An Abstract of the Thesis of

Bryan Blackburn for the degree of Master of Science in Horticulture presented on June 5, 1992. Title: A Closed Root Environment for Plant Production

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Thesis research focused on: 1) Water and fertilizer gradients within the plant root zone, and effects of chemical and physical gradients on *Trichoderma harzianum* populations. 2) Regulation of root growth physically with permeable fabric containers and chemically with copper compounds. 3) Effects of copper coatings for fabric containers on *Glomus intraradix* vesicular-arbuscular mycorrhizal fungus and plant growth.

Notable results were: 1) Potassium and nitrate movement in the medium was slower than would occur by diffusion in water; therefore, movement can be explained by diffusion alone. Lack of visible water-stress symptoms, plant height, and shoot dry weights and fresh weights indicate that water flow rates were adequate to meet the demand of plant transpiration. Populations of *T. harzianum* increased with increasing concentrations of K^+ and NO_3^-, and populations were higher when a corn plant was present. 2) A nonwoven polypropylene fabric container physically prevented root penetration, and reduced, but did not eliminate root circling. A $Cu(OH)_2$-latex paint suspension applied to the fabric containers increased root density, and improved root distribution. Neither the fabric container nor the copper treatment had an adverse effect on plant growth. 3) Treating fabric root pouches with either copper treatment, Spinout or Texcide, significantly increased the percentage of corn root length colonized by the VA endomycorrhizal fungus *Glomus intraradix* without adversely affecting plant shoot growth.

Both copper treatments significantly increased the root density and root distribution in the pouches.

A Closed Root Environment For Plant Production

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A CLOSED ROOT ENVIRONMENT FOR PLANT PRODUCTION

Chapter 1

General Introduction

Environmental concerns about water shortages as well as groundwater contamination by nitrates and phosphates create a need for a container system which is water and fertilizer efficient. In the open, free-draining container, as pore size of growing media is increased to improve aeration, more water drains from the containers. As irrigation is increased, water-use efficiency decreases, and the amount of fertilizers in the leachate increases. The moisture content of the growing media fluctuates with the irrigation schedule and the weather; changes in water content causes the concentrations of dissolved salts, pH, and other factors in the root environment to change. Leaching and water fluctuations create an unstable root environment which may increase dependence on chemical pesticides--another source of pollution. A fluctuating root environment may affect the survival of beneficial microorganisms antagonistic to soilborne plant pathogens.

The Closed, Insulated Pallet System (CIPS) for plant production is an innovative system for growing plants in containers (Kaplan, 1992). The CIPS addresses the needs to reduce the quantities of water, fertilizers, and chemical pesticides used and to contain the root system.

An enclosed root environment with a water reservoir in the pallet base and nutrient supplies or reservoirs on the top surface of the growing media may reduce the quantities of water and fertilizer applied and discharged as waste (Figures 1.1 and 1.2). The pallet top and insulated collars seal around the plant stems to prevent water exchange by evaporation or precipitation. Upward movement of

water within the enclosed root zone is maintained by capillary flow and is directly related to plant uptake. Neither water nor fertilizer can leave the impermeable pallet base so the quantities applied can be reduced and discharge eliminated.

One hypothesis to be tested is that efficiency of water and fertilizer use can be increased without a reduction in plant growth by confining fertilizer movement within the media: The quantity of water required can be reduced by elimination of evaporative and leaching losses; capillary flow of water upward from the base reservoir will be adequate to meet plant requirements for transpiration and growth. Because leaching will be prevented, it should be possible to reduce the quantity of fertilizer applied and increase fertilizer-use efficiency. The rate of nutrient ion movement in the protected root zone will be similar to effective diffusion rates; rates of ion movement will not exceed the diffusion rates for these ions in water.

A second hypothesis is that dependence on chemical pesticides can be reduced by designing systems which provide a stable environment for beneficial, root-zone microorganisms. The inconsistent results of biocontrol agents in the past may have been due to fluctuating environmental conditions. Relatively stable gradients of water and nutrients in the CIPS create varied combinations of moisture/air ratios, pH, salinity and concentrations of specific ions. The gradients should result in unique biological niches. If a means of biological control could be incorporated into a plant production system which provides consistent, reliable results, dependence on chemical pesticides will be reduced.

We hypothesize that beneficial microorganisms can be established in these biological niches. By initially inoculating the root media uniformly with *Trichoderma harzianum*, and then periodically assaying horizontal strata of the growing media along

the established gradients for fertilizer ions and moisture, the research tests the hypothesis that *T. harzianum* establishment and maintenance will be greater in certain niches or combinations of physical, chemical and biological factors.

A third hypothesis is that regulation of root growth, either by physical or chemical means, is required to develop a dense, branched root system without root deformities such as circling roots. However, if root growth inhibition or physical root pruning occur at the wrong stage of plant growth, then there could be a reduction in plant growth or possibly plant death. The research tests the hypothesis that root growth can be regulated in the CIPS by physical containment or by chemical means without reduction in plant growth or quality. It is further hypothesized that the application of a chemical root control agent, in addition to not reducing plant growth, will not reduce mycorrhizae formed by the VA mycorrhizal fungus, *Glomus intraradix*.

A general review of relevant literature is presented in Chapter 2. The major research is presented in chapters 3, 4, and 5. Chapter 3 presents research related to establishment of water and fertilizer gradients and *Trichoderma harzianum* populations within a closed plant root zone. Chapter 4 covers research on physical and chemical control of roots. Research on the effect of copper on VA mycorrhizal colonization and plant growth is presented in Chapter 5.

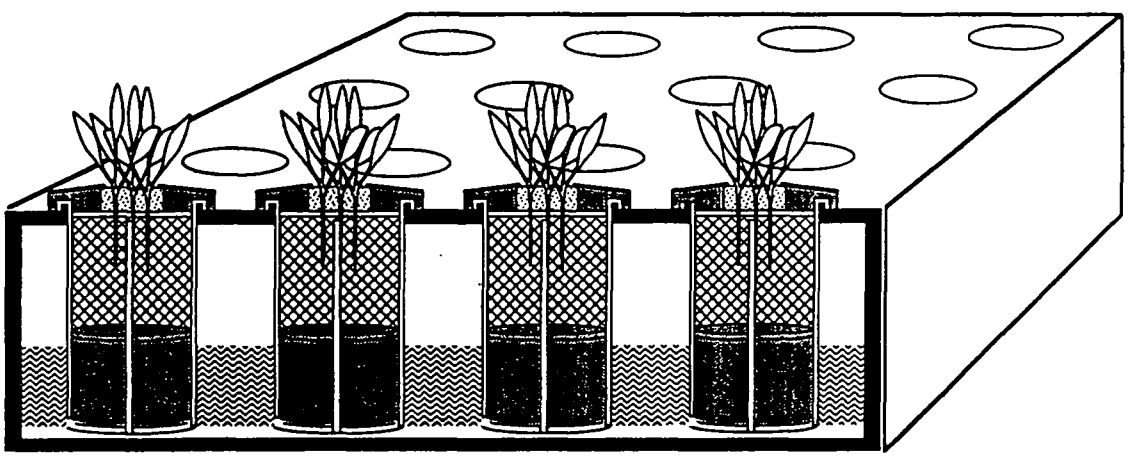

Figure 1.1. Cross-section of a Closed, Insulated Pallet System (CIPS) pallet showing an array of plant units. Plant shoots extend upward through a seal in the pallet top which is continuous, water-impermeable, light reflective, solar and thermal radiation opaque, and insulating.

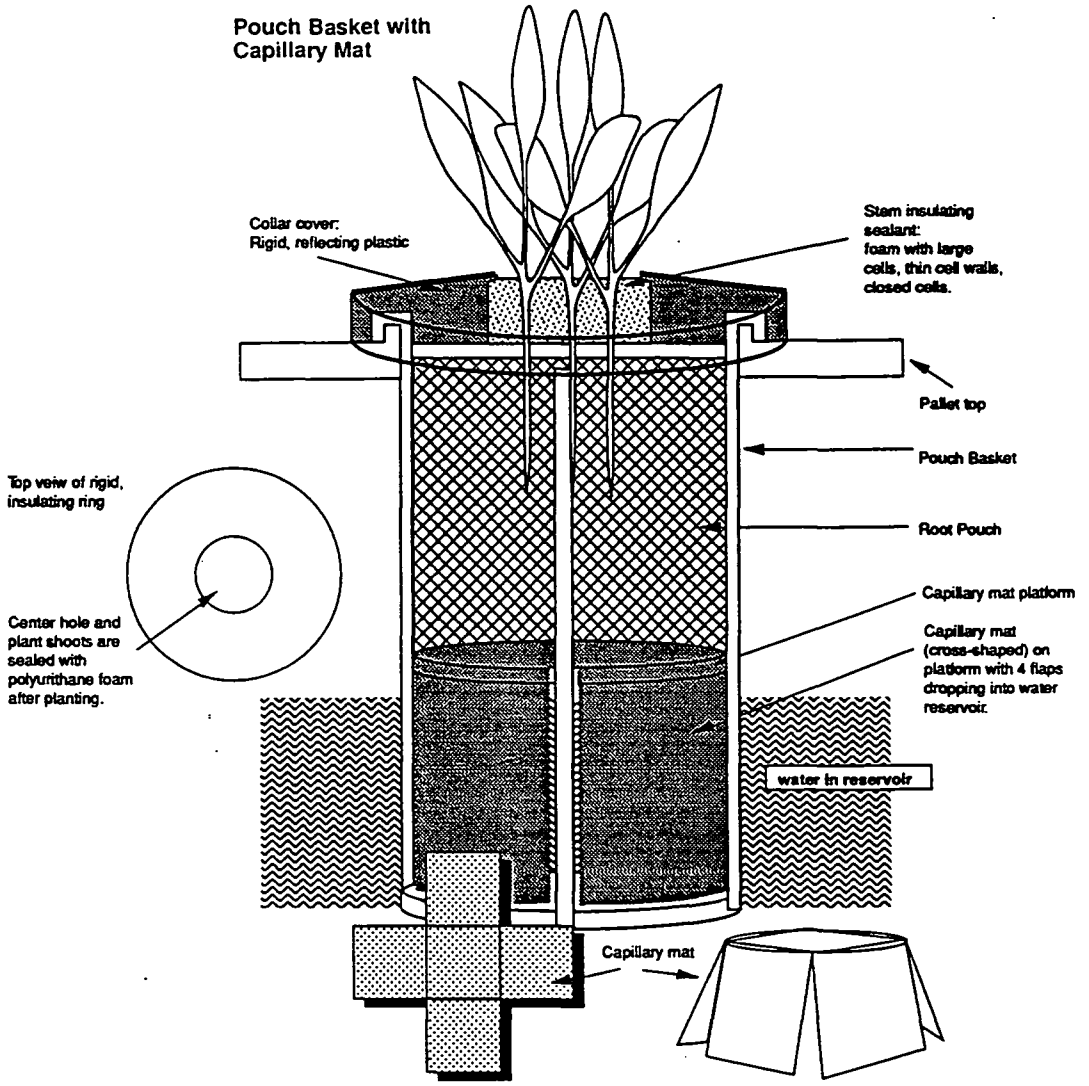

Figure 1.2. Diagram of a plant unit in the Closed, Insulated Pallet System (CIPS) showing the root pouch, pouch basket, plant-stem collar, and capillary wick.

Chapter 2

Review of the Literature

Water- and Fertilizer-Use Efficiency

Plant Available Water

The amount of water available to plants is determined by the volume of water present within a range of energy potentials at which plant uptake of water can occur, and the rate at which it can be transported to the plant. Water may be present, but if it is not within the energy potential range for plant uptake, it is not available to the plants. Accordingly, the energy potential of water has been used to classify water availability.

DeBoodt and Verdonck (1972) defined easily available water as the volume of water released between 10 and 50 cm H_2O (-0.001 and -0.005 MPa) water potentials, plant-available water as the volume of water released between 50 and 100 cm H_2O (-0.005 and -0.01 MPa), and difficultly available water as the amount released between 100 and 15,000 cm H_2O (-0.01 and -1.5 MPa).

Other researchers have related plant growth responses to plant water potentials. They found that different plants experience water stress at different water potentials. Maize leaves showed a temporary reduction in cell elongation at -0.02 MPa and an irreversible reduction in growth at -0.25 MPa (Acevedo et al., 1971). Havis (1980) measured stomatal resistance as an indicator of water stress in Cornus, Magnolia, Rhododendron, and Viburnum plants, and found that increased stomatal resistance occurred at potentials between -0.025 and -0.039 MPa. When wax leaf privet was grown in three soils amended with different organic materials, only soils amended with 60% peat contained enough water between -0.03 and -0.05

MPa during the intervals between surface irrigations to meet plant needs (Richards et al., 1964). Thus watering was recommended when the soil water potential reached -0.03 MPa to avoid water stress. The threshold for water stress may be less negative for herbaceous plants. *Phaseolus vulgaris* plants began to wilt at a potential of -0.01 MPa (Juncher and Madison, 1967).

Water availability is related to the water potentials of the plant and to the water potentials of the soil. However, these potentials are measurements taken at different points on a continuum. Water moves from the soil through the plant to the atmosphere. The amount and rate of water uptake required by the plant depends on weather conditions, plant growth stage, the amount of water in the soil, and the properties of the soil which affect movement of water.

With intermittent irrigation, the volume of water retained after gravitational drainage (container capacity) is finite and may be exhausted between irrigations. With intermittent irrigation, the plant-available water decreases between irrigations and the availability of water at the end of the irrigation cycle becomes the determining factor for plant growth (Karlovich and Fonteno, 1986). However, with continuous subirrigation, the volume of plant available water at any given time does not fluctuate widely and is not as critical as the capillary flow rate or rate of replenishment in response to plant uptake.

Applying the concept of water availability to the CIPS, it should be possible to manipulate the texture and structure of a media to provide a continuous flow of water to the plant within the -0.001 to -0.005 MPa range (10 to 50 cm H_2O). In coarse peat media, approximately 80% of the volume of water is held between -0.001 and -0.01 MPa (Puustjarvi and Robertson, 1975). These values should apply

to coarse, peat-based horticultural media as well. If available water is replaced at a rate greater than or equal to the rate of plant uptake, water stress will not occur.

Critical values for air-filled porosity derived from experiments in open, surface-irrigated media may not be applicable to root matrix conditions in the continuously subirrigated CIPS. The air-filled porosity of a media is inversely proportional to the amount of water contained in its pore space (Spomer, 1975; Bugbee and Frink, 1986). In open surface-irrigated containers, air space in the media can be increased by increasing the average pore size. But, because water drains more readily from the larger pores, and less plant-available water is retained. Optimal aeration values for free-standing containers represent a compromise between the optimal air-filled porosity for the plants and the volume of water available for plant growth.

Bugbee and Frink (1986) defined air-filled porosity as the amount of air in media after free water had drained, but before evaporation had occurred. They reported optimal plant growth when media air space was from 11% to 20% of the pore volume. In their research, plants were watered when tensiometers measured potentials equivalent to -0.03 MPa; so, aeration increased from the value measured at container capacity until water was added to the media again. Optimal plant growth in the rigid-walled containers probably occurred at higher aeration values.

The method for determining air space may produce values which are not truly reflective of the average conditions plants experience. Root pouches in CIPS sit on capillary mats; stable water content and aeration gradients are maintained in the root zone. As long as the capillary flow rate can maintain the amount of water needed by the plant, pore size and air space can be increased without decreasing the amount of water available to the plant.

The oxygen diffusion rate for a media is better correlated to plant growth than its air-filled porosity (Paul and Lee, 1976). The porous fabric root pouches permit air movement through the walls as well as the tops of the pouches. The continuous oxygen diffusion through short distances reduces the air-filled pore space required for optimal root growth.

Nutrient Movement

To reduce the quantity of fertilizer applied and to increase the efficiency of fertilizer use by the plant, several areas need to be addressed . Areas of concern are fertilizer application efficiency, movement of nutrients inside the container and movement of nutrients out of the containers.

Traditional, intensive plant-production systems such as greenhouse plant production, nursery production of container-grown plants, and intensive field production of nursery plants involve inefficient application of large quantities of water, fertilizer, pesticides, labor, and energy to a relatively small production area. For example, in nursery production of container-grown plants, 110 acre-inches of irrigation water may be applied annually during a nursery growing season. If plants are fertigated (application of fertilizer through the overhead sprinkler irrigation system) with 200 ppm N at each irrigation, then application of 1 acre-inch of fertigation water is equal to 43 lbs N per acre. If 110 acre-inches are applied annually, 4,730 lbs N are applied per acre per year (Whitcomb, 1984). Overhead applications of water and fertilizer to widely spaced containers are extremely inefficient. If containers are spaced in the production area on a center-to-center spacing equal to twice the container diameter, containers cover only 20% of the production bed, and can only intercept up to 20% of the overhead-applied materials . At any time in a container nursery,

approximately 75% of the containers are widely spaced and 25% are closely spaced, edge-to-edge.

Direct application of fertilizer to the container media is markedly more efficient than fertigation or broadcast applications. Even so, annual applications of incorporated and topdressed fertilizer to container-grown viburnum and taxus range from 1,036 to 1,459 lbs N/acre and 45-96 lbs P/acre (Taylor et al., 1983). In production of container-grown, herbaceous perennials, 630 lbs N/acre and 275 lbs P/acre are applied annually (Taylor et al., 1990). Annual applications of 1,560 lbs N/acre and 340 lbs P/acre are used in the production of cut and potted chrysanthemums (Waters and Conover, 1969). In comparison, only 100-350 lbs N/acre are applied annually in production of field-grown, agronomic crops.

The loss between containers is eliminated with surface applied, micro-irrigation (trickle or drip irrigation) combined with fertilizer application directly to the container media (incorporated, topdressed, or fertigated). However, with the reduced volume of irrigation water applied per acre and consequent reduced volume of leachate discharged from each container, the concentration of nutrients in the discharge is higher. The quantity of chemicals lost in leachate from surface-applied irrigation and from flood-and-drain irrigation is more directly related to the quantities of soluble chemicals within the container, such as nitrate, than to the volume of effluent, i.e. rate of leaching (Rathier and Frink, 1989).

In many container culture systems, operators leach away the excess nutrients; about 40% to 60% of fertigation nutrients are leached from flood-and-drain rockwool cultures (Dutch greenhouses) in an effort to maintain a desired nutrient/water balance (van Noordwijk, 1990).

Nutrients move through the media to the roots in three ways: by root contact, mass flow, and diffusion. Root contact is influenced

by fertilizer placement, the rate of growth of new roots, and by mycorrhizal associations. Mass flow, with its advective component, refers to the transport of ions in the flow of water. Water moves to the root in response to plant transpiration. Diffusion is the movement of ions from regions of higher chemical potential to those where the chemical potential is lower.

When the supply of a particular ion to the root by interception and by mass flow does not equal or exceed the amount absorbed by the root, a concentration gradient (diffusion gradient) is established around the root. The rate of diffusion is affected by the magnitude of the chemical diffusion gradient, the volumetric water content of the media, the tortuosity of the diffusion path, and the chemical and physical properties of the solid phase. Diffusion coefficients (D_l) in water and effective diffusion coefficients (D_e) of specific ions in soil reported by Barber (1974) are given in Table 2.1.

The volumetric water content of the media affects the rate of ion movement. As the number of water-filled pores decreases, the total cross-sectional area of water in the pores decreases, the total area available for diffusion decreases, and the paths from the soil to the plant root become less direct, more tortuous. Mengel and Kirkby (1982) suggest that restricted nutrient mobility rather than plant-available water may be the factor limiting growth during dry periods.

In the CIPS, an enclosed root zone, noncirculating system with plant-driven uptake of fertilizer ions and water, losses from evaporation, leaching, and misapplication would be prevented. Fertilizer is applied directly to the top surface of the root-matrix media, and water moves by capillarity through the media from the reservoir. The system is sealed; water losses from evaporation from the media and leaching are precluded. Diffusion should be the main force by which nutrients move. If the nutrients are applied in forms

with solubilities that keep pace with plant needs, or if the physical-chemical components of the growing media (pore size/water content, buffering capacity, length of the diffusion pathway, etc.) are manipulated, nutrients may be contained within the root matrix. Essentially 100% could be available to the plant.

There is evidence that specialization of root functions occurs; some roots primarily absorb water while other roots primarily absorb nutrients. In a split-root system, tomato roots preferentially took up water from the root compartment containing solution with low EC and nutrients from the compartment containing a high EC solution (Sonneveld and Voogt, 1990).

When roots grow into a high concentration of nutrients, the roots adjust to the osmotic potential of the soil solution. Duncan and Ohlrogge (1958) banded phosphorus fertilizer along rows of corn plants in the field. As corn roots grew into the band, the tips of the main roots were killed, but lateral roots developed and grew into the band unharmed.

Based on this evidence, specialization of root functions will probably occur in the nutrient gradients of the CIPS as well. Roots growing into or near the fertilizer compartments would become osmotically adjusted and serve primarily to take up nutrients by direct contact or interception. (Growth of roots into an area of concentrated salts is not comparable to sudden immersion of the whole root system into a highly saline environment.) Roots in the lower strata of the root media in the CIPS where the concentration of nutrients is low would specialize in water uptake.

Other Systems for Production of Container-Grown Plants

Commercial plant production systems usually include some means to alleviate plant stress from temperature, water, and/or nutrients outside the optimum range for plant growth. Production systems, to

some degree, control water and nutrient application and shoot and root environments. Neither overhead irrigation nor trickle irrigation address the problem of fluctuating conditions in the root zone caused by evaporation or the problem of groundwater pollution. Evaporation from an open container decreases water use efficiency, and causes fluctuation in the root zone temperature, salt concentrations, moisture levels, and pH. Plant physiological stress associated with fluctuating root zone factors could predispose plants to disease. With increasing concern about environmental pollution, some growing systems contain, treat and/or recycle effluent to prevent fertilizers and/or pesticides from contaminating surface water and groundwater.

Regardless of water conservation measures taken, such as more efficient sprinkler heads (Smucker, 1985), individual trickle emitters (Ross, 1988), or computer-controlled fertigation systems (Whitesides, 1989), the water and nutrient supply systems are not controlled by plant demand. In open systems, where water and fertilizer leach out of the bottom of the pot, environmental pollution is likely.

The potential for overhead irrigation to contaminate the groundwater with effluent depends on fertilizer application rates and on impermeability of collection and storage facilities. Trickle irrigation can reduce the volume of leachate, but does not necessarily reduce the absolute quantity of nutrients in the leachate.

Aeroponics, hydroponics, nutrient film technique, and ebb and flow methods can be more efficient means of applying water compared to surface application. The major loss of applied water is through plant transpiration; however, if the top surface of the root zone is open, evaporative losses will also occur. Collection and recirculation of leachate prevents groundwater and surface water

contamination, but increases the potential for spread of root pathogens. If application of water and nutrients is continuous, root zone factors will be stable. If application is intermittent, then pH, ion concentrations, aeration, etc. will fluctuate. In liquid systems such as hydroponics or aeroponics without a solid growth media, there is little chemical buffering, and rhizosphere conditions may fluctuate widely.

Subirrigation is efficient. However, there is still a large surface area for evaporation and growth of algae and weeds. Perforated black polyethylene covering the surface of capillary beds and standing grounds reduces much of the evaporation from the bed surface, but not from the open tops of containers. Subirrigation of open containers does not address evaporation, root zone stability, nutrient loss from leaching, or containment of roots.

The concept of establishing a relatively constant water-nutrient gradient in the plant root zone by placement of soluble fertilizer at the soil bed surface in conjunction with subirrigation to establish a vertical gradient in the root zone has been evaluated by Geraldson (1970, 1972, 1973, 1977, 1980). The water-nutrient gradient in the field beds was protected by a full-bed plastic mulch. However, even with a mulch cover, the gradient is vulnerable to fluctuating water due to change in water table height associated with periodic rain or variations in irrigation management. With time, the surface applied fertilizer nutrients move downward in this open system to the water table (Geraldson, 1990).

Geraldson (1990) has attempted to integrate the water-nutrient gradient concept into plant production in containers (a "containerized" gradient). An individual plant is planted into a hole toward one end of the top of a plastic bag (35 cm long x 15 cm wide by 17.5 cm high) containing 15 liters of peat-based media. Soluble fertilizer has been placed in a vertical section at the end

of the bag opposite the plant. Drip irrigation is applied through the planting hole. An overflow, drainage hole is located on the sidewall of the bag. With this container system, water moves downward from the top-placed irrigation dripper, and collects in the bottom of the bag below the drainage hole. The water in the bottom of the bag creates a water table from which water moves upward and throughout the bag by capillarity. Excess solution containing dissolved fertilizer overflows as waste discharge from the sidewall drain opening. No data were presented to confirm establishment and maintenance of a horizontal water-nutrient gradient over time from one end to the other of the planting bag. Quantitative budgets delineating fate of applied fertilizer over time in this open system were not reported. Root growth and proliferation in relation to the water-nutrient gradients was not reported.

No plant production system has alleviated all sources of plant stress. Some have incorporated computers to control water and fertilizer inputs; although efficiency has been improved, none of the systems are controlled by the plant.

Beneficial Microorganisms in the CIPS

The potential for soilborne disease outbreaks within the CIPS was a concern. The impracticality of applying soil drenches after the pallet is sealed and/or after root disease occurs necessitates effective methods of prevention before plants are enclosed in the CIPS.

Biological controls for root pathogens would provide non-polluting, disease protection in the enclosed root matrix of the CIPS. Although biocontrol agents may be applied singly, a mixture of beneficial microorganisms would fill more biological niches and provide a wider spectrum of plant protection than a single organism.

Within the root matrix in the CIPS, water, nutrient, and pH gradients exist which may provide biological niches for a range of antagonists.

Two beneficial microorganisms were selected for study. The literature was reviewed to assess their potential for survival in the CIPS root matrix.

Vesicular-Arbuscular Mycorrhizae

Glomus intraradix, a vesicular-arbuscular (VA) mycorrhizal fungus is a logical choice as a component of the biotic community to be established in the CIPS root matrix. VA mycorrhizae may increase plant nutrient uptake, primarily nutrients present in low concentration or with low diffusion rates such as phosphorus and some trace elements (Cooper, 1984; Linderman, 1988). Extraradical VA mycorrhizal hyphae extend the zone of nutrient uptake around plant roots, and increase the effective soil volume that plants can exploit. Mycorrhizal plants may have a more efficient nutrient uptake system compared to nonmycorrhizal plants; the extended nutrient-uptake network could intercept fertilizers applied to the top surface and diffusing down through the growing media, and decrease the potential for ions to move into the reservoir water below the CIPS root matrix. A more efficient uptake system would allow smaller amounts of fertilizer to be applied.

The advantages of mycorrhizae may not be apparent for plants grown in the CIPS, i.e. mycorrhizae may not increase plant growth if nutrients are not limiting (Rhodes and Gerdemann, 1980). However, when plants are removed from CIPS, the improved nutrient-uptake potential of mycorrhizal plants and other physiological changes that affect tolerance to drought stress (Davies et al., 1992) and transplanting injury (Biermann and Linderman, 1983a; Menge et al., 1978a) would be advantageous.

The plant-fungus relationship is affected by factors such as nutrient levels, organic amendments, pH, and other microorganisms. Beneficial effects of mycorrhizae may not be apparent with fertilizer application rates used in greenhouse and nursery production. High nutrient levels, phosphorus in particular, have been reported to generally decrease VAM colonization and production of extraradical hyphae (Hayman and Mosse, 1971; Sanders, 1975; Menge et al., 1978b; Biermann and Linderman, 1983a and b). However, several papers have reported growth enhancement from mycorrhizae in high-phosphorus soils (Angle and Heckman 1986, Lamar and Davey 1988, Davis et al. 1984, Plenchette et al. 1983, Sylvia and Schenck 1983).

Organic amendments and organic matter in general have been associated with having detrimental effects on mycorrhizae (Angle and Heckman, 1986; Biermann and Linderman, 1983b; Menge et al. 1982). Yet mycorrhizal infections and growth enhancement can occur in organic media (Giovannetti and Avio, 1984). Addition of organic and inorganic materials could influence mycorrhizae indirectly by changing the availability of heavy metals, concentration of available phosphorus, and/or microbial populations.

Many researchers have studied the effects of pH on VAM, reporting varying results (Abbott and Robson 1984; Johnson et al. 1984; Angle and Heckman 1986; Danielson and Visser 1989; El-Kherbawy et al. 1989). Germination of VAM spores are affected by pH (Green et al. 1976; Daniels and Trappe 1980; Porter 1982; Hepper 1984), as well as hyphal growth (Mosse and Hepper 1975; Porter 1982), and formation of mycorrhizae (Mosse and Hepper 1975; Davis et al. 1983).

Several studies provide evidence, direct and indirect, that soil microorganisms influence mycorrhizal development (Sutton and Sheppard, 1976; Ames et al., 1989; Mayo et al., 1986; Wilkinson et al., 1989.). These findings suggest that when a mycorrhizal fungus is separated from its naturally associated "helper" microorganisms or

placed in an artificial environment where function of these helper organisms is impaired, the fungal symbiosis with its host will be adversely affected. Also, from the results of Wilkinson et al. (1989), naturally associated organisms which inhibit ercoid mycorrhizal growth need to be removed while maintaining the presence of helper organisms. VA mycorrhizal fungi would probably benefit from similar treatment.

Trichoderma harzianum

The nonparasitic fungus, *Trichoderma harzianum* Rifai, offers a biological means of disease control (Papavizas, 1985; Chet and Elad, 1982) which could be incorporated into the CIPS root-media matrix. Application of *T. harzianum* has reduced the incidence of disease caused by *Rhizoctonia solani* and *Sclerotium rolfsii* (Chet and Elad, 1982), the take-all fungus *Gaeumannomyces graminis* var. *tritici* (Ghisalberti et al., 1990), *Phytophthora cactorum* (Roiger and Jeffers, 1991), and a *Pythium* sp. (Lifshitz et al., 1986).

Several mechanisms by which *T. harzianum* biologically controls plant pathogens have been reported: mycoparasitism (Chet and Elad, 1980; Laing and Deacon, 1991), competition for nutrients in exudates (Sivan and Chet, 1989), and antibiosis (Lifshitz et al., 1986). One mechanism does not preclude the existence of the others. Mechanisms of control may be different against different pathogens (Lifshitz et al., 1986), or mechanisms may differ between isolates of the fungus (Ghisalberti et al., 1990). Ghisalberti and Sivasithamparam (1991) identified four chemically distinct types of *T. harzianum*, noting production of volatile and nonvolatile compounds.

Incorporation of *T. harzianum* into the root matrix may increase mycorrhiza formation. Two *T. harzianum* isolates stimulated production of vegetative spores by *Glomus mosseae* (Calvet et al., 1989) *in vitro*.

Trichoderma is relatively tolerant of a wide range of conditions. *Trichoderma* is normally considered to be acidophilic; but, in a very wet, alkaline (pH 8.2) environment, *Trichoderma* species abounded, representing 29% of the total fungal populations (Pugh and Van Emden, 1969). *T. harzianum* was more effective against *Pythium* at pH 3.7 than at pH 5.3 (Harman and Taylor, 1988); yet, *T. harzianum* was still effective against *Pythium* at pH 7.3 (Lifshitz et al., 1986). The optimal temperature range for growth of *T. harzianum* is 15 to 21C (Eastburn and Butler, 1991; Knudsen and Bin, 1990). An isolate of *T. harzianum* was reported to tolerate a wide range of soil salinities, effectively reducing disease incidence at 1, 3, and 5 mS/cm soil EC (Park and Kim, 1989). Some isolates capable of withstanding high osmotic shock have been applied to soil with liquid fertilizer (J.M. Kraft and G.C. Papavizas, unpublished data, p.36 in Papavizas, 1985). *Trichoderma* spp. are also tolerant of many chemical compounds (methyl bromide, carbon disulfide, sodium azide) and can be integrated into pest management programs (Papavizas, 1985).

Populations of *Trichoderma* (added as inoculum) increased when added to the root zone with a bran food base, but did not increase without being added in intimate contact with a food base (Lewis and Papavizas, 1984). A 10% amendment of wheat bran to autoclaved soil significantly increased the populations of *Trichoderma harzianum* and *Pseudomonas cepacia* and decreased growth of *Phytophthora capsici* (Nam et al., 1988). Similar results were obtained by Chet and Elad (1982) who reported control of damping off disease when *T. harzianum* was applied in the form of wheat bran culture to soil infested with *Rhizoctonia solani* and *Sclerotium rolfsii*. Baker et al. (1986) reported a 270% increase in growth of petunias in soils supplemented with a peat-bran culture of *T. harzianum* strain T-95. They found performance was optimum when T-95 mycelium associated with peat-bran

culture media was added to soil at 10^5 to 10^6 cfu/g soil. Application of conidial preparations to soil was ineffective in preventing disease or in stimulating population increases in colony-forming units of the biocontrol (Lewis and Papavizas, 1985; Lewis et al., 1990).

Lewis and Papavizas (1984), added *T. harzianum* to soil as a mycelial preparation (sterile bran-sand-water media inoculated with conidia and allowed to incubate 1-3 days before addition to soil), and the population increased to 10^6 cfu/g soil by week 3. Populations did not increase when mycelium without any food base was added to soil.

It may be necessary to add inoculum to soil in the form of mycelial preparations in contact with inoculum substrate to enhance the activity of biocontrol agents other than those of the genus *Trichoderma*. The substantial increases in populations of *Gliocladium*, *Talaramyces*, and *Aspergillus* resulting from additions of mycelial inoculum with nutrients (Lewis and Papavizas, 1984) suggest the feasibility of using this approach to increase numbers and activity of a wide range of potential biocontrols.

Root Control

Prolonged feeder root destruction or massive feeder root losses at one time may severely affect a plant's ability to absorb nutrients and water to support plant growth and to produce food reserves. Reduction of these reserves causes starvation and quick or slow decline (Campbell et al., 1974). Reserves are needed for periodic seasonal demands such as leaf and fruit formation. Maggs (1964, 1965) found that pruning half of the root system of young apple trees in summer reduced growth of all parts except the root; leaf growth was disproportionately reduced. If pruning was carried out in autumn, stem growth was increased. Root growth is synchronized with

the morphological stage of the shoot. In the vegetative phase, shoot and root growth proceeds concurrently in a linear fashion. With the advent of flowering and fruiting, however, root growth slows or ceases due to shortage of photosynthates from the shoot.

When plants potted in traditional rigid-walled plastic containers were grown in the CIPS, plant roots grew out of the drainage holes into the capillary wicks (both capillary mats and rockwool pedestals) and reservoir. To be able to remove a plant from a pallet without injury requires a means of keeping the roots within the containers. A review of the literature revealed chemical but not physical means of containing roots.

Physical Containment of Roots

Woven growbags or fabric containers are permeable to air and water, desirable traits for containers used in CIPS. However, fabric bags do not prevent root penetration (Ingram et al. 1987, Chong et al. 1989, James 1987, Wilson 1986). Chong et al. (1989) found that roots of poplar cuttings were girdled where the roots penetrated the fabric bags; translocation of nitrogen into the bags and starch and soluble sugars to roots outside of the bags was restricted. Where the roots penetrated the fabric bags, roots were swollen on either side of the fabric bags. The roots outside the bags were "considerably smaller" than roots inside the bags (Chong et al., 1987). Interference with translocation of nutrients and photosynthates, as well as failure to contain plant roots, make fabric root pouches as reported in the literature unsuitable for use in CIPS.

Chemical Root Control

Use of copper-treated containers in forestry nurseries to control root growth was reported by Saul, (1968). Saul (1968)

covered the bottoms of trays used to hold tree seedling tubes with copper-containing paint or sheets of copper metal or copper-coated fabric. Copper sheets and copper-treated fabric "more or less" confined the roots within the tubes for four species: red pine (*Pinus resinosa*), white pine (*Pinus strobus* L.), white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* (Mill) BSP). Copper naphthenate painted on the bottoms of planting trays reduced the lengths of primary roots of Jeffrey pine (*Pinus jeffreyi*), eucalyptus (*Eucalyptus polyanthemos*), and mesquite (*Prosopis tamarugo*) seedlings (Nussbaum, 1969). Compared to the control, seedlings grown in treated trays had more secondary laterals per unit of primary root length. Copper sulfate also effectively inhibited root growth (Furuta et al. 1972, Pellett et al. 1980). Phytotoxicity symptoms due to uptake of excess copper were not reported for any of the copper treatments.

The copper-treated containers eliminated the need for mechanically pruning the roots without killing the root tips (Arnold and Struve, 1989a; Burdett and Martin, 1982; Arnold and Young, 1991). Coating the inside of containers with cupric carbonate in a latex paint carrier prevented root circling by inhibiting growth at root tips. Higher order laterals then proliferated and in turn stopped growing when they came in contact with the treated container wall (Burdett 1978, Burdett and Martin 1982, McDonald et al. 1984). Inhibited root tips were not killed, but resumed elongation rates similar to non-treated roots within three to six days after the treated container was removed (Arnold and Struve 1989a). Root systems of seedlings grown in treated containers had a greater number of total roots (Wenny and Woollen, 1989; Arnold and Young, 1991) and more higher-order lateral roots (Arnold and Struve, 1989a) compared to seedlings grown in untreated containers. Struve and Rhodus (1990) reported the use of copper hydroxide as a root inhibitor, and

indicated that it worked "even better than cupric carbonate, especially on vigorous-rooted species like forsythia."

The altered root morphology may have beneficial effects on root growth, shoot growth, and survival after transplanting. After transplanting from copper-treated containers, lodgepole pine (*Pinus contorta* Dougl.) developed root systems similar in form to naturally established seedling root systems (Burdett, 1978). For one-year-old green ash and two-year-old red oak seedlings transplanted from copper-treated containers, dry weights of roots beyond the original root ball were greater than root weights of seedlings with roots pruned immediately before being transplanted from untreated containers. Trees transplanted from $CuCO_3$-treated containers had greater shoot growth and fewer symptoms of stress than controls (Arnold and Struve, 1989b). Similar increases in shoot growth were reported for apple seedlings transplanted from copper-treated containers (Arnold and Young, 1991), but not for ponderosa pine (*Pinus ponderosa* Laws. var. *ponderosa* Engelm.), western white pine (*Pinus monticola* Dougl.), or Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) (Wenny, 1988).

Under some conditions, copper-treated containers may have toxic effects on plant growth. Burdett and Martin (1982) grew 10 species of tree seedlings in containers treated with 0, 100, or 500 $g \cdot liter^{-1}$ cupric carbonate in latex paint. Results varied with species, container size, growing media, and concentration of $CuCO_3$. At 500 $g \cdot liter^{-1}$, shoot heights and root dry weights were reduced. The high concentration was lethal to most of the Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tested. Wenny and Woollen (1989) reported that 30 to 300 $g \cdot liter^{-1}$ $CuCO_3$ in latex paint had no phytotoxic effects on shoots, but effects on total length of new roots varied with concentration, containers, and tree species. Rooted cotoneaster (*Cotoneaster divaricata* Rehd. & Wils.) cuttings were wrapped in jute

burlap treated with solutions of 0.05% to 4.0% actual Cu (w/w) as $CuSO_4$ (Kuhns and Sydnor, 1975). Burlap treated with concentrations of 0.2% or greater Cu as $CuSO_4$ was phytotoxic and resulted in decreased root growth. In addition, stress resulted in plant death. The effects of copper may be related to the solubility of the compound (Kuhns and Sydnor, 1975; Wenny and Woollen, 1989), as well as other factors which affect bioavailability such as the amount of organic matter in the growing media (Graham et al., 1986), the latex coating, and the pH of the media.

Copper is highly toxic to roots, but there is negligible translocation of copper from roots to shoots. Accumulation of large quantities of copper in plant roots with little translocation to the shoots is reported by Ishizuka (1942) and Struckmeyer et al. (1969). Symptoms of copper toxicity to roots are blackened root tips and darkening of the root system; numerous laterals appear on shortened roots. The level of copper that is toxic to plant roots varies with the growing media as well as with species or even varieties of plants. A concentration of less than 1.0 ppm Cu in solution is highly toxic to growing roots of most crops (Bennett, 1974).

Fabric root pouches treated with copper would provide several advantages for use in the CIPS. The fabric pouches are porous to air and water yet retain the growing media. Copper treatment would not kill root tips, but would result in plants with more fibrous root systems; plants grown in treated pouches would have an intact root system, and would not be subjected to as great a shock from transplanting as root-pruned plants grown in open containers or bare-root, field-grown plants.

Effect of Copper Compounds on Mycorrhizae

Several papers reported ectomycorrhizal fungus colonization of tree roots was increased by copper treatment. Short roots, roots which are not elongating rapidly, and have small, poorly differentiated steles, are primary sites of ectomycorrhizal colonization (Bogar and Smith, 1965; Wilcox, 1967). Greater numbers of short roots and greater ectomycorrhizal colonization occurred on ponderosa pine seedlings grown in copper-latex coated containers than on seedlings grown in untreated containers (McDonald et al., 1984). Likewise, the ectomycorrhizal fungus *Thelephora terrestris* produced more extraradical mycelium and mycelial strands on lodgepole pine when seedlings were planted in Cu-treated containers (Hunt, 1990). However, the effect of $CuCO_3$-latex-treated containers on colonization by the ectomycorrhizal fungus *Pisolithus tinctorius* was related to the specific host-tree species (Ruehle 1985). Similar responses are expected with endomycorrhizae.

Although no published reports on the effects of Cu-treated containers on vesicular-arbuscular mycorrhizae were found, there have been several studies on VAM in soils with toxic concentrations of heavy metals. Mycorrhizae decreased plant uptake of heavy metals (Koslowsky and Boerner, 1989) and decreased phytotoxic effects (Dueck et al., 1986). Decreased phytotoxicity may have resulted from improved phosphorus nutrition of mycorrhizal plants compared to nonmycorrhizal controls, thereby lowering the concentration of the metal in plant tissues by dilution because the mycorrhizal plants were larger. Graham et al. (1986) showed that in mycorrhizal and nonmycorrhizal Carrizo citrange seedlings of similar size and P-status, mycorrhizal seedlings were more sensitive to Cu toxicity than uninoculated seedlings. When 0 to 300 $\mu g \cdot g^{-1}$ copper sulfate was uniformly incorporated into soil, colonization by *Glomus intraradix* and seedling growth were reduced logarithmically with Cu

concentration. Copper concentrations of 19 and 34 $\mu g \cdot g^{-1}$ of soil were phytotoxic to inoculated and uninoculated seedlings, respectively.

The availability of copper in soils, and therefore its effect on VAM and on plants, is dependent upon the pH, CEC and organic matter. At a soil pH below 5.0, high soil concentrations of copper reduced VAM colonization and caused phytotoxicity symptoms on citrus, but when pH was near 7.0, tree productivity and mycorrhizae increased (Graham et al., 1986). Copper is tightly bound to cation exchange sites between pH 7.0 and 8.0 (Reuther and Labanauskas, 1966). High concentrations of copper are less toxic in organic media than in mineral soils since it is rapidly fixed by organic matter (Hesse, 1972; Kuhns and Sydnor, 1976).

Fabric root pouches are a component of an innovative, new plant production system, the Closed, Insulated Pallet System (CIPS). The CIPS provides an enclosed root environment with a water reservoir in the pallet base. Fabric pouches allow water movement and air exchange; however, a chemical treatment is necessary to prevent roots from penetrating some fabrics. A coating of $Cu(OH)_2$-latex paint prevented root penetration (Blackburn et al., 1992a), but the effect of Cu on VAM in fabric root pouches and organic potting media was unknown.

Table 2.1. Diffusion coefficients for diffusion of ions in water (D_l) and soils (D_e).

Ion	Diffusion Coefficient ($cm^2 s^{-1}$)	
	D_l Water, 25C	D_e Soils
$H_2PO_4^-$	0.89×10^{-5}	10^{-8} to 10^{-11}
K^+	1.98×10^{-5}	10^{-7} to 10^{-8}
NO_3^-	1.9×10^{-5}	10^{-6} to 10^{-7}

Adapted from Barber, 1974.

Chapter 3

Establishment of Water and Fertilizer Gradients and *Trichoderma harzianum* within a Closed Plant Root Zone

Additional index words. Diffusion, subirrigation, water capillary flow, biocontrol(s), potassium, nitrate, *Zea mays*, corn, production system, pollution, waste discharge, leachate, conservation, fertilizer-use efficiency, peat-vermiculite media.

Abstract

Vertical gradients of water, EC, and K^+ and NO_3^-, and acidity established in the root zone over 120 days. Movement of K^+ and NO_3^- in the media was slower than would occur by diffusion in water. *Trichoderma harzianum* populations were higher in media strata where higher K^+ and NO_3^- concentrations were higher. *T. harzianum* populations were higher and declined more slowly in the treatment containing corn plants than in the treatments without plants. In all treatments, *T. harzianum* populations remained greater than 10^5 cfu/g soil at Day 120.

Introduction

The Closed, Insulated Pallet System (CIPS) provides an enclosed root environment which offers the potential for complete containment of water and applied fertilizers. In the CIPS, shoots of container-grown plants extend upward through a seal in the pallet top which

results in a continuous, water-impermeable, light reflective, solar and thermal radiation opaque, insulating lid. The top in conjunction with the bottom tray or pallet form an enclosed root chamber.

Water movement upward from the water reservoir in the base of the pallet is by absorption into the pores of the wick and media and by capillary flow. After absorptive and capillary equilibria are achieved, further movement of water by capillary flow is in response to plant uptake to support plant growth and transpiration.

The water-impermeable pallet top prevents moisture from leaving as evaporation or from entering as free or gravitational water which would result in leaching. Fertilizer applied to the media surface immediately beneath the pallet top is protected from evaporative and gravitational movement of water. Fertilizer movement should be primarily by ion diffusion, not convection.

Research has been conducted on ion diffusion in mineral soils, but at lower water contents than that of the CIPS root matrix. Barber (1984) reported effective diffusion coefficients (D_e) of 10^{-7} to 10^{-8} cm^2/s for K^+ and 10^{-6} to 10^{-7} for NO_3^- in soils. When the water potential of soils are greater than -1.5 MPa, diffusion coefficients for salts will usually be greater than 10^{-7} cm^2/s (Olsen and Kemper, 1968).

In the CIPS, the volumetric water content of the media decreases with increasing height above the water surface; the rate of movement of NO_3^- and K^+ by diffusion should increase with increasing depth and volumetric water content. In water at 25 C, the diffusion coefficients are 1.98×10^{-5} and 1.9×10^{-5} cm^2/s for K^+ and NO_3^- respectively (Barber, 1984). Since the media at the bottom of the root matrix is near saturation, D_e of the bottom stratum may approach, but should not exceed, the D_l in water.

Disease control within the CIPS is another area of concern. Because of the impracticality of applying chemical treatments to the enclosed root media after the pallet is sealed, effective methods of prevention are needed. The nonparasitic fungus, *Trichoderma harzianum* Rifai, offers a biological means of disease control (Papavizas, 1985) which could be incorporated and maintained in the media.

Trichoderma appears to be relatively tolerant of a wide range of conditions. *Trichoderma* is normally considered to be acidophilic but it has been found in slightly alkaline environments (Pugh and Van Emden, 1969). In a study of microhabitat variables, changes in the fungal population could not be correlated to soil water content, electrical conductivity, or pH (Eastburn and Butler, 1988).

The objectives of this study were to determine if stable chemical and physical gradients are established, if nutrient movement can adequately be explained by diffusion alone, and if *Trichoderma harzianum* can persist in the CIPS environment.

METHODS AND MATERIALS

Experimental Design and Statistical Analysis:

Three treatments: 1) +Plant +Fertilizer, 2) -Plant +Fertilizer, and 3) -Plant -Fertilizer were established in CIPs and harvested on five dates at monthly intervals. Samples of media from four strata of each root pouch and one water sample from the individual reservoir of each pouch were collected.

Treatment and Day were primary factors in a complete random design replicated six times. Strata were treated as subplots. The treatments +Plant +Fertilizer and -Plant +Fertilizer were compared in a split-plot analysis of variance to determine the effect of a plant on the dependent variables. The effect of fertilizer was determined by comparing -Plant +Fertilizer and -Plant -Fertilizer treatments (SAS, 1987). Analytical values of samples collected at Day 1 to determine baseline values before water and fertilizer gradients established in CIPS are not included in the statistical analyses.

Setup:

CIPS Root Pouches: Three-liter fabric pouches were constructed from a light-weight composite of spunbonded and meltblown poly-propylene fibers, Kimberly Clark NW401. A permeable coating of a copper hydroxide-latex suspension (100 $g \cdot liter^{-1}$ Kocide 101 wettable powder in latex paint diluted 1:1 v/v with water) was applied to the containers to inhibit growth of root tips contacting the coated fabric thereby preventing root penetration and to promote higher order branching of the root system.

Container Media: Dolomite (3.0 $kg \cdot m^{-3}$) and gypsum (1.8 $kg \cdot m^{-3}$) were uniformly incorporated into a 1 peat : 1 vermiculite (v/v) media prior to uniform incorporation of *Trichoderma* inoculum.

Inoculation of root media: The horticultural media in all treatments, premoistened to 100% water content (by weight), was uniformly inoculated with the nonparasitic, beneficial fungus, *Trichoderma harzianum*. The isolate WT-6-6 was provided by G.C. Papavizas, Beltsville, MD. WT-6-6 is a biotype genetically modified by exposure to UV irradiation resulting in increased tolerance to benomyl and enhanced biocontrol capabilities (Papavizas, et. al., 1982)

Trichoderma harzianum inoculum was prepared by the procedures of Lewis and Papavizas (1984) with the following modifications: 1) Washed river sand was used. 2) A suspension of spores, mycelium, and agar (50 ml) was used to inoculate the wheat bran and sand; since both spores and mycelium were used, number of spores/ml were not counted. 3) The *Trichoderma* inoculum was partially dried at room temperature (23C), passed through a 2.8-mm sieve, then uniformly incorporated into the peat-vermiculite container media at a rate of 5.6 $kg \cdot m^{-3}$. The *Trichoderma* inoculum was incorporated five days prior to the initiation of the experiment, 16 Apr 1991.

Plant Material: Seeds of *Zea maize* cv. 'Golden Jubilee' were planted in 1:1 peat:vermiculite (v/v) on 3 Apr 1991. Plants were grown in a greenhouse under ambient light conditions, and watered as needed, but not fertilized. Corn seedlings were transplanted into fabric pouches 17 days later, 20 Apr 1991.

Establishment of Capillarity: After the corn was transplanted, the media in the pouches was saturated with water and allowed to drain for 24 hours.

CIPS: Pouches were set in the CIPS 21 Apr 1991. The pallets were modified so that each fabric pouch-reservoir was an independent experimental unit; each of the 16 reservoirs in a pallet was a 7.6-liter plastic container.

Fertilizer Treatments: Potassium nitrate (1.2 kgm^{-3} N, 24.3 g or 0.240 mol KNO_3/pouch) and single superphosphate [3 $Ca(H_2PO_4)_2$-H_2O + 7 $CaSO_4$-$2H_2O$ + 2 HF] (0.18 kgm^{-3} P, 7.26 g superphosphate/pouch) were applied in +Plant +Fertilizer and -Plant +Fertilizer treatments. The fertilizers for each pouch were divided equally among three 15 mm x 60 mm petri dishes without lids. Kimberly Clark NW401 fabric, without copper treatment, was secured to the open faces of the petri dishes by a rubber band to retain the solid fertilizer in the petri dishes. The petri dish-fabric containers (fertilizer compartments) were inverted and placed on the media surface at the perimeter of the root pouches away from the stems of the young seedlings--root tips were not immediately subjected to high salt concentrations. Three inverted petri dishes without fertilizer were placed on the media surface of the -Plant -Fertilizer treatment. Prior to chemical analyses of the media, the fertilizer compartments with the residual fertilizer were removed.

Irrigation: Water content of the peat-vermiculite mix in the fabric pouches was maintained by adsorption and capillary flow of water upward from the individual reservoirs through synthetic fiber capillary wicks (Troy Flowthru capillary mat, 153 gm^{-2} polyester, Troy Mills Inc., Troy, NH) to the permeable fabric pouches (Figure 3.1).

Data Collection:

The experiment was initiated 21 Apr 1991, in an Oregon State University greenhouse with no supplemental lighting. Six replicates of each treatment were harvested on Day 1 Day 30, Day 59, Day 88 and Day 120 respectively. The media in each pouch was divided into 4 strata: 0 to 2.5 cm, 2.5 to 5.0 cm, 5.0 to 10.0 cm, and 10.0 to 15.0 cm from the top surface. The samples were stored in plastic bags at 4C until analyzed. A 100-ml water sample was taken from each reservoir and frozen until analyzed. Media and water samples were

analyzed for K^+, NO_3^-, pH, EC and water content. *Trichoderma harzianum* populations were quantified in each media sample by dilution plate methods.

K^+, NO_3^-, pH and EC: Extractable potassium (K^+ on the exchange sites plus K^+ in solution) was determined using a modification of the neutral ammonium acetate extraction procedures of Knudsen et al. (1982) as described by the OSU Soil Testing Lab (Horneck et. al., 1989). Soil Testing Lab procedures were altered as follows: Moist samples were used to minimize the amount of exchangeable K fixed. Due to substantial differences between the densities of the horticultural potting mix and mineral soils, sample weight was reduced from the equivalent of 2 g to the equivalent of 1 g dry weight. Based on previous experiments, the fractional gravimetric water contents were initially estimated to be 3.00, 3.60, 3.98, and 4.90 g H_2O·g dry media, for Strata 1, 2, 3, and 4, respectively. Gravimetric water contents were later determined on separate samples; subsamples were oven-dried at 60C for 3 days. Analyses were corrected to their actual sample dry weight.

KCl-extractable NO_3^- was determined following the methods of Keeney and Nelson (1982) as modified by Horneck et al. (1989) on a 4-g sample of air-dried media. After water contents were determined from subsamples, deionized water was added to 80 to 200 grams of wet media to make a 1:5 media:water extract (w/w). EC and pH were measured from the extract. EC values in the individual reservoirs were measured using a Myron hand-held TDS meter. TDS (ppm) was converted to EC (mS/cm) by the equation, TDS (ppm) = 640 EC (mS/cm) (USDA Handbook No. 60, 1954)

Trichoderma Recovery: Immediately after harvesting samples, a 1:100 (w/w) potting mix:water dilution was made by adding moist media equivalent to 1 g dry weight to 100 g water. Serial dilutions (0.5 ml) of the potting media were plated onto *Trichoderma* Media E with

benomyl (Papavizas and Lumsden, 1982). Colony-forming units (cfu) were counted after incubating in the dark at room temperature for 6 days. Values were reported as cfu/g dry soil.

Water Use: Water in individual plant reservoirs was maintained at a constant level. Volumes of water added were recorded. The water use was calculated as the weight of the water at the end of the experiment subtracted from the sum of the initial amount of water added plus the amounts of water added during the experiment.

RESULTS AND DISCUSSION

The significant effects of surface-applied fertilizer and interactions among the experimental factors (+/- fertilizer, media strata, and time) on the measured parameters (K^+, NO_3^-, EC, pH, H_2O and *Trichoderma harzianum* populations as cfu/g dry media) are shown in Table 3.1. The additional effects of adding a plant with its water and fertilizer ion uptake and root exudates and significant interaction among plant, media strata, and elapsed days are shown in Table 3.2.

Chemical and Physical Gradients

K^+, NO_3^-, and EC gradients. Concentrations of K^+, NO_3^-, and EC were lower in the +Plant treatment than in the -Plant treatment (Figures 3.2, 3.3, and 3.4, respectively) ($P = 0.0001$). Values changed over time in each stratum (Figures 3.5, 3.6, and 3.7, respectively) (Day x Stratum $P = 0.0001$). K^+, NO_3^-, and EC decreased in stratum 1 and increased in the lower strata as the fertilizer in solution moved downward.

pH gradients. The pH in all strata of the growing media (-Plant -Fertilizer) initially decreased (Figure 3.8, comparison of Day 1 and Day 30), and then pH values increased with greatest increase occurring in stratum 4, then 3. The change in pH was apparently related to moisture content of the respective strata increasing the solubility of the uniformly incorporated dolomite.

The same trends were evident in the fertilizer treatment (Figure 3.9), but the pH of all strata of the media decreased more dramatically and was slower to increase than in the unfertilized treatment. The fertilizer diffusing from the fertilizer compartments on the top of stratum 1 interacted with the media, eg. K^+ ions

displaced H^+ ions from cation exchange sites, thereby increasing active H^+ ion concentration.

In the plant treatment (Figure 3.10), there was a general increase in pH with time in all strata. The increase in pH in strata 2 and 3 of the +Plant treatment (Figure 3.10) may have been due to plants releasing bicarbonate ions in these strata. Nye and Tinker (1977) reported that roots generally take up more anions than cations when nitrate-nitrogen is supplied and release bicarbonate ions to maintain electrical neutrality.

Water gradients in the media. In all treatments, a water content gradient was established with the greatest water content in the bottom stratum and least in the top stratum. Total water content in all strata increased with time (Figures 3.11 and 3.12). Increased water content with time was likely due to increased bulk density of the media as a result of media decomposition over time and gravitational settling. Water content in media strata 2 through 4 was greater with a plant than without (Figure 3.13). Expansion of the volume of the plant root system might have increased the density of the growing media in these strata, thereby increasing capillarity and water uptake. Higher initial water contents in the +Plant +Fertilizer treatment may have also resulted from the media being compacted during transplanting.

Moisture content of the media in strata 2, 3, and 4 was greater with a plant present than without a plant, indicating plant uptake of water did not exceed supply (Figure 3.13). There was no increase in water content of the media when fertilizer was surface-applied; after Day 30 (Figure 3.11), water content was higher in the media receiving no surface-applied fertilizer.

Nutrient Movement by Diffusion

The following equation, assuming simple initial and boundary conditions, is one solution of Fick's second law:

$$C_j = \frac{M_j}{2(\pi D_e t)^{1/2}} e^{-x^2/4D_e t} \quad [1]$$

Barber (1984) used the equation to explain diffusion in soils. The boundary conditions for the equation are: 1) There are no obstructions along the x-axis. 2) A finite amount of solute is initially placed (t = 0) in a plane at a point (X_0) on the x-axis. 3) The solute is allowed to diffuse along the x-axis in the plus or minus direction. 4) No additional solute is added at times t > 0. 5) The diffusion coefficient remains constant (Nobel, 1983). Conditions in this experiment do not meet the boundary conditions; nevertheless, it is used here to express the general relationship of the distance of K^+ and NO_3^- ion diffusion in a given time period. Effective diffusion coefficients for K^+ and NO_3^- were calculated (Tables 3.3 and 3.4) using the linear form of Eq. [1]:

$$\ln [C_j t^{1/2}] = [-1/4D_e][x^2/t] + \ln [M_j / 2(\pi D_e)^{1/2}] \quad [2]$$

The slope is $-1/4D_e$ and $D_e = -1/4m$. For conservation of mass, fertilizer applied to the system must remain in the system. When N and K are taken up by a plant, the nutrients are lost from the system. Therefore, no effective diffusion coefficients were calculated for the +Plant +Fertilizer treatment. No fertilizer was applied in a plane at x = 0 for the -Plant -Fertilizer treatment, so diffusion coefficients were not calculated for this treatment.

The effective diffusion coefficient, D_e, at a given volumetric water content and bulk density of soil is assumed to be constant. Since volumetric water content of media in the pouch increased with distance from the origin, to reflect differences in volumetric water, effective diffusion coefficients were calculated between strata 1 and 2, 2 and 3, and 3 and 4 (Table 3.7). A calculated D_e between strata 1 and 4 (Table 3.7) was approximately equal to the mean of the D_e between adjacent strata; therefore, the values in Table 3.7 are assumed to be a reasonable reflection of the rate nutrients move in different strata of the media. The calculated D_e follow expected patterns, increasing with increasing soil moisture, and do not exceed diffusion coefficients for K^+ and NO_3^- in water.

***Trichoderma* Populations in the CIPS.**

Populations of *Trichoderma harzianum* were higher in the +Plant treatment than in the -Plant treatment (Figure 3.14) ($P = 0.0073$), decreased over time (Figure 3.15) ($P = 0.0142$), and decreased from stratum 1 to stratum 4 (Figure 3.16) ($P = 0.0001$). In the +Plant treatment, cfu in stratum 1 remained stable from Day 30 until termination of the experiment at Day 120 (Figure 3.17). This stability may be due to the presence of plant root exudates and/or dead roots in stratum 1 providing a food base for the cellulolytic saprophyte.

The +Fertilizer treatment (Figure 3.18) tended to have higher cfu/g in strata 1 and 2 than the -Fertilizer treatment (Figure 3.19). However, cfu/g soil in the -Fertilizer treatment were somewhat higher in stratum 3 (1.07×10^6, -Fertilizer vs 6.99×10^5, +Fertilizer), and much higher in stratum 4 (1.89×10^7, -Fertilizer vs 4.73×10^5, +Fertilizer) than in the +Fertilizer treatment. We can offer no explanation for the higher population of *Trichoderma* in stratum 4 of the -Fertilizer treatment.

In the absence of plants, *Trichoderma* populations were negatively correlated with soil water; cfu decreased as water content increased (Table 3.8). In the presence of plants, *T. harzianum* populations were positively correlated with K^+ and NO_3^- concentrations and EC.

At the beginning of the experiment, we did not know if the copper hydroxide-latex coating on the fabric pouches might have an adverse effect on the *T. harzianum*. Papavizas (1985) reported *Trichoderma* to tolerate broad-spectrum biocides, but the effect of copper on the fungus was not mentioned. We noticed mycelium growing on the outside of the treated fabric pouches at Day 120. When we plated the mycelium on selective media, *Trichoderma* were recovered. *Trichoderma* populations in the peat:vermiculite media in the fabric pouches (treated with copper) in the CIPS were compared to populations in rigid plastic containers (not treated with copper) which were the TOSS controls on the bench (Table 3.9). Populations of the fungus were higher in CIPS.

Water and Fertilizer Use.

The water use of a corn plant presented in Table 3.10 is the average daily water use of six plants averaged over the period the plants were in the pallets, i.e. the average daily use during the 30, 59, 88, or 120 day period. Maximum water uptake per day was higher. The greatest 24-hour water use per plant in this experiment, 1.0 liter, occurred at Day 42. Mature corn plants in Kansas in July used 3.4 L $plant^{-1}$ day^{-1} (Miller and Coffman, 1918, as reported by Miller, 1938). Since presence of a plant in our research did not decrease the water content of the media (Figure 3.13) and plants did not wilt or show other signs of water stress, the capillary flow rates of water were probably not limiting.

An estimated budget for K^+ and NO_3^- based on specified assumptions was calculated (Tables 3.3-3.6). Distribution of K^+ changed with time (Table 3.4). The percentage of the applied K^+ in the plant shoot increased from 5% at Day 30 to 40% at Day 88 (plant maturity). Increased root uptake of K^+ was associated with less K^+ moving into the water reservoir when compared to the -Plant +Fertilizer data. In the +Plant treatments, only 10% of the applied K^+ had moved into the reservoir by Day 120 compared to 65% in the reservoir of the -Plant treatment. Note that in the +Plant treatment, K^+ did not move from the root media into the reservoir until after Day 88; after Day 88 (90 days to maturity for cv Golden Jubilee), uptake of water and interception/uptake of K^+ decreased with resultant K^+ movement downward to the reservoir. In the -Plant treatment, 27% of the surface-applied K^+ had moved downward to the reservoir by Day 59 and 65% had reached the reservoir by Day 120.

In the open, surface-irrigated container-grown plants, only 5% of the applied potassium remained in the media and 30% in the plant shoot at Day 120. Over 67% of the applied K^+ was unaccounted for at Day 120, and a large portion of that is assumed to have been lost in leachate. Less K^+ was intercepted/taken up by the plant and less K^+ was retained in the media in the open, surface-irrigated container compared with CIPS.

Likewise, distribution of NO_3^- in the plant shoot increased from 40% at Day 59 to 70% at Day 88 (maturity) (Table 3.6). This increase in N in the plant resulted from root interception and uptake of the ion with consequent reduction in the quantity of NO_3^- in the root media, and in the quantity of NO_3^- moving downward unintercepted to the water reservoir (48% of NO_3^- was the reservoir of the -Plant +Fertilizer treatment at Day 120 compared with 6.5% in the +Plant +Fertilizer treatment). In surface-irrigated, container-grown

plants, only 14% of the applied NO_3^- remained in the media, and only 52% in the plant shoots; 34% was unaccounted for and a large portion is assumed to have been lost in the leachate.

Conclusions

The Closed, Insulated Pallet System provides a protected root environment with stable growing conditions. The water content and the pH of the media increased slightly over time; but, overall, were stable. Although concentrations of nutrients decreased over time, concentration gradients persisted the duration of the experiment. Nutrient movement occurred at rates which increased with increasing water content, but the rates of movement did not exceed reported rates of diffusion of the respective ions in water. Nutrients may have moved by other means than diffusion, but such means were not detectable in this experiment. A high rate of a completely soluble fertilizer, KNO_3, (1.2 kgm^{-3} actual N) was used in this experiment to determine the rate of movement in a worst-case scenario. Diffusion is related to solution concentration which is related to the quantity and solubility of the salt. Use of slow-release fertilizers and/or lower rates of fertilizers may decrease the steepness of the gradient and slow nutrient movement.

Trichoderma harzianum populations persisted in the enclosed root environment of the CIPS for the duration of the experiment. Colony-forming units of the beneficial fungus decreased over time and with increasing depth and water content. Populations tended to increase with increasing concentrations of K^+ and NO_3^-. The populations of the fungus were higher when a corn plant was present. In all treatments, cfu of *Trichoderma* were greater than 10^5 at Day 120.

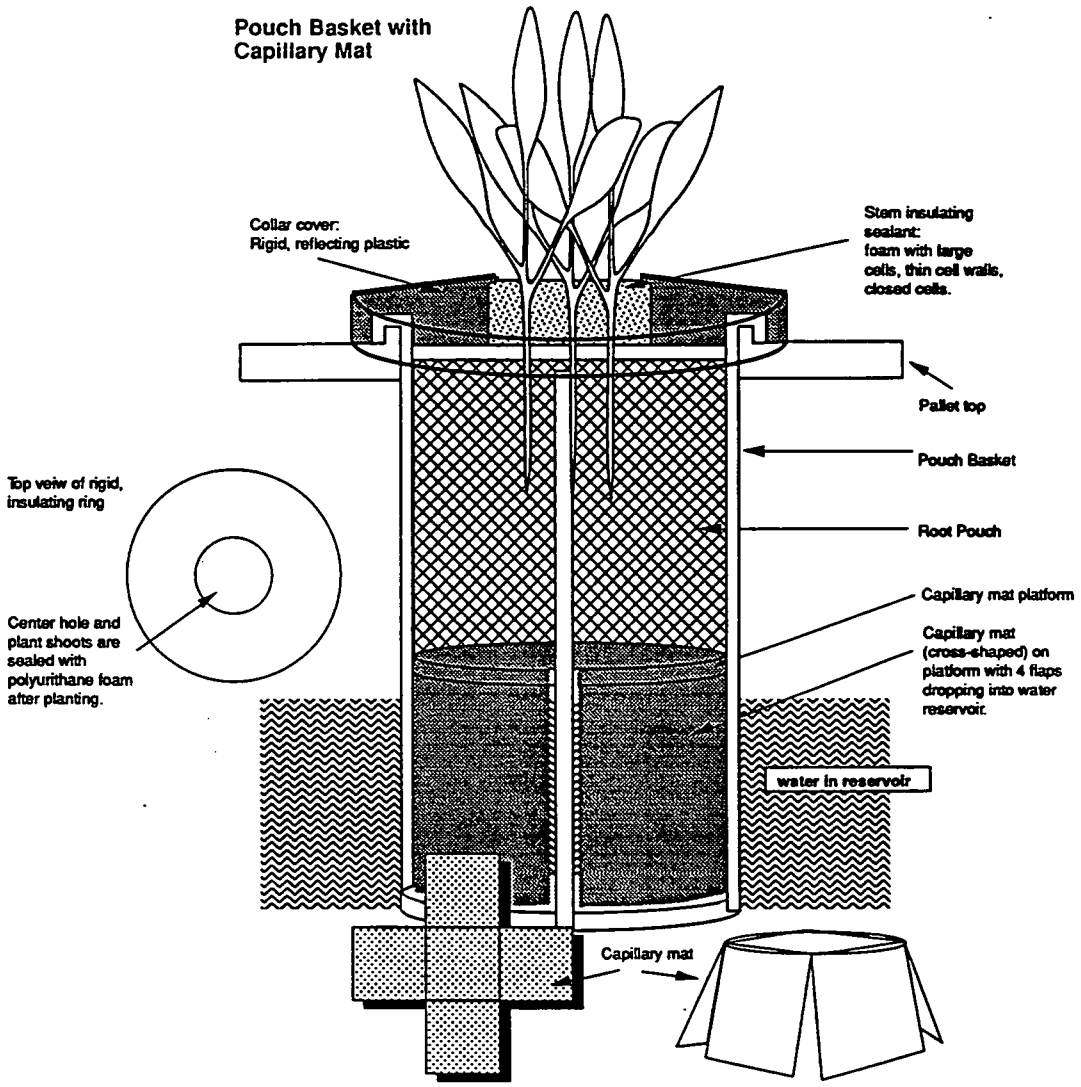

Figure 3.1. Diagram of a plant unit in the Closed, Insulated Pallet System (CIPS) showing the root pouch, pouch basket, plant-stem collar, and capillary wick.

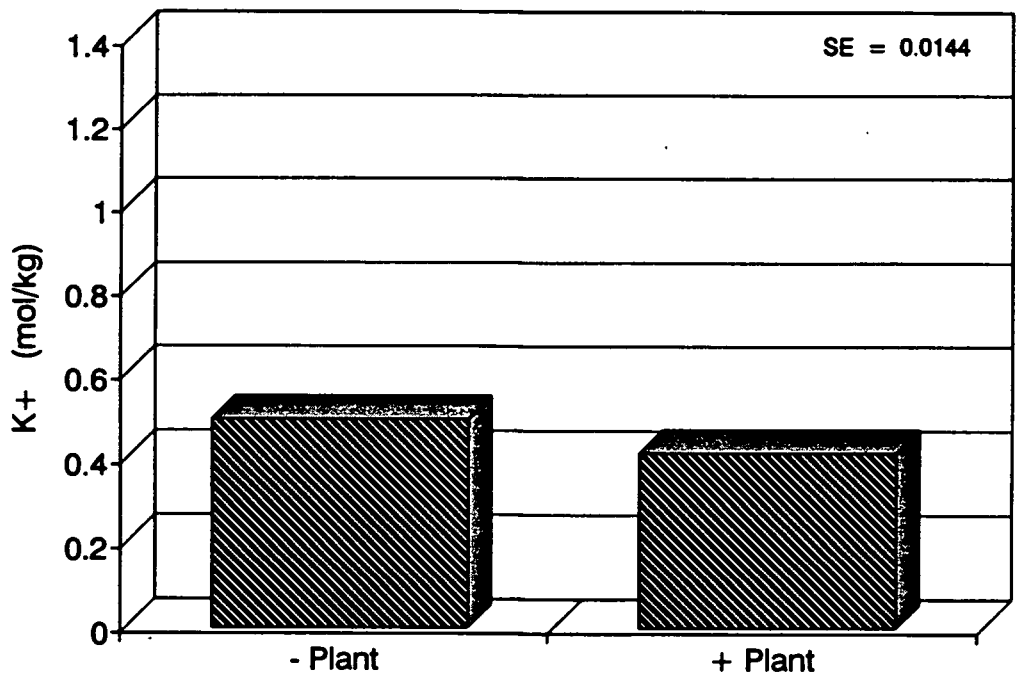

Figure 3.2. Effect of plant uptake on K^+ concentration in the media of +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 96 values.

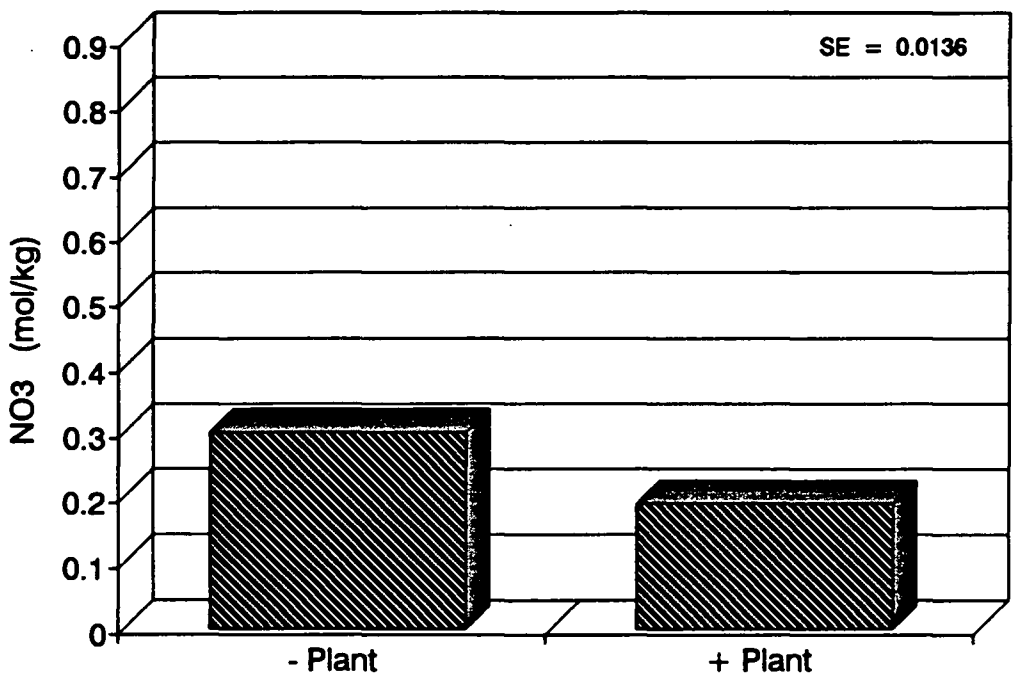

Figure 3.3. Effect of plant uptake on NO_3^- concentration in the media of +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 96 values.

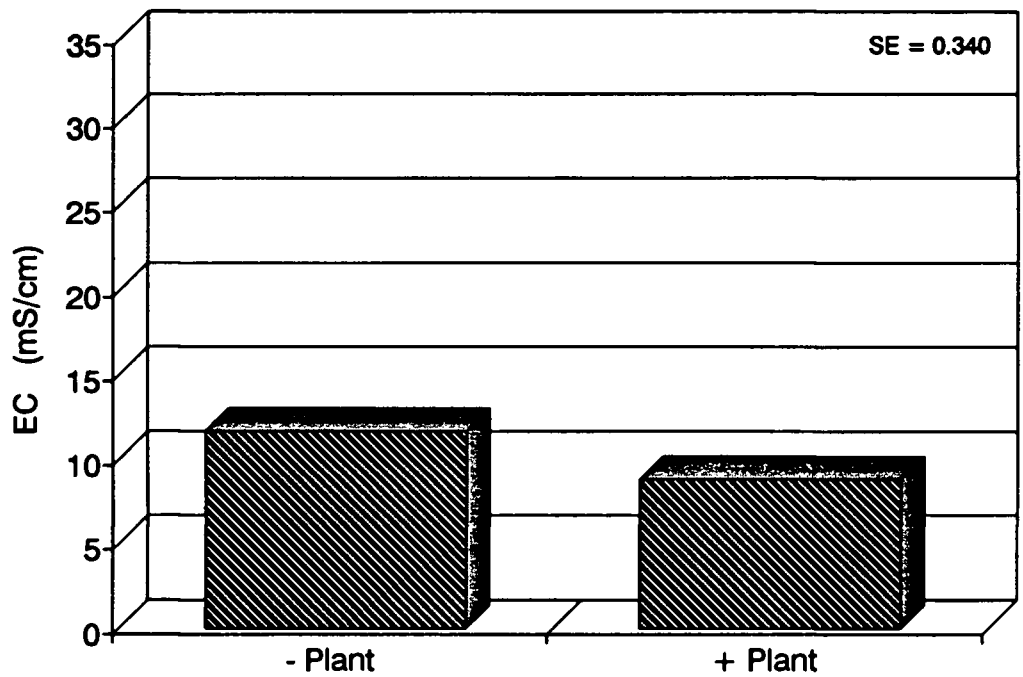

Figure 3.4. Effect of plant uptake on EC values of the media in +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 96 values.

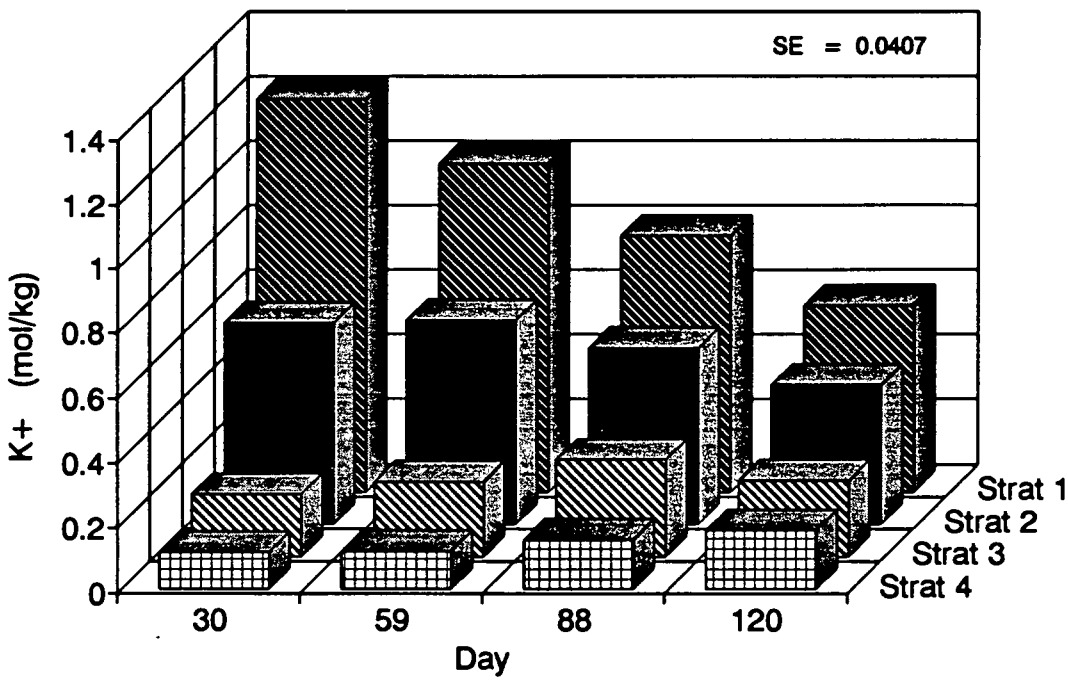

Figure 3.5. Effect of the Day x Strata interaction on K^+ concentrations averaged over +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 12 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

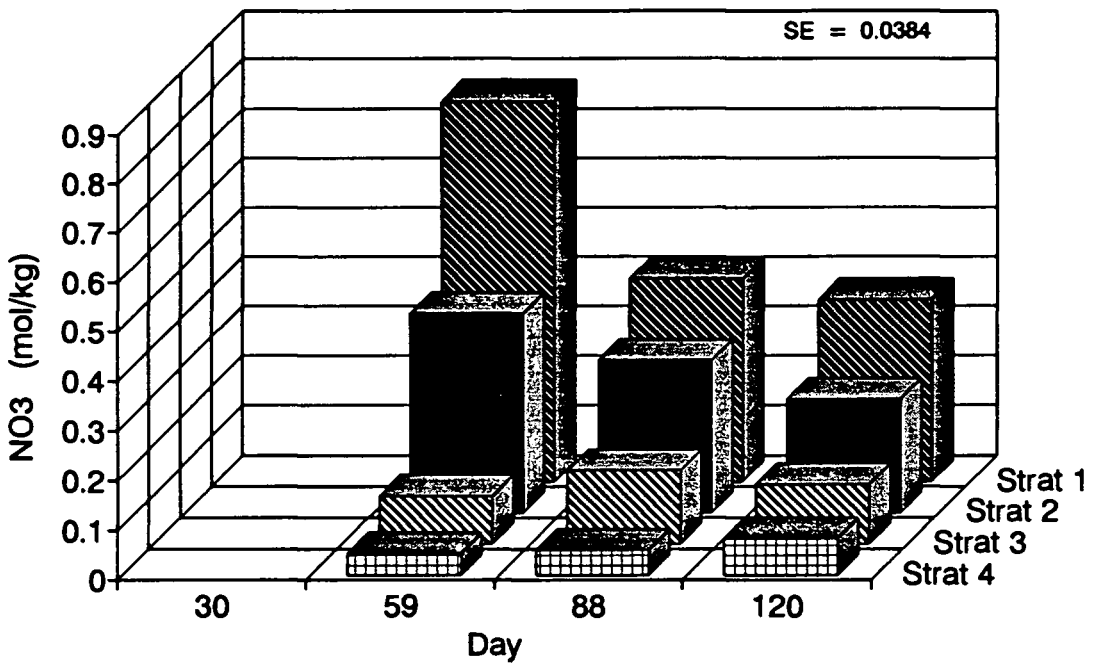

Figure 3.6. Effect of the Day x Strata interaction on NO_3^- concentrations averaged over +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 12 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

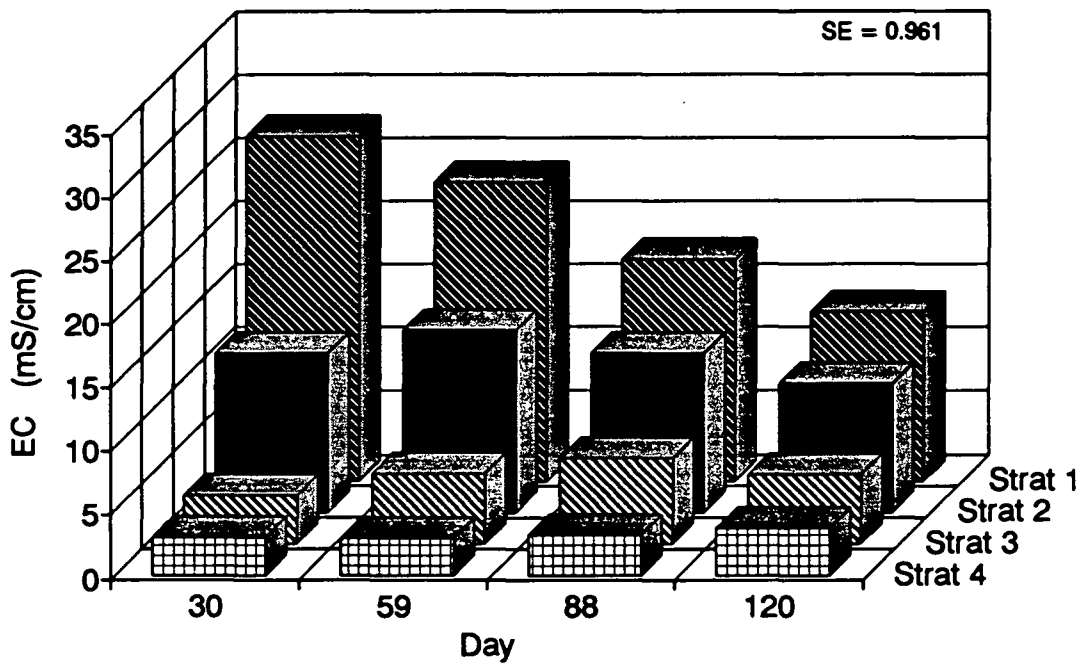

Figure 3.7. Effect of the Day x Strata interaction on EC averaged over +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 12 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

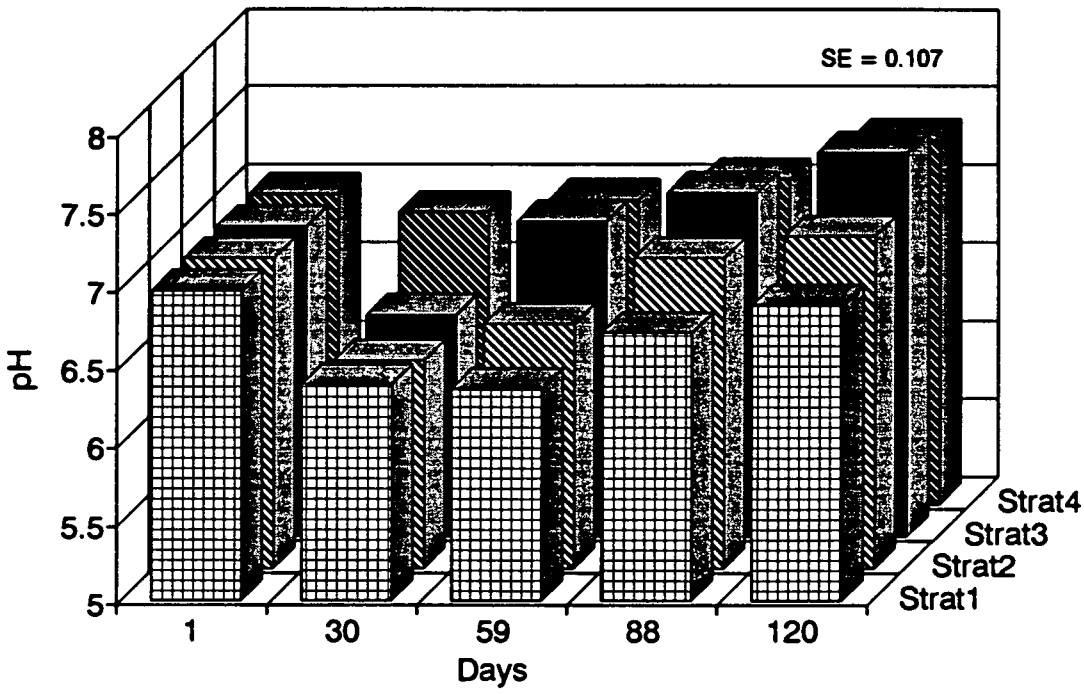

Figure 3.8. PH values of media strata for the -Fertilizer (-Plant-Fertilizer) treatment. Each bar represents the mean of 6 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

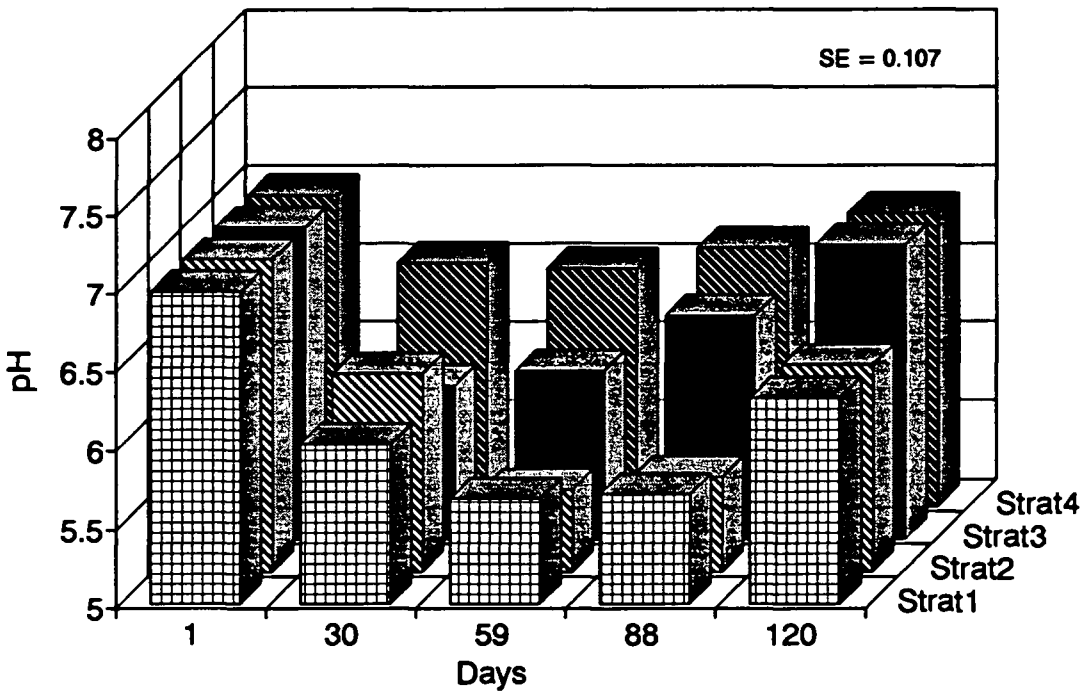

Figure 3.9. PH values of media strata for the +Fertilizer (-Plant+Fertilizer) treatment. Each bar represents the mean of 6 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

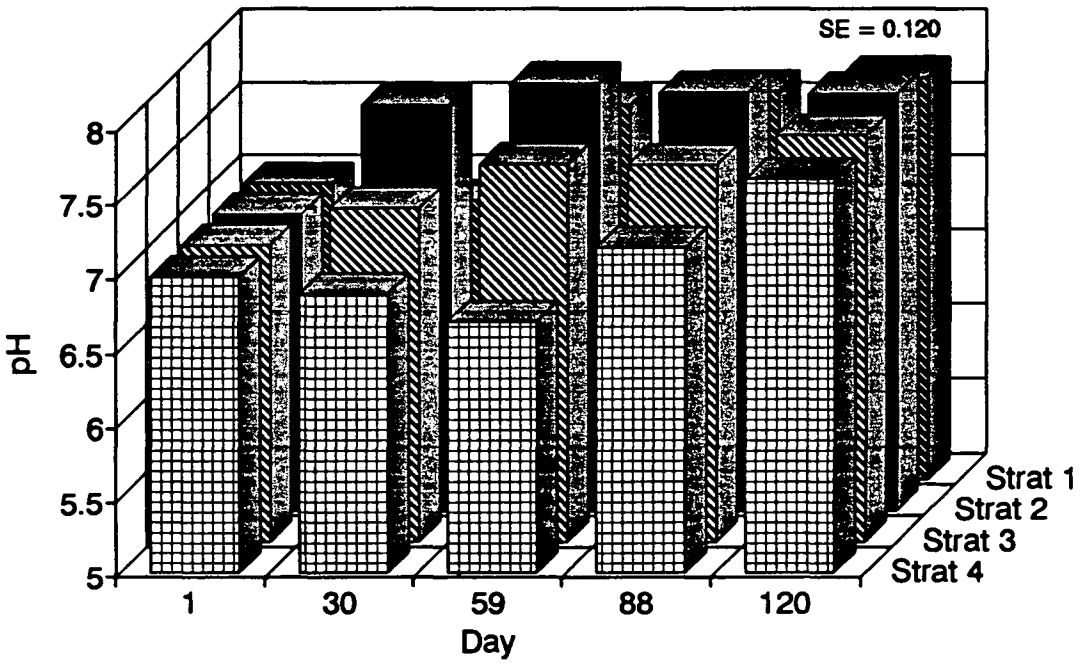

Figure 3.10. PH values of media strata for the +Plant (+Plant+Fertilizer) treatment. Each bar represents the mean of 6 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

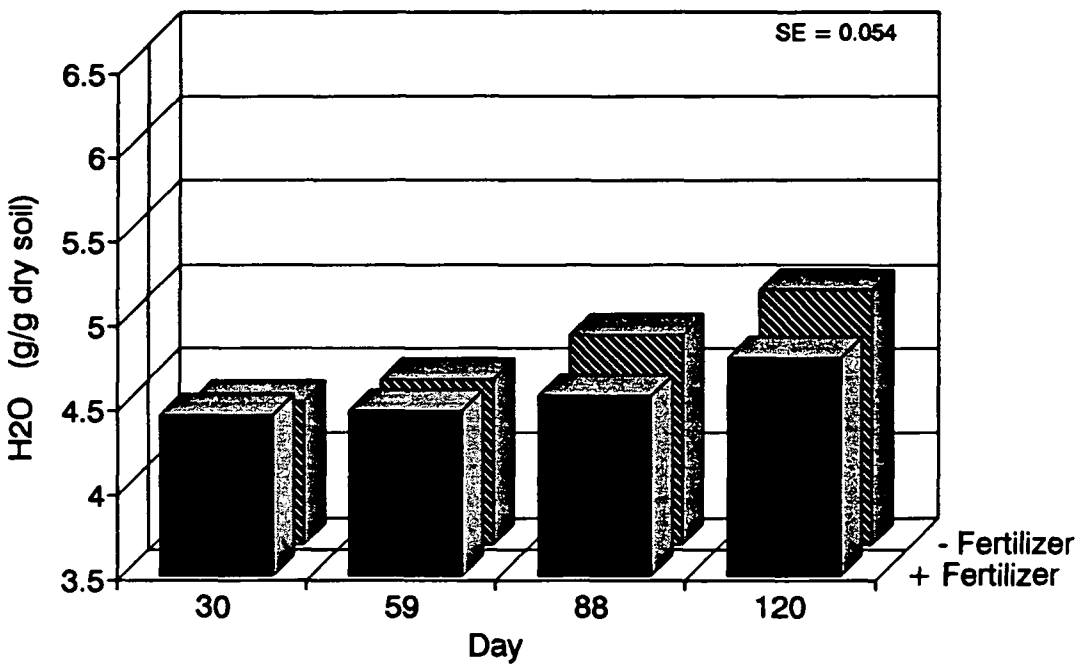

Figure 3.11. Interaction of Day x Fertilizer on water content of the media in -Plant +Fertilizer and -Plant -Fertilizer treatments. Each bar represents the mean of 24 values.

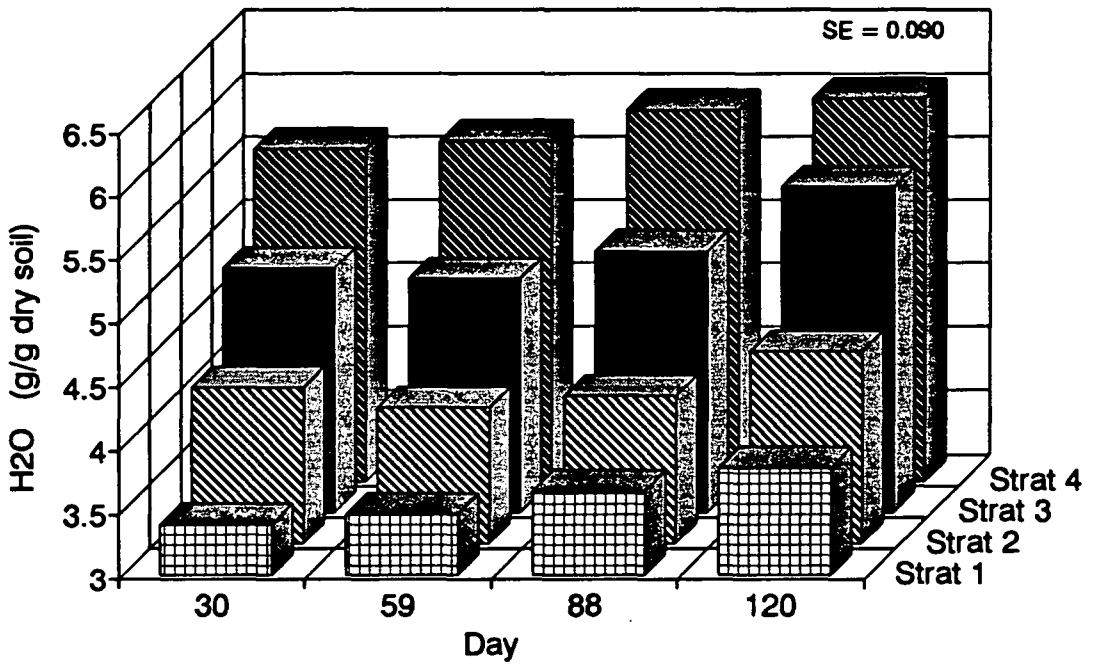

Figure 3.12. Interaction of Day x Strata on the water content of media in the +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 12 values. Strata 1 to 4 are 0-2.5, 2.5-5.0, 5.0-10.0, 10.0-15.0 cm from the top surface of the media, respectively.

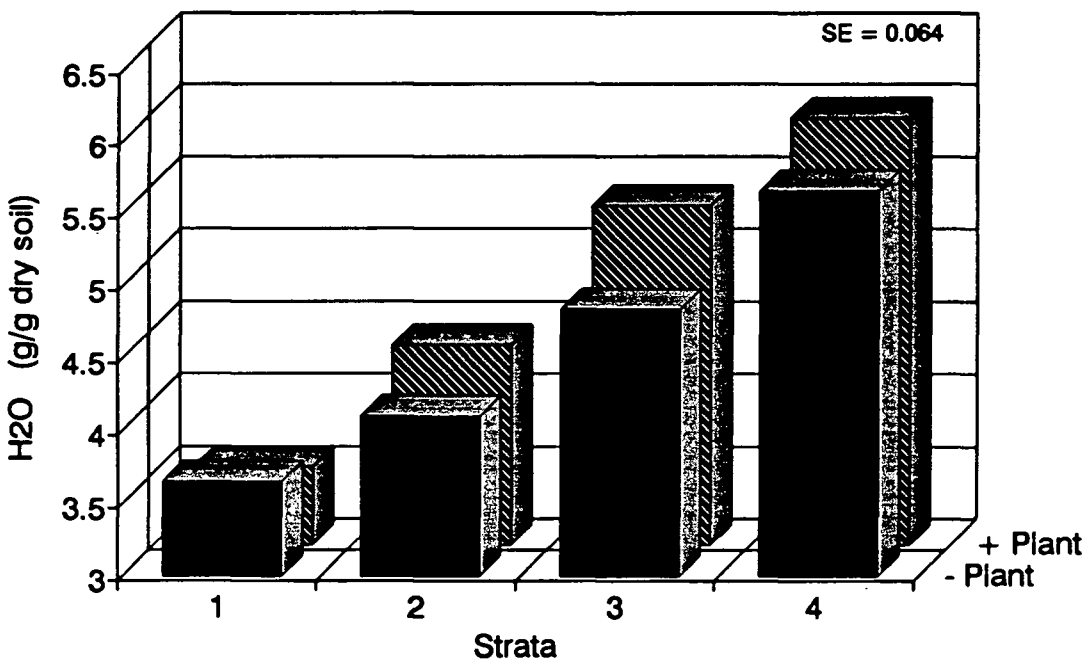

Figure 3.13. Interaction of +/-Plant x Strata on the water content of the media in the +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 24 values.

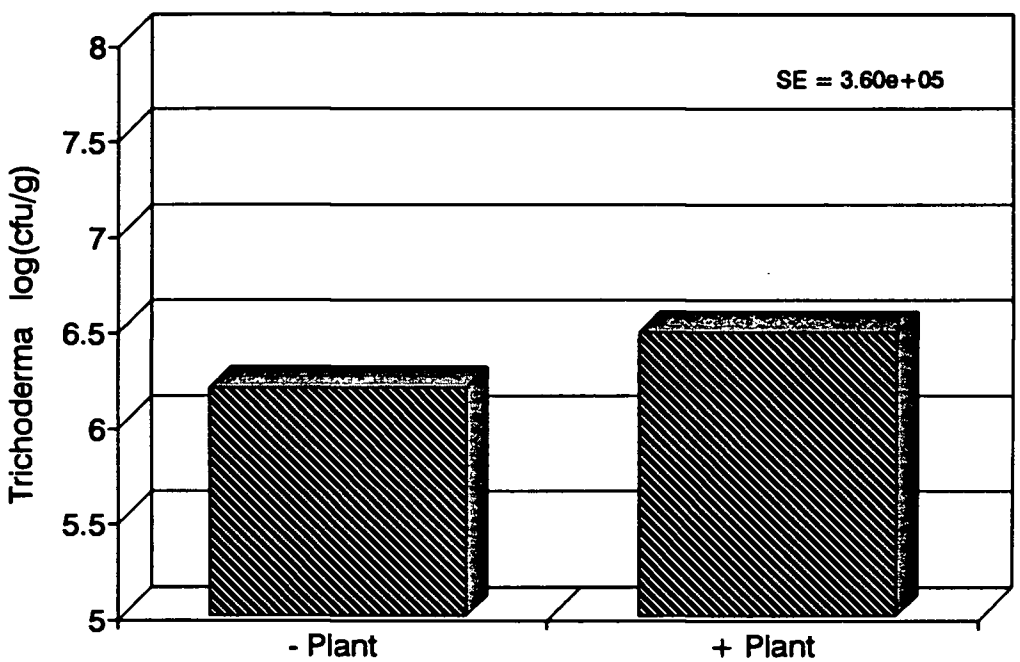

Figure 3.14. Effect of a plant on *Trichoderma* populations in +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 96 values.

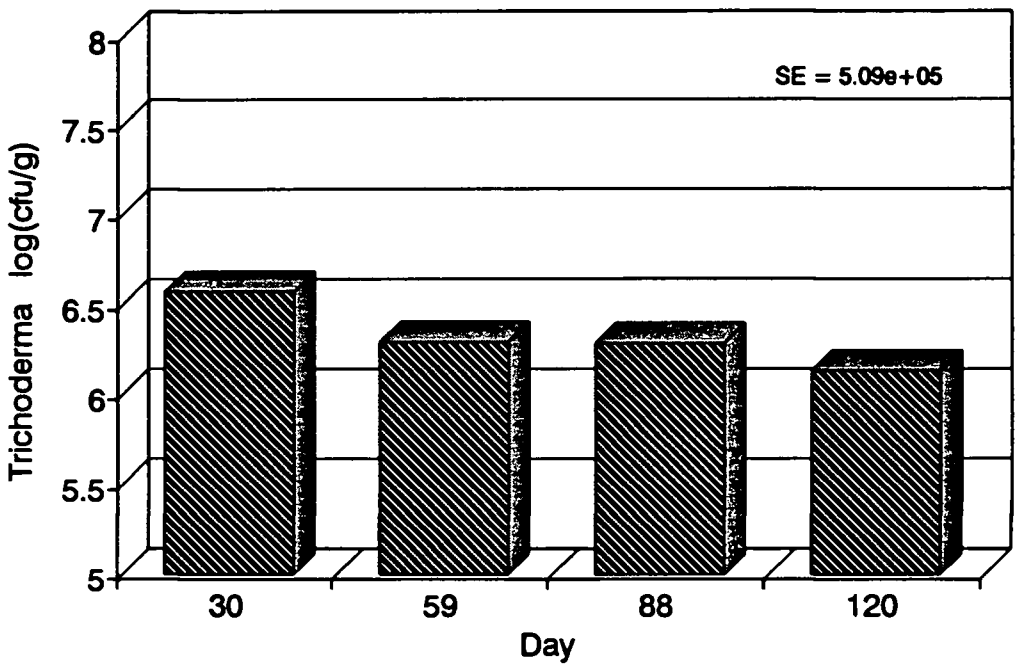

Figure 3.15. Effect of Day on *Trichoderma* populations in +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 48 values.

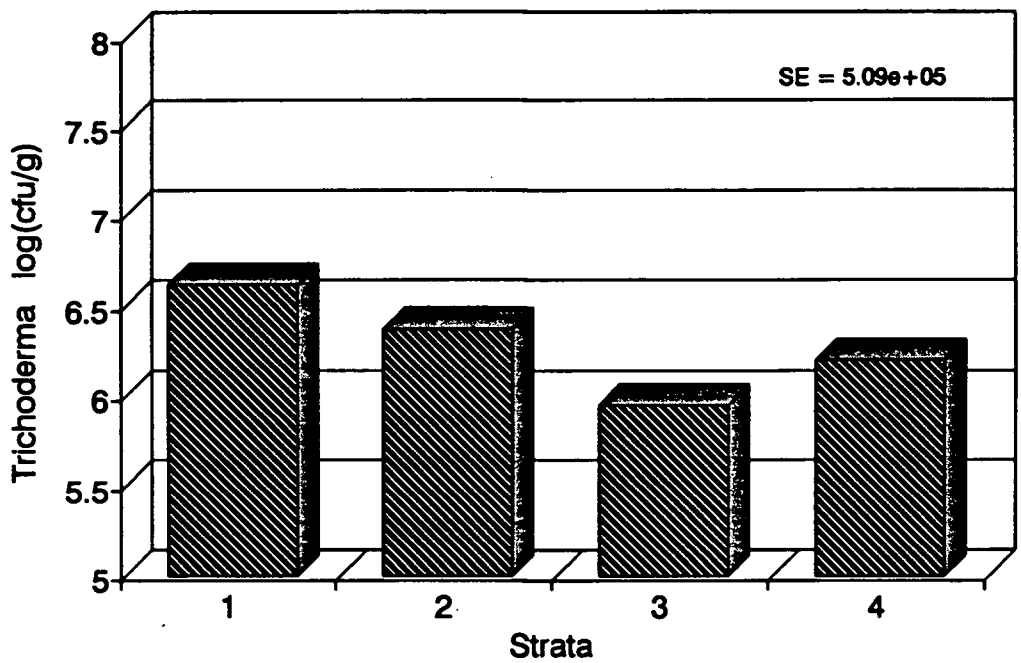

Figure 3.16. Effect of Strata on *Trichoderma* populations in +Plant +Fertilizer and -Plant +Fertilizer treatments. Each bar represents the mean of 48 values.

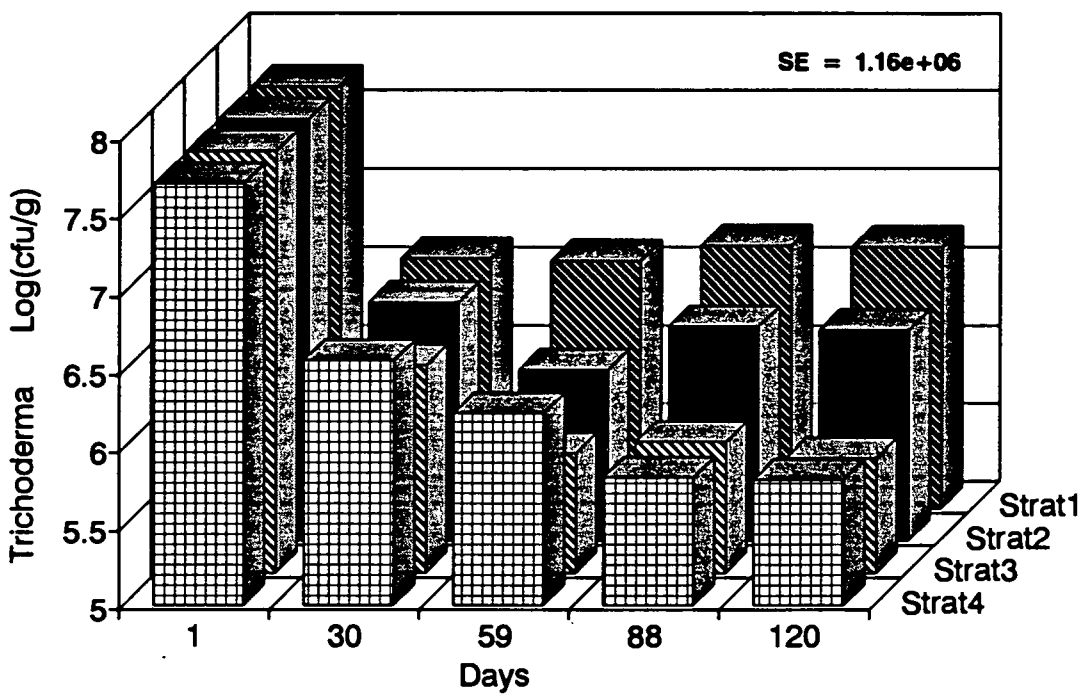

Figure 3.17. Effect of Plant on *Trichoderma* populations in the +Plant +Fertilizer treatment. Populations in stratum 1 remained stable for the duration of the experiment. Each bar represents the mean of 6 values.

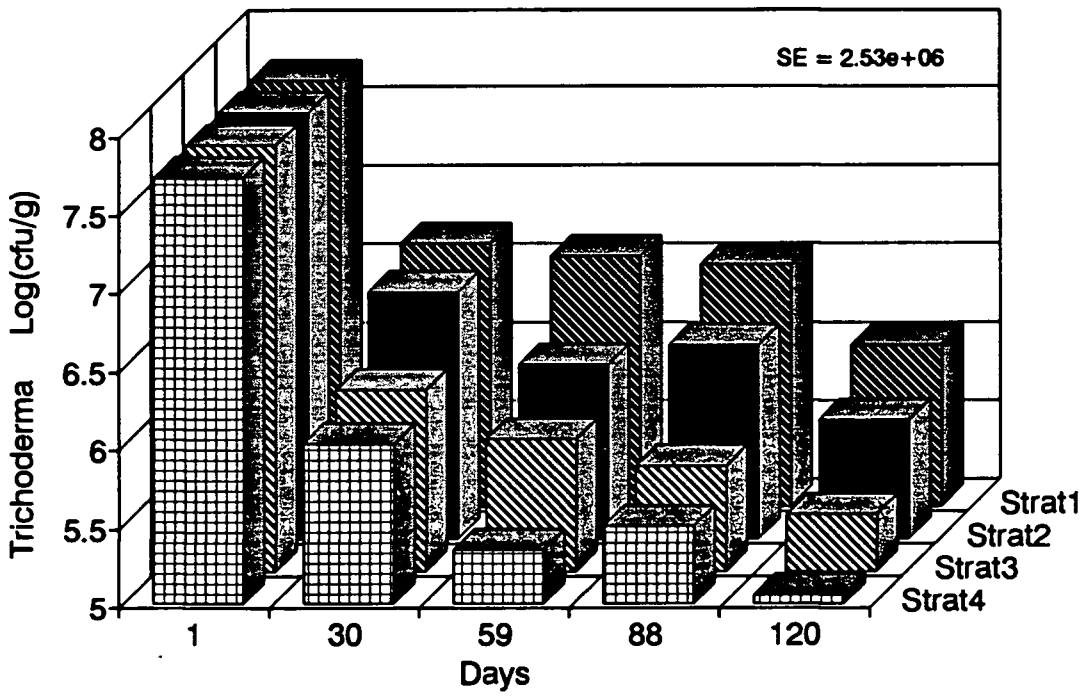

Figure 3.18. Changes in *Trichoderma* populations in the -Plant +Fertilizer treatment. Each bar represents the mean of 6 values.

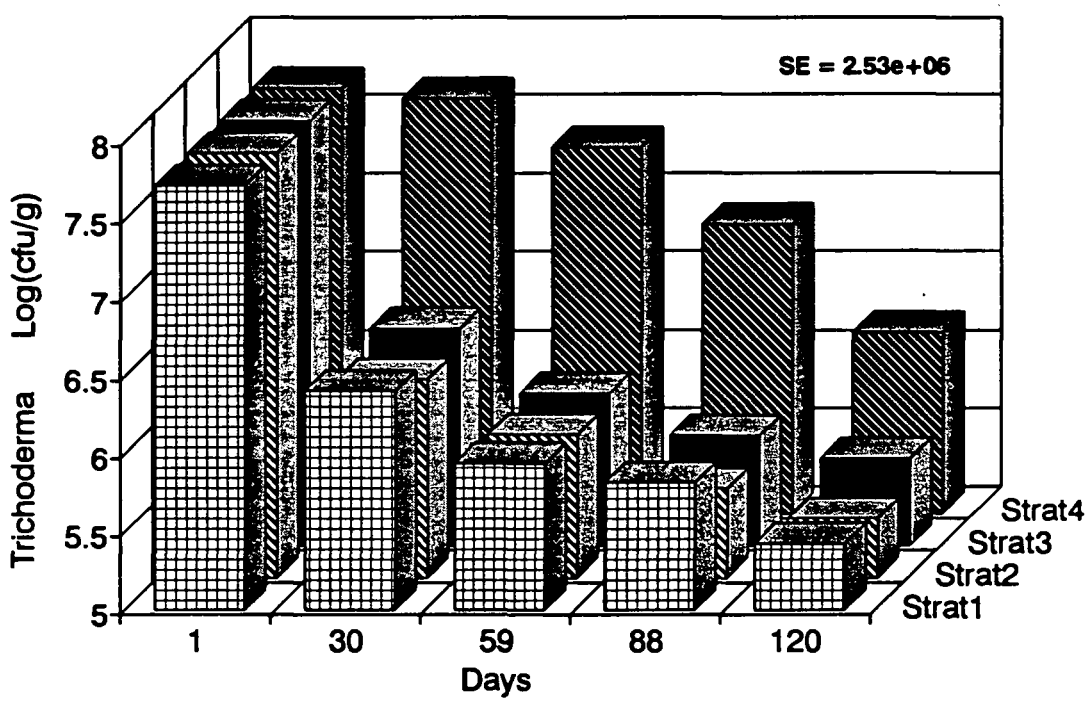

Figure 3.19. Changes in *Trichoderma* populations in the -Plant -Fertilizer treatment. Each bar represents the mean of 6 values.

Table 3.1. Summary analysis of variance table for the effect of applied fertilizer on measured variables.

	K^+	NO_3^-	pH	EC	H_2O	cfu/g
+/- Fertilizer(T)						
Day (D)						
Stratum (S)						
T x D					***	
T x S					*	
D x S					***	
T x D x S	***	***	***	***		***

*,**,***Significant at P = 0.05, 0.01, or 0.001, respectively.

Table 3.2. Summary analysis of variance table for the effect of a plant on measured variables.

	K^+	NO_3^-	pH	EC	H_2O	cfu/g
+/- Plant (T)	***	***		***		**
Day (D)						**
Stratum (S)						***
T x D						
T x S					***	
D x S	***	***		***	***	
T x D x S			***			

*,**,***Significant at P = 0.05, 0.01, or 0.001, respectively.

Table 3.3. Estimated potassium budget (as mol K^+) comparing the fate of potassium in CIPS and a traditional growing system.[z]

Day	Root Pouch	Water Reservoir	Fertilizer Compartment	Plant Shoot	Total - Control
+Plant +Fertilizer					
30	0.1685	0.0005	0.0491	0.0118	0.2175
59	0.1139	0.0024	0.0113	0.0555	0.1711
88	0.1029	0.0027	0.0019	0.0956	0.1925
120	0.0836	0.0290	0.0013	0.0901	0.1927
-Plant +Fertilizer					
30	0.1890	0.0526	0.0281		0.2573
59	0.1668	0.0905	0.129		0.2582
88	0.1133	0.1361	0.0020		0.2409
120	0.0977	0.1600	0.0019		0.2483
Background of control group, -Plant -Fertilizer					
30	0.0091	0.0033	0.0000		
59	0.0087	0.0033	0.0000		
88	0.0069	0.0036	0.0000		
120	0.0067	0.0046	0.0000		
Traditional Overhead-watered Plants					
120	0.0189	N/A	N/A	0.0718	0.0794

[z]Values are the mean of six replicates in mol $K^+ \cdot unit^{-1}$ (mol·pouch^{-1}, mol·reservoir^{-1}, etc.). Replicates were destructively sampled at the end of 30, 59, 88, or 120 days. Moles of K^+ in the fertilizer compartments (FC) were estimated from dry weights of fertilizer remaining in the FC. Potassium concentrations in plant shoots were estimated to be 2% of shoot dry weights (Larson and Hanway, 1977). The quantity of K in the roots was not determined or included in the media analyses. (Root weight measurements were not taken.) The unaccounted for K was probably in the roots.

[y]Treatments: +Plant +Fertilizer = + corn plant + 24.3 g KNO3 fertilizer (0.240 mol N/pouch). -Plant +Fertilizer = no plant + fertilizer (0.240 mol N/pouch). -Plant -Fertilizer = no plant and no fertilizer. Traditional Overhead-watered Plants = + plant in open container on bench receiving overhead watering (traditional production method) + 19.8 g (0.240 mol N/pot) Osmocote 18N-2.6P-9.9K.

Table 3.4. Estimated potassium budget (as % of applied K^+) comparing the fate of potassium in CIPS and a traditional growing system.[z]

Day	Root Pouch	Water Reservoir	Fertilizer Compartment	Plant Shoot	% Un-Accounted
+Plant +Fertilizer					
30	66.42	-1.17	20.46	4.92	9.38
59	43.83	-0.38	4.71	23.13	28.69
88	40.00	-0.38	0.79	39.83	19.78
120	32.04	10.17	0.54	37.54	19.71
-Plant +Fertilizer					
30	74.96	20.54	11.71		-7.22
59	65.88	36.33	0.05		-7.60
88	44.33	55.21	0.83		-0.37
120	37.92	64.75	0.79		-3.45
Traditional Overhead-watered Plants					
120	5.08	N/A	N/A	29.92	66.92

[z]Values are the mean of six replicates in mol $K^+ unit^{-1}$ (% $pouch^{-1}$, % $reservoir^{-1}$, etc.). Replicates were destructively sampled at the end of 30, 59, 88, or 120 days. Amounts of K^+ in the fertilizer compartments (FC) were estimated from dry weights of fertilizer remaining in the FC. Potassium concentrations in plant shoots were estimated to be 2% of shoot dry weights (Larson and Hanway, 1977). The quantity of K in the roots was not determined or included in the media analyses. (Root weight measurements were not taken.) The unaccounted for K was probably in the roots.

[y]Treatments: +Plant +Fertilizer = + corn plant + 24.3 g KNO3 fertilizer (0.240 mol N/pouch). -Plant +Fertilizer = no plant + fertilizer (0.240 mol N/pouch). -Plant -Fertilizer = no plant and no fertilizer. Traditional Overhead-watered Plants = + plant in open container on bench receiving overhead watering (traditional production method) + 19.8 g (0.240 mol N/pot) Osmocote 18N-2.6P-9.9K.

Table 3.5. Estimated nitrate-nitrogen budget (as mol NO_3^-) comparing the fate of nitrate in CIPS and a traditional growing system.[z]

Day	Root Pouch	Water Reservoir	Fertilizer Compartment	Plant Shoot	Total - Control
+Plant +Fertilizer[y]					
59	0.1050	0.0002	0.0113	0.0968	0.1987
88	0.0695	0.0014	0.0019	0.1668	0.2297
120	0.0551	0.0171	0.0013	0.1573	0.2235
-Plant +Fertilizer					
59	0.1470	0.0677	0.0129		0.2129
88	0.1131	0.1091	0.0020		0.2144
120	0.1019	0.1163	0.0019		0.2127
Background of control group, -Plant -Fertilizer					
59	0.0123	0.0024	0.0000		
88	0.0080	0.0019	0.0000		
120	0.0059	0.0015	0.0000		
Traditional Overhead-watered Plants					
120	0.0402	N/A	N/A	0.1254	0.1582

[z]Values are the mean of six replicates in mol $N \cdot unit^{-1}$ (mol·pouch^{-1}, mol·reservoir^{-1}, etc.). Replicates were destructively sampled at the end of 30, 59, 88, or 120 days. Moles of N in the fertilizer compartments (FC) were estimated from dry weights of fertilizer remaining in the FC. Nitrogen concentrations in plant shoots were estimated to be 1.25% of shoot dry weights (Larson and Hanway, 1977). The quantity of N in the roots was not determined or included in the media analyses. (Root weight measurements were not taken.) The 21% of applied N which was unaccounted for may have been in the roots or converted to other form, i.e. NH_4^+ and organic acids.

[y]Treatments: +Plant +Fertilizer = + corn plant + 24.3 g KNO3 fertilizer (0.240 mol N/pouch). -Plant +Fertilizer = no plant + fertilizer (0.240 mol N/pouch). -Plant -Fertilizer = no plant and no fertilizer. Traditional Overhead-watered Plants = + plant in open container on bench receiving overhead watering (traditional production method) + 19.8 g (0.240 mol N/pot) Osmocote 18N-2.6P-9.9K.

Table 3.6. Estimated nitrate-nitrogen budget (as % of applied NO_3^-) comparing the fate of nitrate in CIPS and a traditional growing system.[z]

Day	Root Pouch	Water Reservoir	Fertilizer Compartment	Plant Shoot	% Un-Accounted
+Plant +Fertilizer[y]					
59	38.63	-0.92	4.71	40.33	17.22
88	25.63	-0.21	0.79	69.50	4.29
120	20.50	6.50	0.54	65.54	6.89
-Plant +Fertilizer					
59	56.13	27.21	53.75		11.28
88	43.79	44.67	0.83		10.69
120	40.00	47.83	0.79		11.36
Traditional, Overhead-watered Plants					
120	14.29	N/A	N/A	52.25	34.10

[z]Values are the mean of six replicates in mol N·unit^{-1} (mol·pouch^{-1}, mol·reservoir^{-1}, etc.). Replicates were destructively sampled at the end of 30, 59, 88, or 120 days. Amounts of N in the fertilizer compartments (FC) were estimated from dry weights of fertilizer remaining in the FC. Nitrogen concentrations in plant shoots were estimated to be 1.25% of shoot dry weights (Larson and Hanway, 1977). The quantity of N in the roots was not determined or included in the media analyses. (Root weight measurements were not taken.) The 21% of applied N which was unaccounted for may have been in the roots or converted to other form, i.e. NH_4^+ and organic acids.

[y]Treatments: +Plant +Fertilizer = + corn plant + 24.3 g KNO3 fertilizer (0.240 mol N/pouch). -Plant +Fertilizer = no plant + fertilizer (0.240 mol N/pouch). -Plant -Fertilizer = no plant and no fertilizer. Traditional Overhead-watered Plants = + plant in open container on bench receiving overhead watering (traditional production method) + 19.8 g (0.240 mol N/pot) Osmocote 18N-2.6P-9.9K.

Table 3.7. Effective diffusion coefficients (D_e, $cm^2 s^{-1}$) calculated from the concentrations of NO_3^- and K^+ in CIPS.

Strata[z]	K^+	NO_3^-
S1-S2	1.64E-06	1.41E-06
S2-S3	3.31E-06	2.13E-06
S3-S4	1.10E-05	5.92E-06
S1-S4	6.68E-06	3.56E-06

[z]Strata 1 to 4 are 0 to 2.5, 2.5 to 5.0, 5.0 to 10.0 and 10.0 to 15.0 cm in depth, respectively.

Table 3.8. Correlation of *Trichoderma* populations with independent variables.

+/- Fertilizer			+/- Plant		
Variable	Interaction	R^2	Variable	Interaction	R^2
H_2O	T x D x S[z]	0.74	H_2O	T x S	0.40
			H_2O	D x S	0.49
EC	T x D x S	0.29	EC	D x S	0.54
K^+	T x D x S	0.26	K^+	D x S	0.55
NO_3^-	T x D x S	0.18	NO_3^-	D x S	0.68
NH_4^+	T x D x S	0.22	NH_4^+	T x D x S	0.11
pH	T x D x S	0.13	pH	T x D x S	0.01

[z]T = Treatment, D = Day, and S = Strata.

Table 3.9. Comparison of *Trichoderma* populations (cfu/g) in CIPS and a traditional growing system (TOSS) at Day 120.

	Treatments			
Stratum	-Plant -Fertilizer	-Plant +Fertilizer	+Plant +Fertilizer	TOSS[z]
1	2.66E+05[y]	1.06E+06	5.11E+06	9.18E+03
2	2.41E+05	5.71E+05	2.46E+06	3.29E+04
3	3.58E+05	2.28E+05	6.60E+05	5.94E+04
4	1.44E+06	1.14E+05	6.56E+05	3.22E+04

[z]TOSS were corn plants in traditional open containers on the bench receiving overhead water.
[y]Values are the means of six replicates.

Table 3.10. Average water use of corn plants[z] in CIPS.

Day	Average grams water used per day per:		
	Plant	g Fresh Wt.	g Dry Wt.
30	144	0.404	5.006
59	205	0.339	1.577
88	171	0.373	0.992
120	128	0.407	0.728
Mean	152	0.382	2.097
Std Dev	44	0.038	1.763

[z]Values represent the mean water use of 6 plants. Corn was transplanted into CIPS 17 days after seeds were planted. Seedlings were approximately 15 cm tall. The plants were grown in the pallets from 21 April to 19 August 1991 in an OSU greenhouse.

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Chapter 4

Physical and Chemical Containment of Roots

Additional index words. Grow bags, root pruning, copper hydroxide, corn, *Zea mays*, root growth regulation, polypropylene fabric, copper phytotoxicity, lateral roots, root branching.

Abstract

Physical and chemical means to contain corn (*Zea mays* L.) roots were evaluated. A nonwoven polypropylene fabric container physically prevented root penetration, and reduced, but did not eliminate root circling. A $Cu(OH)_2$-latex paint suspension applied to the fabric containers increased root density, improved root distribution, and prevented root penetration of the fabric. Neither the fabric container nor the copper treatment had an adverse effect on plant growth.

Introduction

The Closed Insulated Pallet System (CIPS) is an experimental plant production system designed to address several factors which cause plant stress (Kaplan, 1992). When plants potted in traditional rigid-walled plastic containers were grown in the CIPS, plant roots grew out of the drainage holes into the capillary mats and reservoir.

To be able to remove a plant from the CIPS without root injury requires containing the roots.

Woven growbags or fabric containers are permeable to air and water, desirable traits for containers used in CIPS. However, fabric bags do not prevent root penetration (Ingram et al. 1987, Chong et al. 1989, James 1987, Wilson 1986).

Several copper compounds were found to be effective in controlling root growth: sheets of metallic copper, coated fibers, and paint (Saul, 1968); copper naphthenate (Nussbaum 1969, Furuta et al. 1972); and copper sulfate (Furuta et al. 1972, Pellett et al. 1980). Coating the inside of containers with cupric carbonate was found to prevent root circling by inhibiting growth at root tips. Higher order laterals then proliferated and in turn stopped growing when they came in contact with the treated container wall (Burdett 1978, Burdett and Martin 1982, McDonald et al. 1984). Inhibited root tips were not killed, but resumed elongation rates similar to non-$CuCO_3$-treated roots within three to six days after the treated container was removed (Arnold and Struve 1989). Copper-pruned lodgepole pine seedlings had better vertical and radial root distribution after transplanting from the treated containers and growing in the field for five years than did pine seedlings grown in untreated containers (Kooistra, 1991). Struve and Rhodus (1990) reported use of copper hydroxide as a root inhibitor. In their preliminary studies, copper hydroxide worked "even better than cupric carbonate, especially on vigorous-rooted species like forsythia."

In a series of experiments, we evaluated the ability of several fabrics to physically contain plant roots as well as the ability of several copper compounds to chemically control root growth.

Materials and Methods

Physical containment of plant roots

Several fabrics were evaluated for their suitability for use as root pouches in the CIPS. Following preliminary tests, three fabrics were evaluated further.

Preliminary experiments. Twelve fabrics were initially tested for their ability to physically prevent root penetration: five polypropylene fabrics, a high-loft cellulose/polypropylene fabric, and six other nonwoven fabrics. Fabrics are described in Table 4.1. Root pouches (3-liter volume) made from the fabrics were tested. Nine fabrics tested were penetrated by roots of *Zea maize* 'Golden Early' within 12 days. The Duon fabric was penetrated by roots more quickly than were other fabrics. Tyvek 1622e appeared to restrict water movement. A few small roots penetrated the material, but by Day 12, the corn plant growing in it had wilted. Results showed that three KC fabrics could potentially retain roots: KC 10NW400, KC 10NW403, and KC 10NW430 (Table 4.2). These three fabrics were further evaluated in experiment 1.

Experiment 1: Fabric tests. Three Kimberly Clark fabrics (KC 10NW400, KC 10NW403, and KC 10NW430) were evaluated. Zea maize 'Golden Jubilee' seedlings were transplanted into 3-liter root pouches made from the fabrics. Pouches with corn plants, four replicates each, were placed in CIPS in a complete random design. Rockwool pedestals extending upward from the reservoir supported the root pouches, and served as capillary wicks.

The horticultural media in the pouches was a 1 peat : 1 perlite (v/v) amended with 3.0 $Kg \cdot m^{-3}$ finely ground dolomite. Each root pouch was topdressed with 1.2 Kg $N \cdot m^{-3}$ Osmocote (14N-6.2P-11.6K), 3 month controlled release fertilizer.

Two control treatments were included: 1) CIPS-grown plants in root pouches made of Duon fabric, a fabric that does not restrict root growth or water movement, were used as a baseline in scoring the effects of the KC fabrics on root penetration and plant growth. 2) Plants grown in open 3-liter rigid plastic containers with surface-applied irrigation were used as a conventional production system.

The experiment ran for 14 days from 30 July 1990 to 12 Aug. 1990. At harvest, shoot height and shoot fresh weight were recorded. Fabric root pouches were scored for root penetration (1 = more than 5 roots, 2 = 1 to 5 roots, and 3 = no roots penetrated). The average gravimetric water content of the media in each pouch was determined after drying samples in an oven at 60C for 3 days.

Chemical containment of plant roots

Two experiments (experiments 2 and 3) were conducted to identify a chemical means of root control for plants grown in CIPS. The first tested effects of different copper compounds and copper concentrations on root containment and plant growth. The second evaluated the effectiveness of copper hydroxide in three container systems.

Experiment 2: Evaluation of the effects of different copper compounds and concentrations on growth of *Zea mays* 'Golden Jubilee'. There were five combinations of treatments: 1) control treatment without copper--An untreated disk (16.2 cm diameter) of a high-loft cellulose/polypropylene fabric (KC COF3, Kimberly-Clark, Roswell, GA) placed inside the bottom of the pot, 2) copper-napthenate-treated burlap disk secured to the exterior of the pot bottom with a rubber band, 3) 1X copper concentration, a fabric disk presoaked in a $Cu(OH)_2$ suspension (100 g·liter^{-1} technical grade powder, 57.3% metallic copper, suspended in water) placed in the bottom of the pot, 4) 2X copper concentration, a fabric disk presoaked in a $Cu(OH)_2$ suspension (200 g·liter^{-1} water) placed in the bottom of the pot, and 5) Copper

naphthenate in a mineral spirit solvent (ATCO Woodlast 2, American Tar Co., Tacoma, WA) placed in the pot bottom. An additional control treatment outside of the CIPS, was a traditional, overhead-watered treatment (TOW); plants in open, rigid plastic containers were placed on the greenhouse bench with no disk and surface-irrigated. Six replicates per treatment, completely randomized, were grown for 33 days in CIPS. On 23 July 1990, the treatments were scored for root containment and root distribution.

Experiment 3: Effect of container systems. Three container systems received two copper treatments applied at two rates, four replicates each. The experiment was conducted as an incomplete factorial in a complete random design. Treatments were: 1) 1/4X Kocide 101 wp (25 grams/liter water), 2) 1X Kocide 101 wp (100 grams/ liter water), 3) 1/4X Texcide and 4) 1X Texcide [Texcide is Kocide 101 wp at the 1/4X or 1X rates in a latex carrier (Glidden wall and ceiling 9020 white vinyl acrylic latex paint diluted 1:1 with water).] Container systems consisted of: 1) root pouches made from a polyolefin fabric, 36 gm^2, (Agryl, Beghin-Say, Kaysersberg, France), 2) plastic florist pots (15 cm, 1.5 liter), 3) florist pots with an Agryl fabric disk secured over the drainage holes of the pots.

Copper-treated burlap and latex paint treatments and a traditional, overhead-watered (TOW) treatment on the greenhouse bench were included as controls. Except for the surface-irrigated control, plants in the treated container systems were grown in CIPS.

Corn seedlings (*Zea mays* cv Golden Jubilee) were transplanted into the containers and grown for 23 days from 16 Aug, 1990 to 8 Sept, 1990. Shoot heights were measured, and root containment was scored. Root balls were visually divided into three strata and scored for vertical and horizontal root distribution on scales of 1 to 3. Vertical and Horizontal scores were then multiplied together to give overall root scores.

Results and Discussion

Physical control of plant roots

Experiment 1: Fabric tests. In experiments on physical containment of plant roots, the mean shoot weights of plants grown in pouches were not different regardless of fabric and mean shoot weights of plants grown in Kimberly-Clark (KC) fabrics were not different from the TOW controls (Table 4.3). Mean shoot heights showed the same pattern except that plants grown in both KC NW400 and Duon fabrics were taller than TOW controls. There were no differences in water contents of the media in the fabric root pouches. While plant growth does not appear to be affected by the type of fabric, KC 10 NW400 was the only fabric which was not penetrated by plant roots (Table 4.3).

Chemical control of plant roots

Experiment 2: Effects of different copper treatments. Kocide and ATCO copper treatments prevented corn roots from penetrating the fabrics in the bottom of the pots. A few roots were not contained in two replicates of copper-treated burlap (Table 4.4). However, the Kocide and ATCO treatments which prevented root penetration were also detrimental to root growth as shown by the lower rating for root distribution in the bottom stratum which was in contact with the treated disks. The rating for the untreated disk was the same as that for copper-treated burlap. Roots had less direct exposure to the copper-treated burlap than to the Kocide treated disk. Roots were only exposed to the burlap through the drainage holes in the bottom of the container; the Kocide-treated disks inside the container was in direct contact with the lower portion of the roots and media. Kocide did not adversely affect plant height. ATCO was phytotoxic. Five replicates of the ATCO treatment died and one was stunted. Heights of the dead plants were included to indicate the amount of growth that occurred before death.

Copper-treated burlap could be used to contain plant roots within a pot. If a Kocide treatment could be bound to a fabric so that it was less mobile, it may also be suitable for use.

Experiment 3: Effect of container systems. Of the three container systems tested, rigid-walled containers painted with 1X Texcide did not contain the roots (Table 4.5). Although the copper coating on the inside of the plastic pots improved root distribution, as reported by others (Wenny and Woollen 1989, Ruehle 1985, McDonald et al. 1984), roots escaped through the drainage holes in the bottoms of the pots. Both Kocide and copper-treated burlap disks covering the bottoms of plastic pots contained the corn roots; however, root distribution scores were low for both treatments. Fabric root pouches treated with Texcide provided root containment as well as the greatest root growth and greatest uniformity of root distribution within the media.

Conclusions

Although one fabric prevented the corn roots from penetrating the fabric pouch during the experiment, root distribution within the media was not uniform. A fabric root pouch treated with Texcide contains the roots by inhibiting root tips, and increases root density and uniformity of root distribution. Rigid containers treated with Texcide improve root distribution, but fail to prevent roots from escaping through the drainage holes. Treated fabric secured to the bottom of a rigid-walled pot contains the roots, but without the improvements in root distribution. Only the treated fabric pouches provide the root containment needed for use in the Closed, Insulated Pallet System, as well as the beneficial effects on root morphology.

Table 4.1. Description of fabrics.

Fabric	Description
	Nonwoven spunbonded polypropylene fibers
Agryl P17	17.4 gm^{-2} (Beghin-Say, Kaysersberg, France)
KC 10NW400	Spunbonded/Meltblown/Spunbonded (SMS) Laminate White HP bond pattern, 76.4 gm^{-2} (Kimberly-Clark, Roswell, GA)
KC 10NW401	SMS Laminate treated with Triton x-102 wetting agent. Black pigmented HP bond pattern, 44.1 gm^{-2} (Kimberly-Clark, Roswell, GA)
KC 10NW403	SMS Laminate untreated, White RHT bond pattern, 51 gm^{-2} (Kimberly-Clark, Roswell, GA)
KC 10NW430	SMS Laminate untreated, White RHT bond pattern, 85 gm^{-2} (Kimberly-Clark, Roswell, GA)
KC 1RSI	Spunbond/Meltblown (SM), wettable White HP pattern 28 gm^{-2} (Kimberly-Clark, Roswell, GA)
	A High-loft cellulose/polypropylene fabric
KC COF3	Coform/tissue composite, untreated, 151 gm^{-2} Unbonded tissue = 21 gm^{-2} Coform = 134 gm^{-2} (1:1 pulp:polypropylene) (Kimberly-Clark, Roswell, GA)
	Other fabrics
Reemay	34.6 gm^{-2} nonwoven polyester. (Reemay, Inc., Old Hickory, TN)
Vispore 5042	29.9 gm^{-2} polyethylene (Visqueen, city, state)
Tyvek 1622e	Spunbonded polyolefin (DuPont, Wilmington, DE)
Typar 3401	(Reemay, Inc., Old Hickory, TN)
Duon	100% nonwoven polypropylene 200 gm^{-2} (Phillips Fiber Corporation, Greenville, S.C.)

Table 4.2. Preliminary experiment. Effects of fabric on growth and root containment.

Fabric	Root Control[z]	Shoot Fresh Wt (g)	Moisture Content (g·g dry wt)
Duon, Control	1.0	113.8	4.57
KC NW400	3.0	123.9	4.26
KC NW403	2.0	131.9	4.08
KC NW430	2.0	87.0	3.71

[z]Each fabric pouch was scored for root penetration: 1 = more than 5 roots, 2 = 1 to 5 roots, and 3 = no roots penetrated the fabric.

Table 4.3. Effects of fabric on growth and root containment[z], Experiment 1.

Fabric	Root Control[y]	Shoot Fresh Wt (g)	Shoot Height (cm)	Moisture Content (g·gdry wt)
DUON, Control 1	1.0 ab[x]	107.2 a	107.8 a	4.17 a
KC 10NW400	3.0 c	97.7 ab	103.2 a	4.40 a
KC 10NW403	1.0 ab	93.0 ab	94.6 ab	5.03 a
KC 10NW430	1.5 b	89.9 ab	80.6 ab	4.23 a
TOW[w], Control 2		73.3 b	77.0 b	1.88 b

[z]Each value in the table is the average of 4 replicates except for the TOW control which is the average of 6 replicates.
[y]Each fabric pouch was scored for root penetration: 1 = more than 5 roots, 2 = 1 to 5 roots, and 3 = no roots penetrated the fabric.
[z]Mean Separation within columns by a multiple range test in Statgraphics, P = 0.05.
[w]TOW (Traditional Overhead Watering) is a control treatment included for comparisons of plant growth.

Table 4.4. Chemical containment of corn roots by copper, Experiment 2.

Treatment[z]	Plant Ht. (cm)[y]	Root Control[x]	Root Distribution (V)[w]			
			S1[v]	S2	S3	Mean V
Untreated	171.2	1.0	1.3	2.3	3.0	2.2
Cu-burlap	176.2	2.7	1.7	2.8	3.0	2.5
Kocide 1X	176.7	3.0	1.2	3.0	2.0	2.1
Kocide 2X	179.3	3.0	1.3	2.0	2.0	1.8
ATCO	42.0	3.0	1.0	1.2	1.0	1.1
TOSS	116.5	1.7	1.2	1.8	2.0	1.7

[z]Root containment treatments:

#1 Untreated 16.2 cm diameter disk of Kimberly-Clark high-loft cellulose/ polypropylene fabric (KC COF3) inside pot bottom.

#2 Copper-treated burlap secured to the exterior of the pot bottom with a rubber band.

#3 KC COF3 disk presoaked in Kocide 101 wp (100 grams per liter of water) and placed in the bottom of the pot.

#4 KC COF3 disk presoaked in Kocide 101 wp (200 grams per liter of water) and placed in the bottom of the pot.

#5 KC COF3 disk presoaked in copper napthenate in a mineral spirits carrier (ATCO Woodlast 2, American Tar Company, Tacoma, WA) and placed in the pot bottom.

#6 Surface-irrigated, control treatment with no disk

[y]Each value is the mean of 6 replicates (plants).

[x]Root containment ratings: 1 = many roots escaped, 2 = few roots escaped, 3 = no roots escaped. Each value is the mean of 6 ratings.

[w]Vertical distribution of roots on the outer edge of the rootball: 1 = no or few roots, 2 = 50% of the periphery of root matrix, 3 = up to 100% peripheral coverage. Each value is the mean rating for 6 replicates (plants).

[v]Horizontal strata: S1 = 0 to 5 cm, S2 = 5 to 10 cm, and S3 = 10 to 15 cm from the top surface of the growing media.

Table 4.5. Chemical root containment in CIPS, Experiment 3.

Container	Treatment	Shoot Ht (cm)	V*H[x]	Root Control[y]
Pot	1X Texcide[x]	81.9	7.5	2.0
	Latex	65.5	4.6	1.0
	Control	81.0	3.0	1.0
Pot+Disk	1/4X Texcide	74.4	3.0	1.5
	1X Kocide	88.4	3.8	3.0
	Cu-Burlap	83.3	3.0	3.0
	Control	70.1	3.0	1.0
Fabric[w]	1/4X Texcide	94.0	9.0	3.0
	1/4X Kocide	84.8	9.0	1.0
	Latex	89.9	6.2	1.0

[x] V*H = mean of the vertical x horizontal visual rating of root quantity-distribution in 3 root matrix strata (top 5 cm, middle 5 cm, and bottom 5 cm). Each V and H value was the mean of 3 strata/root matrix x 4 replicates.

[y] Root containment: 3 = no roots outside of root container (rigid container or fabric pouch). Each value is the mean of 12 values (3 strata/root matrix x 4 replicates).

[x] The 1X rate of Kocide for root control was 100 grams of Kocide 101 wp per liter water (Kocide 101 contains 50% copper). Texcide was latex paint carrier (1 Glidden was and ceiling 9020 white vinyl acrylic latex paint : 1 water) containing 100 grams/liter (1X) or 25 grams/liter (.25X) Kocide 101 wp.

[w] Root pouches were fabricated from Agryl spun-bonded polyolefin (36 $g \cdot m^2$).

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Chapter 5

Copper Increased VA Mycorrhizal Colonization of Corn.

Additional index words. Copper hydroxide, $Cu(OH)_2$, copper toxicity, VAM, *Glomus intradix*, peat-vermiculite media, corn, *Zea mays* L., root growth and development or regulation.

Abstract

Fabric root pouches were treated with either of two copper hydroxide paint mixtures, Spinout or Texcide, and the effects of the treatments on mycorrhizal colonization of corn (*Zea mays* L.) roots by the vesicular-arbuscular mycorrhizal fungus *Glomus intraradix* and on plant growth were evaluated. Treating fabric root pouches with either copper treatment significantly increased the percentage of corn root length colonized by the VA endomycorrhizal fungus without adversely affecting plant shoot growth. Both copper treatments significantly increased root density and uniformity of root distribution in the root pouches.

Introduction

Fabric root pouches are a component of an innovative, new plant production system, the Closed, Insulated Pallet System (CIPS) (Kaplan, 1992). The CIPS provides an enclosed root environment with a water reservoir in the pallet base. Fabric pouches allow water movement and air exchange; however, a chemical treatment is necessary to prevent

roots from penetrating some fabrics. A coating of $Cu(OH)_2$-latex paint prevented root penetration (Blackburn et al., 1992a), but the effect of Cu on VAM in fabric root pouches and organic potting media was unknown.

Use of copper-treated containers in forestry nurseries to control root growth was reported by Saul (1968). Several papers reported ectomycorrhizal fungus colonization of tree roots was increased by copper treatment. Short roots, roots which are not elongating rapidly, and have small, poorly differentiated steles, are primary sites of ectomycorrhizal colonization (Bogar and Smith, 1965; Wilcox, 1967). Greater numbers of short roots and greater ectomycorrhizal colonization occurred on ponderosa pine seedlings grown in copper-latex coated containers than on seedlings grown in untreated containers (McDonald et al., 1984). Likewise, the ectomycorrhizal fungus *Thelephora terrestris* produced more extraradical mycelium and mycelial strands on lodgepole pine when seedlings were planted in Cu-treated containers (Hunt, 1990). However, the effect of $CuCO_3$-latex-treated containers on colonization by the ectomycorrhizal fungus *Pisolithus tinctorius* varied; depending on host-tree species, colonization was increased, decreased, or unaffected (Ruehle 1985). Since the effect of copper-treated containers on ectomycorrhizae varied, one might expect the effect on endomycorrhizae may also vary.

There have been several studies on VAM in soils with toxic concentrations of heavy metals, although no published reports on the effects of Cu-treated containers on vesicular-arbuscular mycorrhizae were found. Mycorrhizae decreased plant uptake of heavy metals (Koslowsky and Boerner, 1989) and decreased phytotoxic effects (Dueck et al., 1986). Decreased phytotoxicity may have resulted from improved phosphorus nutrition of mycorrhizal plants compared to nonmycorrhizal controls; increased growth of mycorrhizal plants lowered or diluted the concentration of the metal in plant tissues. However, Graham et al. (1986) showed that in mycorrhizal and nonmycorrhizal Carrizo citrange

seedlings of similar size and P-status, mycorrhizal seedlings were more sensitive to Cu toxicity than uninoculated seedlings. When 0 to 300 $\mu g \cdot g^{-1}$ copper sulfate was uniformly incorporated into soil, colonization by *Glomus intraradix* and seedling growth were reduced logarithmically with Cu concentration. Copper concentrations of 19 and 34 $\mu g \cdot g^{-1}$ of soil were phytotoxic to inoculated and uninoculated seedlings, respectively.

The availability of copper in soils, and therefore its effect on VAM and on plants, is dependent upon the pH, CEC and organic matter. At a soil pH below 5.0, high soil concentrations of copper reduced VAM colonization and caused phytotoxicity symptoms on citrus, but when pH was near 7.0, tree productivity and mycorrhizae increased (Graham et al., 1986). Copper is tightly bound to cation exchange sites between pH 7.0 and 8.0 (Reuther and Labanauskas, 1966). High concentrations of copper are less toxic in organic media than in mineral soils since it is rapidly fixed by organic matter (Hesse, 1972; Kuhns and Sydnor, 1976).

The objective of this experiment was to test the effects of copper applied to the root-container interface on plant growth and colonization of roots by a VA mycorrhizal fungus.

METHODS AND MATERIALS

The experiment included three copper root regulation treatments (2 copper formulations and an untreated control) and two mycorrhizal treatments (+/- mycorrhizal plants) in a completely randomized factoral design with two replications and four samples per replication; media strata were subplots within each replication. The effects and posible interactions of copper and mycorrhizal inoculum on plant shoot growth (shoot height, grams fresh shoot weight), plant root growth (root density and distribution by strata), and on mycorrhizal colonization (% root length infected within each stratum) were statistically evaluated using a factorial analysis of variance.

The experiment was conducted in a greenhouse under high-pressure sodium-vapor lights with a minimum photon flux density of 200 $\mu mol\,m^{-2}s^{-1}$. Air temperatures were 21/18C (day/night). Seeds of *Zea mays* L. cv Golden Jubilee were planted on 15 October, 1991 in trays with 21-cm^3 cells filled with a 1 peat: 1 vermiculite (by volume) media with 3.0 $kg\,m^{-3}$ dolomite. The tray receiving the mycorrhizal treatment had 100 $g\,liter^{-1}$ *Glomus intraradix* preplant incorporated uniformly into the media. *Glomus intraradix* inoculum (Nutri-Link, Native Plants, Inc., Salt Lake, UT) is spores of the fungus embedded in a clay matrix which disintegrates in water. The inoculum rate used was based on previous experiments (Blackburn et al., 1992b). Thirty days after germination (13 November, 1991), seedlings were evaluated for mycorrhizal colonization. The terminal one centimeter of each root ball was clipped and the roots washed free of media. Root samples were cleared and stained following the procedures described in the **Data Collection** section. Twenty-five random root segments were examined; seedlings were considered to be mycorrhizal or nonmycorrhizal based on presence or absence of hyphae and vesicles in one or more of the root segments.

Seedlings were transplanted on Day 35 (18 November, 1991) into 3-liter volume fabric pouches constructed from a spunbonded/meltblown/spunbonded laminate of a nonwoven, polypropylene fabric treated with a wetting agent (KC 10NW401, 44.1 $g m^{-2}$, Kimberly-Clark, Roswell, GA) containing a 1 peat : 1 perlite (by volume) media amended with 3.0 $kg m^{-3}$ dolomite. The pouches had previously received one of three copper treatments: 1) a "Texcide" coating, a solution of 0.5 liter latex paint and 0.5 liter water with 104.8 g technical grade $Cu(OH)_2$ suspended in it, 2) a Spinout coating (Spinout, Griffin Corp., Valdosta, GA), or 3) an untreated fabric pouch (Control). After transplanting seedlings into pouches, water was applied to the top surface of the media to thoroughly moisten the media; gravitational water was allowed to drain from the pouches for 24 hours prior to placement into the CIPS.

Styrofoam chests were modified to serve as closed, insulated pallet systems (CIPS) to provide a stable root environment. The inside dimensions of the CIPS were 80 cm long x 25 cm wide x 25 cm high. The CIPS had 3.5 cm thick sidewalls and bottom and 4.0 cm thick lids. Four 15-cm diameter holes were cut in the lids to accommodate the plants/pouches. Two observation ports, 15 cm long x 5 cm high, were cut 15 cm above the bottom of each CIP to monitor the water level. The CIPs were covered with opaque, solar-reflective aluminum foil. Rockwool blocks were placed in the chests as platforms for the plant pouches and as capillary wicks, and the CIPs were filled with approximately 30 liters of water. Based on previous experience, the water levels were initially raised one centimeter above the tops of the rockwool platforms to insure that capillarity was established between the rockwool and the root pouches. As capillary uptake of water in the media reached equilibrium, the water level in the CIPs fell to just below the tops of the platforms.

On Day 35, after root pouches with corn plants were placed in the CIPS (four plants/CIP), 19.8 g fertilizer (Osmocote 18N-2.6P-9.9K), was placed on the top surface of the media. A 2.5 cm styrofoam disk was placed around each plant's stem, and the top of each CIP was sealed with adhesive tape around each of the corn plants extending through it to minimize moisture and heat exchange between the root and shoot environments.

Data Collection

The experiment was terminated on Day 43, 31 December, 1991. Plant shoot growth (grams dry weight and height), root development and mycorrhizal colonization were determined.

The root media was divided into three strata (strata 1 = 0 to 5 cm, 2 = 5 to 10 cm, and 3 = 10 to 15 cm from the top surface of the media, respectively). Vertical root quantity on the periphery of each stratum sample was visually rated on a scale of 1 to 3, with 3 being the greatest number of roots and 1 being the least. Horizontal distribution uniformity across each stratum was visually rated on a scale of 1 to 3, with 3 being the most uniform distribution across the stratum. The vertical (V) and horizontal (H) ratings for each stratum were multiplied to give a V*H root rating, the highest possible VH score being 9.0.

Roots in each of the three strata were washed free of potting media, then cut into 1 cm segments to evaluate mycorrhizal colonization. Root samples were cleared and stained following the procedures of Phillips and Hayman (1970) and Kormanik et al. (1980) as modified by Davis et al. (1983) using lacto-glycerol in the place of lacto-phenol. Mycorrhizal colonization was estimated by the percentage of the root segment length with vesicles present (Biermann and Linderman (1981); 50 root segments were scored per sample.

Results and Discussion

There were no significant interactions among copper and mycorrhizal treatments. Copper had a significant effect on uniformity and density of root distribution ($P < 0.05$), Table 5.1. Root distribution in pouches treated with Texcide and Spinout were not significantly different from each other (V*H = 8.1 and 7.8, respectively on a scale of 1 to 9). But, root density was greater and roots were more evenly distributed in pouches receiving either of the copper treatments than in pouches not receiving a copper treatment (V*H for Control = 5.2). Similar effects of copper on root development were noted by others (Arnold and Struve, 1989a; Burdett and Martin, 1982; McDonald et al., 1984; Ruehle, 1985).

Roots at the periphery of the root ball were in contact with the copper-treated fabric pouch and exhibited symptoms of minor copper phytotoxicity, i.e. darkened, bulbous root tips which curled away from the treated fabric. Similar symptoms were reported by Reuther and Labanauskas (1966). When root tip growth was inhibited, higher-order lateral roots developed and, in turn, their growth was arrested when the laterals reached the treated surface, as was also reported by Burdett (1978) and McDonald et al. (1984). In research by Arnold and Struve (1989b), none of the inhibited root tips were dead. Within 3 to 6 days after removal from the treated containers, the roots resumed normal elongation rates.

Root distribution varied significantly among strata ($P < 0.001$). Strata 1 and 2 root ratings were not different from each other, but both were significantly greater than stratum 3 (V*H = 7.7, 7.4, and 6.1, respectively). The difference in root distribution between strata may be related to the change in water content of the media and/or the change in nutrient concentration. In the CIPS, the volumetric water content of the media decreased with increasing height above the water surface; the

slow-release fertilizer topdressed on the surface of the potting media diffused downward, creating a nutrient gradient. Roots proliferate in increments of the gradient where optimal conditions for root development occur (Geraldson, 1970).

The effect of copper treatments on mycorrhizal formation (Table 5.2) was not significant ($P = 0.058$). However, very little colonization occurred in stratum 3 (S3 = 1.1%). When stratum 3 was removed from the analysis of variance, the effect of copper treatment was significant ($P = 0.029$). Spinout significantly increased the root length colonized compared to the control (24.7% vs 19.9%), and Texcide increased colonization more than did Spinout (29.2% vs 24.7%). Colonization was significantly different among strata: 35.4% in stratum 1 was significantly greater than 7.2% in stratum 2, $P < 0.01$).

Based on the report by Hatch and Doak (1933) that mycorrhizal fungi can only successfully colonize unlignified roots, McDonald et al. (1984) hypothesized that the increased numbers of lateral roots which result from the Cu treatment provide a greater number of unlignified root initials for mycorrhizal fungus colonization. Pine seedlings in the $CuCO_3$ treatment had 1.4 times more roots colonized by an ectomycorrhizal fungus than seedlings in the control treatment. In the present experiment, plants grown in copper-treated containers had higher percentages of root lengths colonized by a VA mycorrhizal fungus than plants grown in untreated containers. Compared to controls, plants grown in treated containers also had higher root density and uniformity of root distribution. However, our data does not prove that the increase in mycorrhizae resulted from an increase in the number of root tips since numbers of roots and root tips were not counted.

Neither main factors, mycorrhizal fungus inoculation or copper treatments, affected plant shoot growth. There were no differences in shoot dry weight or plant height regardless of treatment combination. Because the plants were grown under conditions of high fertility, a

plant growth response to the mycorrhizae was not expected. The seedling root plug containing colonized roots and inoculum was planted in Stratum 2. When the root-media matrices were divided into strata, the peat-vermiculite media of the seedling plugs was consistently found in Stratum 2. Yet, the greatest amount of mycorrhizal colonization occurred in Stratum 1 (35.4%, 7.2%, and 1.1%, respectively, for S1, S2, and S3; $P = 0.001$). Plant roots extended beyond the inoculated root plug after the corn seedlings were transplanted, and development of mycorrhizae was significantly greater in new root growth in Stratum 1 which had the highest concentrations of plant nutrients. We do not know if vesicles and hyphae developed internally as the roots extended from Stratum 2 into Stratum 1, or if extraradical hyphae grew through the potting media, then colonized the roots. A similar pattern of greater mycorrhizae in strata 1 and 2 and no significant mycorrhizal formation in stratum 3 is consistent with VAM colonization patterns of *Cotoneaster repens* grown in CIPS (Briggs, 1991).

Both copper treatments significantly increased the percentage of corn root length colonized by the VA endomycorrhizal fungus *Glomus intraradix* without adversely affecting plant shoot growth. Both copper treatments also significantly increased the root density and uniformity of root distribution in the pouches.

Growing plants in copper-treated root pouches may increase survival and growth of plants after transplanting. Arnold and Struve (1989a) reported more root and shoot growth and fewer symptoms of plant stress in green ash transplanted to the landscape from $CuCO_3$-treated containers than in root-pruned plants transplanted from untreated containers.

Table 5.1. Effects of copper and strata on root development (VH).

Effect of Cu		Effect of Strata	
Cu Treatment[z]	Mean VH[y]	Stratum[x]	Mean VH
Control	5.2 a[w]	1	7.7 a
Spinout	7.8 b	2	7.4 a
Texcide	8.1 b	3	6.1 b

[z]Control = a fabric root pouch, 3-liter, without copper treatment. Spinout = a fabric pouch treated with Spinout, a copper-containing liquid formulation. Texcide = a fabric pouch treated with Texcide, 100 $g \cdot liter^{-1}$ $Cu(OH)_2$ suspended in a solution of 1 latex paint : 1 water (v/v).

[y]The vertical root density, from 1 to 3, 3 being the most dense, multiplied by the horizontal root distribution, from 1 to 3, 3 being the most uniformly distributed across the stratum horizontally. The maximum V*H rating is 9. Each value represents the mean of two replicates, four plants per replicate.

[x]Strata 1, 2, and 3 were 0 to 5 cm, 5 to 10 cm, and 10 to 15 cm from the upper surface of the media, respectively.

[w]Means within a column followed by different letters are significantly different from each other (P = 0.05)

Table 5.2. Effects of copper and strata on mycorrhizal colonization (ANOVA of data from strata 1 and 2 of inoculated treatments.)

Effect of Cu		Distribution by Strata	
Cu Treatment[x]	% Colonized[y]	Stratum[z]	% Colonized
Control	19.9 a[w]	1	35.7 a
Spinout	24.7 b	2	13.5 b
Texcide	29.2 b		

[x]Control = a fabric root pouch, 3-liter, without copper treatment. Spinout = a fabric pouch treated with Spinout, a copper-containing liquid formulation. Texcide = a fabric pouch treated with Texcide, 100 g·liter^{-1} $Cu(OH)_2$ suspended in a solution of 1 latex paint : 1 water (v/v).

[y]The mean percentage of root length colonized with vesicles and hyphae present in 50 one-cm root segments. Each value represents the mean of two replicates, four plants per replicate. 100 g·liter^{-1} *Glomus intraradix* inoculum added (+M) or not added (-M) to media in planting trays.

[z]Strata 1, 2, and 3 were 0 to 5 cm, 5 to 10 cm, and 10 to 15 cm from the upper surface of the media, respectively.

[w]Means within a column followed by different letters are significantly different from each other (P = 0.05)

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APPENDICES

Appendix A

Tables of Values Corresponding to Graphs in Chapter 3

Table A.1. The effect of a plant on the electrical conductivity and concentrations of nitrogen as nitrate and potassium in the media. Values are averaged over Day and Strata. This table contains the data graphically presented in Figures 3.2, 3.3, and 3.4.

Dependent Variables	Units	Treatments[z]	
		-Plant	+Plant
K	$mol \cdot kg^{-1}$	0.4950[y]	0.4182
NO_3-N	$mol \cdot kg^{-1}$	0.3017	0.1968
EC	$mS \cdot cm^{-1}$	11.67	8.87

[z]Both treatments received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. In the +Plant treatment, a 17-day old 'Golden Jubilee' corn seedling was transplanted into each of the pouches.
[y]Each value is the mean of 96 replicates.

Table A.2. Potassium concentrations of different media strata and days averaged over Treatment (+ or - plant). This table contains the data graphically presented in Figure 3.5.

Stratum[z]	Days until harvested			
	30	59	88	120
	$mol \cdot kg^{-1}$			
1	1.2150[y]	1.0109	0.7913	0.5748
2	0.6216	0.6273	0.5433	0.4284
3	0.1893	0.2214	0.2953	0.2326
4	0.1120	0.1102	0.1523	0.1799

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Each value is the mean of 12 replicates.

Table A.3. Concentrations of nitrogen as nitrate for different media strata and days averaged over Treatment (+ or - plant). This table contains the data graphically presented in Figure 3.6.

Stratum[z]	Days until harvested			
	30	59	88	120
	$mol \cdot kg^{-1}$			
1	---	0.7609[y]	0.4083	0.3667
2	---	0.4002	0.3068	0.2262
3	---	0.0928	0.1471	0.1196
4	---	0.0390	0.0508	0.0724

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Each value is the mean of 12 replicates.

Table A.4. Electrical conductivity of media averaged over treatment (+ and - plant). This table contains the data graphically presented in Figure 3.7.

Stratum[z]	Days until harvested			
	30	59	88	120
	$mS \cdot cm^{-1}$			
1	27.16[y]	23.43	17.44	13.30
2	12.47	14.27	12.44	10.15
3	3.80	5.35	6.66	5.43
4	2.98	2.70	3.10	3.69

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Each value is the mean of 12 replicates.

Table A.5. The pH values of media with or without fertilizer topdressed on the surface. This table contains the data graphically presented in Figures 3.8 and 3.9.

Stratum[z]	Days until harvested			
	30	59	88	120
	-log[H^+]			
-Fertilizer[y]				
1	6.38[x]	6.35	6.72	6.89
2	6.33	6.57	6.98	7.13
3	6.42	7.02	7.20	7.46
4	6.87	6.95	7.08	7.33
+Fertilizer				
1	6.02	5.67	5.70	6.31
2	6.25	5.52	5.60	6.31
3	5.97	6.07	6.42	6.87
4	6.55	6.52	6.65	6.87

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Values in the -Fertilizer treatment indicate the native concentration of hydrogen ions in the growing media. The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch.
[x]Each value is the mean of six replicates.

Table A.6. The pH values of media with or without a corn plant in the root pouch. This table contains the data graphically presented in Figures 3.9 and 3.10.

Stratum[z]	Days until harvested			
	30	59	88	120
	-log[H^+]			
	-Plant[y]			
1	6.02[x]	5.67	5.70	6.31
2	6.25	5.52	5.60	6.31
3	5.97	6.07	6.42	6.87
4	6.55	6.52	6.65	6.87
	+Plant			
1	6.87	7.52	7.62	7.71
2	7.73	7.88	7.82	7.81
3	7.23	7.54	7.53	7.73
4	6.87	6.68	7.18	7.65

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Both treatments received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. In the +Plant treatment, a 17-day old 'Golden Jubilee' seedling was transplanted into each of the pouches.
[x]Each value is the mean of six replicates.

Table A.7. Water content of media of different strata in root pouches with or without fertilizer topdressed on the media surface averaged over Strata. This table contains the data graphically presented in Figure 3.11.

Day[y]	Treatment[x]	
	-Fertilizer	+Fertilizer
	g $H_2O \cdot g^{-1}$ dry wt.	
30	4.34[x]	4.44
59	4.47	4.47
88	4.74	4.56
120	5.00	4.78

[z]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO3 placed on the media surface of the root pouch.
[y]Days from the beginning of the experiment until harvested.
[x]Each value is the mean of 24 replicates.

Table A.8. Water content of media in different strata in root pouches with or without fertilizer topdressed on the media surface averaged over Day.

Stratum[y]	Treatment[z]	
	-Fertilizer	+Fertilizer
	g $H_2O \cdot g^{-1}$ dry wt.	
1	3.95[x]	3.65
2	4.17	4.10
3	4.77	4.84
4	5.66	5.66

[z]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch.
[y]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[x]Each value is the mean of 24 replicates.

Table A.9. Water content of media for different strata and days averaged over Treatment (+ or - fertilizer[z]).

Stratum[y]	Days until harvested			
	30	59	88	120
	g $H_2O \cdot g^{-1}$ dry wt.			
1	3.64[x]	3.73	3.82	4.01
2	3.95	4.03	4.13	4.42
3	4.53	4.60	4.78	5.32
4	5.45	5.53	5.86	5.81

[z]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch; the -Fertilizer treatment received no KNO_3.
[y]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[x]Each value is the mean of 12 replicates.

Table A.10. Water content of media for different strata and days averaged over Treatment (+ or - plant). This table contains the data graphically presented in Figure 3.12.

Stratum[z]	Days until harvested			
	30	59	88	120
	g $H_2O \cdot g^{-1}$ dry wt.			
1	3.39[y]	3.47	3.66	3.84
2	4.22	4.06	4.15	4.50
3	4.91	4.83	5.04	5.55
4	5.61	5.68	5.92	6.00

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]Each value is the mean of 12 replicates.

Table A.11. Water content of media of different strata in root pouches with or without a corn plant present averaged over Day. This table contains the data graphically presented in Figure 3.13.

Stratum[y]	Treatment[z]	
	-Plant	+Plant
	g $H_2O \cdot g^{-1}$ dry wt.	
1	3.65[x]	3.53
2	4.10	4.37
3	4.84	5.32
4	5.66	5.94

[z]Both treatments received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. In the +Plant treatment, a 17-day old 'Golden Jubilee' corn seedling was transplanted into each of the pouches.
[y]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[x]Each value is the mean of 24 replicates.

Table A.12. The effects of the presence of a plant, time, and media depth on the population density of *Trichoderma harzianum* WT6-6 in CIPS. This table contains the data graphically presented in Figures 3.14, 3.15, 3.16.

Main Effects		n[x]	*T. harzianum* Population
			cfu·g^{-1} dry wt.
Treatment[y]		96	
	-Plant		1.56E+06
	+Plant		3.00E+06
Day[x]		48	
	30		3.80E+06
	59		2.02E+06
	88		1.94E+06
	120		1.36E+06
Strata[w]		48	
	1		4.22E+06
	2		2.35E+06
	3		8.98E+05
	4		1.65E+06

[x]Sample size for the means.
[y]Both treatments received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. In the +Plant treatment, a 17-day old 'Golden Jubilee' corn seedling was transplanted into each of the pouches.
[x]Days from the beginning of the experiment until harvested.
[w]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.

Table A.13. Populations of *Trichoderma harzianum* WT6-6 in the media with or without fertilizer[y] topdressed on the surface. This table contains the data graphically presented in Figures 3.18 and 3.19.

Stratum[z]	Days until harvested			
	30	59	88	120
	cfu·g^{-1} dry wt.			
-Fertilizer[y]				
1	2.52E+06[x]	8.68E+05	6.49E+05	2.66E+05
2	1.87E+06	8.24E+05	3.74E+05	2.41E+05
3	2.45E+06	9.50E+05	5.03E+05	3.58E+05
4	4.50E+07	2.17E+07	6.98E+06	1.44E+06
+Fertilizer				
1	4.78E+06	3.92E+06	3.48E+06	1.06E+06
2	3.73E+06	1.28E+06	1.70E+06	5.71E+05
3	1.43E+06	6.82E+05	4.62E+05	2.28E+05
4	1.03E+06	2.20E+05	3.12E+05	1.14E+05

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch.
[x]Each value is the mean of six replicates.

Table A.14. Concentrations of nitrogen as nitrate in the media with or without fertilizer topdressed on the surface.

Stratum[z]	Days until harvested			
	30	59	88	120
	mol N·kg^{-1}			
-Fertilizer[y]				
1	---	0.0571[x]	0.0492	0.0347
2	---	0.0440	0.0232	0.0184
3	---	0.0185	0.0069	0.0044
4	---	0.0032	0.0007	0.0012
+Fertilizer				
1	---	0.8370	0.4674	0.4708
2	---	0.4133	0.3821	0.2841
3	---	0.1421	0.1877	0.1635
4	---	0.0776	0.0943	0.1007

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. Values in the -Fertilizer treatment indicate the native concentration of the nutrient in the growing media.
[x]Each value is the mean of six replicates.

Table A.15. Concentrations of potassium in the media with or without fertilizer topdressed on the surface.

Stratum[z]	Days until harvested			
	30	59	88	120
	mol $K \cdot kg^{-1}$			
-Fertilizer[y]				
1	0.0433[x]	0.0403	0.0377	0.0378
2	0.0282	0.0310	0.0303	0.0295
3	0.0215	0.0220	0.0235	0.0235
4	0.0170	0.0162	0.0175	0.0160
+Fertilizer				
1	1.3382	1.0427	0.8068	0.5913
2	0.6170	0.6235	0.5615	0.4318
3	0.2123	0.2958	0.3310	0.2905
4	0.1817	0.1720	0.2105	0.2133

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. Values in the -Fertilizer treatment indicate the native concentration of the nutrient in the growing media.
[x]Each value is the mean of six replicates.

Table A.16. Electrical conductivity of the media with or without fertilizer topdressed on the surface.

Stratum[z]	Days until harvested			
	30	59	88	120
	$mS \cdot cm^{-1}$			
-Fertilizer[y]				
1	3.86[x]	3.89	3.41	3.36
2	3.48	3.11	2.41	2.19
3	2.48	1.80	1.48	1.33
4	1.63	1.05	0.78	0.82
+Fertilizer				
1	30.55	24.65	19.42	14.52
2	15.53	14.49	13.39	10.63
3	5.56	6.56	7.23	6.54
4	4.70	4.06	4.43	4.55

[z]Strata 1 to 4 are 0 to 2.5 cm, 2.5 to 5 cm, 5 to 10, and 10 to 15 cm from the top surface of the media in the root pouches, respectively.
[y]The +Fertilizer treatment received 24.3 g (0.240 mol) soluble KNO_3 placed on the media surface of the root pouch. Values in the -Fertilizer treatment indicate the native concentration of the nutrient in the growing media.
[x]Each value is the mean of six replicates.

Appendix B

Analysis of Variance Tables for Chapter 3.

Table B.1. ANOVA of the effect of fertilizer on colony-forming units of *Trichoderma harzianum* WT6-6 per gram of dry media.

Source	df	MS	F	P
Treatment (T)	1	7.201E+14	18.76	0.0001
Day (D)	3	4.903E+14	12.77	0.0001
T x D	3	2.619E+14	6.82	0.0008
Error	40	3.838E+13		
Strata (S)	3	8.072E+14	21.75	0.0001
T x S	3	1.132E+15	30.50	0.0001
D x S	9	2.418E+14	6.52	0.0001
T x D x S	9	2.780E+14	7.49	0.0001
Error	120	3.711E+13		

Table B.2. ANOVA of the effect of fertilizer on water content of the growing media.

Source	df	MS	F	P
Treatment (T)	1	0.2723	3.86	0.0564
Day (D)	3	2.3525	33.35	0.0001
T x D	3	0.2687	3.81	0.0172
Error	40	0.0705		
Strata (S)	3	32.3837	906.56	0.0001
T x S	3	0.3144	8.80	0.0001
D x S	9	0.1662	4.65	0.0001
T x D x S	9	0.0641	1.79	0.0761
Error	120	0.0357		

Table B.3. ANOVA of the effect of fertilizer on the pH of the growing media.

Source	df	MS	F	P
Treatment (T)	1	20.2735	294.73	0.0001
Day (D)	3	3.2995	47.97	0.0001
T x D	3	0.8093	11.77	0.0001
Error	40	0.0688		
Strata (S)	3	3.8277	231.69	0.0001
T x S	3	0.3667	22.19	0.0001
D x S	9	0.2689	16.28	0.0001
T x D x S	9	0.1602	9.70	0.0001
Error	120	0.0165		

Table B.4. ANOVA of the effect of fertilizer on the EC of the growing media.

Source	df	MS	F	P
Treatment (T)	1	4204.1845	506.42	0.0001
Day (D)	3	77.3924	9.32	0.0001
T x D	3	35.5417	4.28	0.0103
Error	40	8.3019		
Strata (S)	3	1010.5434	333.74	0.0001
T x S	3	583.4972	192.71	0.0001
D x S	9	32.8785	10.86	0.0001
T x D x S	9	36.1611	11.94	0.0001
Error	120	3.028		

Table B.5. ANOVA of the effect of fertilizer on nitrate-nitrogen concentration in the growing media.

Source	df	MS	F	P
Treatment (T)	1	28210.282	239.43	0.0001
Day (D)	2	535.657	4.55	0.0189
T x D	2	306.727	2.60	0.0907
Error	30	117.821		
Strata (S)	3	5374.309	82.90	0.0001
T x S	3	3747.177	57.80	0.0001
D x S	6	377.294	5.82	0.0001
T x D x S	6	355.874	5.49	0.0001
Error	90	64.825		

Table B.6. ANOVA of the effect of fertilizer on the potassium concentration in the growing media.

Source	df	MS	F	P
Treatment (T)	1	105037.94	796.73	0.0001
Day (D)	3	932.48	7.07	0.0006
T x D	3	918.21	6.96	0.0007
Error	40	131.84		
Strata (S)	3	14483.77	230.51	0.0001
T x S	3	12889.51	205.14	0.0001
D x S	9	842.44	13.41	0.0001
T x D x S	9	812.78	12.94	0.0001
Error	120	62.83		

Table B.7. ANOVA of the effect of a plant on colony-forming units of *Trichoderma harzianum* WT6-6 per gram of dry media.

Source	df	MS	F	P
Treatment (T)	1	9.918E+13	7.99	0.0073
Day (D)	3	5.360E+13	4.32	0.0100
T x D	3	4.141E+12	0.33	0.8012
Error	40	1.242E+13		
Strata (S)	3	9.740E+13	24.27	0.0001
T x S	3	9.699E+12	2.42	0.0697
D x S	9	4.150E+12	1.03	0.4170
T x D x S	9	7.287E+12	1.82	0.0720
Error	120	4.013E+12		

Table B.8. ANOVA of the effect of a plant on water content of the growing media.

Source	df	MS	F	P
Treatment (T)	1	2.4820	25.40	0.0001
Day (D)	3	2.1957	22.47	0.0001
T x D	3	0.2019	2.07	0.1200
Error	40	0.0977		
Strata (S)	3	44.9406	1209.79	0.0001
T x S	3	0.7587	20.42	0.0001
D x S	9	0.1352	3.64	0.0005
T x D x S	9	0.0561	1.51	0.1519
Error	120	0.0372		

Table B.9. ANOVA of the effect of a plant on the pH of the growing media.

Source	df	MS	F	P
Treatment (T)	1	75.6891	869.82	0.0001
Day (D)	3	2.4422	28.07	0.0001
T x D	3	0.6794	7.81	0.0003
Error	40	0.0870		
Strata (S)	3	0.5595	21.55	0.0001
T x S	3	4.4897	172.90	0.0001
D x S	9	0.2463	9.49	0.0001
T x D x S	9	0.3723	14.34	0.0001
Error	120	0.0260		

Table B.10. ANOVA of the effect of a plant on the EC of the growing media.

Source	df	MS	F	P
Treatment (T)	1	376.6650	34.00	0.0001
Day (D)	3	124.6313	11.25	0.0001
T x D	3	25.7206	2.32	0.0897
Error	40	11.0785		
Strata (S)	3	2899.5663	688.25	0.0001
T x S	3	6.7068	1.59	0.1949
D x S	9	128.1652	30.42	0.0001
T x D x S	9	3.7869	0.90	0.5287
Error	120	4.213		

Table B.11. ANOVA of the effect of a plant on nitrate-nitrogen concentration in the growing media.

Source	df	MS	F	P
Treatment (T)	1	3964.507	22.36	0.0001
Day (D)	2	2094.947	11.81	0.0002
T x D	2	26.196	0.15	0.8633
Error	30	177.337		
Strata (S)	3	15323.622	173.79	0.0001
T x S	3	127.900	1.45	0.2334
D x S	6	1522.991	17.27	0.0001
T x D x S	6	56.373	0.64	0.6984
Error	90	88.175		

Table B.12. ANOVA of the effect of a plant on the potassium concentration in the growing media.

Source	df	MS	F	P
Treatment (T)	1	2832.54	14.22	0.0001
Day (D)	3	2882.08	14.47	0.0006
T x D	3	59.05	0.30	0.0007
Error	40	199.22		
Strata (S)	3	56788.81	510.49	0.0001
T x S	3	270.25	2.43	0.0001
D x S	9	2562.57	23.04	0.0001
T x D x S	9	121.49	1.09	0.0001
Error	120	111.24		

Appendix C

Water Release Characteristics of Different Media.

The amount of water contained in a growing media affects plant growth. Water release characteristics of 10 components and mixtures of horticultural potting media were evaluated (Table C.1).

Pieces of cheesecloth were secured with a rubberband to soil rings, 3 cm x 5 cm dia, which were then packed with media, three replicates each, following the procedures of Bilderback et al. (1982). Grodan rockwool slabs were cut to the size of the soil rings. Soil cores and rockwool slabs were allowed to saturate for 24 h in a tray filled with water to a 1-cm depth. A Chehalis silt loam (9% clay, 12% sand, 79% silt), which had known water release characteristics and was routinely used in the Soil Physics Lab, was included as a control. An empty soil ring with cheesecloth and rubberband (Blank) was included so that the volumetric water content of samples could be corrected for the amounts of water adhering to the metal soil ring and the cheesecloth.

Water-release characteristics for the potting mixes were determined at water potentials of -0.0003, -0.001, -0.005, -0.01, -0.08, -0.2, and -1.5 MPa. The five less negative water potentials (-0.0003, -0.001, -0.003, -0.005, and -0.007 MPa) were ran on a tension table similar to the low-range system described by Klute (1986) but at atmospheric pressure. Negative tension was applied by changing the elevation of the reference bottle (DeBoodt et al., 1974). Water-release characteristics for the more negative water potentials (-0.01, -0.08, and -1.50 MPa) were measured using porous ceramic plates in pressure chambers following the procedures of Klute (1986).

After determining water release characteristics of the media, the soil rings and media were dried in an oven at 60C for 3 days. Bulk densities and volumetric water contents were then calculated.

Table C.1. Volumetric water contents[z] of different media components and mixes at various free energies.

Media and Components[y]	Free Energy State[x] (cm H_2O)						
	3 cm	10 cm	30 cm	50 cm	70 cm	100 cm	800 cm
1:1 Peat:Perl	0.55	0.36	0.33	0.24	0.25	0.21	0.17
2:1 Peat:Perl	0.57	0.39	0.35	0.24	0.24	0.21	0.15
1:1 Peat:Vermic	0.65	0.45	0.41	0.31	0.30	0.28	0.22
2:1 Peat:Vermic	0.64	0.46	0.40	0.29	0.28	0.27	0.20
2:1 Peat:RW	0.70	0.52	0.44	0.21	0.20	0.18	0.12
Chehalis	0.56	0.52	0.42	0.43	0.39	0.40	0.31
Blank	0.03	0.02	0.01	0.01	0.01	0.01	0.00
Rockwool	0.94	0.67	0.52	0.03	0.07	0.01	0.00
Perlite	0.51	0.35	0.28	0.31	0.27	0.23	0.30
RW	0.53	0.37	0.22	0.13	0.07	0.02	0.00
1:1 Perl:RW	0.61	0.42	0.31	0.30	0.23	0.17	0.20
2:1 Perl:RW	0.56	0.39	0.30	0.30	0.24	0.18	0.23
Bark media	0.77	0.46	0.41	0.39	0.35	0.31	0.28
Chehalis	0.55	0.46	0.49	0.42	0.39	0.37	0.31
Blank	0.06	0.04	0.03	0.03	0.02	0.02	0.02

[z]Volumetric water contents are the volume of water/ the total volume. Each value is the mean of three replicates.
[y]Media and components: Peat = Sunshine peat moss, Perl = coarse, horticultural grade perlite, Vermic = TerraLite vermiculite, RW = Grodan rockwool cut into 1-cm^3 cubes, Rockwool = Grodan rockwool slab cut to fit the soil ring, Chehalis = a Chehalis Silt Loam with known characteristics used as a reference soil, Blank = a brass soil ring, approximately 5 cm diameter x 3 cm high, with cheese cloth secured by a rubberband. All mix ratios are by volume.
[x]Free energy states: 3, 10, 30, 50, 70, 100, and 800 cm are equivalent to 0.0003, 0.001, 0.003, 0.005, 0.007, 0.01, and 0.08 MPa.

Table C.2. Bulk densities of media and components.

Media and Components[z]	Bulk Density[y] ($g \cdot cm^3$)
1:1 Peat:Perlite	0.074
2:1 Peat:Perlite	0.065
1:1 Peat:Vermiculite	0.077
2:1 Peat:Vermiculite	0.070
2:1 Peat:Rockwool cubes	0.062
Chehalis silt loam reference soil	1.012
Rockwool slab	0.068
Perlite	0.108
Rockwool cubes	0.030
1:1 Perlite:Rockwool cubes	0.102
2:1 Perlite:Rockwool cubes	0.100
Bark media	0.155
Chehalis silt loam reference soil	0.965

[z]Media and components: Peat = Sunshine peat moss, Perl = coarse, horticultural grade perlite, Vermic = TerraLite vermiculite, RW = Grodan rockwool cut into 1-cm^3 cubes, Rockwool = Grodan rockwool slab cut to fit the soil ring, Chehalis = a Chehalis Silt Loam with known characteristics used as a reference soil, Blank = a brass soil ring, approximately 5 cm diameter x 3 cm high, with cheese cloth secured by a rubberband. All mix ratios are by volume.
[y]Each bulk density is the mean of three replicates.

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Appendix D

Mycorrhizal Colonization of Golden Jubilee Corn Roots Resulting From Addition of Different Amounts of *Glomus intraradix* Inoculum

Introduction

Most VA endophytes have not been reported to be host-specific (Mosse, 1973). Mosse (1968) found *Endogone mosseae* (*Glomus mosseae*) to be able to colonize many different hosts. Gerdemann (1961) reported an unnamed *Endogone* species of fungi which formed vesicular-arbuscular mycorrhizas in corn. Although mycorrhizal fungi have been reported to have a wide host range which includes corn, one of the purposes of this initial experiment was to verify that the species *Glomus intraradix* can colonize *Zea mays* cv. Golden Jubilee. We also wanted to determine the amount of inoculum and the time required to establish mycorrhizae in corn seedlings.

Objectives of the experiment were: 1) to test the ability of *Glomus intraradix* to colonize roots of *Zea mays* cv. Golden Jubilee, 2) to determine the time required for *G. intraradix* to colonize the corn (assuming it can colonize this cultivar), 3) to determine the amount of inoculum required for production of a well-colonized transplant. (Does the amount of inoculum used affect the time required to establish mycorrhizae and does it affect the amount of roots colonized?)

Materials and Methods

Corn seeds, *Zea mays* cv. Golden Jubilee, were rinsed under warm running water to remove Captan fungicide. Each treatment was planted in a separate planting tray, 1 seed per 21-cm^3 cell. Treatments consisted of four rates of *G. intraradix* inoculum (Table D.1) incorporated in a horticultural media of equal volumes of bark, peat, perlite, and sand amended with 3.0 $kg \cdot m^3$ dolomitic lime. No preplant fertilizer was added to the media. The experiment was conducted in a greenhouse from 6 June, 1990 to 18 July, 1990 with 21C day temperatures and 18C night temperatures under ambient light conditions.

At Week 4, all treatments were topdressed with a slow-release, low phosphate fertilizer, Sierra Blend plus Minors (17N-2.6P-9.9K). Ten plants were harvested from each treatment at Weeks 2, 3, 4, and 6. Roots from each plant were cut into 1 cm segments then cleared and stained following the procedures of Phillips and Hayman (1970) and Kormanik et al. (1980) as modified by Davis et al. (1983) using lacto-glycerol in the place of lacto-phenol. Twenty-five randomly selected root segments--those root pieces at grid line intersections--were scored for the percent root length colonized based on the presence of vesicles. The average root length with vesicles and hyphae present were calculated for each plant and for each treatment (Table D.2).

Results and Discussion

At Weeks 2, 3, and 4; no roots were colonized. At Week 4, corn plants in all treatments showed symptoms of nitrogen deficiency. No differences in growth or nutrition were visible among the treatments, no growth responses from mycorrhizal colonization. After fertilizer application at Week 4, the corn plants became greener but never dark green, never completely healthy in appearance.

Corn roots were colonized with *G. intraradix* at Week 6 (Table D.2). One plant in the control treatment (0X) had 4% of its root length colonized. This probably resulted from splash while watering and should be disregarded. The three plants in the 1/2 X treatment (Table D.2) that were recorded as 0 percent colonized actually had vesicles present in some root segments but these colonized root sections did not fall on the intersections of grid lines and were not counted.

Conclusions

G. intraradix was able to colonize *Zea mays* cv. Golden Jubilee, but measurable colonization of the roots did not occur until after 4-6 weeks. It was difficult to maintain adequate moisture and fertilizer to support corn plant growth in the small planting cell, 21 cm3 volume, as the plant became larger. More frequent application of water and fertilizer at rates not deterimental to mycorrhizal development or a larger volume of media with greater moisture and fertilizer reserves might have alleviated the observed nutrient-stress symptoms.

When the percentage of roots colonized at Week 6 for inoculated treatments were compared by regression analysis (Table D.3), there was no significant differences among inoculum rates ($P > 0.05$). These results are similar to results of others (R.G. Linderman,

personal communication). Although there was no differences among inoculation rates, 3 out of 10 plants in the 1/2 X rate were weakly colonized. When I use an inoculated plant in a CIPS experiment, I want to feel certain that mycorrhizae are well established; therefore, I would want to use plants inoculated with the 1 X rate rather than the 1/2 X rate.

Table D.1. Application rate for *Glomus intraradix* inoculum.

Rate	g·liter media
0X	0.0
1/2X	15.0
1X	30.0
2X	60.0

Table D.2. Percent of root length colonized by *G. intraradix* at different rates of inoculum at Week 6.

Rep	0X	1/2X	1X	2X
1	0	0	15.6	49.6
2	4.4	40.4	12.0	14.8
3	0	4.4	63.2	42.8
4	0	43.7	38.8	22.8
5	0	29.2	53.2	44.8
6	0	21.2	53.2	22.8
7	0	18.0	45.6	20.4
8	0	53.2	5.2	14.4
9	0	0	32.4	61.2
10	0	0	48.0	75.6
Mean	0.44	21.01	36.72	36.92
SD	1.39	20.01	19.79	21.11

Table D.3. Analysis of variance for the linear regression of percentage of root length colonized on inoculum rate.

Source	SS	df	MS	F-ratio	P
Model	1265.6	1	1265.6	3.07	0.0906
Error	11539.6	28	412.1		
Total	12805.2	29			

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Appendix E

Clearing and Staining Procedure

Procedure:

Wash roots to remove planting media.
Cut roots into 1 cm segments.
Soak in 10% KOH at ambient room temperature overnight.
Rinse with water.
Soak roots in 1% HCl for approx. 2 h (> 1 h)
Pour off HCl solution
Cover with 0.05% trypan blue in lactoglycerine until stained.
(usually 30 min to 2 h at room temp)
Destain in lactoglycerine if necessary.

Solutions:

10% KOH

100g KOH pellets into 1000 ml DI water

1% HCl

56 ml conc. HCl into 1000 ml DI water

0.05% Trypan Blue Stain

437 ml 85% lactic acid
315 ml glycerin
310 ml DI water
0.5 g trypan blue

Lactoglycerine

87.5 ml 85% lactic acid
63 ml glycerine
62 ml DI water