Bioassay Measurements of Mycorrhizal Inoculum in Soils from Eucalyptus Plantations of Varying Ages in Western Australia

Chen Yinglong Brundrett Mark Dell Bernie Gong Mingqin Malajczuk Nicholus

ABSTRACT Bioassay measurement was used to estimate the inoculum potential of mycorrhizal fungi in intact soils collected from *Eucalyptus* plantations of varying ages in Western Australia. The results showed that both ectomycorrhizal (ECM) and vesicular-arbuscular mycorrhizal (VAM) fungi existed in soils from most plantation sites according to two bait plants (clover for VAM and *Eucalyptus* for ECM). The levels of ECM or VAM fungal inoculum were considered to be moderate. Analysis of roots sampled from plantations suggest that *Eucalyptus* stands are more associated with VAM fungi compared to ECM fungi that occupied most fine roots in older stands. Information from the bioassay experiment suggests that there may be a need for the introduction of a wider range of inoculum to assist the establishment of new *Eucalyptus* plantations in this region. Further work is required on below-ground ECM fungal diversity in these plantations.

KEY WORDS bioassay measurement, *Eucalyptus* plantations, mycorrhizal inoculum, ectomycorrhiza, vesicular-arbuscular mycorrhiza

1 Introduction

Ectomycorrhiza (ECM) and vesicular-arbuscular mycorrhiza (VAM) are the two major associations predominating in most natural ecosystem with a wide variety of habitats (Brundrett 1991). It would be expected that these associations play a valuable role in plant nutrient uptake in nature, as recently documented by Smith and Read (1997). The nature and abundance of propagules of mycorrhizal fungi in soil determine their persistence in soil during periods of inactivity, their response to disturbance, their resistance to predation by other soil organism and their capacity for dispersal to new locations, but none of these processes are well understood (Brundrett *et al*. 1996). Inoculum potential is defined as the energy for growth of an organism at the surface of its host, and is a consequence of the numbers of active propagules of that organism and their nutritional status (Garrett 1956). The total mycorrhizal inoculum potential of soils can be tested by growing bait plants in intact cores of soil to measure the rate of mycorrhiza formation (Abbott and Robson 1991). Bioassays allow "typical levels" of mycorrhizal activity in different soils to be compared and the relative contribution of different types of fungus to be determined from their colonization patterns within roots (Brundrett and Abbott 1995). This paper addresses an estimation of the inoculum potential of propagules of VAM and ECM fungi in intact soils collected from *Eucalyptus* plantations of varying ages in Western Australia, and two rem-

Chen Yinglong[1], **Gong Mingqin.** Research Institute of Tropical Forestry, Chinese Academy of Forestry, Longdong, Guangzhou 510520, P. R. China

Brundrett M. CSIRO Forestry and Forest Products, CCMAR, Private Bag, PO Wembley, WA 6014, Australia

Dell B, Malajczuk N. School of Biological Sciences and Biotechnology, Murdoch University, Perth WA 6150, Australia

[1]Author to whom all correspondence should be addressed

E-mail: gzritfg@public.guangzhou.gd.cn

Phone: (+86) 20 87725613; Fax: (+86) 20 87725622

Received June 17, 1999

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

nant areas of forest, using bioassay measurement.

2 Methods and materials

2.1 Field sampling procedure

A bulk soil sample was collected from the surface horizon (1～10 cm) by taking shovels of soil from 5 equally spaced locations across a 100 transect in each plot in 9 commercial blue gum (*Eucalyptus globulus* Labill.) plantations aged from 0 to 11 years. The soils were brought to a glasshouse in Perth for bioassay experiment and a combined soil sample from each site was used for chemical analysis. Ten cores (2 cm in diameter, 15 cm in length) were taken at each site and roots from cores were processed to determine mycorrhizal formation under natural conditions.

2.2 Glasshouse bioassay experiment

Soil (approx. 2500 g) from each transect location was placed in pots lined with a plastic bag. There were 5 replicate pots for each site. Sterilized Karrakatta Yellow Sand from the Spearwood Dune System north of Perth was used as the control soil type for the duration of the experiment. Two relatively uniform seedlings of *Eucalypus globulus* (3 months old) grown in a nursery of Bunning Treefarms were transplanted into each pot as bait plants for ectomycorrhiza (ECM). Clover seeds, pre-germinated by soaking them in aerated water overnight and inoculated with rhizobium, were sown in the same pots of the *Eucalyptus* to assay for vesicular-arbuscular mycorrhiza (VAM). The clover seeds were inoculated with 1.2 g rhizobium inoculum in peat. After 2 weeks clover seedlings were thinned to 4 per pot. No mineral nutrients were applied. Plants were kept in a glasshouse where temperature and light were adequate for plant growth. Water was applied to the non-draining pots by watering them to field capacity using a balance.

2.3 Harvesting and root processing

Four weeks after planting, one *Eucalyptus* and two clover seedlings were carefully extracted using soil cores to provide an early assessment of mycorrhizal formation. The holes were back filled with sterilized Yellow Sand. At 8 weeks, the remaining plants were harvested by washing the soil from the roots. The fresh weight of roots and shoots were determined and the shoots were dried at 70 °C in an oven. To assess mycorrhizal colonization, roots collected from field sites and the glasshouse trial were cleared and stained with trypan blue in lactoglycerol. The infective rates of VAM was determined using the grid-line intersect method (Brundrett *et al*. 1996; Gong *et al*. 1997) and the number of ectomycorrhizal root tips per m in root was counted. Morphotypes of ectomycorrhiza in field root samples were observed with a compound microscope.

2.4 Data analysis

Data from mycorrhizal colonization were subjected to one-way analysis of variance and Duncan's Multiple Range Test (Duncan 1955) with SAS System software (Release 6.12). Percentages of VAM infective rates were transferred by arcsin (sqrt) for statistical analysis.

3 Results

3.1 Mycorrhizal formation under natural conditions

Site locations and soil properties are described in Table 1. Soil chemical properties, especially phosphorus and nitrogen, varied with site location and plantation age. There were significant differences ($p < 0.001$) in mycorrhizal colonization on *Eucalyptus* roots between sites (Table 2). Multiple comparison between sites for either VAM infective rates or numbers of ECM tips per m in root was assessed using Duncan's mul-

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

tiple range test (Table 3). Relatively high VAM colonization rates were observed in roots from plantation sites 7 and 10 (above 50 % each), while sites 2 ,5 and 9 had low colonization ,less than 10 % (Table 3). By contrast, *Eucalyptus* roots from sites 2 ,5 ,and 9 were well associated with ectomycorrhizal fungi with more than 120 infected tips per m. Roots collected from sites 7 and 8 were poorly colonizated by ECM fungi. VAM associations were observed on roots of clover and other herbs from intensively managed pasture (site 6) where no ectomycorrhizae were recorded. There was an interaction between colonization by the two types of mycorrhizal fungi ($y = 98.43 - 1.434\,7x$, $r^2 = 0.61$). About 5 morphotypes of ectomycorrhizal in field root samples were observed under microscope. A view image of the 3 morphytypes are shown in Figure 1. The unbranched morphotypes with yellow to brown in colour on the surface are the typical type of *Eucalyptus* mycorrhizae. The black Cenococcum-like mycorrhizae were also presented (Figure 1)

TABLE 1 Site location and soil properties

Samples				Site location and land history				Soil property *					
Site	Code	Plantation age	Site name	Location	Lat(S)	Long(E)	Site History	Texture	pH	OM/ ppm	P/ ppm	NO_3/ ppm	NH_4/ ppm
1	CARP 86	11	Carpenters	18km SW of Manjimup	34°20′	116°00′	Pasture	Loamy clay	5.5	5.0	24	12	20
2	WRENS 88	9	Wrens RD	12km NW of Manjimup	34°11′	116°02′	Planted in 1988	Loamy clay	5.2	5.0	33	4	20
3	CARP 95	2	Carpenters	see site 1	34°20′	116°00′	Pasture site planted in 1995	Sandy	5.6	2.4	12	2	20
4	CARP 96	1	Carpenters	see site 1	34°20′	116°00′	Forest site planted in 1996	Loamy clay	6.5	3.0	7	4	17
5	DUNN 91	6	Dunnets	Scott River area, 40 km E of Augusta	34°15′	115°21′	Pasture planted in 1991	Sandy	5.1	1.8	5	1	14
6	WARN 97	0	Warenella Farm	Karridale ,12 km N of Augusta	34°12′	115°06′	Intensively managed pasture sampled just before 1997 planting	Sandy clay	5.7	8.8	189	30	20
7	LA 96	1	Landells Farm	Kudardup (Augusta)	34°15′	115°09′	Intensively managed pasture planted in 1996	Sandy	5.7	2.4	35	5	20
8	HART 93	4	Hartridges Farm	Scott River area, 45 km E of Augusta	-	-	Pasture planted in 1993	Sandy loam	-	-	-	-	-
9	HART 95	2	Hartridges Farm	See site 9	-	-	Pasture planted in 1995	Sandy	-	-	-	-	-
10	MAT 91	6	Mathews Treefarm	Mumbellup ,43 km E of Donnybrook	31°34′	116°04′	Jarrah/ Marri forest cleared and planted in 1991	Sandy clay	6.5	3.9	18		

Note : Methods for chemical analysis : pH (water slurry) ,OM (Walkley-Black method) ,NO_3 (extract in 1 mol·L^{-1} KCl ,Salicylate/ Hypochlorite ,Quikchem method No 10-107-04-1-Z) ,P (Bray extractable) ,NH_4 (extract in 1 mol·L^{-1} KCl ,Analysis is performed on Lachat FIA (Colourmetric) ,Quikchem method No 12-107-06-2-B). Some data were not available shown as '-' instead.

TABLE 2 Analysis of variance for VAM percentage and ECM tips per m in roots from Eucalyptus **plantations**

Variable	Source	*DF*	Adj *SS*	Adj *MS*	*F*
VAM	Site	9	37 864.6	4 207.2	15.3***
	Error	87	23 943.1	275.2	
ECM	Site	9	243 909.4	27 101.0	15.0***
	Error	87	157 234.6	1 807.3	

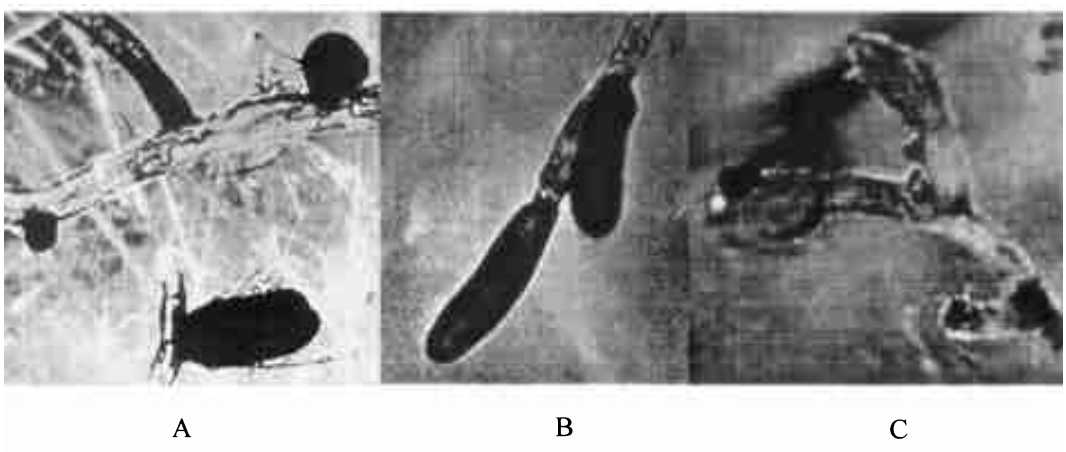
Note : * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

TABLE 3 Mycorrhizal colonization on roots sampled from Eucalyptus **plantation sites (both VAM infective percentage and ECM tips per m in root were counted)**

	Site	1	2	3	4	5	6	7	8	9	10
VAM	Means/ %	20.05	9.52	15.16	36.67	2.30	0	50.72	43.29	5.80	52.82
	Duncan's Test	B-C	C-D	C	B	C-D	D	A	A	C-D	A
ECM	Means/ tips $\cdot m^{-1}$	56.27	123.96	70.70	74.19	147.18	0	15.02	2.10	120.50	35.32
	Duncan's Test	B-C	A	A	A	A	E	D	D-E	A	C-D

Note: Percentages of VAM colonization were tranformed by arcsin(sqrt) for statistical analysis. Means with the same letter are not significantly different (Duncan's Multiple Range Test, $Alpha = 0.05$, $df = 87$). Mycorrhizal associations were also found in root samples of clover or other herbs from site 6 (data were not presented here).



A B C

FIGURE 1 Morphotypes of Eucalyptus-ectomycorrhiza in field root samples
A: cenococcum-like mycorrhizae with radiating external hyphae; B-C: typical unbranched morphological types of *Eucalyptus* mycorrhizae

3.2 Glasshouse bioassay trial

There was a large difference ($P < 0.001$) in VAM colonization in clover plants with site followed by ECM ($P < 0.001$) (Table 4). However, infective rates of both types of mycorrhizal fungi on bait plants were generally low revealing low fungal inoculum levels in soils (Table 5). The average colonizatin of both types of mycorrhizae varied in the range of 14.5 %～63.5 % (VAM) and 3.7 %～32.0 % (ECM tips $\cdot m^{-1}$). No mycorrhizal roots were observed in the sterilised yellow sand. The ECM inoculum level was very low in soils from site 6. Few ectomycorrhizas were observed in *Eucalyptus globulus* sampled at 4 weeks (data not presented). Overall, the results indicate the universal occurrence of VAM inoculum across the plantation estate.

TABLE 4 Analysis of variance for mycorrhizal colonization in soils from bioassay measurement

Variable	Source	DF	Adj SS	Adj MS	F
VAM/ %	Site	10	18 938.5	1 893.8	5.56 ***
	Error	44	14 985.9	340.6	
ECM/ tips $\cdot m^{-1}$	Site	10	4 448.5	444.8	1.97 **
	Error	44	9 911.1	225.3	

Note: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

TABLE 5 Mycorrhizal colonization of clover (VAM %)

	Site	1	2	3	4	5	6	7	8	9	10	11
VAM	Means/ %	22.84	20.52	39.55	14.51	39.64	28.18	63.49	62.07	17.20	34.34	0
	Duncan's Test	B-C	B-C	A-B	B-C	A-B	B	A	A	B-C	B	C
ECM	Means/ tips $\cdot m^{-1}$	6.38	9.82	25.04	18.27	18.71	3.74	18.47	15.94	32.00	18.13	0
	Duncan's Test	B-C	B-C	A-B	A-C	B-C	C	A-C	A-C	A	A-C	D

Note: Site 11 refers to the control soil type (sterilized sand). Percentages of VAM colonization were transferred by arcsin (sqrt) for statistical analysis. Means with the same letter are not significantly different (Duncan's Multiple Range Test. *Alpha* = 0.05, *n* = 44).

4 Discussion and conclusion

The moderate levels of ECM and VAM fungal inoculum in soils of blue gum plantation sites were generally recognized according to the infection rates of roots from both plantations and glasshouse bioassay trial. Eight-week growing of clovers could well detect VAM inoculum levels in intact soils, but may be less enough for *Eucalyptus* associate with ECM fungi. From the bioassay experiment, the overall trend in mycorrhizal colonization showed an early decline for VAM and a later increase for ECM with plantation age. This is consistent with endo- and ectomycorrhizae suggested by other workers for Australia (Lapeyrie and Chilvers 1985) and Brazil (Bellei *et al*. 1992). Oliveira *et al*. (1997) discerned three patterns of VAM and ECM colonization of *Eucalyptus dunnii* in southern Brazil. These were: 1) Pattern A followed the VAM-forming soya bean—the relatively large incidence of VAM 5 months after planting progressively decreased while that of ECM increased; 2) Pattern B followed the VAM/ ECM-forming *E. viminalis*—the incidence of VAM remained minimal while that of ECM relatively rapidly reached a high plateau; and 3) Pattern C followed the ECM-forming *Pinus taeda*—both VAM and ECM progressively increased but were never abundant. Negative associations were also found on the same root system of *Populus deltoides* and *Salix nigra* (Lodge and Wentworth 1990).

In native Australian *Eucalyptus*-dominated forests there is a high diversity of ectomycorrhizal fungi (Bougher 1995). However, in *Eucalyptus* plantations the diversity is greatly reduced. Lu *et al*. (1999) recorded, for plantations of blue gum in the same region as this study, ECM species (from sporocarp collections) increasing from 2 in 1-year-old stands to 12～17 in 6-year-old stands. In our study, we observed 3～5 morphotypes of ECM in the field and glasshouse grown *Eucalyptus* roots. However, no work has been done to compare the flora of vesicular-arbuscular mycorrhizal fungi in *Eucalyptus* plantations in Australia. There is a study on nearby native *E. marginata* forest (Brundrett and Abbort 1991).

In China, a low diversity of VAM and ECM fungi in *Eucalyptus* plantations has been identified (Chen *et al*. 1998a). As a consequence, incoculation programs with ECM fungi are being developed to increase the biodiversity of symbiotic fungi in plantations. In Western Australia, the situation is less clear. Certainly there is some ECM inoculum in plantations being established on ex-farm sites. It is unclear what the origins of this inoculum are or whether the diversity of the ECM fungi in the bioassay is equivalent to that discerned in Lu's study above mentioned. Inoculation with combined types of mycorrhizal fungi could be more efficient than pure inoculum for *Eucalyptus urophylla* (Chen *et al*. 1998b) and *Pinus patula* (Sudhakara Reddy and Natarajan 1997) in the nursery. Further re-

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

search is required to screen mycorrhizal inoculum fungi as optimal candidates with potential for commercial application in forest production.

Acknowledgements

This work was supported by the Australian Centre for International Agriculture Research (ACIAR). The authors wish to thank CSIRO Forestry and Forest Products for access to the laboratory facilities at CCMAR, Perth, Western Australia. We thank Associate Prof. L. Abbott of the University of Western Australia for advice, and acknowledge the technical support from Ms. S. Snelling and Mr. C. Lubcke of CSIRO Forestry and Forest Products, Australia.

Literature cited

Abbott L K and Robson AD. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizae. *Agric. Ecosyst. Environ.* 35:121～150.

Bellei MM, Garbaye J and Gil M. 1992. Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa Catarina (southern Brazil). *For. Ecol. and Mana.* 54:205～213.

Bougher NL. 1995. Diversity of ectomycorrhizal fungi associated with *Eucalyptus* in Australia. In Mycorrhizas for Plantation Forestry in Asia. M Brundrett, B Dell, N Malajczuk and Gong Mingqin (Eds). Austrilia: *Canberra ACIAR Proceedings*. 2:8～14.

Brundrett MC. 1991. Mycorrhizas in natural ecosystems. In: Advances in ecological research. A Macfayden, M Begon, AH Fitter (Eds). London: *Academic Press*. 21:171～313.

Brundrett MC and Abbott L K. 1991. Mycorrhizal fungus propagules in the jarrah forest. Ⅱ. Spatial variability in inoculum levels. *New Phytol.* 131: 461～469.

Brundrett MC and Abbott L K. 1995. Roots of jarrah forest plants. I. Mycorrhizal associations of shrubs and herbaceous plants. *Australian J. Botany* 39:445～457.

Brundrett MC, Bourgher N, Dell B, Grove T and Malajczuk N. 1996. Working with mycorrhizae in forestry and agriculture. Australia: *ACIAR Canberra*. 141～172.

Chen YL, Gong MQ, Wang FZ, Chen Y, Brundrett M, Dell B. 1998 a. Diversity of VA mycorrhizal and ectomycorrhizal fungi in *Eucalyptus* plantations in southern China. In: Programme and abstracts of second international conference on mycorrhiza. Uppsala, Sweden, July 5～10, 1998:42～43.

Chen YL, Gong MQ, Wang FZ, Chen Y, Dell B, Brundrett M. 1998b. Growth promotion and nutrient uptake of *Eucalyptus urophylla* coinoculated with *Glomus* and *Pisolithus isolates*. In: Overcoming Impediments to Reforestation, Proceedings of BIO-REFOR Workshop. Brisbane, Australia. December 2～5, 1997:153～155.

Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics*. 11:1～24.

Garrett SD. 1956. Biology of root infecting fungi. Cambridge: *Cambridge University Press*.

Gong MQ, Chen YL and Zhong CL. 1997. Mycorrhizal research and application (in Chinese). Beijing: *Chinese Forestry Publishing House*. 84～88.

Lapeyrie FF and Chilvers GA. 1985. An endomycorrhizal-ectomycorrhiza succession associated with enhanced growth by *Eucalyptus dumosa* seedlings planted in a calcareous soil. *New Phytol.* 100:93～104.

Lodge DJ and Wentworth TR. 1990. Negative associations among VA-mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. *OIKOS*. 57:347～356.

Lu Xian-Heng, Malajczuk N, Brundrett M and Dell B. 1999. Fruiting of putative ectomycorrhizal fungi under blue gum (*Eucalyptus globulus*) plantations of different ages in Western Australia. *Mycorrhiza*. 8:255～261.

Oliveira VL, Schmidt VDB and Bellei MM. 1997. Patterns of arbuscular-and ecto-mycorrhizal colonization of *Eucalyptus dunnii* in southern Brazil. *Ann. Sci. For.* 54:473～481.

SAS SAS/STAT. 1988. User's guide for personal computers. Release 6.08 Edition. Gary, NC. USA: *SAS Institute*.

Smith SE and Read DJ. 1997. Mycorrhizal symbiosis. *Academic Press, Cambridge*. 126～289.

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

Sudhakara Reddy M and Natarajan K. 1997. Coinoculation efficacy of ectomycorrhizal fungi on *Pinus patula* seedlings in a nursery. *Mycorrhiza*. 7: 133～138.

西澳州桉树林地土壤菌根菌剂接种潜力研究

陈应龙　弓明钦

(中国林业科学研究院热带林业研究所,广州 510520)

Brundrett Mark

(澳大利亚联邦科工组织林业研究所,澳大利亚,佩斯 6014)

Dell Bernie　Malajczuk Nicholus

(麦道克大学生物学院,澳大利亚,佩斯 6150)

摘要　对澳大利亚西澳州桉树人工林菌根类型及其形成情况进行了调查,并采用生物测定法(Bioassay measurement)研究了桉树林地土壤菌根菌剂的接种潜力. 根系菌根检查和生物测定法试验均表明,在自然条件下桉树可以和不同真菌共生而形成三种类型的菌根,即外生菌根、VA 菌根和混合菌根;林分成熟程度与菌根形成有一定的相关性,与共生体的类型也有一定影响. 在幼林中,桉树根系主要与内囊霉菌共生形成 VA 菌根,而成熟林主要与担子菌共生形成外生菌根,混合菌根表现为一种中间类型. 在收集的林地土壤中移植的菌根诱饵植物(三叶草和蓝桉)分别检测出土壤中存在有一定量的 VA 菌根菌和外生菌根菌,但菌根菌繁殖体数量及接种潜力相对较小,并且也揭示了按树年龄对土壤菌剂的相对接种潜力影响较大. 本文对桉树人工林土壤菌剂接种潜力进行了评价,并就引进优良菌根菌对桉树人工林生产的重要性进行讨论.

关键词　外生菌根,VA 菌根,桉树人工林,生物测定法,菌根菌剂

© 1994-2010 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net