# Changes in Susceptible and Resistant Red Clover Epidermal Cells After Infection with Erysiphe polygoni

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ABSTRACT

Susceptible and resistant red clover epidermal cells stain with Azure B immediately after infection with *Erysiphe polygoni*, whereas normal host cells do not. The susceptible infected cells and, in some instances, the adjoining cells stain light blue, then change to purple after 2 or 3 weeks. The periphery of the infected resistant cells become greenish 1 to 2 days after infection. After 4 to 7 days, the whole cell becomes deep blue. Benedict's solution turned resistant cells, which had been infected, a yellowish color, while healthy epidermal clover cells remained uncolored. In susceptible infected cells only a faint yellowish color was observed during the 1st week of infection. Resistant infected cells were a yellowish brown 1 or 2 days after infection. These results indicate that specific cells rather than diseased and healthy areas should be compared. Phytopathology 60:681-683.

Recently, J. Benada, Kromeriz, Czechoslovakia, found that distinct limits of redox potential in host tissues were needed for successful infection of barley and wheat powdery mildew (*personal communication*). We report a change in the quantity of reducing compounds in susceptible and resistant infected cells, and also an alteration in their staining with Azure B.

MATERIALS AND METHODS.—La Salle, a locally-grown variety of red clover, *Trifolium pratense* L., served as susceptible host, and the variety Lakeland, which was obtained from J. Watterson, Madison, Wis., served as the resistant host. A local strain of *Erysiphe polygoni* DC was the pathogen used. One-month-old clover plants were maintained in an illuminated, temperature-controlled room at 20 C after inoculation.

Strips of epidermis were removed from the lower surface of susceptible and resistant clover leaves by fine forceps at selected times after inoculation. Some strips, fixed in formalin-acetic acid-alcohol, were stained in Azure B, 0.25 mg/ml of solution, in a citrate buffer at pH 4 from 1 to 12 hr. Other strips, some fresh, some fixed in formalin-acetic acid-alcohol, were boiled from 2 to 5 min in Benedict's quantitative solution (1). The strips were observed by means of the light microscope.

Sunflower and barley epidermal strips, infected with *Erysiphe cichoracearum* DC ex Merat and *E. graminis* f. sp. *hordei* Em Marchal, respectively, were treated in a similar way.

RESULTS AND DISCUSSION.—At 20 C, penetration of the host epidermal wall may begin 8 hr after inoculation. The first staining of the infected epidermal cell with Azure B occurred at the 9th hr (Fig. 1). Thus the infection peg, within 1 hr after it begins to form, stimulates the host cell either mechanically or chemically; the latter being more probable (3).

Usually the blue color in the susceptible epidermal cells disappeared during the 2nd day after infection, indicating recovery, but reappeared 4 to 10 days later and very gradually increased in intensity, finally becoming a deep blue or blue-green after approximately 3 weeks.

The infected epidermal cells in the resistant Lakeland variety became more deeply stained than in the susceptible variety. At 9 to 12 hr, a light-blue color was present, and at 48 hr, a deep blue-green color was present around the margin of the cell (Fig. 4). By the 6th day, the color intensity had increased and the whole cell was completely colored (Fig. 5). The cytoplasm had coagulated and the cell had died.

Not only did the resistant cell display a gradual increase in coloration until death, but also differed in staining with Azure B, a metachromatic dye. The orthochromatic shade, blue-green, was more characteristic in the resistant cell, and the metachromatic shade was more characteristic in the susceptible cell. This indicates, according to Jensen (2), that different high-molecular-weight substances, having free anionic groups, are present in the susceptible and resistant cell.

The cells adjoining the infected cells showed either no color change or a less intense coloration (Fig. 2, 3), or were a very faint pink color. More distant cells were not altered.

Resistant cells which had been boiled in Benedict's solution were a yellow color 48 hr after inoculation. The intensity of coloration increased until death resulted at or prior to the 6th day (Fig. 8, 9). The susceptible infected cells were a light yellow 3 days after inoculation (Fig. 6), and the coloration gradually increased up to 3 weeks (Fig. 7).

The yellow-brown coloration of the infected and adjoining cells after treatment with Benedict's solution shows that reducing compounds appear soon after infection occurs. These reducing substances become much more abundant in the resistant clover cells and may be responsible directly or indirectly for resistance. Obviously the redox potential of the cells changes as more reducing compounds form in cells. Benada (*personal communication*) has found that distinct limits of redox potential in host tissues were needed for successful infection in barley and wheat powdery mildew. Perhaps a particular redox potential range is necessary for a compatible mildew-clover relationship.

Because there is little or no response when barley- and sunflower-infected cells are stained with Azure B or boiled in Benedict's solution, it is evident that each host responds in a specific fashion. One might have

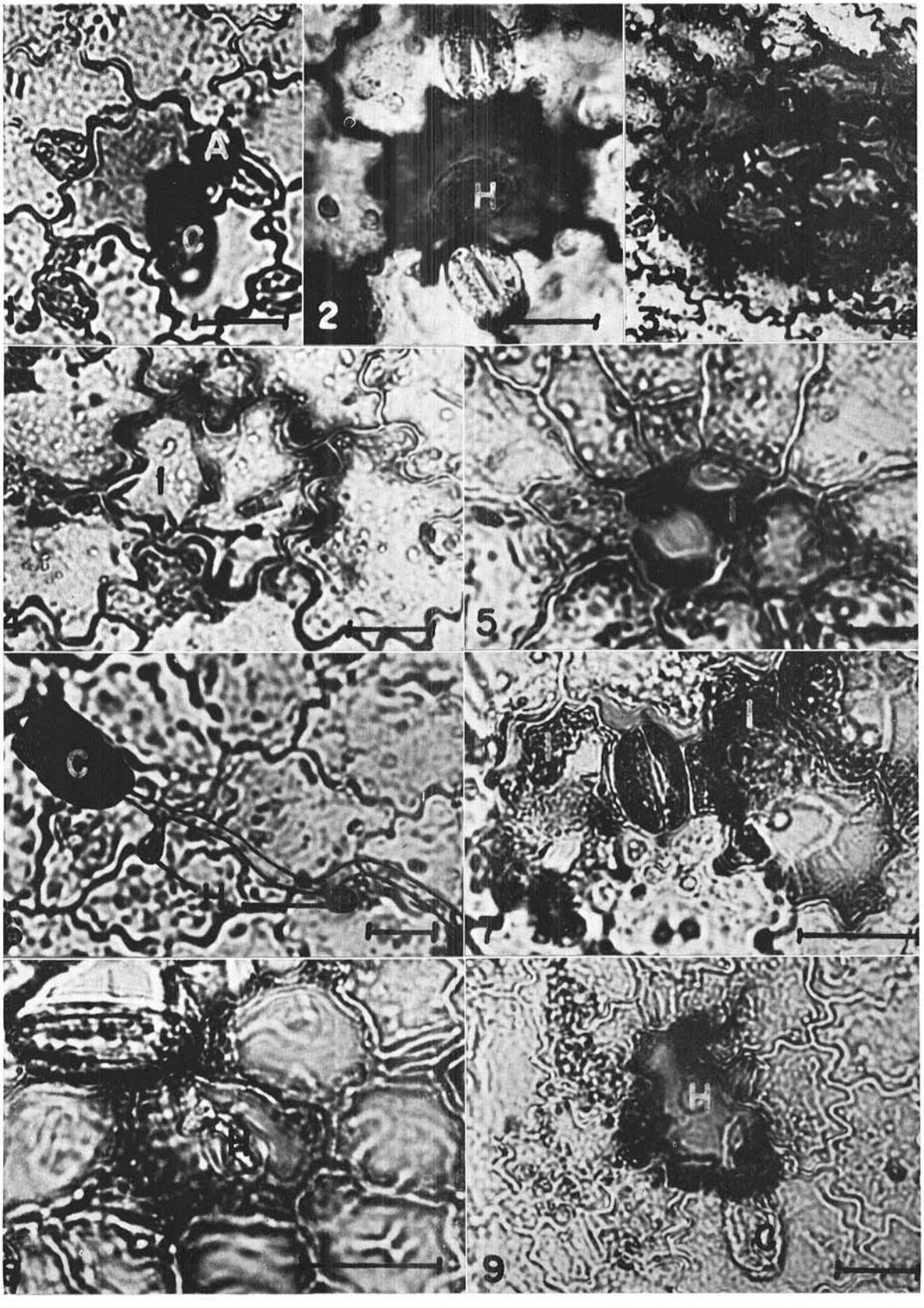
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**Fig. 1-9.** Micrographs of a few epidermal cells from susceptible and resistant red clover leaves which have been infected with *Erysiphe polygoni*. A = appressorium; C = conidium; H = haustorium; I = infected cell. **1)** Epidermal cells from a susceptible red clover leaf upon which a conidium of *E. polygoni* is resting. The central cell inoculated 18 hr previously shows an appressorium and is stained light blue with Azure B. The noninfected cells are not stained (×670). **2)** A susceptible infected epidermal red clover cell surrounded by healthy cells. The cell containing the haustorium is deeply stained with Azure B; the healthy cells are unstained. The epidermal cells had been infected for 2 weeks (×900). **3)** A group of susceptible epidermal red clover cells which have been infected for 7 days. The infected and adjoining cells are stained with Azure B, but the more distant cells are unstained (×470). **4)** Resistant red clover epidermal cells stained with Azure B, 2 days after inoculation. The central cell is infected and is stained blue-green around its margin (×650). **5)** Resistant red clover epidermal cells stained with Azure B, 5 days after inoculation. The dead central cell was infected and stained deep blue, while the neighboring cells are only slightly stained adjacent to the infected cell (×570). **6)** Susceptible red clover epidermal cells treated with Benedict's solution 3 days after inoculation. Two of the cells contain a haustorium each, but all cells are uncolored (×590). **7)** A few susceptible red clover epidermal cells which have been treated with Benedict's solution. The two central cells have been infected for 3 weeks and are an orange color. All cells appear to have been living (×980). **8)** A group of resistant red clover epidermal cells treated with Benedict's solution. The central cell contains a haustorium, and was inoculated 2 days prior to treatment with Benedict's solution. It is a yellowish brown color (×1080). **9)** A group of resistant red clover epidermal cells treated with Benedict's solution 5 days after inoculation. The central cell contains a haustorium (×610) and is a deep orange color and was dead when fixed.

expected an increase in reducing compounds in infected sunflower cells because large quantities of simple carbohydrates must be used in the formation of the collar around the infection peg (4).

Pathologists (5, 6) have shown previously that chemicals and radio-active substances accumulate in diseased host tissues, but this work reveals that alterations occur in the infected cell and sometimes in the neighboring cells in some types of infections. It is apparent that cells rather than tissues need to be studied.

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