

Occurrence of *Glomerella cingulata* in Pecan Nut Shucks and Its Association with Fungal Leaf Scorch

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ABSTRACT

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Twenty-three pecan cultivars were tested for susceptibility to *Glomerella cingulata*. The incidence of *G. cingulata* from nut shucks ranged from 35.5% on Elliott to 94.0% on Cherokee. Isolation of *G. cingulata* from pecan leaves with fungal leaf scorch symptoms ranged from 36.9% on Desirable to 82.5% on Choctaw. Correlation coefficients from 1992 data indicated a positive and significant relationship ($r = 0.92$, $P < 0.05$), over cultivars, between the frequency of isolation of *G. cingulata* from pecan shucks with anthracnose and leaves with fungal leaf scorch symptoms. Typical symptoms of fungal leaf scorch were reproduced with *G. cingulata* on Schley pecan trees in the greenhouse.

Additional keywords: pecan diseases

Pecan anthracnose, caused by *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz.), causes a late-season shuck and kernel rot of pecan (*Carya illinoensis* (F. A. Wagenheim) K. Koch) (5,15,16). Anthracnose symptoms usually occur on green pecan nuts during late summer when rainy weather prevails (9). Symptoms on shucks appear as shiny, dark brown, sunken lesions that may envelope the entire fruit (15). Lesions are initiated at wounds where a cluster of nuts are in contact, or along the suture of the shuck. When infection by *G. cingulata* occurs early in the growing season, pecan shells may be nearly hollow at harvest. If infection occurs later, the cotyledons fail to develop and only waferlike kernels are found when the nuts are shelled. Rand (14) described anthracnose symptoms on leaves as irregular, reddish to grayish brown blotches varying greatly in size and often covering the whole leaf. Black perithecia were found scattered within the blotches. Although anthracnose was reported on pecans in 1914 (14), its importance was not recognized until recently, when it was found on several cultivars in Georgia (5).

A closely related problem on pecans, known as fungal leaf scorch (FLS) (12), is considered by growers as one of the major causes of premature leaf drop and reduced fruit quality in the southeastern

United States (10,12). According to Littrell and Worley (12), FLS lesions are characterized by a dark brownish black margin separating the brown necrosis from healthy leaf tissue. Fungal leaf scorch symptoms usually begin at the leaflet apex and necrosis progresses basipetally, or lesions begin at the leaf margin and progress toward the midrib. In addition, Littrell and Worley (12) associated black fruiting structures, which may have been perithecia, with FLS symptoms. Perithecia were observed by Rand (14) in his description of anthracnose symptoms on leaf tissue. Despite the similarity of anthracnose symptoms on leaves and FLS, the etiology of FLS is still unclear (11,13).

Leaf drop from pecan trees occurring in September and October is often thought to be the result of normal senescence. However, Littrell and Worley (12) observed that trees sprayed with the fungicides benomyl, captafol, and fentin hydroxide had significantly less defoliation than trees sprayed only with insecticides. Thus, premature defoliation is likely due to foliage diseases, including FLS. Maintenance of pecan leaves on trees as late into the season as possible was shown to be important by Worley (18), who hand-defoliated pecan trees at 15-day intervals from 15 August through 1 November. Worley found that trees defoliated prior to 1 October failed to set nuts in the following 2 yr. Worley (19) also showed that defoliation at any time prior to 1 November reduced a tree's carbohydrate reserves, which resulted in reduced yield the following season. Therefore, it is essential for pecan growers to provide optimal growing conditions through an adequate fertility and pest management program to prevent leaf drop prior to 1 November.

To effectively control premature leaf

drop due to FLS, the etiology of this disease must be more clearly understood. Therefore, the objectives of these studies were to evaluate pecan cultivars commonly grown in the Southeast for susceptibility to infection by *G. cingulata*, and to determine the possible relationship between this fungal pathogen and FLS. A preliminary report has been published (9).

MATERIALS AND METHODS

Investigations were conducted in the pecan orchard at the Gulf Coast Substation, Fairhope, Alabama, in 1992 and 1993. The orchard was arranged in a randomized complete block design, with five trees of each of 15 pecan cultivars (Table 1) in each of four replications. At least three trees of an additional eight pecan cultivars were completely randomized in a group of trees adjacent to the rest of the orchard. Alabama Cooperative Extension Service recommendations (8) were followed for orchard management and control of pecan diseases. Propiconazole (Orbit 3.6 EC) was applied on 26 March and 6 April at 185 g a.i./ha. Fentin hydroxide (Super-Tin, TPTH) at 0.42 kg a.i./ha was then applied three times at 14-day intervals followed by four applications at 21-day intervals.

Location of shuck infections. Portions of the nut shuck that *G. cingulata* preferentially attacks and differences in susceptibility among cultivars were initially determined in 1992. Ten green nuts with suspect anthracnose lesions were collected arbitrarily from each of five randomly selected trees (50 nuts total) of each of 15 cultivars (Table 1). Suspect anthracnose lesions were dark brown and sunken with regular margins. Nuts were transported on ice to Auburn on 18 September. Tissue samples, 6 × 3 mm, were excised from suspect lesions located on basal, middle, or distal portions of shuck tissue. Sampled tissue was surface-disinfested with 0.0325% NaOCl solution for 5 min, rinsed with sterile water, and placed in petri dishes containing potato-dextrose agar (PDA). Petri dishes with tissue were incubated at 24 C under UV light with a photoperiod of 12 hr for a minimum of 14 days. A number of common fungi were observed on culture dishes, but only colonies of *G. cingulata*, identified by microscopic examination of spores, were counted. Differences among means of the shuck portions infected and means of cultivar infections were

determined by least significant difference ($P < 0.05$).

Cultivar differences. Differences in cultivar susceptibility to *G. cingulata* infection were determined from 50 green nuts collected from each cultivar in each of the four replicate plots (200 nuts total) on 27 and 28 September 1993. Nuts were disinfested with 0.0325% NaOCl for 5 min, rinsed with tap water, treated with parpargite (Omite 30W) at 0.38 g a.i./L to suppress mite populations, and placed on a moist paper towel on aluminum trays. Green nuts were incubated in dew chambers for 14 days at 25 C. Sporulation of *G. cingulata* on nuts was visually assessed, and frequency of nuts infected for each replicate cultivar was recorded. Differences among cultivars were determined by least significant difference ($P < 0.05$).

Fungi associated with FLS. Fungi associated with FLS symptoms on leaves were determined in 1992 and 1993. Twenty leaves with FLS symptoms were collected from each of 23 pecan cultivars and transported on ice to the laboratory on 2 November 1992. Thirty disks (7 mm diameter) were cut arbitrarily from the periphery of lesions on leaves of each cultivar. Disks were cut with a sterile cork borer, surface disinfested with 0.0325% NaOCl solution for 5 min, rinsed with sterile water, and plated on PDA. Petri dishes were incubated at room temperature under UV light for 14 days. Pure cultures were made of fungal colonies by hyphal tip or single spore transfer. Fungi isolated were identified to genus through microscopic examination and comparison with published descriptions (2,6,7,17). The incidence of predominant fungi was recorded from leaf disks from each cultivar.

On 1 and 2 November 1993, 40 pecan leaves showing FLS symptoms and 40 leaves without symptoms were collected arbitrarily from trees of each of 23 cultivars. Leaves were transported in an ice chest to the laboratory. Forty disks, 5 mm diameter, were cut from the periphery of leaf lesions, surface-disinfested as described earlier, and plated on PDA. The dishes with leaf disks were incubated at room temperature for 14 days, and the incidence of fungi was determined.

Correlation coefficients between incidence of *G. cingulata* in shucks and isolation frequency from FLS leaf lesions were calculated. Data used were means of cultivars from previously described tests (see also 9). A perfect correlation ($r = 1$) between shuck anthracnose and FLS over cultivars would show that every cultivar had a similar reaction to both diseases and would be evidence of similar etiology of these two diseases.

Koch's postulates. Koch's postulates (1) were conducted in order to determine possible relationships between *G. cingulata* and FLS. A water suspension of 1.2×10^5 *G. cingulata* conidia per milliliter

was collected from a culture originally isolated from pecan shucks. Six-month-old pecan leaves approaching senescence on 8-yr-old Schley trees growing in 26.7×50.8 cm plastic tree containers were sprayed to runoff with the conidial suspension. Trees were incubated in dew chambers at 24 C and 100% relative humidity (RH) for 24 hr with 12 hr of darkness. Subsequently, trees were maintained on a 12-hr light schedule at 100% RH for 48 hr. At the end of this incubation period, they were moved to the greenhouse. After 60 days, 7-mm-diameter disks were cut from FLS-like lesions on inoculated leaves. Leaf disks were surface-disinfested with 0.0325% NaOCl for 5 min, rinsed with sterile water, and plated on PDA. After incubation at 24 C for 14 days, fungi were identified.

Colletotrichum acutatum and *C. gloeosporioides* isolates, originally collected from pecan shucks in the orchard and morphologically identical to *G. cingulata* except for development of perithecia, were distinguished from one another by conidial shape (3). In order to determine whether there were differences in symptom development due to spore type, Koch's postulates were repeated as described above using these fungal isolates and ascospores from an isolate of *G. cingulata*. Trees were inoculated with conidial or ascospore suspensions (3.8×10^5 conidia per milliliter and 1.9×10^5 ascospores per milliliter) of each isolate. Inoculated trees were incubated in dew chambers for 48 hr, as described earlier, and moved to the greenhouse. After 12 wk, FLS-symptomatic leaves were sampled and cultured, and fungal colonies were identified as described previously.

RESULTS

Location of shuck infections. A significantly higher frequency of *G. cingulata* was determined on the basal portion than on the other portions (Table 1). The lowest frequency of *G. cingulata* was isolated from the distal portion of shucks.

Cultivar differences. Cultivars tested differed in susceptibility to *G. cingulata* based on isolation frequency from nut shucks. Choctaw and Cheyenne cultivars had consistently greater incidence of infection than did other cultivars (Table 1). Those cultivars having the lowest frequency of *G. cingulata* incidence in 1992 were Forkert, Jackson, Surprise, Cape Fear, Jubilee, and Sumner. Isolation frequencies from other cultivars were intermediate.

In 1993, the isolation frequency of *G. cingulata* from nut shucks of the 23 cultivars sampled ranged from 35.5% on Elliott to 94% on Cherokee (Table 2). Nuts from the cultivars Cherokee, Cheyenne, Choctaw, Mohawk, and Schley-Harris showed the highest isolation frequency of *G. cingulata* from shuck tissue; while Kiowa, Desirable, Maramec, Gloria Grande, Jackson, and Elliott had the lowest frequency. However, *G. cingulata* isolation frequency from symptomatic leaves of pecan cultivars showed slightly different patterns. For example, the cultivars Kiowa and Gloria Grande, which had lower levels of infection of shucks, were among those with the highest isolation frequency for *G. cingulata* from leaves (Table 2). The frequency of isolation of *G. cingulata* from nonsymptomatic leaves ranged from 8 to 72% and was lowest for the cultivars Cape Fear, Davis, Stuart, and Maramec.

Table 1. Isolation frequency (%) of *Glomerella cingulata* from suspect anthracnose lesions on differential portions of pecan nut shucks^x for cultivars at the Gulf Coast Substation, Fairhope, Alabama, 1992

Cultivar	Isolation frequency (%), by location on shuck ^a				Total
	Basal	Middle	Distal		
Forkert	0	4	0		4 a ^y
Jackson	4	2	0		6 a
Surprise	2	2	2		6 a
Cape Fear	6	2	0		8 a
Jubilee	6	4	0		10 a
Sumner	6	2	2		10 a
Kiowa	10	4	2		16 ab
Desirable	12	6	0		18 abc
Maramec	14	4	0		18 abc
Pawnee	8	10	0		18 abc
Davis	12	6	2		20 abc
Mohawk	18	8	2		28 bc
Melrose	18	14	2		34 c
Cheyenne	48	22	2		72 d
Choctaw	50	42	18		100+ e
Mean	14.0 A ^z	8.8 B	2.3 C		

^xFive samples of 10 nuts (50 nuts total) were collected arbitrarily from each cultivar.

^yTotal isolation frequencies followed by the same letter are not significantly different according to the least significant difference test ($P < 0.05$).

^zNumbers followed by the same letter are not significantly different according to the least significant difference test ($P < 0.05$).

Fungi associated with FLS. In 1992, predominant fungi isolated from leaves with FLS symptoms were *Pestalotia* (35.4%), *Alternaria* (27%), *Glomerella* (19.3%), *Nigrospora* (8.2%), and *Fusarium* (5.2%) (data not shown). *Curvularia*, *Epicoccum*, and *Phomopsis* also were isolated from leaves with FLS symptoms,

but at frequencies less than 3%. Over all cultivars, *Pestalotia* and *Alternaria* were isolated most frequently.

The predominant fungi isolated from leaves with FLS symptoms in 1993 were *Glomerella* and *Pestalotia* (Table 3). *Alternaria*, *Fusarium*, and *Phomopsis* also were consistently (10–20%) isolated

from leaves with FLS symptoms. Other fungi that were isolated included *Botryosphaeria*, *Curvularia*, *Epicoccum*, and *Nigrospora*, but these were isolated at frequencies less than 4%.

Correlation coefficients indicated a positive and significant relationship ($r = 0.92$, $P = < 0.05$) between the incidence of *G. cingulata* isolated from affected shucks and from FLS-symptomatic leaves over 15 cultivars in 1992. Correlation coefficients between *G. cingulata* isolations from shucks and leaves collected in 1993 were also positive but not significant.

Koch's postulates. After 60 days in the greenhouse, trees inoculated with ascospores of *G. cingulata* had developed brown necrotic areas with dark margins on leaves, similar to the symptoms previously described for FLS. *G. cingulata* was isolated from 84% of inoculated symptomatic leaves. Isolation frequency of *G. cingulata* was 8% from pecan leaves of trees sprayed with sterile water, and no FLS symptoms developed on these trees. *Alternaria*, *Curvularia*, *Fusarium*, and several unknown fungi and bacteria were also isolated from leaves sprayed with sterile water. Similar pathogenicity tests with *C. acutatum* and *C. gloeosporioides* conidia, and *G. cingulata* ascospores, revealed 81.3, 68.8, and 81.3% recovery, respectively, of fungi morphologically identical to *G. cingulata*. Leaves of trees sprayed with the water controls for each of these pathogenicity tests remained asymptomatic. However, *Alternaria* spp. were recovered from 6.0, 25, and 38% of trees inoculated with conidia of *C. gloeosporioides*, ascospores of *G. cingulata*, and the water control, respectively. In addition, *Fusarium* and *Nigrospora* were recovered from 12 and 6%, respectively, of pecan leaves inoculated with ascospores of *G. cingulata*.

DISCUSSION

G. cingulata was isolated from basal portions of shuck tissue in greater frequency than from other portions of the shuck. This may be due to the relatively close microenvironment of nut clusters, and possibly higher humidity in this area, compared to the distal end of the fruit. Also, the higher isolation frequency from the basal area may be indicative of inadequate deposition of fungicides into such areas, or because of a higher frequency of wounds caused by nut contact at the basal end of the nut.

In 1992, *G. cingulata* was isolated significantly more frequently from nut shucks of Choctaw and Cheyenne cultivars than from all other cultivars. In 1993, however, isolation frequencies of *G. cingulata* from these two cultivars did not differ from those of Melrose and Mohawk. The greater isolation frequency and presumably infection frequency of *G. cingulata* from all cultivars

Table 2. Frequency (%)^w of *Glomerella cingulata* sporulating from nut shucks and isolated from leaves with and without symptoms of fungal leaf scorch of pecan cultivars at the Gulf Coast Substation, Fairhope, Alabama, 1993

Cultivar	Nut shucks ^x	Symptomatic leaves ^y	Nonsymptomatic leaves ^y
Cherokee	94.0	68.8	71.9
Schley-Harris	89.3	58.3	40.0
Cheyenne	87.0	45.6	27.5
Choctaw	87.0	82.5	15.6
Mohawk	87.0	62.5	18.8
USDA 61-6-67 ^z	86.0	61.3	30.0
Melrose	84.5	48.1	20.0
Shosoni	81.3	66.7	71.7
Pioneer	80.6	57.5	59.2
Jubilee	75.3	45.8	23.3
Davis	74.0	46.3	14.4
Cape Fear	72.5	43.1	13.8
Pawnee	71.5	75.6	36.3
Sumner	70.5	51.9	24.4
Surprize	68.0	55.8	35.0
Stuart	65.5	55.6	14.4
Forkert	63.5	56.9	25.0
Kiowa	59.5	66.9	20.6
Desirable	50.0	36.9	18.1
Maramec	43.5	61.9	8.1
Gloria Grande	43.3	64.2	37.5
Jackson	42.5	43.8	36.9
Elliott	35.5	63.8	16.3
LSD (0.05)	7.8	6.2	7.6

^wFrequency was based on the proportion of nuts or leaves on which *G. cingulata* was identified based on spore development.

^xAverage percentage of 50 green nuts on which *G. cingulata* sporulated.

^yAverage percentage of 40 leaf disks on which *G. cingulata* sporulated.

^zUSDA accession number.

Table 3. Fungi isolated from leaves of various pecan cultivars showing symptoms of fungal leaf scorch at the Gulf Coast Substation, Fairhope, Alabama, 1993

Cultivar	Isolation frequency (%) ^y				
	<i>Alternaria</i>	<i>Fusarium</i>	<i>Glomerella</i>	<i>Pestalotia</i>	<i>Phomopsis</i>
Cape Fear	10.0	15.0	43.1	56.9	14.4
Cherokee	2.5	11.9	68.8	32.5	8.8
Cheyenne	12.5	16.3	45.6	45.0	11.9
Choctaw	6.9	8.8	82.5	20.6	7.5
Davis	25.0	18.1	20.6	48.8	16.9
Desirable	16.9	21.9	36.9	28.1	21.9
Elliott	11.9	18.1	63.8	33.1	10.0
Forkert	14.4	21.3	56.9	41.9	10.6
Gloria-Grande	10.8	13.3	64.2	57.5	9.2
Jackson	17.5	16.9	43.8	46.3	8.8
Jubilee	11.7	21.7	45.8	56.7	9.2
Kiowa	7.5	11.9	66.9	43.8	10.1
Maramec	18.1	15.0	61.9	33.1	10.0
Melrose	13.8	10.6	48.1	60.0	13.8
Mohawk	11.9	18.8	62.5	43.8	11.3
Pawnee	6.3	8.1	75.6	27.5	13.8
Pioneer	22.5	19.2	57.5	38.3	19.2
Schley-Harris	10.0	24.2	58.3	58.3	10.0
Shoshoni	10.0	7.5	66.7	48.3	9.2
Stuart	27.5	9.4	55.6	46.3	15.0
Sumner	25.0	16.3	51.9	38.8	5.0
Surprize	15.0	24.2	55.8	40.8	14.2
USDA 61-6-67 ^z	8.8	23.1	61.3	45.0	10.6

^yFrom 40 leaf disks cut from the lesion periphery of 40 symptomatic leaves from each cultivar on 1 and 2 November 1993.

^zUSDA accession number.

in 1993 was probably due to a more favorable environment.

In 1993, the prevailing weather at the Gulf Coast Substation was much more conducive to disease development than in 1992. During 1992, average temperatures for August through October were below 26.7 C, and rainfall was 3.7 and 9.8 cm during September and October, respectively. Average temperatures in 1993 were at least 2 C higher in August and September than in 1992, and no less than 12.7 cm of rain fell in either of these months. Pecan anthracnose has been associated with high temperatures and rain (9,11), as occurred in 1993.

Anthrachnose diseases, such as those caused by *Glomerella* spp., are characterized by long latent periods (1), and this could explain the high isolation frequencies of *G. cingulata* from nonsymptomatic leaves in 1993. In addition, the more favorable environment and greater potential for disease development in 1993 may have caused the differential responses of cultivars to *G. cingulata* infection. The correlation between isolation frequency of *G. cingulata* from nut shucks with suspect anthracnose lesions and leaves symptomatic for FLS was consistently positive in the two study years, but the relationship was only significant in 1992. The conducive environment of 1993 may have allowed the disease to overwhelm any low levels of resistance in the cultivars, which were distinguishable in 1992. In addition, resistance may be manifested differently in leaves than in nut shucks. For example, the isolation frequencies of *G. cingulata* from nut shucks of the cultivar Pawnee were relatively moderate in both study years, but *G. cingulata* isolation frequencies were consistently high from leaves of Pawnee symptomatic for FLS. Over both years, isolation frequencies of *G. cingulata* from nut shucks and FLS-symptomatic leaves were low from the cultivars Jackson, Forkert, and Desirable, while frequencies from Choctaw, Cheyenne, Melrose, and Mohawk were high.

Environmental differences may also account for differences in the fungi associated with FLS. Littrell and Worley (12) found that *Pestalotia* was most frequently isolated from FLS-symptomatic leaves, which agrees with our 1992 results. However, based on statistical analysis, Littrell and Worley (11) concluded that *Curvularia*, *Epicoecum*, and

Fusarium, as well as *Pestalotia*, were primarily associated with FLS. Our results in 1993 indicated that *Glomerella*, *Pestalotia*, and *Alternaria* were most often associated with FLS. Thus, predominant fungi associated with FLS differed between years. In addition, the significant and positive relationship found between *G. cingulata* isolations from shucks and FLS-symptomatic leaves in 1992 indicated that FLS occurrence increases with infection and sporulation on shucks by *G. cingulata*, which supports the hypothesis that *G. cingulata* may be a contributing factor to FLS. Another hypothesis is that an occurrence of a stress factor predisposes trees to both FLS and infection by *G. cingulata*.

The frequencies of *Alternaria* in our isolations from leaves showing FLS symptoms in the field were similar to those reported from senescing leaves (4). Blakeman (4) explained that some species of saprophytic filamentous fungi, under appropriate environmental conditions, behave as minor pathogens and attack living leaves shortly before senescence. This could explain the relatively high (38%) isolation frequency of *Alternaria* from sterile water-sprayed trees in our pathogenicity tests. The preferential attack of senescing leaves by *Alternaria* may also explain how more than one fungus apparently may contribute to FLS.

Confirmation of the causal relationship between *G. cingulata* and FLS was accomplished by following Koch's postulates for establishing proof of pathogenicity. *G. cingulata* was reisolated from nearly all inoculated, symptomatic leaves of potted pecan plants. Disinfestation procedures probably prevented re-isolation of *G. cingulata* from every symptomatic leaf. Pathogenicity tests with conidia of two species of *Colletotrichum* and one ascospore suspension of *G. cingulata* yielded comparably high isolation frequencies from leaves showing the dark-edged necrotic lesions typical of FLS. These results indicate that *G. cingulata* and its anamorphs not only are pathogens of pecan nut shucks, but also can contribute to early leaf degradation and necrosis manifested as FLS. Since no symptoms of FLS developed on any of the trees sprayed with sterile water, *G. cingulata*, *Alternaria*, *Curvularia*, *Fusarium*, and other fungi and bacteria isolated from the controls were presumably caused by

airborne contamination following incubation in the dew chamber.

While it is possible that in the field there is a complex of fungi or of biotic and abiotic agents that causes FLS, our data indicate that *G. cingulata* is primarily responsible for this disease.

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