Relationship of Callose Deposition to Resistance of Lettuce to *Plasmopara lactucae-radicis*

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ABSTRACT

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Resistance of lettuce to *Plasmopara lactucae-radicis,* an obligate root-infecting fungus, was attributed to host deposition of callose around fungal haustoria and production of septumlike intrahyphal callose walls by the fungus. Treatment of a genetically resistant cultivar with 2-deoxy-D-glucose, an inhibitor of callose synthesis by the plant, resulted in susceptibility to the root pathogen. Nutrient deprivation that results from callose deposition may be the basis of resistance of lettuce to the pathogen.

Additional keywords: induced resistance, *Pythium*.

During our investigations of the pathology of *Plasmopara lactucae-radicis* Stang. & Gilbn., a pathogen of lettuce (*Lactuca sativa* L.) roots, we observed that resistant and susceptible cultivars were colonized by the fungus (11,14). Intercellular hyphae and intracellular haustoria occurred in all colonized roots, but the fungus was unable to sporulate on roots of resistant cultivars. Apparently, sufficient nutrients were present in the resistant host to support vegetative hyphal growth and production of haustoria. We also observed that haustoria in resistant cultivars were larger than those in susceptible cultivars. Preliminary studies (14) indicated that haustoria in resistant cultivars, but not those in susceptible cultivars, were encased in callose.

Callose has been implicated numerous times as a provoked structural resistance response by plants to attempted penetration by fungi (1,3–6,10,12). This hypothesis was further strengthened by a recent investigation (3) in which inhibition of callose synthesis with 2-deoxy-D-glucose (DDG), a known inhibitor of callose production in plants (8), resulted in increased penetration efficiency of a resistant plant by the pathogenic fungus. No evidence was presented, however, regarding fungal sporulation or development of disease symptoms on resistant plants treated with DDG. Both of these latter processes are obligatory and characteristic responses indicative of host susceptibility. The objective of this investigation was to determine the effect of DDG on callose deposition and resistance of lettuce to *P. lactucae-radicis*.

MATERIALS AND METHODS

Fungus cultures. Cultures of *P. lactucae-radicis,* an obligate root-infecting parasite, were maintained on lettuce plants (cv. Ostinata) grown under hydroponic conditions as previously described (11,14). Infected plants served as the source of inoculum in studies described below.

Callose inhibition and detection tests. Three 1-wk-old seedlings, one of a susceptible lettuce cultivar (Ostinata) and two of resistant cultivars (Cobham Green and an F$_3$ progeny from a cross between a resistant cultivar, Cobham Green, and a susceptible cultivar, Calmar), were transplanted into separate hydroponic chambers, each containing 1 L of a nutrient solution (14). Previous studies showed that resistance was determined by a single recessive gene (14).

One week after the seedlings were transplanted, an Ostinata plant infected with *P. lactucae-radicis* was placed into each chamber to serve as a source of inoculum. After incubation for 4 days, microscopic examination of fresh, excised portions of

the root system (four 2-cm-long apical root segments with a mean root diameter of 0.8 mm) of each cultivar revealed the presence of intercellular hyphae and intracellular haustoria. Furthermore, the fungus was sporulating on roots of the susceptible cultivar but not on roots of the resistant cultivars. After root colonization was verified, the hydroponic nutrient solution in some chambers was amended with various concentrations of DDG. Treatments included 1) susceptible cultivar without DDG, 2) resistant cultivars without DDG, 3) resistant cultivars with DDG, and 4) susceptible cultivar with DDG.

Portions of the root systems of the plants were periodically excised, gently squashed, and stained for microscopic observation of the presence or absence of callose and sporulation. Callose deposits were stained with resorcinol blue for bright field microscopy and with basic aniline blue for fluorescence microscopy (7). No autofluorescence was observed in our study. Diameters of haustoria and thickness of callose deposits were measured in roots stained with resorcinol blue. The mean of three counts was determined (25 haustoria per count for each treatment). Sporulation (number of sporangia-bearing sporangiophores per centimeter of root) was recorded for each treatment (10 1-cm-long root segments per treatment), and data were presented as follows: 0 = −, 1–3 = +, 4–7 = ++, and 8–15 = +++. The experiment was repeated two times, and results from a single representative experiment were chosen for presentation.

In addition to these studies, which focused on young roots of susceptible and resistant cultivars, callose deposition and fungal sporulation were observed on older portions of the root system of the susceptible cultivar, which were in various stages of necrosis.

RESULTS

Within 96 h after inoculation, intercellular runner hyphae and intracellular haustoria were observed microscopically within young roots of resistant and susceptible cultivars. In the susceptible cultivar, haustoria, which had a mean diameter of 5 ± 1 μm, exhibited only a faint fluorescence (Fig. 1A and C) that was localized around haustorial necks (Fig. 2). In the resistant cultivar, however, approximately 94% of the haustoria exhibited intense fluorescence when stained with aniline blue, and they appeared to be totally encased in callose (Fig. 1D). Callose-encased haustoria had a mean diameter of 8 ± 1 μm, and the callose deposits in the resistant cultivar ranged from 1.5 to 2.5 μm in thickness (Fig. 3B). The latter measurements were made in colonized roots stained with resorcinol blue.

Septumlike callose walls were frequently observed within the intercellular runner hyphae of the fungus in resistant cultivars but not in the susceptible cultivar (Fig. 3A). These callose walls

occurred at irregular intervals (5–100 μm) within any single intercellular hypha. The observable condition of the fungal cytoplasm between two given walls was in some cases similar in appearance to the cytoplasm of fungal hyphae in the susceptible cultivar; in some cases the cytoplasm was in various stages of lysis; and in other cases, the area was totally devoid of cytoplasm.

Amendment of the nutrient solution with DDG altered sporulation by the fungus and callose deposition in the resistant cultivar but not in the susceptible cultivar (Table 1). Without DDG, the fungus sporulated extensively on roots of the susceptible cultivar (Fig. 1A), but no sporulation occurred on the resistant cultivar. The fungus did sporulate on roots of the resistant cultivar, however, approximately 60 h after the nutrient solution was amended with 0.5×10^{-5} M DDG. Higher concentrations of DDG (1×10^{-4} and 1×10^{-5} M) were phytotoxic. The extent of sporulation on resistant plants treated with DDG was, however, considerably less than that on the susceptible cultivar. Haustoria in roots of resistant plants treated with DDG were not encased in callose
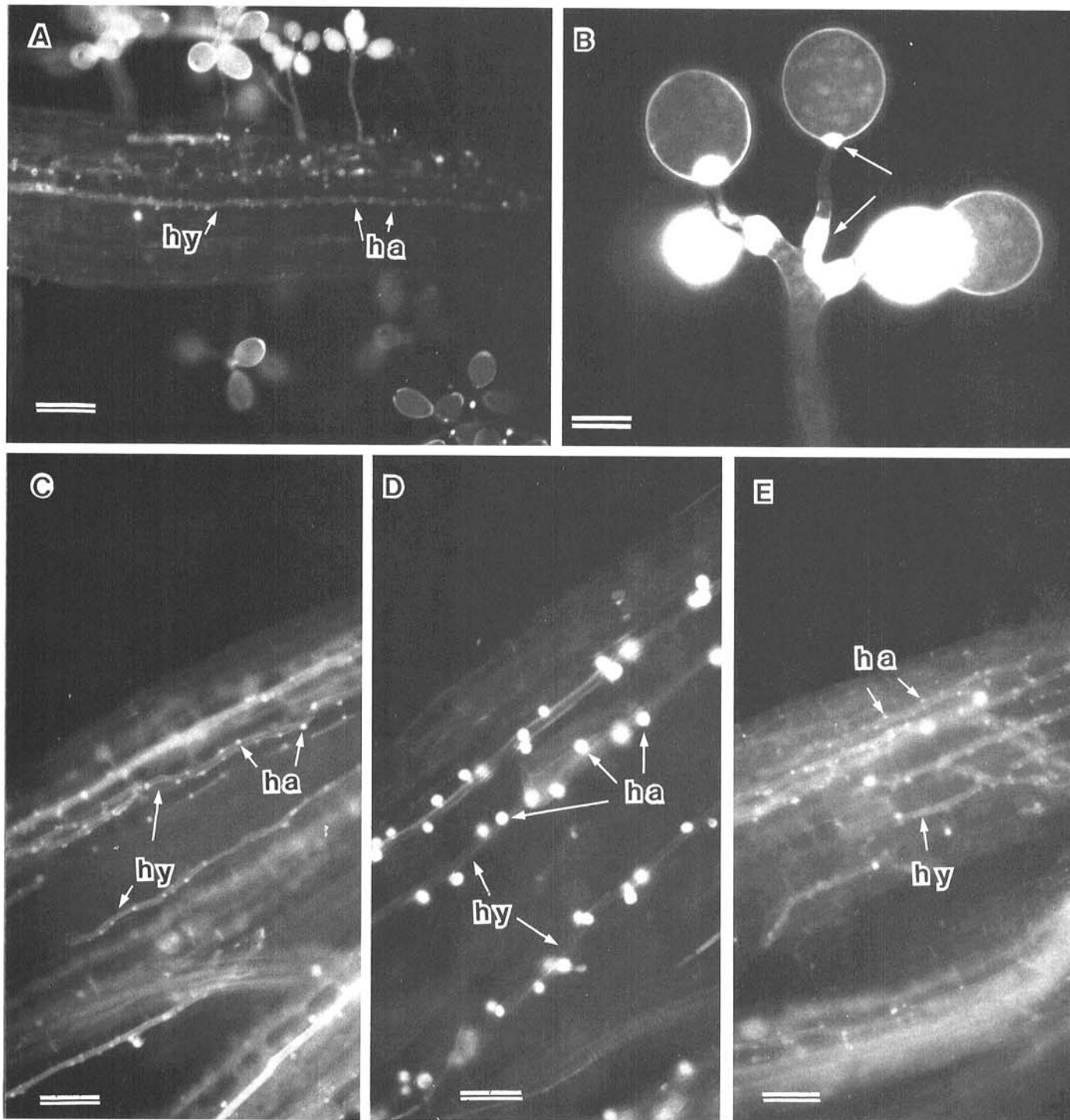

Fig. 1. Light micrographs of *Plasmopara lactucae-radicis* and lettuce roots stained with aniline blue. Callose deposits are indicated by the intensity of fluorescence with ultraviolet illumination. **A,** Sporulation of the fungus on a root of a susceptible lettuce plant, runner hypha (hy) and numerous haustoria (ha) in a root, faint fluorescence around haustoria (scale bar = 100 μm); **B,** septumlike callose walls (arrows) within a sporangiophore of the fungus (scale bar = 20 μm); **C,** hyphae (hy) and haustoria (ha) in a root of a susceptible lettuce plant, faint fluorescence around haustoria (scale bar = 50 μm); **D,** hyphae (hy) and haustoria (ha) in root of a resistant lettuce plant, intense fluorescence around haustoria (scale bar = 50 μm); **E,** hyphae (hy) in root of a resistant lettuce plant treated with 2-deoxy-D-glucose, faint fluorescence around haustoria (ha) (scale bar = 50 μm).

(Fig. 1E). Furthermore, necrosis of roots, typical of a susceptible reaction, occurred on resistant plants treated with DDG.

Older portions of the root system of the susceptible cultivar were in various stages of necrosis, and 20–40% of the haustoria were encased in callose. The degree of sporulation declined relative to that which occurred on younger and nonnecrotic portions of the root system. Septumlike callose walls also were observed occasionally in intercellular hyphae and within mature sporangiophores of the fungus (Figs. 1B and 3C).

DISCUSSION

Our study provides direct evidence that callose deposition by the host is the mechanism associated with resistance to pathogen sporulation. Two lines of evidence, formulated into the following scenarios, are presented.

The first line of evidence concerns callose deposition by the host. In a susceptible interaction, callose is deposited initially only around haustorial necks. Nutrients apparently are absorbed by the haustoria and are translocated via intercellular hyphae in sufficient quantities to support fungal sporulation. Haustoria are slowly but eventually encased in callose, and sporulation then declines. In a resistant interaction, however, callose deposition around haustoria occurs rapidly and apparently blocks fungal absorption of host nutrients necessary to support sporulation.

Fig. 2. Light micrograph of *Plasmopara lactucae-radicis* stained with aniline blue. Callose deposits are indicated by the intensity of fluorescence with ultraviolet illumination. Fluorescence is localized around the necks of haustoria (ha) on hypha (hy) in a root of a susceptible lettuce plant. Scale bar = 25 μm.

This scenario has been hypothesized for some resistant host-fungus interactions (6,10). If deposition of callose by the host acts as a physical barrier to nutrient absorption by haustoria in a resistant host, inhibition of callose synthesis in a resistant host may be expected to result in haustorial absorption of nutrients sufficient to support fungal sporulation. Our results conclusively demonstrate this effect for the first time.

The second line of evidence concerns callose deposition by the pathogen. Hyphae of *P. lactucae-radicis* have aseptate hyphae, as do all other members of the Peronosporales. As hyphae age, however, intrahyphal septumlike callose walls are commonly formed. Such walls may function as a mechanism of cytoplasmic conservation (2). Wall formation is not restricted to vegetative hyphae but also occurs in mature sporangiophores (2). In our study, intrahyphal walls of callose were consistently observed in young hyphae in resistant cultivars but not in the susceptible cultivar. Such walls were also observed in mature intercellular hyphae and in mature sporangiophores of the fungus in the necrotic susceptible cultivar. The occurrence of these walls in young intercellular hyphae indicates that insufficient nutrients are being absorbed by the haustoria and that nutrient deprivation triggers a defensive response by the fungus that conserves cytoplasm. This response results in compartmentalization of the intercellular hyphae and prevents translocation of the nutrients necessary for sporulation. This hypothesis is not without precedence. Under experimentally induced starvation conditions, the cytoplasm in aseptate hyphae of young germlings of *Pythium ultimum* Trow is retracted into the sporangium. During the process of retraction, intrahyphal walls are formed. These walls, which have been identified subsequently as callose deposits (*unpublished data*), acted as a physical barrier to regrowth of the fungus upon the addition of nutrients (13).
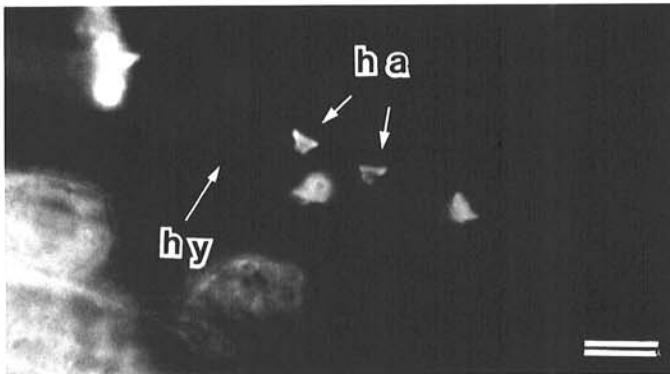
TABLE 1. Relationships of callose deposition, sporulation, and 2-deoxy-D-glucose (DDG) treatments to susceptibility and resistance of lettuce to *Plasmopara lactucae-radicis*

Lettuce cultivar and treatment	Host genotype	Host-pathogen interaction	Callose[a]	Sporulation[b]
Ostinata	Susceptible	Susceptible	−	+++
Ostinana + DDG	Susceptible	Susceptible	−	+++
F3 progeny	Resistant	Resistant	+	−
F3 progeny + DDG	Resistant	Susceptible	−	+

[a]− = Absent; + = present.
[b]Sporangiophores per centimeter of root. − = 0; + = 1–3; ++ = 4–7; and +++ = 8–15.
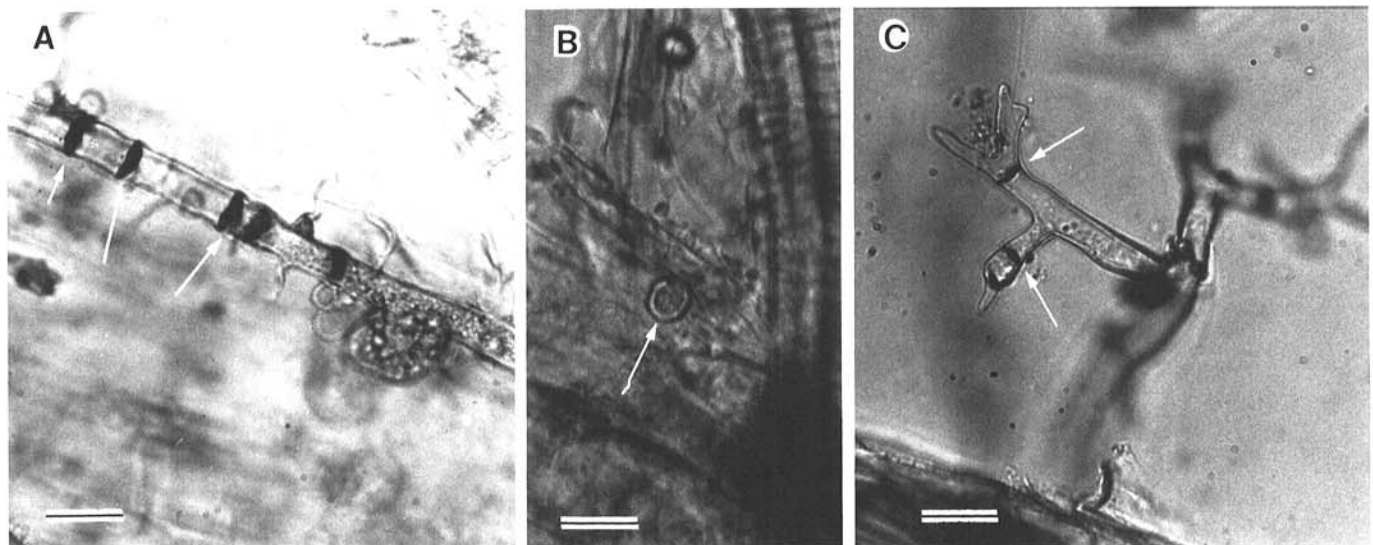

Fig. 3. Light micrographs of *Plasmopara lactucae-radicis* and lettuce roots stained with resorcinol blue. **A,** Septumlike callose walls (arrows) within a hypha of the fungus in a resistant lettuce plant; **B,** a callose sheath (light-colored area, arrow) around a haustorium in a root of a resistant lettuce plant; **C,** septumlike callose walls (arrows) in a sporangiophore of the fungus. Scale bars = 20 μm.

The cumulative results of our investigation indicate that nutrient deprivation, resulting from an initial and early deposition of callose by the host and followed by a callose response in the pathogen, is the basis of resistance of lettuce to *P. lactucae-radicis*. As mentioned previously, resistance to this fungus is determined by a single recessive gene (14). The precise location of this resistance gene has been mapped recently (9).

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