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Abstract approved: _____

Mary L. Powelson

Redacted for Privacy

Fred J. Crowe

A greenhouse experiment was conducted in 1991 and in 1992 to determine the effect of soil water pressure on potato (cv Russet Burbank) plant growth and infection by Verticillium dahliae. Soil water pressures of -0.01, -0.03, -0.08, or -0.15 MPa were combined factorially with two inoculum concentrations, 0 or 10 and 0 or 25 colony forming units per gram of soil in 1991 and 1992, respectively. Plants were sampled destructively over a 4 wk period. Dry weight of foliage, fresh weight of below ground plant material, root length, and root and vascular colonization by V. dahliae were determined. In weeks 3 and 4, change in foliar dry weight in response to the level of one independent variable depended on the level of the

other. This interaction between soil water pressure and inoculum density was such that foliar dry weight was reduced in infested compared to noninfested soil at -0.01 MPa but not at other soil water pressures. There were no significant main effect interactions on below ground fresh weight or on root length. There was, however, a main effect of soil water pressure on root length in which length decreased as soil water pressure increased. In both years, colony density in roots (cfu/cm or cfu/g) was reduced at -0.01 MPa compared to lower soil water pressures. Colonization of vascular tissue (cfu/ml stem sap) was significantly reduced at -0.01 MPa compared to lower soil water pressures. Although V. dahliae and soil water pressure at -0.01 MPa interactively reduced foliar growth of plants, there were no corresponding significant increases in population densities of the pathogen in roots or stems.

Soil Water Pressure and *Verticillium dahliae* Interactions on Potato

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Suzanne M. Gaudreault

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Redacted for Privacy

Professor of Botany and Plant Pathology

Redacted for Privacy

Associate Professor of Botany and Plant Pathology

Redacted for Privacy

Head of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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Typed by Suzanne M. Gaudreault for Suzanne M. Gaudreault

TABLE OF CONTENTS

LIST OF FIGURES

Soil Water Pressure and *Verticillium dahliae* Interactions on Potato

Chapter I. **Literature Review**

Pathogenic soilborne fungi are major problems in the production of most crops. They are the causal agents of many different seedling, root, crown, tuber, and wilt diseases. After completing their life cycle in diseased plants, they are re-introduced to the soil and may undergo further dispersal within soil through autonomous movement and/or farming activities. They survive by saprophytic growth and/or by resting structures. Because certain critical phases of the life cycle of soilborne pathogens occur in soil, they are affected by various parameters of the soil environment. Soil texture, temperature, bulk density, pH, fertility, and moisture are among these parameters. It is soil moisture that is central to this thesis. This review will first cover the biology of pathogenic species of *Verticillium* and their interactions with host plants and then describe the effects of soil moisture on *Verticillium* and selected other soilborne pathogens.

VERTICILLIUM BIOLOGY

Symptom expression. Symptoms most commonly associated with Verticillium wilt diseases are apparent premature senescence, involving foliar chlorosis which often appears unilateral in leaves (Schnathorst, 1981), necrosis, and wilt. Plant growth may be retarded (Rowe et al., 1987), and, in response to foliar necrosis, root deterioration has been reported (Kotcon and Rouse, 1984). Vascular tissue is often discolored, a symptom referred to as vascular browning (Schnathorst, 1981). In addition, stem hypertrophy on hop (Beckman and Talboys, 1981) and red currant (Seemuller, 1972) has been reported. Interestingly, general growth stimulation has been observed in infected compared to noninfected resistant tomato plants (Blackhurst and Wood, 1963) and in peppermint (F. Crowe, personal communication).

Leaf invasion by V. dahliae is associated with reduced transpiration, resulting in water stress, and ultimately leading to wilt (Hall et al., 1975). Garber and Houston (1966 and 1967) and Temple et al. (1973) postulated that water stress was the result of vascular occlusions blocking water flow. Misaghi et al. (1978) observed a direct correlation between extent of occlusion of xylem elements in leaves and extent of chlorosis, necrosis, and wilt.

Verticillium albo-atrum and V. dahliae. The most important pathogenic species of Verticillium are V. albo-atrum Reinke and Berthold and V. dahliae Kleb. These were once considered to be two forms of the same species but are now recognized as separate species (Krikun and Orion, 1979). Both species can survive for several years as resting structures in the soil (Powelson and Rowe, 1993), withstanding adverse environmental conditions (Schnathorst, 1981). They differ primarily by their resting structures, optimal growth temperature, and host range. Melanized hyphae serve as the resting structures for V. albo-atrum, whereas microsclerotia are resting structures for V. dahliae. Optimal growth of V. albo-atrum occurs at about 21 C, whereas optimal growth of V. dahliae occurs at temperatures as high as 27 C (Rowe et al., 1987). Both species have a broad host range which includes various agronomic crops, fruits, vegetables, shade trees, and weeds (Powelson and Rowe, 1993). The host range of V. dahliae, however, includes considerably more plant species than that of V. albo-atrum (McCain et al., 1981).

Host defense mechanisms. In soil, Verticillium infection begins with invasion of the host's root system. This process is initiated when melanized hyphae of V. albo-atrum or microsclerotia of V. dahliae germinate in response to the exudates of a nearby growing root (Schnathorst,

1981). Systemic infection is achieved when the pathogen reaches the host's vascular system and its conidia move upward through the vascular stream.

There are several different types of defense mechanisms that occur in response to invasion by Verticillium. These include: phytoalexin production, increased growth regulator production, hypersensitive browning, formation of cell wall appositions and lignitubers, suberization of cells, formation of vascular occlusions, and other anatomical distortions. Whether or not a plant exhibits a particular response and in what part of the plant defense mechanisms are employed, may depend on the species of plant and the cultivar involved.

Hoyos and Anderson (1990) made reciprocal grafts of stem and root cuttings of a susceptible (Kennebec) and a resistant (Reddale) potato cultivar. Plants with 'Kennebec' roots and 'Reddale' stems exhibited significantly higher population densities of V. dahliae in the stem than plants with 'Reddale' roots and 'Kennebec' stems. This suggests that in 'Reddale' resistance occurs primarily in roots and involves the prevention of the pathogen from entering or moving upward through the vascular tissue.

There is evidence that the importance of phytoalexins in resistance to Verticillium spp. is minimal. Elgersma and Liem (1989), for example, found that susceptible tomato

plants produced higher concentrations of phytoalexins in response to V. albo-atrum than resistant plants. They hypothesized that the larger amounts of phytoalexins were due to more extensive exposure of tissue of the susceptible plants to the pathogen. They also suggested that mechanical prevention of pathogen spread is the most important mechanism distinguishing plants resistant to V. albo-atrum from those that are susceptible.

In support of this hypothesis, Vaughn and Lulai (1991 and 1992) demonstrated that, in response to V. dahliae, potato tuber tissue from the resistant cultivar, Reddale, exhibited more rapid and extensive hypersensitive browning of cells, and faster suberization of cells and formation of wound periderm, than tuber tissue from the susceptible cultivar, Kennebec. Furthermore, high concentrations of the phytoalexin, rishitin, were produced by the 'Kennebec' tissue, and only trace amounts were produced by 'Reddale' (Vaughn and Lulai, 1991). Because rishitin production by the two cultivars in response to the standard elicitor, arachidonic acid, was similar, the differential response to V. dahliae was evidently not due to a mere difference in the capacity of the two cultivars to produce rishitin.

In potato, development of potato early dying (Verticillium wilt) symptoms does not generally occur until after tuber initiation (Busch and Edgington, 1967). In fact, potato cultivars and Solanum species that mature and

begin tuber production late in the season often exhibit a high level of resistance (Corsini and Pavek, 1983; Corsini et al., 1988). In response to infection by V. dahliae increased production of growth regulators has been known to occur (Pegg, 1981). Corsini et al. (1989) demonstrated that applications of indole acetic acid (IAA), naphthalene acetic acid (NAA), and gibberellic acid (GA) decreased wilt severity, and IAA and NAA reduced stem colonization in highly susceptible (Norgold Russet), moderately susceptible (Russet Burbank), and highly resistant (selection A66107-57) potato cultivars infected with V. dahliae. This suggests that production of growth regulators in response to V. dahliae infection may occur in order to prolong the resistance expressed by immature plants. Pennypacker and Leath (1993) discovered dissimilarities in the responses of two different resistant clones of alfalfa to V. albo-atrum. Due to the production practices for alfalfa, V. albo-atrum acts as an aerial pathogen, and infection originates on the plant stub near the crown. With clone 1079, the pathogen was restricted to the crown and inoculated stub for the first 4 wk of regrowth, whereas the pathogen had moved into the stem after only 2 wk on clone WL-5. Many anatomical distortions, occurring in response to the pathogen, were similar in the two clones. One response observed exclusively in WL-5 was hypertrophy of parenchyma cells

surrounding infected vessel elements which resulted in the crushing of these elements.

In roots, the resistance response probably begins in the outer cell layers. Hyphae of V. dahliae penetrating the outer cells are thought to seldom enter the vascular tissue. Using cotton roots dipped in a conidial suspension of V. albo-atrum, Garber and Houston (1966) observed colonization of cotton roots by direct staining. Penetration occurred in taproots, lateral roots, and root hairs, but never at the point of emergence of lateral roots from taproots, an area known to be susceptible to attack by other fungi. Invasion of tissue occurred both inter- and intracellularly. They found that the fungus seldom penetrated beyond the epidermal layer. In cases where penetration of the epidermis did occur, hyphae were impeded by the first few layers of cortical cells. Impedance of the fungus was apparently accomplished by the swelling of cell walls and the formation of a dark gumlike substance on the walls. Often, when the substance had not formed, hyphae were able to penetrate into the inner cortical cells. At this point, mycelial strands that penetrated intracellularly formed appressoria and thin penetration pegs at cell walls. Hyphae that penetrated the cortex seldom penetrated endodermal cells which were filled with a gumlike material and possessed thick outer walls. Hyphae that had reached vessel members passed unimpeded into

adjacent vessel members. No differences were detected between a resistant variety and a susceptible variety of cotton in the mode or extent of fungal penetration of root tissue up to entry of the xylem. Development and spread of the fungus within the xylem, however, was much more extensive in the susceptible compared to the resistant variety.

In a similar study, Perry and Evert (1983a) used electron microscopy to study infection of potato (cv Russet Burbank) roots by V. dahliae. Although penetration of epidermal cells was extensive, they, like Garber and Houston (1966), observed that the fungus rarely entered the cortex. Pathogen-mediated hydrolytic enzymes apparently eroded outer epidermal cell walls prior to hyphal entry. In a few cases epidermal cells produced a callose-like material that surrounded hyphae at the point of contact, but mostly response to epidermal penetration was absent. Once inside the epidermis, the pathogen spread easily through radial walls, from one epidermal cell to another.

The pathogen was usually prevented from penetrating the common epidermal-exodermal cell wall by the formation of lignified appositions which the authors classified as lignitubers. These lignitubers apparently formed on cell walls in response to contact with the fungus. All lignitubers contained remnants of hyphae or live hyphae. Hyphae, however, were never seen emerging from lignitubers.

Occasionally hyphae penetrated into the second cortical layer where they were usually prohibited from further penetration by lignitubers. Exodermal and cortical cell walls that had been successfully penetrated lacked lignitubers.

Although fungal penetration beyond the two outer cortical layers was extremely rare, vascular infection was observed as early as 9 days after inoculation. Symptom development in similarly treated plants occurred 11 days after inoculation. Hyphae from root invasions that achieved vascular penetration proceeded, virtually unimpeded, throughout the tracheal elements, xylem parenchyma, phloem and, eventually, back into the cortex. Occlusions, including tyloses, tylose-like membranes, and gums, were observed in vascular tissue but only in association with wounds. The authors speculated that these reactions were a response to wounding rather than to V. dahliae. Stems were not observed in this study.

Gerik and Huisman (1985), using an immunoenzymatic staining technique on cotton roots grown in infested soil, observed that penetration of vascular tissue rarely occurred, which is in agreement with the findings of Perry and Evert (1983a and 1983b). Huisman and Gerik (1989) have concluded that, in fact, cortical colonies are in approximately 5000-fold excess of successful vascular invasions by V. dahliae. In contrast to the observations

of Perry and Evert (1983a), Gerik and Huisman (1985) observed extensive colonization of the root cortex, including inner layers.

In alfalfa stems, Pennypacker and Leath (1993) observed extensive vascular occlusions, ranging from globular deposits on cell walls to complete plugging of xylem cells in stub and crown tissue infected with V. albo-atrum. Root plugging also occurred in the crowns and stubs of noninoculated plants, probably as a response to wounding. These observations are in agreement with those of Perry and Evert (1983a) on potato roots. Vascular occlusions also occurred in regrown stem tissue of inoculated alfalfa plants, apparently in response to V. albo-atrum (Pennypacker and Leath, 1993). In addition, the authors observed frequent encasement of fungal hyphae in what appeared to be lignitubers in xylem cell walls, similar to those which Perry and Evert (1983a) found exclusively in cortical cells of potato roots.

Most defense mechanisms distinguishing resistant from susceptible cultivars or clones involve anatomical and/or physiological responses within the plant to the pathogen. Resistance, however, may be associated with soil microflora in some cases. Studies on this type of defense mechanism are rare. There are reports, however, that resistance in one potato cultivar, A66107-51, may involve increases in populations of antagonistic bacteria in the rhizosphere.

Azad et al. (1985 and 1987) found that populations of bacteria antagonistic to *V. dahliae* were larger in the rhizospheres of A66107-51 than in the rhizospheres of the more susceptible cultivars, Butte and Russet Burbank.

EFFECT OF SOIL MOISTURE ON SOILBORNE PATHOGENS

Soil moisture. Soil moisture may be expressed in terms of % soil moisture content, % water holding capacity, % field capacity, or water pressure or potential (usually expressed in terms of bars, mbars, kPa, or MPa). Because water pressure is most precise in terms of expressing water availability to organisms, it is the measure of soil moisture that is of most interest in current research. Water pressure consists primarily of two components, osmotic pressure and matric pressure. Although osmotic pressure may be an important factor influencing the pathogen within host tissue, matric pressure is the component of available moisture that is of primary significance to pathogens in most soils.

Verticillium. Based on available literature the effect of soil water pressure on diseases caused by Verticillium appears to be rather complex. Moisture stress enhances Verticillium wilt on mint (Nelson, 1950) and maple (Caroselli, 1957). Soil water deficits may enhance disease by compounding the reduced internal water content already affecting infected plants. This hypothesis is supported by a study in which leaf water content of potato plants (cv King Edward) infected with V. 2albo-atrum was reduced by up

to 10% compared to that of noninfected plants (Harrison, 1971).

Pullman and Devay (1981) demonstrated that flooding of soils for at least 6 wk reduced V. dahliae populations in soil and suppressed disease on cotton. This effect increased with increasing duration of flooding and with the presence of paddy rice culture during flooding. The authors attributed this effect to anaerobic conditions and increases in the activity of antagonistic microflora.

The effect of soil moisture status on Verticillium, apart from the extremes considered above, will be described in detail in Chapter 2. In general, relatively excessive soil moisture enhances disease and reduces yields of various plants, but the mechanism for this effect is unknown.

Phytophthora. Soil moisture is recognized as one of the most important environmental factors affecting diseases caused by the Oomycete, Phytophthora (Duniway, 1979). The influence of soil moisture on the diseases caused by this fungus is primarily due to the impact of soil water pressure directly on the pathogen. Predisposition of host plants to disease, at soil water pressures that favor the pathogen, may also play a role in the increase in severity of some diseases.

Periodic flooding (0 MPa) of soils has been widely demonstrated to increase severity of diseases caused by soilborne species of Phytophthora (Duniway, 1979; Duniway, 1983). In several studies, the precise soil moisture regimes under which disease was most enhanced were also optimal for the release and dispersal of zoospores and for their efficiency in infecting roots (Duniway, 1976; Duniway, 1983; Macdonald and Duniway, 1978; Shew, 1983; Wilcox and Mircetich, 1985). This suggests that zoospores are the most important propagules involved in the development of Phytophthora root rot epidemics. When released in soil, zoospores disperse by swimming through water-filled soil pores. Duniway (1976) showed that zoospores of P. cryptogea can readily swim up to 35 mm through standing water or through a coarse textured potting mix at a soil water pressures of -0.1 kPa or higher. Zoospore movement in the potting mix decreased, however, as the soil water pressure was reduced. Because zoospores travel through soil pores, it is presumed that their movement is facilitated by increases in the size and number of pores that become filled with water as soil water pressure increases.

The release of zoospores by sporangia is also generally favored by saturation of soil (Duniway, 1979; Gisi, 1983; Wilcox and Mircetrich, 1985; Bernhardt and Grogan, 1982). Macdonald and Duniway (1978) found that

zoospore discharge was more profoundly diminished by reductions in the matric component of soil water pressure than by reductions in the osmotic component. This suggests that sporangia, like many fungal structures, can compensate for low osmotic potential by taking up solutes from the external environment. They hypothesized that the effect of water matric pressure on zoospore discharge was due to swelling of a gel matrix surrounding the zoospores contained within the sporangium. Although a sporangium containing such a matrix could adapt to decreasing external osmotic pressure by building up internal solutes, decreasing matric pressure would force the water-holding matrix to expel water. In contrast, a very high water matric pressure (at or near saturation) would cause the matrix to absorb water, swell, and finally burst the wall of the sporangium, causing zoospores to be forcefully released. Soil water pressure also influences the production of sporangia by mycelia of Phytophthora, but these effects vary, depending on the species. P. parasitica, for example, formed sporangia at soil water pressures as low as -30 kPa. Production was maximized after drained soils became saturated (Bernhardt and Grogan, 1982), but incubation of mycelia in drained soil was necessary for germination to occur in saturated soil. Wilcox and Mircetrich (1985) found that P. cambivora, P. dreschleri, and P. megasperma form sporangia almost

exclusively after drained soil (-2.5 kPa) became saturated but that saturation was unnecessary for the production of sporangia by P. cryptogea. P. cactorum and P. palmivora also can produce sporangia in both saturated soil and drained soil (Gisi, 1983). Mycelia of P. cinnamomi must be on the soil surface rather than buried for significant sporangia production to occur under saturated conditions (Duniway, 1983; Gisi, 1983), suggesting that certain gas exchanges are needed for sporangium production in this species.

Although high soil moisture clearly increases the severity of Phytophthora-caused root diseases by favoring reproduction and movement of the pathogen, several researchers have examined the possibility of a predisposing effect of high soil water pressure on plant hosts. It has been demonstrated, for example, that flooding induces stress on alfalfa (Alva et al., 1985; Kuan and Erwin, 1980) and avocado (Ploetz and Schaffer, 1989) plants which predisposes them to infection by Phytophthora and/or to the effects of those infections. Fraser fir seedlings, however, are not predisposed to Phytophthora root rot infection by flooding (Kenerly et al., 1984). Using scanning electron microscopy, Kuan and Erwin (1980) found that breaks occurred on the surface of alfalfa roots grown in saturated soil but not on roots grown in nonsaturated soil. They also found that roots maintained

for 7 days in saturated soil exuded approximately 2 and 4.5 times the amount of amino acids and carbohydrates, respectively, than roots maintained in nonsaturated soil.

Furthermore, many more zoospores were chemotactically attracted to roots maintained in soil saturated for as little as 1 day than to roots maintained in nonsaturated soil. They suggested that this was due to the increase in root exudates present under saturated conditions which, in this case, was essentially equivalent to increasing the inoculum density in the soil.

Thus, the enhancement of Phytophthora root rot diseases under very wet watering regimes can be a very complex phenomenon. The role of excess water stress on the plant's predisposition to disease and the particular developmental stages of the pathogen involved in the enhancement mechanism, depend on the particular plant and pathogen species involved.

Pythium. Another Oomycete whose members may be affected by soil water pressure is Pythium. Species of Pythium are not as consistently favored by conditions of excessive soil moisture as species of Phytophthora (Bratoloveanu and Wallace, 1985). This is probably due, in part, to the more limited importance of zoospores in the life cycles of pathogenic species of Pythium. For example, two species of Pythium, both causal agents of root necrosis

of peach, were differentially affected by watering regimes in which periodic soil saturation was employed (Biesbrock and Hendrix, 1970a). Stunting caused by P. vexans was most severe when soil was periodically saturated. Effects of P. irregulare, on the other hand, were not influenced by soil water conditions. This difference, also observed on holly (Biesbrock and Hendrix, 1970b), was attributed to the predominance of zoospores in the pathogenic activity of P. vexans as opposed to P. irregulare, the sporangia of which germinated and infected roots directly rather than producing zoospores.

Another species that apparently does not produce zoospores readily is P. ultimum (Lifshitz and Hancock, 1983). Excessive soil moisture, however, is reported to increase saprophytic development of P. ultimum (Lifshitz and Hancock, 1983) and severity of the diseases it causes (Pieczarka and Abawi, 1978; Vivoda and Davis, 1991). Lifshitz and Hancock (1983) found that increases in the population size of this species under conditions of excessive soil moisture were absent when the soil was sterilized. From this they concluded that the favorable effect of excessive soil moisture on P. ultimum in nonsterilized soil was due to an inhibitory effect on microbial competitors and antagonists of the fungus. Stanghellini and Burr (1973) studied the effect of soil moisture on P. aphanidermatum, a species for which oospores

rather than zoospores served as primary inoculum, and concluded that excessive soil moisture favored germination of oospores by increasing nutrient availability.

Fusarium. The genus Fusarium includes species that cause either vascular wilts or root rots. Bhatti and Kraft (1992) studied the effect of soil moisture on four pathogens of chickpea, individually and in combination. These included two species of Fusarium, F. oxysporum f. sp. ciceri and F. solani f. sp. pisi which individually cause wilt and root rot, respectively, of chickpea. Severity of both diseases increased as soil water pressure increased (greatest severity at -20 to -40 kPa, lowest severity at -1060 to -260 kPa), an effect which the authors attributed to increased chlamydospore germination due to increased root exudation at higher soil water pressures. This study agrees with other reports, reviewed by Cook and Papendick (1972), of soil moisture effects on wilt diseases caused by F. oxysporum. It is not consistent, however, with reports of root rot of red clover (Nan et al., 1991), caused by F. solani, and root rot of pea (Cook and Flentje, 1967), caused by F. s. pisi, in which disease severity and activity of the pathogen were greatest in relatively dry soil. This may be explained by the fact that, in contrast to other studies (Nan et al., 1991; Cook and Flentje, 1967), Bhatti and Kraft (1992) autoclaved the soil used in

their experiment, a procedure which may have eliminated antagonistic microflora that would otherwise have restricted growth of the pathogen under wet conditions. This possibility is supported by the fact that Cook and Flentje (1967) observed a higher proportion of chlamydospore germination in relatively wet soil (above 8.7% water content) than in dry soil, but germ tube survival was reduced in wet compared to dry soil, ultimately resulting in increased pathogen activity under dry conditions.

There is strong evidence that a similar mechanism occurs in seedling blight and crown, foot, and root rot of wheat, barley, and oats, caused by F. culmorum, which are most severe under dry conditions (Cassell and Herring, 1982; Colhoun and Park, 1964; Cook, 1968). In sterile soil, hyphal growth is maximum when soil water pressure is high and decreases as soil water pressure decreases (Cook et al., 1972). In nonsterile soil, chlamydospores germinate over a wide range of soil water pressure, but germ tubes only continue to grow at soil water pressures ranging from -0.8 to -1.0 MPa (Cook and Papendick, 1970). This upper soil water pressure limit for survival of F. culmorum germ tubes coincides with the minimum soil water pressure at which bacterial activity occurs (-1.0 to -1.5 MPa) (Cook and Papendick, 1971), suggesting that germ tube survival and growth depend on a the dramatic reduction

or total absence of bacterial antagonists that occur only in dry soil.

Fusarium graminearum, which also causes root and foot rot in grains, survives not primarily as chlamydospores but as mycelia in crop residues (Sitton and Cook, 1981). Like F. culmorum, however, its activity is enhanced under dry soil conditions, an effect also attributed to decreases in bacterial antagonism. In addition, it is thought that when soil conditions are dry enough to cause water deficit stress on the plant the effect of both these pathogens is enhanced (Cook and Christen, 1976).

Early studies on Fusarium wilt diseases found that wilt of tomato (Clayton, 1923; Strong, 1946), celery (Ryker, 1935), cotton (Tharp, 1939), and alfalfa (Weimer, 1930) were more severe under relatively wet than dry soil conditions. Cook and Papendick (1972) suggested that this was due to an increase in evapotranspiration in wet soil which would distribute the pathogen through the plants vascular system more rapidly.

Emberger and Welty (1983) compared the effect of soil water pressure on Fusarium wilt of alfalfa in a susceptible verses a resistant variety. In an outdoor experiment performed using wooden flats, they found that wilt severity was significantly higher under a wet soil water pressure range (0 to -0.002 MPa) than under a moist soil water pressure range (0 to -0.1 MPa) for both the susceptible and

the resistant variety at 31 and 56 days after inoculation, respectively, but not at other sampling dates. In a similar experiment performed in the greenhouse, however, there were no significant differences in disease severity. They suggested that the effect of soil water pressure on disease severity occurred in the outdoor but not in the greenhouse experiment because evaporative demand in the greenhouse was probably lower than outdoors. Thus, evapotranspiration differences that might have occurred outdoors and resulted in a moisture effect on disease severity, as proposed by Cook and Papendick (1972), would not have occurred in the greenhouse. Apart from this, however, there is very little evidence for evapo-transpiration effects as the cause of enhanced wilt symptoms under wet soil conditions.

Streptomyces scabies. Common scab of potatoes, caused by the Actinomycete, Streptomyces scabies, is favored by dry soil conditions, specifically during tuber formation when the pathogen infects tubers through lenticels, prior to suberization (Lapwood, 1966). Proper irrigation management has been established as a means of controlling losses to this disease (Lapwood et al., 1970).

At least three different mechanisms may be involved in suppression of common scab in wet soil. First, Actinomycetes are generally most active in dry soils which

could give S. scabies a competitive advantage under such conditions (Cook and Papendick, 1972). A more widely recognized mechanism for control of common scab in wet soil is reduced success of the pathogen due to increased populations of bacterial antagonists that occur under wet conditions (Lewis, 1970; Lapwood and Adams, 1975). Another possible mechanism involves the fact that calcium reduction in tuber tissue has been correlated with increased resistance to common scab (Horsfall et al., 1954). In wet soil the calcium content of tuber tissue is reduced relative to that in drier soils (Davis et al., 1976). Therefore, it is suggested, that a reduction in calcium in tubers, resulting from excessive soil moisture, could limit invasion by the pathogen.

Concluding remarks. Clearly, soil moisture is extremely influential in development of disease in many host-pathogen systems. Because of the complexity of host-pathogen interactions, there are many elements of disease that may be affected by water. Considering the diversity of mechanical and physiological processes involved in the development of Verticillium wilt, the potential effects of water on these processes are numerous. Efforts to understand these effects could greatly assist in developing water management practices that will reduce crop losses by Verticillium-caused diseases.

Chapter II. Soil Water Pressure and *V. dahliae* Interactions on Potato

Potato early dying (PED) reduces yield of potatoes in many production areas of the world (Davis, 1981; Krikun and Orion, 1979; Martin et al., 1982; Nachmias and Krikun, 1985; Powelson, 1979; and Rowe, 1983). In Oregon, for example, some growers have reported yield declines of up to 30% in fields that have been cropped several times to potato (Johnson et al., 1988). These declines have been attributed primarily to PED (Powelson, 1980).

Two species of the soilborne fungus, *Verticillium*, are the primary causes of PED, *V. dahliae* Kleb and *V. albo-atrum* Reinke and Berthold. Crop rotation and soil fumigation, commonly used strategies for controlling PED, are aimed at reducing the amount of inoculum in soil (Rowe et al., 1987; and Davis 1985). Because of the long survivability in soil (Wilhelm, 1955) and broad host range of *V. dahliae* (McCain et al., 1981), the practicality of crop rotation for controlling *Verticillium*-caused diseases is limited (Davis and McDole, 1979; Huisman and Ashworth, 1976). This and the financial costs and environmental concerns associated with chemical fumigants, accentuate the need for development of alternative management practices.

Certain cultural manipulations may be employed to reduce losses to PED. Studies in Idaho have shown that the

optimization of soil fertility, specifically concentrations of phosphorus and nitrogen, can suppress PED in cv Russet Burbank (Davis and Everson, 1986; Davis et al., 1990). This effect is presumably the result of enhanced plant health with a resulting increase in resistance to the disease. In the same study, the authors reported that severity of PED was lower in furrow compared to sprinkler irrigated fields, an effect attributed to enhanced nutrient availability to plants in furrow irrigated fields.

In irrigated potato production systems, another factor that may influence the development of PED is the amount of applied water. Reports on the effect of soil moisture on diseases caused by Verticillium are sparse and apparently conflicting. Moisture stress enhanced Verticillium wilt in studies on mint and maple (Nelson, 1950; Caroselli, 1957), for example. On the other hand, studies on guayule (Schneider, 1948) and antirrhinum (Isaac, 1956) demonstrated that moist and wet (waterlogged) soil enhanced disease development compared to dry soil. In addition, separate studies on Verticillium wilt in cotton have reported that disease can be suppressed and yield enhanced by delaying the first irrigation of the season (El-Zik, 1985; Huisman and Gerik, 1989). El-Zik (1985) also reported that disease was significantly reduced and yield significantly enhanced by irrigation treatments with 30 or

43.2 cm of water compared to treatments with 55.9 cm of water.

In microplot studies in Oregon, Cappaert et al. (1992a) compared the combined effects of a range in both inoculum density of V. dahliae and amounts of irrigation water on severity of PED and tuber yield. The ranges in amount of applied water were related to estimated consumptive use (ECU). Disease severity in infested soil was greater under the excessive (150% ECU) than under the moderate (100% ECU) irrigation regime. Also, as inoculum density increased, reductions in tuber yield were greater under excessive than under moderate or deficit (50% ECU) irrigation regimes. This was demonstrated at both a cool, short season site and a warm, long season site. Another microplot study demonstrated that disease severity was 17% greater under an excessive, early season (prior to tuber initiation) irrigation regime compared to a moderate or deficit early season irrigation regime (Cappaert et al., 1992b). Irrigation regimes after tuberization had no effect on disease severity. In both studies, petiole nutrient analyses indicated that the effect of irrigation regimes on disease was not associated with nutrient leaching. Therefore, the mechanism for this effect is unknown.

In culture, V. dahliae is active at osmotic pressures as low as -12 MPa (Ioannu et al., 1977a) and matric

pressures as low as -10 MPa (Mandanhar and Bruehl, 1973). In addition, V. dahliae establishes itself under low osmotic pressures in host tissue and ultimately grows saprophytically in very dry, moribund plant tissue. We have estimated that the excessive, moderate, and deficit irrigation regimes of Cappaert et al. (1992a and 1992b) represent average water matric pressures of approximately -0.01 to -0.03, -0.08, and -0.15 MPa, respectively. Therefore, it is unlikely that an excessive irrigation regime would directly favor pathogen activity over moderate or deficit soil moisture. It may affect, however, one or more of the following parameters: the pathogen's ability to colonize host roots, the pathogen's ability to infect the vascular tissue of the host root and become systemic, the host's ability to resist vascular and systemic infection, and the host's tendency to express symptoms. Insights into the role of these factors in enhancing disease might be gained by understanding the dynamics of root and vascular colonization by V. dahliae in association with the growth dynamics of the host.

It is the purpose of this study to monitor early foliar and root growth of potatoes (Solanum tuberosum L 'Russet Burbank') planted in soil noninfested or infested with V. dahliae over a range of soil water pressures and to quantify colonization of roots and stems in these plants by the pathogen.

MATERIALS AND METHODS

Soil. Soil (Quincy fine sandy loam; mixed, mesic xeric Torripsamment), fumigated with methyl bromide/chloropicrin (3:2, v/v) at 488 kg per hectare, was obtained from the Hermiston Agricultural Research and Extension Center in Hermiston, OR. Prior to use, the soil was steam pasteurized at 60 to 71 C for 1.25 h and air-dried. In 1992, soil also was sieved through a 4 mm mesh screen to increase its uniformity and to remove rocks and organic debris.

Inoculum. Four isolates of V. dahliae, obtained from symptomatic potato plants in Umatilla County, Oregon, were used in this study. Single spore isolates of these were obtained and subcultured onto potato dextrose agar (PDA). Cultures used to produce inoculum were chosen for absence of sterile sectors. These cultures were flooded with sterile distilled water and scraped gently with a glass rod to release conidia. Aliquots (1 ml) of the resulting conidial suspension were tranferred onto minimal agar medium (Puhalla, 1979) overlain with sterile, uncoated cellophane (Bio-Rad Laboratories, Richmond, CA). After 3 wk of growth, the cellophane, now covered with microsclerotia, was removed and processed in a blender with distilled water. The resulting suspension was washed

through a nested series of three screens (1.18, 0.425, and 0.075 mm mesh) to separate microsclerotia from the cellophane and to remove conidia and mycelial fragments. A more detailed protocol of this procedure is contained in Appendix I. The inoculum, which was air dried at room temperature for 2 to 3 days, was mixed with dried soil in a twin shell blender. This blend of soil and inoculum was used as an inoculum concentrate. Infestation of the soil was accomplished by mixing the inoculum concentrate with dried soil in a cement mixer. Noninfested soil was not mixed in the cement mixer in 1991 which may have resulted in a bulk density difference between the noninfested and infested soil. To eliminate this possible variable, infested and noninfested soil were mixed in the cement mixer in 1992. Plastic 5 L pots (21 cm diameter X 19.5 cm height) were filled with approximately 7100 g of dry soil. Water, in amounts appropriate to achieve desired soil water pressures, and a liquid fertilizer were added to soil. The fertilizer consisted 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) per kg of soil (Appendix II). Soil was allowed to equilibrate for 5 days between initial watering and planting. Nuclear seed potatoes were obtained from Alpine Spuds, Enterprise, OR. Melon ball seed pieces were scooped from around potato eyes with a 29 mm diameter melon ball scooper, air dried for 18 h, and pre-sprouted in moist vermiculite for 1 wk (Appendix

III). For planting, a 6 cm hole was made in the center of each pot using a calibrated planting stick. One melon ball seed piece was placed in each hole and covered with soil. A layer of perlite, approximately 3 cm deep, was then spread evenly over the soil to reduce evaporative water loss. Soils were maintained at desired water pressures by weighing pots periodically (daily by 1 wk after emergence) and adding water as necessary. Greenhouse temperatures were maintained at 13 to 27 C, and plants were grown under natural daylight.

Treatments and experimental design. Three (1991) and four (1992) soil water pressures were combined factorially with two inoculum densities of V. dahliae for a total of six (1991) and eight (1992) treatments. The four soil water pressures, determined by a soil water release curve (determined by Soil Analysis Laboratory, Department of Crop and Soil Science, Oregon State University) (Fig. 1), were -0.01 MPa (very excessive), -0.03 MPa (excessive), -0.08 MPa (moderate), and -0.15 MPa (deficit). These soil water pressures correspond to 18.5, 15.4, 12.7, and 7.8% water per total weight of soil, respectively. In 1991, the soil water pressures were -0.01, -0.08 and -0.15 MPa. The -0.01 MPa treatment was replaced with -0.03 MPa after 3 wk due to evidence of anaerobic conditions at -0.01 MPa. In 1992, both -0.01 and -0.03 MPa were employed throughout the

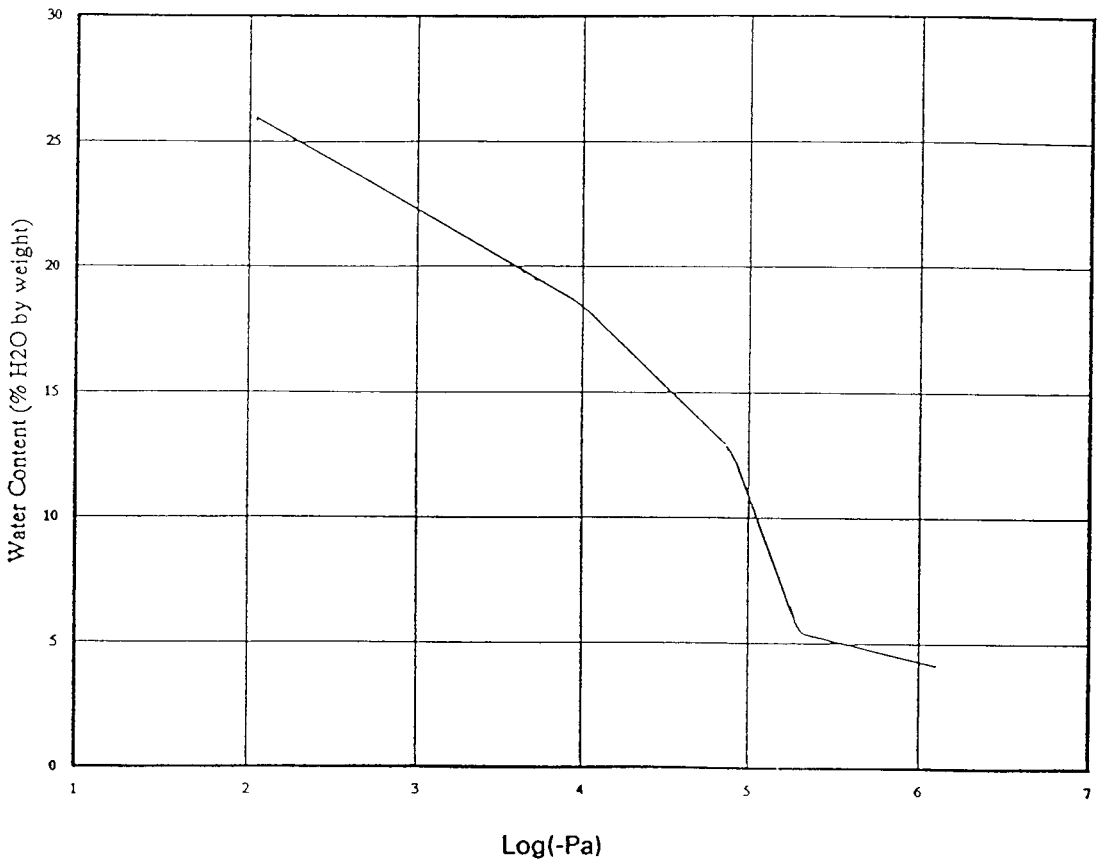

Fig. 1. Soil water release curve for Quincy fine sandy loam.

experiment. Inoculum densities of V. dahliae were established at 0 and 10 colony forming units per gram (cfu/g) of soil in 1991, and at 0 and 25 cfu/g of soil in 1992. In 1991 each of the six inoculum X soil water pressure treatments was replicated 10 times for each of four harvest weeks. In 1992, the inoculum X soil water pressure treatments were replicated 10 times and the noninfested X soil water pressure treatments were replicated eight times for each of four harvest weeks. Treatments were completely randomized within each of four blocks which represented harvest weeks.

Sampling and assays. After 90% emergence, plants were harvested weekly for 4 wk. Foliage of each plant was cut at the soil line, dried at 40 C, and weighed. The below ground portion of each plant (roots and below ground portion of the stem) was removed from the soil, pre-washed in running tap water, blotted dry, weighed, placed in a plastic baggie containing a moist paper towel, and refrigerated until processed. In 1991, root systems were placed in separate sacks made of nylon screen, and washed overnight in a running water bath. In 1992, root systems were washed individually by hand under running tap water over a fine mesh sieve.

Root lengths were estimated using the line-intercept method of Newman (1966) as modified by Marsh (1971). In

1991, all root systems from weeks 1, 2, and 3 were spread over the window of a Xerox copying machine and copied onto paper. Root copies were then overlain with a grid transparency to determine number of line-intercepts and subsequently, approximate total length of the root system of each plant.

In 1992, root length was determined for 100, 20, and 10% of the root system in weeks 1, 2, and 3 to 4, respectively. As in 1991, root lengths for all plants were measured at weeks 1, 2, and 3. Root lengths also were measured at week 4 for plants from infested soil so that number of colony forming units per gram of root could be converted to number of colony forming units per cm of root (see below). Each root system (week 1) or a subsample of each root system (weeks 2, 3, and 4) was spread over a metal 1.5 mm mesh screen set in a pan of water to facilitate even dispersal. The screen with roots spread on it was removed from the pan and placed over a grid, and root line-intercepts were counted directly.

For subsampling, the 90 squares (4 cm^2 each) contained within a 36 by 40 cm grid drawn on plexiglass were randomly divided among 10 colors. Each color on the grid represented 10% of the grid space. Root systems from weeks 2, 3, and 4 were spread evenly over the subsampling grid. Sections of the root system, covering the squares corresponding to a previously randomly chosen color, were

cut out with a razor blade and placed on a metal screen in a pan of water for line-intercept measurement as described above.

Both stems and roots were assayed for V. dahliae on streptomycin water agar (Nadakavukaren and Horner, 1959) in 1991 and Sorensen's NP-10 medium (Sorensen et al., 1991) in 1992.

Roots were cut into 1.5 to 2.5 cm long segments and plated onto a medium to determine number of colony forming units per centimeter and/or gram of root. In 1991, three plates of roots (estimated to be a total of approximately 90 cm), and in 1992 a maximum of six plates of root pieces were prepared. In 1992, plates were weighed before and after addition of roots so that weight of the plated roots could be determined. Using a ratio of root weight to root length, colony forming units per gram of roots were mathematically converted to colony forming units per cm of roots.

In 1991, wafer thin cross-sections from the basal portion of each stem were plated onto agar (four cross-sections/plate). After 10 days incubation at room temperature, sections were examined microscopically for presence of microsclerotia. In 1992, sap was extracted from stems with a garlic press, serially diluted when necessary, and aliquots spread onto agar plates. Plates

were incubated at room temperature for approximately 10 days before counting number of colonies.

Data analysis. Significance of treatment differences was determined on SAS version 6.04 using Analysis of Variance for balanced data and General Linear Models for imbalanced data (including data sets in which values were missing). Two-way analyses were performed on plant growth data with inoculum density and soil water pressure as the independent variables. Because V. dahliae was not recovered from plants grown in noninfested soil, one-way analysis was performed on colony forming unit data with soil water pressure as the independent variable. Means were separated by Fischer's Protected Least Significant Difference (LSD) analysis. In 1991, 0 values were not included in analysis of root colony density due to the frequency with which no colonies were recovered from root systems. Incidence of root system colonization in 1991 is presented separately. Because of the exponential increase in colony forming units per milliliter of stem sap between weeks 1 and 4 of 1992, analysis was performed on ln (natural log) transformed data. ANOVA summaries are presented in Appendix IV.

RESULTS

Foliar and root growth. Effects of inoculum density on foliar dry weight were not significant at week 1 but were significant in week 2 (Fig. 2). At week 2 of 1991, weights of plants from noninfested soils were 20% greater than those from infested soils ($P \leq 0.05$). This effect was reversed for the same sampling date in 1992. Also at week 2 of 1992, plant weights were 32 and 44% smaller at -0.01 MPa compared to -0.03 and -0.08 MPa, respectively; also, plant weights at -0.15 MPa were 27% lower than those at -0.08 MPa. There were main effect interactions at weeks 3 and 4 of both years: plants grown in infested soil at -0.01 MPa weighed an average of 36% less than those from noninfested soil. Otherwise, plant weights appeared to be directly proportional to soil water pressure in weeks 3 and 4.

Although there were no interactive effects of treatments on below ground fresh weight in either year, there were significant main effects (Fig. 3) ($P \leq 0.05$). In 1991, mean weights were lowest at -0.01 MPa for the first three sampling dates. In week 4, after this soil water pressure was reduced to -0.03 MPa, below ground weights were directly proportional to soil water pressure. In 1992, the effect of soil water pressure was only significant at week 1 where below ground fresh weight was reduced at -0.01 MPa compared to -0.08 and -0.15 MPa. There were effects of

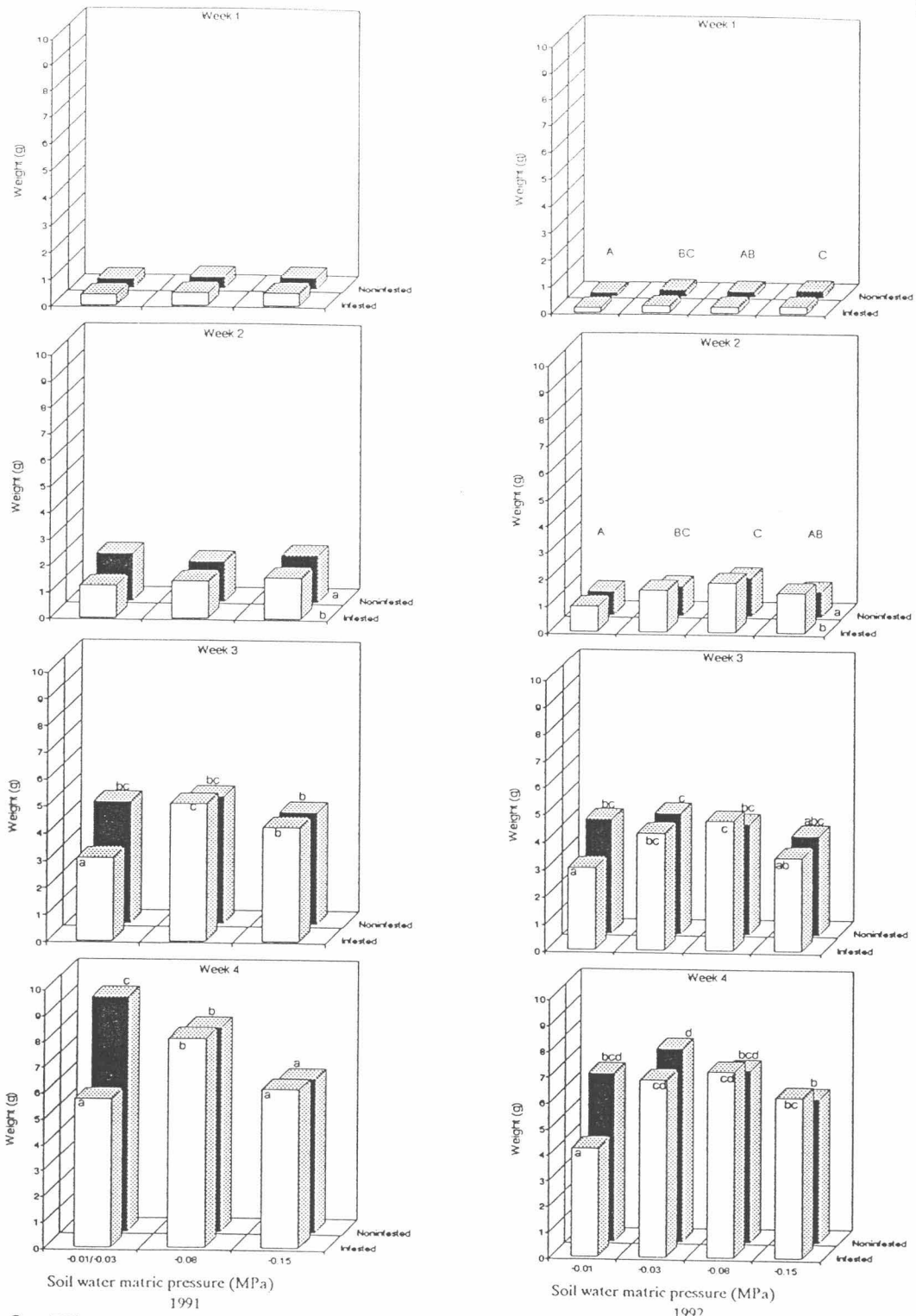

Fig. 2. Effect of soil water pressure and inoculum density of Verticillium dahliae on mean foliar dry weight of potato. Bars with the same letter are not significantly different ($P \leq 0.05$ except in week 3 of 1992 where $p = 0.11$), according to Fischer's protected LSD test. Significant soil water pressure effects are represented by upper case letters above bars, inoculum density effects by lower case letters beside bars, and interactions by lower case letters on or on top of bars.

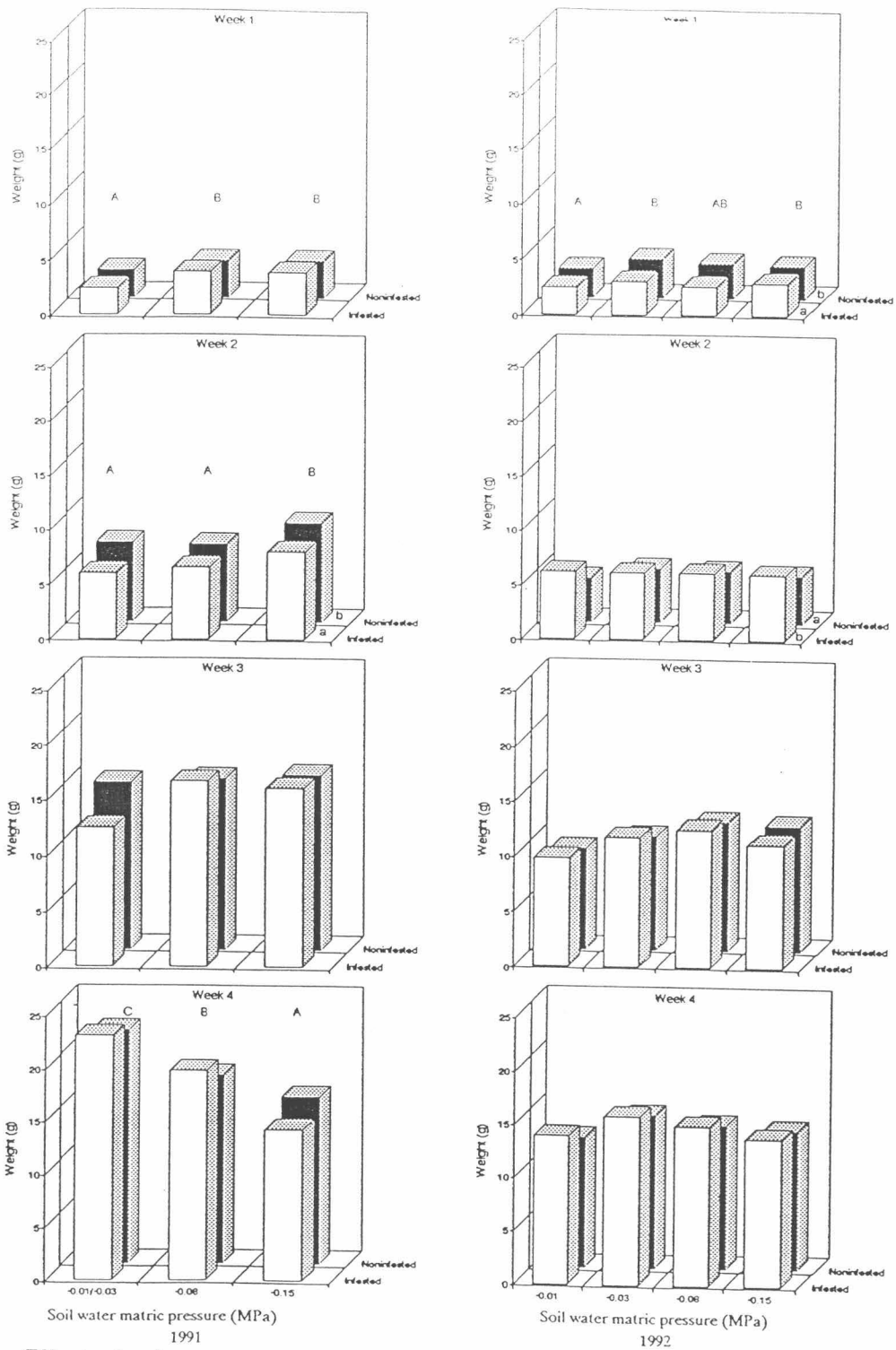

Fig. 3. Effect of soil water pressure and inoculum density of Verticillium dahliae on mean below ground fresh weight of potato. Within weeks, bars with the same letter are not significantly different ($P \leq 0.05$), according to Fischer's Protected LSD test. Significant soil water pressure effects are represented by upper case letters above bars and inoculum density effects by lower case letters beside bars.

inoculum at week 2 of 1991 and at weeks 1 and 2 of 1992. At week 2 of 1991 and week 1 of 1992 below ground weights were significantly higher in noninfested than infested soil. By week 2 of 1992, however this effect was reversed.

In all weeks except week 2 of 1992, root length was inversely related to soil water pressure (Fig. 4). Inoculum had an effect, similar to that on root weight, of reducing root length in 1991 and increasing it in 1992 at week 2.

Root colonization. V. dahliae was not recovered from roots of plants grown in noninfested soil. In 1991, V. dahliae was first recovered from roots of plants grown in infested soil at week 2 at -0.08 and -0.15 MPa and at week 3 at -0.01/-0.03 MPa (Fig 5). The proportion of root systems from which V. dahliae was recovered was consistently lower at -0.01/-0.03 MPa compared to the other soil water pressures, and it never approached 100% at any soil water pressure. In contrast, the pathogen was recovered from virtually all root systems in 1992, beginning at week 1.

In both years, colony density (cfu/cm or cfu/g) generally increased over time until week 3 regardless of soil water pressure (Fig. 6). After week 3, colony density either leveled off or continued to increase, depending on soil water pressure. In 1991, the colony density was generally highest at -0.15 MPa (an effect which was significant at week 2) and lowest at -0.01/-0.03 MPa

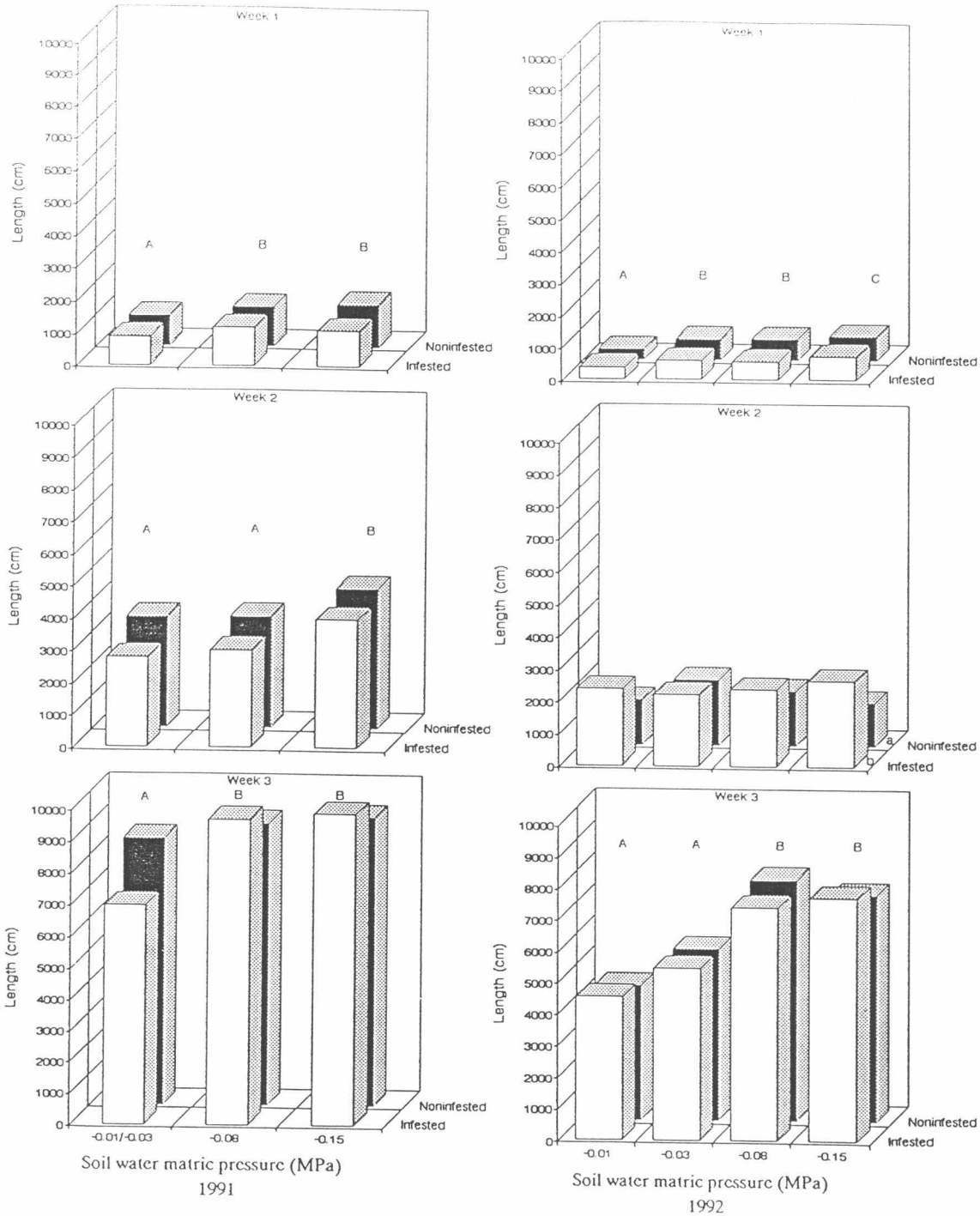

Fig. 4. Effect of soil water pressure and inoculum density of Verticillium dahliae on mean root length of potato. Within weeks, bars with the same letter are not significantly different ($P \leq 0.05$), according to Fischer's Protected LSD test. Significant soil water effects are represented by upper case letters above bars and inoculum density effects by lower case letters beside bars.

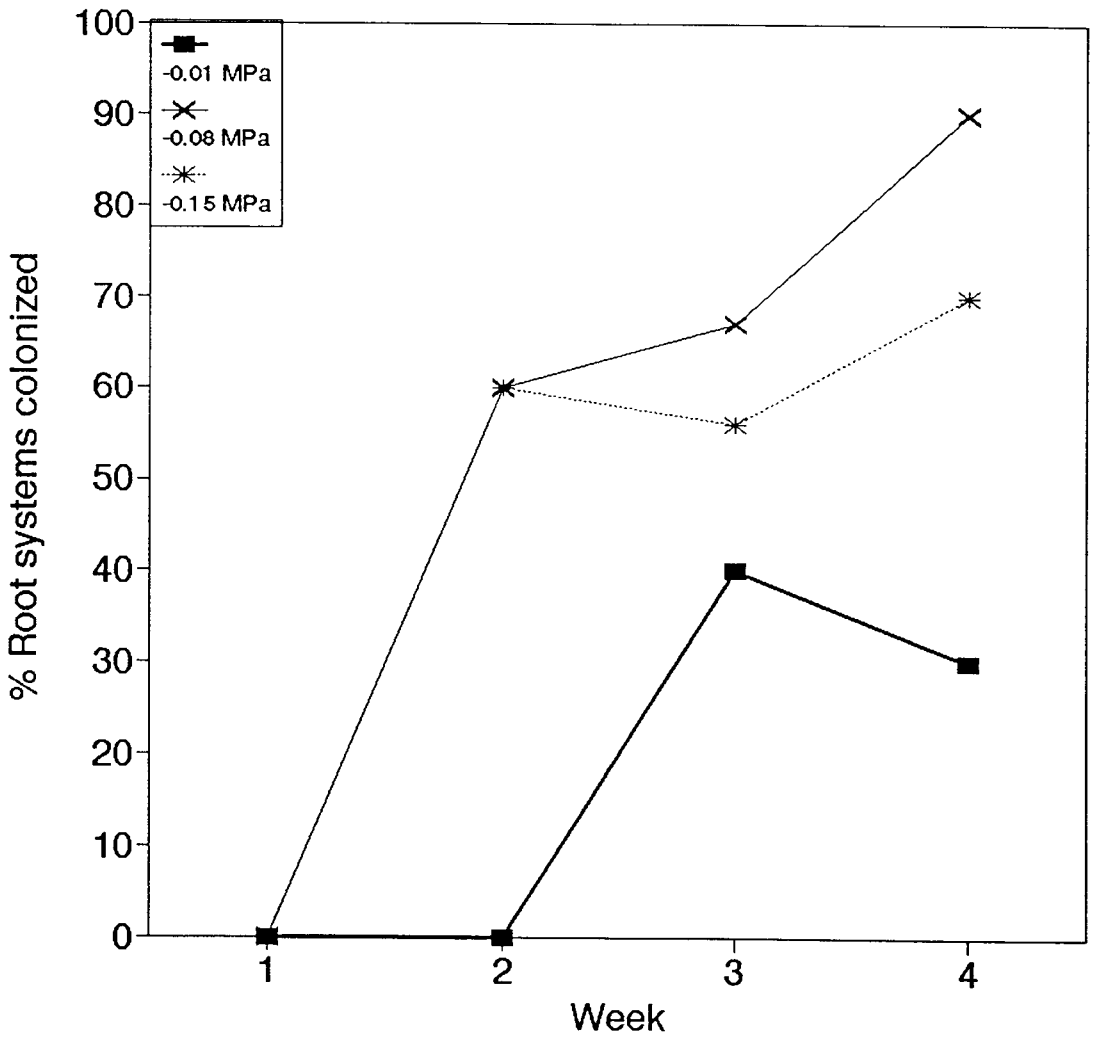

Fig. 5. Influence of soil water pressure on incidence of colonization of potato roots by Verticillium dahliae in 1991. Incidence was measured as the proportion of plants from which V. dahliae was detected in roots.

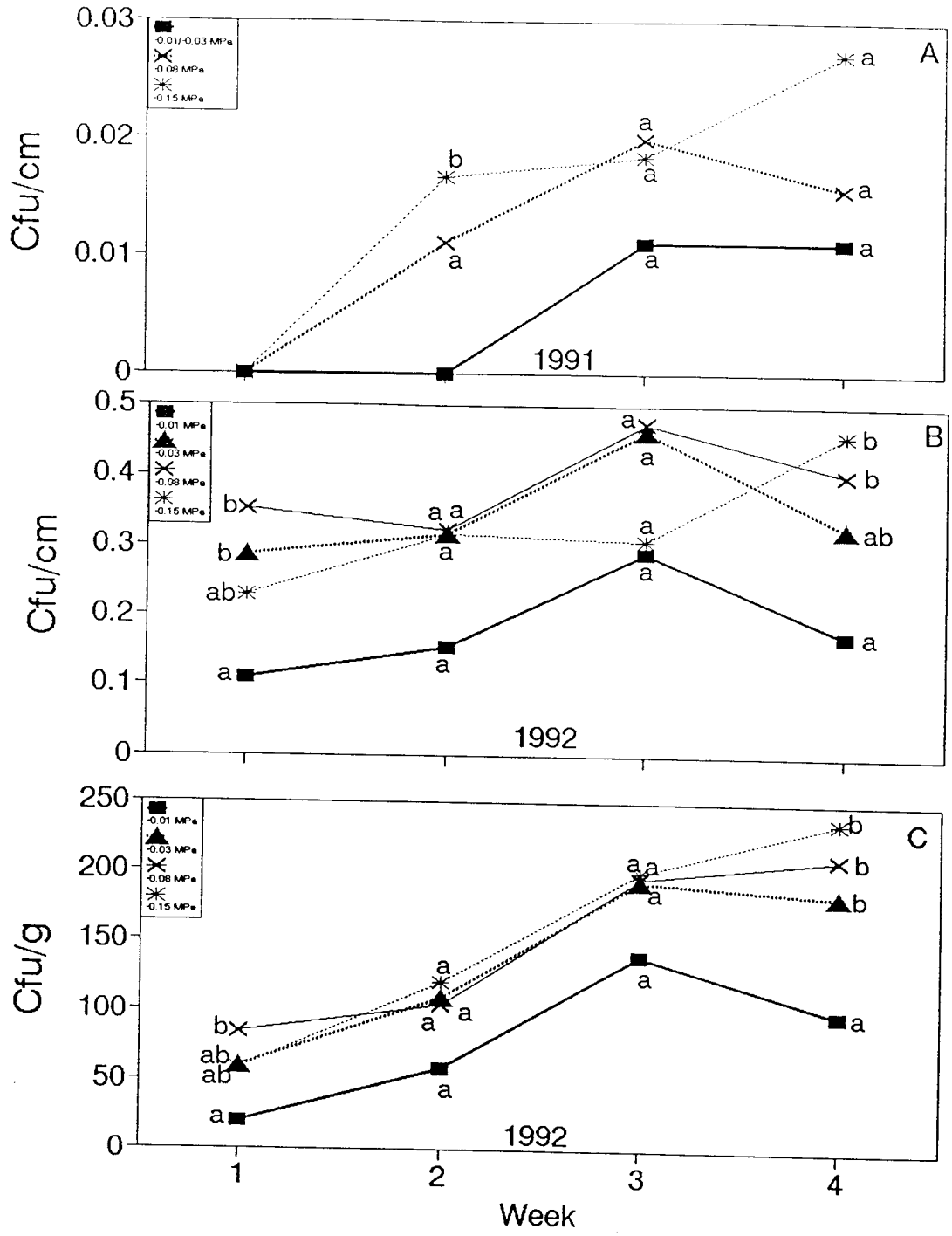

Fig. 6. Effect of soil water pressure on potato root colonization by Verticillium dahliae. Mean colony density is presented as colony forming units (cfu) (A) per cm root in 1991, (B) per cm root in 1992, and (C) per g root in 1992. Within weeks, means with the same letter are not significantly different ($P \leq 0.05$) according to Fischer's Protected LSD test.

(Fig. 6A). In 1992, colony density was approximately 40-fold higher than in 1991, but in both years there was a trend in which colony density was reduced in wetter soils. In 1992, colony density was reduced at -0.01 MPa compared to lower soil water pressures throughout the experiment, an effect which was significant at weeks 1 and 4 (Fig. 6B and C).

Stem colonization. In 1991, recovery of V. dahliae from plated stem cross sections was negligible. Colony forming units recovered from stem sap in 1992 revealed an exponential pattern of increase in vascular colonization over time (Fig. 7). At week 3 vascular colonization was significantly reduced at -0.01 MPa compared to lower soil water pressures. At all other weeks, however, effects of soil water pressure were not significant.

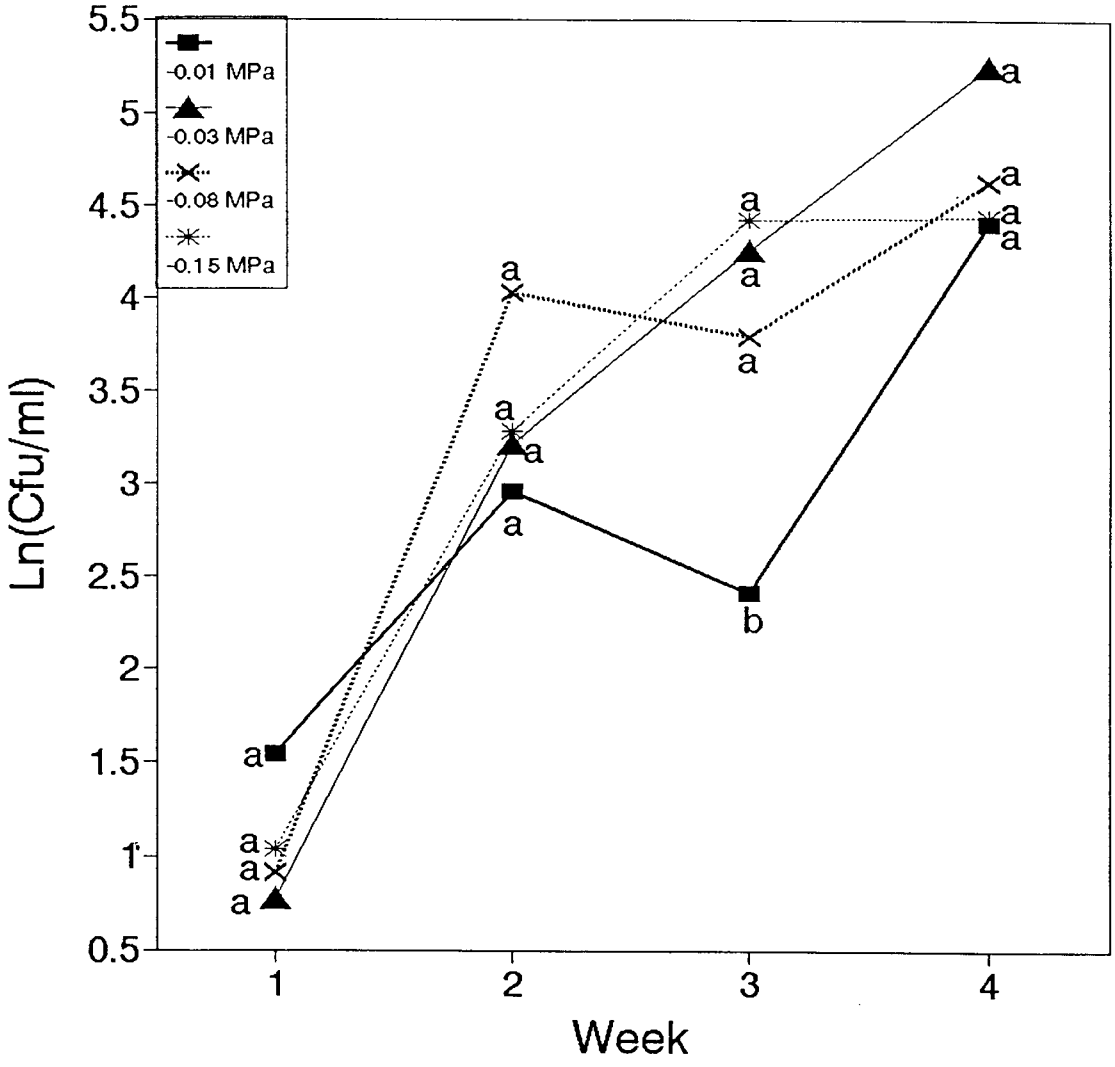

Fig. 7. Effect of soil water pressure on vascular colonization of the lower stem of potato by Verticillium dahliae. Within weeks, bars with the same letter are not significantly different ($P \leq 0.05$), according to Fischer's Protected LSD test.

DISCUSSION

Our study demonstrated that, soil water pressure and infestation of soil with V. dahliae interacted to reduce foliar growth of potato under conditions of very excessive soil moisture (-0.01 MPa); however there were no corresponding significant increases in population densities of the pathogen in either roots or stems.

Soil infested with V. dahliae appeared to inhibit foliar growth relative to growth of plants in noninfested soil, only at the highest soil water pressure. Although reduction of plant growth is a symptom commonly associated with diseases caused by Verticillium (Gutierrez et al., 1983; Isaac and Harrison, 1968), symptoms in the field, including growth inhibition, are not observed in potatoes prior to tuberization (Busch and Edgington, 1967). In this study, tuberization did not occur until 4 wk after plant emergence. This could explain the absence of growth inhibition at -0.03, -0.08, and -0.15 MPa despite it's occurrence at -0.01 MPa. Harrison and Isaac (1969) reported significant reductions in potato plant dry weight prior to tuberization due to V. dahliae and V. albo-atrum. In contrast to our experiments, their study used the cultivar, King Edward, and was performed in a rainfed field environment. Cultivar resistance and parameters such as inoculum density and pathogen virulence may influence

symptom development (Schnathorst, 1981). Because these factors were homogenous among treatments in our study, reduction in foliar weight in infested soil that occurred only at -0.01 MPa was apparently due to soil water pressure rather than to some other factor.

There was no interactive effect of soil water pressure and inoculum on root length or weight. The relative effect of soil water pressure on root length generally corresponded to that on root weight, suggesting that variation in root mass among treatments was affected more by variation in length than in diameter. The most pronounced deviation from this trend was at week 3 of 1992 where there was a main effect of soil water pressure on root length but not on root weight. This deviation may be attributed to proliferation of fine roots in drier soils to allow for maximum water access (Vartanian, 1981), a phenomenon that would contribute significantly to root length but not root weight.

V. dahliae is known to cause reductions in root mass in association with foliar symptom development (Kotcon and Rouse, 1984; Kotcon et al., 1984). In our study inoculum did not have a consistent effect on root growth. In 1991, the inoculum effect may have been confounded by the effect of mixing infested but not noninfested soil in the cement mixer. By reducing the availability of pore space, the higher bulk density of the infested (mixed) soil could result in slower root growth than in non-mixed soil (Glinski

and Lipiec, 1990). This could explain the disparity between the apparent inoculum effects on root length and below ground fresh weight in week 2 of 1991 verses the same week in 1992. In 1992, when both infested and noninfested soils were mixed, root lengths from infested soil were significantly greater than those from noninfested soil in week 2. This effect also occurred for root weight in weeks 2 and 4, suggesting a hypertrophic response of the plants to invasion by the fungus. Although root hypertrophy is not a symptom frequently associated with V. dahliae., stem hypertrophy has been reported to occur on red currant (Seemuller, 1972) and hop (Beckman and Talboys, 1981). If hypertrophy occurred it was not a significant enough effect in 1991 and in weeks 1 and 3 of 1992 to offset other factors affecting root growth.

Colonization of potato roots by V. dahliae was not significantly affected by soil water pressures ranging from -0.03 to -0.15 MPa. At -0.01 MPa, however, there was a significant reduction in root colonization. Mandanhar and Bruehl (1973) and Ioannou et al. (1977c) demonstrated that V. dahliae can grow at water pressures (osmotic and matric) as low as -10 MPa (-100 bars). Ioannou et al. (1977c) also reported that colony growth in culture was reduced at the highest osmotic pressure tested (-0.2 MPa) relative to maximum growth which occurred at osmotic pressures ranging from -1 to -2 MPa, though the mechanism for this was not

understood. In field experiments flooding has been shown to reduce populations of microsclerotia in soil (Pullman and Devay, 1981). This effect was attributed to anaerobic conditions, specifically, antagonism by anaerobic soil microorganisms and changes in the gaseous composition of the soil (eg. a decline in O_2 and buildup in CO_2 concentration). Decreases in colony growth, sporulation, and microsclerotia production were associated with increased CO_2 and decreased O_2 concentrations in vitro (Ioannu et al., 1977b). Anaerobic conditions in soils maintained at -0.01 MPa could, therefore, account for the reductions in root colonization that we observed.

It is paradoxical that despite reduced root colonization at -0.01 MPa, a significant reduction in plant weight due to V. dahliae was observed at this soil water pressure. Evidently, the very wet soil enhanced the ability of the fungus to detrimentally affect the plant, in a manner not directly related to the extent of root colonization. In response to infection by vascular wilt pathogens, plant respiration is accelerated (Dimond, 1970). This may be required for maximum expression of resistance mechanisms. By not meeting the increased respiratory demands of infected plants, anaerobic soil conditions may have resulted in a decrease in resistance to fungal infection and/or symptom (reduced foliar growth) development. Furthermore, stress resulting from competition between plant roots and invading

fungi for a very limited quantity of oxygen could increase the likelihood of the fungus penetrating the stele.

The disparity between amount of root colonization and apparent effect of V. dahliae on foliar weight at -0.01 MPa might be understood, in part, by considering the possible histological origins of the colonies recovered in our root assays. In order for Verticillium to systemically infect its host and cause symptoms, it must gain entrance into the host's stele. It has been widely demonstrated, however, that most root infections by V. dahliae do not successfully enter the vascular tissue (Perry and Evert, 1983a; Perry and Evert, 1983b; Gerik and Huisman, 1985; J. Bowers, personal communication). In fact, the susceptibility of a plant to systemic invasion by V. dahliae does not appear to be a function of the fungus' ability to colonize the root cortex (Evans and Gleeson, 1973; Evans et al., 1974). Huisman and Gerik (1989) estimates that in cotton there are approximately 5000 cortical invasions of the root by V. dahliae for every vascular infection.

In histopathological studies in which staining and electron microscopy were used to examine artificially inoculated Russet Burbank roots, V. dahliae seldom penetrated beyond the epidermis (Perry and Evert, 1983a). In response to invasion by the fungus, the exodermis of plant roots produced lignitubers which were highly effective at preventing further penetration by the fungus. In fact,

where V. dahliae succeeded in penetrating the cortex, the endodermis did not appear to be an effective barrier against further invasion into the vascular tissue.

Although the root systems in our study were washed in running water, a procedure which should have removed most fungal tissue from the root surface, they were not surface sterilized. Depending on the concentration of the sterilant and the duration of sterilization, sterilizing can eliminate fungal growth from subsurface root tissues (Huisman, 1988). Huisman (1988) reported a 50% reduction in colony growth from plated cotton roots that had been surfaced sterilized in NaOCl for 5-15 sec. Because our roots were not sterilized, colonies appearing on the medium could have grown from any layer of root tissue, including the epidermis. According to current knowledge about root colonization by V. dahliae, it is almost certain that the vast majority of colonies recovered in our assays did not originate from the vascular tissue. Nevertheless, it is reasonable to presume that a higher incidence of colonization of root tissue in general should lead to a higher incidence of vascular infection and consequently, accelerated symptom development. This relation could be altered, however, by conditions which enhance fungal invasion of the vascular tissue.

An important factor to consider with regard to the impact of environmental parameters on soilborne pathogens is

the interrelation between pathogen ecology and root growth dynamics. In our experiments, very excessive soil water not only reduced root colonization and interacted with inoculum to reduce foliar weight, it also reduced root growth (especially apparent in week 3; Fig. 4).

In situations where inoculum is randomly mixed with soil V. dahliae is randomly distributed along the root system (Evans and Gleeson, 1973; Huisman and Gerik, 1989). This is consistent with our observations of potato root colonization. Assuming that microsclerotia of V. dahliae germinate in response to growing roots at a fairly constant rate, the oldest colonies appearing in a root plating assay are those growing from the oldest sections of root. In studies on V. dahliae infection of a variety of plants using immunofluorescent staining, Fitzel et al. (1980) reported that initial contact of the fungus with root tissue occurred directly by germ tubes (usually one and no more than three per propagule) growing from microsclerotia, without involvement of conidia.

There has been considerable controversy over the role of the root tip in infection. Nutrient leakage occurring at the growing root tip, specifically the zone of elongation, greatly exceeds that of older root tissue (Rovira, 1969; Schroth and Snyder, 1961). Furthermore, nutrients that are released by the root are used up rapidly by the microbial community of the rhizosphere, dramatically reducing the

sphere of influence of root exudates around the mature root (Newman and Watson, 1977). Therefore, stimulation of a fungal propagule is most likely to occur when the growing root, specifically the root tip, first reaches the vicinity of the propagule. On a plate assay, the center of each colony along the length of the root should approximate the point at which the young, growing root first encountered the microsclerotial germ tube which produced the colony (Huisman and Gerik, 1989). If an approaching root stimulates a microsclerotium to germinate, the proximity of the point of initial contact between the two organisms should be a function of the rate of root elongation and the speed with which the propagule responds to root exudates. Where G = the germination period of a microsclerotium, E = the elongation rate of a root, and D = the distance of the initial contact point from the root tip:

$$D = G \times E.$$

Therefore, anything that causes G and/or E to increase will also increase D.

Roots of most cultivated plants grow at a rate of 3 to 10 mm/day depending on the plant species and the type of root, eg. taproot, lateral root, etc. (Huisman, 1982). In addition, environmental and nutritional factors can affect root elongation rate. In our experiments, total root length was reduced at -0.01 and -0.03 MPa compared to roots of plants grown at lower water potentials, an effect which was

especially apparent at week 3 (Fig. 4). If this reduction in total root length reflected an actual reduction in E, the point of initial contact of the fungus with the root could have been closer to the root apex, possibly in a region of increased vulnerability.

Due to an increased tendency of cells to rupture with age, vulnerability of cortical tissue to penetration increases with time whereas resistance in endodermal tissue improves with age due to suberization. Therefore, the portion of the root most vulnerable to invasions that will ultimately lead to vascular infection is the region of undifferentiated tissue just behind the root apex where resistance mechanisms have yet to take effect (Beckman and Talboys, 1981). In cotton, for example, this region extends approximately 0.25 mm behind the root apex (Huisman and Gerik, 1989).

Observations on the mode of infection of roots by V. dahliae are limited and rather conflicting. Bowers et al. (1992; personal communication) examined potato (cv. Superior) roots using an immunoenzymatic stain and observed that invasion of the vascular tissue by V. dahliae occurred exclusively via the root apex. Using an assay, similar to the one employed in our study, in which cotton roots were plated on agar, Huisman and Gerik (1989) found that colonies were detected as close as 1 mm from the root apex, but colonies this close to the apex were rare. They were

detected with increasing frequency farther away from the apex, reaching a plateau at approximately 1 cm from the root tip, a region well behind the region of undifferentiated tissue. Colony density then remained stable for most of the remaining root length. Fitzel et al. (1980) observed a similar pattern of V. dahliae colony density in wheat and thornapple, with a plateau reached at 2-3 cm from the root apex. Therefore, the region of most extensive colonization and the region of greatest vulnerability to infection are evidently not equivalent in most cases.

The root locus or loci at which contact with the pathogen will most likely result in vascular infection and the probability that the fungus will reach that locus will depend primarily on the plant species and cultivar involved and secondarily on environmental conditions.

Direct observations on root colonization of Russet Burbank, a comparatively resistant cultivar, are limited to experiments in which roots were inoculated by artificial means. Perry and Evert (1983a and 1983b) cultured the roots in a liquid suspension containing V. dahliae conidia, hyphae, and microsclerotia, thus directly exposing the entire root system to nondormant inoculum. After direct staining, they examined the roots with light and electron microscopy. In response to massive attack by the fungus, the meristematic region of the root lapsed into early maturity. The endodermis and exodermis became extended

completely around the apical meristem, and protoxylem matured 200 um closer to the apical meristem than in healthy controls. In addition, sieve plate pores, which developed in the protophloem of healthy roots, were absent in the early maturing protophloem of inoculated roots. Despite massive colonization of the root cap and epidermis, no invasion of the xylem was observed in this region. In a similar type of study on cotton, Garber and Houston (1966) observed penetration of roots through the root cap. Because the roots in these studies were exposed to unnaturally large quantities of inoculum in a very artificial manner, the plant's response may have been very unlike what would occur in a natural situation. In addition, the accuracy of direct observation is restricted by limitations of the staining technique employed.

In a natural soil environment, microsclerotia must germinate, and the germ tube must reach the root before penetration can begin. Because the sphere of influence of a root tip on a microsclerotium is small; eg., only 30-100 um for cotton (Huisman, 1982), the speed at which the fungus can begin colonizing a root is more restricted by the amount of time it requires to germinate than by the time required for the germ tube to reach the root. It has been shown that germination periods are greatly reduced when soils are amended with plant extracts (Fitzell et al., 1980; Timofeeva, 1976). Kuan and Erwin (1980) demonstrated the

leakage of amino acids and carbohydrates from alfalfa roots increased by 2 to 4.5 times in saturated soil. If increased soil water in general facilitates leakage of nutrients from roots, thereby increasing the concentration of germination stimuli reaching a microsclerotium, G could be reduced. This would reduce D, possibly placing the initial point of contact between root and fungus at or near the location of maximum vulnerability of the root to vascular infection.

If vascular infection was indeed enhanced in wetter soils, a relative increase in number of colonies arising from plated basal stem sap would be expected at some point in time. Although this phenomenon was observed in week 4 for plants maintained at -0.03 MPa (Fig. 7), it was absent in plants maintained at -0.01 MPa.

In field studies, we estimate that soil maintained at the wettest watering regime (Cappaert et al., 1992a; Cappaert et al., 1992b) was irrigated to approximately -0.01 MPa and allowed to dry to approximately -0.03 MPa before irrigating. In our study plants at both -0.01 and -0.03 MPa were kept much closer to their designated soil water pressures. Anaerobic soil conditions, that probably ensued from a consistently high soil water pressure of -0.01 MPa and the confining conditions of the pots, may have been a factor in reducing foliar growth in infested soils.

It is doubtful that anaerobic conditions play a role in the significantly greater amount of PED that occurs in wet

compared to dry soils under field conditions. Soil in pots maintained at -0.03 MPa was probably more similar to soil in plots maintained at an irrigation regime of excessive water in the field (Cappaert et al., 1992a; Cappaert et al., 1992b) than soil in pots maintained at -0.01. Thus, the increased stem colonization observed in week 4 of our experiment at -0.03 MPa may truly reflect a trend toward an increase in vascular infection due to relatively wet soil conditions. These conditions in the field could ultimately result in enhanced PED. Whether the mechanism for this phenomenon is due to increases in G or E as described above or to some other factor is unknown.

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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 g
$NaNO_3$	2 g
KH_2PO_4	1 g
$MgSO_4 * 7H_2O$	0.5 g
KCl	0.5 g
Trace element solution	0.2 ml
Difco agar	20 g

2. Trace element solution:

Distilled water	95 ml
Citric acid	5 g
$ZnSO_4 * 7H_2O$	5 g
$FeSO_4 * 7H_2O$	4.75 g
$Fe(NH_4)_2(SO_4)_2 * 6H_2O$	1 g
$CuSO_4 * 5H_2O$	0.25 g
$MnSO_4$	0.05 g
H_3BO_3	0.05 g
Na_2MoO_4	0.05 g

A. Autoclave and pour into 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 have dried. Using sterile technique (eg. use 2 pairs of tweezers, flaming them with alcohol frequently) lay cellophane over agar.

D. Addition of inoculum

1. Ideally, inoculum should come from fresh cultures that were prepared using single spore isolates to insure that cultures are fertile (ie. 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; the amount of water may be varied according to the size of the culture.

3. Then, using a flamed, L-shaped glass stirring rod rub the surface of the culture. This will release conidia into the water resulting in a spore suspension.
4. With sterile pipette tips, pipette about 1 ml of the suspension onto cellophane/VIM plates. Spread the suspension evenly on the cellophane with sterile stirring 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 three sieves: 4 mm, 425 um, and 75 um. Material of size ranging from 425um to 75um should be saved and dried.

C. Optional - If it is necessary to maximize the inoculum obtained, the water that passes through the 75um mesh sieve will contain a significant amount of small microsclerotia. To seperate 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 "supernatent" is then siphoned off, and the remaining slurry of inoculum is centrifuged for a few minutes at low speed in large centrifuge jars.

Appendix II

NUTRIENT WATERING SOLUTION

In 1991 and 1992 Greenhouse Experiment the following nutrient fertilizing solution was used for initial watering of pots. Stock solutions were prepared individually.

	STOCK SOLUTIONS	
Dry compound	mol. wt.	g/L of H_2O
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. - eg. 120, 60, 60, and 12 ml of respective stock solutions were added to approx. 11748 ml of water in a large bucket (totalling 12 L final solution) and mixed. A total of 600 ml of the final solution was added to each pot of approx 7100 g soil.

Consequently, nutrients were added to each pot in the following approximate concentrations:

Element	mmole/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

II. Vermiculite preparation.
   A. Autoclave moistened vermiculite in autoclave bags for 1 h.
   B. Clean out crisper boxes with 10% bleach solution then rinse with distilled water.
   C. Pour vermiculite into boxes in 2.5 cm layers.

III. Potatoes.
   A. Obtain appropriate potato seed tubers.
   B. Using heavy duty 29 mm diameter 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.

IV. Place seed pieces in crisper box between layers of vermiculite, and let them sprout for a week or more (depending on the variety) at room temperature. Refrigerate for storage.

Appendix IV

ANOVA SUMMARIES

1991 Dry Foliar Weight

Source of variation	df	MS	F	P
Week 1				
Model	5	0.01371	0.84	0.5290
ID	1	0.0040	0.24	0.6230
SWP	2	0.0293	1.79	0.1768
ID X SWP	2	0.0030	0.18	0.8345
Error	54	0.0164		
Week 2				
Model	5	0.5457	3.12	0.0152
ID	1	1.6401	9.37	0.0034
SWP	2	0.2412	1.38	0.2606
ID X SWP	2	0.3030	1.73	0.1867
Error	54	0.1750		
Week 3				
Model	5	4.6877	9.13	0.0001
ID	1	1.7725	3.45	0.0687
SWP	2	6.1793	12.04	0.0001
ID X SWP	2	4.6862	9.13	0.0004
Error	53	0.5132		
Week 4				
Model	5	18.2654	32.9	0.0001
ID	1	13.8965	25.03	0.0001
SWP	2	19.4517	35.04	0.0001
ID X SWP	2	19.7810	35.63	0.0001
Error	51	0.5552		

1992 Dry Foliar Weight

Source of variation	df	MS	F	P
Week 1				
Model	7	0.0184	2.33	0.0352
ID	1	0.0002	0.03	0.8609
SWP	3	0.0410	5.19	0.0029
ID X SWP	3	0.0014	0.17	0.9147
Error	62	0.0079		
Week 2				
Model	7	1.1211	4.92	0.0002
ID	1	2.2354	9.82	0.0026
SWP	3	1.5139	6.65	0.0006
ID X SWP	3	0.2228	0.98	0.4086
Error	63	0.2276		
Week 3				
Model	7	2.9393	2.8	0.0134
ID	1	0.6008	0.57	0.4519
SWP	3	3.7154	3.54	0.0196
ID X SWP	3	2.2319	2.13	0.1057
Error	61	1.0482		
Week 4				
Model	7	10.1489	8.29	0.0001
ID	1	2.9579	2.42	0.1251
SWP	3	12.4262	10.15	0.0001
ID X SWP	3	7.994	6.53	0.0006
Error	63	1.2243		

1991 Below Ground Weight

Source of variation	df	MS	F	P
Week 1				
Model	5	4.1583	4.56	0.0016
ID	1	1.2672	1.39	0.2436
SWP	2	8.7430	9.59	0.0003
ID X SWP	2	0.8846	0.97	0.3855
Error	53	0.9115		
Week 2				
Model	5	11.6945	4.01	0.0037
ID	1	14.9353	5.12	0.0277
SWP	2	21.3818	7.33	0.0015
ID X SWP	2	0.9156	0.31	0.7319
Error	53	2.9158		
Week 3				
Model	5	20.5733	1.93	0.1049
ID	1	1.5256	0.14	0.7068
SWP	2	31.1021	2.92	0.0629
ID X SWP	2	19.9153	1.87	0.1646
Error	53	10.6656		
Week 4				
Model	5	116.8799	6.84	0.0001
ID	1	2.1254	0.12	0.7258
SWP	2	275.4163	16.12	0.0001
ID X SWP	2	16.2368	0.95	0.3933
Error	52	17.0893		

1992 Below Ground Weight

Source of variation	df	MS	F	P
Week 1				
Model	7	1.3768	3.45	0.0035
ID	1	2.7384	6.87	0.0110
SWP	3	1.5735	3.95	0.0122
ID X SWP	3	0.568	1.42	0.2441
Error	62	0.3987		
Week 2				
Model	7	6.0508	2.95	0.0097
ID	1	39.1658	19.12	0.0001
SWP	3	0.6217	0.3	0.8228
ID X SWP	3	0.8779	0.43	0.7333
Error	63	2.0489		
Week 3				
Model	7	9.547	1.04	0.416
ID	1	6.4278	0.70	0.407
SWP	3	18.1825	1.97	0.1275
ID X SWP	3	1.7988	0.2	0.8993
Error	61	9.2184		
Week 4				
Model	7	9.7991	1.29	0.2676
ID	1	26.5415	3.51	0.0658
SWP	3	13.5914	1.8	0.157
ID X SWP	3	0.6513	0.09	0.9674
Error	63	7.5682		

1991 Root Length

Source of variation	df	MS	F	P
Week 1				
Model	5	338560.031	3.91	0.0043
ID	1	49506.389	0.57	0.4529
SWP	2	743591.647	8.58	0.0006
ID X SWP	2	78055.235	0.9	0.4121
Error	54	86621.843		
Week 2				
Model	5	3429550.33	3.29	0.0115
ID	1	3567545.01	3.42	0.0699
SWP	2	6665600.69	6.39	0.0032
ID X SWP	2	124502.64	0.12	0.8877
Error	54	1042977.83		
Week 3				
Model	5	11174635.76	2.49	0.0423
ID	1	12675.45	0.00	0.9578
SWP	2	19667894.88	4.38	0.0173
ID X SWP	2	8262356.78	1.84	0.1686
Error	54	4490461.22		

1992 Root Length

Source of variation	df	MS	F	P
Week 1				
Model	7	186983.325	6.28	0.0001
ID	1	23654.798	0.79	0.3762
SWP	3	415424.162	13.95	0.0001
ID X SWP	3	14074.378	0.47	0.7024
Error	61	29770.68		
Week 2				
Model	7	1871092.1	2.84	0.0122
ID	1	9814540.629	14.92	0.0003
SWP	3	165900.665	0.25	0.8595
ID X SWP	3	1044318.204	1.59	0.2013
Error	63	658001.52		
Week 3				
Model	7	16588958.7	3.43	0.0038
ID	1	180018.7	0.04	0.8476
SWP	3	37503776.8	7.76	0.0002
ID X SWP	3	457135.1	0.09	0.9628
Error	59	4834160.5		

1991 Root Colony Density (cfu/cm)

Source of variation	df	MS	F	P
Week 2				
Model	1	0.00009075	5.0	0.0493
SWP	1	0.00009075	5.0	0.0493
Error	10	0.00001815		
Week 3				
Model	2	0.0000968	1.57	0.2489
SWP	2	0.0000968	1.57	0.2489
Error	12	0.00006184		
Week 4				
Model	2	0.00041205	1.46	0.2609
SWP	2	0.00041205	1.46	0.2609
Error	16	0.00028164		

1992 Root Colony Density (cfu/cm)

Source of variation	df	MS	F	P
Week 1				
Model	3	0.1	3.1	0.0393
SWP	3	0.1	3.1	0.0393
Error	35	0.03229		
Week 2				
Model	3	0.0663	1.96	0.1368
SWP	3	0.0663	1.96	0.1368
Error	36	0.0338		
Week 3				
Model	3	0.0913	0.95	0.4294
SWP	3	0.0913	0.95	0.4294
Error	34	0.0966		
Week 4				
Model	3	0.1525	3.64	0.0219
SWP	3	0.1525	3.64	0.0219
Error	35	0.0419		

1992 Root Colony Density (cfu/g)

Source of variation	df	MS	F	P
Week 1				
Model	3	6496.0904	3.18	0.0359
SWP	3	6496.0904	3.18	0.0359
Error	35	2043.8858		
Week 2				
Model	3	7551.7706	2.0	0.1311
SWP	3	7551.7706	2.0	0.1311
Error	36	3772.8863		
Week 3				
Model	3	7232.6879	0.67	0.5766
SWP	3	7232.6879	0.67	0.5766
Error	33	10795.8978		
Week 4				
Model	3	36069.4072	3.97	0.0155
SWP	3	36069.4072	3.97	0.0155
Error	35	9087.4422		

1992 Stem Sap Colonization (cfu/ml)

Source of variation	df	MS	F	P
Week 1				
Model	3	1.0176	0.48	0.7006
SWP	3	1.0176	0.48	0.7006
Error	36	2.1355		
Week 2				
Model	3	1.6363	0.65	0.5903
SWP	3	1.6363	0.65	0.5903
Error	34	2.5294		
Week 3				
Model	3	7.2830	3.94	0.0163
SWP	3	7.2830	3.94	0.0163
Error	34	1.8488		
Week 4				
Model	3	1.4936	0.58	0.63
SWP	3	1.4936	0.58	0.63
Error	35	2.5611		