THE ROLE OF MYCORRHIZAE IN SRC WILLOW PLANTATIONS

A report written for the Lake Taupo Development Company Limited as part of the SFF project

Energy Farming to Protect Lake Taupo using *Salix* (Willow)

SFF Project 05/058

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INTRODUCTION

A literature review of current knowledge of the role of mycorrhizae in crop yield for SRC willow and the influence of common agricultural practices on the effectiveness of the association.

This review is in two parts.

Part 1 (page 2) is a lengthy discussion of mycorrhizae to familiarise readers with the nature of these fungi and the associations they have with living plants. It deals with concepts of symbiosis, the exchange of nutrients between host plant and the mycorrhizal fungus, and how the mycorrhizal fungi can influence nutrient availability to the host plant. The differences between ectomycorrhizal fungi (EM) and arbuscular mycorrhizal fungi (AM) are explained, and some reports of experimental findings on the effects of tillage and fertilisation on host-mycorrhizal associations are presented briefly. Part 1 has been copied almost in its entirety from the website listed at the end (page 11).

Part 2 deals with mycorrhizae in *Salicaceae* (willows and poplars).

To those more familiar with mycorrhizal associations with plants a read of the general summary on page 1 may suffice, before moving on to Part 2 (page 12 onwards).

PART 1

GENERAL SUMMARY OF MYCORRHIZAE

Mycorrhizae are symbiotic associations that form between the roots of most plant species and fungi. These symbioses are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. In infertile soils, nutrients taken up by the mycorrhizal fungi can lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are non-mycorrhizal plants.

Mycorrhizal associations vary widely in form and function. Ectomycorrhizal fungi are mostly basidiomycetes that grow *between* root cortical cells of many tree species, forming what is described as a Hartig net. Arbuscular mycorrhizal fungi belong to the order Glomales and form highly branched structures called arbuscules, *within* root cortical cells of many herbaceous and woody plant species.

Plant responses to colonization by mycorrhizal fungi can range from dramatic growth promotion to growth depression. Factors affecting this response include the mycorrhizal dependency of the host crop, the nutrient status of the soil, and the inoculum potential of the mycorrhizal fungi. Management practices such as tillage, crop rotation, and fallowing may adversely affect populations of mycorrhizal fungi in the field. Where native inoculum potential is low or ineffective, inoculation strategies may be helpful. With the current state of technology, inoculation is most feasible for transplanted crops and in areas where soil disturbance has greatly reduced the native inoculum potential.

INTRODUCTION TO MYCORRHIZAE

Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth. The association is characterized by the movement of plant-produced carbon to the fungus and fungal-acquired nutrients to the plant

The term mycorrhiza, which literally means *fungus-root*, was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank. Since then we have learned that the vast majority of land plants form symbiotic associations with fungi: an estimated 95% of all plant species belong to genera that characteristically form mycorrhizae.

The benefits afforded plants from mycorrhizal symbioses can be characterized either agronomically by increased growth and yield or ecologically by improved fitness (i.e., reproductive ability). In either case, the benefit accrues primarily because mycorrhizal fungi form a critical linkage between plant roots and the soil. Mycorrhizal fungi usually proliferate both in the root and in the soil. The soilborne or extramatrical hyphae take up nutrients from the soil solution and transport them to the root. By this mechanism, mycorrhizae increase the effective absorptive surface area of the plant. Mycorrhiza provide a much larger root system, 100 times longer, and up to 2000 times more surface area. In nutrient-poor or moisture-deficient soils, nutrients taken up by the extramatrical hyphae can lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are non-mycorrhizal plants.

TYPES OF MYCORRHIZAE

Ectomycorrhizae

The diagnostic feature of ectomycorrhizae (EM) is the presence of hyphae between root cortical cells producing a netlike structure called the Hartig net, after Robert Hartig who is considered the father of forest biology. Many EM also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots). The mantle can vary widely in thickness, colour, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in root bifurcation and clustering. Contiguous with the mantle are hyphal strands that extend into the soil. Often the hyphal strands will aggregate to form rhizomorphs that may be visible to the unaided eye. The internal portion of rhizomorphs can differentiate into tubelike structures specialized for long-distance transport of nutrients and water.

Ectomycorrhizae are found on woody plants ranging from shrubs to forest trees. Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae. Over 4,000 fungal species, belonging primarily to the Basidiomycotina, and fewer to the Ascomycotina, are known to form ectomycorrhizae. Many of these fungi produce mushrooms and puffballs on the forest floor. Some fungi have a narrow host range, such as *Boletus betulicola* on *Betula* spp., while others have very broad host range, such as *Pisolithus arhizus (*also called *P. tinctorius*) which forms ectomycorrhiza with more than 46 tree species belonging to at least eight genera).

Arbuscular Mycorrhizae

The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows

between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is **endomycorrhiza**. In this association neither the fungal cell wall nor the host cell membrane are breached. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. This apoplastic space prevents direct contact between the plant and fungus cytoplasm and allows for efficient transfer of nutrients between the symbionts. The arbuscules are relatively short lived, less than 15 days, and are often difficult to see in field-collected samples.

Other structures produced by some AM fungi include vesicles, auxiliary cells, and asexual spores. Vesicles are thin-walled, lipid-filled structures that usually form in intercellular spaces. Their primary function is thought to be for storage; however, vesicles can also serve as reproductive propagules for the fungus. Auxiliary cells are formed in the soil and can be coiled or knobby. The function of these structures is unknown. Reproductive spores can be formed either in the root or more commonly in the soil. Spores produced by fungi forming AM associations are asexual, forming by the differentiation of vegetative hyphae. For some fungi (e.g., *Glomus intraradices*), vesicles in the root undergo secondary thickening, and a septum (cross wall) is laid down across the hyphal attachment leading to spore formation, but more often spores develop in the soil from hyphal swellings.

The fungi that form AM are currently all classified in the order Glomales (Morton, 1988). The taxonomy is further divided into suborders based on the presence of: (i) vesicles in the root and formation of chlamydospores (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae or (ii) absence of vesicles in the root and formation of auxiliary cells and azygospores (spores resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae.

The term **vesicular-arbuscular mycorrhiza** (VAM) was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, AM is now the preferred acronym.

The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type. However, it is important to distinguish between *specificity,* innate ability to colonize, **infectiveness**, amount of colonization, and **effectiveness**, plant response to colonization. AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth.

Mixed Infections

Several fungi can colonize the roots of a single plant, but the type of mycorrhiza formed is usually uniform for a host. In some cases, however, a host can support more than one type of mycorrhizal association. *Alnus* (alders), *Salix* (willows), *Populus* (poplars*),* and *Eucalyuptus* can have both AM and EM associations on the same plant.

An intermediate mycorrhizal type can be found on coniferous and deciduous hosts in nurseries and burned forest sites. The **ectendomycorrhiza** type forms a typical EM structure, except the mantle is thin or lacking and hyphae in the Hartig net may penetrate root cortical cells. The ectendomycorrhiza is replaced by EM as the seedling matures. The fungi involved in the association were initially designated "E-strain" but were later shown to be ascomycetes and placed in the genus *Wilcoxina*.

UPTAKE AND TRANSFER OF SOIL NUTRIENTS

When a nutrient is deficient in soil solution, the critical root parameter controlling its uptake is surface area. Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of the root. For example, Rousseau et al. (1994) found that while extramatrical mycelia (aggregates of hyphae) accounted for less than 20% of the total nutrient absorbing surface mass, they contributed nearly 80% of the absorbing surface area of pine seedlings. It is also important to consider the distribution and function of the extramatrical hyphae. If the mycorrhiza is to be effective in nutrient uptake, the hyphae must be distributed beyond the nutrient depletion zone that develops around the root. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion. For a poorly-mobile ion such as phosphate, a sharp and narrow depletion zone develops close to the root. Hyphae can readily bridge this depletion zone and grow into soil with an adequate supply of phosphorus. Uptake of micronutrients such as zinc and copper is also improved by mycorrhizae because these elements are also diffusion-limited in many soils. For more mobile nutrients such as nitrate, the depletion zone is wide and it is less likely that hyphae grow extensively into the zone that is not influenced by the root alone. Another factor contributing to the effective absorption of nutrients by mycorrhizae is their narrow diameter relative to roots. The steepness of the diffusion gradient for a nutrient is inversely related to the radius of the absorbing unit; therefore, the soil solution should be less depleted at the surface of a narrow absorbing unit such as a hypha. Furthermore, narrow hyphae can grow into small soil pores inaccessible to roots or even root hairs.

Another advantage attributed to mycorrhizal fungi is access to pools of phosphorus not readily available to the plant. One mechanism for this access is

the physiochemical release of inorganic and organic phosphorus by organic acids through the action of low-molecular-weight organic anions such as oxalate which can (Fox et al. 1990): (i) replace phosphorus sorbed at metal-hydroxide surfaces through ligand-exchange reactions, (ii) dissolve metal-oxide surfaces that sorb phosphorus, and (iii) complex metals in solution and thus prevent precipitation of metal phosphates.

Some EM fungi produce large quantities of oxalic acid, and this may partially explain enhanced nutrient uptake by EM roots. Another mechanism by which mycorrhizal fungi release inorganic phosphorus is through mineralization of organic matter. This occurs by phosphatase-mediated hydrolysis of organic phosphate (C-O-P) ester bonds. Significant phosphatase activity has been documented for mycorrhizal fungi grown in pure cultures and for excised and intact EM short roots. In the field, a positive correlation has been reported between phosphatase activity and the length of fungal hyphae associated with EM mantles (Haussling and Marschner, 1989). Care must be exercised in interpreting these data because plant roots and the associated microflora also produce organic acids and phosphatases; however, mycorrhizal fungi certainly intensify this activity.

Ericoid and EM have a special role in the mineralization of nitrogen (Read et al. 1989). Most plant litter entering the soil has a high C:N ratio and is rich in lignin and tannins. Only a few mycorrhizal fungi can mobilize nutrients from these primary sources. However, a wide range of ericoid and EM fungi can obtain nitrogen and other nutrients from secondary sources of organic matter such as dead microbial biomass. A wide range of hydrolytic and oxidative enzymes capable of depolymerising organic nitrogen have been demonstrated. These types of mycorrhizae may have an important role in nitrogen cycling in the acidic and highly organic soils where they predominate.

CARBON FLUXES IN MYCORRHIZAL PLANTS

Mycorrhizal fungi range from **obligate symbionts,** which can only obtain carbon from the plant host as in the case of AM fungi to **facultative symbionts**, which can also mineralize organic carbon from nonliving sources as in the case of some EM species. In nature the heterotrophic mycorrhizal fungi obtain all or most of their carbon from the autotrophic host plant. Ectomycorrhizae and ericoid mycorrhizae transform host carbohydrates into fungal-specific storage carbohydrates, such as mannitol and trehalose, which may produce a sink for photosynthate that favours transport of carbohydrate to the fungal partner. In AM, lipids accumulate in vesicles and other fungal structures and provide an analogous sink for host photosynthate.

As much as 20% of the total carbon assimilated by plants may be transferred to the fungal partner. This transfer of carbon to the fungus has sometimes been

considered a drain on the host. However, the host plant may increase photosynthetic activity following mycorrhizal colonization, thereby compensating for carbon "lost" to the soil. Occasionally plant growth suppression has been attributed to mycorrhizal colonization, but usually this occurs only under low-light (photosynthate limiting) or high-phosphorus conditions.

In an ecosystem, the flow of carbon to the soil mediated by mycorrhizae serves several important functions. For some mycorrhizae, the extramatrical hyphae produce hydrolytic enzymes, such as proteases and phosphatases that can have an important impact on organic matter mineralization and nutrient availability. Extramatrical hyphae of mycorrhizae also bind soil particles together and thereby improve soil aggregation. Typically there are between 1 to 20 m of AM hyphae g^{-1} of soil (Sylvia, 1990). Another important consequence of carbon flow to the fungal partner is the development of a unique rhizosphere microbial community called the **mycorrhizosphere,** which we will discuss shortly. Soil scientists now realize that carbon flow to the soil is critical for the development of soil aggregation and the maintenance of a healthy plant-soil system. Enhanced carbon flow to the soil should be considered an important benefit of mycorrhizal colonization.

INTERACTIONS WITH OTHER SOIL ORGANISMS

Mycorrhizal fungi interact with a wide assortment of organisms in the rhizosphere. The result can be either positive, neutral, or negative on the mycorrhizal association or a particular component of the rhizosphere. For example, specific bacteria stimulate EM formation in conifer nurseries and are called **mycorrhization helper bacteria**. In certain cases these bacteria eliminate the need for soil fumigation (Garbaye, 1994).

The interaction between rhizobia and AM fungi has received considerable attention because of the relatively high phosphorus demand of N_2 fixation. The two symbioses typically act synergistically, resulting in greater nitrogen and phosphorus content in combination than when each is inoculated onto the legume alone. Legumes are typically coarse-rooted and therefore inefficient in extracting phosphorus from the soil. The AM fungi associated with legumes are an essential link for adequate phosphorus nutrition, leading to enhanced nitrogenase activity that in turn promotes root and mycorrhizal growth.

Mycorrhizal fungi colonize feeder roots and thereby interact with root pathogens that parasitize this same tissue. In a natural ecosystem where the uptake of phosphorus is low, a major role of mycorrhizal fungi may be protection of the root system from endemic pathogens such as *Fusarium* spp. Mycorrhizae may stimulate root colonization by selected biocontrol agents, but our understanding of these interactions is meager. Much more research has been conducted on the potential effects of mycorrhizal colonization on root

pathogens. Mycorrhizal fungi may reduce the incidence and severity of root diseases. The mechanisms proposed to explain this protective effect include: (i) development of a mechanical barrier-especially the mantle of the EM-to infection by pathogens, (ii) production of antibiotic compounds that suppress the pathogen, (iii) competition for nutrients with the pathogen, including production of siderophores, and (iv) induction of generalized host defense mechanisms.

MANAGEMENT OF MYCORRHIZAE

The dramatic plant growth response achieved in pot studies following inoculation with mycorrhizal fungi in low-fertility soils led to a flurry of activity in the 1980s aimed at using these organisms as biofertilizers. Field responses were often disappointing, especially in high-input agricultural systems, and many concluded that mycorrhizae had little practical importance in agriculture. Further studies, however, have confirmed that most agricultural plants are colonized by mycorrhizal fungi and that they can have a substantial impact, both positive and negative, on crop productivity (Johnson, 1993). Certainly, agriculturists should appreciate the distribution of mycorrhizae within their systems and understand the impact of their management decisions on mycorrhizal functioning.

Factors that should be considered when assessing the potential role of mycorrhizae in an agroecosystem include:

Mycorrhizal dependency (MD) of the host crop. This is usually defined as the growth response of mycorrhizal (M) versus non-mycorrhizal (NM) plants at a given phosphorus level; MD = ((M - NM) / NM) x 100. Although most agricultural crops have mycorrhizae, not all benefit equally from the symbiosis. Generally, coarse-rooted plants benefit more than fine-rooted plants.

Nutrient status of the soil. Assuming that the major benefit of the mycorrhizal symbiosis is improved phosphorus uptake, the management of mycorrhizal fungi will be most critical when soil phosphorus is limiting. Many tropical soils fix phosphorus and proper mycorrhization of plants is essential to obtain adequate phosphorus nutrition. In temperate zones, phosphorus is sometimes applied in excess of crop demand. However, with increased concerns about environmental quality, phosphorus use in developed countries may be reduced, resulting in increased dependence on native mycorrhizae for nutrient uptake. Another factor to consider is the interaction of water stress with nutrient availability. As soils dry, phosphorus may become limiting even in soils that test high in available phosphorus.

Inoculum potential of the indigenous mycorrhizal fungi. Inoculum potential is a product of the *abundance* and *vigour* of the propagules in the soil and can be quantified by determining the rate of colonization of a susceptible host under a standard set of conditions. Inoculum potential can be adversely affected by

management practices such as fertilizer and lime application, pesticide use, crop rotation, fallowing, tillage, and topsoil removal.

Examples of how management practices affect mycorrhizal populations in soil and subsequent growth of the host crop:

Soil disturbance such as tillage can dramatically affect the function of mycorrhizae in an agricultural system. M.H. Miller and co-workers from the University of Guelph, Canada, documented an interesting case where disturbance of an arable, no-till soil resulted in reduced AM development and subsequently less absorption of phosphorus by seedlings of maize in the field (Miller et al., 1995). They hypothesized that soil disturbance reduced the effectiveness of the mycorrhizal symbiosis. To confirm this, they conducted a series of growth chamber studies with nondisturbed and disturbed soil cores collected from long-term field plots. Disturbance reduced both mycorrhizal colonization and phosphorus absorption by maize and wheat roots, but did not reduce phosphorus absorption by two non-mycorrhizal crops, spinach and canola. The authors concluded that under nutrient-limited conditions, the ability of mycorrhizal seedlings to associate with intact hyphal networks in soil may be highly advantageous for crop establishment.

Crop rotation and fallow systems can affect the diversity and function of mycorrhizal fungi. J.P. Thompson described the role of AM fungi in a long-fallow (more than 12 months) disorder of field crops in the state of Queensland, Australia (Thompson, 1987). In semiarid cropping systems, clean fallows conserve soil moisture and nitrate for the subsequent crop. Since the 1940s, some crops sown immediately after long fallow grew poorly and had phosphorus and zinc deficiencies. The Australian researchers found that the fallow resulted in a decline in propagules of AM fungi in the soil and reduced colonization of the crop plants in the field. Furthermore, they conducted inoculation trials and found that increasing inoculum abundance in the soil overcame the deleterious impact of fallow. They recommended that farmers avoid planting mycorrhizal-dependent crops, such as linseed, sunflower, and soybean, following periods of fallow or after a nonhost plant such as canola that lead to reduction in AM propagules.

PROBLEMS AND POTENTIAL FOR INOCULUM PRODUCTION AND USE

In situations where native mycorrhizal inoculum potential is low or ineffective, providing the appropriate fungi for the plant production system is worth considering. With the current state of technology, inoculation is best for transplanted crops and in areas where soil disturbance has reduced native inoculum potential.

The first step in any inoculation program is to obtain an isolate that is both **infective,** or able to penetrate and spread in the root, and **effective,** or able to enhance growth or stress tolerance of the host. Individual isolates of mycorrhizal fungi vary widely in these properties, so screening trials are important to select isolates that will perform successfully. Screening under actual cropping conditions is best because indigenous mycorrhizal fungi, pathogens, and soil chemical and physical properties will influence the result.

Isolation and inoculum production of EM and AM fungi present very different problems. Many EM fungi can be cultured on artificial media. Therefore, isolates of EM fungi can be obtained by placing surface-disinfested portions of sporocarps or mycorrhizal short roots on an agar growth medium. The resulting fungal biomass can be used directly as inoculum but, for ease of use, inoculum often consists of the fungal material mixed with a carrier or bulking material such as peat. Obtaining isolates of AM fungi is more difficult because they will not grow apart from their hosts. Spores can be sieved from soil, surface disinfested, and used to initiate "pot cultures" on a susceptible host plant in sterile soil or an artificial plant-growth medium. Inoculum is typically produced in scaled-up pot cultures. Alternatively, hydroponic or aeroponic culture systems are possible; a benefit of these systems is that plants can be grown without a supporting substratum, allowing colonized roots to be sheared into an inoculum of high propagule number. Sylvia (1994) summarized methods for working with AM inoculum.

Examples where inoculating with either EM or AM fungi is beneficial when planting a mycorrhizal-dependent crop in an area where native inoculum potential is low:

Pines were not native to Puerto Rico, and their fungal symbionts were absent from the soil (Vozzo and Hacskaylo, 1971). As far back as the 1930s , attempts to establish pine on the island were unsuccessful. Typically, the pines germinated well and grew to heights of 8 to 10 cm in a relatively short time, but then rapidly declined. Phosphorus fertilizers did not substantially improve plant vigor. In 1955, soil from under pine stands in North Carolina was transported to Puerto Rico where it was incorporated as inoculum into soil around 1-year-old "scrawny" pine seedlings growing at Maricao in the western mountains. Thirty-two seedlings were inoculated, and an equal number were monitored as noninoculated control plants. Within one year, inoculated plants had abundant mycorrhizal colonization and had achieved heights of up to 1.5 m, while most of the noninoculated plants had died. Further trials with mixtures of surface soil containing mycorrhizal fungi and with pure inocula, consisting of fungi growing in a peat-based medium, confirmed that inoculated seedlings were consistently more vigorous and larger than non-mycorrhizal ones. Subsequent surveys more than 15 years after inoculation indicated that the inoculated fungi continued to grow and sporulate in the pine plantations.

Beach erosion is a problem in many coastal areas and replenishing the beaches with sand dredged from offshore is often the method of choice for restoring them. Native grasses are planted in the back beach to reduce further erosion and to initiate the dune-building process. In native dunes, beach grasses are colonized by a wide array of AM fungi. However, when these grasses are propagated in nurseries, they do not have mycorrhizae. Furthermore, the replenishment sand is typically devoid of AM propagules. In a series of studies (Sylvia, 1989) AM fungi were isolated from grasses growing in native dunes. The fungi were screened for effectiveness with the given host/soil combination and for compatibility with the nursery production system, and the effect of inoculation was documented on transplants placed on newly restored beaches. **In the nursery**, moderate amounts of colonization were achieved, even with high levels of pesticide and fertilizer use. After transfer of these plants to a low-nutrient beach environment, AM colonization spread rapidly and enhanced plant growth significantly compared to noninoculated control plants even though plants were equal size when they left the intensively managed nursery. Compared to noninoculated plants after 20 months on the beach, AM-colonized plants had 219, 81, 64 and 53% more shoot dry mass, root length, plant height, and number of tillers, respectively. In most cases the objective of nursery inoculation is not to achieve a growth response, but rather to establish the symbiosis with the plant so that it can be effectively transferred to the field.

The above information has been condensed from an article entitled 'Overview of Mycorrhizal Symbioses' written by mycologist D.M. Sylvia and located at the website address given below.

http://cropsoil.psu.edu/sylvia/mycorrhiza.htm

PART 2

MYCORRHIZAE IN *SALICACEAE*

At present, there is very little information about the mycorrhizal communities in SRF or SRC plantations, and about the contribution of mycorrhizae to yields in SRC plantations. Most of the studies reported in the scientific literature have been carried out in Germany or Sweden, and to a lesser extent in Estonia and United Kingdom. There have been collaborative studies between researchers in these countries

It is known that roots can respond to patches of fertility; however, root proliferation is often too slow to exploit resources fully, and organic nutrient patches may be broken down and leached, immobilized or chemically fixed before they are invaded by the root system. *Salix* sp. seedlings inoculated with a mycorrhizal species were grown for a year in pots and then fed an organic nutrient patch B (=killed bean seeds). Plants with mycorrhizas and nutrient patch addition rapidly acquired denser, darker foliage, while non-mycorrhizal plants without nutrient addition remained thin and spindly with pale, partially senescent leaves, and were clearly under nutrient stress. Treatments with either nutrient patches or mycorrhizas only showed intermediate responses. N and P leaf concentrations were significantly higher for +M+B plants. Leaf biomass was only slightly greater in the +M+B treatment compared with –M treatments (Tibbett and Sanders, 2002).

A study by Jones et al (1991) measured P uptake over 90 days for *S. viminalis* rooted cuttings (2.5 cm long and 3 mm in diameter), with and without EM inoculation. They found that mycorrhizal colonisation of willow roots caused a two-fold increase in growth due to substantially higher P uptake. The major increase occurred over the first 50 days, suggesting that the early stages of mycorrhizal infections are particularly effective in supplying P to the plant. This was due to higher inflow rates of P. Hypotheses accounting for the higher inflow are: EM alter the soil chemistry so more exchangeable P comes into solution, being able to use different forms of P such as organic P, increasing the volume of the soil to which the plants have access via their external hyphae. This confirmed findings of an earlier study by Backhaus et al (1986) who showed that rooted cuttings of *S. dasyclados* and *S. daphniodes* inoculated with VAM produced a significantly higher shoot mass after 3 months than uninoculated cuttings in peat and sand but not clay soil despite all receiving the same commercial fertiliser addition. It appeared that the VAM were able to increase plant growth in nutrient poor soils (sand and peat) but not nutrient rich soil (clay). Cutting shoot mass gain was greater in the clay soil than in the other two despite VAM influences.

Two willow clones, *Salix viminalis* L. and *S. dasyclados* Wimm., growing in separate blocks in an SRC plantation in Estonia were found to have 75% and 94% ectomycorrhizal (EM) colonisation respectively (Puttsepp et al, 2004). A tendency towards higher EM levels with higher N and organic matter concentrations, and low pH, P and K concentrations were observed when the mean levels of EM colonisation were compared with soil variables. However no statistically significant relationship was found between the mean level of EM colonisation on the root tips of *S. viminalis* and *S. dasyclados* and any of the measured soil variables (pH, N, P, K, OM, ash). Other workers have found that increasing nutrient status and pH often reduces EM colonisation levels. Puttsepp et al. identified up to nine fungal taxa represented by the EM colonisers, and between 40 and 60% of the root length colonised. Root litter production by the host plant species can be equal to and even higher than above ground litter production (Fogel, 1991; Berg et al, 1998 ref. in Puttsepp et al 2004). Considering these findings and an unpublished study by Puttsepp it is likely that EM colonisation could be affected by the quality of soil litter, e.g. N and phenolic content, ease of decomposition.

Baum et al (2000) used *Populus trichocarpa* cv. Weser cuttings as the host plant in a pot experiment to determine the effect of inoculation by EM on leaf nutrient concentrations, shoot lengths, root and shoot biomass production and N accumulation in the biomass. Two arable soils chosen for the experiment were assumed to have a very low native EM due to a lack of host plants under arable management. They inoculated the soils in pots containing the rooted cuttings with two EM species which had previously been shown to readily colonise this host species. The plants were kept at 60% water holding capacity, i.e. under some water stress. After 3 months leaf nutrient concentrations were measured and after 6 months biomass measurements were taken. Inoculation was found to increase EM colonisation from 10% contributed by native species to 35-40% in the nutrient rich(RNS) soil (due to former slurry applications) and from 5% to 13-15% in the soil with lower contents of organic matter and N (PNS). Shoot lengths, shoot dry matter and shoot:root ratio of the poplar clone were increased by inoculation by the mycorrhizal strains on the RNS soil, whereas on the PNS soil only the shoot:root ratio was increased. The root dry weight and the total N accumulation in the biomass of shoot and root were only affected by the substrate, not by the inoculation. All leaf concentrations of P, K, Ca and Mg were sufficient in all treatments. The leaf concentrations of K, Ca and Mg showed no effects of inoculation at all. The Ca and Mg concentrations were soil dependent rather than EM dependent and N and P concentrations for plants grown in RNS soil were reduced by EM inoculation. This was explained by an increased biomass production as determined later in the season. On the PNS soil inoculation by one of the EM species increased leaf N concentration but not the other. For the effect of inoculation on shoot biomass $P<0.05$ i.e. inoculation significantly increased shoot biomass in both soils. In an associated piece of research Baum and Makeschin (2000) assessed the effects of N and P

fertilisation on mycorrhizal formation of two poplar clones *P. trichocarpa* and *P. tremula x tremuloides*. The outcome of the research was difficult to assess since N- and P-nutrition of both poplar clones, recorded by foliar analysis, was optimal on all the test plots, both fertilised and unfertilised. Fertilisation caused increased foliar nutrient concentrations, but no changes in the growth rates. N-addition depressed EM colonisation of *P. tremula x tremuloides* but had no significant effect on EM colonisation of *P. trichocarpa.* Likewise P-addition had similar effects to N-addition on EM colonisation in both species. Studies using other host species have produced varying conclusions about the effect of fertilisation on EM colonisation. Large amounts of available P in the soil can reduce EM colonisation (Jones et al., 1990). In such situations P is then unlikely to be a limiting factor for plant nutrition. It was considered that fertilisation decreased spore formation in the EM species in the *Populus* study, a feature reported in other, but not all, comparable trials.

Baum et al (2002) investigated the effects of nitrogen fertilisation and soil properties on mycorrhizal formation of *Salix viminalis* in SRC plantations in Germany and Sweden. They found EM colonisation was lower on clayey soils than sandy soils, and that similar amounts of N (100kg N/ha/yr) caused different responses in EM formation by *S. viminalis* under different site conditions. At low soil N fertilisation increased EM colonisation, whereas at higher soil N fertilisation decreased EM colonisation. In the years following fertilisation EM colonisation fluctuated and a common pattern did not emerge. Soil N was not monitored after the initial measurements were made. In the Swedish trial with a high N soil, N fertilisation decreased EM colonisation. The measured EM colonisation of *S. viminalis* roots varied from 5-28% across the different soils and treatments.

These findings lead to the conclusion that different species host a different range of mycorrhizal fungi. Despite the different species associations the gains to the host plant in terms of increased nutrients and water provision would be expected to be similar. It is reasonable to expect a consequent increase in biomass production, particularly in situations of nutrient or water shortage such as would occur in phosphate deficient soils and in dry summers. The findings of pot studies with small willow cuttings over a three month period have yet to be confirmed for field trials of large willow plants over a three year period. Current research findings do not give helpful guidance on the usefulness of inoculating SRC willow plantations with EM spores. It is clear that different species host different collections of EM, and that many species are able to successfully colonise *Salix* host root tips. It appears that low soil organic nutrients can be limiting for EM colonisation, though the limiting mechanism is not yet clear, and high available soil nutrients may nullify any gains offered by EM associations. Colonisation could be expected to increase with the age of the plantation as roots extend in the soil and as the EM multiply, as a consequence of improved host presence and consequent nutrient status.

A review paper entitled 'Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003' by Lekberg and Koide (2005) particularly looked at trials addressing the following questions: What are the effects of soil disturbance on % mycorrhizal colonisation? Is an increased mycorrhizal colonisation associated with changes in harvestable yield, biomass, and P concentration of the host plant? In cases where mycorrhizal colonisation is increased, is there a correlation between increased P uptake and increased harvestable yield or biomass? What factors affect whether increased mycorrhizal colonisation results in benefit to the host plant? They focussed on P but not other nutrients because of the well-researched connection between mycorrhizal fungi and plant P uptake and the importance of this element for crop production. Of 290 studies reviewed, reported increased yield from increased colonisation was 23% across all management practices. Biomass at harvest and shoot P concentration in early season were increased by inoculation (57% and 33%, respectively). Reduced disturbance increased shoot P by 27%, but biomass was not significantly affected. Mycorrhizal colonisation was increased most by inoculation (29% increase), followed by shortened fallow (20%) and reduced tillage (7%). Irrespective of management practice, an increased mycorrhizal colonisation was less likely to increase biomass if either soil P or indigenous inoculum potential was high.

Because a large number of these studies were carried out in pots in glasshouse conditions I have reported two recent examples of field inoculation trials below, one for garlic and the other for three bean species. In both situations, rainfall is low and the regions are prone to drought.

The effect of mycorrhizae species and phosphorus (P) fertilizer on garlic (Allium sativum L.) growth, yield–quality, and P uptake under high P accumulated non-sterile field conditions were studied. Experiments were conducted for two successive years under field conditions of Menzilat soil series (Typic xerofluvent) at the Research Farm of the University of Cukurova (Turkey). Glomus mosseae arbuscular mycorrhizae (AM) were tested on local Urfa genotype of garlic at 0, 40, 80, and 120 kg phosphorus (P_2O_5) ha^{-1}. In the first year, garlic was inoculated with 1,000 spores per plant, but in the second year, garlic was inoculated with either 1,000 or 2,000 spores per plant. Emergence, plant growth, yield, bulb size, root mycorrhizal infection, and phosphorus uptake of plants were examined. Neither mycorrhizal inoculation nor P_2O_5 supply increased garlic growth and yield. However, at 0 level of P_2O_5 application, mycorrhizal inoculation slightly increased plant P uptake. In the second year of the experiment, mycorrhizae significantly increased clove yield. The results revealed that although garlic is mycorrhizal dependent, mycorrhizal inoculation did not contribute to the plant growth and nutrient uptake (Sari et al, 2002).

A field study was conducted to determine the effect of vesicular arbuscular mycorrhizal (VAM) fungi on growth and nutrient uptake of the drought-hardy legumes, clusterbean (Cyamopsis tetragonoloba (L.) Taub.), mung bean (Vigna radiata (L.) Wilczek) and moth bean (Vigna aconitifolia (Jacq.) Marechal). Nodulation, nitrogenase activity, percent root infection by VAM fungi, and the number of VAM spores in the soil were increased significantly upon inoculation. Phosphatase activity was enhanced significantly due to VAM inoculation. An improvement in dry matter production (20 to 38%) and grain yield (15 to 22%) upon inoculation was obtained. Concentrations of N, P, Cu and Zn in the shoot were found to be significantly higher in inoculated plants. However, in general, concentrations of K, Ca, Mg, Na, Fe and Mn remained unaffected. From the results, a positive interaction between Rhizobium and VAM fungi is evident under arid field conditions. All the legumes showed similar effects upon inoculation with Glomus mosseae and Glomus fasciculatum (Tarafdar and Rao, 1997).

SRC willow was NOT one of the crops included in this large number of pot and field studies. The review focussed on VAM rather than EM and almost all of the species studied were annual crop species. Nonetheless, it is well to ponder on these conclusions before deciding whether field inoculation of SRC willow plantations will produce a greater shoot biomass at the end of a rotation.

Given the moderate fertility in Taupo soils, and water limitation in summer/ autumn, this review indicates a likely response by SRC willow being grown around Taupo to Ectomycorrhizae and Arbuscular mycorrhizae. The response could be from negative to highly positive (90% improvement). Arable and pastoral soils are likely to contain low populations of EM suitable for willow colonisation since pasture grasses will have AM associations if at all. Ex-forest soils will probably have more fungal activity.

Initial studies should establish the degree of colonisation of the willow plants by indigenous EM species, and the levels of soil P currently available to the plants as a result of former fertilising practices. This could be done by taking a few soil cores within 0.5 m of the cutting in the autumn at each site during each year of the trial. The soil would be tested for P and any willow roots present inspected for EM and AM colonisation using a microscope. This work has not been budgeted for so a separate application for funding should be made.

Glasshouse trials where the soil is sterilised and inoculated under controlled conditions should precede any managed **field studies** in which introduced inoculum would supplement the endemic soil EM. Trials using inoculum should be carried out in low P soils first, where the measurable differences would be expected to be greater. The general consensus among experts is that mycorrhizal inoculation can be useful for restoration in degraded sites, but is not needed everywhere.

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