Ecology and Epidemiology of Pecan Downy Spot

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ABSTRACT

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Conidia and ascospores of *Mycosphaerella caryigena* were trapped in a Stuart pecan orchard throughout the season. Ascospores were present before bud break in the spring. Maximum release occurred in late April, after which numbers dropped until few were trapped after mid-June. Conidia were present in low numbers in the spring and early summer. Maximum numbers of conidia were trapped in late June and early July with smaller numbers released the remainder of the season. Maximum conidial production on diseased leaves occurred at 18–25 C and at a relative humidity of 90–98%. Some conidia were produced throughout the range of the test, from 70 to 100% and from 11 to 32 C. Conidial germination and leaf penetration occurred at temperatures of 20, 26, and 31 C and with maximum germination and penetration at a relative humidity of 99–100%. Light was not essential for either conidial production or germination. Disease incidence was highest in the lowest parts of the tree. This difference was most distinct in early season and became less as the season progressed.

Additional key word: etiology.

Downy spot of pecan (*Carya illinoensis* Koch.), caused by the fungus, *Mycosphaerella carvigena* Demaree & Cole, is widespread and destructive throughout the southern United States. For several years after its discovery in 1927 (1,2,6), it was considered a minor disease because it does not attack the nuts and does not cause readily apparent damage. Later it was found to reduce greatly the photosynthetic efficiency of affected foliage (9) and to contribute to premature defoliation, which, in turn, result in decreased yield and quality. Bud break is delayed and yield and quality are reduced the following year, resulting in alternate-year bearing (4,14,15).

Demaree and Cole (7) in 1932 observed the perfect stage of *M. caryigena* in fallen pecan leaves in March and April and demonstrated the connection between the perfect and imperfect stages and that ascospores can serve as primary inoculum. Conidia from infected leaves initiated infection on healthy leaves, but only after conidia were germinated on agar blocks. The incubation period after infection was 6–7 wk. Converse (5) presented good, but circumstantial, evidence that conidia can overwinter on dead pecan leaves when he successfully induced downy spot in trees by hanging overwintered dead leaves from infected trees in healthy pecan trees in the spring. Careful microscopic examination

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revealed no pseudothecia in these overwintered leaves or viable conidia on the leaf surface. Because no pseudothecia could be found and because they are large and easily observed, he concluded that airborne ascospores probably play no role in the disease cycle in Oklahoma, and primary infection probably resulted from overwintered conidia, although he could not identify their source. Boyd (1) was unable to find conidia on lesions after the tissue in the lesions died.

Environmental effects on downy spot development have received little study. Cole (4) noted that downy spot is more destructive in drier regions of Texas and Louisiana than elsewhere. McGlohon (10) and Payne et al (12) reported that downy spot is more severe in bottom lands, along river banks, and in crowded conditions where air circulation is poor. Demaree and Cole (7) observed maximum growth of the pathogen in culture at 23–27 C.

We studied certain aspects of the life cycle of *M. caryigena* on pecan, and the effects of rainfall, relative humidity, temperature, and light on spore production and disease development.

MATERIALS AND METHODS

Field studies were conducted in an orchard of Stuart pecans at Blackville, SC, between 1973 and 1980. These trees, which were planted about 1917, had been neglected for several years before the initiation of the tests and disease incidence was high.

Development and release of ascospores. Leaf disks, 1 cm in diameter, were cut from diseased pecan leaves from a nonsprayed tree in October 1974, placed in a clear mesh bag that was pinned to the orchard floor, and left there through the winter. Five disks were removed on 14 November and 14 December 1974 and 8, 22, 26 January; 13 February; 31 March; and 9, 24 April 1975. On each date the disks were examined microscopically for the presence of pseudothecia and those found were crushed and examined for the presence of ascospores. Disks also were removed from the bags on 6, 11, 12 April; 17, 22 May; and 24 June and placed on wet filter paper in the top of a petri dish containing 1.6% water agar. The dishes were inverted so that the ascospores were discharged upward onto the agar surface above them. The numbers of spores imbedded in the agar from each disk were counted.

Ascospore populations in the orchard air were monitored in 1975, by a Hirst spore trap, and during 1977–1979 by a Kramer–Collins spore sampler, which collected spores continuously from 10 L of air per minute. The samplers were located beneath the canopy of a Stuart pecan tree that received no pesticides and was severely diseased. Spores with the same morphology, size, and color as the ascospores of *M. caryigena* were counted. In 1975 the sampler was in the orchard only from early April until late June. Hourly spore deposits were counted for 24-hr periods from 30 April to 9 May, from 16 to 26 May, and from 24 to 26 June. In the other 3 yr the sampler was left in the orchard from April until late September. In 1980 a single Kramer–Collins trap was placed at each site at 0, 30, 69, and 788 m from the Blackville orchard and under a pecan tree at Clemson, SC, that was free of downy spot to ascertain whether spores of similar morphology that might be confused with the ascospores of *M. caryigena* were present in the vicinity of the orchard. The traps at Blackville operated continuously from 6 June to 14 August. The Clemson trap operated continuously from 12 to 23 May. Data were expressed as ascospores trapped per day over this period.

Overwintered pecan leaves were collected from the orchard floor beneath a nonsprayed Stuart tree at weekly intervals from 11 April to 26 September 1980. Leaf fragments were placed as close together as practical and with the stroma side up on a moist 9-cm-diameter filter paper in the bottom of a 14-cm petri dish. The dish was then inverted over the upper end of a 30.5-cm section of 10.2-cm-diameter plastic pipe. A piece of double-coated Scotch tape covered with a thin layer of Vaseline petroleum jelly was placed at the bottom of the pipe to catch the ascospores. Four of these towers were used on each date. The ascospores released within 24 hr at 25 C were counted microscopically.

Dissemination of conidia. The presence of conidia in the orchard was monitored in 1977, 1978, and 1979 by catching rainwater descending through a Stuart pecan tree in 1,000-ml Erlenmeyer flasks each fitted with a polypropylene funnel with a top diameter of 16 cm. Six flasks were suspended at heights of 1.0, 1.5, 2.1, 2.3, 3.0, and 4.0 m above ground, one flask at each location. A circle of aluminum screen was placed in each funnel to catch foreign material, such as leaves and insects, and to prevent clogging of the funnel stem. Ten milliliters of a 5% copper sulfate solution was added to each flask to prevent spore germination and growth of contaminants. The rainwater was removed from the flasks at weekly intervals. An aliquot of the water in each flask was passed through a Metricel GA-6 grid membrane filter. Spores collected on the filter were stained with lactophenol-cotton blue and counted microscopically. Spores with the same morphology, size, and color as the conidia of *M. caryigena* were counted. The spore counts were converted to number per month per square millimeter of collecting surface.

Diseased leaves bearing numerous conidia were collected from a nonsprayed Stuart tree in August 1973 and stored until February 1974 at room temperature (20–25 C), in a refrigerator (4 C), or frozen (below 0 C). In February 1974 conidia were collected from the leaves and allowed to germinate in drops of tap or distilled water at 23 C under 11.75- or 14.0-hr light regimes. Three samples of conidia were collected from each lot of stored leaves and conidia were observed in three microscope fields from each sample. The percent germination was determined after 12, 15, 24, 37, 60, 70, 80, and 84 hr.

Disease distribution in the canopy. Secondary disease spread was determined by sampling leaves from three nonsprayed Stuart trees on 12 June, 11 July, 8 August, and 12 September 1975. Samples were taken from the four quadrants of the tree, north, east, south, and west; from four heights in each quadrant, 1.8, 5.5, 9.2, and 12.7 m; and from three trees. The right-hand leaflet of the third pair from the terminal was used to determine disease incidence by the Barrett–Horsfall method. These ratings were converted to percentages by use of tables developed by Redman, King, and Brown (13).

Temperature, light, and relative humidity as related to conidia germination and development. To study the effects of temperature and relative humidity on the production of conidia, infected Stuart leaves collected 8 August 1979 were washed in running water to remove as many of the conidia as possible. Leaf disks, approximately 5 mm in diameter and each containing a single downy spot lesion of uniform size, were cut from the leaves and washed twice in tap water for 5 sec in a low-speed Vortex test tube mixer to remove additional conidia. The disks were blotted dry with paper towels and floated on a nutrient solution containing 2% (w/v) sucrose and 10 ppm of streptomycin sulfate. Approximately 120 disks per petri dish were floated on the solution with the lower side of the leaf facing up and remaining dry. Four dishes were placed in each of eight growth chambers, with two chambers set at each temperature, 11, 18, 25, and 32 C. At intervals of 0.5, 1, 2, 3, 5, 7, 10, 13, 17, and 21 days after incubation began, 10 leaf disks were removed from each plate and agitated in 4 ml of water to remove the conidia. A 1-ml aliquot of this conidial suspension was filtered through a Metricel GA-6 grid membrane filter and the conidia trapped on the filter were counted microscopically by counting the spores in four fields. Counts were adjusted to conidia per square millimeter of lesion surface.

Leaf disks, cut and treated as described above, were collected 10 September 1979 and floated on a sucrose-streptomycin sulfate solution in eight petri dishes. The bottoms and tops of the dishes were completely covered with aluminum foil with only enough of the foil extending over the lip of the dish to hold the foil in place but not enough to touch the solution in the dish. On four of the plates the foil was cut away from the bottom and top, so that four cultures were exposed to light and four were held in the dark. The foil around the sides was left in place so that any effects of the foil on aeration were the same in all dishes. The dishes were incubated at 25 C for 23 days. Light was maintained at approximately 140 ft-c with cool-white fluorescent tubes during the 15-hr daily light period. After 23 days the numbers of conidia on 100 disks per dish were determined as described above.

Pecan leaflets with downy spot lesions were exposed to controlled relative humidity from 70 to 100% in sealed 1/2-gal mason jars, by appropriate concentrations of either sodium chloride or glycerol. Leaflets with at least 10 downy spot lesions were detached and washed thoroughly to remove the conidia. The base of each petiolule was placed in water in a test tube and held in place by a cork notched to minimize pressure on the petiolule and midrib. Most of the leaflet was above the cork and was exposed to the relative humidity of the chamber. The experimental design was a complete randomized block with each plot consisting of one chamber containing 15 test tubes. The chambers were incubated at 25 C and with 15 hr of light each day. Light was maintained at approximately 140 ft-c. After 21 days, 100 disks, each 5 mm in diameter and containing a single lesion, were removed from each chamber and the conidia counted as described above.

Clayton's technique (3) was used to determine the effects of temperature and relative humidity on spore germination and leaf penetration. Leaf disks 15 mm in diameter from healthy Stuart pecan leaves were attached to glass cover slips with paraffin so that the upper side of the leaf was attached to the glass. The cover slips were attached to a glass rod suspended from a rubber stopper in a 250-ml Erlenmeyer flask over a solution of sodium chloride or calcium chloride. The solutions were adjusted to give approximately 100, 99, 94, 85, and 65% relative humidity. Freshly collected conidia were transferred to the leaf disks with a camel's hair brush. The flasks were incubated at 20, 26, or 32 C and exposed to constant light at about 60 ft-c. Three flasks arranged in a randomized block design were used in each temperature-relative humidity regime. After 24 hr, disks were removed from the chambers, stained with dilute crystal violet, and scanned microscopically. Percentages of germinated spores and of germ tubes that had penetrated were determined.

RESULTS

Development and release of ascospores. Pecan leaf disks collected in late summer of 1974 produced mature pseudothecia by December, asci by mid-February, and a few mature ascospores by 31 March. Pseudothecia varied greatly in stage of development at each date. On 31 March, for example, 1% had mature ascospores, 12% had asci with immature ascospores, 12% had asci with no ascospores, and 75% had no asci. Leaf disks collected from the orchard floor in 1975 released a few ascospores as early as 6 April–more than 1 wk before bud break. The number of spores released increased rapidly until 23 April, then dropped to 0 by 24 June. The mean numbers of ascospores released per 15-mm disk were 7, 105, 479, 147, 9, and 0 for 6, 11, 23 April; 17, 22 May; and 24 June, respectively. Each leaf disk released ascospores over a period of 2–4 wk. The release of ascospores in the spring of 1975 followed a regular daily pattern of a peak in numbers between 6 p.m. and midnight and a lower peak between 6 a.m. and noon. On days preceded by rainfall approximately 10 times more ascospores were released than on days not preceded by rainfall (Fig. 1).

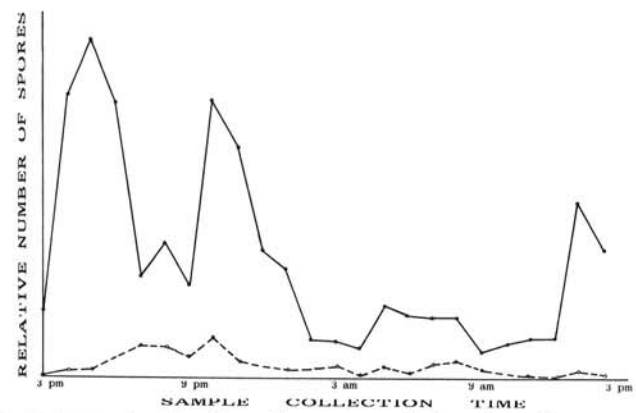

Fig. 1. Average hourly release of ascospores of *Mycosphaerella caryigena* at Blackville, SC, from 30 April to 26 June, 1975, for 6 days preceded by rainfall (——) and 12 days not preceded by rainfall (----).

In contrast to 1975, during 1977–1979 ascospores were trapped from early April until after 1 September. In 1977 (Fig. 2) and 1978 there was a peak of ascospore release in late spring and a second, but much lower, peak in late summer, with few spores trapped in June and early July. In 1979 (Fig. 3) the peak in late spring was followed by moderate numbers trapped in June and July and very few after August 1. In the three seasons there were 22 days when more than 139 ascospores per 1,000 L of air were trapped. Rainfall

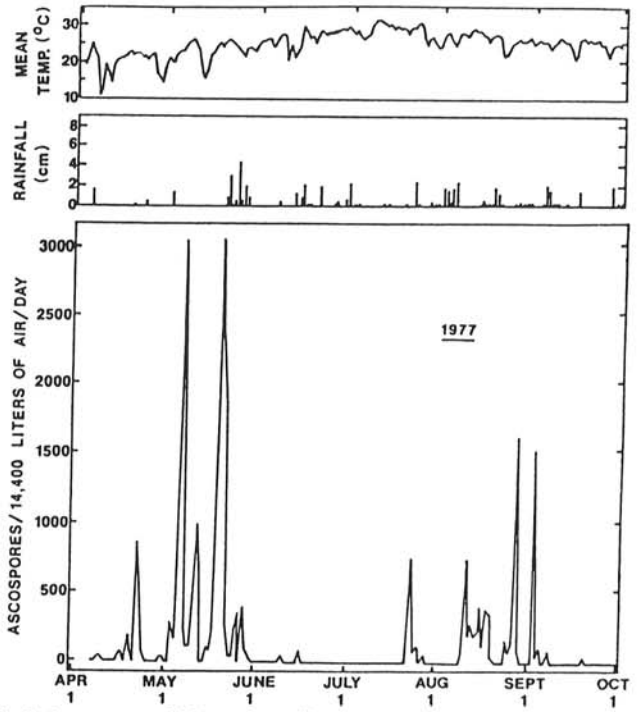

Fig. 2. Ascospores of *Mycosphaerella caryigena* collected daily in 1977 in a downy spot-infested pecan orchard, mean daily temperature and daily rainfall. Sampling was continuous using a Kramer–Collins spore sampler, from 10 L of air per minute.

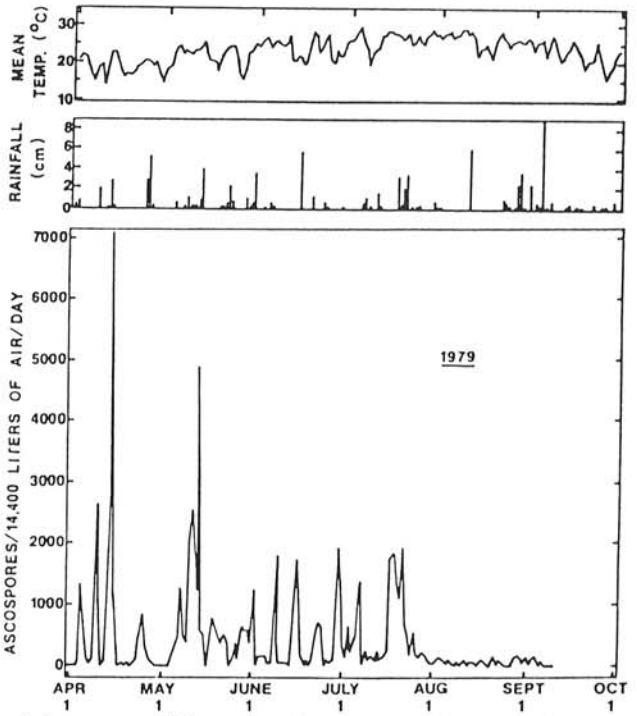

Fig. 3. Ascospores of *Mycosphaerella caryigena* collected daily in 1979 in a downy spot-infested pecan orchard, mean daily temperature and daily rainfall. Sampling was continuous using a Kramer–Collins spore sampler, from 10 L of air per minute.

was recorded the day before or the day of the high spore counts on 17 of the 22 days, but on 3 days no rain fell during the 3 days preceding collection. The mean temperature for the 22 days was 23.1 C, with a range from 13.9 to 26.7 C.

When dead leaves from the orchard floor were suspended above Vaseline-coated adhesive tape, over 70 ascospores per square millimeter were collected on 11 April. The number increased to more than 200 per square millimeter on 25 April, and then decreased until the end of May. No ascospores were collected between 6 June and 11 July and very few during the latter half of July. None was collected after 1 August.

Dissemination of conidia. During 1977–1979, conidia were trapped in the orchard throughout the season. Numbers were low from April until late June, after which they rose sharply, reaching a peak in late July. Thereafter the numbers declined gradually, with smaller peaks and valleys probably correlated with environmental conditions. During 1978 and 1979 the average numbers of conidia collected per square millimeter of collecting surface for the month were 3.2, 9.4, 11.2, and 232.5 for April, May, June, and July, respectively. In 1977 the trap was not obtained and placed in the orchard until July (Fig. 4).

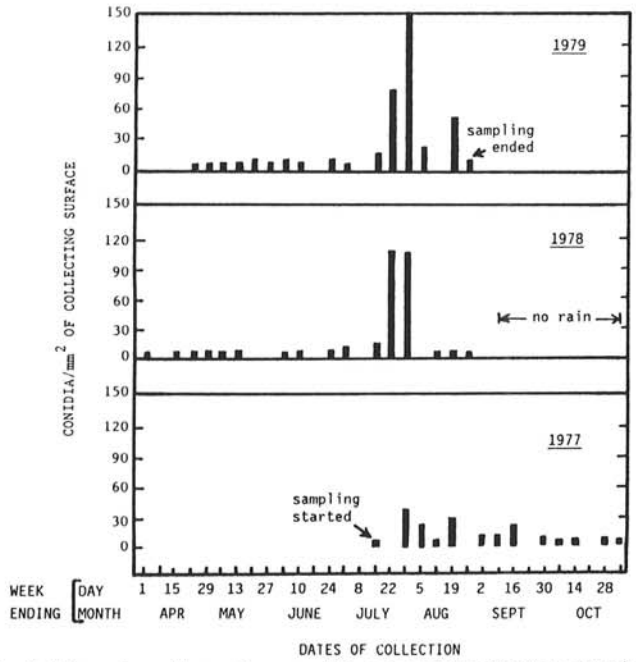

Fig. 4. *Mycosphaerella caryigena* conidia collected in rainwater-collecting flasks hung in a downy spot-infected pecan tree. Collecting surface area represents the area enclosed by the tops of funnels inserted in each collecting flask.

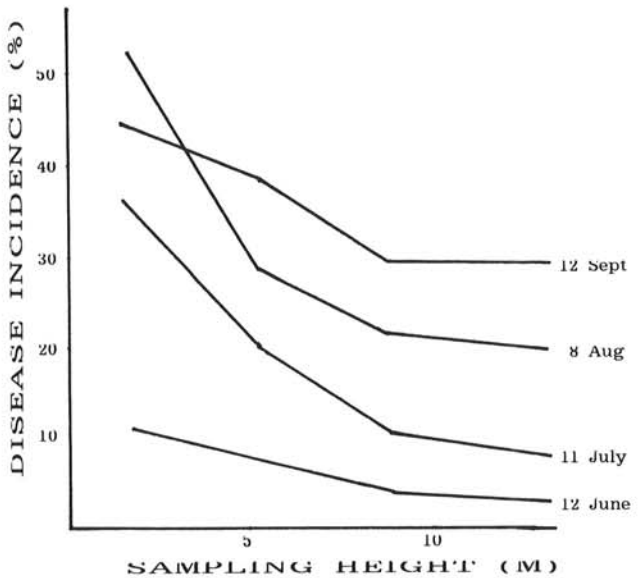

Fig. 5. Incidence of pecan downy spot at various heights in the tree canopy at four dates during the growing season. Lines connect the means of 12 readings at each point.

Nonsprayed infected leaves collected in August 1973 and held 1) at a room temperature of 20–25 C, 2) at 4 C, 3) or in a freezer contained viable conidia in February 1974. The percent germination was 6.3, 4.5, and 9.9, respectively, for the three storage conditions.

Disease distribution in the canopy. Disease incidence was highest in the lowest part of the tree and was progressively less as height increased. Severity increased progressively throughout the season at all heights and the differences between the lower and upper parts of the tree became less distinct (Fig. 5). The differences that occurred among the four quadrants of the trees are not significant.

Effects of temperature and relative humidity on conidial production and germination and leaf penetration. When leaf disks, cut from downy spot lesions and washed to remove the conidia, were floated on a sucrose-streptomycin sulfate solution at different temperatures, significantly more conidia were produced ($P = 0.05$) at 18 or 25 C than at 11 or 32 C (Fig. 6). An average of 11 conidia per square millimeter of lesion surface was produced on the disks held in the light as compared with four per square millimeter on those in the dark, but this difference was not statistically significant ($P = 0.05$). Conidia were produced on leaves at relative humidities from 70 to 100%, with 75, 39, 132, 93, 114, 118, and 57 conidia per square millimeter of lesion surface at 70, 80, 90, 94, 96, 98, and 100%, respectively. Maximum production was at 90–98%, but none of the differences was statistically significant at 5% level.

Conidia germinated and penetrated the leaves through stomata at relative humidity of 85% or more and at temperatures from 20 to 31 C. Maximum germination and penetration occurred at 99 to 100% relative humidity. Only a small percentage of the germinated spores ultimately resulted in leaf penetration (Fig. 7). Germ tubes penetrated stomata of leaves within 14 hr. A few conidia germinated and produced appressoria on the leaf surface and appeared to penetrate directly.

DISCUSSION

Both ascospores and overwintered conidia of *M. caryigena* probably serve as primary inoculum for pecan downy spot in South Carolina, because both were present in the orchard before bud break in the spring, although conidia were few. The presence of conidia in the trees in early April indicates that the fungus probably overwinters at some point in the tree. No search was made for twig cankers and no effort was made to isolate conidia from bark crevasses or bud scales. Conidia were trapped in the

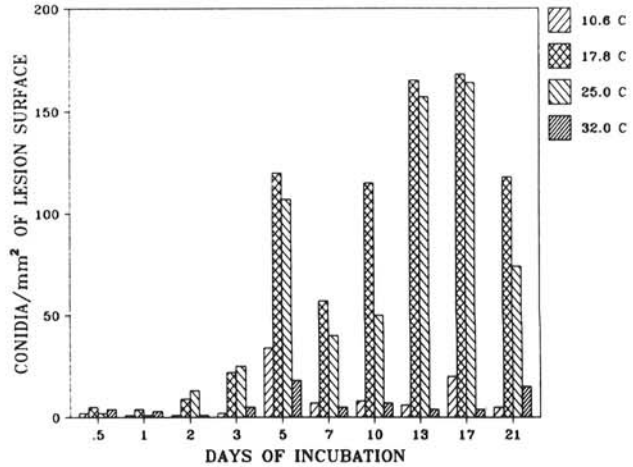

Fig. 6. Conidia of *Mycosphaerella caryigena* produced on infected pecan leaf disks floated on nutrient solutions and incubated at various temperatures.

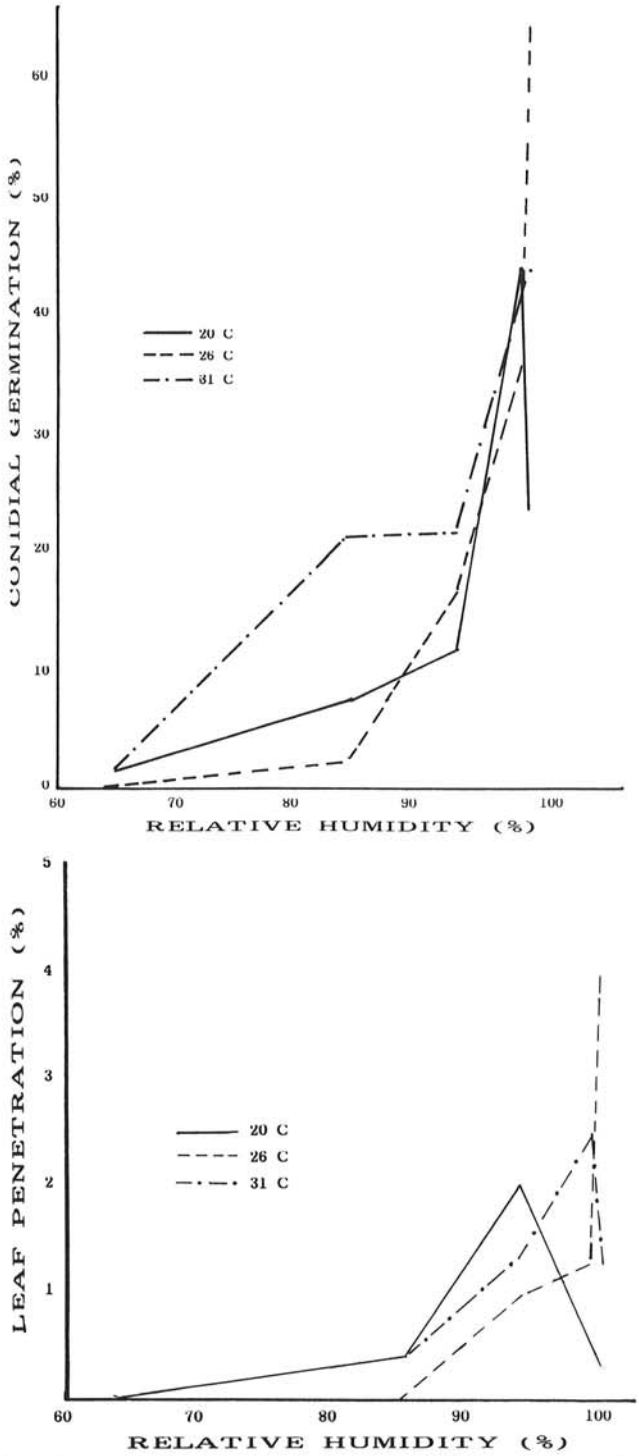

Fig. 7. The effects of temperature and relative humidity on the germination of and penetration of healthy Stuart pecan leaf disks by conidia of *Mycosphaerella caryigena*.

dormant tree after leaves of the preceding year had fallen and no new leaves had appeared. Conidia on leaves collected in August 1974 and stored under controlled conditions in the laboratory were viable for 6 mo, but these optimum conditions do not prevail in orchards. Nelson (11) noted that alternate wetting and drying, typical of outdoor conditions, resulted in fairly rapid death of conidia of *Helminthosporium maydis* (*Cochliobolus heterostrophus*), whereas similar spores stored in a protected location, such as a barn, readily survived from season to season. Converse (5) obtained circumstantial evidence for the presence of conidia in early spring when young pecan leaves just beneath overwintered leaves suspended in the tree became infected. He found neither conidia nor ascospores on the leaves. He concluded that ascospores were not involved because, if present, pseudothecia should have been easily observed.

The large numbers of ascospores and the small numbers of conidia in the spring indicate that ascospores are probably the principal primary inoculum in South Carolina. The small numbers of conidia trapped in late spring and early summer and the peak period of conidial production from late June to late July agrees with the long incubation period of 6–7 wk reported by Demaree and Cole (7).

Mature ascospores were observed in overwintered leaves collected from the orchard floor and were trapped in the air in early April. Cole (4), in south Georgia, also noted the presence of ascospores in pecan orchards before bud break. The presence of primary inoculum in the orchard before bud break in the spring indicates the need for a fungicide application on pecans as soon as bud break begins. This need is supported by results of Latham's field fungicide tests (8) in Alabama. In our tests, inoculum was present throughout the season—ascospores reaching a peak in April and conidia in July. The critical sprays probably are in April and early May and again in late June and July. If spore monitoring is used to predict infection periods, emphasis should be on ascospores in the spring and conidia after mid-June, although both types of spores may be detected throughout the season.

Ascospores and conidia of *M. caryigena* are difficult to distinguish from those of related species. We believe, however, that the spores counted were *M. caryigena* because traps were located beneath or within trees heavily infected with downy spot, pseudothecia of *M. caryigena* were numerous in overwintered leaves, and few spores were collected in traps placed outside the orchard. The only other *Mycosphaerella* species reported on pecan produces ascospores that are sufficiently different in size so that they can be readily distinguished. Similarly, related conidial forms that occur on pecan are sufficiently different in size and/or color so they are readily distinguished.

Environmental requirements for spore production, release, germination, and infection were not clearly delineated, although several relevant conclusions may be drawn from the data. Rainfall increased the release of ascospores, but was not essential for large numbers of spores to be released. Conidia were produced at relative humidities from 70 to 100%, which represented the range included in the test, and at temperatures of 18 and 25 C. Therefore, inoculum is probably present from spring, when the first leaves appear, until early fall, except during very hot or dry periods.

Conidia germinated and penetrated healthy leaves at relative humidities above 85% and at temperature ranges from 20 to 31 C. If leaves remain susceptible, infection probably occurs throughout the summer and early fall and disease spread should continue except during hot, dry weather.

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