The mycorrhizosphere phenomenon

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Summary

The mycorrhizal association of fungi with the roots of land plants has existed for hundreds of millions of years and logically includes associations with other functional groups of soil microbes. In addition to mycorrhizal fungi, the microbial composition of rhizosphere soil surely would involve a myriad of rhizobacteria, other rhizosphere fungi, and diverse fauna. The combination of these organisms in natural, undisturbed ecosystems would seem to contribute to the successful growth and health of plants. We have attempted to characterize qualitative changes in populations of rhizobacteria associated with plants with mycorrhizae in what is called the "mycorrhizosphere". Microbial populations in the mycorrhizosphere can change dynamically over time and are influenced by what microbes are present in the background soil or growth medium, and by the process of selective enrichment of specific functional groups of microbes from that medium due to root and arbuscular mycorrhizal (AM) fungus hyphal exudates.

My perception of the mycorrhizosphere phenomenon includes specific roles that some rhizobacteria might play in combination with mycorrhizal fungi, especially in relation to plant growth enhancement and increased antagonism against soilborne pathogens. Plant diseases are rare in undisturbed ecosystems compared to disturbed agroecosystems where they often cause serious economic loss. Disease suppressive soils occur naturally or due to specific management practices, and are thought to involve soil type and specific bacteria, fungi, or actinomycetes. However, I believe that mycorrhizae play a significant role as well. In our research, we have explored factors that affect AM formation, and have determined that AM formation causes an increase in levels of antagonistic bacteria, provided the background soil contains effective antagonists to be selectively increased.

The evidence increasingly supports a new mycorrhizosphere paradigm that is a microbial hierarchy wherein roots attract mycorrhizal fungi and the latter attract bacterial associates. The result is a "team" system that functions to support plant growth and health. The microbial components of the system must come from inoculation or selection from the bulk soil or potting medium. Optimization of the system comes from having microbes, selected from a medium with high microbial diversity, that are efficacious and compatible and therefore can function in tandem. This mycorrhizosphere paradigm involving plants forming AM that select specific bacterial associates can explain the success of the AM symbiosis in supporting plants for some 460 million years.

Key words: Mycorrhiza, rhizosphere, hyphosphere, mycosphere, plant growth-promoting rhizobacteria (PGPR), biological control, antagonistic potential, arbuscular mycorrhizal fungi (AMF)

Introduction

My interest in understanding the rhizosphere has always been from the perspective of controlling soilborne diseases though some manipulation of the microbial populations therein. My assumptions or beliefs are that root health is the product of microbial activities in the rhizosphere, and that above-ground plant growth is a reflection of the health of the root system. A parallel assumption, based on my observations and those of others, is that root disease is rare in natural ecosystems, due to microbial support systems in the rhizosphere soil associated with plant roots. My goal has always been to characterize the microbial systems involved in normal healthy growth of plants and to incorporate that knowledge into agricultural systems as a means of improving crop productivity and health. This has led me to believe that among the rhizosphere microbial populations with the greatest influence, arbuscular mycorrhizal (AM) fungi are the most important, but only in combination with bacterial associates in what we now call the "mycorrhizosphere". It is this mycorrhizosphere phenomenon that will be discussed.

The mycorrhizosphere concept

The rhizosphere phenomenon, as described by Hiltner (1904), was induced initially by nutrients released from roots. The realization that mycorrhizae altered the microflora in the rhizosphere led to the expanded concept of the mycorrhizosphere (Linderman, 1988) in which mycorrhizae significantly influence, qualitatively and quantitatively, the microflora due to altered root physiology and exudation (Ames et al., 1984; Bagyaraj, 1984; Fitter and Garbaye, 1994; Meyer and Linderman, 1986; Secilia and Bagyaraj, 1987; Gryndler, 2000). But the paradigm of the mycorrhizosphere, as initially described (Oswald and Ferchau, 1968; Rambelli, 1973; Linderman, 1988), is not complete, both temporally and spacially, and in terms of the dynamic processes that occur. Following the initial enrichment by root products that are specific to the plant species, the dynamic process is influenced by the age of the plant, the nature and treatment of the soil, foliar applications, environmental factors, fertilizer applications and host nutrition, and last, but not least, by the microbial interactions that occur therein. Because they establish a persistent interface between the host root and the soil, mycorrhizae become perhaps the only stable microbial system in the rhizosphere. While increases and decreases in the abundance of certain types of microorganisms have been reported, how and when those changes occur has not been determined fully. Further, descriptions of qualitative changes in microbial populations with potential functional activity have only inferred that such activity would occur because of the increased numbers of microbes with that potential. Measurement of actual *in situ* activity, such as antagonistic activity against a specific pathogen, has not been documented.

Consideration of the microbial shifts that can be induced by the formation of mycorrhizae requires examination of the sources of nutrient enrichment within the mycorrhizosphere: (a) root tissue exudates and sloughed cells, and (b) AMF hyphal exudates. Both can have qualitatively specific chemical components that favor some microbes and not others (Andrade et al., 1997, 1998a, b; Olsson et al, 1996; Vancura et al., 1989). When considering the microbial composition of the mycorrhizosphere, the

sum of the two sources must be included. **Thus, rhizosphere soil is soil adjacent to roots and influenced by root exudates, while mycorrhizosphere soil is soil adjacent to mycorrhizae and influenced by exudates from both the root tissue and the fungal hyphae. Both have increased populations of specific microbes selected from the bulk soil.**

Recent studies have physically separated AM fungal (AMF) hyphae from roots or roots + AMF hyphae by means of mesh that restricts root growth but allows AMF hyphae to pass through (Figure 1), and have distinguished microbial changes induced directly by the hyphae due to their specific exudates (Andrade et al., 1997, 1998a, b; Filion et al., 1999; Vancura et al., 1989). Others have examined the interactions of the AMF hyphae with other microbes in a two-compartment *in vitro* system that also separates hyphae from host roots (Fortin et al., 2002). The *in vitro* system, of course, eliminates the dynamic interactions that occur from having different hosts, different AMF symbionts, changing environmental conditions, and from having a myriad of other microbes that would be present in a soil system. Nonetheless, there is information derived from each that sheds light on what the mycorrhizosphere phenomenon is and how it relates to microbial shifts that could affect plants.
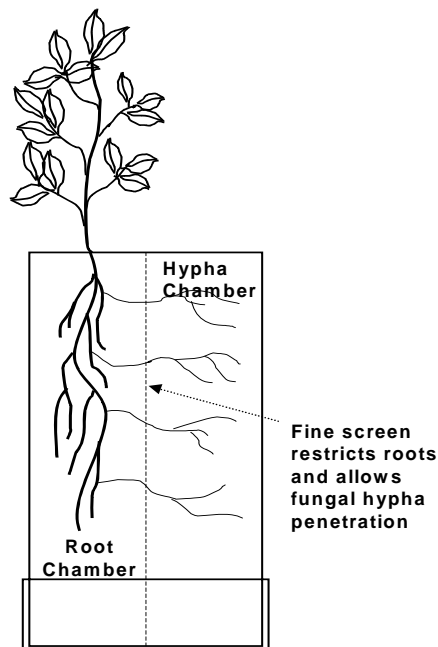

Figure 1. Generalized diagram of an experimental chamber in which plants are inoculated with AM fungi, and roots, but not AMF hyphae, are restricted from the hyphal chamber, allowing microbial analyses of the AMF hyphosphere soil.

Rhizosphere/Mycorrhizosphere microbial composition

A myriad of microbes can be present and functioning in the rhizosphere of plants, including rhizobacteria, rhizosphere fungi, fauna, and mycorrhizal fungi. How these microbes may interact and function in relation to plant growth and health has been the focus of our research.

Rhizobacteria. Bacteria that occupy the rhizosphere/mycorrhizosphere soil can have various functions in relation to plant growth and health. We know that some of those bacteria can be antagonistic to soilborne pathogens, based on *in vitro* tests showing inhibition due to the production of antibiotics or other inhibitors. What is often not appreciated, however, is that many, if not most, of the antagonists are also plant growth-promoting rhizobacteria (PGPR) (Mahaffee and Kloepper, 1994; Pieterse et al., 2003). We have confirmed this in tests with petunia using a range of bacterial or actinomycete antagonists to inoculate young seedlings. All of the antagonists stimulated plant growth
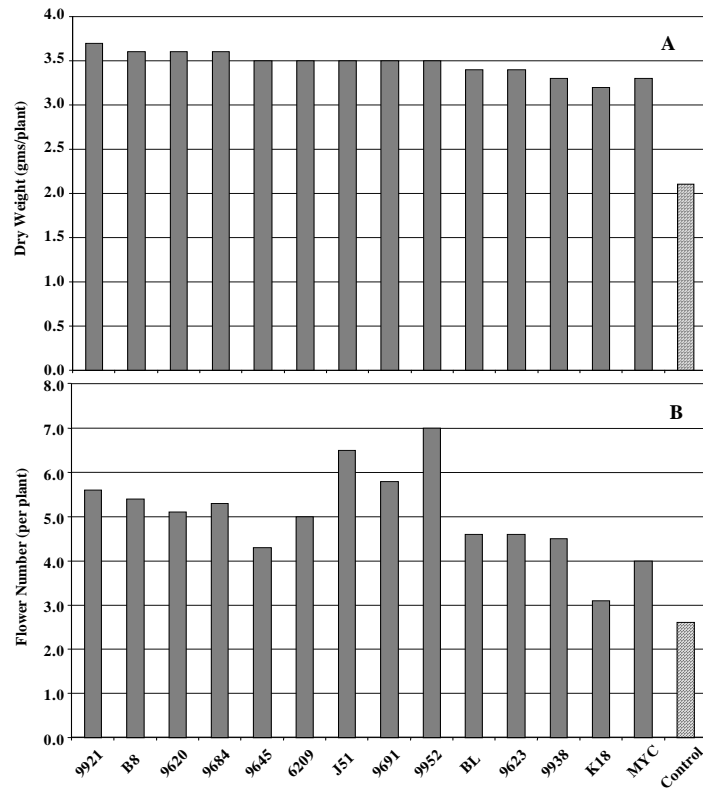

Figure 2. Experimental data showing that rhizobacteria antagonistic toward soilborne pathogens can function as plant growth-promoting rhizobacteria (PGPR) in enhancing the growth (A) and flowering (B) of inoculated petunia plants compared to the water control (Linderman, 1993).

and flowering, and thus would be classified as PGPR (Linderman, 1993) (Figure 2). Of course, other bacteria, such as symbiotic or free-living nitrogen fixing bacteria, can also be considered as PGPR (Bashan et al., 2004). We should not forget, too, that some of the rhizobacteria may have deleterious effects on plant growth (deleterious rhizobacteria, DRB), presumably due to the production of toxic materials that retard plant growth (Nehl et al., 1997; Suslow and Schroth, 1982).

Rhizosphere fungi. Some fungi that occupy the rhizosphere in the form of spores or hyphae can also be antagonistic to fungal pathogens, can be nutrient cyclers (phosphate solubilizers, enzyme producers, etc.), or may just be occupants of that soil with no known function in relation to plant growth and health. In contrast, however, are mycorrhizal fungi that occupy that same space and have profound effects on plant growth and health. Primary among the types of mycorrhizal fungi are the AMF and ectomycorrhizal fungi (EMF). While my discussion will focus on the AMF, the concepts also apply to EMF.

Arbuscular mycorrhizae (AM). We know of many benefits of AM to plant growth and health, due to the unique capacity of AMF to colonize host plant roots internally as well as externally into the surrounding soil. The soil hyphae and spores provide a source of inoculum for new infections as well as uptake of water and nutrients from the soil (Smith and Read, 1997). Exchange of materials within the root takes place by means of the arbuscules. The symbiotic relationship that is established is reported in many publications documenting many benefits to plants. Those benefits include improved plant growth under nutrient (especially P) deficient conditions (Figure 3), improved

Figure 3. Growth enhancement of geranium (a) and watermelon (b) inoculated with *Glomus intraradices* (right hand plants) and grown in P-deficient growth media.

tolerance to soil toxicity from heavy metals and salinity, improved transplant success, improved crop uniformity, improved root development on cuttings and transplants, improved drought tolerance, and improved disease tolerance. Benefits to plant growth

can also be the result of improved soil structure by means of enhanced formation of water-stable aggregates resulting from the entanglement and binding of microaggregates into macroaggregates (Tisdale et al., 1997; Wright and Upadhyaya, 1996). Such aggregates are significant sites within the mycorrhizosphere, providing conditions for microbial activity within the aggregates, such as phosphate solubilization (Andrade, 1998b) as well as the production of other bacterial metabolites and substances that hold the aggregates together. The point to remember, however, is that the microbial products within the aggregates would be immediately available for uptake by the AMF hyphae and translocation to the plant root. Those microbial products may contribute significantly to the overall effects of the mycorrhizae on plant growth and health (Bethlenfalvay and Linderman, 1992).

Effects of AM on diseases

AM formation: The general consensus of mycorrhiza researchers has been that mycorrhizae function primarily as scavengers of nutrients from the soil. However, in addition to that function, mycorrhizae induce significant physiological changes in their host plant, one of which is to alter the quantity and quality of root exudates (Graham et al., 1981). The result of those changes is a shift in the microbial composition in the mycorrhizosphere soil. In defining the mycorrhizosphere, however, one must consider the processes and components that are involved in establishing mycorrhizae in the first place, including the soil or substrate; the microbial dynamics in the rhizosphere over time; and inputs of fertilizers as well as organic matter amendments to soil or to soilless potting media. A myriad of microbes occur in the bulk soil, and every soil or soilless medium has a different composition of microbes and is physically and chemically different, depending on the parent material, geographic origin, and cropping history or plant cover. In artificial substrates or other soilless media, these traits are generally very distinct from those of soil. The substrate variability can, in my opinion, significantly affect the formation and function of AM, thus explaining in part why different studies under different conditions yield different results.

We have investigated the effect of different components of soilless plant growth media used in the nursery industry on the establishment and function of the AM symbiosis. If we hope to employ AM on plants to suppress soilborne plant diseases, or any other beneficial function for that matter, we must first evaluate the most commonly used materials in soilless media to determine which favor and which suppress AM formation. Our work has been a continuation of the work of Menge et al. (1982) who showed that organic matter in soilless nursery media inhibited the establishment of AM. We investigated different peat mosses to determine if they were responsible for the inhibition and found that some inhibited but did not completely suppress AM formation (Linderman and Davis, 2003a). We examined the use of coconut fiber (coir) as a soilless medium component and found that it did not adversely affect AM formation (Linderman and Davis, 2003b). We then examined the use of different commercial organic and inorganic fertilizers to determine which were more compatible with AM. In general, we found that organic fertilizers were more compatible with AM formation, presumably because they require microbial breakdown and thus more slowly release bound nutrients. However, inorganic fertilizers were compatible if the P content was kept low (Linderman

and Davis, 2004). Currently we are investigating the amendment of soilless media with different composts to determine their influence on AM formation. In general, different composts inhibit AM formation, presumably due to their high P content. Some of our results were reported at the 4th ICOM meeting in Montreal, Canada (Linderman et al., 2003). However, we continue to investigate one compost that is fully compatible with AM formation. Preliminary evidence suggests that it has greater P-absorbing or chelating capacity than the other composts. In that regard, it mimics traits of most soils where P can be immobilized largely. Composts in general, however, add to soilless media a more diverse microbial community, some of which could have significant effects on AM formation and function, both negative (Hetrick et al., 1986) and positive. Some may provide microbes that are "helpers" in the formation of AM (Garbaye, 1994).

AM and disease suppression: There are numerous examples of disease suppressive soils, such as the Ashburner system for controlling root rot of avacado caused by *Phytophthora cinnamomi* (Linderman et al., 1983).   Ashburner was a farmer who sought to transfer what appeared to be natural pathogen suppression in the adjacent rain forest into his avocado orchard. He deduced that the key was to create a layer of organic matter around the trees that would simulate the accumulated litter layer in the forest. The intense microbial activity that occurred in the decomposition of the organic matter appeared to be responsible for the disease suppression that he observed. The roots that grew into the decomposing organic matter were free of the pathogen and thus were able to support normal growth of the trees. Work by Australian scientists showed that heat-tolerant

Figure 4. Biological suppression of *Phytophthora cinnamomi* due to activity of specific microbes from Ashburner's avocado orchard soil, demonstrated by means of heat treatment using aerated steam to establish specific temperatures at (left to right) ambient, 120ºF, and 212ºF for 30 min.   Each flat was inoculated with the pathogen and seeded to susceptible jacaranda. Heat tolerant microbes, such as spore-forming bacteria or actinomycetes, were shown to be responsible for the suppression. Photo by P. Broadbent as presented in Linderman et al., 1983.

bacteria or actinomycetes were responsible for the observed pathogen suppression (Figure 4). The component of the microbial community that was not considered by them, however, was the AM fungi that surely had colonized those roots.

Many reviews on the subject of plant disease suppression by mycorrhizae (Azcon-Aguilar and Barea, 1996; Caron, 1989; Dehne, 1982; Filion et al., 1999; Hooker et al., 1994; Jalali and Jalali, 1991; Linderman, 1992, 1994, 2000; Linderman and Paulitz, 1990; Zak, 1964) have focused on the mechanisms of interaction such as (a) enhanced nutrition, (b) competition for nutrients and infection sites, (c) morphological changes, (d) changes in chemical constituents in plant tissues, (e) alleviation of abiotic stress, and (f) microbial changes in the mycorrhizosphere. Depending on the disease and the environmental situation, any or all mechanisms could be involved, but changes in microbial populations in the mycorrhizosphere seems to be the best explanation, yet the least studied. We have addressed a number of horticultural practices in the nursery industry that potentially could influence the establishment and then the function of AM, including and especially biological disease suppression of soilborne pathogens. As mentioned earlier, we have studied effects of different peat mosses, and amendments to soil or soilless media with coir, fertilizers, and composts. Regarding the compost studies, we investigated the microbial changes induced by compost amendments in the presence or absence of AM that can influence the incidence and severity of plant diseases.

We developed an *in vitro* method of assessing the antagonistic potential of bacterial populations that occur in the rhizosphere soil of plants with or without AM against a range of soilborne, root pathogens. We define the antagonistic potential as the sum of the potential of bacteria to suppress any specific pathogen, and the antagonistic potential index (API) as the number generated by summing the widths of the *in vitro* zones of inhibition against a pathogen by all the bacterial antagonists isolated. Bacteria are isolated from dilution plates of rhizosphere or mycorrhizosphere soil extracts. Our results show that, in general, when AM are formed, there is an increase in the number and proportion of bacteria from the mycorrhizosphere soil that can inhibit specific pathogens *in vitro*, compared to those from rhizosphere soil from non-mycorrhizal plants (Figure 5).

A number of factors can influence the potential and magnitude of disease suppression due to mycorrhizosphere microbial populations. One significant factor is the microbial diversity as affected by the amendment of soil or potting mix with composted materials (Figure 6). The host species or genotype within the species can also affect the nature of root exudation and the specifics of the AM association. Any change in the combination of host and fungal endophyte can alter the energy supply to the microbial associates in the mycorrhizosphere. As mentioned before, the soil or growth medium can provide different numbers and kinds of microbes that become AM associates, and different soils have different AMF to form the AM association. It is also important to consider the temporal aspects of AM formation in relation to infection by pathogens: time to establish the mycorrhizal association, to effect physiological change, and to establish a fully functional extraradical mycelial network will affect the effectiveness of the mycorrhizosphere microbial community to suppress root pathogens. For many annual crop plants, time required for disease onset is often too short for AM to become established. This fact strongly suggests the need for establishing AM and their
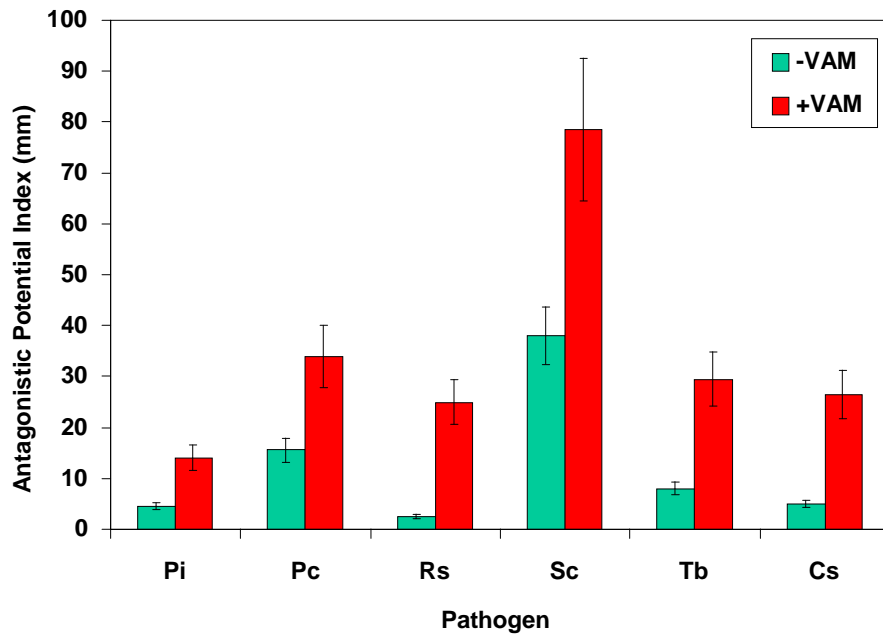
Figure 5. Antagonistic potential index (API) of rhizobacteria from rhizosphere soil around roots of plants with or without AM (VAM) against the soilborne pathogens *Pythium irregulare* (Pi), *Phytophthora cinnamomi* (Pc), *Rhizoctonia solani* (Rs), *Sclerotium cepivorum* (Sc), *Thielaviopsis basicola* (Tb), and *Cylindrocladium scoparium* (Cs). (Linderman, 2000)
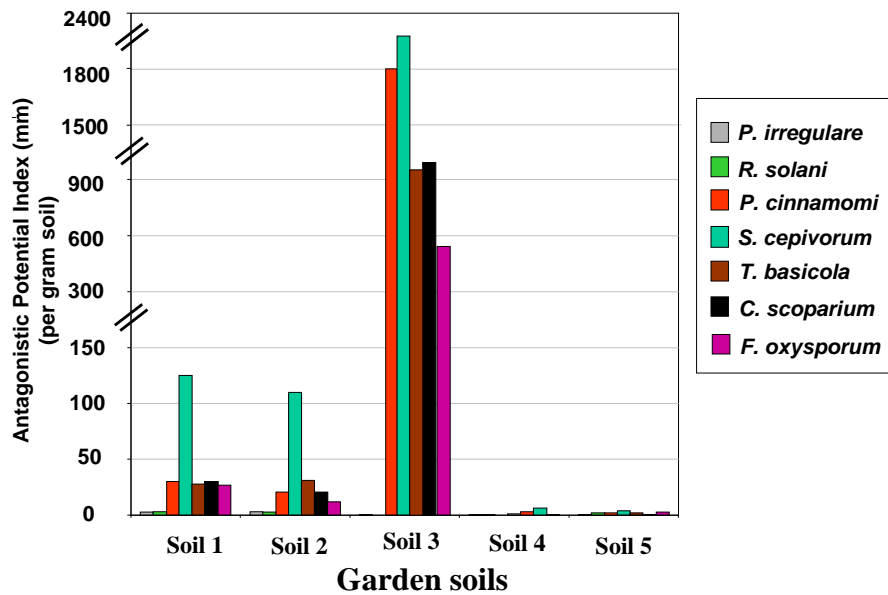
Figure 6. Antagonistic potential of garden soils amended with composts for 1 year (soils 1 and 2), 3 years (soil 3), or non-amended (soils 4 and 5). The antagonistic potential index (API) was determined against a series of soilborne pathogens: *Pythium irregulare*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotium cepivorum*, *Thielaviopsis basicola*, *Cylindrocladium scoparium*, and *Fusarium oxysporum*.

antagonistic associates as early in the production cycle as possible, even by preinoculating transplants before outplanting into the field.

In our studies, inoculating marigold seedlings with the AMF *Glomus intraradices* and transplanting them into soil, amended or not with compost, increased the API dramatically only on plants with AM (Figure 7).
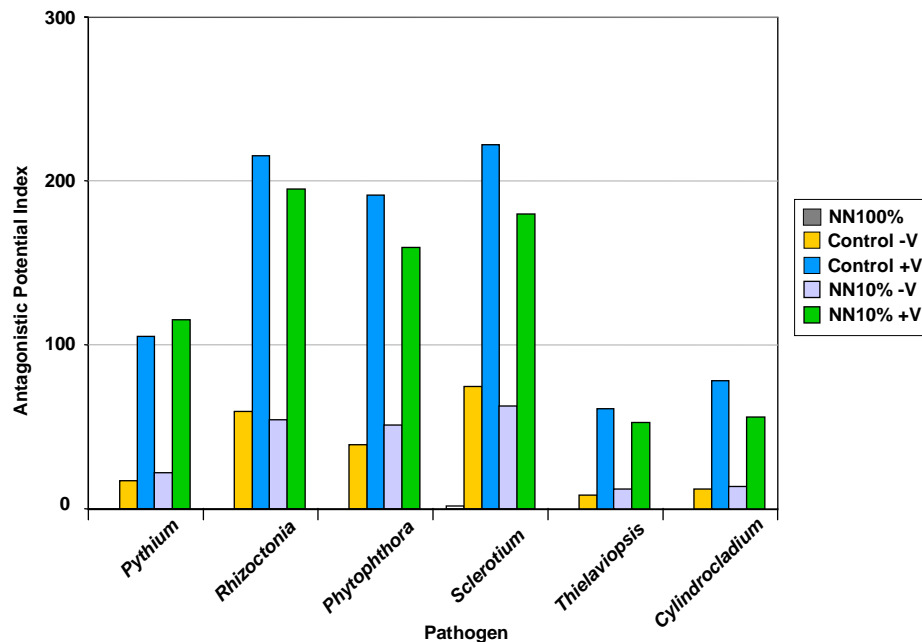

Figure 7. Antagonistic potential index (API) of soil amended or not with compost (10% Natures Needs Compost (NN) or non-amended control) and inoculated or not with the AMF *Glomus intraradices* (V). The data indicate that the API increases dramatically against all pathogens in mycorrhizosphere soil compared to rhizosphere soil from non-mycorrhizal marigold plants. Pathogens used were: *Pythium irregulare*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotium cepivorum*, *Thielaviopsis basicola*, *Cylindrocladium scoparium*.

Other roles of AM bacterial associates?

While our studies have focused on antagonistic bacterial associates of AM in the mycorrhizosphere, we should consider other possible roles that bacterial associates may play in plant growth and health. If one considers the list of benefits attributed to AM such as (a) improved plant growth, improved tolerance to soil toxicity (heavy metals,

salinity), improved transplant success, improved crop uniformity, improved root development, improved soil drought tolerance, as well as improved disease tolerance, it seems reasonable to think about how bacterial associates of AM (AMBA) contributed. For example, are AMBA involved in nutrient cycling or conversion to forms available for absorption by AM fungal hyphae or roots? Are they involved in plant growth promotion as PGPR? Are they involved in bioremediation of metals (Cu, Zn, Cd) contaminated soil (Gonzalez-Chavez et al., 2005) or tolerance to soil salinity (Cantrell and Linderman, 2001)? Many other examples could be presented that suggest the possible or unknown roles of bacteria that only increase in population because AM are present. Without AM, these microbes may reside in the bulk soil but never reach high enough populations to have any substantial effect on plant performance under stressful conditions.

Summary and Conclusions

Formation of an effective AM symbiosis in production agriculture can be important under a number of stressful situations, including the growth-limiting effect of P deficiency, soil salinity, drought stress, and disease pressure. Several management strategies must be considered in order to assure AM formation and the prospect of having any effect on plant performance in early growth stages or after transplanting. Preinoculation of transplants seems to be a logical approach in order for AM to effectively address any future stresses. Nursery practices for production of transplants with AM should include organic fertilizers or inorganic fertilizers with low P, could include peat or coir as an amendment to the soilless growth media commonly used, and could include the use of compost to increase the microbial diversity of the medium that could contribute to potential disease suppression. Without that diversity, there might be too few of the needed bacterial associates to complete the "team", the members of which function in tandem to support or enhance plant growth and health. This means that the mycorrhizosphere paradigm is actually a hierarchy wherein the plant roots select and allow formation of AM, and the extraradical hyphae, along with modified host root exudates changes (Graham et al., 1981; Lynch and Whipps, 1990), select specific bacterial associates and sustain them, in part, by means of specific hyphal exudates (Bago et al, 1996; Bansal amd Mukerji, 1994). The specificity of AM function that we see could be explained in terms of quality and completeness of the mycorrhizosphere team that can vary with different AM fungi and the soil/growth medium and the microbial populations contained therein. I believe that all soils contain microbial components capable of performing needed functions that aid "normal" plant growth. This mycorrhizosphere paradigm could explain the success of the AM system for some 460 million years (Remy et al., 1994; Smith and Read, 1997; Taylor et al., 1995; Simone et al., 1993).

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