Influence of Temperature, Lesion Water Potential, and Cyclic Wet-Dry Periods on Sporulation of *Cercospora arachidicola* on Peanut

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ABSTRACT

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Sporulation (conidia per millimeter of lesion area) of *Cercospora arachidicola* on peanut (*Arachis hypogaea*) leaflets incubated under 100% RH was greatest at 24 and 28 C, intermediate at 20 C, and least at 16 or 32 C. Sporulation decreased as the water potential of lesions decreased from −0.05 through −6.0 MPa. In cyclic wet/dry regimes of 24/0, 20/4, 16/8, 12/12, 8/16, 4/20, or 0/24 hr, in which the wet period was at saturation and dry period was at 75% RH, sporulation increased with increased wet period. Under well-watered conditions for plants, dawn or midday water potentials of lesions were significantly lower than adjacent healthy tissue. Diffusive resistance was higher and transpiration was lower for leaves with lesions than for those without lesions.

Additional key words: Cercospora leaf spot, early leaf spot.

Early leaf spot of peanut (*Arachis hypogaea* L.), caused by *Cercospora arachidicola* Hori, is an important disease of peanut wherever the crop is grown. Early leaf spot epidemics are favored when minimum daily temperatures exceed 19 C and the period of relative humidity 95% per day is longer than 6 hr (6). In aeromycology studies large catches of conidia in spore traps generally were preceded by several days with periods of warm temperatures and high relative humidity (8,9,13). However, the role of temperature and moisture in regulating conidial production has not been well defined.

In a preliminary report, Gobina and Melouk (5) indicated that 30 C is the optimal temperature for spore production on detached peanut leaves incubated under 100% relative humidity, although they did not quantitatively define the influence of temperature on sporulation. Melouk and Ketring (10) reported abundant sporulation of *C. arachidicola* on detached peanut leaves incubated at 100% RH and no sporulation from those incubated at 90% RH. However, it is not known if peanut leaf water status influences sporulation of *C. arachidicola*, or vice versa, or if lesion tissue equilibrates with ambient atmospheric moisture levels. Disruption of leaf water status during fungal sporulation is well documented in other host-pathogen interactions (2).

The objectives of this study were to determine the influence of temperature, lesion water potential, and cyclic wet-dry periods on sporulation of *C. arachidicola* and to examine the influence of lesions induced by *C. arachidicola* on peanut leaf water status.

MATERIALS AND METHODS

Production of lesions. An isolate of *C. arachidicola* obtained from naturally infected peanut leaves, collected from a field near Lewiston, NC, in 1985, was used throughout this study. The fungus was maintained on 1- to 2-mo-old peanut plants (cultivar NC2) grown in 15-cm-diameter clay pots in a greenhouse.

For inoculation, 1- to 2-mo-old peanut plants (cultivar NC2) were sprayed to near runoff with a suspension of conidia (1×10^4 conidia per milliliter) of *C. arachidicola*, then enclosed in plastic bags to maintain high humidity, and incubated in a controlled temperature chamber at 24 C. The chamber was housed in a greenhouse such that plants received indirect daylight. The bags were opened daily for misting to maintain high humidity during the first 4 days and were removed after 2–3 wk. After an additional 2–3 wk, leaves with lesions were removed, rinsed under running tap water, and blotted dry. Peanut leaf disks containing a 3–4 mm diameter lesion (lesion disks) were excised with a 5-mm-diameter cork borer immediately before use in controlled temperature or moisture studies. Preliminary studies indicated that, following 3 or 4 days' incubation under near saturated humidity at 24 C, sporulation from lesions on leaves attached or detached from plants, or from lesion disks, were similar.

Quantification of sporulation. Sporulation was quantified as conidia/lesion area of three lesion disks (a replicative unit comprised three disks). Lesion disks supporting conidia were placed in vials (three disks per vial) containing 2 ml of water amended with 0.05 ml Tween 20 (polyoxyethylenesorbitan, monolaurate) per 100 ml of water. Conidia were dislodged by rapid stirring with a microstirbar (Fisher Scientific, Raleigh, NC) for 1 min, and numbers of conidia per vial were based on an average of six hemacytometer counts. Lesion area was calculated from lesion radius using the equation for the area of a circle.

To quantify the sporulation on lesion disks with respect to time, 120 lesion disks were removed at random from peanut plants, placed on moist filter paper (Whatman No. 2) in petri dishes (three disks per dish), and incubated under continuous fluorescent light (6.5 $\mu E/m^2/sec$) at 24 C. At daily intervals after incubation (through 10 days) conidia from lesion disks were counted and lesions areas were measured. The experiment was arranged in a completely randomized design with four replications. The experiment was conducted three times and analysis of variance was conducted on pooled data.

Effect of temperature on sporulation. Sixty lesion disks were excised from leaves collected at random from three peanut plants. Disks were placed in petri dishes containing moist filter paper (Whatman No. 2) and incubated at 16, 20, 24, 28, or 32 C. After 4 days, conidia were counted and lesions were measured. The experiment was arranged in a completely randomized design with four replications. The experiment was conducted twice and analysis of variance was conducted on pooled data. The experiment also was repeated using lesions from naturally infected

leaves collected from field plots near Lewiston, NC.

Effect of water potential on sporulation. Molten 2% water agar, amended to various water potentials with NaCl, was poured into 9-cm-diameter petri dishes; before solidification one-half of the dishes were inverted. Lids containing solid agar were placed over bottoms containing agar. Lesion disks were positioned on 1- × 3-cm metal screens in a 5-mm gap between upper and lower agar slabs. Screens were bent at the ends to support disks above the lower agar surface, permitting isopiestic adjustment of lesion water potential (1). Water potentials of the agar were adjusted to −0.05, −1.0, −2.0, −4.0, and −6.0 MPa, respectively (corresponding to 99.9, 99.3, 98.6, 97.1, or 95.7% RH, respectively), based on data of Robinson and Stokes (12). Sixty lesion disks were excised from leaves collected at random from three plants, mixed, and distributed at random among dishes (three disks per dish). After 4 days at 25 C, sporulation was quantified. The experiment was completely randomized in design with four replications.

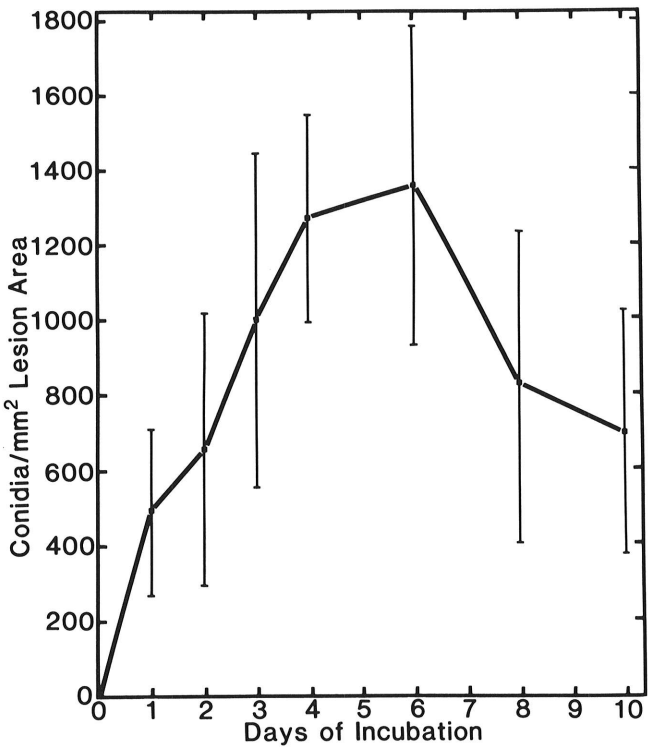

Fig. 1. Sporulation of *Cercospora arachidicola* in excised lesions from NC 2 peanut leaves incubated at 24 C under 100% RH. Means and standard deviations were based on four replications.

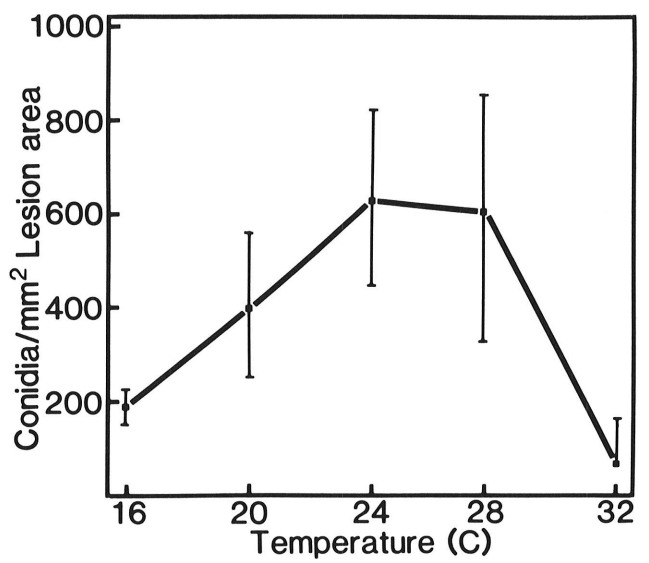

Fig. 2. Effect of temperature on sporulation of *Cercospora arachidicola* in excised lesions of NC 2 peanut leaves incubated for 4 days. Means and standard deviations were based on four replications.

Effect of cyclic wet-dry periods on sporulation. Two cyclic wet-dry experiments were conducted. In the first, lesion disks were placed under a 3-day wet/dry regime of 24/0, 20/4, 16/8, or 12/12 hr in which the wet period was at saturation and dry period was at 75% RH. In the second, lesion disks were placed under an initial 24-hr wet period followed by a 3-day wet/dry regime of 4/20, 8/16, 12/12, or 0/24 hr in which the dry period was at 75% RH. Within each experiment there were four replications per treatment.

Lesion disks were placed on moistened filter paper for wet period treatments. For dry period treatments lesion disks were placed in small screen cages (three disks per cage) and enclosed in a 1.6-cm-diameter drying tube. An air flow of 50 cm^3 sec^{-1}, generated by an aquarium pump, was bubbled through a series of flasks containing saturated NaCl solution and then across leaf disks in the drying tube. The time required to reach equilibration of the lesion disks was determined by weighing leaf disks to within 0.1 mg after various durations within the system. Conidia per square centimeter of lesion area was determined daily over the 3-day experimental period and used to calculate conidia per square millimeter of lesion area per day.

Determination of leaf water status. Water potentials of leaf disks or lesion disks from 2- to 3-mo-old plants were determined with a dew point hygrometer (Wescor MJ 55 microvoltmeter and C-51 sample chambers; Wescor, Inc., Logan, UT). Leaf or lesion disks were placed in chambers and equilibrated at 24 C until constant readings were obtained (2 hr). Filter paper disks saturated with NaCl solutions of known molalities were used to prepare a standard curve for the hygrometer and to check the instrument before and after disk readings. Water potential readings of leaf and lesion disks were taken at dawn and midday on each of 10 days.

Diffusive resistance and transpiration were measured with a Li Cor LI-1600 steady-state diffusive resistance porometer (Li Cor, Inc., Lincoln, NE). Readings were taken at midday on leaves of five plants (2- to 3-mo-old) with lesions and five plants without lesions. Plants with lesions were inoculated 3–5 wk previously.

RESULTS

Sporulation under 100% RH. Conidia per square millimeter of lesion area increased with increasing duration of wet period through day 6, then declined through day 10 (Fig. 1). An increase in aerial mycelia and germinated conidia was observed on stromata after 6 days and appeared to coincide with the decreased conidial numbers obtained from leaf washings.

During the course of experimentation, several hundred lesions, collected from field or greenhouse sources, were examined for the presence of conidia, using a dissecting microscope. In every case where sporulation was observed, conidia developed on stromata that emerged through the cuticle, external to the leaf epidermis. In addition, we did not observe a tearing-away or gross rupturing of the epidermis around erumpant stromata.

Effect of temperature on sporulation. Conidial production increased from 16 to 24 C and declined sharply between 28 and 32 C (Fig. 2). A similar response was observed when the experiment was repeated using lesions collected from a naturally infected field plot at Lewiston.

Effect of water potential of sporulation. Sporulation in lesions was greatest near moisture saturation but declined with decreasing water potential to −4 MPa (Fig. 3). Conidia were not detected at −6 MPa. A similar response was observed on lesions collected from naturally infected field plots at Lewiston.

Effect of cyclic wet-dry periods on sporulation. Lesion disks placed in drying chambers equilibrated within 1 hr (Fig. 4). Leaf disks without lesions placed in drying chambers lost water at a slower rate than did lesion disks.

Data from the two cyclic wet-dry experiments were combined and analyzed by linear regression analysis. Rate of conidial production per day was directly porportional to duration of wet period (Fig. 5). Maximum rate of sporulation occurred under the

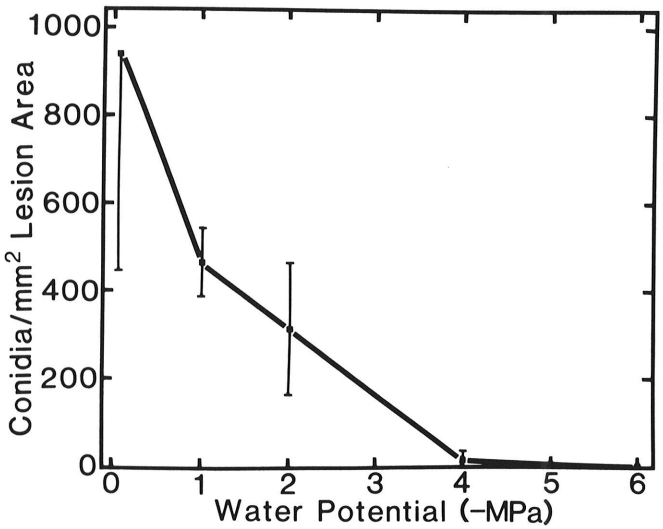

Fig. 3. Effect of water potential on sporulation of *Cercospora arachidicola* in excised lesions. Means and standard deviations were based on four replications.

24-hr wet period per day.

Influence of lesions on host-water status. Mean and standard deviation of leaf and lesion water potentials taken at dawn were -0.86 ± -0.07 and -2.22 ± -0.35 MPa, respectively. Mean and standard deviation of leaf and lesion water potentials taken during midday were -0.98 ± -0.12 and -2.81 ± -1.60 MPa, respectively.

Mean and standard deviation of midday leaf and lesion diffusive resistance were 0.35 ± 0.05 and 0.71 ± 0.03 sec cm^{-1}, respectively. Mean and standard deviation of leaf and lesion transpiration were 0.27 ± 0.08 and 0.48 ± 0.05 μg cm^{-2} sec^{-1}, respectively.

DISCUSSION

Field studies (7) indicated that temperatures at or below 16 C are not favorable for early leaf spot epidemics. We observed poor sporulation at 16 C, which is consistent with these observations. Although we observed poor sporulation at 32 C, temperatures this high generally do not occur in the peanut production areas of North Carolina during late evening or early morning hours when humidities are sufficiently high to support development of *C. arachidicola*. Temperatures at or lower than 16 C, however, can occur and may limit spore production.

Humidity in excess of 95% is required for germination and infection of peanut leaves (1). Once the fungus is inside leaves, its growth would be regulated by internal leaf water potentials. Leaf water potentials of water-stressed peanut plants (3,4,11) are within the 0.0 to -0.8 MPa range required for growth of *C. arachidicola* (1). Sporulation, however, required higher leaf water potentials than that required for fungal growth (1). By the time stromata have matured, lesion water potentials (under well-watered conditions for the plant) are below that required to support abundant sporulation, unless external relative humidities are in excess of 98%.

We did not observe a decreased diffusive resistance as has been observed with fungi such as rusts (2), which disrupt the cuticle during sporulation. *C. arachidicola* sporulated on stromata that emerged through the epidermis of necrotic leaf tissue without disrupting the cuticle, suggesting that *C. arachidicola* does not disrupt the cuticle sufficient to significantly lower diffusive resistance. This could be advantageous to *C. arachidicola* from the standpoint of maintaining conditions favorable for development and maturation of stroma.

Excised lesions lost water at a greater rate than surrounding tissue. On intact leaves, water potential of lesions was lower than surrounding healthy tissue but higher than would have occurred if the lesions had equilibrated with ambient humidities. Relative humidities at midday, when water potentials were taken, were 40–80%, indicating that lesion tissue had not equilibrated with low

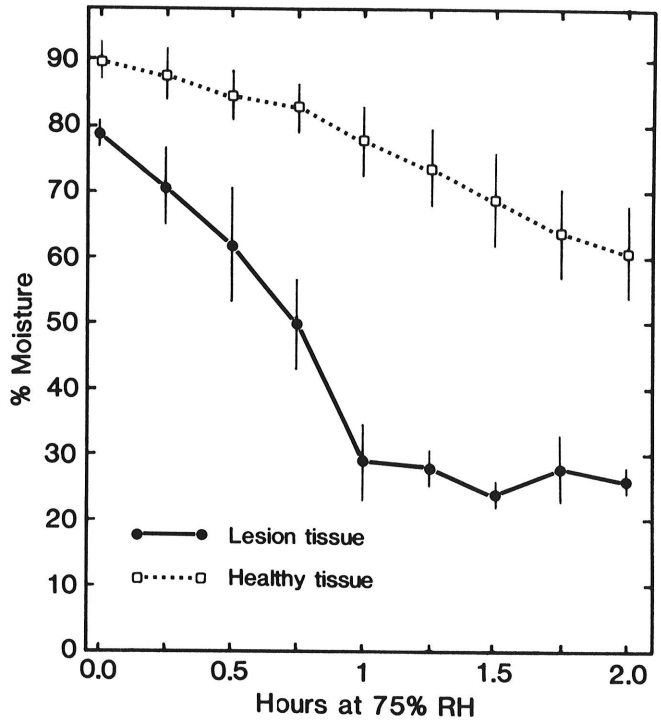

Fig. 4. Effect of duration of incubation at 75% RH, following exposure at 100% RH, on moisture content of peanut leaf tissue with or without lesions caused by *Cercospera arachidicola*. Means and standard deviations were based on four replications.

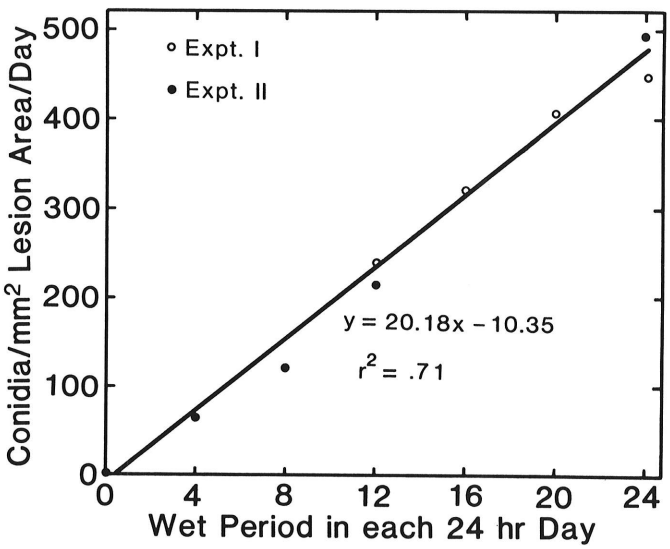

Fig. 5. Influence of wet period duration per day on rate of sporulation of *Cercospora arachidicola*. Rates were calculated from conidia collected over three consecutive days. Regression is based on pooled data from two experimental runs (experiment I and experiment II).

ambient humidities. Relatively high leaf and lesion water potentials probably are maintained by movement of water from adjacent healthy tissue, a continuous cuticle across leaf and lesion tissue (including stromata), and increased diffusive resistance in lesions.

Conidia developed in response to high humidity conditions. Relatively high lesion water potentials may be advantageous to *C. arachidicola* in maintaining varying stages of conidial development in between periods of dew or high humidity. Conidia in varying stages of development were observed on lesions collected on 10 separate occasions on naturally infected leaves at Lewiston during 1985.

Forecasting of peanut leaf spot is based on a running 2-day average of duration of RH > 95% and of minimum temperature

(7). We observed that periods of high (98%) relative humidity RH or dew periods less than 8 hr in duration supported less than 25% maximum rate of sporulation. Duration of leaf wetness or dew greater than 8 hr at favorable temperatures may be more influential in supporting abundant spore production than periods of high RH.

In the peanut forecasting model of Jensen and Boyle (7), periods of RH > 95% for less than 4 hr and minimum temperature during the high RH period < 16 C are defined as unfavorable for leaf spot development. Our results suggest that such conditions would support few conidia, thus the lower limits of RH and temperature, as defined by Jensen and Boyle (7), could be explained in terms of decreased sporulation under these conditions. However, a mean temperature, rather than minimum temperature, as suggested by Jensen and Boyle (7), may offer greater precision in defining conditions favorable for leaf spot.

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