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Note: Detailed location data of indigenous orchid and endangered species sightings presented at the Conference have been removed from the papers in these Proceedings as a precautionary measure. Should you require access to this information please contact the respective speakers.

Demystifying orchid mycorrhizal interactions

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Mycorrhizal fungi

Ancestrally mycorrhizal fungi have interacting with plants for over 550 million years and are regarded as being instrumental in the establishment of terrestrial plants. These associations persist with mycorrhizal fungi forming a symbiotic relationship with the roots of majority of plant. The associations are broadly divided into ectomycorrhizal (ECTO) and endomycorrhizal (ENDO) depending on the fungi and host plans involved (Smith and Read, 2008). In South Africa exotic trees such as those that are grown for forestry products (e.g. pines, Hawley et al., 2008) are ECTO while the majority of our indigenous plants and trees are ENDO associated (Hawley and Dames, 2004). The most ubiquitous ENDO associations are the Arbuscular Mycorrhizal which have a broad host range; Ericoid Mycorrhizal fungi associate with members of Ericales (Bizabani and Dames, 2016a), while orchids associate with the Orchid Mycorrhiza (OM). These different associations can be distinguished when observing roots microscopically (Figure 1).

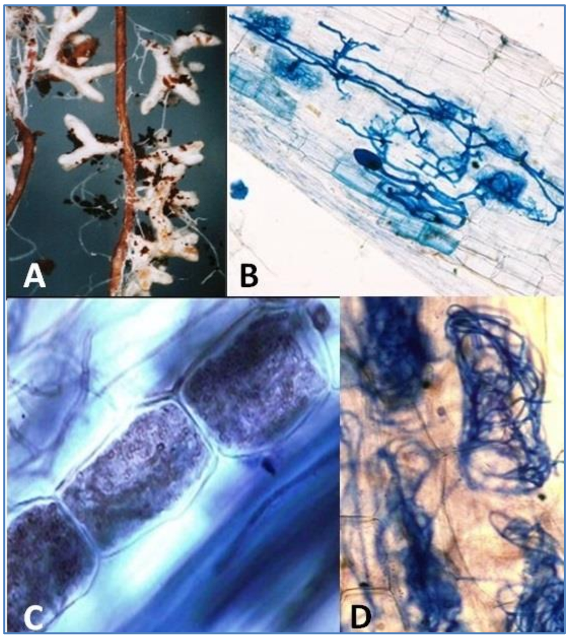

Figure 1: Different mycorrhizal structures around and within roots that distinguish between; A) Ectomycorrhizal, B) Arbuscular Mycorrhizal, C) Ericoid Mycorrhizal and D) Orchid Mycorrhizal interactions.

The mycorrhizal relationship is regarded as mutually beneficial enhancing the host plants ability to access nutrients particularly from harsh environments (Adeoyo et al., 2017; Bizabani and Dames, 2016b; Dames et al, 2002), improving plant establishment and growth and increasing tolerance to stress such as drought and pathogens. In return, the fungi obtain photosynthetic carbon from the host plant. All orchid species form a unique relationship with fungi belonging to the Ascomycota and Basidiomycota (Smith and Read, 2008) these mycorrhizal relationships are not well studied in South Africa.

Orchid mycorrhiza

The OM association is unique in that it varies depending on the stage of growth and mode of nutrition. Autotrophic orchid species are able to produce their own carbon (C) compounds through the process of photosynthesis. Achlorophyllous orchid species, which lack autotrophic ability, are mycoheterotrophic, meaning that they are highly dependent on the mycorrhizal fungi for C compounds. Mixotrophy is a combination of these two physiological types where orchid species, utilise the mycorrhizal fungi to supplement their C requirements (Dearnaley et al, 2007; Dearnaley et al, 2012). Under natural conditions a continuum of these relationships are likely to exist (Selosse and Roy, 2009). Regardless of the physiological type all orchids are unified by their 'dust seed' morphology and mycorrhizal interactions, which are interdependent. The lack of food reserves to support seed germination makes orchids dependent on mycorrhizal fungi for the provision of nutrients and development of the protocorm (Dearnaley et al, 2007; McCormick et al, 2006; Selosse and Roy, 2009) (Figure 2).

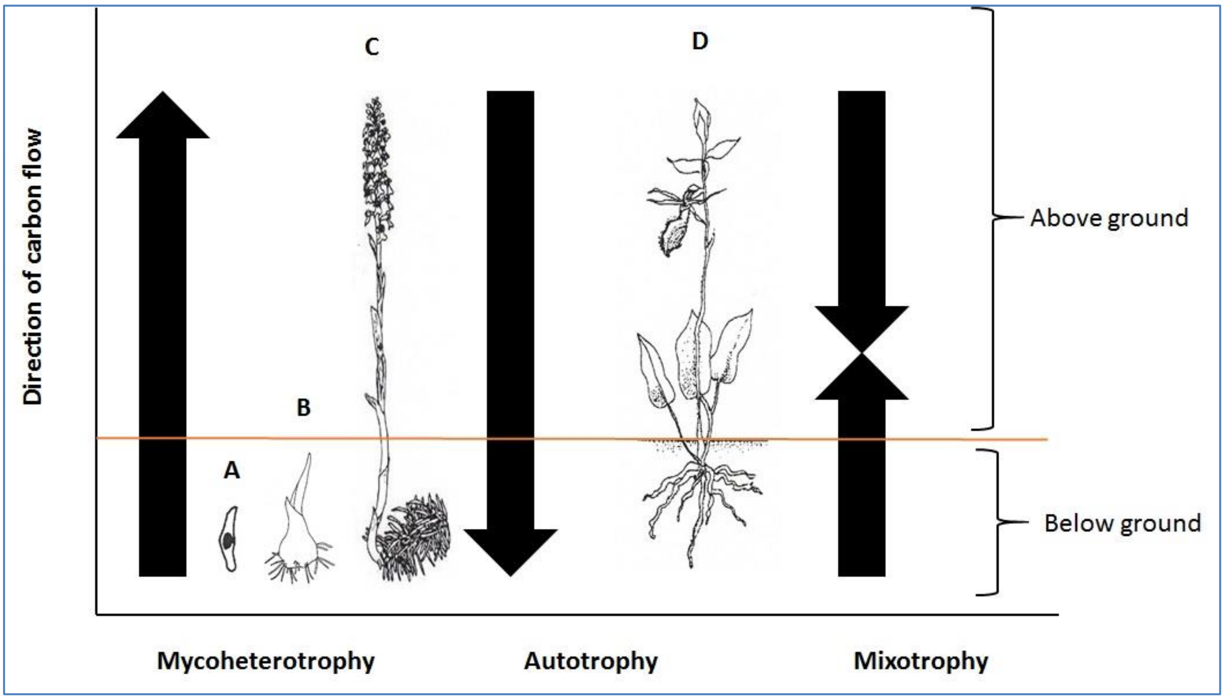

Figure 2 Carbon physiological types of orchids and levels of dependence on mycorrhizal fungal partners. A) seed, B) protocorm development, C) achlorophyllous orchid and D) adult photosynthesising orchid (modified from Smith and Read, 2008).

Orchid mycorrhizal fungal diversity

Because of the increased saprotrophic abilities *in vitro* isolation of the OM associated fungi has facilitated the identification of some fungal associates as well as determination of ecological function. Use of OM fungal isolates has also increased our understanding of symbiotic seed germination. Orchids were generally regarded as interacting with fungi in the *Rhizoctonia* complex, however other fungi are now recognised as belonging to the Sebacinales, Caratobasidiaceae and Tulasnellaceae which have little similarity to *Rhizoctonia*. Sebacinales encompasses two groups (Weiβ et al, 2011) that occur as endophytes in many plant species. Group B are found as mycorrhizas of chlorophyllous orchids and ericaceous plants, while Group A forms ECTO associations and associate with mycoheterotrophic orchid species (Selosse et al, 2009). Fungal members of the Tulasnellaceae and Ceratobasidiaceae also form ECTO associations. Recent studies by Bonnardeaux et al, 2007; Motomoura et al, 2010 and Waterman et al, 2011 suggest that fungal associates are much more complex. Waterman et al., 2011 (the only published South Africa study) investigated mycorrhizal diversity on oil-secreting species within the Coryciinae (genera included *Disperis*, *Pterygodium*, *Corycium*, *Ceratandra* and *Evotella*). Results indicated association with six fungal clades belonging to the Ceratobasidaeae, *Tulasnella*, Sebacinales group B, *Tricharina,* Sebacinales group A and *Peziza*.

A number of fungi, such as those belonging to the Basidiomycota, which associate with plant roots are uncultivable or difficult to culture (Bougoure et al, 2005; Selosse et al, 2009). The Sebacinales fungi probably consist of several thousands of species, mostly undescribed and are very frequently revealed through molecular analysis (Oberwinkler et al, 2013). Such findings highlight the limitations of the culture based methods for identification emphasising the need to combine culture dependent and independent techniques for assessing orchid mycorrhizal fungal diversity in roots. Dearnaley et al, 2012 listed 23 fungal genera in 3 Basidiomycotean sub phyla as being associated with orchid mycorrhizal interactions. The level of specificity of these fungal partners varies between orchid species and those required for symbiotic germination may differ from those that associate with adult plants (Dearnaley et al, 2012; Smith and Read, 2008). Germination of seeds symbiotically *in vitro* requires a compatible fungal isolate to ensure success; these fungal species may differ from those required under field condition, indicating the complex nature of the mycorrhizal association (Smith and Read, 2008).

Life cycle

The germination of seeds requires either an exogenous source of carbon (asymbiotic) or a mycorrhizal fungal partner to provide nutritional support during seedling development. Depending on the fungal Seeds respond favourably to the fungal species forming a mycorrhizal interaction as indicated by the formation of pelotons, or negatively resulting in either parasitism or rejection (Dearnaley et al, 2012; Smith and Read, 2008) (Figure 3).

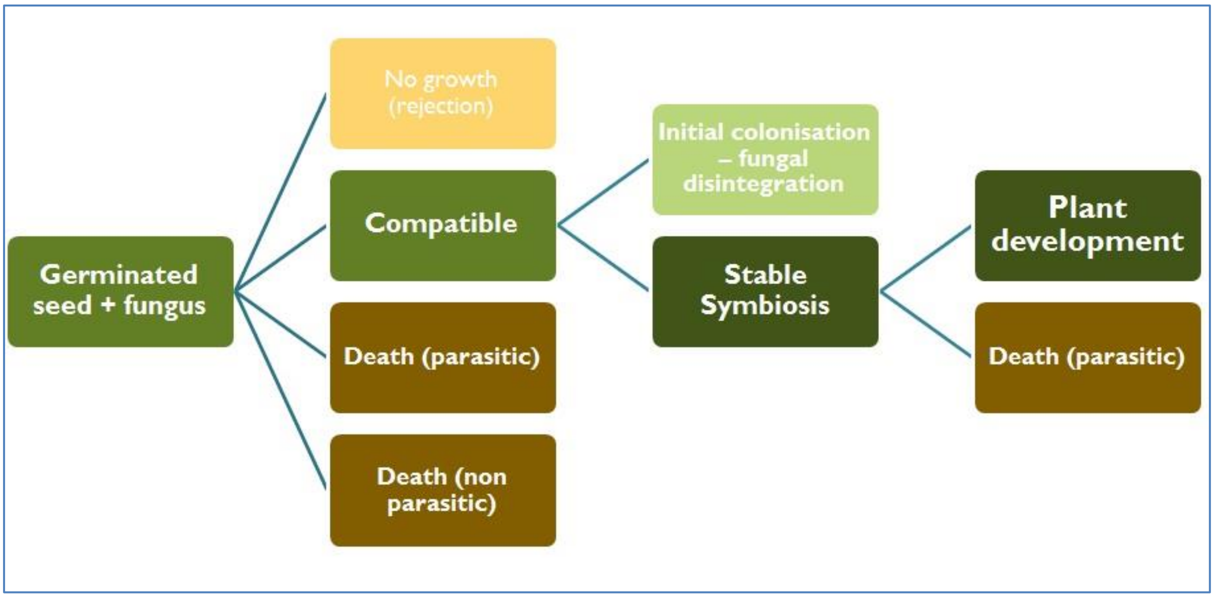

Figure 3: Development outcomes between mycorrhizal fungal and orchid interactions.

Soil mycorrhizal fungi colonise cortical root cells through the embryo or epidermal hairs. As with all ENDO interactions the fungi enter roots cells but never breach the plant cell membrane. Thus the integrity of the plant root cell remains intact. The fungi produce hyphal intracellular coils between the plant cell wall and membrane called pelotons in embryos of developing seedlings, rhizomes and roots of adult plants (Smith and Read, 2008). Pelotons increase the surface between the symbionts and are regarded as the site of nutrient transfer. Their collapse and hyphal digestion further aid carbon and nutrient transfer in some species. The life span of an individual peloton is approximately 11 days (Dearnaley et al, 2012; Smith and Read, 2008). As the plant matures new roots will rapidly become colonised with cells containing more than one fungal species. At any one time there will be different proportions of uncolonised roots cells, active mycorrhizal cells and cells containing degenerating hyphae (Smith and Read, 2008).

Nutrient transfer

Orchid mycorrhizal associations are unique in that during the protocorm developmental stage the fungus supplies C compounds to the plant, unlike other mycorrhizal associations. Fungi are absorptive organism which release enzymes into the soil environment in order to access nutrients from complexed polymers. This requires intact hyphal growth. Polymers such as starch and cellulose are hydrolysed and the smaller subunits are transported via the hyphae to the root cells. As the adult plant starts to photosynthesis the direction of C transfer is reversed with some of the C being allocated to the roots and fungal hyphae (Ramuusen, 2002; Selosse and Roy, 2009). Studies by Cameroon et al, (2006) demonstrated the mixotrophic nature of *Goodyera repens* using labelled C (^{14}C) applied to the shoots and transferred to the mycorrhizal fungal hyphal network, with a subsequent transfer of double labelled glycine (C and N) in the reverse direction. Further studies also demonstrated the transfer of ^{33}P to plant through the mycorrhizal fungi (Cameroon et al, 2007).

Conservation

As many of the mycoheterotrophic and mixotrophic physiological types are dependent on mycorrhizal fungi that may associate with other non-orchid hosts it is important to maintain plant diversity in the environment. The presence of organic material in the soil profile is an important source of mycorrhizal mediated C required for seed germination and protocorm development. The requirement for a relatively limited range of mycorrhizal fungal partners due to specificity results in the inability of orchids to tolerate habitat disturbance as fungi differ in their ability to grow under certain environmental conditions (McCormick et al, 2006; Swarts and Dixon, 2009). Research on the isolation and identification of mycorrhizal fungal partners, their compatibility with various orchid species in order to promote symbiotic germination and their interaction with other soil micro-organisms which may assist in both mycorrhizal and orchid establishment is required. The conservation of South Africa's diverse wild orchid populations cannot be successfully achieved without an understanding of these important plant-soil-microbe interactions.

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