

Effect of Resistance on Growth of *Cercospora beticola* Race C2 on the Leaf Surface and within Leaf Tissue of Sugar Beet

E. D. Whitney and N. F. Mann

Plant pathologist and biological technician, respectively, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, U.S. Agricultural Research Station, Salinas, CA 93915.

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ABSTRACT

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When the open-pollinated sugar beet cultivar FC 701/2 was inoculated with *Cercospora beticola*, race C2, individual plants responded with either a susceptible reaction, a large fleck reaction, a small fleck reaction, or no visible reaction. For a given plant, these symptoms were consistent from one time of inoculation to another. On resistant plants, the number of germ tubes per conidium, width of mycelium, and appressorium length were reduced, and the appressorium configuration was altered, compared to those parameters on susceptible plants. We interpret these changes as a fungal response to the resistance of the plants rather than as an

environmental effect. Histopathological studies showed that, although not usually visible to the unaided eye, necrosis of host tissue was present at or near the site of hyphal penetration about one third the depth of the leaf thickness. The amount of necrosis was similar in the large and small fleck host response. The hyphae within the resistant host were usually limited to a single mycelium with one or two branches, while in the susceptible hosts a dense stroma developed in the necrotic tissue. Chloroplasts, nuclei, and nucleoli were degenerate in infected cells near hyphae compared with those from healthy cells.

Additional key words: host-parasite relationship, genetic variation, *Beta vulgaris*.

Cercospora leaf spot incited by *Cercospora beticola* Sacc. is one of the most serious foliar diseases of sugar beet (*Beta vulgaris* L.). Genetic resistance to control *C. beticola* has received much attention. Solel and Minz (11) described the infection processes of *C. beticola* in sugar beet inoculated with two races of *C. beticola* that were differentiated by quantitative resistance of the host. They found no effects of cultivar or race on spore germination. However, they did not test the effects of plant resistance on mycelial diameter or suggest any effect of resistance on appressorium development. In their study, resistance was expressed as either fewer infections per spore or less hyphal development within the host. In only one published study have physiological races of *C. beticola* been differentiated on the basis of a single host-reaction gene in sugar beet (6, 14, 15). Whitney and Lewellen (14) designated the new race as C2 to distinguish it from the common race, C1. They found that some plants of the heterogeneous, open-pollinated cultivars FC 701/2 are resistant to race C2. Inoculation of these plants with race C2 results in flecks that vary in size from plant to plant (6). This investigation was undertaken to study this interaction of FC 701/2 with race C2. Preliminary information has been published (13).

MATERIALS AND METHODS

Cultivar FC 701/2 (4), an open-pollinated type, was greenhouse grown and used exclusively in these studies. Isolate CA-1 of race C2 of *C. beticola* (15) was maintained on sugar beet leaf extract agar (SBLEA) (1). Inoculum was produced by culturing the isolates on SBLEA at 15 C with a fluorescent light intensity of 8,600 lx for 7 days. The inoculum was prepared by adding 10 ml of distilled water to the culture and gently agitating the surface of the colonies with an L-shaped glass rod. Enough polyoxyethylene sorbitan monolaurate was added to each inoculum to produce a 0.001% solution. Spore concentration of the inoculum was estimated with a hemacytometer, and the concentration was adjusted by dilution with water to 30,000 spores per milliliter. The inoculum was applied

with an atomizer until leaves were wet; the inoculated plants were placed in a humidity chamber (95–100% relative humidity) for 72 hr before being returned to open greenhouse benches (15). Plants varied in age but were usually 6–8 wk old when inoculated; some were re inoculated after defoliation and regrowth.

For sporulation studies, leaf disks 5 mm in diameter, each including a single spot or fleck, were cut from appropriate plants. Disks were then placed on SBLEA in petri dishes and incubated for at least 10 days at 15 C with continuous fluorescent light, 8,600 lx (1). With a dissecting microscope, each spot was observed daily for spore production and necrosis of living tissue. Four tests were conducted that comprised a total of 60 disks.

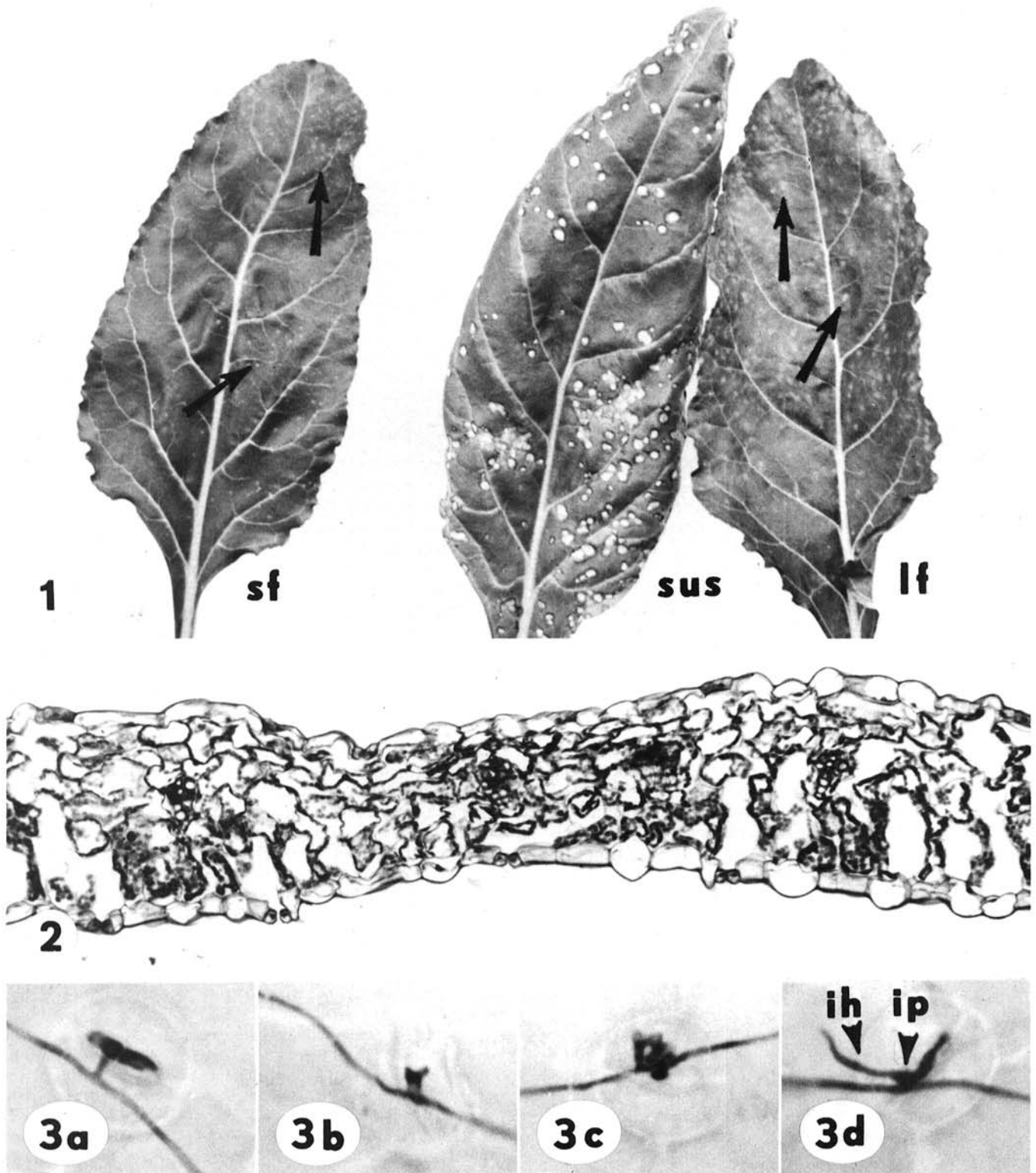
For morphological studies, infected and healthy leaves were cut from plants 3, 6, and 11 days after inoculation. Each leaf was cut into strips about 1 cm wide and 2 cm long. These strips were boiled gently in 70% ethyl alcohol for 30 min to clear the tissue, stained for 5 min in lactophenol cotton blue at 45 C, and destained in either lactophenol or water. From each strip semipermanent mounts were made with glycerin and water (1:1, v/v) as the mounting medium. Measurements of mycelia and appressoria on the leaf surface were made with a light microscope with an ocular micrometer.

For cytological studies, individual chlorotic flecks or necrotic spots were cut from leaf tissue 1 mo after inoculation and fixed in formalin-acetic acid-alcohol or Karnovsky's fixative (5) (2 hr). Those to be embedded in paraffin were dehydrated in an ethanol series and embedded in Paraplast (mp 56 C; Sherwood Medical Industries, St. Louis, MO 63103). Serial sections 10 μ m thick were cut perpendicular to the leaf surface, fixed to slides with Haupt's adhesive, and triple stained by the hematoxylin-safranin-fast green technique. Those specimens to be embedded in plastic were rinsed in water, dehydrated in an acetone and propylene oxide series, and embedded in Epon 812. Serial sections 1 μ m thick were cut paradermal to the leaf surface, fixed to slides by warming, and triple stained as with paraffin embedded sections. Composite photomicrographs were made by fitting appropriate sections from a series together from large- and small-flecked infection sites (inset in Figs. 4 and 5). Figures 7–12 are from the paradermal sections selected from a representative series of a large fleck host-pathogen interaction.

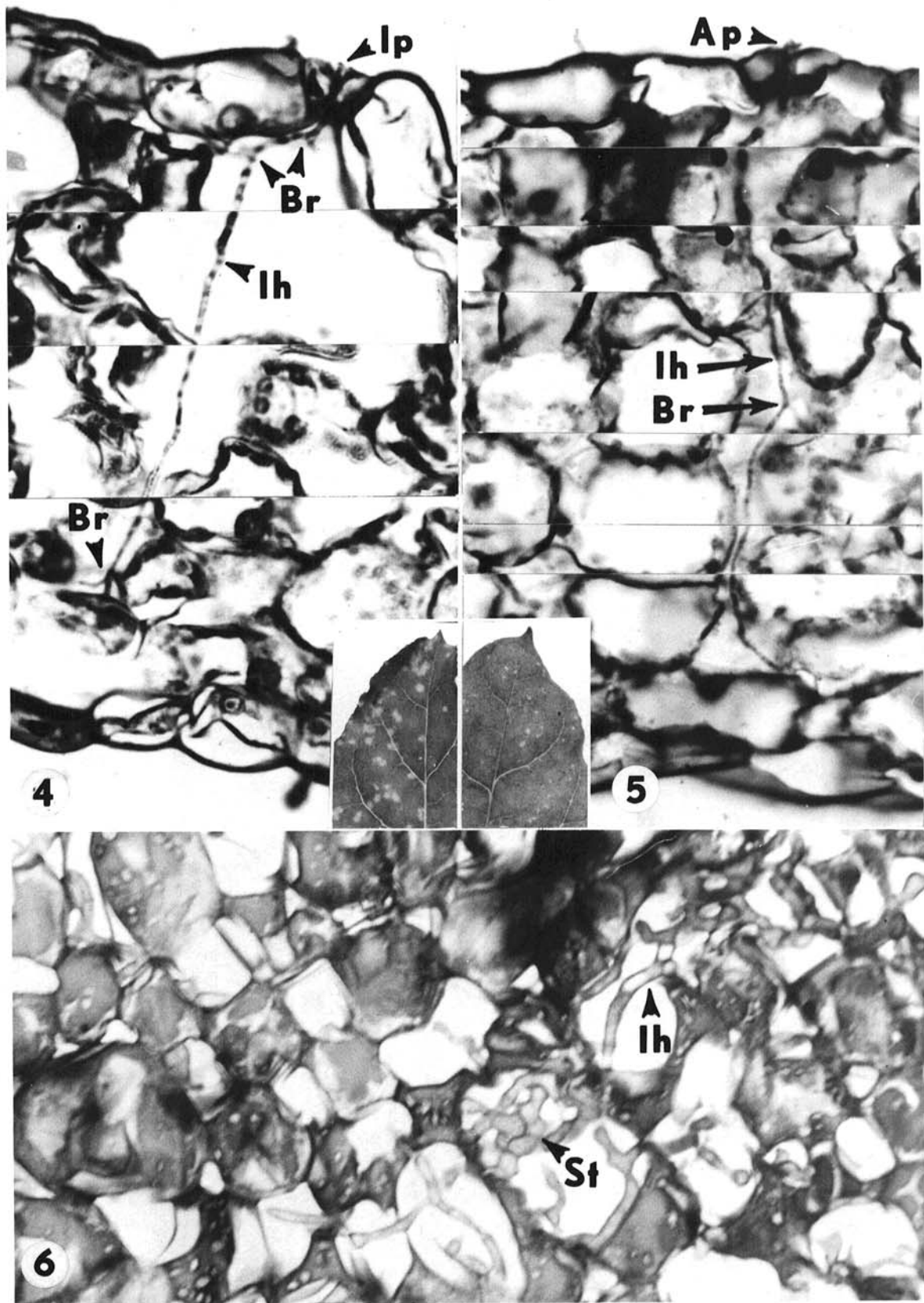
RESULTS

Individual plants of cultivar FC 701/2 showed four different discrete reaction types to *C. beticola* inoculations: a necrotic spot (considered to be a susceptible reaction), a large chlorotic fleck, a small chlorotic fleck (Fig. 1), or no visible reaction. The percentage of plants in each category and the mean diameter of the visible

host-pathogen response are listed in Table 1. Only 4 of 104 inoculated plants showed no visible disease response. Flecking type was found to be consistent from one inoculation to the next and from one leaf to the next in a single plant. The susceptible reactions, small, round, light-brown necrotic spots, were typical *Cercospora* leaf spot symptoms. In areas of concentrated infections, spots coalesced to produce characteristic symptoms of severe leaf spot



Figs. 1-3. *Cercospora beticola* Race C2 infection of sugar beet. **1**, Small fleck (sf), susceptible reaction (sus), and large fleck (lf) caused by *C. beticola* on sugar beet cultivar FC 701/2 ($\times 0.6$). **2**, Sunken leaf tissue associated with infection by *C. beticola* Race C2 on cultivar FC 701/2 ($\times 210$). **3**, Appressoria of *C. beticola* on sugar beet. **A**, Normal appressorium showing three cells. **B-D**, Deformed appressoria on resistant plants. Ih = infection hypha, Ip = infection peg ($\times 1,100$).

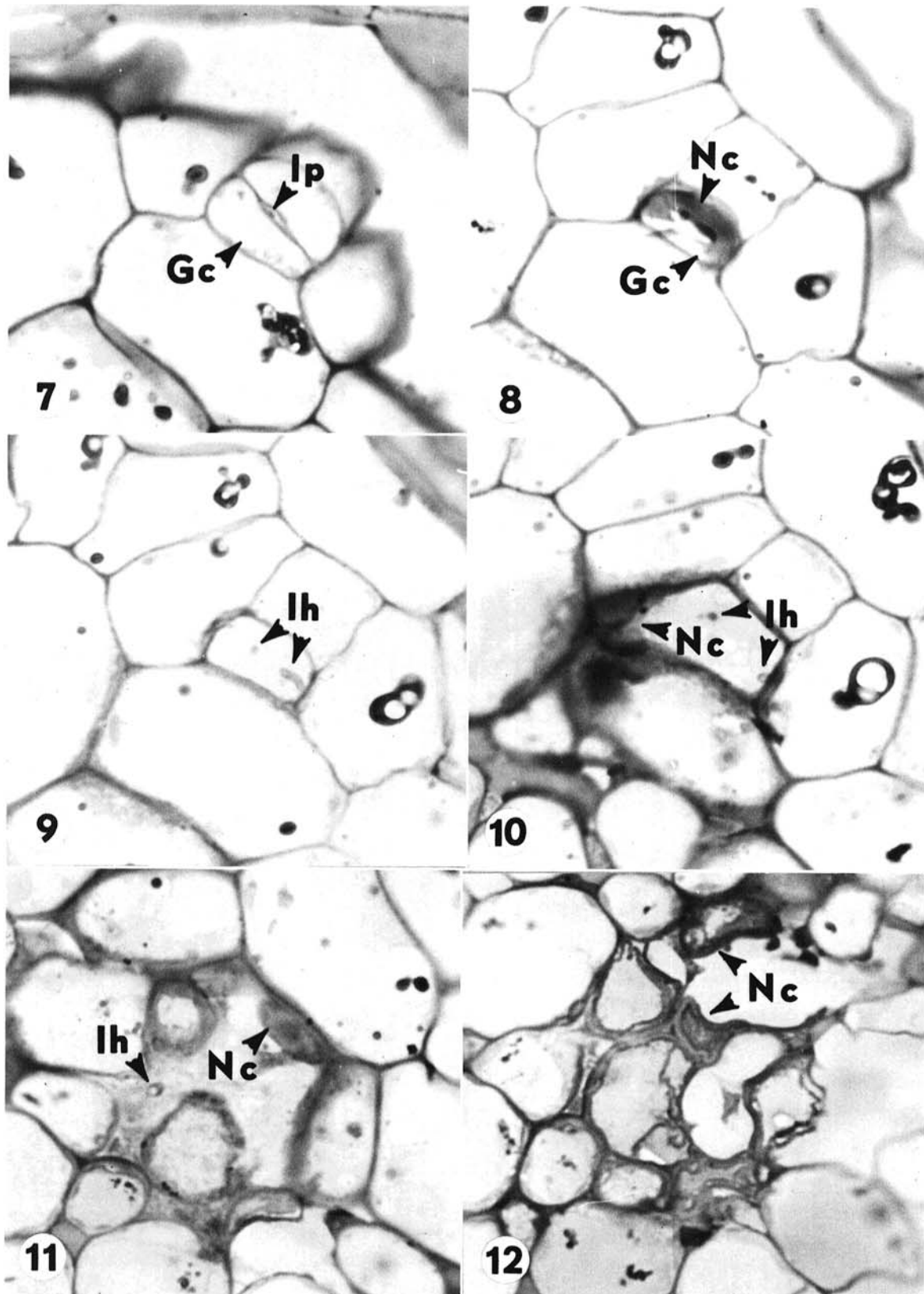


Figs. 4-6. Resistant (Figs. 4-5) and susceptible (Fig. 6) sugar beet plants infected with *Cercospora beticola*. **4**, Composite of perpendicular serial sections of a large fleck reaction showing limited hyphal growth and general cell deterioration and collapse ($\times 760$). Ip = infection peg, Br = branching of infectious hyphae, Ih = infectious hyphae. Inset large fleck symptoms ($\times 0.75$). **5**, Composite of perpendicular serial sections of a small fleck reaction showing less cell deterioration and collapse than the large fleck reaction in Fig. 4 ($\times 760$). Ap = appressorium, Ih = infectious hyphae, Br = branching of infectious hyphae. Inset small fleck symptom ($\times 0.75$). **6**, Paradermal view of a susceptible reaction showing robust hyphal growth, stroma, and cell collapse ($\times 760$). St = stroma and Ih = infectious hyphae.

(Fig. 1). In resistant plants a few necrotic leaf spots occurred on older leaves but usually not until the leaves had become chlorotic or senescent, and occasionally necrotic spots occurred on the leaf margin or near an injury. Sporulation of the fungus from incubated flecks and spots produced a velvety growth of conidiophores and

conidia typical of *C. beticola*. Flecks were accompanied by chlorosis, and both surfaces of the leaf were sunken (Fig. 2). The flecks arose through cellular collapse of the internal tissue of the leaf.

Incubation showed that infectious hyphae remained alive in



Figs. 7-12. Paradermal sections showing *Cercospora beticola* infection of a resistant sugar beet. Serial sections number 5, 10, 11, 14, 19 and 23 in a sequence of 1- μ m-thick sections, showing hyphal ingress and necrosis of adjacent cells in a large fleck reaction ($\times 760$). Ih = infectious hyphae, Ip = infection peg, Gc = guard cell, Nc = necrosis.

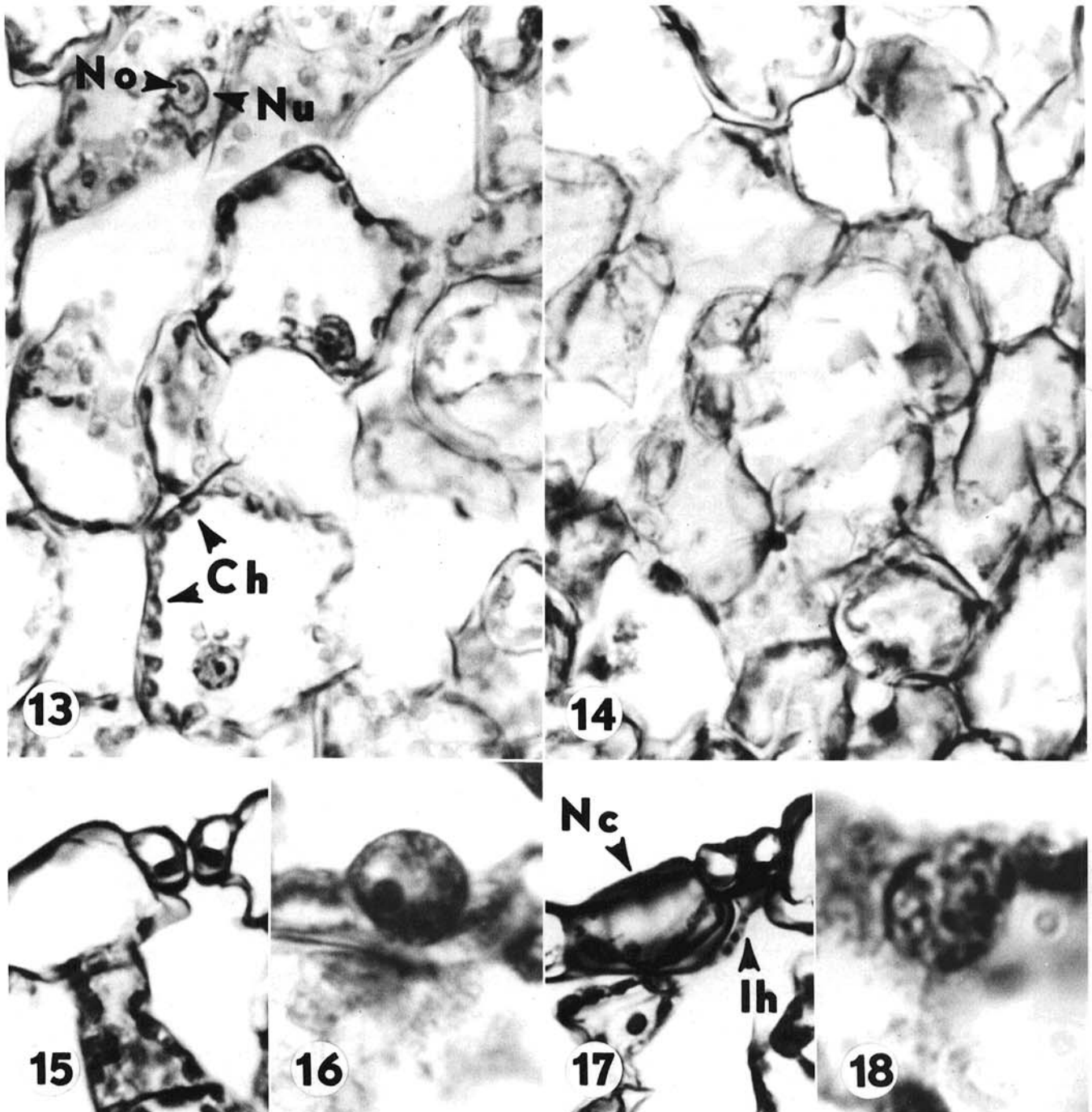
some flecks, but apparently in an arrested state. Individual infection centers (flecks or spots) differed in the length of time required for sporulation at an incubation temperature of 15 C. The mean length of time required for sporulation was 3.1 days for spots and 8.0 days for large or small flecks. Sporulation of the fungus did not occur until the leaf tissue of the isolated disk had become necrotic. In some leaf disks with small flecks, no evidence of fungal growth was found after 10 days of incubation, even though necrosis of the disk tissue was complete (Table 1).

The numbers of germ tubes per conidium were reduced slightly on resistant plants exhibiting small flecks or no reaction, compared with susceptible plants (Table 1). The numbers were similar on susceptible plants and plants that showed the large fleck symptom

(Table 1).

Length of mycelial growth per colony of fungus on the leaf surface ranged from 33 to 1,135 μm on susceptible plants. The range was 13.2–4,797 μm for plants that had large flecks, 13.2–686 μm for plants that had small flecks, and 33–686.4 μm for plants that showed no response. An analysis of variance showed no differences in mean length of mycelial growth among reaction types. Mycelial growth on the surface of the leaf did not continue after the 72-hr incubation period in the humidity chamber when the plants were removed from the chamber and placed in the greenhouse.

Fungi growing on susceptible and resistant plants differed in width of mycelium and length of appressorium (Table 1). Compared with appressoria on susceptible plants (Fig. 3A),



Figs. 13–18. Cytological comparisons of healthy and infected sugar beet tissue. **13,** Paradermal view of section from healthy portion of the same leaf as in Fig. 14 from a large fleck reaction ($\times 760$), 1 μm thick. Nu = nucleus, No = nucleolus, Ch = chloroplast. **14,** Paradermal view of section from large fleck reaction showing cell deterioration. **15,** Guard cells from healthy plant tissue ($\times 760$). **16,** Nucleus from healthy plant tissue ($\times 3,020$). **17,** Guard cells from diseased plant tissue with large flecks showing necrosis and infectious hyphae ($\times 760$). Nc = necrosis, lh = infectious hyphae. **18,** Nucleus from diseased plant with large flecks ($\times 3,020$).

TABLE 1. Characteristics of responses to infection with *Cercospora beticola* Race C2 on the open-pollinated sugarbeet cultivar FC 701/2

Characteristic	Host-pathogen reaction			
	Susceptible	Large fleck	Small fleck	None
Percentage of plants ^a	40	22	34	4
Percentage sporulation ^b	100	100	88	
Mean reaction diameter (mm) ^c	2.1	0.8	0.3	
Number of germ tubes/spore ^d	2.9	3.0	2.7	2.6
Mean mycelial width (μm) ^{e,f}	1.6	1.3	1.4	1.2
Mean appressorium length (μm) ^g	11.0	6.7	8.5	7.3
Mean stomatal length (μm) ^{h,i}	24.4	24.9	25.5	24.9

^aPercentage from two tests of 52 plants each.

^bSixty spots per host-pathogen reaction were incubated at 15 C in continuous light; data represent percentages of spots with spores.

^cMean of 100 measurements, 1 mo after inoculation.

^dLSD ($P = 0.05$) = 0.15.

^eMean of single measurements from each of 20 colonies.

^fHighly significant ($P = 0.01$) correlation with appressorium length ($r = 0.99$).

^gLSD ($P = 0.01$) = 1.85.

^hMean of 120 stomata per host-pathogen reaction.

ⁱNot significant, $P = 0.05$.

appressoria that formed on resistant plants occasionally were deformed or appeared aborted (Fig. 3B–D). The mycelium of race C2 was 1.6 μm in diameter and branched perpendicularly on the susceptible hosts (Fig. 3A). The appressoria were septate and had two, or occasionally three, cells (Fig. 3A).

Fungal penetration was usually limited to a single hypha with one or two branches (Figs. 4 and 5). In some chlorotic spots, the fungus could not be observed even though cytological damage was visible. Hyphae were intercellular and often appeared closely associated with cell membranes (Figs. 4, 5, and 17). The fungus in a susceptible plant formed a loose to tightly packed stroma with profuse branching (Fig. 6). Fungal development was associated with cell collapse and necrosis of cells adjacent to hyphae.

Hyphal width also decreased with depth of hyphal ingress into the leaf. This reduction in hyphal growth (width) was associated with a reduction in cell damage, ie, necrosis. Although necrosis usually is not visible to the unaided eye in resistant plants, the guard cells (Figs. 4, 5, 8; also compare 15 with 17), epidermal cells (Fig. 17), and the spongy parenchyma cells (Figs. 9–12) often were necrotic when adjacent to or closely associated with the fungus. Necrosis usually was limited to the first one third of the tissue below the stomatal opening. Chlorosis that resulted from chloroplast deterioration and cell collapse was much less severe in small flecks than in large ones (Figs. 4 and 5). Large differences in necrosis between large and small flecks were not evident in serial sections.

Cytological differences were noted between healthy and infected tissues. Chloroplasts lost their rounded integrity and became fused or twisted ribbonlike in resistant plants (compare Figs. 4, 5, and 14 with 13). Nuclei lost their integrity, chromatin became more diffuse, and the nucleolus disappeared or was less evident in infected plant cells (Figs. 16 and 18).

DISCUSSION

The resistance response has been shown to be consistent from one inoculation to another and among the leaves of the same plant. Vigor of the pathogen is reduced during its association with a resistant host plant. Therefore, we interpret the resistance responses as being under genetic rather than under environmental control. Further, resistance does not appear to be merely the manifestation of a mechanical barrier because the pathogen is affected adversely by association with the resistant hosts; the pathogen shows slightly reduced germ tube initiation, reduced mycelial width, reduced length of appressoria, and changes in appressorium morphology. In contrast to our findings, Solel and Minz (11) found no effects of host resistance on the fungus. Sizes of stomata were similar in resistant and susceptible leaves (Table 1), as reported by Ruppel (10), so this characteristic does not appear to be associated with resistance. Resistance may be associated with phytoalexins (3,7) because sporulation of the fungus is delayed until the host cells die, are injured, or senesce. The interactions between pathogen and host in the large and small flecks were similar except for the greater amount of chlorosis in large flecks,

which suggested that differences in resistance could result from differences in the amount of phytoalexins. We have interpreted the deformed appressorium in Fig. 3D as an aborted infection peg and hypha because these structures appear to be identical to those observed in *C. beticola* by Rathaiah (8,9).

Cunningham (2) in 1928 reported that a cicatrice was formed between the infected and healthy tissue in sugar beet infected with *C. beticola*. We did not find any evidence of a cicatrice in this study, nor have others (11,12) found any such evidence in recent studies.

The limitation of fungal growth associated with resistance in leaf tissue appears to depend upon a single dominant gene in cultivar FC 702/2 (6) and upon additional factors or incomplete dominance that affect the interaction in cultivar FC 701/2. Studies are in progress to determine the inheritance of these modifying genes. These differences in plant response to the fungus could have some significance in selecting plants with higher levels of resistance to *C. beticola*.

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