Influence of Temperature, Leaf Wetness Period, Leaf Age, and Spore Concentration on Infection of Pecan Leaves by Conidia of Cladosporium caryigenum

T. R. Gottwald

Research plant pathologist, USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, P. O. Box 87, Byron, GA 31008. The author greatly appreciated the technical assistance of Raymon R. Pate and James Stuckey. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply approval to the exclusion of other products that also may be suitable. Accepted for publication 8 September 1984.

ABSTRACT


To develop a method for rapidly screening a large number of pecan seedlings in a breeding program for resistance to pecan scab, a study was conducted to determine the greenhouse environmental optima for infection by the pathogen, Cladosporium caryigenum. Optimum temperature for infection of pecan foliage ranged from 15 to 25°C. Maximum lesion development occurred with 48 hr of continuous free foliar moisture. Foliar susceptibility decreased with increasing leaf age. Maximum foliar susceptibility occurred 7-21 days after bud break. Foliage became relatively resistant to infection upon reaching full expansion. Lesion number and disease rating increased directly with inoculum concentration; the highest concentrations were adequate and more practical to produce for large-scale greenhouse scab screening.

Additional key words: breeding for resistance.

Scab of pecan [Carya illinoensis (Wang.) Koch] is a devastating disease of most commercial cultivars throughout the southeastern United States. The scab pathogen, Cladosporium caryigenum (Ell. et Lang.) Gottwald (5) causes severe foliar necrosis, twig dieback, lesions on the nut shuck (involucre), and reduces crop quality (8,9). Although conidia of C. caryigenum are dispersed primarily by wind, rain-splash of conidia is a major contributor to localized inoculum dispersal (6,7,11). Moisture in the form of rain, fog, or dew is required for successful infection (3,7,11,13,14).

The requirement of foliar moisture for infection has been demonstrated previously. Demaree and Cole (4) were able to initiate infections successfully by brushing a conidial suspension of inoculum collected from the field onto pecan leaflets and enclosing the leaflets in double glassine bags. Converse (1) later demonstrated the requirement for free moisture when he sprayed or dripped an inoculum suspension collected from leaf or shuck lesions onto potted trees (1). Symptoms developed after the inoculated trees were atomized with water for 48 hr at 22°C. He reported infection provided by fluorescent lights (Westinghouse F40/Agro-lite, mounted ~28-32 cm above the petri plates) that provided a photon flux density of 1,050 μE·m⁻²·sec⁻¹ at the colony surface. Humidity was held at ~85%. Five milliliters of sterile distilled deionized water were used onto the surface of each petri plate and the colony surface was brushed lightly with a stiff artist's brush to loosen and suspend the conidia. The suspension was centrifuged at 10,000 rpm for 5 min, the supernatant was decanted, and the spore pellet was resuspended in sterile, distilled, deionized water. Centrifugation was repeated twice to remove culture nutrients, staining products, etc. The final spore concentration was determined via hemacytometer and adjusted to that needed for specific experiments.

Isolate 81-1F, a pathovar especially adapted to cultivar Wichita pecan, was used in all experiments because it was highly virulent on greenhouse-grown pecan seedlings and sporulated more profusely in culture than other isolates.

MATERIALS AND METHODS

Inoculum preparation. Conidia of C. caryigenum were harvested from 2-wk-old cultures on oatmeal agar grown at 24°C with a 12-hr light/dark regime in a walk-in growth chamber. Light was provided by fluorescent lights (Westinghouse F40/Agro-lite, mounted ~28-32 cm above the petri plates) that provided a photon flux density of 1,050 μE·m⁻²·sec⁻¹ at the colony surface. Humidity was held at ~85%. Five milliliters of sterile distilled deionized water were used onto the surface of each petri plate and the colony surface was brushed lightly with a stiff artist's brush to loosen and suspend the conidia. The suspension was centrifuged at 10,000 rpm for 5 min, the supernatant was decanted, and the spore pellet was resuspended in sterile, distilled, deionized water. Centrifugation was repeated twice to remove culture nutrients, staining products, etc. The final spore concentration was determined via hemacytometer and adjusted to that needed for specific experiments.

Isolate 81-1F, a pathovar especially adapted to cultivar Wichita pecan, was used in all experiments because it was highly virulent on greenhouse-grown pecan seedlings and sporulated more profusely in culture than other isolates.

Temperature versus leaf wetness period. A suspension of 2.65 × 10¹⁰ conidia per milliliter was atomized with an artist’s air brush operated at 1.4 kg/cm² (20 psi), onto both adaxial and abaxial leaf surfaces of 600, 2-wk-old greenhouse-grown pecan seedlings (seed source, cultivar Wichita) to run-off. Inoculated seedlings were placed in dew chambers (model F-60DL; Percival Manufacturing Co., Boone, IA), 100 per chamber, programmed for an ambient
temperature of 10, 15, 20, 25, 30, or 35 C, 100% RH, free dew formation, and total darkness. Trees, selected at random for position in the chamber, were removed from the chambers after 0, 2, 4, 6, 9, 12, 18, 24, 36, or 48 hr in replicates of 10 greenhouse grown. Following this leaf wetness period all trees were returned to the greenhouse and placed on a sand bed where they were held in air-conditioned temperatures of 24–28 C in a polyethylene high-humidity enclosure (85–90% RH) to promote lesion development. After a 21-day incubation period, trees were removed from the enclosure and the second, third, fourth, and fifth (second leaf oldest, fifth leaf youngest) leaves were rated on a 1–5 scale for percent disease (1 = no lesions; 2 = few small brown flecks <1.0 mm; 3 = numerous lesions, consisting of brown flecks mixed with small black lesions, 1–2 mm; 4 = small well-developed black lesions, 1–2 mm; and 5 = large well-developed spreading black lesions, >2.0 mm). These leaves were then removed from the seedlings, the lesions on each leaf were counted, and the leaf area was measured with a leaf-area meter (model Li-3100; Li-Cor, Inc., Lincoln, NE) and number of lesions per square centimeter were calculated. The first or bottom-most leaf was much smaller than other leaves and often distorted; therefore, it was not included. Experimental design was a 6 X 10 factorial (six temperatures and 10 leaf wetness periods) replicated 10 times.

Effect of leaf age and infection. Numerous pecan seeds were planted in 7.5-cm-diameter peat moss cups and germinated in an air-conditioned greenhouse, 24–28 C. Seedlings were grouped by date of emergence into groups of 20 plants each and only the most uniform plants were utilized in the study. Each group of plants was then inoculated with conidia (concentration 2.5 X 10^5 spores per milliliter collected and treated as described above) at 1, 7, 14, 21, or 28 days postemergence. Age of individual leaves (days from bud-break) on each plant at time of inoculation was determined by referencing each plant to its individual date of bud break. Plants were randomized in Percival dew chambers following inoculation for 48 hr at 25 C, 100% RH, free dew formation, and total darkness. Following the leaf wetness period, all plants were returned to the greenhouse and held for 3 wk in the high-humidity enclosure, rated for disease, and the leaf areas were measured as described above.

Effect of spore concentration on infection. To determine the optimum inoculum spore concentration for infection, groups of 20 3-wk-old greenhouse-grown pecan seedlings (seed source, cultivar Wichita) were inoculated with 0, 32, 160, 800, 4.0 X 10^4, 1.0 X 10^5, 5.0 X 10^5, or 2.5 X 10^6 spores per milliliter of sterile distilled water resulting from a dilution series prepared by using the inoculum preparation techniques described above. All plants were atomized with inoculum via the airbrush techniques discussed previously. Immediately following inoculation, all plants were randomized in Percival dew chambers at 25 C, 100% RH, for 48 hr. Plants were then returned to the greenhouse sandbed, incubated, rated for disease, and the leaves were measured as previously described.

Statistics. All three studies were replicated at least once. Data were subjected to analysis of variance and differences among treatment means were detected by using Duncan's multiple range test. Data on the effect of spore concentration were also subjected to linear regression. Graphic analysis was performed with SAS-Graphics (Graphics package of Statistical Analysis System, SAS Institute, Cary, NC).

RESULTS

Lesion development. In all studies, pecan scab lesions first became noticeable 7–9 days after inoculation. Lesions continued to develop for 14–21 days at which time they had attained full size and stopped expanding. After 21 days, lesions ranged from minute brown flecks with no apparent disruption of the leaf epidermis and no external evidence of hyphae and conidiophores laden with numerous catenulate chains of blastoconidia (disease rating = 2) to luxuriant erumpent outgrowths of hyphae and conidiophores laden with numerous catenulate chains of blastoconidia (disease rating = 5).

Effect of temperature and leaf wetness period on infection. Disease developed at all temperature-leaf-wetness-period combinations tested. Lesion numbers generally increased with increasing leaf wetness period. A few lesions developed after only 2 hr of continuous dew; however, maximum lesions per square centimeter leaf surface area (0.365) occurred after 48 hr of leaf wetness (Fig. 1). Total numbers of lesions produced at 10, 15, 30, and 35 C were statistically the same (P = 0.05) for all leaf wetness periods. Maximum lesion production occurred after 32–48 hr of wetness at 20 C and 48 hr of wetness at 25 C.

Data from plants rated for disease intensity and data of actual lesion counts produced very similar results; however, temperature appeared less critical for maximum disease production.

![Fig. 1. The effect of temperature and leaf wetness period on the total number of pecan scab lesions per square centimeter of leaf surface area of 2-wk-old greenhouse-grown pecan seedlings (seed source, cultivar Wichita). Note some infection took place after 2 hr of leaf wetness, but maximum lesion numbers resulted from 20 C and 48 hr of continuous wetness.](image-url)
Temperatures of 15, 20, and 25°C during 48 hr of continuous wetness all resulted in similar maximum disease ratings of 3.2–3.67 (Fig. 2). Few lesions (Fig. 1) and little disease (Fig. 2) developed during wetness periods of <9 hr. Increasing the wetness period up to or beyond 9 hr tended to stimulate an overall increase in lesion numbers and disease ratings at all temperatures tested (Figs. 1 and 2, note steplike increase at 9 hr of wetness); however, there was no statistical difference (P = 0.05) between 9 and 36 hr of wetness at any temperature tested. A definite disease increase was demonstrated when wetness periods were extended beyond 36 hr within the optimum temperature range of 15–25°C.

Effect of leaf age on infection. The first inoculation to test the susceptibility of leaves of various ages to optimum conditions of temperature and wetness (25°C and 48 hr of wetness) was conducted when the first (terminal) seedling bud had been broken for ~1 day. The outermost leaf at this time was often repressed in size and oddly shaped and was therefore not used. The second and third outermost leaves were moderately susceptible to inoculum of C. caryigenum at this time while the fourth and fifth leaves were generally unexposed and closely appressed to the short apex. They were more protected and smaller in leaf surface area (Table 1) and therefore appeared slightly less susceptible. Inoculations 7 days after bud break when second and third leaves were about one-half expanded demonstrated increased susceptibility to inoculum of C. caryigenum. Fourteen to 21 days after bud break, the first leaves to unfold (second and third leaves) were nearly fully expanded and somewhat less susceptible, however, those leaves just slightly later to unfold (fourth and fifth leaves) were in their most susceptible condition. The outermost leaves (second and third) of the terminal bud became completely resistant to infection (after 14 and 28 days, respectively) while the inner leaves were still highly susceptible (Table 1). When the susceptibility of entire plants was considered, there was no statistical difference among groups of plants inoculated at various days after bud break due to the variability of individual leaf susceptibility over time and ultimate surface area (Table 1). However, there was a trend of increased disease susceptibility between 7 and 21 days after bud break which is a reflection of this same trend for individual leaf positions.

Effect of inoculum concentration on infection. A roughly fivefold dilution series from 2.5 × 10⁶ to 32 conidia per milliliter of sterile, distilled water was sprayed on pecan seedling plants at the optimum temperature-wetness period of 25°C-48 hr described above. The number of lesions per square centimeter leaf surface area increased with inoculum concentration for each leaf position and for the plant as a whole (Table 2). The relationship between the natural logarithm of inoculum concentration and number of lesions per square centimeter of leaf surface area was fairly linear (R² = 0.78) for concentrations of 0 to 5 × 10⁵ conidia per milliliter. Lesion numbers increased more than fivefold when the inoculum concentration increased from 5 × 10⁵ to 2.5 × 10⁶ conidia per milliliter. The data were therefore subjected to two regression analyses. The first demonstrated a significant response of numbers of lesions per plant to increasing inoculum concentrations from 0 to 5.0 × 10⁵ conidia per milliliter. The second analysis demonstrated the increase in response (steeper slope) between 5.0 × 10⁵ and 2.5 × 10⁶ conidia per milliliter (Fig. 3). The average disease rating was linearly related to the natural log of spore concentration over the entire range of spore concentration tested (r² = 0.79) (Fig. 4).

DISCUSSION

The requirement of leaf wetness periods caused by dew, fog, and misting rain for infection of pecan by C. caryigenum has been suggested many times (1,10–12). My results indicate that infection increases with duration of leaf wetness. Converse described the optimum temperature for conidial germination as ~25°C (2). My experiments with seedling pecans have demonstrated that optimum temperature for infection was 20°C when combined with a 48-hr leaf wetness period. Successful infections were obtained within the temperature range of 15–25°C when leaves were wet for 48 hr. Although continuous 48-hr leaf wetness periods are unusual in the orchard, 2–3 days of intermittent showers are not uncommon and can create leaf wetness periods approaching 48 hr.

Extended postinoculation leaf wetness periods apparently did not wash spores off the foliage to any appreciable extent as the longest wet period promoted the most lesions. An increase in lesion numbers with increasing leaf wetness periods could be a response to delayed spore germination. Not all spores of C. caryigenum washed from petri dish cultures may be in a germinable stage when dislodged from their conidiophores. These latent spores may

![Fig. 2](image-url)
germinate only after residing for a period of time on the leaf surface in the presence of free moisture. For the purpose of screening large numbers of greenhouse seedlings, the temperature range for maximum infection of 15–25°C is most useful and not difficult to maintain in an air-conditioned greenhouse bay. The presence of free foliar moisture can be easily provided by commercial greenhouse fogging equipment.

The relative resistance of older pecan foliage to infection by C. caryigenum has been previously noted (10). My results indicate that susceptibility of pecan foliage decreased rapidly with age. Foliage was most susceptible ~7–21 days after bud break. Prior to 7 days after bud break, the foliage has limited surface area, and therefore, even though highly susceptible, only a few infections can occur and these become spread out as the leaf expands. After 21 days, the leaves are approaching full size, cuticle formation catches up with expansion, and there is a deposition of phenolic substances in the palisade parenchyma layers of the leaf blade (15). Any or all of these changes may make the leaf resistant to infection. In addition, the chemical composition of leaf surface exudates change with leaf age (T. R. Gottwald and B. W. Wood, unpublished). This change in water-soluble chemical constitution may well affect the germination and/or viability of spores of C. caryigenum on the phylloplane.

When susceptibility of the entire seedling is considered rather than that of the individual leaf, optimum infection can be achieved when inoculations are made ~7 days after bud break. This is a most fortunate circumstance because large numbers of seedlings can be rapidly screened for scab susceptibility with a very short turn-around time. Although field inoculum has been used successfully in the past in greenhouse studies (12), it is not always readily available. In addition, since such inoculum is collected from numerous lesions, its viability and pathogenicity is often quite variable and may represent a mixture of several strains or genotypes. In a screening program, it is desirable to screen against pretested, highly virulent, consistent inoculum from a pathovar (or pathovars) pathogenic to the seed parent cultivar (or cultivars). For such screening, inoculum

![Fig. 3. The effect of inoculum concentration (logarithmically transformed) on the number of pecan scab lesions per square centimeter of leaf surface area. Note increase between last two points on curve which correspond to $5.0 \times 10^5$ and $2.5 \times 10^6$ conidia per milliliter. Trees were subjected to 25°C and a leaf wetness period of 48-hr postinoculation. Note that the regression coefficient is significantly greater than 0, indicating that there is a significant response in the number of scab lesions per square centimeter of leaf surface area to spore concentrations.](image_url)

![Fig. 4. The effect of inoculum concentration (logarithmically transformed) on pecan scab disease rating of seedling pecan cultivar Wichita. Postinoculation temperature was 25°C during a 48-hr leaf wetness period. (Rating scale: 1 = no lesions; 2 = few small brown flecks, <1.0 mm; 3 = more numerous lesions-brown flecks mixed with small black lesions; 4 = small well-developed black lesions, 1–2 mm; and 5 = large well-developed spreading black lesions, >2.0 mm.) Note that the regression coefficient is significantly greater than 0, indicating that there is a significant response in number of lesions per square centimeter leaf surface to disease rating.](image_url)

### TABLE 1. The effect of pecan leaf age on infection by *Cladosporium caryigenum*

<table>
<thead>
<tr>
<th>Days after bud break</th>
<th>Lesions per square centimeter</th>
<th>Average per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd leaf from bottom&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3rd leaf from bottom&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.208 a (%) 0.242 a (%) 0.119 c (E)</td>
<td>0.039 c (E) 0.206 a</td>
</tr>
<tr>
<td>7</td>
<td>0.419 a (%) 0.366 a (%) 0.583 abc (%) 0.390 bc (%) 0.442 a</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.000 b (%) 0.266 a (%) 0.879 ab (%) 1.982 a (%) 0.374 a</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.000 b (%) 0.057 a (%) 1.196 a (%) 1.397 ab (%) 0.311 a</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.000 b (%) 0.000 a (%) 0.455 bc (%) 0.943 bc (%) 0.140 a</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Greenhouse grown pecan seedlings (seed source, cultivar Wichita) were inoculated 1, 7, 14, 21, or 28 days after first bud break. Disease determinations were made ~7 days after bud break. Disease determinations were made for each leaf 3 wk after inoculation. Means followed by the same letter are not significantly different at *P* = 0.10.

<sup>2</sup>Terms in parenthesis following lesion counts denote general overall leaf stages, i.e., E = newly emergent leaf; ½ = one-quarter fully expanded leaf, ½ = half fully expanded leaf, ¾ = three-quarter fully expanded leaf, and F = fully expanded leaf.

### TABLE 2. The effect of inoculum concentration on infection of pecan foliage by *Cladosporium caryigenum*<sup>3</sup>

<table>
<thead>
<tr>
<th>Inoculum concentration (spores per ml)</th>
<th>Lesions per square centimeter</th>
<th>Leaf rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th leaf</td>
<td>5th leaf</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.001</td>
</tr>
<tr>
<td>32</td>
<td>0.0</td>
<td>0.008</td>
</tr>
<tr>
<td>160</td>
<td>0.016</td>
<td>0.010</td>
</tr>
<tr>
<td>800</td>
<td>0.044</td>
<td>0.046</td>
</tr>
<tr>
<td>$4 \times 10^3$</td>
<td>0.085</td>
<td>0.102</td>
</tr>
<tr>
<td>$2 \times 10^4$</td>
<td>0.207</td>
<td>0.099</td>
</tr>
<tr>
<td>$1 \times 10^5$</td>
<td>0.063</td>
<td>0.117</td>
</tr>
<tr>
<td>$5 \times 10^5$</td>
<td>0.051</td>
<td>0.051</td>
</tr>
<tr>
<td>$2.5 \times 10^6$</td>
<td>0.500</td>
<td>0.708</td>
</tr>
</tbody>
</table>

<sup>3</sup>Greenhouse-grown pecan seedlings (seed source, cultivar Wichita) were inoculated with nine concentrations of conidia of *C. caryigenum* when ~14 days old. Disease determinations were made ~3 wk after inoculation.
produced in the laboratory may therefore be superior to inoculum collected directly from the field.

Although this study indicated that the highest inoculum concentrations tested resulted in the highest lesion counts, production of large quantities of inoculum at this concentration (2.5 × 10⁶ conidia per milliliter) is extremely time consuming and difficult to achieve with present techniques. More easily obtained concentrations of 1.0–2.5 × 10⁵ conidia per milliliter are quite adequate to produce sufficient inoculum pressure for practical large scale screening of pecan seedlings.

Results of this study can be used to develop standard techniques to assess the susceptibility of progeny from the USDA/ARS pecan breeding program to pecan scab. Such a screening program would allow selection of resistant plants and serve to eliminate large numbers of plants prior to planting in the field for further evaluation. However, further studies including the effects of mixed inoculum from several different pathovars and the development of entire greenhouse bay climate and leaf wetness period control will be needed before such a screening program can be implemented.

LITERATURE CITED