Ectomycorrhizal fungi in the mounds and other constructs of tropical Australian termites (Isoptera)

A.V. Spain, V. Gordon, and P. Reddell

Technical Report **25/98**
December 1998

ECTOMYCORRHIZAL FUNGI IN THE MOUNDS AND OTHER CONSTRUCTS OF TROPICAL AUSTRALIAN TERMITES (ISOPTERA)

ALISTER V. SPAIN, VICTORIA GORDON* and PAUL REDDELL*

CSIRO Land and Water, Private Mail Bag, Aitkenvale, Qld 4814, Australia

*Tropical Forest Research Centre, CSIRO, P.O. Box 780, Atherton, Qld 4883, Australia

CSIRO LAND AND WATER

TECHNICAL REPORT 25/98

December 1998

INTRODUCTION

The materials incorporated into termite mounds, particularly those of grass-harvesting and litter-feeding termites, are often nutrient-enriched compared with the surrounding soils. In many situations, both tree and grass roots have been noted within mounds, and it was postulated that the mycorrhizal fungi normally associated with the roots of most species could play an important role in the extraction of nutrients from these structures. Previous studies of this phenomenon are limited to records of dead spores of arbuscular mycorrhizal (AM) fungi in the mounds of Indian macrotermitine termites (Harinkumar and Bagyaraj, 1994).

This report records the regular association of ectomycorrhizal (ECM) fungi with certain termite constructs. Specifically, we report:

(i) the regular presence of the spores of ECM fungi in termite mounds and their less-consistent occurrence in the earthen/organic materials sheathing the stems of certain trees;

(ii) communities of ECM fungal taxa in termite mounds overlapping in generic composition but more diverse than those surrounding adjacent host species;

(iii) the presence of viable inocula of ECM fungi in the mounds of termites of differing ecological strategies;

(iv) the occasional presence of the sporocarps of ECM fungi on the surfaces of epigeal termite mounds.

METHODS

Field Collections

Observations were made and sampling was conducted between 1989 and 1996 in a diverse range of savanna and wooded environments in tropical Australia including such disturbed environments as roadsides and rehabilitated minesites.

Mean annual precipitation at the sampling sites ranged between *ca*. 587 to 2000 mm, all with summer maxima. Most were open forests or woodlands dominated by *Eucalyptus* spp. and were concentrated in northeastern Queensland between the latitudes of (16° 30'S and 20°S), although samples were also taken in northwestern Queensland (18° 45'S, 138° 37'E), in central Queensland (23° 46'S, 149° 12'E), in the Northern Territory between Darwin and Jabiru (12° 42'S, 132° 55'E) and Nhulunbuy (12° 16'S, 136° 51'E), and in the vicinity of Weipa (12° 38'S, 141° 53' E) on Cape York Peninsula. Samples were also taken from a number of closed rainforest sites in humid tropical northeastern Queensland (Mt Lewis, Davies Creek, Kuranda Range).

Soils ranged from alfisols at most sites to ultisols (Kuranda Range) and oxisols (Weipa, Nhulunbuy). Two disturbed sites were sampled in central Queensland, namely a roadside environment (Maraboon Dam area) and a rehabilitated minesite environment (Blackwater Mine).

All the Cape York Peninsula collections were made in the vicinity of Weipa. Samples were taken from a number of ecosystem types (Gunness *et al.*, 1987) which differ in their soils, drainage status and the compositions of their plant and termite communities. The 2b type is dominated by *Eucalyptus tetrodonta* and has a very sparse herbaceous layer. The 2c type is dominated by *Eucalyptus nesophila* and *E. tetrodonta* and has a well-developed grassy herbaceous layer; it is less well drained than the 2b type. The seasonally inundated 7b ecosystem type occurs on the periphery of a permanent swamp and is dominated by water-logging tolerant species of *Melaleuca, Lophostemon* and *Asteromyrtus* (Myrtaceae). The Arnhem Highway sites occurred in open forest and woodland environments dominated by sclerophyllous species and were located between Jabiru and Darwin in northern Australia.

The vegetation of the Mt Lewis and Davies Creek sites consisted, respectively, of simple microphyll vine-fern forests (vegetation type 9) and simple notophyll vine forest (vegetation type 8) (Tracey, 1982) which are both typically non-ECM. The two Kuranda Range sites consisted of vine forests (type 12c, Tracey, 1982) both containing mainly non-ECM species. However, this forest type also includes *Acacia mangium* and *Acacia aulacocarpa,* both of which are capable of forming ECM associations.

Samples were also collected from dry and wet sclerophyll forests at Davies Creek which are dominated by species that are typically ectomycorrhizal. The dry sclerophyll collections were made at two sites, both of which consisted of vegetation type 16i (Tracey, 1982) (one site was more heavily dominated by *Allocasuarina littoralis*). The wet sclerophyll collections were made at one site which was a vine forest with sclerophyllous species as emergents and in the upper canopy (vegetation type 13c, Tracey, 1982).

In the field, mound materials were sampled from the lower halves of the above-ground parts of mounds built by termites of different ecological groupings, here including grass-harvesting, litter-feeding, wood-feeding termites and the polyphagous species *Nasutitermes longipennis*. For comparative purposes, four individual surface (0–5 cm) soil samples were taken at points less than 30 cm from the bases of the nearest putative ECM host trees. These were mixed prior to extracting, counting and identifying the spores present. Samples were also taken from the above-ground earthen:organic workings of *Schedorhinotermes actuosus* from the stems of *Asteromyrtus symphyocarpa* and *Eucalyptus* spp. at locations near Weipa. Termites forage beneath largely earthen, protective workings that form extensive sheathings over the bark of a number of tree species.

Laboratory and glasshouse methods

Spores were separated from the soil and mound materials using a sequence of sieving, centrifugation and differential flotation on sucrose solutions of different strengths (Gordon and Comport, 1998). Spore densities were calculated to reflect numbers per gram of either soil or mound material. Spores of putative ectomycorrhizal fungi were classified to genus level, where possible, on the basis of spore morphology and size (Largent, 1977; Largent and Theirs, 1977; Largent *et al.*, 1977; Stuntz, 1977; Aberdeen, 1979; Castellano *et al.,* 1989; and Mueller, 1992). Those that we were unable to identify were listed as unknown.

The presence of viable propagules in the mound materials was demonstrated by germinating surface-sterilised seed of *Eucalyptus setosa* on the disaggregated mound materials of different termite species. For *Amitermes laurensis* and *Amitermes vitiosus* from a number of locations, the above-ground parts of the mounds were divided into three parts: the lower, the centre and the apical third; *Eucalyptus setosa* specimens were grown on each part separately. *Eucalyptus setosa* specimens were also grown separately on materials from the earthen external capping and the carton centre materials of a mound of *Coptotermes acinaciformis* collected from Jabiru, Northern Territory.

The spores of *Pisolithus* sp. were collected from a basidiocarp found on the surface of a mound of *Amitermes laurensis* and were applied in suspension to sterile soil within which seedlings of *Eucalyptus setosa* were growing. These plants together with appropriate controls were grown for approximately 14 weeks and roots were subsequently examined for evidence of infection.

RESULTS

Table 1 summarises the spore counts for mound materials and other termite constructs from all locations and includes counts for surface soils sampled adjacent to the ECM host species closest to the mounds.

Spores were found in the mounds of all termite species sampled, except for those from certain closed forest environments where ECM host species are absent. Excluding the latter sites, spore population densities in mound materials were higher than those in the surface soils adjacent to ECM hosts in nine of the eleven grass-harvester mound–soil pairs sampled and in all other termite mound–soil pairs studied.

Table 2 presents the spore counts for the largely earthen materials with which *S. actuosus* builds the extensive sheathings on the stems of trees sampled in the Weipa environment. Few or no spores were found in workings from the materials sampled in well-drained sites while materials from two trees at the seasonally inundated sites had higher spore densities. This may occur because the termites are constrained to use near-surface soil materials in building their constructions, at least for the wetter part of the year.

As with the spore counts, the mean numbers of spore types found in the mound materials were generally higher than those in the adjacent surface soils (Table 1), except for sites lacking ECM hosts. This was so in nine of the eleven mound–soil pairs of the grass-harvesting species and in all mound–soil pairs of the litter-feeding, wood-feeding and polyphagous termites examined. In contrast, the mean numbers of spore types present in the sheathing formed over the tree stems by *S. actuosus* were lower than those of the surrounding soils.

Table 3 presents the percentages of fine root lengths colonised by ECM fungi on *Eucalyptus setosa* plants grown on the mound materials of a number of termite species of differing ecological strategies. Most of the mound materials contained viable inoculum, including six of the eight samples from grass-harvesting species and all samples from those of the litter-feeding species tested. The external capping of the single mound of *C. acinaciformis* contained viable inoculum while the carton centre material did not. The intermediate nest material of the decayed-wood feeder *Ephelotermes melachoma* (Miller, 1991) also contained viable inoculum.

Colonisation of the fine roots of *Eucalyptus setosa* seedlings grown in materials sampled from different positions in the mounds of *Amitermes laurensis* and *Amitermes vitiosus* showed that viable inoculum may occur in all parts of the mounds. This may not be consistent, as shown by the *Amitermes laurensis* mound materials from Nhulunbuy where only those from the apical third were able to initiate colonisation.

Table 4 presents the genera of spores identified from the mounds and over-bark workings of seven species of termites. The most common spore taxon was *Pisolithus* which occurred in the mounds of all species – and workings on tree stems – from all open forest and woodland locations. Other common taxa were those of the family Boletaceae – largely *Boletus* – and *Amanita*, *Nothocastorium* and *Scleroderma*. While considerable overlaps occurred in the taxa found in mounds compared with soils adjacent to nearby host species, many unknown spore types were found and it is considered premature to judge how closely the communities of the mounds resemble those of the surrounding soils.

Species of the common ECM fungal genera *Pisolithus* and *Scleroderma* have been noted to fruit on the surfaces of the mounds of the termites *Amitermes laurensis* and *Nasutitermes longipennis*, even though the nearest putative host plants may have been located some metres away. The *Eucalyptus setosa* seedlings grown in sterilised soil and inoculated with a spore suspension made up from one of these sporocarps developed the root sheathing characteristic of ECM colonisation. This also indicates the presence of hyphae within the mound and connection to the roots of an ECM host tree although such roots need not necessarily occur within the mounds.

CONCLUDING REMARKS

It is concluded that ectomycorrhizal fungi occur regularly in the mounds of termites of a wide range of ecological strategies, whenever suitable host plant species occur. Populations of viable ECM and AM fungi have now been found in the mounds of a range of termites and it seems likely that they play an important role in assisting to extract nutrient elements from these 'hot spots' to the surrounding vegetation. It will be of interest to discover whether these fungi occur within the mounds of termite species belonging to strategies not represented in the present sampling, notably the Macrotermitinae.

ACKNOWLEDGEMENTS

Dr L. Miller, Division of Entomology, CSIRO, identified a number of the termites. We also acknowledge the able technical assistance of Ms S. Joyce, CSIRO Land and Water, during the course of the study.

REFERENCES

Aberdeen, J.E.C. (1979) *Introduction to the Mushrooms, Toadstools and Larger Fungi of Queensland,* Queensland Naturalist Club, Australia.

Castellano, M., Trappe, J.M., Maser, Z. and Maser, C. (1989) *Key to Spores of the Genera of Hypogeous Fungi of North Temperate Forests with Special References to Animal Mycophagy*. Mad River Press Inc., Eureka, California. pp. 4-5.

Gordon, V. and Comport, S. (1998) A comparison of three methods for extraction of spores of ectomycorrhizal fungi from mammal scats. *Mycologia*, *90*, 47-51.

Gunness A.G., Lawrie J.W. and Foster, M.B. (1987) *Land Units of the Weipa Environs, 1:24 000 Map and Explanatory Notes*, Unpublished Report to Comalco Mineral Products, Weipa.

Harinikumar K.M. and Bagyaraj D.J. (1994) Potential of earthworms, ants, millipedes, and termites for dissemination of vesicular-arbuscular mycorrhizal fungi in soil. *Biology and Fertility of Soils,* **18**, 115-118.

Largent, D.L. (1977) *How to Identify Mushrooms to Genus I: Macroscopic Features*, Mad River Press Inc., California.

Largent, D.L. and Thiers, H.D. (1977) *How to Identify Mushrooms to Genus II: Field Identification of Genera,* Mad River Press Inc., California.

Largent, D.L., Johnson, D. and Watling, R. (1977) *How to Identify Mushrooms to Genus III: Microscopic Features,* Mad River Press Inc., California.

Miller, L.R. (1991) A revision of the *Termes-Capritermes* branch of the Termitinae in Australia. *Invertebrate Taxonomy*, **4**(6), 1147-1282.

Mueller, G.M. (1992) Systematics of *Laccaria* (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types. *Fieldiana Botany*, **30,** 1-158.

Stuntz, D.E. (1977) *How to Identify Mushrooms to Genus IV: Keys to Families and Genera*, (eds D.L. Largent and R. Watling), Mad River Press Inc., California.

Tracey, J.G. (1982) *The Vegetation of the Humid Tropical Region of North Queensland,* CSIRO, Melbourne.

Table 1. Spore population and species richness in the mounds of selected termites and in their surrounding surface soils close to the nearest putative host species (CQ, central Queensland; NQ, northeastern Queensland; NWQ northwestern Queensland; NT Northern Territory, Jabiru and Arnhem Highway sites, the Nhulunbuy sites are on the Gove Peninsula, on the western side of the Gulf of Carpentaria; Weipa is located on the western side of Cape York Peninsula).

			Termite mound		Adjacent soil	
Species	**Location**	**Environ-ment**	**Mean number of spores g^{-1}**	**Mean number of spore types**	**Mean number of spores g^{-1}**	**Mean number of spore types**
Grass-harvesting species						
Drepanotermes rubriceps	Blackwater area (CQ)	open forest	2400	4.0	7083	5.9
D. rubriceps	Moranbah-Clermont road (CQ)	open forest	39 500	4.7	12 000	3.0
D. rubriceps	Century Zinc lease, NWQ	open woodland	25 000	2.0	117 000	3.0
Nasutitermes triodiae	Arnhem Highway(NT)	open woodland	51 000	4.7	36 000	3.2
N. triodiae	Arnhem Highway(NT)	open woodland	45 000	3.5	35 000	2.0
N. triodiae	Arnhem Highway(NT)	open woodland	49 333	4.3	17 333	3.0
N. triodiae	Arnhem Highway(NT)	open woodland	64 000	3.0	16 000	2.7
N. triodiae	Arnhem Highway(NT)	open woodland	66 500	3.2	8 000	1.0
N. triodiae	Arnhem Highway(NT)	open woodland	150 000	6.0	54 000	2.2
N. triodiae	Weipa area (2c)	open forest	74 000	5.0	6 000	1.2
N. triodiae	Weipa area (2c)	open forest	120 000	2.0	0	0.0

Table 1 (cont.)

			Termite mound		Adjacent soil	
Species	**Location**	**Environ-ment**	**Mean number of spores g^{-1}**	**Mean number of spore types**	**Mean number of spores g^{-1}**	**Mean number of spore types**
Litter-feeding species						
Amitermes laurensis	Century Zinc lease (NWQ)	disturbed woodland	48 000	4.0	117 000	1.0
A. laurensis	Andoom Mine (Weipa)	open forest	121 600	2.0	0	0.0
A. laurensis	Weipa Mine	open forest	54 000	5.2	48 000	4.5
A. laurensis	Weipa Mine	open forest	104 000	2.0	9600	1.2
Polyphagous species						
Nasutitermes longipennis	Blackwater area (CQ)	open forest	7592	9.0	7083	5.9
N. longipennis	Blackwater Mine (CQ)	rehabilitated minesite	967	3.0	0	0.0
N. longipennis	Maraboon Dam Road (CQ)	unvegetated road verge	250 000	4.0	124 000	2.0
N. longipennis	Maraboon Dam Road (CQ)	unvegetated road verge	1 368 000	3.8	124 000	2.0
N. longipennis	Weipa township	workings on base of tree	104 000	2.0	96 000	1.2

Table 1 (cont.)

			Termite mound		Adjacent soil	
Species	**Location**	**Environ-ment**	**Mean number of spores g^{-1}**	**Mean number of spore types**	**Mean number of spores g^{-1}**	**Mean number of spore types**
Wood-feeding species						
Microcerotermes turneri	Davies Creek (NEQ)	dry sclerophyll with *Allocasuarina*	101 250 000	2.2	38 658	1.6
M. turneri	Davies Creek (NEQ)	dry sclerophyll	4 557 500	2.5	16 500	1.4
M. turneri	Davies Creek (NEQ)	wet sclerophyll	250	1.0	192	0.0
M. turneri	Davies Creek (NEQ)	closed forest	946	1.0	0	0.0
M. turneri	Mt Lewis (NEQ)	closed forest	0	0	0	0
M. turneri	Kuranda Range (NEQ), site 1	closed forest	15 796	1.7	9583	0.7
M. turneri	Kuranda Range (NEQ), site 2	closed forest	0	0	0	0
Coptotermes acinaciformis	Weipa	open forest	capping: 16 000	capping: 2.0	17 000	1.0
			centre: 136 000	centre: 4.0		

Table 2. Mean spore populations and spore types recorded from workings created by the wood-feeding termite *Schedorhinotermes actuosus* on the stems of trees in the Weipa area.

Location	**Environment**	**Workings on tree stems**		**Adjacent soil**	
		Mean spore count	**Mean number of spore types**	**Mean spore count**	**Mean number of spore types**
Andoom Mine	Open forest, 5k	0	0	12 000	3.00
Weipa township	Urban parkland	0	0	0	0
Weipa Mine	Open forest (7b)	12 800	2.60	52 000	2.80
Andoom Mine	Open forest (2c)	66 800	2.00	42 400	3.80

Table 3. Presence of ECM infection on the roots of *Eucalyptus setosa* grown on the mound materials of selected termites. Unless otherwise indicated, all mound samples were collected from northeastern Queensland between the latitudes of 17° and 20° S.

Termite species	Mound type	Site name	% of root length infected
Grass-harvesting species			
Drepanotermes rubriceps	mound	Toomba 1	45
D. rubriceps	mound	Toomba 2	36
D. rubriceps	mound	Antill Plains	4
D. rubriceps	mound	Mt Cutheringa	0
Nasutitermes triodiae	mound	Arnhem Highway (NT)	35
N. triodiae	mound	Arnhem Highway (NT)	5
N. triodiae	mound	Mt Molloy area	0
Tumulitermes pastinator	mound	Jabiru (NT)	54
Amitermes laurensis (1)	mound apex	Nhulunbuy (NT)	52
A. laurensis (1)	mound centre	Nhulunbuy (NT)	0
A. laurensis (1)	mound base	Nhulunbuy (NT)	0
A. laurensis (2)	mound apex	Antill Plains	66
A. laurensis (2)	mound base	Antill Plains	25
A. laurensis (3)	mound apex	Townsville	52
A. laurensis (3)	mound base	Townsville	1
Amitermes vitiosus (1)	mound apex	Toomba (1)	5
A. vitiosus (1)	mound centre	Toomba (1)	16
A. vitiosus (1)	mound base	Toomba (1)	2
A. vitiosus (2)	mound base	Toomba (2)	2
A. vitiosus (2)	mound centre	Toomba (2)	1
A. vitiosus (2)	mound base	Toomba (2)	10

Table 3 (cont.)

Termite species	**Mound type**	**Site name**	**% of root length infected**
A. vitiosus (3)	mound	Toomba (2)	36
A. vitiosus (3)	mound apex	Toomba (3)	32
A. vitiosus (3)	mound centre	Toomba (3)	35
A. vitiosus (3)	mound base	Toomba (3)	25
Wood-feeding termites			
Coptotermes acinaciformis (1)	carton centre	Jabiru (NT)	0
C. acinaciformis (1)	earthen capping	Jabiru (NT)	10
*Ephelotermes melachoma**	intermediate nest	Arnhem Highway (NT)	64

* Note that this species feeds on partially weathered and decaying wood (Miller, 1991).

Table 4. The presence of spores of the selected genera in the mounds and workings of seven termite species (**Al** *Amitermes laurensis* ; **Dr** *Drepanotermes rubriceps* ; ***Ca*** *Coptotermes acinaciformis* ; **Mt** *Microcerotermes turneri* ; **Nl** *Nasutitermes longipennis* ; ***Nt*** *Nasutitermes triodiae*; **Sa** *Schedorhinotermes actuosus*).

Genera of spores	**Termite species**						
	Al	**Dr**	**Ca**	**Mt**	**Nl**	**Nt**	**Sa**
No. of mounds sampled	5	3	2	7	5	8	4
Amanita	2	2	1	0	1	3	1
Boletaceae	2	2	1	1	2	4	0
Cantharellus	1	0	0	0	0	1	0
Elaphomyces	0	0	0	1	0	0	0
Gautieria	0	0	0	1	0	0	0
Gummiglobus	0	0	0	0	0	0	0
Laccaria	0	1	0	0	2	0	0
Leccinum	0	0	0	0	1	0	0
Mesophelia	1	0	0	0	0	0	0
Nothocastorium	2	0	0	0	0	5	1
Pisolithus	4	2	1	1	3	7	2
Ramaria	0	0	0	0	0	0	0
Russula	1	0	0	0	1	0	0
Scleroderma	1	2	2	0	1	2	0
Zelleromyces	0	0	0	2	0	0	0
Unknown taxa	3	2	2	2	4	6	1