Response of Detached Apple Leaves to Venturia inaequalis

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ABSTRACT

Hypersensitive, resistant, and susceptible symptoms of scab infection were expressed at the same time and in the same manner on detached as on attached apple leaves. Symptoms were confined within the borders of inoculation sites except in susceptible interactions where extensive stromatic growth of the fungus occurred. The hypersensitive response was characterized by the complete collapse and necrosis of inoculation sites within 2 to 10 hr after the initial hypersensitive pit response.

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In many disease interactions it is difficult to determine the location and extent of host tissue affected until symptoms are expressed. This may prohibit the careful evaluation of physiological changes preceding visible symptoms. In the susceptible interaction of apple with *Venturia inaequalis* (Cke.) Wint., the fungal stroma may eventually cover an extensive area of the leaf blade. Less host tissue is involved in the resistant interaction due to limited development of the fungal stroma. In the hypersensitive interaction a single reaction site may include as little as three or four epidermal cells and occasionally a few cells of the palisade parenchyma (1, 2, 3, 4). Thus, the majority of tissue sampled prior to symptom expression is probably not involved in the host-parasite interaction.

The purpose of this study was to develop a procedure of inoculation and incubation which would increase the relative proportion of leaf tissue involved in the host-parasite interaction per unit area of tissue sampled.

MATERIALS AND METHODS.—*Plant material.*—Clonal plants of the following apple selections (5) were grown in the greenhouse: *Malus atrosanguinea* '333-9', hypersensitive to races 1 to 4 and susceptible to race 5 of *V. inaequalis*; 'Russian 384-1', resistant to races 1, 3, 4, 5 and susceptible to race 2; *M. floribunda* '612-1', resistant to the five races of *V. inaequalis*; 'Red Delicious', susceptible to the five races of *V. inaequalis*.

Young succulent leaves were selected for inoculation, washed with distilled water, and their surfaces were air dried. Leaves were then detached by an angular, abaxial cut at the base of the petiole and placed immediately on filter paper supports in petri dishes with their petioles submerged in water.

Inoculum.—Isolates representing each of the five races of *V. inaequalis* were grown in wick culture (4) on 4% malt extract at 19 C for 14 days. Spores were centrifuged and resuspended three times in distilled water. Spore concentrations ranged from 1 to 4 × 10^6 spores/ml.

The inoculum was applied at random to the adaxial leaf surface in 20- to 30-μliter drops. That surface area covered by the inoculum droplet is termed the inoculation site. The petri dishes were closed, placed in clear plastic bags and incubated in the greenhouse under supplementary lighting (150 ft-c, Sylvania 20W Cool White fluorescent lamps at 61 cm). Leaves inoculated by the same method, but not detached, served as controls.

RESULTS.—No differences in symptom expression of hypersensitive, resistant and susceptible scab interactions were observed between attached and detached leaves. The first appearance of symptoms on detached leaves occurred at the same time as on attached controls; however, not all inoculation sites on either detached leaves or controls exhibited symptoms at the same time. Symptoms were restricted to the area of the inoculation site except in the susceptible interaction where extensive stromatic growth of the fungus occurred.

Hypersensitive symptoms first occur as minute necrotic pits (4) (Fig. 1). The term 'pit' describes the area of host tissue which collapses forming a depression or pit on the leaf surface. Lesion coalescence begins shortly after pit formation and within 2 to 10 hr after the appearance of pits the entire inoculation site is necrotic (Fig. 2, 3). The necrosis extends from the upper to the lower epidermis but is always restricted in surface area to the boundary of the inoculation site.

Complete necrosis is prevented in both excised and attached leaves if the water droplet covering the inoculation site is removed and the leaf surface is allowed to dry prior to pit formation. Symptoms then occur only as necrotic pits as in Fig. 1. Complete necrosis is reinstated if the original inoculation site is washed and dried, and the area covered with fresh sterile water. Symptoms of resistant and susceptible interactions are shown in Fig. 4 and 5.

DISCUSSION.—Symptoms of hypersensitive, resistant and susceptible apple scab interactions were shown to be the same on detached leaves as on leaves

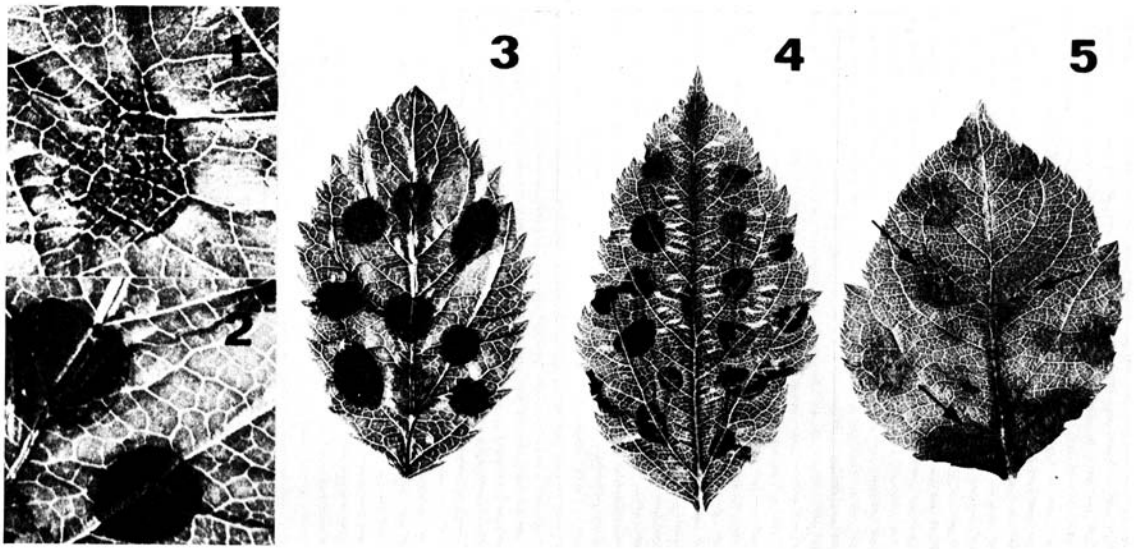

Fig. 1-5. Symtpoms of infection by *Venturia inaequalis* on detached apple leaves. **1)** An inoculation site showing necrotic pits of the hypersensitive response 36 hr after inoculation. **2 and 3)** Complete collapse and necrosis of inoculation sites 42 hr after inoculation, following the hypersensitive pit response. **4)** Collapse and necrosis of inoculation sites in the resistant response; 7 days after inoculation. **5)** Susceptible apple scab interaction. Arrows indicate areas of sporulation by *V. inaequalis* 11 days after inoculation.

not detached from shoots. In the hypersensitive response, pit formation is confined to the inoculation site, indicating that penetration only occurs within the boundary of the inoculum droplet. Extensive collapse and necrosis of the inoculation site occurs when the inoculum droplet is left in situ throughout the incubation period. However, if droplets are removed prior to symptom expression, only pits are formed. Complete necrosis of the inoculation site again occurs if droplets are removed and replaced with fresh water. These data suggest that by-products of individual reaction sites diffuse into the inoculum droplet and back into the host, initiating additional collapse and necrosis.

The use of detached leaves eliminates the need for costly space and equipment required for the inoculation of whole plants. Application of inoculum in droplets allows for the selection of tissue samples with a high probability of involvement in the host-parasite interaction.

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