

Invasive Fungi Research Priorities, with a Focus on *Amanita muscaria*

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Summary

Project and Client

This report provides research priorities for addressing the risks that ectomycorrhizal fungi pose to natural ecosystems, in particular the impacts of non-native ectomycorrhizal fungi and options for management. The report was produced by Landcare Research for Northland Regional Council under FRST EnviroLink small-advice grant funding in October 2008.

Objectives

- To develop a research strategy for understanding ectomycorrhizal invasions, with a focus on *Amanita muscaria*.

Methods

- We arranged a focus group meeting on 13 June 2008 with participation by Landcare Research, Scion, Auckland Regional Council, and the Department of Conservation. We identified a comprehensive list of research questions and prioritised based on professional opinion.

Results

- Three main topics emerged as the key high-priority research areas:
 1. *What are the impacts of invasive ectomycorrhizal fungi on native ectomycorrhizal fungi?*
 2. *What are the potential native hosts for Amanita muscaria?*
 3. *What is the relationship between sporocarp production and below-ground mycelium?*
- Two further research priorities were ranked as high-to-moderate priority:
 4. *Impacts on non-native plants (Do non-native fungi promote non-native tree invasion into intact Nothofagus forest?).*
 5. *What are the vectors of spread, and in particular can A. muscaria spread in soil on boots or camping equipment?*

Conclusions

- There are no major technological barriers to addressing the key research priorities. A targeted research programme around our identified key research priorities is an essential first step to establishing the necessity and utility of further research.

Recommendations

- We recommend that research proceed around the identified key research priorities listed above.
- Given the high probability that *Amanita muscaria* and other non-native fungi will continue to establish and spread, research should be funded and conducted in a timely fashion.

1. Introduction

This report provides research priorities for addressing the risks that ectomycorrhizal fungi pose to natural ecosystems, in particular the impacts of non-native ectomycorrhizal fungi and options for management. The report was produced by Landcare Research for Northland Regional Council under FRST EnviroLink small-advice grant funding in October 2008.

Most land plants (>80% of angiosperms and almost all gymnosperms) form mycorrhizas: symbiotic associations between plant roots and fungi that increase plant nutrient uptake. Several types of mycorrhizas occur, including arbuscular mycorrhizas (most grassland species and podocarp forests), ectomycorrhizas (native *Nothofagus*, *Kunzea*, *Leptospermum*, *Pomaderris*, and *Pisonia* and many introduced trees such as *Pinus*, *Eucalyptus*, *Quercus*), and ericoid mycorrhizas (native and introduced plants in the family Ericaceae). Mycorrhizas play a critical role in plant nutrient uptake, particularly of P, NH_4^+ and organic nutrients. In return for increased nutrient acquisition, plants direct 10–30% of total fixed carbon to supporting mycorrhizas. While relatively few native plant species form ectomycorrhizas, these plants are the ecosystem dominants of around 60% of New Zealand forests. Introduced ectomycorrhizal plants such as pine (*Pinus radiata*) and Douglas-fir (*Pseudotsuga menziesii*) are the basis for virtually the entire forestry industry in New Zealand.

The fungi that form ectomycorrhiza are highly diverse, with an estimated 150 ectomycorrhizal fungal species in a typical New Zealand *Nothofagus* forest site (Dickie et al. unpubl. data). Many of these species are under conservation threat – of 49 ‘Nationally Critical’ fungal species (the highest level of threat), at least 21 are probable ectomycorrhizal species; a further 1445 fungal species are ‘Data Deficient’ (Hitchmough 2007).

2. Background

The introduction of forestry species such as *Pinus*, *Pseudotsuga*, and *Eucalyptus* along with garden trees such as *Quercus* and *Betula* has introduced many species of ectomycorrhizal fungi to New Zealand. These present a risk of becoming invasive. Invasive species go through a series of stages:

- 1, Transport
- 2, Release
- 3, Establishment
- 4, Spread

In the case of ectomycorrhizal fungi, it makes sense to further subdivide the process of spread into four levels:

- 4a, Only on non-native host plants, spreading slower than non-native plant
- 4b, Only on non-native host plants, co-invading with non-native plants
- 4c, On native host plants, only in disturbed environments
- 4d, On native host plants, in relatively undisturbed environments

Within New Zealand there are numerous ectomycorrhizal fungi that have been transported,

released, and established in plantation forestry, urban parks, and non-native urban plantings (stages 1–3). A few of these appear to be common on invasive pines – most notably *Suillus*, which appears to co-invade with wilding pines, but does not occur on native hosts (stage 4b). *Suillus* is unlikely to further spread onto native hosts, as *Suillus* spp. are generally host-specific to the Pinaceae.

Only a very few ectomycorrhizal fungi are known to have spread onto native hosts, with the most notable being *Amanita muscaria*. This species is the dominant fungus in *Pinus radiata* plantations in New Zealand (K. Walbert pers. comm.); is one of the four most dominant fungi on invasive *P. contorta* in Canterbury (Dickie unpubl. data); is common on native *Nothofagus* in disturbed environments (e.g. urban plantings); and has established in intact *Nothofagus* forests along trails, and at road edges (stage 4c). Recent observations of *A. muscaria* in undisturbed *Nothofagus* forest (N. Singers pers. comm.) suggest that it will continue to spread into undisturbed *Nothofagus* forest (stage 4d). There is no confirmed evidence of *A. muscaria* spreading onto native *Leptospermum* or *Kunzea* in New Zealand (two herbarium records are believed to be incorrect; one anecdotal report from the Nelson region needs to be confirmed), but we believe such spread is entirely possible given the known ability of *A. muscaria* to associate with other genera in this plant family.

While *A. muscaria* is the only known invasive ectomycorrhizal fungus that has spread onto native hosts in undisturbed habitats, there may be others. *A. muscaria* is unusually apparent when present – its bright red colour makes it stand out from a considerable distance and its appearance is both iconic and distinct from any native species. If other non-native fungi have established and spread, they may not be as quickly detected.

While other ectomycorrhizal fungi may be or may become invasive, *A. muscaria* is an ideal case study for understanding these other invasions. *A. muscaria* produces highly visible, easily identified sporocarps. In contrast, many other ectomycorrhizal fungi produce inconspicuous or difficult-to-identify sporocarps. This makes *A. muscaria* much easier to track, particularly as non-specialist identification is quite straightforward. *A. muscaria* is also widely established (stage 3) in New Zealand, but has only spread into native forest (stages 4c – 4d) in a limited number of locations, providing scientific replication as well as a possibility of effective control if managed in a timely fashion.

3. Objective

Given the paucity of knowledge regarding ectomycorrhizal invasions, there is a nearly limitless list of possible research directions that could be endorsed, yet given limited funding and a degree of urgency there is a clear need to prioritise research needs. Our objective was:

- To develop a research strategy for understanding ectomycorrhizal invasions, with a focus on *Amanita muscaria*.

4. Methods

We arranged a focus group meeting on 13 June 2008 with participation by Landcare Research, Scion, Auckland Regional Council, and the Department of Conservation. Presentations from that meeting are attached (Appendix 1). A survey of recent literature related to invasive ectomycorrhiza, with emphasis on *Amanita muscaria*, was carried out (Appendix 2).

5. Results

The group first identified possible research directions (Table 1). We divided research into three main categories: (1) impacts, (2) spread, and (3) control. Within each category we identified a wide range of possible research directions, and then ranked these into categorical priority groups. These rankings represent professional opinion, and are hence open to some interpretation. Nonetheless, our discussions and rankings involved scientists from a range of disciplines (Forestry, Plant Ecology, Mycology, Entomology) and had input from regional councils and the Department of Conservation. Our rankings were guided by:

- *Logical sequence.* Research was ranked higher priority if it either (1) was necessary to justify whether further research was needed, or (2) was a necessary prerequisite for other high-ranked research priorities.
- *Feasibility.* Research was ranked lower priority if the cost or difficulty of achieving an outcome was high relative to potential benefit.
- *Prior evidence.* Where prior evidence suggests a likely impact, this increased the priority of research as compared with areas where prior evidence suggested less likely impacts.

A comprehensive list with background information and ranking is presented in Table 1. From our discussions three main topics emerged as the key high-priority research areas, and two further research priorities were ranked as high-to-moderate priority.

Table 1 Comprehensive list of research topics and priorities for invasive ectomycorrhizal fungi in New Zealand

Research topic	Key background information	Priority	Rationale for research priority
1. What are the impacts of <i>A. muscaria</i> and other invasive ectomycorrhizal fungi in New Zealand?			
1.1 Impacts on native and non-native fungi, fauna, and flora			
1.1.1 What are the impacts on native ectomycorrhizal fungi?	• There are 49 ‘Nationally Critical’ species of fungi in NZ, including at least 21 probable ectomycorrhizal species; a further 1445 species are ‘Data Deficient’ (Hitchmough 2007) • Ectomycorrhizal fungi compete for root (plant host, source of carbon) and soil (mineral nutrient) resources (Kennedy et al. 2006) • The high density of <i>A. muscaria</i> sporocarp production implies a high level of resource capture	HIGH	• Impacts on nationally critical species are of the highest concern to DOC • Showing impacts on native species underpins most further research
1.1.2 Impacts on native plants	• While ectomycorrhizal fungi are generally beneficial, the degree of benefit may vary considerably and novel associations (e.g. non-native fungus on native plant) are more likely to be unstable	MODERATE	• At present, <i>A. muscaria</i> occurs only on common plants; impacts are likely to develop slowly
1.1.3 Impacts on non-native plants (Do non-native fungi promote non-native tree invasion into intact <i>Nothofagus</i> forest?)	• Some non-native ectomycorrhizal plants are invading into <i>Nothofagus</i>, <i>Kunzea</i>, and <i>Leptospermum</i> dominated areas • The presence of a non-native fungus may increase the relative competitive ability of non-native trees	HIGH / MODERATE	• Although possible, no current evidence to support this for <i>A. muscaria</i> • Higher risk may be <i>Suillus</i> or <i>Rhizopogon</i> spore bank establishment (Ashkannejhad & Horton 2006) • Ranked ‘moderate’ as can be combined effectively with 1.1.2 (above)
1.1.4 Impacts on fungivores	• Many endemic insects depend on native fungi as food source • High diversity and very high endemism rate of native fungivorous insects in NZ • Some possibility that <i>A. muscaria</i> sporocarps may be lethal to native insect larvae?	LOW	• At present, no evidence of <i>A. muscaria</i> impacting native fungivores at more than a local scale

1.2 Impacts on carbon cycles			
1.2.1 Photosynthetic carbon fixation	Ectomycorrhizal fungi can directly influence photosynthetic carbon fixation (Dickie et al. 2007)	LOW	Logical first-step would be 1.1.2 with detailed physiological experiments secondary
1.2.2 Soil carbon sequestration	Co-invasion of Ecuador by <i>Pinus</i> and <i>Suillus</i> has resulted in up to 30% loss of soil C	LOW	Area impacted by <i>A. muscaria</i> too limited to have major effect on C sequestration in NZ
1.3. What micro-niche(s) within sites are invaded by <i>A. muscaria</i> ?	- Different ectomycorrhizal fungi preferentially occupy different niches within sites, one study suggests <i>Amanita</i> most common in mineral soil (Dickie et al. 2002) - Knowledge of niche preference would permit more focused sampling, and perhaps prediction of impact on native fungi	LOW	- Knowledge of niche preference would be of value primarily in context of native fungi niche knowledge, which is lacking - High-cost relative to potential gain
1.4. Invasional melt-downs / invasional system	Will <i>A. muscaria</i> invasions contribute to further invasions by plants, ectomycorrhizal fungi, other fungi, or other organisms?	LOW	- A broader view of 1.1.3 (whole system view) - Costs are high for broad-view research; focus on invasive plants first
1.5. Direct human impacts			
1.5.1 Māori	Māori have limited but significant cultural usage of fungi (Fuller et al. 2004)	LOW	Although possible, no current evidence to support impacts on Māori cultural use of native fungi or other forest resources
1.5.2 Health risks (particularly regarding <i>Amanita phalloides</i>)	<i>A. phalloides</i> is present in NZ and has caused hospitalisations but no fatalities to date - In other regions <i>A. phalloides</i> has significant health costs, including around 10% lethality even with treatment. Treatment is very expensive (can include liver transplantation – cost in 2001 for a single liver transplant was around \$180,000) <i>A. muscaria</i> is poisonous but rarely fatal; can be used as a hallucinogen	MODERATE	- Research on <i>A. phalloides</i> health impacts would be timely as this non-invasive species may be controllable for relatively low cost if performed BEFORE spread - Impacts of <i>A. muscaria</i> on health are likely to be limited
1.5.3 Visual impact	<i>A. muscaria</i> is a major visual change to the character of native forest	LOW	Visual impact is large, but value in research on such impacts is limited

2. Understanding the spread of ectomycorrhizal fungi (with <i>Amanita muscaria</i> as case study)			
2.1 What is the potential native host-range of <i>Amanita muscaria</i> ?			
2.1.1 Which native <i>Nothofagus</i> species support <i>Amanita</i> ?	• At present there is no known establishment of <i>Amanita</i> on <i>Nothofagus menzeisii</i> in NZ • In Tasmania, <i>A. muscaria</i> is invading <i>N. cunninghamii</i> forest, which suggests that at least some populations of <i>A. muscaria</i> are compatible with the <i>Lophozonia</i> subgenus (including <i>N. menzeisii</i>)	HIGH	• Given the extensive range of <i>Nothofagus</i> in NZ (an ecosystem dominant in 60% of NZ forests), understanding the potential of <i>A. muscaria</i> to invade this range is the single best predictor of potential spread
2.1.2 Are native tea trees, <i>Kunzea</i> and/or <i>Leptospermum</i> , potential hosts?	• The only existing records of <i>A. muscaria</i> on native tea tree are believed to be incorrect; however, <i>A. muscaria</i> is known to associate with <i>Eucalyptus</i> (in the same plant family)	HIGH	• Tea tree is a widespread ecosystem-dominant in NZ; any potential spread of <i>A. muscaria</i> onto tea tree is of critical concern. • 2.1.1 and 2.1.2 (and 2.1.3) could be logically integrated with 22 below
2.1.3 Other native hosts (e.g. <i>Pomaderris</i> , <i>Pisonia</i>)	• It is now recognised that <i>Pomaderris</i> is ectomycorrhizal in NZ • <i>Pisonia</i> may also be ectomycorrhizal • Both tree genera may be potential hosts	LOW	• <i>Pomaderris</i> is not a widespread dominant, and frequently co-occurs with other ectomycorrhizal hosts. Not likely to contribute significantly to spread of <i>A. muscaria</i> • <i>Pisonia</i> is mainly restricted to islands, and is therefore at lower risk of being exposed to invasive ectomycorrhizal fungi.
2.2 Do different <i>Amanita</i> populations have different host-specificity for natives and non-native trees?	• The clustered distribution of <i>A. muscaria</i> invasions and the currently limited number of these invasion sites suggests that <i>A. muscaria</i> invasion is presently limited by unknown factors • Relatively little is known about host-specificity and fungal population genetics in ectomycorrhizal fungi	MODERATE	• If host-specificity varies with populations, this would have very important implications for managing the future spread of the species • Identifying and mapping populations is high-cost

2.3 How are new populations of <i>Amanita</i> spread?			
2.3.1 Human vectors, particularly adhering to soil on boots	• A small number of novel populations have established in intact <i>Nothofagus</i> forest where DOC staff and hunters have had access (N. Singers pers. comm.) • Controlling the spread is contingent on knowing the vectors of spread • Need to understand recreational land use effects, and mitigating strategies	HIGH / MODERATE	• Direct ramifications for control of spread • DOC presently undertakes control for <i>Didymo</i> spread, which could serve as learning model for other microbes
2.3.2 Spread from plantations, timber-harvesting equipment, personnel	• <i>A. muscaria</i> is most common fungus in <i>P. radiata</i> plantations (K. Walbert pers. comm.) • Personnel move from plantations (work) to indigenous forests (hunting) regularly	MODERATE	• Answering 2.3.1 will cover timber harvesting potential as well
2.3.3 Animal vectors	• Animals, particularly deer, are important vectors in the spread of some ectomycorrhizal fungi (Ashkannejhad & Horton 2006)	LOW	• No current evidence for animal dispersal
2.4 What is the rate and extent of vegetative spread once a population establishes?			
2.4.1 What is the relationship between sporocarp production and below-ground mycelium?	• In some fungal species individual fungal genets can spread up to several hectares in size, although many species produce much smaller genets • The clustered distribution of <i>A. muscaria</i> sporocarps suggests few, spreading genets rather than multiple small genets	HIGH	• Control of established individuals is contingent on knowing size of individuals • This research underpins work on impacts
2.4.2 Do all individuals produce sporocarps, and how soon are sporocarps produced following establishment of an individual?	• The extent of <i>A. muscaria</i> invasion is unknown, as it is not known if all individuals produce sporocarps	MODERATE	• Necessary for understanding current range and spread • Potentially very challenging to achieve
2.5 Do urban plantings of native tree permit alien fungi to adapt to native hosts and subsequently spread into natural populations?	• <i>A. muscaria</i> commonly associates with urban plantings of <i>Nothofagus</i> • Urban plantings have played a significant role in the spread of sudden oak death in California	LOW	• No current evidence for this
2.6 What is the current extent of spread (resurvey of populations)?	• Existing survey data are nearing 10 years old • We know of several new populations that were not included in earlier survey	MODERATE / LOW	• Existing public databases should be sufficient to capture this (but need improvement)

			• Might be a logical approach towards understanding spread onto <i>Kunzea</i> or <i>Leptospermum</i> [2.1.2 above]
2.7 What are the specific site requirements of <i>Amanita</i> – are some sites more invisable (disturbance, climate, soil type)?	• Spread has been slow, with most populations in disturbed habitats	MODERATE	• Important to understanding control of spread and possible impacts
2.8 What is the origin of the <i>Amanita</i> populations in New Zealand?	• Believed to be at least 2 different origins of <i>A. muscaria</i> in NZ	LOW	• Some application in understanding the biology of <i>A. muscaria</i> in NZ, but not a high priority for management at this point
2.9 Other than <i>Amanita muscaria</i> , what are the risks of other ECM fungi invading NZ?			
2.9.1 <i>Amanita phalloides</i>	• <i>A. phalloides</i> is present in urban settings, and has high health risks • <i>A. phalloides</i> is invasive in other countries	MODERATE / LOW	• Control of <i>A. phalloides</i> is still possible in NZ (presently restricted in establishment) • Human impact of invasion is potentially higher than for most invasive ectomycorrhizal fungi
2.9.2 <i>Chalciporus piperatus</i>	• <i>Chalciporus</i> appears to be co-invading with <i>A. muscaria</i> and may be parasitic on <i>A. muscaria</i>	LOW	• Impact of <i>C. piperatus</i> likely low as it appears restricted to <i>Amanita</i>-invaded areas
2.9.3 <i>Boletus edulis</i>	• <i>Boletus edulis</i> believed to be expanding in NZ, but no records with native hosts • Anecdotal evidence of human-aided spread (choice edible species)	LOW	• Impact likely low • Some economic/cultural benefit to spread of this species

3. Control and management			
3.1 Measures to prevent the spread	• There is virtually no current knowledge of how to prevent the spread of ectomycorrhizal fungi • May be possible to apply knowledge from other invasive species with small propagules (e.g. <i>Didymo</i>)	MODERATE	• Not a high priority unless impacts are shown • Contingent on understanding methods of spread (2.3 above)
3.2 Measures to control once present	• There is virtually no current knowledge of the control of ectomycorrhizal populations	MODERATE	• Not a high priority unless impacts are shown • Contingent on knowing how large individual are likely to be (2.4 above) and whether large numbers of undetectable populations are present (2.5 above)
3.3 Nursery management	• Restoration nurseries are propagating large numbers of native trees, including <i>Kunzea</i> and <i>Leptospermum</i> • No current guidelines regarding soil micro-organisms present on these seedlings	MODERATE	• General research on eco-sourcing of soils for restoration plantings would be useful
3.4 Public knowledge / perceptions	• Amateur mycology reasonably popular in NZ among the public • High visual impact of invasive ectomycorrhizal fungi	VERY LOW	• No clear use for this research

5.1 High-priority research priorities

1. What are the impacts of invasive ectomycorrhizal fungi on native ectomycorrhizal fungi?

Ectomycorrhizal fungi compete for root (plant host, source of carbon) and soil (mineral nutrient) resources (Kennedy et al. 2006). As *A. muscaria* frequently produces a high density of sporocarps when present on native *Nothofagus*, it is logical to hypothesise that it is capturing a significant portion of available resources, and hence making these resources unavailable to native fungi. This could seriously impinge on native fungi, including nationally critical species. We ranked this research as a high priority as it would justify and underpin further research on mycorrhizal invasions.

Identifying impacts on native ectomycorrhizal fungi requires below-ground surveys of fungi on plant roots. While such research was impossible until the last decade, recent molecular advances have made below-ground research on ectomycorrhizal fungi reasonably routine (Anderson & Cairney 2004; Dickie & FitzJohn 2007). Understanding the impacts of invasive ectomycorrhizal fungi on native fungi is therefore feasible given existing technology (Dickie & FitzJohn 2007).

2. What are the potential native hosts for *Amanita muscaria*?

The single best predictor of the potential spread of *A. muscaria* is native host-compatibility. At present, there are no confirmed observations of *A. muscaria* in association with *Nothofagus menziesii* (which is the only native *Nothofagus* in the subgenus *Lophozonia*) nor with *Kunzea* or *Leptospermum*. These tree species are dominants across wide areas of New Zealand. If *A. muscaria* spreads onto native *Kunzea* or *Leptospermum* it would dramatically increase the potential invaded range.

Research on this topic would involve both greenhouse or field plantings of native trees, and might include updating and confirming observational data [2.7 in Table 1]. It would be a logical and efficient extension to include impacts on native and non-native plant growth as part of this research [Topics 1.1.2, 1.1.3]. This research is entirely feasible with existing methodology and in-country expertise (Dickie et al. 2004).

3. What is the relationship between sporocarp production and below-ground mycelium?

Understanding the size of invasive *A. muscaria* populations is critical both as underpinning research on impacts and for control. Where sporocarps of invasive fungi are observed they represent only a small fraction of the individual fungus. Mushrooms (or sporocarps) are only the fruiting bodies of a larger individual. In some cases fungal individuals may extend many metres through the soil – the largest known fungal individual spreads over at least 15 ha (Smith et al. 1992). In order to understand and study the impacts of *A. muscaria* on native fungal species it is essential to know how large individuals are.

This research was also ranked a high priority because it underpins further research. Studies on the impact of invasive fungi clearly depend on knowing how large the individuals are. It is also essential to know if the invasive fungus is already ubiquitous below-ground – in which case management may be impossible – or whether it remains restricted to limited areas associated with visible sporocarps. This research priority could be effectively addressed in tandem with the first (impacts on native ectomycorrhizal fungi) in a single below-ground survey.

5.2 High-to-moderate research priorities

4. Impacts on non-native plants (Do non-native fungi promote non-native tree invasion into intact *Nothofagus* forest?)

We considered impacts on plant invasion to be high-to-moderate priority, with a particular consideration of *Suillus* and *Rhizopogon* invasions as well as *Amanita*. *Suillus* and *Rhizopogon* are highly unlikely to occur on native plants, but are found on invasive Pinaceae at the earliest stages of invasion and appear to facilitate the establishment of these species in many early-successional habitats (Ashkannejhad & Horton 2006; Wiemken & Boller 2006). The role of *Amanita* in plant invasions is less clear, but *Amanita* is a dominant on invasive *Pinus contorta* in Canterbury (Dickie unpubl. data).

Research on this topic could be logically initiated by planting native and non-native tree seedlings in soil within and adjacent to *Amanita*-invaded locations. This research could be logically integrated with the second research priority (potential native hosts) in a single experiment.

5. What are the vectors of spread, and in particular can *A. muscaria* spread in soil on boots or camping equipment?

Observations of *A. muscaria* associated with tracks, campsites, and other areas where human activity is present suggest that human activity may be involved in spread. Knowledge of dispersal vectors is an important prerequisite for controlling spread. This research would require some limited new methods development, but is likely to be feasible at moderate cost.

6. Conclusions

The impacts of invasive ectomycorrhizal fungi remain highly uncertain, as very little research has been done in this area. Uncertainty should not be confused with irrelevance – it is impossible to prejudge the impacts of invasive fungi or the potential for control without further knowledge. There are no major technological barriers to addressing the key research priorities. A targeted research programme around our identified key research priorities is an essential first step to establishing the necessity and utility of further research.

7. Recommendations

- We recommend that research proceed around the identified highest research priorities, particularly impact on native fungal species and potential native plant host range.
- Appendix 3 provides some thoughts about possible funding sources.
- Given the high probability that *Amanita muscaria* and other non-native fungi will continue to establish and spread, research should be funded and conducted in a timely fashion.

8. Acknowledgements

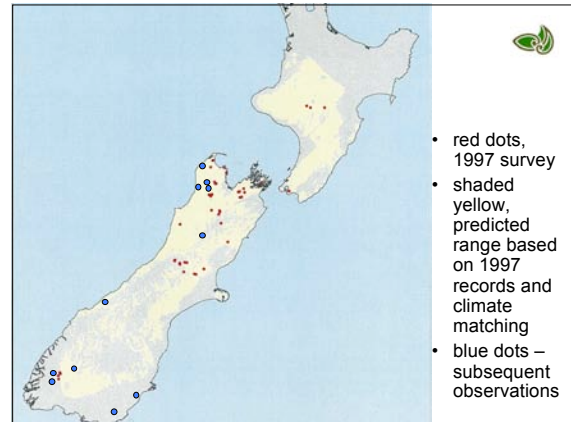
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9. References

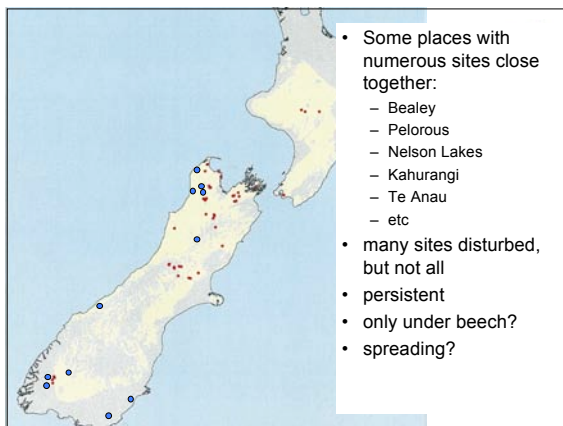
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- Hitchmough R 2007. *New Zealand Threat Classification System lists 2005*. 194 p.
- Kennedy PG, Bergemann SE, Hortal S, Bruns TD 2006. Determining the outcome of field-based competition between two *Rhizopogon* species using real-time PCR. *Molecular Ecology* 16: 881–890.
- Smith M, Bruhn J, Anderson J 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* 356: 428–431.
- Wiemken V, Boller T 2006. Delayed succession from alpine grassland to savannah with upright pine: Limitation by ectomycorrhiza formation? *Forest Ecology and Management* 237: 492–502.

Appendix 1 Copies of presentations from workshop

- introduced a long time ago, late 1800's, and perhaps many times
- where from?
 - no idea
 - Oda et al. (2004) include a single NZ specimen, part of a geographically broad, Eurasian clade. Genetically close to Japanese specimens, but really matches nothing in particular. Recognise 2 other clades, one N American the other alpine Eurasian. Putatively allopatric.
 - Geml et al. (2006), carried out intensive sampling in Alaska, found Alaskan specimens representative of all 3 clades. Suggest sympatry with recent dispersal from Beringia.
 - pers. comm. from Tanaka that NZ specimens representative of both Eurasian and N American clades (unpublished)
- host range in NZ wider than expected, based on behaviour in natural range (includes Eucalyptus)
- perhaps multiple introductions of genetically and biologically slightly distinct populations??



- red dots, 1997 survey
- shaded yellow, predicted range based on 1997 records and climate matching
- blue dots – subsequent observations



- Some places with numerous sites close together:
 - Bealey
 - Pelorous
 - Nelson Lakes
 - Kahurangi
 - Te Anau
 - etc
- many sites disturbed, but not all
- persistent
- only under beech?
- spreading?

research needs



- Taxonomy and dispersal
 - What genotypes do we have here?
 - Where did they come from?
- Factors driving naturalisation
 - Population structure – are the invasive populations genetically distinct?
 - How does it spread – locally through soil movement versus multiple introductions through spore dispersal?
 - Local environment - climate, soils, etc.
 - Effect of disturbance (include something on longevity of propagules with respect to movement of soil)
 - can it form mycorrhizae with teatree as well as beech? [Pomaderris?]
- Impacts
 - On the diversity of native mycorrhizal fungi
 - On native mushroom-feeding invertebrates
 - On host tree environmental resilience
 - Potential to facilitate invasion by exotic mycorrhizal trees
 - Impact on soil Carbon

research needs



- Taxonomy and dispersal
 - What genotypes do we have here?
 - Where did they come from?
- Factors driving naturalisation
 - Population structure – are the invasive populations genetically distinct?
 - **How does it spread – locally through soil movement versus multiple introductions through spore dispersal?**
 - Local environment - climate, soils, etc.
 - Effect of disturbance (include something on longevity of propagules with respect to movement of soil)
 - **can it form mycorrhizae with teatree as well as beech?** [Pomaderris?]
- Impacts
 - **On the diversity of native mycorrhizal fungi**
 - **On native mushroom-feeding invertebrates**
 - On host tree environmental resilience
 - Potential to facilitate invasion by exotic mycorrhizal trees (too much?)
 - Impact on soil Carbon

funding sources



- IIOF (International Investment Opportunities Fund, objective 2)
 - joint NZ-Japanese research projects
 - up to \$250k for 2 years
 - full FRST proposal, due early August
- Envirolink
 - 'Tools' proposals (possible to develop through \$20k medium advice grant)
 - \$150-250k for 2 years
- FRST through current ECO round
 - Landcare extremely unlikely to buy into it
- DOC?
 - in 1999 tried unsuccessfully with Nick Singers for DOC research funding to support a MSc student
- less formal collaborations/student projects to try to keep things moving?
 - ENSIS?
 - build into current Landcare below-ground projects?
 - Otago University?
 - Australians (Teresa Lebel, Melbourne Botanic Garden)?

IIOF



- Japanese interested in:
 - taxonomy
 - host specialised populations?
 - potential for hybridisation [sympatry, allopatry questions]
 - impact on fungal feeding invertebrates
- New Zealand
 - factors driving naturalisation
 - impacts (insects, fungi, carbon)

EnviroLink



- 'tools' proposal (\$150-250k, 2 years)
 - Don McKenzie will push
 - need buy in from other Regional Councils
 - need very clear research priorities
 - could use \$20k EnviroLink medium advice grant to develop a bid. Likely to get such a grant if Don can find support in principle from other Regional Council biosecurity managers for a 'tools' project.

some recent EnviroLink 'tools' projects

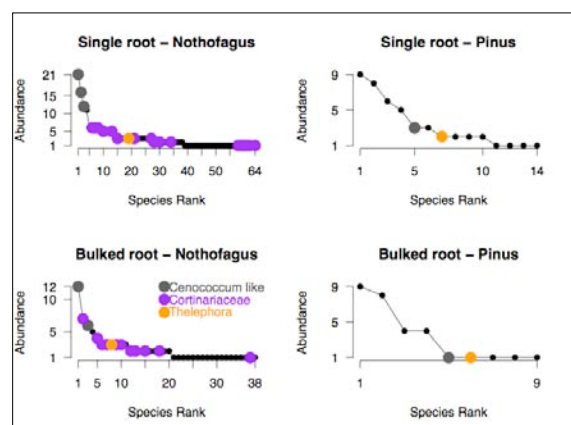
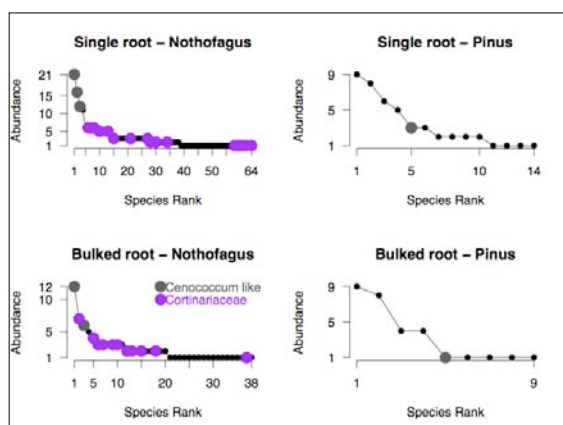
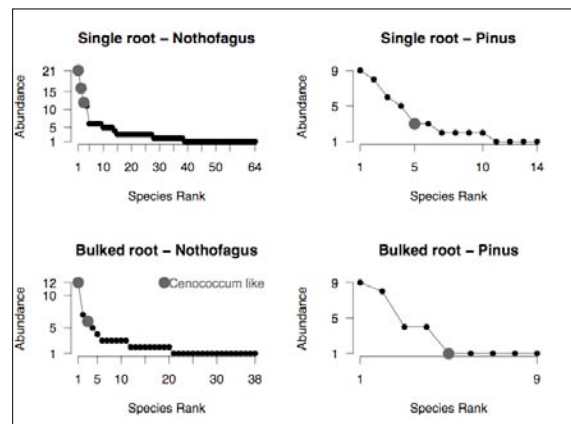
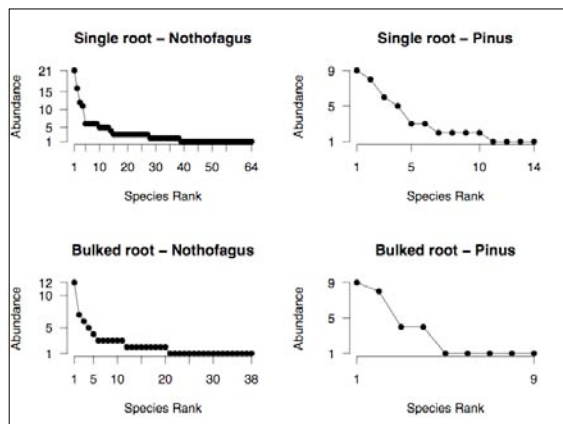
- Sedimentation effects on in-stream values
 - protocols to quantify sedimentation
 - guidelines or standards to maintain a range of desired in-stream values
- Incentives for air quality
 - identify high emission areas, the kinds of domestic heating used in those areas, and identify incentives needed to change to something better
- Guidelines for design and management of constructed lakes and ponds
 - many developments include formation of lakes and ponds, those developed are often dodgy
 - guideline document covering lake formation limnological processes and issues, information for developers of water bodies, assist councils to consider, approve and control such developments
- Assessment tool for identifying coastal habitats of national importance
 - criteria for identifying and mapping such habitats
 - a case study applying the criteria
 - Regional Council workshops
- Uses and values of water bodies
 - what things are important in deciding a 'value'
 - criteria and methods for assigning significance or value
 - methods to manage water bodies to address those values
- Value of abstracted water
 - assessing water value in terms of security of supply, allocation, etc, for multiple potential users of the water (farms, industries, communities, etc)



Invasive Ectomycorrhizal Fungi in New Zealand

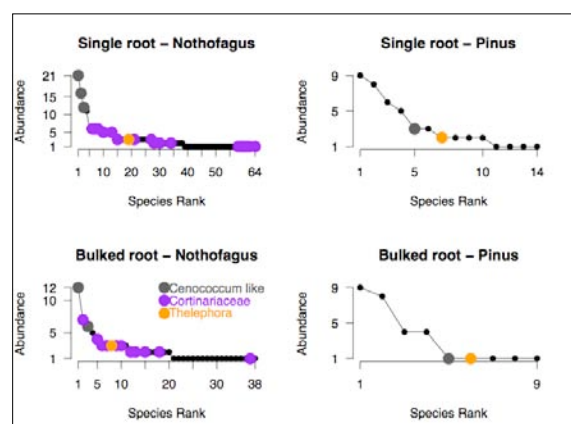


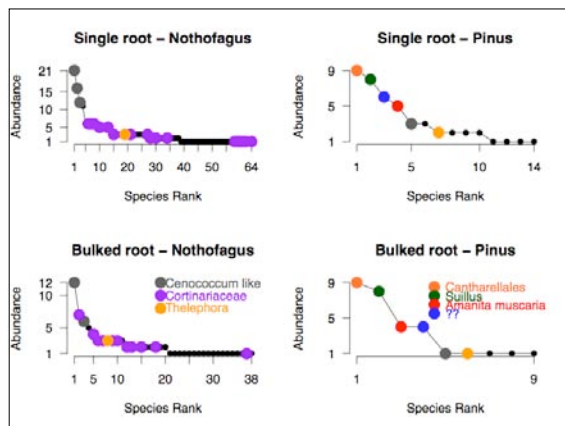
- 25 Soil cores from under Pine and Nothofagus
- Terminal-Restriction Fragment Length Polymorphism
- Individual roots (all distinct types)
- Bulk roots (60 root tips)



Dunstan et al. 1998

- Pine in Western Australia shared 2 ECM with native flora:
 - *Cenococcum geophilum*
 - *Thelephora*





Invasive plants...

- Co-invading with both known and unknown ectomycorrhizal fungi
- Some native fungi on invasive plants
- Little evidence of non-natives on native trees
- Mycorrhizal invasion includes both partners



Stages of Invasion

- Transport
- Release
- Establishment
- Spread

Mycorrhizal invasion

- Does a species need to occur on a native host to be invasive?
- Does it need to occur on a native host in an undisturbed ecosystem to be invasive?

Pine is not invasive!

- Does not occur on native ectomycorrhizal fungi...
- Rarely invades intact native habitat...

Stages of Invasion

- Transport
 - Release
 - Establishment
 - Spread
-
- On non-native
 - Slower spread
 - Concurrent spread
 - Facilitating invasion
 - On natives
 - Disturbed habitat
 - Undisturbed habitat
 - Is there any undisturbed habitat?

Invasive ECM in NZ

- On non-natives, slower spread
- On non-natives, concurrent spread
 - Suillus, Rhizopogon
- On natives
 - *Amanita*
 - (*Chalciporus piperatus*)

Lessons from the world...

...or lessons to the world.

- Reviews outnumber data by 2 X
- *Amanita* in NZ becoming a “classic case”
- Impact factor bias
 - Data: *Australian mycological newsletter*
 - Reviews: *Ecology Letters*, *Trends in Ecology and Evolution*...

Pringle and Vellinga 2006

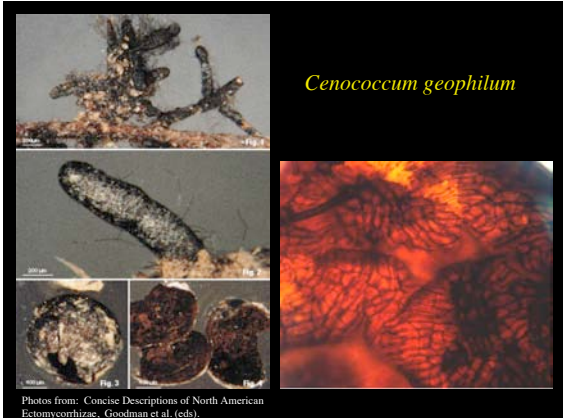
- *Amanita phalloides* CA and East Coast, USA.
- Poor species concept
- East coast invasive
- California equivocal (found in intact native forest)

Amanita phalloides in NZ?

- Will *Amanita phalloides* become invasive?
- What are human health consequences?
 - ~10% fatal with intensive treatment

Pringle and Vellinga 2006

- *Amanita phalloides* CA and East Coast, USA.
- Poor taxonomic records
- East coast invasive
- California equivocal (found in intact native forest)



NZ *Cenococcum geophilum*

- In indigenous forest and on invasive pine
 - At least 3 “T-RFLP” species on natives
- Mejsrik, V. (1970). *Cenococcum graniforme* in New Zealand. *Mycologia* 62(4): 585.
- One collection in PDD, 2006 (my collection)
- MAF “Regulated Pest”

What other NZ fungi are unknown?

Diez 2005

- Eucalyptus mycorrhiza in Spain
- Eucalyptus not invasive until its ectomycorrhizal associates became established.
- *Laccaria fraterna* now invading onto native *Cistus*



Ashkannejhad & Horton 2006
Best matching *Cantherallales* sequence
Suillus dominated

Suillus

- *P. contorta* in Oregon sand dunes
 - Ashkannejhad & Horton 2006
- Early succession of *Larix* on Mt. Fuji
 - Nara 2006
- *Pinus* in alpine grasslands in Switzerland
 - Weimken & Boller 2006
- *Pinus* invasion in Ecuador
 - Chapela et al. 2001

Chapela et al. 2001

- Loss of 30% of soil C following *Suillus* invasion

Suilloid fungi

- *Suillus* and *Rhizopogon*
- Largely restricted to Pinaceae
- Animal dispersed, High spore longevity

Commercial Forestry

- Will increased *Rhizopogon* spore banks make *Pseudotsuga* more invasive?
- Should the spread of *Rhizopogon* be restricted?



Eucalyptus

- Highly invasive in California, Spain
- Promote fire / positive feedback
- Why is Eucalytus rarely invasive in NZ?

Research priorities...

- Origin of *Amanita*
- Spread
 - Host compatibility and populations
- Impacts
 - Fungal communities
 - Plant growth and health
 - Other organisms
 - Ecosystem outcomes (C sequestration)

Fly agaric (*Amanita muscaria*) in Tongariro Taupo Conservancy

Nicholas Singers

Threat

- There is a high diversity of ecomycorrhizal fungi associated with *Nothofagus* forests, over 800 species which represent approximately 20% of the known diversity.
- Several threatened and uncommon fungi are present within beech forests
- We presently do not know what the effect of *Amanita muscaria* invasion is having on the diversity of native fungi of *Nothofagus* forests

History

- 1999 with PJ unsuccessfully applied to DoC for some research funding "Threat of the exotic mycorrhizal fungus, *A. muscaria* to indigenous mycorrhiza fungi in beech forests"
- Pre 2005 there were no records of *A. muscaria* within beech forests, only on forest edges, but widespread in South Island forests
- 2006-2008 Increasing records of *A. muscaria* within Kaimanawa Ranges.
- Possibility of humans as "primary" vectors? as mushrooms have been discovered near campsites

Amanita muscaria in the Waipakihi Valley, Kaimanawa Ranges



April 2006

- Most of valley walked.
- 1 mushroom found in clearing , along un-formed track
- 1 mushroom found on campsite
- This valley has moderate use by trampers and hunters

Upper Rangitikei Valley

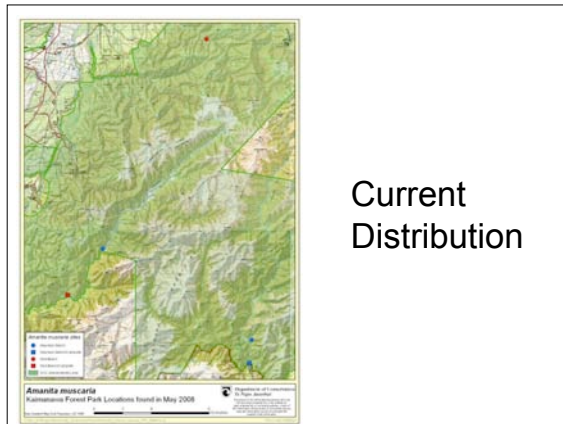


April 2008

- 1 fruiting body seen near campsite
- 2 others within 200m from this along possum bait-station track
- 1 fruiting body found in very remote area on ridge-line

Amanita muscaria at Rangitikei –Otamateanui campsite

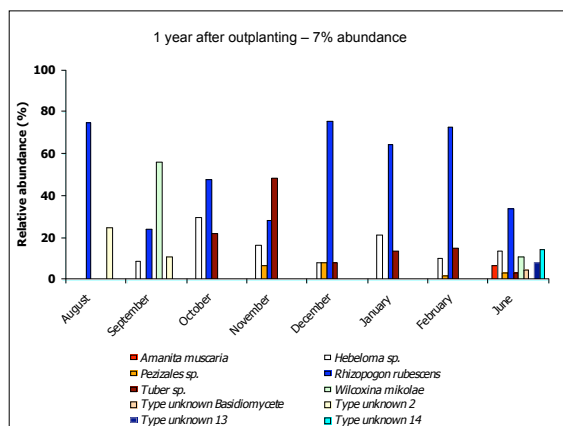
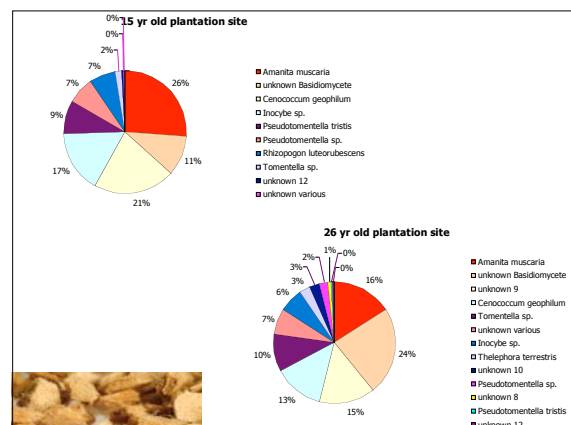
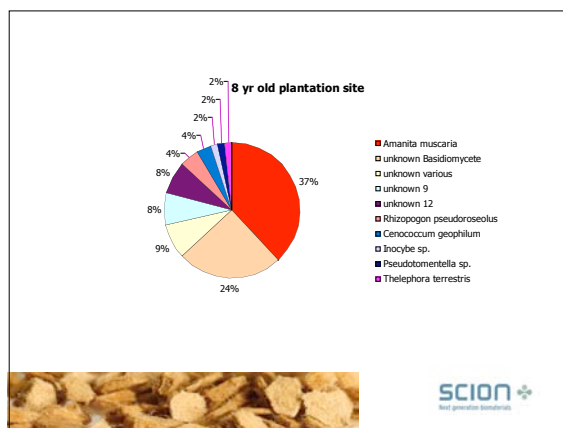






The Forestry view of things?

- Sorry, we're the bad ones...
- Need for *A. muscaria* in radiata pine and Douglas fir plantations
- *A. muscaria* not present in nurseries, but of importance in plantation sites
- In Chu-Chou and Grace literature not found in stands <10yrs
- In PhD study – of importance and abundance in >8yrs old stands
- ! Present already 1 yr after outplanting



What are our interests?

- We need it, we want it! Yea, we're the bad ones!
- Nurseries – could it be of importance there for us?
- What are means of dispersal?
- Spore viability in the soil?
- Do forestry practices & traffic resulting from forestry spread the species?
- What could we do to minimise spread?



Appendix 2 Survey of recent literature related to invasive ectomycorrhiza, with emphasis on *Amanita muscaria*

General literature on invasive ectomycorrhizal fungi

- Orlovich DA, Cairney JWG 2004. Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. *New Zealand Journal of Botany* 42: 721–738.
- Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A 2005. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters* 9: 501–515.
- Desprez-Loustau M-L, Robin C, Buée M, Courtecuisse R, Garbaye J, Suffert F, Satche I, Rizzo DM 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* 22: 472–480.
- Van der Putten et al. 2007. Microbial ecology of biological invasions. *ISME Journal* 1: 28–37.
- Reinhart KO, Callaway, RM 2006. Soil biota and invasive plants. *New Phytologist* 170: 445–457.
- [Scant coverage of ectomycorrhiza, but general discussion and review of arbuscular-mycorrhiza and plant invasions. References work on ericoid mycorrhiza and invasive plants in NZ]

Amanita-specific literature

- Pringle A, Vellinga VC 2006. Last chance to know? Using literature to explore the biogeography and invasion biology of the death cap mushroom *Amanita phalloides* (Vaill. Ex Fr. :Fr.) Link. *Biological Invasions* 8: 1131–1144.
- Oda T, Tanaka C, Tsuda M 2004. Molecular phylogeny and biogeography of the widely distributed *Amanita* species, *A. muscaria* and *A. pantherina*. *Mycological Research* 108: 885–896
- Johnston PR, Buchanan PK 1997. invasive exotic fungi in New Zealand indigenous forests- you can help! *New Zealand Botanical Society Newsletter* 47: 8–10.
- Johnston P, Buchanan P, Leathwick J, Mortimer S 1998. Fungal invaders. *Australasian Mycological Newsletter* 17: 48–52
- Sawyer NA, Chambers SM, Cairney JWG 2001. Distribution and persistence of *Amanita muscaria* genotypes in Australian *Pinus radiata* plantations. *Mycological Research* 105: 966–970.
- Bagley SJ, Orlovich DA 2000. Genet size and distribution of *Amanita muscaria* in a suburban park, Dunedin, New Zealand. *New Zealand Journal Botany* 42: 939–947.
- McKenzie EHC, Johnston PR, Buchanan PK 2006. Checklist of fungi on teatree (*Kunzea* and *Leptospermum* species) in New Zealand. *New Zealand Journal of Botany* 44: 293–335.
- [‘*Amanita* Pers.: An ectomycorrhizal genus. *A. phalloides*, an adventive species usually found under introduced trees, is known from a single collection underneath regenerating *K. ericoides* and *L. scoparium* (Ridley 1991). Although *A. muscaria* has been found fruiting close to teatree on at least three occasions, *Pinus radiata* or *Quercus* trees were always present as well, and there is no evidence that *A. muscaria* has become an invader forming mycorrhiza with teatree, as it has done with *Nothofagus* (Johnston & Buchanan 1998). The [7] other species are endemic, associated with teatree and some also with *Nothofagus* spp.’]

Human impacts

- Trim GM, Lepp H, Hall MJ, McKeown RV, McCaughan GW, Duggin GG, Le Couteur DG

1999. Poisoning by *Amanita phalloides* (“deathcap”) mushrooms in the Australian Capital Territory. MJA 171: 247–249

[Report results of 7 admissions: 3 cases developed severe hepatic dysfunction, 1 fatality. This is around typical fatality rate for California as well]

de Brunhoff J 1934. The story of Babar the little elephant. Methuen Children’s Books. 1954 English translation (8th edn). London, W S Cowell.

‘Alas! That very day the King of the Elephants had eaten a bad mushroom. It had poisoned him. He had been very ill, and then had died. It was a terrible misfortune.’

[Image clearly shows *Amanita muscaria*]

Other invasive ectomycorrhizal fungi

Diez J 2005. Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian peninsula. Biological Invasions 7: 3–15.

Plant–fungus co-invasions

Ashkannejhad S, Horton TR. 2006. Ectomycorrhizal ecology of *Pinus contorta* on the Oregon dunes. New Phytologist 169: 331–339.

[*Suillus* and *Rhizopogon* dominate in deer-dispersed ECM]

Chapela IH, Osher LJ, Horton TR, Henn MR. 2001. Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biology and Biochemistry 33: 1733–1740.

Other invasive fungi relevant to New Zealand

Johnston PR, Whitton SR, Buchanan PK, Park D, Pennycook SR, Johnson JE, Moncalvo JM 2006. The basidiomycete genus *Favolaschia* in New Zealand. New Zealand Journal of Botany 44: 65–87.

Vizzini A, Zotti M, Mello A 2008. Alien fungal species distribution: the study case of *Favolaschia calocera*. Biological Invasions (in press; DOI 10.1007/s10530-008-9259-5)

Other recent and relevant literature

Murat C, Zampieri E, Vizzini A, Bonfante P 2008. Is the Perigord black truffle threatened by an invasive species? We dreaded it and it has happened! New Phytologist 178: 699–702.

Tedersoo L, Suvi T, Beaver K, Kõljalg U 2007. Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriaopsis seychellarum*

(Dipterocarpaceae) and *Intsia bijuga* (Caesalpinaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). New Phytologist 175: 321–333.

[Introduced pine only associated with 3 introduced fungi: 2 *Rhizopogon* and 1 *Pisolithus* species; *Eucalyptus* associated with native fungi]

Appendix 3 Funding sources to support research

Envirolink + larger regional councils

Have good relationships with several regional councils, can further develop with Nelson [R. Toft to develop], Horizons [N. Singers can work on]

- Link into restoration plan / planting of native *Leptospermum/Kunzea*
- Risk assessment protocol – model to assess
- Vectors / risk of invasion / preventing spread / phytosanitary advice
- Risk of land use/recreation – SOPs to alleviate risk

FRST – International Investment Opportunity Funds for collaboration with Japan

Significant pool of funds

Application deadline very soon

We have good linkages to Japanese collaborators who have interest in the topic

MAF/Native Biosecurity [Melanie Newfield contact] – risk assessment

Risk of exotic pathogens/pests on natives

DOC – Important support/advocacy link. Direct DOC funding is likely to be limited to small co-funding, particularly around impacts on threatened species. Weed science fund?

Universities – Several of the research topics might be achievable as student research. Ability of universities to support students should be looked into [David Orlovich at Otago a good contact here]

FRST – ECO round. Conflicts with other research priorities of Landcare Research groups. Would be politically difficult to move a proposal forward

ERMA – Unknown possibility. Would be good to discuss with Geoff Ridley

Marsden Fund – Unlikely. Focus of Marsden is on fundamental research, not applied

MFE Sustainable Management Fund – believe focus is more on carbon, not a good probability of success

CRI/Capability Funds

Scion – Difficult to sell. Focus of Scion is on applied management, unlikely to support.

Landcare Research – Possible, particularly to support visiting fellowships. Moderate probability of limited funding