Characteristics of Pecan Scab Lesions on Mockernut Hickory and Pecan Cultivars of Differing Susceptibility

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ABSTRACT

Diehl, S. V., and Graves, C. H., Jr. 1994. Characteristics of pecan scab lesions on mockernut hickory and pecan cultivars of differing susceptibility. Plant Dis. 78:512-516.

Leaves of pecan (*Carya illinoensis*) cultivars Schley, Stuart, Success, and Stevens and of mockernut hickory (*C. tomentosa*) plus nut husks from Schley and Stuart were compared by scanning electron microscopy for differences in surface morphology. Internal tissue structure was also compared by freeze-fracture of some selected samples. Scab lesions on leaves of susceptible Schley pecan were made up of dense, compact mats of mycelium with prolific sporulation. There was complete breakdown of host spongy and palisade parenchyma cell structure. Leaf lesions on resistant mockernut hickory had sparse mycelial growth, no sporulation, and minimal disruption of surface and internal tissue structure. The reactions of other cultivars varied between these extremes. Both Stuart and Schley nut husks appeared to support fungal development and sporulation and had similar internal tissue breakdown. Differences seemed to be in lesion size and were not related to the extent of fungal development. Mycelium appeared to be closely associated with noncollapsed plant trichomes, whereas conidiophores and mycelium often appeared to emerge from the trichome base. Few trichomes were collapsed. Trichome density does not seem to be a factor in the resistance of hickories other than pecan.

Additional keywords: *Cladosporium caryigenum*, phenolic compounds

Pecan scab, caused by *Cladosporium caryigenum* (Ellis & Langl.) Gottwald, is the most serious disease of pecan (*Carya illinoensis* (F.A. Wangenheim) K. Koch) and a major limiting factor in pecan production in the humid southeastern United States. Even with a good fungicide spray schedule, substantial crop losses can result if inoculum levels are high and environmental conditions favor scab development. Scab on leaves may cause stunting and deformation and may influence bud set for the following year on susceptible cultivars. On nut husks, the disease may cause premature drop, nuts that do not fall free of the shuck at harvest, and a reduction in crop quality (9). Native pecan populations exhibit a high incidence of scab, whereas native stands of other hickory species are rarely infected. These hickories may possess chemical and/or structural resistance factors not found in pecan or not found in concentrations needed to confer resistance (4). Due in part to the presence of physiological races of the pathogen, scab disease is not uniformly distributed on the same pecan cultivars in all locations. Thus the distribution of the disease on one cultivar is independent of that on other cultivars and in the same cultivar at different locations (2). Pecan cultivars do, however, exhibit differing degrees of susceptibility.

Resistance to any pathogen is often the result of multiple factors (11). Physical and chemical deterrents to pathogen spore germination, penetration, and infection are all components of a plant's defense system. Wetzstein and Sparks (12) correlated pecan leaf resistance with *C. caryigenum* and the presence of fewer glandular trichomes, a greater frequency of collapsed trichomes, and abundant phenols in the palisade parenchyma and bundle sheath cells. Susceptible cultivars also showed a greater diversity in trichome diameter than did resistant cultivars. Latham and Rushing (8) found that when conidia of *C. caryigenum* landed near trichomes, a majority (82.2%) of those that germinated grew to the base of trichomes, where penetration occurred. Influence of trichomes may result in altered patterns of leaf wettability, humidity at the leaf surface, solution retention, and conidial penetration (6,12).

For pecan scab, it appears the stages of early infection in leaves are similar for both susceptible and resistant cultivars (13). Resistant cultivars develop pinpoint scab lesions, but the fungus is confined to a small area where the host cells die within several days (13). Susceptible cultivars develop much larger lesions, and longer periods following penetration are necessary before degradation is evident (13). All mycelial growth during early infection is intercellular, and no hyphae are observed on the leaf surfaces (10).

Pecan tissues infected with *C. caryigenum* contain higher concentrations of the phenolic compounds juglone, isoquercitrin, and condensed tannin, in situ, than do uninfected tissues (3,4). The total phenolic concentration appears to be a better indicator of resistance than that of a single phenol (4). Hickory species other than pecan consistently contain higher concentrations of all three phenols in both infected and uninfected material (4).

Reduced (but not eliminated) spore germination and penetration in addition to chemical confinement may all be important components of pecan resistance to scab. There has been no work, however, that examined fungal development and host structural components of fully developed scab lesions. The purpose of this study was to compare host surface morphology and fungal development in scab-infected and uninfected leaves and nut husks and to note differences between resistant and susceptible pecan cultivars and mockernut hickory (*C. tomentosa* (Poir.) Nutt.); mockernut was the only hickory to have scab lesions on its leaves at the time of this study. Internal tissue structure and the degree of host deterioration were also compared.

MATERIALS AND METHODS

Small branches with *C. caryigenum*-infected leaves from pecan cultivars Schley, Stevens, Success, and Stuart and from mockernut hickory were collected from the field in July 1989, several months after initial infections occurred. All trees were located on the Mississippi State University campus and subjected to the same environmental conditions. Branches with scab-infected nut husks from Schley and Stuart pecan were also collected. Leaflets containing scab lesions were submerged in 2.5% glutaraldehyde in 0.05 M potassium phosphate buffer, pH 7.2, with 0.1% Triton X-100 (Sigma, St. Louis, MO) and then cut from the leaf rachis. Nuts were cut in half under the same fixative. Samples remained in the fixative overnight at 4 C. Host samples (20 per cultivar) containing individual lesions plus samples of uninfected tissue of equal size were then excised and placed in vials containing buffer (0.05 M potassium phosphate, pH 7.2). After 10 buffer rinses over 7.5 hr, samples were placed in 2% osmium tetroxide in potassium phosphate buffer and stored overnight at room temper-

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Accepted for publication 14 February 1994.

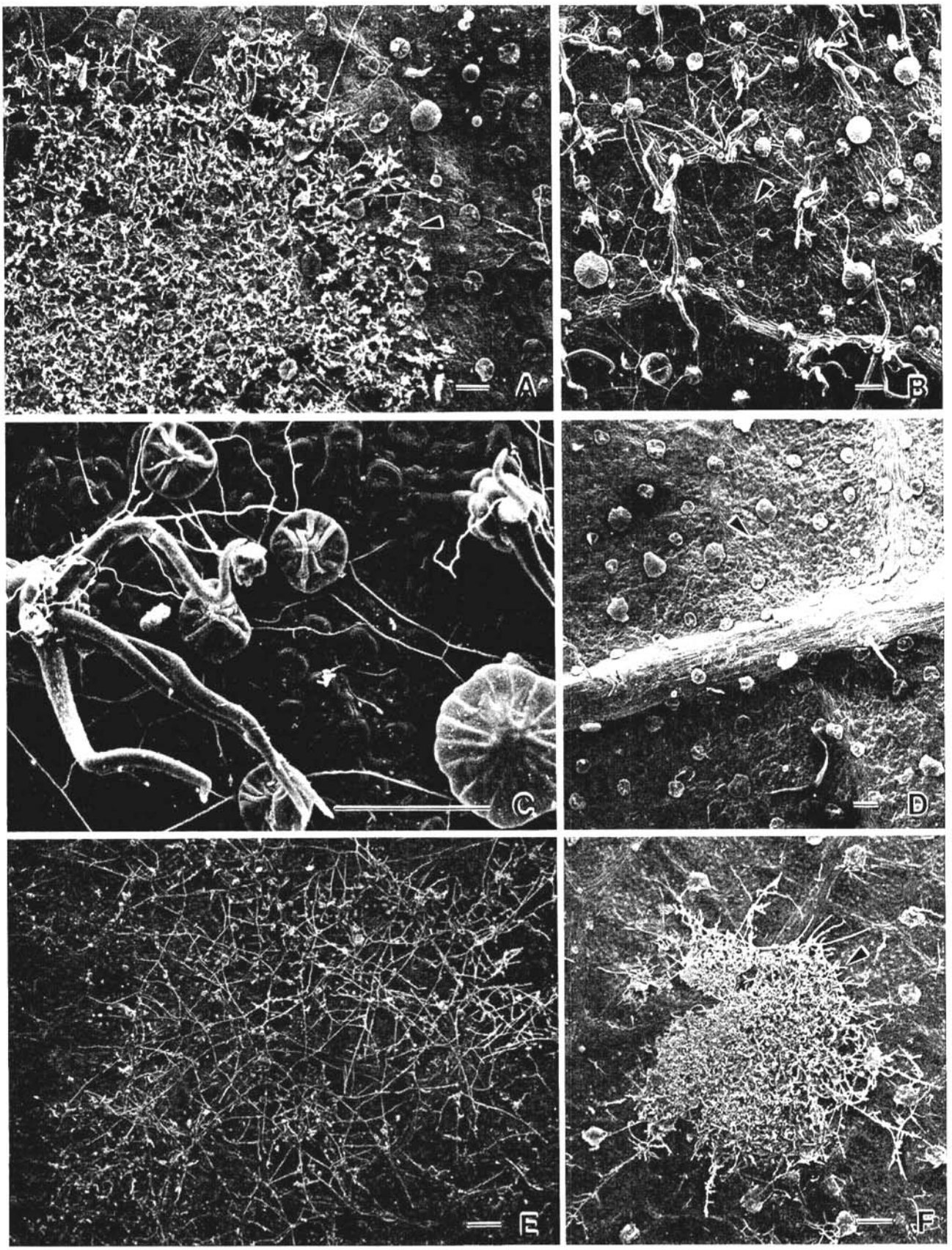

Fig. 1. Scanning electron micrographs of *Cladosporium caryigenum*-infected pecan and mockernut hickory leaves: **(A)** Lesion area (arrow) on susceptible Schley pecan showing dense mycelial growth and sporulation. **(B and C)** Resistant mockernut hickory showing (B) an indistinct lesion area (arrow) and (C) sparse mycelial growth on and protruding out of fasciculate trichomes. **(D and E)** Moderately resistant Stuart pecan (D) with sunken lesion area (arrow) and little mycelial growth and (E) with loosely growing mycelia. **(F)** Moderately resistant Stevens pecan with small, compact lesion. Scale bars = 100 μm.

ature. After five rinses in buffer, samples were dehydrated through an ethanol series (35, 50, 70, 95, 100%). Part of each sample was freeze-fractured in liquid nitrogen. All samples were critical-point dried with liquid CO_2, mounted on aluminum stubs with silver paste, coated with gold/palladium, and viewed in a Joel JSM-35CF scanning electron microscope (SEM) at 20 kV. A minimum of five each of infected and uninfected intact samples and of infected and uninfected freeze-fractured samples per cultivar were examined.

Scab lesions were removed from leaf and nut tissues of each pecan cultivar and of mockernut hickory, surface-sterilized, plated on potato-dextrose agar, and maintained in the dark at 28 C until colony growth occurred to confirm the presence of *C. caryigenum*.

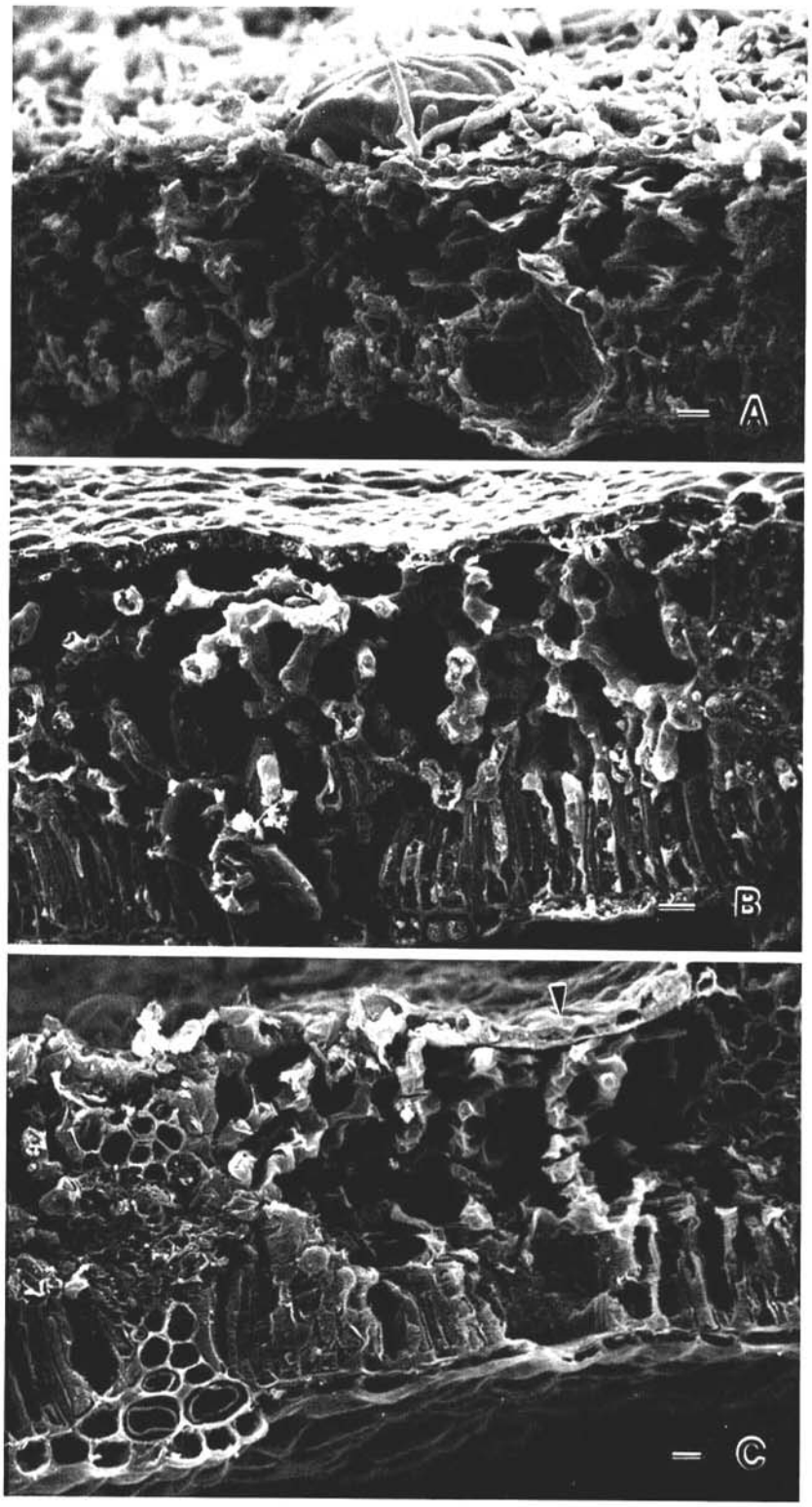

Fig. 2. Scanning electron micrographs of freeze-fractured pecan and mockernut hickory leaves: **(A)** *Cladosporium caryigenum*-infected susceptible Schley pecan with mycelial growth on abaxial surface. Note collapse of internal tissue structure. **(B)** Uninfected Schley leaf. Note intact palisade and spongy parenchyma cells. **(C)** *C. caryigenum*-infected resistant mockernut hickory lesion area (arrow) with minimal collapse of cells and tissue structure still intact. Scale bars = 10 μm.

RESULTS

Susceptible Schley pecan displayed distinctly delineated scab lesions on leaves with the most prolific and densely packed mycelial development and the most prolific sporulation of any cultivar/species examined. This pecan cultivar exhibited a large number of discoid peltate scales, few vesicular peltate scales, and very few collapsed trichomes. Mycelia appeared to grow on and around the trichomes (Fig. 1A).

In contrast, indistinct lesions on the resistant mockernut hickory leaves consisted of sunken areas of tissue where the cells had collapsed, but the cell walls remained intact, with sparse mycelial development and no observed sporulation. Mockernut hickory had the largest number of fasciculate trichomes and discoid and vesicular peltate scales (Fig. 1B). Few collapsed trichomes were observed. The mycelium present appeared to be associated with the fasciculate trichomes, growing on and out of the trichome bases (Fig. 1C).

Other cultivars varied between these extremes. Infected areas on the moderately resistant Stuart pecan leaves were small and indistinct and consisted of sunken tissue with little mycelial growth (Fig. 1D) to loosely growing mycelia (Fig. 1E) and little sporulation. Leaves of Stuart had a medium number of both types of peltate scales and of collapsed trichomes. The moderately resistant Stevens, in contrast, contained small distinct leaf lesions with moderately packed mycelial growth (Fig. 1F) and sporulation. Stevens had the lowest number of discoid trichomes, a low number of vesicular trichomes, and the fewest number of collapsed trichomes. Moderately resistant Success had indistinct leaf lesions, little mycelial growth, and no observed sporulation. Success had a medium number of discoid and vesicular scales and a large number of collapsed trichomes.

Cultivar variation was apparent in the freeze-fractured leaf samples, with Schley and mockernut hickory as the extremes. In the lesion area, the internal tissue structure of Schley exhibited complete collapse (Fig. 2A); both the palisade and spongy parenchyma were in disarray and the tissue thickness had decreased because of cell collapse. Uninfected freeze-fractured leaf samples of Schley had intact palisade and spongy parenchyma cells (Fig. 2B), and infected mockernut hickory exhibited no tissue disarray and minimal shrinkage of cells

and leaf thickness (Fig. 2C). Similarly infected Stevens, Success, and Stuart exhibited some tissue shrinkage and minor disarray. No mycelium was seen growing within the leaf tissue in any of the cultivars examined.

Scab-susceptible Schley nut husks contained large, sunken infected areas (Fig. 3A) and densely packed mycelium and prolific sporulation (Fig. 3B). Lesions on Stuart nut husks consisted of patches of dense mycelium interspersed with areas of sparse mycelium (Fig. 3C); all lesions were somewhat sunken and sporulation was observed (Fig. 3D). Freeze-fractured tissues of infected Schley and Stuart husks revealed collapse of the area beneath the lesion two to three cell layers deep (Fig. 4A), whereas uninfected tissue showed normal tissue structure (Fig. 4B). The mycelium was densely packed but did not appear to grow into the collapsed tissues. Schley appeared to have more peltate glands than Stuart, while Stuart had more capitate glands. The mycelium appeared to grow over the top of and out from the base of the peltate scales (Fig. 4C).

C. caryigenum was isolated from lesions of all pecan cultivars tested and from mockernut hickory.

DISCUSSION

The susceptibility of pecan leaves to *C. caryigenum* is related to their age. The fungus primarily infects young expanding leaves, and mature leaves are much more resistant or immune (1). Conidia of *C. caryigenum* germinate, form appressoria, and penetrate young leaves of both resistant and susceptible cultivars (13). Lesion development typically is maximum between 7 and 10 days after infection (2,5,8). In this study, we examined naturally infected tissues of fully mature leaves. Infection would have occurred on the young leaves of all cultivars months prior to collection. Lesion development and size and sporulation should be directly related to resistance or susceptibility, not to the age of the infection. All infections were in our study were "old."

The extent of fungal development and host disruption among the samples studied correlated well with known field resistance. It has been proposed that the moderate resistance of Stuart to the scab fungus is due to the resistance of the leaves, not the nut husks. In this study, both Stuart and Schley nut husks appeared to support fungal development and sporulation and had similar internal tissue breakdown, but lesion size was reduced in Stuart.

Grauke et al (6) described three types of pecan trichomes: nonglandular, peltate scales, and capitate glands. Wetzstein and Sparks (12) correlated resistance to scab with fewer glandular trichomes and a greater frequency of collapsed trichomes. These authors did not distinguish between discoid and peltate scales but referred to them both as glandular trichomes (12). The present study supports their hypothesis in part. Susceptible Schley pecan leaves exhibited the largest number of discoid peltate scales, fewer vesicular scales, and few collapsed trichomes. Moderately resistant Stevens pecan leaves had the fewest peltate and vesicular scales and the fewest collapsed trichomes. However, the most resistant sample studied, mockernut hickory, exhibited by far the largest number of vesicular, discoid, and fasciculate trichomes and few collapsed trichomes. Thus, if trichome density plays a role in resistance or susceptibility in pecan, it does not appear to be a factor in the resistance of hickories other than pecan.

Studies have provided evidence that pecan trichomes contain tannins, juglone, and other flavonoids (6,7), but if these trichomes are not affected by the presence of the fungus, their phenolic compounds would not be available in a resistance response. Collapsed trichomes may release phenols and inhibit *C. caryigenum* germination and/or infection, which would support the correlation of resistance to a greater number of collapsed trichomes (12). This study, however, found mycelium growing on and around the trichomes but few collapsed trichomes. There did not appear to be a distinct correlation between resistance and a greater number of collapsed trichomes.

Mycelium of *C. caryigenum* appeared to be closely associated with the noncollapsed plant trichomes; conidiophores and mycelium often appeared to emerge from the trichome base. It is possible that there is some chemical attraction to plant trichomes. Wetzstein and Sparks (12) observed secretions of the glandular trichomes, although the chemical nature of these secretions is unknown. Latham and Rushing (8) noted a zigzag pattern of germ tube growth and suggested attraction to a specific penetration site. The consistent close association of mycelia with these structures found in this study support this hypothesis. Perhaps trichomes play a role in increased spore germination and/or penetration, but other internal factors determine the extent of fungal development and disease severity.

Scab leaf lesions were very difficult to distinguish with the SEM from uninfected tissue on resistant cultivars. In the field, however, these lesions are visibly distinct as black necrotic areas. Rushing and Latham (10) observed no major ultrastructural changes as the result of early leaf infection and no intracellular

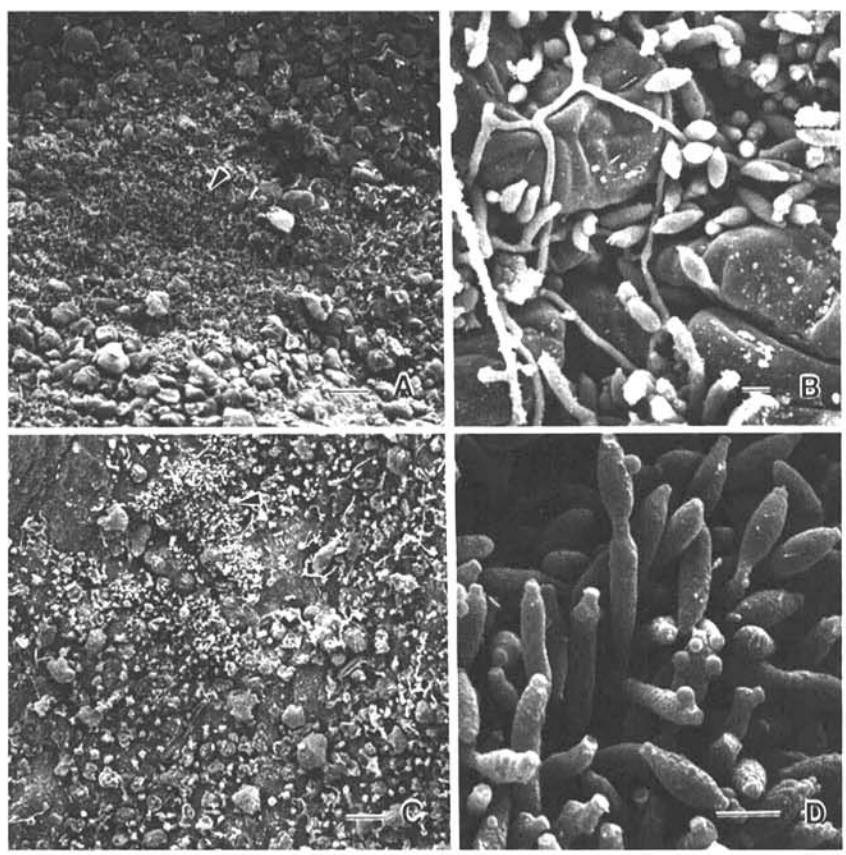

Fig. 3. Scanning electron micrographs of *Cladosporium caryigenum*-infected pecan nut husks: Susceptible Schley pecan with **(A)** large sunken lesion areas (arrow) showing densely packed mycelial growth and **(B)** prolific sporulation. Stuart pecan with **(C)** patches of dense mycelial growth (arrow) interspersed with areas of sparse mycelial growth and **(D)** prolific sporulation. Scale bars = 100 μm for A and C and 10 μm for B and D.

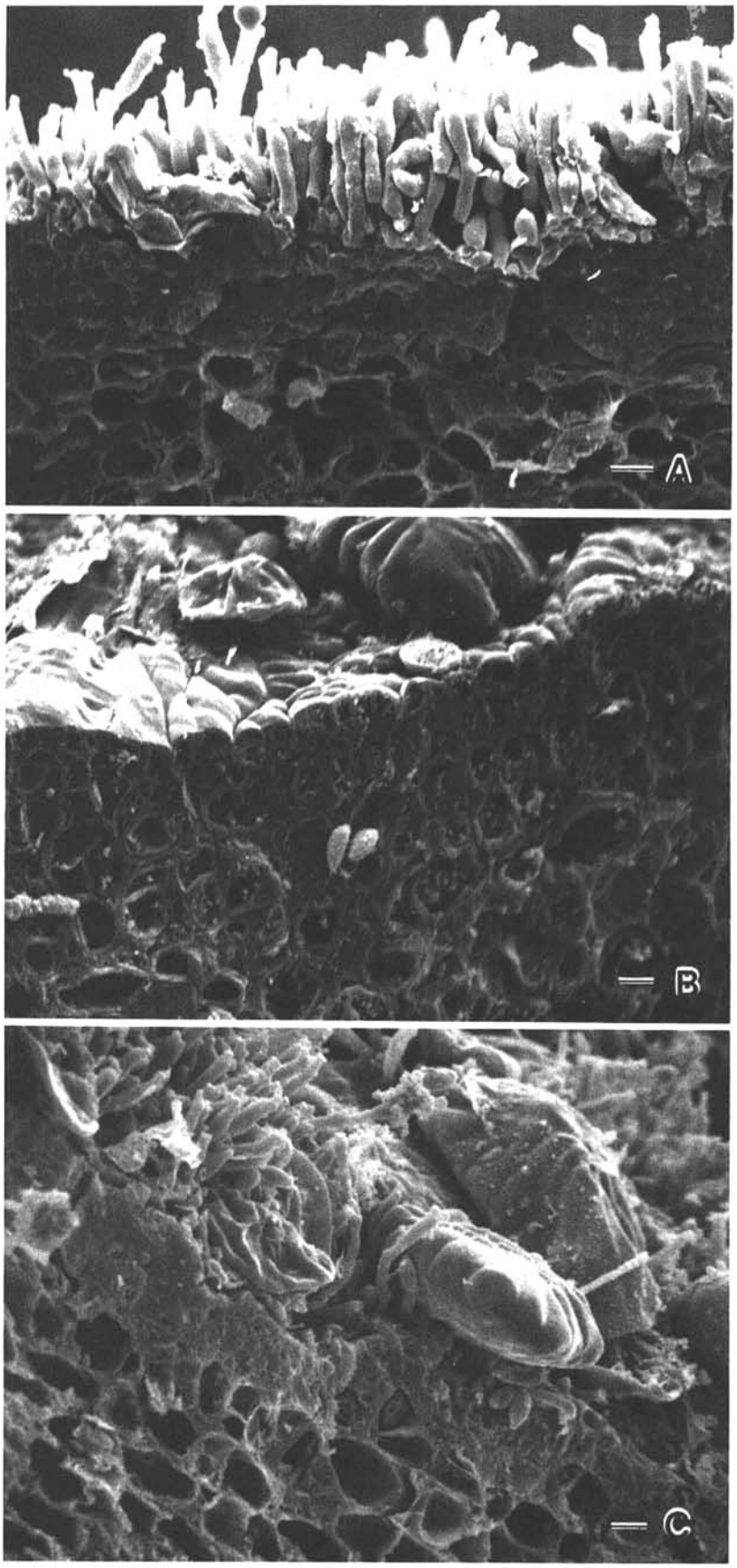

Fig. 4. Scanning electron micrographs of freeze-fractured, scab-susceptible Schley pecan nut husks: **(A)** *Cladosporium caryigenum*-infected area with mycelial growth on the surface and collapse of the area beneath the lesion two to three cell layers deep. **(B)** Uninfected area showing normal tissue structure. **(C)** *C. caryigenum*-infected area with mycelial growth and conidiophores emerging from beneath the base of the peltate scales. Scale bars = 10 μm.

hyphae. In our SEM study of fully developed scab infection, leaf cells still appeared intact, although sunken, in resistant cultivars, whereas host cells in susceptible cultivars were destroyed. Additionally, some sporulation was observed on the moderately resistant pecan cultivars. Scab-infected tissues contained, in situ, significantly greater concentrations of phenolic compounds than did uninfected tissues (4). This suggests a physiological response to the presence of *C. caryigenum*. When comparing the species/cultivars examined in this SEM study, mockernut hickory contained greater concentrations of all three compounds in infected and uninfected tissues, whereas Schley pecan contained the lowest concentration of phenols (4). SEM showed mockernut to have the least developed *C. caryigenum* mycelium, minimal to no sporulation, and the least internal tissue damage, whereas Schley had the most. It is possible that total levels of phenolic compounds play a significant role in reducing the growth and development of *C. caryigenum*.

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