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Ultrastructure of *in vivo* interactions of the antagonistic bacteria *Bacillus cereus* X16 and *B. thuringiensis* 55T with *Fusarium roseum* var. *sambucinum*, the causal agent of potato dry rot

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Summary. The interaction of *Fusarium roseum* var. *sambucinum*, the causal agent of potato dry rot, with two antagonistic bacteria, *Bacillus cereus* X16 and *B. thuringiensis* 55T, was studied on wounded potato tubers using light and electron microscopy. Application of *B. cereus* X16 or *B. thuringiensis* 55T to potato wounds before challenge with the pathogen suppressed dry rot and restricted fungal growth in plant tissues to the first few cell layers beneath the site of inoculation. Both bacterial antagonists penetrated into potato tissues and established themselves through intercellular and intracellular proliferation. The extent of *Fusarium* colonization was appreciably reduced in the bacterized tubers, and most fungal cells in these tubers were severely damaged, with appreciable morphological and structural changes. In potato tubers bacterized by *B. thuringiensis* 55T, *Fusarium* invasion of the host tissues did not stimulate structural host reactions, and direct parasitism, which operates by degradation of the fungal cell walls and disintegration of the fungal cytoplasm, seemed to play a key role in the antagonism against *Fusarium* hyphae. In potato tubers inoculated with *B. cereus* X16 and challenged with the pathogen, on the other hand, a set of defense reactions, were triggered, including modifications of the primary cell walls and the occlusion of some cells and vascular tissues with different types of electron-opaque materials. Fungal hyphae in the vicinity of these barriers, apparently containing higher than usual levels of phenol-like compounds, usually showed advanced stages of disorganization, suggesting the existence of a fungitoxic environment. The results presented here show that the two antagonistic bacilli use different biocontrol strategies to suppress Fusarium dry rot development.

Key words: *Bacillus*, biocontrol, induced resistance.

Introduction

Dry rot caused by *Fusarium roseum* var. *sambucinum* is a serious threat to potatoes and causes important crop losses under cold and traditional storage conditions (Daami-Remadi and El Mahjoub, 1996; Carnegie *et al.*, 1998; Chérif *et al.*, 2000). Since few practical methods for controlling this postharvest pathogen are available, studies were conducted in our laboratory to isolate and select *Bacillus* spp. effective in the biocontrol of potato dry rot (Sadfi *et al.,* 2001). Accumulating evidence from recent studies indicates that *Bacillus* spp. have the potential to be effective against different pathogenic fungi, and are readily amenable to formulation owing to their resistant endospores, as well as being safe for the environment (Pleban *et al.*, 1995; Sholberg *et al.*, 1995; Podile and Laxmi, 1998; Walk-

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er *et al.*, 1998). However, the mechanisms by which these bacteria achieve their antagonistic effect are not fully understood. The biological activity of antagonistic bacteria may involve nutrient competition, site exclusion, antibiosis, production of antifungal factors and the induction of plant resistance to pathogen attack (Liu *et al.*, 1995; Tuzun and Kloepper, 1995; Benhamou *et al.*, 1996a; Glick and Bashan, 1997). Benhamou *et al.* (1996b) reported that the endophytic bacterium *Bacillus pumilus* strain SE34 induced various host reactions, including strengthening of plant cell walls and the accumulation of phenolics, in pea roots infected with the root-rot fungus *F. oxysporum* f. sp. *pisi*. Indeed, it has become more and more apparent that resistance induced by bacterial biocontrol agents is associated with marked metabolic changes in the host, involving physical and biochemical responses restricting pathogen development in the plant tissues (Liu *et al.*, 1995; Podile and Laxmi, 1998; Raupach and Kloepper, 1998). However, in spite of the large research effort expended, little has been reported about cytological and cellular host–pathogen interactions following bacterization with *Bacillus* spp. Moreover, the mechanisms by which these bacteria trigger the plant defense system are not fully elucidated. A better understanding of these mechanisms will enable more effective methods of selecting, formulating and applying *Bacillus* antagonists to be developed.

We recently reported that treatment with two antagonists, *B. cereus* X16 and *B. thuringiensis* 55T, significantly reduced dry rot caused by *F. roseum* var. *sambucinum* on potato tubers (Sadfi *et al.*, 2001). Antagonism tests in dual cultures on agar plates seemed to indicate that *B. cereus* X16 was more antagonistic to the pathogen than *B. thuringiensis* 55T, as shown by the formation of a strong inhibition zone by the former (Sadfi *et al.,* 2001). Nevertheless, more recent ultrastructural and cytochemical investigations revealed that *B. thuringiensis* 55T, which had appeared macroscopically ineffective, resulted in extensive damage to fungal cells on an agar medium, leading to complete degradation of the *Fusarium* cell walls and cytoplasm depletion (Chérif *et al.*, 2002). Such microscopic alterations were seldom seen with *B. cereus* X16, suggesting that the metabolites excreted by this antagonist in the agar medium had a merely fungistatic effect on the pathogen. However, Chérif *et al.* (2002) showed that both *Bacillus* antagonists greatly altered the pathogen when antagonism tests were performed in a liquid medium, where intimate contact between the two organisms is ensured. These fungal alterations suggested that there was production of antibiotics and hydrolytic enzymes, particularly chitinases, by the two bacterial antagonists. To further define to what extent these mechanisms observed *in vitro* are implicated in pathogen control *in planta*, it seemed of interest to undertake similar cytological and ultrastructural investigations on potato tubers. This will also bring answers to questions regarding the relationship between the plant cell, the antagonistic bacteria, and the challenging pathogen.

Materials and methods

Fungal culture and growth conditions

The isolate of *F. roseum* var. *sambucinum* used in the present study was obtained from potato tubers showing typical symptoms of Fusarium dry rot. This isolate was virulent on potato tubers (Chérif *et al.*, 2000), and was routinely grown on potato dextrose agar (PDA) medium at 25°C.

Bacterial culture and growth conditions

The strain of *B. cereus* X16 was selected among a collection of 83 *Bacillus* spp. isolated from samples of salty soils collected from different locations in the south of Tunisia. This strain strongly inhibited mycelium growth of *F. roseum* var. *sambucinum* on nutrient agar (NA, Oxoid, Basingstoke, UK) by forming a very conspicuous inhibition zone, presumably due to the excretion of diffusible inhibitory metabolites by the bacterium (Chérif *et al.*, 2002). *B. cereus* X16 was also very effective in controlling dry rot on potato tubers (Sadfi *et al.*, 2001; Chérif *et al.*, 2002).

Strain 55T of *B. thuringiensis* was selected among five *B. thuringiensis* strains kindly provided by A. Boudabous from the Microbiology Laboratory of the Faculté des Sciences de Tunis, Tunisia. Although it appeared that none of the five *B. thuringiensis* strains inhibited the growth of *F. roseum* var. *sambucinum* on agar medium,

since inhibition zones were not observed with them in dual cultures, strain 55T significantly reduced Fusarium dry rot on wounded potato tubers (Sadfi *et al.*, 2001; Chérif *et al.*, 2002).

Both bacterial antagonists were maintained on NA slants at 4°C and subcultured at two-month intervals. To produce bacterial suspensions for potato tuber inoculation, bacteria were streaked on NA and incubated at 25°C. After incubating *B. cereus* X16 and *B. thuringiensis* 55T for 24 h and 48 h respectively, bacterial cells were suspended in sterile distilled water and the concentration adjusted to 10^6 cfu ml^{-1}.

Potato tuber inoculation

Potato tubers cv. Spunta were surface-sterilized (2% aqueous sodium hypochlorite for 10 min), rinsed with sterile distilled water, wounded by removing a plug 3 mm in diameter and 3 mm in depth with a sterile cork borer, and inoculated by depositing 20 ml of one of the bacterial suspensions on each wound. Potato wounds challenged with the pathogen received 20 ml of a conidial suspension (10^5 macroconidia ml^{-1}) of *F. roseum* var. *sambucinum*. Controls consisted in inoculation of wounded potato tubers with sterile distilled water, the fungal pathogen or either of the bacterial antagonists alone. The treated wounds were sealed with adhesive tape and the tubers placed in plastic bags to maintain high humidity and were incubated at 20°C. Each treatment was applied to four replicates of 5 potato tubers, and the experiment was repeated twice.

Light and transmission electron microscopy (TEM)

Samples (2 mm^2) were carefully excised from control and inoculated potato tubers at sites of inoculation and of potential pathogen penetration after 7 days of incubation, by which time well-developed macroscopic dry rot lesions approximately 20 mm in diameter were visible in the *Fusarium*-inoculated controls. Samples were immersed overnight in 3% (v:v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. They were subsequently postfixed with 1% (w:v) osmium tetroxide in the same buffer for 1 h at ambient temperature and dehydrated in a graded ethanol series prior to embedding in Epon 812. For light microscopy, thin sections (0.25 to 0.5 mm) were collected on microscope slides and stained with methylene blue. All slides were examined and photographed with a Zeiss Axioscope microscope (Carl Zeiss, Thorwood, NY, USA). For TEM, ultrathin sections were collected on 200-mesh nickel grids coated with formvar and stained with uranyl acetate and lead citrate. Grids were examined with a JEOL 1200 EX transmission electron microscope (JEOL Ltd., Tokyo, Japan) operating at 80 kV. For each treatment (bacterium alone, pathogen alone, combination of bacterium and pathogen), an average of five samples from four potato tubers were examined, using more than four sections per sample.

Results

Potato tubers inoculation

Inoculation of potato tubers with *F. roseum* var. *sambucinum*, with or without bacterial antagonists, showed results similar to those described in earlier reports (Sadfi *et al.*, 2001; Chérif *et al.*, 2002). In the control tubers infected with the pathogen alone, brown lesions were visible by the third day of storage at 20°C; on the seventh day the diameter of these lesions ranged from 20 to 25 mm. Treatment of potato wounds with *B. cereus* X16 or *B. thuringiensis* 55T prior to inoculation with the fungal pathogen significantly reduced dry rot development as compared with the controls. With *B. cereus* X16 antagonist treatment, no sign of *Fusarium* infection was detected by the seventh day of incubation. By that time, fungus-inoculated potatoes bacterized with *B. thuringiensis* 55T had some small, brownish lesions, but their frequency and diameters never reached those observed in the controls. Potato tubers inoculated with *B. cereus* X16 or *B. thuringiensis* 55T, and not infected with the pathogen, showed no symptoms and appeared healthy throughout the experiment.

Microscopic observations in the controls

Under the light microscope, sections from potato tubers bacterized with *B. cereus* X16 or *B. thuringiensis* 55T, and not challenged with *Fusarium*, showed that the bacteria grew actively at the wound site and colonized a few intercellular spaces in the outer potato cell layers. Electron microscopy examination of these tissues revealed structural features similar to those of the

unbacterized and unchallenged controls. Bacteria were often and mainly observed in the intercellular spaces of the outermost host tissue layers, and their presence was not associated with particular cell reactions as compared to control potato tubers which were wounded but not inoculated with the bacteria.

Light microscope examination of thin sections from lesions that developed on potato tubers infected with the pathogen alone showed massive colonization of the tissues by fungal hyphae. Invading *Fusarium* hyphae ramified inter- and intracellularly throughout potato tissues beneath the wound site. These hyphae appeared healthy and were densely stained with methylene blue. Ultrastructural observations showed that the cell walls of the invaded host cells and those adjacent to them were often severely damaged, with swelling, shredding and breakdown (Fig. 1A, B). Fungal colonization of vascular tissues was often associated with a noticeable degradation of the secondary walls and with marked disorders of the paratracheal parenchyma cells (Fig. 1C). During the whole process of colonization, fungal hyphae, whether in the parenchymatic tissues or in the vascular area, appeared normal, with a well-preserved and densely stained cytoplasm, and without signs of cellular disorganization (Fig. 1A-D). Invasion of tissues and vessels was not accompanied by the formation of defense barriers such as wall appositions and thickenings, or the occlusion of xylem elements.

Microscope examination of bacterized potato tissues challenged with the pathogen

Bacterization with Bacillus thuringiensis *55T*

Light microscope examination of *Fusarium*-infected samples bacterized with *B. thuringiensis* 55T showed that fungal hyphae in potato tissues beneath the site of inoculation were severely damaged, as shown by their failure to stain densely with methylene blue. In spite of this, however, the pathogen was able to escape bacterial control and invade the potato tissues. Nevertheless, this fungal invasion remained restricted to the 10–12 outermost cell layers beneath the site of inoculation.

The ultrastructural examination under the light microscope showed that *Fusarium* hyphae penetrated the potato tissues (Fig. 2A, B) and, by the seventh day of infection, abundantly colonized the topmost layers of cells beneath the site of infection. Even so, most of these fungal cells were severely damaged, with appreciable morphological and structural changes (Fig. 2B–E). *B. thuringiensis* 55T penetrated potato tissues and multiplied intercellularly (Fig. 2A), intracellularly (Fig. 2B, D), and even within the vascular tissue (Fig. 2E). Most pathogen hyphae in the vicinity of bacterial cells exhibited pronounced alterations, including cytoplasm disorganization (Fig. 2B, E), cell wall degradation (Fig. 2C), and often loss of the protoplast (Fig. 2B, C, D).

Bacillus thuringiensis 55T was also observed in infected potato tissues as resistant endospores (Fig. 3A, B, arrows). Endospores were generally oval, central, darkly stained and covered with a typical spore coat and exosporium (Fig. 3A). Fungal cells near these endospores were completely destroyed (Fig. 3A, B).

In potato tubers bacterized with *B. thuringiensis* 55T, *Fusarium* invasion of the host tissues failed to trigger host reactions such as wall appositions, intercellular plugging, intracellular deposits, or xylem vessel occlusions (Fig. 2A–E).

Bacterization with Bacillus cereus *X16*

Seven days after inoculation, light microscope observations on about 50 sections from more than 10 potatoes revealed that fungal growth in potato tissues bacterized with *B. cereus* X16 was restricted to the first four to six outer cell layers beneath the site of inoculation. In these invaded tissues hyphae of the pathogen were not stained with methylene blue as they were in the controls.

Electron microscopy showed that *B. cereus* X16 cells, like *B. thuringiensis* 55T, were frequently found in bacterized potato tissues, either in the form of vegetative cells (Fig. 4B), or as darkly stained endospores (Fig. 4C). *Fusarium* hyphae in bacterized potato tissues generally exhibited advanced stages of cytoplasm disorganization and damage (Fig. 4A–C). These alterations were generally correlated with the formation and deposition of a thick layer of osmiophilic polymorphic material at the level of the parenchymatic tissues (Fig. 4C). This electron-dense material usually separated the pathogen from the host wall (Fig. 4C and 5A) and in many in-

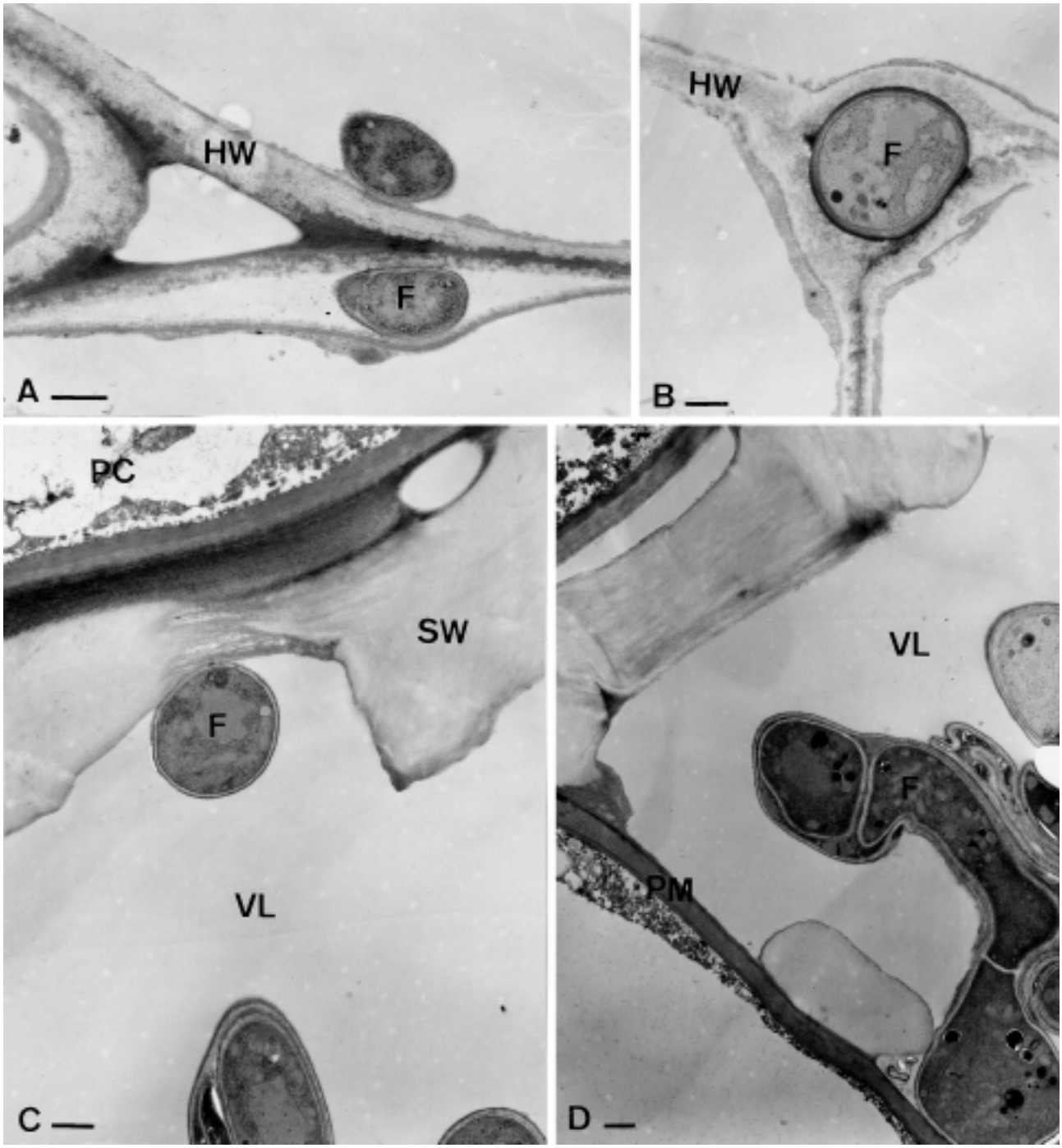

Fig. 1. TEM sections of control potato cv. Spunta tissues 7 days after inoculation with *Fusarium roseum* var. *sambucinum*. (A) Fusarium hyphae invading potato tissues intracellularly and intramurally; (B) intercellular colonization accompanied by severe damage to potato cell walls; (C, D), colonization of vessels of potato tubers by *Fusarium* hyphae. Fungal hyphae appear healthy with well-preserved cell walls and densely stained cytoplasm. Bar = 1 mm. F, fungus; HW, host wall; PC, parenchyma cell; PM, pit membrane; SW, secondary wall; VL, vessel lumen.

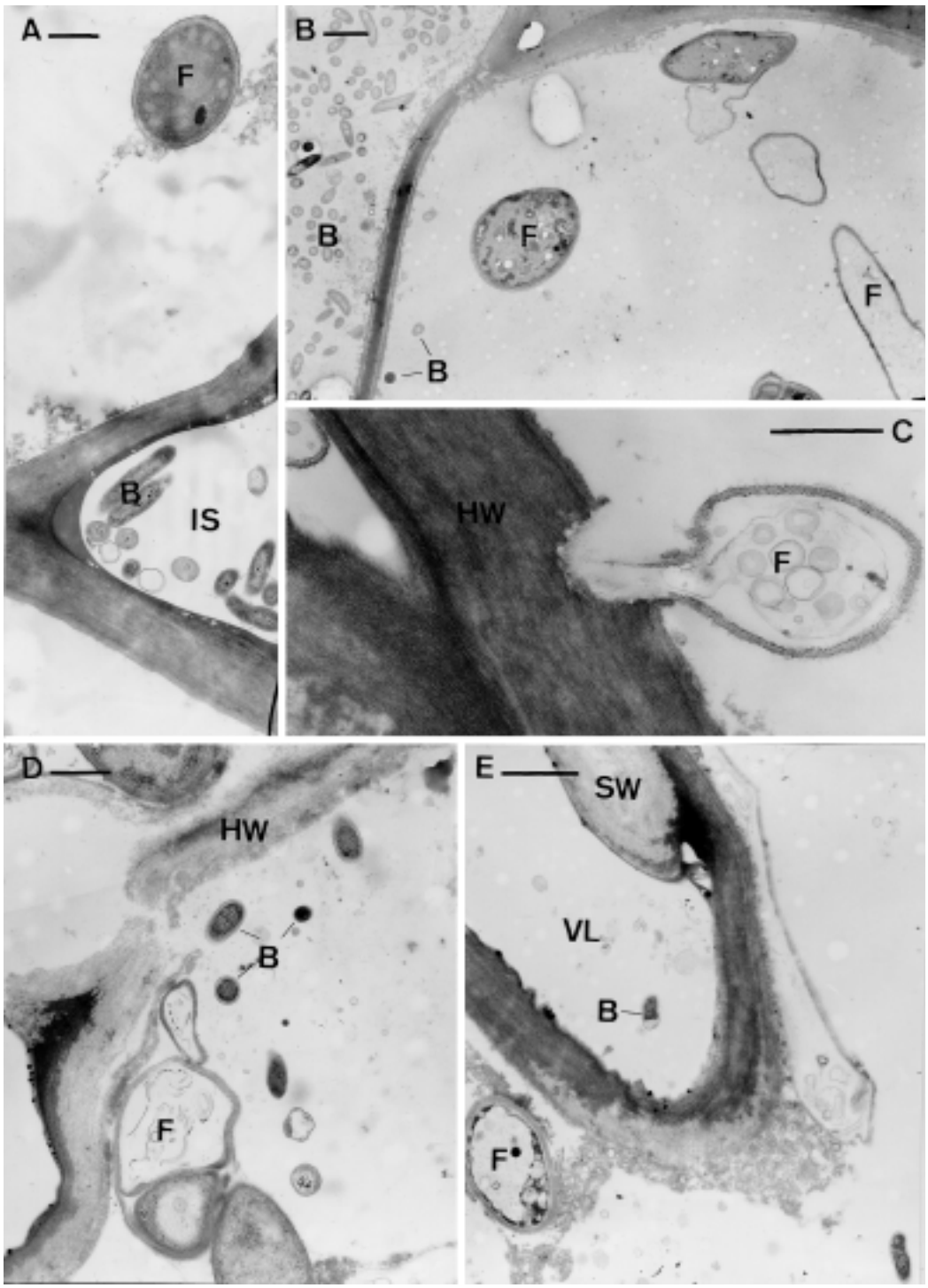

Fig. 2. TEM sections of potato cv. Spunta tissues 7 days after bacterization with *Bacillus thuringiensis* 55T and challenge with *Fusarium roseum* var. *sambucinum*. Bacterial vegetative cells are present intercellulary (A), intracellularly (B, D), and in the vascular tissues (E). Most fungal cells near the bacterium show severe damage (B–E) and cell-wall breakdown (C). Note the absence of typical structural host reactions (A–E). Bar = 1 mm. B, bacterium; F, fungus; HW, host wall; IS, intercellular space; SW, secondary wall; VL, vessel lumen.

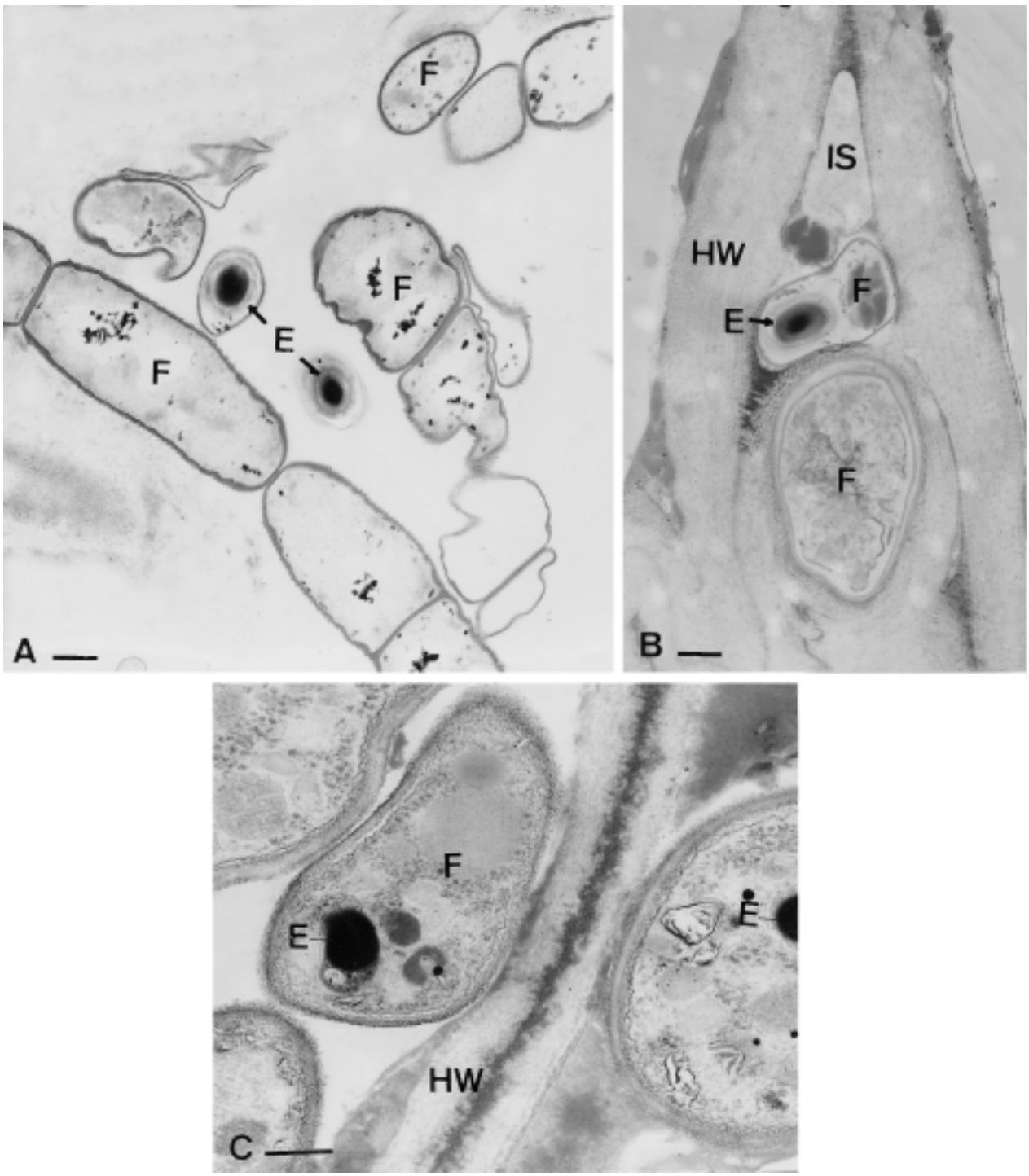

Fig. 3. TEM sections of potato cv. Spunta tissues 7 days after bacterization with *Bacillus thuringiensis* 55T and challenge with *Fusarium roseum* var. *sambucinum*. (A, B, C) Bacterial endospores in potato tissues colonized by the pathogen. Bar = 500 nm. E, endospore; F, fungus; HW, host wall; IS, intercellular space.

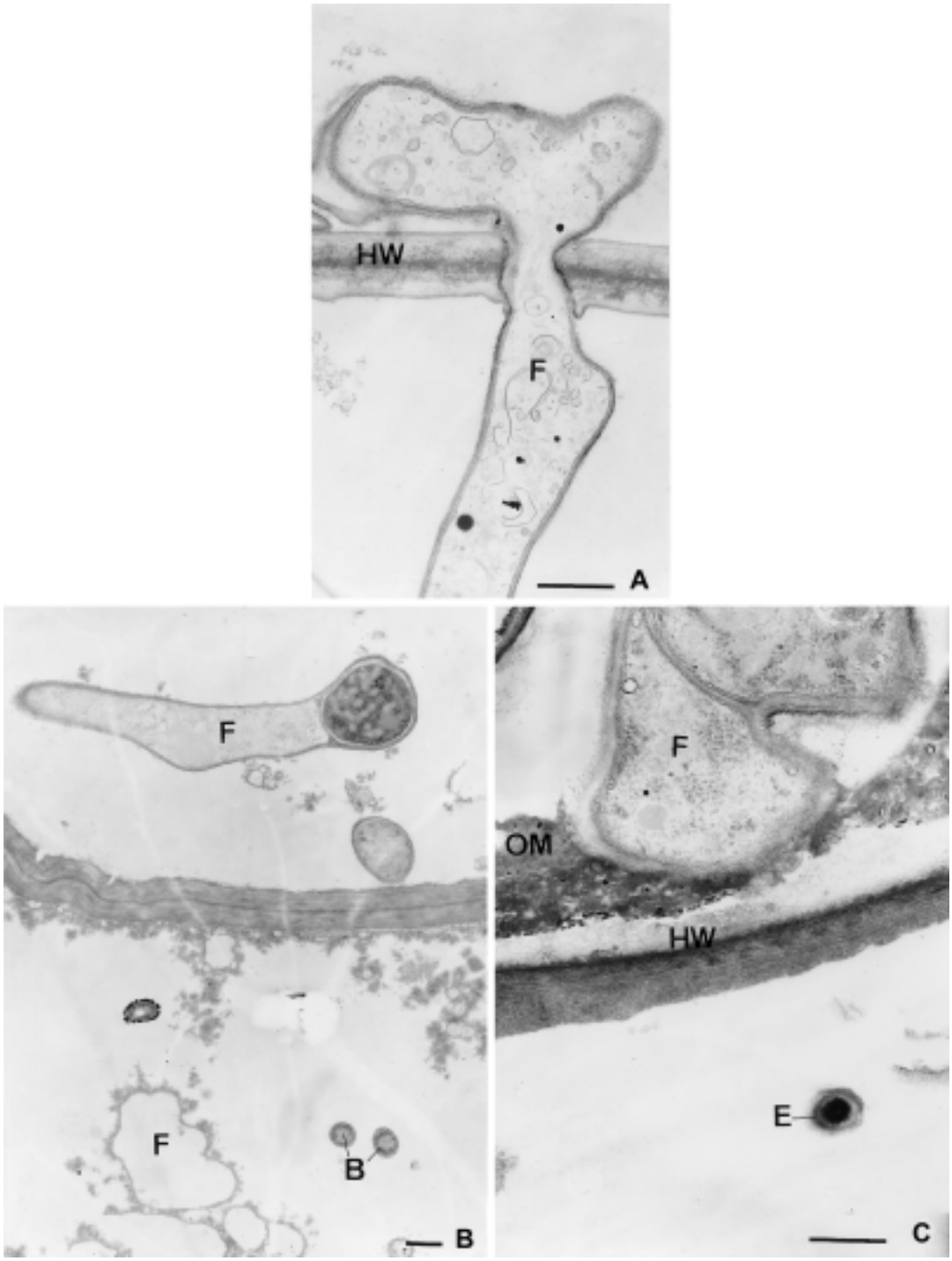

Fig. 4. TEM sections of potato cv. Spunta tissues 7 days after bacterization with *Bacillus cereus* X16 and challenge with *Fusarium roseum* var. *sambucinum*. An antagonistic bacterium observed as vegetative cells (B) or as a resistant endospore (C) in host tissues colonized by the fungal pathogen; (A) fungal hyphae exhibiting marked alterations; (C) a thick layer of osmiophilic polymorphic material deposited between the fungus and the primary host cell wall. Bar = 1 mm. B, bacterium; E, endospore; F, fungus; HW, host wall; OM, osmiophilic material.

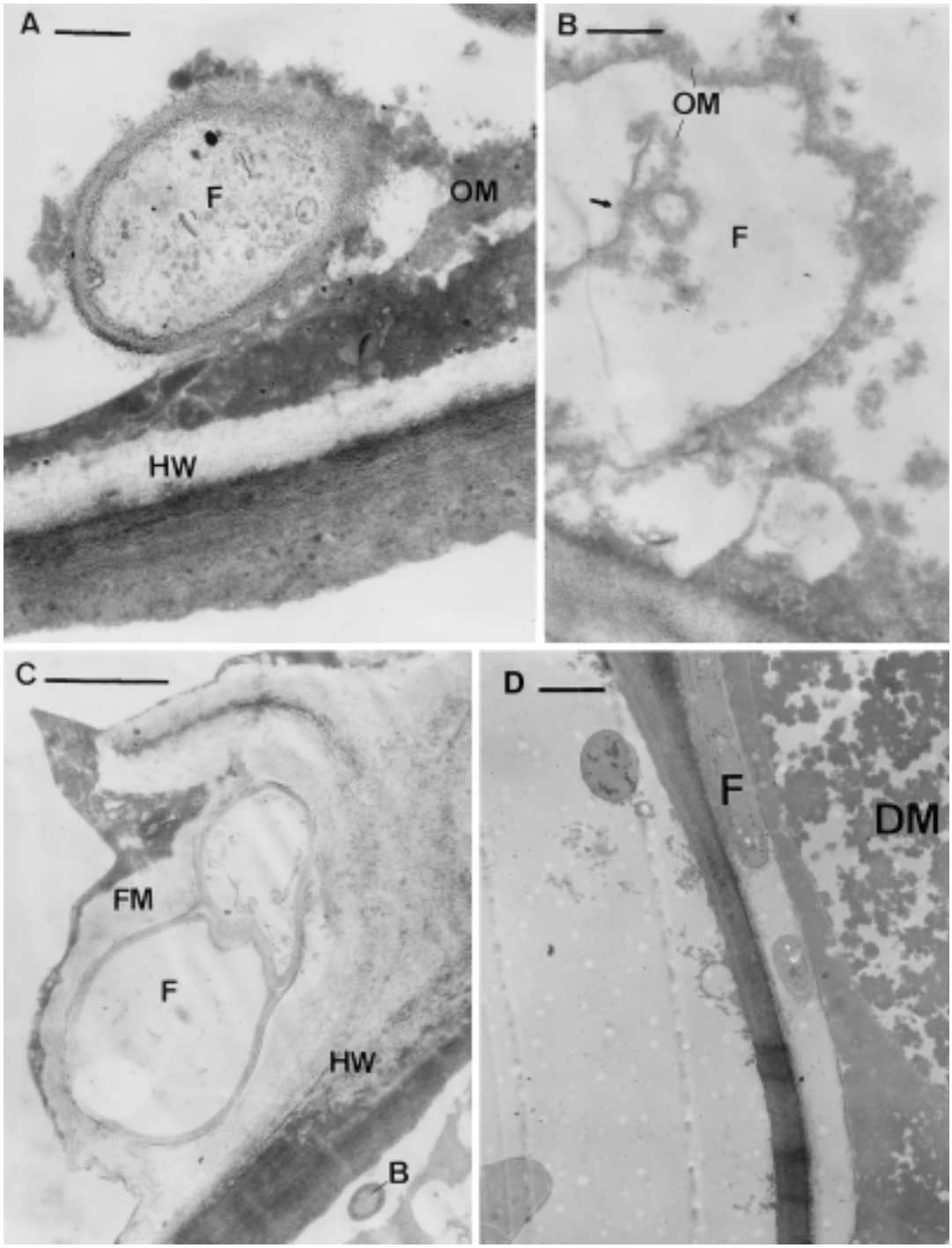

Fig. 5. TEM sections of potato cv. Spunta tissues 7 days after bacterization with *Bacillus cereus* X16 and challenge with *Fusarium roseum* var. *sambucinum*. (A) An electron dense layer of material between the fungus and host primary cell wall; this material partially encircles the fungal cell; (B) dead cells of *Fusarium* completely surrounded by osmiophilic material, which also occurs in the cells; (C) dead *Fusarium* hyphae trapped by fibrillar material; (D) a host cell occluded by densely stained material. A and B, bar = 500 nm; C and D, bar = 2 mm. B, bacterium; DM, dense material; F, fungus; FM, fibrillar material; HW, host wall; OM, osmiophilic material.

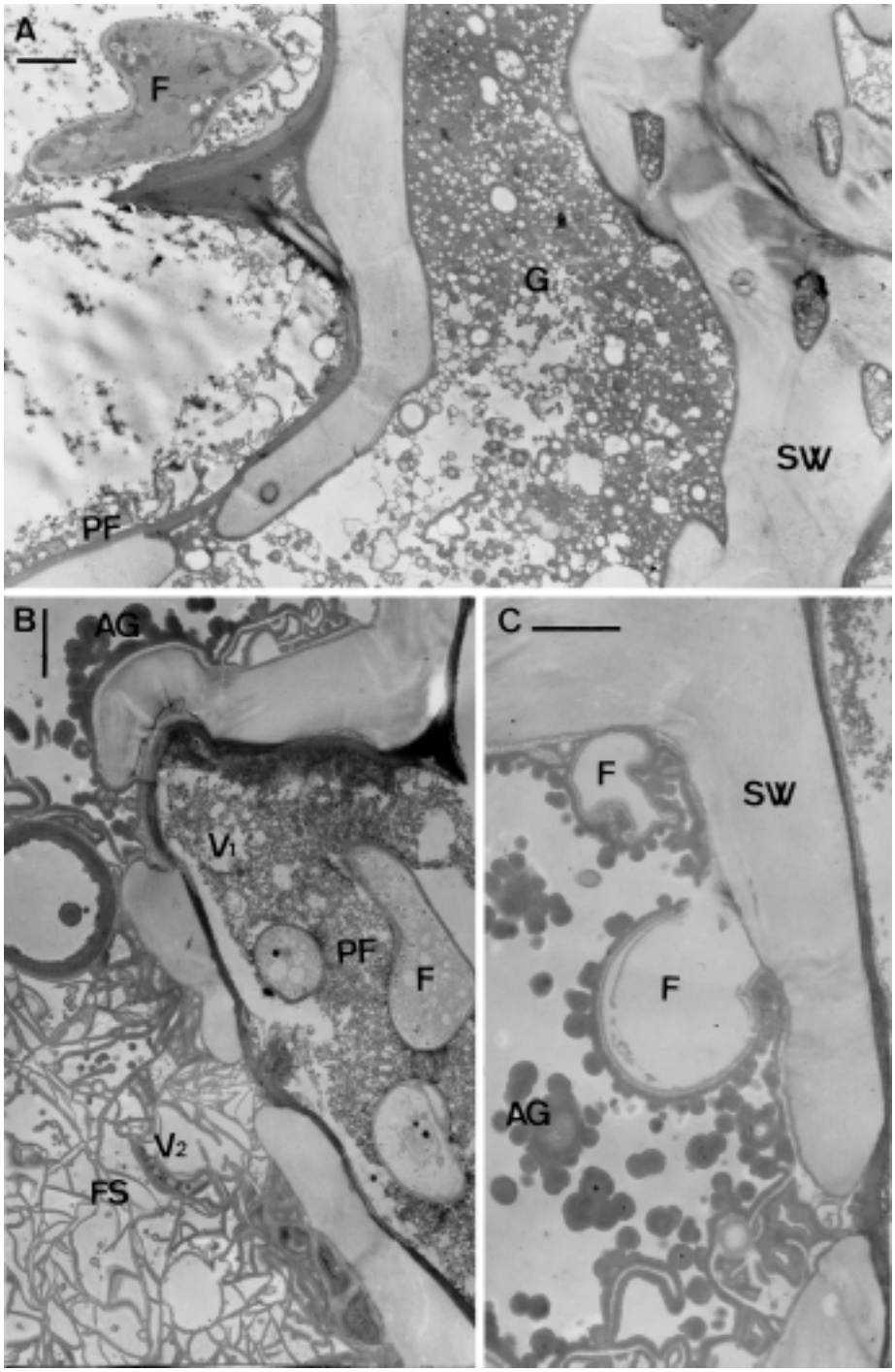

Fig. 6. TEM sections of vascular tissues of potato 7 days after bacterization with *Bacillus cereus* X16 and challenge with *Fusarium roseum* var. *sambucinum*. (A) Densely packed globules in potato vessels; (B) vessel lumina occluded by polymerized polymorphic flecks in V1 and by filamentous densely stained structures and amorphous osmiophilic aggregates in V2; (C) aggregates apparently responsible for damage caused to dead fungal hyphae in the vessel lumen. Bar = 2 mm. AG, aggregates; F, fungus; FS, filamentous structures; G, globules; PF, polymorphic flecks; SW, secondary wall; V, vessel.

stances encircled the fungal cells partially (Fig. 5A) or completely (Fig. 5B). The dense material was also observed in severely damaged *Fusarium* cells (Fig. 5B, arrow). Severely damaged *Fusarium* hyphae were frequently trapped by a fibrillar material (Fig. 5C). Among other features of the host-response to *Fusarium* in tissues bacterized with *B. cereus* X16 was the plugging of some host cells by a densely stained material, which seemed to be the result of the accumulation and polymerization of polymorphic electron opaque flecks (Fig. 5D).

When the wounds made on the potato tubers coincided with vascular areas, so that *B. cereus* X16 and the pathogen were inoculated near the vessels, various reactions occurred (Fig. 6A–C). Pathogen penetration of the vessels was accompanied by an accumulation of densely packed globules, as well as by the deposition of polymorphic electron opaque flecks in the vessel lumen (Fig. 6A). Secondary walls and pit membranes of xylem vessels were coated with a thick layer of dense material. These vessels were usually occluded by materials that included polymerized polymorphic flecks, osmiophilic amorphous aggregates, and densely stained filamentous structures (Fig. 6B). *Fusarium* hyphae trapped in this accumulating material usually displayed marked changes in their ultrastructure (Fig. 6B). Aggregated amorphous deposits often accumulated at the cell surface of such altered invading hyphae (Fig. 6C). These deposits resulted in protoplasm depletion of *Fusarium* cells (Fig. 6C).

Structural reactions similar to those observed in *B. cereus* X16-bacterized potato tissues challenged with the pathogen were lacking in the controls, or in potato tubers inoculated with the bacterium alone.

Discussion

The data reported here provide evidence for the first time that inoculation of potato wounds with *B. cereus* X16 increases the resistance of potato tubers to *F. roseum* var. *sambucinum*, with decreased pathogen growth and viability, and the formation of physical barriers and a marked accumulation of new apparently fungitoxic products in the host. However, the possibility of antibiosis and parasitism of *Fusarium* hyphae by *B. cereus* X16 in potato tissues cannot be ruled out. A fungistatic effect of the bacterium, similar to that observed *in vitro* on an agar medium, or a fungitoxic effect causing fungal cell wall degradation, similar to that observed in a liquid medium (Chérif *et al.*, 2002), may be functioning *in planta* as well.

Although cells of both bacteria penetrated and colonized the outermost potato tissues, they did not induce degenerative changes in the host cells or defense reactions of any type. Expression of structural defense reactions occurred only in tubers treated with *B. cereus* X16 and challenged with the fungal pathogen. Nevertheless, since these reactions did not occur in the controls infected with the pathogen alone, it is likely that the bacterium sensitise the plant to respond to a potential attack without causing an accumulation of defense products. Similar conclusions have been reached by different authors studying the effect of endophytic bacteria, mycorrhizal fungi and abiotic agents such as chitosan and silicon on plant diseases (Benhamou *et al.,* 1994a, b; Chérif *et al.,* 1994; M'Piga *et al.,* 1997).

The current investigation demonstrated that *B. thuringiensis* 55T invades and colonizes potato tissues challenged with *F. roseum* var. *sambucinum*. Bacterial proliferation was often accompanied by extensive alteration of the fungal hyphae near the bacterial cells. Most fungal cells in any case showed marked structural changes, including wall disintegration, cytoplasm disorganization, and often the loss of the protoplast. Although the basic mechanisms behind such alterations are not clearly defined, it is possible that *B. thuringiensis* 55T antagonizes the fungal hyphae by competition, antibiosis and parasitism operating synergistically *in planta*. Recently, we showed that this antagonist produced different types of chitinases, N-acethyl-β-D-glucosaminidases, chitobiosidases and endochitinases (Sadfi *et al.*, 2001) and caused appreciable fungal cell wall degradation and particularly chitin breakdown (Chérif *et al.*, 2002).

This is the first report indicating that bacterization of potato wounds with *B. cereus* X16 induces various structural defense responses following challenge with the potato dry rot agent, *F. roseum* var. *sambucinum*. Our finding that the potato defense response was not induced after

bacterization with *B. thuringiensis* 55T is completely consistent with the idea that bacterial-mediated induced resistance is dependent upon the inducing bacterial species and strain (Tuzun and Kloepper, 1995). Our cytological investigations seem to indicate that direct antimicrobial activity and the parasitism of fungal hyphae are less common as biocontrol mechanisms with *B. cereus* X16 than with *B. thuringiensis* 55T. Several investigations have shown recently that direct antibiosis, parasitism and competition are not involved in the biocontrol that various bacterial antagonists exercise against different pathogenic fungi, including *Fusarium oxysporum* f. sp. *cucumerinum* (Liu *et al.*, 1995) and *Colletotrichum orbiculare* (Wei *et al.*, 1991), causing respectively Fusarium wilt and anthracnose of cucumber. This was revealed by experiments in which the pathogen and the bacterial biocontrol agent were kept spatially separated throughout the bioassay (Kroon *et al.*, 1991; Liu *et al.*, 1995). More recent studies have confirmed that several endophytic and plant-growth promoting bacteria elicit structural and biochemical responses in plant tissues challenged with *Fusarium* species, such as *F. oxysporum* f. sp. *radicis-lycopersici*, the agent of crown and root rot of tomato (M'Piga *et al.*, 1997), and *F. udum*, the agent of pigeonpea wilt (Podile and Laxmi, 1998).

The results demonstrated that pronounced changes in the primary cell walls, consisting in the deposition of new barriers occurred in potato tubers inoculated with *B. cereus* X16 and challenged with *Fusarium*. Deposits similar to those observed in the present study were also seen in *Fusarium*-infected pea roots bacterized with the antagonistic bacterium *Pseudomonas fluorescens* (Benhamou *et al.*, 1996a). Those deposits were found to be rich in phenolic compounds by labeling with a gold-complexed laccase. Among the other structural reactions observed was the occlusion of some cells with an electron-opaque amorphous material. The intense staining of this material suggested that it was likewise enriched with phenolics, especially those containing the O-dihydroxy groups known to react readily with osmium tetroxide and to reveal a high electron density under TEM (Scalet *et al.*, 1989). Similar materials have been reported by Chérif *et al.* (1992) in silicon-treated cucumber plants, and by Benhamou *et al.* (1994b), in chitosan-treated tomato, and are believed to contain fungitoxic phenol-like compounds, that help plant tissues to restrict the spread of invading pathogens.

Treating potato wounds with *B. cereus* X16 also triggered several structural defense reactions in the vascular tissues invaded by *Fusarium* hyphae. These reactions included the deposition of thick layers of osmiophilic material, along secondary thickenings and pit cavities of the xylem vessels, and the partial or complete occlusion of vessel with darkly stained polymorphic compounds. These phenomena have long been thought to occur mainly in genetically resistant plants infected with vascular wilt fungi (Ouellette, 1978; Brammal and Higgins, 1988). The presence of dead fungal hyphae in potato xylem vessels showing these reactions, though without any bacterial cells, provides evidence for the belief that the polymorphic compounds were deposited as a protective response to infection. Opaque compounds similar in structure to those detected in this study were also found in the gums and gels sealing off xylem vessels, where they were again associated with phenolic substances (Ouellette, 1978). It is well known that such secondary metabolites and the free radicals formed during oxidative polymerization reactions are highly toxic and deleterious to the fungal metabolism (Southerton and Deverall, 1990).

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