Cotyledon and Leaf Ultrastructure of a Bacterial Blight-Immune Cotton Line Inoculated with a Low Level of *Xanthomonas campestris* pv. *malvacearum*

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ABSTRACT

Al-Mousawi, A. H., Richardson, P. E., Essenberg, M., and Johnson, W. M. 1982. Cotyledon and leaf ultrastructure of a bacterial blight-immune cotton line inoculated with a low level of *Xanthomonas campestris* pv. *malvacearum*. Phytopathology 72:1230-1234.

Cotyledons and foliage leaves of the cotton line Im 216, which is immune to bacterial blight, were inoculated with a low level inoculum (5×10^5 cells/ml) of *Xanthomonas campestris* pv. *malvacearum*, and examined by light microscopy and transmission electron microscopy. Degenerative changes in some of the mesophyll cells were apparent 2 days after inoculation. Bacteria were found in samples taken 5 and 6 days after inoculation and were located in intercellular spaces near collapsed mesophyll cells. Fibrillar material was associated with all bacteria observed but did not completely envelop all the bacteria. Clusters of bacteria were larger than observed in an earlier ultrastructural study of Im 216 cotton cotyledons inoculated with a higher cell number but were similar to compact masses of bacteria observed previously in light microscopic studies of Im 216 cotyledons inoculated with 7×10^5 cells per milliliter of *X. campestris* pv. *malvacearum*.

Envelopment of pathogenic and saprophytic bacteria by fibrillar material at the surfaces of host cells has been observed by transmission electron microscopy (TEM) in leaves of tobacco (12,13), cotton (2,4), rice (10), and bean (5,9). In these studies, leaves were inoculated with bacterial suspensions sufficiently concentrated (10^8–10^9 cells per milliliter) to elicit hypersensitive responses (HR), resulting in confluent necrosis (5,7,11) in incompatible hosts. Such high concentrations are probably rare under field conditions. Our bacterial blight-immune cotton (*Gossypium hirsutum* L.) line Im 216 has been grown in field rows adjacent to rows of diseased susceptible lines for more than 10 yr without showing macroscopically visible HR to naturally transmitted infection.

In tobacco, cotton, and rice, envelopment of bacteria has been observed with incompatible pathogens and saprophytes, but not with compatible pathogens (2,4,10,12,13). It has been proposed that attachment and envelopment are essential steps in the expression of host resistance (8,13). In bean, however, envelopment is less specific (5,9).

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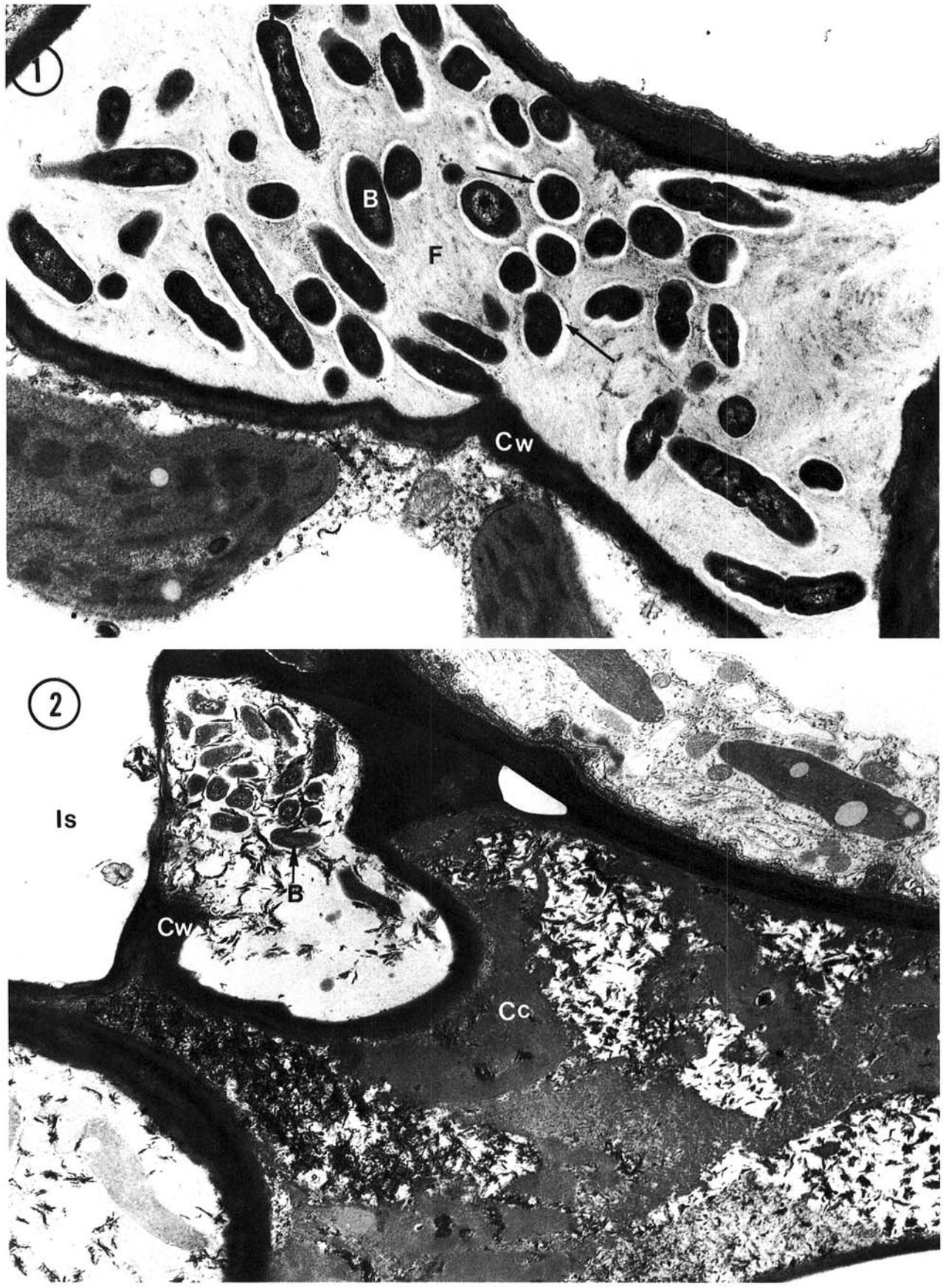
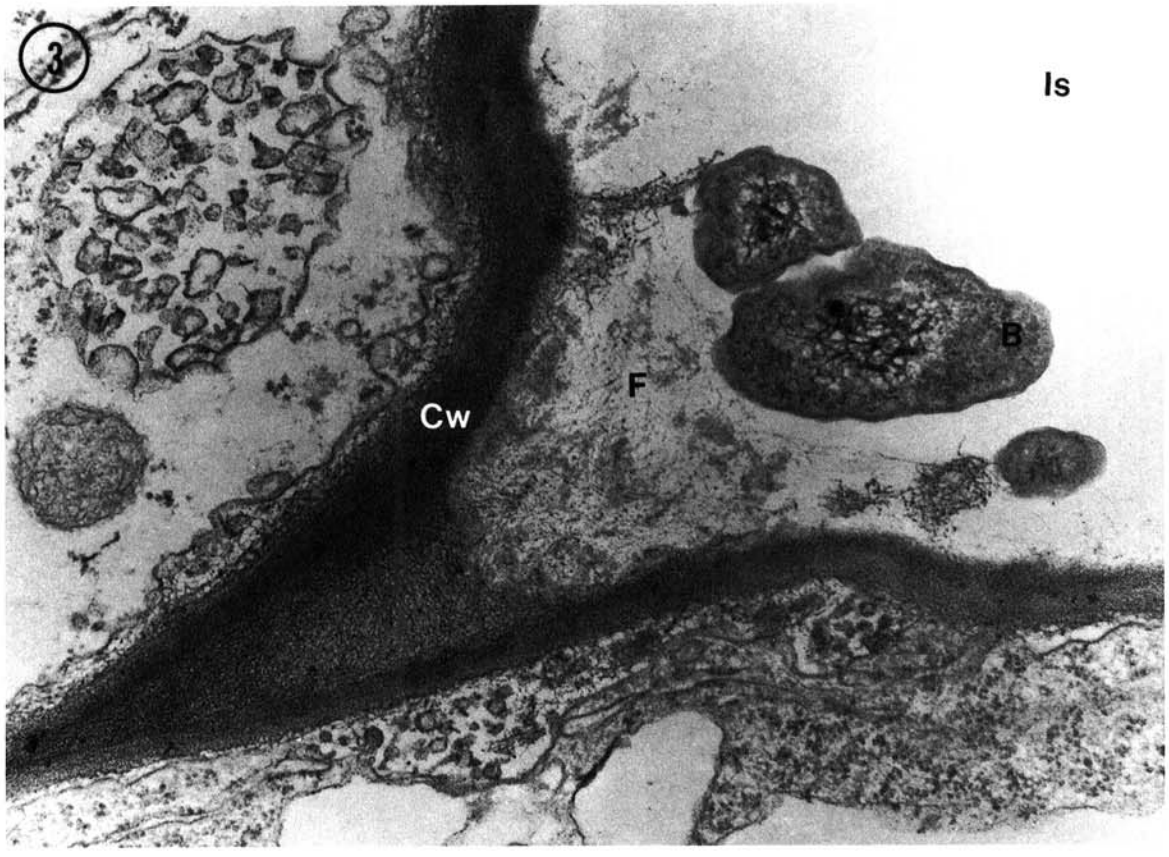
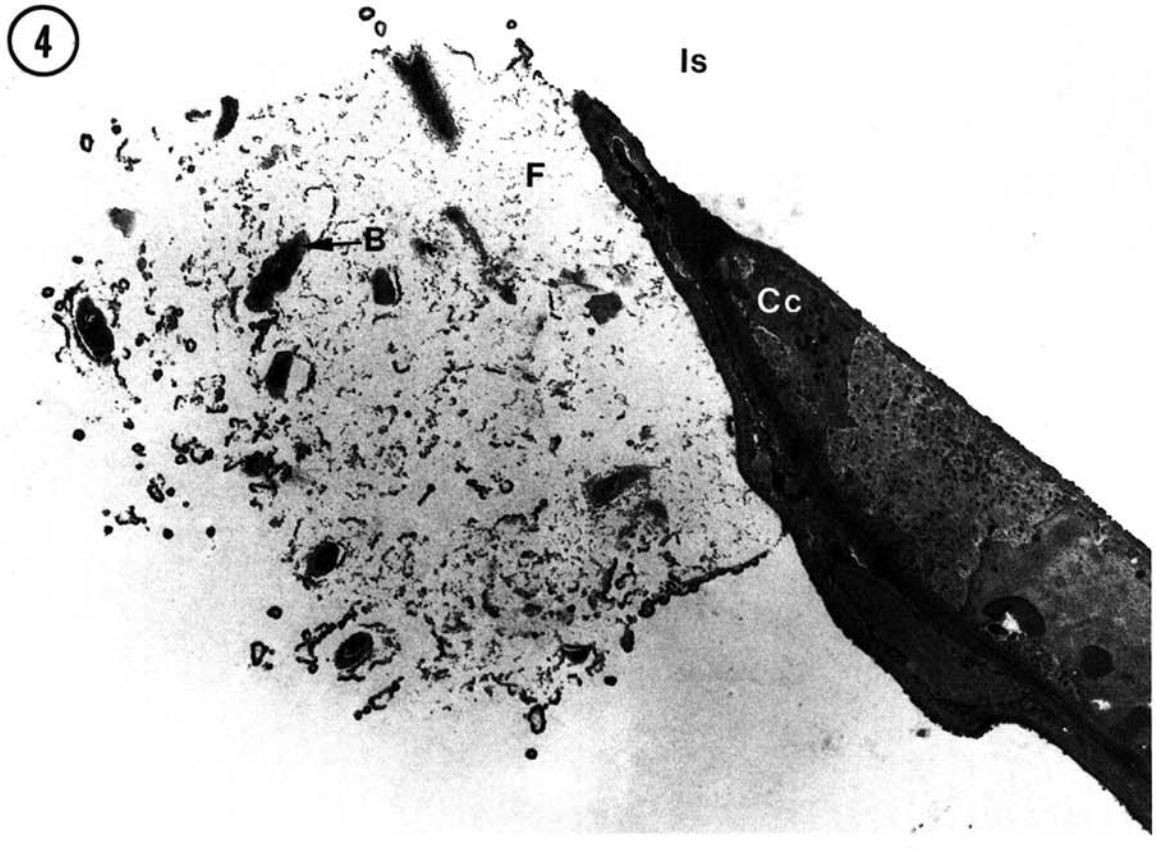
Infiltration of leaves of a cotton line Im 216, which is immune to bacterial blight, with suspensions of greater than 10^7 cells per milliliter of *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye caused confluent necrosis (7). Following infiltration with low concentrations of *X. campestris* pv. *malvacearum*, leaves remained alive and turgid, and only small clusters of mesophyll cells became brown and necrotic (7). Bacteria were observed by light microscopy (LM) in the intercellular spaces adjacent to the necrotic cells. Some of these bacteria were in compact masses on the surfaces of mesophyll cells (7, plates 11–13) or between collapsed mesophyll cells (7, plate 15).

We report here TEM observation of Im 216 cotton line leaves and cotyledons inoculated with 5×10^5 cells of *X. campestris* pv. *malvacearum* per milliliter, which introduced about 2×10^3 cells per square centimeter of leaf area (7). At this inoculum concentration, resultant bacterial colonies were widely separated, and growth was inhibited 3–4 days after inoculation by the host's localized resistance response (7). Other workers have considered that studies with such dilute inocula would be infeasible for TEM studies (5). The objectives of the present study were to determine if envelopments previously described (4) with an artificially high inoculum level also occurred with lower levels and to demonstrate with TEM whether compact masses of bacterial cells produced after introduction of dilute inocula were similar to those described in a previous LM study (7).



Figs. 1–2. 1, Mesophyll cells of immune cotton cotyledon 5 days after inoculation with *Xanthomonas campestris* pv. *malvacearum*. Bacterial cells within fibrillar material in the intercellular space, bacteria surrounded by capsular material (arrows) (×15,120). **2,** Mesophyll cells of foliage leaf 5 days after inoculation; bacteria apparently trapped in mass of fibrillar material near collapsed mesophyll cell (×11,350). B = bacterium, Cc = collapsed cell, Cw = cell wall, F = fibrillar material, Is = intercellular space.





Figs. 3–4. Mesophyll cells of foliage leaf 6 days after inoculation. **3,** Bacterial cells between two mesophyll cells. The upper mesophyll cell has abnormal cytoplasmic contents (×43,800). **4,** Bacterial cells surrounded by fibrillar material attached to two collapsed mesophyll cells (×10,100). B = bacterium, Cc = collapsed cell, Cw = cell wall, F = fibrillar material, Is = intercellular space.

MATERIALS AND METHODS

Cotton line Im 216 was inoculated with a highly aggressive isolate of *X. campestris* pv. *malvacearum* race 3 (1). The Im 216 cotton line (3) possesses a high resistance to all known races of *X. campestris* pv. *malvacearum*. Growth conditions for host plants and bacteria were as described in Al-Mousawi et al (1) and Essenberg et al (6), respectively. The inoculum was a suspension of 5×10^5 bacteria/ml in sterile, saturated calcium carbonate solution. Cotyledons of four 2-wk-old plants were infiltrated with the inoculum using a hypodermic syringe without a needle. Nine fully expanded leaves of six 3-wk-old plants were inoculated by vacuum infiltration. Samples were taken from cotyledons and leaves every 24 hr for 6 days after inoculation. The tissues were fixed and embedded as previously described (1). Sixty blocks prepared from eight cotyledons and nine leaves were sectioned and examined. Thick sections (0.4–0.5 μm) were taken from the blocks, stained with Azure B bromide (7), and examined with a light microscope. Blocks containing darkly stained mesophyll cells that were characteristic of cellular responses to bacterial infection (7) were selected, trimmed to include them, and thin sectioned for TEM (1).

RESULTS

Two days after inoculation, some mesophyll cells showed collapsed, concave cell walls, and disrupted organelles and membranes. During the following days after inoculation, affected mesophyll cells contracted further and became more densely stained.

Bacteria were detected by light microscopy in 46 of the 60 blocks. Approximately 250 grids with thin (silver-reflective) sections were examined by TEM. Bacteria were found in a few grids containing sections of five cotyledons and three leaves, taken 5 and 6 days after inoculation. All bacteria observed were associated with collapsed mesophyll cells and were located in intercellular spaces.

Groups of bacteria were surrounded by fibrillar material and appeared to be trapped by collapsing mesophyll cells (Figs. 1 and 2). Other clusters of bacteria occurred in intercellular spaces at the junction of two mesophyll cells (Fig. 3) or on the surfaces of cells (Figs. 4 and 5). Fibrillar material was always observed to be associated with bacteria.

DISCUSSION

In earlier studies of Im 216 cotton cotyledons after inoculation with 10^8 cells/ml of *X. campestris* pv. *malvacearum*, bacterial cells were observed to be completely enveloped by fibrillar material that was connected to mesophyll cell walls (4). In the present study of Im 216 cotton leaves after inoculation with 5×10^5 cells/ml, fibrillar material was associated with all bacteria observed, but it did not appear to envelop all bacteria completely. This is true except in the case of Fig. 1, which is totally enclosed space, completely filled with the fibrillar material. In most cases, with the exception of the bacterial mass in Fig. 5, the fibrillar material extended to mesophyll cell walls. It is possible that the bacterial mass of Fig. 5 was attached to the host wall in a plane that was not sectioned.

The larger groups of bacteria seen in this study were consistent with the larger bacterial growth yield from inocula of 5×10^5 cells/ml (approximately 600-fold), which produce widely spaced bacterial colonies to which cotton leaves appear to respond locally, requiring 3–4 days to inhibit bacterial growth (7). In contrast, an inoculum of 10^8 cells/ml elicited a more rapid and general HR which inhibits bacterial growth within one day, permitting a growth yield of only approximately sixfold (7). The shape, location, and number of bacteria in the groups appeared similar to compact masses of bacteria demonstrated previously by

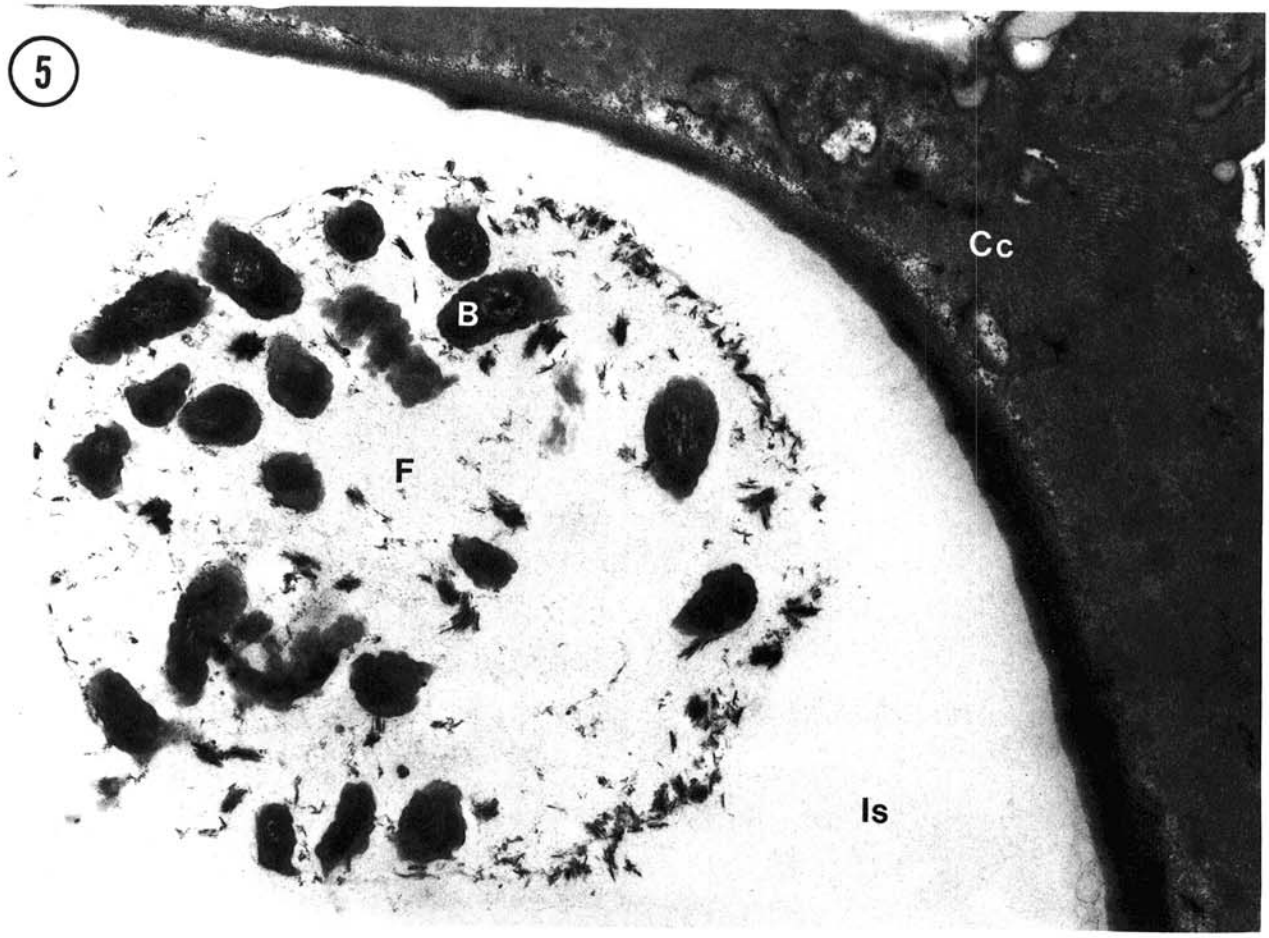


Fig. 5. Bacterial cells enveloped by fibrillar material. Bacteria close to collapsed foliage leaf mesophyll cell (×24,500). B = bacterium, Cc = collapsed cell, Is = intercellular space.

photomicrography of Im 216 leaves that had been inoculated with 7×10^5 bacteria per milliliter (7).

In our earlier ultrastructural study of the general HR to a concentrated inoculum (4), fibrillar materials were associated with *X. campestris* pv. *malvacearum* and appeared to attach it to host cell walls. We conclude from the current study that fibrillar material is also present during the localized resistance response of Im 216 cotton leaves to individual bacterial colonies. The greater looseness of the fibrillar material in the observations presented here causes us to doubt that it directly restricts bacterial multiplication, as has been suggested by others (8).

In leaves and cotyledons of susceptible cotton, no attachment of *X. campestris* pv. *malvacearum* to host cell walls by enveloping fibrillar material has been observed (1); however, in two cotton lines that possess lower levels of genetically determined resistance than Im 216, we have observed *X. campestris* pv. *malvacearum* enveloped and attached to host cell walls within a few hr after inoculation. Later, however, the envelopes ruptured as the bacteria multiplied (*unpublished*).

We cannot at present reject either of the hypotheses that the attachment observed in resistant cotton plays a role in the incompatible interaction or, alternatively, that it plays no essential role in resistance, but for some reason is not observed during a compatible interaction.

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