conjunction with other characteristics to resolve biologically meaningful species or subspecies.

Monitoring fungal hotspots - Stringybark Walking Trail, Deep Creek Conservation Park – a South Australian example

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A number of macrofungal hotspots in South Australia have been identified from surveys conducted in Parks in seven Regions of the State since 1997. These data, together with historic collections deposited in the State Herbarium of South Australia (AD) enable estimation of the conservation status of macrofungal taxa present at these hotspots.

One hotspot, Stringybark Walking Trail, an 18 ha site in Deep Creek Conservation Park, approximately 100 km south of Adelaide, has a particularly rich macrofungal flora. The number and diversity of fungal species recorded at this small site exceeds that at all other locations surveyed over the last twelve years in South Australia, with the exception of the much larger and ecologically more diverse Flinders Chase National Park on Kangaroo Island.

A Bunch of No-Good Rotters

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Sustainable forestry in Tasmania aims to retain all elements of a natural forest cycle in its management plan. In the wet *E. obliqua* forests of southern Tasmania, wildfire at different intervals has produced a mosaic of multi-aged stands with a successional climax of temperate rainforest after 400 years in the absence of fire. There are several heart rot polypores found in these forests that appear to be either host specific or confined to fruiting on large diameter eucalypts. Such trees are found in the older forests (> 250 years) or as legacies of wildfire disturbance in the younger stands. The logging of the old growth forests, the silviculture treatment of CBS (clearfell, burn and sow) and current rotation lengths of 80–100 years will lead to a loss of the large diameter *E. obliqua* and mature *Nothofagus cunninghamii* and *Atherosperma moschatum*. The fruiting bodies of the macrofungal species *Phellinus wahlbergii*, *Fomes hemitephrus* and *Australoporus tasmanicus* found in this study were exclusively in older forests and could be at risk of local disappearance. This has implications for forestry managers in Tasmania.

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Recently completed research theses in mycology

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Coarse woody debris, macrofungal assemblages, and sustainable forest management in a *Eucalyptus obliqua* forest of southern Tasmania

Genevieve Maria Gates

PhD thesis, The University of Tasmania, Australia.

2009

Supervisors: Assoc. Prof.Caroline Mohammed, School of Agricultural Science UTAS; Dr Neil Davidson, School of Plant Science UTAS; Dr Tim Wardlaw, Forestry Tasmania

Abstract

This study focussed on two components of the forest ecosystem at a small spatial scale: coarse woody debris (CWD), defined as fallen dead wood ≥ 10 cm diameter and ≥ 1 m length, and the macrofungal assemblages found on wood, soil and litter in native forest at different times of regeneration since the natural disturbance of wildfire.

The CWD on the forest floor and standing dead wood (stags) in four 50 × 50m plots (= 1 ha total area) with differing wildfire histories in a *Eucalyptus obliqua* dominated native wet sclerophyll forest in southern Tasmania, Australia, were quantified and mapped. The CWD volumes obtained were amongst the highest in the world.

Analyses showed that although a plot size of 0.25 ha was too small to give an accurate measurement of volume, it was large enough to contain dead wood having attributes that reflected the stand structure resulting from wildfire disturbance. Therefore, a plot's wildfire history can be deduced from the CWD and stags of a 0.25 ha plot.

The substrates wood (dead wood and standing trees), soil and litter in each plot were surveyed for macrofungal fruit bodies at approximately fortnightly intervals for 14 months. A total of 849 macrofungal species was recorded from 1ha of native forest.

Wood supported 410 species of which 295 were on CWD but not exclusively, i.e., a few species were found on CWD and soil or on CWD and litter. The majority of the remaining species on wood was supported by 'other dead wood' (a category containing dead wood that did not fit into CWD), which contained many species not in common with those on CWD.

It was concluded that macrofungal species richness on CWD is not affected by decay class; however, length or surface area explained between 45–48% of the variation in species richness.

Of the 495 species found fruiting on soil, 330 were known to be ectomycorrhizal and 165 were considered decomposers. In addition, 146 species of macrofungi were associated with litter. It was found, using temperature and rainfall data, that the appearance of fruit bodies is seasonal but not directly attributable to rainfall events. There was a better correlation using the indigenous peoples' concept of three seasons than when using the four European-based seasons.

In essence, each plot contained a distinctive mycota, reflecting its chronosequence history, site characteristics (e.g., soil type, soil pH) or microclimate. To maintain the macrofungal diversity associated with the differing plots, a mosaic of multi-aged stands in the managed forest landscape is needed to provide inoculum for the reestablishment of macrofungal communities in forests at different times of regeneration. In addition, reserves should be as large as possible (at least 1ha) to encompass the variability (due to site characteristics, vegetation type, etc.) in the forest landscape and

the associated macrofungal diversity as evidenced by the appearance of fruit bodies. This has particular implications for the silvicultural treatment of ARN (aggregated retention) where the retained aggregates provide refugia for macrofungal assemblages associated with the pre-treatment forest type. The results of the study also suggest that there should be some coupes assigned to longer rotations to provide a continuum of dead wood sizes and decay classes in the forest landscape, thereby maintaining associated macrofungal diversity.

Evaluation of *Metarhizium anisopliae* for biopesticide control of livestock ectoparasites

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PhD thesis, School of Veterinary Science, The University of Queensland

June 2009

Abstract

Current control strategies for livestock ectoparasites are limited by problems associated with chemical resistance and residues. Fungal biopesticides could provide an alternative control without these problems. However, a strategic approach is needed to first evaluate the suitability of selected fungal isolates for fungal biopesticide development. Two ectoparasites of significance to cattle and sheep are the cattle tick *Rhipicephalus (Boophilus) microplus* (Canestrini) and the Australian sheep blowfly *Lucilia cuprina* (Wiedmann). The fungus *Metarhizium anisopliae* (Metsch.) Sorokin) was evaluated for its potential to control these livestock ectoparasites.

The growth characteristics of 30 isolates of M. *anisopliae* were investigated. Radial growth measurements were used to identify vigorous isolates that grew well at 30°C and were capable of growing at 35°C. A qualitative assessment of sporulation capacity further refined the candidate isolate group. A possible nutritive role of oil in the formulation was also investigated. However, there was no clear support for the theory that oil as a formulation additive could boost the germination and growth of the fungal conidia *in vitro*.

Quantal response bioassays were conducted with cattle ticks and sheep blowflies using a range of conidial doses of three different isolates of *M. anisopliae* and different methods of inoculation. Ticks were either dosed with 2 µl or immersed in the conidial doses. Blowflies were either dosed with 2 µl of the conidial doses or fed conidia mixed with sugar. Probit analyses were carried out on the mortality data to compare the virulence of these isolates to ticks and blowflies and look for indications of different virulence mechanisms employed by *M. anisopliae* isolates when invading these hosts. One isolate (ARIM16) showed high virulence to both hosts killing 95 % of ticks after two days and 88 (±2) % of blowflies after four days. Strikingly different mortality patterns indicated quite different virulence mechanisms operating when *M. anisopliae* invades ticks or blowflies. The mortality pattern seen with ticks suggested that the number of conidia adhering per unit area of the cuticle was more important for rapid tick death than the total number of conidia contacting the entire tick surface. Blowflies fed conidia mixed with food died rapidly after an initial lag phase regardless of dose.

Microscopic investigations were carried out to resolve the basis of the virulence patterns observed. The spatial and temporal aspects of the invasion of ticks and blowflies by *M. anisopliae* isolate ARIM16 were investigated with different types of microscopy. The scanning electron microscope and stereo light microscope were used to record surface changes and events and the compound light microscope revealed internal changes. Two distinctly different patterns of invasion were found in ticks and blowflies. Fungal conidia germinated on the surface of ticks then hyphae simultaneously penetrated into the tick body and grew across the tick surface. There was extensive fungal degradation of the tick cuticle with a preference for the outer endocuticle. While large numbers of conidia adhered to the surface of blowflies, no conidia were recorded germinating on external surfaces. One germinating conidium was seen in the entrance to the buccal cavity. Investigations of the fly interior revealed a higher density of hyphal bodies in the haemolymph surrounding the buccal cavity than in haemolymph from regions of the upper thorax. This pattern suggested that fungal invasion of the blowfly is through the buccal cavity. Plentiful extracellular mucilage was seen around the hyphae on ticks, and crystals of calcium oxalate were seen amongst the hyphae on the surface of ticks and in the haemolymph of blowflies killed by *M. anisopliae* isolate ARIM16. It was considered that cattle ticks are more suited for control with fungal biopesticides than adult blowflies.

Three field trials were conducted over twelve months to assess the pathogenicity of *M. anisopliae* to parasitic stages of *R. microplus* on dairy heifers under different environmental conditions. Two isolates were selected based on their high optimal growth temperature (30°C), good conidial production characteristics and ability to kill adult engorged ticks in the laboratory in minimum time. Conidia were formulated in an oil emulsion and applied using a motor driven spray unit. Surface temperatures of selected animals were monitored, as were the ambient temperature and relative humidity. Unengorged ticks sampled from each animal immediately after treatment were incubated under laboratory conditions to assess the efficacy of the formulation and application. Egg production by engorged ticks collected in the first 3 days after treatment was monitored. Side counts of standard adult female ticks were conducted daily, before and after treatment to assess the performance of the fungus against all tick stages on the animals. At each trial the formulation caused 100% mortality in unengorged ticks that were removed from cattle and cultured under laboratory conditions. A significant reduction in egg production was recorded for engorged ticks collected in the three days post treatment. In the field, the fungal formulation had an inconsistent effect on ticks, which might be due to the influence of environmental temperature and humidity.

Surrogates for cryptogam conservation - associations between mosses, macrofungi, vascular plants and environmental variables.

Sapphire J. M. McMullan-Fisher

PhD thesis, School of Geography and Environmental Studies, University of Tasmania, Hobart, Australia.

2008

Abstract

Cryptogams are rarely included in conservation planning and management. This study aims to improve the data available for cryptogam conservation by focusing on two groups of cryptogams, mosses and macrofungi, to test the usefulness of vegetation type, vascular plants and environmental variables, including substrate, as surrogates for cryptogams in achieving satisfactory conservation outcomes.

Sites from four vegetation types (wet forest, heathy woodland, grassy woodland and alpine heath) in the Hobart region of Tasmania were surveyed over a period of several years for vascular plants, mosses and epigeous macrofungi using permanent plots. Repeated sampling of the macrofungi ensured that a reasonable proportion of the taxa likely to be present were recorded. A total of 284 vascular plants, 71 mosses and 233 macrofungi were recorded.

Ordination and analysis of similarity both showed that the four vegetation types were significantly different from each other; this pattern occurred for vascular plants, mosses and macrofungi. Congruence between the three taxonomic groups was tested using Partial Mantel tests; all pair-wise associations

were highly significant, showing highly predictive r-values. Significant and predictive associations occurred between environmental and substrate variables and biotic groups (vascular plants, mosses and macrofungi, and their various subsets). Canopy cover was the best single predictor of most biotic groups. Particular combinations of significant environmental variables had higher correlations with biotic groups than single variables, for example the combination of altitude, canopy cover and geology had higher r-values than any of these factors individually. Mosses and macrofungi exhibited high substrate fidelity across time and space. Substrate preferences of macrofungi did not vary among vegetation types, but mosses in wet forest occurred on a wider range of substrates than the same species in other vegetation types.

Iterative, optimisation, fully random and stratified random methods were compared for their effectiveness in the selection of sites for the conservation of vascular plants, mosses and macrofungi. When 10% of sites were selected for reservation there was little commonality in site selection between the three taxonomic groups. When 30% of sites were selected, at least 48% of all taxa were reserved by all approaches tested. The most useful data sets for selecting sites representative of the three taxonomic groups were vascular plants, named species from all three taxonomic groups and sites selected randomly with equal proportions of each vegetation type.

The results suggest that coarse scale conservation of vegetation types with reservation of at least 30% of their area should conserve common mosses and macrofungi. However, at the site scale, uncommon taxa (i.e., taxa only found on a single site) of mosses and macrofungi are not concordant with vascular plants. Associations of moss and macrofungal species with particular substrates and microhabitats may assist with site selections for reservation. For adequate management, further research is required on the occurrence and substrate and habitat specificity of rare taxa.

Fungal diversity in remnant vegetation patches along an urban to rural gradient

Mark Gavin Newbound

PhD thesis, School of Botany, The University of Melbourne, Australia.

September 2008

Supervisors: Michael McCarthy, Australian Research Center for Urban Ecology, and School of Botany, University of Melbourne; Teresa Lebel, Royal Botanic Gardens, Melbourne; Pauline Ladiges, School of Botany, University of Melbourne.

Abstract

Remnant vegetation patches in urban areas are valuable for biodiversity conservation, as well as recreation and community education. Fungi are a functionally important, and often overlooked, aspect of the diversity of remnants. Fungi are highly diverse, accounting for around 8% of the world's species. They are critical in food webs and for nutrient cycling. Symbiotic mycorrhizal relationships between plants and fungi are important for plant nutrition, seedling establishment, and can affect the composition of plant communities. This thesis presents an investigation into how the distribution and occurrence of fungi in remnants are influenced by the level of surrounding urbanization. The research was set in the city of Melbourne, Victoria.

Surveys were made of both above and below ground fungal structures in remnant vegetation patches along an urban to rural gradient. Above ground, species of macrofungi were surveyed over two years by the collection and identification of fruiting bodies. Two below ground surveys were made. In the first, mycorrhizal root tips of two eucalypt species were sampled along a gradient. The second used a molecular method, terminal restriction length polymorphism (TRFLP), to assess diversity in bulk soil samples taken from sites.

The fruiting body surveys produced the most informative results. Distinctions were made between properties affecting saprotrophic species, which derive carbohydrates from decaying organic matter, and ectomycorrhizal (ECM) fungi, which obtain carbohydrates from symbiotic plant hosts. A total of 199 species were found, with close to four times more saprotrophic than ECM species. Urbanisation appeared to have little effect on diversity, which was influenced more by particular site properties. Saprotrophic species richness decreased with increasing canopy openness; ECM richness decreased with higher soil pH and available phosphorus; and the ratio of saprotrophs to ECM increased with greater soil nitrate. Some management practices based on the findings are suggested to promote fungal diversity within urban remnants.

A second topic in the thesis is an investigation of methods used to survey fungi. Assessments of fungal diversity are problematic because fungi are cryptic, highly diverse and patchily distributed. To help improve the efficiency of fruiting body surveys, Bayesian models were made to identify the environmental factors that influence fruiting. These suggested that to increase the probability of detecting species, frequent fruiting body surveys should be made in late autumn to early winter when the average value for rainfall minus evaporation for the previous 28 days is above -1 mm per day. Analyses were also made to compare the efficiency of the fruiting body and TRFLP survey methods. These found that species present were four times more likely to be found using TRFLP. However the TRFLP results did not correlate with environmental properties, probably because different fungal functional groups could not be differentiated. Thus, although less efficient for finding the total number of species present, fruiting body surveys provided a more representative sample of the fungal community.

In conclusion, remnant vegetation in urban settings appears to be a valuable repository of macrofungal diversity, although doubts remain because of the limits of the surveying methods used.

Molecular characterization of the Vancouver Island *Cryptococcus gattii* outbreak strains and their placement within the *Cryptococcus* species complex

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PhD thesis, Sydney Medical School –Western, The University of Sydney, Australia.

2008

Supervisor: Associate Professor Dr. rer. nat. Wieland Meyer, Sydney Medical School - Western, University of Sydney; Co-supervisors: Prof. Tania C. Sorrell, CIDM, Sydney Medical School -Western, The University of Sydney, A/Prof. Ariya Chindampon, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Abstract

The *Cryptococcus* species complex is composed of two basidiomycetous yeasts: *Cryptococcus neoformans* (var. *grubii* and var. *neoformans*) and *Cryptococcus gattii*. Three haploid molecular types, VNI, VNII and VNIV, and four haploid molecular types, VGI, VGII, VGIII and VGIV have been recognized in *C. neoformans* and *C. gattii,* respectively. *C. gattii* mainly infects immonocompetent individuals and its VGII molecular type has been the cause of the ongoing cryptococcosis outbreak on Vancouver Island, BC, Canada, since 1999. Two subtypes of this molecular type, VGIIa and VGIIb, have been recognised on the island with VGIIa being the major population. The VGIIa population has also been shown to be hyper-virulent, in a comparative study of a

representative strain from each subgroup, namely R265, VGIIa, and R272, VGIIb. In our study, we found a correlation between the VGIIa subtype with fertility and melanin production. The comparative transcriptome study has identified several genes that encode putative virulence factors (e.g. *LAC1, LAC2, CAS3* and *MPK1*) and proteins involved in cell wall assembly, carbohydrate and lipid metabolism being induced in R265 comparing to R272. Gene characterizations showed that *LAC1* revealed defects in melanin synthesis where *CAS3* was not essential for the capsule production. *MPK1* was not only required for cell integrity but also for melanin and capsule production, despite a mild effect on thermotolerance in *C. gattii*. Phylogenetic investigation using *ACT1*, *URA5*, *PLB1* and *IDE* revealed highly-supported seven haploid molecular type clades with similar variations and topologies observed between *C. neoformans* and *C. gattii*. Hence, raising each clade to at least a variety level should now be considered. In conclusion, a uniqueness of the VGII molecular type over the other molecular types was shown and was manifested in the phylogenetic analysis. However, due to the limitation in the number of the strains studied further investigations with more strains are warranted.

Pseudocyphellaria crocata: Investigations into this symbiosis.

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Postgraduate Diploma in Science thesis, Dept of Botany, University of Otago, Dunedin, New Zealand.

2009

Supervisors: Drs David Orlovich and Tina Summerfield, Dept of Botany, University of Otago, Dunedin, New Zealand.

Abstract

A lichen is a symbiotic association between a fungus (mycobiont) and a photosynthetic partner (photobiont) which may be a green alga or cyanobacterium or the symbiotic association of all three organisms. This is a complex association where the fungus produces a thallus structure distinct from its free-living form, and this thallus "houses" the photobiont. *Pseudocyphellaria crocata* is a cyanolichen, with the cyanobacterium *Nostoc* providing both photosynthate and fixed nitrogen to the fungal partner. Little is known about the interactions between the symbiotic partners nor the role of one symbiont in different parts of the thallus. For example, the cyanobacterium is present as a continuous layer throughout the thallus but levels of photosynthesis and nitrogen fixation across this photobiont layer have not been determined. The aims of this project were (i) to measure gene expression levels throughout the lichen thallus in order to determine whether or not the metabolic potential of the cyanobiont varied in different parts of the thallus and (ii) to use culturing techniques to isolate the mycobiont partner and other fungi growing in close association with this symbiosis. The main results showed an increase in transcript abundance of photosynthetic genes in the growing margins of the thallus, compared to the centre samples and an increase mRNA levels of a nitrogen fixation gene in the thallus centre compared to growing margins. These results indicate that at the margins there may be an increase n photosynthetic capacity, whereas there could be an increase in nitrogen fixing ability in the centre. This represents the first use of gene expression to study metabolic compartmentalization of any lichen. A wide range of fungi associated with this lichen species were isolated into axenic culture, however, the mycobiont was not cultured. However other fungi were isolated into axenic culture that may represent new species and which were associated with the lichen, which raised some questions such as do these fungi have any role in the lichen symbiosis. Further research is needed to provide insight into the communication and coordinated growth of the respective symbionts, in order to gain a deeper understanding of this symbiosis.

Molecular phylogenetics, divergence dating, and species delimitation in New Zealand *Menegazzia* (Parmeliaceae, lichenized ascomycetes)

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Bachelor of Science (Hons) thesis, Dept of Botany, University of Otago, Dunedin, New Zealand

2009

Supervisor: Dr David Orlovich, Dept of Botany, University of Otago, Dunedin, New Zealand.

Abstract

Menegazzia is a genus of ca. 70 described lichen species. Modern molecular phylogenetic methods have not been used to investigate this genus before, and so a number of questions relating to its evolutionary history have remained unanswered. For the first portion of this study, molecular data from four genes (nuclear ITS, 28S, eflα, and GPDH) was employed to test the monophyly of 20 species and both major subgenera of *Menegazzia*. Bayesian Inference, Maximum Likelihood, and Maximum Parsimony methods were used to build the phylogenies. The second part of the study involved fitting the phylogeny to a historical timeline. Lichen phylogenetic studies have so far largely avoided using molecular clock methods to estimate divergence dates, so it seemed worthwhile to find out if there was a valid reason for this, or if they had simply not been tried. The final part of this study revolved around species delimitation. A model specifically designed to delimit lichen species based on a combination of DNA sequence data and morphological/chemical/ecological data was developed and tested. The main conclusions of the study were: (1) The two main *Menegazzia* subgenera were found to be paraphyletic, and should therefore be abandoned. (2) It is possible to use modern divergence dating techniques on lichens (at least parmelioid lichens) and result in confident estimates, and tMRCA for *Menegazzia* was found to be 94 Ma (±11 m.y.). (3) One previously unknown Evolutionary significant Unit, and four potentially new species were discovered using a novel approach to lichen species delimitation.