

## MICROBIAL RELATIONSHIPS IN SURFACE-MINE REVEGETATION

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### Abstract

The establishment and interrelationships of microorganisms with soil and plant processes during reclamation are greatly influenced by the composition of the planting medium and vegetation practices. While in some instances the parent material may be used as the vegetation medium, the practice of topsoiling, particularly the direct haul method, may be beneficial in introducing microorganisms and improving the quality of the plant growth medium of spoils that are chemically or physically less desirable than the native soils. The influence of different vegetation types on soil development on surface mines may be a reflection of physiological differences that affect microbial development in the rhizosphere. Such differences include levels of carbohydrate translocated to the root system and/or released into the surrounding soil; the plant's effectiveness as a mycorrhizal host; and the rate of degradation of plant residues. It has become apparent that microbial interactions are an important part of plant and soil processes in reclamation. While some of the microorganisms important in plant growth and soil development can be introduced readily by management practices, the majority usually are disseminated by natural means and only gradually become a part of the microbial population. More research is needed on developing new methods or refining current procedures for early introduction of these microorganisms in reclamation practices.

## INTRODUCTION

Microorganisms have long been known as important factors in surface-mine reclamation in the Eastern United States. The significance of microbial processes, particularly those of bacterial and fungal symbionts, was early recognized in the reforestation of mine spoils and in the pioneering of woody species on anthracite wastes.<sup>(1,2)</sup> Following the enactment of recent laws, herbaceous vegetation has been used increasingly in reclamation and, in fact, is beneficial in the surface stabilization of graded minesoils. Concomitant with the use of a greater variety of plant species, there has been an increase in the diversity of microorganisms. Many of these have been identified with numerous physiological activities important in soil development as well as plant interactions considered essential for the survival and growth of most plant species on surface-mined lands.<sup>(3-6)</sup>

The establishment and rate of development of microorganisms associated with plant and soil processes in minesoils are greatly influenced by the composition of the materials used in forming the planting medium and the availability of biodegradable organic matter. The following discussion covers various aspects of topsoiling and revegetation practices in the Eastern United States and their influence on the establishment and interrelationships of microorganisms with plant and soil processes during reclamation. In this paper, the term "topsoil" is considered synonymous with the zone of organic accumulation in the native soil.

## TOPSOILING

The process of removing topsoil from areas to be surface mined adversely affects the soil ecosystem, whereby the organization of microbial communities and

physical integrity of native soils are severely disrupted. Regulations state that "all topsoil shall be removed as a separate layer from the area to be disturbed, and segregated. Where the topsoil is of insufficient quantity or poor quality for sustaining vegetation, the materials approved by the regulatory authority...shall be removed as a separate layer from the area to be disturbed, and segregated."<sup>(7)</sup>

The primary benefit of using topsoil is to increase the availability of beneficial microorganisms and to improve the quality of plant growth medium on areas where the spoils or minesoils are chemically or physically less desirable than the native soils.<sup>(6)</sup> The physical structure of topsoil may aid in decreasing runoff and evaporation, and result in increased surface moisture compared to spoil materials.<sup>(8)</sup> However, in the mountainous parts of the Appalachian Coal Province, there is evidence that substitute materials sometimes are equal to or better than the highly leached and severely eroded surface materials commonly used as topsoil.<sup>(9-11)</sup> Microorganisms can be introduced into spoils by management practices as well as by natural processes. Where topsoil does not occur in sufficient quantities to cover the mined area, mixing the thin surface layer of soil with a suitable topsoil substitute material will serve to introduce microorganisms into the biologically deficient overburden material.

It is important to realize that microbial activity is greatly influenced by available oxygen and moisture. With increased time of storage, both oxygen and moisture decrease, and there is a concomitant loss in organic matter and soil structure, and a reduction in the microbial population, including viable spores of vesicular-arbuscular endomycorrhizal (VAM) fungi.<sup>(12,13)</sup> Therefore, the

storage of topsoil in deep, narrow piles for periods greater than a few months reduces the functional capacity of these organisms.<sup>(14)</sup> Shallow, wide piles are preferable if topsoil must be stored; however, the best practice is the direct haul method which eliminates stockpiling, saves costly rehandling, and helps maintain an active microbial population.

## VEGETATION

Shafer<sup>(15)</sup> has suggested that while the parent material exerts an overriding influence on soil properties early in soil development, the selection of vegetation species may offer an opportunity to alter or modify soil development on surface mines. The establishment of vegetation is known to improve the structural properties of most soils, particularly the formation and stabilization of soil aggregates. Aggregate stability is one of the most significant soil properties influencing surface erosion;<sup>(16)</sup> on minesoils it is known to vary with the vegetation types.<sup>(17)</sup> In most instances, annual and perennial grasses are used to achieve a quick ground cover to help reduce water runoff and soil erosion.<sup>(6)</sup> The extensive fibrous root system of the grasses not only presents a physical barrier to soil erosion but also releases organic materials that function either directly in aggregating soil particles (i.e., rhizosheath)<sup>(18)</sup> or indirectly as carbon and energy sources during microbial synthesis of mucilaginous polysaccharide soil-binding agents. During active vegetative growth, both annual and perennial plants appear to be equally effective in providing nutrients for microbial activities leading to aggregate formation, though aggregate stability is more characteristically observed under perennials.<sup>(19)</sup>

Physiological properties that differ in some grasses may quantitatively or

qualitatively influence the development of soil structure. Plants with the C-4 photosynthetic pathway are used in revegetation reclamation in some parts of the Eastern United States. Among other characteristics, C-4 plants such as Bermudagrass (*Cynodon dactylon*) have a rapid rate of photosynthesis and a high growth rate, and are thought to translocate more of their photosynthate (carbohydrates) to the roots and exude more of the carbohydrates to the surrounding rhizosphere.<sup>(20)</sup> Of the bacterial species known to be capable of polysaccharide formation, most are found in the rhizosphere; the greater abundance of carbohydrates in the rhizosphere of C-4 plants would result in an obvious advantage. Also, since vesicular-arbuscular mycorrhizal fungi derive most of their energy directly from plant roots, C-4 host plants may have a greater influence than other hosts on the physiological response of the fungal endophyte. In addition to the use of seed mixtures, various application and management practices in topsoiling may influence vegetation diversity,<sup>(21)</sup> particularly where locally adapted native species are compatible with the reclamation plan.<sup>(22)</sup>

#### ORGANIC AMENDMENTS

Various types of mulching materials are used on minesoils to help control erosion until plant growth is established. In conjunction with their known physical benefits, many of the woody residues -- freshly prepared whole tree chips, shredded bark, hay, straw -- are the source of water-soluble organic materials that percolate into the planting media and are readily utilized by soil microorganisms.<sup>(23)</sup> In most instances, hay and straw mulches undergo a relatively rapid decomposition by cellulose degrading fungi. These organisms commonly are found in minesoils where such organic amendments have been

used.<sup>(5)</sup> The characteristic mucilaginous material on the hyphal surfaces of most decomposers has been observed to bind minesoil particles and may be important in the early phases of surface stabilization as a result of improved aggregation.

Woody residues used as mulch are perhaps of greater significance as a long-term reservoir for lignin. Plant lignins represent an essential source of aromatic compounds for humus formation<sup>(24)</sup> and, as suggested by Kirk<sup>(25)</sup>, the structural differences of lignins in different plants may to a great extent influence their degradation potential. An obvious inference can be made that the resultant compounds released after degradation may differ in available coupling sites in the formation of soil humic acid molecules. In addition to plant lignins, a number of soil fungi are known to synthesize lignin-like compounds that are involved in humus formation.<sup>(24)</sup> It has been shown that *Penicillium* species isolated from a sandy loam minesoil can produce such compounds in culture, and undoubtedly are contributing factors in soil formation on the site.

Of the organic amendments commonly used, wood chips and bark are two of the more resistant to microbial degradation; they are generally decomposed primarily by the basidiomycete fungi (Figure No 1). Under natural conditions, many of these fungi (including ectomycorrhizal fungi) produce and excrete substantial quantities of organic acids as part of their normal metabolic activity.<sup>(26)</sup> Oxalic acid is one of the organic acids that may accumulate in large amounts as the oxalate salt of calcium, which is important in both the geochemical and plant nutrient cycles.<sup>(27)</sup> One of these basidiomycetes was observed on the surface and interior of hardwood chips and bark fragments collected from mulched

plots. In both instances, copious quantities of the white crystalline material attached to the mycelial strands was confirmed by X-ray diffraction as the weddellite (dihydrate) form of calcium oxalate (Figure No. 2). The small, relatively insoluble crystals may percolate into the rhizosphere where they are either decomposed by microorganisms, or undergo dissolution where vigorous root growth has reduced calcium levels in the soil solution. Under these conditions the calcium that is released may function in plant metabolism, while the chelating effect of the oxalate anion may effectively prevent the precipitation of iron and the removal of phosphate from solution.<sup>(27)</sup> While both microbial and plant activities undoubtedly were operative in the degradation of the oxalate crystals, only the bacterial features have been confirmed. It has been shown that large number of **Streptomyces** species isolated from the rhizosphere soil can degrade both calcium and potassium oxalates. Preliminary evidence that might indicate the complexing of iron by oxalate under these conditions was observed in samples of 4-mm granular aggregates collected from the rhizosphere soil. Within the aggregates, the surfaces of siliceous soil particles were found to be remarkably free of iron deposits compared with particles in the nonrhizosphere soil.

#### MICROBIAL FUNCTIONS

In some instances, microbial functions known to be important in agronomic soils also are those readily recognized as significant in plant and soil development on mined lands. Nitrogen-fixation represents one of these essential functions, and is associated primarily with **Rhizobium**, the bacterial symbiont of both woody and herbaceous legumes used on these sites. This symbiotic organism has a well-established role in the accumulation of nitrogen and subsequent soil building processes in minesoils.<sup>(6,3,28)</sup> These organisms are most readily

introduced by seed inoculation, though they may be present in topsoils previously used in legume crop production.

Other nitrogen-fixing bacteria that are introduced primarily by topsoil additions include **Azotobacter**, **Azospirillum**, and **Bacillus polymyxa**, all of which are free living or nonsymbiotic but are usually found in close association with the plant root system. These organisms are found infrequently in agronomic soils and are thought to contribute little to the total nitrogen economy of such soils; however, when present in minesoils they may be much more significant, particularly when nitrogen is a limiting factor. Due to their relatively short life span, the nitrogen incorporated by these organisms is available to the N pool within days or weeks after fixation as opposed to a much greater time period for symbiotic N-fixation.<sup>(29)</sup> Also, the polysaccharide capsules of **Azotobacter** and **Bacillus** (Figure No 2) may function in soil aggregation. Phytotoxic substances produced by herbaceous species growing in minesoils may influence the physiological activity of these rhizosphere organisms. However, no adverse effects on the growth of **Azospirillum** were observed when phenolic compounds were incorporated in a culture medium at levels greater than those produced by herbaceous species.

As noted for the nitrogen-fixing symbionts, mycorrhizal fungi may well be considered one of the essential microorganisms involved in minesoil revegetation.<sup>(30,31)</sup> Mycorrhizae are specialized plant roots formed as a result of a mutualistic symbiosis between certain soil fungi and compatible host plants. Endomycorrhizae are produced primarily by various genera of the VAM fungi (Endogonaceae). Vesicles are the fungal storage organs and may be either intercellular or intracellular. The arbuscles are highly branched projections



of the intracellular fungal hyphae and are believed to be the sites for reciprocal exchange of mineral nutrients translocated by the fungus and photosynthates produced by the plant. Although endomycorrhizae are physiologically distinct, they differ little in appearance from nonmycorrhizal roots. Most of the grass and legume species, as well as many of the hardwood tree species used for minesoil reclamation in the Eastern United States, are hosts for the vesicular-arbuscular mycorrhizal fungi. Ectomycorrhizae are produced by a great number of basidiomycete and, to a lesser extent, ascomycete fungi, commonly referred to as "mushrooms." In contrast to the endomycorrhizae noted previously, ectomycorrhizae formed on trees such as conifers and oaks are both physiologically and morphologically distinct. They are covered with an external mantle of fungal hyphae which penetrates the epidermal layer and forms intercellular growth (Hartig net) in the cortex tissue. Again, the internal hyphae represent the exchange sites of nutrients and carbon compounds between the fungus and the plant.

In addition to their well-established role in plant interactions, VAM fungi are involved in many beneficial soil reactions,<sup>(32,33)</sup> including the binding of soil particles into stable aggregates.<sup>(34,35)</sup> As part of a separate study characterizing minesoil properties, Fugill and Sencindiver<sup>(36)</sup> determined the percentage of water-stable aggregates in surface samples of a predominantly coarse sandy loam minesoil. They observed that individual aggregates comparable to those obtained from a leguminous host survived the slaking and agitation procedure as small clusters of sand grains held tenaciously by anastomosing hyphal strands. Because their soil analyses indicated the binding mechanism associated with clay was, for the most part, lacking in the minesoil, they suggested that fungal aggregation could have had an affect on the formation of

water-stable aggregates.

Other than through the redistribution of topsoil, there is currently no practical method available for introducing VAM fungi into large acreages of herbaceous vegetation on minesoils. Where economically feasible, inoculated soil plugs and dry soil pellets incorporating spores of the VAM fungi<sup>(37,38)</sup> can be used for limited operations such as critical area stabilization.

#### FIELD PLANTINGS

Nursery-grown seedlings of commercially important forest trees such as sweetgum (*Liquidambar styraciflua*), ash sp. (*Fraxinus*), tulip poplar (*Liriodendron tulipifera*), eastern cottonwood (*Populus deltoides*), and American sycamore (*Platanus occidentalis*) that are frequently used in afforestation of surface mine sites are hosts for the VAM fungi. In many instances, the nursery stocks are lacking or have low levels of the endophyte. The use of highly effective VAM host cover crops in rotation with tree seedling crops has been suggested as a method increasing the inoculum potential of nursery soils for tree seedlings.<sup>(39)</sup>

*Telephora terrestris* is the most commonly found ectomycorrhizal associate on nursery seedlings used for reforestation throughout the United States and in many other parts of the world.<sup>(40)</sup> It is highly beneficial to seedlings planted on routine sites, but is not well adapted to the harsh soil conditions normally encountered on abandoned mined lands.

During the past several years, the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia, has conducted extensive research on *Pisolithus tinctorius* (P.t.), and its application to forest tree

nurseries and field forestation. Significant research results leading to the practical application of **P.t.** to nursery seedling production, field forestation, and mine land reclamation have been obtained. Data from plots in Alabama, Kentucky, Tennessee, and Virginia with Virginia pine, loblolly pine (**Pinus taeda**), pitch pine (**Pinus rigida**), shortleaf pine (**Pinus echinata**), and pitch x loblolly pine hybrid showed that survival of all **P.t.**-treated pine seedlings was not significantly different. Average seedling survival was approximately 10 percent greater, height growth was increased by 56 percent, and stem diameter was increased by 66 percent when **P.t.** seedlings were compared with **T. terrestris** seedlings (Table 1).<sup>(12)</sup>

During the spring of 1981, a research application and demonstration project was jointly undertaken by the Office of Surface Mining, the States of Ohio and Indiana, and the USDA Forest Service. The project was designed to evaluate the potential benefits of using **P.t.**-inoculated red (**Pinus resinosa**), Virginia (**Pinus virginiana**), and eastern white (**Pinus strobus**) pine tree seedlings in abandoned mine reclamation. Specific objectives of the project were to demonstrate the required nursery procedures for producing inoculated seedlings in quantity and to monitor their survival and growth on abandoned mine sites.

As shown in Table 2, the survival of the **P.t.**-inoculated seedlings on all experimental plots was significantly greater than that of the control seedlings. Overall, the **P.t.**-inoculated seedlings planted in 1981 had 9 percent better survival than the control seedlings; those planted in 1983 had 33 percent better survival. The most probable cause for higher mortality in the 1981 plots was the absence of significant precipitation during the 15 days following planting. These differences in survival are expected to widen with time.

## SUMMARY

For many years, reclamation efforts were concerned primarily with plant species adaptation. In recent years, reclamation requirements have been more concerned with establishment of a thriving plant cover for site stabilization and erosion control. Many of the plant-microbial relationships have been identified that are beneficial in helping to accomplish this. One of the more significant of these relationships is the symbiotic nitrogen-fixing activity of legumes, for which a bacterial inoculum is commercially available and can be introduced with established procedures. Mulches and soil amendments also support or stimulate the growth of microorganisms involved in minesoil stabilization. However, these beneficial organisms usually are disseminated by natural means, and only gradually become a part of the microbial population. Practical methods are needed for the early introduction of these organisms. Such methods may include the composting of woody residues with basidiomycetes known to be important in geochemical and plant nutrient cycles and with cellulose decomposers effective in soil aggregation. More research is needed in developing or refining current methods used for introducing endomycorrhizal associates of herbaceous and woody species. Most of the asymbiotic nitrogen-fixing bacteria can be cultured readily and applied by techniques such as hydroseeding. In direct seeding, polysaccharide polymers of these nitrogen-fixing and other bacteria may be suitable as the encapsulating material for introducing fungal associates of small-seeded host tree species. Many of the commercial forest tree species used are ectomycorrhizal hosts. With proper nursery management practices, tree seedlings for outplanting can be produced with adequate levels of indigenous or introduced associates. The only commercial inoculum available is *P.t.*, which is best suited for adverse sites or abandoned mine lands. It has become

increasingly apparent that microbial interactions are an integral part of plant and soil processes in reclamation. Application of these suggestions may increase the effectiveness of these microorganisms for early establishment of vegetation and soil development in reclamation.

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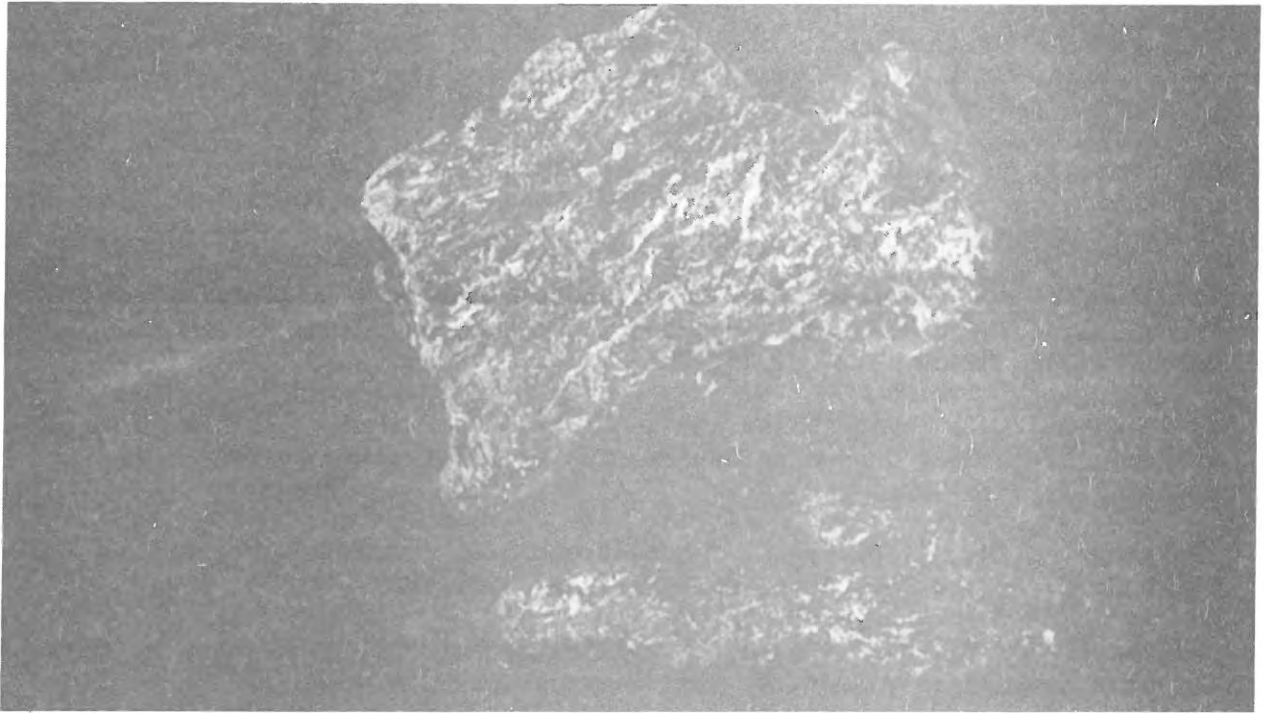
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**A**



**B**

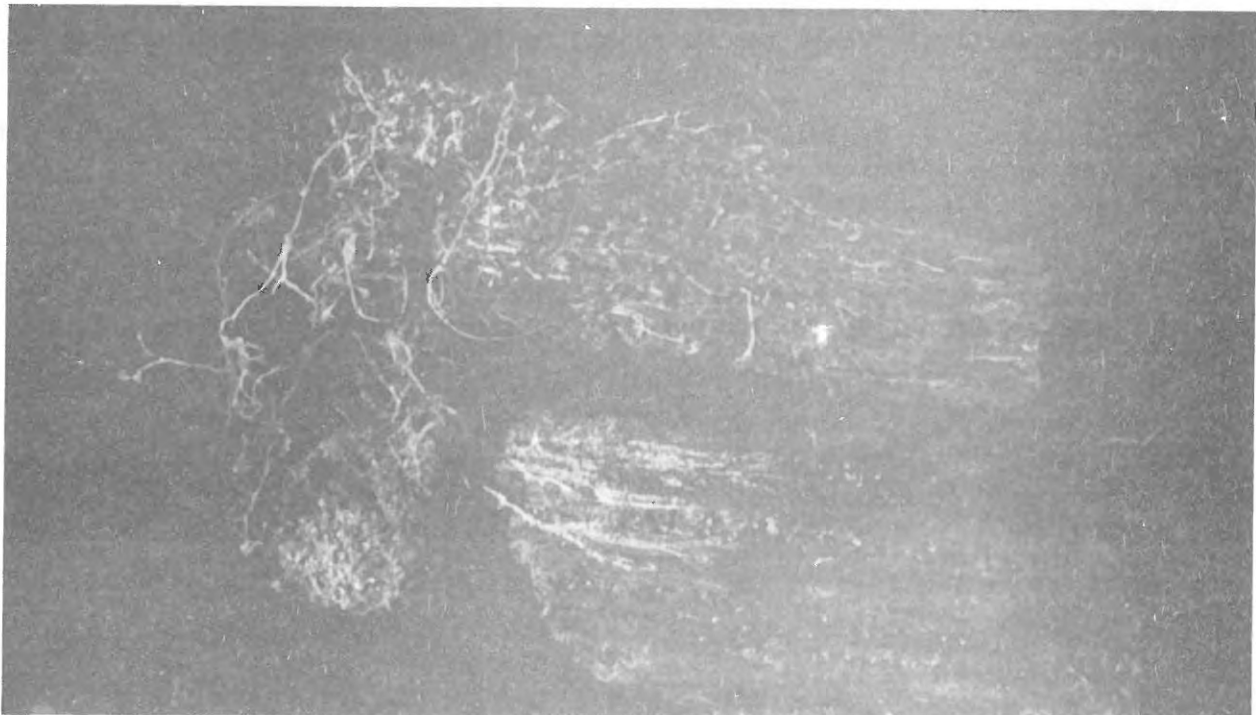


Figure 1. Crystals of calcium oxalate associated with growth of a basidiomycete on bark (A) and wood chips (B) taken from mulched plots.

A



B

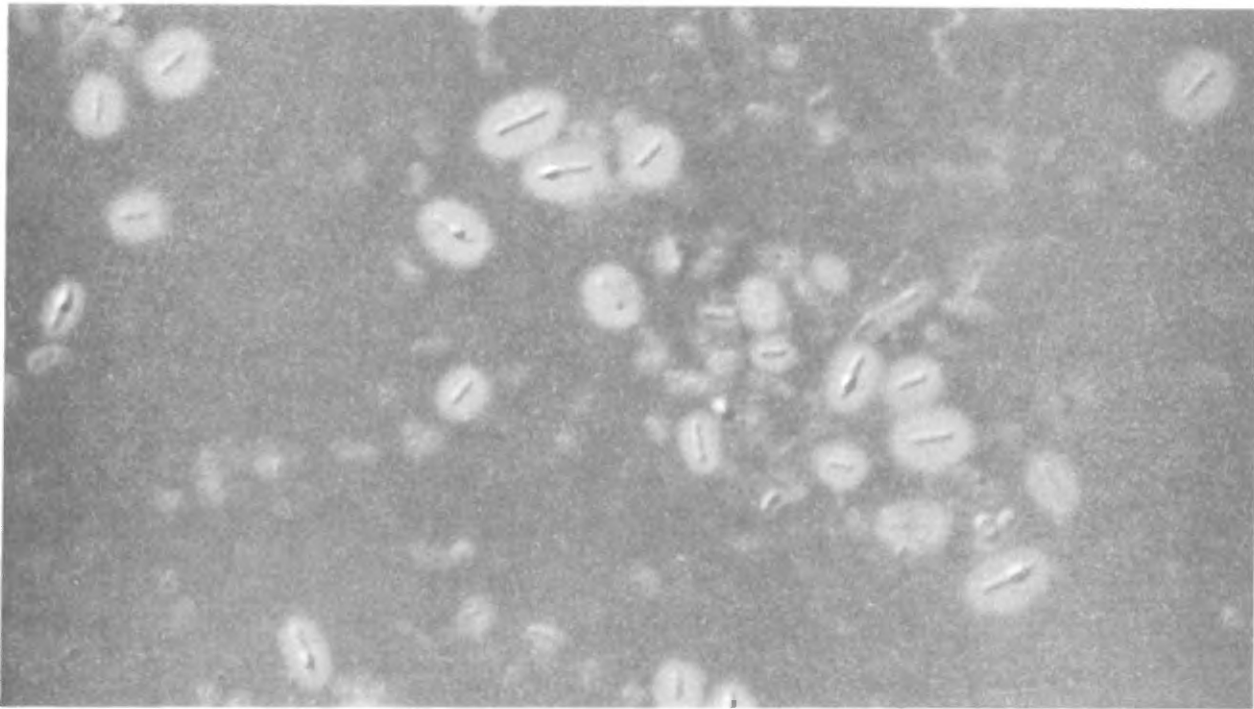


Figure 2. A. Crystals of the weddellite form of calcium oxalate adhering to hypha of the basidiomycete growth shown in plate 1.

B. Negative stain view of polysaccharide capsules of *Bacillus polymyxa*.

Table 1. Performance of *Pisolithus tinctorius* (P.t.) Treated and Untreated Pine Seedlings Planted on Abandoned Coal Minesoils

State	Tree Species	Mycorrhizal Condition at Planting	% Surv.	Height (cm)	Stem Dia. (cm)	Date Estab.	Date Sampled
KY	Virginia Pine	P.t.	49	49	1.6	1973	1975
		Control	1	19	0.4	1973	1975
KY	Loblolly Pine	P.t.	80	145	3.84	1974	1977
		Control	75	83	2.15	1974	1977
KY	Shortleaf Pine	P.t.	64	106	3.08	1974	1977
		Control	61	65	1.82	1974	1977
VA	Loblolly Pine	P.t.	81	299	7.6	1974	1978
		Control	67	214	5.3	1974	1978
AL	Loblolly Pine	P.t.	79	76	2.9	1977	1980
		Control	73	51	1.7	1977	1980
AL	Pitch Pine	P.t.	55	55	2.8	1977	1980
		Control	45	38	1.7	1977	1980
AL	Pitch x Loblolly Hybrid pine	P.t.	82	85	3.3	1977	1980
		Control	70	53	1.85	1977	1980
TN	Loblolly Pine	P.t.	38	79	2.5	1977	1980
		Control	48	40	1.3	1977	1980
TN	Pitch Pine	P.t.	87	54	2.5	1977	1980
		Control	94	45	1.9	1977	1980
TN	Pitch x Loblolly Hybrid Pine	P.t.	90	84	3.4	1977	1980
		Control	79	55	2.1	1977	1980

Table 2. Survival of *Pisolithus tinctorius* (P.t.)-inoculated and Control Pine Seedlings on Experimental Plots, Southeastern Ohio, Fall 1983

Site	County	Pine Species	P.t.-inoculated	Control
			Percent Survival	
Little Kyger Creek	Gallia	Virginia <sup>a</sup>	60.0	45.6
		White <sup>b</sup>	80.0	57.6
Margaret Creek	Athens	Virginia <sup>a</sup>	52.8	45.6
		White <sup>b</sup>	68.0	29.6
Meigs No. 1	Meigs	Virginia <sup>b</sup>	84.0	17.6
		White <sup>b</sup>	58.4	35.2
Snowville	Meigs	Virginia <sup>a</sup>	41.6	37.6
Little Leading Creek	Meigs	White <sup>b</sup>	100.0	95.0

<sup>a</sup> Planted, Spring 1981

<sup>b</sup> Planted, Spring 1983