Colonization of Roots of Strawberry Cultivars with Different Levels of Susceptibility to *Phytophthora fragariae*

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Research was funded in part by the North Carolina Agricultural Research Service, Raleigh 27695-7643. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.
We thank L. F. Grand, J. B. Ristaino, and H. D. Shew for critical review of the manuscript.
Accepted for publication 2 February 1993.

ABSTRACT

Milholland, R. D., and Daykin M. E. 1993. Colonization of roots of strawberry cultivars with different levels of susceptibility to *Phytophthora fragariae*. Phytopathology 83:538-542.

Roots of strawberry cultivars Tennessee Beauty (susceptible), Surecrop (partially resistant), and Climax (highly resistant) were examined microscopically at 2, 4, 6, 8, and 10 days after inoculation with *Phytophthora fragariae* race Pf-2. Cultivar Climax had no infection hyphae or reproductive organs of *P. fragariae* at any time period or distance from the root tip. Cultivar Surecrop roots were colonized at a slower rate and had fewer reproductive organs than had Tennessee Beauty. The lack of sporangial production (secondary inoculum) on Surecrop substantiates previous findings of few or no sporangia produced on Surecrop roots 14 days after inoculation with *P. fragariae* race Pf-2. Restriction or reduction in sporangial production may be a component of partial resistance to *P. fragariae*.

Investigations into the nature of red stele disease of strawberry (*Fragaria* × *ananassa* Duchesne), caused by *Phytophthora fragariae* C.J. Hickman, have been carried out since it was first described in Scotland in 1926 (14). This root rot disease is a major factor limiting fruit production in North Carolina and causes serious losses to strawberry growers in areas of the world where temperature and soil-moisture conditions are favorable for its development (6,11).

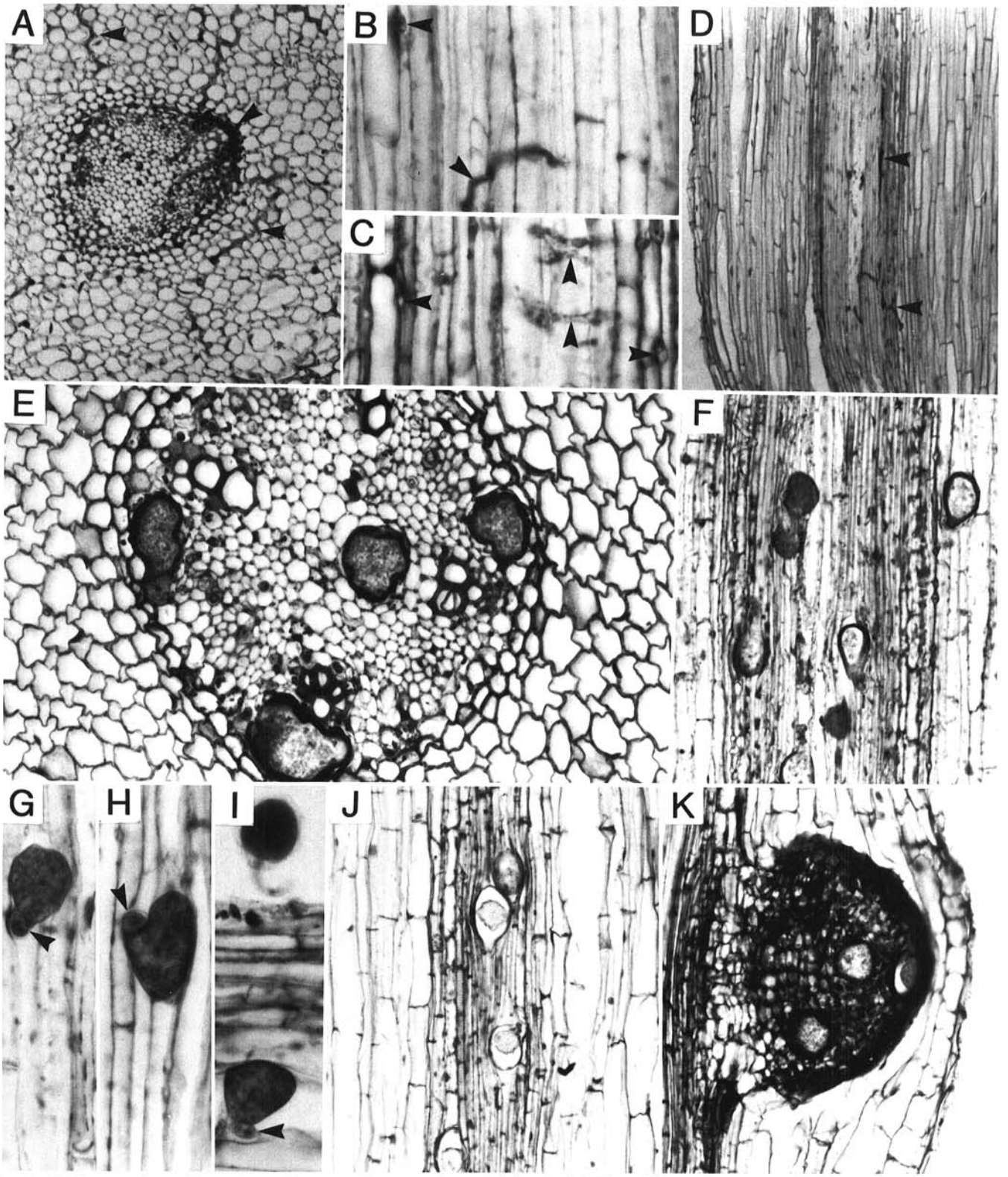
In our studies, susceptibility is defined as the inability of a host plant to resist disease or attack by a particular pathogen. Resistance is the inherent ability of a host plant to prevent or retard the development of a disease. Partial resistance is the incomplete or quantitative resistance through which host plants become infected by the pathogen but differ in the rate or amount of disease development. The level of host susceptibility to a particular *P. fragariae* race is quantified by the number of oospores produced in infected roots (10). A disease-severity index (DSI) developed to evaluate susceptibility of strawberry to *P. fragariae* has been very effective and reliable. Recent studies (9,10) have shown that the level of susceptibility of strawberry genotypes to infection and colonization by seven *P. fragariae* races varies widely. Cultivars such as Darrow, Delite, Earliglow, and Midway had complete resistance to six of seven *P. fragariae* races tested. Analysis of susceptible cultivars that have variable DSIs revealed the partial resistance of 12 strawberry cultivars responding to five races, and the response appeared to be race dependent (9). In controlled environment studies (9), growth responses, such as foliar and root dry weight and number of crowns per plant, of highly susceptible cvs. Albritton, Apollo, Atlas, Earlibelle, Titan, and Tennessee Beauty (DSI = 41, 95, 66, 60, 75, and 172, respectively) appeared to be correlated with levels of susceptibility to infection and colonization.

Goode (5) reported that encystment and penetration of root epidermal cells by zoospores of *P. fragariae* were similar in the resistant and susceptible strawberry cultivars she tested. However, no further development of the fungus occurred in resistant cv. Climax, whereas mycelium grew and oospores formed in the roots of susceptible cv. Huxley. Goode's test included only strawberry cultivars with complete resistance or no resistance. It is possible that *P. fragariae* may colonize only on a limited scale on cultivars with partial resistance to the pathogen. One of the effects of partial resistance can be the limitation of spore production (13). The objectives of the present study were to examine histologically the amount of colonization by *P. fragariae* in roots of strawberry cultivars with different levels of susceptibility and to determine the temporal and spatial relationships of spore production among these cultivars.

MATERIALS AND METHODS

Strawberry cvs. Tennessee Beauty, Surecrop, and Climax were used in these experiments. Their susceptibility to *P. fragariae* race Pf-2 (the predominant race in North Carolina) was determined in previous tests (10). A DSI, determined by multiplying the mean number of oospores per root segment by the percent roots with oospores and dividing by 100, of 172, 15, and 0 for Tennessee Beauty, Surecrop, and Climax, respectively, was calculated. Primary-runner plants were removed from stock plants in the greenhouse, potted in 5-cm-diameter clay pots containing Metro-Mix 220 (W. R. Grace & Co., Cambridge, MA), sand, and soil (1:1:1, v/v/v), and placed under intermittent mist at 25–30 C for 10 days to promote root formation. Plants were removed from the soil and roots were rinsed thoroughly in tap water and spray-inoculated according to the procedure of Milholland et al (10). Zoospores of isolate NC-1 representing *P. fragariae* race Pf-2 were used as inoculum. Inoculum was produced according to the procedure of George and Milholland (4). A suspension of nonmotile (encysted) zoospores at 2×10^4 zoospores per milliliter was used to inoculate five 10-day-old roots per plant. Sixteen plants of each cultivar were inoculated. Spores were placed on glass slides inside a moist petri dish to determine the percentage of spores germinated after 24 h. Spore germination 24 h after inoculation was 82%. Plants were maintained in round, plastic containers (25-cm diameter × 9-cm deep) filled to a depth of 8 cm with Metro Mix 220 moistened with 1,750 ml of cool tap water. Each container was placed inside a plastic bag, sealed, and placed under a 12-h photoperiod at 15 C in a walk-in growth chamber. Roots from each cultivar were selected for histological examination 2, 4, 6, and 8 days after inoculation. Later, additional Surecrop plants were inoculated as described previously, and their roots were examined histologically 10 days after inoculation. Spore germination 24 h after inoculation was 90%. A 5-mm-

long section was excised from each root 5–10, 15–20, 25–30, and 35–40 mm from the root tip. The sections were labeled and fixed separately in formalin (5 ml), propionic acid (5 ml), and 50% propanol (90 ml) for 1 wk. Sections were dehydrated for 1 wk in an isopropyl alcohol series then infiltrated and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO) (7). The specimens were softened for 48 h in a solution of 90 ml of 1% sodium lauryl sulfate (the household detergent Dreft) and 10 ml of glycerol, and 12-μm-thick sections were cut with a rotary microtome. Sections were mounted on slides with Haupt's adhesive and stained with Triarch Quadruple Stain (Triarch, Inc., Ripon, WI). The experiment was repeated once.



Figs. 1A–K. Root sections of strawberry plants (cv. Tennessee Beauty) infected with *Phytophthora fragariae*. **A,** Hyphae (arrows) in the cortex and stele 4 days after inoculation (×200). **B,** Intercellular hyphae (arrows) in cortex (×400). **C,** Hyphae and holes (arrows) made by penetrating hyphae in cortex (×400). **D,** Hyphae (arrows) in stele 40 mm from root tip (×200). **E,** Oogonia in stele 6 days after inoculation (×400). **F,** Ooogonia scattered throughout the stele and in the cortex 6 days after inoculation (×200). **G–H,** Paragynous antheridia (arrow) (×400). **I,** Amphigynous antheridium (arrow) and sporangium (×400). **J,** Oospores in roots of cv. Surecrop 8 days after inoculation (×200). **K,** Oospores in young lateral roots of Surecrop 10 days after inoculation (×200).

RESULTS AND DISCUSSION

The process by which zoospores of *P. fragariae* establishes contact with its host, encysts, germinates, and then penetrates epidermal cells has been described by several investigators (5,6,15). Our results describe the process by which the pathogen invades and colonizes the root of strawberry.

Histological examination of inoculated roots of cv. Climax revealed no infection hyphae or reproductive structures at any time period or distance from the root tip. This agrees with Goode's results (5); zoospores encysted at the root tip of cv. Climax germinated within 3 h but failed to develop further. The inability to produce sporangia (8) and oospores (10) in roots inoculated with *P. fragariae* race Pf-2 in previous experiments also supports these findings.

Root sections of susceptible cv. Tennessee Beauty examined 2 days after inoculation had a few scattered hyphal strands in the cortex 10 mm from the root tip, but none were observed beyond this distance. Hyphae were not observed in the stele. Goode (5) indicated she could see hyphae in the cortex behind the root cap of susceptible cv. Huxley and in the differentiating stele as early as 3 days after inoculation.

After 4 days, hyphae ramified throughout the cortex of Tennessee Beauty and into the stele 10 mm from the root tip (Fig. 1A). Long strands of hyphae grew tangentially in the cortex and were stained the darkest red at the growing tip. The fungus was scattered throughout the vascular tissue, with large amounts of mycelium accumulating in the endodermis and pericycle regions. Some disruption of these tissues as well as of the phloem was observed. No reproductive organs or sporangia were observed at this time or distance from the root tip (Fig. 2A). Colonization of the tissue 15–20 mm from the root tip was similar to that at 5–10 mm. Abundant hyphae were observed both in the cortex and stele. Hyphae grew inter- and intracellularly, frequently moving from one cell to another by penetrating directly through the cell walls, leaving small holes denoting their path (Fig. 1B and C). There was light to moderate colonization of the stele at 25–30 mm but very little hyphal growth in the cortex. All hyphae observed in the cortex at this distance were adjacent to or near the stele. At 35–40 mm, there were a few strands of hyphae in the stele; however, hyphae were not observed in the cortex at this distance and time after inoculation (Fig. 1D). As the hyphae grew away from the penetration site toward the crown, they moved primarily through the vascular tissue. Goode (5) also

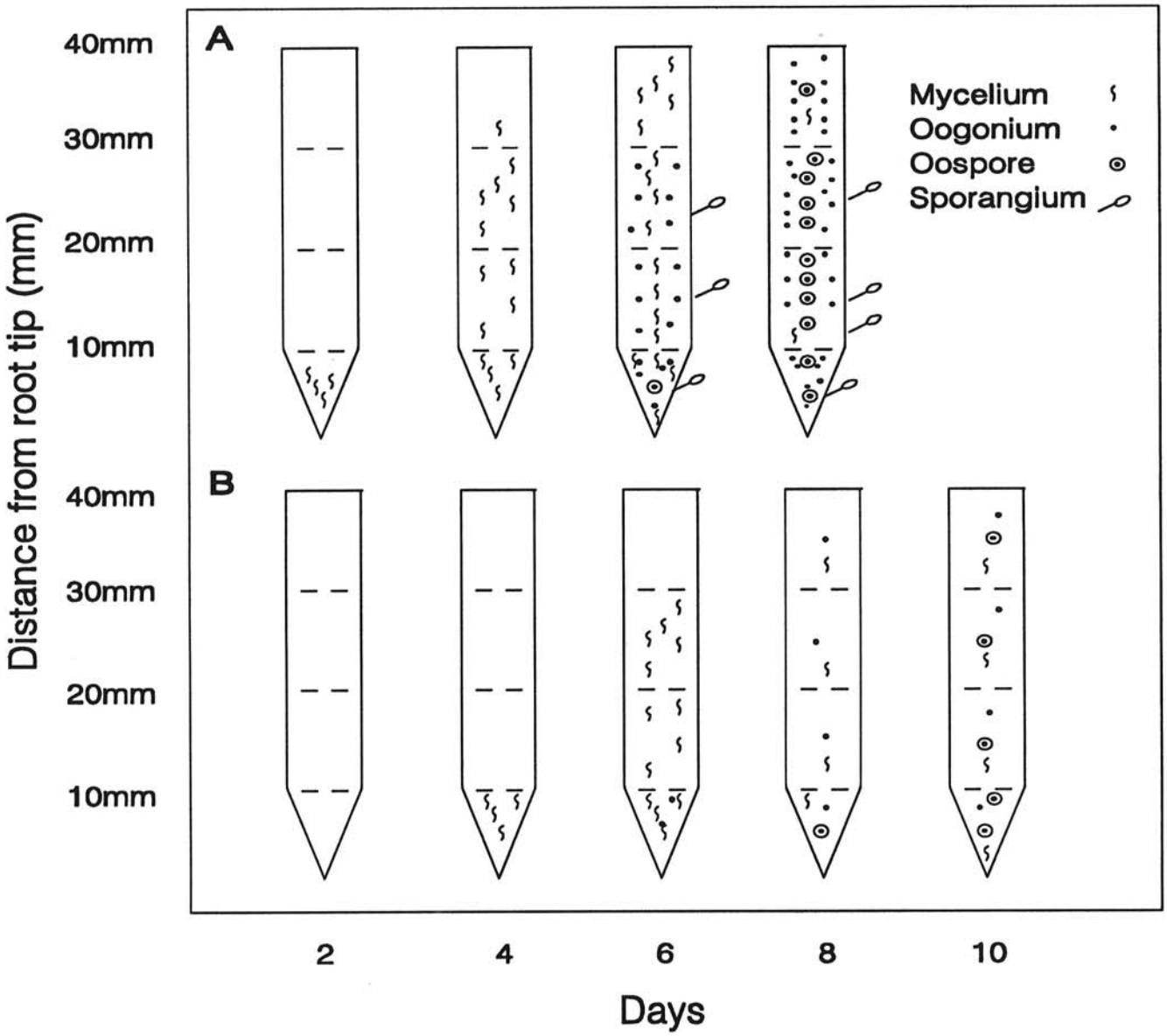


Fig. 2. Diagrams of strawberry cvs. **A,** Tennessee Beauty and **B,** Surecrop roots denoting distances from root tip of mycelia, reproductive organs, and sporangia of *Phytophthora fragariae* 2, 4, 6, 8, and 10 days after inoculation. Distances examined microscopically included 5–10, 15–20, 25–30, and 35–40 mm from the root tip. The numbers of oogonia, oospores, and sporangia depicted in each section are an average of five different 5-mm sections.

noted this trend.

After 6 days, reproductive organs and sporangia of *P. fragariae* were observed in roots of inoculated Tennessee Beauty (Fig. 2A). Numbers of oogonia, oospores, and sporangia depicted in each section of Figure 2 are an average taken from five different root sections at each distance and time period. Oogonia and antheridia developed in all areas of the stele, as well as in the cortex, 30 mm from the root tip (Fig. 1E and F). Large granular masses stained yellow to yellow-brown (early stages of reproductive structures) initially developed in the phloem but also were observed in the xylem and pith areas. The oogonia varied in shape and size and developed a thick, brown wall with age. Amphigynous and paragynous antheridia were observed in equal numbers (Fig. 1G, H, and I). Earlier studies indicated the antheridia of *P. fragariae* were predominantly amphigynous (1,3,6). Later studies (2) showed that the two types of fertilization take place in equal proportions. Regardless of the type of fertilization, the expansion of the oogonium and maturation of the oospore are similar. A detailed description of the reproductive organs of *P. fragariae* is presented by Hickman (6). A few (average of one per 5-mm root section) thick-walled oospores were observed in the roots of Tennessee Beauty 10 mm from the root tip (Fig. 2A). Sporangia were observed as far as 30 mm from the root tip 6 days after inoculation. At 40 mm, the fungus had not completely colonized the stele. Only a few scattered strands of hyphae were observed in the cortex. No reproductive organs or sporangia were observed (Fig. 2A). Mussel and Fay (12) observed sporangia on roots of strawberry seedlings 4–6 days after inoculation when day temperatures were held at 14–16 C and night temperatures at 11–13 C. Wynn (15) noted that sporangia formed on roots of cultivar Blakemore 5 days after inoculation. Those studies did not quantify sporangia number over time or indicate sites of sporangial production.

After 8 days, all root sections of Tennessee Beauty examined were colonized by *P. fragariae* (Fig. 2A). The reproductive organs that formed in the stele frequently caused cellular disruptions, leaving cavities in the vascular tissue. The average number of reproductive organs per 5 mm of root tissue ranged from nine to 14, with some sections having as many as 25 oogonia (Fig. 2A). Sporangia were observed in all root sections except the 40-mm section. Previous tests (8) showed that the production of sporangia by *P. fragariae* race Pf-2 was greatest when roots of Tennessee Beauty were incubated after inoculation for a total of 8 days.

Law and Milholland (8) described and quantified the chronological development of sporangia and oospores in roots of strawberry plants infected with *P. fragariae*. The site of infection by *P. fragariae* on susceptible strawberry roots (Tennessee Beauty) appeared to be within 10 mm of the root tip because more sporangia were produced in this area of the root, although the entire root was inoculated. Our histopathological studies support the results of Law and Milholland (8) and those of Goode (5). Although the entire root was inoculated, the initial site of infection was 5–10 mm from the root tip. No hyphae were observed beyond 10 mm from the root tip of Tennessee Beauty after 2 days (Fig. 2A).

In previous studies (9), growth responses of highly susceptible cv. Tennessee Beauty, partially resistant cv. Surecrop, and highly resistant cv. Earliglow appeared to be correlated with levels of susceptibility to infection and colonization by *P. fragariae* race PF-2. In those tests, root necrosis for Tennessee Beauty, Surecrop, and Earliglow was 75, 25, and 20%, respectively. For Tennessee Beauty, percent root necrosis was a fairly accurate assessment of its susceptibility to *P. fragariae*. However, there was little or no difference in percent root necrosis between partially resistant Surecrop and highly resistant Earliglow. Therefore, percent root necrosis did not distinguish these two types of resistance. In the past, disease-assessment procedures for red stele disease generally were based on the amount of root necrosis and frequently led to inconsistent results. Bain and Demaree (3) stated that the most dependable proof of infection is the microscopic demonstration of oospores of *P. fragariae* in diseased strawberry roots. Based on our histological studies and previous infection studies (4,9,10), evaluation of strawberry genotypes susceptible to *P. fragariae* should not be based entirely on visual assessment but also should include the percentage of roots infected and the number of oospores in infected roots.

When roots of cv. Surecrop were examined microscopically, hyphae of *P. fragariae* were not observed until 4 days after inoculation (Fig. 2B) nor were they observed at any distance beyond 10 mm from the root tip. Again, this strongly suggests that the primary infection site is in the meristematic region behind the root cap.

At 6 days, there was light to moderate colonization in the cortex and vascular tissue. A few oogonia and antheridia were observed in the 5- to 10-mm root section. At 20 mm from the root tip, hyphae were scattered through the stele, with some hyphae in the cortex near the endodermis. Only a few hyphal strands were observed in the stele at 30 mm, with no hyphae in the cortex. No hyphae were observed in the 40-mm section.

Oospores were observed for the first time in Surecrop 8 days after inoculation (Fig. 1J). The oospores were located near the root tip (5–10 mm) and were limited to an average of one oospore per 5 mm of root section. Colonization in the cortex was light to moderate and was moderate to severe in the stele. At 20 and 30 mm from the root tip, hyphae were light and scattered in the cortex, with a general colonization of the stele. At 40 mm, oogonia were observed in the stele but were sparse. Hyphal colonization in the stele was light to moderate, with no hyphae in the cortex. No sporangia were observed in any root section 8 days after inoculation (Fig. 2B).

After 10 days, the reproductive organs had developed throughout the stele, as far as 40 mm from the root tip. Oospores were not observed in the cortex of Surecrop plants, as was noted in highly susceptible Tennessee Beauty. Oospores also were observed in young lateral roots of Surecrop that had developed 30 mm from the root tip (Fig. 1K). Sporangia were not observed on any inoculated Surecrop roots (Fig. 2B).

P. fragariae race PF-2 colonized the roots of partially resistant cv. Surecrop much more slowly than it colonized Tennessee Beauty and produced fewer reproductive organs. The absence of sporangia on Surecrop roots in these studies substantiates previous test results (8) in which there were very few or no sporangia produced on Surecrop roots 14 days after inoculation with *P. fragariae* race PF-2. Without the production of secondary inoculum, extensive proliferation of roots can occur in those cultivars that have partial resistance to *P. fragariae*, allowing them to grow and produce a good crop. Additional studies are needed to examine the importance of secondary inoculum in strawberry genotype resistance to *P. fragariae* and to determine if other mechanisms are responsible for partial resistance to the red stele pathogen.

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