Ed. NH Scarlett. La Trobe University, Bundoora.
- Stuwe, J. 1986. An Assessment of the Conservation Status of Native Grasslands on the Western Plains, Victoria and Sites of Botanical Significance. Department of Conservation, Forests and Lands, ARI Technical Report Series No. 48, Melbourne.
- Thompson, D. 2002. Orchid cultivation for conservation. In 'Abstracts, Mutual Gains, Co-operative Orchid Conservation in South-eastern Australia'. Royal Botanic Gardens, Melbourne.
- Tonkinson, D. 1985. Progress Report on Research Carried out on the Rare Orchid (*Diuris punctata* var. *albo-violacea*). La Trobe University Botany Department (Unpublished), Bundoora.
- TORT 2002. Cultivation guidelines for the Sunshine Diuris (*Diuris fragrantissima* D.L. Jones & M.A. Clem.). Threatened Orchid Recovery Team Department of Sustainability and Environment, Melbourne.
- Walsh, N.G. and Entwisle, T.J. 1994. Flora of Victoria Volume 2. Inkata Press, Melbourne.
- Warcup, J.H. 1971. Specificity of mycorrhizal associations in some Australian terrestrial orchids. *New Phytologist* 70: 14–46.
- Webster, A. and McKay, J. 1993. Sunshine Diuris (*Diuris fragrantissima*) Action Statement No. 50. Department of Conservation and Natural Resources, Melbourne.
- Willis, J.H. 1951. Sydenham Sanctuary for Vanishing Basalt Flowers. Walkabout 17: 36-7.

Duration of Recovery Plan and Estimated Costs

Action	Description	Priority	Feasibility	Responsibility*	Cost estimate						Cost estimate			
					Year 1	Year 2	Year 3	Year 4	Year 5	Totals				
1.0	Maintain & enhance population at Sunshine													
1.1	Monitor plants and habitat	1	100	DSE	\$3,000	\$3,000	\$3,000	\$3,000	\$3,000	\$15,000				
1.2	Undertake pest control	1	100	DSE	\$4,000	\$4,000	\$4,000	\$4,000	\$4,000	\$20,000				
1.3	Undertake ecological burning	2	100	DSE	\$0	\$4,000	\$0	\$0	\$4,000	\$8,000				
2.0	Maintain & enhance population at Altona													
2.1	Monitor plants and habitat	1	100	DSE	\$5,000	\$5,000	\$5,000	\$5,000	\$5,000	\$25,000				
2.2	Undertake pest control	1	100	PV	\$4,000	\$4,000	\$4,000	\$4,000	\$4,000	\$20,000				
2.3	Undertake ecological burning	2	100	PV	\$0	\$0	\$3,000	\$0	\$3,000	\$6,000				
3.0	Maintain a population in cultivation													
3.1	Produce seedlings using in vitro propagation techniques	2	100	Zoo / RBG	\$3,000	\$3,000	\$3,000	\$0	\$0	\$9,000				
3.2	Develop techniques for symbiotic inoculation	3	95	Uni Melb / RBG	\$30,000	\$20,000	\$0	\$0	\$0	\$50,000				
2.3	Increase plant numbers in cultivation	3	100	Zoo/ RBG	\$20,000	\$20,000	\$10,000	\$10,000	\$10,000	\$70,000				
4.0	Reintroduce cultivated plants to the wild													
4.1	Reintroduce plants to Altona reserve	2	55	Recovery Team	\$0	\$30,000	\$30,000	\$0	\$0	\$60,000				
4.2	Establish a new reintroduction site	3	55	Recovery Team	\$0	\$30,000	\$30,000	\$0	\$0	\$60,000				
	Totals				\$69,000	\$123,000	\$92,000	\$26,000	\$33,000	\$343,000				