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The Prize Lecture: Part I of II
Pt Ectomycorrhizal Fungi

By Donald H. Marx, Ph.D.

Forest Application of the Ectomycorrhizal Fungus *Pisolithus Tinctorius.*

On September 26, 1991, Dr. Donald H. Marx made the presentation of a lifetime. On that day, in a ceremony in Stockholm, Dr. Marx of the USDA Forest Service, Institute of Tree Root Biology, Athens, GA received The Marcus Wallenberg Prize. The Prize is awarded by the King of Sweden on behalf of The Marcus Wallenberg Foundation for Promoting Scientific Research in the Forest Industry to recognize, encourage and stimulate pioneering scientific achievements which significantly contribute to broader knowledge and/or technological development within the broad fields of interest to the forest industry. Specifically, an international committee of eight members chose Dr. Marx for The Prize for his "path-breaking development of a process for the selective mycorrhizal inoculation of tree nursery soils which greatly increases the growth and survival rates of conifer seedlings used in the reforestation of inhospitable soils."

This is the first of a two-part series of the text presented by Dr. Marx on that September day in Stockholm. (Part II continues immediately following this article)

Your Majesties, Your Royal Highness, Your Excellencies, Distinguished Guests, Ladies and Gentlemen:

I have been very fortunate during my career to have received a number of accolades recognizing my research. I am very grateful for each of them. The ultimate honor, however, is The Marcus Wallenberg Prize. This prestigious award—the highest in forestry—marks the culmination of my scientific research career. I am deeply honored and humbled by this recognition. I will be forever grateful to those who nominated me and to those who considered me worthy.

The award recognizes the product of nearly 20 years of basic and applied research on tree mycorrhizae and their fungi. In the early years, serendipity led to success. In the later years, success came from an unprecedented amount of cooperation between scientists in the USDA Forest Service, state forestry commissions, land reclamation agencies, mining companies, forest products industries, and various forestry groups in different countries.

In this lecture, I will describe how the research evolved from the discovery stage through the development and application phase for *Pisolithus tinctorius*, just one of many species of ectomycorrhizal fungi that occur in forests of the world. In the telling of this story, I hope that two messages will be clear. One is the importance of long-term research programs with adequate funding for risk-taking scientists. The second, and perhaps the most important, is that research administrators must be willing to take the same risks in support of long-term research. Near-term results must be of lesser importance than long-term goals. Without a willingness to take and share risks, new technologies will seldom be forthcoming. We were very fortunate in our program to have had both ingredients.

Intro to Mycorrhizae

Since the term "mycorrhizae" is not a household word and since the significance of this symbiotic relationship to plant health is not well known, it is appropriate that I furnish a basic understanding of mycorrhizal

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A Story of Discovery, Part I CONTINUED

associations before I discuss our research development and application program. The term mycorrhiza (fungus-root) is used to describe a structure that results from a mutually beneficial association between the fine feeder roots of green plants and highly specialized, root-inhabiting fungi.

In this association, each partner contributes to the well-being of the other but, also, takes from its partner what it needs to survive. Mycorrhizae are active, living components of the soil. They have some properties like those of roots and some like those of microorganisms. The mycorrhizal fungi derive most of their organic nutrition (carbohydrates, vitamins, amino acids) from the primary tissues of fine feeder roots. Evidence suggest that the mycorrhizal habit evolved as a survival mechanism for both partners of the association, allowing each to survive low fertility, drought, disease, temperature extremes, and other forms of stress. Because of this co-evolutionary process, many green plants have obligate requirements for mycorrhizae while others benefit greatly from the association but can survive without them. Mycorrhizae are as common on the roots of trees and other plants as chloroplasts are in their leaves. In examining plants in their natural environment, the question is not "are these plants mycorrhizal" because as a rule they are, but rather "which type of mycorrhizae is present and what is the degree of their development on the root system?"

There are two major types of mycorrhizae of plants in nature. Endomycorrhizae, the most wide-spread type, has three subgroups. The most common subgroup is vesicular-arbuscular mycorrhizae (VAM) which occur on over 1,000 genera of plants representing some 200 families. Over 90 percent of the 100,000 species of vascular plants in the world form VAM. These fungi are present in all natural soils throughout the world except where they have been eliminated by man's activities or severe natural disturbances. VAM increase the green plant's uptake of nutrients (especially phosphorus), reduce the effects of various fungal pathogens and parasitic nematodes, enhance water uptake, increase plant tolerance to heavy metals, saline soils and drought, decrease transplant shock, and bind soil into semi-stable aggregates.

The other major type is the ectomycorrhizae which occurs on about 10 percent of the world's flora. Trees belonging to the *Pinaceae* (pine, fir, larch, spruce, hemlock), *Fagaceae* (oak, chestnut, beech), *Betulaceae* (alder, birch), *Salicaceae* (poplar, willow), *Juglandaceae* (hickory, pecan), *Myrtaceae* (eucalyptus), and a few others form ectomycorrhizae. Numerous fungi have been identified as forming ectomycorrhizae. In North America alone we estimate that more than 2,100 species of fungi form ectomycorrhizae on some 2,000 species of woody plants. Most of these fungi produce mushrooms, puffballs or truffles as their reproductive structures or fruiting bodies. Unlike the VAM fungi, these fungi produce spores in their fruiting bodies which are disseminated great distances by wind, rain and by small mammals that eat the fruiting bodies. Ectomycorrhizal colonization changes the shape and color of feeder roots. They may be unforked, birfurcate, nodular, multi-forked, or in other shapes. Their color may be black, red, yellow, brown, white or blends of these colors. An important aspect of the ecology and physiology of mycorrhizal fungi of both types is that they cannot grow independent of their host plants in natural soils. Spores or other resistant structures may survive long periods in soil without a plant host, but the fungi from these propagules will not grow in soil without plant roots. This latter understanding is paramount to any practical application of these fungi.

Ectomycorrhizal fungi aid the growth and development of trees. For many tree species they are indispensable for growth under natural conditions. The obligate requirement of certain tree species for ectomycorrhizae in a natural environment has been clearly demonstrated many times in tree regeneration trials in former treeless areas and in countries without native

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ectomycorrhizal trees that harbor the required fungi. Mycorrhizae, especially ectomycorrhizae, appear to be the first line of biological defense of trees against stress. Trees with abundant ectomycorrhizae have a much larger, physiologically active, root-fungus area for nutrient and water absorption than trees with few or no ectomycorrhizae. This increase in surface area comes from the branching habit of most ectomycorrhizae and from the extensive vegetative mycelial growth of the fungal symbionts from the ectomycorrhizae into the soil. Mycelia function as additional water and nutrient absorbing organs. Ectomycorrhizae also increase the tolerance of trees to drought, high soil temperatures, soil toxins, and extremes of soil acidity. Ectomycorrhizae deter infection of feeder roots by root pathogens. Hormone production induced by fungal symbionts causes ectomycorrhizal roots to maintain juvenility which greatly extends the periods of physiological activity in feeder roots.

Many species of fungi are normally involved in ectomycorrhizal associations in a forest ecosystem, a forest stand, a single tree species, an individual tree, or even a small segment of lateral root. Three species of fungi have been isolated from a single ectomycorrhiza. A single fungus can enter into ectomycorrhizal association with numerous tree species on the same site at the same time forming fungal bridges between trees. While some fungi are apparently host-specific, others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families. An ecological succession of ectomycorrhizal fungi apparently occurs during the aging of forest stands. Increasing age of stands and an increasing number of tree species in the stands are associated with an increase in the diversity of ectomycorrhizal fungi in the stands. Much of the above information was obtained from research done over the last 25 years.

From Disasters to Discoveries

Early Research and Field Observations. Research on ectomycorrhizae was begun in the southern United States in the late 1950s by Dr. Bratislav Zak and William C. Bryan in Athens, GA. I became their technician in 1958. The research priority was to determine the species of fungi forming ectomycorrhizae with commercially important southern pine species, particularly shortleaf, slash and loblolly pines. The technique used to confirm the identity of suspected fungal symbionts was the aseptic synthesis test developed by Melin in Sweden in the early 1920s. Several species of fungi, including *Pisolithus tinctorius*, were eventually identified using a modified version of Melin's technique.

The mycorrhizal research program was transferred to the USDA Forest Service's Research Triangle Park Laboratory in North Carolina in 1963. The objective was to study the effect of ectomycorrhizae on a feeder root disease of shortleaf pine. Our entire program was based on five species of ectomycorrhizal fungi—*Amanita muscaria*, *Laccaria laccata*, *Lactarius deliciosus*, *Leuco-paxillus cerealis* var. *piceina* and *Pisolithus*—which had been identified by Bryan and Zak in Georgia as being ectomycorrhizal with shortleaf pine. The only reliable technique to form ectomycorrhizae on shortleaf pine was still the modified Melin's aseptic method.

Because of high temperatures in the south, it was common practice to immerse the bottoms of the flasks containing test seedlings in a circulating bath of refrigerated water in the greenhouse to reduce temperatures in the closed containers. In one of our studies, over 300 aseptic seedlings inoculated with different fungi were incubating in the greenhouse water bath. The resulting ectomycorrhizae were going to be inoculated with a feeder root pathogen to determine their resistance to infection. Halfway through the incubation, there was a power failure and cooling capacity was lost. The result of the high temperature in the seedling containers was devastating. Within two days all the seedlings died except those inoculated with *Pisolithus*. They were in perfect

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health! Nearly six months of research was lost and had to be repeated because we needed many seedlings with different ectomycorrhizae to inoculate with the pathogen. But why did the *Pisolithus* seedlings survive while all others died? This disaster was our first significant example of serendipity. We learned that pine seedlings with *Pisolithus* ectomycorrhizae appeared to tolerate high temperatures but we did not know how high these temperatures were. We had to set aside this observation since it had no bearing on the objectives of our sanctioned research at the time.

Research on the effect of ectomycorrhizae on feeder root disease was advanced by our second serendipitous observation. It was necessary to growth the ectomycorrhizal fungi in pure liquid culture in temperature-controlled incubators in the laboratory in order to examine their physiological traits. During one study, mites were accidentally brought into the laboratory by another research project. Mites have bacteria and fungi on their bodies and are feared sources of contamination in any microbial laboratory working with pure cultures. The mites got into all of our pure cultures in the incubator and within two to three days all cultures were obviously contaminated except those of *L. cerealis* var. *piceina*. Why were these cultures not contaminated? Eventually, we discovered that the mycorrhizal fungus produced very strong antibacterial and antifungal antibiotics that inhibited these contaminants. This serendipitous discovery eventually led to a new concept of the value of ectomycorrhizae to forest trees. We eventually learned that ectomycorrhizal fungi can ward off pathogenic attacks on feeder roots by producing potent antibiotics and elucidate their role in control of feeder root disease. Following this discovery, the National Institute of Health developed an interest in screening forest fungi for new sources of antibiotics for control of human and animal diseases.

The ectomycorrhizal research program was transferred back to Athens, GA in 1966 following completion of studies in North Carolina. Bryan and I started our collaboration at this time. Our research mission was to investigate more intensely the role of ectomycorrhizae in the biological control of feeder root diseases of forest trees. We did that, but we did not forget the high temperature tolerance of seedlings with *Pisolithus* ectomycorrhizae. The results of subsequent temperature studies proved that *Pisolithus* could grow and form ectomycorrhizae on pine seedlings at temperatures as high as 34º C and that pine seedlings with *Pisolithus* ectomycorrhizae survived and grew vigorously at soil temperatures as high as 40º C. Seedlings with other mycorrhizae or no ectomycorrhizae either grew poorly or died at 34º C. All of those temperature studies were done under controlled conditions in the laboratory or greenhouse. It was now imperative to determine if this physiological trait was functional in the natural world. About this time, as luck would have it, Schramm published a classic work on plant colonization of anthracite coal wastes in Pennsylvania. He found fruiting bodies of *Pisolithus* to be common under pine and oak on this waste material. He traced the extensively developed mycelial strands formed by *Pisolithus* from ectomycorrhizae of various tree species through large waste volumes to the base of fruiting bodies. These mycelial strands were brilliant gold-yellow and were easily traced through the contrasting dark anthracite wastes. The ectomycorrhizae were also gold-yellow and prolifically branched and were associated with the most vigorously growing seedlings. In most cases, *Pisolithus* was the first detectable ectomycorrhizal fungus on seedling roots. Other species of ectomycorrhizal fungi, including *Thelephora terrestris*, appeared on roots and produced fruiting bodies primarily after litter had accumulated under the canopies of older trees. *T. terrestris* is the most common ectomycorrhizal fungus on pine seedlings in nurseries, which accounts for its presence on planted coal spoils. It was on the seedling roots from the nursery prior to their planting on the spoil. A second significant observation made by Schramm was soil temperatures between 35º C and 65º C in bare wastes

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at a depth of 6 cm. We concluded that perhaps *Pisolithus* was dominant because it was ecologically and physiologically adapted to these high soil temperatures, which slowed or eliminated establishment of other fungi.

Prompted by Schramm's observations and our research findings on temperature effects, we made examinations of various strip-mined spoils in the eastern United States. We confirmed Schramm's observations. *Pisolithus* fruiting bodies and its unique, gold-yellow ectomycorrhizae and mycelial strands were the predominant, if not the only, ectomycorrhizal structure on roots of young pine, oak and birch seedlings on coal spoils in Alabama, Indiana, Kentucky, Ohio, Pennsylvania, Tennessee, Virginia and West Virginia, as well as on southern pines on strip-mined kaolin clay spoils in Georgia. In addition to high temperatures during summer, some of these spoils also had a soil reaction as low as pH 2.9.

Research Inoculum Production and Testing. We hypothesized that *Pisolithus* ectomycorrhizae were instrumental in tree establishment and maintenance on these strip-mined sites and initiated research to develop techniques to "tailor" seedlings in the nursery with *Pisolithus* ectomycorrhizae. The working premises were simple—Why wait for erratic but natural processes to establish *Pisolithus* on trees on these adverse sites? Most planted or volunteer seedlings die before *Pisolithus* colonization can occur naturally. Tree survival and growth should be improved by having *Pisolithus* ectomycorrhizae pre-formed on the seedlings in the nursery prior to their planting on the strip-mined spoil.

Research began in an electronically, air-filtered growth room we constructed in the greenhouse. Air filtration was needed to eliminate the air-borne spores of *T. terrestris* which routinely contaminated our test seedlings with undesirable ectomycorrhizae. Following success here, we graduated to open-air greenhouse studies and then into our microplot nursery. Final testing was done in conventional tree nurseries. In these various studies we learned a great deal. We learned how to identify *Pisolithus* ectomycorrhizae and to accurately quantify them on root systems. Many scientists questioned our ability to do this so we had to develop a specific fluorescent antibody technique to prove that our assessments using the unaided eye were reproducible and as accurate as the biochemical method. We also learned that many factors such as soil fertility, pesticides, organic matter, and competition from other soil microorganisms, which many scientists thought would negate our success, were not insurmountable problems.

It took a few years, but techniques were eventually developed for tailoring the root system of pine with abundant *Pisolithus* ectomycorrhizae. Briefly, the techniques involved the production of pure-culture, vegetative inoculum of *Pisolithus* in a vermiculite-peat moss-nutrient substrate or the use of spores collected from *Pisolithus* fruiting bodies. Both inocula were used successfully to artificially infest fumigated nursery soil. Effective soil fumigation shortly before soil infestation and viable inoculum appeared to be the only prerequisites for successful "tailoring" of seedlings with *Pisolithus* ectomycorrhizae. This research was the beginning of our collaboration with Dr. Ed Cordell, State and Private Forestry, USDA Forest Service.

An unexpected benefit of *Pisolithus* ectomycorrhizae was improved growth of pine seedlings in the nursery. Growth increases of between 100 and 150 percent in the nursery occurred with various pine species following development of *Pisolithus* ectomycorrhizae. The introduction of this fungus into the nearly sterile rooting substrate of containerized seedlings was also developed at this time.

For Part II, see next page.

The Prize Lecture: Part II of II
Pt Ectomycorrhizal Fungi

By Donald H. Marx, Ph.D.

Forest Application of the Ectomycorrhizal Fungus *Pisolithus Tinctorius*, continued.

This is the conclusion of a two-part series of the text presented by Dr. Marx on September 26, 1991 in Stockholm.

By the mid 1970s, a great deal of interest was being generated on the practical use of *Pisolithus* in forestation efforts around the world. Because of this and the implications of plant quarantine in introducing *Pisolithus* to other countries, we had to determine its geographic distribution and tree host range. We confirmed that it naturally occurred in Europe, Asia, Africa, Central and South America and the Middle East. It has also been found in most states of the U.S. Its tree host range is also quite impressive. Over 100 species of pine, birch, eucalyptus, poplar, cedar and oak have either been experimentally confirmed as hosts or have been associated with this fungus consistently in the field. We have concluded that *Pisolithus* can form ectomycorrhizae, at least experimentally, with all species of trees that normally form ectomycorrhizae and can do so under a wide array of environmental conditions. Following the development of effective research inoculum production methods, we initiated cooperative nursery research studies and confirmed its value on a variety of tree species in Brazil, Canada, The Congo, France, Ghana, India, Liberia, Malawi, Mexico, Nigeria, South Africa, South Korea and Thailand.

Reclamation Site Studies. In 1974 we began a series of outplanting trials with tailored seedlings on adverse sites throughout the eastern U.S. Over 25 field studies involving tens of thousands of seedlings of several pine species grown in our microplot nursery were installed on abandoned acid coal and kaolin clay spoils, borrow pits, and severely eroded sites. In addition to high summer temperatures and severe acidity (pH 2.8-3.5) of many coal spoils, they also contained very high concentrations of soluble aluminum, manganese and sulfur which are normally toxic. They were also very droughty due to poor water retention properties. Most of the other adverse sites were not toxic but were infertile and droughty. In all tests, our control or comparison seedlings had naturally occurring ectomycorrhizae because this is the normal condition of pine roots. These seedlings had abundant *T. terrestris* ectomycorrhizae from the nursery. Results have been dramatic. On many coal spoils, survival and growth of tailored seedlings were 4 to 5 times greater than controls. On certain extremely toxic sites, survival of control seedlings was near zero, while that of seedlings with *Pisolithus* ectomycorrhizae was 50 to 60 percent. On less adverse sites, such as clay spoils or eroded sites, *Pisolithus* routinely doubled survival and growth, even on sewage sludge amended sites. Plant tissue analyses showed that tailored seedlings on coal spoils had more nitrogen and phosphorus and less heavy metals than control seedlings. These data suggested a selective absorption of desirable nutrients and the rejection of undesirable elements by this special root system. These studies could not have been installed and maintained without the cooperation of several federal and state forestry and reclamation agencies and mining companies.

Routine Reforestation Site Studies. Shortly after we initiated the reclamation research, we expanded the field research to included routine reforestation sites. Many such previously forested sites, especially those that have been intensively prepared (stump shearing, root raking, slash removal, burning, disking, etc.), are temporarily adverse. Until vegetation is reestablished by either natural or artificial means, the exposed mineral soil is subject to broad fluctuations of temperature, moisture and fertility, as well as to erosion and compaction. These soil conditions are adverse for the root systems of newly planted seedlings. When these soil conditions are sufficiently extreme, survival and growth of nursery seedlings with *T. terrestris* ectomycorrhizae are poor.

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A Story of Discovery, Part II CONTINUED

Our early efforts in this cooperative program involved field tests of tailored seedlings of loblolly, slash, eastern white, Virginia, shortleaf and sand pines throughout the south on sites of different quality. These studies were done in cooperation with state forestry commissions and with several forest products companies. We found great differences in survival and growth on certain sites while in others we found minimal or no significant effect. We found as much as 250 percent volume differences in some studies.

In studies with great differences in growth, control seedlings survived and grew poorly. In studies with little or no difference, the control seedlings survived and grew well. In these instances, regardless of site quality, the *Pisolithus* seedlings survived and grew at similar rates. These findings suggested to us that the *Pisolithus* seedlings were able to compensate for stresses that depressed survival and growth of control seedlings. Later we found that one site stress factor was available soil water (ASW). Even on high-quality sites, ASW was limited during periods of prolonged drought which plagued the southern U.S. during these test periods.

Researchers in other parts of the U.S. confirmed our findings and reported significant improvements in field performance of tailored seedlings of various tree species on a variety of test sites. By the early 1980s, significant results were also coming from Brazil, Mexico, South Korea, Philippines, The Congo, Nigeria, Ghana and France. One of the most interesting was done in Liberia in cooperation with the Liberia Forest Corporation, a group organized in Sweden. We created a "bush" container seedling nursery and inoculated Caribbean pine seedlings with *Pisolithus*, *Thelephora*, and native forest soil inoculum, or with no inoculum. Seedlings were outplanted in a cleared jungle site void of any native ectomycorrhizal fungi. We measured the trees at the end of each seven-month rainy season and each five-month dry season for three years. The results were significant. Seedlings with *Pisolithus* ectomycorrhizae and those formed by a fungus in the soil inoculum grew as much or more during the dry season as seedlings with other ectomycorrhizae grew during the longer rainy season. Inadvertently, seedlings we wanted nonmycorrhizal became contaminated with *T. terrestris* in the nursery and we lost the nonmycorrhizal comparison. We were able to identify the fungus in the soil inoculum as an *Alpova* species, which is biologically related to *Pisolithus*. These results confirmed our earlier suspicion that *Pisolithus* was able to manage water relations for the seedlings more effectively than other fungal species. Recent evidence suggests that this water relationship is mediated by the *Pisolithus* hyphal strands which occupy large soil volumes and enhance exploitation of available water.

Technology Transfer Efforts. Early in our research program we found that few scientists or foresters understood the importance of ectomycorrhizae, much less the potential value of manipulating and managing for a specific ectomycorrhizae like *Pisolithus* ectomycorrhizae. We began a lecturing and publishing campaign throughout the U.S. at scientific meetings, regional workshops, universities, conservation groups, and just about anywhere people would sit and listen to us. Our international efforts were greatly enhanced by workshops, sponsored by the International Foundation for Science of Stockholm, held in Ghana and Malaysia and by various international cooperative forestry programs of the USDA Forest Service initiated in Brazil, China, India, Morocco, Pakistan, Taiwan and Venezuela. Each of these occasions furnished us with opportunities to teach and instruct young scientists on the concepts and methods needed for applying the ectomycorrhizal technology. From these contacts came our international cooperation.

We were also able to bring graduate students and scientists to our laboratory from various universities in the U.S. and abroad to work with us on various research studies. We gained a great deal of insight into the unique problems and seedling production procedures in other parts of the world. It was challenging to devise modifications of our methods to accommodate particular needs.

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A Story of Discovery, Part II CONTINUED

Commercial Inoculum Production and Application. By the late 1970s, we had obtained sufficient results from nursery and field tests to know that the management and application of *Pisolithus* showed promise in practical forestation efforts. It was necessary to determine the feasibility of producing commercial volumes of inoculum and to test its effectiveness in nursery and field tests. Biologically, we knew it would not be easy to shift from producing a few hundred liters of *Pisolithus* inoculum in our laboratory to producing thousands of liters for commercial application using industrial protocols.

In 1976, USDA Forest Service administrators in Washington made a risky decision. They approved our proposal to form the Institute for Mycorrhizal Research and Development with an international mission. They basically agreed to let us follow our research discoveries wherever in the world they might lead. This was unprecedented.

The newly formed Mycorrhizal Institute, State and Private Forestry represented by Dr. Cordell, and Abbott Laboratories, North Chicago, Illinois, began a cooperative program of research and nursery evaluation to develop commercial volumes of vegetative inoculum of *Pisolithus* using industrial fermentation techniques. From 1977 through 1981, different formulations of Abbott inoculum, and for comparison our research inoculum, were tested on 13 species of trees in containers at six locations in the U.S. and one in Canada and on 14 species of trees in 33 bare-root tree nurseries in 25 states in the U.S. Six federal, 13 state and 11 forest products industries cooperated in this program. Results showed that industrial fermentation technology could be modified to produce viable vegetative inoculum of *Pisolithus* in large volumes. The results also proved that *Pisolithus* would form ectomycorrhizae on numerous tree species from vegetative inoculum under a wide array of environmental conditions, soil types, fertility regimes, and pesticide applications in conventional tree nurseries.

Unfortunately, Abbott Laboratories decided for economic reasons not to commercially produce the inoculum. In 1980, we initiated a similar research program with Sylvan Spawn Laboratory in Pennsylvania. Novel formulations of vegetative inoculum were tested using a modification of a method to produce mushroom spawn. Various formulations were tested on pine and oak in several southern nurseries and in container systems. By 1986 a highly effective formulation grown and shipped in 8-liter plastic bags was developed. A biotechnology company, Mycorr-Tech Inc. in Pittsburgh, PA, was formed and is the producer of this inoculum today. [Editor's Note: Mycorr-Tech Inc. was acquired by Plant Health Care, Inc.]

In order to develop this vegetative inoculum and methods to apply it, we performed over 85 discrete nursery tests on nearly 2 million tree seedlings between 1977 and 1986 in 41 nurseries located in 31 states in the U.S. During this time, Dr. Cordell also designed and perfected a tractor-drawn nursery seedbed applicator. This machine places inoculum at specific soil depths in bands directly under where seed will be planted. This applicator has reduced the amount of inoculum needed by 75 percent compared to other methods. Also, during this period we devised spore pellets for broadcasting onto soil, and developed methods for encapsulating pine seeds with spores for direct sowing application. The seed research was done in cooperation with Hilleshog company, Landskrona, Sweden.

In the last six years, over 40 million seedlings of several tree species have been produced with *Pisolithus* ectomycorrhizae from commercial inoculum in nurseries in the eastern U.S. These seedlings have been operationally outplanted on a variety of problem sites as well as routine forest sites. Results from these plantings are similar to those we obtained from our research plots. Tailored seedlings significantly improved survival and growth on routine sites, and on acid coal spoils tailored seedlings are now growing where ordinary seedlings did not grow at all. As discussed earlier, these data suggest that *Pisolithus* ectomycorrhizae may not be stimulating growth potential of seedlings but instead may be removing or managing a factor(s) that inhibits growth. The

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A Story of Discovery, Part II CONTINUED

inhibitory effects of certain stress factors like low amounts of ASW, acute acidity, very high soil temperatures, and high concentrations of normally toxic elements such as aluminum, manganese and sulfur apparently are minimized by the tailored root system.

Among the most gratifying consequences of our work are the research and development programs initiated by scientists in other locations in the U.S. and abroad. Recently the world literature was catalogued on the practical aspects of the ectomycorrhizal technology. This report showed that over 66 species of fungi have been used experimentally to form ectomycorrhizae on 49 tree species. Over 40 percent of the publications dealt with *Pisolithus* on 29 different tree species. USDA Forest Service scientists in the Pacific Northwest of the U.S. have developed a successful spore inoculation program with several species of fungi. Results with *Rhizopogon vinicolor* and *R. colossus* ectomycorrhizae on Douglas fir seedlings are very positive. A private company has been formed to collect and process spores from the forest for operational use in bare-root and container seedling nurseries.

In France, nursery inoculations are underway to improve field performance of seedlings in reforestation programs and to enhance production of edible fungi. *Laccaria laccata* and *Hebeloma crustuliniforme* show the most promise for improving field performance of seedlings, and the fungi, *Tuber melanosporum* and *T. uncinatum* are being used for truffle production. The French are currently developing commercial fermentation techniques to produce large quantities of these fungi.

Although their work is still in an early developmental stage, Canadian scientists are making rapid progress in developing inoculation techniques for *L. laccata*, *H. cylindrosporum*, *T. terrestris* and *Pisolithus* for container production of pine, spruce and larch seedlings. These tailored seedlings will be used in their national program to reforest cutover lands.

Researchers in the Philippines have developed a mycorrhizal fungal tablet made of a mixture of spores of *Pisolithus* and *Scleroderma cepa*. Each container gets one tablet before sowing in the nursery. The procedure has proven successful with pine, eucalyptus, dipterocarps and *Casuarina*. Growth in the nursery is dramatically improved, and volume growth in the field after three years is increased by as much as 200 percent. These tablets are commercially available from the Philippine government.

These practical ectomycorrhizal programs represent only the beginning of a much needed and organized worldwide effort. Much of the technology can be applied now without further research to solve many forestation problems in the world. Other locations with unique problems obviously need more research to find solutions to specific problems.

Conclusion

It took 20 years of continuous research by us and many other scientists to progress from the serendipitous observation of high temperature effects of *Pisolithus* to its practical application in forestation practices. Many risks were taken but that is the nature of research. Along the way many basic facts were found concerning new and important ecological and physiological aspects of ectomycorrhizae. These successes stimulated other scientists worldwide to examine the role of other mycorrhizae in growth and development of agricultural and other plants with renewed vigor.

In closing I would like to quote a statement made by Calvin Coolidge, our 30th U.S. President: “Nothing in the world will take the place of persistence. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent. The slogan ‘Press on’ has solved and will always solve the problems of the human race.”

It worked for us.

Stopping to Smell the Roses

By Jerry Roche, Guest Columnist, and Editor, *Landscape & Irrigation*

By and large, landscapers and arborists are harried people—sometimes even frazzled, due to the extremely time-intensive nature of your work. Client A needs his lawn mowed today; Client B needs his trees pruned yesterday. So you hop in the pick-up and go here…do your thing…zip there…do your thing…and do it some more—quick, before the sun sets!

If you fall into that category, you might feel like you're spending your life battering your head into brick walls or going around in endless circles. So busy *planting* the roses that you don't have time to *smell* the roses, so to speak.

It's not that you don't have high hopes, because most of you do. It's just that the 24 hours in each day never seems enough to achieve what you want to achieve. But Albert Einstein had 24 hours; Colin Powell and Donald Trump and Hugh Hefner have 24 hours. If they can achieve a measure of success in the 24-hour time frame, you can, too. You just have to know how to use your time effectively.

Over the years, I've attended dozens of seminars designed to help landscapers and arborists find a better way to live their hectic lives. Though the specifics are different, the general message is that *balance is the key*. The speakers always make interesting, useful suggestions, some of which I'd like to share with you here. Not all might be applicable to your specific situation, but I'll bet that at least one or two will be useful:

- Simplify your business and your life. Sort out the unnecessary from the necessary. Find someone to handle the mundane tasks while you concentrate on doing the things you like to do (which should be the things you do best). Start the process by ditching profit centers that don't turn a profit, then concentrate on building the more lucrative parts of your business. Whittle down your avocations, too, keeping only those that you truly enjoy. (Haven't touched that stamp collection in ages? Post it on e-Bay!)

- Write down your priorities in business and life. Life's joys come from recognizing who you are, what you are, where you're headed, and from achieving your goals—no matter how large or small they may be. Keep the list close to your heart, check off each goal as you reach it, and then go on to the next one.

- Pursue your passions. Define what your passions are, and then pursue them fervently. Your passions have nothing to do with what society might consider failure or achievement. They're your own personal portal to happiness. For instance, is a person a failure because he loves to sing, but is a lousy singer and can't make a buck from it? No, he's happy because when he sings, he's pursuing a passion.

- Slow down. "Slow" doesn't kill companies, "fast" does. The hare self-destructed, while the slow, steady tortoise crossed the finish line first. Our Japanese colleagues learned long ago that success might not even be achieved in one person's lifetime, but can stretch over generations. Nothing has to be done today, but—by the same token—you shouldn't put everything off until tomorrow. Don't try to lop off too much: take baby steps toward your final goal, if necessary. Whatever you choose to do, budget yourself enough time to do it right the first time.

- Plan ahead. As our drill sergeant used to say, "Prior Planning Prevents Poor Performance." A little planning prevents a lot of headaches (and heartaches). Make a realistic list every day of the things you want to get done. Don't quit until they're done, and when they're done, quit.

continued

Balance Your Work and Life CONTINUED

■ Avoid making ill-informed decisions. Throwing ideas against the wall to see what sticks is a great way to waste valuable time and money. Better to try one new idea with a 90 percent certainty that it will work than 10 new ideas with no clear indication of how many or which ones will be profitable. The more important the decision, the more studied it must be.

■ Avoid the temptation to become big, bigger, biggest. Growth patterns must be calculated long beforehand, using every piece of information available. Acceleration must be controlled. Yes, a certain rate of growth will exactly fit into your long-range plans—but don't get caught up in the hectic, thankless task of trying to grow beyond your resources and your ability; if you do that, you'll only run a higher risk of everything crashing down on you.

■ Accept the fact that not everyone can show the world how to build a heavier-than-air flying machine; or be the standard-bearer for truth, justice and the American way; or create a worldwide network of drive-through eateries serving microwaved beef in a wide variety of styles. You can't be Superman, no matter how hard you try, so don't be afraid to settle for what might seem like less. Helping your employees lead more fulfilling lives and/or helping paint a more beautiful American landscape are honorable, achievable goals.

■ Live for the moment. Take things one at a time, as they come. Drift with the natural flow of life, but enjoy each moment for what it is. When you're working, *really* work. Find something constructive to do every minute of the workday. When you choose to have fun, *really* have fun. Go play golf, and leave the cell phone in the car. Take a week's vacation with your family, don't tell anyone where you're going, and fight the temptation to call the office. Separate your professional life from your personal life whenever possible. And enjoy both.

■ Get plenty of sleep. Eight hours is said to be the minimum for people involved in physical labor. If necessary, work up to it: if you're a five-hour-a-night person, try for six or seven and see what a difference it makes the next day. You'll be pleasantly surprised.

■ Don't look back. It's a waste of time. If it happened yesterday, it's history. Learn from yesterday, live for today, plan for tomorrow. The future holds no bounds.

■ Remember that you control your job and your life, no matter what is going on around you. As "The Desiderata" says, *"Go placidly amid the noise and haste, and remember what peace there may be in silence...and whatever your aspirations, in the noisy confusion of life, keep peace with your soul."* Walk the path you've chosen with your head held high and your chest proud.

■ Have fun. Learn to focus on things you can change rather than banging your head against the wall on things you can't change. Never undertake more than one stressful activity or project at a time. Delegate authority. Take a portion of the day to get away from things, whether it's by playing basketball with the guys at lunch (as I do) or reading a chapter in a favorite book. Your mind and body are your most important tools. Take care of them.

■ Take 30 minutes a day doing nothing. Make it a time of quiet and solitude, in your office or later at home. Use 10 minutes to reflect on the day's events. Use 10 minutes to plan for the next day by creating a visual appointment calendar in your mind. Finally, take 10 minutes to totally clear your head, rejoice in your blessings, and thank your favorite "higher power" that you've been lucky enough to survive another day.

Photosynthesis: Let There Be Light

By Michael J. Kernan, Ph.D.

I've heard people say that photosynthesis is a dry and boring topic. Nothing could be further from the truth.

Significance to Life on Earth

Photosynthesis–its first occurrence on the planet–was an astonishingly Earth-changing event. It is the biological Big Bang. Photosynthesis transformed the planet into the jewel of nature that it is today. Without photosynthesis, we would not have our fantastic diversity of life. Photosynthesis is the basis for nearly all life on Earth.

Chemistry of Photosynthesis

Most molecules that are natural to the early Earth are all very simple, very small molecules, such as water, carbon dioxide, hydrogen and nitrogen gas. Chemically, many things can be produced if these molecules are bound or chained together, but the binding requires chemical reactions and the reactions require energy. Plants devised photosynthesis to take ambient energy coming to Earth in the form of sunlight, and harness it to produce complex molecules. Plants then use these molecules as building blocks to create plant tissues and cellular structure, and as sources of stored chemical energy to fuel the process. Most other life is totally dependent on this plant tissue, either directly or indirectly, for survival.

Plants are very elaborate chemical labs that use photosynthesis to chemically convert simple minerals into complicated, energy-containing, organic molecules that can be used for plant structure and fuel. Plants take carbon in the form of carbon dioxide from the air and hydrogen from water to construct sugar molecules. The energy required to accomplish this comes from the sunlight and the process is called photosynthesis. Plants use the sugar molecules both as fuel and building blocks. The sugar molecules are stored in plant tissue for later use. That's why certain fruits taste sweet. Or plants will chain the sugar molecules together to make a sugar polymer called starch, which isn't sweet but still has the energy of sugar in it and can be metabolized and used as a fuel. Or plants link the sugar molecules into a slightly different polymer form to make what is called cellulose–the main structural component of plant cells. All plant cell walls are made of cellulose. The most familiar commercial cellulose product is paper. Paper is made from cellulose by pressing the fibers into fine sheets.

That is what plants have accomplished. They have developed a method to trap the energy from sunlight and put it to work making sugar molecules which they can then use for food or for building blocks.

Once plants have trapped the energy from sunlight, they can use that energy to build other necessary things. Plants make amino acids by taking nitrogen from the soil and adding it to some of the precursors of sugar. The amino acids can then be used to make proteins. Proteins are important structural parts of plant and animal bodies. Many proteins function as enzymes which run complicated reactions, including photosynthesis and respiration. Of course, animals get their proteins by eating plants, and converting the plant proteins into their own proteins.

Early Life Prior to Photosynthesis

On Earth millions of years ago, there were early animals and microbes that gathered energy through a process called chemosynthesis. Some chemosynthetic organisms remain today living deep within the ocean near volcanic vents that continuously spew sulfur or other minerals. The organisms process these minerals to retrieve what little energy they require to live. Eons ago, it is possible that chemosynthetic organisms developed photosynthesis as a method to gain more energy.

Photosynthesis gave plants the means to take simple energy-less molecules and, using the energy from the sun, combine those molecules together to achieve not only building blocks but also energy storage molecules–sugars–as storable food. In this way, plants devised a method to store energy for use in the day or at night. With this ability to store energy, life on Earth changed forever.

Plants Are Elaborate Chemical Labs CONTINUED

Impact of the Major Byproduct of Photosynthesis—Oxygen

The oxygen we breathe is a by-product of the photosynthetic process. Plants split water molecules (H2O) to take the hydrogen and use it to build their sugar molecules. The oxygen from these water molecules is released as a by-product of photosynthesis.

Early Earth had little oxygen in its atmosphere. And, since ozone is a form of oxygen, there was no ozone layer to filter the ultraviolet rays. Therefore, the ultraviolet (UV) rays of the sun were able to pass unhindered right to the ground. As a result, the surface of the land was very inhospitable; organisms were easily burned by UV light. After millions of years of photosynthesis by phytoplankton in the sea, oxygen accumulated in the air as a type of beneficial pollutant. Eventually enough oxygen was built up to produce the ozone layer that provides us with our shield against the majority of UV light. Today, air is about 21 percent oxygen, as O2. Ozone is O3, and is formed naturally by O2 in the presence of ultraviolet light or lightning. Photosynthesis not only provided a means for bringing energy into the biosphere and provided replenishable energy as the source of food and structural molecules, but it also provided a shield for UV light. This made the land surface habitable, so early plants and animals could move from the sea to land.

Photosynthesis as the Support for Most Life on Earth

Organisms in the soil are not exposed to sunlight. They have to get their energy from plants or from dead organic material in the soil, which stored its energy when it was alive, made directly or indirectly from photosynthesis. Many of the microbes, like the rhizosphere bacteria, get their energy-containing molecules–their food–from plant root exudates and sloughed root tissues. Similarly, mycorrhizal fungi obtain sugars and other organic molecules from plants for their energy needs. Herbivores rely on photosynthetic plants for their food. Carnivores rely on the plants to support the herbivores—their food. Ultimately, almost all life and energy can be traced to photosynthesis.

Significance of Photosynthesis to Modern Industry

The fossil fuels that we use today–coal, natural gas and oil–are all left over from photosynthesis performed by plants millions of years ago. The energy-containing sugar molecules were trapped underground, and over the millennia, they chemically changed into fossil fuels that still contain the energy captured from sunlight. Today, as we use these fuels, we are using the energy of the sunlight from millions of years ago. Virtually everything that is fueled on Earth essentially comes from photosynthesis. Even our industrial society. (Nuclear energy is an exception.)

The Electrochemical Leaf

The leaf is the organ in which photosynthesis is carried out. It's actually similar to electronics. Each leaf cell is like a photoelectric cell, so plant leaves are like a whole series of photoelectric cells hanging out in the air. When the light shines on them, certain electrons in the chlorophyll pigment are energized. But instead of the electrons being captured in a current to run a calculator or a flashlight, the electrons are harnessed to run chemical reactions which link carbon molecules together into sugars. When leaves are damaged by disease, pests, or storms, this directly impacts the plant's ability to produce sugars for food.

Magnesium—An Important Component of Chlorophyll

Plants require water for photosynthesis, from which they will extract hydrogen. But there are certain minerals, notably magnesium, that are also required to keep the photosynthesis process going. Magnesium is needed because it is central to the chlorophyll molecule, much like the way that iron is central to human hemoglobin. Various other minerals are required to support the chemistry of the photosynthetic processes. These are extracted from the soil, as micronutrients, and can be recycled.

So is photosynthesis a bland subject? I don't think so, and I believe the topic opens new ways for us to appreciate plants and the major role they played in the evolution of our own existence.

Michael Kernan is a scientist at Plant Health Care, Inc.

About Planthealthcare.com Online Magazine

PlantHealthCare.com Online Magazine is posted at www.planthealthcare.com for professionals who produce, design and maintain plant material in the arbor, landscape architecture/design, landscape maintenance, nursery/greenhouse, and parks and recreation industries.

Published as an educational service by Plant Health Care, Inc., the PlantHealthCare.com Online Magazine is designed to engage, educate and inform professionals about new technologies that promote the health of plants, specifically those that create "sustainable" landscapes that cost less, provide more value and last longer. The magazine also seeks to open discussion about issues that impact the many businesses that serve the plant health industry.

Meet Your Editors

Felicia L. Gillham
Managing Editor

Felicia Gillham is owner of Gillham & Associates Marketing Communications, a San Diego, CA firm she established in 1989 to service the needs of turf and ornamental, agricultural and biotechnology companies. Articles written by Gillham on behalf of her clients have appeared in more than 100 Green Industry and farm trade publications. She is a 1980 graduate of the University of Missouri—Columbia with a degree in agricultural journalism. Gillham is a member of the Turf & Ornamental Communicators Association, American Agricultural Editor's Association and the National Association of Farm Broadcasters.

Dr. Donald H. Marx
Winner, 1991 Marcus Wallenberg Prize

Dr. Marx, considered the leading authority on mycorrhizae and their use in reforestation, is the scientific backbone of PHC, managing the university and field testing of PHC products and working with PHC's forestry, horticulture and land reclamation customers. Dr. Marx came to PHC with more than 37 years of experience with the USDA Forest Service, where he conducted extensive research on the practical use of mycorrhizal fungi to improve forests worldwide, in addition to work in air pollution, stress relationships in trees, use of organic soil amendments in reclamation, exotic tropical forestry and nursery management. Dr. Marx founded the Institute for Mycorrhizal Research and Development in 1974 and the Institute of Tree Root Biology in 1990 for the Forest Service. He has authored more than 230 scientific papers in forest microbiology and has presented more than 300 invitational lectures in 21 countries, as well as at most major universities in the U.S.

Jerry Roche
Guest Columnist

Jerry Roche is editor of *Landscape & Irrigation*. He is employed by Adams Business Media with a home office in Strongsville, Ohio. Roche is former editor of *Lawn Care Industry, Weeds Trees & Turf* and *Landscape Management* magazines. He's been involved in the green industry since 1982, following 11 years in the daily newspaper business. The graduate of Ohio University is a member of the Turf & Ornamental Communicators Association.

Calendar of Industry Events

July

10-15
ANLA Convention, American Nursery & Landscape Association, Cleveland, OH
202-789-2900

19-21
Turfgrass Producers International Summer Convention, Toronto, Canada,
800-405-8873

19-22
ALCA Summer Leadership Meeting, Associated Landscape Contractors of America, Amelia Island, FL,
800-395-2522

20-22
EXPO 2001, Louisville, KY,
800-558-8767

24
Midwest Turf Field Day, Purdue University, West Lafayette, IN
765-494-8039

August

4-8
Annual Conference of the Soil and Water Conservation Society, Myrtle Beach, SC
515-289-2331

17-18
Nursery-Landscape Expo, Dallas, TX
530-458-3191

17-19
ALCA Design/Build Workshop, Atlanta, GA, 800-395-2522

September

9-11
American Nursery & Landscape Association Legislative Leadership Conference, Washington, DC
202-789-2900

13-14
Southwest Horticultural Trade Show, Arizona Nursery Association, Phoenix, AZ, 480-966-1610

22-25
American Society of Landscape Architects Annual Meeting, Montreal, Canada, 202-898-2444

October

3
Washington Landscape Field Day and Trade Show, Puyallup, WA
800-833-2186

12-13
Plant Health Care Plant Biology Workshop, Instructor: Dr. Donald H. Marx, Frogmore, SC
888-290-2640

November

9-10
Plant Health Care Plant Biology Workshop, Instructor: Dr. Donald H. Marx, Frogmore, SC
888-290-2640