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SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF ROOT COLONIZATION OF LEAFY SPURGE (*EUPHORBIA ESULA* L.) SEEDLINGS BY RHIZOBACTERIA[1]

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Abstract

The colonization of roots of hydroponically-grown leafy spurge seedlings inoculated with deleterious rhizobacteria was studied using scanning and transmission electron microscopy. Scanning electron microscopy revealed that colonization and attachment of the rhizobacteria, *Pseudomonas fluorescens* isolate LS102 and *Flavobacterium balustinum* isolate LS105, to root surfaces were associated with microfibrils likely anchoring and/or entrapping bacterial cells. Ultrastructural observations with transmission electron microscopy revealed considerable alterations of root cells including vesiculation and convolution of plasmalemma, partial cell wall degradation, disorganization of cytoplasm, and cell plasmolysis. Inoculation of leafy spurge roots with isolates LS102 and LS105 in the hydroponic system inhibited root growth by 80 and 65%, respectively, compared to the control. This study showed that selected rhizobacteria colonized root surfaces of leafy spurge and apparently released phytotoxins resulting in cellular aberrations and, consequently in growth inhibition of leafy spurge seedlings.

Key Words : Biological control, *Euphorbia esula* L., rhizosphere, root colonization, Scanning electron microscopy, Transmission electron microscopy.

Leafy spurge is an aggressive and persistent perennial plant of Eurasian origin. Its vigorous growth and deep root system make it a persistent weed problem in pastures and rangelands (Best et al. 1980). Leafy spurge infests millions of hectares in the Northern Great Plains of USA and the Prairie Provinces of Canada. The weed expands its infestation by 10% annually, doubling its original area about every seven years (Senft 1944). The expense of chemical controls and their relative ineffectiveness on leafy spurge has led to investigations of strategies for biological control with plant pathogens.

Pathogens that curtail plant growth and reproduction may be useful for biological control of weeds. Manipulation of naturally-occurring plant pathogens to suppress growth of

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weeds in crops has only recently been intensively investigated. Such biological control strategies are based on initiating disease epidemics by pathogens of the host within the target weed population. Little is known about the pathogenesis of selected pathogens on host weeds. An understanding of the factors affecting pathogenesis of potential biocontrol microorganisms is needed if the manipulation of such factors for biological control is to be realized.

Rhizobacteria that colonize the roots of plants have been classified by Schroth & Hancock (1982) as beneficial, neutral or deleterious, but the distinction between these types is often obscure. For example, colonization of the roots by *Pseudomonas fluorescens* and *P. putida* groups resulted in a significant stimulation of yield in potato (Burr et al. 1978) and sugarbeet (Suslow & Schroth 1982b), whereas root colonization by other species such as *P. virdiflava*, induced a series of degradation events including distortion of root tissue and increased susceptibility to bacterial and fungal pathogens (Suslow & Schroth 1982a). In many bacterial diseases of plants caused by "traditional or major pathogens", the pathogenic principles are well established (Goodman et al. 1986). For instance, syringomycin, a toxin produced by plant pathogenic isolates of *P. syringae* pv. *syringae* was associated with the development of some diseases caused by this pathogen (Surico & Devay 1982). In contrast, with "minor pathogens" (Suslow 1982), the pathogenic principles are more subtle and much less understood. Such microorganisms, generally regarded as saprophytic, may reduce and/or suppress plant growth with no obvious cellular damage. In such situations, production of chelating agents such as siderophores (Teintze et al. 1981), alteration of root cell permeability (Bowen & Rovira 1976) and production of toxic compounds (Suslow 1982) have been implicated.

Rhizobacteria have previously been isolated from roots of leafy spurge seedlings and screened for their deleterious effects using tissue cultures (Souissi & Kremer 1994). Two isolates, *Pseudomonas fluorescens* isolate LS102 and *Flavobacterium balustinum* isolate LS105, reduced root growth and emergence of seedlings by as much as 50% of the control in greenhouse studies. The effectiveness of isolates LS102 and LS105 in inhibiting leafy spurge growth depend on their ability to grow in the root environment and to colonize the root surface of seedlings. This study was undertaken to examine the attachment to and colonization of the root surfaces of leafy spurge seedlings by isolates LS102 and LS105 and determine their impact on root growth. Factors, such as bacterial enzyme and polysaccharide productions, that may be involved in the attachment of bacteria to the roots and colonization or that may enhance their virulence were also examined during this study. Leafy spurge seedlings were inoculated with isolates LS102 and LS105 and the rhizobacterium-plant cell interaction was examined over time using scanning and transmission electron microscopy.

Material and Methods

BACTERIAL CULTURES — *Pseudomonas fluorescens* isolate LS102 and *Flavobacterium balustinum* isolate LS105, previously isolated from roots of leafy spurge seedlings (Souissi & Kremer 1994) and tested for their deleterious effects on leafy spurge growth, were used during this study. Bacteria were grown on King's B agar medium for two days and a loop of the fresh cultures was used to inoculate 50 ml flasks containing King's Broth. Flasks were shaken overnight on a rotary shaker at 140 rpm. Cultures were harvested by centrifugation

at 7,000 x g for 20 min at 4°C. Cell pellets were resuspended in phosphate-buffered saline (PBS; 10mM K_2PO_4-KH_2PO_4, 0.14 M NaCl; pH 7.2) and spectrophotometrically adjusted to 10^8 CFU/ml. One ml of bacterial inoculum was used to inoculate seedlings of leafy spurge.

PLANT MATERIAL — Leafy spurge seeds, provided by Dr B. Masters, were collected from sub-irrigated meadow near Ainsworth, NE in 1992. Seeds were surface disinfected first in 1.25% sodium hypochlorite for 2 min, then 70% ethanol for 30 sec, followed by repeated washings in sterile distilled water (SDW). Pregerminated seeds on 1% agar were used to determine the effects of bacteria on root growth and for microscopic studies.

THE HYDROPONIC SYSTEM — The effect of isolates LS102 and LS105 on leafy spurge was monitored in a hydroponic system by measuring root elongation of seedlings growing in nutrient solution. The assembly consisted of a 200 X 25 mm glass test tube containing a sterile paper towel wick and 20 ml of nutrient solution (Hoagland & Arnon 1938). Potassium nitrate (1.5 mM) was added to the nutrient solution and the pH was adjusted to 6.7. Two pregerminated leafy spurge seeds were aseptically placed on the paper towel wick and inoculated with 1 ml of bacterial inoculum prepared as described above. Control tubes received 1 ml of PBS. Tubes stoppered with foam and covered with aluminum foil were placed at 28°C under continuous light provided by fluorescent lamps at an intensity of 250 μE $sec^{-1}m^{-2}$. Five replications per treatment arranged in a completely randomized design were used. Six seedlings per treatment were randomly sampled and their root elongation was recorded periodically. The experiment was repeated three times and data presented were averaged and variation expressed as standard error.

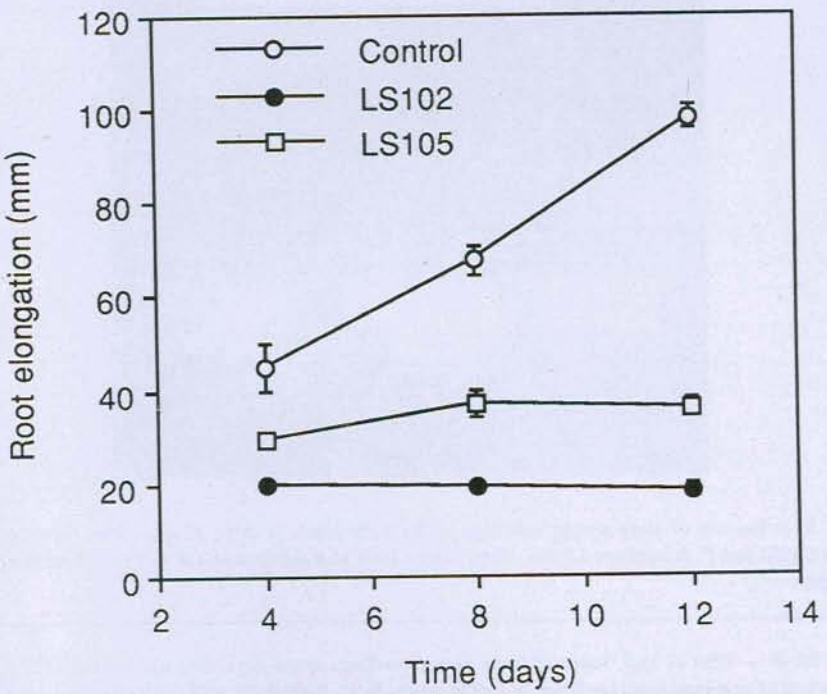

Fig. 1 — Time course of root elongation of leafy spurge seedlings exposed to *P. fluorescens* LS102 and *F. balustinum* LS105 in the hydroponic system. Values are mean of 3 replications. Vertical bars indicate SEM.

ENZYME ASSAYS — Isolates LS102 and LS105 were assayed for enzyme activities. Extracellular pectinase activity was scored on polygalacturonate-yeast extract agar (PYA) by the development of a precipitate around the colony after flooding the medium with 6N HCl (Chatterjee & Starr 1977). Protease activity was tested on nutrient gelatin agar (NGA) medium by the production of a clearing around the colony after incubation of plates at 27°C (Barras er al 1986). Ten single bacterial colonies per plate were patched on either medium and two plates per treatment were used.

DETECTION OF POLYSACCHARIDES IN ROOT TISSUE — The presence of polysaccharides of bacterial origin within the inoculated root tissue was detected using the Periodic Acid-Schiff (PAS) staining system, as described in Procedure No. 395 (Sigma Diagnostics, P.O. Box 14508, St. Louis, MO). Sections of 3.5 μm thickness were made from a 7-d-old root tissue inoculated with isolate LS102 and stained with PAS. The reagent stains the glycol-containing components in the tissue pink to red. Non-inoculated leafy spurge root tissue was used as control. Four slides with four sections per slide were used per treatment. The whole experiment was repeated two times.

ELECTRON MICROSCOPIC STUDIES — Tissue samples from 7- and 12-d-old inoculated and non-inoculated seedlings of leafy spurge grown in the hydroponic system were fixed in 2.5%

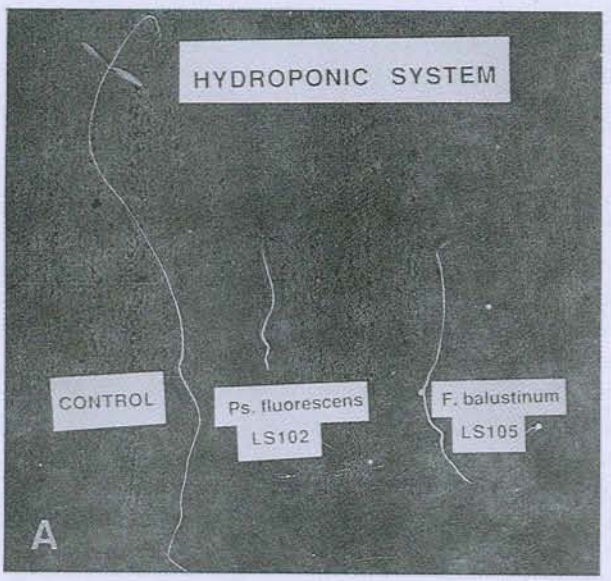

Fig. 2 — Growth of leafy spurge seedlings in the hydroponic system, 12 days after inoculation with *P. fluorescens* LS102 and *F. balustinum* LS105. Note inhibition of root development in inoculated seedlings compared to the control.

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Fig. 3A-D — SEM of root tissue of leafy spurge seedlings seven days after inoculation with rhizobacteria. **A.** Root surface of non-inoculated seedlings free of bacteria. **B, C.** Root surface of seedlings inoculated with LS105 and LS102, respectively. Bar = 10 μm. **D.** Formation of small clusters of bacteria as a result of the entrapment of LS102 cells by fibrillar material (arrowhead). Bar = 2.0 μm.

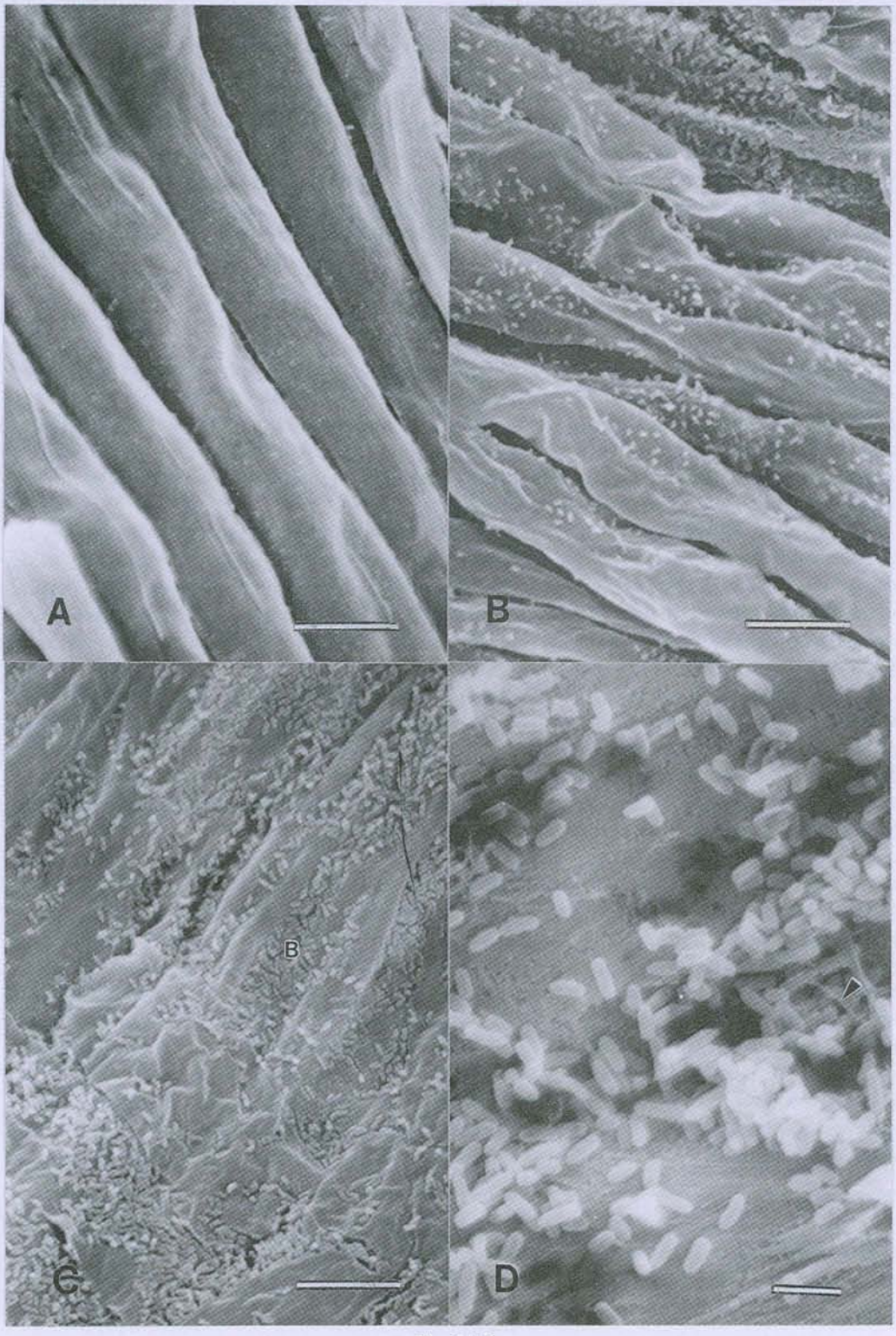

Fig. 3A-D

glutaraldehyde (100 mM phosphate buffer, pH 7) for 4 hr at room temperature. Samples were washed in the same buffer over 1 hr and postfixed in 1% osmium tetroxide for 4 hr. For SEM, samples were dehydrated through a graded ethanol series (20, 40, 60, 80, 95 and 100%), critical-point dried in liquid CO_2 and examined in a JEOL JSM-35 scanning electron microscope operating at 20 kv. Tissue samples taken for the TEM were fixed as above, dehydrated in acetone and embedded in Epon. Sections were made with an ultramicrotome equipped with a diamond knife. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL JEM-100B transmission electron microscope operating at 100 kv.

Observations

ALTERATION OF ROOT DEVELOPMENT IN THE HYDROPONIC SYSTEM — When surface-sterilized leafy spurge seeds were inoculated with isolates LS102 and LS105 and allowed to germinate in the hydroponic system, seedling root growth was inhibited about 80% and 65%, compared to the control, respectively (Fig. 1). Twelve days after inoculation, a significant growth inhibition was observed in seedlings inoculated with LS102 and LS105 compared to control non-inoculated seedlings (Fig. 2).

ENZYME PRODUCTION — When isolates LS102 and LS105 were tested for enzyme production, neither isolate produced pectinases, however both of them developed clear halos on nutrient gelatin agar medium which indicated the production of proteases. Visual examination revealed that isolate LS102 possessed the highest protease activity since halos on nutrient gelatin agar were about twice the size of those produced by isolate LS105 (data not shown).

MICROSCOPIC EXAMINATION OF INOCULATED LEAFY SPURGE SEEDLINGS — Thin sections of infected tissues examined by light microscopy for the presence of bacteria showed an extensive colonization of the epidermal and cortical cells. Additionally, the presence of bacteria in the root tissue was associated with the deposition of thick material on the surface of the epidermal cells. This material stained pink when treated with PAS staining system and was identified as bacterial polysaccharides (data not shown).

Rhizobacteria inoculated on seeds of leafy spurge colonized the root surface of seedlings seven days after inoculation, as revealed by SEM (Fig. 3A-D). Figure 3A shows the typical topography of root seedlings where cells of the epidermal layer formed a series of parallel ridges and valleys. No detectable bacteria were observed on the root surface of uninoculated seedlings. Root surfaces from inoculated seedlings showed a large surface bacterial population with bacteria "intruding" deeply into surface furrows (Fig. 3B, C). Cells of isolate LS105 appear to be individually attached to the cell surface (Fig. 3B) whereas LS102 formed large clusters of cells and microcolonies (Fig. 3C). Unlike the uninoculated tissue where surfaces of cells were smooth, root surfaces of colonized tissue were rough and wrinkled. The attached bacteria synthesized fibrillar material that entrapped more bacteria to form adherent clusters of bacteria (Fig. 3D). Areas of the root surface were mostly covered by *P. fluorescens* isolate LS102 by 12 days after inoculation (Fig. 4A). Entrapped bacteria in

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Fig. 4A,B — SEM of root tissue of leafy spurge seedlings 12 days after inoculation with rhizobacteria. **A.** LS102 completely covered the root surface. Note the formation of large clumps of bacterial fibrils and cells (inset). Bar = 10 μm. **B.** Few cells of LS105 were observed at the root surfaces of inoculated tissue. Bar = 2.0 μm.

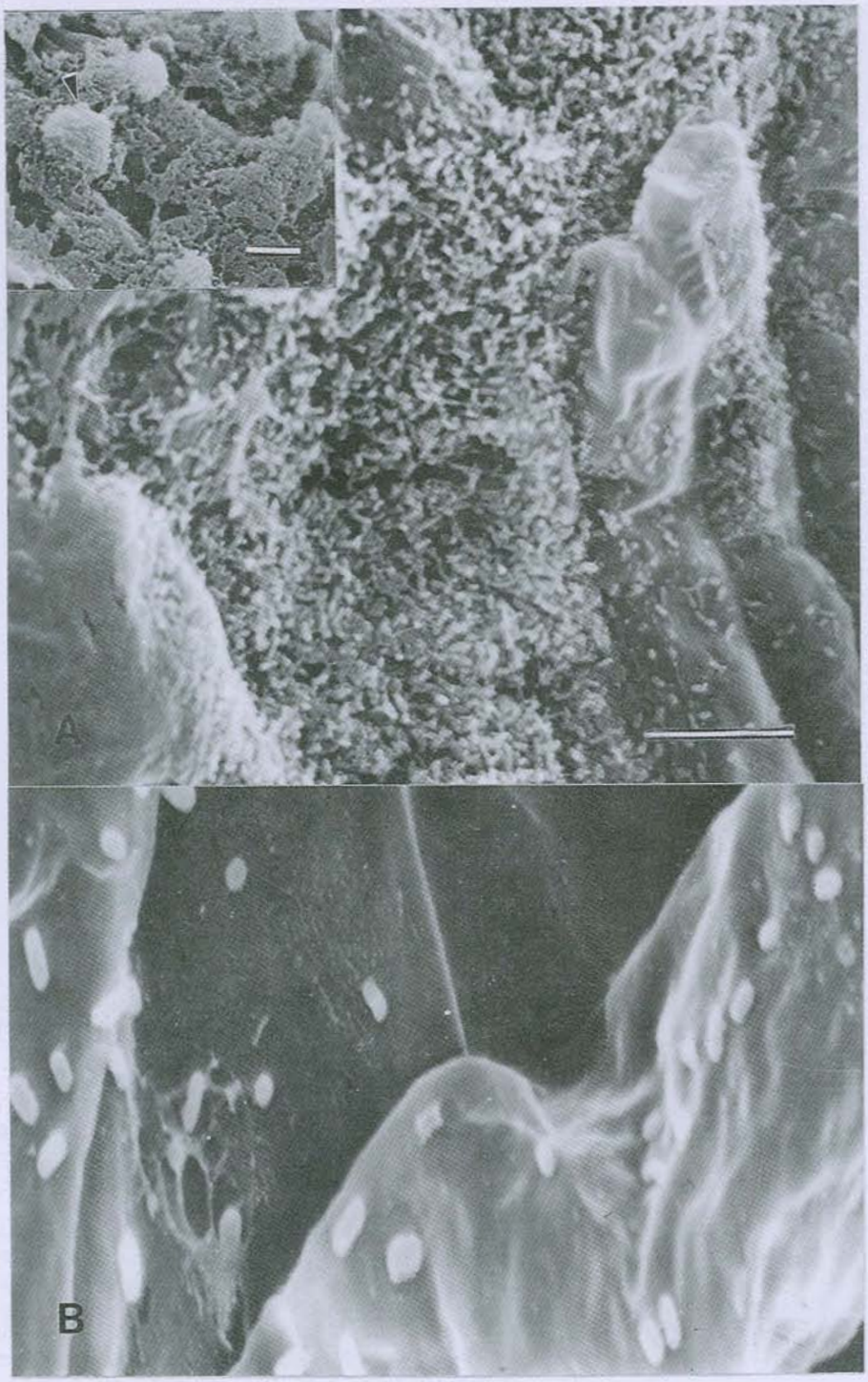

Fig. 4A,B

the fibrillar material continued to divide, increasing the number of bacteria in the clusters and resulting in the formation of large clumps of bacterial fibrils and cells (Fig. 4, inset). In tissue inoculated with isolate LS105, few bacterial cells were observed at 12 days, with little synthesis of associated fibrillar material (Fig. 4B).

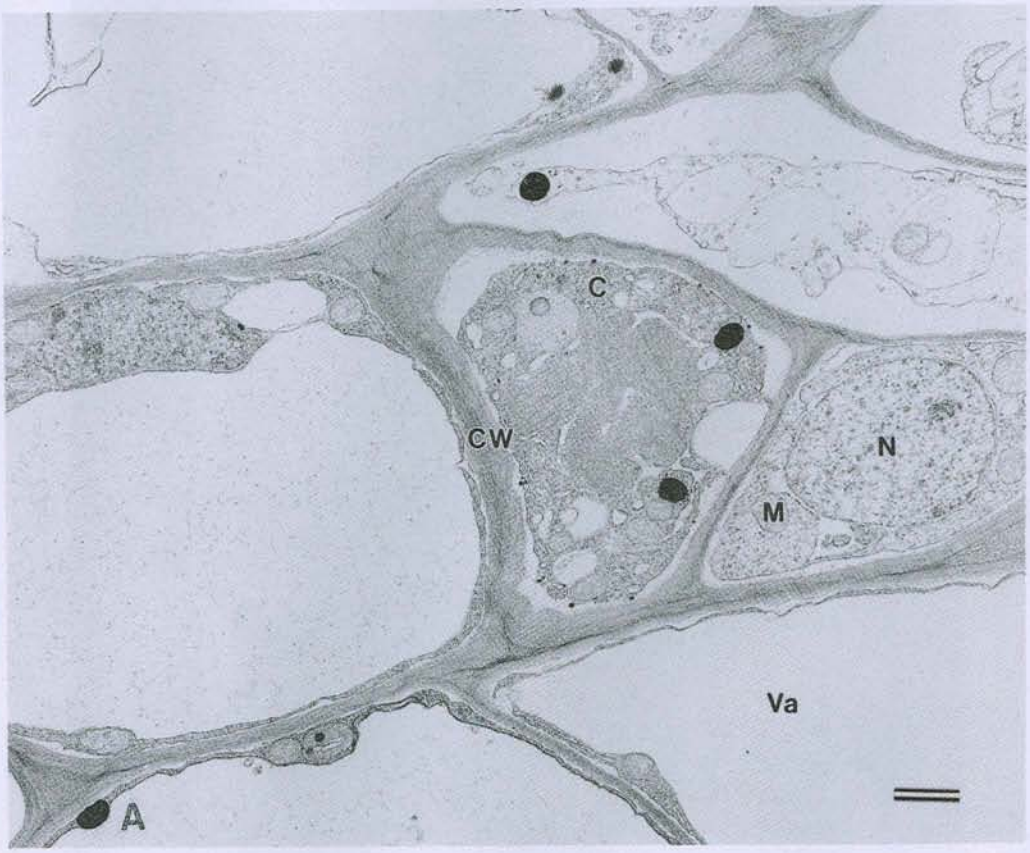

Fig. 5A — TEM of non-inoculated leafy spurge root tissue (*C*, cytoplasm; *CW*, cell wall; *M*, mitochondrion; *N*, nucleus; *Va*, vacuole). Bar = 1.0 μm.

Fig. 6A-D — *P. fluorescens* LS102 and *F. balustinum* LS105 in the intercellular spaces of leafy spurge root tissue seven days (**A**, **B**) and 12 days (**C**, **D**) after inoculation (*Am*, amorphous material; *B*, bacterium; *FM*, fibrillar material; *IS*, intercellular space; *Va*, vacuole). **A.** LS102 in microcolonies surrounded by a network of electron dense particulate (arrowhead) and fibrillar material, probably bacterial extracellular polysaccharide. Note the presence of a distinct film delimiting bacteria from the intercellular space (arrow). **B.** LS102 cells surrounded by abundant fibrillar material. **C.** Partial wall dissolution (arrowhead) in tissue inoculated with LS105. Note the deposition of amorphous material on cell walls. **D.** Group of LS102 cell showing vesicles projecting from bacterial surfaces (arrowheads). Bar = 1.0 μm.

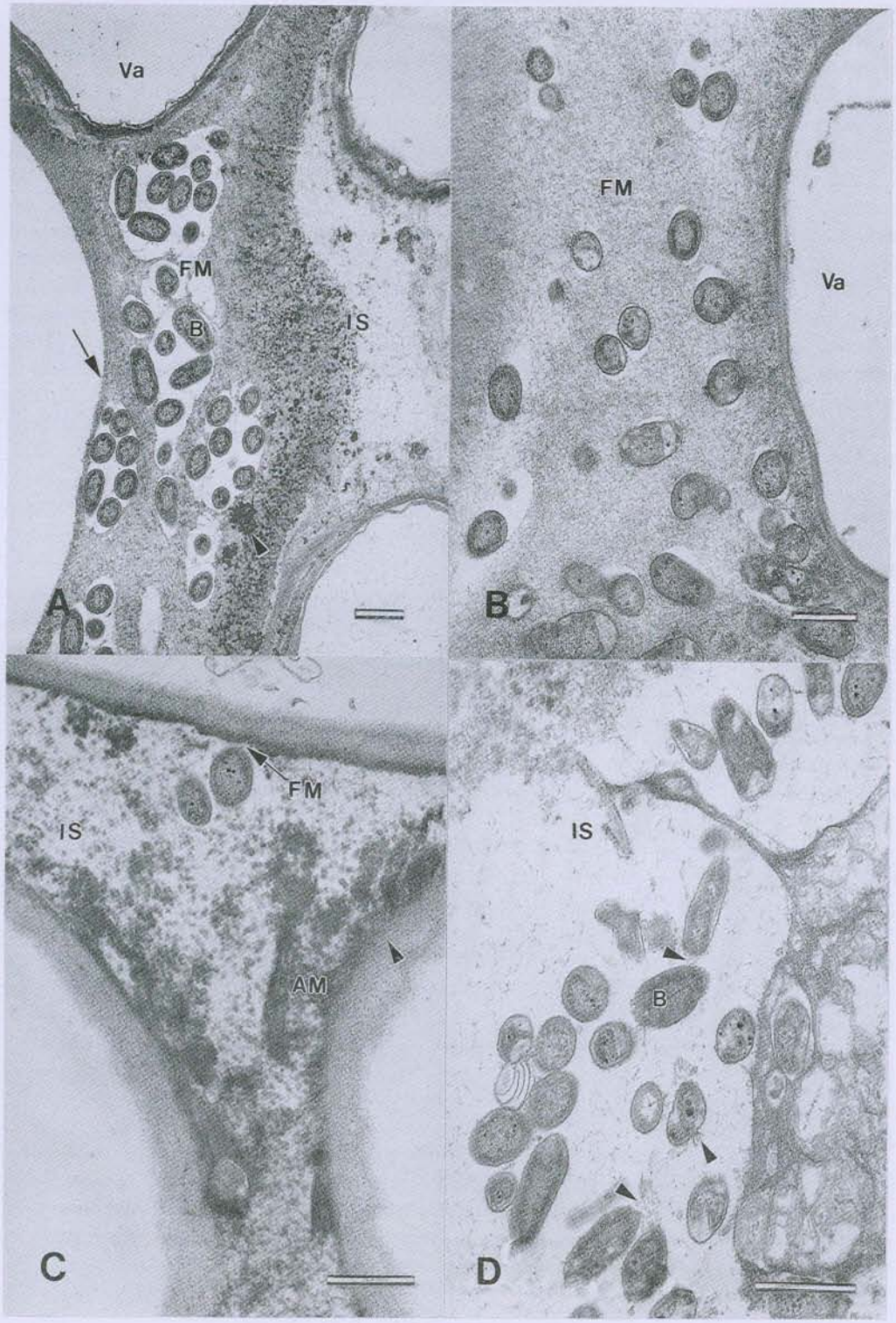

Fig. 6A-D

ULTRASTRUCTURAL OBSERVATIONS WITH TEM — Control and infected leafy spurge tissues collected seven and 12 days after inoculation were examined by TEM. Cell damage was not observed in control tissues. Cell walls and membranes were generally distinct and continuous. Organelles, including mitochondria, did not show signs of alteration as judged by the preserved structure of cristae and lamellae (Fig. 5A).

In infected tissue, there was no intimate association of bacteria with cell walls. In contrast, most of the bacteria were free and evenly distributed throughout the intercellular spaces, although local accumulations or microcolonies of bacteria were frequently observed (Fig. 6A). The microcolonies of bacteria were surrounded by a network of fibrillar material, probably bacterial extracellular polysaccharide (EPS), and by granular material (Fig. 6A, B). Some amorphous and darkly-stained material also accumulated in the intercellular spaces of infected tissues (Fig. 6C). The microcolonies of bacteria were often delimited from the intercellular space by a film of electron dense material (Fig. 6A). Bacterial cells were seen attached to plant cell walls through fibrillar material which appeared to originate from the cell walls, which was eroded to some extent (Fig. 6B, C), suggesting possible degradation of host wall by bacterial-induced host enzymes.

Twelve days after inoculation, cells of isolate LS102 appeared to liberate numerous vesicles from their bacterial surfaces into the interbacterial matrix (Fig. 6D). Some vesicles burst, liberating their contents into the matrix. The residual membranes of ruptured vesicles were observed between bacteria and in some cases attached to bacterial surfaces.

The movement of bacteria was not restricted to the epidermal cells. Indeed, bacteria were found in the intercellular space between cortical cells where severe damage to plant cells occurred. One week after inoculation, tissues infected with isolate LS102 showed severe plasma membrane convolution and cytoplasmic disorganization (Fig. 7A). Secondary vacuoles were seen in the cytoplasm, apparently fusing with the tonoplast. The endoplasmic reticulum became dilated and occasional changes in the fine structure of mitochondria were observed. Twelve days after inoculation, infected tissues were severely damaged with complete collapse of cells and darkly stained cytoplasm. Some of the sections showed dissolution of the middle lamella (Fig. 7B).

One week after inoculation, damage caused by isolate LS105 on leafy spurge root tissues was less pronounced than that caused by isolate LS102. Most of the cellular organelles were present. However, the plasma membrane separated from the cell wall and numerous vesicles were found in the cytoplasm (Fig. 8A). By twelve days after inoculation, cell compartmentation was lost. There was a striking change in the shape, location and structure of mitochondria. Most of the organelles degenerated leaving fragments of membranes in the cytoplasm. The tonoplast ruptured and most of the cytoplasm moved into the cell lumen. Apparent deposition of papilla-like structure also occurred in cell walls of inoculated tissues (Fig. 8B).

Discussion

A hydroponic system was developed where pregerminated leafy spurge seeds were inoculated with either isolate LS102 or LS105. Root elongation of treated seedlings was significantly inhibited (Fig. 1). This hydroponic system allows root growth inhibition to be monitored easily and may be very useful in further investigations of the mechanism(s) by which rhizobacteria inhibit root growth (Tranel et al. 1993a,b).

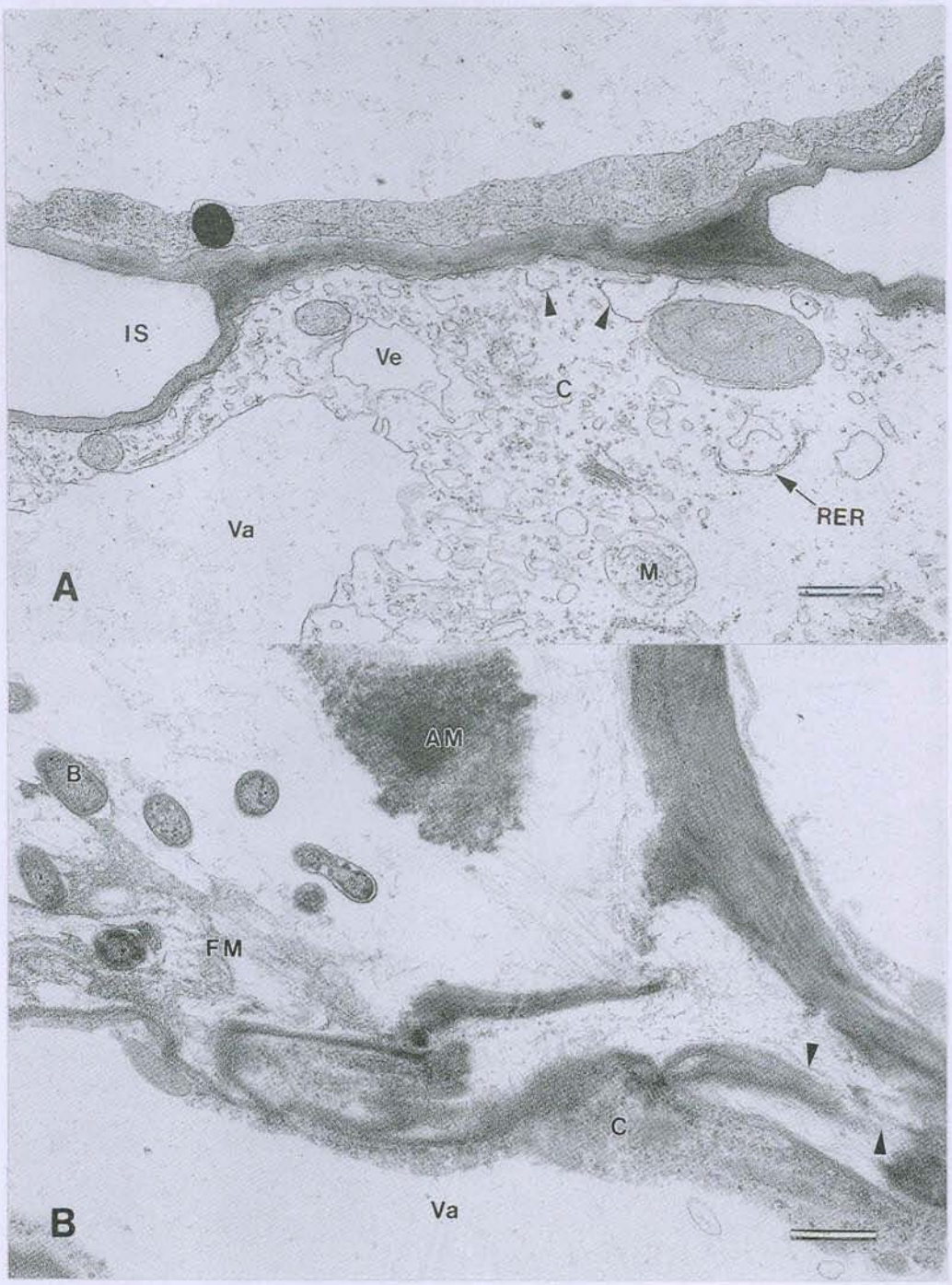

Fig. 7A,B — Leafy spurge responses of *P. fluorescens* LS102 inoculations after 7d (**A**) and 12 d (**B**) (*AM*, amorphous material; *B*, bacterium; *C*, cytoplasm; *FM*, fibrillar material; *IS*, intercellular space; *M*, mitochondrion; *RER*, rough endoplasmic reticulum; *Va*, vacuole; Ve, vesicle). **A.** Convolution of the plasmalemma (arrowhead) and vesiculation of the cytoplasm. **B.** Dissolution of the middle lamella (arrowheads). Bar = 1.0 μm.

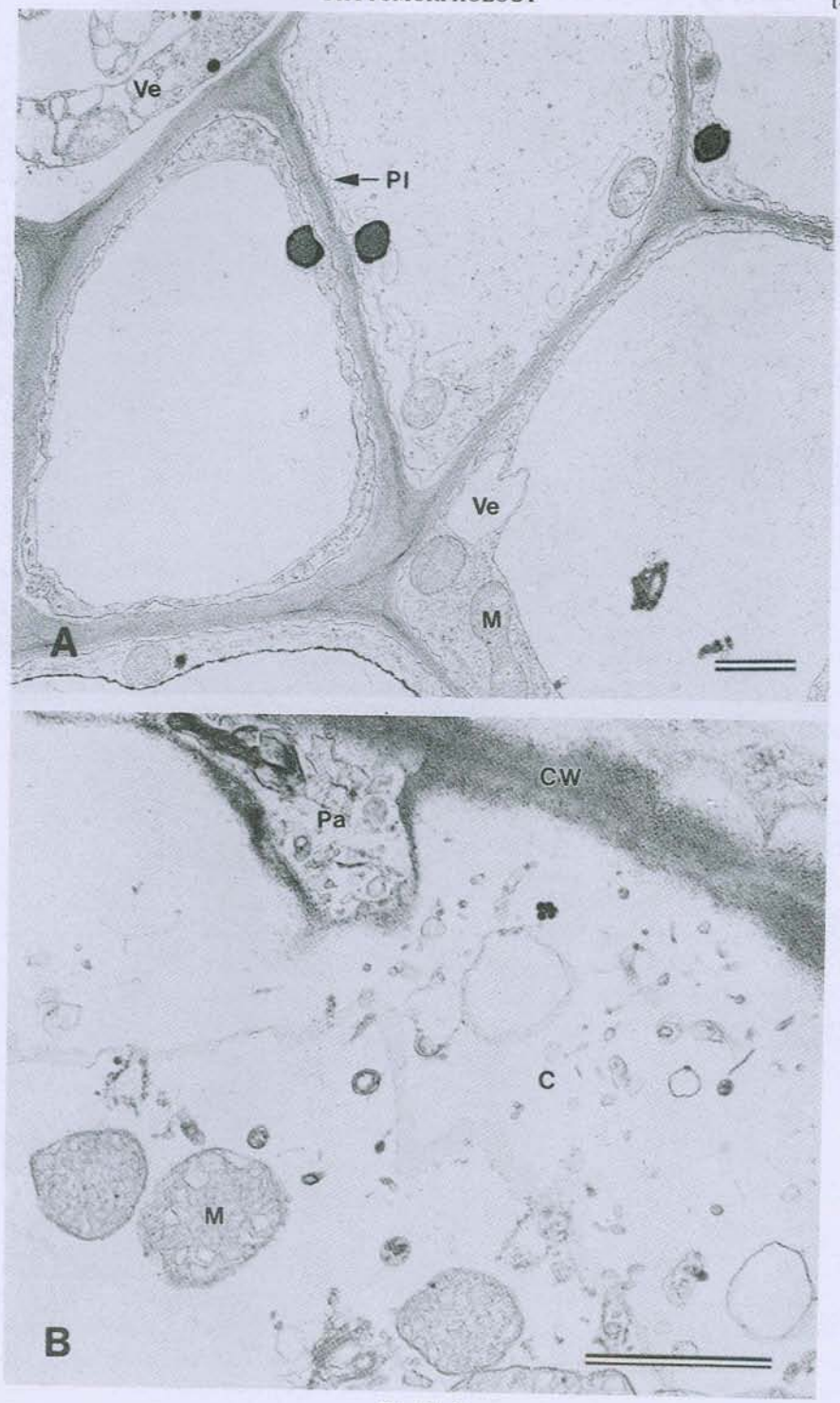

Fig. 8A,B

Colonization of the root surface by bacteria is an essential step to subsequent penetration of the pathogen between cells and to subsequent host damage. Attachment is one of the factors that may contribute to colonization of plant roots. In general, bacterial attachment to plant surfaces begins with a reversible phase, where the microorganism migrates to its host via chemotaxis (Begonia & Kremer 1994). Chemotaxis is followed by a second phase which is irreversible, in which the attached microorganism synthesizes exocellular polymers to anchor the cell to the host plant (Goodman et al. 1986). The occurrence of a lectin-mediated, site-specific attachment of *Rhizobium trifolii* to clover has been reported by Dazzo et al. (1984). Also, evidence for the pili-mediated attachment of bacteria to plant cells is accumulating. In studying the mechanism of *Pseudomonas fluorescens* attachment to plant roots, Vesper (1987) presented evidence for the occurrence of pili on *P. fluorescens* strains, and suggested that their occurrence is correlated with the ability of the bacterium to attach to corn roots. The possible involvement of an outer-membrane (OM) protein, adhesin, from a rhizosphere isolate of *P. fluorescens* in root attachment was reported (DeMot & Vanderleyden 1991). This OM protein selectively adheres to roots and, therefore, may have a potential role in root colonization by *P. fluorescens*. Our results on the effects of isolate LS102 on leafy spurge growth suggests the involvement of one of the above mechanisms in the attachment of LS102 to leafy spurge cells. Such mechanisms would enhance the ability to colonize and adversely affect host tissue as observed by SEM and TEM. The ability of a rhizosphere strain to establish a significant population size along a root system is a key determinant in its effect on plant growth (Suslow 1982). A previous study (Souissi 1994) showed that LS102 was able to colonize the rhizosphere of leafy spurge roots and inhibit their growth by more than 50% in non-sterile soils in greenhouse. The ability of isolate LS102 to establish on developing roots will enhance its rhizosphere competence and increase its efficiency as a biocontrol agent in the field.

Rhizobacteria may affect their hosts in a way similar to classical phytopathogenic bacteria through production of enzymes, toxins, or growth regulating substances (Goodman et al. 1986). At some points of contact between rhizobacteria and leafy spurge cells, the cell wall of the host appeared to be dissolved or eroded. Such alteration possibly indicates the hydrolytic action of specific enzymes produced by the rhizobacteria. Production of an array of wall-degrading enzymes during pathogenesis by pathogenic bacteria has been documented and reported by several investigators (Collmer & Keen 1986, Goodman et al. 1986). When isolates LS102 and LS105 were tested for enzyme production, both isolates produced proteases on nutrient gelatin agar. The role of proteases as virulence factors in pathogenesis has not been intensively examined in the literature. Reddy et al. (1971) detected potential protease activity in alfalfa infected by *Xanthomonas alfalfae*, a leaf-spotting pathogen. The enzyme was active in vitro and its activity increased sharply with the development of the disease in the susceptible clone. Proteases may contribute to virulence by augmenting the action of other enzymes such as those involved in tissue-maceration caused by *Erwinia* spp., or by providing

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Fig. 8A,B — Leafy spurge responses to *F. balustinum* LS105 inoculations after 7 d (A) and 12 d (B). (*C*, cytoplasm; *CW*, cell wall; *M*, mitochondrion; *Pa*, papilla-like structure; *Pl*, plasmalemma; *Ve*, vesicle). **A.** Vesiculation of the cytoplasm. **B.** Loss of structural integrity in organelles and membranes. Note the deposition of papilla in the cell wall. Bar = 1.0 μm.

the bacterium with readily utilizable sources of C and N through the degradation of polymeric substances (Goodman et al. 1986). Enzymes of bacterial origin may serve to weaken and loosen the wall structure enabling rhizobacteria to spread within the tissue. However, the possibility that cellulases and/or pectinases of plant origin may also have been activated at the onset of bacterial attack cannot be ruled out.

Inoculation of leafy spurge seedlings with rhizobacteria in the hydroponic system resulted in root lengths significantly shorter than the control seedlings (Figs 1,2). Additionally, previous results using the host-pathogen interaction system, which exposed leafy spurge callus tissue to bacteria with no physical contact (Souissi & Kremer 1994), suggested the possible involvement of phytotoxins in causing the deleterious effects of rhizobacteria (Suslow & Schroth 1982a, Tranel et al. 1993a,b). Tranel et al. (1993) found that a phytotoxin produced by *Pseudomonas fluorescens* strain D7 may inhibit downy brome root elongation by disrupting lipid synthesis and membrane integrity. Similarly, in the present study, electron micrographs revealed rupture of cell membranes in root tissues of leafy spurge (Fig. 8B).

The formation and liberation of surface vesicles from isolate LS102 observed 12 days after inoculation (Fig. 6D) has also been observed in leaves of french beans (*Phaseolus vulgaris* L.) inoculated with *P. phaseolicola* (Sigee & Epton 1975). Liberation of these vesicles may result in the release into the host tissue of large amounts of material of bacterial origin which would otherwise be retained within the bacterial cells. The possibility of a membrane-associated toxin is worth considering, in view of previous reports implicating extracellular polysaccharides (EPS) as a bacterial toxin and linking it to pathogenesis (Goodman et al. 1986). EPS has been implicated in pathogenicity by contributing to water-soaking (El-Banoby & Rudolph 1979) and by preventing direct cell-cell contact between host and bacteria (Smith & Mansfield 1982).

Closer examination of scanning electron micrographs of leafy spurge root tissues inoculated with isolates LS102 and LS105 revealed the association of these isolates with fibrillar material, probably EPS, on cell surfaces (see Fig. 4A, B). The latter may be involved in the attachment of rhizobacteria to root cells of leafy spurge. Microfibrils elaborated by certain bacterial species may serve to anchor bacteria to plant cell surfaces and entrap additional bacteria (Matthyse et al. 1982). The multiplication of entrapped and attached bacteria may result in the establishment of high populations at the surface of root cells. Microfibrils isolated from *Rhizobium trifolii* and identified as cellulose, enhanced infection by aggregating infective cells and mediating adsorption to the root hair surface with the aid of lectin (Napoli et al. 1975).

Microcolonies were observed on the epidermal surface of root tissue inoculated with isolate LS102. These microcolonies are relatively common on an infected root and can be detected early in the infection. Formation of microcolonies suggests the occurrence of preferred sites of bacterial replication and subsequent invasion. The occurrence and attraction of aggregates of *Rhizobium meliloti* to preferred sites on the surface of alfalfa roots has been described and found to have a role in the infection of host legume roots by rhizobia (Gulash et al. 1984). Preferred sites of replication have also been reported for the association of *P. fluorescens* with canola seedlings (Campbell et al. 1987) and for association of *P. putida* with velvet leaf seedling roots (Begonia et al. 1990).

The accumulation of cells of isolates LS102 and LS105 in microcolonies surrounded by electron dense granular-fibrillar material (Fig. 6A) was similar to the physical entrapment of

bacteria in bean leaves described by Hildebrand et al. (1980). Physical entrapment of *P. phaseolicola* cells was observed in both resistant and susceptible bean cultivars (Nesbat & Slusarenko 1983). However, cells of *P. phaseolicola* were in general more evenly distributed throughout the intercellular spaces in the susceptible host than the resistant host. This agrees with the observed distribution of bacteria in the intercellular space of infected leafy spurge tissue. Smith & Mansfield (1982) similarly found greater amounts of electron dense fibrillar material filling the intercellular space in the compatible interaction between pseudomonads and oat leaves. They attributed this to the bacterially-produced acidic EPS.

Accumulation of the fibrillar granular material surrounding bacteria has been reported as a typical feature of infection in plants invaded by vascular wilt bacteria (Figs 4A,B, 7A, B). Infection of tomato leaf tissues with *Clavibacter michiganense* subsp. *michiganense*, a vascular wilt pathogen, was accompanied by marked cell damage and the formation of granular or fibrillar material at sites where bacteria were actively growing (Benhamou 1991). The literature available on the origin and nature of these accumulating substances is highly controversial. While some authors consider this material to be composed mainly of EPS of bacterial origin (Brown & Mansfield 1988), others reported that the material surrounding invading bacteria was, at least partly of host origin (Benhamou 1991, Huang & Van Dyke 1978). The amount of cell wall disruption observed during the present study appears insufficient to conclude that all the fibrillar and granular material present originated from the host. It is probable that a proportion of this material is bacterially-produced EPS and that interaction of EPS with plant polysaccharide leads to bacterial attachment to the root cell wall surface (Nesbat & Slusarenko 1983).

The origin and nature of the dark-granular material associated with the fibrillar material is unknown (see Fig. 6A, C). Cytochemical analysis of similar material in tobacco leaf inoculated with *P. solonacerum* indicated the presence of phenolics and possibly tannin (Obukowicz & Kennedy 1981). These authors postulated a role for polyphenoloxidase acting on phenolic metabolites at the host cell surface. Numerous reports exist on the accumulation of phenolics in plant tissue during pathogenesis or hypersensitive reaction (HR) (Sutic & Sinclair 1991). Root-colonization of bean plants with *P. fluorescens* REW1-l-1, a plant growth promoting rhizobacterium, resulted in the alteration of defense responses of plants and in accumulation of phenolics and phytoalexins in bean cotyledons (Zdor & Anderson 1992).

Attempts to analyze phenolic acids in leafy spurge tissue inoculated with isolate LS102 showed formation of greater quantities of vanillic acid than found in the control tissue (data not shown). Increased vanillic acid concentration in leafy spurge cells affected by isolate LS102 may be an indicator of host/pathogen interaction and suggests that phenolic compound content could be useful in assessing effectiveness of biocontrol pathogens on host weeds. Further, Hoagland (1990) suggests that secondary metabolites such as phenolic acids could improve biological weed control by acting as "pathogen synergists".

In the present study, the effects of selected deleterious rhizobacteria on growth of leafy spurge at the cellular level have been demonstrated. The rhizobacteria colonize plant cell surfaces and apparently release phytotoxic substances that cause cellular aberrations, that result in seedling growth inhibition. A previous study where biocontrol activity of isolates LS102 and LS105 on leafy spurge seedlings was evaluated in non-sterile soil in greenhouse experiments, showed reductions in root length and shoot dry weights by over 65 and 70%, respectively and a decrease in seedling emergence by more than 50% of the control

(Souissi 1994). Results from the present and previous study indicate that rhizobacteria are able to establish and colonize root surfaces of leafy spurge seedlings. Bacterial growth along and colonization of the root are important traits that may enhance the efficiency of rhizobacteria in suppressing plant growth under field conditions.

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