

Using native soil fungi to improve sustainability of woodland revegetation



Valema farms putting
sustainability to the test
Corrigin LCDC
Genaren Hill LCDC



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Have you ever wondered:...

How native woodland trees grow in our soils which are infertile for agricultural crops?

How do the plants manage without fertilizer applications?

In agriculture, we fertilize crops

In woodlands, fungi do most of this job

Just as we put back plants, we need to put back the original fungi in our revegetation

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Executive Summary

Fungi are critical biodiversity in maintaining soil fertility. They drive major nutrient cycling processes in woodlands by decomposing dead plant and animal material, supplying nutrients to ensure the healthy growth of plants, and providing food for insects, small mammals and soil microbes. A diverse array of native fungi exists in woodland remnants but these fungi take a very long time, if they ever do, to self-establish in revegetation sites. The fungi need to be assisted back to promote sustained soil functional values for greater long-term sustainability of woodland revegetation, increased nature conservation, and improved landscapes.

This project has developed a unique sustainability strategy for woodland revegetation incorporating practical on-farm methods for landholders to reintroduce native macrofungi into revegetation schemes. Field demonstrations in two catchments (Swan-Avon, WA and Lachlan, NSW) have confirmed the practicality of successful reintroduction of native fungi as part of revegetation.

Communication of new methodologies to landholders and extension workers by field days and workshops not only encouraged a willingness to incorporate fungi in revegetation programs but also revealed some major strategies for encouraging wide-scale adoption. These include simple guides to enable identification and selection of native fungi appropriate for local conditions of the wheatbelt catchment regions. Step-by-step guidelines with pictures are needed to demonstrate how to re-introduce fungi to new plantings of native trees and other plants. Training of local bushcare/landcare officers for each region is needed, using the methods developed in the project. Long-term monitoring of demonstration sites and progressive enhancement of local skills via information through workshops is needed.

Resources of native fungi are dwindling as the size and quality of Australia's woodland remnants are contracting, and there is a pressing need to maximise the use of these fungal resources with minimal disturbance of remaining remnants. To service large-scale revegetation schemes, practices enabling use of large-scale inoculation of native fungi need to be developed.

Broad Objectives of Project

Demonstrate in two catchments the application of new information on the role of fungi in supporting plant growth and the reintroduction of fungi as part of revegetation schemes, to assist the success of sustainable woodland revegetation across Australia.

Communicate these new methodologies at a national level to encourage wide scale adoption.

Focus Catchments

Corrigin in Western Australia – Swan Avon Catchment (Upper Kunjin Catchment), Lat. 32.23 S x Long. 117 46' E.
Parkes/Peak Hill in New South Wales - Lachlan Catchment. Lat. 32.40 S x Long. 148.10' E

CSIRO Forestry and Forest Products

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NEW SOUTH WALES - Mike Sutherland of Genaren Farm, Greening NSW Molong, Greening NSW Forbes, Alan & Monica McDonald of McDonalds Farmtrees, staff at Forbes State Forests NSW, many farmers of the Lachlan catchment.

TASKS

TASK 1 Sources of fungal biodiversity in vegetation remnants

Background

The purpose of this task was to identify sources of fungal biodiversity in vegetation remnants. Fungi in remnants were documented according to protocols developed at CSIRO for collecting, processing and identifying Australian fungi.

Task 1	Milestone	Reporting Date	Performance Indicator
Identify sources of fungal biodiversity in vegetation remnants	Documentation of fungi in remnants by 31 October 2000	31 May 2001	Identified and listed sources of fungi in remnant vegetation sites in Corrigin and Lachlan catchments
ACTIVITY	ACTION	DATE	PERFORMANCE MEASURE
Vegetation remnants and fungi identified in Lachlan and Upper Kunjin catchments and representative collections made from each site	Vegetation remnants for fungal resources cooperation of NPWS, catchment coordinators and farmers in Lachlan and Upper Kunjin catchments making remnant vegetation on private land available.	December 1999- May 2001	12 sites identified and monitored for fungi in Lachlan and Corrigin catchments. Estimated 200 and 168 species of fungi obtained at respective catchments.

Key Findings

- Sources of fungi in remnant vegetation sites in Upper Kunjin and Lachlan catchments have been identified
- Large diversity of native fungi occurs in woodland remnants. A total of 257 collections of fungi were obtained in remnant vegetation in the Upper Kunjin catchment and Wallitin Creek catchment. These represent an estimated 200 species in 95 genera. In the Lachlan catchment remnants 168 species were found.
- Large diversity of fungi occurs in small remnant vegetation patches on farmland and roadside remnant vegetation.
- Few native fungi are present in woody revegetation on farmland. eg. 10 in the Upper Kunjin catchment .
- Many unique, unidentified species including some new to science were found within remnant vegetation patches.
- Many native truffle-like fungi occur in woodland remnants but virtually none in revegetation sites.
- Some macrofungi are potentially rare and endangered species e.g. apparently restricted to associations with particular plants or only occur in several patches of remnant vegetation.
- Functional roles of macro fungal species evident, e.g. the fungi are associated with native trees and shrubs which are dominantly ectomycorrhizal at all stages from young seedling age to mature trees in remnant woodland.

Implications for revegetation

- Native fungi need assistance to encourage their establishment in revegetation sites for biodiversity and functional purposes.
- Fungal food for small native mycophagous marsupials eg. woylies, potoroos, bandicoots and other small mammals, is not available in revegetation and needs to be re-introduced
- A diverse choice of native fungi is available to select from for putting back into revegetation and degraded woodlands , but information to help landholders identify and select appropriate fungi is not available.
- Important to match fungi to soil types and plant communities in revegetation sites as fungal communities differ in different plant communities. Ideally fungal and plant communities should be matched to soil type for revegetation.

TASK 2 Grow seedlings with fungi in soil from native vegetation sites

Production of ectomycorrhizal seedlings under nursery conditions using local native plants from the Swan-Avon (Upper Kunjin) and Lachlan catchments.

Background

The purpose of this task was to test sources of inoculum in relation to soil type and vegetation community.

Task 2	Milestone	Reporting Date	Performance Indicator
Grow seedlings with fungi in soil from these source sites	Production of seedlings by 28 February 2001	31 May 2001	Production and assessment of seedlings from the 2 catchments using local seed, soil and fungi
ACTIVITY	ACTION	DATES	PERFORMANCE MEASURE
Source seed from Upper Kunjin and Lachlan Catchments	Seed local to the demonstration sites collected or accessed through an experienced collector	July 1999-May 2000	80 species of seed available for selection of shrubs and trees
Source soil with fungi from Upper Kunjin and Lachlan Catchments	Soil with fungi matched to seed source vegetation community types collected	December 1999-January 2000	Soil sources confirmed to have complexity of fungi for incorporating into nursery potting mixes.
Grow seedlings with soil from Lachlan catchment.	Prepare glasshouse facilities at CSIRO Floreat WA to quarantine standards for handling interstate soils	July – August 2000	Glasshouse and experiment methodology passed inspections by AQIS. Soil handling procedures included restricted access, designated glasshouse footwear and clothing, gloves, autoclaving contaminated equipment and rubbish, capture and decontamination of water flow through.
Source soil with spores from Upper Kunjin and Lachlan Catchments	Soil with spores matched to seed source vegetation community types collected.	December 2000–January 2001	Soil and complex mixes of spores incorporated into nursery potting mixes.
Grow seedlings with fungi with remnant vegetation inoculum in soil	2000 seedlings from 39 plants species measured for soil nutrient cycling fungal associations	January-July 2000	Produced seedlings from local seed from Corrigin (Swan-Avon catchment) and Lachlan catchments of 38 species of <i>Eucalyptus</i> , <i>Allocasuarina</i> , <i>Acacia</i> , <i>Gastrolobium</i> and <i>Melaleuca</i> with diverse fungal associations on roots. Seedlings produced in a practical nursery system appropriate for outplanting using standard equipment.
Grow seedlings with spores from fruit bodies in soil	1200 seedlings from 12 plant species assessed for soil nutrient cycling fungal associations.	January-August 2001	Produced seedlings from local seed from Corrigin of 7 <i>Eucalyptus</i> and 5 <i>Acacia</i> with diverse fungal associations on roots.
Set up two field demonstrations at Corrigin	Selected soil type from paddock site – sandy friable soil. Planted out two demonstrations based on randomised block design with <i>Acacia</i> buffer zone	January-December 2002	Seedling sampling and root analysis one year after planting out. Successful field demonstration of diverse fungal associations on roots.

Grow seedlings with seed from the Lachlan catchment: Persistence of fungal inoculum in fenced and unfenced grazed lands and proximity to remnant vegetation.

Background

A large remnant patch of native vegetation protected from sheep and cattle grazing was recently fenced to contain a remnant patch specifically set aside for nature conservation. This area was fenced under an initiative by the Genaren Hill Landcare Group. The patch on private property is located within the broader Lachlan Catchment area of NSW. The fencing extends to 200m out from the remnant patch and encompasses a total area of 6 km². This fenced area is now considered as prime remnant grassland and woodland and is a sanctuary for native fauna including re-introduced woylies. The area beyond the fencing continues to be used for grazing as it has since clearing.

To determine the implications for revegetation strategies, soil inoculum persistence was compared within the fenced and unfenced areas and in proximity to remnant woodland. Soil was collected adjacent to the edge of the remnant patch and out towards the fence, at 50m intervals, along two transects (F1 and F2). Soil samples in the unfenced (grazed) area were collected at 100m intervals extending out from the fence, along three transects (G1, G2, G3). We then mixed a portion of each soil sample with potting mix including fertiliser developed for native revegetation and supplied by Men of the Trees Nursery, WA.

Bimble box (*Eucalyptus populnea*), grey box (*E. microcarpa*) and Dwyers gum (*E. dwyeri*) are dominant ectomycorrhizal tree species in the remnant patch. These trees were transplanted as seedlings into each soil mixture and raised under glasshouse conditions. After 4 months these plants were harvested and assessed for number of ectomycorrhizal types and extent of ectomycorrhizal development.

Key findings

- Development of ectomycorrhizal colonisation demonstrated inoculum persisted at the margin of remnant vegetation and only occurred sporadically from 100-500m from the remnant edge. A total of 18 different morphological types of ectomycorrhizal roots were described, each representing a different fungal species or related group of species.
- Remnant woody vegetation has a high diversity and amount of native soil macrofungi and grassland has moderate to negligible amounts and types.
- Amount of ectomycorrhizal development is highest in the soil samples from the remnant edge indicating soil inoculum quantity is greatest at the remnant edge.
- Number of ectomycorrhizal types is high at the remnant edge indicating higher diversity of soil macrofungi is present. The diversity of these fungi is less and more patchy at 50m from the remnant edge. Tree roots appear not to spread beyond 15m from the remnant margin into the grassland.
- Generally, treatments with soil from the fenced areas have greater ectomycorrhizal diversity than the unfenced (grazed) areas. This may be due to the closer proximity to the remnant patch, or spore dispersal in scats due to the presence of native vectors such as the woylie within the fenced area. Due to the positioning of F1 & F2 transects extending from projections in the remnant patch contour it is likely that these projections have provided suitable cover for the woylie and may have increased opportunity for spore dispersal via scats. Quantity and importantly types of macrofungi are increased by scat dispersal.
- Dwyers gum has greater ectomycorrhizal development than bimble and grey box, however all three tree species have similar amounts of ectomycorrhizal fungal types (diversity). Data indicates that each tree species has some specific symbiotic fungal associations.
- In treatments with soil from the unfenced (grazed) area ectomycorrhizal development is low and numbers of ectomycorrhizal types are low to moderate.

Implications for revegetation

- Soil nutrient cycling macrofungi associated with remnant woody trees and shrubs are needed to kickstart soil biodiversity associated with sustainable soil nutrient cycling processes. Fungi need to be restored with revegetation. Even in native grasslands surrounding remnant woodland having a good diversity of native mammals which transport fungi in their scats, the diversity of inoculum is low and distribution spasmodic beyond the remnant vegetation margin.
- Proximity of revegetation to remnant vegetation is important in influencing establishment of native macrofungi on trees. Revegetation strips or corridors adjoined or alongside remnant vegetation patches may encourage better establishment of native fungi than plantings in relative isolation. Numbers and types of macrofungi are moderate to negligible beyond the tree roots of native vegetation.

- Using local trees in revegetation programs will encourage establishment of mycorrhizal relationships with local native fungi.
- A mixture of tree species increases the diversity of mycorrhizal relationships formed.
- Fenced areas where fungal vectors such as the woylie are present may encourage establishment of mycorrhizal relationships.
- Nursery methods, which increase soil inoculum levels available to seedlings, could give more consistent and uniform levels of diversity over whole areas of revegetation. These methods could encourage mycorrhizal diversity where revegetation sites are remote from native vegetation fragments.

TASK 3 Demonstration scale plantings of seedlings grown with native fungi

3 Field demonstrations in Upper Kunjin Catchment, Corrigin, WA.

3 Field demonstrations in Genaren, Lachlan Catchment, NSW.

Background

The purpose of this task was to set up three long-term demonstration sites to monitor the restoration of macrofungi in revegetation on farmland

Task 3	Milestone	Reporting Date	Performance Indicator
Establish demonstration scale plantings of seedlings with fungi	1st demonstration established in Upper Kunjin catchment by 1 December 2000	31 May 2001	Demonstration trees, N-fixing shrubs and macrofungi from source sites established at 1 site
ACTIVITY	ACTION	DATES	PERFORMANCE MEASURE
Demonstration site Upper Kunjin Catchment, WA.	Site for demonstration planting selected, ripped, fenced (farmer contribution all costs). Site is integrated into a 2 km by 50m wide width of revegetation corridor of local trees and shrubs, matched to soil type, linking 2 remnants.	December 1999 - March 2000	Demonstration site prepared for planting.
Source seed and soil nutrient cycling macrofungi	Seed and fungi collected from a local salmon gum-wandoo-mallee remnant tall tree woodland community matched to community type growing in the soil type in a paddock cropped to wheat/lupin/ canola at Valema farms	July 1999- January 2000	Seeds of 12 <i>Eucalyptus</i> and 3 <i>Acacia</i> species and diverse fungi collected produced in a nursery planting
Production and outplanting seedlings for 1 st demonstration in the Upper Kunjin Catchment.	1500 seedlings of trees, mallees, acacia produced with macrofungi in a standard nursery. Germination of 10 <i>Eucalyptus</i> and 3 <i>Acacia</i> species sufficient for outplanting in demonstration.	January-July 2000	Demonstration site set up with 1300 seedlings of tree and nitrogen fixing shrubs produced in 6 different fungal soil and potting mixes. Seedlings out planted in a 2 km x 55m revegetation corridor.
Out planted seedlings attacked by plague locusts	Substantial numbers of locusts from WA's worst ever locust plague were not destroyed by spraying. Out planted seedlings individually covered with fine mesh on wire frames. 20 people days of volunteer labour contributed to enable rapid design and manufacture and placement of net protection.	November 2000 – February 2001	Management of locust plague at demonstration site by covering trees and shrubs Revegetation belt with demonstration fenced by farmer to exclude stock
Production and out planting of seedling for 2 nd and 3 rd demonstration demonstrations in the Upper Kunjin Catchment	1800 seedlings, 1 <i>Acacia</i> , 2 tall trees and 4 mallee species in 5 different soil treatments produced under glass house conditions for out planting.	January-July 2001	Two demonstration scale sites selected, relative uniform soil site within prepared revegetation corridor.

Task 3 continued.	Milestone	Reporting Date	Performance Indicator
Monitoring of field sites	Demonstration 2 and 3 scored for seedling survivors, most deaths occurring 6-8 months after out planting due to insect attack, most likely locusts and summer drought conditions.	October 2001 and May 2002	More than 50% of the planted seedling survived drought and insect damage. Excellent growth and establishment of those surviving seedlings particularly <i>E. loxophleba</i> .
Establish demonstration scale plantings of seedlings with fungi	1st demonstration established in Lachlan catchment by 1 December 2000	31 May 2001	Demonstration trees, N-fixing shrubs and macrofungi from source sites established at 1 site
ACTIVITY	ACTION	DATES	PERFORMANCE MEASURE
Demonstration site Lachlan Catchment, NSW.	Site for three transects within the Genaren Sanctuary under the project management of Genaren Hill Landcare Group selected.	January 2002	Transects and plots at 50m, 100m, 150m and 200m measured and pegged out. Demonstration site sprayed for weeds in August & September 2001 and May 2002
Source seed and soil nutrient cycling macrofungi	Seed and fungi collected from a local box and ironbark remnant woodland communities matched to community type growing in the soil type of a wheat / canola grazing farm, adjacent to grazed and ungrazed land at Genaren.	December 2001	Seeds of 3 <i>Eucalyptus</i> , 4 <i>Acacia</i> 1 <i>Allocasuarina</i> , <i>Dodonea</i> , <i>Senna</i> and <i>Hakea</i> species grown with diverse fungi in a nursery.
Production and outplanting of deomonstration at Genaren.	Inoculated and uninoculated grey box seedlings produced by local commercial nursery under scientific supervision and direction. A further 8 local understorey plant species for buffer also produced by the nursery	January-May 2002	A total of 2700 of plants seedlings produced under nursery conditions for out planting May / early June 2002.

Re-introduction of native macrofungi into woodlands.

Demonstration at Valema farms.

Background

The Valema Farms project on integrated farm systems trials a number of innovative projects in tree plantings and agricultural, biological surveys and conservation of natural ecosystems with a view to increasing biodiversity. The project is managed in association with Corrigin Land Conservation District Committee (LCDC) and incorporates a number of privately owned farms 20km west of Corrigin in the central southern wheatbelt area of Western Australia.

The integrated farm system project aims to significantly increase numbers of tree plantings including local native tree species and commercial tree crops such as oil mallees and nut trees. These projects endeavour to combine environmental issues such as salinity, declining diversity and land degradation with economic issues such as declining returns, global pressure for sustainable agricultural, consumer pressure for organic produce and increasing markets for 'gourmet' products.

The soil fungi project funded by the Natural Heritage Trust was divided into two components. Twelve remnant vegetation patches on Valema Farms were surveyed to establish an inventory of ectomycorrhizal and decomposing fungi. Both these fungi types have an important role in soil nutrient cycling and providing nutrients to plant species. The second component investigated the types of fungi occurring with native revegetated trees one year after out planting. These trees were grown in six different soil media as seedlings prior to planting out.

Fungal surveys showed *Tricholoma*, *Amanita* and *Cortinari* ectomycorrhizal fungi and *Agaricus*, a decomposer commonly occur (fruit) on remnant patches on the farm. The spores of these fungi were collected separately in inoculum trays. These trays were subsequently

pooled and mixed together into three different inoculum mixes to demonstrate a method of re-introducing fungi into revegetation. 1. Mycorrhizal inoculum, 2. Decomposer inoculum, and 3. Mycorrhiza and decomposer inoculum mix. These inoculum mixes were finally mixed with soil. To demonstrate that diversity of fungi occurring in soil from the remnant patches near to the demonstration site was much greater than the paddock site and that the paddock site would allow this broad and diverse spectrum of fungi to develop and persist with outplanted seedlings, the soils used to raise seedlings were collected from a paddock used for sheep and wheat farming, small amounts of remnant vegetation soil and a potting mix supplied from Men of the Trees in Western Australia.

Tree, mallee and *Acacia* species native to local remnant patches were raised for 6 months in these soil treatments under glasshouse conditions, then out-planted. The presence and types of fungi present among roots were measured prior to out-planting. Plants assessed for ectomycorrhizal fungi colonization prior to out planting showed that no mycorrhizal development occurred in the MOT (a potting mix used as a standard by Men of The Trees) and very small amounts in the P (soil from paddocks) treatments. One year after out planting, a further 120 plants from the 6 different treatments were sampled and assessed for ectomycorrhizal fungi colonisation and diversity as a measure of persistence of ectomycorrhizal fungi associations.

The field demonstration site chosen for out-planting was integrated within a 2km long corridor prepared in a paddock after the first winter rains in 2000 by mechanical ripping of lines (rows) along topographic contours. Eleven lines were spaced approximately 4 metres apart. Pre- and post-emergence herbicides were applied for weed control. This preparation method and row spacing for tree plantings is usual practice for revegetation strips or corridors in Western Australia. Treatment plants were planted along 9 rows of ripped lines. Trees and *Acacia* species were planted 1m apart and mallees were planted 0.5m apart in view of later thinning. The demonstration was based on a random block design, which effectively randomised treatments and plants in blocks along designated rows. Buffer rows of *Acacia* species outside the demonstration area were planted with seedlings from the Valema farm revegetation scheme.

Key Findings

- Ectomycorrhizal colonization occurs on seedlings raised under glasshouse conditions in soil mixes with inoculum from remnant vegetation prior to out planting.
- *Acacia* showed no mycorrhiza development across all 6 treatments prior to out planting.
- No seedlings in potting mix and only two seedlings in paddock soil treatments developed mycorrhiza relationships before out planting.
- Addition of decomposer inoculum did not suppress ECM (ectomycorrhiza) development in comparison with other treatments both prior to out planting and 1 year after out planting.
- A total of 25 morphotypes of ectomycorrhizal roots were categorized after out planting.
- 25 morphotypes were found for fungi described from remnant vegetation patches. They were all found on tree seedlings grown with inoculum.
- Adding spore inoculum of symbiotic fungi did not change the overall amount and types of fungi present on seedlings grown in the various soil types.
- 7 morphotypes were found in small parts of root systems on all treatments.
- No unique association was found between any particular *Eucalyptus* species and fungal morphotype.
- No arbuscular mycorrhizas (AM) colonisation was found 1 year after out planting showing that the eucalypts are strongly preferentially ectomycorrhizal.
- Ectomycorrhizal colonization is often good to extensive on eucalypts one year after out planting.

Implications for revegetation.

- Eucalypts used in revegetation schemes on paddock soils are strongly ectomycorrhizal, harbouring a diverse range of morphotypes.
- Many diverse ectomycorrhizal fungi associated with remnant vegetation and can be re-introduced into and persist in paddock soils by using small quantities of inoculum.
- Diversity of macrofungi in revegetation is increased by diverse tree plantings.
- Soils which have been ploughed, fertilized, recently min-tilled, cropped and grazed over 5 decades can support and sustain extensive colonization of ectomycorrhizal fungi which benefit native plants.

TASK 4 Monitor and compare establishment of plant-fungal relationships

Task 4	Milestone	Reporting Date	Performance Indicator
Monitor and compare establishment of plant-fungal relationships with seedlings grown by conventional techniques	Assessment of plant-fungal relationships in demonstration plantings by 28 May 2002	30 May 2002	Establishment of trees, N-fixing shrubs with fungi assessed and analysed
ACTIVITY	ACTION	DATES	PERFORMANCE MEASURE
Source spores from Genaren.	Spores of seven native fungi harvested.	30 May 2002	Spores of seven fungi stored in readiness for application .
Grow seedlings with spores from Genaren.	Two dilutions of spores applied to seedlings.	30 May 2002	Produced seedlings with fungi.
Monitoring of fungal fruit bodies.	Fruiting monitored at Corrigin at 17 sites with different vegetation and soil type.	30 May 2002	Fungal species, vegetation and soil data captured .
Mycorrhizal development in different soil types.	Test 17 soils with 2 <i>Eucalypts</i> species and <i>Gastrolobium</i> .	30 May 2002	Mycorrhizal formation by 2 eucalypts and <i>Gastrolobium</i> assessed .

Nursery stock receptivity to native fungal inoculum.

Can ectomycorrhizal development by local native fungi be promoted on eucalypt seedlings in standard potting mixes?

Background

Ectomycorrhizal inoculation of eucalypts in nurseries can produce vigorous seedlings in low nutrient potting mixes. As yet seedlings produced for native revegetation programs in Australia are not inoculated with ectomycorrhizal fungi. Inoculation with local native fungi of seedlings used for revegetation will after outplanting have the added benefit of replacing vital soil nutrient recycling components back into the revegetated ecosystems. Inoculation in nurseries may also minimise colonisation by weed fungi. Weed fungi are favoured by disturbance and lack of competition with a wide diversity of local native fungi.

Various techniques to inoculate plants with ectomycorrhizal fungi have been used in Australia and North America such as hydrogel bead technology in blue gum plantations and broad scale spore dispersal in pine plantations. However these techniques lack diversity of fungi and are mostly inappropriate for less commercially focussed applications such as multi-purpose revegetation schemes. A main impediment to inoculation of plants for revegetation is that the amount of inoculum required as sources of native fungi are becoming limited. Small amounts of inoculum need to be effectively introduced to large numbers of seedlings so that the macrofungi can be efficiently put back in revegetation.

We have shown, in the remnant woodland patch recently fenced through the Genaran Hill Landcare Group initiative, NSW, that local fungi present in remnant vegetation can form ectomycorrhizal relationships with native tree species. However, the diversity and abundance of fungi varies spatially, especially in regard to edge effects. To ensure more uniform inoculation of plants in revegetation it is desirable to inoculate nursery stock with propagules of native fungi.

In this demonstration we sourced native fungi as sporocarps from the remnant patch at Genaren. We applied spores of native fungi from remnant woodlands to 3 dominant eucalypt species under standard nursery conditions to test the receptivity of these trees to 7 native fungal species.

Eucalyptus species tested were grey box (*E. microcarpa*), Dwyers gum (*E. dwyerii*) and river red gum (*E. camaldulensis*). Seed was raised in standard potting mix used in revegetation programs, which includes a standard level of fertiliser.

Sporocarps of these selected fungi were homogenised separately in a water solution and diluted to two levels of spore concentrations. Each plant species was transplanted to a separate cell and supplied with an aliquot of inoculum one fungus or a mixture of all species in solution. A small amount of water was used to wash the solution into the potting mix.

Key Findings

- Approximately 50% of seedling inoculated with spore inoculum formed ectomycorrhizas and they rarely had weed fungi, which were not inoculated.

- Fungal diversity of the ectomycorrhizal relationships formed was low suggesting that the potting mix substrate is not conducive to formation of ectomycorrhizal relationships of many local native fungi. Some of the inoculated fungi did not develop on any of the tree species and may reflect the quality of the inoculum which was not tested for viability.
- In contrast to inoculated seedlings, 11% of uninoculated seedlings had ectomycorrhizas formed by weed fungi (fungi which thrive in heavily disturbed situations).
- One fungus produced fruitbodies with up to 7% of the seedling pots suggesting that this fungus was a predominant ectomycorrhizal species on seedling roots

Implications for revegetation

- Nursery stock used in revegetation can be successfully inoculated with native macrofungi.
- The extent of inoculation success for individual macrofungi is likely to be variable in the nursery. Inoculation with a mixture of many native fungi in nurseries may ensure multiple fungi on root systems by time of outplanting.
- Weed ectomycorrhizal fungi (of unknown or known identity) can colonise roots and may inhibit the native fungi. Nursery techniques and methods (eg partial potting mixture sterilisation) can minimise the presence of these fungi.
- Persistence and fluctuations in levels of inoculated and weed fungi after outplanting is unknown and needs to be monitored.
- Choice of potting mixes is crucial as it affects establishment of ectomycorrhizal relationships with a diversity of local fungi in the nursery. An optimal potting mix has the following characteristics:
 1. promotes growth of inoculum fungi and their mycorrhizal formation
 2. promotes seedling growth of various tree species
 3. promotes establishment of both seedling and fungi after outplanting

Monitoring of fungal fruiting and development in 17 different woodland communities.

How is fungal fruiting related to soil and vegetation type?

Background

Soil types in the wheatbelt area of Western Australia have an irregular and mosaic-like distribution and soil types can change drastically within a small area; some soil types are in patches less than a few metres in length. Patchiness of soil type distribution is reflected in the presence of plant community types and vegetation structure and composition. This project monitored macrofungi on 17 different remnant woodland patches having different soil and vegetation types within the Valema farms area. These soil types were further monitored under glasshouse conditions for development of ectomycorrhizal relationships with two species of *Eucalyptus* and one species of *Gastrolobium* occurring in the area. Over a period of one year *Eucalyptus salmonophloia* and *E. wandoo* were grown with *Gastrolobium* sp. in two pots of each soil type and then assessed for ectomycorrhizal development.

Key findings

- Different soil types are associated with highly diverse and varying groups of vegetation and macrofungi.
- Soil types and disturbance affects fruiting of different macrofungi. For example *Scleroderma cepa* fruited abundantly in association with oil mallees adjacent to the woodland community on soil site 12 but was not found in the remnant vegetation patch.
- Several *Agaricus* species including a secotiod type are the predominant and most conspicuous decomposing macrofungi for many soil sites.
- Soil site 1 produced a diverse range of ectomycorrhizal fungi genera commonly associated with undisturbed forest sites. For example *Russula*, *Amanita*, *Cortinarius* and *Boletus* were recorded during the monitoring period.
- Diversity of fungal fruiting particularly the ectomycorrhizal fungal component may be indicative of soil type and quality and status of remnant woodland patches in Western Australia.
- Ectomycorrhizal associations can develop in soils types with low or negligible fungal fruiting.
- Risk of disease affecting seedlings such as damping off is increased through soil and litter transfer.

Implications for revegetation

- Success of revegetation programs is increased where vegetation is matched to soil type. This leads to greater success of plant and also ectomycorrhizal relationships.
- Soil disturbance increases risk and spread of disease.
- Degraded and low quality remnant woodland patches can be characterised by their low diversity of fungal fruiting as compared to better quality sites.
- Low quality remnant patches may benefit from restoration techniques involving the re-introduction of fungal diversity. If the macrofungi had been fit for the degraded conditions they would have persisted. Any restoration of fungi would have to be concurrent with site enhancement measures eg fencing and often targeted revegetation is needed.

TASK 5 Simple guidelines for re-introduction of fungi

Background The purpose of this task was to assess the efficiency of methods for re-introducing macrofungi. These methods were the introduction of a wide diversity of spores to seedlings so that local native fungi and plants are outplanted together.

Task 2	Milestone	Reporting Date	Performance Indicator
Grow seedlings with fungi in soil from these source sites	Production of seedlings by 28 February 2001	31 May 2001	Production and assessment of seedlings from Corrigin soils with and without inoculum spores
ACTIVITY	ACTION	DATES	PERFORMANCE MEASURE
Source seed from Corrigin	Seed local to the Corrigin area collected or accessed through an experienced collector	July 1999-May 2000	Seed of 80 species available for selection of shrubs and trees.
Source soil from Corrigin	Soil from 17 sites / types collected and seeded with 3 local species measured for soil nutrient cycling fungal associations	December 1999-August 2001	All sites confirmed to have complexity of macrofungi, even those where no fruiting bodies were found.
Source soil with spores from Corrigin	Soil with spores matched to seed source vegetation community types collected.	June -December 1999	Soil with complex mixtures of spores incorporated into nursery potting mixes.
Grow seedlings with spores from fruitbodies of remnant woodland	500 seedlings from 36 plants species measured for soil nutrient cycling fungal associations	January-July 2000	Produced seedlings from local seed from Corrigin (Swan-Avon catchment) 36 species of <i>Eucalyptus</i> , <i>Allocasuarina</i> , <i>Acacia</i> , <i>Melaleuca</i> , <i>Callistemon</i> and <i>Atriplex</i> with varying fungal associations on roots.
Source soil with spores from Corrigin	Soil with spores matched to seed source vegetation community types collected.	January-July 2000	Soil and complex mixtures of spores incorporated into potting mixes for field demonstration seedlings 9 Eucalypts, 3 <i>Acacia</i> for demonstration.
Source soil with spores from Corrigin	Soil with spores matched to seed source vegetation community types collected.	January-July 2001	Soil and complex mixtures of spores incorporated into nursery potting mixes for demonstration 2 with 7 Eucalypts and 5 <i>Acacia</i> species.
Source soil with spores from Corrigin	Soil with spores matched to seed source vegetation community types collected.	January-July 2001	Soil and complex mixtures of spores incorporated into nursery potting mixes for demonstration 3 with 2 Eucalypts and 1 <i>Acacia</i> species.
Source soil with spores from Lachlan Catchment	Soil with spores matched to seed source vegetation community types collected.	January-May 2002	Soil and complex mixtures of spores successfully incorporated into nursery potting mixes for seedlings in field demonstrations 4 & 5 with 1 Eucalypt, 4 <i>Acacia</i> , 1 <i>Allocasuarina</i> , <i>Dodonea</i> , <i>Senna</i> & <i>Hakea</i> species.

Using local native fungi to produce plants with diverse mycorrhizas in nurseries

Which woodland plant species form mycorrhizas?

Background

A wide range of local plant species are used in revegetation to combat degradation and salinity in many agricultural areas of Australia. Ectomycorrhizal fungi have an important role in nutrient cycling between the soil and plants and may have other important functions in the health of dryland ecosystems. The fruiting bodies of these types of fungi have been collected in patches of remnant vegetation in the wheatbelt area of Western Australia, which falls in the rainfall zone of 300-400mm annually. However the relationship between ectomycorrhizal fungi and many local plant species has not been determined.

This demonstration tests the mycorrhizal status of 36 plant species local to the southern central wheatbelt district of Corrigin, Western Australia. Information about these relationships may have important implications for nursery production of plant species for revegetation as a management strategy to conserve biodiversity and increase it through the re-introduction of local fungi.

Soil was collected from a fenced patch of remnant vegetation on private property within Valema Farms. Soil was sub-sampled, pooled and then mixed with standard potting mix (supplied by Men of the Trees, WA.) in a 1:1 volume ratio. Only small volumes of soil were required for the task and collection by sub-sampling a number of small soil quantities minimised soil disturbance. Soil disturbance in dryland systems is inimical to the establishment and survival of both seedlings and established trees as the soil ecosystem and soil-root interface recover slowly. It cannot be emphasised enough that soil from remnant patches is a valuable and delicate resource and should be treated conservatively.

The remnant woodland patch selected for the demonstration is dominated by tall trees red morrel (*E. longicornis*), salmon gum (*E. salmonophloia*) and kondinin blackbutt (*E. kondininensis*) as well as mallees and *Acacia erinaceae*

Collections of ectomycorrhizal fungi were made from wheatbelt locations. The spores from these air-dried fruit bodies were added to the soil and potting mix and mixed for a further 5 minutes in an electric cement mixer. Soil was then transferred to seedling cell trays in the glasshouse. Seeds were sown directly into these cells (8 replicates of each) and plants were harvested after 7 months.

Key Findings

- Thirty five of 36 plant species assessed formed ectomycorrhizal associations in inoculum from remnant vegetation in the glasshouse.
- All *Acacia* species tested formed ectomycorrhizal associations excepting *A. hemiteles*. Low-level AM mycorrhiza colonisation was seen on 4 of the *Acacia* species, which also formed ectomycorrhizal relationships. *Rhizobium* nodules formed on all except *A. hemiteles* and *A. colletioides*.
- *Allocasuarina* species formed moderate levels of ectomycorrhizal association. No AM association formed.
- Strong ectomycorrhizal associations formed on all eucalypts tested and in all replicates consisting of individual plants. AM association was mostly low.
- *Melaleuca* species formed predominantly ECM associations. *Callistemon phoeniceus* formed very low association (<4%) with both ECM and AM.
- *Atriplex amnicola* formed very low levels of ectomycorrhizal association in only two of seven replicates and developed no AM association.

Implications for revegetation

- Many plants species native to dryland ecosystems are strongly ectomycorrhizal., AM associations form to a much lesser degree and *Rhizobium* sp. form associations with *Acacia*. Promotion of dominant symbiotic associations will benefit seedlings and revegetated areas.
- Soils from remnant woodland patches harbour a diverse range of soil organisms as inoculum for development of symbiotic relationships on many tree species. These can develop in nurseries before seedlings are planted out.
- Revegetation of areas previously under agriculture may benefit from potting mixes blended with small quantities of diverse symbiotic organisms to re-introduce soil nutrient cycling and nitrogen fixing species into soils.
- Mixed plantings of a variety of local native plant species may benefit plant species that may not readily form associations with ectomycorrhizal and AM fungi by providing reservoirs of inoculum for a long time.
- Plants may be selected and placed strategically in revegetation plantings to benefit surrounding species. For example, *Acacia* species can form three symbiotic associations simultaneously; ectomycorrhizae, arbuscular mycorrhiza and rhizobium nodules. These plants may provide a source of symbionts for other *Acacia* and nitrogen fixing species in revegetation which may not colonise as readily.
- Spore inoculum added to soil mixes can form ectomycorrhizal relationships on seedlings at the nursery stage and persist as an inoculum source after out planting.

TASK 6 Communication/workshops

Background

The purpose of this task was to conduct workshops for landholders and extension workers to demonstrate methods to practitioners.

Task 6	Milestone	Reporting Date	Performance Indicator
Conduct workshops for landholders and extension workers to demonstrate methods to practitioners	Workshops organised for Western Australia and NSW in 2001 and 2002 by 28 May 2002	30 May 2002	Workshops held
ACTIVITY	ACTION	DATE	PERFORMANCE MEASURE
YEAR 1999			
Catchment and Landcare coordinators in Lachlan, Upper Kunjin, Blackwood and Dumleyung catchments	Communication with key catchment groups in WA and NSW	July-September 1999	Provided information about incorporating soil nutrient cycling fungi into bush management, bush regeneration and rehabilitation
Australian Association of Bushland Regenerators WA. Kings Park, WA	How to ID fungal sources, their role in ecosystems	July 1999	Workshopped biodiversity of fungi and their functional role in bushland regeneration
Friends of Kings Park, and for Friends of Bold Park, and for Friends of Hollywood Bushland.	4 workshops focussed on sourcing fungi, fungal ID and role in ecosystems	July 1999	Demonstrated fungi and their role in bushland management
XIth International Congress of Mycology. Sydney	Disparity in biodiversity: sequester fungi in natural and modified vegetation types in Western Australia.	August 1999	Symposium paper about the significance and diversity of truffle like fungi in the Australian ecosystem
WWF	Workshop with managers of landcare and bush for wildlife	August, October, December 1999	Supplied and workshopped information about how to incorporate soil nutrient cycling fungi into bush management, bush regeneration and rehabilitation
Greening Australia	Workshop with managers of landcare coordinators	August & Sept. 1999	Workshopped practical information about sourcing and incorporation of soil nutrient cycling fungi into revegetation projects for sustainable landmanagement
National, international and community biodiversity experts	Australian fungi: much to learn about their taxonomy and ecology	December 1999	Dampier 300 Biodiversity in Australia Conference, Perth, Australia.
Ecological Society of Australia	Fungi - the root cause of tree success	December 1999	Paper in 'Ecological Connections' (ed. R. Wills, C. Yates, R.J. Hobbs)
YEAR 2000			
Land for Wildlife	Discussions with coordinator on exchange of information about practicalities of revegetation with broad biodiversity and sustainability qualities	February, April 2000	Provide practical information on how to promote widescale incorporation of practical soil nutrient cycling fungi into bush management, bush regeneration and rehabilitation
Dowerin Field Days Swan Avon Integrated Catchment Management Working Group's	Value of fungi for ecosystem services, integral part of display which our project manned for 3 days to interact with community members.	August 2000	Natural Resource Management Display – Prize winning display. Visited by 2,000 people. 1 on 1 discussions with 420 famers, land-management agents and officers.
Agroforestry Expo 2000,	Similar to above.	August 2000	400 people through the display;

Boyup Brook			detailed discussions with 55 landcare/bushcare workers, farmers, TAFE landcare course coordinators, state land management agencies and rural industry representatives
Ecoplan, Swan Avon Catchment Workshop	Workshop; Fungi in Habitat and Bushland Management	August 2000	
Bungendore Park, Shenton Bushland, Bold Park, Kings Park.	Fungi in bushland	June 2000	Talks and workshops for bushcare groups
Falls Park Hovea, Warwick Open space (Fungimap foray).	Fungi in bushland	July 2000	Talks and workshops for bushcare groups
Corrigin Shire. Valema farms	Workshop + field days Putting Sustainability to the Test Field Days; Extending genetic resources; Conservation/Biodiversity Challenges, Including Native Fungi.	September 2000	100 Landcare, government agencies and farmers attended workshop and field days
YEAR 2001			
Fungi. for Nature Photography Group, Kings Park.	How to ID fungal sources	April 2001	Workshops and talks delivered
'Introduction to Bushcare.' Apace Aid (Inc.) and City of Joondalup.	Fungi and bushland management	May 2001	Talk at workshop delivered
Nature Reserves Preservation Group (Kalamunda).	Fungi in bushland management	June 2001	Talk and workshop for bushcare group delivered
Fungimap Inaugural Conference. Denmark, W. Australia.	Collecting, documenting and processing fungal specimens for scientific study	June 2001	Talks and workshops delivered
"Skills for Nature Conservation" training program run by Greening Australia WA; EcoPlan, and the Swan Catchment Centre; Warwick Bushland.	3 Fungi in bushland workshops with talks and field activities	July 2001	Talk and training workshops for community conservation volunteers and land managers delivered
Marchagee Priority Catchment	Coorow Land for Wildlife	September 2001	On site demonstrations and discussions during workshop with 40 regional landcare/bushcare workers and landholders.
YEAR 2002			
Corrigin WA	Explanation/demonstrations about using native soil fungi to improve farm revegetation and sustainability.	April 2002	Workshop, organised and delivered by CSIRO, with sponsorship from NHT Bushcare, and Valema Farms. Extensive feedback from participants. See workshop booklet, and summary of feedback.

Key Findings

- There was a low level of awareness, but a high level of interest. We have raised the awareness levels for example being contacted and asked to give workshops (see above).

Implications for revegetation

- Farmers wanting to use practical guidelines / methods to integrate fungi in their revegetation and preserve and feel proud of their own remnant patch. For example if they have rare and endangered macrofungi.

Using native fungi to improve sustainability of woodland revegetation

MAJOR OUTCOMES and CONCLUSIONS

This project has initiated a unique sustainability strategy for woodland revegetation by developing practical on-farm methods for landholders to reintroduce native macrofungi into revegetation.

Key achievements include:

1. **The sourcing of fungi:**

Recognition and definition of a highly biodiverse resource of native nutrient cycling macrofungi on private land and on public lands, reserves, and roadsides. Absence of native fungi in revegetation. The native fungal resource is dwindling and under threat as are many trees and other plants.

2. **The reintroduction of macrofungi for community revegetation programs:**

- ***Demonstrations***

On site demonstrations in two catchments - Swan Avon (Upper Kunjin) WA, Lachlan, NSW - confirmed the practicability of successfully reintroducing fungi as part of revegetation.

- ***Simple practical guide lines***

Communication of the new methodologies to landholders and extension workers by field days and workshops. This communication drew strong interest in the current project, particularly its sourcing of fungi, and its nursery and on-ground success for revegetation. Workshops encouraged a willingness to adopt fungi in revegetation programs.

3. **Feedback about issues raised by landholders:**

Our strategy to obtain substantial feedback from landholders during the project revealed some major issues and strategies for encouraging wide-scale incorporation of fungal introduction methods in revegetation schemes. Some of these include:

- A major requirement for readily accessible information to enable landholders to use fungi in revegetation. e.g. simple guide-book information to help landholders recognise, select and source native fungi suitable for the local conditions. Such information is needed to ensure that fungi are compatible with plant types targeted for revegetation, and that those fungi which are introduced into revegetation persist long-term. *Fungibank* could be established as a web-based source of information akin to that of *Florabank* which was developed for sourcing and selecting native plants.
- Need for monitoring of demonstrations to determine how much and how quickly soil nutrient cycling fungi, once introduced, proliferate and spread within revegetated areas and reconstructed plant communities.
- Need to maximise the use of dwindling resources of native fungi, with minimal disturbance of woodland remnants and enhance methods for large-scale inoculation for many different local native fungi simultaneously.
- Need to develop a range of guidelines to suit different requirements of revegetation programs ranging in scale from small on-farm or local groups to large commercial operations.
- Need to apply the guidelines developed for revegetation to restoration of degraded native woodland remnants and to increasing the size of existing remnant patches. This will promote the long-term viability of woodland communities and maintenance of native genetic resources.
- Need to communicate and demonstrate the guidelines regionally. Community groups involved in this project have emphasized the need to know about and to apply guidelines for establishment and maintenance of healthy revegetation. This is a goal of rural community groups in farming catchments throughout the Australian temperate woodland region.

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