Etiolated Apple Hypocotyls: a Useful Host Tissue in Apple Scab Research

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ABSTRACT

A technique using etiolated apple hypocotyls was developed to observe developmental changes in the living apple-*Venturia inaequalis* interaction. These changes cannot be observed in living leaves because of tissue thickness and presence of pigments. Fungal growth and symptom expression of susceptible and hypersensitive interactions corresponded to those of green leaves. Microscopic symptoms in the hypersensitive interaction appeared as early as 30 hr before macroscopic symptoms. Cytoplasmic granulation and the accumulation of yellow-orange globules preceded extensive discoloration and death of host cells. As seedlings age, the extent of cell browning in the hypersensitive response is lessened and is often absent although stromatic growth of the fungus is inhibited. This suggests that inhibition of fungal growth cannot be completely accounted for by the oxidation of phenols.

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Pellizzari (8) first reported the use of etiolated seedlings in apple scab research. Subsequently, Maeda (4) observed the ultrastructure of the susceptible interaction at the time of sporulation. Neither author determined whether the hypersensitive interaction of etiolated tissue is comparable to that of green leaves.

The purpose of this study was to determine the extent of fungal development coincident with host responses leading to symptom expression in susceptible and hypersensitive hypocotyls.

MATERIALS AND METHODS.—*Culture of the fungus.*—Isolates representing each of the five races of *Venturia inaequalis* (Cke.) Wint. were grown in wick culture (1) on 4% malt extract at 19 C for 14 days. Spores were centrifuged (2,000 *g*) and resuspended 3 times in distilled water. Spore concentrations were determined with a hemacytometer.

Plant material.—Progeny of the following crosses were used: McIntosh × 1197-1 (527-1 = *Malus micromalus* pit type × 317-22 = *Malus atrosanguinea* 804 pit type), hypersensitive to races 1 to 4 and susceptible to race 5 of *V. inaequalis*; 1197-1 (open-pollinated), hypersensitive to races 1 to 4; and McIntosh (open-pollinated), susceptible to the five races of *V. inaequalis*. Additional reference to these progeny will be made using the terms "pit type-hypersensitive" to denote the hypersensitive interaction of races 1 to 4 with seedlings possessing hypersensitive resistance, "pit type-susceptible" to denote the susceptible interaction of race 5 with pit type seedlings, and "McIntosh-susceptible" to denote the susceptible interaction of all race isolates with McIntosh seedlings.

Seed were removed from fruits, dried, and stratified in sterile, moist sphagnum at 4 C for 3 months. Following stratification, seeds were planted in a mixture of 60% vermiculite and 40% soil, watered once with a 0.25% (w/v) Tersan 75 solution (E. I. DuPont de Nemours & Co., Inc., Willmington, Del.) and placed in the dark at 19 C for 8 to 14 days. Seedlings were washed in tap water followed by distilled water and were placed in moisture chambers with their roots covered by absorbent paper moistened with distilled water. Seedlings were sprayed with spore suspensions (2-4 × 10^6 spores/ml) and the chambers were closed. The inoculated plants were incubated at 19 C in the dark. Water mounts of hypocotyl epidermis were prepared at intervals after inoculation to observe fungal growth and host responses.

RESULTS.—*Pit type-hypersensitive interaction.*—The time of macroscopic symptom expression depended on the fungus isolate and ranged from 48 to 96 hr after inoculation. Red-brown necrotic lesions developed on both abaxial and adaxial surfaces of cotyledons and lesions coalesced (Fig. 7). Symptoms on hypocotyls appeared as red-brown streaks which coalesced (Fig. 6). Pits characteristic of the hypersensitive reaction of green leaves (11) did not occur on etiolated seedlings.

Microscopic symptoms on hypocotyl epidermis appeared 10 to 30 hr prior to macroscopic symptoms. The first host response consistently observed was a granulation of the cytoplasm (Fig. 2, 3, 4). An apparent bubbling of the cytoplasm (Fig. 1) was occasionally observed in cells that exhibited no granulation but were in contact with or close to the pathogen. The first evidence of cell discoloration was the appearance of yellow-orange globules (Fig. 2). Globules were also found in cells without apparent cytoplasmic granulation, and this phenomenon was often observed in epidermis from hypocotyls which had not elongated extensively.

Extreme granulation of the cytoplasm eventually occurred, often beginning at one or both ends of the affected cell (Fig. 4). In conjunction with this, the

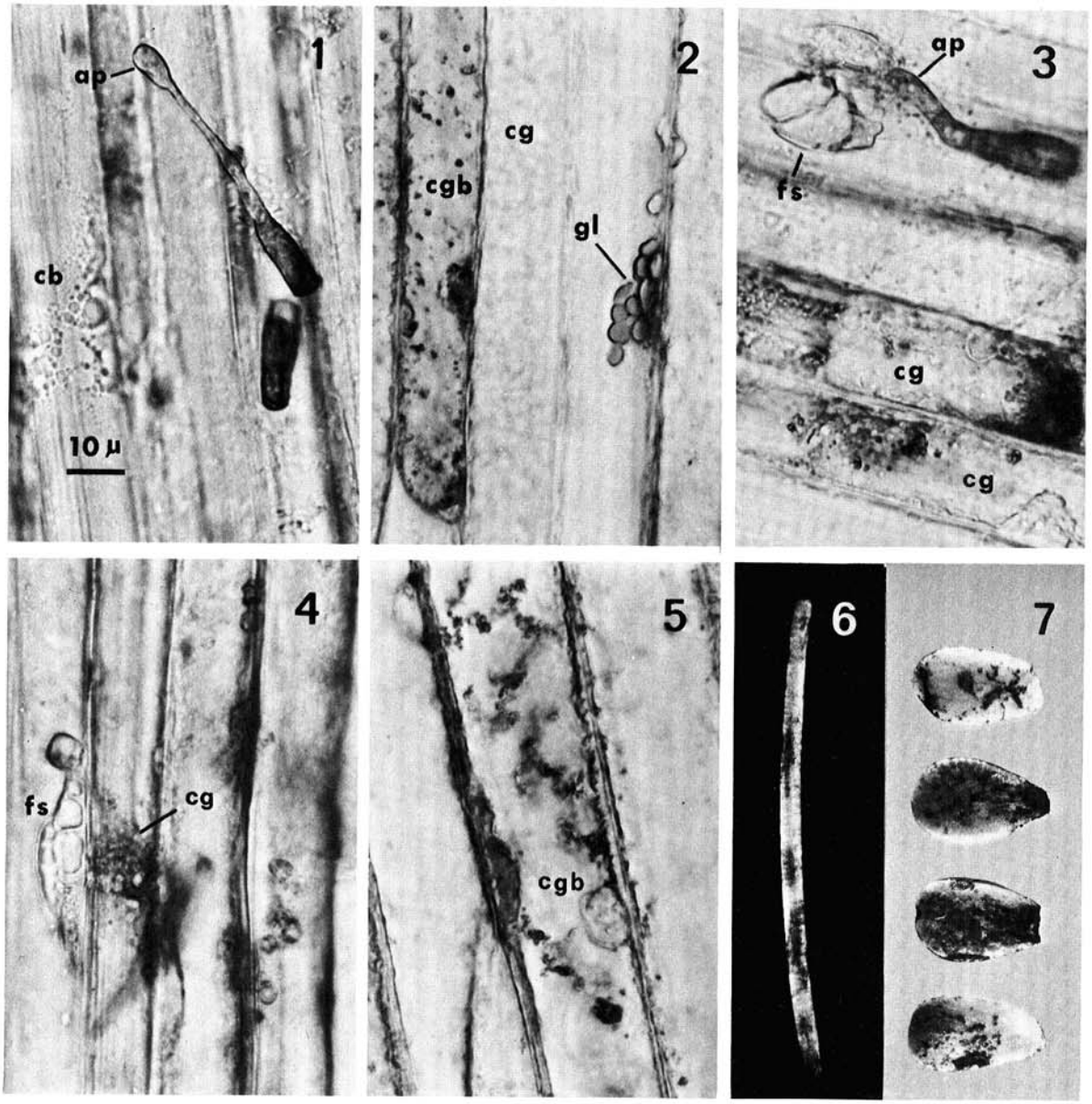

Fig. 1-7. The hypersensitive interaction of *Venturia inaequalis* with etiolated hypocotyls of apple. 1) Cytoplasmic bubbling (cb) sometimes observed in epidermal cells before cytoplasmic granulation. Germinated spore with an appressorium (ap). 2) Host cytoplasmic granulation (cg), yellow-orange globules (gl) that appear before extensive cell discoloration, and extreme granulation and browning (cgb). 3) Host cytoplasmic granulation (cg) and limited stromatic growth of the fungus (fs) extending outward from the appressorium (ap). 4) Cytoplasmic granulation (cg) at the interface of two epidermal cells directly beneath a fungal stroma (fs). 5) Extensive cytoplasmic granulation and browning (cgb). 6) Coalesced hypersensitive lesions on an etiolated apple hypocotyl. 7) Coalesced hypersensitive lesions on apple cotyledons.

yellow-orange globules either disappeared or were broken up, resulting in a browning of the entire cell contents (Fig. 2, 5). The reaction to infection extends beyond the infected cell, since several uninfected cells in the area of a single infection site exhibit the hypersensitive response.

Germination, appressorium formation, and penetration by the fungus are as described by Nusbaum & Keitt (7), although penetration pegs are not easily observed (probably due to thinness of the cuticle). Subcuticular development beyond the primary hypha may or may not occur, and the extent of stroma formation is apparently isolate-dependent. Generally, no more than four or five stromatic cells were formed (Fig. 3, 4) and, irrespective of the extent of subcuticular coverage, the stroma did not exceed

one cell in depth. Sporulation did not occur. When little or no subcuticular development occurred, the host response was often restricted to one or two cells which exhibited only a limited browning.

Seedlings with extensively elongated hypocotyls rarely exhibited macroscopic symptoms except on the cotyledons. Microscopic symptoms were also less severe. Cytoplasmic granulation was present, although discoloration rarely occurred. The absence of cell browning apparently had some effect on the fungus, since stromatic growth was often more extensive without browning. The stroma remained one cell thick, and the fungus did not sporulate.

McIntosh-susceptible and pit type-susceptible interactions.—No differences between the pit type- and McIntosh-susceptible interactions were observed. Sporulating lesions were formed 5 to 8 days after inoculation. Symptoms on cotyledons appeared as limited areas of sporulation, and were not as distinctive as hypersensitive symptoms. Small, dark areas of sporulation that coalesced appeared on hypocotyls (Fig. 11). A limited browning of the tissue may occur after sporulation.

Development of the subcuticular stroma was detected within 5 to 10 hr after the formation of primary hyphae. The stroma was at first one cell in depth (Fig. 8), but eventually became several layers thick (Fig. 9). Sporulation only occurred from the thickened areas of stroma (Fig. 10). Extensive subcuticular growth and sporulation occurred on seedlings of all ages in both the pit type- and McIntosh-susceptible interactions.

The first observed host response was a limited cytoplasmic granulation present in cells associated with well-developed stroma or in cells associated with numerous infection sites. The formation of yellow-orange globules was, as in the hypersensitive response, the first evidence of cell discoloration. Granulation and discoloration were not as extensive as in the hypersensitive interactions, and generally did not occur before sporulation.

DISCUSSION.—The subcuticular development of *V. inaequalis* on etiolated hypocotyls in the susceptible and hypersensitive interactions corresponds to that on green leaves (4, 7). Microscopic observations are facilitated because the epidermis lacks pigments and is easily removed from the hypocotyl. A single layer of host cells with accompanying fungal stroma may be observed in the living state because *V. inaequalis* develops subcuticularly.

Maeda (4) observed the accumulation of

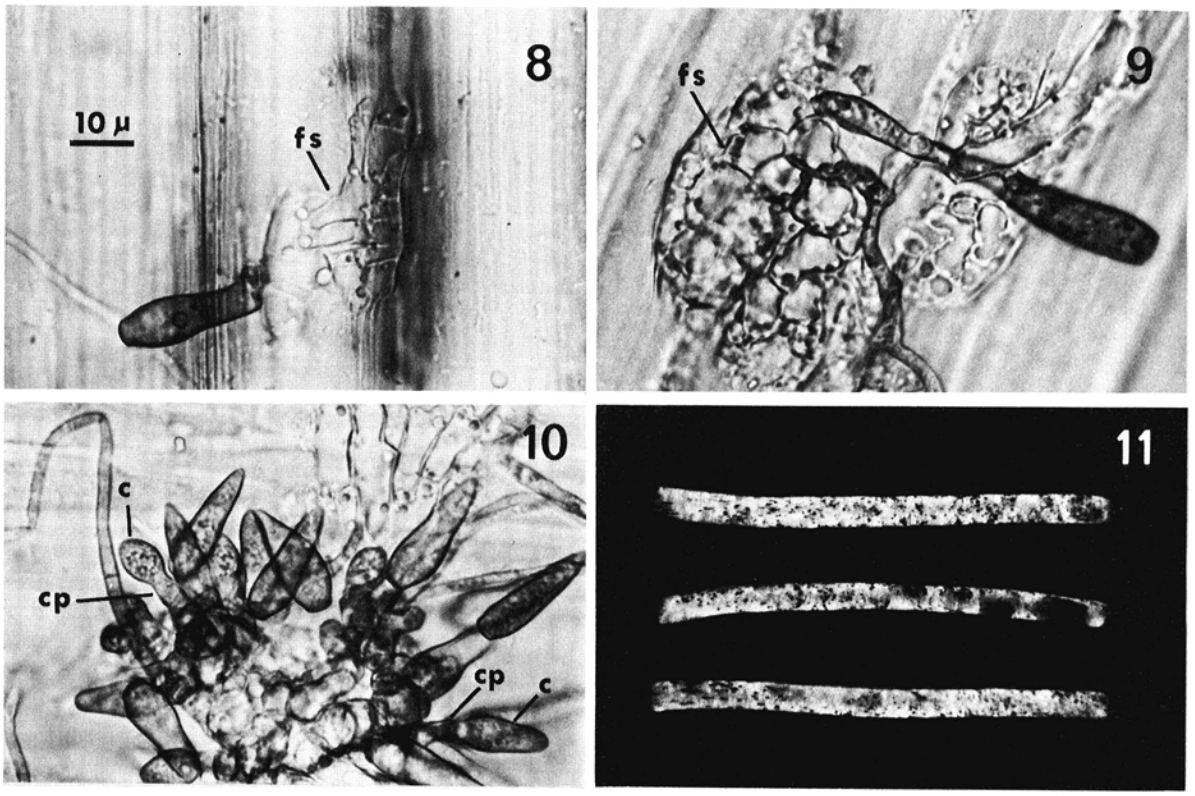

Fig. 8-11. The susceptible interaction of *Venturia inaequalis* with etiolated hypocotyls of apple. **8)** Developing subcuticular fungal stroma (fs) which is one cell thick. **9)** Fungal stroma (fs) several cells in thickness. **10)** Sporulation of *V. inaequalis* from an area of thickened stroma. Conidia (c) and conidiophores (cp) are present. **11)** Sporulating lesions on etiolated apple hypocotyls.

osmiophilic bodies in host cells of hypersensitive reaction sites in green leaves. These bodies may correspond to the yellow-orange globules observed in etiolated epidermis. The globules may represent the accumulation of phloridzin (the major phenolic in apple) and/or other phenolic compounds preceding extensive cell discoloration.

The oxidation of phenolics, principally phloridzin and phloretin, has been suggested to account for inhibiting development of the fungus in the hypersensitive and resistant interactions (1, 2, 3, 5, 6, 9, 10, 12). However, cell browning (a result of phenolic oxidation) does not occur in epidermis from elongated (older), etiolated hypocotyls, and yet stromatic growth of the fungus is inhibited. More stromatic growth may occur in the absence of browning; however, the stroma remains as a single layer of cells and sporulation does not occur. Containment in the absence of browning could result from an inadequate flow of nutrients to the fungus, but this is unlikely as pit type seedlings of the same age and stage of hypocotyl elongation exhibit the normal susceptible interaction when inoculated with race 5 isolates. These data suggest that oxidation of phenolics is not the primary reason for containment of *V. inaequalis* in the hypersensitive interaction.

LITERATURE CITED

1. BARNES, E. H., & E. B. WILLIAMS. 1961. The role of phloridzin in the host-parasite physiology of apple scab disease. Can. J. Microbiol. 7:525-534.
2. KIRKHAM, D. S. 1957. Studies of the significance of polyphenolic host metabolites in the nutrition of Venturia inaequalis and Venturia pirina. J. Gen. Microbiol. 17:120-134.
3. KIRKHAM, D. S. 1957. The significance of polyphenolic metabolites of apple and pear in the host relations of Venturia inaequalis and Venturia pirina. J. Gen. Microbiol. 17:491-504.
4. MAEDA, K. M. 1970. An ultrastructural study of Venturia inaequalis (Cke.) Wint. infection of Malus hosts. M.S. Thesis, Purdue Univ., Lafayette, Indiana. 112 p.
5. NOVEROSKE, R. L., J. KUĆ, & E. B. WILLIAMS. 1964. Oxidation of phloridzin and phloretin related to resistance of Malus to Venturia inaequalis. Phytopathology 54:92-97.
6. NOVEROSKE, R. L., E. B. WILLIAMS, & J. KUĆ. 1964. β-Glycosidase and phenoloxidase in apple leaves and their possible relation to resistance to Venturia inaequalis. Phytopathology 54:98-103.
7. NUSBAUM, C. J., & G. W. KEITT. 1938. A cytological study of host-parasite relations of Venturia inaequalis on apple leaves. J. Agr. Res. 56:595-618.
8. PELLIZZARI, E. D. 1970. A biochemical study on the defense mechanisms of apple plants to Venturia inaequalis (Cke.) Wint. Ph.D. Thesis, Purdue Univ., Lafayette, Indiana. 135 p.
9. RAA, J. 1968. Polyphenols and natural resistance of apple leaves against Venturia inaequalis. Netherlands J. Plant Pathol. 74:37-45.
10. RAA, J., & J. C. OVEREEM. 1968. Transformation reactions of phloridzin in the presence of apple leaf enzymes. Phytochemistry 7:721-731.
11. SHAY, J. R., & L. F. HOUGH. 1952. Evaluation of apple scab resistance in selections of Malus. Amer. J. Bot. 39:288-297.
12. WILLIAMS, E. B., & J. KUĆ. 1969. Resistance in Malus to Venturia inaequalis. Annu. Rev. Phytopathol. 7:223-246.