MPMI Vol. 8, No. 6, 1995, pp. 825–836. © 1995 The American Phytopathological Society

hrp Genes in *Xanthomonas campestris* pv. *vesicatoria* Determine Ability to Suppress Papilla Deposition in Pepper Mesophyll Cells

Ian Brown[1], John Mansfield[1], and Ulla Bonas[2]

[1]Department of Biological Sciences, Wye College, University of London, Ashford, Kent, TN25 5AH, U. K., and [2]C.N.R.S. Institut des Sciences Végétales, 91198 Gif-sur-Yvette Cedex, France

Received 23 May 1995. Accepted 24 July 1995.

Electron microscopy was used to examine the responses of pepper mesophyll cells to strains of *Xanthomonas campestris* pv. *vesicatoria* with insertion mutations in each of the clustered *hrp* loci *hrpA, B, C, D, E,* and *F*. All *hrp* mutants caused the localized formation of large papillae in adjacent cells. Similar deposits also accumulated after inoculation with the saprophytic strain T55 of *X. campestris,* which lacks the entire *hrp* cluster. Immunocytochemistry with antibodies to tomato extensin revealed the accumulation of hydroxyproline-rich glycoproteins in the amorphous electron-dense matrix that encapsulated *hrp* mutant bacteria onto the plant cell wall and also within paramural deposits. Callose was also detected at reaction sites. By contrast, only minor cell wall alterations were observed in response to inoculation with wild-type strain 85-10 or a mutant deficient in extracellular polysaccharide production (85-10::454). Unlike other *hrp* mutants, the *hrpE*$^-$ strain 85-10::*E*75, when harboring plasmids overexpressing the avirulence gene *avrBs3*, caused the hypersensitive reaction in pepper cv. ECW30-R in addition to inducing deposition of large papillae. If cells of strains 85-10 or 85-10::454 were heat-killed or treated with chloramphenicol (Cm) before inoculation they also caused formation of large papillae. Treatment with Cm in the plant, 1 to 4 h after inoculation, also led to papilla deposition adjacent to cells of 85-10 or 85-10::454 but if antibiotic treatment was delayed for 8 h, although bacterial cells were killed, no papillae developed. An *hrp* also induced papilla formation in *Arabidopsis*, bean, and lettuce mesophyll cells. Our results suggest that localized cell wall modification and associated papilla deposition represent a defense response of plant cells to strains of *X. campestris* and that suppression of the reaction by *X. c.* pv. *vesicatoria* requires each of the clustered *hrp* loci.

Additional keywords: Capsicum, elicitor, pathogenicity, xanthan.

Genes in bacteria that determine basic pathogenicity to the host and ability to cause the hypersensitive reaction (HR) in resistant plants (*hrp* genes) have been described in several plant pathogens (see recent reviews by Willis et al. 1991; Bonas 1994). In the pepper and tomato pathogen *Xanthomonas campestris* pv. *vesicatoria* six clustered *hrp* loci, *hrpA, B, C, D, E,* and *F,* have been characterized (Bonas et al. 1991; Schulte and Bonas 1992; Bonas 1994). Sequence analyses have revealed similarity between predicted Hrp proteins and proteins of known function. For example, HrpA1, HrpB3, and HrpC2 from *X. c.* pv. *vesicatoria* share homology with proteins involved in secretionary functions in animal pathogens (Fenselau et al. 1992; Van Gijsegem et al. 1993). The role of *hrp* genes in protein secretion has recently been demonstrated by the finding of *hrp*-dependent secretion of HR-inducing proteins; the "harpins" from *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae,* and PopA1 from *P. solanacearum* (Wei et al. 1992, 1993; He et al. 1993; Arlat et al. 1994). The apparent involvement of Hrp proteins in a specialized secretion apparatus associated with the delivery of signal molecules to the plant provides an explanation for the characteristically pleiotropic Hrp$^-$ phenotype in plant pathogens. For example, mutation in any *hrp* locus in *X. c.* pv. *vesicatoria* leads to inability to multiply in planta, or to cause macroscopic symptoms in susceptible or resistant plants (Bonas et al. 1991).

It has been suggested that *hrp* mutants of phytopathogenic bacteria are unable to secrete pathogenicity factors that might cause the controlled release of nutrients from the challenged host plant (Mansfield et al. 1994). The inability of *hrp* mutants to multiply within plants would therefore be due to starvation caused by the lack of freely available nutrients in the apoplast rather than through the activation of a defense response that does not involve cell collapse characteristic of the HR. However, Jakobek and Lindgren (1993) have shown that *hrp* mutants of *Pseudomonas syringae* pv. *tabaci,* although failing to induce the HR in the nonhost bean, did activate transcription of genes possibly associated with plant defense such as phenylalanine ammonia lyase and chitinase. Following challenge with an *hrpD* mutant of *P. s.* pv. *phaseolicola,* lettuce cells have been shown to undergo cell wall alterations including deposition of hydroxyproline-rich glycoproteins (HRGPs), phenolics, and callose (Bestwick et al. 1995). The implications of the detection of responses to *hrp* mutants are that signal molecules produced by bacteria and recognized by the nonhost plant have an alternative route rather than through the Hrp protein–based export machinery, and that growth of the mutants may be actively inhibited by the plant. The ex-

Corresponding author: John Mansfield; E-mail: J.Mansfield@wye.ac.uk

periments with bean and lettuce dealt with responses of nonhost plants. The *X. c.* pv. *vesicatoria*/pepper interaction is well characterized in relation to race-specific varietal resistance and provides a valuable model for the analysis of Hrp protein function during compatible and incompatible interactions in the host plant.

In leaves of susceptible cultivars, cells of *X. c.* pv. *vesicatoria* multiply rapidly causing water-soaked lesions (Stall and Cook 1966). Production of extracellular polysaccharides (EPS) that contain xanthan is thought to have a role in symptom development (Brown et al. 1993) but low molecular weight toxins or cell-wall-degrading enzymes are not major determinants of pathogenicity (Beaulieu et al. 1991). Several genes for resistance to bacterial spot have been identified in pepper and found to determine resistance to races of *X. c.* pv. *vesicatoria* carrying matching avirulence genes *avrBs1*, *avrBs2*, and *avrBs3* (Bonas et al. 1989; Minsavage et al. 1990). In all cases, resistance is expressed by the HR. The avirulence gene *avrBs3* is constitutively expressed in *X. c.* pv. *vesicatoria* but requires all of the clustered *hrp* loci to be functional for induction of the HR (Knoop et al. 1991).

Microscopical studies of the *X. c.* pv. *vesicatoria*/pepper interaction reported by Brown et al. (1993) using immunocytochemistry to locate components of EPS and the AvrBs3 protein have defined the structural and temporal framework within which recognition and response occur in pepper. Cells of pathogenic strains secreted EPS rapidly during colonization of intercellular spaces. During compatible and incompatible interactions the formation of small paramural papillae in mesophyll cells adjacent to bacterial colonies was occasionally observed. No other major changes in ultrastructure were detected in plant cells during the first 48 h after inoculation of a susceptible cultivar but the onset of membrane dysfunction leading to the HR was apparent after 20 h at some sites during the incompatible interaction.

The initial aims of the studies described here were to examine (i) the response of pepper cells to strains of *X. c.* pv. *vesicatoria* with mutations in each of the *hrp* loci, (ii) the production of EPS by *hrp* mutants, and (iii) the accumulation of AvrBs3 protein in mutants overexpressing the *avrBs3* gene. We found that *hrp* mutants all induce production of large papillae within adjacent plant cells. Our findings prompted comparative studies with EPS$^-$ strains, a saprophytic xanthomonad, and heat-killed cells, and an investigation of the failure of wild-type strains to induce extensive cell wall alterations. Results obtained suggest that wild-type *X. c.* pv. *vesicatoria* suppresses rapid and nonspecific cell wall modification.

RESULTS

Symptom development.

The appearance of symptoms at sites inoculated with wild-type strain 85-10 , EPS$^-$, and *hrp* mutants and strains harboring the cloned avirulence gene *avrBs3*, all of which were examined by electron microscopy, is summarized in Table 1. No macroscopic symptoms developed at sites inoculated with 85-10 mutagenized in *hrpA, B, C, D,* or *F* loci, or in these mutants harboring pL3XV1-6. The *hrpE* mutant 85-10::*hrpE75* containing *avrBs3* expressed from pL3XV1-6 or pD36 caused a very patchy or almost confluent HR, respectively, characterized by dark brown pigmentation within necrotic tissue at the site of infiltration. Mutations in the other *hrp* loci prevented induction of the HR even if *avrBs3* was overexpressed. The EPS$^-$ mutant caused water-soaking indicative of susceptibility but the lesions produced developed about 24 h later than those caused by wild-type 85-10.

hrp mutants induce striking cell wall alterations.

Bacterial multiplication and the development of responses in adjacent mesophyll cells were observed following the inoculation of suspensions of cells of strains of *X. c.* pv. *vesicatoria* into young leaves of pepper cv. ECW-30R. With each interaction, tissues were sampled for electron microscopy 0.5, 4, 8, 24, and 48 h after inoculation. Observations made at early time-points showed that bacteria were deposited onto plant cell walls as inoculum droplets evaporated within the intercellular space. In agreement with data obtained from population counts (Bonas et al. 1991) the numbers of *hrp* mutant bacteria remained low, usually one or two being ob-

Table 1. Characteristics of strains of *Xanthomonas campestris* examined

Strain or transconjugant	Feature[a]	Reactions in leaves of pepper cv. ECW-30R[a]	Reference
X. c. pv. *vesicatoria* 85-10	Wild-type race 2	S[b]	Bonas et al. (1989)
85-10::*hrpA*22		–	Bonas et al. (1991)
85-10::*hrpB*35	*hrp* mutants with Tn*3-gus* insertions in *hrp* loci	–	Bonas et al. (1991)
85-10::*hrpC*17	*A, B, C, D, E,* or *F*	–	Bonas et al. (1991)
85-10::*hrpD*140		–	Bonas et al. (1991)
85-10::*hrpE*75		–	Bonas et al. (1991)
85-10::*hrpF*312		–	Bonas et al. (1991)
85-10::*hrpF*440	*hrpF* mutant; Tn*3-gus* insertion, EPS$^-$	–	Bonas (unpublished)
85-10::454	EPS$^-$, *hrp*$^+$	s	Bonas (unpublished)
85-10 (pD36)	85-10 with plasmid overexpressing *avrBs3*	HR	Brown et al. (1993)
85-10::*hrpA*22 (pD36)	*hrpA* and *hrpC* mutants overexpressing *avrBs3*	–	Bonas (unpublished)
85-10::*hrpC*17 (pD36)		–	Bonas (unpublished)
85-10::*hrpE*75 (pL3XV1-6)	*hrpE* mutant expressing *avrBs3* regulated by its own promoter	hr	Knoop et al. (1991)
85-10 E75 (pD36)	*hrpE* mutant overexpressing *avrBs3*	Hr	Bonas (unpublished)
X. campestris			
T55	Nonpathogenic, saprophyte; lacks the *hrp* cluster	–	Stall and Minsavage (1990)

[a] Reactions were scored over a period of 5 days after inoculation with 5×10^8 CFU ml^{-1}.

[b] S, susceptible reaction, water-soaked lesions; s, susceptible but less rapid development of water-soaking; HR, hypersensitive reaction; Hr, hypersensitive reaction confluent at most sites; hr, patchy hypersensitive reaction; –, no symptoms.

served in sections from each site of contact with the plant cell wall. Unlike the pathogenic strains, the *hrp* mutants, including an EPS⁻ *hrp* mutant, 85-10::*hrpF*440, all caused the localized formation of large papillae in adjacent cells (Fig. 1).

In order to compare reactions to *hrp* mutants and pathogenic strains objectively, the size and frequency of papilla deposition were quantified. Results obtained, summarized in Table 2, confirmed that the deposition of papillae next to *hrp* mutants was in clear contrast to the general lack of response to wild-type 85-10 or the EPS⁻ strain 85-10::454. Time-course studies indicated that the papilla response was most rapid to the *hrpA* mutant but differences between strains with mutations at different *hrp* loci were minor and all had induced significant papilla deposition by 8 h after inoculation. The development of papillae involved an initial deposition of amorphous material at sites of plasma membrane convolution and the subsequent formation of layers of osmiophilic and electron translucent material. Where deposits were observed next to pathogenic bacteria they always remained small and typically lacked structural complexity, as previously reported (Brown et al. 1993).

Components of papillae and wall modifications observed next to *hrp* mutants were characterized by immunocytochemistry and histochemistry. Immunogold staining with polyclonal antiserum to the tomato extensin (HRGP), which is secreted into the supernatant of tomato cell suspension cul-

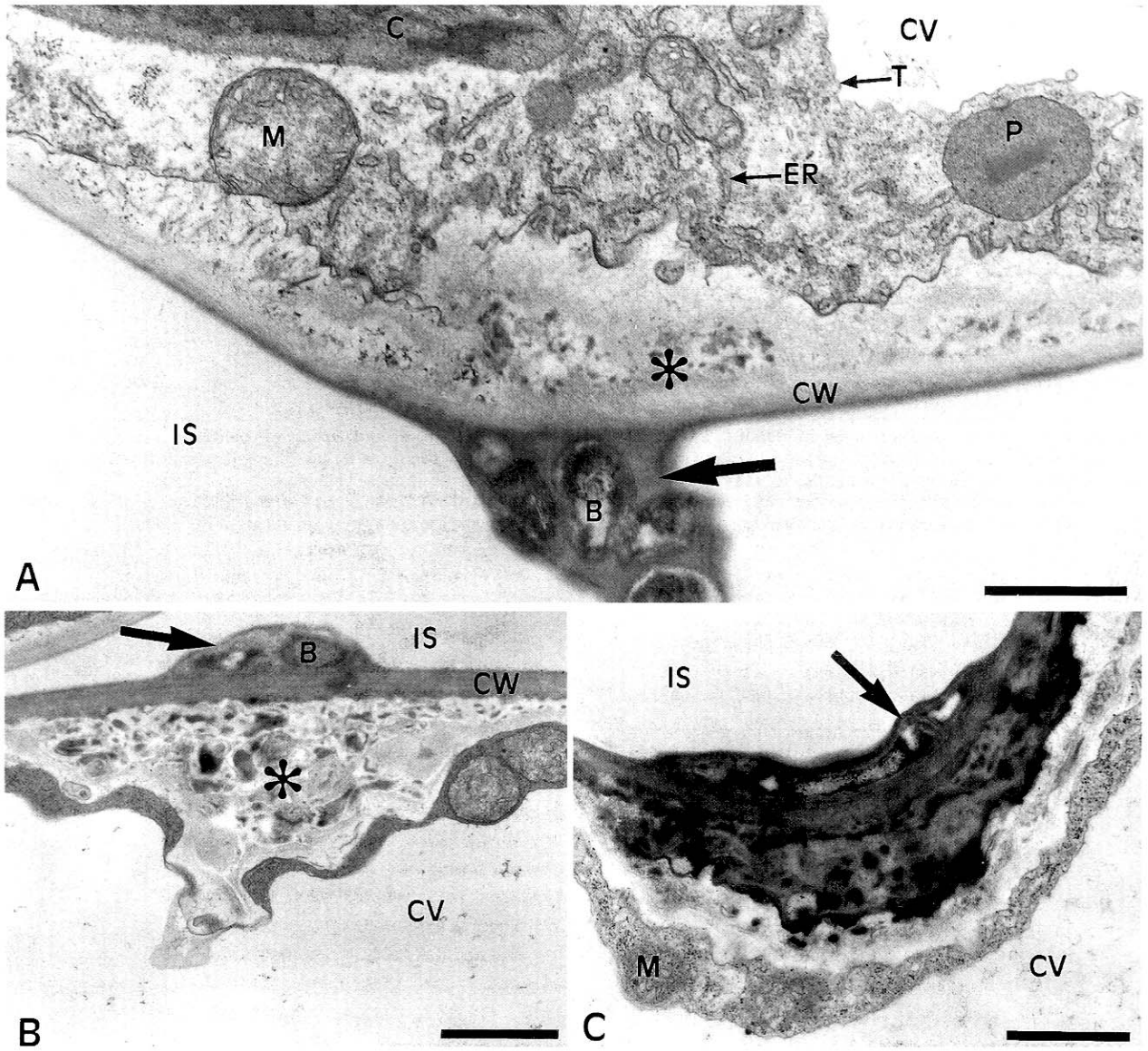

Fig. 1. Deposition of papillae in spongy mesophyll cells adjacent to *hrp* mutants of *Xanthomonas campestris* pv. *vesicatoria*. Note that bacterial cells are embedded in amorphous material (large arrows) and that papillae (asterisks) contain electron-dense vesicles within an opaque matrix. Cytoplasm adjacent to papillae is rich in endoplasmic reticulum. **A** and **B**, 24 h and 48 h after inoculaton with 85-10::*hrpD*140, showing category 3 and 4 papillae respectively. See Table 2 for definitions of categories. **C**, 48 h after inoculation with 85-10::*hrpF*440 with category 4 papilla. Bars, 1 μm; B, bacterium; C, chloroplast; CV, central vacuole; CW, plant cell wall; IS, intercellular space; M, mitochondrion; P, peroxisome; T , tonoplast; ER, endoplasmic reticulum.

tures (Brownleader and Dey 1993), demonstrated the accumulation of HRGPs in the amorphous material that appeared to encapsulate *hrp* mutants on the apoplastic face of the plant cell wall and also, to a lesser extent, within papillae (Fig. 2). Accumulation of HRGPs was apparent at both sites by 8 h after inoculation. After 24 h, callose was detected at reaction sites by fluorescence microscopy using the aqueous aniline blue staining procedure (data not shown, appearance as reported in Bestwick et al. 1995). Deposition of HRGPs or callose was rarely detected in cells adjacent to pathogenic strains.

Only the *hrpE* mutant delivers signals for both cell wall modification and the HR.

Although the *hrpE* mutant 85-10::*hrpE*75 fails to cause the HR in the resistant pepper cv. ECW-10R or in nonhost plants, transconjugants carrying *avrBs3* on pL3XV1-6 do cause a weak reaction on ECW-30R (Knoop et al. 1991). We confirmed these responses and found that, if *avrBs3* was overexpressed on pD36 in 85-10::*hrpE*75, not only formation of papillae but also an almost confluent HR were induced in leaves of ECW-30R (Tables 1 and 2). The first ultrastructural indication of the onset of decompartmentalization during the HR caused by 85-10::*hrpE*75 (pD36) was the appearance of numerous large vesicles within the cytoplasm (Fig. 3). A similar response was observed during the HR to 85-10(pD36) as described by Brown et al. (1993). Interestingly, with the *hrpE* mutant, changes associated with the HR occurred in addition to the increased activity of ER and Golgi at sites of papilla formation. Our observations suggested that there was no interference between the two responses induced by 85-10::*hrpE75*(pD36).

Table 2. Formation of papillae adjacent to cells of *Xanthomonas campestris* pv. *vesicatoria*

	Percentage of sites with papillae in categories 0 through 4, 24 or 48 h after inoculation[a]									
	24 h					48 h				
Strain	0	1	2	3	4	0	1	2	3	4
Pathogenic strains										
Wild-type 85-10	43.3	53.3	3.3	0	0	26.7	70.0	3.3	0	0
EPS$^-$, *hrp*$^+$ 85-10::454	30.0	63.3	6.7	0	0	40.0	60.0	0	0	0
hrp mutants										
85-10::*hrpA*22	0	3.3	33.3	50.0	13.3	0	10.0	30.0	30.0	30.0
85-10::*hrpB*35	6.7	46.7	16.7	20.0	10.0	6.7	16.7	53.3	13.3	10.0
85-10::*hrpC*17	6.7	33.3	36.7	16.7	6.7	3.3	10.0	33.3	36.7	16.7
85-10::*hrpD*140	0	50.0	36.7	10.0	3.3	0	16.7	36.7	26.7	20.0
85-10::*hrpE*75	0	3.3	56.7	33.3	6.7	0	6.7	50.0	40.0	3.3
85-10::*hrpF*312	0	20.0	26.7	43.3	10.0	6.7	3.3	26.6	46.7	16.7
85-10::*hrpF*440	10.0	30.0	16.7	26.7	16.7	0	0	10.0	53.3	36.7

[a] At least 30 sites were examined at each time for each strain. Categories based on the size and electron density of papillae: 0 = no deposit; 1 = small mainly fibrillar apposition less than 0.25 µm thick; 2 = as 1 but 0.25 to 0.75 µm thick; 3 = deposit at least 0.75 µm thick and with some osmiophilic inclusions; 4 = large papilla containing mostly electron-dense material. Categories 3 and 4 are illustrated in Figure 1.

[b] Analyses of these date using χ^2 contingency tests on combined numbers recorded in low (0 and 1) and high (2, 3, and 4) categories of response showed that differences between responses to the pathogenic strains 85-10 or 85-10::454, and the *hrp* mutants, were significant at $P < 0.0001$.

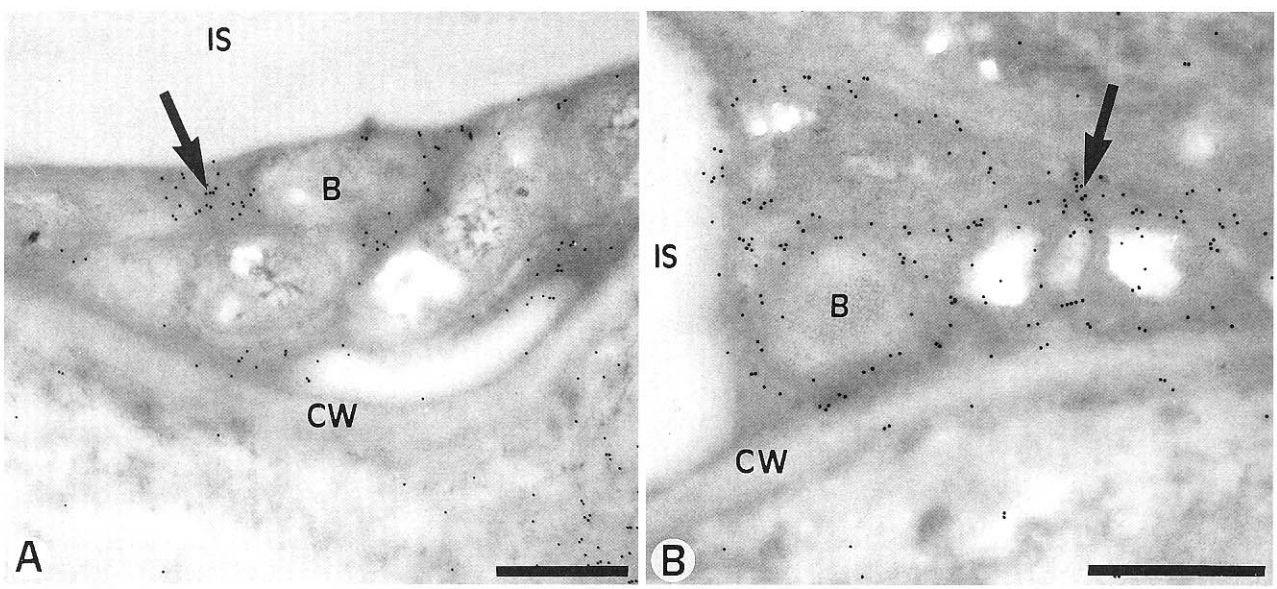

Fig. 2. Immunocytochemical localization of hydroxyproline-rich glycoproteins at reaction sites using polyclonal antiserum raised to tomato extensin. **A,** 24 h after inoculation with 85-10::*hrpA*22 and **B,** 48 h after inoculation with 85-10::*hrpD*140. Note the accumulation of label in the extracellular matrix surrounding bacterial cells (arrows). Bars, 0.5 µm; B, bacterium; CW, plant cell wall; IS, intercellular space.

Immunogold labeling with polyclonal antiserum to the AvrBs3 protein showed that the protein accumulated to similar levels in different *hrp* mutants overexpressing *avrBs3* even though the *hrp* mutants became encapsulated at the cell surface (Fig. 4). Mean numbers (± SEM) of gold particles in longitudinal sections of cells of *hrpA*, *hrpC,* and *hrpE* mutants recorded 4 h after inoculation were 8.9 ± 1.1, 7.5 ± 0.9, and 7.7 ± 1.1, respectively.

Mutants produce EPS in the plant.

The production of EPS was examined using the monoclonal antibody A6 to components of the xanthan side-chain, and the polyclonal antiserum XV1, raised to whole bacteria, which has a high affinity for commercial xanthan (Haaheim et al. 1989; Brown et al. 1993). The similar patterns of immunogold labeling observed around *hrp* mutants using each antiserum indicated that they produced some EPS in the plant. As illustrated in Figure 5, however, the small numbers of Hrp$^-$ bacteria found within colonies rarely became dispersed within a fibrillar matrix, as was characteristically observed with wild-type 85-10.

The spontaneous EPS$^-$ mutant 85-10::454 examined here fails to produce extracellular slime when grown in culture. In the plant, although cells of 85-10::454 multiplied they re-

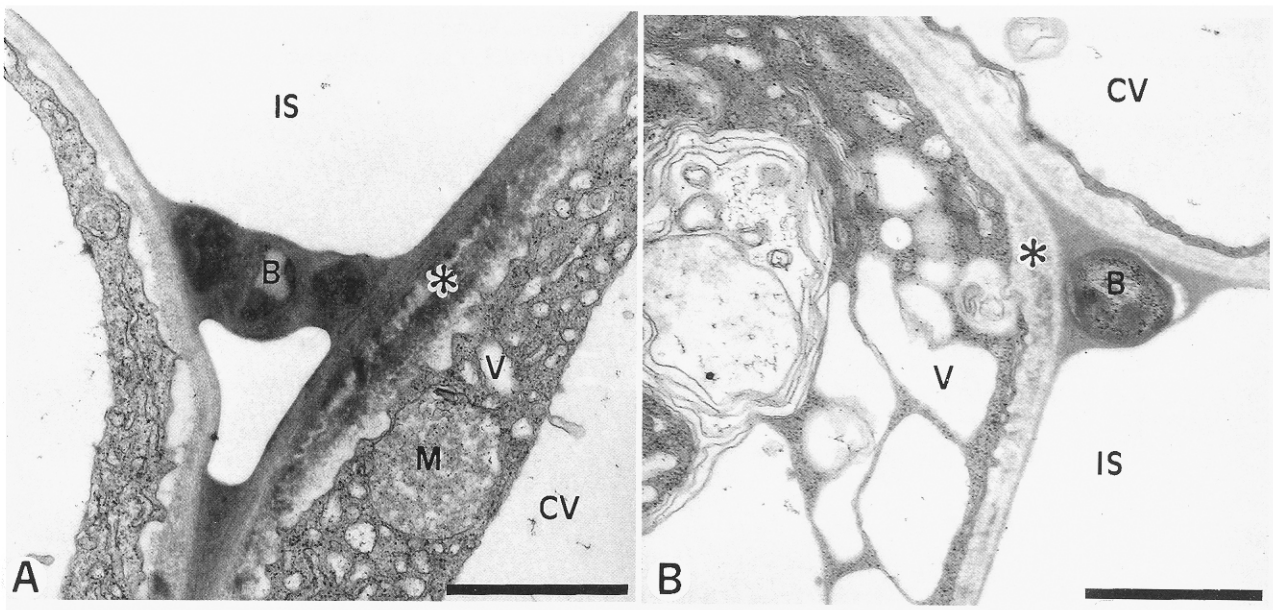

Fig. 3. Induction of papilla formation and the hypersensitive reaction by 85-10::*hrpE*75 harboring pD36, which allows overexpression of the avirulence gene *avrBs3*. Note the presence of papillae (asterisks) in cells that are undergoing cytoplasmic collapse. **A,** 24 h and **B,** 48 h after inoculation. Bar, 1 μm; B, bacterium; CV, central vacuole; M, mitochondrion; IS, intercellular space; V, small vacuole.

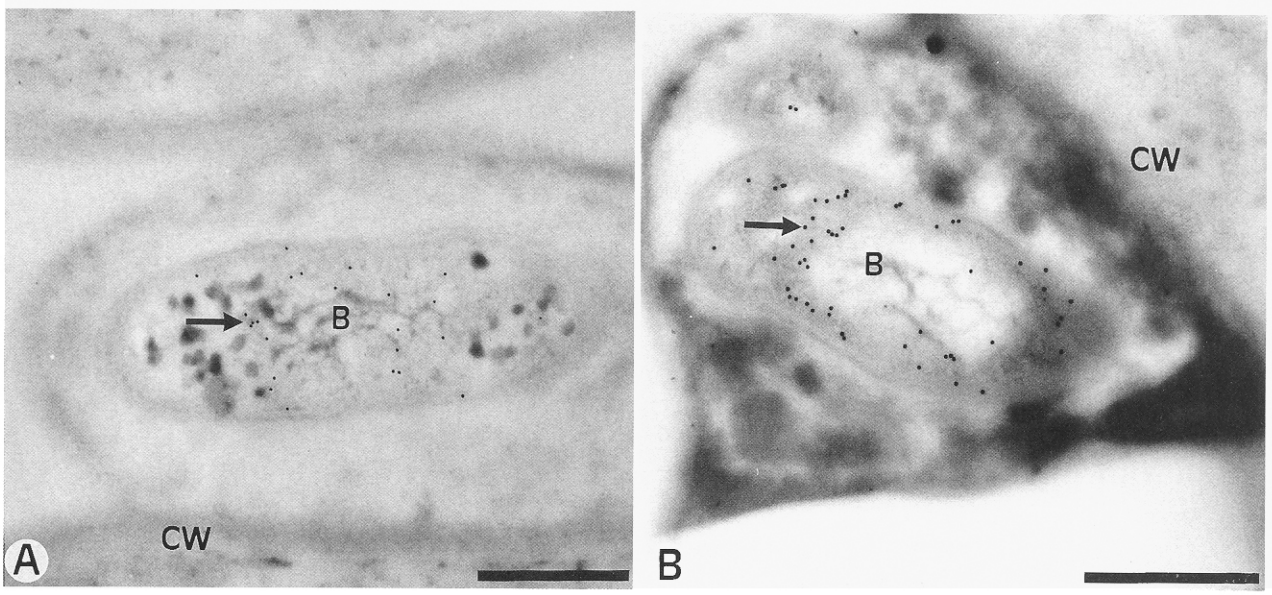

Fig. 4. Immunocytochemical localization of AvrBs3 protein within *hrp* mutants. **A,** 85-10::*hrpA*22 and **B,** 85-10::*hrpE*75 mutants both overexpressing *avrBs3*, 48 h after inoculation into pepper leaves. Immunogold label (arrowed) is present within the cytoplasm of *hrp* mutants, which are encapsulated onto the plant cell wall. Bar, 0.5 μm; B, bacterium; CW, plant cell wall.

mained closely packed together. Immunogold labeling revealed the localized presence of EPS around bacterial cells but not associated with fibrils (compare Fig. 5A and B). Thus, although the EPS$^-$ mutant appeared to retain the ability to synthesize a form of xanthan, and despite rapid bacterial multiplication, the polymer did not spread away from the cell surface.

Treatment with chloramphenicol causes pathogenic strains to induce papilla formation.

A characteristic of the HR is the requirement for an "induction time," discovered by Klement and Goodman (1967). The induction time is the time after inoculation into plant tissue during which bacteria need transcription and translation in order to elicit the HR. If bacteria are inactivated by secondary infiltration with antibiotics affecting mRNA or protein synthesis during the induction time then no HR develops. We were interested to find out if there was a similar induction time for ability to cause papilla deposition. Chloramphenicol (Cm) at 150 μg ml^{-1} was used to block protein synthesis in wild-type 85-10, the EPS$^-$ 85-10::454, and *hrpA* mutants either before inoculation or by a second injection, 1, 2, 4, or 8 h after infiltration of bacteria into leaves. Results obtained were the converse of those expected. Instead of detecting an induction time for papilla deposition, we found that the *hrp* mutant and the pathogenic strains induced the formation of large papillae if they were treated with Cm before inoculation. Antibiotic infiltration 1 to 4 h after inoculation also led to papilla formation. However, if Cm treatment of strain 85-10 was delayed until 8 h after inoculation, no large papillae were pro-

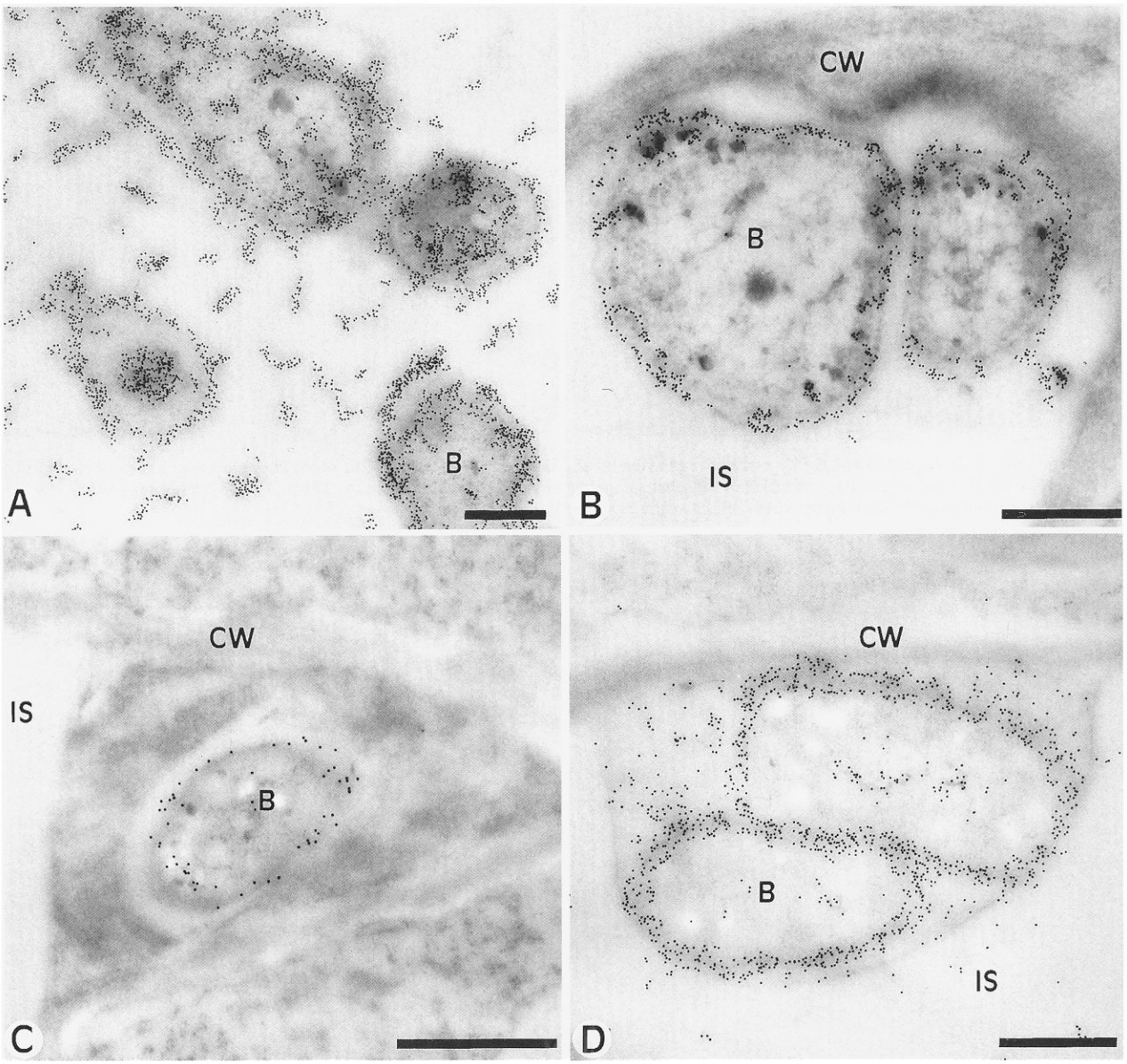

Fig. 5. Immunocytochemical localization of extracellular polysaccharides (EPS). **A,** Wild-type 85-10, and **B,** EPS$^-$ 85-10::454, 24 h after inoculation, labeled with monoclonal antibody A6. **C,** 85-10::*hrpA*22, 48 h after inoculation, labeled with monoclonal antibody A6. **D,** 85-10::*hrpD*140, 24 h after inoculation labeled with XV1 antiserum. The *hrp* mutants are embeddded in an amorphous matrix that is largely unlabeled. Bar, 0.5 μm; B, bacterium; CW, plant cell wall, IS, intercellular space.

duced during the following 48 h, despite bacteria showing signs of distortion characteristic of antibiotic action (Fig. 6). A similar pattern, effectively indicative of an induction time for the *suppression* of the papilla response, was also observed with the EPS$^-$ strain 85-10::454. Quantitative analyses of the effect of Cm on papilla formation by the pathogenic strains are summarized in Table 3.

Papilla deposition is induced by a saprophyte and heat-killed cells.

The induction of cell wall alterations by Cm-treated cells (Fig. 6 and Table 3), prompted experiments with heat-killed cells of 85-10 and the saprophytic *X. campestris* strain T55 that lacks the *hrp* cluster (Bonas et al. 1991). Large papillae were produced next to cells of the saprophyte that were embedded in a dense amorphous matrix (Fig. 7A). Injection with a suspension of heat-killed 85-10 caused several responses as illustrated in Figure 7B, ranging from the accumulation of layers of osmiophilic material along the inner face of the cell wall to papilla deposition. Papillae were often associated with fragments of dead bacteria.

Cell wall alterations are produced in other plants challenged by *X. c.* pv. *vesicatoria*.

In order to test if the induction of large papillae was a common feature of the reaction of plants to *hrp* mutants of *X. c.* pv. *vesicatoria,* suspensions of 85-10 and 85-10::*hrpA*22 were injected into leaves of *Arabidopsis thaliana* ecotype Keswick 1, French bean cv. Canadian Wonder, and lettuce cv. Diana. Infection sites were examined 16 and 48 h after inoculation. The *hrpA* mutant did indeed cause accumulation of papillae that were very similar in appearance in each plant, as illustrated in Figure 8. The wild-type strain 85-10 did not induce papilla formation in bean or lettuce but caused hypersensitive tissue collapse during the second day after inoculation. By contrast, in *Arabidopsis*, the wild-type strain 85-10, like the *hrpA* mutant, did induce papilla formation and failed to cause a confluent HR (Fig. 8). The ability of 85-10 to cause the HR in nonhosts appeared to be associated with absence of the papilla response.

DISCUSSION

Under the electron microscope, the response of pepper cells to *hrp* mutants of *X. c.* pv. *vesicatoria* appears almost identical to that observed to the saprophytic xanthomonad strain T55. Similar reactions were also observed to heat-killed *X. c.* pv. *vesicatoria.* A mutation in each *hrp* locus led to a very similar reaction phenotype in terms of the striking and highly localized alterations induced to the plant cell wall and the encapsulation of bacteria. Although many *hrp* mutant bacteria were found to be distorted, their viable numbers within pep-

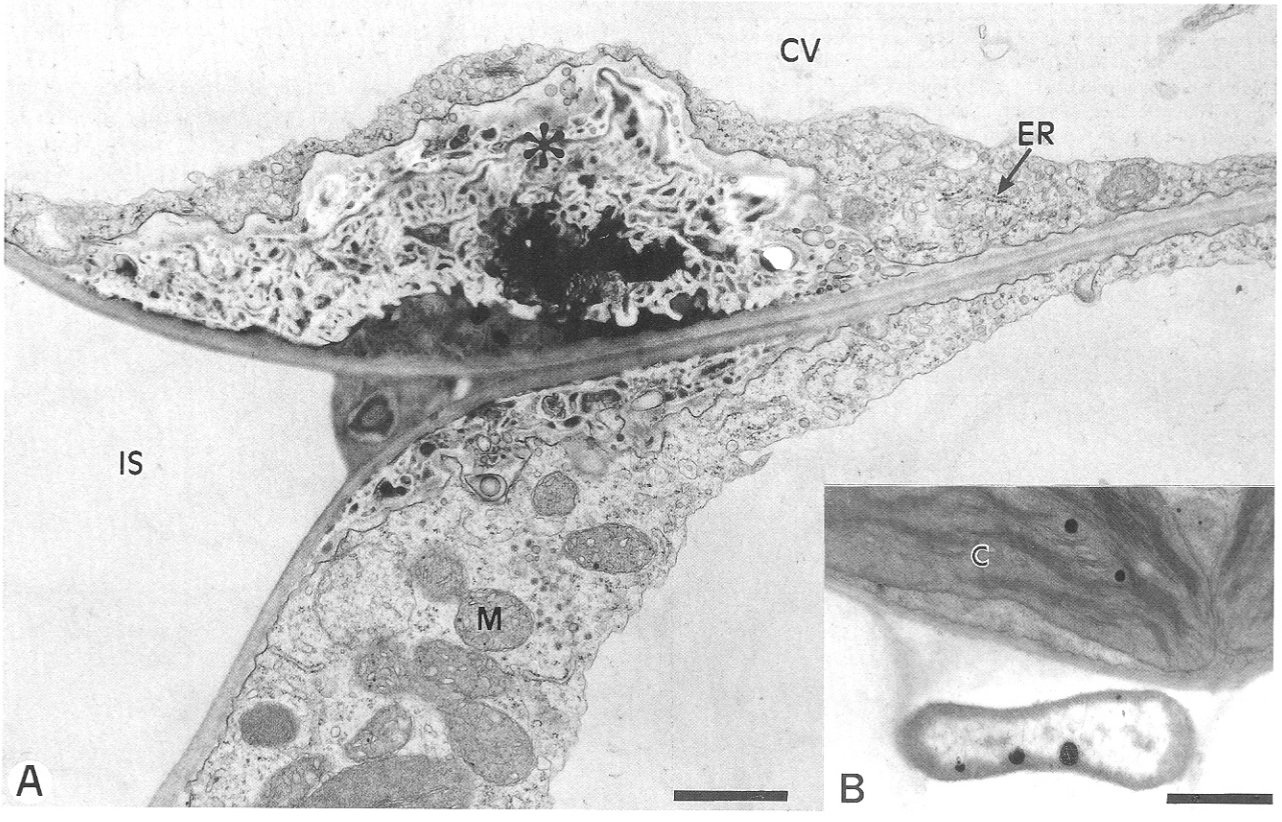

Fig. 6. Response of pepper cells to wild-type *Xanthomonas campestris* pv. *vesicatoria* strain 85-10 treated with chloramphenicol; tissue sampled 48 h after injection with the antibiotic. **A,** deposition of papillae in spongy mesophyll cells adjacent to 85-10 treated with chloramphenicol 4 h after inoculation. The bacterium is distorted and embedded in amorphous material, and large papillae (asterisks) have developed in adjacent cells. **B,** lack of reaction to 85-10 when treatment with antibiotic was delayed until 8 h after inoculation. Bar, 1 μm; IS, intercellular space; C, chloroplast; CV, central vacuole; M, mitochondrion; ER, endoplasmic reticulum.

per leaves do not decline rapidly during the first 2 days after inoculation (Bonas et al. 1991). The elicitors of papilla deposition are, therefore, probably not merely components released after bacterial cell death. Clearly, the signals from *X. c.* pv. *vesicatoria hrp* mutants or strain T55 that elicit the plant's response do not require the proposed Hrp protein–based secretion system for export from bacteria (Bonas 1994).

Restriction of colony development was associated with the accumulation of an amorphous matrix containing HRGPs around bacterial cells. A possible role for HRGPs in agglutinating invading bacteria has been proposed (Leach et al. 1982; Swords and Staehelin 1993). The intercellular location of the epitope(s) recognized by the anti-extensin antibodies is consistent with the predominately extracellular location of the HRGP in tomato cell suspension cultures (Brownleader and Dey 1993; Brownleader et al. 1993). Changes in the cell wall and papilla deposition next to bacterial colonies may restrict the diffusion of nutrients from plant cells. The deposition of HRGPs and their incorporation into plant cells has been proposed to result from the formation of isodityrosine cross-links mediated by a specific peroxidase/H_2O_2 system (Fry 1986; Bolwell 1993; Brownleader and Dey 1993). The H_2O_2 required for cross-linking reactions may result from NAD(P)H oxidation (Halliwell 1978; Mäder and Amberg-Fisher 1982; Bradley et al. 1992; Levine et al. 1994). Deposition of HRGPs has also been associated with the deployment of a less specific oxidative cross-linking defense response identified by Bradley et al. (1992). Interestingly, however, Brisson et al. (1994), on the basis of data from experiments with an undefined Hrp mutant of *P. s.* pv. *tabaci,* suggested that activation of oxidative cross linking in bean is dependent on Hrp functions. Analysis of more defined mutants and the localization of responses in the bean/*Pseudomonas* interaction examined by Brisson et al. (1994) would clarify the general significance of the activation of oxidative cross-linking that may contribute to the type of cell wall alterations we have observed in bean challenged by an *hrpA* mutant of *X. c. pv. vesicatoria.* As proposed by Peng and KuΔ (1992), the localized increases in H_2O_2 that probably occur at sites of papilla deposition may generate antibacterial conditions within the encapsulated colony.

The secretory processes that lead to the formation of large paramural deposits in pepper were not clearly identified by our ultrastructural studies. In contrast to the formation of large papillae in lettuce cells (Bestwick et al. 1995), no obvi-

Table 3. Effect of chloramphenicol on the deposition of papillae at reaction sites in pepper leaves examined 48 h after treatment of *Xanthomonas campestris* pv. *vesicatoria* with the antibiotic

Strain and treatment with chloramphenicol	Percentage of sites with papillae[a]				
	0	1	2	3	4
Wild-type 85-10					
No antibiotic	26.7	70.0	3.3	0	0
Co-infiltration	3.3	16.7	40.0	33.3	6.7
1 h after inoculation	15.4	23.1	15.4	23.1	23.1
2 h after inoculation	0	4.8	19.1	38.1	38.1
4 h after inoculation	10.0	30.0	16.7	20.0	23.3
8 h after inoculation	30.4	60.9	8.7	0	0
EPS$^-$, 85-10::454					
No antibiotic	40.0	60.0	0	0	0
Co-infiltration	0	0	23.3	56.7	20.0
1 h after inoculation	0	6.7	43.3	43.3	6.7
2 h after inoculation	0	0	26.7	63.3	10.0
4 h after inoculation	0	0	30.0	66.7	3.3
8 h after inoculation	0	80.0	20.0	0	0

[a] At least 30 sites were examined in two repeated experiments with each strain. Categories (0 through 4) are described in Table 2. Statistical analyses were performed using χ^2 contingency tests as described in Table 2. The differences observed in responses to bacteria treated with chloramphenicol up to 4 h after infiltration and to untreated 85-10, 85-10::454, or these strains treated 8 h after inoculation, were significant at $P < 0.0001$.

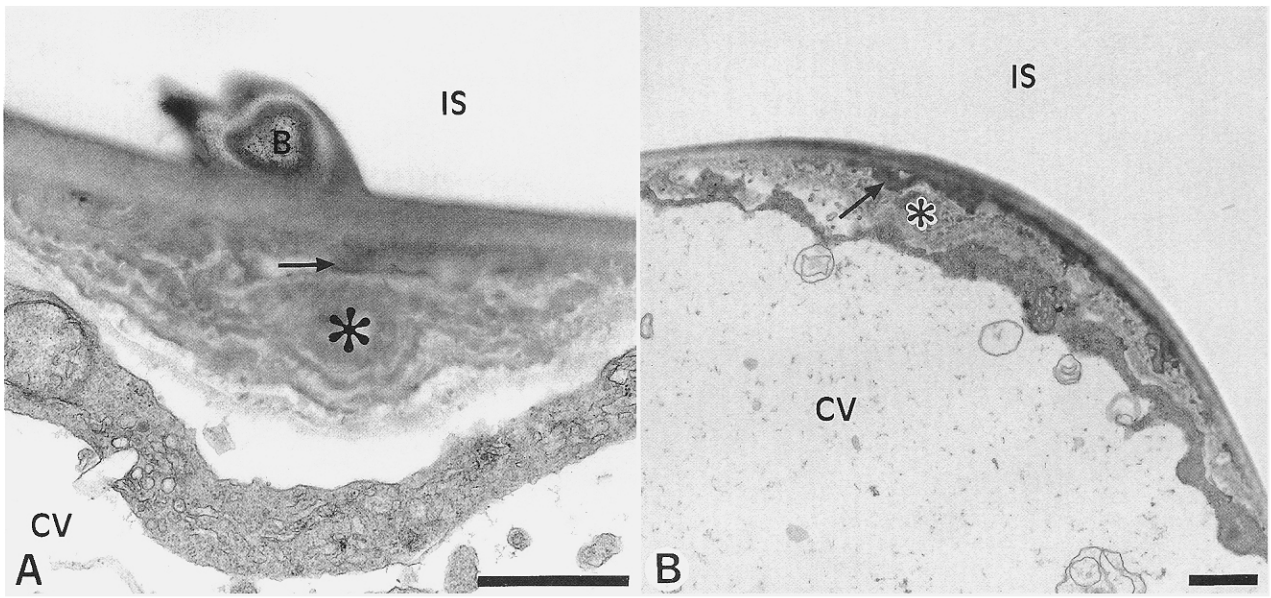

Fig. 7. Reactions of pepper cells 48 h after inoculation with **A,** the saprophytic *Xanthomonas campestris* strain T55 and **B,** heat-killed cells of *X. c.* pv. *vesicatoria* 85-10. Deposition of papillae marked by asterisks, appearance of osmiophilic deposits by arrows. In **A,** the mesophyll cell was partially plasmolysed during fixation and the plasma membrane has pulled away from the paramural deposit. Bar, 1 μm; B, bacterium; IS, intercellular space; CV, central vacuole.

ous exocytosis of materials from ER-derived vesicles was apparent in pepper. The dense plant cell cytoplasm often found next to papillae was, however, typically rich in ER and ribosomes that are likely to be involved in the active synthesis of material for incorporation into deposits.

A characteristic feature of the *X. c.* pv. *vesicatoria* wild-type strain 85-10 is the rapid production of a fibrillar matrix of EPS, containing xanthan, within the intercellular spaces in pepper leaves (Brown et al. 1993). Immunogold labeling revealed the localized presence of EPS around bacterial cells of the mutant 85-10::454 that was isolated as EPS^- in vitro but not associated with dispersed fibrils. Lack of dispersal may be due to altered xanthan structure, for example because of the action of a defective repeating unit polymerase leading to reduction in the internal chain length (Vanderslice et al. 1989). The EPS^- strain was pathogenic, causing water-soaked lesions, but the lesions developed more slowly than those caused by the wild-type strain 85-10. Like 85-10, however, mutant 85-10::454 did not induce the formation of major cell wall alterations including the deposition of large papillae. It is difficult to assess the production of EPS by *hrp* mutants because of their failure to multiply significantly within the plant. Labeling with antibodies A6 and XV1 confirmed that each of the *hrp* mutants accumulated a thin coating of xanthan. It seems probable, therefore, that the secretion of EPS in the plant does not require Hrp functions.

Mutations in *X. c.* pv. *vesicatoria hrp* loci *A, B, C, D,* and *F* prevent delivery of the signal leading to the HR determined

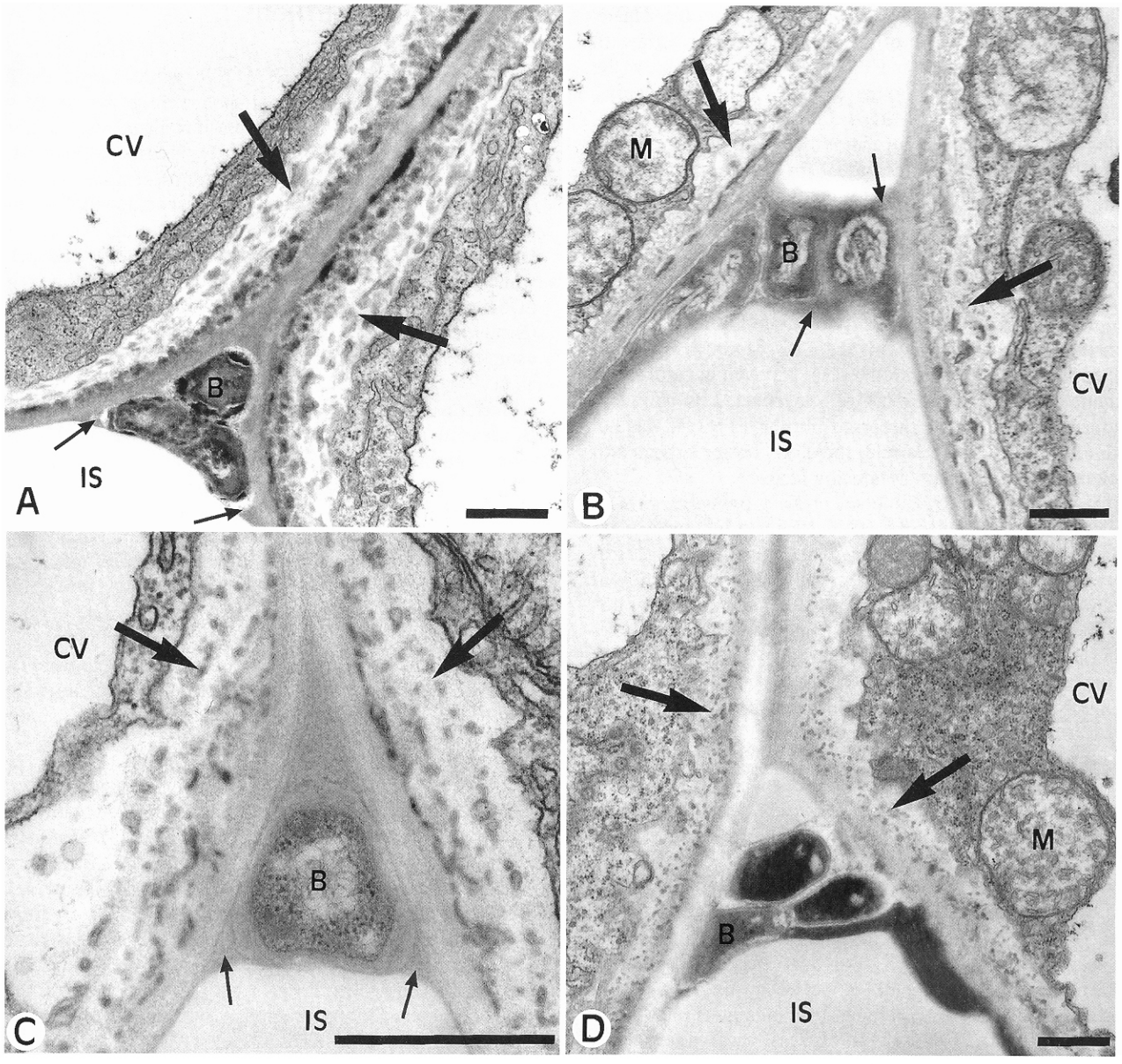

Fig. 8. Reactions of nonhost plants to *Xanthomonas campestris* pv. *vesicatoria.* **A**, French bean; **B**, *Arabidopsis* and **C**, lettuce, 48 h (**A, B**) and 16 h (**C**), after inoculation with the *hrp* mutant 85-10::*hrpA*22, and **D**, *Arabidopsis* 16 h after inoculation with wild-type 85-10. Bacteria (B) are embedded in amorphous material (small arrows) and extensive papilla deposition (large arrows) has occurred in adjacent cells. Bar, 0.5 μm; CV, central vacuole; IS, intercellular space; M, mitochondrion.

by the *Bs3* gene for resistance in pepper. Immunocytochemical localization has confirmed that the protein product of the matching avirulence gene *avrBs3* accumulates within cells of the Hrp$^-$ strains containing the pD36 plasmid. Despite the presence of significant amounts of AvrBs3 protein in, for example, *hrpA* and *hrpC* mutants, no reactions characteristic of the HR occurred in pepper cells. Interestingly, with the *hrpE* mutant 85-10::*hrpE*75, containing *avrBs3* on pD36, both papilla deposition and the HR were induced. However, an almost confluent HR is caused only if *avrBs3* is overexpressed, suggesting that, in the *hrpE* mutant in particular, the quantity of AvrBs3 protein present may have to reach a critical threshold level that can lead to signal transfer. The signaling cascade leading to the HR does not seem to override or interfere with the coordinated secretion of papillae in pepper; the two reactions appear to be controlled independently.

Our interpretation of the results obtained from the experiment to examine the effect of the inactivation of bacteria with Cm is that the wild-type and EPS$^-$ strains both suppress the deposition of papillae and other cell wall alterations. During the HR determined by the *avrBs3/Bs3* interaction, avirulent strains do not induce the formation of large papillae (Brown et al. 1993). Delivery of the signal for induction of the HR must therefore occur alongside suppression of the papilla response. It has often been argued that one of the functions of EPS is somehow to prevent the recognition of invading bacteria by the plant (Brown et al. 1993; Newman et al. 1994; Rudolph 1993). In the interaction with the wild-type strain, accumulation of EPS does rapidly separate invading bacteria from the plant cell wall. With the EPS$^-$ strain, bacteria remained in close contact with the plant cell wall but still failed to induce papillae unless they were inactivated by antibiotic treatment (Table 3). The dispersed fibrillar EPS that was not produced by 85-10::454 would, therefore, not be expected to block transfer of the papilla-inducing factor.

The ability of the *hrpE* mutant of *X. c.* pv. *vesicatoria* to deliver signals leading to the HR determined by *avrBs3* suggests that some mutations in *hrpE* may not impair the Hrp-dependent secretion process in the pathogen. The function of *hrpE* in *X. c.* pv. *vesicatoria* remains to be determined but it is possible that the corresponding protein may itself act as a secreted pathogenicity factor contributing to the suppression of the papilla response.

The production of suppressors of resistance has been proposed for a number of biotrophic fungal plant pathogens (Heath, 1980; 1981a, 1981b; Doke and Tomiyama 1980; Bushnell and Rowell 1981) and the bean halo-blight bacterium *Pseudomonas syringae* pv. *phaseolicola* (Gnanamanickam and Patil 1977; Jakobek et al. 1993). Suppression of the HR and phytoalexin accumulation in bean by the tripeptide vivotoxin phaseolotoxin produced by *P. s.* pv. *phaseolicola* was proposed by Gnanamanickam and Patil (1977). Jakobek et al. (1993) found that the suppression of accumulation of possibly defense-related transcripts was not caused by phaseolotoxin but an unidentified factor. Our findings, including the first analysis of a pathogen with mutations in each *hrp* locus, support the proposal put forward by Jakobek et al. (1993) that production or delivery of the suppressor is determined by clustered *hrp* loci. The identity of the proposed suppressor produced by *X. c.* pv. *vesicatoria* is unknown but it probably represents a compatibility factor essential for basic pathogenicity toward pepper. Our studies with nonhost plants suggest that the *hrp*-dependent suppressor of papilla deposition may also operate in bean and lettuce but not in *Arabidopsis*.

The hypothesis put forward by Bushnell and Rowell (1981) to explain the evolution of parasitism envisaged production of a nonspecific elicitor of active resistance that was blocked by a host-specific suppressor. The elicitor(s) of papilla deposition in pepper, being present in the saprophyte and dead bacterial cells, are good candidates for such a nonspecific elicitor, which is not involved in the HR. Clearly, further clarification of various hypotheses concerning regulation of the plant's response will require identification of both the suppressor and elicitor of the papilla deposition active during the pepper/*X. c.* pv. *vesicatoria* interaction.

MATERIALS AND METHODS

Plants and bacteria.

Plants of the pepper cv. ECW-30R, which carries the *Bs3* allele for resistance to bacterial spot, were grown to the six- to eight-leaf stage and leaves were inoculated with bacterial suspension by injection as previously described (Bonas et al. 1989; Minsavage et al. 1990). The strains of *X. c.* pv. *vesicatoria* used are given in Table 1. Transconjugants harboring pL3XV1-6, a pLAFR3-based clone containing *avrBs3* regulated by its own promoter (Bonas et al. 1989), and pD36, which contains the coding region of *avrBs3* cloned into pDSK602 resulting in overexpression of the AvrBs3 protein (Brown et al. 1993), were obtained from triparental matings using the helper plasmid pRK2013 as described by Knoop et al. (1991).

Bacteria were prepared for inoculation as described by Bonas et al. (1989) and washed three times in 1 mM $MgCl_2$ to remove nutrients and EPS before resuspension at 5×10^8 cells ml^{-1}. For certain experiments suspensions were treated in a microwave oven (750 watt) for 2×30 s, which killed all bacteria. Following injection, plants were maintained at 28°C day/22°C night under a 16-h photoperiod, with relative humidity not less than 80%. Samples were taken for electron microscopy at various time points from inoculation sites in leaves from two plants, with each interaction examined.

Arabidopsis, bean, and lettuce plants were grown and inoculated as described by Dangl et al. (1992) and Bestwick et al. (1995).

Antibodies.

Polyclonal antiserum XV1, which has affinity for commercial xanthan, was raised in rabbits injected with whole cells of *X. c.* pv. *vesicatoria* (Brown et al. 1993). Monoclonal antibody A6 was obtained as mouse ascitic fluid from Lars R. Haaheim, University of Bergen. The antibody was raised to purified xanthan from *X. c.* pv. *campestris*; and is directed against the nonsubstituted trisaccharide side-chain of xanthan (Haaheim et al. 1989). The AvrBs3-specific polyclonal antiserum is directed against a major part of the protein including the N terminal and repeat regions. The serum was affinity purified as described by Knoop et al. (1991). Polyclonal antibodies to tomato extensin (glycosylated form of HRGP, Brownleader and Dey 1993) were raised in rabbits and kindly provided by Michael Brownleader, University of the South Bank, London.

Electron microscopy and immunogold labeling.

Pieces of inoculated leaf tissue (ca. 1.5 mm^2) were fixed in 2.5% glutaraldehyde in 45 mM sodium cacodylate buffer (CAB) pH 7.2 for 16 h at 4°C. Vacuum infiltration of fixatives was not used as this displaced bacterial colonies from microsites in leaves. For conventional staining and immunogold localization of EPS, samples were then washed in CAB and post fixed for 1 h in 1% osmium tetroxide in CAB. After washing in water the samples were divided, half were dehydrated in ethanol and embedded in LR white resin (London Resin Co.) medium grade for immunocytochemistry and the remainder dehydrated in acetone and embedded in epon-araldite. For labeling of AvrBs3 or HRGPs, within inoculated leaves post-fixation with osmium tetroxide was omitted and samples were embedded in LR white resin. Polymerized blocks were sectioned on a Reichert UltraCut-E microtome using a diamond knife and sections (70 to 100 nm) mounted on 300-mesh copper or gold grids. Epon-araldite sections were stained in uranyl acetate and lead citrate (Brown et al. 1993).

For immunogold localization all solutions were prepared in 20 mM Tris buffered saline (pH 7.4) containing 0.1% bovine serum albumin (BSA) and 0.05% Tween 20 (TBST). After blocking, routinely in 2% BSA or for HRGP localization in 1% BSA, 5% nonimmune goat serum (Sigma Chemical Co., St. Louis, MO), and 0.5% nonfat milk powder in TBST for 0.5 h at room temperature, sections on uncoated gold grids were transferred to a 20-µl droplet of the primary antibody and incubated for 16 h at 4°C. The rabbit polyclonal antibody raised to whole bacteria (XV1) was diluted 1:500, the AvrBs3 antiserum diluted 1:50, mouse monoclonal ascites (A6, D1) were diluted 1:2,000, and antiserum to extensin diluted 1:50. After washing in a stream of TBST from a wash bottle, grids were incubated in the appropriate second antibody, either goat anti-rabbit IgG 10 nm gold (Amersham Bucks) or goat anti-mouse IgM 10nm gold (Sigma) diluted 1:25 for 0.5 h at room temperature. After washing in TBST followed by distilled water, grids were dried and examined without further staining.

Controls for the specificity of immunogold labeling were as follows: incubation in the absence of the primary antibody; replacement of the immune serum by nonimmune rabbit serum (as no pre-immune serum was available); and, for HRGPs, pre-absorption of antiserum with pure tomato extensin (Brownleader and Dey 1993). For pre-absorption, 20 µl of extensin (100 µg ml^{-1}) was added to 20 µl of a 1:50 dilution of antiserum and the solutions were incubated at 20°C for 2 h.

All sections were viewed in a Hitachi H-7000 transmission electron microscope with an accelerating voltage of 75 kV.

Callose.

Semi-thin sections (1 µm) of LR-white embedded tissue were cut and mounted on glass slides. A 20-µl droplet of aqueous aniline blue (0.05% wt/vol) in 50 mM phosphate buffer pH 8.0, was added to the section and a coverslip placed over it. The section was immediately viewed under UV excitation, a bright light blue fluorescence being indicative of callose (O'Brien and McCully 1981).

ACKNOWLEDGMENTS

We wish to acknowledge support from the U.K. Agricultural and Food Research Council and to thank Shelagh Reardon for technical assistance with electron microscopy.

LITERATURE CITED

Arlat, M., Gough, C. L., Zischek, C, Berberis, P. A., Trigalet, A., and Boucher, C. A. 1992. Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum*. Mol. Plant-Microbe Interact. 5:187-193.

Arlat, M., Van Gijsegem, F., Huet, J. C., Pernollet, J. C., and Boucher, C. A. 1994. PopA1, a protein which induces a hypersensitive-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBO J. 13:543-553.

Beaulieu, C., Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1991. Biochemical and genetic analysis of a pectate lyase gene from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Plant-Microbe Interact. 4: 446-451.

Bestwick, C. S., Bennett, M. H., and Mansfield, J. W. 1995. Hrp mutant of *Pseudomonas syringae* pv. *phaseolicola* induces cell wall alterations but not membrane damage leading to the HR in lettuce (*Lactuca sativa*). Plant Physiol. 108:503-516.

Bolwell, G. P. 1993. Dynamic aspects of the plant extracellular matrix. Int. Rev. Cytol. 146:261-324.

Bonas, U. 1994. *hrp* genes of phytopathogenic bacteria. Pages 79-98 in: Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. J. L. Dangl, ed. Current Topics in Microbiology and Immunology. Vol. 192. Springer-Verlag, Berlin.

Bonas, U., Schulte, R., Fenselau, S., Minsavage, G. V., Staskawicz, B. J., and Stall, R. E. 1991. Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Mol. Plant-Microbe Interact. 4:81-88.

Bonas, U., Stall, R. E., and Staskawicz, B. J. 1989. Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. 218:127-136.

Bradley, D. J., Kjellbom, P., and Lamb, C. J. 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell 70:21-30.

Brisson, L. C., Tenhaken, R., and Lamb, C. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6:1703-1712.

Brown, I., Mansfield, J., Irlam, I., Conrads-Strauch, J., and Bonas, U. 1993. Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Mol. Plant-Microbe Interact. 6:376-386.

Brownleader, M., Golden K. D., and Dey, P. M. 1993. An inhibitor of extensin peroxidase in cultured tomato cells. Phytochemistry 33:755-758.

Brownleader, M. D., and Dey, P. M. 1993. Purification of extensin from cell walls of tomato (hybrid of *Lycopersicon esculentum* and *L. peruvianum*) cells in suspension culture. Planta 191:457-469.

Bushnell, W. R., and Rowell, J. B. 1981. Suppressors of defense reactions: A model for roles in specificity. Phytopathology 71:1012-1014.

Dangl, J. L., Ritter, C., Gibbon, M. J., Mur, L. A. J., Wood, J. R., Goss, S., Mansfield, J. W., Taylor, J. D., and Vivian, A. 1992. Functional homologs of the Arabidopsis *RPM1* disease resistance gene in bean and pea. Plant Cell 4:1359-1369.

Doke, N., and Tomiyama, K. 1980. Suppression of the hypersensitive response of potato tuber protoplasts to hyphal wall components by water soluble glucans isolated from *Phytophthora infestans*. Physiol. Plant Pathol. 16:177-186.

Fenselau, S., Balbo, I., and Bonas, U. 1992. Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant-Microbe Interact. 5:390-396.

Fry, S. C. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu. Rev. Plant Physiol. 37:165-186.

Gnanamanickam, S. S., and Patil, S. S. 1977. Phaseolotoxin suppresses bacterially induced hypersensitive reaction and phytoalexin synthesis in bean cultivars. Physiol. Plant Pathol. 10:169-179.

Haaheim, L. R., Kleppe, G., and Sutherland, I. W. 1989. Monoclonal antibodies reacting with the exopolysaccharide xanthan from *Xanthomonas campestris*. J. Gen. Microbiol. 135:605-612.

Halliwell, B. 1978. Lignin synthesis: The generation of hydrogen peroxide and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols. Planta 140:81-88.

He, S. Y., Huang, H. C., and Collmer, A. 1993. *Pseudomonas syringae*

pv. *syringae* $Harpin_{Pss}$: A protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell 73:1-20.
Heath, M. C. 1980. Effects of infection by compatible species or injection of tissue extracts on the susceptibility of nonhost plants to rust fungi. Phytopathology 70:356-360.
Heath, M. C. 1981a. The suppression of the development of silicon-containing deposits in French bean leaves by exudates of the bean rust fungus and extracts from bean rust-infected tissue. Physiol. Plant Pathol. 18:149-155.
Heath, M. C. 1981b. A generalized concept of host-parasite specificity. Phytopathology 71:1121-1123.
Jakobek, J. L., and Lindgren, P. B. 1993. Generalized induction of defense responses in bean is not correlated with the induction of the hypersensitive reaction. Plant Cell 5:49-56.
Jakobek, J. L., Smith, J. A., and Lindgren, P. B. 1993. Suppression of bean defense responses by *Pseudomonas syringae*. Plant Cell 5:57-63.
Klement, Z., and Goodman, R. N. 1967. The role of the living bacterial cell and induction time in the hypersensitive reaction of the tobacco plant. Phytopathology 57:322-323.
Knoop, V., Staskawicz, B., and Bonas, U. 1991. Expression of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria* is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.
Leach, J. E., Cantrell, M. A., and Sequeira, A. L. 1982. Hydroxyproline-rich bacterial agglutinin from potato. Plant Physiol. 70:1353-1358.
Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583-593.
Mäder, M., and Amberg-Fisher, V. 1982. Role of peroxidase in the lignification of tobacco cells I. Oxidation of nicotinamide adenine dinucleotide and formation of hydrogen peroxide by cell wall peroxidases. Plant Physiol. 70:1128-1131.
Mansfield, J. W., Brown, I. R., and Maroofi, A. 1994. Bacterial pathogenicity and the plants response: Ultrastructural, biochemical and physiological perspectives. Pages 85-107 in: Biotechnology and Plant Protection. D. D. Bills and S.-d. Kung, eds. World Scientific Publishing Co., Singapore.
Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearney, B., Bonas, U., Staskawicz, B. J., and Stall, R. E. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. Mol. Plant-Microbe Interact. 3:41-47.
Newman, M. A., Conrads-Strauch, J., Scofield, G., Daniels, M. J., and Dow, J. M. 1994. Defense-related gene induction in *Brassica campestris* in response to defined mutants of *Xanthomonas campestris* with altered pathogenicity. Mol. Plant-Microbe Interact. 7:553-563.
O'Brien, T. P., and McCully, M. E. 1981. The Study of Plant Structure: Principles and Selected Methods. Termarcarphi Pty. Ltd., Melbourne, Australia.
Peng, M., and KuΔ, J. 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology 82:696-699.
Rudolph, K. 1993. Infection of the plant by *Xanthomonas*. Pages 193-264 in: Xanthomonas. J. G. Swings and E. L. Civerolo, eds. Chapman and Hall, London.
Schulte, R., and Bonas, U. 1992. Expression of the *Xanthomonas campestris* pv. *vesicatoria hrp* gene cluster, which determines pathogenicity and hypersensitivity on pepper and tomato, is plant inducible. J. Bacteriol. 174:815-823.
Stall, R. E., and Cook, A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. Phytopathology 56:1152-1154.
Stall, R. E., and Minsavage, G. V. 1990. The use of *hrp* genes to identify opportunistic xanthomonads. Pages 369-374 in: Plant Pathogenic Bacteria. Z. Klement, ed. Proc. Int. Conf. Plant Pathol. Bacteriol., 7th. Akademiai Kiadó, Budapest.
Swords, K. M. M., and Staehelin, L. A. 1993. Complementary immunolocalization patterns of cell wall hydroxyproline-rich glycoproteins studied with the use of antibodies directed against different carbohydrate epitopes. Plant Physiol. 102:891-901.
Vanderslice, R. W., Doherty, D. H., Capage, M. A., Betlach, M. R., Hassler, R. A., Henderson, N. M., Ryan-Graniero, J., and Tecklenburg, M. 1989. Genetic engineering of polysaccharide in *Xanthomonas campestris*. Pages 145-156 in: Recent Developments in Industrial Polysaccharides: Biomedical and Biotechnological Advances. V. Crescenzi, I. C. M. Dea, and S. S. Stivola, eds. Gordon & Breach Science Publishers, New York.
Van Gijsegem, F., Genin, S., and Boucher, C. 1993. Evolutionary conservation of pathogenicity determinants among plant and animal pathogenic bacteria. Trends Microbiol. 1:175-180.
Wei, Z.-H., and Beer, S. V. 1993. HrpI of *Erwinia amylovora* functions in secretion of harpin and is a member of a new protein family. J. Bacteriol. 175:7958-7967.
Wei, Z.-M., Laby, R. J., Zumoff, C. H., Bauer, D. W., He, S. Y., Collmer, A., and Beer, S. V. 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85-88.
Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. *hrp* genes of phytopathogenic bacteria. Mol. Plant-Microbe Interact. 4:132-138.