Fate of *Xanthomonas campestris* Infiltrated into Soybean Leaves: An Ultrastructural Study

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ABSTRACT


Leaves of soybean cultivars Clark (susceptible) and Clark 63 (resistant) were inoculated by infiltration with virulent or avirulent strains of *Xanthomonas campestris* pv. *glycines*, the causal agent of bacterial pustule disease of soybean, and with *X. campestris* pv. *campestris*, a pathogen of crucifers. Ultrastructure of infected leaves was studied at 26, 48, and 72 hr after inoculation. Virulent strains of *X. c. pv. glycines* exhibited rapid growth in vivo and remained free in the intercellular spaces of cultivars Clark and Clark 63. The incompatible strains exhibited restricted growth, induced a moderate to strong hypersensitive response evidenced by browning of leaf laminae, and were immobilized to varying degrees by fibrillar or electron-dense amorphous material on the surface of leaf mesophyll cells. The extent of bacterial immobilization was inversely related to bacterial growth. Heat-killed cells of virulent or avirulent strains of *X. c. pv. glycines* were also immobilized. Other host cell responses to incompatible strains of *X. campestris* included invagination of host plasmalemma, accumulation of vesicles and amorphous material between host plasmalemma and cell wall, migration and disorientation of thylakoid membrane systems in chloroplasts, and host cell necrosis. Host cell responses to virulent strains of *X. c. pv. glycines* included dilation of endoplasmic reticulum, accumulation of electron-dense amorphous material within the cytoplasm, a change in chloroplast morphology to a more rounded shape, and migration of chloroplasts away from the host plasmalemma. The results indicated that active immobilization is a primary or secondary defense response of soybean against incompatible xanthomonads.

Additional key words: bacterial growth, *Glycine max*, host cell organelles, immobilization, pustule.

Incompatible strains of phytopathogenic erwinias, pseudomonads, xanthomonads, and saprophytic bacteria have been shown by electron microscopy to be surrounded by fibrillar or electron-dense material at the surface of plant cell walls in apple (22), bean (32), cotton (6), oats (35), rice (21), and tobacco (16, 20). This phenomenon has variously been called immobilization (16), engulfment (6, 30), encapsulation (24), or entrapment (19). The commonality of the observation of enveloping fibrils, often interspersed with an amorphous electron-dense substance, is striking and has led to the hypothesis that envelopment, or its corollary, attachment, is responsible for restriction of bacterial growth and/or is a requirement for induction of the hypersensitive response (HR) (6, 21, 28, 30, 34). In contrast, engulfment of bacteria in some host plants has been considered to be a nonspecific physical phenomenon (10, 19) or even to be required for bacterial survival and multiplication (19, 24).

*Xanthomonas campestris* pv. *glycines* causes bacterial pustule disease of soybean. Commercially available soybean cultivars that carry a recessive gene pair *rpm* (18) are resistant to the disease, i.e., develop only limited symptoms in greenhouse trials (9). It has recently been proven that soybean phytoalexins are not responsible for resistance of cultivar Clark 63 to a virulent strain of *X. c. pv. glycines*, or for the resistance of susceptible soybean cultivar Clark to avirulent strains of *X. c. pv. glycines* and to the species nonpathogen *X. campestris* (9).

The present study was undertaken to evaluate bacterial immobilization as a defense mechanism of cultivars Clark and Clark 63 against pathogens of *X. campestris*. A prior study (13) showed that cells of an avirulent strain (S-9-8) of *X. c. pv. glycines* were immobilized at leaf mesophyll cell walls in cultivar Clark, while those of a virulent strain (XP175) remained free. A preliminary report has been published (12).

MATERIALS AND METHODS

Bacterial strains and inoculation of soybean leaves. Source, geographic origin, and pathogenicity of bacterial strains used in this study have been reported (9). *X. c. pv. glycines* strains XP175 and A are virulent on soybean; *X. c. pv. glycines* strains 1716, 1136, and S-9-8 are avirulent. *X. c. pv. campestris* strain XC42 is a pathogen of cabbage and is not a pathogen of soybean.

Seeds of cultivars Clark and Clark 63 were kindly supplied by R. L. Bernard, United States Regional Soybean Laboratory, Urbana, IL. Plants were grown as described previously (9). Plants used for inoculation were approximately 21 days old and had one fully opened, but not yet fully expanded, trifoliate leaf per plant.

Bacteria were grown on nutrient agar (NA) (Difco) for 24 hr at 28°C. Cells were washed from the agar surface, suspended in sterile water, and washed three times by centrifugation and resuspension.

Final bacterial suspensions in sterile water were adjusted turbidimetrically (OD₅₆₀nm = 1.5) to approximately 3 × 10⁴ colony-forming units (cfu) per milliliter. Trifoliate leaves were inoculated by forcibly spraying inoculum onto the abaxial side of leaflets until water-soaking appeared. Leaves were then rinsed well under running tap water. After water-soaking disappeared (approximately 2 hr), inoculated plants were returned to the growth chamber and maintained at 28°C day, 24°C night. Growth of bacteria in vivo was monitored by removing leaf "dinks" from inoculated areas, triturating them in buffer, and determining the number of bacteria by standard dilution plating techniques (9).

For experiments with heat-killed bacteria, bacterial suspensions (OD₅₆₀nm = 2.0) were heated in a boiling water bath for 45 min. Killing of the bacteria was verified by plating 0.1-ml aliquots of the heated suspensions on NA.

Electron microscopy. Leaf tissue was excised and vacuum infiltrated with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. Tissue was fixed 3-4 hr at 4°C, rinsed in the same buffer plus 1.5% surose, and postfixed in 2% osmium tetroxide in veronal acetate buffer for 4 hr. Fixed tissue segments were dehydrated in a graded acetone series, including en bloc staining with 2% uranyl acetate in 70% acetone for 1 hr, and embedded in Spurr's low viscosity resin. Sections were stained with uranyl
acetate and lead citrate. Water-infiltrated leaf tissue was processed as a control. Electron micrographs were from two or three inoculation trials. All interactions were examined 48 hr after inoculation, and selected interactions were examined also at 26 and 72 hr after inoculation.

RESULTS

Symptomology. Table 1 summarizes the interactions tested and the symptomology observed. On cultivar Clark, virulent strains XP175 and A of *X. c. pv. glycines* caused yellow-green chlorosis of leaf laminae within 24–48 hr. By 48–72 hr, chlorosis intensified to strong yellowing and pustules developed at the margins of affected leaf areas.

In cultivar Clark, avirulent strains of *X. c. pv. glycines* and the species nonpathogen *X. c. pv. campestris* all induced an HR, which was evidenced by varying degrees of browning. Strain 1716 caused slight browning and yellow-green chlorosis by 24 hr. Chlorosis did not intensify, but browning became severe by 72 hr. Similar symptomology was induced by strains 1716, S-9-8, 1136, and XC42, except that strain 1716 produced more intense browning by 72 hr.

On resistant cultivar Clark 63, both the virulent strain XP175 and the avirulent strain S-9-8 induced yellow-green chlorosis and slight browning of leaf laminae by 48 hr. By 72 hr, tissue inoculated with strain XP175 developed strong yellowing and moderate to strong browning, while tissue inoculated with strain S-9-8 developed only moderate browning and chlorosis.

No water-soaking of inoculated leaves occurred as the result of any interaction.

Bacterial growth. In cultivar Clark (Fig. 1), strains A and XP175 reached populations of approximately $2 \times 10^6$ cfu/cm² leaf area by 48–72 hr. The four incompatible strains exhibited varying degrees of growth restriction. Strain S-9-8 was the most severely restricted with less than $2 \times 10^6$ cfu/cm² in 72 hr, while strain 1716 reached final populations intermediate between the other incompatible strains and the virulent strains. The growth of strains S-9-8 and XP175 in the resistant host Clark 63 was similar to their growth in cultivar Clark.

Observations of bacteria within leaf cells. At 26 hr, cells of virulent strain XP175 were in microcolonies bridging mesophyll cells, usually at host cell junctions (Fig. 2a). The bacteria were surrounded by fibrillar material, probably bacterial extracellular polysaccharide (EPS). A dense film often covered the microcolony surface. By 48 hr, microcolonies were absent and bacteria were free in the intercellular space, intermingled with electron-dense material (Fig. 2b). At 48 and 72 hr, cells of virulent strain A were surrounded by abundant EPS that filled the intercellular space (Fig. 2c). Heat-killed strain A cells were always sequestered at the host cell wall by an amorphous substance (Fig. 2d).

At 26 hr, cells of avirulent strain S-9-8 were either free (Fig. 3a) or immobilized (Fig. 3b and c) in cultivar Clark. Spaces around

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**TABLE 1. Symptomology and extent of bacterial immobilization in leaves of soybean cultivars Clark and Clark 63 after infiltration with pathovars of Xanthomonas campestris**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Soybean cultivar</th>
<th>Color of affected leaf laminae at 72 hr</th>
<th>Extent of bacterial immobilization at 48 hr</th>
</tr>
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<tbody>
<tr>
<td><em>Xanthomonas campestris</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>pv. glycines</em> strain XP175&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Clark&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Strong yellowing&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>pv. glycines</em> strain A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Clark</td>
<td>Strong yellowing with moderate-strong browning</td>
<td></td>
</tr>
<tr>
<td><em>pv. glycines</em> strain 1716&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Clark</td>
<td>Strong yellowing&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>pv. glycines</em> strain S-9-8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Clark</td>
<td>Strong browning</td>
<td></td>
</tr>
<tr>
<td><em>pv. glycines</em> strain 1136&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Clark</td>
<td>Yellow-green with browning</td>
<td></td>
</tr>
<tr>
<td><em>pv. campestris</em> strain XC42&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Clark</td>
<td>Yellow-green with browning</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Bacterial cells covered by fibrillar or electron-dense amorphous material in the leaf intercellular space.

<sup>b</sup>Virulent.

<sup>c</sup>Susceptible.

<sup>d</sup>Pustules occurred at margins of affected areas.

<sup>e</sup>Symbols: = immobilization absent or rare; ++ = part of bacterial population immobilized with the remainder free in the intercellular space; ++ = all or nearly all bacteria immobilized.

<sup>f</sup>Resistant.

<sup>g</sup>Avirulent.

<sup>h</sup>Pathogen of crucifers; nonpathogen of soybean.

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Fig. 2. Virulent *Xanthomonas campestris* pv. *glycines* in the intercellular spaces of mesophyll of susceptible soybean cultivar Clark. a, Virulent strain XP175 in microcolonies surrounded by bacterial extracellular polysaccharide at 26 hr. Host mesophyll cells (MC) exhibited dilated endomembranes. IS = intercellular space (×20,000). b, Strain XP175 at 48 hr. Microcolonies were absent and bacteria were free in the intercellular space (IS). Large lucent vesicles appear in the host cytoplasm, especially in the cell on the right. Chloroplasts were often rounded and displaced away from the host cell membrane (×12,000). c, Virulent strain A surrounded by abundant bacterial EPS at 48 hr (×20,000). d, Strain A, heat-killed, at 48 hr. Heat-killed bacteria (B) were always immobilized. IS = intercellular space (×12,000).
bacteria contained fibrils that apparently originated at the plant cell wall, suggesting degradation of the host wall possibly by bacterial or induced host enzymes. Various images considered together suggested a process that included elaboration of fibrils which eventually enveloped the bacteria and filled in with amorphous, electron-dense material (Fig. 3a–c). The methodology, however, did not allow unequivocal discrimination between wall microfibrils and bacterial EPS. All cells of strain S-9-8 were wholly embedded in a heavy-staining cementum 48 hr after inoculation (Fig. 4). Some bacteria contained coagulated cytoplasm, while others appeared to be normal.

A similar involvement of fibrils was occasionally observed around cells of virulent strain XP175 in cultivar Clark. Fig. 5 shows strain XP175 cells at 48 hr apparently situated in a narrow space between host cells. The bacteria are surrounded by fine fibrils. Moreover, two structures with the general appearance of wall appositions appear in one adjacent host cell.

Results (unpublished) with avirulent strain 1136 on cultivar Clark were similar to those with strain S-9-8. At 24 hr, live bacteria were in cell junctions surrounded by fibrillar material; at 48 hr, bacterial cells were immobilized by cementum and a few free bacteria were observed.

At 48 hr, cells of avirulent strain 1716 were rarely immobilized; the large bacterial population was nearly all free in the intercellular space (Fig. 6a and b). However, when strain 1716 was heat-killed before inoculation, immobilization of dead cells was total (unpublished). At 48 hr, cells of X. c. pv. campesiris strain XC42 were about equally free and immobilized (Fig. 6c). The structures immobilizing strain XC42 were fibrillar with an amorphous heavy-staining component.

Cultivar Clark 63 was inoculated only with strains S-9-8 and XP175. At 48 hr, most bacteria of avirulent strain S-9-8 were immobilized (unpublished), with no immobilization of virulent strain XP175 (Fig. 6d) occurring up to 72 hr after inoculation.

Ultrastructure of host cells. Changes in mesophyll cell structure in cultivar Clark that were characteristic of the response to virulent strains A and XP175 (Figs. 2a–c and 5) included dilation of the endoplasmic reticulum and formation of many lentic vesicles (possibly Golgi vesicles) within the cytoplasm. Chloroplasts tended to assume a more rounded shape and move away from the plasma membrane. Fig. 2a shows invagination of the host plasmalemma in apposition to the bacterial colony.

The response of cultivar Clark to avirulent strain S-9-8 appeared to be mild and limited to small areas. Fig. 4 shows alterations in chloroplast structure in cells near the mass of immobilized bacteria.

Strain 1716 caused extensive host cell necrosis and collapse in cultivar Clark (Fig. 6a), as well as invagination of host plasmalemma and accumulation of vesicles and amorphous material between the plasmalemma and cell wall (Fig. 6b). Chloroplasts tended to remain in position close to the plasma membrane, but thylakoid membranes were disoriented and displaced toward the inner half of the organelle, leaving a large parietal area of stroma.

Strain XC42 affected cultivar Clark severely (Fig. 6c), causing extensive invagination of the cytoplasm into the cell vacuole (plasmolysis), swollen mitochondria, and minor displacement of chloroplast membranes. The principle host response induced by strain XC42 was plasmolysis rather than host cell collapse.

In Clark 63, virulent strain XP175 caused considerable cell necrosis (Fig. 6d). In addition, some cells exhibited invagination of plasmalemma and changes in chloroplast membrane arrangements as commonly seen in the incompatible interactions in cultivar Clark, while occasional cells had endomembrane dilation (unpublished) typical of the compatible interaction.

No effects on host cells were noted in response to heat-killed bacteria.

**DISCUSSION**

Virulent phytopathogenic bacteria multiply rapidly in compatible hosts (11,34) and, in thin sections observed in the electron microscope, tend to be dispersed in the extensive...
Fig. 4. Avirulent strain S-9-8 of *Xanthomonas campestris* pv. *glycines* totally immobilized in leaf mesophyll of soybean cultivar Clark at 48 hr. Bacteria appeared to be a mixture of live and dead cells; some showed signs of cell division. Contents of nearby chloroplasts were disorganized. MC = mesophyll cell, EC = epidermal cell (×8,000).
intercellular spaces of the leaf (2.7,30). X. c. pv. glycines strains XP175 and A were typical of compatible bacteria. Both strains appeared to generate EPS, shown to be correlated with virulence of some bacteria (8,30). The extensive EPS of strain A eventually occluded most of the intercellular space in cultivar Clark. With strain XP175, EPS was evident at 26 hr, the earliest observation, surrounding the bacteria in microcolonies. At 48 hr, the microcolonies and the structured EPS of XP175 were absent, although considerable material was mixed with bacteria in the intercellular space. Sample preparation may have contributed to collapse of the highly hydrated EPS. EPS has been implicated in pathogenicity by contributing to water-soaking (5,8) and by preventing direct cell-cell contact between host and bacteria (8,24,30,35).

Incompatible bacteria usually reach lower final populations in inoculated plants (11,34). The failure of avirulent strains, species nonpathogens, and saprophytes to attain high populations in host or nonhost plants may result from several factors, singly or in combination, such as drying out of the intercellular space (8,31,35), immobilization and consequent inactivation of bacteria at the mesophyll cell surface (16,21,27,30) or the leaf surface (25), or accumulation of phytoalexins by host cells during the incompatible interaction (14,23). The soybean phytoalexin glyceollin has been implicated in the resistance of soybean to incompatible races of Pseudomonas syringae pv. glycinea (20); however, phytoalexins are not responsible for soybean resistance to incompatible strains of X. c. pv. glycines or to X. c. pv. campesiris, since comparable kinds and levels of soybean isoflavonoids and isoflavone glucosides with antibacterial activities were found in both compatible and incompatible interactions (9).

On the other hand, all four of the incompatible strains of Xanthomonas tested in the present experiments demonstrated some tendency to be immobilized at the mesophyll cell surface, either by an amorphous heavy-staining material or by fibrils, or by a combination of the two. The immobilization ranged from the total entrapment of strain S-9-8 at 48 hr to minor entrapment of strain 1716 after the same time. The strains that had the smallest proportion of immobilized bacteria reached the highest population levels.

Sequeira et al (30) and Huang et al (22) reported immobilization of avirulent strains of Pseudomonas solanacearum in tobacco leaves and Erwinia amylovora in apple xylem vessels, respectively, and concluded that immobilization served to restrict bacterial population growth and translocation within the tissue. The avirulent strains used in those two studies differed from the corresponding virulent strains in their reduced ability to produce bacterial EPS in vitro. Sequeira (29) proposed that specific host recognition of bacterial cell surface components and consequent immobilization were able to occur when EPS was absent. All of the strains of X. c. pv. glycines used in the present study produced similar amounts of EPS in vitro (9; W. F. Fett, unpublished) and the EPS of virulent and avirulent strains was chemically identical (S. F. Osman and W. F. Fett, unpublished). If differential EPS production is a factor in host recognition in this interaction, then the difference must be expressed only in vivo.

It has been pointed out that entrapment of bacteria in bean (Phaseolus vulgaris L.) leaves may be a passive phenomenon brought about by evaporation of intercellular water after inoculation with bacteria (19). Our results with heat-killed X. c. pv. glycines support the hypothesis that at least some aspect of envelopment in soybean is passive. Heat-killed bacteria were always heavily covered by an amorphous substance, usually at cell junctions. At 48 hr, we observed no structural effects on the host cells, although dead bacteria were observed to produce local plasmolysis and vesiculation of the host plasma membrane in cotton and tobacco (3,30). If surface tension and capillarity were wholly responsible for bacterial envelopment, the expectation would be to observe envelopment at cell junctions or other small gaps that would accommodate water droplets or bridging by a presumably "sticky" substance. We observed apparently live bacteria immobilized in this way, suggesting the possibility of passive entrapment. However, we sometimes observed strain S-9-8 at 24 hr on mesophyll cell surfaces away from cell junctions where capillary effects would be minimal. At these sites, the host cell surface appeared to be disrupted and microfibrils were released from the cell wall matrix. Similar phenomena have been described in tobacco, cotton, and bean (6,16,32). We conclude that in addition to passive envelopment, an active immobilization occurs in soybean that involves interaction of the host with live bacteria. By 48 hr all strain S-9-8 cells were immobilized by profuse amounts of electron-dense material. These results could be used to support a role for immobilization in the severe growth restriction observed for this strain. However, equally plausible is the possibility that immobilization was due to poor growth of strain S-9-8 and, consequently, more contact between bacteria and host cells. For each of the incompatible strains of Xanthomonas that were tested, the extent of immobilization was inversely related to the final population levels reached. For example, leaves inoculated with avirulent strain 1716, which grew almost as well as the two compatible strains, contained few immobilized bacteria. The intense HR caused by strain 1716 appeared to be a consequence, not of bacterial immobilization, but of the large bacterial population. A massive HR induced by a large inoculum plus rapid growth may mask the role of immobilization. Politis and Goodman (27) reported a more intense immobilization response in tobacco when the concentration of bacterial inoculum was 10⁶ cfu/ml than when it was at 10⁶ cfu/ml and observed that the HR induced by the lower concentration was delayed and milder, permitting more sustained contact between bacterial cell and host.

The findings with X. c. pv. campesiris strain XC42 are more typical of the putative relationship between bacterial immobilization and HR. Strain XC42 exhibited moderate growth in vivo, about equal numbers of immobilized and free bacteria, and caused a moderate macroscopic HR. Strain XC42 was similar to strain 1716 in growth characteristics and effect on host tissue except...
Fig. 6. *Xanthomonas campestris* strains that caused hypersensitive leaf browning in soybean cultivars Clark or Clark 63. a and b, Cells of avirulent strain 1716 of *X.c. pv. glycines* in cultivar Clark at 48 hr were almost always free in the intercellular spaces. Necrotic host cells (NC) and invagination of host plasma membranes (arrow, in b) were common. Vesicles accumulated between host wall and invaginated plasma membranes (×10,000). c, *X.c. pv. campestris* (pathogen of cabbage) in cultivar Clark at 48 hr. The principle host symptom was plasmolysis rather than cell necrosis. Bacteria (B) were immobilized or free (×6,000). d, Virulent strain XP175 of *X.c. pv. glycines* in resistant cultivar Clark 63. No bacterial immobilization was observed. Necrotic host cells (NC) were common (×10,000).
for a lower incidence of host cell collapse and higher incidence of host cell plasmolysis with strain XC42. We could not conclude that cell collapse was the sequel to plasmolysis because possible intermediate structures were rarely observed. Al-issa and Sige (1) observed transient plasmolysis in tobacco leaf cells in the first 6 hr after inoculation with *Pseudomonas pisi* and attributed it to water congestion in the leaf. In our experiments, we concluded that plasmolysis was not an artifact, although it could be exacerbated by buffer concentrations greater than 0.1 M. Water leakage from the vacuole during the HR (15) could be expected to produce plasmolysis as observed. If sustained plasmolysis caused extensive breakage of plasmodesmata, then turgor could not be restored and cell desiccation and collapse would ensue. Thus, the severity of the macroscopic HR may reflect the rate of ion (hence, water) efflux from the mesophyll cells and the number of cells simultaneously induced to plasmolyze. This would explain the massive HR caused by rapidly growing strain 1716. It is interesting that with strain S-9-8 at 26 hr, plasmolysis was frequently observed, while at 48 hr there was no plasmolysis in the area of the immobilized bacteria. This suggests that the plasmolysis of the early HR is reversible and does not necessarily lead to cell collapse.

Resistance of cultivar Clark 63 to virulent strain XP175 of *X. pv. glycines* was not correlated with bacterial immobilization since there was little evidence of immobilization. The absence of immobilization could be predicted on the basis of the high bacterial growth in vivo reported here and elsewhere (9,17). The origin and nature of the occasionally large amounts of dark coloration involved in immobilization, such as that observed with strain S-9-8, is unknown. Ultrastructural analysis of similar material in a tobacco leaf inoculated with an avirulent strain of *P. solanacearum* indicated the presence of phenolics and possibly tannin (26). The authors postulated a role for particulate polyphenoloxidase acting on phenolic secondary metabolites at the host cell surface.

The capability for in situ agglutination of bacteria in plant leaves has been amply demonstrated, although not specifically shown to be involved in resistance. Lectins, pectins, galacturonide, and uncharacterized plant leaf extracts agglutinate bacteria in vitro (4,16,21,29,33). The evidence suggests that, depending on their surface hydrophobicity and/or charge, bacteria in contact with the mesophyll cell surface are likely to adhere. Depending on the growth rate, the bacteria either stay there long enough to elicit a full, immobilizing event, or they escape as cell division progresses. When cells of virulent strain XP175 were located in an apparently restrictive intercellular location, enveloping fibrillar, wall appositions (27), and host cell plasmolysis developed (Fig. 5), suggesting the immobilization may be a generalized response of the mesophyll cell to the sustained presence of live bacteria at the surface. The invagination of the host plasmalemma near cells of strain XP175 in Fig. 2a is possibly a weak manifestation of the same phenomenon. Active immobilization may be a second defense reaction after bacterial growth or EPS production is inhibited by other means. Al-Mousawi et al (3) concluded that entrapment is the normal fate of bacteria within cotton leaf intercellular spaces unless countervailing circumstances occur. In our experiments, *X. campestris* was entrapped unless the bacteria generated EPS that prevented sustained contact between host and bacteria, or the bacterial growth rate was high enough so that sustained contact with the host cell surface was minimized.

We have examined at the ultrastructural level the interaction of soybean leaves with virulent and avirulent soybean pathogens; with nonpathogens of soybean, including *Pseudomonas*, *Xanthomonas*, *Erwinia*, and *Corynebacterium*; and with saprophytic bacteria (10,11,15, unpublished). Several envelope phenomena occur in soybean leaves after inoculation with bacteria. The earliest is passive envelopment as observed after 4 and 24 hr with incompatible pseudomonads (10) and with heat-killed bacteria. Second, there is active immobilization of certain incompatible bacteria (*C. flaccumfaciens* pv. *flaccumfaciens*, *E. carotovora* subsp. *atroseptica*, *X. pv. campestris*) and avirulent bacteria (*X. pv. glycines*). The degree of immobilization correlates with observed bacterial growth restriction, but a causal relationship has not been firmly established. Third, there is envelopment of virulent compatible bacteria (*X. pv. glycines*) by bacterial EPS which provides a favorable environment for bacterial survival and growth. We also conclude that bacterial envelopment or immobilization is not required for induction of HR in soybean leaves (10,11).

We propose that the term envelopment be used to describe structures that appear to be too fragile or in insufficient amount to inhibit bacterial growth and movement, e.g., envelopment by bacterial EPS. The term immobilization should be used only when bacteria are completely covered by electron-dense material that appears capable of restricting bacterial growth and movement. The term encapsulation should not be used because of possible confusion with the polysaccharide capsules produced by some bacteria.

**LITERATURE CITED**


