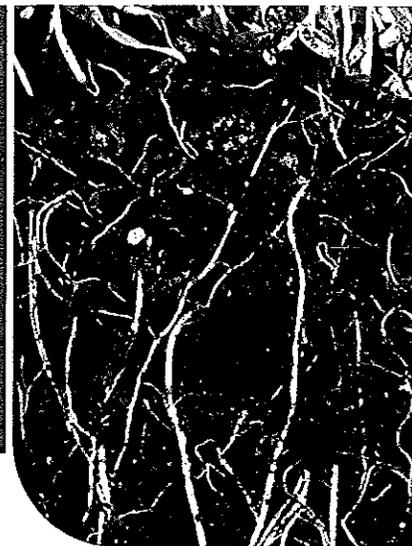


Who's On Your Roots?

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Background

Most crops form symbiotic associations with mycorrhizal fungi that can influence plant nutrient uptake, growth, and health. Small fruit and nursery crops that are members of the Ericaceae (e.g. blueberry, cranberry, rhododendron) form specific associations with ericoid mycorrhizal fungi (EMF), yet there is almost no information concerning how these EMF influence the physiology of their host plants in horticultural production systems. Knowledge of the physiological responses of blueberry to mycorrhizal symbiosis will increase how effectively they are used in blueberry production systems. Knowledge of the genetic and functional diversity of EMF is extremely important for furthering our understanding of these fungi and their interactions with *Vaccinium* under production conditions and with native ericaceous plants. Although Australia has several native ericaceous plants, members of the genus *Vaccinium* are not native to Australia. Comparing the diversity and attributes of isolates of EMF that exist in blueberry plantings in Australia and the USA would increase our understanding of the role that these fungi play in blueberry production in both countries. Identifying the mycorrhizal fungi present in roots of blueberries grown in Australia will allow comparisons to be made to known ericoid mycorrhizal fungi (EMF) from blueberries grown in the northern hemisphere and EMF found on native Australian ericaceous plants. Preliminary investigations conducted

by McLean (unpublished) showed some mycorrhizal isolated from Australian blueberry plants are similar to EMF found on northern hemisphere blueberries; however, there are other fungal species which have not been reported on northern hemisphere ericaceous plants.

In November 2006 Drs Cassandra McLean and Carolyn Scagel collected samples of blueberry roots from fields in Victoria, Tasmania, and New South Wales. Samples of roots collected from blueberry farms from New South Wales (Coffs Harbour and Alstonville) were used for the undergraduate research project of Lana Smart at the University of Melbourne, Burnley. Ms Smart cultured a total of 102 fungal isolates and used these fungi in experiments to (1) identify the fungi present in roots of field-grown blueberry plants, (2) determine whether the fungi formed mycorrhizae with blueberry plants, and (3) establish whether the fungi preferred specific sources of nitrogen (N) for growth.

Identification of fungi from blueberry roots

The fungal isolates that Ms Smart obtained from roots were separated to 15 groups based on macroscopic characteristics in culture including, culture colour, texture, exudate, growth rate, similarity of culture characteristics to that of known EMF. Specific isolates from each of these 15 groups were selected for further characterization based on DNA. To determine the identity of the fungal isolates Ms Smart isolated and sequenced specific areas of

Svetlana Smart provides a summary of her thesis research with Doctors Carolyn Scagel and Cassandra McLean on mycorrhiza work in northern New South Wales.

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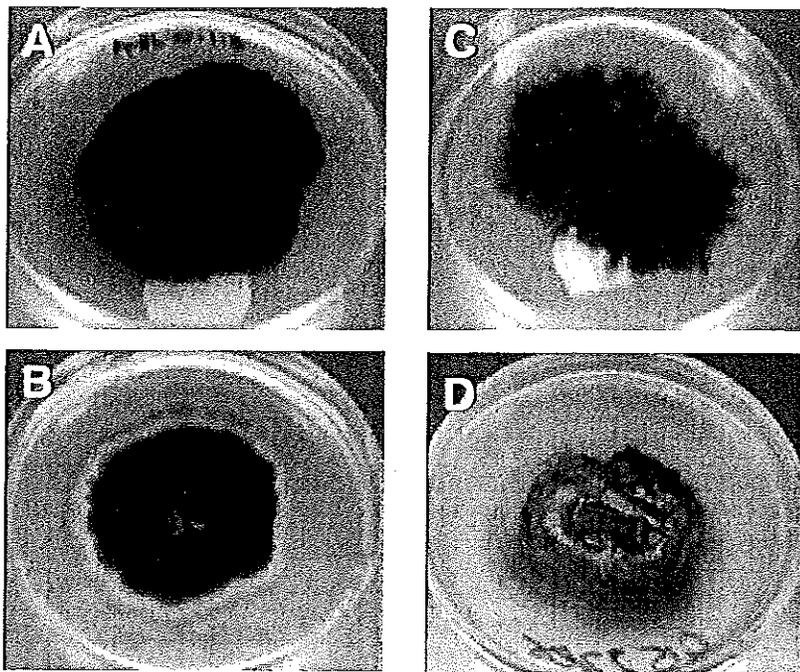


Figure 1: Photograph four different groups based on culture characteristics. A and B were identified as *Rhizoscyphus ericae* based on DNA sequences and C and D were identified as *Phialocephala scopiformis* based on DNA sequences.

DNA from the fungi in culture and compared the sequences she obtained to a worldwide database of DNA sequences (GenBank) to identify the fungi.

There was quite a diverse range of culture characteristics although most isolates were dark sterile isolates, which is typical of EMF from Australia and the rest of the world (McLean et al. 1999). Culture characteristics were used to define the initial groups of isolates; however, even though culture characteristics varied between groups in some cases isolates from different groups were identified as the same species based on DNA results. Fungi can be identified using traditional methods based on morphological characteristics; however, many characteristics such as spore length and width overlap for closely related species, which makes species delimitation difficult using morphology alone. Also, traditional identification techniques are heavily biased towards spore morphology and the majority of EMF do not produce spores in culture. Therefore sequencing DNA is a much more reliable method for identification.

The results from the DNA analyses identified approximately one-third of the isolates to the species level, and one-third of the isolates to the genus level. The taxonomy of the remainder of the isolates was uncertain. The DNA sequences from approximately 80% of the groups were similar to those of known EMF or endophytes associated with epacrid roots but never identified. A phylogenetic tree was developed as part of this research in an attempt to determine how isolates were related to previously identified sequences from fungi. It was not possible to accurately identify all of the isolates because the taxonomic information or knowledge was not available in the Genbank database. Taxonomic knowledge in the GenBank database needs to be expanded before these isolates can be accurately identified by sequence analysis.

Blueberry plants in Australia share some fungi with blueberry plants in other countries

The DNA sequences from two of the 15 groups were closest to sequences from *Rhizoscyphus*

ericae. The fungus *R. ericae* is one of the most widely studied EMF and has previously been isolated from cranberry (*Vaccinium macrocarpon*) and blueberry (*V. corymbosum*) in North America, lingonberry (*V. vitis-idaea*) and bilberry (*V. myrtillus*) in Northern Europe, *Rhododendron lochiaie* in Australia, and the leafy liverwort *Cephaloziella varians* in Antarctica. This fungus has not been isolated from members of the Epacridaceae in Australia even though *R. ericae* is able to form Typical Ericoid Mycorrhizal Structures (TEMS) with epacrids, such as *Epacris impressa* in culture.

Isolating *R. ericae* from roots of blueberry in Australian production fields is significant and it raises several questions since this species is present in Australia but has not been isolated from native epacrids. The presence of this fungus in Antarctica, in Australia on *Rhododendron lochiaie*, as well as in the northern hemisphere suggests *R. ericae* has a global distribution and *R. ericae* was present in Australia prior to the introduction of blueberry plants. Potential native host plants for *R. ericae* in Australia would include members of the Epacridaceae, which are distributed throughout Australia, and members of the Ericaceae, which currently have very localized distributions within Australia (e.g. *Gaultheria appressa* or *Rhododendron lochiaie*). Additionally, common non-native landscape plants (*Rhododendron sp.*, *Pieris sp.*, etc.) in the Ericaceae may be hosts for *R. ericae*. After assessing over 200 fungi isolated from two species in the Epacridaceae, McLean et al. (1999) concluded fungi isolated from epacrids did not belong to the same species as those reported to form TEMS in the Ericaceae from the northern hemisphere. Interestingly, epacrids are thought to be closely allied to the Vaccinioid tribe of the Ericaceae, so there may be a good potential for compatibility between EMF from epacrids and plants in the genus *Vaccinium*.

Fungi in the *R. ericae* groups were more frequently isolated from blueberry farms around Coffs Harbour than those close to

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Alstonville. Increased sampling at a greater number of blueberry farms as well as from native epacrids and ericaceous plants grown for the horticultural industry may help to determine the geographical distribution of *R. ericae* in Australia. It is unknown whether different isolates of *R. ericae* are better suited for the warmer blueberry growing regions in northern NSW compared to those in cooler regions like Tasmania.

The DNA sequences from one of the 15 groups were closest to the sequence of *Oidiiodendron maius* var. *maius*. This is the only species of this genus confirmed to form beneficial relationships with members of the Ericaceae and is consistently isolated from ericaceous plants in North America and Europe where it is reported to tolerate relatively high salt concentrations. This species is able to use cellulose, gelatine, lipid, pectin, starch and tannic acid for growth and its optimum conditions for growth are lower than the optimum soil pH reported for blueberry production in NSW. This may explain why this fungus was only isolated three times from NSW blueberry plants. *O. maius* var. *maius* has been isolated from *Rhododendron lochiaie* in Queensland. *R. lochiaie* is one of the very few members of the Ericaceae which is native to Australia. *R. lochiaie* is more closely related to *Vaccinium* sp. than epacrids.

Assessment of mycorrhizal ability

Specific isolates from each of these 15 groups were selected by Ms. Smart to determine their ability to form mycorrhizae in culture. It was essential to determine if isolates were able to form TEMS with blueberry plants because this is a good predictor of a beneficial mycorrhizal association. The formation of TEMS provides an interface for nutrient exchange to occur between the plant and fungus; if TEMS do not form the fungus may not be any benefit to the host plant. A vast majority of early research with EMF confirmed that fungi isolated from ericaceous plants were actually

mycorrhizal. To broaden our ability to manage EMF in any horticultural production system, it is essential to prove that suspected EMF isolates form TEMS with their hosts. After validating the ability to form TEMS, long-term studies can be used to assess benefits to both partners in the association. Due to time constraints, it was not possible to determine if the presence of the isolates were able to benefit the host plants, instead the formation of TEMS was used as evidence to support a claim that an isolate was an EMF. Formation of TEMS was evaluated on blueberry plants grown from seed in sterile media. Seedlings were inoculated after germination and the ability of the fungi to form TEMS was evaluated after 6 weeks.

Validated EMF

Some of the isolates that were used to inoculate blueberry seedlings produced TEMS in the root cells and there was no difference in the degree of colonisation between isolates that produced TEMS. Two isolates that produced TEMS were identified as *Rhizoscyphus ericae*

which is a known EMF. One isolate that produced TEMS was similar to an unidentified endophyte of *Epacris microphylla*. This is the first documented evidence that both the fungi commonly found on ericaceous plants in the northern hemisphere and those found on native epacrids are not only present in blueberry roots in NSW Australia but also can form mycorrhizae with blueberry plants.

In culture conditions native Australian EMF can form mycorrhizal associations with non-native ericaceous plants. For example, an unidentified EMF from the Australian epacrid *Woolisia pungens* was able to form TEMS with cranberry roots in culture. However, past DNA comparisons between EMF from the Epacridaceae and Ericaceae in native habitats has resulted in separate clusters of EMF from the two host types. Ms. Smart's results are significant because it shows that Australian blueberry EMF are not only composed of the common northern hemisphere blueberry EMF, such as *R. ericae* and *O. maius*, but part of a genetically different group of EMF which is similar to Australian EMF.

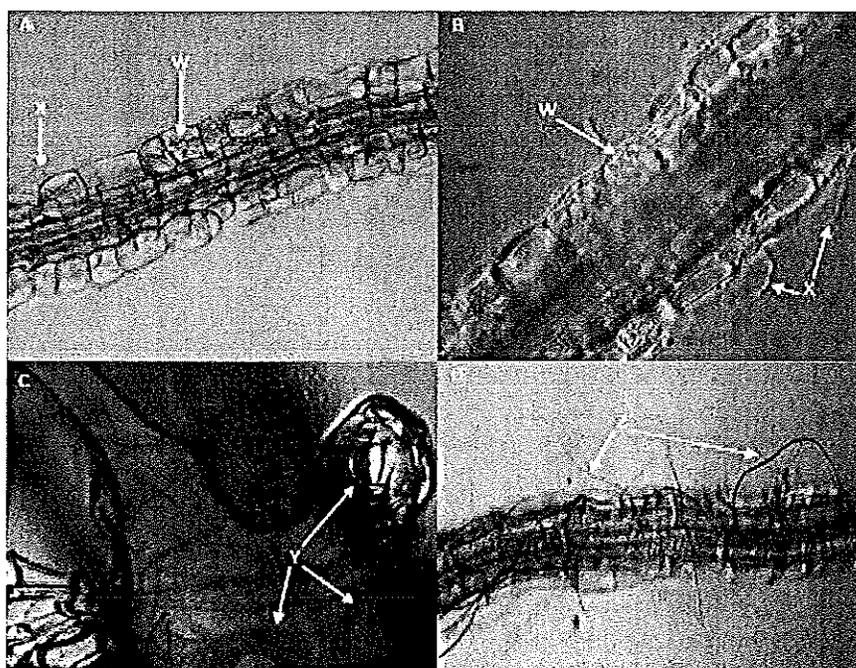


Figure 2. Photomicrograph of blueberry seedling roots inoculated with fungi isolated from field-grown blueberry plants. (A) *Rhizoscyphus ericae*, (B) isolate similar to endophyte from *Epacris microphylla*, (C) unidentified fungus, and (D) isolate similar to endophyte from an epacrid root. W = typical ericoid mycorrhizal structures (TEMS) inside root epidermal cells; X = hyphae growing externally to root Y = hyphal coils outside root; and Z = hyphae growing along roots.

Who's On Your Roots?

Some of the fungi Ms Smart isolated from roots of field-grown plants did not produce TEMS, and only grew along roots. The sequences from two groups of these isolates were more similar to EMF isolated from *Epacris impressa* and *Epacris microphylla* than EMF isolated from northern hemisphere blueberry plants. Other researchers have isolated fungi from roots of ericoid mycorrhizal plants and these fungi did not form TEMS with the original plant host. There are two possible reasons why TEMS were not produced by these isolates on blueberry seedlings: (1) the isolates are not EMF; therefore, they are either another type of endophyte (non-pathogenic or pathogenic) or a rhizosphere contaminant; and (2) the isolates are EMF but the conditions for establishment of the symbioses were not present under the experimental conditions, for example temperature, time, growing substrate, or blueberry cultivar.

Together, Ms Smart's results suggest there may be some level of host-fungus specificity or selectivity in EMF associations with Australian

plants not accounted for during *in vitro* tests. Others have reported environmental conditions, such as higher temperatures, may drive the specificity/selectivity of mycorrhizal associations in other plant systems. This indicates the ability of EMF to form associations in experimental systems may not mean the fungus is capable of forming similar associations in the plants native habitat or in a production field.

Potential EMF and other endophytes

Isolates from three other groups that have potential to be EMF did not form TEMS with blueberry roots. The DNA sequences from two of the 15 groups were closest to sequences from *Phialocephala scopiformis* and *Phialocephala* spp. which are commonly found as endophytes and EMF of native ericoid mycorrhizal plants in alpine and subalpine areas of North America and Europe. Very little research has been published on EMF of native ericoid mycorrhizal plants from alpine and subalpine

areas in Australia. The EMF from these areas may be more suitable or likely to form mycorrhizal associations with blueberries because of the original habitat of the host plant. The DNA sequence from one of the 15 groups was closest to the sequence of *Geomyces pannorum*. Other researchers have shown *G. pannorum* can produce loops of hyphae similar to TEMS in *Vaccinium* microcuttings but this fungus is not currently regarded as an EMF. The ability of this fungus to produce TEMS in *Vaccinium* sp. warrants further investigation of the potential role of this fungus in blueberry production. *G. pannorum* is able to degrade polyester and polyurethane in the soil and therefore might be useful in bioremediation of landfill sites.

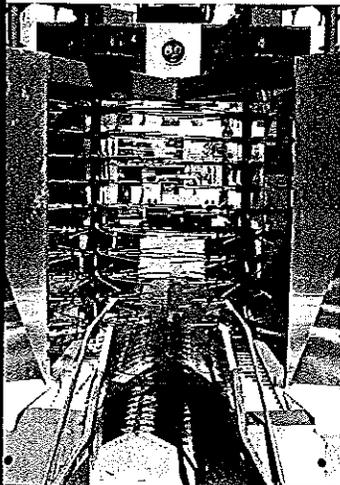
Inoculating roots with one isolate resulted in a distorted root system. The DNA sequences from this group were closely related to species in the genus *Phoma*. *Phoma* spp. are very common soil fungi commonly isolated from declining or diseased plants as primary or secondary pathogens. The stunted roots on inoculated seedlings



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could have been an indication of a pathogenic relationship between this isolate and blueberry. This highlights the importance of not only validating the mycorrhizal ability of fungi isolated from root systems, but also indicates why using roots from field-grown blueberry plants as EMF inoculum is a dangerous practice because the roots may contain deleterious organisms.

Nitrogen preference

Specific isolates from each of these 15 groups were selected to determine their nitrogen use in culture. To determine the nitrogen preference of each isolate, ammonium, nitrate or chitin was provided as the sole N source to fungi growing in liquid media. Ammonium and nitrate occur frequently in many inorganic fertilizers commonly used in food production systems. Mycorrhizal fungi are also able to use organic sources of N such as amino acids or proteins. In natural environments N is likely to be contained in more complex polymers, such as chitin, which may be the most important naturally occurring N source in soil that is exploited by mycorrhizal fungi.

Growth of the fungi varied with N source. Approximately one-third of the fungi equally preferred ammonium or nitrate compared to chitin. Isolates identified as *P. scopiformis* (one of which formed TEMs) exhibited this preference for inorganic sources of N over chitin. One unidentified fungus that formed TEMs (similar to an unidentified endophyte of *Epacris microphylla*) preferred nitrate over ammonium and one fungus identified as *R. ericae* that formed TEMs preferred ammonium over nitrate. All these fungi were isolated from root systems of blueberry plants grown with inorganic fertilizers, it would be interesting to determine whether EMF isolated from blueberry plants grown with organic nutrient sources exhibited the same preference for inorganic N sources.

Fungi identified to the same species varied in their preference for N sources. For example, one isolate identified as *R. ericae* grew more on media containing

inorganic N sources than on medium containing chitin and the other isolate identified as *R. ericae* grew a similar amount with all N sources. Two of the fungi which were similar to those found on epacrid roots also showed no preference between N sources. Obviously there is variation in N preference between EMF that could potentially be exploited to improve management of EMF in different blueberry production systems (e.g. organic versus traditional production systems). Others have shown that EMF colonization of blueberry plants varies between cultivars and EMF colonization and is influenced by fertilizer application rate but there is no information on how nutrient form influences EMF colonization or function in blueberry production.

Other thoughts

This research on EMF in blueberry plants from NSW represents only a single snapshot in time through a small window. Only one isolation method was used in this research and it is highly likely some endophytes were not isolated with this method. Each location was visited on a single day to collect root samples during November 2006 and it is highly likely sampling during different times of the year would detect more species of fungi. Root samples were taken from the top layers of soil and represented only a small proportion of the total plant root system and it is highly likely more species of fungi would be found if complete root systems had been sampled. Considering the above listed constraints, and others, Ms Smart's research has provided an excellent starting point for examining the potential impact of EMF in Australian blueberry production.

Genetic diversity is a primary driver for functional diversity. The diversity of EMF present in an individual plant or in a production field is representative of the potential to select or exploit the benefits of EMF for use in blueberry production. This research shows that (1) fungi similar to EMF reported from northern hemisphere blueberry plants are present in Australia

and can form EMF with blueberry plants in Australia; and (2) fungi similar to EMF found on native epacrids are present in blueberry roots in Australian production fields and these fungi can form EMF with blueberry plants. The fungi that are currently present in roots of blueberry plants in Australia production fields are probably adapted to the specific environmental conditions of these fields. The environment of these fields is quite different compared to areas inhabited by native epacrid plants and it is highly likely that EMF species and function would be different for these two environments. Ms Smart's results have highlighted that research on EMF on Australian epacrids in natural environments has direct relevance for the blueberry industry.

ABGA Diary Dates

4 May 2010

**Victorian Farmers Federation
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Details to be confirmed