AN ABSTRACT OF THE THESIS OF

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Population size of Clavibacter michiganensis subsp. sepedonicus in potato cv Russet Burbank and plant response as affected by drought were assessed in a greenhouse experiment. Water deficit stress and no stress treatments, and inoculum densities of 0 or 2 X $10^7$ cfu C. m. sepedonicus/seed piece were arranged factorially. Stem populations of C. m. sepedonicus were significantly lower in the water deficit stress treatment compared to the non-stressed treatment at every sampling date in both experiments. In seven of the eight harvests the number of C. m. sepedonicus cells/g of stem tissue for the water deficit stress treated, infected plants was a factor of 10 lower than the non-stressed treatment. Foliar symptoms of bacterial ring rot were not observed, but symptoms

developed in tubers. Compared to the noninoculated control inoculum reduced aerial biomass from 12 to 21% and tuber yield from 15 to 38% in samples taken four times after the drought was terminated. Reduction of these same variables due to water deficit stress ranged from 17 to 21% and 15 to 41%, respectively, compared to the non-stressed control. Therefore, both water deficit stress and C. m. sepedonicus had similar effects on growth and tuber yield of potato.

Water Deficit Stress Effects
on Bacterial Ring Rot of Potato Caused
by <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u>

by

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## Chapter I.  Literature Review

Pathogenic vascular inhabiting bacteria, causal organisms of vascular wilts, can be serious yield limiting factors in many agronomic crops.  One such xylem inhabiting bacterium is _Clavibacter michiganensis_ subsp. _sepedonicus_, cause of bacterial ring rot of potato.  C. m. _sepedonicus_ survives the non-cropping period in protected environments, such as tubers in storage, dried slime on organic or inorganic surfaces, or as quiescent cells in host debris (Westra and Slack, 1992).  Soil properties such as fertility, potassium and nitrogen in particular (Sakai, 1992), temperature, and water interact with potato and C. m. _sepedonicus_.  These interactions have an effect on all phases of the disease.

Soil moisture content is central to host colonization for many vascular pathogens, including C. m. _sepedonicus_. Extent of water movement in the vascular bundles regulates the degree of vascular colonization by C. m. _sepedonicus_ and subsequent development of disease symptoms.  Soil moisture, as it affects the internal water pressure of potato and thereby the water flow in the vascular bundles and the bacterial progression within, is the focus of this

thesis. This review will cover the biology of Clavibacter, the effects of soil and vascular water pressure on potato growth, and plant water relations as effected by vascular inhabiting bacteria.


## CLAVIBACTER BIOLOGY


**Pathogen.** Clavibacter is a division of the previously larger genus Corynebacterium and now contains most of the plant pathogens of the xylem-inhabiting coryneform group. These are slow growing, fastidious, Gram positive, non-motile, short rods with no spore forming ability which generally produce copious quantities of extracellular polysaccharides (EPS). There are five species of Clavibacter and various subspecies, one of which, Clavibacter michiganensis subsp. sepedonicus, is central to this thesis.

C. m. sepedonicus has an optimum growth temperature of 20-24C in vitro, and, unlike the other plant pathogenic bacteria (most of which are Gram negative), has difficulty growing at 27C (Klement et al, 1990). Growth is slow even with optimum conditions, taking approximately one wk to see growth on nutrient broth plus yeast extract (NBY) agar. Bishop and Slack (1982) have investigated the effects of temperature on in planta development of C. m.

<u>sepedonicus</u> and found that warm nights (24C) were more conducive to symptom development than cool nights (5C), $\underline{P}<0.001$, although the stem populations at soil level were not significantly different, $\underline{P}>0.25$.

The cells of <u>C</u>. <u>m</u>. <u>sepedonicus</u> normally produce large quantities of EPS, composed of capsule and loose slime layers. The outer most layer, the loose slime, is composed of water soluble polysaccharides, as is the case for most phytopathogenic bacteria (Sequeira, 1982). Some phytopathogenic bacteria also have polypeptides or glycopeptides incorporated into this layer (Klement et al, 1990; Sutherland, 1977), however <u>C</u>. <u>m</u>. <u>sepedonicus</u> apparently does not (van den Bulk, 1991). This EPS is an important virulence factor in pathogenesis, although Bishop et al (1988) have a reported virulent nonfluidal strain isolated from potato. Henningson and Gudmestad (1993) found both quantitative and qualitative differences in the EPS of the different colony morphologies. Sugar residue analyses performed on EPS layers from mucoid, intermediate, and non-mucoid strains of <u>C</u>. <u>m</u>. <u>sepedonicus</u> demonstrated differences in their compositions. Of the three, the non-mucoid strains appeared to have only one type of EPS molecule, and this was of low molecular weight. The mucoid strains had more than one type of EPS molecule, some of high and some of low molecular weight. Intermediate strains had proportionately more glucose than

mucoid strains, but they have a diversity of polysaccharide molecules in both size and sugar composition.

Rai and Strobel (1969b), Reis and Strobel (1972b), Strobel (1970), and Strobel and Hess (1968) have researched a phytotoxic glycopeptide produced by C. m. sepedonicus. Although the origin and function of this glycopeptide as set forth by Reis and Strobel (1972) are debated, respectively, by van den Bulk (1991) and Bishop and Slack (1992).

**Pathogenesis.** The interaction between a host and its bacterial pathogen involves a series of molecular and cellular recognition processes. The exchange of information between host and pathogen, and the correct combination of events induced by pathogen invasion determines whether an interaction will be compatible or incompatible. Many Clavibacter-host interactions are highly specific. This specificity depends on a cell to cell recognition where complimentary molecules on the cell surfaces of both organisms interact in particular ways to allow certain communications to occur. Bacterial EPS, lipopolysaccharides (LPS) (present in Gram negative bacteria), and outer membranes appear to interact with plant cell wall structure and cell surface components, particularly the hydroxyproline-rich structural glycoproteins (Benhamou, 1991; Sequeira, 1980). EPS

components may themselves do the plugging (van den Bulk, 1991) or they may partially compose the blockage as in the apple/_Erwinia amylovora_ combination (Suhayda and Goodman, 1981).

Plant cell wall alteration appears to be a process by which certain bacterial pathogens are enabled to move out of the xylem. The Benhamou study (1991) found that in tomato infected with <u>C</u>. <u>m</u>. <u>michiganensis</u> or <u>C</u>. <u>m</u>. <u>sepedonicus</u> the bacterial cells were not restricted to the xylem elements, but were distributed throughout the plant stem tissues, especially at the junctions between mesophyll cells. Swelling, shredding and partial wall dissolution are typical features in areas adjacent to sites of high bacterial accumulation, eventually leading to stem cankers. These alterations indicate that hydrolytic enzymes are among the array of chain splitting enzymes produced by <u>Clavibacter</u> during pathogenisis which move out ahead of the bacterial growth to weaken and loosen the wall structure. Beckman (1987) indicated that cell wall degradation in vascular wilt diseases may be due to the decreased pH in the vessels. This acidification supplies the optimal pH (2.5) for the action of cell wall degrading enzymes and may stimulate the activity of bacterial and/or plant hydrolases (Collmer and Keen, 1986).

The glycopeptide from C. m. sepedonicus, has been shown to be capable of inducing wilt in potato plant foliar cuttings in the absence of bacterial cells (Strobel, 1970). Optimum pH for operation of Strobel's glycopeptide is 2.1, which correlates with the acidic pH generated in the xylem during the disease process (Beckman, 1987; Benhamou, 1990). Van den Bulk et al (1991), based on previously performed analyses of EPS components, indicated that this glycoprotein cannot be a component of the EPS for C. m. michiganensis and have extrapolated this to include C. m. sepedonicus. It is possible that this glycopeptide is manufactured in the interior of the cell and transported to the exterior via the Golgi apparatus as are other polysaccharide materials (Benhamou, 1990).

It is likely that the in planta EPS production by phytopathogenic bacteria, including the Clavibacter species, is related to pathogenesis. Many functions have been attributed to EPS in this regard, including the prevention of bacterial attachment to host cells which prevents recognition and the hypersensitive response (Sequeira et al, 1977), the inhibition of bacterial agglutination with host agglutinins causing immobilization, water retention in intercellular spaces (water soaking) for bacterial establishment, replication, and increased distance from host cell wall and recognition

responses (Beckman, 1987 p.68), protection against
bacteriostatic compounds, and induction of host wilting
through the restriction of water movement. EPS layers are
generally composed of neutral and acidic sugars with
esterified substituent groups formed into hetero-
polysaccharides of high molecular weight, minor amounts of
protein and other possible constituents and complexes (van
den Bulk et al, 1991). Clavibacter appears to have no
glycoproteins in its EPS (van den Bulk et al, 1991).
Although Strobel and Hess (1968) and Rai and Strobel
(1969) claim that the toxic effects of Clavibacter are due
to a toxic glycopeptide, they do not state that it is a
component of the EPS. Pathogenic Clavibacter species
produce serologically related phytotoxic compounds (Westra
and Slack, 1992). In 1967 Rai and Strobel showed the
phytotoxic polysaccharides of C. m. insidiosus, C. m.
michiganensis and C. m. sepedonicus to be non-specific in
their ability to cause wilt in various dicot plants and to
be antigenic in action. Strobel and Hess (1968) then
followed this with studies which strongly suggested that
the primary effect of the toxin formed by C. m.
sepedonicus was to destroy the integrity of cellular
membranes, including chloroplasts, mitochondria and
plasmalemma, as well as the structural integrity of the
cell wall.

**Host defense.** There are many specific and non-specific responses a host may use to defend itself against an invading microorganism. Constitutive structural and biochemical defenses are innate and ever present as the plant grows. These normally form the first line of defense (Beckman, 1987), however C. m. sepedonicus is a tuber-borne pathogen so that the constitutive defenses of a potato plant have already been bypassed when the sprouts begin growth. Potato plants also possess inducible structural and biochemical means of defense, such as tyloses and phenolics or phytoalexins, respectively. If any inducible defenses are to be activated the host must recognize that a harmful agent or pathogen is invading. The recognition process is triggered in various ways by the changing chemistry as invasion begins. For other diseases this occurs during ingression, but for ring rot of potato this occurs during replication and movement of the cells in the vascular channels, and culminates in the acceptance or rejection (ie. exclusion responses) of the introduced organism (Beckman, 1987).

As the invasion advances, cell wall hydrolysis by Verticillium albo-atrum or Pseudomonas solanacearum is often the first signal to the host. This releases cell wall fragments which can be sensed within the plasmalemma (Beckman, 1987). Pectic wall fragments of tomato cells were able to induce proteinase inhibitor activity in plant

tissues at some distance from their source (Ryan et al, 1981). Phytoalexin synthesis can be elicited by the glucan degradation of Phytophthora megasperma var. soyae by a constitutive glucanase in the walls of soybean cells (Cline and Albersheim, 1981). The breakdown of plant cell walls by C. m. michiganensis exo-enzymes, in addition to being damaging to host cells, may release potent elicitors of plant defense mechanisms (Darvill and Albersheim, 1983). These defenses involve, in part, the deposition of fibrillar-granular material (galactose residues and pectin-like molecules) around the invading bacterial cells, which are overlaid with pectin-like molecules and hydroxyproline-rich glycoproteins (Benhamou, 1991). A particular hydroxyproline-rich glycoprotein from potato is capable of agglutinating strains of the bacterial wilt pathogen, Pseudomonas solanacearum (Leach et al, 1982), therefore it also may have a role in the defense against other vascular invading bacterial pathogens, such as Clavibacter.

The basic responses by which infections are localized in vascular elements appear to be initiated by pathogens and nonpathogens alike but differences in success or failure of invasion depend on quantitative differences in the recognition itself or in the rate or extent of host responses (Beckman et al, 1982). There is recognition and interaction of surface polymers when bacterial cells

adhere to vessel walls (Sequeira et al, 1977). Callose material can be synthesized, excreted through the plasmalemma, and deposited onto the inner surface of paravascular parenchyma cell pits first, and then onto the entire wall surfaces adjacent to infected vessels (Beckman, 1987; Beckman et al, 1982; Beckman et al, 1989). This callose may then be infiltrated with secondary metabolites to form lignified barriers to lateral pathogen progression (Beckman et al, 1982; Beckman et al, 1989). There are many dynamic interactions which are triggered and coordinated by recognition events.

The interior of the xylem elements also can be blocked. A number of different induced defense strategies may be involved in this blockage process. Tyloses protruding into the elements from contact parenchyma cells through pits, and gels for vessel occlusion, phytoalexins manufactured in contact parenchyma cells and phenolics infused into the element lumen (vascular browning) may all be involved. These are all under host genome control and have been termed "stress metabolism" (Beckman, 1987). When bacteria enter the vascular pathway the potato plant is induced by bacterial EPS (Westra and Slack, 1992) to form two types of occluding material (Gardner et al, 1983). The amorphous, low density carbohydrate is intended to envelope the bacterial cells slowing their replication, while the fibrous, high density material

blocks the transpiration stream and further progression of the pathogen (Gardner et al, 1983). As host enzymes lyse pathogen walls not only are the numbers of the pathogen reduced but molecular fragments also may be liberated in the process which act as recognition messenger molecules to the host (Young and Pegg, 1982).

A phytotoxic glycopeptide, probably not in association with the EPS, found in spent culture fluid and infected potato tissue is also suspected of participation in the plugging and pathogenisis process of C. m. sepidonicus (Strobel, 1970; Strobel and Hess, 1968). Similar phytotoxic substances as studied by Strobel (1970) are involved in initiating the wall coating response by tomato to Verticillium albo-atrum and Fusarium oxysporum f. sp. lycopersici, which is a host directed response to vascular invasion against lateral spread of the pathogen (Robb et al, 1987; Street et al, 1986). In contradiction, Westra and Slack (1992) suggest that the EPS is the stimulus for host plug and wall coating material production against C. m. sepedonicus in potato.

EPS components also can trigger formation of vascular occlusions. Benhamou (1991) indicated the composition of Clavibacter EPS is devoid of pectin-like molecules and galactose residues which are present in fibrillar-granular plug material. The occurrence of these substances in plant cell walls and not in bacteria supports Benhamou's

view that these plugs are, at least partially, of host origin. Similar substances are involved in the wall coating response of tomato to _Verticillium albo-atrum_ and _Fusarium oxysporum_ f. sp. _lycopersici_, which is a host directed response to vascular invasion against lateral spread of the pathogen (Robb et al, 1987; Street et al, 1986). The suggestion is that the EPS is the stimulus for host plug production (Westra and Slack, 1992). Gardner et al (1983) state that plug material appears rapidly at vessel walls in response to bacterial invasion, and that the material is of two types, fibrous and amorphous. Fibrillar material appears in about 3 h limiting bacterial multiplication while the amorphous type takes around 10 days to be formed. They also observed both types of chemically complex occlusions originating from middle lamellae along border pits. Gardner et al (1983) also were able to induce formation of plug materials with polystyrene beads and nonpathogenic bacteria; however, the materials never became dense enough to impede water passage. Obstruction was only completed when the pathogen was present. They, therefore, concluded that a critical mass of occluding material may be required to close off water flow and that the density required for occlusion would likely only be stimulated by a pathogen, but that the general response of occlusion formation is a non-specific response. The work of Gardner et al (1985)

supported their conclusions by showing that both nonpathogenic and weakly pathogenic rhizobacteria could also incite vascular plugging. Braun (1990) made observations that suggested that although EPS plays a critical role in wilt induction and aids the pathogen in movement in the xylem vessels, it may be much less important in the initial infection process than previously supposed. His experimentation supports van Alfen's (1982) hypothesis that the physical pressure exerted by the expanding, hydrated EPS matrix is very important in facilitating the movement of bacteria within the vessels.

**Symptom expression.**    Potato plants infected with C. m. sepidonicus may or may not express disease symptoms characteristic of ring rot. When symptoms are expressed they include whole plant wilting or unilateral wilting of leaves or branches, leaf chlorosis progressing from the base to the apex (Hooker, 1981), stunting or rosetting (Guthrie, 1959), upward rolling of leaf margins and marginal necrosis (DeBoer and Slack, 1984), and vascular browning in stems (Hooker, 1981). Infected tubers may exhibit stem-end vascular necrosis (Hooker, 1981) or the symptom which is the name-sake for the disease: a creamy, cheese-like, odorless bacterial ooze which exudes like ribbons from the vascular ring when tubers are cut transversely and squeezed (Hooker, 1981; De Boer and Slack, 1984). Further degradation of the vascular ring

and surrounding tissue by secondary invaders creates a fluidal rot.

Bishop and Slack (1992) have shown that infection of potato with C. m. sepidonicus results in reduced transpiration and that the reduction in transpiration and associated wilting appear to be the result of reduced xylem flow. The transpiration pull is reduced by occluded vessels, plugged as a part of the host's response to infection, thereby creating a water deficit stress (drought) within the host and eventually causing wilt.

Symptoms of wilt also have been reported to be induced by EPS (Westra and Slack, 1992; Van Alfen et al, 1987; van den Bulk et al, 1991). From the investigations of Bishop et al (1988) it seems that only certain portions of the EPS are responsible for the wilting symptom. From plants which exhibited symptoms of stunting but no wilting, only nonmucoid strains were isolated, and from wilted hosts mucoid strains were recovered.

The wilting symptom produced in potato cultivars susceptible to C. m. sepedonicus is one for which there are a few possible explanations. Plugging of the vascular bundles is one likely explanation. This has been attributed to the pathogen's EPS layer components (Westra and Slack, 1992; Van Alfen et al, 1987; van den Bulk et al, 1991) or to the presence of bacterial cells in the xylem, regardless of EPS presence (Gardner et al, 1983).

A phytotoxic glycoprotein, "vivotoxin" produced by $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$ both in culture and $\underline{in}$ $\underline{planta}$ (Mazars et al, 1989; Strobel, 1970) is another proposed explanation.

Latent or quiescent infections are common among infected potato plants (Lelliott and Sellar, 1976; Schuld et al, 1992). Up to 10% of infected plants may not show outward symptoms (Nelson, 1985). These symptomless infections have been associated with low inoculum levels (Nelson, 1982; DeBoer and McCann, 1990), late-season infection, high nitrogen fertility (Easton, 1979) and cool, wet environmental conditions that suppress or mask symptom expression (Bishop and Slack, 1982; De Boer and Slack, 1984). Symptomless stems and tubers may support bacterial populations up to $10^9$ and $10^7$ cfu/g tissue, respectively (Bishop and Slack, 1982; DeBoer and Slack, 1984) and can remain latent after three generations of tuber propagation (Nelson, 1982). Nelson (1982) also demonstrated that when as few as 30 cfu (and possibly only 3 cfu) of $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$ were injected into seed pieces latent infections developed which were undetectable by IFAS, however tuber progeny from these plants produced some plants with symptoms the following year as well as an increased incidence of latent infections. Dykstra (1942) conducted an experiment in which 100% of the inoculated tubers produced asymptomatic plants; however, when the

progeny tubers were planted 57% of the plants expressed symptoms.

The environment in which the plants grow is another factor to be considered in the symptom expression of ring rot of potato. Infected plants usually do not show aboveground symptoms untill they are fully grown, or the symptoms may show so late in the season that they are masked by senescence, late blight, or other diseases. However, in years with cool springs and warm summers one or more of the stems in a hill may appear stunted to one degree or another while the rest of the plant appears normal (Agrios, 1988).

**Disease detection and diagnosis.** Certification programs have not been successful in eradicating ring rot from North America. This lack of success has been attributed to occurrence of symptomless or latent infections in seed fields which have gone undetected (Easton, 1979; DeBoer and McNaughton, 1986). _Clavibacter_ is not the only gram positive bacterium that may be found internally in potato plant tissue. Other soil inhabiting Gram positive bacteria, such as _Clostridium_ spp., _Bacillus_ spp., and saprophytic coryneforms can be found internally in stems and tubers (De Boer and Slack, 1984). Therefore, Gram stain is not effective when used as the sole diagnostic tool in pathogen identification. Eggplant is a sensitive bioassay host for _C_. _m_. _sepidonicus_ (Hooker,

1981), developing marginal or sectorial wilting of the first one or two true leaves followed by chlorosis and necrosis (Bishop and Slack, 1987). Although the eggplant bioassay offers a relatively sure diagnosis for ring rot it also requires greenhouse space and time.

The current methods of pathogen detection and disease diagnosis are far more sensitive than visual detection of foliar symptoms in the field by certification agents. These involve serological techniques which employ polyclonal or monoclonal antibodies overcome the problems of detecting symptomless infections (DeBoer et al, 1989). However, the pathogen populations within host tissues may be below the detectable threshold of the test being used (Nelson, 1982). Detection limits involve both pathogen population thresholds and serological similarity of pathogen strains to other bacteria.

The most effective and widely used serological techniques are indirect immunofluorescent antibody staining (IFAS) and enzyme linked immunosorbent assay (ELISA). These tests give more reliable results when monoclonal antibodies are used due to the possibility of cross-reaction with polyclonal antibodies (Gudmestad et al,1990; DeBoer and Copeman,1980). Although cross-reaction is also possible with monoclonal antibodies (DeBoer and Copeman, 1980), it is much less likely than with polyclonal antibodies (Baer and Gudmestad, 1992;

DeBoer et al, 1989). However, serological tests also can be inaccurate and the results must be carefully considered due to the possibility of false negatives and false positives because of the variable sensitivity to, and specificity for, the target organism (DeBoer et al, 1989).

Variations in molecular size and sugar composition of some polysaccharides (Henningson and Gudmestad, 1993) led to differences in the ability of ELISA to detect the presence of C. m. sepedonicus cells. These variations altered the quantities of antigenic sites present for antibody attachment. Among the three colony morphology groups, mucoid, intermediate, and non-mucoid, which are based on quantity and type of EPS produced, the only ones which could not be adequately detected using any privately or commercially available C. m. sepedonicus polyclonal or monoclonal antisera were those strains classified as non-mucoid (Baer and Gudmestad, 1993). The EPS carbohydrate content of these non-mucoid strains was of significantly different composition from that of the mucoid and intermediate strains (Henningson and Gudmestad, 1993). Non-mucoid strains both fluoresced with reduced intensity in IFAS and gave lower optical density readings in ELISA when compared with fluidal strains (A.A.G. Westra, personal communication). Non-fluidal strains are, therefore, difficult to detect with ELISA, showing a sensitivity of 40% or less (Baer and Gudmestad, 1993). To

obtain good visibility of either fluidal or non-fluidal strains in IFAS, careful adjustment of the quantity of conjugated antibody applied is essential for the proper balance between the brightness of the cells to contrast with the brightness of the background (DeBoer and Copeman, 1980), due to the quantity of dissolved extracellular antigen in solution. The sensitivity of ELISA is difficult to interpret in terms of cells per gram of tissue because the antibody reacts with dissolved as well as attached extracellular antigens (DeBoer et al, 1988). IFAS may be the only existing serological technique capable of detecting fluidal and non-fluidal strains with equivalent sensitivity (Baer and Gudmestad, 1993).

The studies of Westra and Slack (1992) bear different, but not necessarily conflicting, results. Their work with EPS of C. m. sepidonicus indicates the quantity of EPS produced is a function of the presence or absence of in vitro aggregations of the third of three components of its EPS. However, loss of the components resulting from aggregation affects neither the organism's ability to infect a susceptible host nor the development of disease symptoms.

**Epidemiology and Disease management.** C. m. sepedonicus causes a disease so devastating to the potato industry that a zero tolerance has been established (Shepard and Claflin, 1975) within the seed production

program in an effort to eliminate the disease from North America (De Boer and Slack, 1984). Through the disease certification program a strong effort has been made to eradicate bacterial ring rot of potato from North America (Slack and Darling, 1986). "Zero tolerance" means if one diseased plant or one infected tuber is found the entire seed lot will be rejected by the certifying agency (De Boer and Slack, 1984). The seed lot can then be either sold as commercial table stock or for processing or destroyed (De Boer and Slack, 1984). Eradication has been unsuccessful primarily due to latent infections.

Until eradication is accomplished other measures must be taken to reduce spread of ring rot. Mandatory flushing of all seed lots from a farm where any seed lot has been found positive for C. m. sepedonicus (Gudmestad, unpublished data, as cited by Gudmestad, 1994), use of certified seed coupled with a limited generation system, and proper sanitation of equipment and storage areas with scraping, washing and application of recommended disinfectants (Gudmestad, 1994) are among the most important precautions.

C. m. sepedonicus can survive and maintain virulence in dried slime on any porous surface under cool, dry outdoor conditions (Easton, 1982). Survival on burlap storage bags was reported for up to 5 yr by Nelson and Kozub (1990), on storage walls and floors, equipment, and

miscellaneous other surfaces. Overwintering also can
occur on insects (Christie et al, 1991), in plant debris
stuck to equipment or in the soil (De Boer and Slack,
1984), although Dykstra (1942) indicates that this
organism survives poorly in the soil. Survival is best
if it is kept constantly frozen at -20 or -40 C (5 yr),
but at more normal temperature fluctuations a 1-2 yr
survival period was observed (Nelson and Kozub, 1990).
Relative humidities of 50-70% were more detrimental to the
ring rot bacterium than 12% at 5 C (Nelson, 1980), showing
that cool, dry conditions are more conducive to long term
survival.

Dispersal of C. m. sepedonicus takes place primarily
by means of cutting tools, pick planters and other
mechanical means. Splashing and flowing water, birds and
mammals (De Boer and Slack, 1984), movement of plant
debris or soil on equipment, and true seed (Easton, 1979),
play a very minor role in dispersal. Colorado potato
beetle and the green peach aphid, (Christie et al, 1991),
the potato flea beetle (Christie and Gudmestad,
unpublished, as cited by Gudmestad, 1994) and black
blister beetle (List and Kreutzer, 1942) were found to
transfer C. m. sepedonicus from one potato plant to
another in the greenhouse. Duncan and Genereaux (1960)
and Christie et al (unpublished, as cited by Gudmestad,
1994) have shown all but the black blister beetle to be

effective vectors of the ring rot pathogen under field conditions as well. DeBoer et al (1988), using ELISA, found that fruit flies which had been in association with infected tubers in storage produced a positive test result for the bacterium. In turn, stored tubers which had previously tested negative became positive after being exposed to fruit flies which carried the pathogen.

Resistance to C. m. sepedonicus infection has been bred into commercial cultivars. However, fear within the industry that these may be symptomless carriers of the disease has prevented widespread use of these cultivars (Gudmestad, 1994).

C. m. sepedonicus can establish an endophytic relationship in sugar beet roots (Bugbee et al, 1987) and be moved long distances in sugar beet seed (Bugbee and Gudmestad, 1988). Strains of the pathogen recovered from sugarbeet roots responded identically to potato strains in physiological, biochemical and serological tests, and caused wilt symptoms in potato (Bugbee et al, 1987). Therefore, this symptomless haven has serious implications in the management of ring rot.

**Conclusion.** The biology of Clavibacter michiganensis subsp. sepedonicus and its interaction with potato plants is a complex relationship with agression and defense from both partners of the pathogenic relationship. Asymptomatic infections are currently the road block to

removal of ring rot from North American potato production.
More investigation is required for improved understanding
of the physiology of the latent infection.


### EFFECTS OF SOIL AND VASCULAR WATER POTENTIAL ON SOLANUM TUBEROSUM

Water is essential to all plant growth. Compared to
other plant species potatoes are particularly sensitive to
water stress. The reduction of marketable yield as a
result of water deficit stress may be due to reduced leaf
area and/or reduced photosynthesis per unit area of leaf
surface in addition to the direct effect of water
deficiency. Water shortage during the tuber bulking
period decreases yield to a greater extent than a water
deficit stress during any other time (van Loon, 1981).

The amount of water required by a potato crop depends
on climate, soil and the variety (van Loon, 1981);
therefore, what constitutes a drought varies as well.
Among potato varieties Russet Burbank is especially
sensitive to conditions of reduced water, therefore,
requiring less of a deficit to create drought conditions.
Epstine and Grant (1973) determined the stomatal diffusion
resistance of Russet Burbank plants to be 2-3 times
greater than those of cv Katahdin, a drought resistant
variety. Burrows (1969) showed that an increasing water
deficit in the soil caused the transpiration rate of

potato plants to decrease at a more rapid rate than sugarbeet. Potato also exhibited a much slower rate of leaf water potential recovery overnight than cotton or sorghum (Ackerson et al, 1977). This may relate to its shallower and less extensive root system than other crops (Corey and Blake, 1953) and thus its greater sensitivity to drought.

Durrant et al (1973) found that potato extracted considerably less water from the soil than barley or sugarbeet indicating a relatively weak root system. The amount and distribution of the root system could influence the amount of abscisic acid produced in the root tips as the soil dries and this may affect the stomatal conductance (Zhang and Davies, 1990). Jefferies (1993) suggested that root systems may affect the response of the plant to water stress by hydraulic effects and the generation of chemical signals in response to water stress. Jefferies (1990) and Turner (1986) found that potato root:shoot ratios were increased by drought indicating that root growth was enhanced over shoot growth by decreased water availability. He also found that root length was increased while root diameter was decreased by drought. This could increase the hydraulic pressure in the vessels and increase the water stress in the leaves but conserve the water supply.

When potato plants are subjected to drought the relative water content of their leaves gradually decreases. The leaf water potential represents the energy status of the water within a plant, and is one of two parameters which describe plant water deficit (van Loon, 1981). Even though living plant tissues are composed of approximately 90% water, only about 1% of the water required by a plant is used in its metabolic pathways. The remaining 99% of the water moving through a plant is used for transpiration. Water stress may inhibit or stop transpiration, which in turn inhibits or stops any of the physiological processes such as photosynthesis, cell enlargement, and enzymatic activities (van Loon, 1981). The stomatal conductance, as mentioned earlier, is a reflection of the water status within the plant (increasing stomatal resistance indicates the closure of the stomates (van Loon, 1981)) and is related to the leaf water potential. Decreases in leaf water potential and relative water content of leaves were associated with a decline in photosynthetic rate. Photosynthesis is reduced when stomatal closure results in transpiration reduction during plant water stress (Campbell et al, 1976). One field experiment showed a photosynthesis reduction of 50% in water stressed potato plants compared to nonstressed plants (Witsch and Pommer, 1954). Rijtema and Aboukhaled (1973) used -0.35 MPa as the critical leaf water potential

for nonstressed conditions of the potato crop. Gander and

Tanner (1976) measured leaf water potentials of -0.2

to -0.3 MPa in the well watered plants compared to -0.6 to

-0.7 MPa in the droughted plants, while Ackerson et al

(1977) found leaf water potentials as low as -1.9 to -2.0

MPa (lower than most reports indicate) in stressed plants.

Significant reductions on photosynthesis occurred at these

leaf water potentials due to stomatal closure.

Differences in evaporative demand may explain these

differences. Stomatal sensitivity is greater for

greenhouse plants than for plants grown in the field

(Davies, 1977), therefore greenhouse grown plants would

suffer reduced photosynthesis at higher leaf water

potentials than field grown plants. In combination, the

studies of Moorby et al (1975) and Ackerson et al (1977)

indicate that closure of the stomates in potato is

associated with a decrease in the photosynthetic rate but

that there is no reduction in carbon dioxide fixing

enzymes in younger leaves, however older leaves show

reduction of the photosynthetic carboxylating enzymes.

Reduction of leaf area (via reduced cell enlargement)

due to water deficit was studied by Krug and Wiese (1972).

They found that soil moisture at 20-30% of its holding

capacity during the first 24 days after emergence

initially decreased the leaf area; however, after being

well watered, these same plants showed a higher foliage

weight than those which had sufficient water continually.
Gander and Tanner (1976) found potato leaf elongation to
be reduced at a leaf water potential of -0.3 MPa, and
growth cessation at -0.5 MPa. This has an unfavorable
effect on tuber bulking due to lack of full canopy cover
over the soil surface (van Loon, 1981).

Tuberization is decreased by water shortage,
particularly at tuber initiation. For optimum yields soil
moisture should never drop below approximately 65% of crop
available water in the densely rooted soil layer during
the tuber bulking period (Curwen, 1993). However, high
soil water content early in the growing season causes
early senescence of the plants, and, therefore, reduced
tuber production (Krug and Wiese, 1972). Water stress
during the period when the canopy is closed or after
flowering also causes early senescence (van Loon, 1981).
Potato plants grown under low water conditions initially
seem to support higher yields if also water stressed
during the tuber bulking period. Both Necas (1974) and
Cavagnaro et al (1971) came to the conclusion that as a
result of water stress either before emergence or between
emergence and flower bud formation the plants were
hardened to drought at the critical tuber bulking stage
allowing for a better total yield. For the grower,
however, it is not the total yield but the marketable
yield which is important. Water deficit stress can

decrease the number of tubers initiated so that there are
few but large tubers, or distort the tubers creating
knobby, dumb-bell shaped, or second growth tubers. Soil
moisture deficit during tuber bulking causes cell
maturation so that when the crop is rewatered the tubers
do not resume normal growth. Growth will then be
restricted to the axillary bud (eye) zones (van Loon,
1981) causing knots to form.

The percent of crop available water that can be used
before stress occurs (Rijtema and Aboukhaled, 1973) varies
for differing soil types and profiles. The pattern by
which a soil will allow water to be accessible to a plant
can be shown with a soil moisture retention/release curve
(such as Fig. 1). These curves show the relation between
soil moisture suction and soil moisture content. The
shape of the soil moisture retention curve determines the
quantity of available water in the root zone of the plant
at any particular water tension, that is, the amount of
water between field capacity and wilting point as long as
sufficient oxygen is available for proper root function
(van Loon, 1981).

**Conclusion.** The effects of excessivly negative
soil and vascular water pressure on the stomatal function
and photosynthesis of potato are very similar. With the
possible exception of toxic effects from the glycopeptide
isolated by Strobel (1970), the effects of heavy vascular

populations of <u>Clavibacter</u> <u>michiganensis</u> subsp.

<u>sepedonicus</u> (suspected value of $\geq 10^9$ cfu/g stem tissue)

closely simulate those of low soil water pressure due to

blockage of transpiration flow.

## INTERACTIONS OF VASCULAR INHABITING BACTERIA
## WITH PLANT WATER RELATIONS

Vascular plant pathogens generally cause water

imbalances in the host by interfering with water

transportation (Bishop and Slack, 1992; Duniway, 1971) or

with stomatal regulation by changing the water holding

capacity of cellular membranes (Turner, 1972). Host

internal water pressure due to external factors such as

soil water potential, relative humidity, and sun exposure

can influence the pattern of pathogen advancement, and

thereby, disease progression (Schouten, 1990).

High-molecular-weight EPS, which are produced by a

large number of pathogenic bacteria, are known to

interfere with water transport in the vascular tissue of

host plants (Buddenhagen and Kelman, 1964; Husain and

Kelman, 1958; van Alfen and Turner, 1975). The molecular

weights of these polysaccharides determine the specific

plant tissues that will be blocked to water passage

(Suhayda and Goodman, 1981; van Alfen and Allard-Turner,

1979). This selectivity is the result of variation in

diameter of the vessel elements at various locations

within the plant (Bowden and Rouse, 1991) as well as possible variations in biochemical responses of different organs and tissues.

There is good evidence in the work of Woods (1984) with P. solanacearum that the water shortage resulting from the vascular plugging of banana is associated with wilting (Beckman et al, 1962). Their evidence indicates that a continuously declining water supply to the leaves causes stomate closure and photosynthetic process failure (chlorosis) and finally laminar wilt and petiole collapse. Vascular occlusion was described as the primary cause of wilting (Beckman et al, 1962) for banana.

Erwinia stewartii, causal organism of Stewart's wilt of corn, is another vascular wilt disease where the EPS brings about changes in the water potential within the plant (Braun, 1990). EPS may cause wilt by increasing the viscosity of the xylem fluid (Husain and Kelman, 1958), or by blocking the vessels and plugging pit membranes (van Alfen, 1982). The gums that block the vessels are secreted by the xylem parenchyma in response to infection then ooze through the cell walls to fill the lumens of the vessels. Strains without much EPS were unable to move well through the vessels (Braun,1990).

Clavibacter michiganensis subsp. sepedonicus and C. m. insidious are closely related vascular wilt pathogens and cause similar symptoms in their respective hosts

(Bishop and Slack, 1992). The glycopeptide isolated from C. m. sepedonicus by Strobel (1967) was shown to cause membrane disruption in potato plants and increase the rate of water loss. Van Alfen and Turner (1975), in further work with this glycopeptide, found it to decrease the water movement through the xylem of alfalfa by >38-44% and to decrease abaxial stomatal conductance and transpiration. Alfalfa cuttings were wilting after a 1 h exposure to the glycopeptide. Their conclusion was that the toxin acted by interfering with the flow of water through the vascular system and not by any direct toxicity to plant cells since the cellular membranes of toxin-treated stems were intact. This would indicate that its mode of action is different from that of the C. m. sepedonicus glycopeptide. In 1979 van Alfen and Allard-Turner showed how these macromolecules, previously classed as phytotoxins, can physically (not biochemically) block and stop vascular conductance in alfalfa at levels of activity characteristic of plant hormones. Physical size is apparently the most important characteristic for this activity.

However, Bishop and Slack (1992) have shown that the C. m. insidious "cell membrane toxin" does not have as great an involvement in symptom expression as was previously suspected. They found infected potato plants to exhibit reduced transpiration and depressed xylem

function, which they say is contrary to what would occur if the toxin were fully responsible for the wilting. Therefore, they (1992) concluded that the primary cause of wilting was physical obstruction of xylem flow which was not associated with the toxin's effect. Glycopeptide toxin effect in causing wilt might be expected to be similar to one of the fungal wilt producing toxins in Turner's work (1972). From the work with victorian (Turner, 1972) host transpiration was reported to be significantly decreased due to stomatal closure at all toxin concentrations, and stomatal reopening, with time, at higher toxin concentrations. His experiments with fusicoccin (Turner, 1972) showed the opposite effect, a permanent opening of the stomata at all toxin concentrations and increased transpiration so that water loss exceeded water uptake. Turner's conclusion that each toxin uses a different mode of action in causing wilt is well supported, therefore indicating that wilt producing toxins can function in different ways to achieve the same result. Bishop and Slack (1992) and Turner (1972) have both generated evidence to refute van Alfen and Allard-Turner's (1975, 1979) conclusion that the phytotoxic glycopeptide causes physical blockage of the vascular system.

Dey and van Alfen (1979), working with alfalfa under both water deficit stress and nonstressed conditions,

found C. m. insidious infected plants to achieve a more negative water potential than healthy plants during the day and to recover less well at night, regardless of the water treatment. As the soil became drier, stomatal conductance decreased much more in diseased than in healthy plants and xylem water potentials dropped more drastically in infected plants. They found no evidence that cellular membrane damage was a factor in water stress of diseased plants, which is further indication that a toxin is not the major cause of tissue desiccation.

Schouten (1990) found that E. amylovora progresses through the plant by mechanical pressure in relation to the water potential within the host. When water potential is low (drier) the bacteria replicate to fill the available space. When water becomes more plentiful within the host, increasing the water potential, the EPS layers around the bacterial cells hydrate. This causes the same number of cells to occupy more space, forcing them up the xylem vessels or through degraded vessel walls into surrounding tissues and through intercellular spaces, eventually bursting through the epidermis. Continual positive pressure forces the Erwinia cells out in columns or as ooze. The ideas of Goodman and White (1981) and Schouten (1990) may be combined together. These same principles likely apply to Clavibacter infections in tomato and potato.

**Conclusion.** It is easy to see that there is much concerning the relationships among host, pathogen, and environment that remain to be discovered in the effort to understand and control losses due to ring rot of potato. The harboring of C. m. sepedonicus populations in asymptomatic foliage and tubers is the most significant impass remaining to overcome in the efforts to bring the zero tolerance regulation to it intended goal of elimination of ring rot from North America. Understanding of the means of alteration of water relations within potato plants by C. m. sepedonicus appears to be the pivotal point in the visualization of symptoms. If this were more fully understood effective methods could be devised to bring infections past the latent phase to symptom expression and elimination.

# Chapter II.

## Water Deficit Stress Effects on Bacterial Ring Rot of Potato Caused by _Clavibacter michiganensis_ subsp. _sepedonicus_

**Introduction.** Pathogen induced water deficit has been implicated in several vascular wilt diseases. Tzeng and DeVay (1985) found evidence of reduced leaf water pressure in cotton infected with _Verticillium dahliae_. Similar results have been reported for tomato infected with _Fusarium oxysporum_ f.sp. _lycopersicon_ (Duniway, 1971a; Duniway, 1971b) and alfalfa infected with _V. dahliae_ (Pennypacker et al, 1990). Decreases in stomatal conductance, transpiration, and photosynthetic rates of potato infected with _V. dahliae_ also have been demonstrated (Havercourt et al, 1990). Pathogen induced drought also is indicated with the bacterial vascular wilt pathogen _Clavibacter michiganensis_ subsp. _sepedonicus_, cause of bacterial ring rot of potatoes. Bishop and Slack (1992) showed that _C. m. sepedonicus_ reduced transpiration in potato plants prior to and during wilting by interfering with water flow from soil to leaf, and that xylem function was significantly reduced in petioles of infected plants. Their findings are inconsistent with those of Rai and Strobel (1969b), Reis and Strobel (1972b), Strobel (1970), and Strobel and Hess (1968) who

stated that wilting in potato infected with C. m.
sepedonicus was primarily caused by the action of a toxin
which increased water loss from the leaves.

Potatoes show an adverse response to abiotic water
stress at a less negative soil water pressure than other
crops such as cotton, corn, barley and alfalfa (Coleman,
1988; van Loon, 1981). This enhanced sensitivity to water
stress is due, in part, to the shallowness of the root
system which disallows the sequestering of available water
at the soil depths other crops can reach. Growth of
leaves and tubers are particularly sensitive to
retardation with even mild drought stress at early stages
of development (Gander and Tanner, 1976a; Gander and
Tanner, 1976b; Jefferies, 1989; Levy, 1983; Levy, 1985).
Drought stress may inhibit or stop any of the
physiological processes of the plant, such as
transpiration, photosynthesis, cell enlargement and
enzymatic activities (Campbell et al, 1976; van Loon,
1981). These same processes also are affected by vascular
wilt diseases (Bishop and Slack, 1992; Havercourt et al,
1990).

Panton (1965) found that expression of Verticillium
wilt symptoms in alfalfa was intensified after a period of
limited precipitation, as did Morehart and Melchoir (1982)
in their work with Verticillium wilt of yellow-poplar.
However, Pennypacker et al (1991) described the opposite

effect of water deprivation on expression of Verticillium wilt symptoms in alfalfa. The abiotically-induced drought stress apparently altered some facet of the host/pathogen interaction in favor of the host as shown by her measurements of plant growth parameters. Colonization of alfalfa stems by V. dahliae began 1 wk earlier for droughted plants than well watered plants; however, the disease ratings were lower under drought stress than non-drought stressed conditions. Water deficit stress also reduced the suppressive effect of the pathogen on stem dry weight of alfalfa. Havercourt et al (1990) working with potato found that the combination of V. dahliae and water deficit stress had less effect on stomatal conductance and transpiration than the two separately. However, this interaction was only observed occasionally.

The effect of combined stresses on plant growth is not fully understood, and this is especially true for the combined effects of biotic and abiotic stresses (Pennypacker, 1991). Effect of water deprivation on the progression of vascular wilt diseases caused by bacterial pathogens has not been reported. The object of this study, therefore, was to assess the effect of C. m. sepedonicus and water deficit stress, both separately and in combination, on leaf water pressure, symptom expression, foliar growth, and tuber yield of Russet Burbank potatoes.

Bishop and Slack (1982) in their investigations of stem populations and Nelson (1982) in his work with tuber populations have indicated, respectively, that low populations of C. m. sepedonicus in the stems and tubers are associated with detectable and undetectable latent infections. The effect of variations in quantity of available water on pathogen population size and plant growth parameters has been reported for other vascular pathogens such as Verticillium dahliae (Cappaert et al, 1992; Gaudreault, 1993) and other soilborne fungal pathogens (Cook, 1973). To our knowledge this is the first report on the effects of variations in soil water availability on C. m. sepedonicus stem populations or symptom expression in potato.

## MATERIALS AND METHODS

**Treatments and experimental design.** The experiment was a factorial with inoculum concentration (2) and water stress (2) as the main treatments. Treatments were arranged in a completely randomized design with nine replications per treatment for each of four harvest dates. The experiment was performed twice in a greenhouse where the daytime temperature was held at 22-26 C and night temperature at 16-20 C.

Inoculum densities were 0 and 2 X $10^7$ colony forming units (cfu) of C. m. sepedonicus per seed piece. Water stress treatments were non-stressed and stressed. The non-stressed treatment consisted of not allowing the soil water pressure to exceed -0.05 MPa. The stress treatment was initiated when 95% of the plants had formed visible flower buds. Water was withheld until half of the plants reached a leaf water pressure of -1.4 MPa or less as determined with a Scholander pressure chamber (Appendix III, Figs. 19 and 20) (Soilmoisture Equipment Corp., Santa Barbara, CA).

**Pots and soil.** Pots were fashioned from PVC sewer pipe (25.4 cm diameter) cut into 76.2 cm long segments. Plywood, 3.8 cm thick, was cut into 25.4 cm diameter circles. Each circle was drilled vertically to create 12 equally spaced holes for water drainage. PVC pipe segments were fitted with the plywood bottoms which were held in place with wood screws. Fiberglass window screen was cut into 25 cm diameter circles and placed into the bottom of each the pot to prevent the soil mix from plugging the drainage holes.

The soil mixture was designed to have a water release curve (water retention curve) (Fig. 1) specific for slow imposition of drought conditions when water was withheld (Pennypacker et al., 1990). The water release curve as determined by the Soil Analysis Laboratory, Department of

Crop and Soil Science, Oregon State University, was similar to the water release curve reported for the soil mix used by Pennypacker (1990). Formulation of the mix was 2:1 (v:v) of Redi Earth Peat-lite Mix (W.R. Grace Co., Cambridge, MA) and Monterey beach sand (RMC Lone Star, Pleasanton, CA). Added to the mix were 8.9 g Osmocote 14-14-14 120 day release fertilizer (Sierra/Grace Crop Protection Co., Milpitas, CA) and 1.2 g Esmigran micronutrients (Sierra/Grace Crop Protection Co.)/kg soil mix. The soil mixture with amendments was blended by hand. Once the pots were filled they were watered on three successive days to hydrate the mix prior to planting of seed pieces.

**Seed pieces and inoculum.** Seed tubers of potato (Solanum tuberosum L.) cv Russet Burbank (Foundation seed class) were washed with tap water and kept at room temperature for 7 days. Active sprouts were removed with a 2.5 cm melon ball scoop, rinsed with tap water, dipped in 20% commercial bleach for 2 min, followed by a Captan (Dow Chemical Co.) suspension (8 g/L water) dip to inhibit the growth of surface fungi during sprout growth. Seed pieces were air-dried overnight at room temperature, placed into transparent, covered plastic boxes, and kept at room temperature for 2-3 wk to promote shoot development.
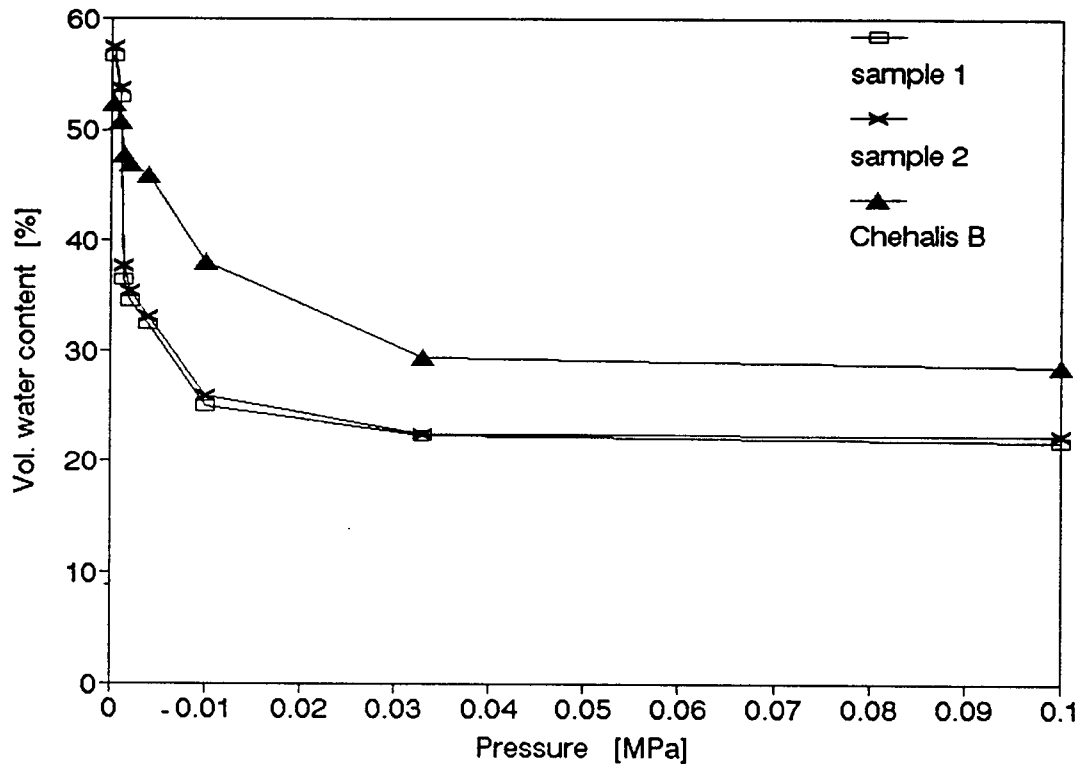
**Figure 1.** Water release/retention curve for soil mix. Formulation of the soil mix was 2:1 (v:v) of Redi Earth Peat-lite mix and Monterey beach sand. Each sample represents the average of two subsamples. The standard, Chehalis B, is a loam from the B horizon of Chehalis, WA, and used as a standard because it is not overly influenced by presence of clay or sand.

Inoculum was produced by streaking Petri plates of nutrient broth yeast extract agar (NBY) with one of two strains of $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$ (CIC31 non-fluidal and CIC132 fluidal, obtained from Carol Ishymura, CO) which carry plasmids for rifampicin resistance. Cells from two 7-day-old cultures of each strain were suspended together in 100 ml sterile distilled water. Concentration was determined with a spectrophotometer (Spectronic 20, Bausch and Laumb, Germany) which was set at 600 nm and the bacterial suspension was diluted with sterile distilled water until the absorbance reading was between 0.5 and 1.0 to approximate $10^9$ cfu/ml. The true final concentration was determined by dilution plating of the cell suspension on NBY. Seed pieces with sprouts of approximately 2.5-3 cm in length were inoculated with 20 uL of either sterile distilled water or a $10^9$ cfu/ml bacterial suspension (2 X $10^7$ cfu/seed piece). Treatments were applied underneath the sprout into a hole made with an automatic pipet tip.

Seed pieces were planted in the pots at a depth of 10 cm on 22 March and 6 April 1993. There was one noninoculated and one inoculated seed piece per pot. Plant emergence occurred in 10-12 days. At that time the photoperiod was set at 16 h light/8 h darkness to induce flowering. The growing plants were spiraled around cotton string which was hung from wires running 3 m above the pots to support upright growth.

**Soil water and leaf water pressures.** Tensiometers
with -0.1 MPa capacity were placed into the pots with the
porus tip 46 cm below the soil surface. A pressure
transducer with attached syringe needle and digital
readout (Tensimeter™ from Soil Measurements Systems,
Tucson, AZ) (Appendix II, Fig. 18B) was used for soil
moisture measurements. Measurements were made 4 to 5
times per week.

Leaf water pressure measurements were taken
throughout the drought period with a Scholander pressure
chamber following the procedures of Gander and Tanner
(1976). Leaves were severed and placed into the chamber
within 10-15 sec of severance. Due to the close proximity
of pressure bomb and experimental plants it was
unnecessary to protect the leaves from desiccation between
detachment and measurement. Initially, the fourth or
fifth apical leaf was selected for measurements, but as
growth slowed among the droughted plants it became
necessary to sample lower leaves. Approximately the tenth
leaf below the apex was then selected as more indicative
of the water stress within the plant since the lower
leaves began to wilt first. On any given day samples were
taken from the same location on each plant. Measurements
were taken on a daily basis through the first run of the
experiment, and on an every 1 to 3 day basis during the
repeat of the experiment beginning at 1300 and ending by

1600 h.  Leaf water pressure was determined on 33% of the plants at each reading.  These three groups were rotated so each was measured every third reading date.

**Sampling and assays.**  After half of the plants in the water stress treatment had a leaf water pressure of at least -1.4 MPa, all the plants were watered.  The first harvest began 1 wk or 2 wk following termination of the water deficit treatment for the first and second experiments, respectively.  The three subsequent harvests were at weekly intervals.

Plant height, number of branches longer than 2.5 cm, number of internodes, internode length, aerial biomass (leaf dry weight + stem dry weight), number of tubers, and tuber yield (wet weight of tubers) were determined at harvest.  Tubers went into cold storage (2-5 C) for 6 mo.

Population densities of C. m. sepedonicus within the basal stem of each inoculated plant was determined by indirect immunofluorescent antibody staining (IFAS) following the proceedures of Agdia, Inc.  Stem segments 3.8 cm in length, removed from just above the seed piece, were placed into heavy plastic bags containing 5.0 ml 0.01 M phosphate buffer plus normal saline (PBS), and pulverized with a sledge hammer to suspend the vascular contents.  The suspension was serially diluted four times with sterile distilled water.  Twenty microliters of each dilution were placed serially in the wells of a

toxiplasmosis slide (Belco Glass, Inc., Vineland, NJ),
dried at 45 C for 1 h, fixed in acetone for 10 min, rinsed
with distilled water and air-dried in a fume hood. A 20
uL aliquot of diluted 100X antiboby concentrate, mouse
anti-Cs clone 9A1 (Agdia, Inc., Elkhart, IN) was added to
each well, incubated in a humid container at 37 C for 1 h,
rinsed with distilled water and air-dried in a fume hood.
Twenty microliters of diluted 100X FITC concentrate,
fluorescein isothiocyanate conjugated goat anti-mouse IgG
plus IgM solution (Agdia, Inc.) were then added to each
well, incubated in a humid container for 1 h at 37 C,
rinsed with distilled water and air dried in a fume hood.
These stained slides were observed under a fluorescent
(dark field) microscope at 1000X (oil immersion) and the
number of fluorescing cells in at least 10 fields was
counted. Number of cells per well was converted to cells
per gram of stem tissue using the following formula:
cells/g tissue = (avg no. of cells/field) (dilution) (20
ul/no. of fields in well area) ($10^6$ ul/ml) (1 ml/1 g)
(sample weight/(sample weight + 5 ml)).

Incidence of tubers with symptoms of bacterial ring
rot after storage was determined. The stem end of each
tuber was removed and the tuber was visually assessed for
yellowing and/or bacterial ooze from the vascular ring.
Two grams of symptomatic vascular tissue were removed to a

plastic bag and pulvarized with a hammer in 2 ml 0.01 M PBS to suspend the vascular contents. The suspension was evaluated by IFAS as described above to confirm the visual diagnosis.

**Data analysis.** Significance of treatment differences was determined with SAS version 6.04 (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC) (Appendix VI). Procedures for analysis of variance (ANOVA) for balanced data and general linear models (GLM) for unbalanced data were used. Fisher's Protected Least Significant Difference (LSD) procedure was used for comparing means when ANOVA or GLM showed a significant difference. Two-way analyses were performed on the dependant variables using inoculum and water treatments as the independent variables. Data from those plants which did not reach a leaf water pressure of -1.4 MPa or less were excluded from the analysis (Experiment 1 = 40 plants excluded, 36 retained; Experiment 2 = 27 plants excluded, 24 retained). Many of the residual patterns indicated the need of square root or natural log transformation of the data to obtain a normal point distribution curve to make the assumptions valid and the analyses accurate. Square root transformations were performed on plant height, tuber weight, and aerial biomass whereas log transformations were performed on leaf water pressure and IFAS data. GLM summaries are presented in Appendix IX.

RESULTS


Harvest occurred weekly for four successive weeks beginning at 1 or 2 wk following the termination of the drought treatment. For each harvest date there was no significant interaction between water and inoculum treatments for any of the measured parameters (Appendix IX, GLM summaries). Drought resulted in symptoms of wilting in both trials, and defoliation of the lower half of several plants occurred in the second trial (no recorded data). No classical foliar disease symptoms were observed; however, infected plants tended to wilt before the noninfected plants within the drought treatment, and the lower leaves of the infected plants scenesced somewhat earlier than the noninfected plants in both drought and nondrought treatments.

**Inoculum.** Infection of potato seed pieces with $\underline{C}$. $\underline{m}$. sepedonicus did not result in foliar symptoms of bacterial ring rot. However, infection resulted in a significant ($\underline{P}<0.05$) reduction in plant height, aerial biomass, and number of branches, internodes and tubers, and tuber yield compared to the healthy controls. In the first experiment plant height was reduced by 3% (Fig. 2), number of branches was reduced by 17% in the second harvest (Fig. 3); and number of internodes (Fig. 4) was reduced 5 and 6% in the second and third harvests, respectively.
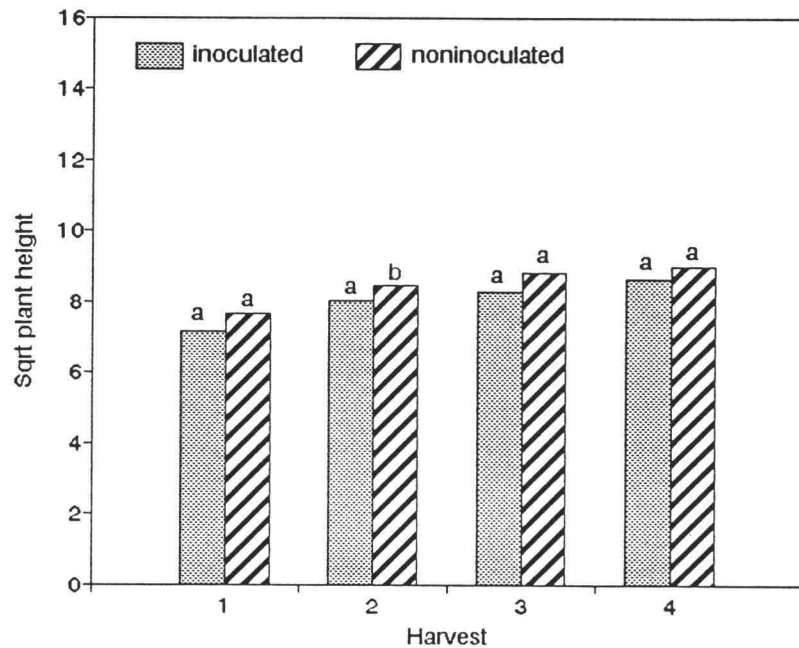
**Figure 2.** Effect of inoculum of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> on height of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different (P ≤0.05) according to Fisher's Protected LSD test.
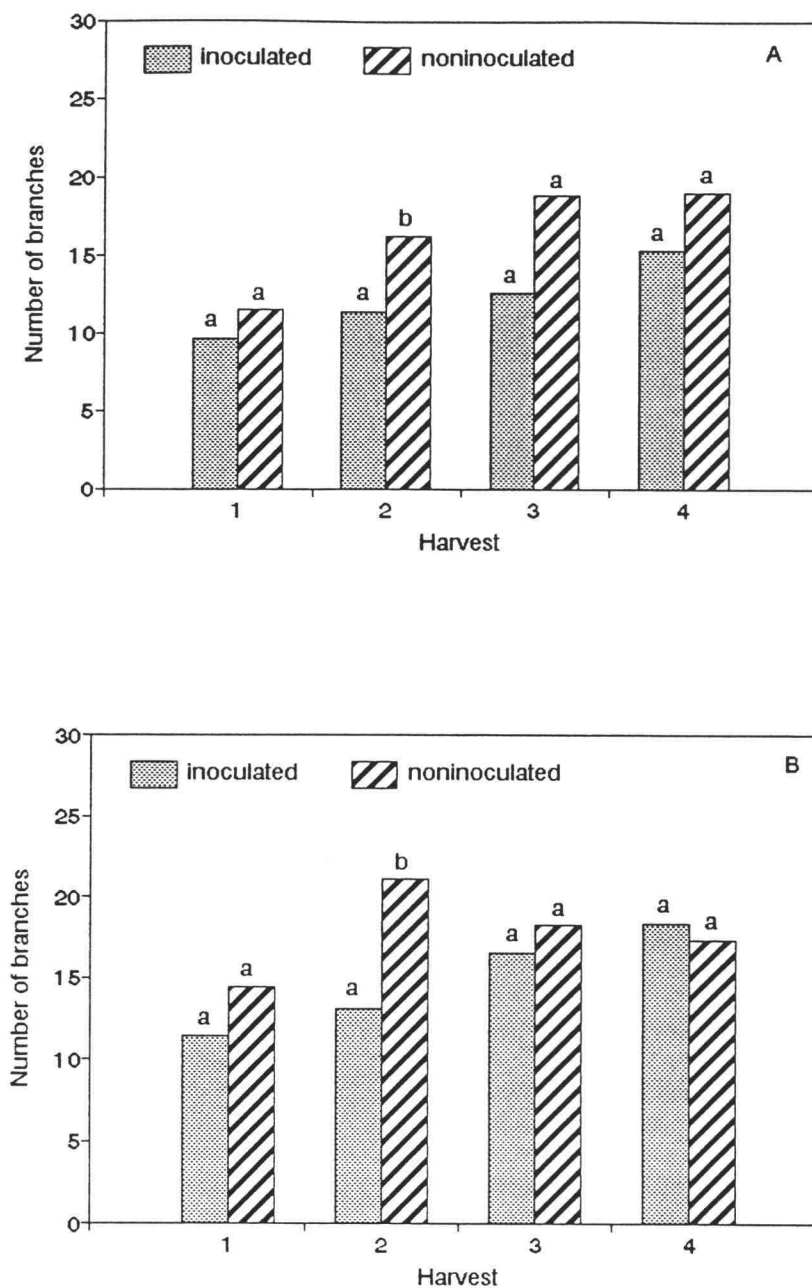
**Figure 3.** Effect of inoculum of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> on number of branches of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test.
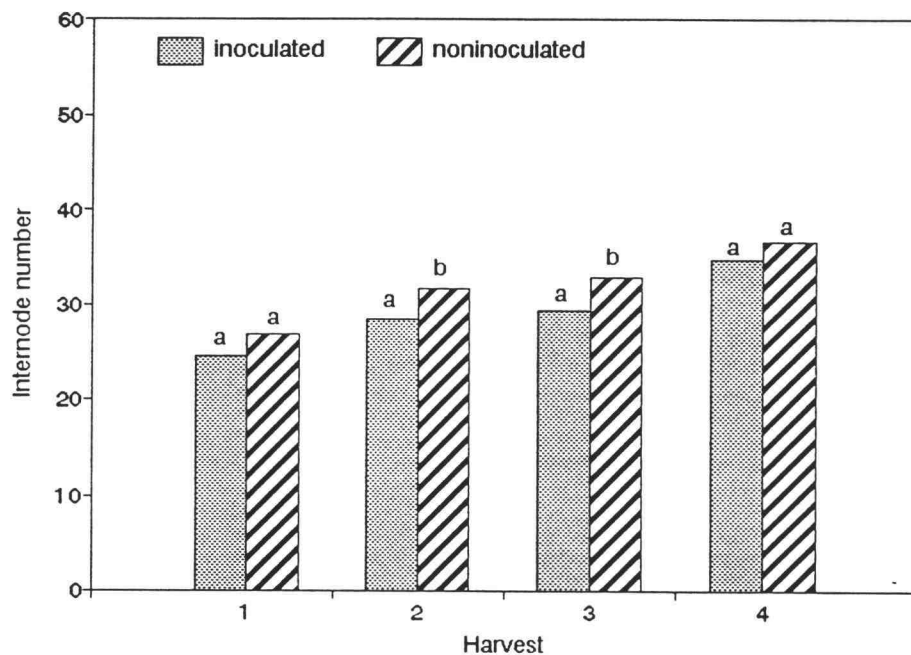
**Figure 4.** Effect of inoculum of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> on number of internodes of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test.

In three of four harvests aerial biomass was reduced by 12-21% (Fig. 5A), tuber number was reduced from 20-29% (Fig. 6A), and tuber yield was decreased by 14-38% (Fig. 7A).

There were fewer significant effects of inoculum in the second experiment. Number of branches (Fig. 3B) was decreased by 21% (harvest 2), aerial biomass (Fig. 5B) was reduced by 14% (harvest 2), tuber number (Fig. 6B) was decreased by 19 and 16% (harvests 2 and 3), and tuber yield (Fig. 7B) declined by 15 and 18% (harvests 2 and 3).

**Water deficit.** Water deficit stress significantly ($P$<0.05) reduced plant height, number of internodes, aerial biomass, tuber number, and yield in at least one harvest of the first experiment; however, plant height was the only parameter which was significantly reduced in the second experiment.

Plant height (Fig. 8A) and number of internodes (Fig. 9) were reduced 8 and 3% and 12 and 10%, respectively, in harvests 1 and 2 of the first experiment. Aerial biomass (Fig. 10) and tuber number (Fig. 11) were decreased 21 and 38%, respectively, in the first harvest, and reduction in tuber yield ranged from 15-41% in three of the four harvests (Fig. 12). Percent reduction in tuber yield in the third harvest was 36%, but was not significant.
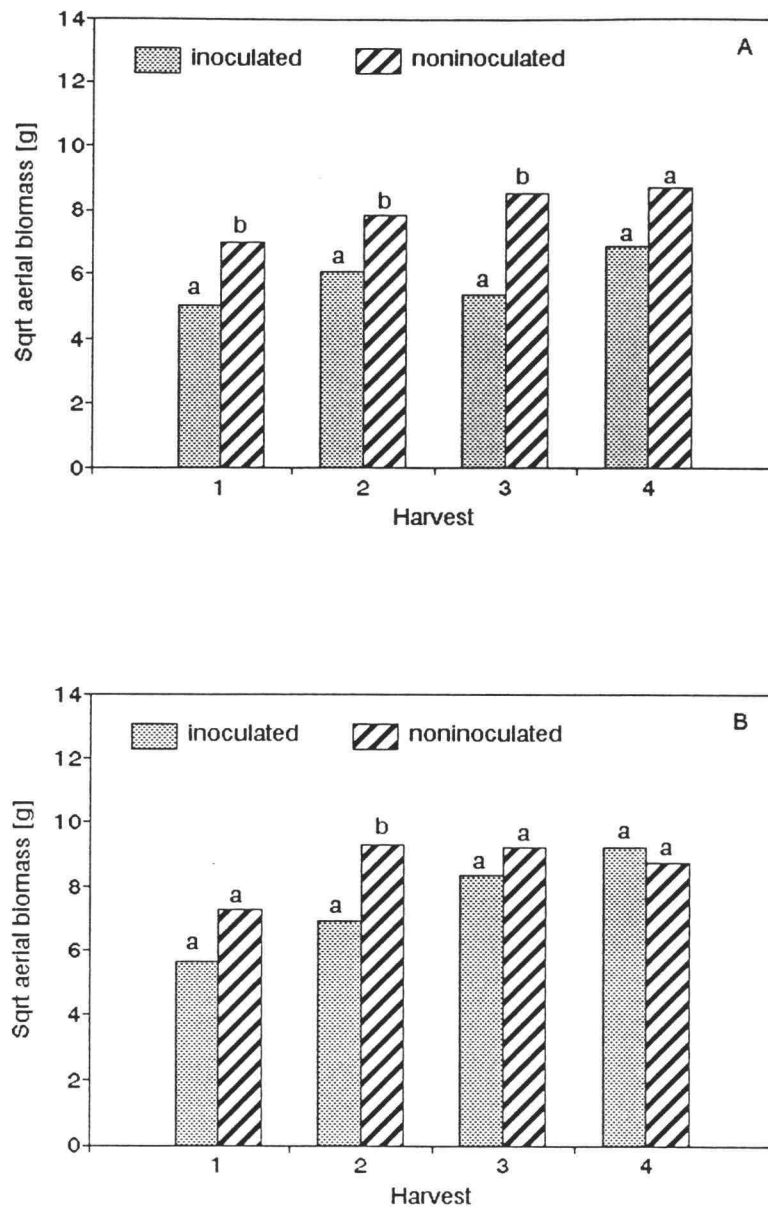
**Figure 5.** Effect of inoculum of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> on aerial biomass of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test.
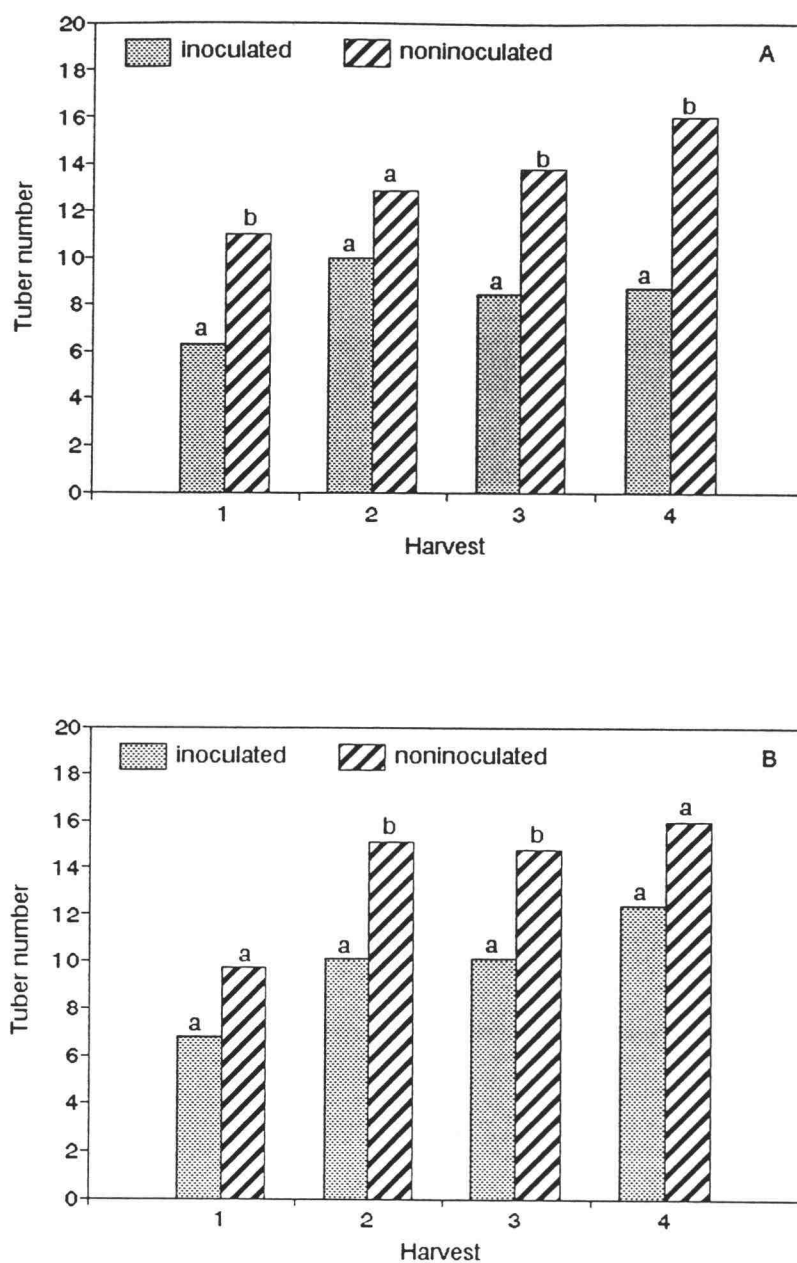
**Figure 6.** Effect of inoculum of <u>Clavibacter michiganensis</u> subsp. <u>sepedonicus</u> on tuber number of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test.
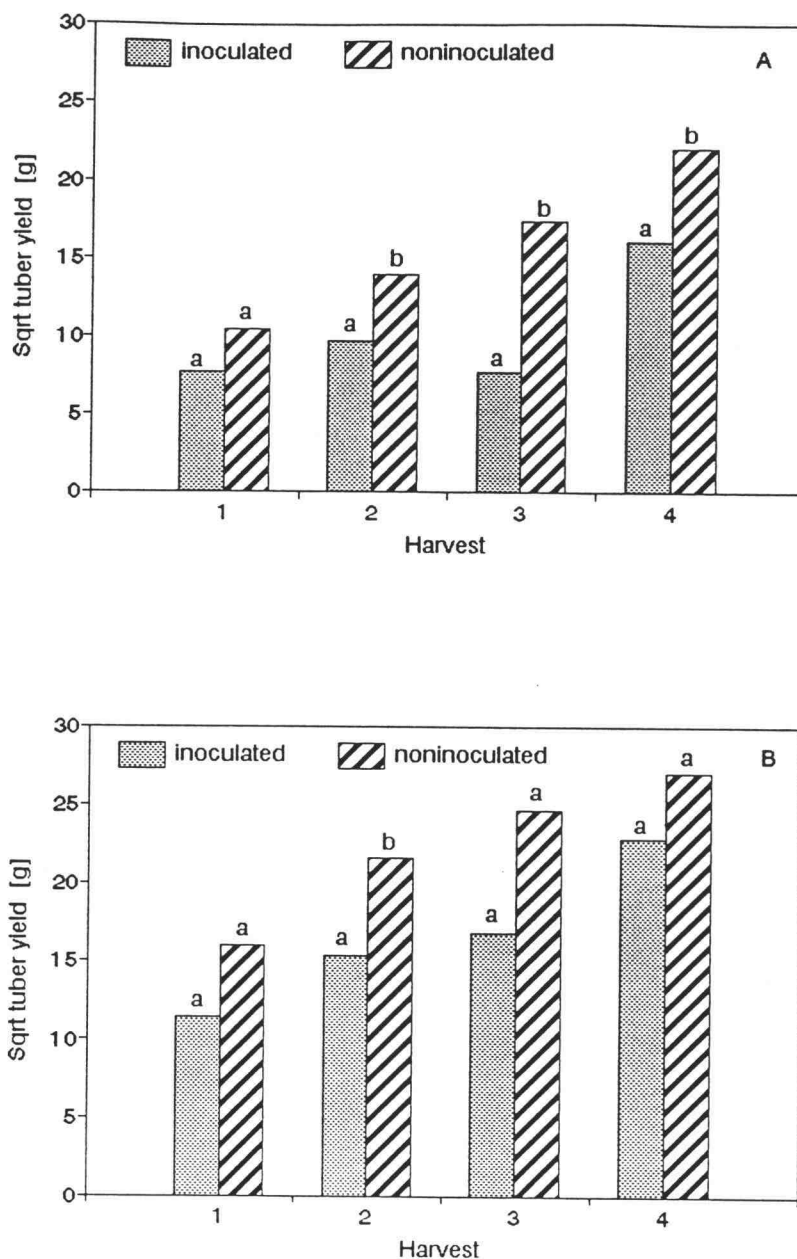
**Figure 7.** Effect of inoculum of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> on tuber yield of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different (<u>P</u> ≤0.05) according to Fisher's Protected LSD test.

**Figure 8.** Effect of water deficit stress on height of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at flowering. Plants were rewatered when leaf water pressure was -1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit, in experiments 1 and 2, respectively.

**Figure 9.** Effect of water deficit stress on internode number of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at flowering. Plants were rewatered when leaf water pressure was -1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively.

**Figure 10.** Effect of water deficit stress on aerial biomass of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different ($\underline{P} \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at flowering. Plants were rewatered when leaf water pressure was -1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively.

**Figure 11.** Effect of water deficit stress on tuber number of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different ($\underline{P} \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at flowering. Plants were rewatered when leaf water pressure was -1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively.
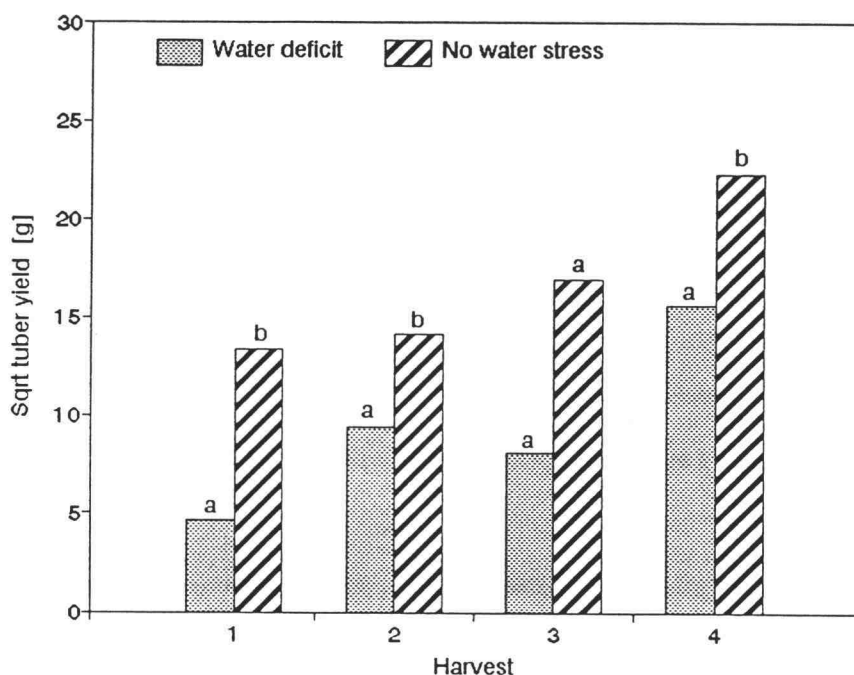
**Figure 12.** Effect of water deficit stress on tuber yield of Russet Burbank potatoes on four harvest dates in experiment 1. Within harvest date, bars with different letters are significantly different ($P \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at flowering. Plants were rewatered when leaf water pressure was -1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively.

In the repetition of the experiment, water deficit reduced plant height (Fig. 8B) by 6 and 4%, respectively in the first and third harvests.

Typical ring rot symptoms were observed in tubers of harvest four after a 6 mo storage (Fig. 13A). In the first experiment plants with no water stress showed a 100% increase in the number of symptomatic tubers over those with a water stress deficit. However, in the repeat of the experiment both water treatments had approximately the same percentage of tubers with symptoms in the fourth harvest.

**Leaf water pressure.** Inoculum of C. m. sepedonicus had no significant effect on water pressure of the sampled leaves. Leaf water pressures of plants just prior to rewatering averaged -0.87 and -1.20 MPa for noninoculated and -0.85 and -1.24 MPa for inoculated in the first and second experiments, respectively (Figs. 14A and 14B). In contrast, leaf water pressures were 3.2 and 3.5 times higher (less negative) in the nonstressed compared to the water deficit stressed plants in the first and second experiments (Figs. 14A and 14B) just prior to the termination of the drought periods.
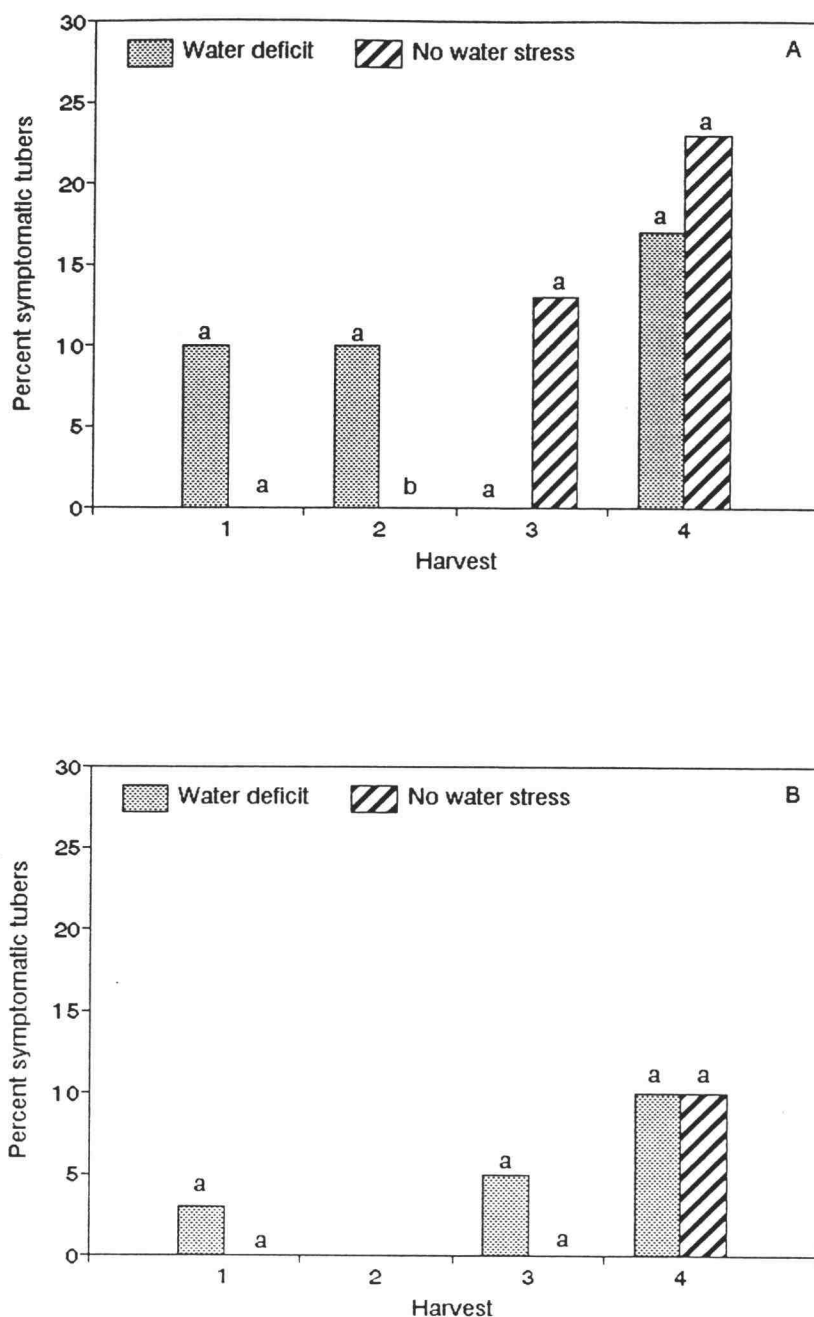
**Figure 13.** Effect of water deficit stress on tuber symptoms of bacterial ring rot in Russet Burbank potatoes in **A**) experiment 1 and **B**) experiment 2. Water deficit was imposed by termination of irrigation at peak flowering. Plants were rewatered when leaf water potential was $\leq$-1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively. Tubers were stored at 2-5C for 6 mo prior to symptom assessment.
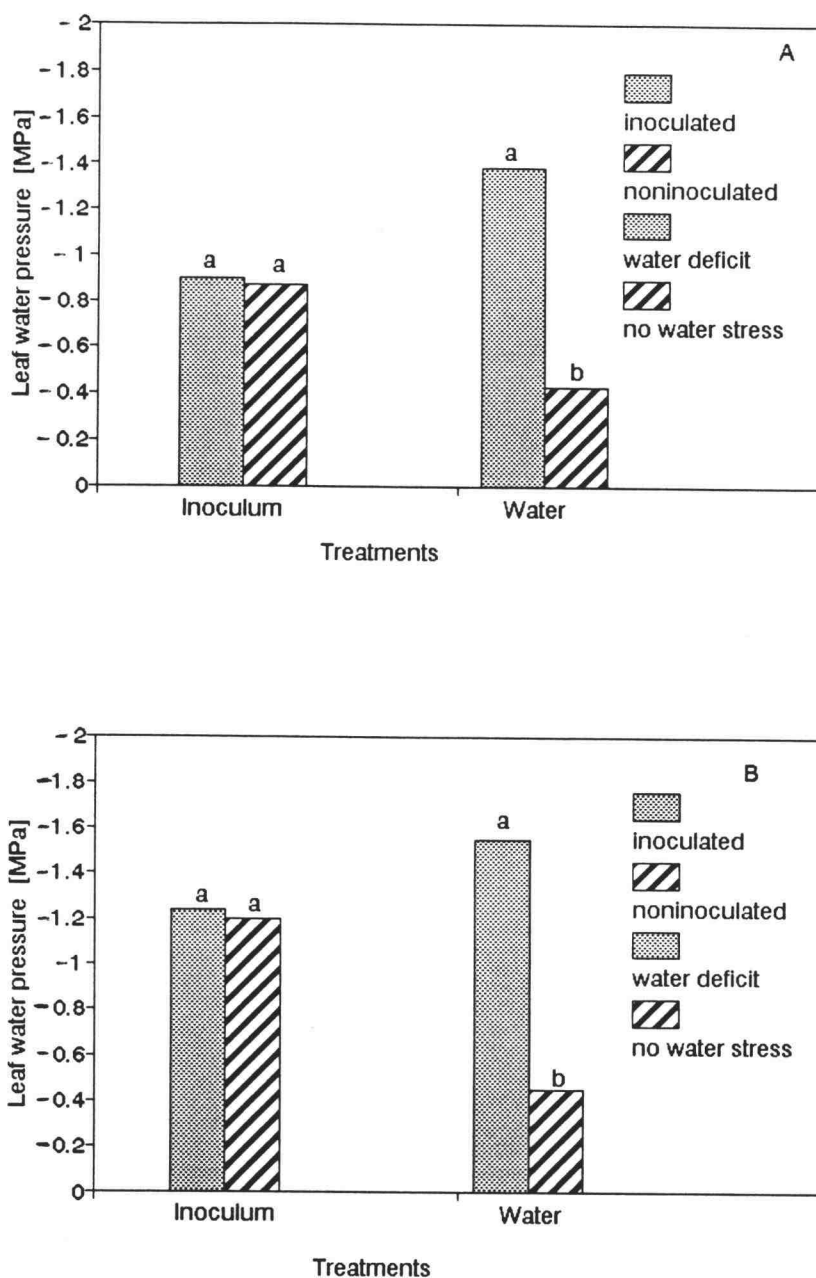
**Figure 14.** Effect of inoculum density of <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> and water deficit stress on leaf water pressure of Russet Burbank potatoes in **A**) experiment 1 and **B**) experiment 2. Within treatment, bars with different letters are significantly different ($\underline{P} \leq 0.05$) according to Fisher's Protected LSD test. Inoculum treatment was 0 or 20ul $10^9$ cells/ml $\underline{C}$. $\underline{m}$. <u>sepedonicus</u>. Water deficit was imposed by termination of irrigation at peak flowering. Plants were rewatered when leaf water pressure was $\leq$-1.4 MPa. Final leaf water pressure was recorded just prior to reirrigation. Harvest began 1 or 2 wk following termination of water deficit in experiments 1 and 2, respectively.

**Stem populations of <u>Clavibacter michiganensis</u> subsp.**
**<u>sepedonicus.</u>** Plants that were water deficit stressed had

significantly fewer cells of $\underline{C}$. $\underline{m}$. <u>sepedonicus</u> ($\underline{P}$=0.01)

than did nonstressed plants at each harvest in both

experiments (Fig. 15A). Population density of $\underline{C}$. $\underline{m}$.

<u>sepedonicus</u> in stem tissue of nonstressed plants ranged

from 4 X $10^4$ to 3 X $10^8$ cfu/g and from 7 X $10^5$ to 2 X $10^7$

cfu/g in water deficit stressed plants of the first

experiment (Fig. 15A). In the second experiment,

variation in population size was 2 X $10^3$ to 4 X $10^8$ cfu/g

for the nonstressed plants and 1 X $10^3$ to 2 X $10^8$ cfu/g

among the water deficit stressed plants (Fig. 15B).

Population size of $\underline{C}$. $\underline{m}$. <u>sepedonicus</u> in infected plants

never exceeded $10^9$ cfu/g in either experiment.
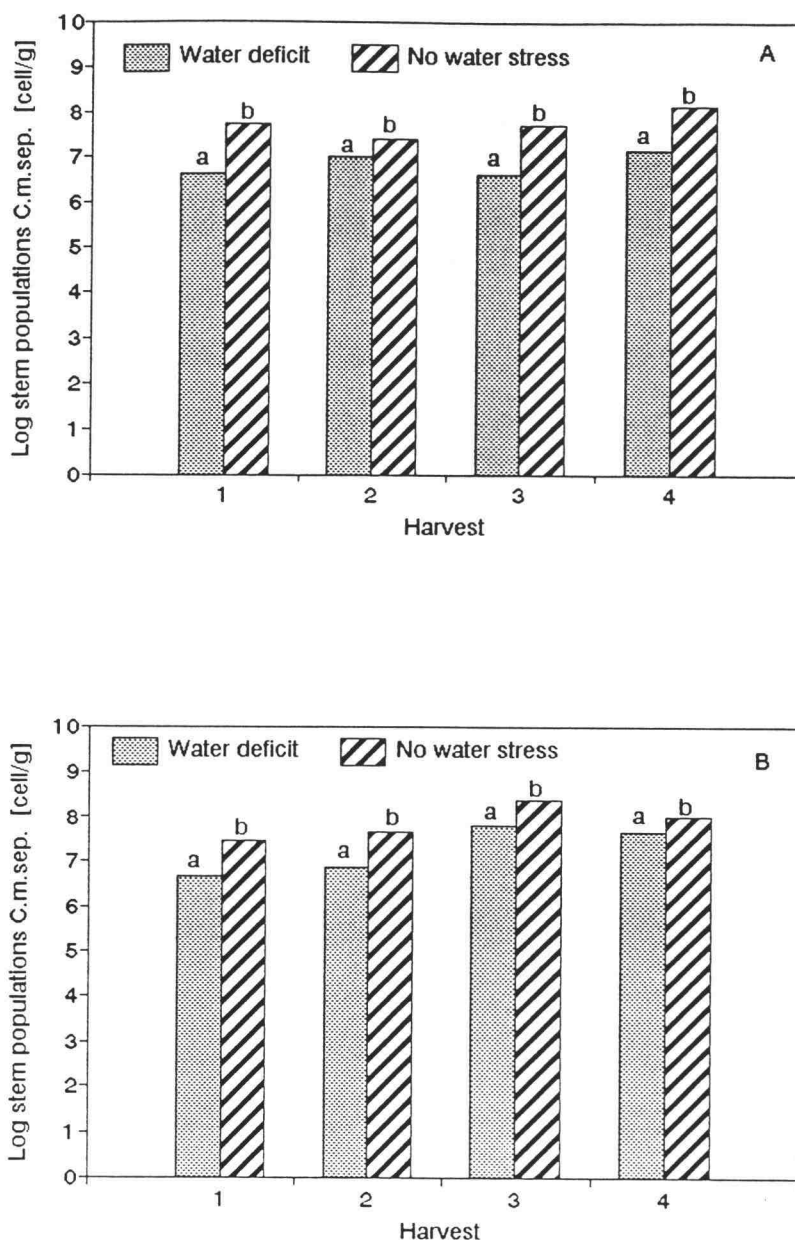
**Figure 15.** Effect of water deficit stress on stem population size of <u>Clavibacter michiganensis</u> subsp. <u>sepedonicus</u> in stems of Russet Burbank potatoes on four harvest dates in **A**) experiment 1 and **B**) experiment 2. Within harvest date, bars with different letters are significantly different 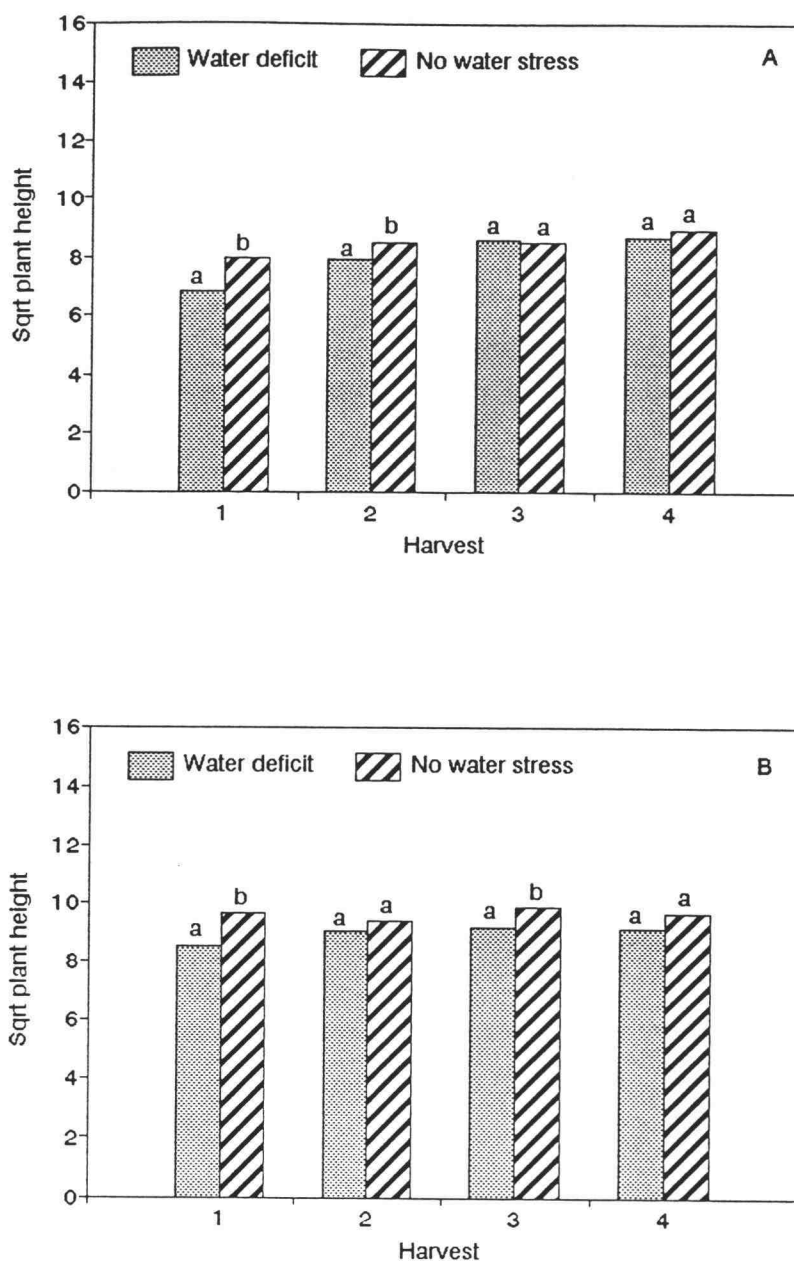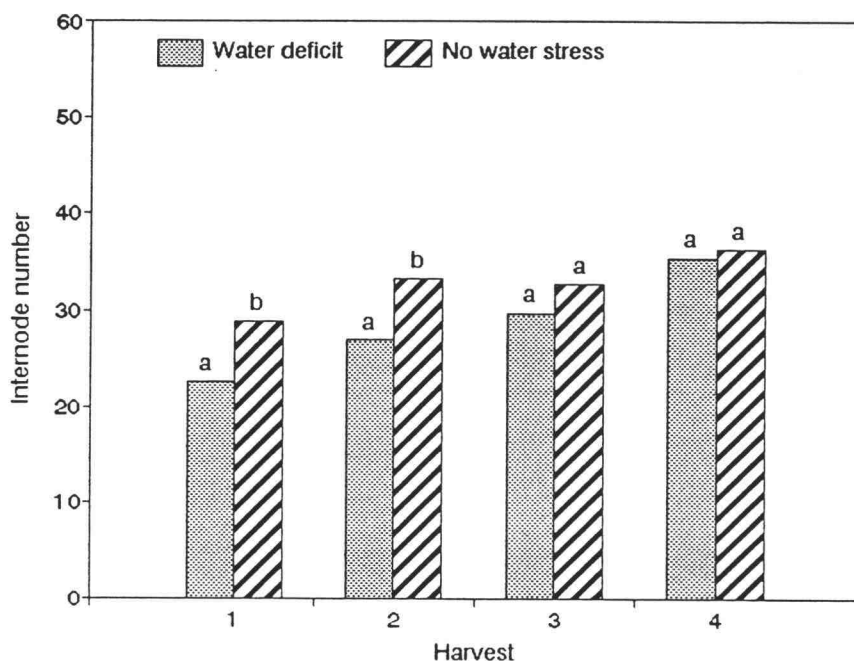($\un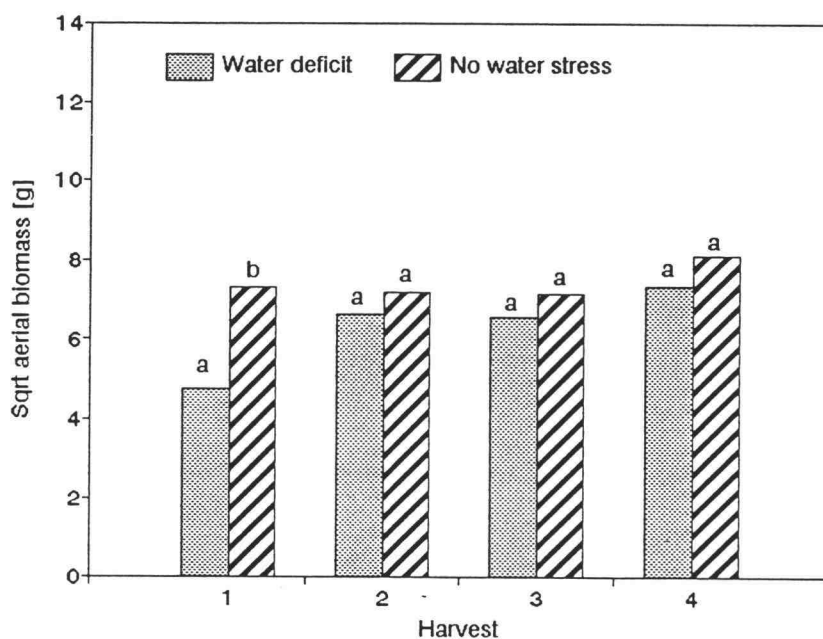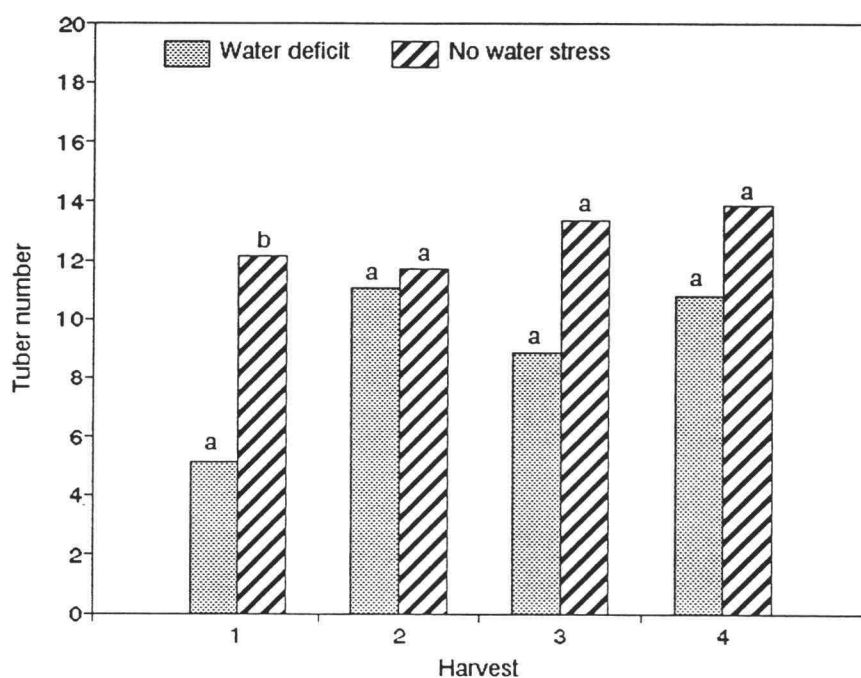derline{P} \leq 0.05$) according to Fisher's Protected LSD test. Water deficit was imposed by termination of irrigation at peak flowering. Plants were rewatered when leaf water potential was $\leq$-1.4 MPa. Harvest began 1 wk or 2 wk following termination of water deficit in experiments 1 and 2, respectively.

DISCUSSION

Since the establishment of the zero-tolerance
regulation for bacterial ring rot of potato (Shepard and
Claflin) prior to 1975 there has been increased research
activity with <u>Clavibacter michiganensis</u> subsp. <u>sepedonicus</u>
and its pathogenic relationship with potato, <u>Solanum
tuberosum</u>. The conditions under which <u>C</u>. <u>m</u>. <u>sepedonicus</u>
populations increase and translocate <u>in planta</u>, and the
mechanism by which symptom expression is initiated remain
poorly understood. This understanding is important if we
are to succeed in eradicating this disease from North
America. One factor that has been suggested to affect the
development of vascular wilt diseases is water.

Results of the present study demonstrate that both
inoculum of <u>C</u>. <u>m</u>. <u>sepedonicus</u> and abiotically induced
water deficit stress suppressed foliar growth and tuber
yield of potato under greenhouse conditions; however,
these two treatments did not interact to affect these
measured parameters. With vascular wilts such as those
caused by <u>C</u>. <u>m</u>. <u>sepedonicus</u> (Bishop and Slack, 1992), <u>C</u>.
<u>m</u>. <u>michiganensis</u> (Benhamou, 1991; van den Bulk, 1991),
<u>Fusarium oxysporum</u> (Beckman, 1987; Beckman et al, 1989),
<u>Pseudomonas solanacearum</u> (Beckman et al, 1962; Buddenhagen
and Kelman, 1964), and <u>Verticillium dahliae</u> (Havercourt et
al, 1990), water availability can be decreased

sufficiently by the pathogen to cause stomatal closure and the resulting reduced leaf and vine expansion (Curwen, 1993). The result is that yield is limited in the same manner as would be caused by low water availability in the soil. Bishop and Slack (1992) have shown that infection of potato with C. m. sepedonicus causes vascular occlusion which reduces xylem flow. This reduction in water flow creates a water deficit stress within the plant which, when severe, results in wilting of the foliage (Bishop and Slack, 1992; Dey and van Alfen, 1979).

In our study, however, no significant change in leaf water pressure was detected due to infection by C. m. sepedonicus. Nevertheless, reductions in foliar growth and tuber yield did occur among the inoculated plants. Subtle changes in moisture level due to vascular presence of C. m. sepedonicus can be sufficient to trigger stomatal closure, especially under greenhouse conditions. Davies (1977) indicated that stomatal sensitivity is greater for greenhouse than field grown plants causing greenhouse plants to suffer reduced photosynthesis at higher leaf water pressures. Stomatal closure in potato is associated with reduced photosynthesis with or without a reduction in carbon dioxide fixing enzymes (Moorby et al, 1975; Ackerson et al, 1977). Stomatal closure and reduced photosynthesis are then followed by reduced leaf expansion, vine growth, and tuber bulking (Curwen, 1993).

We suggest that this process occurred in our study to an extent great enough to initiate stomatal closure and thereby affect plant growth, but not sufficiently to cause a decrease in leaf water pressure beyond incipient plasmolysis which would be detectable with a pressure chamber (Krug and Wiese, 1972).

The soil water pressure at which potato leaf stomates close and transpiration becomes limiting to growth was -0.08 MPa in the field studies of Campbell et al (1976). Our soil water pressure curve during the drought episodes (Fig. 16) correlates well with that of Campbell et al (1976). Based on this combined information, we observed the point at which the stomates began to close to be approximately -0.077 MPa on the eleventh day of water deficit stress in both experiments. This point coincided with a sudden decrease in the rate of moisture removal from the soil.

Decrease in leaf water pressures in our study (Fig. 17) followed a pattern similar to those of Campbell et al (1976). However, in their study stomatal closure and transpiration reduction began at a leaf water pressure of -0.34 MPa on day 4 of the drought, differing considerably from the corresponding leaf water pressure of -0.73 MPa on day 11 of our drought period. This difference may be due to the difference in the composition and structure of our mix and the field soil of the Campbell (1976) study.
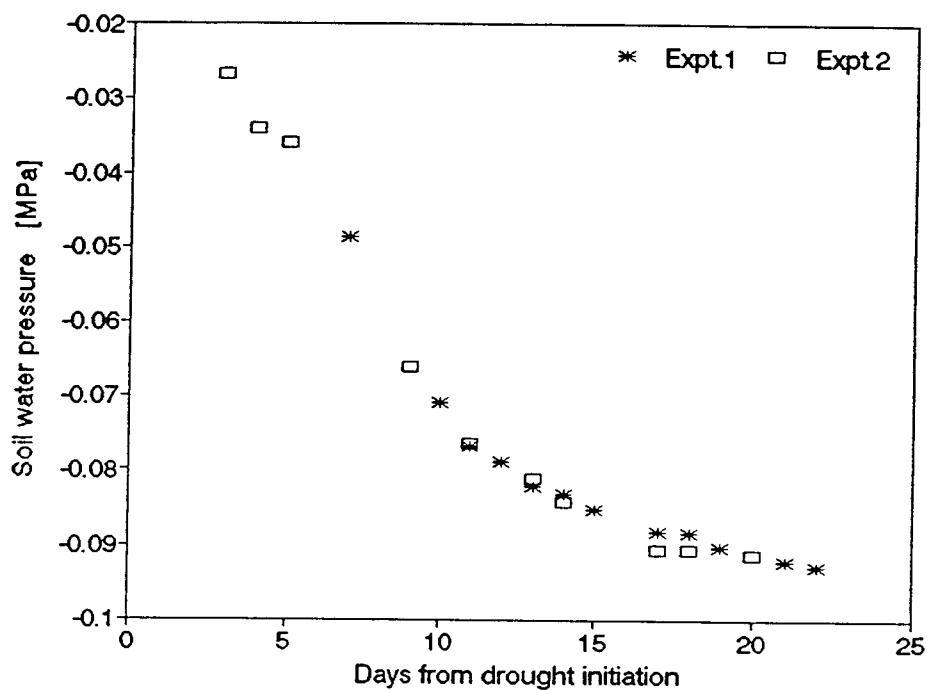
**Figure 16.** Soil water pressure as measured through the water deficit stress period in experiments 1 and 2 . The point of stomatal closure was estimated to occur at -0.077 MPa which coincided with day 11 of water deficit stress period in both experiments. The duration of the single occurance drought was 22 days.
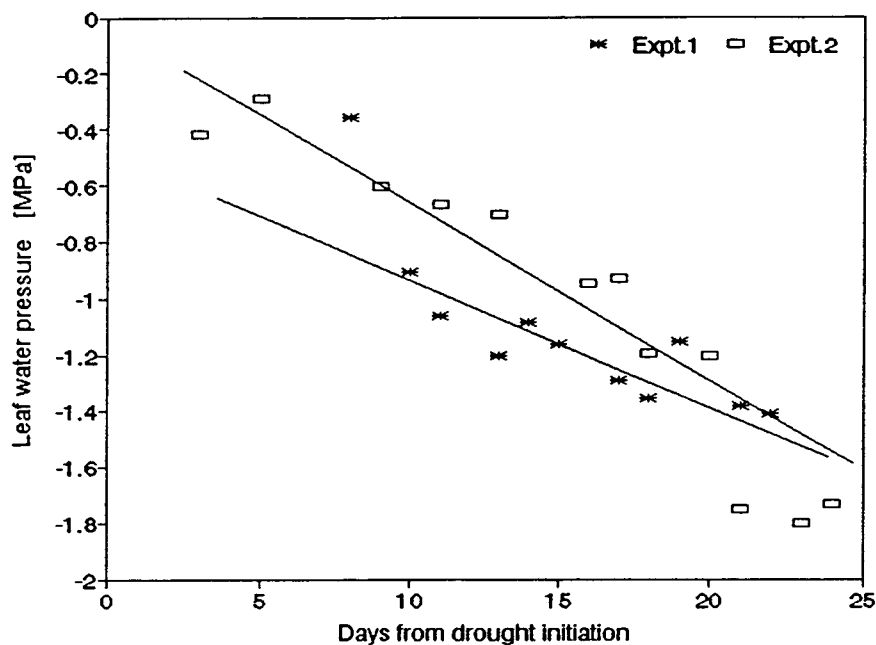
**Figure 17.** Leaf water pressures of Russet Burbank
potatoes obtained with a Scholander pressure
chamber throughout the water deficit stress
periods of experiments 1 and 2. Median lines
drawn by eye.

This same effect can be expected to result from

infection by $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$. If the pathogen

population size becomes large and vascular occlusions

become sufficiently dense transpiration flow is reduced

(Gardner et al, 1983), the plant would be expected to

respond with stomatal closure and foliar wilt as when soil

moisture is low.

Tubers were the only plant organs which developed

symptoms of bacterial ring rot. Incidence of tubers with

symptoms of bacterial ring rot after a 6 mo storage
increased progressively from the first to the fourth
harvest. The lack of pathogen induced foliar wilt and
chlorosis in the non-drought plants is most likely due to
the low ($\leq 4$ X $10^8$ cells/g tissue) pathogen populations in
the stems and/or slowed transport through the xylem by the
high greenhouse humidity (50-80%). It is possible for
symptomless stems to support bacterial populations up to
$10^9$ cfu/g tissue (Bishop and Slack, 1982; DeBoer and Slack,
1984).

After the culmination of the drought, the abiotically
stressed plants, began abundant growth of the lateral
axillary buds. This removal of apical dominance and burst
of axillary bud growth is typical of stressed plants when
the causal stress is relieved (Beckman, 1987) and could be
due to an increase of internal cytokinin concentrations.
An increase in cytokininswould also account for the darker
green color of the water deficit stressed leaves. The
reduced leaves of the droughted plants had fully expanded
by the third and fourth harvests. This was expected in
light of the research of Krug and Wiese (1972) which
showed that potato plants, after being droughted then well
watered, would have a higher foliage weight than plants
which had received sufficient water continually.

In contrast, symptoms of reduced tuber number and
yield persisted in inoculated-droughted plants, as did the

ten-fold difference between water stressed and water non-stressed stem populations of C. m. sepedonicus even after the abiotically induced water stress was alleviated. Pathogen population differences and yield reductions are likely due to the presence of xylem occlusions which would maintain the slight water stress and thereby continue to maintain reduced photosynthesis and storage carbohydrate production. Since the period of tuber initiation occured during the duration of the water deficit stress few new tubers would have been initiated post-drought thereby maintaining the previously reduced tuber quantity.

Of equal importance was the 10 fold decrease in stem populations of C. m. sepedonicus in water deficit stressed compared to well watered plants (Fig. 14). This pattern persisted for seven of the eight harvests dispite the alleviation of the drought. Samples were not collected prior to termination of the water deficit stress, therefore it is not known to what degree stem populations were supressed during the water stress. However, the effect of the water deficit stress was to reduce the rate of increase in population size and/or decrease the dispersal of the pathogen in the stems. Eventhough there was no significant interaction between inoculum and water treatment, there was definitely a very significant biological interaction between the two. Therefore we

suggest that a mild water deficit stress may be a contributing factor in the persistence of latent infections of C. m. sepedonicus. The findings of this research suggest that latent infections of bacterial ring rot may be related to a reduction in transpiration under drought conditions. Efforts to confirm the relevance of abiotically induced water stress on development of potatoes under field conditions is warranted.

In our study the abiotically induced water deficit stress significantly decreased leaf water pressure whereas C. m. sepedonicus had no significant effect on this parameter. This dissimilarity of the effect of drought and inoculum on leaf water pressure, however, may be true more often for potato plants with latent infections of C. m. sepedonicus than symptomatic plants. In addition there was no interaction between inoculum and water to exacerbate the effect of the pathogen on leaf water pressure. Drought and inoculum had similar effects on growth parameters of potato immediately following termination of the water deficit, however this similarity did not persist through the subsequent harvests.

Abiotically induced drought symptoms in the foliage and classic ring rot symptoms are externally very similar, consisting of leaf edge roll and wilting of first the lower leaves which progresses to all the leaves in a

basipetal direction, and eventually even the stems become flaccid. During the wilting phase of symptom development ring rot may differ from abiotic drought by producing some unilateral wilting of leaves or stems because of uneven plugging within the vascular bundles. Lower leaves may become partially chlorotic (early senescence) just prior to or after initiation of wilt and eventually necrotic. The absence of foliar drought symptoms in infected plants in this study may be due to the relatively low pathogen population within the stems (Nelson, 1982; DeBoer and McCann, 1990), reduced pathogen transport throughout the xylem, and fertility levels (Easton, 1979). Population size of $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$ in stem tissue samples never exceeded 4 X $10^8$ cfu/g (Fig. 16). These populations may never have become great enough to cause the dense vascular plugs (Gardner et al, 1983) which would reproduce foliar drought conditions, nor were the populations large enough to result in other disease symptoms. The chlorotic response to pathogenesis is related to the effect of reduced transpiration flow and leaf water pressure on photosynthesis and scenescence. Phytotoxic glycopeptides (Rai and Strobel, 1969b; Reis and Strobel, 1972b; Strobel, 1970; Strobel and Hess, 1968) may also be involved in wilt and reduction of photosynthesis. Because plant water stress is the basis for the observed chlorotic symptoms, leaf edge roll and necrosis follow due to lack of water.

Bishop and Slack (1982; DeBoer and Slack, 1984) suggested that population size of less than $10^9$ cfu/g tissue is within the range of latent infections of C. m. sepedonicus. Average stem populations for infected plants exibiting foliar symptoms have not been published (Westra, personal communication, 1994). Under conditions of high relative humidity the rate of transpiration is reduced. Within the greenhouse the relative humidity ranged from 50 to 80% (sling psychrometer readings). This may have been sufficient to affect the transpiration flow and thereby the upward movement of the Clavibacter cells to locations where xylem blockages could be formed, ie. narrow diameter xylem vessels of petioles and leaves. This also could have slowed bacterial replication. Finally, Easton (1979) noted that high levels of fertilizers (Osmocote and Esmigran, Sierra Chemical Co.), especially nitrogen, suppressed or masked symptoms of bacterial ring rot. With the slow release N,P,K and micronutrient fertilizers used in our study it is probable that nitrogen was never limiting, although no plant tissue or soil nutrient analyses were performed.

Incipient plasmolysis in leaves of field grown herbaceous crops occurs in the range of -1.3 to -1.6 MPa (Bradford and Hsiao, 1982). Therefore, the value -1.4 MPa was chosen as a reasonable point at which to terminate the drought treatment. However, the rate at which the water

stress increased varied widely among experimental units.
Gander and Tanner (1976) also had difficulty with this
situation. Havercourt et al (1990) indicated that this
variation was the result of soil water loss at different
rates proportional to the leaf area of the plants. It,
therefore, became necessary to terminate the water deficit
stress when only half of the experimental units within the
water stress treatment had reached the target leaf water
pressure of -1.4 MPa or less.

Because vascular plug formation is a fairly non-
specific defense response by the host to foreign material
in the xylem (Gardner et al., 1983), it was suspected that
a drought stress might both decrease the available water
for uptake in the partially blocked vessels and weaken
host defenses via reduced photosynthesis and reduced
nutrient uptake. The end result would be an increase in
disease severity. However, the reduced water availability
appears to have decreased the pathogens rate of
replication and dispersal within the host so that the
water restricting blockages were not formed well enough to
create drought within the infected but well watered
plants.

**Conclusions.** The results of this research suggest
two general conclusions. First, abiotic water deficit
stress supresses vascular populations of $\underline{C}$. $\underline{m}$. $\underline{sepedonicus}$
in potato plants, thereby enhancing the occurrance of

latent infections.  Second, infection of potato with C. m.

sepedonicus causes water deficit within the host plants

[proportional to population density within the stem

tissue] which reduces plant growth  parameters in the same

manner as abiotic water deficit stress, particularly

aerial biomass, tuber number and tuber yield.

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APPENDICES

## APPENDIX I

## ELISA


Populations of C. m. sepedonicus were determined using two different serological methods, indirect immuno-fluorescent antibody staining (IFAS) and enzyme linked immunosorbent assay (ELISA). All inoculated plants and three to five of the noninoculated plants from each harvest date were sampled. The lower most 7.6 cm of main stem was romoved and split into upper and lower halves, each 3.8 cm in length. The lower segments were refrigerated overnight and shipped the following day via Federal Express overnight service to Steve Slack at Cornell University for the ELISA evaluation. These samples for ELISA were frozen at 0 C upon arrival at Cornell. The upper 3.8 cm segments for IFAS were also frozen at 0 C until processing at Oregon State University.

The ELISA procedure was performed using the Agdia Reagent Set with alkaline phosphatase labled conjugates. Eight 96 well ELISA plates were coated with dilute IgG in coating buffer, 100 ul/well incubated at room temp for 4 h or overnight at 4 C in a humid chamber. Following incubation the coating solution was removed from the wells and the plate was rinsed 3-4 times with PBS buffer plus Tween-20 (PBST). One gram samples were pressed to extract

the sap which was diluted 1:10 in extraction buffer. A

100 ul aliquot of the 1:10 dilution was added to the wells

in duplicate for each sample and incubated 16 h at 4-10 C

in a humid chamber. The standard curve culture dilutions

were treated in the same manner. The plate was washed as

before. The substrate solution was then made by adding

p-nitrophenylphosphate (PNP) to the substrate buffer at

1 mg/ml, and adding 200 ul to each well. This was then

incubated 30-60 min in the dark at room temperature for

color development. Plates were ready for reading when the

positive controls were yellow-green and the negitive

controls were still colorless to very slightly colored. A

50 ul aliquot of 3M sodium hydroxide was then added to

stop the reaction. The optical density was measured at

405nm.

# APPENDIX II

## Tensiometer, Tensimeter™

Tensiometers (Fig. 18A) were specially constructed after Merthaler et al (1973) for use with a portable pressure transducer (Fig. 18B). Tensiometers were constructed from 50 cm lengths of 1.2 cm diameter PVC 1120 (schedule 80, 850 psi) irrigation pipe with an 8 cm piece of plexiglass tubing cemented into one end so that 6 cm extended from the PVC for a water level viewing during use, and a -0.1 MPa capacity porus ceramic cup cemented into the opposite end for attaining water pressure equilibrium between tensiometer and soil (approximately 3-5 days). Once the body of the tensiometer was filled to 1 cm from the top with deaerated water septum stoppers were inserted into the plexiglass tubing to form an air tight seal. These stoppers are designed to maintain an air tight seal during and following the insertion and removal of a hypodermic needle through the stopper. An extra precaution was taken by filling the central space in the interior of the stopper with silicone to give further protection against air leakage during the measuring process. These tensiometers were refilled with a syringe as the water level deminished in the viewing tube and allowed to re-equilibrate with the soil. The size of the air space normally varied with time, but was maintained at

$\leq 2$ cm$^3$ to prevent error in measurement.

A Tensimeter™ (Soil Measurment Systems, Tucson, AZ) was used for soil water pressure readings. The Tensimeter™ measures the air pressure in the upper end of the plexiglass tubing by inserting a syringe needle attached to a pressure transducer through the septum. A guide tube keeps the transducer system (Fig. 18B) in a vertical position when placed on the tensiometer and centers the needle in the septum (Fig. 18A). The inside diameter fits the outside diameter of the stopper while secured in the clear tubing. A spring guarantees the smooth insertion of the needle into the septum as it is pushed downward. The transducer consists of a steel compartment with a transducer membrane separating the inner space into an upper and lower chamber.

The upper chamber is at atmospheric pressure. Through the syringe needle the air pressure in the lower chamber equilibrates with the pressure in the plexiglass tube causing a small deflection of the membrane. This deflection changes the resistance of silicon simiconductors embeded into the membrane by diffusion. A shielded four-lead wire connects the silicon element with a resistivity meter with a constant current source of 800 uA DC. The pocket sized resistivity meter with liquid crystal display, zero adjustment and adjustable range may be calibrated to read directly in millibars (0.0001 MPa)

or centimeters of water (Marthaler et al, 1983). If the hypodermic needle becomes wet during insertion the output reading will be drastically different from the true value, therefore this must be avoided (W. Austin, 1993, personal communication).

Tensiometers (-0.1 MPa capacity cups) were placed into the pots to a depth of 38 cm for measuring the capillary tension of the soil water (Richards and Gardner, 1936). Measurements were collected 4-5 times weekly after onset of the drought treatment. As the drying of the soil began registering near logarithmic decreases in water potential, daily measurements were made.

Measurement involved the following steps:

1) Adjust the Tensimeter™ to read 0 mbar pressure.

2) Insert the needle through the stopper vertically into the air pocket, avoiding the water surface.

3) Read the resistivity meter.

4) Remove the needle from the tensiometer slowly to assure resealing.

5) Calculate the soil water tension by subtracting the stem length of the tensiometer from the reading on the resistivity meter.

At 11 days into the drought the rate of decrease in soil water pressures slowed and the curve began to level off (Fig. 16) due to the physiological closing of the

stomates. The tension in many of the tensiometers began to exceed the capacity of the ceramic cups soon thereafter, resulting in loss of vacuum prior to the time when the leaf water potential began to indicate any water stress in the plants. Tensiometers with at least -0.2 MPa capacity cups should have been used.
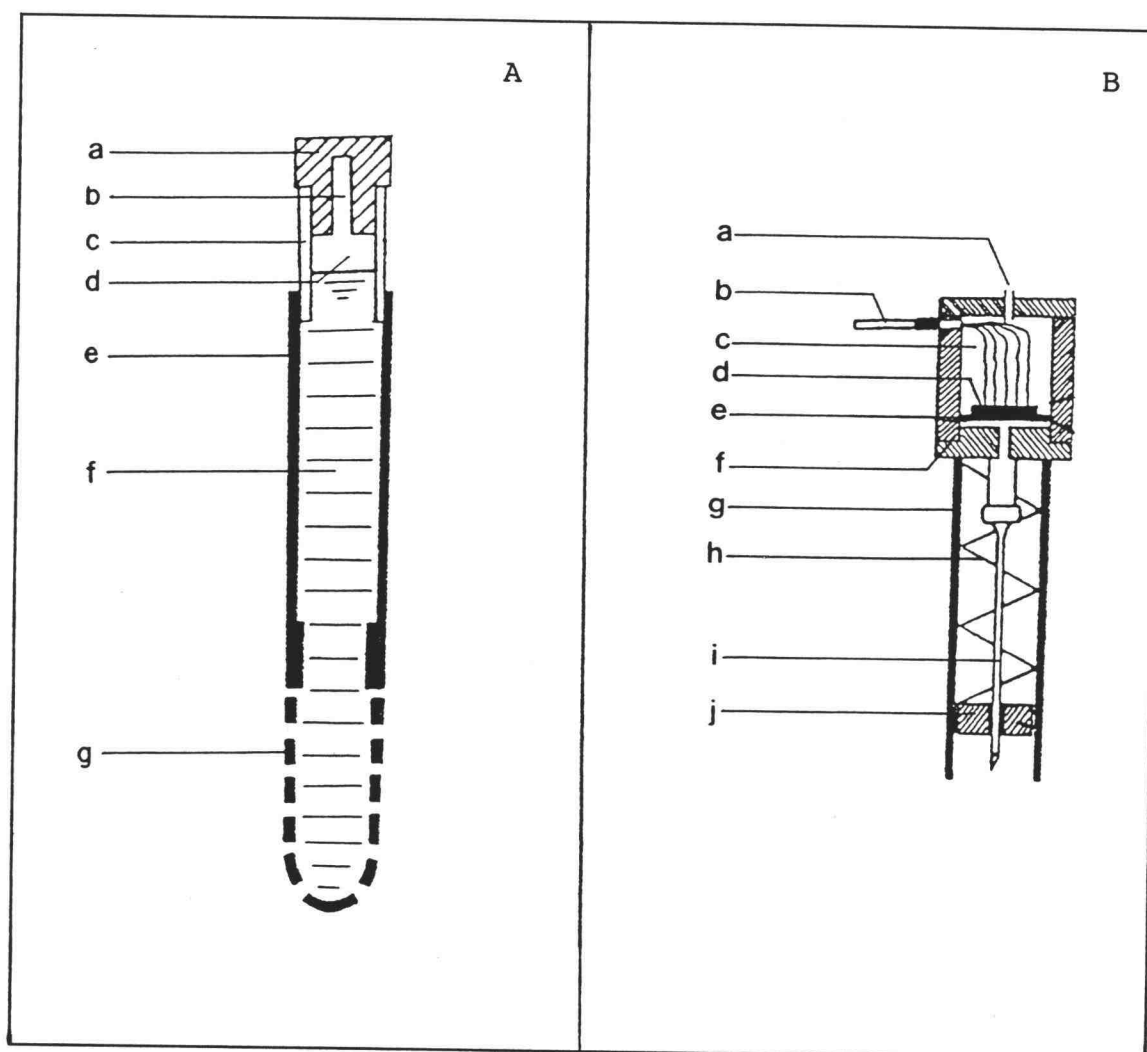
**Figure 18.** Sections of tensiometer and Tensimeter™. A) Longitudinal view of tensiometer; a) nitril-caoutchouc septum stopper, b) silicone filling, c) plexiglass viewing tube (lucid), d) air pocket for end of syringe needle, e) opaque pvc irrigation pipe, f) deaerated water, g) porus ceramic cup. B) Longitudinal view of transducer system; a) air vent, b) shielded four lead wire, c) space at atmosphaeric pressure, d) semiconductor element, e) steel membrane, D = 13mm, f) aluminum container at atmospheric pressure, g) guiding tube, h) guiding spring, i) syringe needle, D = 0.4mm, j) guiding disc for needle.

APPENDIX III.

Scholander Pressure Chamber


Since the work of Scholander (1964, 1965) and Boyer
(1969), measurment of plant water pressure by the pressure
bomb method has been used with increasing frequency.
During this study a Scholander pressure bomb, Plant Water
Status Console 3000 series (Soilmoisture Equipment Corp.,
Santa Barbara, CA) (Fig. 19), was used to determine the
leaf water potential of individual potato leaves.  One
leaf was removed and trimmed to the terminal three or five
leaflets with a razor blade to insure a smooth surface
(where the rachis was both large enough and cylindrical
enough to allow formation of a seal between rachis and lid
O-ring without breaking the cell walls), and sealed it
immediately inside the pressure vessel (Fig. 20).  The
seal was accomplished with one O-ring surrounding the
sample rachis that is pressed into place with a camlock,
and another O-ring between the lid and the inside of the
chamber.  Pressurized nitrogen gas (Fig. 18) was then
slowly released into the cylindrical stainless steel
pressure vessel until the pressure inside the vessel
counter balanced the negative pressure within the leaf.
This equilibrium point was reached when the xylem sap
crowned at the cut surface of the xylem vessels of the
rachis.  This was observed using a bright light fixed

directly above the pressure cylinder for reflection and a hand held magnifying glass while the pressure was being increased. Bars were read directly from the gauge and converted to MPa. The pressure was then released from the pressure vessel and the spent sample removed from the sample holder to ready the pressure bomb for the next sample.
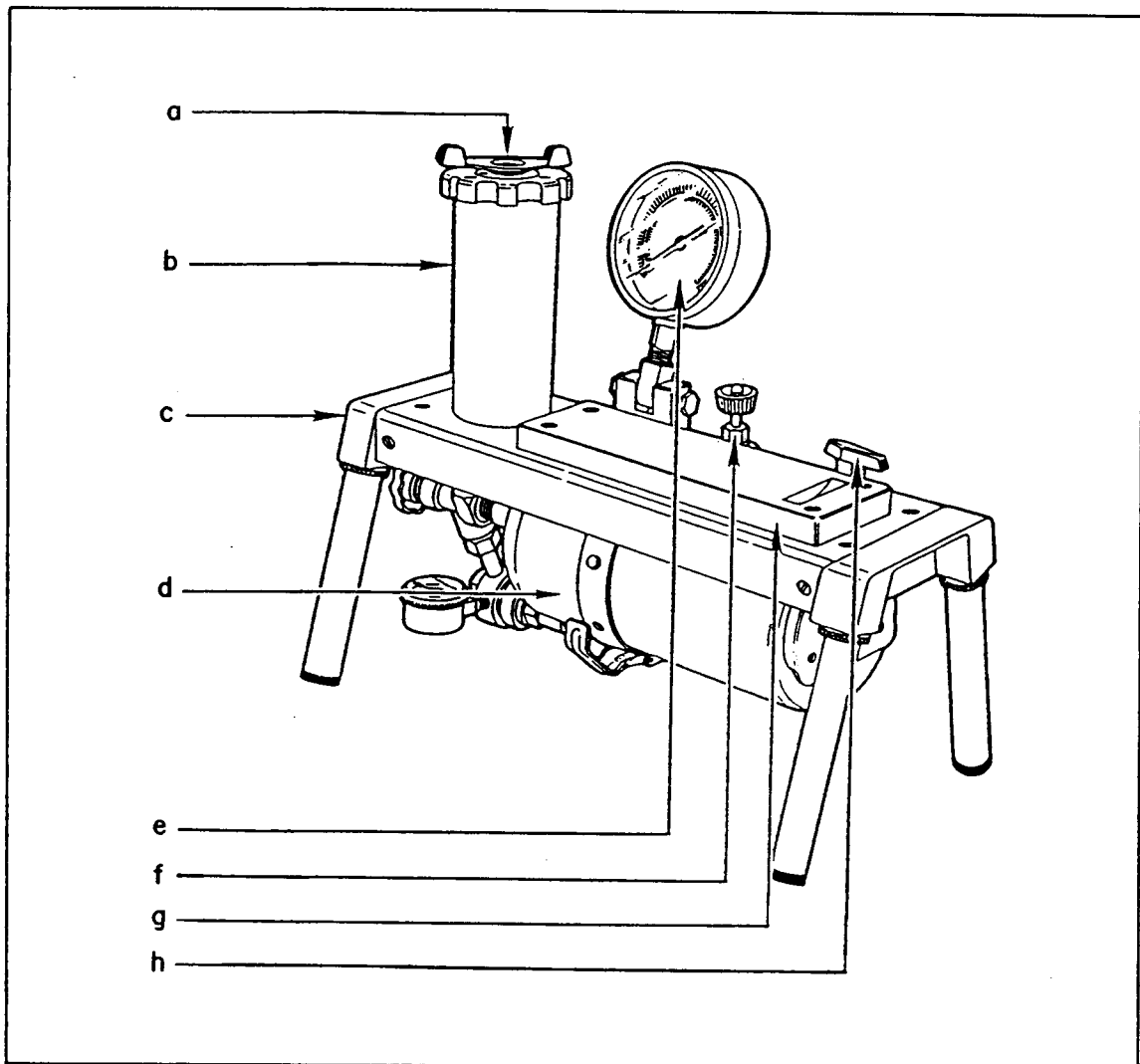
**Figure 19.** Exterior view of Plant Water Status Console™. a) Camlock specimen holder, b) stainless steel pressure vessel, 7.6 cm diameter X 15.2 cm depth, c) aluminum chassis for bench operation, d) refillable 25 ft³ (= 762cc) capacity high pressure nitrogen supply tank attached beneath the chassis, with output pressure regulator and internal plumbing, e) 11.4 cm dial pressure test guage graduated in bars of pressure and psi, f) fine adjustment metering valve for control of pressure increase within the vessel, g) hardwood sample preparation and sample loading board, h) threeway pressure control valve with "pressurize", "exhaust", and "off" positions.
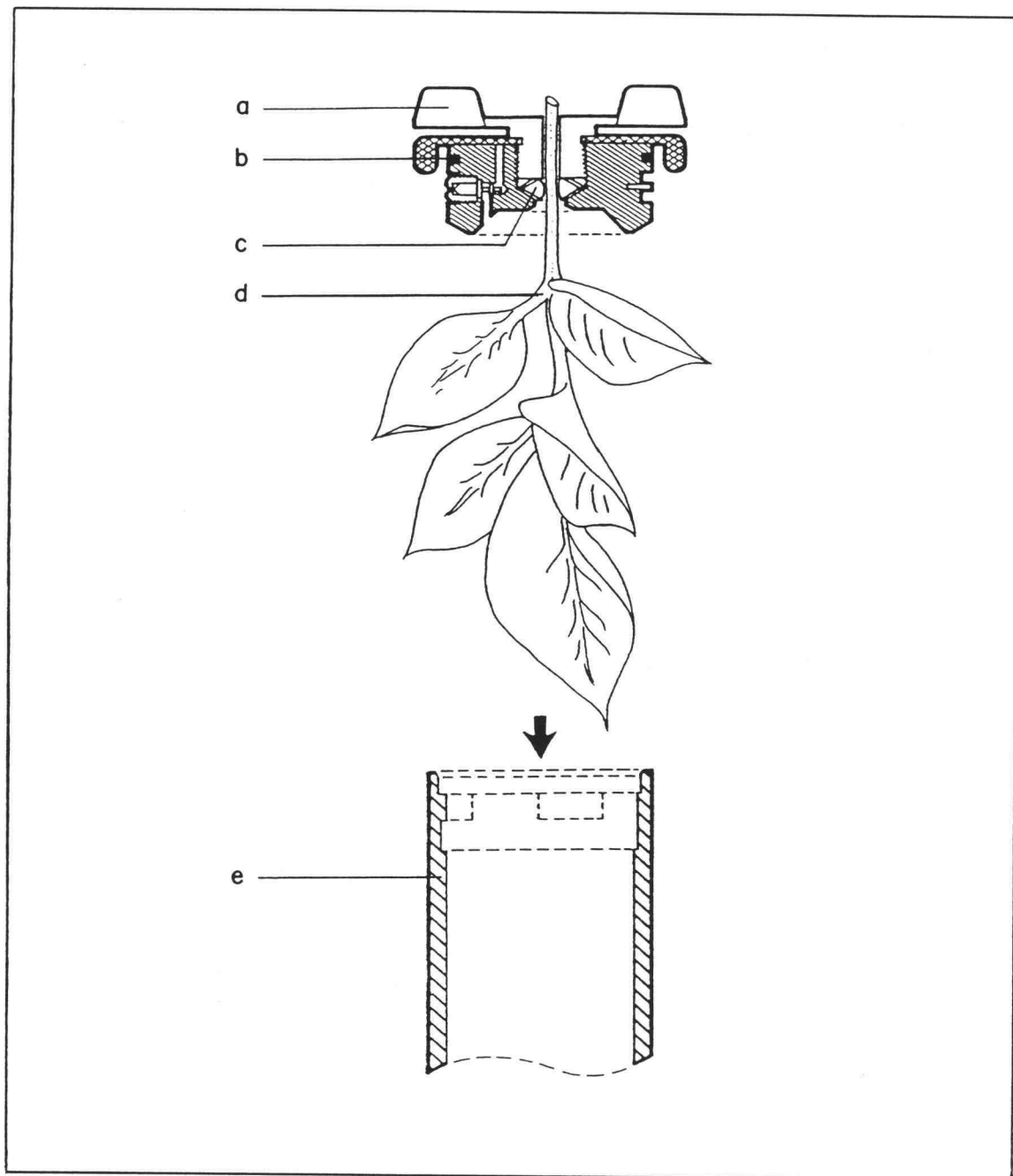
**Figure 20.** Sectional view of specimen holder and pressure chamber. a) sealing mechanism of sample holder/lid, b) outer O-ring, c) inner O-ring, d) mounted potato leaf, e) longitudinal section of pressure vessel.

APPENDIX IV.

**Table 1.** Effect of water deficit stress and <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> treatments on plant height, number of branches, number of internodes, aerial biomass, number and yield of tubers of Russet Burbank potatoes at four sampling dates for the first experiment. (with square root transformations as indicated to create normal distributions so that the assumptions can be made)

| Treatment | n= | Sqrt. Plant Height, cm. | Number of Branches | Number of Internodes | Sqrt. Aerial Biomass, g. | Tuber Number | Sqrt. Tuber Yield, g. |
|---|---|---|---|---|---|---|---|
| | | Experiment 1, Harvest 1 | | | | | |
| Drought | 4 | 6.84 | 8.07 | 22.61 | 4.87 | 5.14 | 4.67 |
| Water | 14 | 7.98 | 13.07 | 28.86 | 7.3 | 12.14 | 13.35 |
| *= Sig. diff. | | * | | * | * | * | * |
| Inoculated | 9 | 7.17 | 9.61 | 24.55 | 5.03 | 6.28 | 7.62 |
| Noninoculated | 9 | 7.65 | 11.54 | 26.91 | 6.99 | 11 | 10.4 |
| *= Sig. diff. | | | | | * | * | |
| | | Experiment 1, Harvest 2 | | | | | |
| Drought | 6 | 7.93 | 12.76 | 26.92 | 6.64 | 11.08 | 9.43 |
| Water | 14 | 8.51 | 14.79 | 33.14 | 7.21 | 11.71 | 14.16 |
| *= Sig. diff. | | * | | * | | | * |
| Inoculated | 10 | 8 | 11.36 | 28.42 | 6.06 | 9.92 | 9.67 |
| Noninoculated | 10 | 8.44 | 16.19 | 31.64 | 7.83 | 12.87 | 13.93 |
| *= Sig. diff. | | * | * | * | * | | * |
| | | Experiment 1, Harvest 3 | | | | | |
| Drought | 2 | 8.58 | 15.43 | 29.64 | 6.56 | 8.86 | 8.05 |
| Water | 14 | 8.51 | 15.93 | 32.64 | 7.15 | 13.36 | 16.94 |
| *= Sig. diff. | | | | | | | |
| Inoculated | 8 | 8.26 | 12.61 | 29.32 | 5.37 | 8.43 | 7.68 |
| Noninoculated | 8 | 8.83 | 18.75 | 32.96 | 8.49 | 13.79 | 17.32 |
| *= Sig. diff. | | | | * | * | * | * |
| | | Experiment 1, Harvest 4 | | | | | |
| Drought | 6 | 8.68 | 17.02 | 35.33 | 7.36 | 10.82 | 15.64 |
| Water | 16 | 8.92 | 17.19 | 36.19 | 8.11 | 13.88 | 22.37 |
| *= Sig. diff. | | | | | | | * |
| Inoculated | 11 | 8.62 | 15.28 | 34.82 | 6.84 | 8.71 | 16 |
| Noninoculated | 11 | 8.98 | 18.93 | 36.69 | 8.7 | 15.99 | 22.01 |
| *= Sig. diff. | | | | | | * | * |

* denotes significant difference, $P < 0.05$ according to Fishers Protected LSD

APPENDIX V.

**Table 2.** Effect of water deficit stress and <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> treatments on plant height, number of branches, number of internodes, aerial biomass, number and yield of tubers of Russet Burbank potatoes at four sampling dates for the second experiment. (with square root transformations as indicated to create normal distributions so that the assumptions can be made)

| Treatment | n= | Sqrt. Plant Height, cm. | Number of Branches | Number of Internodes | Sqrt. Aerial Biomass, g. | Tuber Number | Sqrt. Tuber Yield, g. |
|---|---|---|---|---|---|---|---|
| Experiment 2, Harvest 1 | | | | | | | |
| Drought | 6 | 8.54 | 14.5 | 30.69 | 6.16 | 10.13 | 14.16 |
| Water | 10 | 9.67 | 11.41 | 34.23 | 6.74 | 6.35 | 13.19 |
| *= Sig. diff. | | * | | | | | |
| Inoculated | 8 | 9.06 | 11.44 | 32.19 | 5.63 | 6.75 | 11.34 |
| Noninoculated | 8 | 9.15 | 14.47 | 32.73 | 7.29 | 9.73 | 16.01 |
| *= Sig. diff. | | | | | | | |
| Experiment 2, Harvest 2 | | | | | | | |
| Drought | 10 | 9.03 | 17.84 | 33.36 | 8.05 | 11.59 | 17.06 |
| Water | 10 | 9.14 | 16.36 | 33.76 | 8.1 | 13.66 | 19.88 |
| *= Sig. diff. | | | | | | | |
| Inoculated | 10 | 9.12 | 13.11 | 33.08 | 6.92 | 10.1 | 15.31 |
| Noninoculated | 10 | 9.32 | 21.1 | 34.04 | 9.3 | 15.16 | 21.63 |
| *= Sig. diff. | | | * | | * | * | * |
| Experiment 2, Harvest 3 | | | | | | | |
| Drought | 12 | 9.18 | 20.51 | 35.25 | 8.76 | 13.38 | 21.57 |
| Water | 12 | 9.88 | 14.33 | 37.79 | 8.79 | 11.56 | 19.9 |
| *= Sig. diff. | | * | | | | | |
| Inoculated | 12 | 9.13 | 16.53 | 35.41 | 8.34 | 10.15 | 16.77 |
| Noninoculated | 12 | 9.74 | 18.3 | 37.63 | 9.22 | 14.79 | 24.71 |
| *= Sig. diff. | | | | | | * | * |
| Experiment 2, Harvest 4 | | | | | | | |
| Drought | 8 | 9.14 | 17.44 | 35.72 | 8.65 | 14.83 | 22.7 |
| Water | 12 | 9.67 | 18.19 | 38.47 | 9.29 | 13.58 | 27.21 |
| *= Sig. diff. | | | | | | | |
| Inoculated | 10 | 9.42 | 18.36 | 37.35 | 9.22 | 12.38 | 22.83 |
| Noninoculated | 10 | 9.38 | 17.28 | 36.85 | 8.71 | 16.04 | 27.09 |
| *= Sig. diff. | | | | | | | |

* denotes significant difference, <u>P</u>< 0.05 according to Fishers Protected LSD

APPENDIX VI.

IFAS Data Means

**Table 3.** Effect of drought on population size of
Clavibacter michiganensis subsp. sepedonicus (cells/g stem
tissue) in Russet Burbank potatoes at four sampling dates
following termination of drought.  A) expt. 1, B) expt. 2.
IFAS was performed on a 3.8cm section of lower stem tissue
to obtain these population data.

| A Treatment | Harvest 1 | Harvest 2 | Harvest 3 | Harvest 4 |
|---|---|---|---|---|
| Watered | $5.5 \times 10^7$ | $2.5 \times 10^7$ | $5.2 \times 10^7$ | $1.3 \times 10^8$ |
| Droughted | $4.3 \times 10^6$ | $1.0 \times 10^7$ | $4.2 \times 10^6$ | $1.4 \times 10^7$ |

| B Treatment | Harvest 1 | Harvest 2 | Harvest 3 | Harvest 4 |
|---|---|---|---|---|
| Watered | $2.9 \times 10^7$ | $4.5 \times 10^7$ | $2.4 \times 10^8$ | $1.0 \times 10^8$ |
| Droughted | $4.7 \times 10^6$ | $7.2 \times 10^6$ | $6.6 \times 10^7$ | $4.7 \times 10^7$ |

APPENDIX VII.

**Table 4.** Percent decrease in parameter measurments of Russet Burbank potatoes due to water deficit stress or <u>Clavibacter</u> <u>michiganensis</u> subsp. <u>sepedonicus</u> treatments over four harvest dates within experiments 1 and 2. A negative decrease indicates an increase in the parameter measurement.

| Parameter Measured | Harvest Number | % Decrease by Drought among: | | | | % Decrease by Inoculum among: | | | |
| | | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | |
| | | Inoc. | Noninoc. | Inoc. | Noninoc. | Drought | Water | Drought | Water |
| Number of Observations | 1 | n=9 | n=9 | n=8 | n=8 | n=4 | n=14 | n=6 | n=10 |
| | 2 | n=10 | n=10 | n=10 | n=10 | n=6 | n=14 | n=10 | n=10 |
| | 3 | n=8 | n=8 | n=12 | n=12 | n=2 | n=14 | n=12 | n=12 |
| | 4 | n=11 | n=11 | n=10 | n=10 | n=6 | n=16 | n=8 | n=12 |
| Sqrt. Plant Height | 1 | 8 | 7 | 6 | 6 | 3 | 3 | 1 | 0 |
| | 2 | 4 | 3 | 2 | 2 | 3 | 3 | 1 | 1 |
| | 3 | 0 | 0 | 4 | 4 | 3 | 3 | 2 | 2 |
| | 4 | 1 | 1 | 3 | 3 | 2 | 2 | 0 | 0 |
| Number of Branches | 1 | 22 | 20 | -12 | -11 | 10 | 8 | 10 | 12 |
| | 2 | 8 | 7 | -5 | -4 | 17 | 16 | 20 | 21 |
| | 3 | 2 | 1 | -17 | -16 | 18 | 18 | 5 | 5 |
| | 4 | 1 | 0 | 2 | 2 | 10 | 10 | -3 | -3 |
| Number of Internodes | 1 | 12 | 11 | 5 | 5 | 5 | 4 | 1 | 1 |
| | 2 | 10 | 10 | 1 | 1 | 5 | 5 | 1 | 1 |
| | 3 | 5 | 4 | 3 | 3 | 6 | 6 | 3 | 3 |
| | 4 | 1 | 1 | 4 | 4 | 3 | 3 | -1 | -1 |
| Sqrt. Aerial Biomass | 1 | 21 | 17 | 5 | 4 | 17 | 13 | 12 | 12 |
| | 2 | 4 | 4 | 0 | 0 | 12 | 12 | 14 | 14 |
| | 3 | 5 | 4 | 0 | 0 | 21 | 20 | 5 | 5 |
| | 4 | 5 | 5 | 3 | 4 | 12 | 11 | -3 | -3 |
| Tuber Number | 1 | 38 | 30 | -22 | -19 | 29 | 20 | 15 | 19 |
| | 2 | 3 | 3 | 9 | 7 | 12 | 12 | 19 | 18 |
| | 3 | 21 | 17 | -8 | -6 | 24 | 20 | 16 | 18 |
| | 4 | 14 | 10 | -5 | -4 | 27 | 24 | 12 | 12 |
| Sqrt. Tuber Yield | 1 | 41 | 37 | -4 | -3 | 18 | 12 | 16 | 16 |
| | 2 | 20 | 17 | 8 | 7 | 18 | 15 | 16 | 15 |
| | 3 | 36 | 26 | -4 | -4 | 38 | 28 | 17 | 18 |
| | 4 | 18 | 15 | 9 | 8 | 16 | 14 | 9 | 8 |
| Leaf Water Pressure | just prior to drought end | 70 | 67 | 42 | 68 | 2 | -7 | -51 | -13 |
| Stem Populations | 1 | 92 | | 84 | | | | | |
| | 2 | 60 | | 84 | | | | | |
| | 3 | 92 | | 73 | | | | | |
| | 4 | 89 | | 53 | | | | | |

APPENDIX VIII.
Scatter plots of Aerial Biomass data points
with respect to inoculum treatment
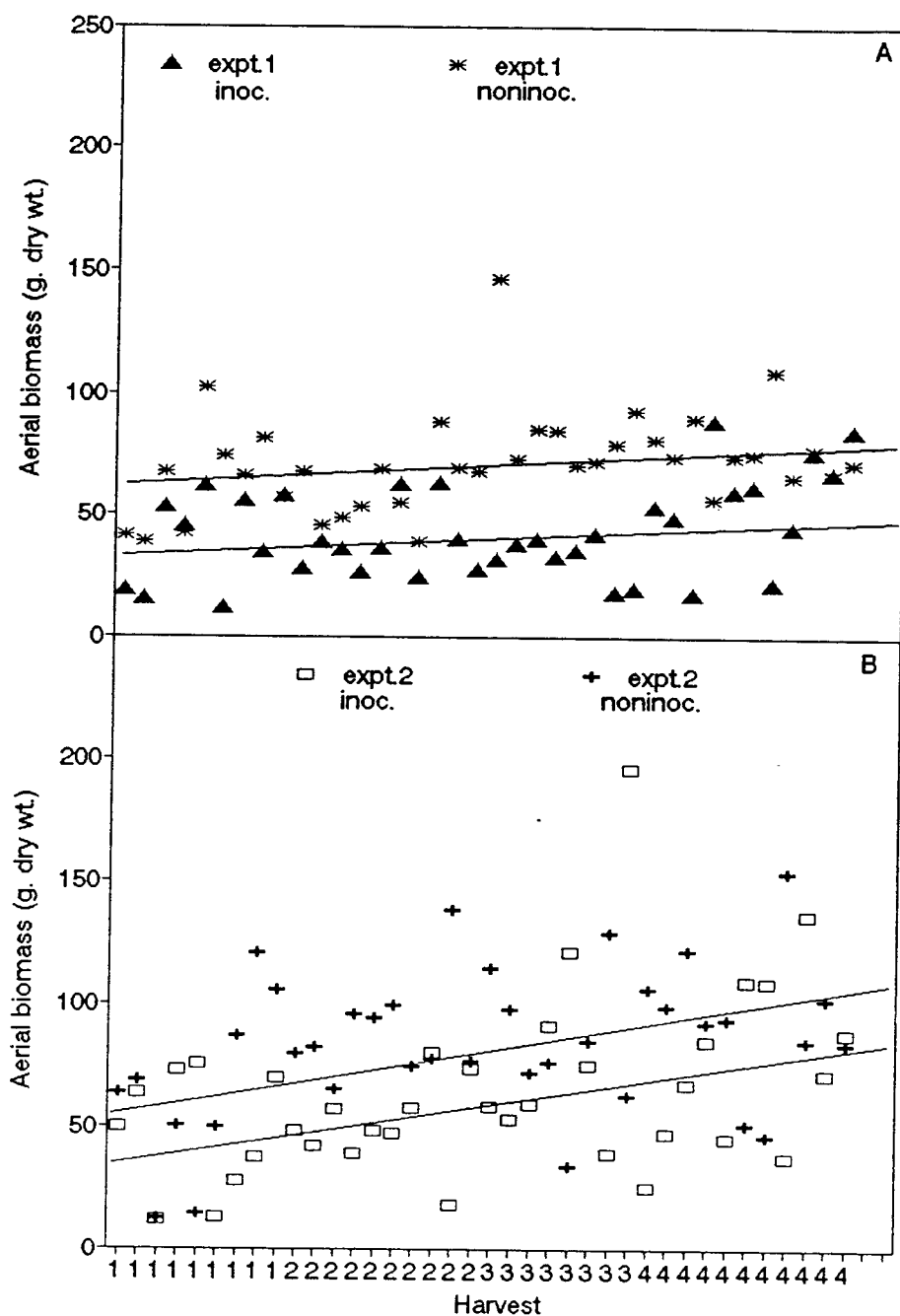


**Figure 21.** Scatter plots of aerial biomass data points
for Russet Burbank potatoes A) experiment 1
and B) experiment 2 over four harvest dates.
Median lines drawn by eye.

APPENDIX IX.
## Table 5. General Linear Model Summaries

| Experiment 1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
| 1 | Sqrt. Plant Height | Model | 9 | 4.9795 | 0.5532 | 2.59 | 0.0975 |
| | | Rep | 6 | 1.5672 | 0.2612 | 1.22 | 0.3854 |
| | | Wetness | 1 | 2.5761 | 2.5762 | 12.05 | 0.0084 |
| | | Inoculum | 1 | 0.7069 | 0.7069 | 3.31 | 0.1065 |
| | | Wet*Inoc | 1 | 0.0098 | 0.0098 | 0.05 | 0.8354 |
| | | Error | 8 | 1.7097 | 0.2137 | | |
| | Number of Branches | Model | 9 | 213.0714 | 23.6746 | 1.87 | 0.1958 |
| | | Rep | 6 | 100.4285 | 16.738 | 1.32 | 0.3484 |
| | | Wetness | 1 | 50 | 50 | 3.94 | 0.0823 |
| | | Inoculum | 1 | 11.5714 | 11.5714 | 0.91 | 0.3674 |
| | | Wet*Inoc | 1 | 13.3492 | 13.3492 | 1.05 | 0.3349 |
| | | Error | 8 | 101.4285 | 12.6785 | | |
| | Number of Internodes | Model | 9 | 160.1607 | 17.7956 | 4.47 | 0.0232 |
| | | Rep | 6 | 51.5892 | 8.5982 | 2.16 | 0.1548 |
| | | Wetness | 1 | 78.125 | 78.125 | 19.63 | 0.0022 |
| | | Inoculum | 1 | 17.2857 | 17.2857 | 4.34 | 0.0707 |
| | | Wet*Inoc | 1 | 1.2857 | 1.2857 | 0.32 | 0.5854 |
| | | Error | 8 | 31.8392 | 3.9799 | | |
| | Sqrt. Aerial Biomass | Model | 9 | 7320.026 | 813.3362 | 3.29 | 0.0541 |
| | | Rep | 6 | 2052.973 | 342.1621 | 1.38 | 0.3267 |
| | | Wetness | 1 | 2032.0312 | 2032.0312 | 8.22 | 0.021 |
| | | Inoculum | 1 | 1712.8928 | 1712.8928 | 6.92 | 0.0301 |
| | | Wet*Inoc | 1 | 2.8928 | 2.8928 | 0.01 | 0.9165 |
| | | Error | 8 | 9298.8644 | 247.3548 | | |
| | Tuber Number | Model | 9 | 256.5873 | 28.5097 | 2.24 | 0.1351 |
| | | Rep | 6 | 154.7142 | 25.7857 | 2.03 | 0.1751 |
| | | Wetness | 1 | 98 | 98 | 7.7 | 0.0241 |
| | | Inoculum | 1 | 69.1428 | 69.1428 | 5.43 | 0.0481 |
| | | Wet*Inoc | 1 | 33.5873 | 33.5873 | 2.64 | 0.143 |
| | | Error | 8 | 101.8571 | 12.7321 | | |
| | Sqrt. Tuber Yield | Model | 9 | 222.0564 | 24.6729 | 2.95 | 0.0713 |
| | | Rep | 6 | 123.5081 | 20.5846 | 2.46 | 0.1191 |
| | | Wetness | 1 | 150.7765 | 150.7765 | 18.02 | 0.0028 |
| | | Inoculum | 1 | 23.9368 | 23.9368 | 2.86 | 0.1293 |
| | | Wet*Inoc | 1 | 0.3871 | 0.3871 | 0.05 | 0.8351 |
| | | Error | 8 | 66.9505 | 8.3688 | | |
| | Symptomatic Tubers | Model | 9 | 0.5694 | 0.0632 | 1.35 | 0.3416 |
| | | Rep | 6 | 0.125 | 0.0208 | 0.44 | 0.8306 |
| | | Wetness | 1 | 0.125 | 0.125 | 2.67 | 0.1411 |
| | | Inoculum | 1 | 0.1944 | 0.1944 | 4.15 | 0.0761 |
| | | Wet*Inoc | 1 | 0.1944 | 0.1944 | 4.15 | 0.0761 |
| | | Error | 8 | 0.9444 | 0.0468 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---|---|---|---|---|---|---|---|
| | | | | **Experiment 1** | | | |
| 2 | Sqrt. Plant Height | Model | 9 | 5.9583 | 0.662 | 4.48 | 0.0113 |
| | | Rep | 6 | 1.5771 | 0.2628 | 1.78 | 0.193 |
| | | Wetness | 1 | 1.2229 | 1.229 | 8.28 | 0.0151 |
| | | Inoculum | 1 | 0.8691 | 0.8691 | 5.88 | 0.0337 |
| | | Wet*Inoc | 1 | 0.3828 | 0.3828 | 2.59 | 0.1358 |
| | | Error | 11 | 1.6253 | 0.1477 | | |
| | Number of Branches | Model | 9 | 230.5337 | 25.6148 | 1.24 | 0.3624 |
| | | Rep | 6 | 60.6765 | 10.1127 | 0.49 | 0.8035 |
| | | Wetness | 1 | 14.8027 | 14.8027 | 0.72 | 0.4153 |
| | | Inoculum | 1 | 104.1091 | 104.1091 | 5.04 | 0.0463 |
| | | Wet*Inoc | 1 | 4.6932 | 4.6932 | 0.23 | 0.643 |
| | | Error | 11 | 227.2757 | 20.6614 | | |
| | Number of Internodes | Model | 9 | 284.5158 | 31.6128 | 5.37 | 0.0056 |
| | | Rep | 6 | 104.6587 | 17.4431 | 2.96 | 0.0565 |
| | | Wetness | 1 | 139.3777 | 139.3777 | 23.69 | 0.0005 |
| | | Inoculum | 1 | 46.3087 | 46.3087 | 7.87 | 0.0171 |
| | | Wet*Inoc | 1 | 2.6981 | 2.6981 | 0.46 | 0.5123 |
| | | Error | 11 | 64.7222 | 5.8838 | | |
| | Sqrt. Aerial Biomass | Model | 9 | 8215.5689 | 912.841 | 1.47 | 0.2709 |
| | | Rep | 6 | 2250.2307 | 375.0384 | 0.6 | 0.724 |
| | | Wetness | 1 | 307.6551 | 307.6551 | 0.49 | 0.4968 |
| | | Inoculum | 1 | 3157.067 | 3157.067 | 5.07 | 0.0458 |
| | | Wet*Inoc | 1 | 408.1665 | 408.1665 | 0.66 | 0.4354 |
| | | Error | 11 | 6850.971 | 622.8155 | | |
| | Tuber Number | Model | 9 | 245.3432 | 27.2603 | 1.49 | 0.2635 |
| | | Rep | 6 | 191.3432 | 31.8905 | 1.74 | 0.2016 |
| | | Wetness | 1 | 1.4694 | 1.4694 | 0.08 | 0.7823 |
| | | Inoculum | 1 | 38.6165 | 38.6165 | 2.11 | 0.1745 |
| | | Wet*Inoc | 1 | 0.0324 | 0.0324 | 0 | 0.9672 |
| | | Error | 11 | 201.6091 | 18.3281 | | |
| | Sqrt. Tuber Yield | Model | 9 | 277.7053 | 30.8561 | 2.71 | 0.061 |
| | | Rep | 6 | 81.2292 | 13.5382 | 1.19 | 0.379 |
| | | Wetness | 1 | 80.6146 | 80.6146 | 7.08 | 0.0221 |
| | | Inoculum | 1 | 80.6854 | 80.6854 | 7.09 | 0.0221 |
| | | Wet*Inoc | 1 | 20.4971 | 20.4971 | 1.8 | 0.2066 |
| | | Error | 11 | 125.1805 | 11.3801 | | |
| | Symptomatic Tubers | Model | 9 | 3.0714 | 0.3412 | 2.5 | 0.0769 |
| | | Rep | 6 | 0.5 | 0.0833 | 0.61 | 0.7177 |
| | | Wetness | 1 | 0.9 | 0.9 | 6.6 | 0.0261 |
| | | Inoculum | 1 | 1.115 | 1.115 | 8.18 | 0.0155 |
| | | Wet*Inoc | 1 | 1.115 | 1.115 | 8.18 | 0.0155 |
| | | Error | 11 | 4.5714 | 0.1363 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---|---|---|---|---|---|---|---|
| | | | | Experiment 1 | | | |
| 3 | Sqrt. Plant Height | Model | 9 | 4.2512 | 0.4724 | 3.23 | 0.0834 |
| | | Rep | 6 | 1.426 | 0.2376 | 1.63 | 0.2845 |
| | | Wetness | 1 | 0.0057 | 0.0057 | 0.04 | 0.8488 |
| | | Inoculum | 1 | 0.5581 | 0.5581 | 3.82 | 0.0984 |
| | | Wet*Inoc | 1 | 0.1875 | 0.1875 | 1.28 | 0.3004 |
| | | Error | 6 | 0.8764 | 0.1461 | | |
| | Number of Branches | Model | 9 | 345.2857 | 38.365 | 1.8 | 0.2437 |
| | | Rep | 6 | 35.4285 | 5.9047 | 0.28 | 0.928 |
| | | Wetness | 1 | 0.25 | 0.25 | 0.01 | 0.9172 |
| | | Inoculum | 1 | 66.0357 | 66.0357 | 3.1 | 0.1287 |
| | | Wet*Inoc | 1 | 17.2857 | 17.2857 | 0.81 | 0.4022 |
| | | Error | 6 | 127.7142 | 21.2857 | | |
| | Number of Internodes | Model | 9 | 128.2232 | 14.247 | 3.6 | 0.0663 |
| | | Rep | 6 | 27.7142 | 4.619 | 1.17 | 0.4274 |
| | | Wetness | 1 | 9 | 9 | 2.28 | 0.182 |
| | | Inoculum | 1 | 23.2232 | 23.2232 | 5.88 | 0.0516 |
| | | Wet*Inoc | 1 | 4.7232 | 4.7232 | 1.2 | 0.3163 |
| | | Error | 6 | 23.7143 | 3.9523 | | |
| | Sqrt. Aerial Biomass | Model | 9 | 9425.6228 | 1047.2914 | 9.83 | 0.0058 |
| | | Rep | 6 | 288.89 | 48.1483 | 0.45 | 0.8216 |
| | | Wetness | 1 | 66.4225 | 66.4225 | 0.62 | 0.4598 |
| | | Inoculum | 1 | 3525.7728 | 3525.7728 | 33.1 | 0.0012 |
| | | Wet*Inoc | 1 | 23.7728 | 23.7728 | 0.22 | 0.6533 |
| | | Error | 6 | 639.0871 | 106.5145 | | |
| | Tuber Number | Model | 9 | 362.2232 | 40.247 | 4.26 | 0.0459 |
| | | Rep | 6 | 74.7142 | 12.4523 | 1.32 | 0.3732 |
| | | Wetness | 1 | 20.25 | 20.25 | 2.14 | 0.1936 |
| | | Inoculum | 1 | 50.2232 | 50.2232 | 5.31 | 0.0607 |
| | | Wet*Inoc | 1 | 19.7232 | 19.7232 | 2.09 | 0.1987 |
| | | Error | 6 | 56.7142 | 9.4523 | | |
| | Sqrt. Tuber Yield | Model | 9 | 607.904 | 67.5448 | 2.58 | 0.1306 |
| | | Rep | 6 | 20.177 | 3.3628 | 0.13 | 0.9877 |
| | | Wetness | 1 | 78.8758 | 78.8758 | 3.01 | 0.1333 |
| | | Inoculum | 1 | 162.7227 | 162.7227 | 6.21 | 0.047 |
| | | Wet*Inoc | 1 | 3.6523 | 3.6523 | 0.14 | 0.7216 |
| | | Error | 6 | 157.0969 | 26.1828 | | |
| | Symptomatic Tubers | Model | 9 | 8.5714 | 0.9523 | 1.67 | 0.2749 |
| | | Rep | 6 | 3.4285 | 0.5714 | 1 | 0.5 |
| | | Wetness | 1 | 0 | 0 | 0 | 1 |
| | | Inoculum | 1 | 0.5714 | 0.5714 | 1 | 0.3559 |
| | | Wet*Inoc | 1 | 0.5714 | 0.5714 | 1 | 0.3559 |
| | | Error | 6 | 3.4285 | 0.5714 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---|---|---|---|---|---|---|---|
| | | | | Experiment 1 | | | |
| 4 | Sqrt. Plant Height | Model | 10 | 2.2961 | 0.2296 | 1.08 | 0.4528 |
| | | Rep | 7 | 1.0655 | 0.1522 | 0.72 | 0.6621 |
| | | Wetness | 1 | 0.1494 | 0.1494 | 0.7 | 0.4214 |
| | | Inoculum | 1 | 0.4351 | 0.4351 | 2.05 | 0.183 |
| | | Wet*Inoc | 1 | 0.0071 | 0.0071 | 0.03 | 0.8586 |
| | | Error | 10 | 2.126 | 0.2126 | | |
| | Number of Branches | Model | 10 | 248.872 | 24.8872 | 1.24 | 0.3708 |
| | | Rep | 7 | 175.1041 | 25.0149 | 1.24 | 0.3638 |
| | | Wetness | 1 | 0.0714 | 0.0714 | 0 | 0.9536 |
| | | Inoculum | 1 | 46.6844 | 46.6844 | 2.32 | 0.18584 |
| | | Wet*Inoc | 1 | 3.66 | 3.66 | 0.18 | 0.6786 |
| | | Error | 10 | 200.9375 | 20.0937 | | |
| | Number of Internodes | Model | 10 | 82.6478 | 8.2647 | 1.01 | 0.4946 |
| | | Rep | 7 | 60.618 | 8.6597 | 1.06 | 0.4526 |
| | | Wetness | 1 | 1.9067 | 1.9067 | 0.23 | 0.6399 |
| | | Inoculum | 1 | 12.2562 | 12.2562 | 1.5 | 0.2493 |
| | | Wet*Inoc | 1 | 1.9392 | 1.9392 | 0.24 | 0.6371 |
| | | Error | 10 | 81.9236 | 8.1923 | | |
| | Sqrt. Aerial Biomass | Model | 10 | 3598.3357 | 359.8336 | 0.65 | 0.7496 |
| | | Rep | 7 | 379.6934 | 54.2419 | 0.1 | 0.9973 |
| | | Wetness | 1 | 185.6643 | 185.6643 | 0.33 | 0.5767 |
| | | Inoculum | 1 | 2279.0805 | 2279.0805 | 4.09 | 0.0708 |
| | | Wet*Inoc | 1 | 688.2536 | 688.2536 | 1.23 | 0.2926 |
| | | Error | 10 | 5577.2356 | 557.7235 | | |
| | Tuber Number | Model | 10 | 298.6746 | 29.8674 | 1.64 | 0.2243 |
| | | Rep | 7 | 73.9722 | 10.5674 | 0.58 | 0.7586 |
| | | Wetness | 1 | 24.0079 | 24.0079 | 1.32 | 0.2778 |
| | | Inoculum | 1 | 186.0271 | 186.0271 | 10.21 | 0.0096 |
| | | Wet*Inoc | 1 | 18.2222 | 18.2222 | 1 | 0.341 |
| | | Error | 10 | 182.2777 | 18.2277 | | |
| | Sqrt. Tuber Yield | Model | 10 | 412.1604 | 41.216 | 2.78 | 0.0609 |
| | | Rep | 7 | 29.616 | 4.2308 | 0.29 | 0.945 |
| | | Wetness | 1 | 116.4843 | 116.4843 | 7.87 | 0.0186 |
| | | Inoculum | 1 | 126.951 | 126.951 | 8.58 | 0.0151 |
| | | Wet*Inoc | 1 | 5.8455 | 5.8455 | 0.39 | 0.5438 |
| | | Error | 10 | 148.0139 | 14.8013 | | |
| | Symptomatic Tubers | Model | 10 | 48.3005 | 4.8301 | 1.47 | 0.278 |
| | | Rep | 7 | 17.6041 | 2.5149 | 0.76 | 0.6296 |
| | | Wetness | 1 | 4.5714 | 4.5714 | 1.39 | 0.266 |
| | | Inoculum | 1 | 16.1722 | 16.1722 | 4.91 | 0.0511 |
| | | Wet*Inoc | 1 | 0.8064 | 0.8064 | 0.24 | 0.6314 |
| | | Error | 10 | 32.9375 | 3.2937 | | |

**Table 5.** Continued

| Experiment 2 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
| 1 | Sqrt. Plant Height | Model | 10 | 6.2079 | 0.6207 | 3.67 | 0.0626 |
| | | Rep | 7 | 0.8713 | 0.1245 | 0.74 | 0.0542 |
| | | Wetness | 1 | 1.9996 | 1.9996 | 11.82 | 0.0138 |
| | | Inoculum | 1 | 0.0238 | 0.0238 | 0.14 | 0.7202 |
| | | Wet*Inoc | 1 | 0.0079 | 0.0079 | 0.05 | 0.8356 |
| | | Error | 6 | 1.0151 | 0.1691 | | |
| | Number of Branches | Model | 10 | 386.0684 | 38.6068 | 0.42 | 0.8925 |
| | | Rep | 7 | 303.7939 | 43.3991 | 0.47 | 0.8263 |
| | | Wetness | 1 | 15.0129 | 15.0129 | 0.16 | 0.7005 |
| | | Inoculum | 1 | 28.0022 | 28.0022 | 0.3 | 0.6014 |
| | | Wet*Inoc | 1 | 7.5578 | 7.5578 | 0.08 | 0.7842 |
| | | Error | 6 | 552.8727 | 92.1454 | | |
| | Number of Internodes | Model | 10 | 121.8994 | 12.1899 | 0.73 | 0.6859 |
| | | Rep | 7 | 41.1984 | 5.8854 | 0.35 | 0.9008 |
| | | Wetness | 1 | 19.7532 | 19.7532 | 1.18 | 0.3186 |
| | | Inoculum | 1 | 0.879 | 0.879 | 0.05 | 0.8262 |
| | | Wet*Inoc | 1 | 4.879 | 4.879 | 0.29 | 0.6083 |
| | | Error | 6 | 100.2181 | 16.703 | | |
| | Sqrt. Aerial Biomass | Model | 10 | 6693.4415 | 669.3442 | 0.38 | 0.9128 |
| | | Rep | 7 | 3509.4063 | 501.3437 | 0.29 | 0.9361 |
| | | Wetness | 1 | 106.6021 | 106.6021 | 0.06 | 0.8129 |
| | | Inoculum | 1 | 1923.3575 | 1923.3575 | 1.1 | 0.3339 |
| | | Wet*Inoc | 1 | 72.4953 | 72.4953 | 0.04 | 0.8451 |
| | | Error | 6 | 10455.759 | 1742.6266 | | |
| | Tuber Number | Model | 10 | 203.8278 | 20.3827 | 1.05 | 0.497 |
| | | Rep | 7 | 105.8954 | 15.1279 | 0.78 | 0.6262 |
| | | Wetness | 1 | 22.3668 | 22.3668 | 1.16 | 0.3235 |
| | | Inoculum | 1 | 27.1676 | 27.1676 | 1.4 | 0.2808 |
| | | Wet*Inoc | 1 | 5.8343 | 5.8343 | 0.3 | 0.6027 |
| | | Error | 6 | 116.0545 | 19.3424 | | |
| | Sqrt. Tuber Yield | Model | 10 | 122.4421 | 12.2442 | 0.29 | 0.9586 |
| | | Rep | 7 | 23.1391 | 3.3055 | 0.08 | 0.9982 |
| | | Wetness | 1 | 1.4681 | 1.4681 | 0.03 | 0.858 |
| | | Inoculum | 1 | 66.6961 | 66.6961 | 1.58 | 0.255 |
| | | Wet*Inoc | 1 | 0.896 | 0.896 | 0.02 | 0.8888 |
| | | Error | 6 | 252.6701 | 42.1116 | | |
| | Symptomatic Tubers | Model | 10 | 203.8278 | 20.3827 | 1.05 | 0.497 |
| | | Rep | 7 | 105.8954 | 15.1279 | 0.78 | 0.6262 |
| | | Wetness | 1 | 22.3669 | 22.3669 | 1.16 | 0.3235 |
| | | Inoculum | 1 | 27.1677 | 27.1677 | 1.4 | 0.2808 |
| | | Wet*Inoc | 1 | 5.8343 | 5.8343 | 0.3 | 0.6027 |
| | | Error | 6 | 116.0545 | 19.3424 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---|---|---|---|---|---|---|---|
| | | | | Experiment 2 | | | |
| 2 | Sqrt. Plant Height | Model | 9 | 2.6094 | 0.2899 | 1.44 | 0.2982 |
| | | Rep | 6 | 1.7724 | 0.2954 | 1.47 | 0.2906 |
| | | Wetness | 1 | 0.361 | 0.361 | 1.79 | 0.2135 |
| | | Inoculum | 1 | 0.1622 | 0.1622 | 0.81 | 0.3929 |
| | | Wet*Inoc | 1 | 0.1193 | 0.1193 | 0.59 | 0.4612 |
| | | Error | 9 | 1.8135 | 0.2015 | | |
| | Number of Branches | Model | 9 | 456.8307 | 50.7589 | 0.97 | 0.5186 |
| | | Rep | 6 | 120.2544 | 20.0424 | 0.38 | 0.8727 |
| | | Wetness | 1 | 5.7697 | 5.7697 | 0.11 | 0.7476 |
| | | Inoculum | 1 | 293.4057 | 293.4057 | 5.6 | 0.0422 |
| | | Wet*Inoc | 1 | 11.8922 | 11.8922 | 0.23 | 0.6452 |
| | | Error | 9 | 471.6955 | 52.4106 | | |
| | Number of Internodes | Model | 9 | 36.8214 | 4.0912 | 0.94 | 0.5376 |
| | | Rep | 6 | 14.6661 | 2.6663 | 0.56 | 0.7527 |
| | | Wetness | 1 | 0.4123 | 0.4123 | 0.09 | 0.7656 |
| | | Inoculum | 1 | 4.2499 | 4.2499 | 0.97 | 0.3495 |
| | | Wet*Inoc | 1 | 12.331 | 12.331 | 2.83 | 0.1271 |
| | | Error | 9 | 39.2838 | 4.3648 | | |
| | Sqrt. Aerial Biomass | Model | 9 | 8074.9068 | 897.2118 | 1.53 | 0.2697 |
| | | Rep | 6 | 377.6776 | 62.9462 | 0.11 | 0.9934 |
| | | Wetness | 1 | 0.8167 | 0.8167 | 0 | 0.9711 |
| | | Inoculum | 1 | 6748.4628 | 6748.4628 | 11.47 | 0.008 |
| | | Wet*Inoc | 1 | 301.1012 | 301.1012 | 0.51 | 0.4925 |
| | | Error | 9 | 5294.0384 | 588.2265 | | |
| | Tuber Number | Model | 9 | 322.1576 | 35.7952 | 2.39 | 0.1052 |
| | | Rep | 6 | 181.5602 | 30.26 | 2.02 | 0.1648 |
| | | Wetness | 1 | 11.2449 | 11.2449 | 0.75 | 0.4087 |
| | | Inoculum | 1 | 117.4467 | 117.4467 | 7.84 | 0.0207 |
| | | Wet*Inoc | 1 | 6.0143 | 6.0143 | 0.4 | 0.542 |
| | | Error | 9 | 134.7897 | 14.9766 | | |
| | Sqrt. Tuber Yield | Model | 9 | 346.3495 | 38.4832 | 2.74 | 0.0744 |
| | | Rep | 6 | 122.2541 | 20.3756 | 1.45 | 0.2948 |
| | | Wetness | 1 | 20.8307 | 20.8307 | 1.48 | 0.254 |
| | | Inoculum | 1 | 183.5843 | 183.5843 | 13.09 | 0.0056 |
| | | Wet*Inoc | 1 | 8.0857 | 8.0857 | 0.58 | 0.4672 |
| | | Error | 9 | 126.2571 | 14.0285 | | |
| | Symptomatic Tubers | Model | 9 | 0 | 0 | 9999.99 | 0 |
| | | Rep | 6 | 0 | 0 | 9999.99 | 0 |
| | | Wetness | 1 | 0 | 0 | 9999.99 | 0 |
| | | Inoculum | 1 | 0 | 0 | 9999.99 | 0 |
| | | Wet*Inoc | 1 | 0 | 0 | 9999.99 | 0 |
| | | Error | 9 | 0 | 0 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---------|--------------------|--------------------|-----|-----|-----|----|----|
| | | | | Experiment 2 | | | |
| 3 | Sqrt. Plant Height | Model | 11 | 5.6294 | 0.5117 | 1.73 | 0.1793 |
| | | Rep | 8 | 2.3689 | 0.2961 | 1 | 0.481 |
| | | Wetness | 1 | 1.7681 | 1.7681 | 5.99 | 0.0308 |
| | | Inoculum | 1 | 1.0489 | 1.0489 | 3.53 | 0.0848 |
| | | Wet*Inoc | 1 | 1.3195 | 1.3195 | 4.47 | 0.0561 |
| | | Error | 12 | 3.5437 | 0.2953 | | |
| | Number of Branches | Model | 11 | 374.2462 | 34.0224 | 0.74 | 0.6858 |
| | | Rep | 8 | 211.4128 | 26.4266 | 0.58 | 0.7792 |
| | | Wetness | 1 | 140.1212 | 140.1212 | 3.06 | 0.106 |
| | | Inoculum | 1 | 17.7083 | 17.7083 | 0.39 | 0.5459 |
| | | Wet*Inoc | 1 | 65.1201 | 65.1201 | 1.42 | 0.2564 |
| | | Error | 12 | 550.2537 | 45.8545 | | |
| | Number of Internodes | Model | 11 | 165.4786 | 15.0433 | 1.22 | 0.3683 |
| | | Rep | 8 | 94.3513 | 11.7939 | 0.96 | 0.5101 |
| | | Wetness | 1 | 23.7575 | 23.7575 | 1.92 | 0.1906 |
| | | Inoculum | 1 | 27.7996 | 27.7996 | 2.25 | 0.1593 |
| | | Wet*Inoc | 1 | 9.2702 | 9.2702 | 0.75 | 0.4032 |
| | | Error | 12 | 148.1486 | 12.3457 | | |
| | Sqrt. Aerial Biomass | Model | 11 | 5822.9626 | 529.3602 | 0.24 | 0.9871 |
| | | Rep | 8 | 3181.5393 | 397.6924 | 0.18 | 0.9889 |
| | | Wetness | 1 | 176.8775 | 176.8775 | 0.08 | 0.7805 |
| | | Inoculum | 1 | 674.9082 | 674.9082 | 0.31 | 0.5879 |
| | | Wet*Inoc | 1 | 1212.1582 | 1212.1582 | 0.56 | 0.47 |
| | | Error | 12 | 26129.457 | 2177.4547 | | |
| | Tuber Number | Model | 11 | 216.4545 | 19.6776 | 1.17 | 0.3961 |
| | | Rep | 8 | 68.9962 | 8.6245 | 0.51 | 0.8265 |
| | | Wetness | 1 | 12.1212 | 12.1212 | 0.72 | 0.4133 |
| | | Inoculum | 1 | 121.8848 | 121.8848 | 7.22 | 0.0198 |
| | | Wet*Inoc | 1 | 5.4142 | 5.4142 | 0.32 | 0.5815 |
| | | Error | 12 | 202.9583 | 16.8753 | | |
| | Sqrt. Tuber Yield | Model | 11 | 571.5153 | 51.9559 | 1.62 | 0.2107 |
| | | Rep | 8 | 149.5017 | 18.6877 | 0.58 | 0.7753 |
| | | Wetness | 1 | 10.2029 | 10.2029 | 0.32 | 0.5835 |
| | | Inoculum | 1 | 355.967 | 355.967 | 11.08 | 0.006 |
| | | Wet*Inoc | 1 | 63.9834 | 63.9834 | 1.99 | 0.1836 |
| | | Error | 12 | 385.6355 | 32.1363 | | |
| | Symptomatic Tubers | Model | 11 | 2.7462 | 0.2496 | 1.59 | 0.2173 |
| | | Rep | 8 | 1.6212 | 0.2026 | 1.29 | 0.3314 |
| | | Wetness | 1 | 0.1212 | 0.1212 | 0.77 | 0.3962 |
| | | Inoculum | 1 | 0.3529 | 0.3529 | 2.25 | 0.1591 |
| | | Wet*Inoc | 1 | 0.3529 | 0.3529 | 2.25 | 0.1591 |
| | | Error | 12 | 1.8787 | 0.1565 | | |

**Table 5.** Continued

| Harvest | Dependent Variable | Source of Variation | df | SS | MS | F | P |
|---------|--------------------|--------------------|----|-----|-----|-----|-----|
| | | | | Experiment 2 | | | |
| 4 | Sqrt. Plant Height | Model | 11 | 3.3524 | 0.3047 | 2.34 | 0.1187 |
| | | Rep | 8 | 3.0551 | 0.3819 | 2.93 | 0.0746 |
| | | Wetness | 1 | 0.5677 | 0.5677 | 4.36 | 0.0703 |
| | | Inoculum | 1 | 0.0062 | 0.0062 | 0.05 | 0.8323 |
| | | Wet*Inoc | 1 | 0.0033 | 0.0033 | 0.03 | 0.8761 |
| | | Error | 8 | 1.0421 | 0.1302 | | |
| | Number of Branches | Model | 11 | 538.6583 | 0.489689 | 1.54 | 0.2761 |
| | | Rep | 8 | 352.0416 | 44.0052 | 1.38 | 0.3287 |
| | | Wetness | 1 | 1.125 | 1.125 | 0.04 | 0.8555 |
| | | Inoculum | 1 | 4.6944 | 4.6944 | 0.15 | 0.7109 |
| | | Wet*Inoc | 1 | 140.0277 | 140.0277 | 4.4 | 0.0692 |
| | | Error | 8 | 254.5416 | 31.8177 | | |
| | Number of Internodes | Model | 11 | 152.425 | 13.8568 | 0.88 | 0.5897 |
| | | Rep | 8 | 148.625 | 18.5781 | 1.18 | 0.411 |
| | | Wetness | 1 | 15.125 | 15.125 | 0.96 | 0.356 |
| | | Inoculum | 1 | 1 | 1 | 0.06 | 0.8075 |
| | | Wet*Inoc | 1 | 1 | 1 | 0.06 | 0.8075 |
| | | Error | 8 | 126.125 | 15.7656 | | |
| | Sqrt. Aerial Biomass | Model | 11 | 6962.6459 | 632.9678 | 0.44 | 0.8956 |
| | | Rep | 8 | 4185.6471 | 523.2059 | 0.36 | 0.9124 |
| | | Wetness | 1 | 173.9113 | 173.9113 | 0.12 | 0.7367 |
| | | Inoculum | 1 | 259.7469 | 257.7469 | 0.18 | 0.6816 |
| | | Wet*Inoc | 1 | 3578.0336 | 3578.0336 | 2.49 | 0.1529 |
| | | Error | 8 | 11473.802 | 1434.2253 | | |
| | Tuber Number | Model | 11 | 595.425 | 54.1295 | 0.83 | 0.6219 |
| | | Rep | 8 | 329.2916 | 41.1614 | 0.63 | 0.7345 |
| | | Wetness | 1 | 3.125 | 3.125 | 0.05 | 0.8321 |
| | | Inoculum | 1 | 53.7777 | 53.7777 | 0.83 | 0.3901 |
| | | Wet*Inoc | 1 | 100 | 100 | 1.54 | 0.2505 |
| | | Error | 8 | 521.125 | 65.1406 | | |
| | Sqrt. Tuber Yield | Model | 11 | 445.9097 | 40.5372 | 1.96 | 0.1745 |
| | | Rep | 8 | 85.9692 | 10.7461 | 0.52 | 0.8134 |
| | | Wetness | 1 | 40.7392 | 40.7392 | 1.97 | 0.1981 |
| | | Inoculum | 1 | 72.6119 | 72.6119 | 3.51 | 0.0979 |
| | | Wet*Inoc | 1 | 127.1549 | 127.1549 | 6.15 | 0.0382 |
| | | Error | 8 | 165.5075 | 20.6884 | | |
| | Symptomatic Tubers | Model | 11 | 23.925 | 2.175 | 1.17 | 0.4222 |
| | | Rep | 8 | 16.7083 | 2.0885 | 1.12 | 0.4367 |
| | | Wetness | 1 | 1.125 | 1.125 | 0.61 | 0.459 |
| | | Inoculum | 1 | 8.0278 | 8.0278 | 4.32 | 0.0714 |
| | | Wet*Inoc | 1 | 0.25 | 0.25 | 0.13 | 0.7234 |
| | | Error | 8 | 14.875 | 1.8594 | | |