AN ABSTRACT OF THE THESIS OF

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Title: Effect of Soil Water Pressures on Population Dynamics of *Fusarium equiseti, Gliocladium virens, Talaromyces flavus and Trichoderma viride*, Biocontrol Agents of *Verticillium dahliae* in Potatoes

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Studies were conducted to determine the effect of different osmotic potentials on time required for 95% spore germination, mycelial growth and sporulation of *Verticillium dahliae* and its four known biocontrol agents including *Fusarium equiseti*, *Gliocladium virens*, *Talaromyces flavus* and *Trichoderma viride*. Parameters investigated were qualitatively similar but quantitatively different over the range of osmotic potentials tested. Time for 95% spore germination increased as osmotic potential of the medium decreased from -0.12 to -8.13 MPa and ranged from 8-29, 16->72, 16-46 and 13-60 h for *F. equiseti*, *G. virens*, *T. viride* and *V. dahliae*, respectively. Ascospores of *T. flavus* did not obtain 95% germination even after heat treatment at 70 C for 1 h. Mycelial growth decreased as osmotic potential of the medium decreased. *Fusarium equiseti*, *T. viride* and *V. dahliae* grew at all osmotic

potentials. In contrast, *G. virens* and *T. flavus* did not grow at -8.13 MPa and below -6.17 MPa, respectively. *Fusarium equiseti* produced macroconidia but no chlamydospores at all osmotic potentials. *Gliocladium virens*, *T. viride* and *T. flavus* did not sporulate below -1.10 MPa whereas *V. dahliae* did not produce microsclerotia below -0.59 MPa.

In a greenhouse study, effect of two soil water pressures in combination with *V. dahliae* either alone or with *G. virens* or *F. equiseti* on potato growth, root and vascular infection by *V. dahliae*, and colonization of roots by biocontrol agents was investigated over time. *Fusarium equiseti* reduced colonization of roots by *V. dahliae* and increased foliar dry weight. Although *G. virens* did not have an effect on colonization of roots by *V. dahliae*, it increased both foliar dry weight and ln root/shoot ratio. Fresh root weight, ln root/shoot ratio and colonization of roots by *G. virens* was greater at -0.15 than at -0.03 MPa whereas infection of roots by *V. dahliae* was reduced at -0.03 MPa. Population density of *F. equiseti* on roots was not affected by soil water pressure. Interactive effect of fungal treatment and soil water pressure were observed for ln root/shoot ratio, foliar dry weight, and vascular colonization by *V. dahliae*. The interaction was such that ln root/shoot ratio increased at -0.15 MPa in soil infested with *V. dahliae* plus *F. equiseti*, and foliar dry weight at -0.15 MPa in soil infested with a combination of *V. dahliae* and *G. virens*. Vascular colonization by *V. dahliae* was reduced at -0.15 MPa in soil infested with *V. dahliae* plus *F. equiseti*.

Effect of Soil Water Pressures on Population Dynamics of *Fusarium equiseti*, *Gliocladium virens*, *Talaromyces flavus* and *Trichoderma viride*, Biocontrol Agents of *Verticillium dahliae* in Potatoes

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Effect of Soil Water Pressures on Population Dynamics of *Fusarium equiseti, Gliocladium virens, Talaromyces flavus and Trichoderma viride*, Biocontrol Agents of *Verticillium dahliae* in Potatoes

Chapter 1. **LITERATURE REVIEW**

In his efforts to manage plant diseases, man has been responsible for polluting the environment through the excessive use and abuse of chemicals. In recent years, however, public awareness of potential ecological hazards due to the use of pesticides has increased. As a result there is interest in identifying alternative methods to suppress plant diseases.

Biological control of plant pathogens through antagonistic microorganisms seeks a solution to the problem. Because biocontrol agents are capable of protecting plants against infection by either destruction of the pathogen or by increasing the ability of the plants to resist infection, biological control could prove a powerful means of improving plant health. In recent years several biocontrol agents have been investigated for their efficacy against many plant pathogens (Davis et al, 1994; Marois et al, 1982; Moody and Gindrat, 1977; Papavizas, 1985). Unfortunately, few of these attempts have been consistently successful. There may be various reasons for this; e.g., prospective antagonists can not survive the relatively severe competition encountered under field conditions, they either lack sufficient efficacy or can not maintain a sufficient inoculum size at the site of attack to compete with other microflora, or they are unable to adjust to the ever changing

environment. What many fail to realize, however, is that biological control is not a panacea. Rather it would be wise to consider it as part of integrated pest management program in which each component contributes to the overall health of the plant.

Biological control of soilborne pathogens through manipulation of cultural practices has a potential to be included as a part of integrated pest management program. Several cultural practices such as green manures, fertilizers and irrigation are known to increase the efficiency of biocontrol agents. This approach not only will enhance the success of biocontrol agents but is likely to help in their ultimate acceptance in management of soilborne plant pathogens.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is an important soilborne disease limiting potato production in many areas of the world (Davis, 1981; Krikun and Orion, 1979; Martin et al, 1982; Nachmias and Krikun, 1985), especially those with intensive cultivation (Rowe et al, 1985). In Oregon, yield losses of up to 30% were reported in fields which have been under potato cultivation for several years (Johnson et al, 1988). The most common symptoms of the disease are chlorosis, which often is manifested unilaterally in leaves (Schnathorst, 1981), and necrosis, which results in retarded growth (Rowe et al, 1987), followed by premature defoliation. Discolorization of vascular tissues (Schnathorst, 1981) and root deterioration (Kotcon and Rouse, 1984) are also commonly associated with the disease. The

net result is the reduction in tuber yield and quality as plants senesce after tuber initiation (Celeti and Platt, 1987).

Management of the disease has been through crop rotation, use of resistant varieties and soil fumigation. However, these practices do not often provide satisfactory management of the disease. Moreover, development of concerns due to the contamination of ground water through indiscriminate use of chemicals has prompted research on biocontrol of the disease. This review focuses on the mode of action of four biocontrol agents with demonstrated efficacy in suppressing Verticillium wilt, and effect of soil water pressure on the population dynamics of soilborne microorganisms including *V. dahliae* and on potato plant growth.

Biocontrol agents and mechanisms of antagonism. A number of fungi including species of *Aspergillus*, *Blastomyces*, *Chaetomium*, *Dendrostilbella*, *Gliocladium*, *Penicillium*, *Pythium*, *Sporidesmium*, *Talaromyces*, *Teratosperma* and *Trichoderma* have been reported to be effective biological control agents of many plant diseases, especially diseases caused by soilborne plant pathogens (Dutta, 1981; Issac, 1954; Papavizas, 1985; Papavizas and Lewis 1981; Xu and Gross, 1986). Species of *Fusarium*, *Gliocladium*, *Talaromyces* and *Trichoderma* have been reported as effective biocontrol agents of *Verticillium albo-atrum* or *V. dahliae* (Davis et al, 1994; Jordan and Tarr, 1978; Keinath et al, 1991; Marois et al, 1984). Competition, mycoparasitism, induced

resistance and antibiosis have been proposed as the mechanisms through which biological control of plant pathogens occurs (Baker, 1968; Cook and Baker,1983; Papavizas and Lumsden, 1980).

Competition among fungi for nutrients (Benson and Baker, 1970; Scher and Baker, 1982) or for infection sites at the root surface (Mandeel and Baker, 1991) is one mechanism involved in the suppression of soilborne plant pathogens. In the former case, biocontrol agents compete and consume nutrients from the soil and root exudates which otherwise could have been used by the plant pathogens. Microbial competition for iron is an example of competition for nutrients in the rhizosphere. Fusarium wilt of cucumber was suppressed when nonpathogenic isolates of *Fusarium oxysporum*, together with *Pseudomonas putida*, were added to soil at a pH value of 7.0. Nonpathogenic *F. oxysporum* isolates increased root exudation which presumably resulted in siderophore production by fluorescent pseudomonads. This resulted in a competition between the pathogen and the bacteria since iron is required by the pathogen for germination and penetration of the host (Parak et al, 1988). A significant reduction in chlamydospore germination of *F. oxysporum* f. sp. *cucumerinum* occurred in the presence of siderophore producing *P. putida* whereas a non-siderophore producing mutant had no effect on spore germination of the pathogen (Simeoni et al, 1987).

Competition for carbon and nitrogen also has been reported (Benson and Baker, 1970; Scher and Baker, 1982). Soils became suppressive when the

supply of nutrients became limiting to the pathogen (Cook and Baker, 1983). Bean root rot, caused by *F. solani* f. sp. *phaseoli,* illustrates competition for nitrogen in the soil between antagonists and the pathogen. Addition of cellulose to the soil resulted in enhanced activities of microorganisms which caused immobilization of nitrogen and thus suppression of disease (Baker, 1968).

Addition of conidia of *Trichoderma harzianum* significantly reduced chlamydospore germination of *F. oxysporum* f. sp. *vasinfectum* and *F. o. melonis* in soils amended with glucose and asparagine at 0.4 and 0.08 mg/g of soil, respectively (Sivan and Chet, 1989). The inhibitory effect was lost when concentration of the two amendments increased. Moreover, a continuous application of germinating cotton seed exudates significantly reduced disease controlling ability of the biocontrol agent against *F. o. vasinfectum* in a cotton field.

Successful biocontrol also can result from the ability of the biocontrol agent to outcompete the pathogen either due to early colonization of infection courts or by displacement of the pathogen. Competition between pathogenic and nonpathogenic isolates of *F. oxysporum* in the rhizosphere and at infection sites resulted in suppression of Fusarium wilt of cucumber (Mandeel and Baker, 1991). The biocontrol agent successfully competed for infection sites, penetrated through intact root tissues, colonized the host, and induced resistance.

Mycoparasitism is a complex process which is initiated by growth of the fungal mycoparasite towards the host. This growth could be either a chemotropic response to some stimulus by the host or in response to a gradient of chemicals excreted by the host (Chet and Elad, 1983). The next step in the process, recognition, is regarded as an important component in the specificity of mycoparasitism and possibly involves agglutination. Elad et al (1983a) studied the host parasite interaction of *Rhizoctonia solani* and *Trichoderma harzianum* and demonstrated the role of a lectin in the process. The lectin present in *R. solani* hyphae attached to galactose residues on *Trichoderma* cell walls. Lectins also are involved in the recognition between *Trichoderma* spp. and *Sclerotium rolfsii* (Barak et al, 1985). Following recognition, the hyphae of the mycoparasite grow along or coil around the host. At this point extracellular enzymes such as ß-1,3-glucanase, chitinase (Hadar et al 1979; Harder et al 1979; and Liu and Baker, 1980) and cellulase (Chet and Baker, 1980; Chet and Baker, 1981) are excreted by the mycoparasite and have been implicated as the mechanism by which the host corpus is degraded.

Trichoderma harzianum and *T. hamatum* were found to grow along hyphae of *S. rolfsii* and *R. solani*. Both lysed sites and penetration holes were observed in the host hyphae (Elad et al, 1983b; Harman et al, 1980; Henis and Chet, 1975; Wells et al, 1972). The result was the vacuolation, collapse and disintegration of hyphal cells (Chet et al, 1981).

A number of species of *Gliocladium*, including *G. catenulatum*, *G. deliquescence* and *G. roseum*, have been documented as mycoparasites of several fungal pathogens (Barnett and Lilly, 1962; Hashioka and Fukita, 1969; Huang, 1978; Walker and Maude, 1975). Tu (1980) reported *G. virens* to parasitize both the mycelium and sclerotium of *Sclerotinia sclerotiorum*. Scanning electron microscopy revealed that infection of sclerotia occurred through appressoria. Once parasitized, the sclerotia were not able to form either mycelia or ascocarps. When *G. roseum* was buried in the mineral soil, it grew profusely on the mycelial mats of *Phomopsis sclerotioides*, cause of black root rot of cucumber, and caused a reduction in disease incidence (Moody and Gindrat, 1977).

Keinath et al (1991) reported that *G. roseum* was effective against *V. dahliae* by reducing the viability of microsclerotia. Viable sclerotia were not recovered from soils amended at 1% (w/w) with the antagonist. The mechanism by which sclerotial viability was reduced was not determined.

Fahima and Henis (1990) reported that *Talaromyces flavus*, when applied as ascospores, multiplied more on *V. dahliae* infected than noninfected aubergine seedlings. Microsclerotia of the pathogen were occupied by hyphae of *T. flavus*, thereby suggesting mycoparasitism as the mechanism. Fravel et al (1986) applied the antagonist either as dust to potato seed pieces or as alginate pyrex pellets to furrows and observed suppression of Verticillium wilt in the field. Moreover, a single application in the form of pellets suppressed the

disease for two growing seasons. Marois et al (1984) observed that germinability of microsclerotia of *V. dahliae* was 32-41% less in *T. flavus* infested soil compared with noninfested soil. When the biocontrol agent was applied as an ascospore drench to rhizosphere or nonrhizosphere soil, population of the antagonist were significantly higher in rhizosphere soil compared to nonrhizosphere soil of potato, eggplant and cotton.

Induced resistance also has been implicated as a possible mechanism of biocontrol resulting in incresed growth of plants. The mechanics of this phenomenon are, however, poorly understood. Biological control agents might excrete a regulatory hormone which may be responsible for increased nutrient uptake and hence increased growth. This in turn may confer resistance against soilborne plant pathogens. Baker et al (1984) reported an increase in height, weight, branch and flower production in *T. harzianum* treated chrysanthemum and peanuts, when the biocontrol agent was added to the soil at a rate higher than 10^5 cfu/g of soil. Elad and Chet (1983) demonstrated that *Trichoderma* propagules were present in plants that showed increased growth responses.

Jordan and Tarr (1978) dipped roots of strawberry runners in suspensions of individual fungi and bacteria and planted them in soil infested with *V. dahliae*. *Trichoderma viride* and three other fungi limited the incidence of wilt and increased plant growth. Biles and Martyn (1989) showed that inoculation of watermelons with either *Fusarium oxysporum* f. sp. *cucumerinum* or *F. o. niveum* provided protection against virulent isolates of

F. o. niveum. A virulent isolate of *F. o niveum*, however, was better than the two biocontrol agents. Moreover, it provided nonspecific and systemic protection since root inoculation with the antagonist resulted in 50% reduction in leaf lesions caused by *Colletotrichum lagenarium*. An interval of 24-72 h between induction and activity of the pathogen provided significant protection. Nonspecific resistance in carnation cuttings was induced by many species of nonrelated fungi against *F. roseum 'avenaceum'*. Moreover, a nonpathogenic isolate of *F. roseum 'gibbosum'* also induced resistance. Production of phytoalexins in the host was suggested as a possible control mechanism (Baker, 1968).

Antibiosis, which involves the production of antibiotic substances/metabolites, is a mechanism of biocontrol manifested by many microorganisms. The substances produced may be volatile or nonvolatile in nature and vary even within isolates of the same species. Production of biologically active secondary metabolites against several plant pathogens have been reported for *G. virens* and closely related species (Brian and Hemming, 1945; Brian and McGown, 1945; Howell and Stipanovic, 1983; Jones and Hancock, 1987; Roberts and Lumsden, 1990). Howell (1987) reported that mutants of *G. virens* unable to parasitize *R. solani* effectively suppressed damping off of cotton in the field. This lack of parasitism coupled with disease suppression indicated the possibility that antibiosis may be involved.

Several different metabolites are known to be produced by *G. virens*. These include gliotoxin, gliovirin, gliocladic acid, viridin, viridol and valinotricin (Taylor, 1986). Gliotoxin, gliovirin, and viridin have been implicated in biocontrol of *R. solani* and *Sclerotinia americana* (Weindling, 1941), *Pythium ultimum* (Howell and Stipanovic, 1983) and *Rhizoctonia solani* (Wright, 1956a and Wright, 1956b), respectively.

Gliotoxin was reported to be produced early during the growth of *G. virens* (Lumsden et al, 1992). Acidic conditions enhanced chemical stability of the toxin whereas rapid breakdown of the metabolite occurred above pH 7 (Brian and Hemming, 1945; Weindling, 1941; Weindling and Emerson, 1936). Production of the toxin may be increased by increasing the level of calcium in organic soils (Wright, 1956b).

Production of gliotoxin in soil and soilless media was associated with the advancing margin of the colony and was detected 4-5 cm away from the source in a peat moss-vermiculite medium (Lumsden et al, 1992). Production of toxin was affected by time, temperature, organic matter, nutrient status and other physical and chemical factors.

Extreme toxicity of gliotoxin to *Pythium ultimum* resulted in protoplast coagulation and hyphal disintegration (Howell and Stipanovic, 1983). Leakage of metabolites from hyphae of *R. solani* treated with extracts from *G. virens* was reported by Lewis and Papavizas (1985).

Culture supernatants from *G. virens*, which contain gliotoxin and the enzymes laminarinase, amylase, carboxymethyl cellulase, chitinase and protease, inhibit sporangial germination and hyphal growth of *P. ultimum* (Roberts and Lumsden, 1990). Culture extracts remained inhibitory after the inactivation of enzymes, which suggests a possible role of the antifungal substance in suppression of *P. ultimum*. Ridout et al (1992) compared gliotoxin producing and nonproducing isolates of the fungus for mycelial polypeptides. Although the two strains produced 146-170 polypeptides, gliotoxin producing isolates consistently and uniquely produced four major polypeptides not produced by nonproducing isolates. Moreover, several major polypeptides were present more abundantly in gliotoxin producing than in nonproducing isolates.

Antagonistic activity of *Talaromyces flavus* to *V. dahliae* by production of antibiotics was reported by Fravel et al (1987). The compound produced by the antagonist killed microsclerotia of the pathogen both *in vitro* and in soil. Kim et al (1987, 1988) identified the compound as glucose oxidase. However, neither a commercial nor a semipurified preparation from cultural filtrates was active against the microsclerotia unless glucose was added to the preparation. They concluded that antibiotic activity was due to hydrogen peroxide, which is a product of a reaction catalyzed by glucose oxsidase.

Many isolates of *Trichoderma* spp. produce volatile and nonvolatile antibiotics (Dennis and Webster, 1971a; Dennis and Webster, 1971b). When

Trichoderma was grown on agar overlain with cellophane, the membrane removed, and agar inoculated with *P. aphanidermatum*, partial growth inhibition of the pathogen was observed (Sivan et al, 1984). Calvet et al (1989) proposed that inhibitory effects of *T. harzianum* on *V. dahliae* were the result of antibiosis. Nonvolatile compounds released by the antagonist inhibited growth of *V. dahliae* on malt agar overlain with cellophane. Heni (1987) reported inhibitory effects of substances produced by *T. harzianum* on microsclerotia of *V. dahliae*. Germination of microsclerotia was inhibited when *T. harzianum* was applied on aubergine plants either at the time of or before inoculation with *V. dahliae*. A reduction in radial growth of *V. albo-atrum* was observed in presence of culture filtrates of *T. viride* and *Penicillium vermaculatum* on Dox's agar. In addition to *in vitro* activity, root dip application of culture filtrates of the two antagonists was effective in suppressing the disease (Dutta, 1981).

Antibiotic production by species of *Fusarium* also has been documented. Suppression of *Sclerotinia homoeocarpa* by *F. heterosporum* through the production of toxic metabolites was reported by Goodman and Burpee (1991). Dutta (1981), in an *in vitro* study, observed a clear zone of inhibition between colonies of *F. culmorum* and *V. albo-atrum*. Cultural filtrates of the antagonist applied to tomato seedlings as a root dip treatment, however, were not effective.

Soil water pressure and soilborne microorganisms. The adoption of a biocontrol agent of a soilborne pathogen depends on the ability of the biocontrol agent to adjust to a wide range of soil conditions. These include temperature, moisture, pH, source of fertility, aeration, soil type and presence of other microorganisms in the soil. It is soil moisture that is central to this thesis.

Fungi with high water requirements cause serious diseases in wet soil whereas those with low water requirements are destructive at low soil water pressures. A drastic change in soil water pressure could either be beneficial or detrimental to the pathogen in relation to the competitive advantage over other organisms. Baker and Cook (1974) refer to this as an effect on "relative competitive advantage" of the organism. A pathogen will be benefitted by unfavorable soil water pressure if that pressure is even more unfavorable to the antagonist. Conversely, a soil water pressure unfavorable to the antagonist but even more unfavorable to the pathogen will result in reduced activities of the pathogen. Bruehl and Lai (1968) reported that during its saprophytic existence on wheat straw, *Cephalosporium gramineum* produced an antibiotic which gave it protection against colonization by other microorganisms. At -7.5 to -10 MPa, however, the fungus was not able to produce the antibiotic and was consequently colonized by other fungi such as *Aspergillus*, *Penicillium* and *Fusarium* which were capable of growth at these water pressures. Similarly, *Fusarium roseum* f. sp. *cerealis* '*culmorum*' can grow at low soil water

pressures. Maximum hyphal growth rate in sterile soil was reported at near 0 MPa and progressively decreased with lower soil water pressures down to -8.0 MPa (Cook and Papendick, 1972). Hyphal growth in nonsterile soil, however, occurred only when soil was drier than -0.08 to -1.0 MPa, above which germ tubes from chlamydospores either lysed or converted back to chlamydospores because of enhanced activity of bacteria. As soil dried, bacterial activity started to decrease and virtually stopped at -1.0 to -1.5 MPa (Cook and Papendick, 1971). This coincided with the highest soil water pressure at which the pathogen could grow in natural soils (Cook and Papendick, 1970).

Soil water pressures for sporulation of pathogens are frequently within the range at which plant growth occurs, which might synchronize pathogen activities with the growth of the host. Prospects of management exist if this relationship is somehow asynchronized. Smith (1972) suggested a biological control system for *S. rolfsii* through sclerotial germination and decay by first allowing the tillage layer to dry followed by irrigation for 2 wk before planting. Drying followed by rewetting stimulates sclerotial germination. Moreover, cracks develop in the rind of sclerotia on drying, and leakage of nutrients occur on wetting, which helps antagonistic organisms to colonize and cause sclerotial death.

Certain plant pathogens are capable of growing at soil water pressures well below the minimum required by most higher plants for their growth

(0 to -1.5 MPa). Activities of *F. solani* f. sp. *pisi* on pea epicotyls were higher in dry soils, chiefly due to decreased activities of antagonists (Cook and Flenntje, 1967). Lapwood and Herring (1968) reported that infection of potato by *Streptomyces scabies* occurred during brief dry periods and could be prevented by keeping the soil wet. Lewis (1970) suggested that increased populations of antagonistic bacteria were responsible for limiting infection in wet soil.

Soil moisture, besides having an effect on pathogens, also affects the activities of antagonists. Liu and Baker (1980) demonstrated that survival of antagonistic *Trichoderma* spp. was greater in moist than in dry soils. However, certain isolates have been shown to grow at -8.0 MPa (Cook and Baker, 1983). A significantly higher hyphal density of *T. harzianum* on wheat bran was found in dry than in wet soils (Knudsen and Bin, 1990). Magan (1988) reported that germination of *Trichoderma* sp., *G. roseum* and *G. virens* on leaf sheaths was significantly lower as compared to that on agar at -0.7 and -2.8 MPa. *Gliocladium roseum* was effective in three different soils at -0.01 and -0.1 MPa (Keinath et al, 1991). Pullman and Devay (1981) reported that Verticillium wilt on cotton was suppressed with a concomitant reduction in *V. dahliae* populations by flooding the soils for at least 6 wk. Control was more effective by increasing the duration of flooding and paddy rice culture due to increased activity of antagonistic microorganisms.

Osmotic potential, soil water pressure, and *Verticillium dahliae*.

Verticillium dahliae is a monocyclic pathogen. The extent to which infection by *V. dahliae* can occur depends upon the population density of the pathogen in the soil, environmental conditions, host cultivar and presence of other pathogens such as *Pratylenchus* spp.

Information on the effect of osmotic potential and soil water pressure on various aspects of *V. dahliae* exists. Growth of *V. dahliae in vitro* occurs over a wide range of osmotic potentials. Conidial germination, radial growth and sporulation of the fungus occur at osmotic potentials as low as -10 to -12 MPa (Ioannou et al, 1977; Manadhar and Bruehl, 1973; Mozumder and Caroselli, 1966). In a liquid medium, adjusted between 0 and -12 MPa, growth, as measured as increase in dry weight, and conidial germination decreased linearly from -0.2 to -12 MPa, whereas on an agar medium, maximum radial growth occurred between -1 to -2 MPa. Conidial production in a liquid medium increased logarithmically between -0.2 to -2 MPa and was maximized at -5 MPa. Microsclerotia production either remained unchanged or increased with reductions in osmotic potential from -0.2 to -2 MPa but decreased with further reductions in osmotic potential to -7 and -8 MPa, where very few or no microsclerotia were produced at all.

Effect of moisture on growth, sporulation and microsclerotia production *in vivo* also was studied. Although microsclerotia were produced in soil at -0.5 and -3.2 MPa at 18, 27, and 30 C, maximum production occurred at 24 C at

-3.2 MPa in infected tomato stems buried in nonsterilized soil. Few microsclerotia were produced in soils that were either saturated or were at -10 MPa, regardless of temperature (Ioannou et al, 1977). According to Menzies and Griebel (1967), microsclerotia became more sensitive to drying after supporting sporulation.

Vascular wilt fungi, including *V. dahliae*, cause the most severe disease under relatively moist soil conditions, although they are capable of growth at extremely low soil water pressures (Bruehl and Cunfer, 1971; Bruehl et al, 1972; Cook and Papendick, 1972; Ioannou et al, 1977; Manandhar and Bruehl, 1973; Mozumder and Caroselli, 1966). A recent study (Cappaert et al, 1992) demonstrated that severity of potato early dying (Verticillium wilt) was greater in excessively irrigated plots than in those receiving deficit irrigation. An increase in inoculum density under deficit water conditions had little effect on tuber yield whereas the same treatment under wet conditions significantly suppressed tuber yield. Similarly, McLean (1955) reported that potato early dying occurred earlier and was more severe in a wet season than in a dry season in Idaho. *Verticillium dahliae* depends on transpiration flow for movement of spores systemically upward in the xylem of the plant (Dimond, 1970). Provided the soil is sufficiently moist to keep pace with evaporative demand, an increase in transpiration rate will increase the rate of distribution of pathogen throughout the plant. Thus without sufficient soil moisture, transpiration and consequently conidial movement will be restricted.

Davis (1981) and Davis and Everson (1986) established a correlation of plant nutrition and irrigation to severity of potato early dying and colonization of potato stems by *V. dahliae*. Disease was more severe in furrow than in sprinkler irrigated fields. The authors suggested that nitrogen was more uniformly distributed in sprinkler irrigated plots whereas it accumulated in the upper few centimeters of soil profile with furrow irrigation. Less availability of nitrogen to the plant root system, therefore, resulted in increased disease severity.

Concluding remarks. The effects of water, both beneficial and detrimental, on root diseases have been well documented (Cook and Papendick, 1971; Griffin, 1963; Griffin, 1969; Stolzy and Van Gundy, 1968). However, the influence of soil water pressure on the competing soil microflora and their effect on root diseases has not been studied in great detail. In view of this, we focused our efforts in two areas. First, the effect of osmotic potential on growth and reproduction of both *V. dahliae* and its biocontrol agents was examined *in vitro*. Second, the influence of soil water pressure and *V. dahliae* alone or in combination with *F. equiseti* or *G. virens* on potato growth, root and vascular colonization by the pathogen, and root colonization by biocontrol agents over time was studied.

Chapter II. **Effect of Soil Water Pressures on Population Dynamics of *Fusarium equiseti*, *Gliocladium virens*, *Talaromyces flavus* and *Trichoderma viride*, Biocontrol Agents of *Verticillium dahliae* in Potatoes.**

Among potato diseases, Verticillium wilt (potato early dying) is a major yield limiting factor. Although it can be caused by a complex of interacting pathogens, the soilborne fungus *Verticillium dahliae* Kleb. is the major cause of the disease in North America (Beckman, 1973; Powelson and Rowe, 1993; Slattery and Eide, 1980). The effect of this disease on yield is extremely variable. Losses up to 30% (6-12 t/ha) have been documented in Idaho (Davis, 1981), New York (Schultz and Cetas, 1977) and Ohio (Rowe et al, 1987). Potato yield and tuber size may be substantially reduced depending upon cultivar, onset of disease symptoms, environmental conditions, and severity of the disease (Davis and Everson, 1986). In view of this, the importance of the disease can not be underestimated and need for its management persists.

Crop rotation, resistant cultivars, and soil fumigation are the primary practices used for disease management. Because of the broad host range of the pathogen and its persistence in soil for many years (Schreiber and Green, 1962), crop rotation has not been very effective. Cultivars with resistance to *V. dahliae* have been grown with varying degrees of success. Due to the long term effects on the environment, including ground water quality and high cost of soil fumigation, concerns have been raised about the use of chemicals

(Schumann, 1991). Because of limitations of the aforementioned strategies and the potential for significant yield losses under conditions conducive to disease, alternative methods for managing this disease warrant investigation.

Biological control, because of its less detrimental effects on environment as compared to chemical control, is one such possibility. An additional benefit of this approach is its use in diverse ways to manage the disease. The antagonists either can be added to the soil to suppress the disease and/or pathogen or in other cases the environment can be modified to increase the efficiency of biocontrol agents. It then follows that intelligent manipulation of cultural practices could provide unlimited opportunities for effective disease management. Moreover, such a strategy stands a good chance of success because of the ease with which it can be incorporated into an integrated program, relatively stable nature of soil microclimate, and cost effectiveness. Cultural practices that influence the biological control of plant pathogens include soil fertility, green manures, irrigation, soil temperature, and pH. Of interest in these studies is soil moisture.

Besides satisfying crop water needs, irrigation also affects disease development directly or indirectly. Whereas direct effects are manifested on the pathogen itself, promotion of activities of antagonists present in the soil is regarded as an indirect effect. For example, *V. dahliae* can grow under a wide range of soil water pressures (Ioannou et al, 1977), however, the disease is more severe under wet soil conditions (Cook and Papendick, 1972). Severity

of the disease, however, can be reduced by proper management of the amount and timing of water application. Reduced irrigation will not only minimize use of water but also will help suppress the disease without affecting tuber yield (Powelson and Rowe, 1993). A two-to-four fold reduction in disease was observed in Oregon and Wisconsin with irrigation treatments that supplied 75 or 100% compared to 125% of the total water use from planting to tuberization (Cappaert et al, 1992). Moreover, low soil water pressure will promote activities of antagonistic fungi such as *Trichoderma* and bring about disease and/or pathogen suppression because *T. harzianum* has been reported to produce a higher hyphal mass under dry conditions (Knudsen and Bin, 1990).

Manipulation of soil water pressure to enhance the efficacy of biocontrol agents, therefore, has potential as a practical disease management strategy. In view of this, present studies were designed to determine the effect of osmotic potential on growth and reproduction of both *V. dahliae* and its biocontrol agents *in vitro* and the influence of soil water pressure and *V. dahliae* alone or in combination with *G. virens* or *F. equiseti* on potato growth, root and vascular colonization by the pathogen, and root colonization by biocontrol agents over time .

MATERIALS AND M

Fungi and inoculum production. Cultu

Miller, Giddens and Foster, *Talaromyces flavu*

and *Trichoderma viride* Pres. ex Gray were pr

culture of *F. equiseti* (Corda) Sacc. Sensu Gordon was provided by O. Huisman. *Verticillium dahliae* Kleb. was a mixture of isolates from symptomatic potatoes grown in Oregon.

Single spore cultures of *V. dahliae* were maintained on Potato Dextrose Agar (PDA) plates at 22 - 24 C. Cultures were flooded with sterile distilled water and scraped with a rubber spatula to collect conidia and mycelia. Aliquots (10 ml) of the resulting conidial and mycelial suspension were transferred to plates containing a modified minimal agar (Puhalla, 1979) overlain with cellophane. Cultures were incubated for 3 - 4 wk at 22 $\pm$ 2 C. The cellophane, covered with microsclerotia, was scraped from each plate and processed in sterile distilled water in a Waring blender for 4 - 5 min. The slurry was filtered through nested 140 and 175 μm mesh sieves. Microsclerotia collected on the 140 μm sieve were washed with tap water and allowed to dry at room temperature for 48 - 72 h. A more detailed protocol of the procedure is contained in Appendix I.

The air-dried inoculum was gathered and mixed with soil in a twin shell blender to obtain the inoculum concentrate. The inoculum concentrate

was assayed by dilution plating on Sorensen's NP10 medium (Sorensen et al, 1991) to determine population density of the pathogen in the concentrate. Enough inoculum concentrate was added to soil to achieve a final inoculum density of 25 colony forming units (cfu)/g of soil.

Fusarium equiseti and *G. virens* were grown on PDA for 1 wk at 25 C. Wheat grains, autoclaved in glass jars (13 cm diameter) at 121 C for 30 min, were inoculated with pieces of agar culture of the biocontrol agents. The jars were incubated at room temperature for 2 wk until the grains were covered with the fungus. The grains were then ground in a Wiley mill for approximately 2 min at a high speed. The resulting concentrate of *F. equiseti* or *G. virens* was assayed on Elad (Elad et al, 1981) or Nash Snyder (Nash and Snyder, 1962) media, respectively. To obtain 25 cfu/g of soil of each biocontrol agent, the inoculum concentrate was mixed at 4 g/7100g of soil.

Soil. Soil was obtained from the Agricultural Research and Extension Center Hermiston, Hermiston OR. The soil was a Quincy fine sandy loam; mixed, mesic xeric Torripsamment. The soil was steam pasteurized for 1 h at 65 C, air-dried on greenhouse benches for 1 mo and then sieved to remove debris and stones.

Mycelial and spore germination studies. For the mycelial growth study, treatments consisted of five fungi, (*F. equiseti*, *G. virens*, *T. flavus*, *T.*

viride, and *V. dahliae*) and 10 osmotic potentials (-0.12, -0.18, -0.59, -1.10, -2.15, -3.06, -5.12, -6.17, -7.16, and -8.13 MPa) for a total of 50 treatments. *Talaromyces flavus* was not included in the spore germination study. The factorial combination of treatments was replicated five or four times for the mycelial growth study and spore germination study, respectively.

Malt yeast extract peptone (MYP) medium (Swart et al, 1992) was adjusted to selected molal concentrations by amending with KCl. Plates lacking KCl served as the control. The actual osmotic potential of the treatments was determined with an osmometer.

For the mycelial growth experiment, a 5 mm diameter agar plug, cut from the periphery of an actively growing colony of the test fungus, was transferred to the center of each MYP plate. Plates were incubated at 24 $\pm$ 2 C for 8 days. Growth was determined by measuring the colony diameter along the two perpendicular lines.

For the spore germination experiment, the colony surface of 2-wk-old cultures of each fungus grown on PDA was flooded with 5 ml of sterile distilled water and scraped with a rubber spatula. The spore suspension was filtered through cheese cloth and adjusted to a concentration of 10^4 propagules/ml. Aliquots (200 μl) of the resulting suspension were spread across the surface of MYP agar with a glass rod. Plates were incubated in dark at 25 C. Numbers of germinated propagules in 15-20 microscopic fields at a 10x magnification were determined every 2 h until 95% of the propagules had

germinated. A propagule was regarded as germinated if the germ tube was approximately twice the width of the propagule. A minimum of 200 propagules per plate was counted.

Data were subjected to regression analysis with osmotic potential as the independent variable and colony diameter or incubation time as dependent variable using SAS version 6.04 (Statistical Analysis Systems, SAS Institute, Inc. Cary, NC). Data values that corresponded to maximum colony diameter were removed from the data set before analysis to avoid censored effects. Linear and curvilinear regression analyses were used to determine the relationship between osmotic potential and mycelial growth or incubation time for each fungus.

Greenhouse study. Treatments consisted of two soil water pressures combined with no pathogen (control), *V. dahliae* alone, or in combination with one of the two biocontrol agents for a total of eight treatments. The experiment was designed as a randomized complete block with a factorial combination of treatments replicated eight times for each of four harvest weeks. Biocontrol agents were *F. equiseti* and *G. virens* and soil water pressures were -0.15 and -0.03 MPa, which corresponded to 7.8 and 15.4% water per weight of dry soil.

Inoculum of each fungus was mixed with soil in a cement mixer. Soil containing neither a biocontrol agent nor *V. dahliae* also was mixed to obtain

an uniform soil bulk density. The cement mixer was disinfected with 95% alcohol between the treatments. Approximately 7100 g of soil were placed in each 22 cm diameter plastic pot. Based on 7.8 and 15.4% water per weight of soil, enough water was added to each pot to achieve the desired soil water pressure. Based on a previous analysis of the soil (determined by Soil Analysis Laboratory, Department of Crop and Soil Science, Oregon State University), liquid fertilizer consisting of KH_2PO_4 (115 mg), $(NH_4)_2SO_4$ (56 mg), $Ca(NO_3)_2.4H_2O$ (100 mg) and $MgSO_4.7H_2O$ (21 mg)/kg of soil (Appendix II) was added to the pots after mixing.

Single eye seed pieces of nuclear seed potatoes (*Solanum tuberosum* L. cv. Russet Burbank) were scooped with a 2.5 cm melon scoop and placed in vermiculite in plastic trays for 1 wk to facilitate germination (Appendix III). Individual seed pieces were planted into the center of each pot to a depth of 6 cm. The surface of the soil was covered with a 3 cm deep layer of perlite to minimize evaporation. Each pot was weighed daily and water was added to maintain the desired water content. Plants were maintained on greenhouse benches under natural daylight at a temperature of 17 to 30 C.

Plants were harvested weekly for 4 wk after 90% of the seed pieces had germinated. At each harvest, plant height from soil line to the tip of the apical bud was recorded. Plants were cut near the soil line, weighed, dried at 40 C for 3 days and then weighed again.

The below ground portion of each plant was removed from the soil, washed in running tap water, blotted dry, weighed, and placed in plastic bags containing moist filter papers. Samples were refrigerated until processed. Fresh root/shoot ratio was calculated from fresh weight of roots and foliage.

Both roots and stems were assayed for *V. dahliae* on Sorenson's NP10 medium (Sorensen et al, 1991), and roots were assayed for *F. equiseti* and *G. virens* on Nash Snyder (Nash and Snyder, 1962) or Elad medium (Elad et al, 1981), respectively. Roots from each treatment were cut into 1 cm long pieces and plated onto their respective medium to determine the number of colony forming units per centimeter of roots. A total of 100 cm of roots per plant was plated. Roots from the control treatment were plated on all three media. For *F. equiseti*, 15% of the colonies were subcultured on water agar for later identification. To determine vascular populations of *V. dahliae*, a garlic press was used to express the sap from the stem of each plant. Aliquots of stem sap from each plant were spread onto a single NP10 plate. Plates were incubated at room temperature (20 - 22 C) for 3, 4 and 7 days for *G. virens*, *F. equiseti* and *V. dahliae*, respectively. At that time colonies of the aforementioned species were enumerated.

SAS version 6.04 (Statistical Analysis Systems, SAS institute, Inc. Cary, NC) was used for statistical analyses. Data on recovery of *G. virens* and *F. equiseti* from roots were analyzed separately to determine the effect of soil water pressure on root colonization. The root/shoot ratio was transformed to

natural logarithms, and data on colonies of *V. dahliae* from stem sap were transformed to square root values in order to stabilize variances as determined by residual plots. Data from plants grown in soil not infested with *V. dahliae* were omitted from the analyses as *V. dahliae* was not isolated from these plants. Procedures on analysis of variance for balanced data and general linear models for unbalanced data (including the data set in which values were missing) were used. Two way analysis of variance was performed on the data with soil water pressures, fungi, and blocks as independent variables. Fischer's protected least significant difference (LSD) procedure was applied for comparing means when the analysis of variance showed significant variation.

RESULTS

Spore germination. In general, spore germination time increased as osmotic potential decreased from -0.12 to -8.13 MPa and ranged from 8-29, 16->72, 16-46 and 13-60 h for *F. equiseti*, *G. virens*, *T. viride* and *V. dahliae*, respectively (Fig. 1A). Regression analysis of time for 95% spore germination versus osmotic potential revealed significantly different slopes of regression lines ($P \leq 0.05$). Regression equations were $Y = 7.57 + 0.21 X$ ($R^2 = 0.90$) for *F. equiseti*; $Y = 13.74 + 0.38 X$ ($R^2 = 0.93$) for *G. virens*; $Y = 11.80 + 0.36 X$ ($R^2 = 0.92$) for *T. viride*; and $Y = 13.61 - 0.25 X + 0.01 X^2$ ($R^2 = 0.99$) for *V. dahliae*, where Y and X represent the time to 95% spore germination and osmotic potential of the medium, respectively. Ascospores of *T. flavus* did not attain 95% germination even after heat treatment at 70 C for 1 h.

Mycelial growth and sporulation. *Fusarium equiseti*, *T. viride* and *V. dahliae* grew at all osmotic potentials tested. In contrast, *G. virens* and *T. flavus* did not grow at -8.13 MPa and below -6.17 MPa, respectively (Fig. 1B). Mycelial growth of the four biocontrol agents decreased as osmotic potential decreased. *Verticillium dahliae*, on the other hand, showed a slight increase in mycelial growth below -0.59 MPa with maximum growth occurring between -1.10 and -3.06 MPa and a linear decrease in growth below -3.06

MPa. When mycelial growth was regressed on osmotic potential for each fungus separately, slopes of regression lines for mycelial growth were significantly different at different osmotic potentials for all the fungi except *F. equiseti* ($P \leq 0.05$). Regression equations were $Y = 8.17 - 0.11 X$ ($R^2 = 0.95$) for *G. virens*; $Y = 10.82 - 0.12 X$ ($R^2 = 0.99$) for *T. viride*; $Y = 5.13 - 0.09 X$ ($R^2 = 0.96$) for *T. flavus*; and $Y = 1.53 + 0.11 X - 0.003 X^2 + 0.00002 X^3$ ($R^2 = 0.88$) for *V. dahliae*, where Y and X represent the colony diameter and osmotic potential of the medium, respectively.

Gliocladium virens, *T. viride* and *T. flavus* did not sporulate below -1.10 MPa whereas *V. dahliae* did not produce microsclerotia below -0.59 MPa. *Fusarium equiseti* produced macroconidia at all osmotic potentials although the number of macroconidia was reduced below -5.12 MPa. Chlamydospores were not produced by this fungus at any osmotic potential.

Plant height and fresh root weight. Soil water pressure had a significant ($P \leq 0.05$) effect on both plant height and fresh root weight. However, its effect on plant height was inconsistent over the course of the study (Fig. 2A-D). Height was reduced at high compared to low soil water pressure at week 1, but the effect was reversed at the last two sampling dates. Plants grown at -0.15 MPa were 1% taller at week 1 but 9 and 14% shorter at weeks 3 and 4, respectively than those grown at -0.03 MPa.

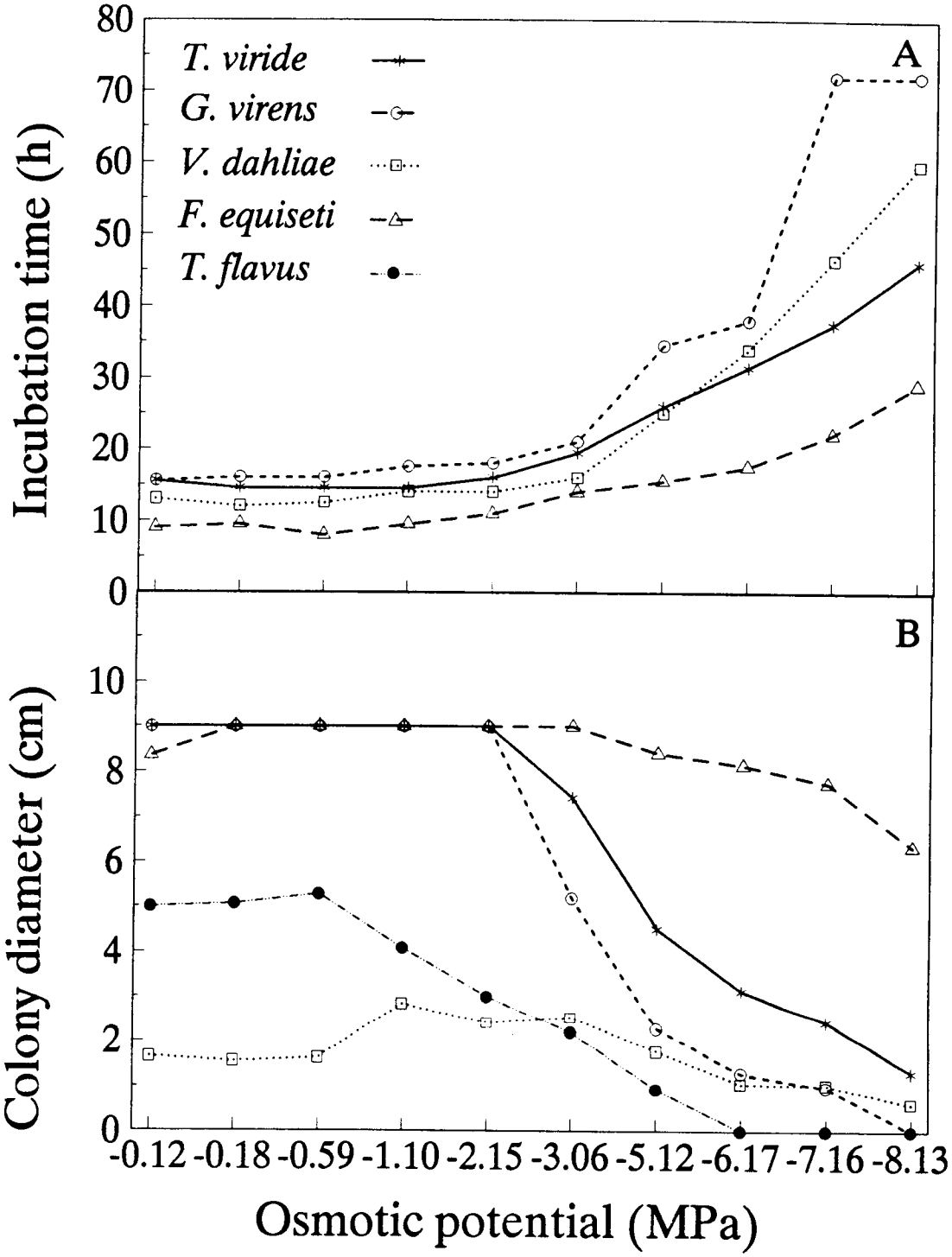

Fig. 1. Effect of osmotic potential on A) time to 95% spore germination and B) colony diameter of *Verticillium dahliae, Fusarium equiseti, Gliocladium virens, Trichoderma viride,* and *Talaromyces flavus.* Ascospores of *T. flavus* did not reach 95% germination.

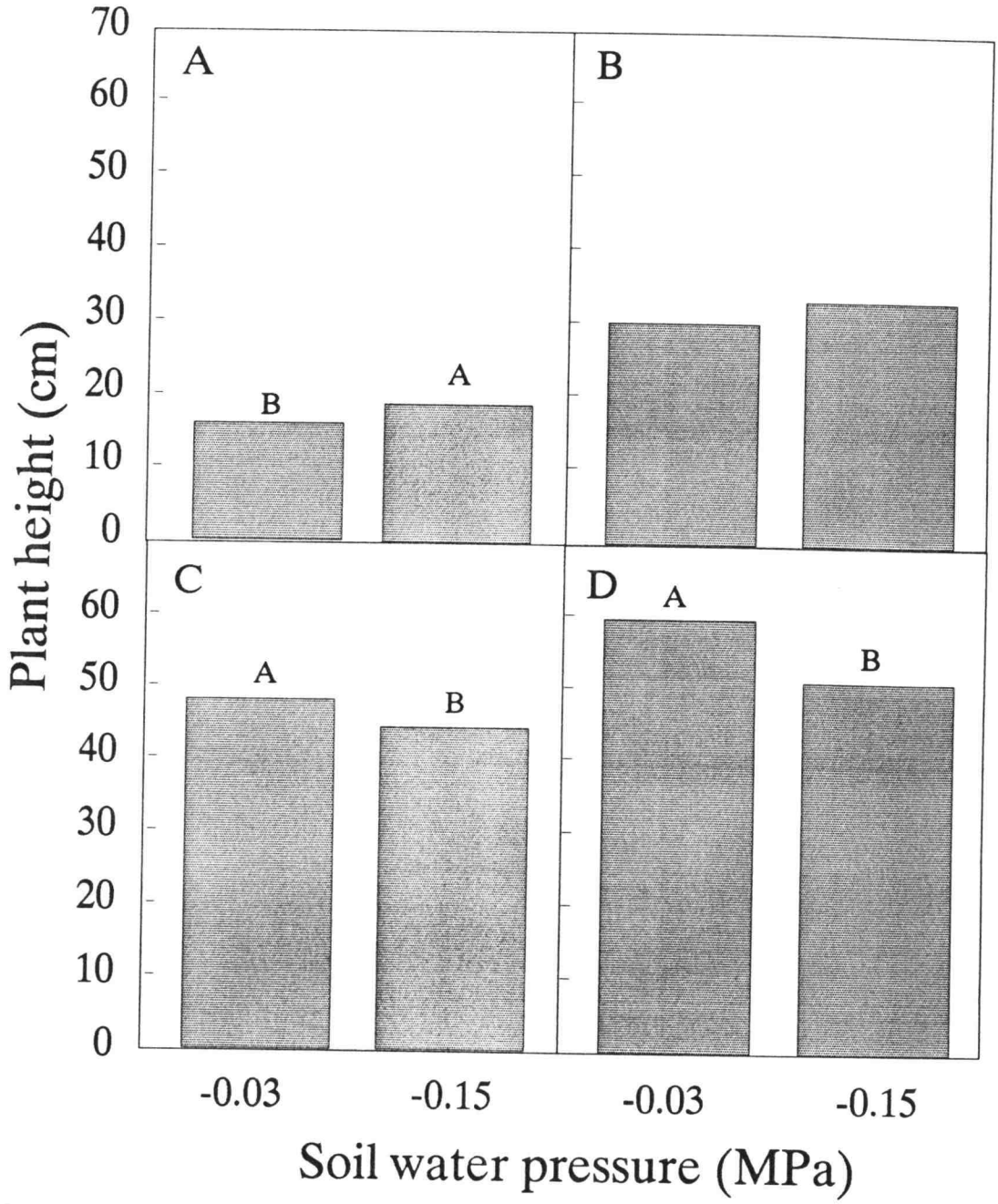

Fig. 2. Effect of soil water pressure on mean plant height of potato grown in soil noninfested or infested with *Verticillium dahliae* alone or in combination with either *Gliocladium virens* or *Fusarium equiseti* at A) week 1, B) week 2, C) week 3, and D) week 4. Within weeks, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test.

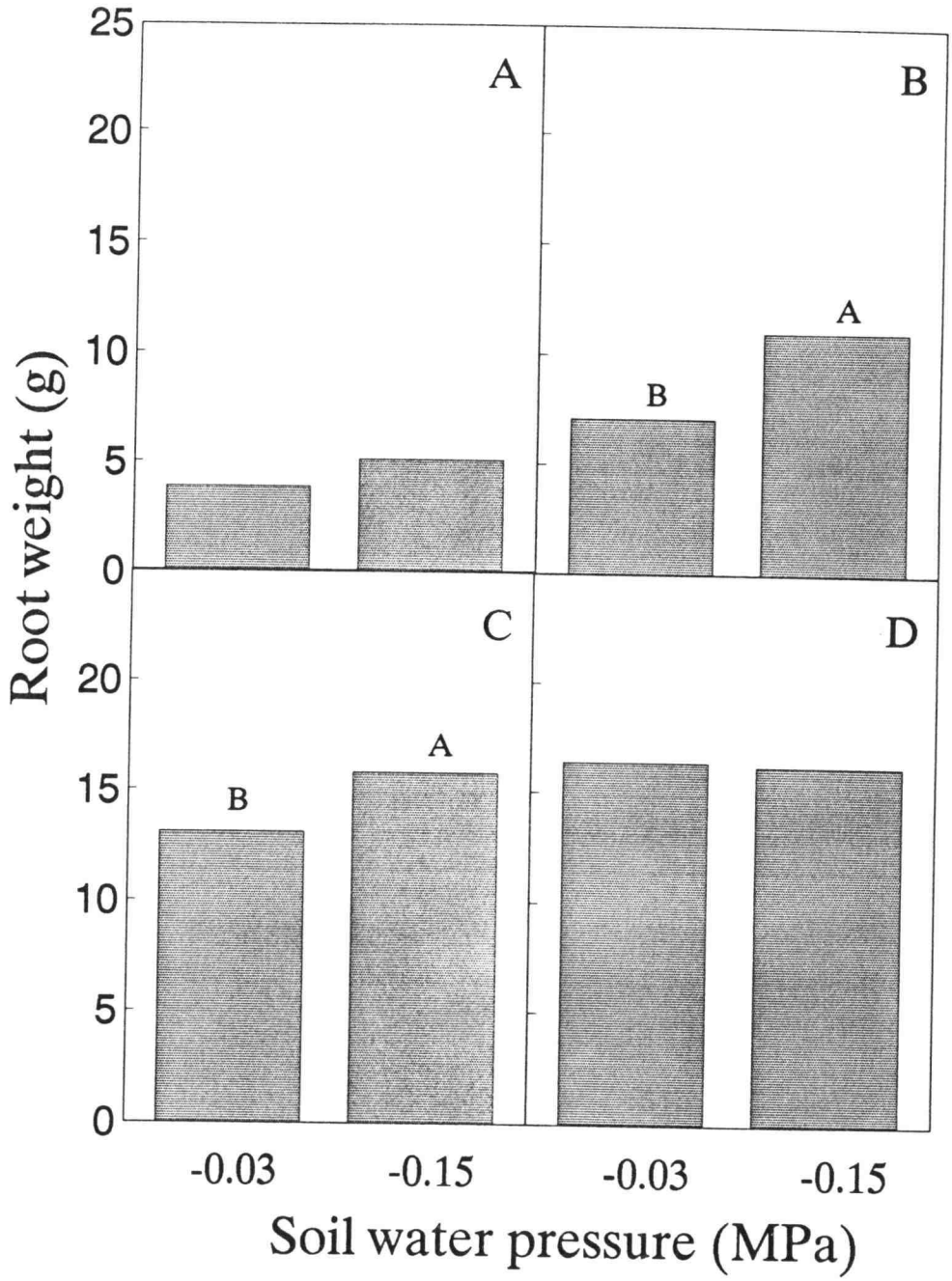

Fig. 3. Effect of soil water pressure on mean fresh root weight of potato grown in soil noninfested or infested with *Verticillium dahliae* alone or in combination with either *Gliocladium virens* or *Fusarium equiseti* at A) week 1, B) week 2, C) week 3, and D) week 4. Within weeks, bars with different letters are significantly different ($P \leq 0.5$) according to Fisher's Protected LSD test.

Main effect of soil water pressure on fresh root weight was significant ($P \leq 0.05$) at weeks 2 and 3 (Fig. 3A-D). Fresh root weight was significantly greater at low compared to high soil water pressure; i.e., 36 and 17% greater for plants grown at -0.15 MPa than those grown at -0.03 MPa.

None of the fungal treatments had an effect on either of these plant responses. In addition, interactions between soil water pressure and fungal treatment were not significant.

Ln root/shoot ratio. A significant ($P \leq 0.05$) interactive effect of soil water pressure and fungal treatments on ln root/shoot ratio was observed at week 3 (Table 1). The ln root/shoot ratio was inversely proportional to soil water pressure for all fungal treatments except *V. dahliae* alone for which it was directly proportional to soil water pressure. The ratio was 26% larger for plants grown in soil infested with *V. dahliae* plus *F. equiseti* at -0.15 compared to -0.03 MPa.

Significant ($P \leq 0.05$) differences among fungal treatments were observed at week 4. The ratio was significantly larger in the control (no fungus) compared to the other treatments. Among biocontrol agents, the ln root/shoot ratio was larger for plants grown in soil infested with *G. virens* plus *V. dahliae* compared to those grown in soil infested with *F. equiseti* plus *V. dahliae*.

Table 1. Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure on ln root/shoot ratio of potatoes.

	ln root/shoot ratio										
	Week1			Week2			Week3		Week4		
	Soil water pressure										
Fungal treatment	-0.15	-0.03	Mean[x]	-0.15	-0.03	Mean	-0.15	-0.03	-0.15	-0.03	Mean
control	-1.62	-0.98	-1.30	-1.46	-1.63	-1.54	-1.68	-2.06	-1.44	-1.61	-1.52 a[y]
V. dahliae	-0.99	-0.56	-0.77	-1.39	-1.55	-1.47	-1.60	-1.50	-1.81	-1.88	-1.84 c
G. virens + *V. dahliae*	-1.29	-1.05	-1.17	-1.47	-1.53	-1.50	-1.72	-1.80	-1.66	-1.70	-1.68 b
F. equiseti + *V. dahliae*	-1.16	-0.97	-1.06	-1.51	-1.80	-1.65	-1.49	-2.02	-1.84	-1.93	-1.88 c
Mean[x]	-1.26	-0.89		-1.45 A		-1.62 B		*	-1.68	-1.78	

[x]Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns
[y]Means followed by different letters are significantly different at $P \leq 0.05$ according to Fisher's Protected LSD test
*indicates the significance of interaction as determined by analysis of variance ($P=0.003$)

Soil water pressure effect on ln root/shoot ratio was significant ($P \leq 0.05$) at week 2. The ratio was 10% larger in plants grown at -0.15 MPa compared to those at -0.03 MPa.

Foliar dry weight. Interactive effects of soil water pressure and fungi were significant ($P \leq 0.05$) at week 2 (Table 2). In plants grown in soil infested with *G. virens* plus *V. dahliae*, foliar dry weight was 59% greater at -0.15 than at -0.03 MPa. Moreover, foliar dry weight was inversely proportional to soil water pressure for all the treatments except *F. equiseti* plus *V. dahliae*.

Significant differences among fungal treatments with respect to foliar dry weight occurred at week 3. Foliar dry weight of plants grown in soil noninfested or infested with *V. dahliae* and either *G. virens* or *F. equiseti* was significantly greater than that of plants grown in *V. dahliae* infested soil. Differences between the biocontrol agents were not significant.

Differences between soil water pressures were significant at week 1 and week 4. Foliar dry weight was 38% greater in plants maintained at -0.15 MPa compared to those at -0.03 MPa at week 1. The effect, however, was reversed at week 4. Foliar dry weight of plants maintained at -0.03 MPa was 15% greater than those at -0.15 MPa.

Recovery of *Verticillium dahliae* from roots. Recovery of *V. dahliae* differed significantly ($P \leq 0.05$) among fungal treatments during the first

Table 2. Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure on foliar dry weight of potatoes.

	Foliar dry weight										
	Week1			Week2		Week3			Week4		
	Soil water pressure										
Fungal treatment	-0.15	-0.03	Mean[x]	-0.15	-0.03	-0.15	-0.03	Mean	-0.15	-0.03	Mean
control	1.09	0.87	0.98	3.36	2.78	8.48	9.63	9.05 a[y]	9.02	11.35	10.18
V. dahliae	1.09	0.50	0.79	3.34	2.07	8.16	6.58	7.37 b	9.66	9.97	9.81
G. virens + *V. dahliae*	1.27	0.61	0.94	5.81	2.37	10.31	8.90	9.60 a	9.28	11.01	10.14
F. equiseti + *V. dahliae*	1.11	0.83	0.97	3.42	3.96	9.92	11.39	10.65 a	11.79	14.71	13.25
Mean[x]	1.14 A	0.70 B		*		9.21	9.12		9.93 B	11.76 A	

[x]Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns
[y]Means followed by different letters are significantly different at $P \leq 0.05$ according to Fisher's Protected LSD test
*indicates the significance of interaction as determined by analysis of variance ($P = 0.003$)

three weeks of the experiment (Fig. 4A-D). *Fusarium equiseti* suppressed the number of root infections by *V. dahliae* at weeks 1 and 2. Plants grown in soil infested with *F. equiseti* plus *V. dahliae* had 32 and 66% fewer root infections by *V. dahliae* compared to those grown in soil infested with *V. dahliae* alone at week 1 and week 2, respectively. At week 3, however, number of *V. dahliae* infections was greater with *F. equiseti* compared to *V. dahliae* alone. Plants grown in soil infested with *F. equiseti* plus *V. dahliae* resulted in 17% more colony forming units of *V. dahliae* per centimeter of root than those grown in soil infested with *V. dahliae* alone. *Gliocladium virens* had no effect on frequency of root colonization by *V. dahliae*.

Effect of soil water pressure on root colonization of *V. dahliae* was significant ($P \leq 0.05$) at weeks 1 and 3 (Fig. 5A-D). For these two respective sampling dates, plants grown at -0.15 MPa had 34 and 27% more root infections by *V. dahliae* than those grown at -0.03 MPa. No interactive effect of main effect treatments was observed.

Recovery of the biocontrol agents from roots. Effect of soil water pressure on root colonization by *G. virens* was significant ($P \leq 0.05$) at week 3 (Fig. 6A-D). Number of root infections by *G. virens* was 42% greater in plants grown at -0.15 MPa than those at -0.03 MPa. Frequency of root colonization by *F. equiseti* was not affected by soil water pressure.

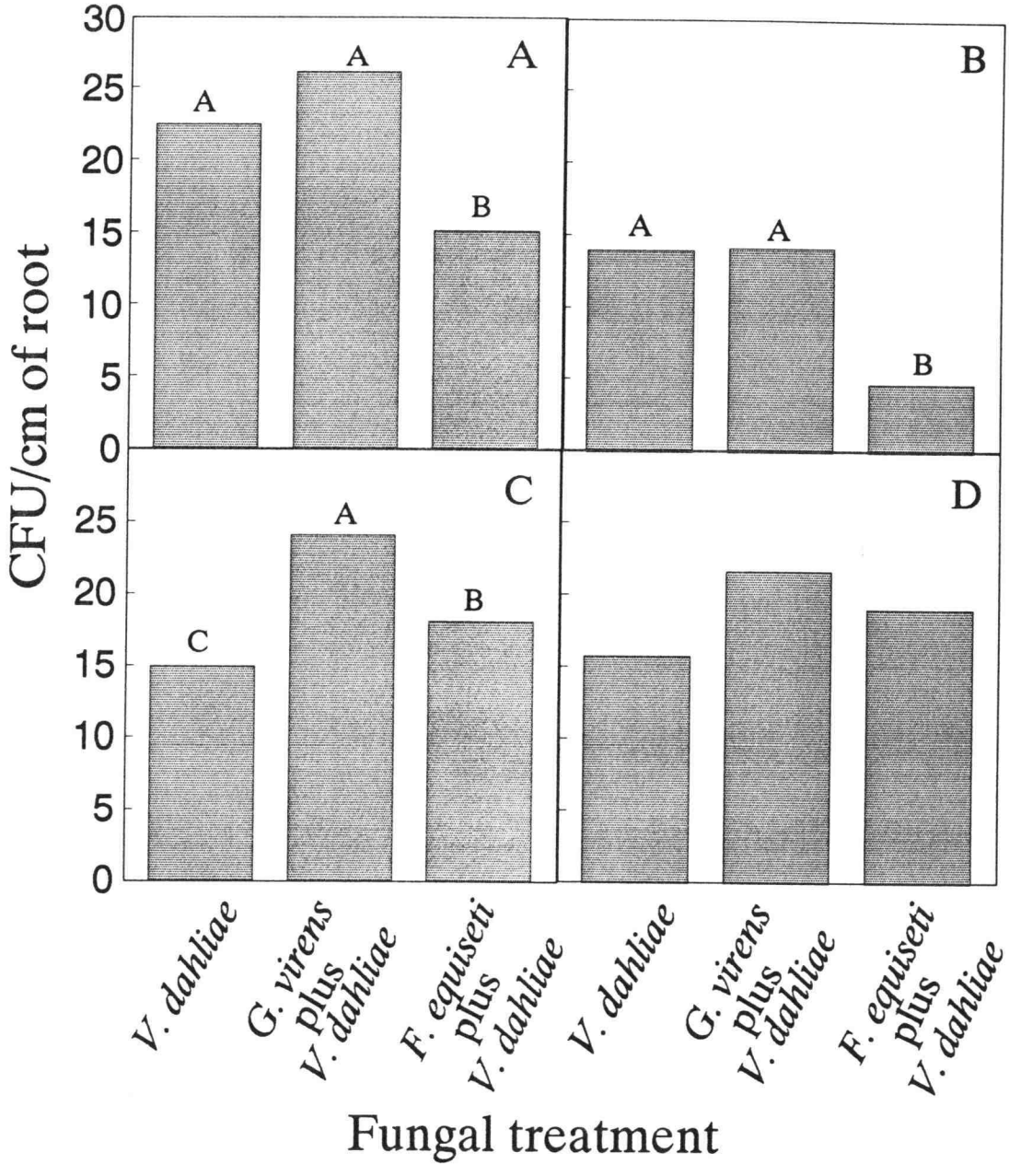

Fig. 4. Effect of fungal treatment on potato root colonization by *Verticillium dahliae* at -0.03 and -0.15 MPa at A) week 1, B) week 2, C) week 3, and D) week 4. Mean colony density is presented as colony forming units (CFU) per centimeter of root. Within weeks, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD.

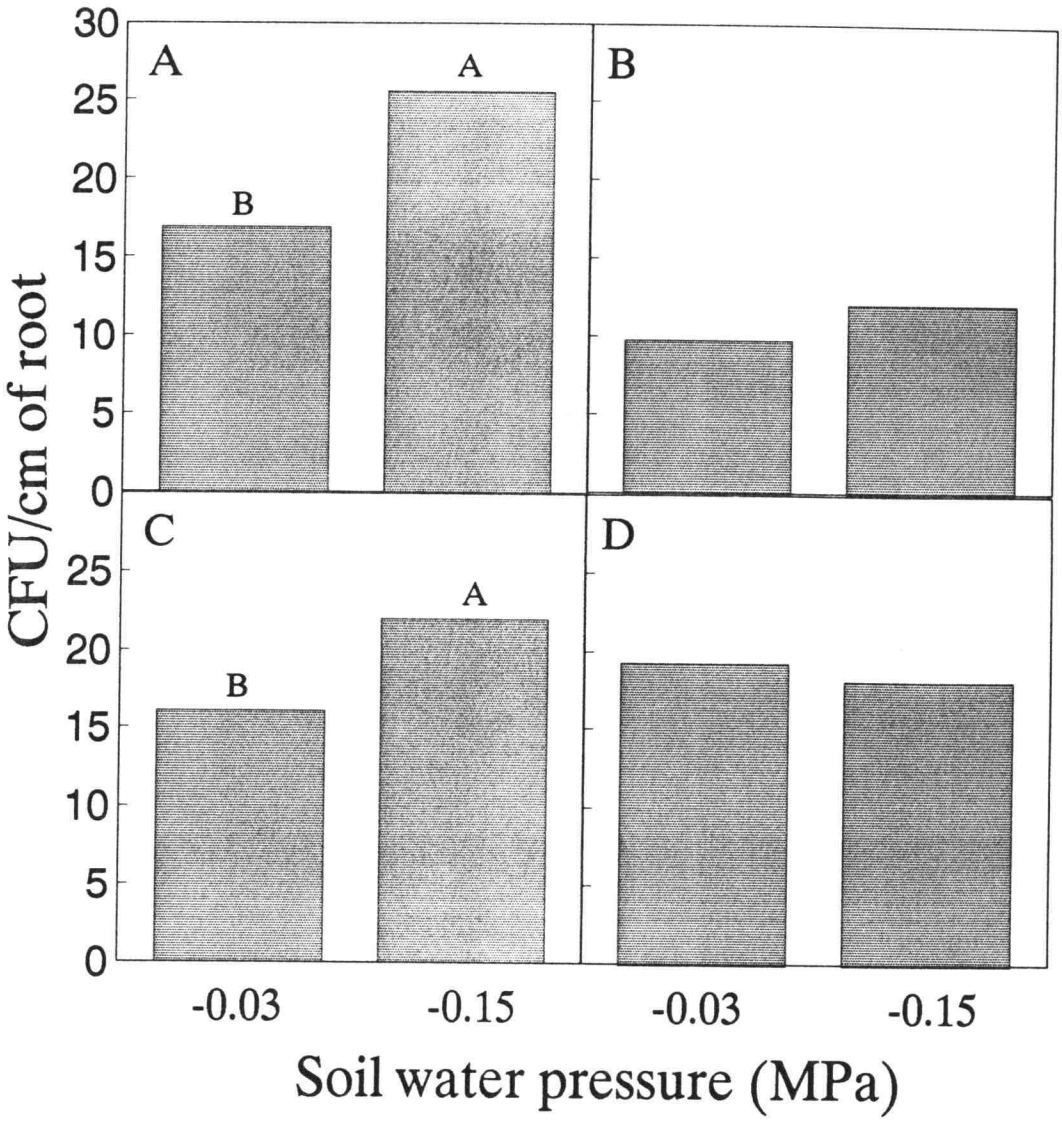

Fig. 5. Effect of soil water pressure on potato root colonization by *Verticillium dahliae* grown in soil infested with *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* at A) week 1, B) week 2, C) week 3, and D) week 4. Mean colony density is presented as colony forming units (CFU) per centimeter of root. Within weeks, bars with different letter are significantly different ($P \leq 0.05$), according to Fisher's Protected LSD.

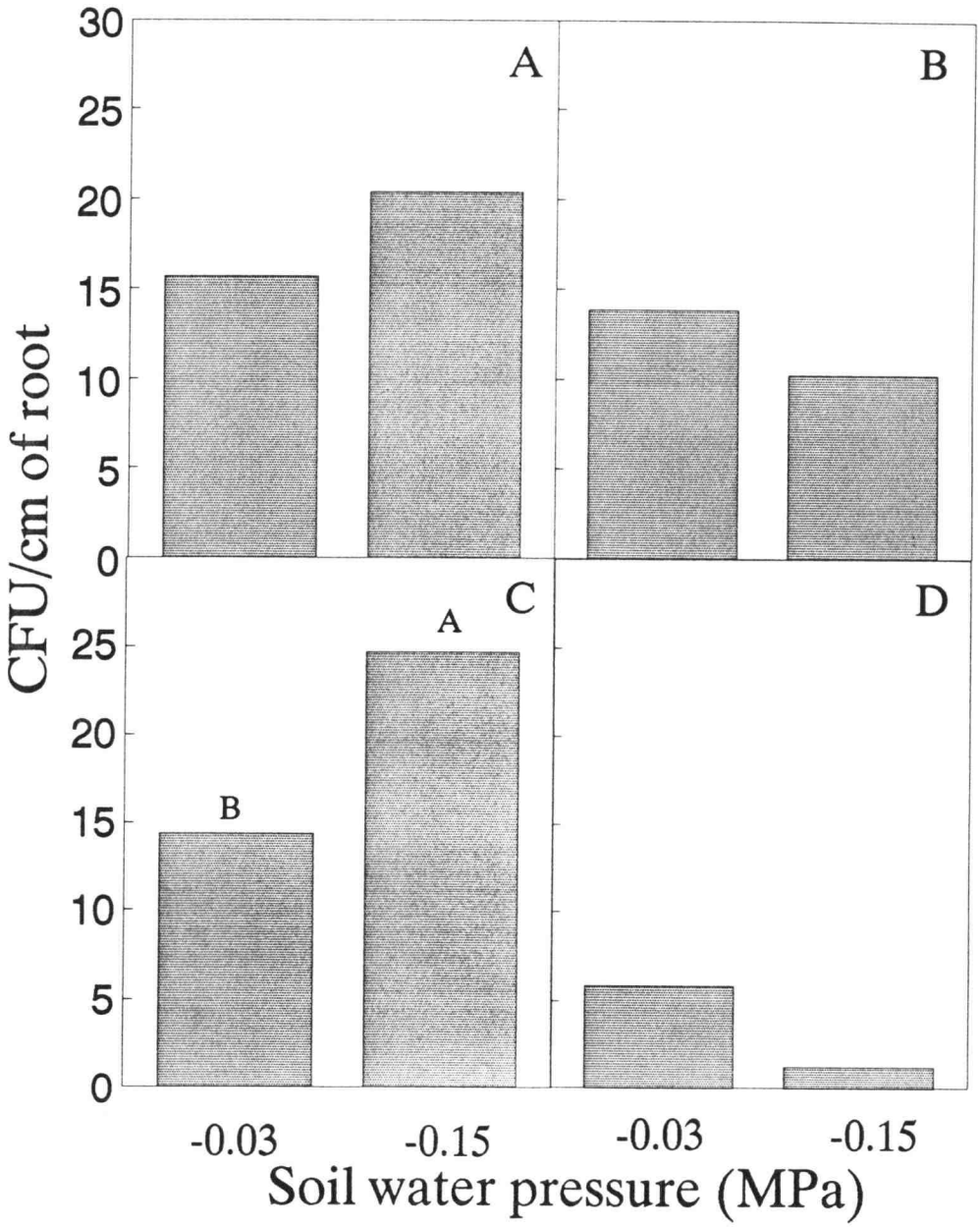

Fig. 6. Effect of soil water pressure on potato root colonization by *Gliocladium virens* grown in soil infested with *Verticillium dahliae* in combination with *Gliocladium virens.* at A) week 1, B) week 2, C) week 3, and D) week 4. Mean colony density is presented as colony forming units (CFU) per centimeter of root. Within weeks, bars with different letters are significantly different ($P \leq 0.05$), according to Fisher's Protected LSD.

Recovery of *Verticillium dahliae* from stem sap. Interactive effect of soil water pressure and fungal treatments on the square root transform data was significant ($P \leq 0.05$) at week 2 of the experiment (Table 3). Number of vascular infections were inversely proportional to soil water pressure for plants grown in soil infested with *V. dahliae* alone or in combination with *G. virens*. The relationship, however, was reversed for plants grown in soil infested with *V. dahliae* plus *F. equiseti*. Plants grown in soil infested with *V. dahliae* plus *F. equiseti* at -0.15 MPa had 72% fewer colony forming units of *V. dahliae* per ml of sap than those at -0.03 MPa. No significant effect of the main effect treatments was observed at any of the sampling dates.

Table 3. Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure on square root of mean vascular population of *V. dahliae* in potatoes.

	Square root vascular population										
	Week1			Week2		Week3			Week4		
	Soil water pressure										
Fungal treatment	-0.15	-0.03	Mean[x]	-0.15	-0.03	-0.15	-0.03	Mean	-0.15	-0.03	Mean
V. dahliae	3.57	0.93	2.25	4.57	1.84	2.21	1.80	2.01	3.84	3.06	3.45
V. dahliae + *G. virens*	1.37	1.00	1.19	2.40	1.00	4.19	1.62	2.91	3.04	1.27	2.98
V. dahliae + *F. equiseti*	0.08	1.50	1.19	0.62	2.21	1.06	1.66	1.36	1.27	2.94	2.11
Mean[x]	1.94	1.14		*		2.49	1.69		1.58	2.42	

[x]Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns
*indicates the significance of interaction as determined by analysis of variance ($P = 0.009$)

DISCUSSION

Time required for 95% germination, mycelial growth and production of reproductive structures by *F. equiseti*, *G. virens*, *T. flavus*, *T. viride*, and *V. dahliae* were qualitatively similar but quantitatively different over the range of osmotic potentials tested. In general, time for 95% spore germination increased whereas mycelial growth decreased as osmotic potential of the agar medium decreased from -0.12 to -8.13 MPa. *Fusarium equiseti* took the least amount of time to complete 95% germination whereas *G. virens* took the longest. Germination of *F. equiseti* spores was two-fold faster than microsclerotia of *V. dahliae* below -3.16 MPa. The ability of *F. equiseti* spores to germinate quickly may prove to be a factor in its success as a biocontrol agent of *V. dahliae*. Spores of *G. virens* did not obtain 95% germination below -7.16 MPa. The spores were either dead, dormant or the osmotic potential of the medium was too low for the spores to germinate. Above -5.13 MPa, spores of *T. viride* completed 95% germination comparatively slowly compared to microsclerotia of *V. dahliae*. *Trichoderma viride*, therefore, could be at a relative disadvantage compared to *V. dahliae* in wet soils. The decreasing rate of germination of microsclerotia of *V. dahliae* with a decrease in osmotic potential of the medium is in agreement with the results of Mozumder et al (1970) who reported a similar trend in germination of conidia of the fungus. Microsclerotia of *V. dahliae* germinate, penetrate and

infect the root cortex. Some of the cortical infections successfully invade the stele where the fungus produces conidia which are disseminated in the xylem. The water potential of xylem fluid, therefore, may affect systemic infection by influencing both production of conidia and their movement in the vascular tissue. *Talaromyces flavus* has been reported as a potential antagonist of many soilborne plant pathogens including *V. dahliae* (Fravel et al, 1986; Katan, 1985; Marois et al, 1982). In our study, however, none of the spores germinated at and below -2.15 MPa, and despite a heat treatment, only 45% germinated at osmotic potentials above -2.15 MPa after 60 h. Katan (1985) reported that if ascospores of the fungus are not heated to a temperature of 70 C for 1 h, only about 5-10% germinate. The lower percent spore germination in our study might be due to the use of MYP instead of PDA, the medium used by Katan.

The reduction in mycelial growth with a decrease in osmotic potential of the medium and/or soil water pressure is in agreement with previous studies (Cook and Papendick, 1972; Ioannou et al, 1977; Swart et al, 1992). Results obtained with KCl are largely attributed to osmotic stress rather than toxicity of the salt (Boddy, 1983; Jacobi and Riffle, 1989; Malajczuk and Theodorou, 1979; Sommers et al, 1970). *Fusarium equiseti*, *T. flavus* and *V. dahliae* showed a trend of slight increase in mycelial growth as the osmotic potential of the medium was lowered up to a certain point, after which each showed a reduction in growth with further lowering of osmotic potential. Cook and

Duniway (1980) reported that growth of many fungi is stimulated by reduction of the osmotic potential of the medium by -0.5 to -2.0 MPa. The optimal osmotic potential, however, depends upon the fungus. Moreover, several other factors such as solute (Bruehl and Cunfer, 1971), and temperature (Manandhar and Bruehl, 1973) also affect the osmotic potential at which optimal growth of a fungus occurs. Maximal radial growth was observed at -0.12 to -3.16 MPa for both *G. virens* and *T. viride*. However, unlike *T. viride* which grew at all osmotic potentials tested, *G. virens* did not grow at -8.13 MPa suggesting that the latter was less tolerant to low osmotic potentials than the former.

Formation of reproductive structures in all the fungi except *F. equiseti* ceased well above the osmotic potential at which mycelial growth continued to occur. Cook and Duniway (1980) reported that water potential requirements for sporulation of fungi were more strict than those for initiation and maintenance of mycelial growth. *Gliocladium virens*, *T. flavus* and *T. viride* did not produce spores below -1.10 MPa, which is in agreement with Cook and Duniway (1980) who reported a range of 0 to -1.5 MPa for the production of sporangia, apothecia or other reproductive structures. *Verticillium dahliae* did not produce microsclerotia below -0.59 MPa. Ioannou et al (1977) reported that production of microsclerotia in *V. dahliae* was more sensitive to decreases in osmotic potential than was mycelial growth. Our results, however, differ from those of Ioannou et al (1977) who reported formation of microsclerotia at osmotic potentials of -7.0 and -8.0 MPa. Water requirements of isolates of a

species or subspecies may be different (Sung and Cook, 1981). Maximal chlamydospore production among isolates of *Fusarium roseum* '*graminearum*', '*culmorum*' and '*avenaceum*', collected from dry areas of Washington state occurs at -1.5 MPa compared with Pennsylvania isolates of *F. roseum* '*graminearum*' which occurs at -0.14 MPa. In addition to different isolates, use of MYP instead of PDA may explain, in part, differences in results between the studies. Production of macroconidia by *F. equiseti* at all osmotic potentials tested is in agreement with Sung and Cook (1981) who reported that *F. roseum* produced macroconidia at osmotic potentials as low as -6 to -8 MPa.

In the greenhouse study, treatments with biocontrol agents were variable in their effects on the population dynamics of *V. dahliae* in roots. Recovery of *V. dahliae* from roots was significantly reduced only during the first two weeks of the experiment with the *F. equiseti* plus *V. dahliae* treatment. *Fusarium equiseti* has been reported as a weak root pathogen (James et al, 1993). Early colonization of infection courts by *F. equiseti* may have resulted in the lower frequency of root infection by *V. dahliae* during the first two weeks. The lack of an effect at the later sampling dates could have resulted from a loss in viability of propagules of *F. equiseti*. In contrast, *G. virens* had no effect on root colonization by *V. dahliae*. *Gliocladium virens* is a soil saprophyte and is not regarded as effective as *F. equiseti* in colonizing roots. Dix (1964) reported *G. virens* as a root surface colonizer of older roots

in beans. By the time roots were old enough to be colonized by *G. virens*, they were already colonized by V. *dahliae*. Thus *G. virens* was not successful in preventing root colonization by *V. dahliae*.

Plant growth responses including foliar dry weight and ln root/shoot ratio were significantly affected by both *G. virens* and *F. equiseti*. In the presence of *F. equiseti*, there was an increase in foliar dry weight at week 3. A similar effect on both foliar dry weight and ln root/shoot ratio was observed with *G. virens* at the last two sampling dates. Biocontrol agents are known to enhance plant growth responses. Baker et al (1984) reported an increase in height, weight, branch and flower production in *T. harzianum* treated chrysanthemum and peanut plants. Similarly, Elad and Chet (1983) demonstrated that *Trichoderma* propagules were present in plants that showed increased growth responses. Similar effects have been documented with plant growth promoting rhizobacteria (PGPR) on potato (Bakker et al, 1989; Frommel et al, 1991). Improved plant growth by PGPR has been attributed to the ability of PGPR to suppress the pathogen by outcompeting it for nutrients (Alstrom and Burns, 1989; Bakker et al, 1989). Moreover, alteration of host physiology resulting in subsequent production of plant growth hormones has also been implicated as one of the mechanisms (Frommel et al, 1991). Thus the increased growth responses seen in our study could be due to suppression of pathogen, increase in plant vigor or induced host resistance. These hypotheses, however, warrant further investigation.

Plant growth responses were affected differentially by soil water pressure. Fresh root weight at weeks 2 and 3 and ln root/shoot ratio at week 2 were significantly lower for plants maintained at -0.03 compared to -0.15 MPa. Poor aeration of the soil due to excessive moisture may have contributed to reduced fresh root weight at this soil water pressure. Our results, however, differ from those of Gaudreault et al (1992) who reported a higher fresh root weight at -0.03 than at -0.15 MPa. A possible reason is that the soil in our experiment was mixed before planting which could have resulted in different bulk densities.

It is paradoxical that despite high fresh root weight at -0.15 MPa, a significant reduction in plant height was observed at this soil water pressure at the last two sampling dates. This could be due to stress resulting from continued exposure of plants to a low water level. Hsiao et al (1976) reported that a reduction from 0 to -0.1 MPa in water potential may reduce the rate of cell division in apical meristems by as much as 50%. Vaadia et al (1961) reported that reduction in growth due to water stress was the result of impaired protein synthesis. Moreover, impaired auxin production resulted in shortened internodes and short stunted plants. A higher fresh root weight at -0.15 than at-0.03 MPa resulted in a larger ln root/shoot ratio at this soil water pressure.

Soil water pressure effect on foliar dry weight varied across sampling dates. Plants maintained at -0.15 compared to -0.03 MPa had greater foliar dry weight at week 1. The effect was reversed at week 4. In another study

Gaudreault et al (1992) concluded that these two soil water pressures had no differential effect on foliar dry weight. A relatively higher greenhouse temperature in our study may have promoted plant growth. Reasons for inconsistent effect of soil water pressure at different harvest dates are, however, unclear.

Colonization of roots by both *V. dahliae* and *G. virens* was affected by soil water pressure. Number of root infections by *V. dahliae* was lower at high compared to low soil water pressure which could be due to anaerobic conditions. Pullman and Devay (1981) reported that flooding resulted in a reduction in population of microsclerotia of *V. dahliae* in soil. Factors contributing to this reduction included antagonism by anaerobic soil microorganisms. Soils at -0.03 MPa could, therefore, be suppressive to *V. dahliae*. A higher rate of root colonization by *G. virens* at -0.15 MPa is in agreement with a previous study in which Keinath et al (1991) reported significant reduction in viability of microsclerotia of *V. dahliae* by *G. roseum* in soil at -0.01 and -0.1 MPa. Colonization of roots by *F. equiseti*, however, was not affected by soil water pressure. Huisman (1988) reported that colonization of cotton roots by *Fusarium* spp. was not affected by soil moisture.

Fungal treatments and soil water pressure interacted to affect foliar dry weight, ln root/shoot ratio and vascular colonization by *V. dahliae*. *Gliocladium virens* and *F. equiseti* at -0.15 MPa increased foliar dry weight

and ln root/shoot ratio, respectively. *Fusarium equiseti* at -0.15 MPa dramatically reduced vascular colonization by *V. dahliae*. Improved growth response could be due to better establishment of the biocontrol agents at low soil water pressure and their subsequent induction of host resistance. Colonization of roots by *F. equiseti* at -0.15 MPa probably prevented *V. dahliae* from colonizing the roots and hence its transport to the vascular system.

Our study showed that the potential for biocontrol of *V. dahliae* exists. However, a better understanding of the effects of moisture on growth and survival of potential biocontrol agents of *V. dahliae* are needed before they can be effectively integrated into normal production systems for suppression of diseases and/or pathogens.

CONCLUSIONS

* Time for 95% spore germination increased as osmotic potential of the medium decreased.
* Spores of *F. equiseti* took the shortest whereas those of *G. virens* the longest amount of time to complete 95% germination.
* Ascospores of *T. flavus* did not obtain 95% germination even after heat treatment at 70 C for 1 h.
* Radial growth of fungi decreased as osmotic potential of the medium decreased.
* *Gliocladium virens*, *T. viride*, and *T. flavus* did not sporulate below -1.10 MPa, whereas *V. dahliae* did not produce microsclerotia below -0.59 MPa.
* *Fusarium equiseti* produced macroconidia but no chlamydospores over the range of osmotic potentials tested.
* *Fusarium equiseti* decreased root colonization by *V. dahliae* and increased foliar dry weight of plants.
* *Gliocladium virens* had no effect on root colonization by *V. dahliae* but increased foliar dry weight and ln root/shoot ratio.
* Neither biocontrol agents had an effect on plant height, fresh root weight or vascular colonization by *V. dahliae*.

* Effect of soil water pressure on plant height and foliar dry weight were inconsistent.

* Fresh root weight, ln root/shoot ratio and colonization of roots by *G. virens* was greater at -0.15 than at -0.03 MPa.

* Infection of roots by *V. dahliae* was reduced at -0.03 MPa.

* Soil water pressure had no effect on root colonization by *F. equiseti* and vascular colonization by *V. dahliae*.

* Interactive effects of main effect treatments were observed for foliar dry weight, ln root/shoot ratio and vascular colonization by *V. dahliae*.

* The interaction was such that ln root/shoot ratio increased at -0.15 MPa in soil infested with *V. dahliae* plus *F. equiseti*, and foliar dry weight at -0.15 MPa in soil infested with a combination of *G. virens* and *V. dahliae*.

* Vascular colonization by *V. dahliae* was reduced at -0.15 MPa in soil infested with *V. dahliae* plus *F. equiseti*.

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Appendices

Appendix I

PREPARATION OF *VERTICILLIUM* INOCULUM USING CELLOPHANE COVERED MEDIUM.

I. *Verticillium* inoculum medium (VIM) (MM/Exp. Mycol. 7:328-335)

1. Add to 1 L distilled water:

Sucrose	30.00 g
$NaNO_3$	2.00 g
KH_2PO_4	1.00 g
$MgSO_4 * 7H_2O$	0.50 g
KCl	0.50 g
Trace element solution	0.20 ml
Difco agar	20.00 g

2. Trace element solution:

Distilled water	95.00 ml
Citric acid	5.00 g
$ZnSO_4 * 7H_2O$	5.00 g
$FeSO_4 * 7H_2O$	4.75 g
$Fe(NH_4)_2(SO_4) * 6H_2O$	1.00 g
$CuSO_4 * 5H_2O$	0.25 g
$MnSO_4$	0.05 g
H_3BO_3	0.05 g
Na2MoO4	0.05 g

A. Autoclave and pour in to Petri plates.

B. Cellophane preparation

1. Cellophane is from Bio-Rad (Bio-Rad Model 43 Gel Dryer, Catalog # 165-0922, Cellophane Membrane Backing, 18 X 34 cm, 50 sheets).
2. Cut cellophane sheets into circles, a size that will allow them to fit over the medium on Petri plates.
3. Wrap cellophane circles in aluminum foil and autoclave.

C. Laying of cellophane: This is done any time after surface of VIM plates has dried. Using sterile technique, lay cellophane circles over the medium with two pairs of tweezers.

D. Addition of inoculum

1. Ideally, inoculum should come from fresh cultures that were prepared using single spore isolates to ensure that cultures are fertile,i.e., produce microsclerotia and to reduce presence of sterile sectors.
2. Each plate of inoculum is flooded with sterile water. It is not critical to use a particular amount of water, rather it can be varied according to the size of the culture.
3. Using a flamed L-shaped glass stirring rod, rub the surface of the

culture. This will release conidia in to the water resulting in a spore suspension.

4. Pipette about 1 ml of the spore suspension onto cellophane/VIM plates with sterile pipette tips. Spread the suspension evenly on the cellophane with sterile glass rod.
5. Each culture plate can be used to inoculate at least 20 plates, depending on the amount of water used to make the spore suspension.

E. Store the plates in the dark for about 3 wk. Cultures grow best at room temperature in an environment that is not excessively humid or warm. Once the cellophane is covered with microsclerotia, the plates are ready to be sieve processed.

II. Sieve-processing

A. Remove sclerotia-covered cellophane from about 30 plates and blend in a blender that is half filled with tap water.

B. Pass this mixture through a series of two sieves: 140 and 175 μm. Material deposited on the sieve of size 140 μm should be saved and dried.

C. Optional- If it is necessary to maximize the inoculum obtained, microsclerotia can be obtained from the water that passes through the 175 μm mesh sieve. To separate these microsclerotia, the water is allowed to sit for a few hours so that most of the inoculum settles to the bottom of the container. Most of the supernatant is then siphoned off, and the remaining slurry of inoculum is centrifuged at low speed for a few minutes to further concentrate the microsclerotia.

Appendix II

NUTRIENT WATERING SOLUTION

The following nutrient fertilizing solution was used for initial watering of pots in the greenhouse. Stock solutions were prepared individually.

	STOCK SOLUTIONS	
Dry compound	mol.wt.	g/L of water
KH_2PO_4	136.07	136.07
$(NH_4)_2SO_4$	132.08	132.08
$Ca(NO_3)_2 * 4H_2O$	236.16	236.16
$MgSO_4 * 7H_2O$	246.49	246.49

To prepare the final solution, these stock solutions were mixed together with water in the following concentrations:

Stock solution	ml/L of H_2O
KH_2PO_4	10
$(NH_4)_2SO_4$	5
$Ca(NO_3)_2 * 4H_2O$	5
$MgSO_4 * 7H_2O$	1

The final solution was prepared in bulk, e.g., 120, 60, 60, and 12 ml of respective stock solutions were added to approximately 11748 ml of water in a large bucket (totalling 12 L of final solution) and mixed. A total of 600 ml of the final solution was added to each pot of approximately 7100 g soil.
Consequently, nutrients added to each pot were approximately in the following concentration:

Element	m mole/pot
N	11.0
P	5.7
K	5.7
Ca	2.8
S	3.4
Mg	0.57

Appendix III

PREPARATION OF POTATO SEED PIECES BY MELON BALLING

I. Vermiculite preparation.
   A. Autoclave moistened vermiculite in autoclave bags for 1 h.
   B. Clean out crisper boxes with 95% alcohol. Rinse with distilled water.
   C. Pour vermiculite in to boxes in 2.5 cm layers.

II. Potatoes.
   A. Obtain appropriate potato seed tubers.
   B. Using a heavy duty (2.5 cm dia) melon ball scooper, scoop out pieces surrounding the eyes. Scoop extra seed pieces because not all of the eyes will sprout adequately.
   C. Leave seed pieces to air dry overnight so that they become somewhat callused.
   D. Place seed pieces in crisper boxes between layers of vermiculite, and let them sprout for a week at room temperature. Refrigerate for storage.

Appendix IV

ANOVA SUMMARIES

95% Spore Germination Time

Source of Variation	df	MS	F	P
Trichoderma viride				
Model	1	1064.8727	98.09	0.0001
OSPOT	1	1064.8727	98.09	0.0001
Error	8	10.8565		
Gliocladium virens				
Model	1	523.4507	78.00	0.0001
OSPOT	1	523.4507	78.00	0.0001
Error	6	6.7113		
Verticillium dahliae				
Model	2	1255.8439	684.80	0.0001
OSPOT	1	2196.5433	1197.76	0.0001
OSPOT x OSPOT	1	315.1445	171.85	0.0001
Error	7	1.8339		
Fusarium equiseti				
Model	1	370.3535	72.01	0.0001
OSPOT	1	370.3535	72.01	0.0001
Error	8	5.1433		

Mean Colony Diameter

Source of Variation	df	MS	F	P
Talaromyces flavus				
Model	1	15.9059	124.78	0.0001
OSPOT	1	15.9059	124.78	0.0001
Error	5	0.1275		
Trichoderma viride				
Model	1	21.8773	202.06	0.0008
OSPOT	1	21.8773	202.06	0.0008
Error	3	0.1083		
Gliocladium virens				
Model	1	10.4559	35.67	0.0269
OSPOT	1	10.4559	35.67	0.0269
Error	2	0.2931		
Verticillium dahliae				
Model	3	1.3385	14.98	0.0034
OSPOT	1	1.7898	20.03	0.0042
OSPOT x OSPOT	1	1.5938	17.84	0.0055
OS x OS x OS	1	0.6318	7.07	0.0376
Error	6	0.0893		
Fusarium equiseti				
Model	1	2.1846	11.86	0.0750
OSPOT	1	2.1846	11.86	0.0750
Error	2	0.1842		

Plant Height

Source of Variation	df	MS	F	P
Week 1				
Model	31	51.1637	1.81	0.0503
Block	3	75.1189	2.66	0.0652
Fungus	3	101.3018	3.58	0.0244
SWP	1	131.9627	4.66	0.0384
Block x fungus	9	63.3932	2.24	0.0452
Block x SWP	3	8.7718	0.31	0.8179
Fungus x SWP	3	29.6081	1.05	0.3854
Blo x fun x SWP	9	26.5745	0.94	0.5057
Error	32	28.2923		
Week 2				
Model	31	65.1522	0.99	0.5160
Block	3	134.0114	2.03	0.1305
Fungus	3	27.2490	0.41	0.7454
SWP	1	160.7424	2.43	0.1291
Block x fungus	9	72.6071	1.10	0.3924
Block x SWP	3	49.8264	0.75	0.5286
Fungus x SWP	3	9.2675	0.14	0.9352
Blo x fun x SWP	9	58.3235	0.88	0.5512
Error	31	66.1035		
Week 3				
Model	31	37.2576	1.00	0.4988
Block	3	47.3385	1.27	0.3009
Fungus	3	32.0260	0.86	0.4718
SWP	1	280.5625	7.53	0.0098
Block x fungus	9	22.7934	0.61	0.7776
Block x SWP	3	20.0521	0.54	0.6594
Fungus x SWP	3	15.8229	0.42	0.7365
Blo x fun x SWP	9	35.9514	0.97	0.4858
Error	32	37.2422		
Week 4				
Model	31	159.6915	1.89	0.0394
Block	3	347.7292	4.11	0.0142
Fungus	3	138.1042	1.63	0.2014
SWP	1	1156.0000	13.66	0.0008
Block x fungus	9	148.1042	1.75	0.1179
Block x SWP	3	37.8333	0.45	0.7211
Fungus x SWP	3	204.6250	2.42	0.0844
Blo x fun x SWP	9	30.7361	0.36	0.9442
Error	32	84.6250		

Fresh Root Weight

Source of Variation	df	MS	F	P
Week 1				
Model	31	9.7355	5.19	0.0001
Block	3	5.0141	2.67	0.0638
Fungus	3	1.0030	0.54	0.6616
SWP	1	26.3682	14.07	0.0007
Block x fungus	9	14.1505	7.55	0.0001
Block x SWP	3	1.9915	1.06	0.3787
Fungus x SWP	3	19.3090	10.30	0.0001
Blo x fun x SWP	9	7.3474	3.92	0.0019
Error	32	1.8745		
Week 2				
Model	31	17.1085	1.43	0.1628
Block	3	14.6678	1.23	0.3172
Fungus	3	4.5443	0.38	0.7684
SWP	1	267.6123	22.35	0.0001
Block x fungus	9	9.3584	0.78	0.6346
Block x SWP	3	10.3960	0.87	0.4680
Fungus x SWP	3	26.6906	2.23	0.1045
Blo x fun x SWP	9	8.1516	0.68	0.7201
Error	31	11.9731		
Week 3				
Model	31	15.5587	1.40	0.1763
Block	3	4.1468	0.37	0.7736
Fungus	3	19.4677	1.75	0.1772
SWP	1	116.9102	10.49	0.0028
Block x fungus	9	16.3984	1.47	0.2007
Block x SWP	3	6.2422	0.56	0.6452
Fungus x SWP	3	14.8581	1.33	0.2808
Blo x fun x SWP	9	9.2979	0.83	0.5902
Error	32	11.1436		
Week 4				
Model	31	25.3277	2.73	0.0030
Block	3	0.8279	0.09	0.9654
Fungus	3	129.7267	14.00	0.0001
SWP	1	0.3600	0.04	0.8450
Block x fungus	9	7.9707	0.86	0.5690
Block x SWP	3	20.5263	2.21	0.1055
Fungus x SWP	3	8.3550	0.90	0.4512
Blo x fun x SWP	9	26.0840	2.81	0.0148
Error	32	9.2675		

Ln Root/Shoot Ratio

Source of Variation	df	MS	F	P
Week 1				
Model	31	0.4490	4.57	0.0001
Block	3	0.3566	3.63	0.0233
Fungus	3	1.0221	10.39	0.0001
SWP	1	2.5883	26.32	0.0001
Block x fungus	9	0.1956	1.99	0.0740
Block x SWP	3	0.4224	4.29	0.0118
Fungus x SWP	3	0.4362	4.44	0.0102
Blo x fun x SWP	9	0.3177	3.23	0.0067
Error	32	0.0983		
Week 2				
Model	31	0.0603	1.92	0.0366
Block	3	0.1388	4.43	0.0105
Fungus	3	0.0920	2.93	0.0488
SWP	1	0.5026	16.04	0.0004
Block x fungus	9	0.0291	0.93	0.5156
Block x SWP	3	0.0086	0.27	0.8442
Fungus x SWP	3	0.0216	0.69	0.5662
Blo x fun x SWP	9	0.0446	1.42	0.2216
Error	31	0.0313		
Week 3				
Model	31	0.1167	2.09	0.0210
Block	3	0.0462	0.83	0.4890
Fungus	3	0.3004	5.38	0.0041
SWP	1	0.8700	15.57	0.0004
Block x fungus	9	0.0529	0.95	0.4993
Block x SWP	3	0.0119	0.21	0.8868
Fungus x SWP	3	0.3153	5.64	0.0032
Blo x fun x SWP	9	0.0276	0.49	0.8673
Error	32	0.0559		
Week 4				
Model	31	0.0740	1.58	0.1013
Block	3	0.0037	0.08	0.9708
Fungus	3	0.4002	8.56	0.0003
SWP	1	0.1799	3.85	0.0586
Block x fungus	9	0.0280	0.60	0.7879
Block x SWP	3	0.0831	1.78	0.1715
Fungus x SWP	3	0.1137	0.24	0.8656
Blo x fun x SWP	9	0.0407	0.87	0.5615
Error	32	0.0468		

Foliar Dry Weight

Source of Variation	df	MS	F	P
Week 1				
Model	31	0.3821	2.42	0.0077
Block	3	0.4855	3.07	0.0418
Fungus	3	0.1220	0.77	0.5188
SWP	1	3.0713	19.42	0.0001
Block x fungus	9	0.4224	2.67	0.0195
Block x SWP	3	0.2086	1.32	0.2855
Fungus x SWP	3	0.1891	1.20	0.3272
Blo x fun x SWP	9	0.2176	1.38	0.2399
Error	32	0.1582		
Week 2				
Model	31	3.9421	1.97	0.0320
Block	3	3.6042	1.80	0.1679
Fungus	3	4.8944	2.44	0.0828
SWP	1	24.9260	12.44	0.0013
Block x fungus	9	1.4900	0.74	0.6667
Block x SWP	3	2.0300	1.01	0.4001
Fungus x SWP	3	11.4224	5.70	0.0031
Blo x fun x SWP	9	2.0930	1.04	0.4286
Error	31	2.0033		
Week 3				
Model	31	6.9370	1.39	0.1820
Block	3	0.2341	0.05	0.9863
Fungus	3	30.1446	6.02	0.0023
SWP	1	0.1305	0.03	0.8728
Block x fungus	9	3.7760	0.75	0.6580
Block x SWP	3	6.5104	1.30	0.2914
Fungus x SWP	3	10.5735	2.11	0.1183
Blo x fun x SWP	9	4.2827	0.86	0.5731
Error	32	5.0082		
Week 4				
Model	31	9.1772	3.36	0.0005
Block	3	3.0725	1.13	0.3531
Fungus	3	41.3478	15.16	0.0001
SWP	1	52.7076	19.32	0.0001
Block x fungus	9	5.8065	2.13	0.0563
Block x SWP	3	1.1736	0.43	0.7328
Fungus x SWP	3	4.9480	1.81	0.1645
Blo x fun x SWP	9	3.1002	1.14	0.3671
Error	32	2.7280		

Root Colony Density of *V. dahliae*

Source of Variation	df	MS	F	P
Week 1				
Model	23	122.8783	2.55	0.0133
Block	3	47.0917	0.98	0.4196
Fungus	2	497.9819	10.34	0.0006
SWP	1	899.6008	18.68	0.0002
Block x fungus	6	36.6577	0.76	0.6073
Block x SWP	3	14.0569	0.29	0.8308
Fungus x SWP	2	131.5515	2.73	0.0853
Blo x fun x SWP	6	44.0234	0.91	0.5018
Error	24	48.1600		
Week 2				
Model	23	78.6651	1.30	0.2680
Block	3	13.0648	0.22	0.8845
Fungus	2	476.5362	7.87	0.0025
SWP	1	76.8800	1.27	0.2715
Block x fungus	6	29.6639	0.49	0.8092
Block x SWP	3	97.9028	1.62	0.2130
Fungus x SWP	2	74.8024	1.23	0.3094
Blo x fun x SWP	6	36.6246	0.60	0.7239
Error	23	60.5739		
Week 3				
Model	23	66.7048	3.76	0.0010
Block	3	13.2211	0.74	0.5359
Fungus	2	338.4400	19.07	0.0001
SWP	1	417.7200	23.53	0.0001
Block x fungus	6	21.5711	1.22	0.3328
Block x SWP	3	26.4444	1.49	0.2425
Fungus x SWP	2	45.6400	2.57	0.0973
Blo x fun x SWP	6	16.6511	0.94	0.4864
Error	24	17.7500		
Week 4				
Model	23	37.9017	0.74	0.7633
Block	3	39.8603	0.78	0.5189
Fungus	2	132.0439	2.57	0.0980
SWP	1	15.6800	0.31	0.5857
Block x fungus	6	13.0039	0.25	0.9528
Block x SWP	3	51.1601	1.00	0.4118
Fungus x SWP	2	74.1839	1.45	0.2562
Blo x fun x SWP	6	8.9664	0.17	0.9810
Error	23	51.3061		

Vascular Colony Density of *V. dahliae*

Source of Variation	df	MS	F	P
Week 1				
Model	23	2.3419	1.08	0.4255
Block	3	0.5869	0.27	0.8454
Fungus	2	4.0204	1.86	0.1784
SWP	1	4.5779	2.12	0.1593
Block x fungus	6	1.8713	0.86	0.5351
Block x SWP	3	0.9341	0.43	0.7323
Fungus x SWP	2	6.9574	3.22	0.0587
Blo x fun x SWP	6	1.5404	0.71	0.6436
Error	23	2.1636		
Week 2				
Model	23	4.6063	1.61	0.1341
Block	3	3.2444	1.13	0.3570
Fungus	2	13.7681	4.81	0.0185
SWP	1	13.8127	4.83	0.0388
Block x fungus	6	2.9889	1.04	0.4239
Block x SWP	3	3.0885	1.08	0.3782
Fungus x SWP	2	16.6624	5.83	0.0093
Blo x fun x SWP	6	0.5081	0.18	0.9801
Error	22	2.8604		
Week 3				
Model	23	3.9070	1.34	0.2393
Block	3	3.4845	1.20	0.3319
Fungus	2	6.2277	2.14	0.1396
SWP	1	11.5796	3.98	0.0575
Block x fungus	6	2.5628	0.88	0.5239
Block x SWP	3	4.3947	1.51	0.2373
Fungus x SWP	2	9.5325	3.28	0.0552
Blo x fun x SWP	6	1.2911	0.44	0.8422
Error	24	2.9102		
Week 4				
Model	23	4.2167	0.96	0.5381
Block	3	6.2764	1.43	0.2598
Fungus	2	10.6944	2.44	0.1098
SWP	1	0.2712	0.06	0.8059
Block x fungus	6	1.5888	0.36	0.8954
Block x SWP	3	2.5828	0.59	0.6289
Fungus x SWP	2	7.0557	1.61	0.2222
Blo x fun x SWP	6	4.2636	0.97	0.4665
Error	23	4.3904		

Root Colony Density Of *Gliocladium virens*

Source of Variation	df	MS	F	P
Week 1				
Model	7	29.2663	0.82	0.5955
Block	3	20.5490	0.58	0.6461
SWP	1	87.8906	2.47	0.1548
Block x SWP	3	18.4423	0.52	0.6815
Error	8	35.5994		
Week 2				
Model	7	43.1702	0.95	0.5187
Block	3	3.9968	0.09	0.9645
SWP	1	52.0202	1.15	0.3151
Block x SWP	3	79.3935	1.75	0.2336
Error	8	45.2902		
Week 3				
Model	7	140.9654	1.85	0.2033
Block	3	160.6225	2.11	0.1773
SWP	1	426.4225	5.60	0.0455
Block x SWP	3	26.1558	0.34	0.7948
Error	8	76.1325		
Week4				
Model	7	43.1105	1.75	0.2392
Block	3	36.6673	1.49	0.2988
SWP	1	80.2222	3.25	0.1143
Block x SWP	3	29.5218	1.20	0.3783
Error	7	24.6571		

Root Colony Density Of *Fusarium equiseti*

Source of Variation	df	MS	F	P
Week 2				
Model	7	0.3147	0.87	0.5683
Block	3	0.5075	1.40	0.3125
SWP	1	0.6123	1.69	0.2302
Block x SWP	3	0.2264	0.06	0.9782
Error	8	0.3631		
Week 3				
Model	7	0.1014	0.20	0.9778
Block	3	0.0633	0.12	0.9447
SWP	1	0.0100	0.02	0.8931
Block x SWP	3	0.1700	0.33	0.8062
Error	8	0.5200		
Week 4				
Model	7	0.1596	0.35	0.9062
Block	3	0.1492	0.33	0.8043
SWP	1	0.3025	0.67	0.4372
Block x SWP	3	0.1225	0.27	0.8449
Error	8	0.4525		