

Influence of Temperature and Moisture on Germination and Germ Tube Elongation of *Cercospora arachidicola*

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ABSTRACT

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Conidia of *Cercospora arachidicola* atomized onto peanut leaves and incubated in dew chambers at 16, 19, 22, 25, 28, or 31 C began germinating after 2 hr; maximum germination (82–85%) occurred by 24–48 hr at 16–25 C. Germ tube elongation increased linearly up to 48 hr; germ tubes were longest at 22 C and shortest at 16 C. On glass slides coated with a chloroform extract from peanut leaves (coated slides), germination and germ tube elongation were similar to those on leaf surfaces at corresponding temperatures. On coated slides, germination declined rapidly as relative humidity (RH) was reduced from 100 through 98%, although some

germination occurred at 94.5% RH after 4 days at 24 C. Germ tubes grew to a greater extent at 100% RH than under free water. In wet-dry-wet postinoculation regimes, with the dry period at various humidities, germ tube elongation declined with declining RH. Under conditions favorable for germ tube growth, 95% of germinated conidia penetrated stomata after 2–12 days and lesions appeared 2–8 days later. After 6 days, a mean of four germ tubes per conidium was present, although an increase in the number of germ tubes did not result in a corresponding increase in the proportion of conidia that penetrated.

Additional key words: *Arachis hypogaea*, early leaf spot.

Cercospora arachidicola Hori causes early leaf spot of peanut (*Arachis hypogaea* L.), a disease that under conditions of prolonged warm temperature and high (>95%) relative humidity (RH) can result in extensive defoliation and significant yield loss (6,12). Prolonged periods of high moisture and moderate temperature are known to favor disease development (12), but the influence of moisture and temperature on conidial germination and germ tube elongation of the pathogen are not clearly established.

Conidia of *C. arachidicola* germinated at near-saturated to saturated humidity conditions at 25–30 C within 3–6 hr on leaf surfaces (1,11) or on glass slides (16). The optimal temperature range for germination was reported to be 20–30 C (16), with very little germination above 30 C. Gobina and Melouk (7) reported that after 48 hr in a water suspension at 15–35 C, germination of conidia was not significantly different. Jenkins (11), however, reported that conidia germinated in a thin film of water but not when immersed in water. Oso (16) observed germination of *C. arachidicola* on glass slides at humidities as low as 96.5%.

Abdou et al (1) reported that germ tubes grew on leaf surfaces for about 4 days before showing stomatal tropism or before appressoria were initiated. Although germination of conidia was similar on leaves of susceptible, resistant, and immune peanut

genotypes, attraction of germ tubes to stomata differed among genotypes 4 days after inoculation. The prolonged period between germination and penetration suggests that germ tubes of *C. arachidicola* can withstand periods of leaf dryness. Germ tubes resistant to dry periods have been noted for such fungi as *Alternaria porri* f. sp. *solani* Neerg. (4), *Cercospora musae* Zimm. (8), and *Stemphylium botryosum* f. sp. *lycopersici* (R. C. & W.) (4); *C. arachidicola* may have a similar tolerance to dry conditions.

The objectives of this study were to determine the influence of temperature, humidity, and leaf wetness duration on germination and germ tube elongation of *C. arachidicola*.

MATERIALS AND METHODS

Inoculum production. An isolate of *C. arachidicola* obtained from naturally infected peanut leaves collected from Lewiston, NC, in 1983 was used throughout this study. The fungus either was maintained in a greenhouse on 1- to 2-mo-old peanut plants (cv. Florigiant or NC2) grown in 15-cm-diameter clay pots or was stored as dry conidia in test tubes at 5 C for up to 5 mo before inoculation of plants. For inoculation, plants were sprayed with a conidial suspension (about 1×10^4 conidia per milliliter), enclosed in plastic bags to maintain near-saturated humidities, and incubated under a greenhouse bench (22–30 C) or in a growth chamber at 24 C. The growth chamber had glass doors and was positioned within a greenhouse so that plants received indirect daylight. Bags were removed after 2 wk.

Three to 4 wk after inoculation, leaves with lesions were

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removed, rinsed in water, blotted dry, and placed in 9-cm-diameter petri plates containing moistened tissue paper. Plates were incubated for 2–3 days at 22–25 C under continuous fluorescent light (6.5 W m^{-2}). Conidia were collected dry (2) from sporulating lesions, suspended in distilled water (about 1×10^4 conidia per milliliter) containing Tween 20 (2 drops/100 ml), and used to inoculate plants.

Inoculation and observation of leaves. Experiments were conducted with 1- to 2-mo-old peanut plants (cv. NC2) grown in 15-cm-diameter pots or with leaf petiole cuttings (from the third or fourth node from the terminus) from similar plant material. Leaf cuttings were rooted in test tubes containing Hoagland's solution (14). The upper surface of each leaflet was inoculated by means of an artist's airbrush at about 0.6 kg/cm^2 air pressure. In all experiments, approximately 25–50 conidia were applied to each leaflet immediately after collection.

After the specified treatments, inoculated leaflets were detached and the midrib excised. Leaflets were fixed in FAA (formalin:50% ethanol:glacial acetic acid, 1:18:1, v/v) and stained with 0.01% trypan blue in 0.05% lactic acid, and the upper surface was examined under a light microscope. All conidia (25–50) on each leaflet were included in germination and germ tube assessments. Germ tube lengths were measured with an ocular micrometer. Conidia adhered tightly to leaf surfaces; less than 2% washed off during fixation and were not counted in assessing germination. All experiments were conducted at least three times.

Preliminary studies indicated that conidia applied dry to leaf surfaces, in suspension, or in suspension and dried onto the leaf germinated similarly when placed in dew chambers at 24 C (*unpublished*). In all subsequent inoculations, conidia were applied in suspension and dried onto leaf surfaces under a fan before being placed in dew chambers.

Influence of temperature and dew period on conidial germination and germ tube elongation. Peanut leaf cuttings (cv. NC2, NC8C, or Florigiant) were inoculated and placed in dew chambers at 16, 19, 22, 25, 28, or 31 C (12 leaves per chamber). After 0, 2, 4, 6, 8, 12, 16, 24, 32, or 48 hr, a leaflet from each of four separate leaves per chamber was removed at random and examined for conidial germination and germ tube production on the upper surface. Because only three dew chambers were used, treatments were divided into two groups that were run in series. Treatments were assigned at random to the groups and chambers.

The dew chambers, measuring $50 \times 25 \times 25$ cm and built of aluminum, were mounted over $62 \times 25 \times 25$ cm water tanks. A heater-stirrer unit, mounted outside each chamber, maintained water temperature within 0.5 C. The dew chamber units were operated in a cold room at 4–5 C. Dew was visible on leaves at 16–32 C within 20–30 min of placement in the chambers. The chamber cover was divided into three close-fitting sections to permit access to the chamber with minimal disturbance of the system. During addition or removal of plants, temperature within the chamber dropped less than 5 C and reequilibrated within 5 min. All temperature measurements were taken with a mercury thermometer at leaf level. Leaves were positioned equidistant from the chamber walls.

Influence of relative humidity and interrupted dew period on conidial germination and germ tube elongation. Twelve peanut plants (cv. NC2) were inoculated with a conidial suspension of *C. arachidicola* and incubated at 22–24 C under 100, 95–100, or 93–97% RH. Saturated (100%) humidity was achieved by misting plants in plastic bags twice a day with distilled water. Bags were also used to create 95–100% RH conditions but without misting. Humidifiers were used to maintain 93–97% RH in the incubation chamber. Humidities in the chamber were monitored with a 7-day recording hygrothermograph. Humidities within plastic bags were measured with a Bendix psychrometer (Bendix Environmental and Processing Instruments Division, Baltimore, MD). After 48 hr, four leaflets at the third or fourth node from the terminus on each of four replicate plants were removed, and germination and germ tube growth were assessed.

To determine the influence of humidity on germ tube elongation during interruptions of the dew period, cuttings were inoculated

and placed in dew chambers at 24 C. After 24 hr, cuttings were removed and placed in a recurring 8-hr dry period followed by 16-hr dew period for 2 days. Dry periods were at low (30–40), medium (65–85), or high (94–98%) humidity. Humidity was adjusted to low levels by passing air through columns containing calcium sulfate, to moderate levels by bubbling air through two flasks of distilled water in series at room temperature, and to high levels by sealing plants in a chamber lined with moistened tissue paper. Airflow through the low and medium humidities was generated by means of small aquarium pumps that created air exchange rates in the chamber of 50 cm^3 per second. Air input and exit ports were at leaf level, and airflow across leaves measured with a hot-wire anemometer was 0.25–2 m per second. Humidities in the chambers during experimental runs were determined with a Bendix psychrometer. The humidity within chambers equilibrated within 15 min after plants were placed in the chambers, as described previously. Additional inoculated plants were maintained under continuous leaf wetness during the 3-day period. After the initial 24-hr dew period, a leaflet from each of four replicate leaves per treatment was removed at the beginning and end of each dry period, and germination and germ tube elongation on the upper leaf surface were assessed.

Influence of temperature and humidity on conidial germination and germ tube elongation on glass slides. Germination and germ tube elongation were assessed on chloroform-extractable peanut leaf material deposited on glass slides. Coated slides were used because germination in preliminary experiments was poorer on plain glass slides than on slides on which leaf extract was deposited. Wax and other leaf materials were extracted from peanut leaflets (1- to 2-mo-old plants, third or fourth leaf from the terminus) for 2 min at 24 C using 1 ml of chloroform per four leaflets, then dried onto a No. 1½ 18 × 18 mm cover glass. In all experiments with coated slides, 5- μ l drops (25–50 conidia per drop) were placed at three sites on each of three replicate slides and air-dried under a fan. All experiments were completely randomized in design and were conducted three times. Germination and germ tube assessments were based on 50 conidia per slide.

To determine the influence of temperature on conidial germination and germ tube elongation, conidia were incubated on coated slides at 100% RH in the dark at 16, 20, 24, 28, or 32 C. After 24 hr, conidia were stained with aniline blue in lactophenol, and germination and germ tube growth were assessed.

The influence of humidity on germination of conidia of *C. arachidicola* was studied using coated slides. Slides were positioned on metal screens in a 5-mm gap between agar slabs located on the top and bottom of 9-cm-diameter petri dishes, a system similar to that described by Harris et al (10). To achieve various humidity levels, the osmotic potential of the agar was adjusted to -2, -4, -6, -8, or -10 MPa (corresponding to 98.6, 97.1, 95.7, 94.4, and 93.0% RH, respectively) using NaCl concentrations, based on data of Robinson and Stokes (17). Plates were sealed with Parafilm and incubated at 16, 24, or 32 C. Concentrations of NaCl were adjusted to provide similar potentials at each temperature. After 24 hr, conidia were stained with aniline blue in lactophenol, and germination and germ tube growth were assessed.

To examine the influence of free water vs. saturated humidity on germination, conidia on coated slides were incubated at saturated (100%) humidity, maintained in a gap between two agar slabs. Free water conditions were created by placing a drop of sterile water over the dried conidia. After 24 hr, conidial germination and germ tube elongation were assessed at 24 C.

Stomatal penetration and lesion induction. Stomatal penetration and lesion induction were examined under conditions favorable for germination and germ tube growth. Peanut plants (cv. Florigiant) were inoculated, placed in plastic bags, and incubated at 24 C under indirect daylight. Plants were misted once daily to maintain near-saturated to saturated humidity. After 2, 4, 6, 8, 10, or 12 days, a leaflet at the third or fourth position from the terminus was removed from each of four plants. Percentages of conidia that germinated, penetrated stomata, and induced lesions on the upper leaf surface were determined. On the sixth day after inoculation, the number of germ tubes per conidium was assessed.

Ten to 50 conidia were present on each leaflet, and all were included in germination and germ tube assessments.

RESULTS

Influence of temperature and dew period on conidial germination and germ tube elongation. At 16–25 C, germ tubes were initiated on leaf surfaces after 2- to 4-hr postinoculation dew periods; maximum germination (82–85%) occurred by 48 hr (Fig. 1). Only 38 and 32% of the conidia had germinated at 28 and 31 C, respectively, after 48 hr. Mean germ tube lengths at 16–31 C increased linearly through 48 hr (Fig. 2). The extent of elongation was greatest at 22 or 25 C and least at 16 C.

Influence of relative humidity and interrupted dew period on conidial germination and germ tube growth. After the 48-hr postinoculation incubation period at 100, 95–100, 93–97, or cyclic 8 hr at 100% and 16 hr at 93–97% RH, mean percentages (and standard deviations) of conidia that germinated were 98 ± 2 , 97 ± 5 , 35 ± 30 , and 64 ± 1 , respectively. The corresponding means (and standard deviations) of the longest germ tube per conidium were 86 ± 5 , 54 ± 10 , 7 ± 1 , and 8 ± 3 , respectively.

Conidia of *C. arachidicola* on plants given a postinoculation 24-hr dew period followed by an 8-hr dry period for 2 days had shorter germ tubes than those maintained under continuous dew (Fig. 3). No increase in length of germ tubes beyond that produced at the initial 24-hr dew period occurred at low (30–40%) humidity. Germ tubes did elongate under moderate- and high-humidity regimes, but little elongation occurred during the dry period of such regimes. At the high-humidity regime, germ tubes appeared to resume growth after the dry period at a rate similar to that under continuous dew.

Influence of temperature and humidity on conidial germination and germ tube elongation on glass slides. Germination was high (84%) when spore suspensions were deposited on wax but was similar when conidia were scraped from sporulating lesions into water droplets on glass slides (82%) or dusted onto glass slides (87%), with or without wax and without subsequent drying, before incubation under saturated humidities.

Germination and germ tube elongation on coated slides were similar to those on leaf surfaces at corresponding temperatures. Means and standard deviations of conidia that germinated at 16, 20, 24, 28, and 32 C were 67 ± 4 , 71 ± 6 , 177 ± 7 , 45 ± 12 , and 18 ± 16 , respectively. Means (and standard deviations) of length of longest

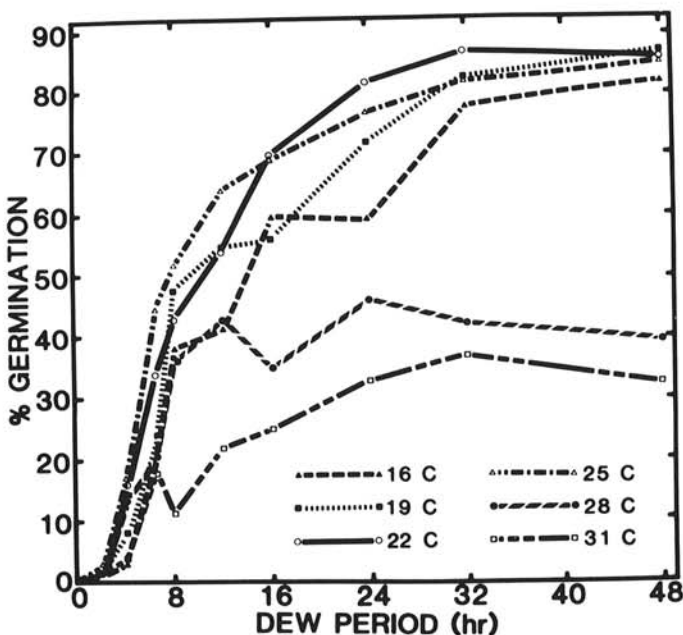


Fig. 1. Influence of postinoculation dew period and temperature on percentage of germination of *Cercospora arachidicola* on peanut leaves. Means were derived from pooled data from three replicate experimental runs.

germ tube per conidium at 16, 20, 24, 28, and 32 C were 26 ± 4 , 31 ± 8 , 41 ± 3 , 30 ± 7 , and 12 ± 10 , respectively.

Germination on coated slides after 3 days of incubation at 16, 24, or 32 C declined with decreasing atmospheric water potentials through -10 MPa (94.5% RH) (Fig. 4). Germination was similar at

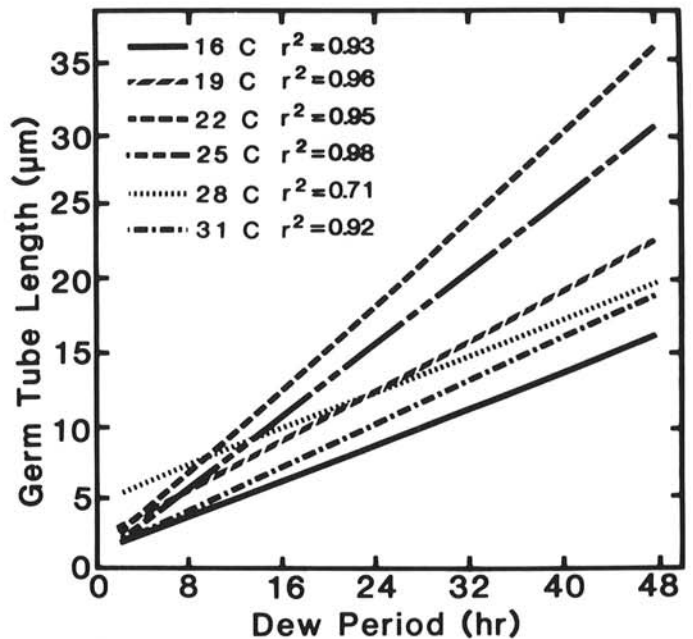


Fig. 2. Influence of postinoculation dew period and temperature on germ tube elongation of *Cercospora arachidicola* on peanut leaves. Germ tube length was based on longest germ tube per conidium. Regression equations for 16, 19, 22, 25, 28, and 31 C were $y = 0.36X + 1.05$, $y = 0.49X + 1.10$, $y = 0.79X + 0.60$, $y = 0.66X + 0.81$, $y = 0.34X + 3.86$, and $y = 0.38X + 0.92$, respectively.

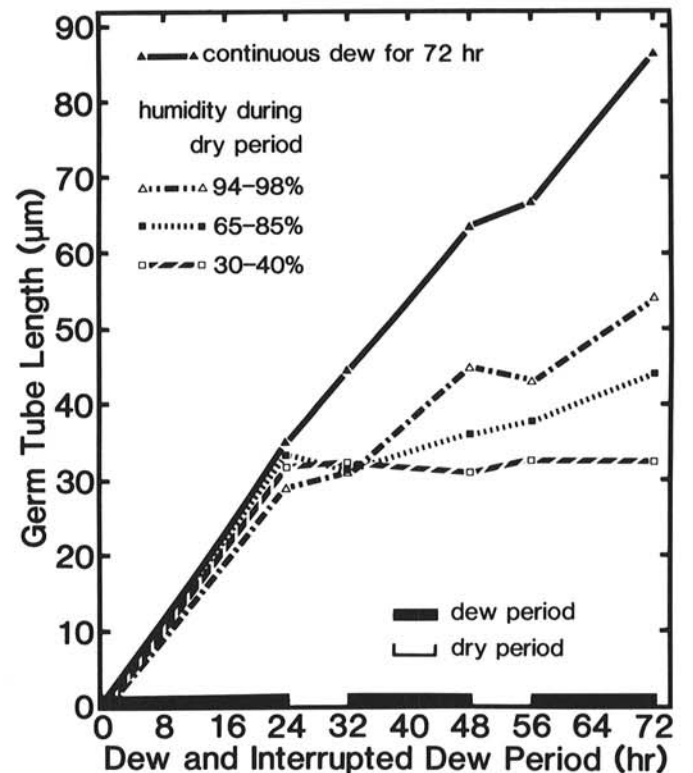


Fig. 3. Influence of an 8-hr dry period at 30–40, 65–85, or 94–98% RH introduced after a 6-hr initial postinoculation dew period and followed by a 16-hr dew period on germination and germ tube elongation of *Cercospora arachidicola* on leaf surfaces. Means were derived from pooled data from three replicate experimental runs. Standard error of mean difference is ± 6.4 μ m.

16 and 24 C and lower at 32 C. Germ tubes were longest at -0.05 MPa; at potentials lower than -4.0 MPa, lengths were similar at 16, 24, and 32 C (Fig. 5).

Germ tubes were longer ($P = 0.05$) at high (100%) RH than with free water, but percentage of germination did not differ. Germination and germ tube lengths were 76% and $3.3 \mu\text{m}$, respectively, under saturated conditions and 69% and $2.2 \mu\text{m}$, respectively, with free water.

Stomatal penetration and lesion induction. Ninety-five percent of conidia on leaf surfaces germinated within 24–48 hr. The percentage of germinated conidia that penetrated stomata

increased sharply after 2 days, reaching 75% after 6 days of incubation (Fig. 6). After 12 days, 95% of germinated conidia had penetrated stomata. A 2- to 8-day delay occurred between stomatal penetration and lesion appearance. After 6 days, the mean (and standard deviation) number of germ tubes per conidium was 4 ± 1.74 . The frequency of germ tube number per conidium followed the Poisson distribution, based on the variance to mean ratio (5). In addition, the frequency of conidia associated with penetration (within classes of germ tube number) also followed the Poisson distribution, based on the variance to mean ratio.

DISCUSSION

Jenkins (11) reported poor germination of *C. arachidicola* when conidia were covered with water. We found that conidia germinated readily on leaves under dew or in water on coated glass slides. Gobina and Melouk (7) also reported that conidia of *C. arachidicola* germinated in water but did not find significant differences in germination after 48 hr at temperatures from 15 to 35 C. We found that germination and germ tube elongation were greatest at cool to moderate (19–25 C) temperatures. Although germination was low at 28–32 C, germ tube elongation continued at a rate similar to that at lower (16–19 C) temperatures. Moderate (19–25 C) night temperatures could favor conidial germination, whereas germ tube elongation could continue during warmer morning or daytime conditions, provided humidities are near saturation.

As reported by Oso (16), we observed germination of *C. arachidicola* under high humidity. Dew or 100% RH was necessary for rapid germination and growth of *C. arachidicola*, but we did not observe stomatal tropism under continuous dew. Germ tubes did show tropism under saturated humidities, as previously reported by Abdou et al (1), but not under free water conditions. Our observation that germ tubes elongate faster under 100% RH than under free water conditions corresponds to reports of stimulation of fungal growth at water potentials of -0.5 to -1.0 MPa (3,9,13,18). It would be advantageous for *C. arachidicola*, a fungus that penetrates via stomata, to have optimal growth during a period when stomata were open.

Jensen and Boyle (12) reported that periods of high (>95%) RH were most favorable for early leaf spot development. High ambient humidities would imply that humidities within a peanut canopy, when leaves are transpiring, would be near 100% at leaf surfaces. Prolonged periods of high humidities would favor stomatal tropism and penetration. Conditions under which dew or rain

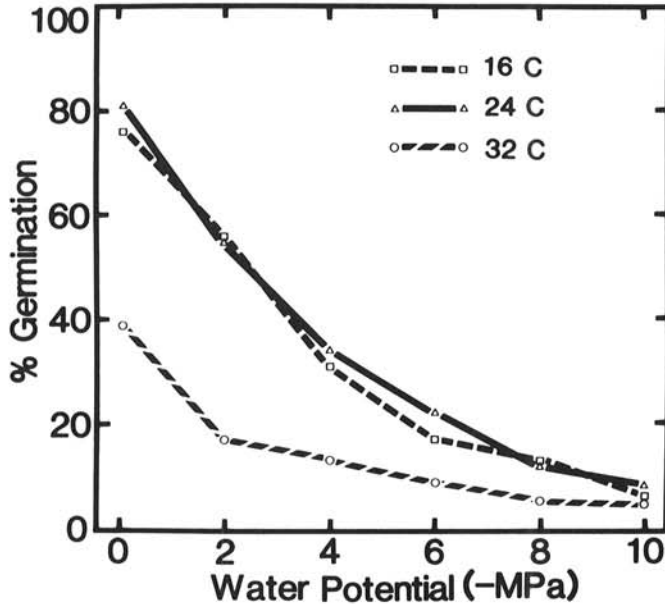


Fig. 4. Influence of temperature and atmospheric water potential on germination of *Cercospora arachidicola* on wax-coated slides at 16, 24, or 32 C. Means were derived from pooled data from three replicate experimental runs. Standard error of mean difference is $\pm 6\%$.

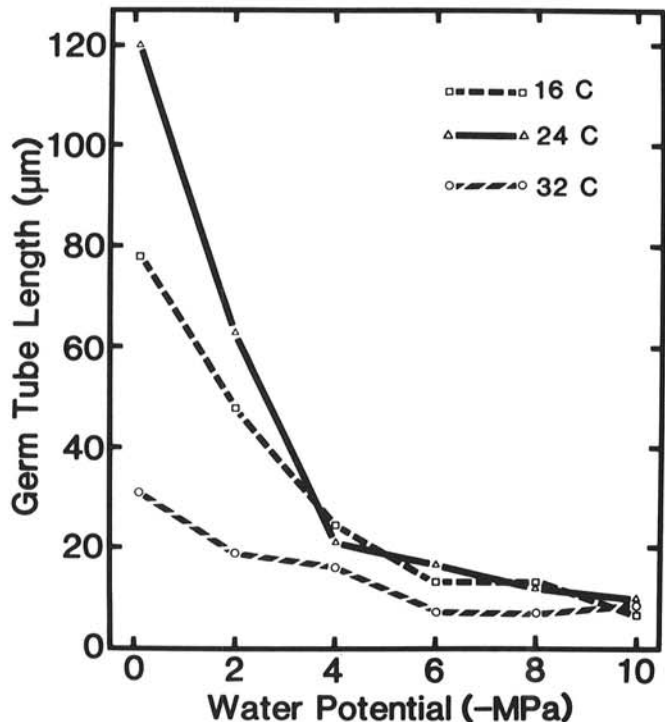


Fig. 5. Influence of temperature and atmospheric water potential on germ tube elongation of *Cercospora arachidicola* on wax-coated slides at 16, 24, or 32 C. Mean values were derived from pooled data from three replicate experimental runs. Standard error of mean difference is $\pm 6.5 \mu\text{m}$.

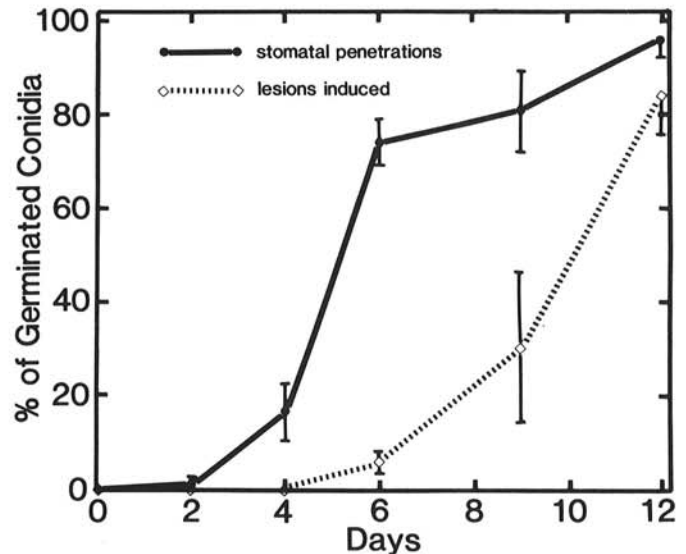


Fig. 6. Percentages of germinated conidia that penetrated and induced lesions after inoculation and incubation under 98–100% RH at 24 C. Means and standard deviations were based on a minimum of 50 conidia observed on four leaflets removed from each of five replicate plants.

evaporated quickly and humidities declined rapidly, on the other hand, would delay growth of germ tubes and stomatal penetration.

C. arachidicola produces one to nine germ tubes, with most having three to five by 6 days after germination. An increase in the number of germ tubes per conidium was not significantly correlated with an increase in the number of penetrations, although we observed multiple penetrations from single conidia. By 12 days after inoculation and incubation (under conditions favorable for germination and germ tube growth), 95% of the conidia that penetrated induced lesions. Thus, multiple germ tubes appear to offer no advantage under prolonged favorable conditions.

Nevill (15) reported an incubation period of 12 days for *C. arachidicola*, which agrees with our observations. Under prolonged favorable conditions, we observed an infection efficiency of 85%, which is considerably higher than the 2% efficiency reported by Nevill (15). *C. arachidicola* requires near-saturated to saturated humidities for growth. Since *C. arachidicola* penetrates via stomata, infection efficiencies could be a function of both humidity and stomatal behavior. A reduction in open stomata in peanut cuttings used by Nevill (15) could account for reduced infection efficiency. We did not observe penetrations when conidia were incubated on leaflets of detached leaves (with petioles in Hoagland's solution) in dew chambers, with or without lights. We did observe penetrations on detached leaves that were rooted in Hoagland's solution and maintained at high humidities after inoculation.

Oso (16) described secondary conidial production in *C. arachidicola* on glass slides under high humidity conditions. We did not observe such a phenomenon, although our use of wax-coated slides may have resulted in germination and germ tube development similar to that on leaf surfaces. We did not observe secondary conidial formation on leaf surfaces under any of our experimental conditions.

The 2- to 4-day delay between germination and penetration suggests that *C. arachidicola* has tolerance to moderate humidity conditions. However, low (40%) humidity during daytime hours could curtail or possibly terminate germ tube elongation and, therefore, infection. Thus, periods of highly unfavorable conditions may be as important in limiting pathogen development as highly favorable conditions are in promoting pathogen development. Understanding the effects of both limits may contribute toward our understanding of early leaf spot on peanut.

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