

## **Microscopic Examination of Race-specific Resistance to Pecan Scab**

From the 2001 Southeastern Pecan Growers Association Meeting

**Patrick J. Conner**

**University of Georgia, Horticulture Department, Coastal Plain Experiment Station, Tifton GA. 31794.**

### **Introduction**

Pecans are attacked by a wide range of disease and insect pests causing substantial losses to the crop. In the humid growing conditions of the southeastern United States, the most economically damaging of these is [pecan scab](#), caused by the fungus *Cladosporium caryigenum*. Scab infection reduces both yield and quality of pecan kernels, and if uncontrolled can result in total crop loss.

Many important high quality cultivars are becoming increasingly susceptible to the scab pathogen. Two such cultivars, 'Stuart' and 'Desirable', comprise over one half of Georgia's total pecan acreage. This increasing susceptibility has often been attributed to the increased prevalence of once rare races of scab that are able to infect these cultivars. Demaree and Cole (1929) were the first to demonstrate pathogenic races of scab. They demonstrated that when inoculum was taken from one cultivar and used to inoculate young leaves of the same cultivar and a different cultivar that a heavy infection was found on the original host and a light or no infection on the other. Converse (1960) found evidence for the presence of at least 4 races in Oklahoma using single-spore isolates and greenhouse inoculations.

In order to help achieve our goal of producing new scab-resistant cultivars, we have been investigating the resistance of cultivars to different scab isolates. This work has centered around obtaining genetically pure strains of the scab fungus and testing them for virulence on different pecan cultivars. Towards this end we have developed a microscopic method using detached leaves to test the virulence of different scab isolates on pecan cultivars. This method has the advantage of allowing us to conduct a large number tests in a relatively short time period. Environmental conditions are controlled by conducting the tests in a growth chamber. In addition, we can use a wide range of isolates collected from diverse growing regions without releasing them into the environment. Determining the resistance of important cultivars to particular races of pecan scab will be vital in designing crosses to produce new cultivars with resistance to multiple races.

### **Materials and Methods**

*Inoculum preparation:* Leaves with sporulating lesions were collected from four cultivars: 'Wichita', 'Desirable', 'Cape Fear', and 'Elliot'. Single conidia were

isolated from lesions on each cultivar and grown in vitro using the procedures of Turechek and Stevenson (1998). For all inoculation tests, conidia were collected from oatmeal agar plates, filtered through cotton swabbing, and the conidial concentration was adjusted to 10<sup>6</sup> conidia per mL with a hemacytometer.

*Inoculation:* Young leaves, from 1/3 to 2/3 of full expansion, were collected, disinfected for 2 min in 0.5% sodium hypochlorite, and rinsed three times in sterile, distilled water. After blotting dry, leaves were placed in petri plates on sterilized moist vermiculite. Leaves were sprayed to runoff with the appropriate conidial suspension using a chromatography sprayer. Plates were placed in plastic boxes and maintained at 100% relative humidity for 48 hours. Plates were then removed from the plastic boxes and opened until the leaf surface was dry. Plates were recovered and the vermiculite kept moist until leaves were harvested. All plates were incubated in a growth chamber at 24 °C and 12 h per day of light.

For field inoculations, expanding shoots were selected with four or five young leaves. Leaflets smaller than 1/3 expansion or larger than 2/3 expansion were removed. The remaining leaflets were sprayed to runoff with a conidial suspension, wrapped with a damp paper towel, and sealed in a plastic bag. Control inoculations were made by spraying leaves with distilled water instead of the conidial suspension. The bag was then wrapped with aluminum foil to reflect the heat and the shoot tagged. After 48 hours the enclosure was removed. After 21 days the leaves were removed and the number of scab lesions and leaf area were determined for 50 leaflets.

*Microscopic examination:* Leaf tissues were examined by light microscopy after staining with chlorazol black E using the procedures of Brundrett et al. (1983). Detached leaves were examined at 4, 8, and 14 days post-inoculation. 50 germinated conidia were examined on 10 different leaflet samples for a total of 500 germinated conidia examined for each host genotype / scab isolate / time period tested.

## **Results and Discussion**

The four cultivars chosen for this experiment, 'Wichita', 'Desirable', 'Cape Fear', and 'Elliot', represent a range of field resistance levels from the extremely susceptible 'Wichita' to the highly resistant 'Elliot'. In addition, 'Desirable' represents a cultivar once considered highly resistant, but is now quite susceptible. Scab isolates were obtained from each of these cultivars and were grown in vitro from single germinated conidia, thus the inoculum prepared from each isolate is genetically homogenous. Ten leaf samples from each cultivar / isolate test were examined 4, 8, and 10 days post-inoculation.

The results of inoculating each cultivar with the 'Wichita' scab isolate is shown in Figure 1. As was shown by Latham and Rushing (1988), penetration of the

cuticle and formation of sub-cuticular hyphae occurs fairly quickly and is evident 3-4 days post-inoculation. By 4 days post-inoculation, about 40% of the conidia had germinated, formed an appressorium, penetrated the cuticle, and formed sub-cuticular hyphae in the susceptible 'Wichita' leaves (fig. 1a). By contrast, no sub-cuticular hyphae were observed in any of the germinated conidia examined on 'Elliot' or 'Desirable' leaves, and only a few were observed in 'Cape Fear'. By 8 days post-inoculation, the subcuticular hyphae had continued to grow radially outward from the penetration site and could be seen both above the and below the epidermal layer on the 'Wichita' leaves. Dark brown basal bulbs which give rise to conidiophores could be seen on 1 % of the colonies (fig. 1b). Less than 1% of the germinated conidia had formed sub-cuticular hyphae on 'Elliot' and 'Cape Fear' leaves, and no subcuticular hyphae were observed on 'Desirable' leaves (fig. 1b). At 14 days post-inoculation, basal bulbs could be seen on 8% of the colonies on 'Wichita' leaves and 5% of the colonies had produced sporulating lesions (fig. 1c). No subcuticular hyphae could be seen on 'Elliot', 'Cape Fear', or 'Desirable' leaves (fig. 1c).

Yates et al. (1996) analyzed germ tube, appressorium, and subcuticular hypha development on host and nonhost leaves and determined that subcuticular hyphal development was the stage specific for host susceptibility. This study is in agreement with those results as only 'Wichita' leaves developed subcuticular hypha to any extent when challenged with the 'Wichita' scab isolate. A very small percentage of conidia were able to form subcuticular hypha on 'Elliot' and 'Cape Fear' leaves, but these colonies always showed very little growth, and the formation of reproductive initials was never observed. No subcuticular growth was seen on these cultivars at day 14, suggesting that the few conidia that were able to produce a small amount of subcuticular growth eventually died. Day 8 was chosen as the optimum time for observing the results of inoculations since by that time the fungus has had time to grow large enough that observation of the hypha is relatively easy. By day 14 fungal colonies are quite large, but a large percentage of leaves will have begun to senesce, making staining more difficult.

The results of inoculation with the 'Desirable', 'Cape Fear', and 'Elliot' scab isolates is shown in figure 2. Over 60% of the germinated conidia of the 'Desirable' scab isolate were able to form subcuticular hypha on 'Desirable' leaves by day 8 (fig. 2a). Only a small percentage of germinated conidia were able to form subcuticular hypha on 'Wichita', 'Cape Fear', and 'Elliot' leaves (fig. 2a). The 'Cape Fear' scab isolate was virulent on 'Cape Fear' leaves and not on 'Desirable' or 'Elliot' leaves (fig. 2b). 'Wichita' was not tested because no young leaves were available. The 'Elliot' scab isolate was able to infect 'Elliot' and 'Cape Fear' leaves, but not 'Desirable' (fig. 2c). 'Wichita' again was not tested.

The results of field inoculations using the 'Wichita' scab isolate are shown in Table 1. 'Wichita' was severely infected by this isolate. Inoculation of 'Desirable' leaves produced only a couple of lesions, which was not statistically different from the control inoculation. No scab lesions were observed on 'Cape Fear' or

'Elliot' inoculations. These results confirm the usefulness of the detached leaf assay in assessing susceptibility to scab isolates. This data also suggests that the small number of subcuticular hyphae observed when 'Cape Fear' and 'Elliot' were inoculated with this isolate in the detached leaf assay would not result in the production of scab lesions in the field.

The results of the detached leaf assays are summarized in Table 2. As can be seen by reading down the columns, each scab isolate varied in its ability to produce scab on the different cultivars, thus each isolate represents a different race of scab. Surprisingly, scab isolates from cultivars generally considered resistant can not always infect cultivars considered more susceptible. For example, the 'Desirable' isolate is not able to produce scab on the 'Wichita' leaf, and the 'Cape Fear' isolate is not able to produce scab on 'Desirable' leaf. Reading across the columns we can see that each cultivar reacts differently to the different scab sources. For example, even though 'Desirable' is now considered fairly scab susceptible, it is highly resistant to all the scab isolates tested except the one isolated from 'Desirable'. It seems likely that the wide planting of 'Desirable' in recent times has led to an increase of the prevalence of this race of scab and consequently a decrease in the apparent resistance of 'Desirable'. However, 'Desirable' may still be a good source of resistance to the other three races of scab.

Future tests with a wider range of scab isolates and pecan cultivars should provide us with a clearer picture of the number of scab races present in most orchards and their virulence on pecan cultivars. This information will be vital in designing crosses to include resistance to as many different races as possible. We will also begin to understand which races are most common, and thus most important to use in screening seedlings for resistance.

**Acknowledgement:** This work was supported in part by grant from the Georgia Commodity Commission for Pecans.

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**Table 1. Field inoculation of four cultivars with a 'Wichita' scab isolate.**

Cultivar	'Wichita' isolate inoculation	Control inoculation <sup>y</sup>
'Wichita'	1.961 <sup>z</sup>	0.012
Desirable	0.001	0.004
Cape Fear	0	0
Elliot	0	0

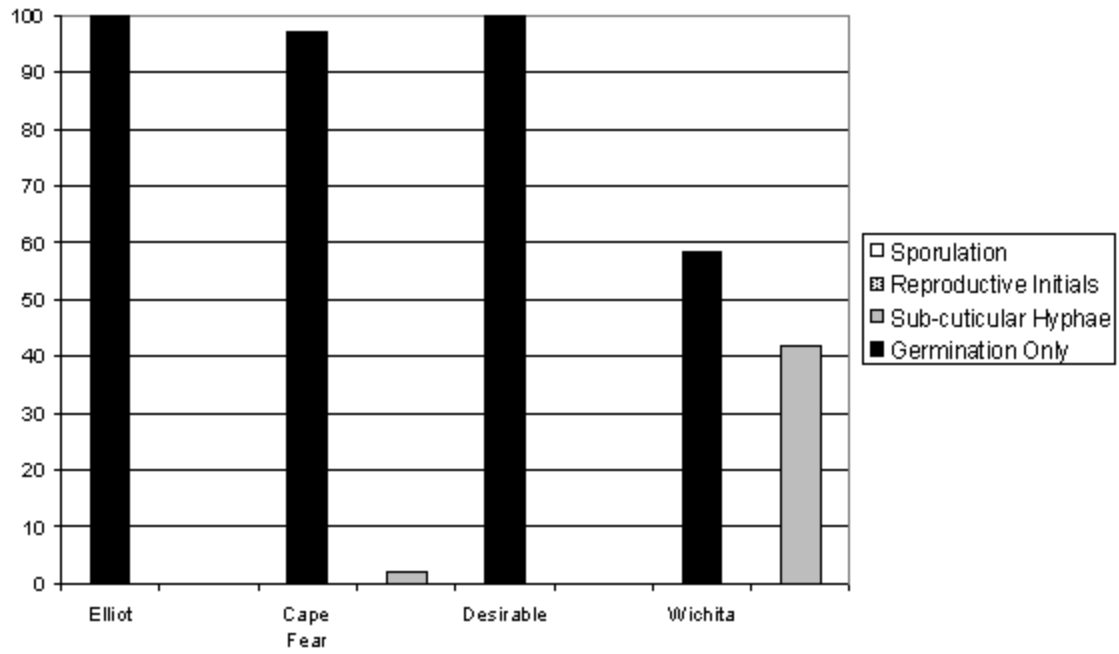
<sup>y</sup>Leaves were misted with sterile water instead of a conidia suspension.

<sup>z</sup>Number of scab lesions on the upper and lower leaf surfaces per cm<sup>2</sup> of leaf area.

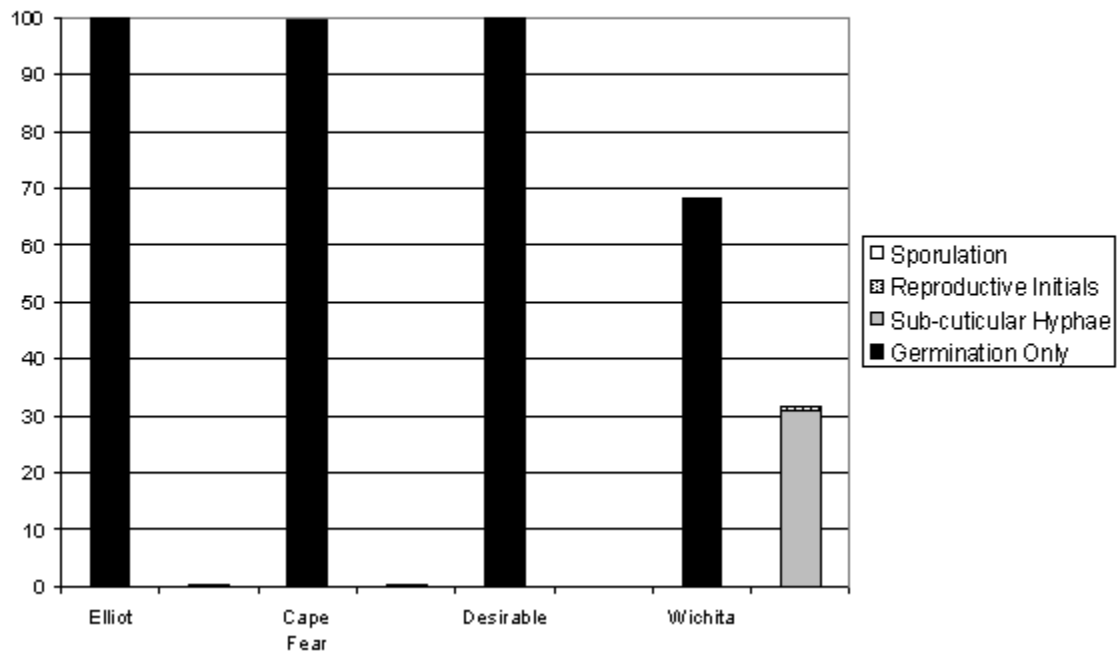
**Table 2. Results of detached leaf assays.**

Cultivar tested	Scab isolate tested			
	Wichita isolate	Desirable isolate	Cape Fear isolate	Elliot isolate
'Wichita' leaf	Scab	No scab	Untested	Untested
Desirable leaf	No scab	Scab	No scab	No scab
Cape Fear leaf	No scab	No scab	Scab	Scab
Elliot leaf	No scab	No scab	No scab	Light scab

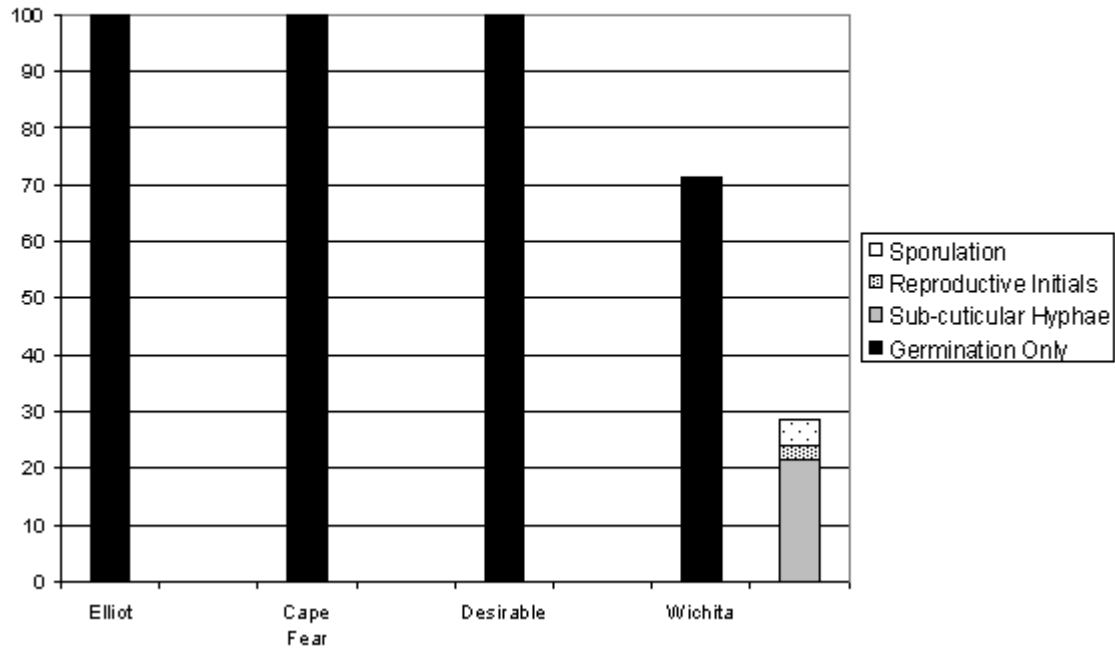
**Fig. 1a**      **Wichita scab isolate, day 4**



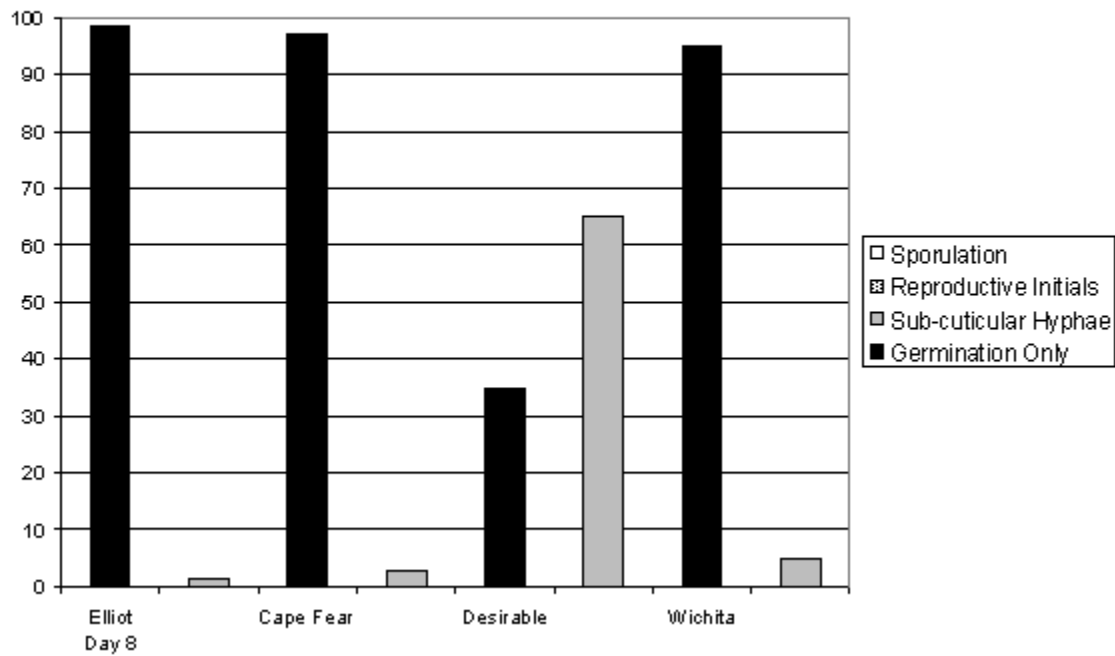
**Fig. 1b**      **Wichita scab isolate, day 8**



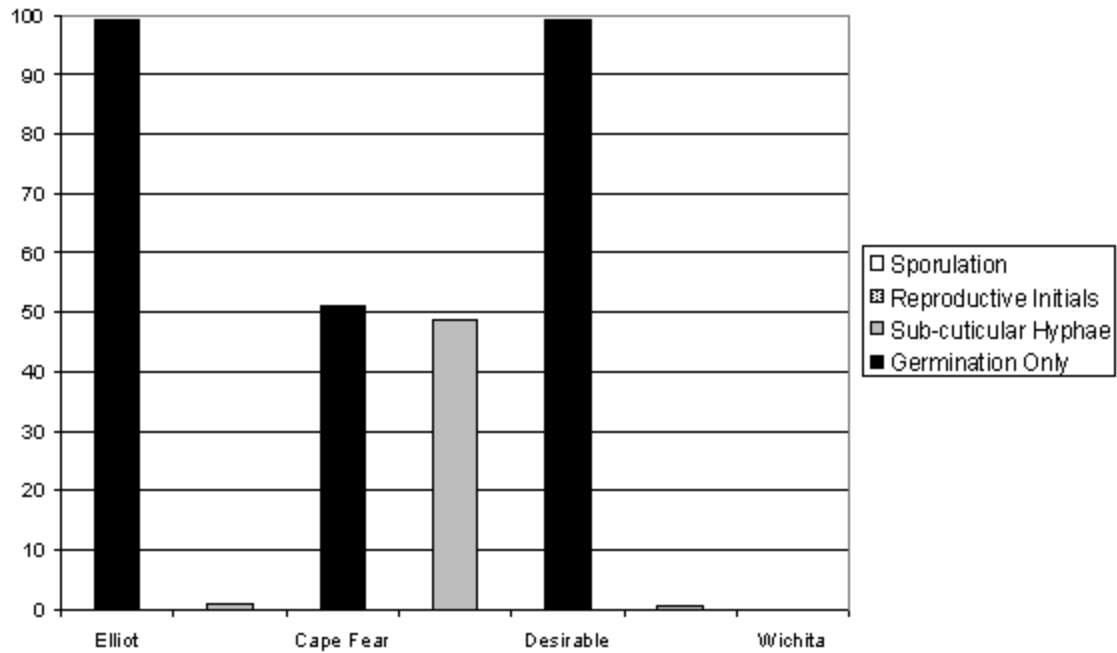
**Fig. 1c** Wichita scab isolate, day 14



**Fig. 2a** Desirable scab isolate, day 8



**Fig. 2b Cape Fear scab isolate, day 8**



**Fig. 2c Elliot scab isolate, day 8**

