Etiology

# Role of the Cuticular Membrane in Ontogenic and Vf-Resistance of Apple Leaves against *Venturia inaequalis*

Claudio Valsangiacomo and Cesare Gessler

Institute of Phytomedicine, Swiss Federal Institute of Technology, 8092 ETH-Zürich, Switzerland.
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**ABSTRACT**

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*Venturia inaequalis* penetrated the cuticular membrane of young, actively growing leaves and fully developed leaves of scab-susceptible (Golden Delicious) and scab-resistant (Liberty) apple cultivars. Initial stromal development was similar in both cultivars; it was most frequent in the young, growing leaves and decreased with their aging. The Vf-resistance in Liberty was expressed as an inhibition of the stroma growth only after 2–3 days.

*Additional keywords*: cuticle, electron microscopy, *Malus*, *Spilocea pomi*.

Most cultivated apple varieties are susceptible to the scab fungus *Venturia inaequalis* (Cke.) Wint., but scab symptoms appear only on leaves that are growing actively and expanding at the moment of inoculation. Fully expanded leaves are resistant to *V. inaequalis* in all the genus *Malus* (ontogenic resistance). Resistance of young, growing leaves was introduced in edible cultivars by Crandall early in this century from *Malus floribunda* (3,19). Two clones created by Crandall, 26829-2-2 and 26830-2, were used as sources of resistance for most of today's resistant cultivars (5,12,20). The Vf-resistance gives cultivars such as Liberty an almost complete field resistance (5,12). As reported earlier (2), Vf-resistance in the cultivars Priscilla and Sir Prize does not lead to a hypersensitive response, but to a reduced formation of stromata. How ontogenic and Vf-resistance is expressed at morphological and physiological levels is not known.

Four general kinds of resistance mechanisms can be recognized: preformed and postinfectional physical barriers, and preformed and postinfectional chemical barriers. The role of the cuticular membrane as a preformed physical barrier against microorganisms' penetration and the mode by which this barrier can be overcome have been questioned for many years. Martin and Juniper reviewed works on plant cuticles (9) and summarized the role of such structures in pathogenicity and resistance to colonization by microorganisms in plants. Considerable evidence is now available that indicates that the cuticular membrane may be enzymatically degraded; extensive illustration of such degradation has been done by van den Ende and Linskens (18) and by Kolattukudy (4). Although Martin pointed out that there is no definitive evidence that the cuticular membrane has more than a limited role in protection of plants in host-parasite relationships (8), several reports indicate that this structure can be an important factor in preventing pathogen infection. Ontogenic resistance in citrus lime leaves to *Gloeosporium limetticola* was correlated with a change in

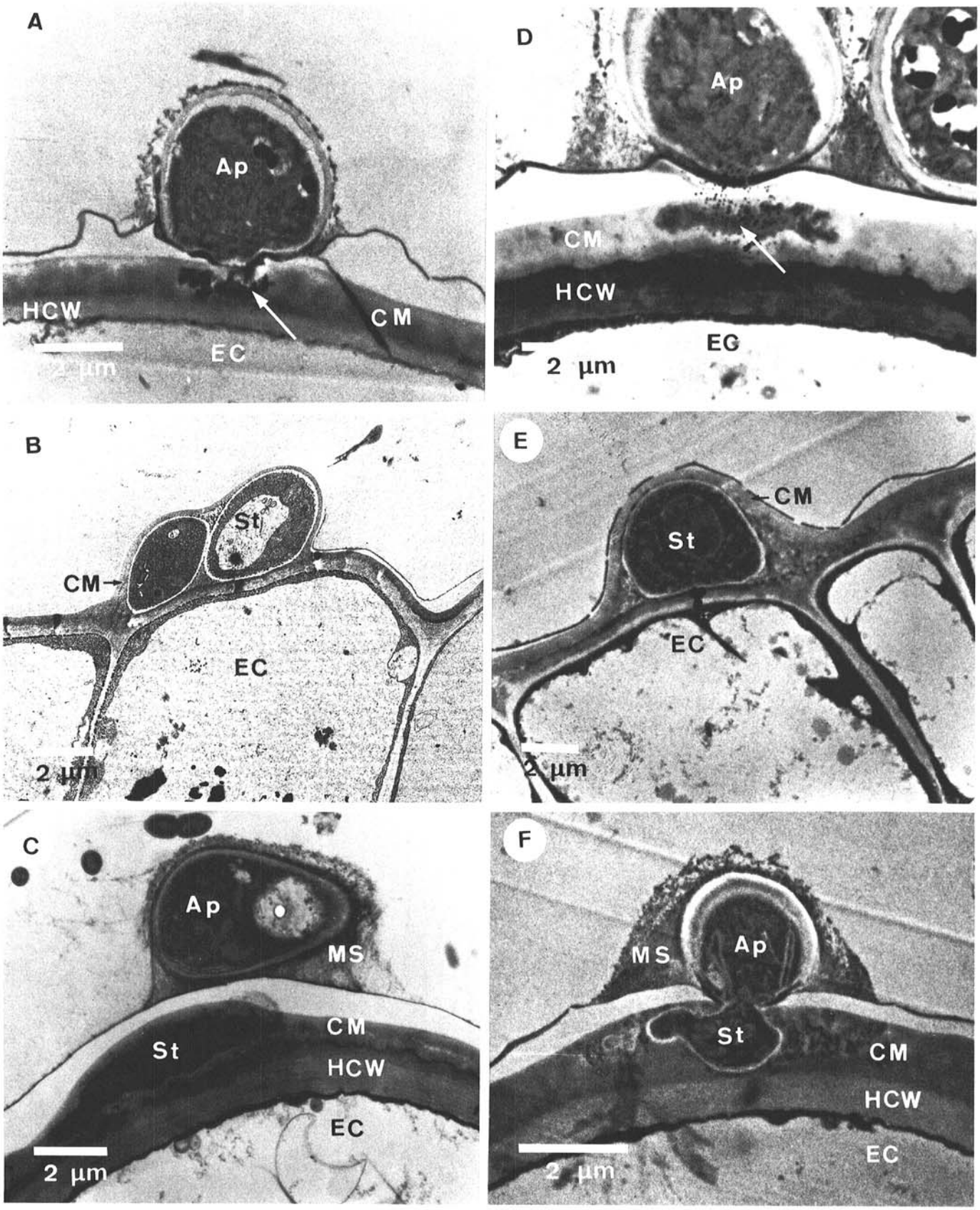


**Fig. 1.** Cross sections of epidermal cells of apple leaves (EC) of cultivars Golden Delicious (left side) and Liberty (right side) with stroma cells (St) of *Venturia inaequalis*. **A** and **D,** old leaf (20 days old) 12 hr after inoculation. No stroma yet formed; the change of electron density in the cuticular membrane (arrow) suggests an enzymatic degradation. **B–F,** 24 hr after inoculation. **B** and **E,** young leaf (4 days old), stroma developed between the cuticular membrane (CM) and the host cell wall (HCW). **C** and **F,** old leaf (20 days old), appressorium (Ap) with a small undifferentiated stroma (St) between the host cell wall (HCW) and the cuticular membrane (CM); the appressoria are surrounded by a moucillaginous sheath (MS); the translucent zone (artifact) in the cuticular membrane is caused by the detachment of the cuticle from the cuticular layers below.

composition of the cuticular membrane that occurs as the leaves age (16). Varietal resistance was linked with difficulty of penetration of the cuticular membrane by *Colletotrichum coffeanum* on coffee leaves (11) and *Sphaerotheca macularis* on strawberry leaves (14). Furthermore, it was shown that the thickness of the cuticular membrane in the bean, tomato, and other host plants is responsible for the different degree of penetration by *Botrytis cinerea* (6).

Both types of resistance analyzed in this paper may be due to a preformed physical barrier, such as the cuticular membrane, which could prevent penetration by the pathogen. Wiltshire (21), Nusbaum and Keitt (10), and Gessler and Stumm (2) state that on young, growing apple leaves, conidial germination and formation of appressoria occur in all cases, without being perceptibly influenced by isolate-variety combinations. No reports include information illustrating the process of penetration of the cuticular membrane on ontogenic or Vf-resistant leaves.

The purpose of this paper is to illustrate qualitatively the first process in infection on hosts with both types of resistance: the penetration of the cuticular membrane by the pathogen (already presented in a preliminary report) (1).

## MATERIALS AND METHODS

**Plants and fungus.** Apple grafts (cultivars Liberty and Golden Delicious on scion M 25), were provided by the agricultural research station in Wädenswil, Switzerland, and the fungal material (sporulating lesions on leaves; the same population mixture used in a previous paper) (2) by Dr. Maag AG Dielsdorf, Switzerland.

**Experimental conditions.** The apple grafts were planted in pots with a potting soil mixture and kept in a greenhouse with a controlled temperature regime (25 C during the day and 20 C at night) with constant humidity of 70% and additional illumination to give 16 hr of daylight. Plants having one shoot with at least 10 leaves were used for inoculations. The age of a leaf at the inoculation time was counted in days from complete unrolling on the sides. A shoot produced a new leaf in 2 days under the given conditions.

Each experiment was carried out by inoculating four plants (two Golden Delicious and two Liberty, susceptible and resistant to scab, respectively). From each leaf two to three disks were punched out by using a cork borer (#6) and were prepared for light microscopy. One single disk from each leaf was further cut to small pieces (1 × 3 mm size) and prepared for electron microscopy. The samples were taken 12 and 24 hr after inoculation. The experiment was repeated three times.

**Inoculation.** The leaves were inoculated with conidial suspensions obtained by stirring leaves carrying sporulating lesions in distilled water for 5 min. The inoculum contained $10^5$ conidia per milliliter for the light microscopical observations and $2 \times 10^6$ conidia per milliliter for the electron microscopical observations. The inoculated plants were kept in a mist chamber with 100% relative humidity at 20 C for at least 48 hr to prevent evaporation.

**Light microscopy.** Samples were taken and prepared as described earlier (2) and examined with an Olympus BH 2 interference contrast microscope.

**Electron microscopy.** Samples of leaf tissue were prefixed with 3% glutaraldehyde in 0.1 M sodium cacodylate (SC) buffer, pH 7.4, for 4–5 hr, then rinsed in the same buffer. After postfixation in buffered (SC) 1% osmium tetroxide for 2 hr, the samples were infiltrated with 1% tannic acid for 2 hr (13) and again fixed for 2 hr with 1% osmium tetroxide in SC buffer. All steps were carried out at 4 C. After en bloc staining overnight in 0.5% aqueous uranyl acetate, fixed tissue segments were dehydrated stepwise in alcohol (30%, 50%, 70%, 90%, 100% twice) and embedded in Spurr's low viscosity resin (17). Ultrathin sections were stained with uranyl acetate and lead citrate according to Reynolds (15) and observed with a Philips 200 TEM.

## RESULTS AND DISCUSSION

Cuticular membranes of apple leaves of any age and from both cultivars were penetrated by *V. inaequalis* (Fig. 1). Observation at the early stages of infection (12 hr after inoculation) showed an electron dense zone below the appressorium in both young and old leaves from the two cultivars (Fig. 1, A and D). Maeda (7) made the same observation about young leaves and attributed it to enzymatic degradation of the cuticular membrane. Recently, a cutinolytic enzyme has been purified and characterized (W. Koeller, *unpublished*).

Twenty-four hr after inoculation the stroma formed from the

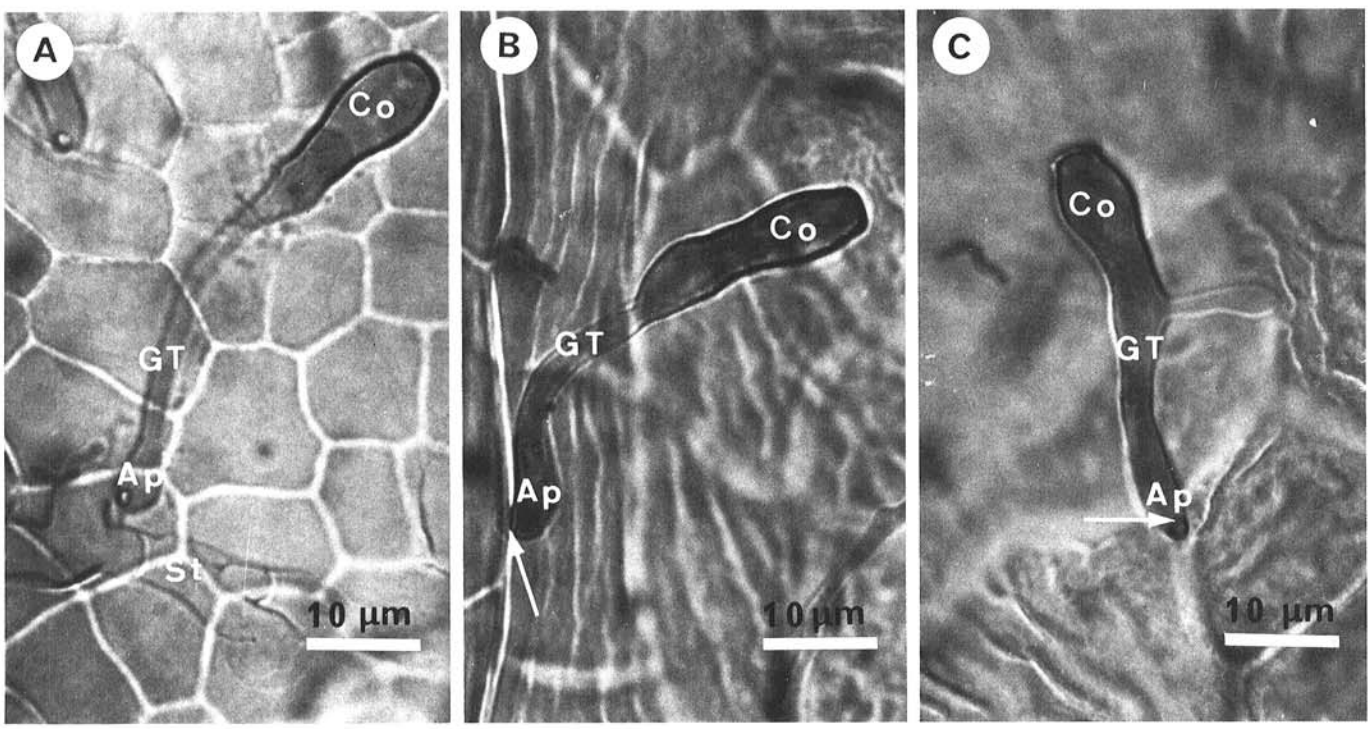


**Fig. 2.** Germinated conidia (Co + GT) with appressoria (Ap) of *Venturia inaequalis* 24 hr after inoculation forming stroma (St) on: **A,** young leaf (2 days old) from Golden Delicious; **B** and **C,** old leaf (20 days old) from Golden Delicious; **B,** stroma just visible (arrow); **C,** only penetration peg visible (arrow).

appressorium was well visible on young leaves from Golden Delicious (Fig. 2A) and to a lesser extent on Liberty. On old leaves of both varieties the stromata were barely visible (Fig. 2B), or more frequently, only the penetration peg could be seen (Fig. 2C). In those cases the stromata could be smaller than the overlying appressoria and therefore not visible with the light microscope. Transmission electron microscopy has been used to investigate the extension of the pentration by the pathogen in those cases in which the stroma formation was not detectable with the light microscope.

Twenty-four to 60 hr after inoculation, the stromata in the young leaves of the susceptible Golden Delicious and of the resistant cultivar Liberty had a thickness between 3 and 6 $\mu$m (Fig. 1, B and E) and were cytologically well structured, with internal cell divisions, vacuoles, and various organelles. In fully expanded leaves (12 days old), the cuticular membrane was entirely penetrated, but the stromata were reduced to a thickness of 1 $\mu$m or less, were cytologically not well developed, and were without internal cell walls and were almost lacking in organelles (Fig. 1, C and F). Moreover, no further development was noted. Unfortunately, statistical evaluation of this type of event is not possible on transmission electron microscopy. However, from samples of resistant leaves, we detected complete penetration as often as in samples from susceptible ones.

We conclude that the cuticular membrane does not represent an effective preformed physical barrier for the pathogen. The expression of both types of resistance, ontogenic and Vf, occurs only after its penetration.

Excluding the cuticular membrane as a preformed factor, the question of the nature of resistance in *Malus* is still unsolved. In our next investigation we shall discuss the postinfectional process of stromal expansion by examining the host cell wall degradation at the interaction point with the pathogen. Further resistance to the fungus will be correlated with differences in stromal expansion and in degradation of host cell walls.

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