

## Ecological relationships among hypogeous fungi and trees: inferences from association analysis integrated with habitat modeling

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**Abstract:** Association analyses by contingency tables and generalized linear modeling were compared to infer relationships among hypogeous (belowground-fruited) ectomycorrhizal fungi and potential host tree species from 136 study plots in forested habitats in southeastern mainland Australia. Results from both types of statistical approaches were highly congruent. As with previous experimental studies, no exclusive fungus-host tree associations were identified. However, the likelihood of occurrence of some species of fungi increased significantly in the presence of particular host tree species, suggesting fungal host preference or shared habitat preferences. Similarly, while most associations among fungal species were nonsignificant, a few taxa were more likely to be found in the presence of certain others. These were termed positively associated and are thought to share common climatic and microhabitat requirements or host preferences. In contrast, other combinations of fungal species were negatively associated with one another, perhaps indicating different habitat preferences. Furthermore, the finding that some fungi occurred more frequently in the presence of certain tree species provides a starting point for selection of

compatible host-fungus combinations that could be used for forest nursery and restoration applications.

**Key words:** *Acacia*, Ascomycetes, Basidiomycetes, ectomycorrhizae, *Eucalyptus*, fungal communities, southeastern Australia

### INTRODUCTION

Ectomycorrhizal fungi are important determinants of ecosystem function. They participate in nutrient cycling and water uptake and may fend off pathogens of feeder rootlets of hosts (Smith and Read 1997). Because of their beneficial effects on plant performance and growth, ectomycorrhizal fungi have been considered pivotal in reforestation and forest restoration programs (Trappe 1977). However, selection of compatible host-fungus combinations is not always simple: Fungi inoculated on nursery seedlings often fail to persist in the root systems and are out-competed by indigenous fungi when the seedlings are planted. Furthermore, ectomycorrhizal fungi easily inoculated in nurseries do not colonize all host species (Lu et al 1998).

Inoculation of eucalypts and introduced ectomycorrhizal trees with selected ectomycorrhizal fungi has been studied in Australia (Burgess et al 1993, Chilvers 1973, Chu-Chou 1979, Dunstan et al 1998, Lu et al 1998, Malajczuk et al 1982, Thomson et al 1994), but the basic ecology, host associations and habitat preferences among Australian ectomycorrhizal fungi and their hosts remain poorly understood. The communities of ectomycorrhizal fungi and host trees in native eucalypt stands need to be identified and the habitat requirements of the component species determined. Candidate fungi then can be experimentally studied for use in reforestation and restoration programs. An understanding of the factors governing species composition of fungal communities also is needed. As a step toward meeting these needs, we have used fungal and host occurrence data from earlier modeling studies to infer potential host-fungus associations that frequently occur under natural conditions in southeastern mainland Australia. We relate these host-fungus associations to tree-tree associations and infer some ecological characteristics of selected fungi based on their occurrence under natural conditions.

TABLE I. Species of hypogeous fungi included in the association analyses preceded by abbreviations (in boldface) used in following tables and figures

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<b>Casrad</b> <i>Castoreum radicum</i> Cooke & Masee
<b>Chavit</b> <i>Chamonixia vittatispora</i> G. W. Beaton, Pegler & T. W. K. Young (species complex) <sup>a</sup>
<b>Corglo</b> <i>Cortinarius globuliformis</i> Bougher
<b>Cysmeg</b> <i>Cystangium megasporum</i> (Rodway) T. Lebel & Castellano
<b>Cyssem</b> <i>Cystangium seminudum</i> (Masee & Rodway) T. Lebel & Castellano
<b>Cysse</b> <i>Cystangium sessile</i> (Masee & Rodway) Singer and A. H. Sm.
<b>Desalbe</b> <i>Descomyces albellus</i> (Masee & Rodway) Bougher & Castellano
<b>Desalbu</b> <i>Descomyces albus</i> (Klotzsch) Bougher & Rodway
<b>Desgia</b> <i>Descomyces giachinii</i> Trappe, Oliviera, Castellano & Claridge
<b>Gymebu</b> <i>Gymnomyces eburneus</i> T. Lebel, sp. ined. <sup>b</sup>
<b>Gymwes</b> <i>Gymnomyces westresii</i> T. Lebel, sp. ined. <sup>b</sup>
<b>Hydcar</b> <i>Hydnangium carneum</i> Wallr.
<b>Hydcon</b> <i>Hydnoplicata convoluta</i> (McAlpine) Trappe & Claridge, comb. ined. <sup>b,c</sup>
<b>Hysaff</b> <i>Hysterangium affine</i> Rodway
<b>Hysin</b> <i>Hysterangium inflatum</i> Rodway
<b>Hysrho</b> <i>Hysterangium rhodocarpum</i> Trappe, Claridge & Castellano <sup>b</sup>
<b>Mesgla</b> <i>Mesophellia glauca</i> (Cooke & Masee) D. A. Reid
<b>Prolev</b> <i>Protoglossum levisporum</i> Trappe, Claridge & Castellano, comb. ined. <sup>b,d</sup>
<b>Stefla</b> <i>Stephanospora flava</i> (Rodway) G. W. Beaton, Pegler & T. W. K. Young
<b>Zelcla</b> <i>Zelleromyces claridgei</i> Trappe, sp. ined. <sup>b</sup>
<b>Zelcor</b> <i>Zelleromyces corkii</i> Trappe, sp. ined. <sup>b</sup>
<b>Zeldau</b> <i>Zelleromyces daucinus</i> G. W. Beaton, Pegler & T. W. K. Young
<b>Zelmic</b> <i>Zelleromyces microsporus</i> Trappe, Claridge & Castellano, sp. ined. <sup>b</sup>

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<sup>a</sup> Referred to by Claridge et al (2001a, b) as *Chamonixia vittatispora* but subsequently determined to be a complex of two or more closely related species by Zdravevski (2000).

<sup>b</sup> Manuscript in preparation.

<sup>c</sup> Referred to by Claridge et al (2001a, b) as *Hydnoplicata whitei* but was earlier described as *Cyclospora convoluta* McAlpine (Trappe, unpublished data); although it belongs in the genus *Hydnoplicata*, McAlpine's species epithet has priority.

<sup>d</sup> Referred to by Claridge et al (2001a, b) as *Hymenogaster levisporus* based on the paper by Beaton, Pegler & Young (1985) but subsequently determined by molecular phylogenetic studies to be more closely related to *Protoglossum* than to *Hymenogaster* (Peintner et al 2001).

TABLE II. Tree species included in the association analyses preceded by abbreviations (in boldface) used in figures. Infra-genic classification of *Eucalyptus* is according to Brooker (2000)

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MIMOSACEAE
<b>Acadea</b> <i>Acacia dealbata</i> Link
MYRTACEAE
EUCALYPTUS
Subgen. <i>Eucalyptus</i>
Sect. <i>Aromatica</i>
<b>Eucrad</b> <i>E. radiata</i> Sieber
Sect. <i>Capillulus</i>
<b>Eucmue</b> <i>Eucalyptus muelleriana</i> A. W. Howitt
Sect. Cineraceae
<b>Eucsie</b> <i>Eucalyptus sieberi</i> L. A. S. Johnson
Sect. <i>Eucalyptus</i>
<b>Eucobl</b> <i>Eucalyptus obliqua</i> L'Hér.
Subgen. <i>Symphiomirtis</i>
Sect. Maidenaria
<b>Euccyp</b> <i>Eucalyptus cyellocarpa</i> L. A. S. Johnson
<b>Eucdal</b> <i>Eucalyptus dalrympleana</i> Maiden var. <i>dalrympleana</i>

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TABLE III. Associations among the 23 truffle species and seven host trees. Confidence intervals (95%) of parameter estimates for odds ratios ( $p/1-p$ ; ratio of truffle occurrence to truffle absence among the 136 experimental plots as explained by host tree presence) from generalized linear models, their significance ( $P > X^2$ ; above dash) and results from two by two contingency table tests ( $P > X^2$ , below dash). Odds ratio  $>1$  indicates greater likelihood of a truffle species (rows) occurrence with a host tree (columns). +++ or --- =  $p < 0.001$ ; ++ or -- =  $0.001 \leq p < 0.01$ ; + or -;  $0.01 \leq p < 0.05$ ; (+) or (-) =  $0.05 \leq p < 0.10$ ; positive association indicated by +; negative association indicated by -. Incongruences between the results from contingency tables and GLIMs are bold-faced

Truffle species	Host species		
	<i>A. dealbata</i> Odds ratio $p > X^2/p > X^2$	<i>E. cybellocarpa</i> Odds ratio $p > X^2/p > X^2$	<i>E. dabrympleana</i> Odds ratio $p > X^2/p > X^2$
<i>Castoreum radicum</i>	0.09–0.96 (-)/(-)	0.23–1.77 ns/ns	0.59–3.97 ns/ns
<i>Chamonixia spp.</i>	0.70–3.74 ns/ns	1.25–8.85 +/+	0.58–3.56 ns/ns
<i>Cortinarius globuliformis</i>	0.38–1.67 ns/ns	0.62–2.8 ns/ns	1.07–5.7 +/+
<i>Cystangium megalosporum</i>	0.54–3.42 ns/ns	0.48–3.19 ns/ns	0.50–3.67 ns/ns
<i>Cystangium seminudum</i>	1.75–8.17 +++/+++	0.54–2.53 ns/ns	0.58–3 ns/ns
<i>Cystangium sessile</i>	1.02–4.62 +/+	0.9–4.22 (+)/(+)	0.86–4.44 ns/ns
<i>Decomyces albellus</i>	1.79–8.41 +++/+++	0.31–1.46 ns/ns	1.14–5.99 +/+
<i>Decomyces albus</i>	0.96–4.85 (+)/(+)	0.53–2.86 ns/ns	0.49–2.97 ns/ns
<i>Descomyces giachinii</i>	0.91–5.26 (+)/(+)	0.16–1.4 ns/ns	<b>0.97–6.11</b> (+)/+
<i>Gymnomyces eburneus</i>	0.68–3.39 ns/ns	0.55–2.86 ns/ns	0.81–2.86 ns/ns
<i>Gymnomyces westresii</i>	0.51–2.77 ns/ns	0.12–1 (-)/(-)	0.17–1.42 ns/ns
<i>Hydangium carneum</i>	2.29–11.47 +++/+++	<b>0.18–0.9</b> (-)/-	1.55–8.58 +/+
<i>Hydnoplicata convoluta</i>	0.35–1.51 ns/ns	1.21–6.17 +/+	0.64–3.32 ns/ns
<i>Hymenogaster levisporus</i>	0.10–0.61 -/-	1.05–4.9 +/+	0.12–0.87 -/-
<i>Hysterangium affine</i>	<b>0.07–0.68</b> -/-	0.48–2.72 ns/ns	<b>0.24–1.79</b> ns/-
<i>Hysterangium inflatum</i>	0.17–0.82 -/-	<b>1.30–6.23</b> +/+	0.14–0.83 -/-
<i>Hysterangium rhodocarpum</i>	0.23–1.36 ns/ns	0.50–2.72 ns/ns	0.28–1.9 ns/ns
<i>Mesophellia glauca</i>	0.26–1.62 ns/ns	0.59–3.25 ns/ns	0.42–2.72 ns/ns
<i>Stephanospora flava</i>	0.07–0.72 -/-	3.39–19.69 +++/+++	<b>NA NA NA</b> -/-
<i>Zelleromyces claridgei</i>	1.11–5.31 +/+	2.08–5.42 +/+	1.15–6.96 +/+
<i>Zelleromyces corkii</i>	0.52–2.44 ns/ns	0.52–2.48 ns/ns	0.09–0.76 -/-
<i>Zelleromyces daucinus</i>	<b>1.79–8.5</b> +++/+++	0.83–3.97 ns/ns	0.41–2.29 ns/ns
<i>Zelleromyces microsporus</i>	0.95–4.35 (+)/(+)	0.58–2.64 ns/ns	0.45–2.27 ns/ns

Host-fungus associations or host growth promoting effects of mycorrhizal fungi often have been studied by experimental inoculation studies (Bougher et al 1990, Burgess et al 1993, Chilvers 1973, Molina 1979, Molina and Trappe 1992, Thomson et al 1994, Thomson et al 1996). However, fungi often perform differently in the laboratory than in their natural environment. For example, certain media may cause atypical colonization of roots by ectomycorrhizal fungi in vitro and result in tissue disorganization more characteristic of pathogenic or parasitic infections (Duddridge 1986, Duddridge and Read 1984). Laboratory data can be difficult to extrapolate to field conditions: Fungi forming mycorrhizae in laboratory may fail to do so in the field. Pure culture studies may indicate host ranges wider than expected from sporocarp occurrence (Grand 1968, Molina 1979, 1981) because of incompatible habitat requirements among the hosts and the fungi or an inability of some fungi to compete with others under certain field conditions. Field trials addressing host and habitat com-

patibility are needed to rationalize application of mycorrhizal fungi in forestation and habitat restoration programs.

Claridge et al (2000a, b) described a modeling approach to understand ecological relationships of hypogeous mycorrhizal fungi in southeastern mainland Australia. They related various habitat parameters to occurrence of several widespread species. Climatic parameters (temperature and moisture) were determined as significant explanatory variables. Additional local microhabitat parameters, such as litter depth and topography, were related to the occurrence of certain fungal taxa indicating possible habitat preferences among fungi. Those results could be used to define the realized niche space in terms of the environmental optima most frequently associated with the occurrence of a given fungus (Morin 1999).

We have used data from systematic censuses of hypogeous fruit bodies and potential host trees to infer associations among ectomycorrhizal fungi and their hosts. Although association analyses have been criti-

TABLE III. Extended

Host species			
<i>E. muelleriana</i>	<i>E. obliqua</i>	<i>E. radiata</i>	<i>E. sieberi</i>
Odds ratio $p > X^2/p > X^2$			
0.44–3.16 ns/ns	<b>0.04–1.05</b> ns/(–)	0.13–1.92 ns/ns	0.42–3.42 ns/ns
0.58–3.56 ns/ns	<b>1.90–44.26</b> ++/++	0.46–3.67 ns/ns	<b>2.97–270.43</b> ++/+++
0.39–1.95 ns/ns	0.69–3.97 ns/ns	0.44–2.8 ns/ns	1.02–6.17 +/ns
0.25–2.25 ns/ns	0.88–6.36 (+)/(+)	1.54–11.59 ++/++	0.21–2.27 ns/ns
0.26–1.49 ns/ns	<b>1.01–5.75</b> +/ns	0.76–4.9 ns/ns	0.35–2.14 ns/ns
0.50–2.64 ns/ns	0.57–3.32 ns/ns	3.16–28.22 ++/++	<b>0.11–0.95</b> ns/–
0.28–1.48 ns/ns	0.25–1.52 ns/ns	0.68–4.35 ns/ns	<b>0–0.18</b> –/–
0.22–1.6 ns/ns	0.28–2.18 ns/ns	0.51–3.78 ns/ns	0.13–1.34 ns/ns
0.88–2.53 ns/ns	<b>0.04–1.05</b> ns/+	0.76–5.93 ns/ns	<b>0.01–0.66</b> (–)/–
0.33–2.1 ns/ns	<b>0.25–1.9</b> ns/(–)	0.32–2.56 ns/ns	0.11–1.16 ns/ns
<b>0.85–4.95</b> ns/(+)	<b>0.01–0.49</b> –/–	0.40–3.25 ns/ns	<b>0.01–0.49</b> –/–
0.22–1.2 ns/ns	0.19–1.2 ns/ns	0.82–5.37 ns/ns	<b>0–0.17</b> –/–
0.54–2.77 ns/ns	<b>2.18–21.98</b> ++/+++	0.63–4.22 ns/ns	0.90–5.7 (+)/(+)
1.22–6.36 ++/+	0.57–3.32 ns/ns	0.38–2.59 ns/ns	0.57–3.32 ns/ns
0.32–2.2 ns/ns	0.57–3.82 ns/ns	0.18–1.92 ns/ns	0.93–5.87 (+)/(+)
1.52–8.76 ++/+++	1.02–6.17 ++/+	0.16–1.15 ns/ns	0.57–3.22 ns/ns
0.59–3.42 ns/ns	0.63–4.01 ns/ns	0.24–2.18 ns/ns	0.37–2.61 ns/ns
0.32–2.2 ns/ns	0.93–5.87 (+)/(+)	0.10–1.42 ns/ns	1.17–7.24 ++/+
<b>1.67–9.58</b> ++/+++	<b>0.98–6.23</b> (+)/+	0.10–1.49 ns/ns	0.59–4.01 ns/ns
0.34–1.73 ns/ns	1.22–9.03 ++/+	1.13–9.78 ++/+	0.37–2.08 ns/ns
0.44–2.41 ns/ns	1.39–8.17 ++/+++	0.68–4.39 ns/ns	0.62–3.6 ns/ns
0.41–2.29 ns/ns	2.92–19.49 +++/+++	<b>1.92–13.33</b> ++/+++	0.19–1.46 ns/ns
0.38–1.9 ns/ns	0.85–5.16 ns/ns	0.72–4.85 ns/ns	0.26–1.49 ns/ns

cized as being correlative, thus precluding inferences on causality (Olofsson et al 1999), they can identify co-occurrence patterns in plant communities (e.g. Blundon et al 1993, Choler et al 2001, Kikvidze 1993, Kikvidze and Nakhutsrishvili 1998, Sohlberg and Bliss 1984, Whitehead 1951). These co-occurrences often relate to positive mechanisms such as facilitation in plant communities (Blundon et al 1993, Choler et al 2001, Kikvidze 1993, Kikvidze and Nakhutsrishvili 1998). Thus, association analyses are valuable starting points. They help decipher relationships in large datasets and guide design of experimental studies.

We have used data on hypogeous sporocarp occurrence from four sampling events (spring and autumn 1996 and autumn 1999 and 2001) across several environmental gradients (Claridge et al 2000b). This dataset comprised 4150 collections of hypogeous fungi representing more than 200 species. Specific goals of our analyses were to infer associations: (i) between hypogeous fungus species and host species, (ii) among the common species of hypogeous fungi, and

(iii) among the common species of host trees. The three types of associations were evaluated individually and together to infer ecological relationships. The conclusions of our study were compared with those from earlier habitat modeling studies (Claridge et al 2000a) to learn more about the habitat and host relationships of those fungi. Finally, we compared results obtained from traditional contingency table analyses with the more flexible, generalized linear modeling analyses.

#### METHODS

Methods of data collection are described in Claridge et al (2000b). In brief, 136 sites were selected in East Gippsland, Victoria, and adjacent New South Wales, Australia, and stratified by climate, geology and topography to represent dominant habitat types in the area. Within each site, a 50 × 20 m rectangle was aligned with the long axis along a contour. Hypogeous fruit bodies were revealed by raking with a four-tined garden cultivator (Castellano et al 1989)

TABLE IV. Associations among the 23 truffle species. Confidence intervals (95%) of parameter estimates for odds ratio ( $p/1 - p$ ; ratio of truffle occurrence to truffle absence among the 136 experimental plots as explained by presence of other truffle species) from generalized linear models, their significance ( $P > X^2$ ; above dash) and results from two by two contingency table tests ( $P > X^2$ ; below dash). Odds ratio  $>1$  indicates greater likelihood of one truffle species (rows) occurrence with another (columns). +++ or --- =  $p < 0.001$ ; ++ or -- =  $0.01 \leq p < 0.01$ ; + or -;  $0.01 \leq p < 0.05$ ; (+) or (-) =  $0.05 \leq p < 0.10$ ; positive association indicated by +; negative association indicated by -. Incongruences between the results from contingency tables and GLIM are bold.

	Casrad	Chavit	Corglo	Cysmeg
<b>Casrad</b>				
<b>Chavit</b>	0.36–2.20 ns/ns			
<b>Corglo</b>	<b>1.35–8.50</b> +/+ +	1.30–6.11 +/+ + +		
<b>Cysmeg</b>	0.05–1.11 ns/ns	0.47–3.29 ns/ns	0.22–1.34 ns/ns	
<b>Cyssem</b>	0.20–1.28 ns/ns	0.79–3.82 ns/ns	0.73–3 ns/ns	1.35–8.17 +/+ + +
<b>Cysses</b>	0.27–1.70 ns/ns	0.88–4.39 ns/ns	0.61–2.48 ns/ns	0.62–3.63 ns/ns
<b>Desalbe</b>	0.18–1.15 ns/ns	0.28–1.23 ns/ns	0.44–1.70 ns/ns	0.42–2.46 ns/ns
<b>Desalbu</b>	0.03–0.69 -/-	0.26–1.30 ns/ns	0.25–1.21 ns/ns	0.54–3.71 ns/ns
<b>Desgia</b>	0.04–1.01 (-)/(-)	0.12–0.76 -/- -/-	0.19–1.12 (-)/(-)	0.31–2.83 ns/ns
<b>Gymebu</b>	0.42–2.75 ns/ns	0.43–2.22 ns/ns	0.83–3.42 ns/ns	0.61–3.94 ns/ns
<b>Gymwes</b>	0.21–1.79 ns/ns	0.13–0.68 -/- -/-	0.40–1.93 ns/ns	0.30–1.57 ns/ns
<b>Hydcar</b>	0.18–1.12 (-)/(-)	0.36–0.73 -/- -/-	0.36–1.43 ns/ns	0.26–1.60 ns/ns
<b>Hydcon</b>	0.45–2.51 ns/ns	1.45–6.55 +/+ + + +	1.84–7.77 +/+ + + + +	0.83–5.31 ns/ns
<b>Hysaff</b>	1.36–8.25 +/+ + + +	0.45–2.51 ns/ns	0.77–3.78 ns/ns	0.08–1.16 ns/ns
<b>Hysinf</b>	0.76–4.31 ns/ns	0.42–1.82 ns/ns	0.64–2.48 ns/ns	0.17–0.92 (-)/(-)
<b>Hysrho</b>	0.74–4.57 ns/ns	0.38–1.82 ns/ns	1.12–5.47 +/+	0.13–1.35 ns/ns
<b>Mesgla</b>	0.68–4.39 ns/ns	0.65–3.97 ns/ns	1.48–8.00 +/+ + + +	0.60–4.10 ns/ns
<b>Prolev</b>	0.65–3.67 ns/ns	0.41–1.90 ns/ns	1.01–4.22 +/+	0.15–1.97 ns/ns
<b>Stefla</b>	0.08–1.08 ns/(-)	0.42–2.39 ns/ns	0.25–1.28 ns/ns	0.82–5.42 ns/ns
<b>Zlecla</b>	0.28–1.52 ns/ns	0.53–2.32 ns/ns	1.03–4.31 +/+	1.13–8.41 +/+
<b>Zelcor</b>	0.04–0.57 -/- -/-	0.68–3.35 ns/ns	0.35–1.48 ns/ns	1.52–9.39 +/+ + + +
<b>Zeldau</b>	0.08–0.85 -/-	0.81–4.31 ns/ns	<b>0.18–0.79</b> -/- -/-	2.64–17.81 +/+ + + + + +
<b>Zelmic</b>	0.16–0.93 -/-	0.34–4.06 ns/ns	0.48–1.86 ns/ns	1.82–15.49 +/+ + + +

and collected during a time-constraint sampling of 100 person-minutes. The species of potential ectomycorrhizal host trees and shrubs also were recorded for each site together with a range of other microhabitat attributes.

Fresh fungal fruit bodies were described with emphasis on size range, shape, surface texture and color, bruising reactions, odor, peridium thickness and the color and general texture of the gleba. These characteristics were used to tentatively assign collections to genera in the field. Collections were dried in a portable dehydrator and stored until identified to species by use of existing published and unpublished taxonomic keys and descriptions. Many of the species and some genera are undescribed and being prepared for publication. Reference collections are available at the National Herbarium, Canberra (CANB), National Herbarium of Victoria (MEL) and Oregon State University Herbarium (OSC).

The dataset from the 136 plots included more than 220 hypogeous fungal species and 26 species of ectomycorrhizal hosts. The fungi and hosts were coded as present or absent for each field site. Twenty-three species of hypogeous fungi (TABLE I) and seven species of hosts (TABLE II) occurred on 25 or more of the 136 plots, 25 being the threshold frequency we set for our association analyses. Resultant presence/absence data were arranged into two-by-two contingency tables that provide a measure of correlation or rel-

ative closeness of association among the taxa (Dighton 1994). The independence of the cell counts was evaluated by  $\chi^2$  tests for fixed margin totals (Sokal and Rohlf 1995), with the number of plots (136) fixed for both hypogeous fungi and trees. These analyses were performed in SAS program PROC FREQ (SAS 1989). When the cell counts were significantly independent as judged by the  $\chi^2$  tests, the direction of observed associations was inferred from comparison of the observed and expected cell counts. If the observed value for a cell for co-occurrence of two taxa was greater than the expected value for that cell, the association was considered positive. If the observed value was less than the expected value, the association was considered negative.

To confirm results from the contingency tables, we also analyzed the data using generalized linear models (GLIM). Generalized linear models use Bernoulli distributed data (binary, i.e., presence/absence) to estimate effects of multiple explanatory variables on probability of response (here, occurrence of another host species or truffle;  $p$ ) or the odds (here, ratio of occurrence of another host species or truffle;  $p/1 - p$ ) (McCullagh and Nelder 1989, Nicholls 1991, Yee and Mitchell 1991). The odds ratio is best interpreted as a chance of an event. Odds ratios of 1 (a 50:50 chance) indicate an equal probability of the two possible outcomes (presence and absence of a taxon). Similarly, an odds ratio of 10 indicates that the estimated event ( $p$ : here

TABLE IV. Extended

Cyssem	Cysses	Desalbe	Desalbu
1.09–4.66 +/+			
1.45–6.17 ++/++	0.80–3.32 ns/ns		
0.31–1.62 ns/ns	0.47–2.34 ns/ns	1.34–6.62 ++/++	
1.57–9.78 ++/++	0.53–3.03 ns/ns	1.15–6.69 +/+	1.22–7.32 +/+
1.01–4.66 +/+	1.05–4.90 +/+	0.79–3.6 ns/ns	0.35–2.01 ns/ns
0.51–2.59 ns/ns	0.64–3.19 ns/ns	1.35–6.96 ++/++	0.84–4.62 ns/ns
1.07–3.86 (+)/(+)	0.67–2.92 ns/ns	7.03–38.47 +++/+++	2.10–11.36 +++/+++
0.38–1.52 ns/ns	0.87–3.67 ns/ns	0.35–1.36 ns/ns	0.39–1.84 ns/ns
0.23–1.31 ns/ns	0.36–1.93 ns/ns	0.11–0.63 --/--	0.14–1.15 ns/ns
0.44–1.79 ns/ns	0.47–1.92 ns/ns	0.80–3.16 ns/ns	0.35–1.65 ns/ns
0.36–1.80 ns/ns	0.63–3.03 ns/ns	0.49–2.29 ns/ns	0.47–2.66 ns/ns
0.29–1.55 ns/ns	0.44–2.27 ns/ns	0.30–1.51 ns/ns	0.35–2.18 ns/ns
0.36–1.57 ns/ns	0.58–2.48 ns/ns	0.27–1.16 ns/ns	0.21–1.21 ns/ns
0.30–1.67 ns/ns	0.57–2.89 ns/ns	0.14–0.81 --/--	0.36–2.34 ns/ns
1.80–8.58 +++/+++	1.02–4.26 (+)/(+)	1.28–5.37 ++/++	0.41–1.92 ns/ns
0.81–3.46 ns/ns	0.64–2.77 ns/ns	0.35–1.49 ns/ns	0.51–2.56 ns/ns
2.59–12.43 +++/+++	1.15–5.10 +/+	1.32–5.42 +/+	1.38–6.89 ++/++
<b>1.26–5.37</b> ++/++	0.55–2.23 ns/ns	0.89–3.49 ns/ns	0.54–2.53 ns/ns

occurrence of a taxon) is 10 times more likely than the nonevent (1 - p: here absence of a taxon). We estimated the odds ratio of hypogeous fungus occurrence as explained by presence of a host tree or another truffle species, and host tree occurrence was explained by presence of another host tree. All GLIM analyses also were performed in SAS program PROC GENMOD (SAS 1997). To evaluate effects of explanatory variables on the response variable we also estimated the parameters with their 95% confidence intervals.

RESULTS

*Choice of the statistical analysis.*—TABLES III, IV and V display results from analyses by contingency tables and generalized linear models. Overall, results from the two types of analyses were highly congruent. In the tests of associations between host trees and hypogeous fungi, 25 of the 161 pairwise comparisons differed between the two approaches in level of significance (TABLE III). Nineteen of these, however, agreed in the direction of the association. In the tests of associations among the truffle species, only four of 243 pairwise comparisons differed in the level of

significance and only one resulted in different conclusions (TABLE IV). In the tests of associations among the host trees, five of the 21 pairwise comparisons differed between the contingency tables and generalized linear models in level of significance. Three of these four agreed in the direction of the association. In summary, the conclusions of the two different analytical methods largely agreed. One main advantage of using GLIM instead of contingency tables is their ability to estimate the magnitude of the response. For example, based on the odds ratio estimated by GLIM analyses, we can conclude that *Zelleromyces daucinus* occurred 3–19 times more frequently on plots with *Eucalyptus obliqua* than on plots without it. However, because results of the analyses by contingency tables largely agreed with those of the generalized linear regression and their interpretation is simple (Dighton 1994), we present our results according to the contingency tables. Furthermore, the contingency tables are well suited for the specific question posed in our analyses: Do two species occur together more frequently than expected by random chance?

TABLE IV. Extended

	Desgia	Gymebu	Gymwes	Hydcar
Casrad				
Chavit				
Corglo				
Cysmeg				
Cyssem				
Cysses				
Desalbe				
Desalbu				
Desgia				
Gymebu	0.68–4.10 ns/ns			
Gymwes	1.36–8.25 ++/+++	1.15–4.66 (+)/(+)		
Hydcar	1.99–13.46 +++/++++	0.76–2.61 ns/ns	0.79–3.90 ns/ns	
Hydcon	0.13–0.81 –/–	0.57–2.61 ns/ns	0.28–1.36 ns/ns	0.19–0.79 ––/––
Hysaff	0.13–1.38 ns/ns	0.17–1.25 ns/ns	0.21–1.60 ns/ns	0.29–1.45 ns/ns
Hysinf	0.44–2.36 ns/ns	0.34–1.54 ns/ns	0.47–2.27 ns/ns	0.76–2.94 ns/ns
Hysrho	0.18–1.54 ns/ns	0.33–1.90 ns/ns	0.33–2.14 ns/ns	0.34–1.62 ns/ns
Mesgla	0.07–.97 (–)/(–)	0.48–2.72 ns/ns	0.10–1.01 (–)/(–)	0.35–1.45 ns/ns
Prolev	0.20–1.38 ns/ns	0.56–2.64 ns/ns	0.76–3.78 ns/ns	0.19–0.85 –/ns
Stefla	0.31–2.39 ns/ns	0.32–1.97 ns/ns	0.40–2.56 ns/ns	0.03–0.34 –––/–––
Zlecla	0.72–4.26 ns/ns	1.26–6.62 +/+	0.35–1.68 ns/ns	1.07–3.74 (+)/(+)
Zelcor	0.46–2.72 ns/ns	0.30–1.55 ns/ns	0.26–1.48 ns/ns	0.51–2.12 ns/ns
Zeldau	0.65–3.74 ns/ns	0.50–2.46 ns/ns	0.18–1.16 ns/ns	1.19–5.21 +/+
Zelmic	0.53–2.92 ns/ns	0.78–3.63 ns/ns	0.33–1.60 ns/ns	0.66–2.56 ns/ns

*Fungus × tree associations.*—The tests for associations between the qualifying fungi and hosts (TABLE III) do not suggest specificity of any single fungus for any host; in no case did fungus species occur only on plots with a particular host species.

Many of the associations were nonsignificant (TABLE III). According to the  $\chi^2$  tests, the cell counts in the contingency tables were dependent and did not suggest associations deviating from what could have been obtained by random chance. For example, *Castoreum radicans*, *Descomyces albus*, *Gymnomyces eburneus*, *Hysterangium rhodocarpum* and *Zelleromyces microsporus* were not significantly associated with any of the seven host species examined.

Some positive associations were significant (TABLE III, FIGS. 1, 2). These can be interpreted as indicating either or both host preferences of the fungi or similar habitat requirements among the fungi and their hosts. For example, *Cortinariopsis globuliformis* was highly significantly associated with *Eucalyptus dalrympleana*; both occur in cool, montane environments. According to our generalized linear model for this relationship, *C. globuliformis* was 1.07–5.70 times more frequent on sites with *E. dalrympleana* than on sites without. *Hysterangium inflatum*, *Protoglossum levisporum* and *Stephanospora flava* were associated positively with *Eucalyptus cypellocarpa* and *E. muelleriana*, all of which occur either in mild, well-drained or coastal environments. *Cystangium seminudum*, *Desco-*

*myces albellus*, *Hydnangium carneum* and *Zelleromyces daucinus* were highly significantly associated with *Acacia dealbata*. The first two showed either negative or nonsignificant associations with the eucalypt species, whereas the second two had significant positive associations with one or more *Eucalyptus* spp.

Some associations were significantly negative (TABLE III, FIGS. 1, 2). *Acacia dealbata* and *Eucalyptus dalrympleana* were negatively associated with *Hysterangium affine*, *P. levisporum*, and *S. flava*. *Acacia dealbata* also was associated negatively with *H. inflatum* and *E. dalrympleana* with *Zelleromyces corkii*. All these negatively associated fungus species (except *H. affine*) were positively associated with at least one of the four host species forming the positively associated host tree cluster (FIG. 2).

Although no exclusive, specific associations between the host trees and hypogeous fungi were detected, some were strong (TABLE III). Detection of *Hydnoplicata convoluta*, for example, was 2.18–21.98 times more likely on plots with *Eucalyptus obliqua* than on plots without. This odds ratio translates to 69–96% probability of detecting *H. convoluta* when *E. obliqua* was present.

*Fungus × fungus associations.*—Results of most of the fungus × fungus tests were nonsignificant, but the 23 taxa did group into two distinct clusters, each characterized by multiple interconnecting positive asso-

TABLE IV. Extended

Hydcon	Hysaff	Hysin	Hysrho	Prolev
0.39–1.90 ns/ns				
0.55–2.12 ns/ns	1.06–5.42 +/+			
0.58–2.72 ns/ns	1.40–7.54 ++/+++	0.71–3.32 ns/ns		
1.77–11.47 +++/++++	0.38–2.44 ns/ns	0.55–2.69 ns/ns	0.42–2.48 ns/ns	
1.28–5.70 ++/+++	0.90–3.71 ns/ns	0.31–1.60 ns/ns	0.76–3.78 ns/ns	0.30–1.62 ns/ns
0.82–4.31 ns/ns	0.10–0.96 (-)/(-)	1.63–9.39 ++/+++	0.55–3.19 ns/ns	1.35–6.96 ++/+++
1.06–3.71 (+)/(+)	0.29–1.43 (ns/ns)	0.52–2.03 ns/ns	0.38–1.75 ns/ns	0.76–3.19 ns/ns
0.88–6.23 ns/ns	0.32–1.77 ns/ns	0.46–1.92 ns/ns	0.34–1.75 ns/ns	0.41–1.82 ns/ns
0.56–2.41 ns/ns	0.29–1.70 ns/ns	0.48–2.05 ns/ns	0.38–1.99 ns/ns	0.25–1.20 ns/ns
0.64–2.48 ns/ns	0.13–0.70 --/--	0.30–1.16 ns/ns	0.37–1.70 ns/ns	0.28–1.17 ns/ns

ciations (TABLE IV, FIG. 3). The two clusters broadly follow the associations between host trees and fungi (FIGS. 1, 2). The exceptions include fungal species that occur in association with hosts from both of the two host clusters (e.g. *Descomyces giachinii*, *Z. corkii*, *Z. daucus*). Significant positive associations emerged in several other cases (TABLE IV, FIG. 3). The *Chamonixia vittatispora* complex, *C. globuliformis* and *Hydnoplicata convoluta* were highly significantly associated. *Castoreum radicans* was highly significantly associated with *C. globuliformis* but not with the other two; *C. radicans* was highly significantly associated with *Hysterangium affine* as well. *Cortinarius globuliformis* also was significantly to highly significantly associated with *P. levisporum*, *Hysterangium rhodocarpum*, *Mesophellia glauca*, and *Zelleromyces claridgei*. *Cystangium megasporum* was significantly to highly significantly associated with *Cystangium seminudum* and the four *Zelleromyces* spp. Noteworthy clustering occurred within genera. The three *Descomyces* spp. were significantly to highly significantly associated with each other as well as with *H. carneum*. Three of the four *Zelleromyces* spp. were highly significantly associated with each other (*Z. claridgei* was highly significantly associated with *Z. daucus* but not with the other two *Zelleromyces* spp.).

Significant negative associations were less common than positive ones. *Castoreum radicans* showed significant negative associations with *Descomyces albus*,

*Z. corkii*, *Z. daucus* and *Z. microsporus*. The *C. vittatispora* complex showed significant to highly significant negative association with *D. giachinii*, *Gymnomyces westresii* and *H. carneum*. Other significant negative associations included *C. globuliformis* with *Z. daucus*, *Descomyces albellus* with *H. affine* and *S. flava*, *D. giachinii* with *H. convolute*, *H. carneum* with *H. convolute*, *P. levisporum* with *S. flava*, and *H. affine* with *Z. microsporus*.

*Tree × tree associations.*—Our primary objective was to identify associations between fungi and hosts and among the fungi. However, analysis of associations among the tree species adds interpretive value to consideration of ecology of the fungi. The analyses of the seven qualifying species revealed one distinct community type and a group of four species linked in various combinations (TABLE V, FIG. 4). *Acacia dealbata*, *Eucalyptus dabrympleana* and *E. radiata* were highly significantly associated with each other. In contrast, these three either were significantly negatively associated or showed no significant associations with the other four species that grouped together. Among the other four species, *E. cypellocarpa* was significantly to highly significantly associated with both *E. muelleriana* and *E. obliqua*. In addition to *E. cypellocarpa*, *E. muelleriana* was significantly associated with *E. sieberi* but showed a significant negative association with *E. obliqua*.

TABLE IV. Extended

	Mesgla	Stefla	Zlecla	Zelcor	Zeldau
Casrad					
Chavit					
Corglo					
Cysmeg					
Cyssem					
Cysses					
Desalbe					
Desalbu					
Desgia					
Gymebu					
Gymwes					
Hydcar					
Hydcon					
Hysaff					
Hysinf					
Hysrho					
Mesgla					
Prolev					
Stefla	0.52–3.16 ns/ns				
Zlecla	1.07–5.00 (+)/(+)	0.62–3.16 ns/ns			
Zelcor	0.69–3.49 ns/ns	0.73–3.74 ns/ns	0.59–2.48 ns/ns		
Zeldau	0.45–2.41 ns/ns	0.84–4.35 ns/ns	1.51–7.54 ++/++	1.48–6.69 ++/++	
Zelmic	0.54–2.61 ns/ns	0.81–4.14 ns/ns	0.67–2.64 ns/ns	2.44–12.18 +++/+++	1.32–6.05 ++/++

## DISCUSSION

Our study identifies significant associations among the hypogeous fungus species encountered in the course of four sampling events on 136 spatially defined plots. The four sampling events spanned 6 yr to provide reliable estimates of the presence or absence of the most common fungal species. Association analyses do not identify causes of positive or negative associations, but they do provide a basis for testable hypotheses on causes, especially when used in conjunction with modeling approaches such as those of Claridge et al (2000a). Choler et al (2001) noted that association analyses, although criticized by some as being only correlative, prove valuable in determining occurrence of positive interactions in plant communities. The results of our analyses provide an understanding of where individual species and communities of species occur. This understanding is a starting point for selection of compatible fungus-host combinations for forest nursery and restoration applications.

*Fungus × tree associations.*—Although some associations between fungi and trees were significant and positive, none of the 23 truffle species occurred exclusively in association with a single host subgenus or species, indicating no strict host specificity. All seven potential host species, in turn, were associated positively with more than one species of hypogeous fun-

gus. The common hypogeous fungus species included here clearly have broad host ranges but share habitat preferences similar to the hosts they are positively associated with. Perhaps the rarer species in the study are the ones to examine for host specificity, but their scarcity precluded inclusion in the association analyses conducted here.

Earlier studies have failed to detect any ectomycorrhizal fungus specificity to particular eucalypt species (Chilvers 1973, Malajczuk et al 1982), although some host-fungus combinations prove incompatible and form no mycorrhizas in synthesis assays (Lu et al 1998, Malajczuk et al 1982). Our analyses found no host specificity, even though the trees included in our study represent two families (Mimosaceae, Myrtaceae), two genera (*Acacia*, *Eucalyptus*), and within *Eucalyptus* two subgenera and five series. Host specificity of ectomycorrhizal fungi occurs at the genus or rarely at the subgenus level of host trees (Molina et al 1992), so no specificity was expected below the subgenus level in *Eucalyptus*. However, the analyses enable inferences beyond simple fungus-host associations to include shared habitats as well. Data on fungal co-occurrences can be used to identify similarities in habitat requirements among ectomycorrhizal fungi. As a result, from the presence of fruit bodies of one species these data predict that certain others are likely to be present or at least be amenable to that habitat.

TABLE V. Associations among the seven host trees. Confidence intervals (95%) of parameter estimates for odds ratios ( $p/1 - p$ ); ratio of host occurrence to host absence among the 136 experimental plots from generalized linear models, their significance ( $P > X^2$ ; above dash) and results from two by two contingency table tests ( $P > X^2$ ; below dash). Odds ratio  $> 1$  indicates greater likelihood of Species 1 (rows) occurrence with Species 2 (columns). +++ or --- =  $p < 0.001$ ; ++ or -- =  $0.001 \leq p < 0.01$ ; + or - =  $0.01 \leq p < 0.05$ ; (+) or (-) =  $0.05 \leq p < 0.10$ ; positive association indicated by +; negative association indicated by -. Incongruences between the results from contingency tables and GLIMS are bold-faced

Species 1	Species 2					
	<i>Acacia dealbata</i>	<i>Eucalyptus cybellocarpa</i>	<i>Eucalyptus dabrympleana</i>	<i>Eucalyptus muelleriana</i>	<i>Eucalyptus obliqua</i>	<i>Eucalyptus radiata</i>
	Odds ratio	Odds ratio	Odds ratio	Odds ratio	Odds ratio	Odds ratio
	$p > X^2/p > X^2$	$p > X^2/p > X^2$	$p > X^2/p > X^2$	$p > X^2/p > X^2$	$p > X^2/p > X^2$	$p > X^2/p > X^2$
<i>E. cybellocarpa</i>	0.12-0.84 -/-					
<i>E. dabrympleana</i>	3.1-17.99 +++/++++	0.02-0.47 --/--				
<i>E. muelleriana</i>	0.04-.58 --/--	3.67-22.20 +++/++++	NA NA NA/--			
<i>E. obliqua</i>	0.1-1.02 (-)/(-)	2.61-16.44 +++/++++	<b>0.04-0.85</b> (-)/-	0.47-3.42 ns/ns		
<i>E. radiata</i>	2.18-15.49 +++/++++	0.14-1.52 ns/ns	2.29-16.28 +++/++++	<b>0.01-0.68</b> (-)/-	0.58-4.90 ns/ns	
<i>E. sieberi</i>	<b>0-0.79</b> -/--	0.91-5.47 (+)/(+)	<b>0.01-0.54</b> -/--	1.03-6.62 +/+	1.39-9.49 +++/++++	0.01-0.88 (-)/(-)

Most of the observed fungus  $\times$  tree associations were nonsignificant, indicating that factors other than any of the seven host species determined occurrence. Claridge et al (2000a) determined environmental parameters that defined habitat preferences for some common hypogeous fungi in southeastern Australia. At a landscape level, climatic factors, including temperature and moisture, best explained occurrence of some fungal species. Additional microhabitat factors such as disturbance history and soil fertility were correlated with fungal occurrence at a more local scale. These factors likely control the occurrence and distribution of both fungi and host trees.

Interpreting significant negative associations is more straightforward than nonsignificant ones. The fungi clearly differ in their environmental requirements. At each trophic level they manifest a range of environmental conditions under which they occur, thereby defining their niche. The negative associations can be inferred from dissimilar habitat preferences. The negative associations among the hypogeous fungi and their ectomycorrhizal hosts merely indicate that the species occur together less frequently than expected by random chance.

*Fungus  $\times$  fungus associations.*—A major conclusion to be drawn from the analyses of associations among fungal species is that most taxa have evolved individual habitat requirements that thwart definition of consistent fungal communities by association analysis. Nonetheless, the highly significant positive association of the *C. vittatispora* species complex with *C. globuliformis* was interesting because it confirmed an inference from our field observations. *Cortinarius globuliformis* forms distinctive, bright yellow hyphal mats in the upper soil layers. *Chamonixia* fruit bodies were observed within these mats, often side by side with fruit bodies of *Cortinarius*. Although no obvious a side-by-side association was noted with *Hydnoplicata convoluta*, that species was collected commonly at the same sites and highly significantly associated with the other two species.

Three *Descomyces* species were associated highly significantly with *H. carneum*. These four species occur over a great diversity of sites. Those species also commonly appear in disturbed sites, such as within regrowth on clearcuts, road verges or plantations of eucalypts abroad (Trappe and Claridge unpubl data, Giachini et al 2001). This generally nonspecific habitat adaptability, as inferred by lack of significant association with other fungal species, may be hypothesized from our data for *G. westresii*, which show a highly significant association with two of the three *Descomyces* species but otherwise is not associated

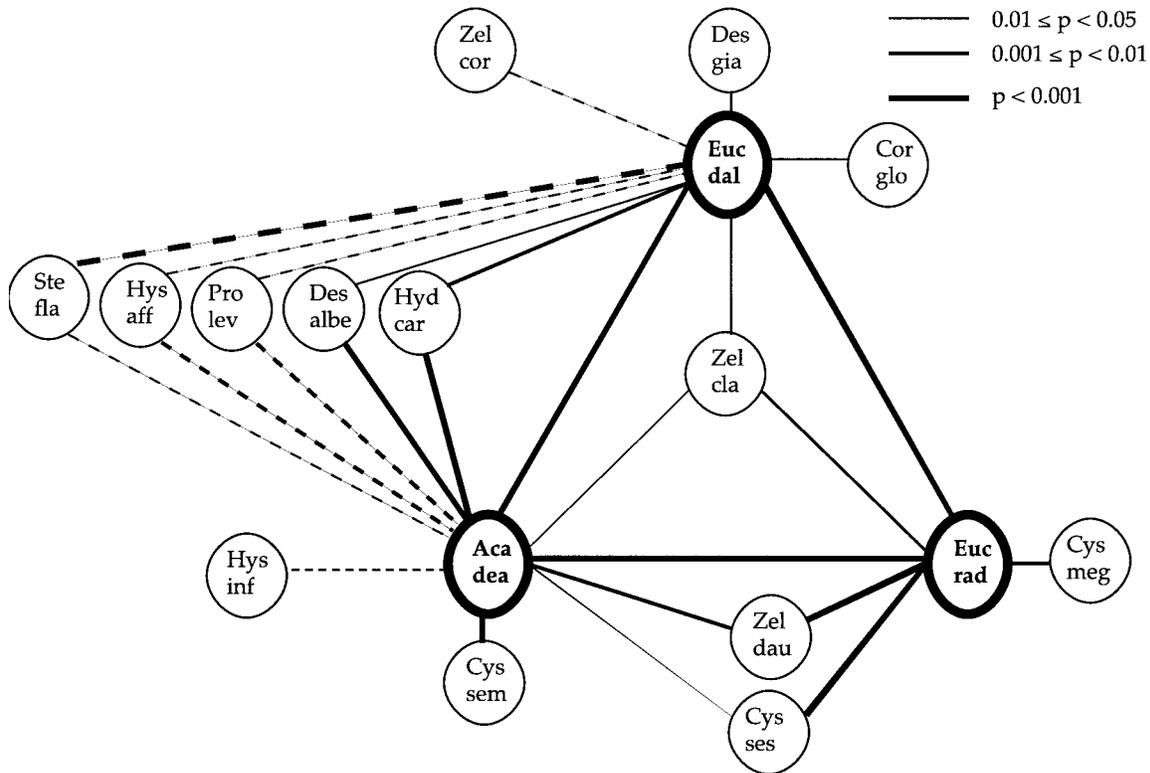


FIG. 1. Positive (solid lines) and negative (dashed lines) associations among *Acacia dealbata*, *Eucalyptus dabrympleana*, *E. radiata* and 14 species of truffles. Thickness of the line indicates the strength of an association as inferred from  $\chi^2$  in the two-by-two contingency tables. Abbreviations for the truffles and their hosts are shown in TABLES I and II.

with other fungi. Fungi with broad habitat adaptability might be good candidates for inoculation of seedlings in container nurseries, especially in ecological restoration programs.

The identification of positive associations among the fungal species also highlight the importance of factors other than the host tree species. *Cortinarius globuliformis*, for example, though associated positively only with *E. dabrympleana*, was associated positively with other fungi that in turn were associated positively with other eucalypt species. Again, nonsignificant, negative and positive associations were observed. The positive associations among the species of fungi suggest either similar host and/or habitat preferences, whereas negative associations infer differences in these preferences.

*Tree × tree associations.*—Although the associations among trees were analyzed particularly to aid in interpretation of fungal associations, the grouping of the seven tree species into two highly significantly negatively associated clusters was unexpected. A close habitat/tree community relationship was revealed between *A. dealbata* and *E. dabrympleana* and *E. radiata*. *Acacia dealbata* is distributed over a wide range of elevations and moisture regimes in southeastern Aus-

tralia, whereas the two eucalypts characteristically inhabit cool, moist and generally montane habitats (Boland et al 1984). The other eucalypts in our analyses mostly inhabit warmer, low-elevation habitats with well drained soils (Boland et al 1984). These data suggest that *A. dealbata* is more habitat specific in our study area than its widespread distribution would have led us to presume. Clearly its habitat requirements involve environmental characteristics beyond inferences from simple association with other tree species.

The other four eucalypt species show a complex set of associations, but all are significantly to highly significantly negatively associated with the *A. dealbata*-*E. dabrympleana*-*E. radiata* cluster. The significantly negative association of *E. muelleriana* and *E. obliqua* indicates differing habitat characteristics of the two, whereas their significant to highly significant association with both *E. cytellocarpa* and *E. sieberi* suggests that the latter two species bridge the habitat ecological gap between the former two.

*Inferences from integrating the three association analyses.*—In general, the fungi positively associated with the *A. dealbata*-*E. dabrympleana*-*E. radiata* cluster either were not significantly or significantly negatively

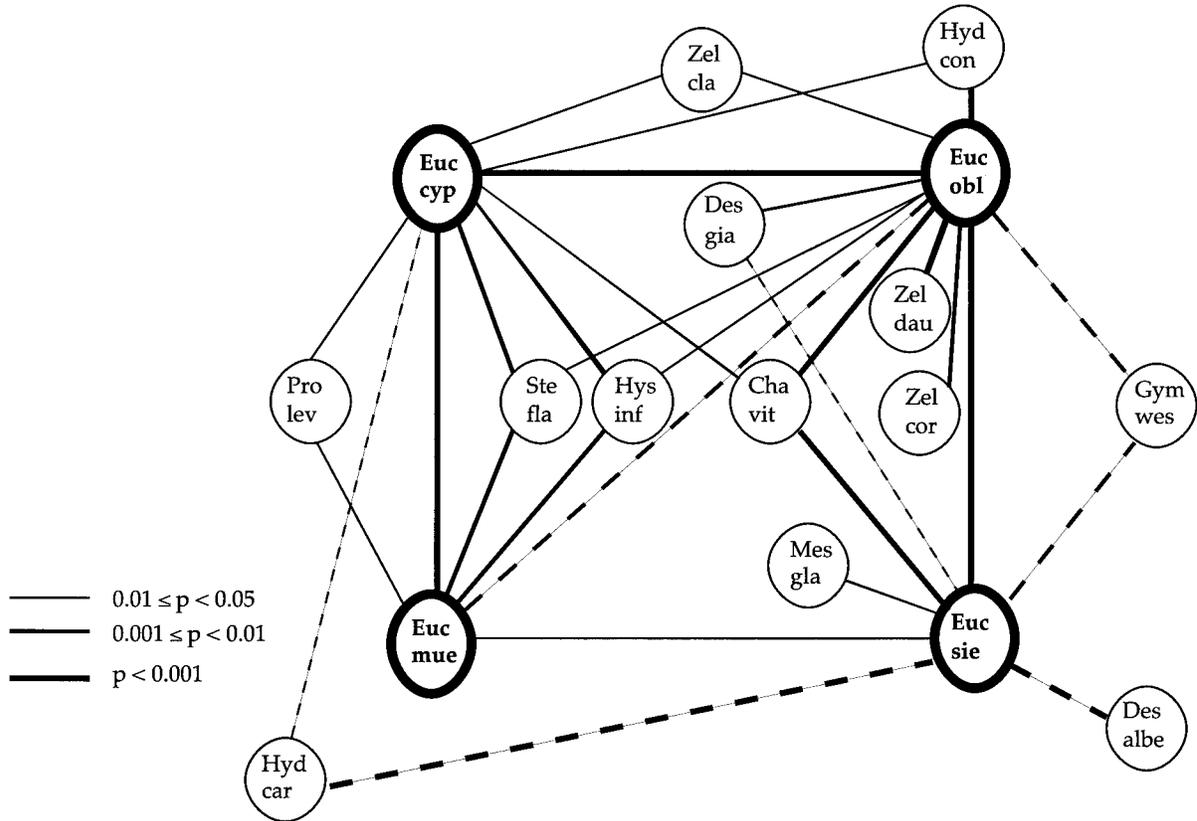


FIG. 2. Positive (solid lines) and negative (dashed lines) associations among *Eucalyptus cypellocarpa*, *E. muelleriana*, *E. obliqua* and *E. sieberi* and 13 species of truffles. Thickness of the line indicates the strength of an association as inferred from  $\chi^2$  in the two-by-two contingency tables. Abbreviations for the truffles and their hosts are shown in TABLES I and II.

associated with the *E. cypellocarpa*-*E. muelleriana*-*E. obliqua*-*E. sieberi* group and vice versa. Of the 23 fungal species analyzed, only three bridged the two tree groups by a significant positive association with at least one tree species in each group: *D. giachinii*, *Z. claridgei* and *Z. daucusinus*. Four species showing one or more significant positive associations with only one of the two tree species groups also showed significant negative association with one or more tree species of the second tree group: *D. albellus*, *H. carneum*, *P. levisporum* and *S. flava*.

*Descomyces albellus* is positively associated with *A. dealbata* and *E. dalrympleana* but negatively with *E. radiata*, even though those three trees are associated positively with each other. One can infer that the site characteristics that permit *E. radiata* to be present with *A. dealbata* and *E. dalrympleana* in some way are inimical to *D. albellus*; we cannot conceive of any reason why *E. radiata* would antagonize *D. albellus*. The last tree species also is associated negatively with the other two tree species, so in that case one can infer that the site characteristics favorable to *E. sieberi* were unfavorable for both *D. albellus* and the other two tree species.

*Inferences from combined habitat modeling and association analyses.*—The association analyses presented here are based on four collection events spread over 6 y. The habitat modeling of individual fungal species by Claridge et al (2000a, b) was based on collections from only the first two samplings of these same plots in spring and autumn 1996, but the models incorporated environmental variables of climate, geology, topography and (among other microhabitat variables) amounts of coarse, woody debris and litter on the forest floor. The data at that time permitted satisfactory modeling of six species (see Claridge et al 2000a). The occurrence of two additional species, *H. convoluta* (designated as *H. whitei* by Claridge et al 2000a) and *Hysterangium rhodocarum* could not be statistically explained by any of the environmental variables measured by Claridge et al (2000a, b).

The explanatory model for the *C. vittatispora* species complex (Claridge et al 2000a) indicated that the probability of its occurrence increased with relatively low temperatures during the hottest month of the year, a moderate annual mean moisture regime (i.e., neither the low or high ends of the moisture scale) and relatively shallow litter. *Cortinarius globu-*

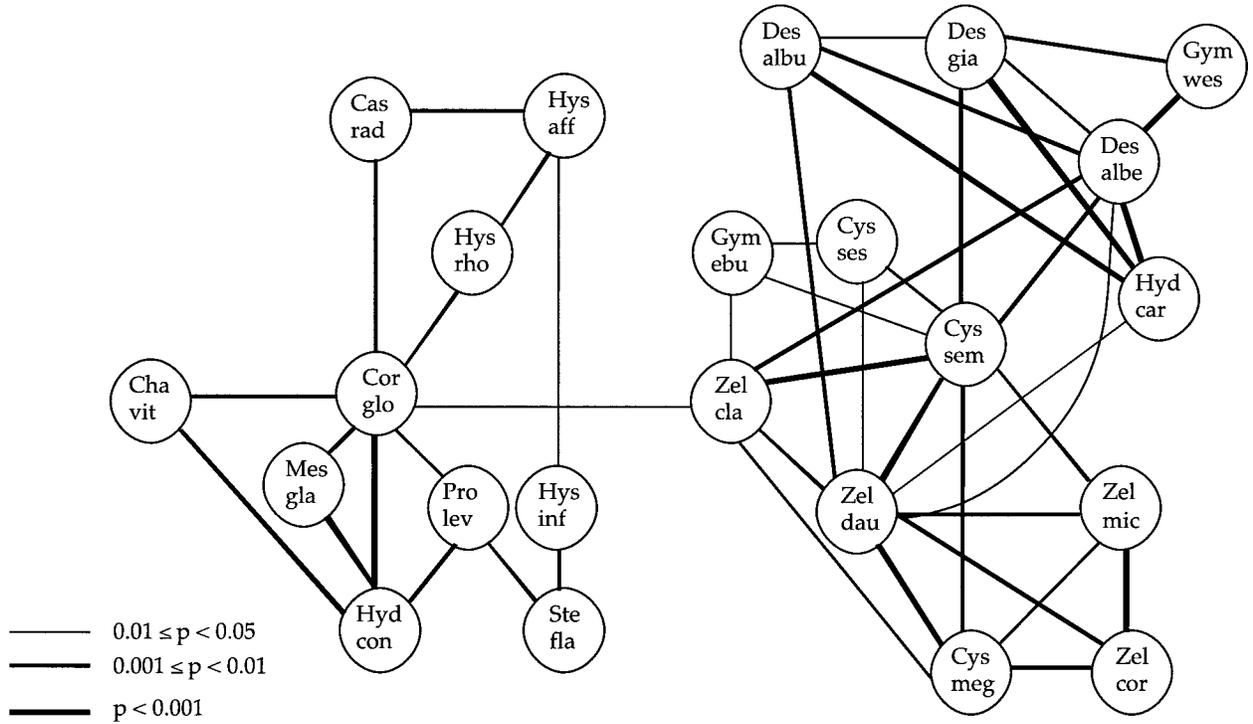


FIG. 3. Positive associations among the 23 species of truffles. Thickness of the line indicates the strength of an association as inferred from  $\chi^2$  in the two-by-two contingency tables. Abbreviations for the truffles and their hosts are shown in TABLE I.

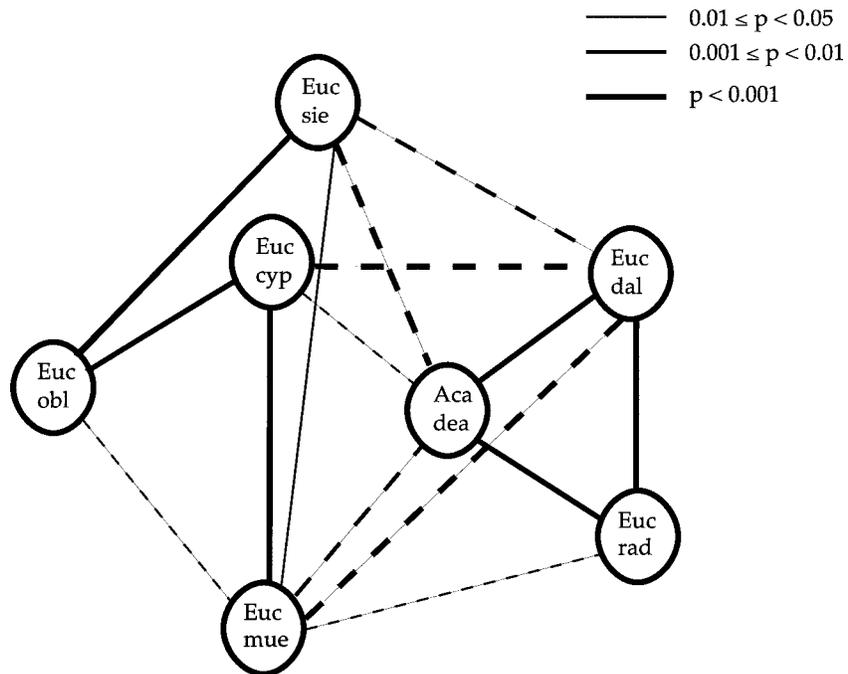


FIG. 4. Positive (solid lines) and negative (dashed lines) associations among the seven host tree species. Thickness of the line indicates the strength of an association as inferred from  $\chi^2$  in the two-by-two contingency tables. Abbreviations for host trees are shown in TABLE II.

*liformis*, highly significantly associated with the *C. vitatispora* species complex, had increased probability of occurrence with falling temperature of the coldest month of the year and less moisture (Claridge et al 2000a). Although the explanatory models for these two species produced significant probabilities in different parameters, both suggest they primarily would occupy relatively cool sites with relatively low to moderate moisture status, as represented in the study area. The association analysis showed a highly significant, positive association between the two species. However, neither the *Chamonixia* nor the *Cortinarius* were associated significantly with the same tree species. The occupation of similar sites evidently does not relate to a coincidence of particular tree species but to other factors. For example those that proved significant in the explanatory models: litter depth (*Chamonixia*) or years since fire (*Cortinarius*).

*Hysterangium inflatum* in the explanatory model showed increased probability of occurrence with higher temperature of the coldest month, sites in gullies and greater litter depth but decreased probability with greater amounts of coarse woody debris (Claridge et al 2000a). In the association analyses among fungi, *H. inflatum* was highly significantly positively associated with *S. flava* and both fungi shared a highly significant positive association with *E. cytellocarpa*, *E. muelleriana* and *E. obliqua*, as well as a significant negative association with *A. dealbata*. Data available when the explanatory models were developed (Claridge et al 2000a) were inadequate to model *S. flava*, but its highly significant association with *H. inflatum* and their congruent positive and negative associations with tree species suggest a strong similarity in habitat requirements.

The explanatory model for *Z. microsporus* showed that its probability of occurrence increased with increasing soil nitrogen and litter depth. This was the only *Zelleromyces* sp. that qualified for modeling at that time. The present association analyses show highly significant positive associations among *Z. microsporus*, *Z. corkii* and *Z. daucus*. These three species, however, do not show similar positive associates with tree species. The habitat factors primarily accounting for their association can be hypothesized as those identified in the explanatory model for *Z. microsporus* (i.e., relatively high soil-nitrogen and litter depth).

Other differences between what could be inferred from the explanatory models versus the association analyses are difficult to interpret at present, except to say that positive associations with tree species do not relate unequivocally to the explanatory habitat models or associations among fungi. In part this is because each fungus species has its own array of hab-

itat relationships, as shown by the explanatory models. For example, in explanatory models the probability of occurrence of the *Chamonixia* complex and *P. levisporum* fell with increasing litter depth, whereas that of *H. inflatum* and *Z. microsporus* rose with increasing litter depth. Litter depth can be affected by site history (i.e., time since last fire) and thus could not be expected to relate directly to tree species, geology or climate of the site.

**Conclusions.**—In experiments on fungal fruit-body distribution related to thickets of *Rhododendron maximum* in the southern Appalachians, Walker and Miller (2002) concluded “Differences within the ectomycorrhizal fungus community were associated only weakly with environmental parameters, as indicated by ordination.” Their data were taken from 4 m<sup>2</sup> experimental plots “on a single hillside with similar aspect and slope.” Our data, in contrast, were taken from time-constraint sampling of 136 plots of 1000 m<sup>2</sup> representing a wide range of measured variables, including those of forest communities, soils, climate, fire history and coarse, woody debris (Claridge et al 2000b). The four sampling events conducted on these plots over 6 yr yielded 4150 collections representing more than 220 species. The data from the first two samplings (1540 collections) permitted habitat modeling of nine of the more common species, with abiotic factors prominent as explanatory factors (Claridge et al 2000a). The association analyses in the present paper, based on all 4150 collections, indicate that biotic factors also can be important, at least in that certain trees and fungi have overlapping environmental requirements, as indicated by their occurrence in similar habitats. Studies such as those of Walker and Miller (2002) combined with ours highlight that each ectomycorrhizal fungus has its own suite of habitat requirements that can be explained only by assessment of a broad range of abiotic and biotic parameters. Through integration of modeling and association analyses, our results expand knowledge and understanding of why certain fungi occur in some habitats but not in others.

Given an adequate database, explanatory models for the occurrence of ectomycorrhizal fungal species and association analyses can be combined to increase our understanding of the ecology of those fungi. Such data can be helpful in defining their habitat requirements, host preferences, successional patterns and community structure. They also can provide insights useful in restoration ecology, both for identifying the fungi with particular promise as inoculum for restoring forests in long-cleared areas and, in the case of the hypogeous species, for establishing the

food base that is important for many animals (Maser et al 1984, Claridge and May 1994).

As a result of our additional sampling, the database for the hypogeous fungal species previously modeled has been strengthened substantially and the added frequency of more species will permit development of additional explanatory models. This progress will sharpen our interpretive capability for these ecologically important fungi.

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