Scanning Electron Microscopy of Apple Blossoms Colonized by *Erwinia amylovora* and *E. herbicola*

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ABSTRACT

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Based on scanning electron microscopy of apple blossoms sprayed with suspensions of *E. herbicola* (strains 159 and 252) and *E. amylovora* (strain 273), we found these strains multiplied mostly on the stigmatic surface. *E. herbicola 252*, which effectively inhibits the development of fire blight, and *E. amylovora* 273 occupied similar niches on stigmas. Bacterial colonies with distinct cells occurred in regions between papillae and in underlying tisssue. *E. herbicola* 159, a less effective antagonist of *E. amylovora*, was more intimately associated with the deteriorating stigmatic cuticle. Bacterial cells usually developed to produce indistinct amorphous aggregates. We suggest that the effective antagonist restricts the pathogen on apple flowers by competing for the same site on the stigmatic surface.

Additional key words: biological control, competition, *Malus pumila*.

The blossom blight phase of fire blight caused by *Erwinia amylovora* (Burr.) Winslow et al has been controlled under experimental and field conditions with different strains of *E. herbicola* (Löhnis) Dye (1). As yet there is no satisfactory explanation to account for the mechanism whereby the antagonist inhibits the pathogen. This information is required for future efforts to screen for more effective antagonists or to improve existing strains by genetic manipulation. No information is available on which floral parts support the growth of *E. herbicola*. The present scanning electron microscope (SEM) study was undertaken to compare the colonization of apple blossoms by *E. herbicola* strain 252 (an effective antagonist), *E. herbicola* 159 (a less effective antagonist), and *E. amylovora* 273 (a pathogenic strain).

MATERIALS AND METHODS

Dormant apple trees (*Malus pumila* Mill. cv. Jonamac) growing in containers were removed from cold storage and transferred to a greenhouse. As flowers began to open, trees were placed in a growth chamber (24 C, relative humidity about 70%, and light period 14 hr). Aqueous suspensions of 24-hr nutrient-yeast extract-glucose agar slant cultures of *E. amylovora* 273, *E. herbicola* 159, and *E. herbicola* 252, containing 10^8 colony-forming units (cfu) per milliliter, were sprayed with a DeVilbiss atomizer onto separate flower clusters until runoff. Control flowers were sprayed with sterile water.

Flowers were removed from trees 2, 24, and 72 hr after being sprayed. Petals still remaining were removed and discarded. A cross section through the bases of styles and filaments of each flower was made with a new razor blade to separate the floral parts. The material was fixed overnight in 5% glutaraldehyde, followed by dehydration successively in 50, 70, 90, and 100% ethanol-water solutions. The material was dried in a Tousimis Autosamdri-814 critical point drier (Tousimis Research Corp., Rockville, MD) under CO_2. Mounted specimens were coated for 2 min with 200 Å of gold-palladium in a Balzers Union Sputter Coater (Balzers High Vacuum Systems, Hudson, NH) and examined in an AMR Model 1000 SEM (Amray, Inc., Bedford, MA) operating at 10 kV.

RESULTS

The stigmas of an uninoculated apple blossom at anthesis are shown in Figure 1. Note the cuticle covering the papillae.

Two hours after we sprayed blossoms with suspensions of the three test strains, bacterial cells were distributed over the stigmatic surface and were also present on other parts of the flowers. Figure 2 shows the stigmatic surface of flowers sprayed with *E. herbicola* 252. Blossoms sprayed with the other two strains had a similar appearance after 2 hr.

Twenty-four hours after application of *E. amylovora* 273,

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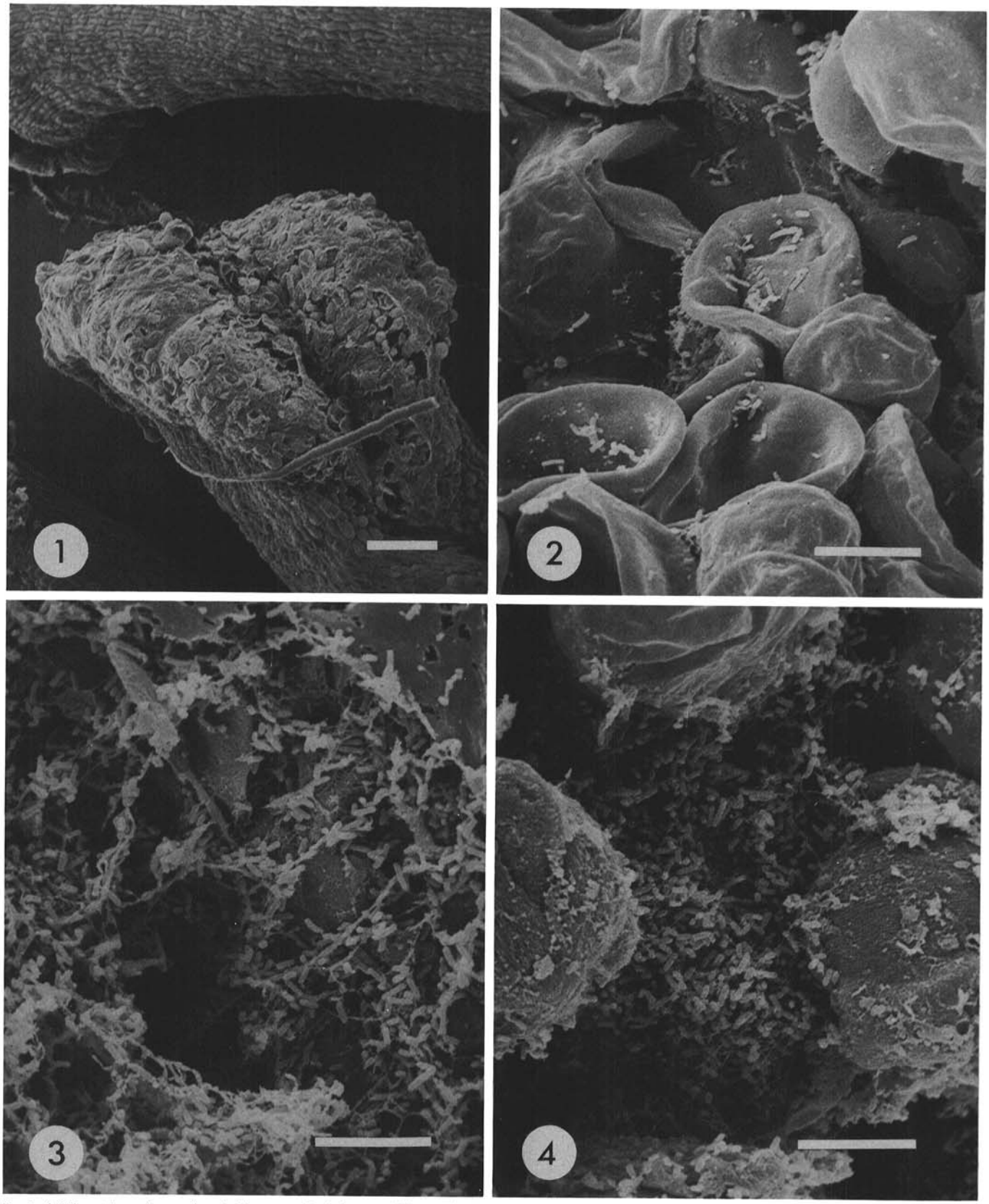
masses of bacterial cells were present on stigmas (Figs. 3 and 4). Despite degeneration of the cuticle (Fig. 3) individual cells of the pathogen were distinct within colonies (Fig. 4). Few or no cells were present on other flower parts. Seventy-two hours after application of this strain, masses of bacterial cells were still confined mainly to the region between papillae and underlying cells (Fig. 5).

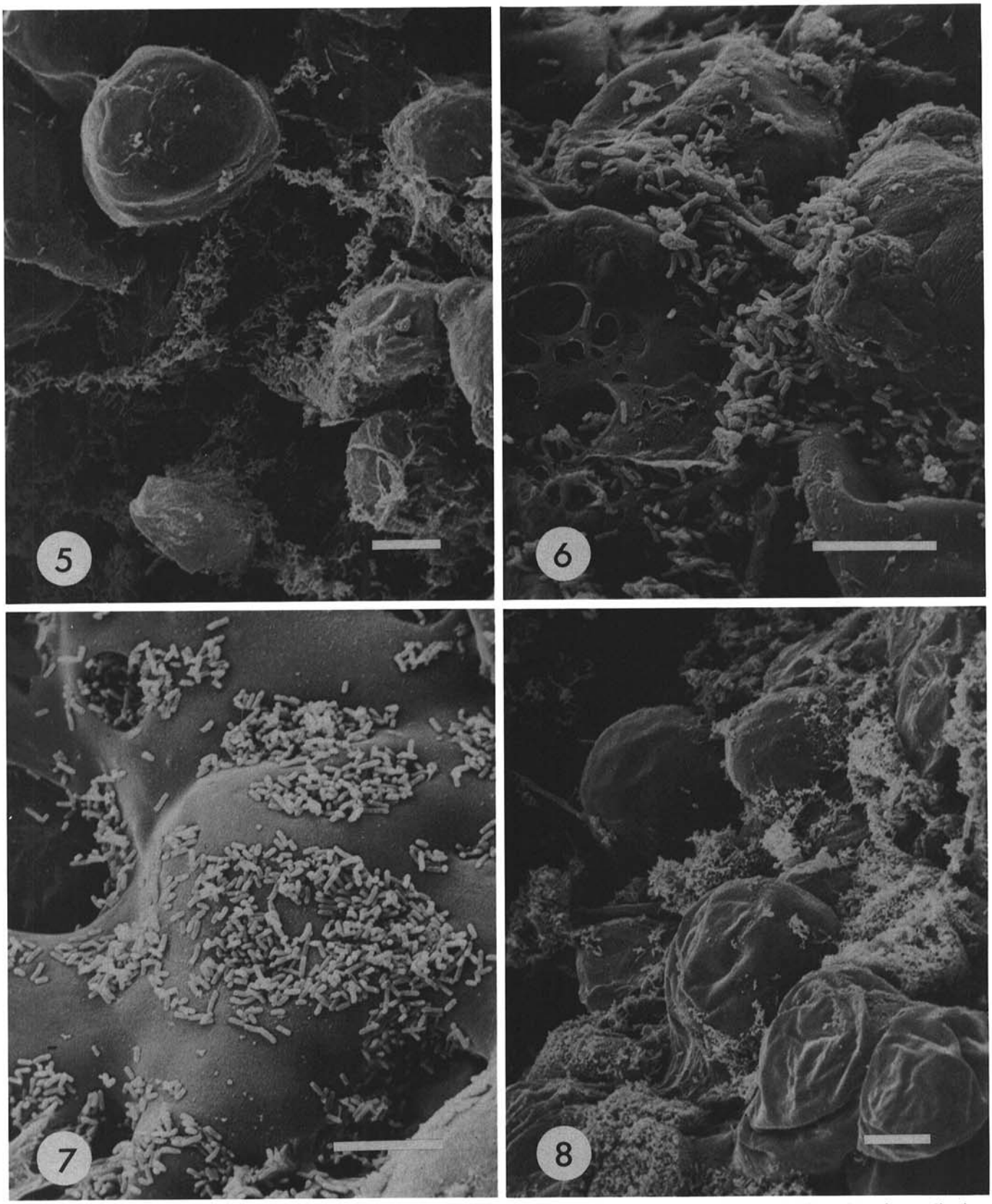
E. herbicola 252 colonized the stigmatic surface in the same way as *E. amylovora* 273, with extensive growth occurring 24 hr after application (Fig. 6). Where the cuticle had not yet undergone



Figs. 1–4. Stigmatic surfaces of apple blossoms. Scale bars = 100 μm (Fig. 1) and 10 μm (Figs. 2–4). **1,** Stigma of uninoculated flower. Note cuticle covering papilla cells. **2,** Stigmatic surface containing scattered bacteria 2 hr after application of *Erwinia herbicola* 252. **3,** Aggregates of bacteria 24 hr after application of *E. amylovora* 273. Deterioration of cuticle is evident. **4,** At 24 hr most cells presumed to be *E. amylovora* 273 were located in the regions between papillae.

marked deterioration, this strain appeared to have multiplied in pores and ruptures of the layer (Fig. 7). On the intact cuticle, no distinct differences were evident between the pattern of colonization by *E. herbicola* 252 (Fig. 8) and *E. amylovora* 273 (Fig. 5) 72 hr after application.

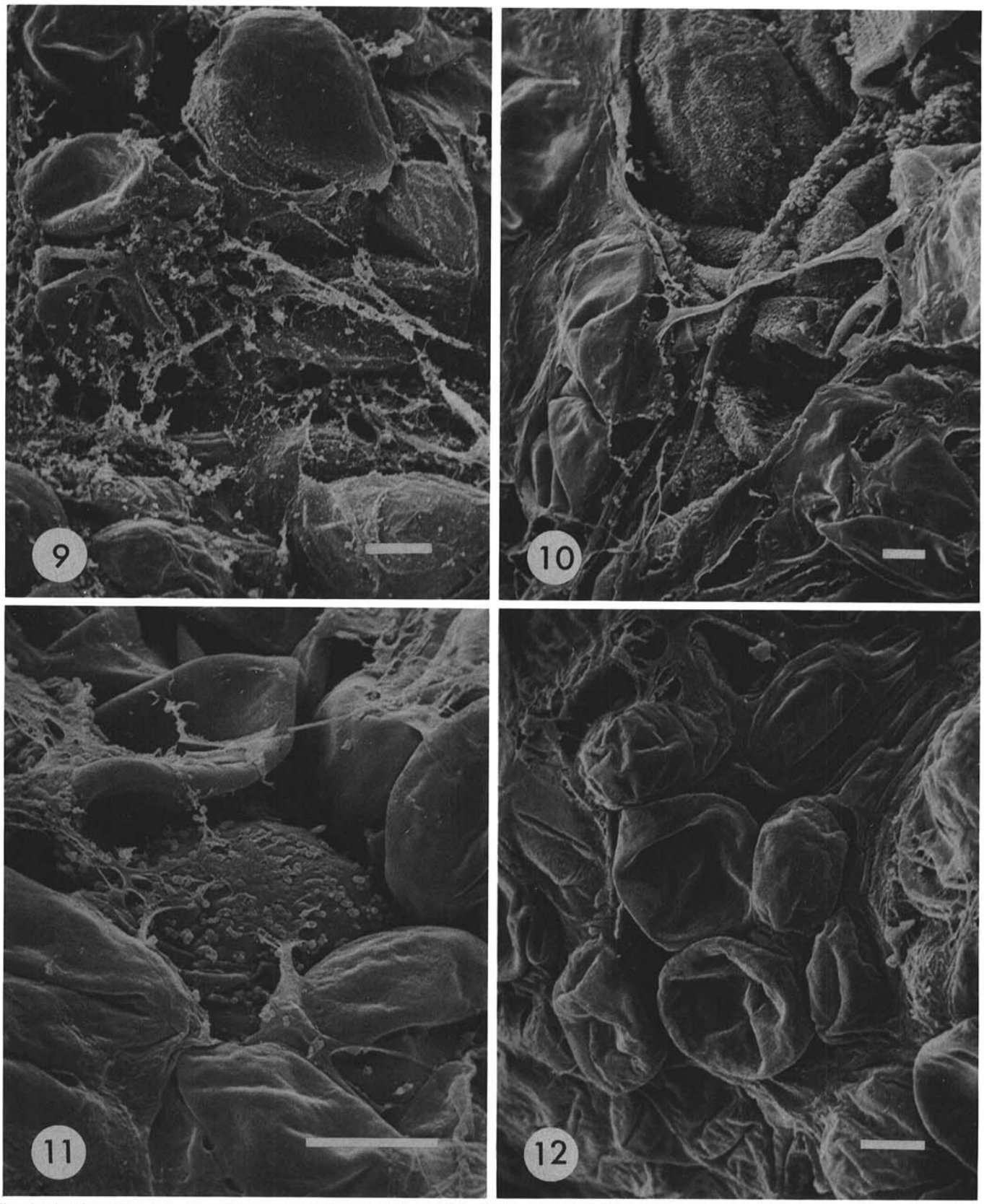
The cuticular layer of the stigmatic surfaces had deteriorated drastically 24 hr after spraying blossoms with *E. herbicola* 159 (Fig. 9). Furthermore, this strain had a different pattern of growth, appearing to be more intimately associated with the deteriorating cuticle than either *E. herbicola* 252 or *E. amylovora* 273. It tended



Figs. 5–8. Bacteria on the stigmatic surfaces of apple blossoms. Scale bars = 10 μm. **5,** Colonization of region between papillae 72 hr after application of *Erwinia amylovora* 273. **6,** Degeneration of cuticle 24 hr after application of *E. herbicola* 252. **7,** Bacteria in pores and concentrated near ruptures of the cuticular layer 24 hr after application of *E. herbicola* 252. **8,** Bacteria massed in region between papillae 72 hr after application of *E. herbicola* 252.

to form amorphous aggregates at or near strands (Fig. 9). Most of the pronounced strands, which were particularly evident 72 hr after application, appeared to be of cuticular origin (Fig. 10). Colonization of the depressions between papillae occurred (Fig. 11), but to a lesser extent than where the cuticle was ruptured.

No bacteria were seen on the stigmatic surfaces of blossoms that were not sprayed with bacteria (Fig. 12). None of the three strains colonized the styles, nectaries, sepals, anthers, or filaments.



Figs. 9–12. Stigmatic surface of apple blossoms. Scale bars = 10 μm. **9,** Marked deterioration of cuticular layer 24 hr after application of *Erwinia herbicola* 159. Bacteria occur in amorphous aggregates. **10,** Strands, apparently of cuticular origin, with closely associated bacteria 72 hr after application of *E. herbicola* 159. **11,** At 72 hr time colonization of depressions between papillae was sparse. **12,** Stigmatic surface of blossoms not sprayed with bacteria.

DISCUSSION

E. herbicola and *E. amylovora* applied to apple blossoms multiplied preferentially on the stigmatic surface. Epiphytic populations of *E. amylovora* occur almost exclusively on the surface of stigmas of pear flowers in California pear orchards (10,11). However, this is the first report that *E. herbicola* develops preferentially on the stigma of rosaceous flowers.

The distal portions of the stigmatic papilla of apple flowers are covered by a cuticle that ruptures after anthesis (2,8). The surface then becomes bathed in a sticky exudate originating from the papilla epidermis and underlying tissue (8). Based on our observations the exposed surface regions were exploited by the three test strains studied. *E. amylovora* 273 and *E. herbicola* 252 appear to colonize stigmas in an identical way. Extensive growth, where individual bacterial cells were clearly visible within the colonies, was observed in the areas between papillae (Figs. 3–6, 8). By contrast *E. herbicola* 159 colonized these areas less profusely. This strain was associated more intimately with the remnants of the deteriorating cuticle. Furthermore, *E. herbicola* 159 became embedded in substances of unknown origin. Strands of putative cuticular material and masses of embedded bacterial cells were confined mostly to the outer surfaces of stigmas.

E. herbicola 252 effectively inhibits the growth of *E. amylovora* 273 if applied to apple blossoms at least 1 day before the pathogen (1). We suggest that this is because these two strains colonize the same niche on the stigmatic surface. If applied first, the antagonist might prevent the pathogen from gaining effective access to these sites. *E. herbicola* 159, which is a less effective antagonist (Beer and Rundle, *unpublished*), apparently occupies a different niche on stigmas.

The importance of stigmas as sites of infection remains unclear. Thomson (10) suggested that *E. amylovora* multiplies epiphytically on the stigmas of healthy pear flowers and that stigmas serve as a reservoir for the bacteria until moisture permits movement to other flower parts where infection occurs. If this is the case, it would seem that instead of preventing infection directly, effective antagonists control disease by reducing the buildup of inoculum on the stigmas.

In considering biological control of the blossom blight phase of fire blight, considerable attention has been given to the significance of nectar and nectaries (3–7,9). Because it now appears that nectaries are not sites of primary colonization, we suggest that future research should focus on stigmatal exudates and the sequential maturation of specific regions on the stigmatic surface (12).

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