Patterns of Diurnal and Seasonal Airborne Spore Concentrations of *Fusicladium effusum* and its Impact on a Pecan Scab Epidemic

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ABSTRACT

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Aerial concentrations of conidia of *Fusicladium effusum*, the causal organism of pecan scab, were monitored in 1980 with a Burkard 7-day recording spore trap to measure dispersal by air. Dispersal by rainwater was measured with funnel traps each with sporulating scab lesions on nut shucks, twigs, and leaf petioles held between screens above the funnel. Rainfall reduced hourly air spore catches. Aerial concentration of conidia was reduced by prolonged periods of drought. Aerial dispersal accounted for most of inoculum recorded. Aerial spore concentration peaked at midday and usually exhibited diurnal periodicity. Appreciable conidia dispersal started in late April and continued through November with maximum air spore concentrations occurring between June and October. Maximum air spore concentration coincided with decreasing periods of vegetative wetness and decreasing relative humidity during drying periods following dew or rainfall.

Additional key words: epidemiology, spore dispersal, inoculation, *Cladosporium*.

Pecan scab, caused by *Fusicladium effusum* Wint., is the most widely prevalent and destructive disease of pecan, *Carya illinoensis* Koch, in the major growing areas (5,10). Overwintering stromata formed in the fall on twigs, nut shucks, and leaf petioles are considered the most important sources of primary inoculum for the following spring (4,5).

Converse (3) was able to incite numerous scab lesions in susceptible scabfree trees by placing diseased nut shucks in the canopy. He was unable to dislodge spores from heavily scabbed nuts in the laboratory with simulated wind velocities of 1.6 km/hr at constant temperature and relative humidity. However, he was able to promote spore dissemination by directing a fine mist on sporulating nut shucks or sporulating agar cultures and allowing the runoff to drip onto pecan leaves. Converse also trapped high numbers of spores on greased microscope slides following rainy periods. Therefore he concluded that conidia of *F. effusum* were dispersed primarily by windblown rain and were not disseminated by dry wind (2,3).

Valli (12) speculated that spores were spread by the washing effect of rain, heavy dew or fog on sporulating lesions. However, he felt that limited spore release could also be achieved by wind whipping of old lesions on leaves, twigs, and old shucks.

The purpose of this study was threefold: to investigate the relative numbers of conidia of *F. effusum* dispersed by wind, which was previously considered negligible, vs rainwater runoff, the preferred explanation for dispersal; to determine the environmental conditions that promote spore dispersal in nature; and to determine possible relationships, if any, between daily spore catches over an entire season and the progress of an associated epidemic.

MATERIALS AND METHODS

Data for this study were collected during the 1980 growing season in a 12-ha, 55-yr-old pecan orchard, located on the grounds of the USDA's Southeastern Fruit and Tree Nut Research Laboratory grounds near Byron, GA. Trees were planted on a 36.6 m × 36.6 m spacing and consisted mainly of pecan cultivars Stuart and Schley.

Experimental design and inoculation. At budbreak (2 April) the entire orchard was treated with dodine (*N*-dodecylguanidine

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acetate) at a rate of 0.64 kg/ha (nearly twice the recommended rate) to inhibit the production of conidia by overwintering stromata. The orchard was then divided into two, 6-ha blocks, hereafter designated "north" and "south." The north block received the Georgia-recommended spray program of 10 fungicide sprays and insecticides applied as required for the remainder of the season. The south block received no fungicide following the initial treatment, but did receive the same insecticide treatment as the north block.

Twenty-five pairs of trees were selected as paired sampling points for disease index determination. One tree of each pair was located in each block and both trees were of the same cultivar, either Schley or Stuart. Both trees of each pair had identical spatial location; ie, distance and direction from the geographical center of each block, with one tree in the north and one in the south block. A single pair of trees of cultivar Schley was selected whose individual locations approximated the geographical center of each block. These trees were designated as the inoculation focal points or sources of secondary inoculum in each block. Thus, the two blocks were identical images of each other with all sampling points spatially identical in relation to the inoculation focal point in each block.

On 29 April 1980, conidia of *F. effusum* were harvested from 20 oatmeal agar cultures (~1 mo old) by spray washing each culture surface with a jet of distilled water. The resulting suspension was diluted to make 2 L of inoculum with a concentration of 6.69×10^5 conidia per milliliter as determined with a hemacytometer. The suspension was prepared in 0.1% Tween-80 to insure even dispersion of the conidia. One liter of the inoculum suspension was sprayed onto each inoculum focal-point tree with a 5.7-L compressed-air hand sprayer. Portions of trees were sprayed to runoff on four main branches located approximately at the four major compass points in the canopy. Inoculations were made ~7–8 m aboveground, and onto a branch area of ~1 M^2 at 1900 hours EST when drying of foliage moistened by inoculum would be relatively slow (temperature 18 C, relative humidity 40% and rising).

Data collection. Leaf samples were collected at 3-wk intervals, from 6 April to 13 November 1980. Each of the paired trees was sampled randomly from all sides at a height of 3–12 m with the aid of a self-propelled hydraulic lift. A sample of 25 leaves was taken from each tree on each sampling date and the number of sporulating scab lesions per leaf was counted. By microscopic examination it was determined that sporulating lesions have a

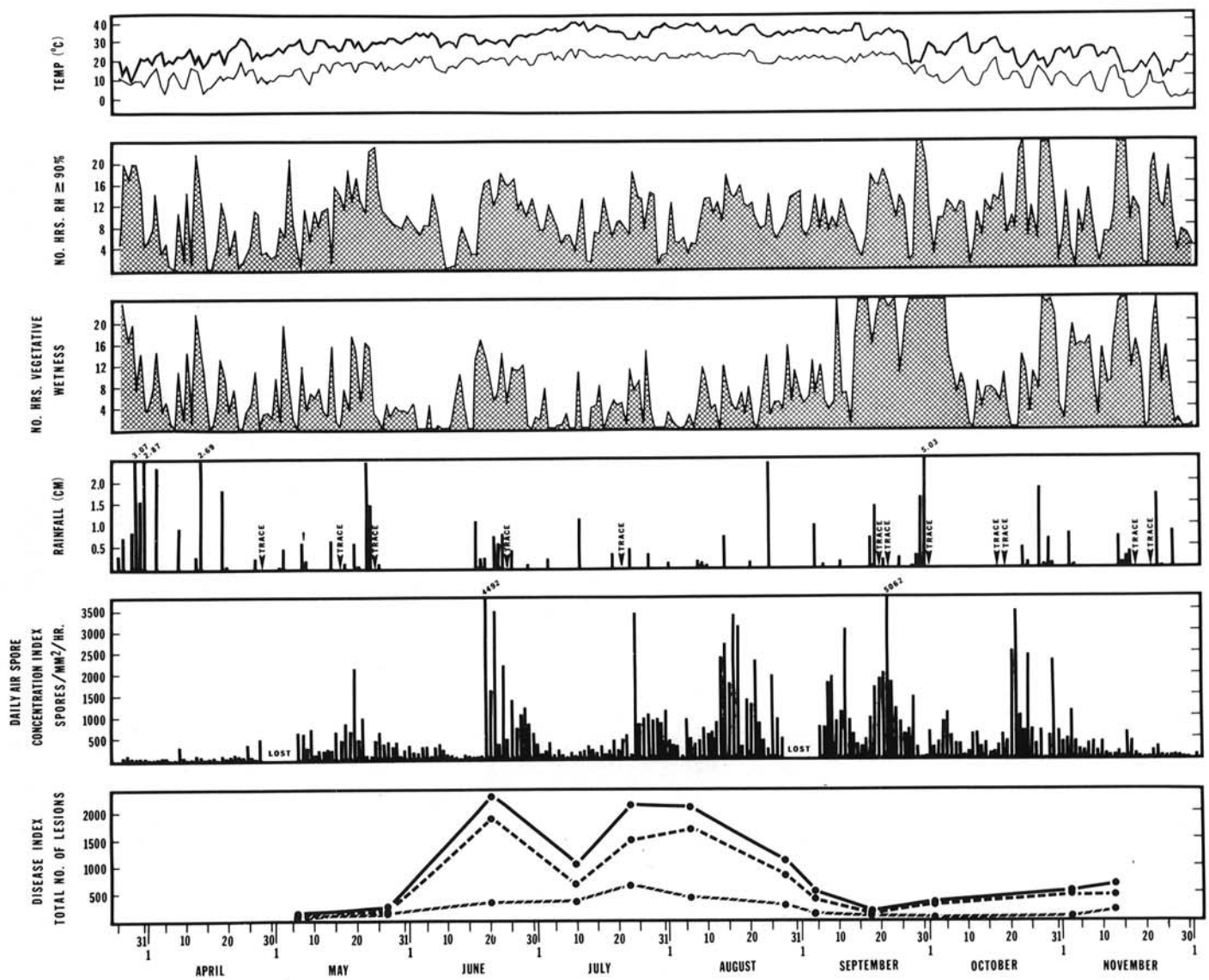

Fig. 1. Relationships of meteorological data, daily concentration of airborne conidia from lesions caused by *Fusicladium effusum* in a 12-ha block of 55-yr-old pecan trees at Byron, GA, and the corresponding disease index during 1980. Conidia were trapped with a Burkard volumetric spore trap. Daily concentrations of airborne conidia and all environmental parameters were measured from 0800 hours EST to 0800 hours EST the following day. Disease index: *solid-line* = total number of sporulating leaf lesions in a sample of 25 leaves from each of 50 trees in a 12-hr block; *broken line* = total number of sporulating leaf lesions in a sample of 25 leaves from each of 25 trees in a 6-ha unsprayed block; *diagonally-broken line* = total number of sporulating leaf lesions in a sample of 25 leaves from each of 25 trees in a 6-ha fungicide-treated block that received the Georgia-recommended spray program (12 fungicide applications). Spore trapping was done only in the unsprayed block.

velvety dark brown to black appearance while older nonsporulating and degenerating lesions have a flat grayish appearance. Only sporulating lesions were considered in lesion counts and disease index determinations because of their ability to add further inoculum to the epidemic. A disease index was calculated for each sampling date by totaling the number of sporulating lesions found in each 6-ha block. A total disease index was calculated by totaling the number of all sporulating lesions found in leaf samples for the entire orchard. The disease index measurement was used to estimate and compare the amounts and fluctuation of disease between the blocks and within the entire orchard on each sampling date and over time. Since the disease index was based on the number of sporulating lesions during each sampling date, it was a fairly accurate estimate of inoculum potential in the block or orchard at the time of sampling.

Spore trapping. A Burkard 7-day recording spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Herts, England) was operated continuously from mid-March to mid-December 1980. The trap was located on a platform built in the canopy of the unsprayed focal point tree and was calibrated to sample 10 L of air per minute. The orifice of the trap was 3.89 m above the ground. Numerous overwintering stromata on infected twigs of pecan cultivar Cherokee were positioned in front of the spore trap orifice to improve detection of low concentrations of airborne spores during the beginning of the growing season. Cut ends of the twigs were sealed against desiccation by dipping in hot paraffin. Twigs were held in place with a folded 25 × 25-cm piece of 1.25-cm hardware cloth bolted on the spore trap bib ~25 cm from the orifice. Only one set of sporulating twigs was used. Viability of overwintering stromata for spore production was checked periodically by visual determination. Degeneration of viability (dark velvety appearance) to nonviability (flat gray appearance) of stromata was complete by mid-June. The nonviable twigs and hardware cloth were left in front of the spore trap orifice after June so as not to alter airflow.

Spore trap tapes were coated with a base mixture of 10% polyvinyl alcohol in distilled water and dried for 24 hr. A second adhesive coat of Vaseline petroleum jelly plus 10% paraffin, thinned to a soft paste with toluene, was applied. The trapping tapes were heated on a hotplate until all brush streaks in the trapping surface were smoothed out, then cooled before placing in the trap. The exposed tapes were cut into daily segments (48 mm) and mounted in acid-fuchsin-lactophenol. All mounts were made permanent by incorporating 1% polyvinyl alcohol into the stain.

Spores were counted at ×400 with phase-contrast optics. Spores of *F. effusum* were identified by staining characteristics, size, and shape.

Hourly spore counts were made by close calibrations of the

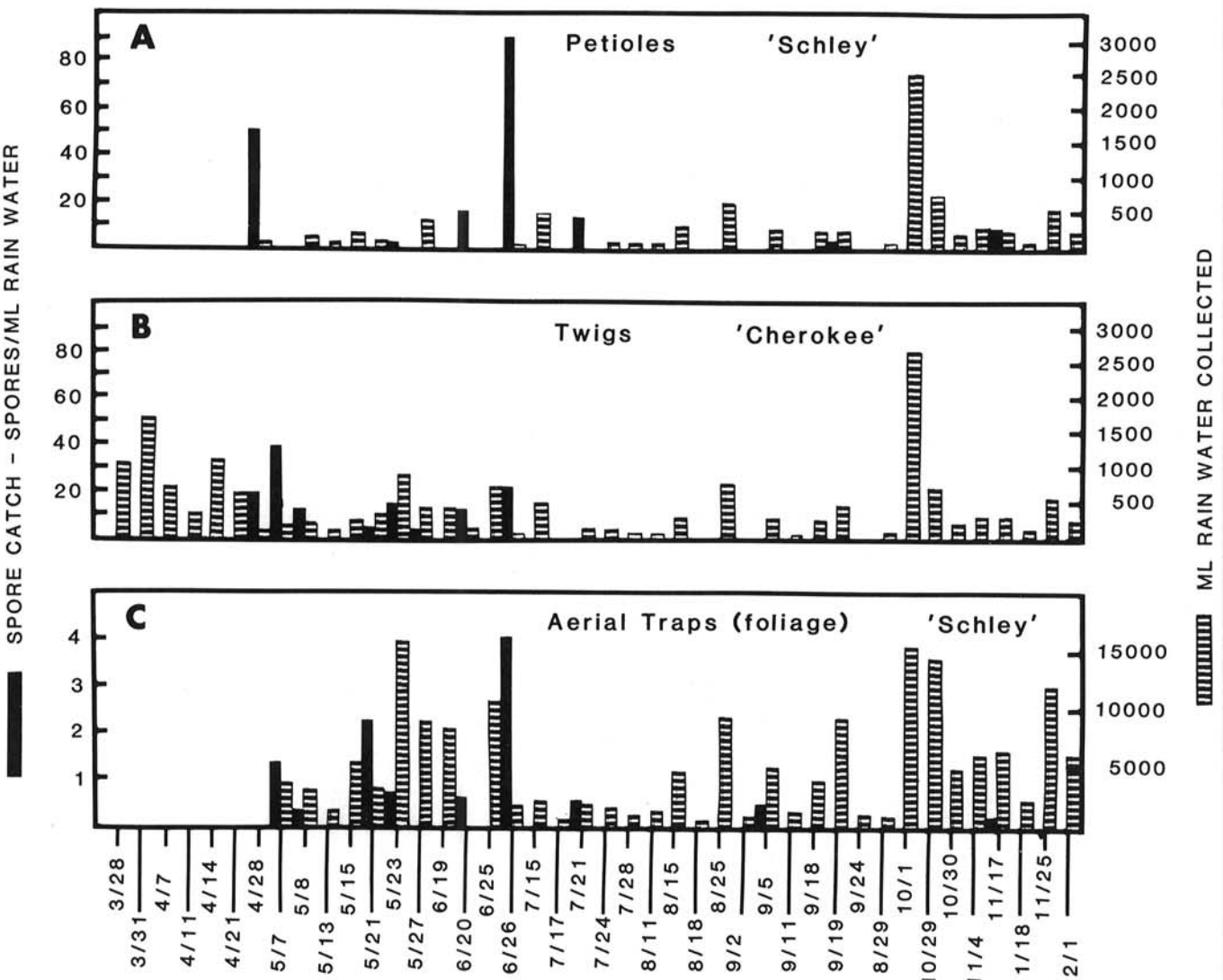

Fig. 2. Number of conidia of *Fusicladium effusum* caught in rainwater runoff traps under pecan trees at Byron, GA. **A,** Total spore catch from an undetermined number of overwintering stromata on infected leaf petioles of cultivar Schley. **B,** Total spore catch from rainwater in five funnel traps containing twig sections of cultivar Cherokee with 250 overwintering stromata per trap. **C,** Total spore catch from rainwater of five aerial funnel traps from leaf lesions resulting from inoculations of branches of a mature tree of cultivar Schley. *Solid bars* = total number of spores caught per milliliter of rainwash; *broken bars* = amount of rainwash in ml.

mechanical stage into 2-mm increments equivalent to 1 hr of spore trap tape movement (2 mm/hr). Aerial spore concentration for each hour was estimated by counting all conidia in a single horizontal pass across each 1-hr (2-mm) segment of slide surface area at ×400. The number of spores per square millimeter per hour was found by multiplying each 1-hr spore count by 1.25. Daily aerial spore concentration indices were calculated by adding the estimated spore catch of five 1-hr periods each day. The five 1-hr periods used were at 0400, 1000, 1400, 1800, and 2400 hours. These periods were determined to best represent the high, medium, and low spore concentration times and serve as a daily index of spore discharge.

Several types of rainwater traps similar to those used by Bertrand and English (1) to study Valsa canker of French prune were used to trap rain-washed spores. Five aerial leaf traps consisting of large funnels 46 cm in diameter were suspended ~10 cm beneath the inoculated branches of the southern focal tree and were connected by lengths of 1.27-cm-diameter garden hose to 3.79-L (1-gallon) plastic milk jugs. Ten other traps of an additional type were also placed on the ground under the canopy of the southern focal tree. Each of these had a 10-cm-diameter plastic funnel fitted through a cork and into the mouth of a plastic milk jug. Each of the funnels was fitted with a piece of window screen and baited only once with either leaf petioles from cultivar Schley in mid-April or twig sections from cultivar Cherokee in late March, with a total of 250 stromata per trap. Twig sections were cut 7–10 cm long, sealed on the ends with paraffin and placed in the traps. Five traps of each type were located on the ground under the canopy of the southern focal tree. The same infected twigs and petioles remained in the traps throughout the entire season to determine the period of spore release from overwintering stromata and the variation in spore number during this period.

Measurement of environmental parameters. All weather measurements were taken at the U.S. Weather Bureau substation, located on the Southeastern Fruit and Tree Nut Research Station, 137 m from the orchard under study. Temperature and relative humidity were recorded with a 7-day recording hygrothermograph (Belfort Instrument Co., Baltimore, MD 21224) located in a weather shelter 1.5 m aboveground. Rainfall was measured with a 7-day recording universal rain gauge (Belfort Instrument Co.). Leaf wetness was recorded on a strip chart recorder connected to a series of wetness sensing elements consisting of a chemically treated printed circuit whose surface resistance changes with hydration. This series of sensors was located, at various angles, in a bush 0.5 m aboveground. Any change in surface resistance from the base line was considered as evidence of vegetative wetness. Wind speed was recorded on a similar strip-chart recorder connected to a three-cup anemometer with four-digit odometer (Model 53498, Belfort Instrument Co.) located ~3 m aboveground. Light intensity (solar radiation) was recorded with a radiometer (Model 8-48, Eppley Laboratory Inc., Newport, RI 02840) and integrator (Model 618.4, Science Associates, Box 230, Princeton, NJ 08540).

RESULTS

Disease increase. During 1980, in both blocks of the orchard under study, pecan scab first appeared with low incidence in late April. Disease severity, measured as the number of sporulating lesions, remained low throughout May and did not increase dramatically until early June (Fig. 1). By mid-June the epidemic had reached a peak, as determined by foliar lesion counts, and started to decline from late June to early July. A second increase in the number of sporulating lesions was recorded in late July. Disease severity remained relatively constant from late July until mid-August when it once again began to fall. Disease severity was low during the 16 September sampling. There was a very gradual increase in the number of sporulating lesions again from late September until leaf fall in November.

Rainwater dissemination of conidia. The highest number of conidia recovered from rainwater traps was during the period, 7 May to 21 July (Fig. 2). Only very few conidia were caught during the remainder of the 1980 season. Conidia catches from rainwater traps were low at all times, never exceeding 90 conidia per milliliter. Rainwater from petioles and twigs contained slightly more conidia than did rainwater from infected leaves. The highest number of conidia found in aerial leaf traps was four conidia per milliliter.

Daily airborne conidia. Spore dispersal was first recorded with the Burkard spore trap on 25 March, when a total of four spores was observed for the entire day. Spore catches did not become appreciable until late April. Trapping continued through early December when spore catches had again decreased to a minimum. Although aerial spore concentrations were minimal after leaf fall, they never reached zero. Apparently, the spore release period for *F. effusum* in middle Georgia in 1980 started in mid-April and continued through late November. Although conidia were often found singly on spore trap tapes, clumps, short chains, and even multibranched chains were often observed (Fig. 3).

Relationship of rainfall to spore catches. Daily airborne spore concentration decreased just before and during moderate to heavy rain periods (Fig. 1, 22–23 May, 18–19 June, 24 August, 28–29 September, 27 October). Spore catches often increased immediately following light rain showers (Fig. 1, 24–25 July, 24–25 August, 10–11 September, 19–20 October) and during prolonged intermittent rainy periods (Fig. 1, 18–25 June, 17–22 September). When intermittent rain periods continued for some time, as in the two examples above, spore catches tended to peak during the first few days and then gradually decreased. Spore catches gradually tapered off after light rains of 23 May to 19 June until it approached the minimal spore release found during the pre- and post-growing season periods. Very few spores where caught during prolonged periods of drought.

Relationship of spore catches to leaf wetness. Periods of 8–10 days, with ≥4 hr of leaf wetness per day, coincided well with peaks of spore release, which were initially high then tapered off (Fig. 1, 16–29 June, 20–28 July, 8–20 August). During a prolonged period of 22 days with ≥12 hr leaf wetness per day, air spore concentration was initially high then decreased (Fig. 1, 14 September to 6 October). Days with ≤4 hrs of leaf wetness coincided well with low air spore concentration index (≤400).

Relationship to relative humidity. Effect of relative humidity could not be completely separated from rainfall. Days with 23–24 hr of relative humidity >90% often coincided with moderate to heavy rain shower activity (Fig. 1, 24 May, 29–30 September, 29 October). During these periods the air spore concentration index was relatively low (<500). In one case relative humidity >90% lasted for 24 hr and was accompanied with light rainfall <0.1 cm (Fig. 1, 30 October). In this exceptional case the spore concentration index was high, 2,062 spores per square millimeter of trapping surface per hour. Days with ≤2 hr relative humidity

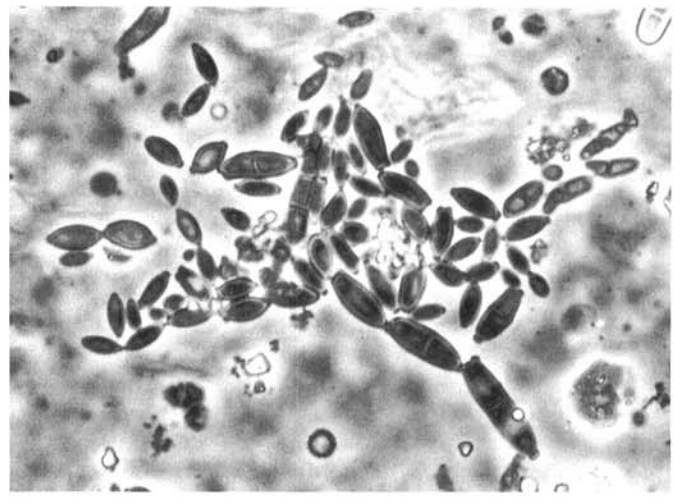

Fig. 3. Branched chain of *Fusicladium effusum* conidia partially broken on impact with spore trap tape surface demonstrating that entire branches of conidia are dislodged from the conidiophore and dispersed. Stained with acid fuchsin/lactophenol. Phase-interference contrast microscopy (×400).

>90% had spore concentration indexes of <500, except in a single case (Fig. 1, 27 October), when the air spore concentration index was 1,487 spores per square millimeter of trapping surface per hour. During this day, a small trace of rain was also recorded. Maximum air spore concentration coincided with periods during which relative humidity remained >90% for 10–18 hr per day for several days in a row.

Relationship to temperature. Air spore concentration did not become substantial until daily minimum/maximum temperatures rose above 10/20 C in early spring. Spore concentration diminished to minimal levels again in late fall when daily minimum/maximum temperatures fell below 5/15 C. Temperatures above 35 C during midseason combined with drought conditions, which resulted in decrease in the number of viable lesions, appeared unfavorable to spore discharge (Fig. 1, 3–22 July).

Hourly airborne conidia. Four separate days were chosen to demonstrate the hourly effects of several environmental parameters on air spore concentration (Fig. 4A to D). Hourly spore concentration began to increase at sunrise each day when an increase in temperature and corresponding drop in relative humidity caused a rapid decrease in leaf wetness. If the decrease in leaf wetness was abrupt, as it was on most days with no rain, a dramatic increase in hourly spore concentration occurred, which often represented the daily peak (Fig. 4B and D). A more gradual decrease in morning leaf wetness gave rise to a more gradual increase in hourly spore concentration. A major nighttime spore peak was seen only once (0400 hours, 20 May) when an unusual 2-hr period of wind gusts caused a temporary rapid decrease in leaf wetness.

Rainfall was found to cause an immediate decrease in hourly spore catches, however spore catches often increased dramatically during drying periods between intermittent rain showers (Fig. 4A) and drying periods following rain shower activity (Fig. 4C).

There was no direct association between air movement and hourly spore catches. The bulk of spore release did occur while there was some air movement, but numerous spores were also caught during periods of relatively still air. Rapid increases or decreases in air movement bore no relationship to hourly conidia catches, except on the night of May 20 as described above.

Hourly spore catches charted for 47 consecutive days (14 April–30 May) demonstrated the presence of a diurnal peak of spore discharge. More than one peak was sometimes observed as was a shift of the peak or peaks from one end to the other of the daylight period in response to rainfall and drying periods.

DISCUSSION

The Byron orchard and surrounding areas of middle Georgia experienced a severe drought in 1980. Drought stress appeared in the orchard in mid-June and intensified throughout most of the remainder of the growing season. This probably accounts for the decline in numbers of sporulating scab lesions observed from 20 June to 10 July and from 20 July to 20 September. *F. effusum* has a 7–14 day latent period for symptom development (6), so up to 2 wk may be required for the effects of hot, dry weather and associated decrease in air spore concentrations to be reflected in reduced numbers of sporulating lesions. We observed a gradual decrease in viability of lesions during mid-June. Many lesions dried, cracked, and fell out of the leaf blade leaving a shot hole appearance. The showers in late June may have been responsible for the brief resurgence of disease in mid-July. In late September, the weather conditions returned to those thought to be conducive to disease increase. The amount of disease increase observed, however, was relatively slight. This may be due to a reduction in susceptibility of leaves as they age as suggested by Nolen (8) and Latham (7).

Conidia catches in rainwater-funnel traps from overwintering

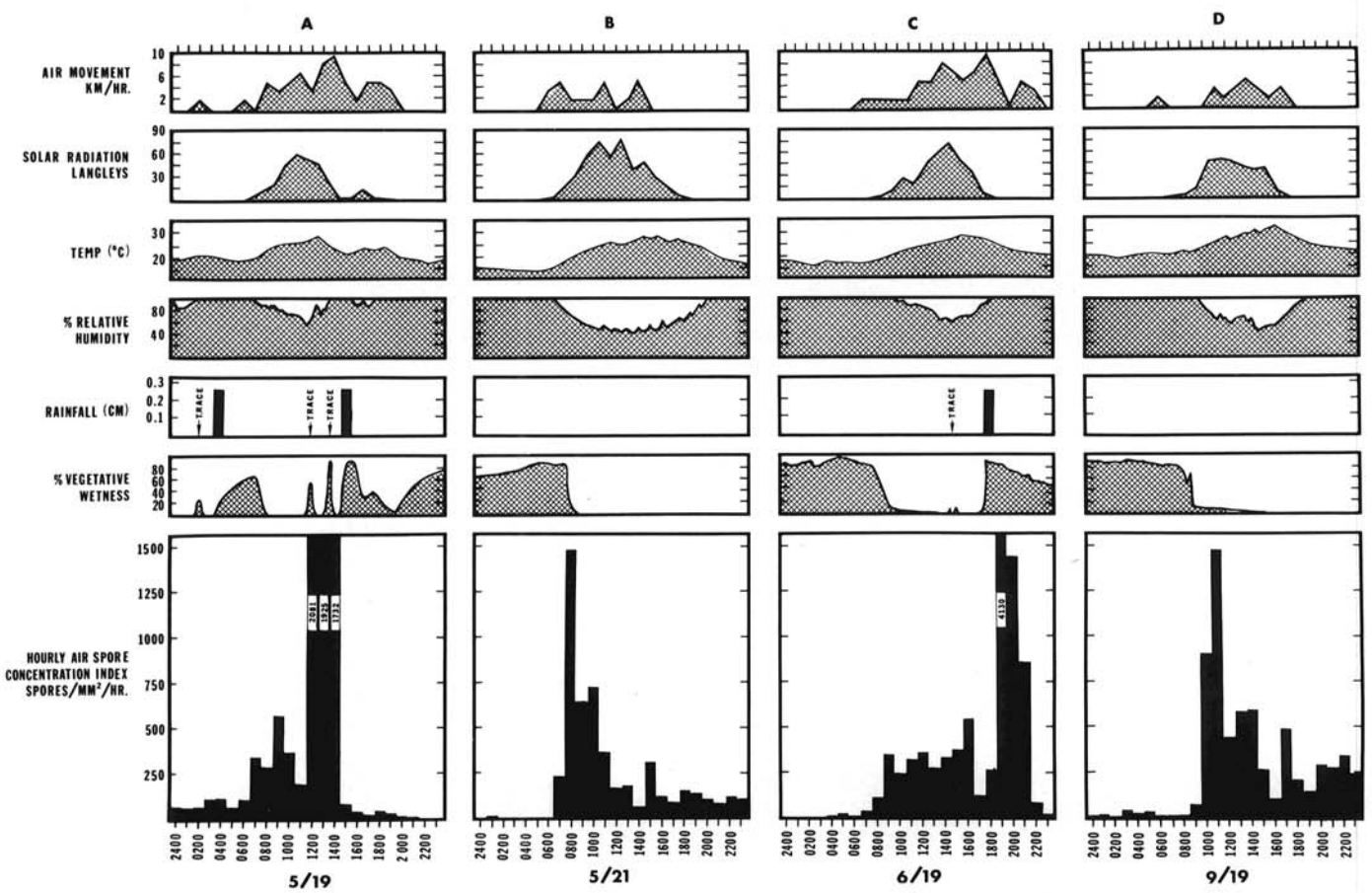

Fig. 4. Hourly concentration of airborne conidia of *Fusicladium effusum* as related to vegetative wetness, rainfall, relative humidity, temperature, solar radiation, and wind speed. Notice the depression of conidial concentration during rain shower activity, 6A and 6C; and peaks of concentration following rapid decreases in vegetative wetness, 6B and 6D.

stromata on twigs and petioles were very low after mid-July. Spore catches in rainwater collected in aerial funnel traps from inoculated foliage were even lower (<5 spores per milliliter). Bertrand and English (1) collected from 2.7×10^5 to 6.1×10^{10} conidia of *Cytospora* per milliliter in similar water traps indicating effective water dispersal for that fungus. Although rainwash of conidia may play a role in localized dispersal of *F. effusum* inoculum within the canopy of a single tree, it appears contrary to previous belief, to be much less effective than air dispersal and of very limited significance as a long-range dispersal mechanism.

Rainfall reduced the air spore concentration of *F. effusum* conidia and it was not until the foliage dried that air spore concentrations again increased. Pady et al (11) found that *Cladosporium herbarum*, which closely resembles *F. effusum* morphologically, also release the maximum number of spores following a rapid decrease in relative humidity. Leach, in his studies of *Drechslera* and *Pyricularia* (8,9), also found that decreasing relative humidity stimulated an increase in spore discharge. Converse believed that *F. effusum* was not air dispersed because of laboratory experiments in which he held temperature and relative humidity constant (2,3). Had he been able to rapidly manipulate either relative humidity or vegetative wetness, he likely would have observed considerable discharge.

The key factor of spore dispersal of *F. effusum* in the orchard was a period of rapid decrease in vegetative wetness. Rapid drying of dew or rainwater on the phylloplane was consistently followed by a rapid increase in the concentration of *F. effusum* conidia in the air (Fig. 4 A to D). More gradual decreases in vegetative wetness were followed by much more gradual increases in hourly spore catches and fewer total spores caught (Fig. 4C). Intermittent showers with intervening rapid-drying periods gave rise to high aerial conidia concentration for periods of up to 3 hr (Fig. 4A); however, each successive spore catch was less than the previous. Under normal conditions, a single drying period gave rise to relatively heavy aerial conidia concentration, which diminished rapidly (Fig. 4B and D). Therefore, it is decreasing vegetative wetness following rain or dew and not rainfall itself as previously thought that causes increases in aerial conidia concentration of *F. effusum*.

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