# Scanning Electron Microscopy of Infection Sites and Lesion Development on Tomato Fruit Infected with *Pseudomonas syringae* pv. *tomato*

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## ABSTRACT

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Tomato ovary and fruit surfaces inoculated with *Pseudomonas syringae* pv. *tomato* (= *P. tomato*) were examined with a scanning electron microscope to observe possible infection sites and to follow lesion development. Bacteria were detected on both glandular and nonglandular trichomes present on ovaries during anthesis. Following anthesis, trichomes were gradually lost, leaving openings in the young fruit epidermis. Swollen areas of the epidermis that resembled trichome bases were filled with bacteria, suggesting that open trichome bases may serve as fruit infection sites. Mature lesions were either sunken or raised, and masses of bacteria were extruded from cracks in the lesion surface.

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.), caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al (= *P. tomato*) (4), often assumes epidemic proportions and may cause considerable crop damage and serious economic losses under favorable environmental conditions (7). The most conspicuous and damaging phase of the disease is on the fruit, where small black lesions are formed. These lesions constitute a severe blemish on fruit for fresh market and are also objectionable to processors (7,9).

Studies of bacterial infection of tomato fruit by *Corynebacterium michiganense* pv. *michiganense* and *Xanthomonas campestris* pv. *vesicatoria* have been reported (2,5), but these are relatively old reports published before the advent of scanning electron microscopy. In addition, the cultivars studied have been replaced by commercial hybrids. There have been no studies of fruit infection by *P. syringae* pv. *tomato*. Fruit infection sites and early stages of bacterial speck lesion development have been difficult to observe with the light microscope due to problems of resolution and depth of focus. Accordingly, this investigation was conducted to determine possible fruit infection sites and to follow lesion development with a scanning electron microscope (SEM).

## MATERIALS AND METHODS

Tomato plants of the susceptible fresh market cultivar Pik-Red (Joseph Harris Co., Inc., Rochester, NY 14624) were greenhouse grown in 2-L plastic pots containing VSP Peat-Lite Mix (Bay-Houston Towing Co., Houston, TX 77081). A 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18100) was applied biweekly. Tomato fruit development was arbitrarily divided into the following developmental stages: closed calyx, open calyx, open corolla, green fruit $\leqslant$3 cm in diameter, green fruit $>$3 cm in diameter, and pink to red fruit.

A naturally occurring rifampicin-resistant isolate of *P. syringae* pv. *tomato* (isolate PtR5) was the pathogen used throughout this study. Inoculum was prepared and applied to each developmental stage as previously described (6).

To observe possible infection sites, ovaries and fruit were sampled at the six developmental stages and prepared for SEM examination (8). Entire ovaries or epidermal blocks (4 × 4 mm) from larger fruit were fixed 2 hr in 4% glutaraldehyde followed by 2 hr in 1% osmium tetroxide. Both solutions were buffered at pH 7.2 with 0.1 M sodium cacodylate. Fixed tissues were dehydrated in a graded ethanol series. All procedures through dehydration were performed at about 4 C. Following dehydration, tissues were critical-point dried using a Bomar critical-point drier with $CO_2$ as the carrier gas. Samples were then mounted on aluminum stubs, sputter-coated with 20–30 nm of gold, and examined in a JEOL JSM - 35C scanning electron microscope.

To follow lesion development, several fruit were inoculated at the green fruit ($\leqslant$3 cm in diameter) stage. Based on initial investigations, greenhouse-grown fruit at this stage was known to be the most susceptible to infection by *P. syringae* pv. *tomato* (6). Blocks (4 × 4 mm) were cut from inoculated fruit surfaces 7, 11, or 21 days after inoculation and prepared for SEM observation as previously described.

## RESULTS

No trichomes were present on tomato ovaries prior to anthesis (closed and open calyx stages) (Fig. 1). The hexamerous nature of the ovary was evident at this early stage of development. Bacteria were distributed over the entire surface with slightly higher populations in depressions between epidermal cells. During anthesis (the open corolla stage), the ovary surface was densely covered with unicellular papillary trichomes, long multicellular nonglandular trichomes, and capitate glandular trichomes (Figs. 2 and 3); no stomata or other natural openings were observed. Bacteria were found primarily on nonglandular (Fig. 4) and glandular (Figs. 5 and 6) trichomes.

On green fruit $\leqslant$3 cm in diameter, few to many trichomes were partially detached (Fig. 7), broken off at their bases (Fig. 8), or completely missing. The actual number of open trichome bases varied according to fruit size, small fruit having more open bases than large fruit. The greatest density of open bases (approximately 12 per square millimeter of epidermis) was observed at the green fruit ($\leqslant$3 cm in diameter) stage, which also was the stage most susceptible to infection by *P. syringae* pv. *tomato* (6). Trichome bases varied in appearance but usually had central openings 10–20 $\mu$m in diameter (Figs. 9 and 10). Only a few apparently randomly dispersed bacteria were observed on the fruit surface.

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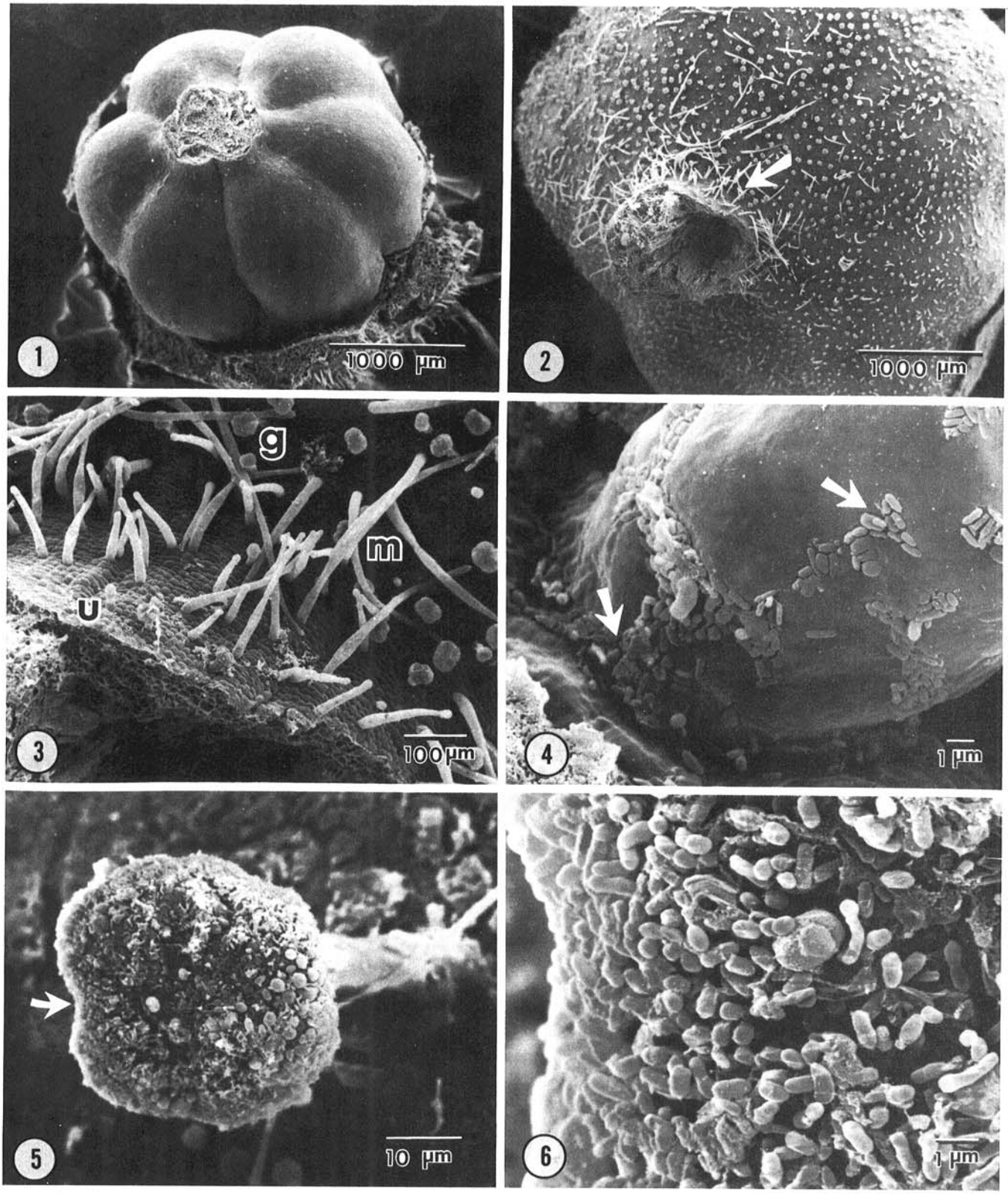
On green fruit >3 cm in diameter, many trichomal openings were filled (Figs. 11 and 12). As in the green fruit (≤3 cm in diameter) stage, very few bacteria were observed on the fruit surface. By the time fruit began to turn color, remnants of trichome bases were barely discernible due to a thick waxy cuticle.

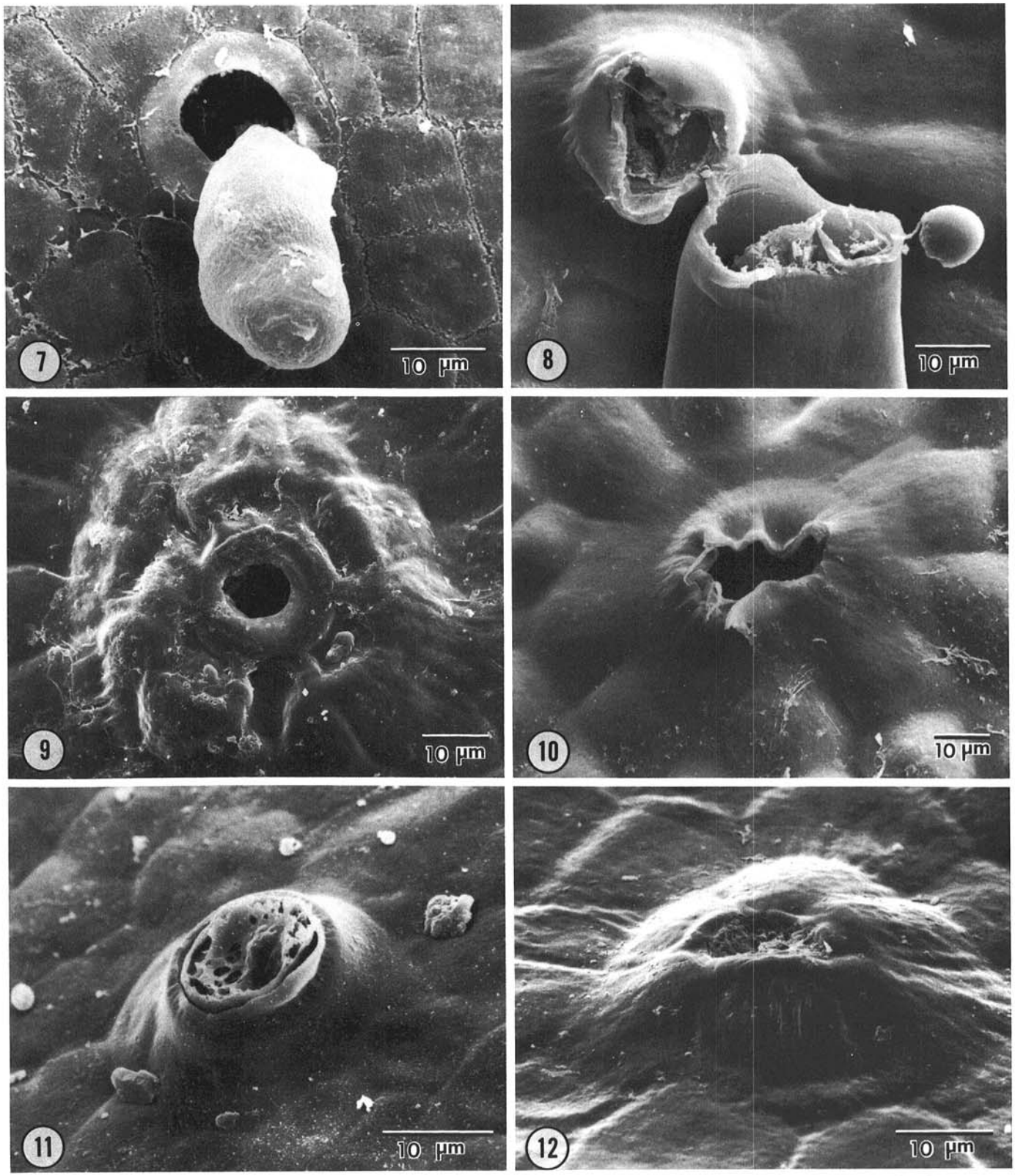
The identity of the bacteria observed with the SEM on fruit surfaces was not positively determined; however, isolations from various inoculated developmental stages yielded rifampicin-resistant colonies while isolations from uninoculated developmental stages did not. Bacteria were never observed with the SEM on uninoculated controls.

In the lesion development study, fruit surfaces were examined 7,




**Figs. 1–6.** Scanning electron micrographs of tomato ovaries inoculated with *Pseudomonas syringae* pv. *tomato*. **1,** Tomato ovary prior to anthesis (×25). **2,** Tomato ovary at anthesis (×22). **3,** Enlargement from Fig. 2 (arrow) showing unicellular papillary trichomes (u), long multicellular nonglandular trichomes (m), and capitate glandular trichomes (g) (×110). **4,** Bacteria (arrows) on the surface and at the base of a long nonglandular trichome (×4,500). **5,** Capitate glandular trichome (×1,355). **6,** Enlargement from Fig. 5 (arrow) showing bacteria on the head of a glandular trichome (×7,505).

11, or 21 days after inoculation. There was no evidence of lesion development and very few bacteria were visible on the fruit surface 7 days after inoculation. Fruit symptoms first appeared to the unaided eye as minute specks 11 days after inoculation. SEM examination of epidermal blocks revealed early stages of lesion development including swollen and ruptured areas (Fig. 13). The similarity between natural openings observed in the epidermis (Figs. 9 and 10) and swollen sites (Fig. 14) was apparent. When one of the epidermal swellings (Fig. 14) was removed from the SEM, artificially ruptured with a glass needle, recoated with gold, and placed back in the SEM, large numbers of bacteria were observed inside the tissue (Fig. 15). Naturally ruptured areas of the epidermis



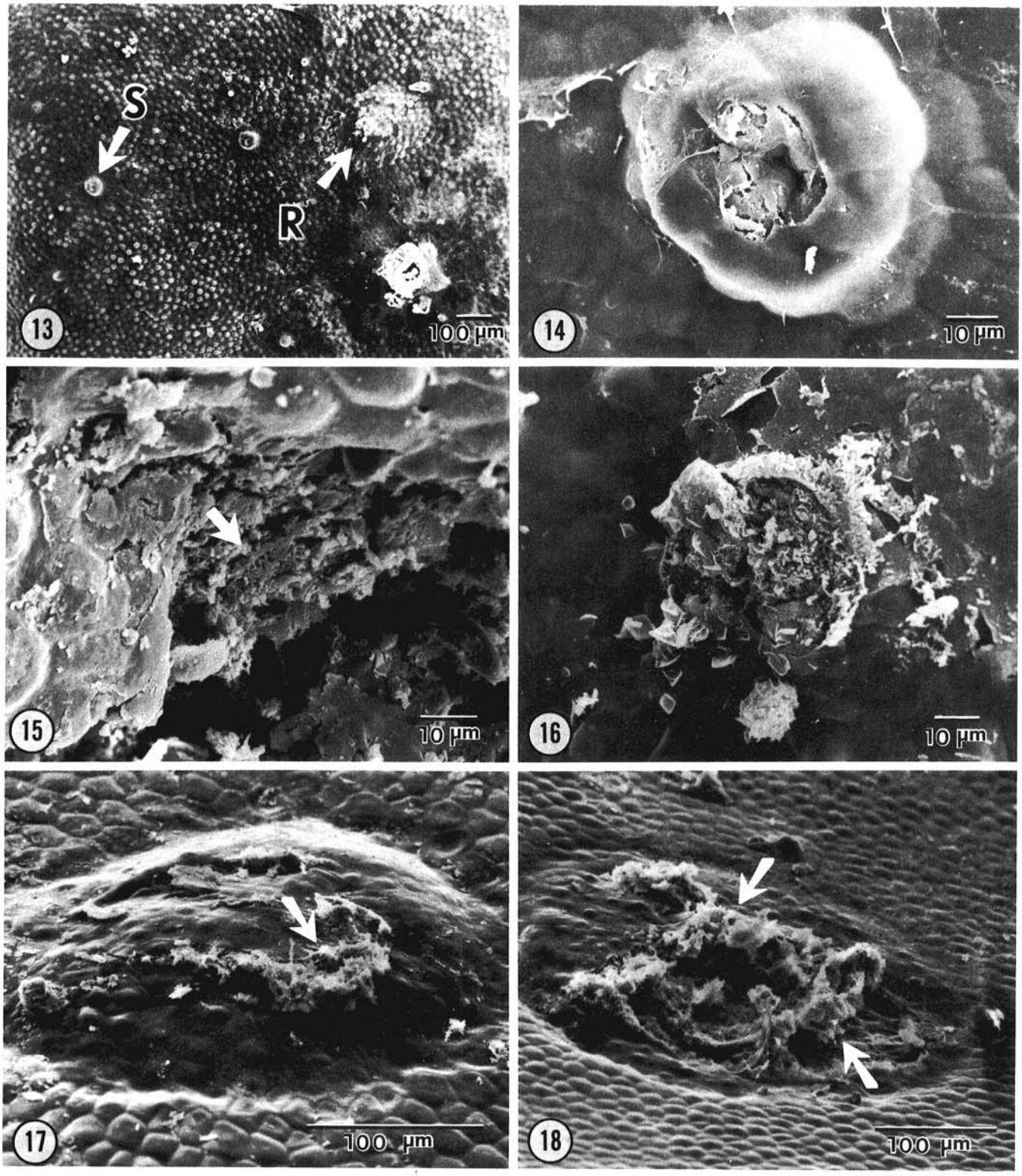

**Figs. 7–12.** Scanning electron micrographs of natural openings in surfaces of green tomato fruit ≤3 cm in diameter (**Figs. 7–10**) and >3 cm in diameter (**Figs. 11** and **12**). **7,** Nonglandular trichome partially detached (×1,715). **8,** Broken nonglandular trichome (×1,350). **9,** Slightly elevated natural opening (×1,210). **10,** Natural opening (×1,035). **11** and **12,** Filled natural openings; **11,** (×1,980); **12,** (×1,735).

exuded copious amounts of bacteria, which made it difficult to identify morphological details of the original infection sites (Fig. 16).

Twenty-one days after inoculation, lesions were clearly visible to the unaided eye and in the SEM appeared either raised (Fig. 17) or sunken (Fig. 18). Masses of bacteria were extruded through cracks in the lesions.

## DISCUSSION

Scanning electron microscopy of tomato ovary and fruit surfaces




**Figs. 13–18.** Scanning electron micrographs of surfaces of green tomato fruit >3 cm in diameter 11 days (**Figs. 13–16**) or 21 days (**Figs. 17** and **18**) after inoculation with *Pseudomonas syringae* pv. *tomato*. **13,** Epidermal block showing swollen (S) and ruptured (R) areas (×61). **14,** Enlargement from Fig. 13 (S) showing swollen area (×895). **15,** Subepidermal view of swollen area in Fig. 14 (×250). The swollen area was ruptured with a glass needle and recoated with gold. Masses of bacteria (arrow) were present directly beneath the swollen area. **16,** Enlargement from Fig. 13 (R) showing a ruptured area (×975). **17,** Raised bacterial speck lesion (×305). Masses of bacteria (arrow) have been extruded from a crack in the lesion surface. **18,** Sunken bacterial speck lesion (×205). Bacterial masses (arrows) have been extruded from the lesion.

revealed a developmental phenomenon that resulted in natural openings in the fruit epidermis. Ovaries were densely covered with trichomes at anthesis. When flowers were inoculated, ovarian trichomes were readily colonized by bacteria. During the rapid fruit expansion following fruit set, many of these trichomes were shed or broken off. Open trichome bases were an obvious feature in all epidermal blocks taken from fruit at the green fruit (≤3 cm in diameter) stage; hence, the possibility of infection through these natural openings was apparent. *P. syringae* pv. *tomato* rods are 0.7–1.2 μm by 1.5–3.0 μm (3). In the presence of free water, *P. syringae* pv. *tomato* residing on or near trichome bases could readily invade the fruit through these openings, which were usually 10–20 μm in diameter.

Tomato trichomes have been shown to be particularly favorable sites for infection by bacterial pathogens. Trichomal infection by *C. michiganense* pv. *michiganense* has been observed on both tomato foliage and fruit (2,10,11). Gardner and Kendrick (5) postulated that *X. campestris* pv. *vesicatoria* penetrates the fruit epidermis through broken trichomes or through minute rifts in the cuticle. Previous reports (1,12) have identified foliar trichomes as habitats and infection sites for *P. syringae* pv. *tomato*.

The process of fruit infection by *P. syringae* pv. *tomato* is envisaged as follows. Initially, trichomes are colonized by the pathogen. As fruit enlarges, these trichomes are lost. In the presence of free water, the pathogen invades the open trichome bases and multiplies subepidermally. Eventually, due to internal pressure from expanding masses of bacteria, the epidermis swells and ruptures, resulting in a lesion. The post-symptomatic egress of *P. syringae* pv. *tomato* from fruit lesions suggests that, in the presence of free water, lesions may be sources of inoculum for secondary infections.

In our study it was clearly demonstrated that artificial inoculation of young tomato fruit (green fruit ≤3 cm in diameter) without wounding ultimately produced typical speck lesions. This confirms earlier reports (2,5) that wounding is not necessary for bacterial infection of tomato fruit. Infection of uninjured fruit probably occurs through open trichome bases that remain after trichomes have been lost.

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