Effect of Conidia Production Temperature on Germination and Infectivity of *Alternaria helianthi*

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Accepted for publication 3 March 1995.

ABSTRACT

Abbas, H. K., Egley, G. H., and Paul, R. N. 1995. Effect of conidia production temperature on germination and infectivity of *Alternaria helianthi*. Phytopathology 85:677-682.

Conidia of an isolate of *Alternaria helianthi* produced under different temperatures were studied for growth and infectivity on hosts and nonhosts. Infective conidia (5×10^4 conidia per ml) caused blight disease on 2- to 3-week-old hosts, including sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), and common cocklebur (*Xanthium strumarium*), within 24 h of treatment. Disease symptoms included necrosis, stunting, wilting, and mortality of susceptible species. The fungus grew well from 18 to 30°C, but growth was more rapid at 28 and 30°C. More conidia were produced at 18 to 26°C than at 28 or 30°C. Infectivity decreased as conidia production temperature increased. The conidia produced at 28 and 30°C were noninfective on their hosts. Infectivity was greatest when conidia had thick cell walls, high percent germination, and high number of germ tubes. Conidia produced at 18 to 22°C germinated more rapidly than did conidia produced at 26 to 30°C. Regardless of production temperature, germination was greater on host plants than on nonhost plants or filter paper. Histochemical studies showed that conidia produced at lower temperatures stained lightly for lipids and proteins and intensely for polysaccharides. Conidia produced at 28°C were either empty or had gutuoles of lipids and only traces of proteins or polysaccharides. The conclusion drawn was that cellular degeneration at the higher temperatures was responsible for the reduced germination and infectivity.

Additional keywords: Alternaria leaf blight, biological control, mycoherbicide.

Alternaria species are important pathogens of a wide variety of weed and crop species (5,6,8,12,25,29,33,34). *A. helianthi* (Hansf.) Tubaki & Nishihara is a pathogen of sunflower (*Helianthus annuus* L.) species (3,4,23,31). Allen et al. (2) suggested that cocklebur (*Xanthium strumarium* L.), also of the Compositae family, may serve as another host to this pathogen, but they provided no experimental data. This observation was substantiated by Quimby (27) in 1989. It was then postulated that *A. helianthi* had potential as a biocontrol agent for common cocklebur plants. Common cocklebur has some resistance to the conventional herbicides MSMA (monosodium salt of methylarsonic acid) (13,24,35) and imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) (7). Consequently, there is interest in developing *A. helianthi* as a mycoherbicide for control of this important weed.

Temperature is an important factor in the development of disease on sunflower caused by *A. helianthi* (1,17). Islam and Marić (16) showed that higher temperatures, 24 and 27°C, favored the development of Alternaria brown spot compared to 17°C. These results supported field studies that showed that development of disease on sunflower was most severe during August when temperature and precipitation were high (16). Because of interest in developing *A. helianthi* for biological control of common cocklebur in regions where sunflower is not grown, the effect of conidia production temperatures of 18 to 30°C on germination and virulence of the fungus was investigated on host and nonhost plants. Determination of optimum temperatures for efficient production of effective inoculum is important to the development of this organism as a mycoherbicide.

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MATERIALS AND METHODS

Fungal cultures. Cultures of an isolate of *A. helianthi* grown on sunflower-leaf agar were obtained from C. Block, USDA, ARS, Ames, IA. The stock cultures were transferred to a modified sunflower-leaf agar medium and then to skim milk and silica gel media (36) and stored at 4°C for further study.

Fungal growth at various temperatures. To study the effect of temperature on radial growth rate, 4-mm^2 plugs were taken from a stock culture of *A. helianthi* grown on sunflower-leaf agar for 10 to 14 days. These plugs were placed in the center of fresh sunflower-leaf agar medium prepared by the following procedure. Sunflower leaves were collected from 2- to 3-week-old plants of the hybrid NK-285 grown in the greenhouse, seeds of which were obtained from T. Gulya, Fargo, ND. The leaves were air-dried for 3 to 5 days under a ventilated hood, and 25 g of leaves was homogenized with 1 liter of distilled water in a Waring blender at high speed for 5 min. The homogenate was centrifuged for 10 min at 10,000 × *g*, and the supernatant was filtered through a double layer of cheesecloth. The filtrate was combined with 20 g of agar, autoclaved, and poured into 9-cm plates, 20 ml each. The plates were inoculated with *A. helianthi* and incubated at temperatures of 15, 18, 20, 22, 24, 26, 28, 30, 33, and 35°C in alternating regimes of 14 h of fluorescent light at 165 E m^{-2} s^{-1} and 10 h of dark. Fungal growth was determined by calculating the area of radial growth for each colony at 3, 7, 14, and 21 days after inoculation. Ten replicate plates for each temperature were measured. The experiments were conducted twice.

Effect of temperature on conidia production. Seven 4-mm^2 plugs were taken from 2-week-old cultures of *A. helianthi* on sunflower-leaf agar medium and placed at equal intervals radially on sunflower-leaf agar plates. The plates were wrapped with Parafilm, and 10 plates were incubated at each temperature of 18, 20, 22, 24, 26, 28, and 30°C in alternating regimes of 14 h of light at 165 E m^{-2} s^{-1} and 10 h of dark for 2 weeks. The experiments were

conducted twice. After 14 days of incubation, 5 ml of autoclaved distilled water was added, and conidia were scraped from each plate. Each milliliter of the conidial suspension represented production from an approximately 12-cm^2 mycelial mat. Conidial suspensions were homogenized by a polytron PT3000 (Brinkmann Instruments, Inc., Westbury, NY) and counted in a hemocytometer.

Effect of temperature on conidia germination. Aqueous suspensions of conidia (5×10^4 conidia per ml) produced at temperatures of 18, 22, 26, and 30°C were applied in 10-µl drops onto 2-cm^2 filter-paper squares wetted previously with distilled water. Filter-paper squares were incubated in 15-cm-diameter petri dishes for up to 24 h in the dark at 15, 24, and 28°C. At 2, 4, 6, 10, and 24 h of incubation, the filter-paper squares were transferred to glass microscope slides. Drops of lactophenol cotton blue were applied to the filter-paper surface, and germination and number of germ tubes per conidium were determined under 100× and 400× magnification (200 conidia in each of three replications for each of the four production temperatures).

Aqueous conidial suspensions (5×10^4 conidia per ml) also were sprayed to run-off on 2-week-old hosts (sunflower, safflower, common cocklebur, ragweed, and musk thistle) and nonhosts (prickly sida, sicklepod, hemp sesbania, and soybean) of *A. helianthi* with an aerosol sprayer. The plants were immediately placed in a dew chamber (air temperature 28 to 30°C). At 4, 6, 10, and 24 h of dew, leaf samples from each plant species were collected and placed on wet filter-paper strips fitted onto glass microscope slides. Drops of lactophenol cotton blue were applied to the leaf surfaces to stain the conidia and prevent further germination and development. The conidia were observed for germination and number of germ tubes per conidium on the leaf surfaces at 400× magnification. Observation of 200 conidia in each of three replications was used to determine the effects of production temperature on the germination of conidia on hosts and nonhosts. The experiments were repeated and representative results are presented as mean ± standard error of the mean.

Effect of temperature on conidia morphology and cytochemistry. Aqueous suspensions of conidia (5×10^4 conidia per ml) were applied in 10-µl drops onto glass microscope slides. A drop of lactophenol cotton blue and coverslip were added, and conidia morphology was determined by observation at 400× magnification. The conidia length and width, number of segments (cells) per conidia, and number of conidia with cellular contents stained by lactophenol cotton blue were determined. Conidia were recorded as stained if at least one cell contained stained material. Each characteristic was determined for 25 conidia in each of three replications for each of the four production temperatures and reported as mean ± standard error of the mean.

To examine the conidia for lipid content, a combination of mycelia and conidia was gently raked from the surface of agar plates and placed in a saturated solution of Sudan IV (Sigma Chemical Co., St. Louis) in 70% ethyl alcohol for 1 h (18). The conidia-mycelia mixture was mounted onto slides in a drop of glycerin and covered with a coverslip.

Total polysaccharides and protein content of conidia also were evaluated by cell staining and microscopic observation. Fresh tissue from spore-producing mycelia grown on sunflower-leaf extract agar medium at 18 and 28°C was fixed in 4% glutaraldehyde in 0.2 M cacodylate buffer. After rinsing in the buffer for 1 h, the tissue was postfixed in 1% OsO_4 for 2 h. The tissues were rinsed in distilled water, dehydrated in a graded acetone series, and embedded in Spurr's medium. Semithin sections (1.0 µm) were obtained with an ultramicrotome and affixed to gelatin-coated glass slides on a slide warmer. These slides were stained for polysaccharides and proteins, described below. All materials were observed and photographed with a Zeiss photomicroscope fitted with Nomarski differential interference contrast optics (Carl Zeiss, Inc., Thornwood, NY).

To stain polysaccharides, the periodic acid Schiff's (PAS) reaction was utilized (21). The slides of sectioned conidia were placed in a saturated solution of 2,4-dinitrophenylhydrazine in 15% acetic acid for 1 h to perform an aldehyde blockade to prevent background staining (10). The solution also provided a yellow counterstain, which contrasts with the red stain of the PAS reaction. The slides were rinsed for 30 min in running tap water. The rinsed slides were placed in 1% periodic acid for 30 min. This step was omitted in control samples. The periodic acid treatment was followed by rinsing in tap water for 30 min. The slides were stained in Schiff's reagent (21) for 1 h, transferred to three quick changes (2 min each) of 2% sodium bisulfite, and rinsed in running water for 10 min. After the sections were dried on a slide warmer, permount (Fisher Chemical Co., Fairlawn, NJ) was applied and a coverslip was mounted.

Other slides containing semithin sections were stained for protein by incubating for 1 h in a solution of 10 g of $HgCl_2$ and 100 mg of bromophenol blue per 100 ml of 95% ethanol (20). The slides were washed for 20 min in 0.5% acetic acid and incubated in 1.0 M cacodylic buffer, pH 7.0, for 3 min, which converted the dye to its blue form. Some slides were rinsed in water for 3 min and dried on a slide warmer; others were dried directly from the buffer. A drop of permount was applied to the slides and a coverslip was mounted.

Host infectivity. Seeds of sunflower (oilseed hybrid NK-285) and safflower were obtained from T. Gulya, USDA, ARS, NCSL, Fargo, ND. Common cocklebur seeds were purchased from Azlin Seed Inc., Leland, MS. Common ragweed (*Ambrosia artemisiifolia* L.) and musk thistle (*Carduus nutans* L.) seeds were purchased from V & J Seed Farms, Woodstock, IL. Hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill), sicklepod (*Cassia obtusifolia* L.), soybean (*Glycine max* (L.) Merr.) and prickly sida (*Sida spinosa* L.) seeds were collected from local fields at Stoneville, MS. Seeds of the above species were planted in a 3:2 (vol/vol) Jiffy mix (Jiffy products of America, Inc., Batavia, IL) soil mixture in plastic pots (350 cm^3). The plants were maintained in the greenhouse (28 to 32°C, 40 to 60% relative humidity). The photoperiod was approximately 14 h at 1,600 to 1,800 mol m^{-2} s^{-1} at midday. When the plants were 2- to 3-weeks-old, 5×10^4 conidia per ml suspended in sterile distilled water was sprayed on plants until run-off with an automatic aerosol sprayer. Up to 300 plates were used to obtain the necessary number of conidia at the higher temperatures. After incubation, the plants (20 per treatment) were subjected to a dew period (air temperature 28 to 30°C) for 20 to 24 h prior to incubation in the greenhouse. Treated and control plants, which received only sterile distilled water, were observed for symptom development (e.g., necrotic lesions, growth inhibition, wilt, and mortality for a 2-week period). After this time, plants were rated for disease symptoms using a 0 to 3 rating scale in which 0 = no symptoms; 1 = slight necrosis; 2 = moderate necrosis and wilting; and 3 = severe necrosis, wilting, and mortality. All experiments were repeated at least twice and reported as mean ± standard error of the mean.

RESULTS

Fungal growth and conidia production. *A. helianthi* grew well from 18 to 30°C but not at 15 or 33°C (Fig. 1). Fungal growth was more rapid at 28 and 30°C than at lower temperatures.

Yields of conidia obtained from cultures washed with 5 ml of water ranged from more than 20,000 conidia per ml (equivalent to an approximately 12-cm^2 mycelial mat) at temperatures from 18 to 26°C to less than 12,000 conidia per ml at temperatures of 28 or 30°C. The ability of the fungus to produce conidia declined at higher temperatures.

Effect of temperature on conidia germination. The germination percentages of conidia produced at 18 and 22°C were

significantly higher than those produced at 26 and 30°C, regardless of the temperature during incubation on filter paper (Table 1). Conidia produced at the two higher temperatures germinated poorly (<7%) in all instances. The highest germination (33%) on filter paper was achieved by conidia produced at 18°C and incubated at 15°C for 24 h. Germination occurred most rapidly with conidia produced at 18 and 22°C (26 and 29% at 4 h) and incubated at 24°C. In preliminary experiments, very few (<2%) of the conidia germinated while suspended in free water, regardless of production or incubation temperatures (data not shown).

Conidia produced at 18°C began germinating after 2 to 4 h of incubation on seven of the nine plant species (Table 2). Germination was most rapid (51% at 4 h) on sunflower. By 24 h after inoculation, the germination percents were 96 to 97% on sunflower, safflower, and common cocklebur leaves and 82 to 86% on common ragweed and musk thistle. At comparable incubation temperatures, a higher proportion of conidia germinated on plant leaves compared to filter paper (Tables 1 and 2). In general, germination percents were higher on hosts (sunflower, safflower, and common cocklebur) than on nonhosts (sicklepod, soybean, and prickly sida) (Fig. 2). In addition, germination percents declined on both filter paper and leaves as conidia production temperature increased (Fig. 2).

Germination of conidia on both sunflower and common cocklebur was greatest for spores produced at 18°C. Germination on the two hosts was delayed and decreased as conidia production temperature was increased (Fig. 2). At 24 h after inoculation, germination of the 18°C conidia on the two hosts was comparable, but the superiority of sunflower in supporting germination became apparent for conidia produced at 22 and 26°C.

The number of germ tubes per germinated conidium was greatest for conidia produced at 18°C compared to those produced at the other three higher temperatures (Fig. 3). For example, conidia produced at 18°C and incubated on sunflower for 24 h had 6.5 ± 0.5 (mean ± standard error) germ tubes per conidium, whereas conidia produced at 22, 26, and 30°C had 3.1 ± 0.4, 2.1 ± 0.5, and 1.3 ± 0.7 germ tubes per conidium, respectively, when incubated on the same species. The conidia also responded differently to various plant species (Fig. 3). For example, at 18°C and 24 h, conidia developed more germ tubes on sunflower (6.5 ± 0.5) than on either common cocklebur (4.9 ± 0.2) or prickly sida (2.6 ± 0.7). The few conidia produced at 26 and 30°C that germinated had statistically equivalent numbers of germ tubes (range was 0.8 to 2.1 germ tubes per conidia) on all plant species tested.

Effect of temperature on conidia morphology and cytochemistry. *A. helianthi* conidial length, width, and the number of cells per conidium progressively decreased as the temperature during conidia production increased from 18 to 30°C (Table 3). The percentage of conidia stained by lactophenol cotton blue was less for those produced at 30°C than for those produced at the three lower temperatures (Table 3), indicating a lack of intracellular materials in nonstained conidia. Conidia produced at the higher temperatures were significantly smaller and less well developed than those produced at the lower temperatures.

The conidia grown at 18°C rarely stained intensely for lipids, indicating that any lipid present may be finely dispersed inside the cells. Some conidial cells, however, did show lipid inclusions as shown in Figure 4A. In contrast, for conidia grown at 28°C (Fig. 4B), heavy staining occurred within a greater proportion of the cells, suggesting that lipid debris from membranes and inclusions had coalesced after the death of the cell. Most of the conidia at this temperature were apparently empty of all cytoplasmic materials. Presumably, the conidium in Figure 4B was in a relatively early stage of disintegration.

Sections of conidia produced at 18°C (Fig. 4C) stained very intensely, indicating relatively high polysaccharide content. Some globular inclusions (Fig. 4C), however, were left unstained. A control procedure that prevents staining of nonpolysaccharide moieties was performed (Fig. 4D). This confirmed that the red stain of Figure 4C and E is indicative of polysaccharide content. The nonpolysaccharide moieties (gutuoles) also are seen in these sections but are not as well differentiated from the rest of the conidium. Sections of conidia produced at 28°C (Fig. 4E) usually showed misshapen cell walls to which some Schiff's reagent adhered as well as lightly staining particulate matter in the cell.

Proteins in conidia grown at 18°C (Fig. 4F) stained lightly throughout the cytoplasm. The cytoplasmic component staining most heavily for proteins appeared to be the same globules (Fig.

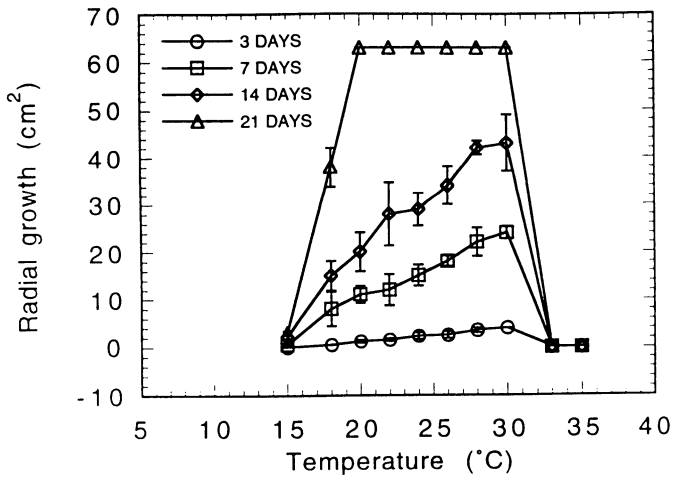

Fig. 1. Effect of temperature on radial growth of *Alternaria helianthi* on sunflower leaf-extract agar.

TABLE 1. Effects of conidia production temperature on germination of *Alternaria helianthi* conidia on filter paper at three incubation temperatures

Temperature (C)		Germination after incubation (%)[a]				
Production	Incubation	2 h	4 h	6 h	10 h	24 h
18	15	0 ± 0	4 ± 1	23 ± 4	23 ± 2	33 ± 1
	24	2 ± 3	26 ± 9	21 ± 7	23 ± 9	21 ± 2
	28	2 ± 1	11 ± 4	18 ± 4	14 ± 2	24 ± 5
22	15	0 ± 0	1 ± 1	10 ± 6	16 ± 5	28 ± 5
	24	3 ± 3	29 ± 6	19 ± 4	26 ± 1	26 ± 1
	28	3 ± 3	15 ± 7	20 ± 12	22 ± 6	23 ± 9
26	15	0 ± 0	1 ± 1	0 ± 0	2 ± 1	4 ± 2
	24	1 ± 1	2 ± 1	4 ± 3	6 ± 3	5 ± 2
	28	0 ± 0	4 ± 1	4 ± 1	3 ± 1	4 ± 2
30	15	0 ± 0	0 ± 0	0 ± 0	4 ± 2	1 ± 1
	24	0 ± 0	1 ± 1	0 ± 0	1 ± 1	1 ± 1
	28	1 ± 1	1 ± 1	0 ± 0	1 ± 1	0 ± 0

[a] Mean ± standard error of mean.

TABLE 2. Germination of *Alternaria helianthi* conidia on several plant species after a 2- to 24-h dew period[a]

	Germination after dew period (%)[b]				
Plant species	2 h	4 h	6 h	10 h	24 h
Host					
Common cocklebur	0 ± 0	1 ± 1	21 ± 5	82 ± 28	97 ± 3
Musk thistle	0 ± 0	3 ± 2	15 ± 1	25 ± 14	82 ± 3
Safflower	0 ± 0	27 ± 8	32 ± 4	58 ± 20	97 ± 1
Sunflower	0 ± 0	51 ± 6	48 ± 12	71 ± 17	96 ± 4
Common ragweed	0 ± 0	1 ± 1	41 ± 14	38 ± 9	86 ± 12
Nonhost					
Hemp sesbania	0 ± 0	7 ± 9	0 ± 0	14 ± 11	44 ± 10
Prickly sida	0 ± 0	0 ± 0	4 ± 2	9 ± 5	9 ± 2
Sicklepod	0 ± 0	0 ± 0	0 ± 0	25 ± 7	29 ± 5
Soybean	0 ± 0	3 ± 1	1 ± 1	1 ± 1	8 ± 3

[a] Temperature of dew chamber ranged from 28 to 30°C. Conidia were produced at 18°C, and spray was applied in a water suspension to leaf surfaces.
[b] Mean ± standard error of mean.

4F, arrows) left unstained by Schiff's reagent. A thin layer of stained material (Fig. 4F) surrounded most of the conidia prepared by this method and may represent the adhesive material necessary for attachment of the conidium to the leaf. Sections of conidia grown at 28°C (Fig. 4G) showed very little staining except in conidia where cell contents were disintegrated. Some of the empty conidia provided evidence that the globules (gutuoles) (Fig. 4G, arrows) are one of the last cytoplasmic components to disappear, although intensity of staining was much reduced.

Host infectivity. Conidia produced at 18°C were highly infective, and infectivity (measured by symptom expression on the host) progressively decreased with increasing temperature during conidiogenesis (Table 4). Conidia produced at 28 and 30°C were noninfective. The symptoms caused by infective conidia included necrotic lesions on stems and leaves that developed into blight, growth inhibition, wilt, and mortality of the host plants sunflower, safflower, ragweed, musk thistle, and common cocklebur. Infective conidia caused a few necrotic lesions on the leaves of the nonhosts hemp sesbania and sicklepod but no mortality. No symptoms were observed from infective or noninfective conidia on the nonhosts soybean and prickly sida. Infective conidia caused visual damage on young leaves of susceptible hosts such as sunflower, safflower, and common cocklebur within 24 h after inoculation. Death of some host plants occurred within 1 week.

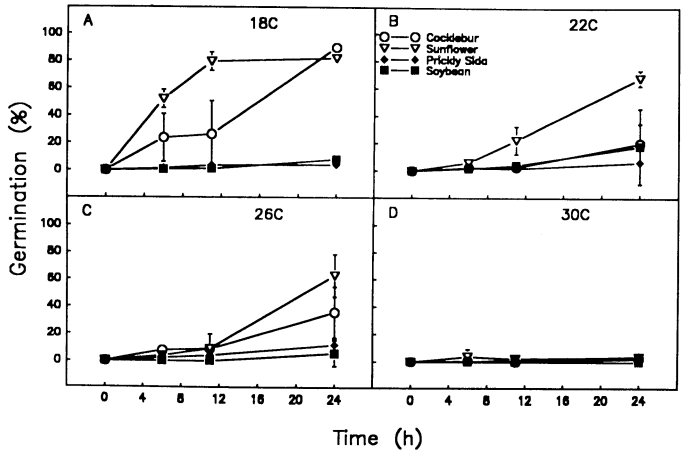

Fig. 2. Percent germination of *Alternaria helianthi* conidia applied on leaves of common cocklebur, sunflower, prickly sida, and soybean plants after a 0- to 24-h dew period. The conidia were produced at **A,** 18, **B,** 22, **C,** 26, and **D,** 30°C. Vertical bars indicate standard error of means.

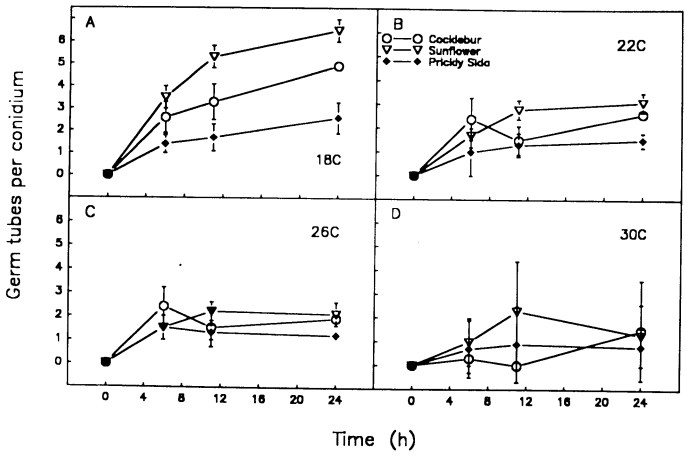

Fig. 3. Number of germ tubes per conidium of *Alternaria helianthi* applied to leaves of common cocklebur, sunflower, and prickly sida after a 0- to 24-h dew period. The conidia were produced at **A,** 18, **B,** 22, **C,** 26, and **D,** 30°C. Vertical bars indicate standard error of means.

DISCUSSION

A. helianthi conidia produced at 18°C were larger, germinated better, and were more infective than conidia produced at 22, 26, and 30°C. In general, conidial vigor and infectivity decreased as production temperature increased. The poor germination of conidia produced at 26 and 30°C was very likely the reason for the failure of these conidia to incite disease on hosts of *A. helianthi* (1).

Histochemical studies confirmed the basis for the increased infectivity of conidia produced at 18°C. Polysaccharide content, indicative of high metabolic activity (11), was greater at the lower temperatures. Proteins were evenly dispersed at 18°C, which indicates widespread enzyme activity. The conidia produced at 28°C showed clumping of lipids, polysaccharides, and proteins, probably due to degenerative processes. When degeneration was advanced, conidia were empty, and no evidence of metabolic activity was observed. Apparently degeneration at the higher temperatures was responsible for reduced germination and infectivity.

The percentages of conidia that germinated and the number of germ tubes that developed per conidium were influenced by the plant species as well as the conidia production temperature. Germination of conidia at comparable temperatures was generally greater on host plants than on nonhost plants or filter paper. One or more characteristics of host plants must have favored germination. The germinated conidia developed more germ tubes per conidium on sunflower compared to common cocklebur or prickly sida. Conidia with the greatest number of germ tubes also had the greatest infectivity. Allen et al. (2) noted that *A. helianthi* conidia germinated on sunflower leaves by producing many germ tubes. Artificial light did not influence germination but increased the number of germ tubes produced per conidium (1). In our study, conidia on all plants were subjected to the same light conditions (alternating regimes of 14 h of fluorescent light at 165 E m^{-2} s^{-1} and 10 h of dark).

Previous research at temperatures within the physiologically active range of several fungi has shown that conidia produced at lower temperatures are more abundant, larger (14,15,19,22,30,32), and more infective (19,26) and germinate faster and at a greater percent (26,37) than conidia produced at higher temperatures. The results reported here generally support these findings and additionally describe the effects of different temperatures on the cytochemistry and infectivity of *A. helianthi*. We also report that conidia produced at lower temperatures germinate better on some hosts than on others. Apparently, some characteristics of the hosts as well as temperature during conidia production influence conidia germination.

The physiological basis for the detrimental effect of 26°C and the beneficial effect of 18°C on conidia production and development was not determined, but Cotty (9) suggested that lower temperatures may benefit conidia production by reducing the amount of respiratory CO_2 produced by the conidia. It is well known that CO_2 inhibits fungal spore production and that respiratory rates increase with temperature (9,22). Thus, a temperature

TABLE 3. Effect of conidia production temperature on morphology of *Alternaria helianthi* conidia

Production temperature[a] (C)	Conidial characteristic[b]			
	Width (μm)	Length (μm)	Cells/ conidia	Stained[c] (%)
18	18 ± 1	73 ± 3	7.5 ± 0.3	75 ± 6
22	16 ± 1	61 ± 6	6.3 ± 0.3	77 ± 12
26	13 ± 1	55 ± 3	5.9 ± 0.3	75 ± 17
30	12 ± 1	42 ± 2	4.6 ± 0.3	12 ± 8

[a] Produced on sunflower-leaf agar plates.
[b] Mean ± standard error of mean.
[c] Stained with lactophenol blue.

of 18°C during conidia production may result in less CO_2 production within the colony than 26°C and correspondingly reduce the possible toxic effects of CO_2 on *A. helianthi* conidia production and development.

The infectivity of *A. helianthi* on common cocklebur is sufficient to warrant further investigation for biocontrol of common cocklebur in areas where sunflower and other desirable agricultural hosts are not grown. Research is needed to develop methods to overcome the lengthy dew period requirements. Formulation of the pathogen with moisture-retaining additives may be one means of overcoming this environmental constraint (28). These results indicate that a temperature of 18 to 22°C

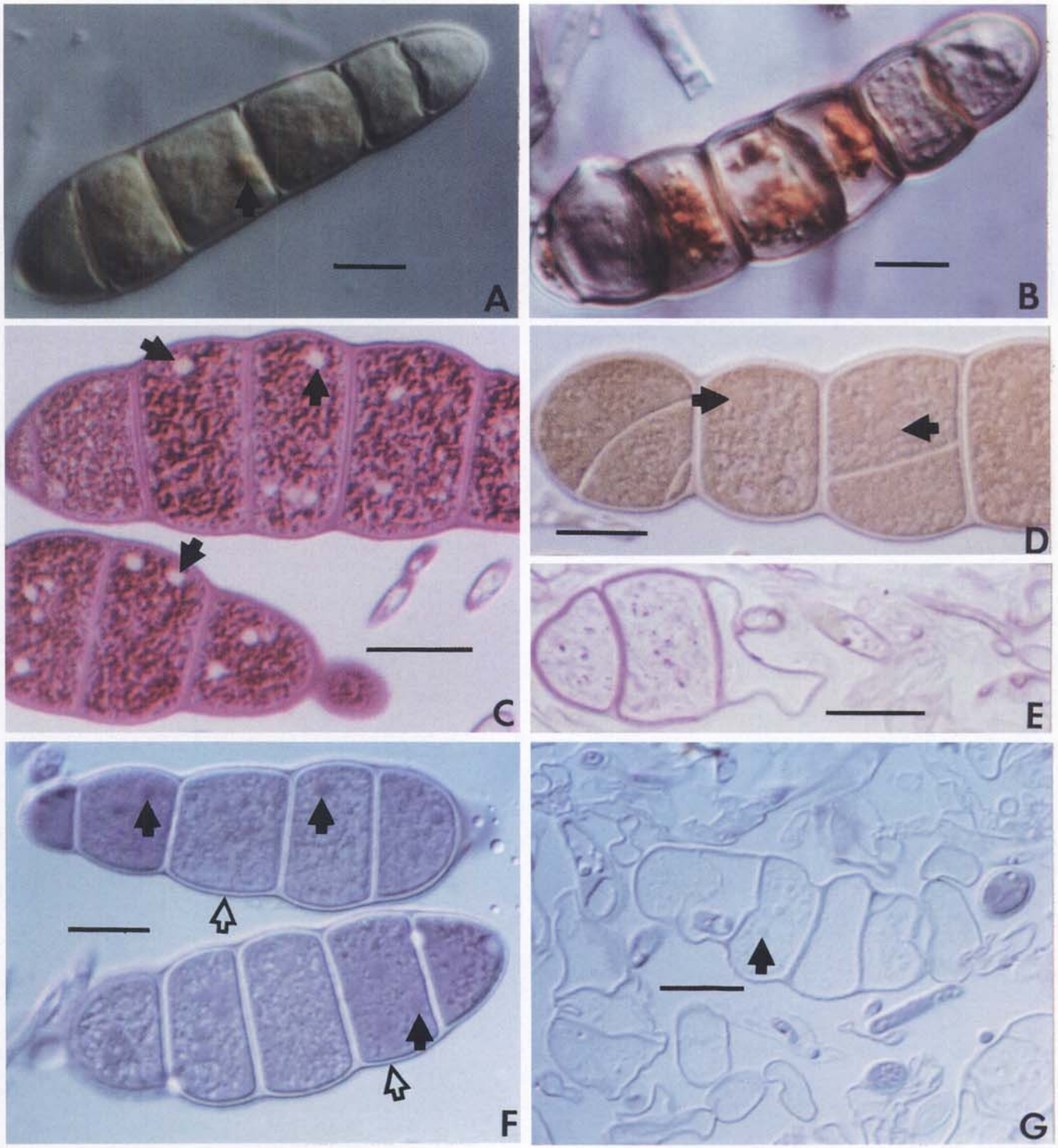

Fig. 4. Conidia of *Alternaria helianthi* examined after application of histochemical stains for lipids, polysaccharides and proteins. **A,** Whole glycerin mount of conidium produced at 18°C and stained positively for lipids. **B,** Whole glycerin mount of conidium produced at 28°C and stained with Sudan IV for lipids. **C,** 1-µm-thick section of conidium stained for polysaccharides by periodic-acid-Schiff's procedure minus the periodic-acid step. **D,** Control to assess nonspecific staining by the Schiff's reagent. Arrows denote globules corresponding to the negative staining globules in **C**. **E,** Section of conidium produced at 28°C and stained by periodic-acid-Schiff's procedure. **F,** Section of conidium produced at 18°C and stained by mercuric bromophenol blue for proteins. Solid arrows denote globules (gutuoles) staining positively for protein; open arrows denote thin layer of proteinaceous material surrounding the conidium. **G,** Section of conidium produced at 28°C and stained for protein. Arrow denotes very lightly staining globules in this degenerating conidium. All photographs made under Nomarski differential interference contrast optics. All bars = 10 µm.

TABLE 4. Effects of *Alternaria helianthi* conidia produced at different temperatures on disease severity[a] on several plant species under greenhouse conditions

Plant species	Conidia production temperature (C)[b]							Control[c]
	18	20	22	24	26	28	30	
Host								
Common cocklebur	3 ± 0.5	1.5 ± 0.2	0.5 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Musk thistle	2.5 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	0.5 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Common ragweed	2.5 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.5 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Safflower	3 ± 0.3	3 ± 0.2	2 ± 0.2	1.5 ± 0.1	0.5 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Sunflower	3 ± 0.1	3 ± 0.1	2 ± 0.1	1.5 ± 0.2	0.5 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Nonhost								
Hemp sesbania	0.5 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Prickly sida	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sicklepod	0.5 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Soybean	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] Disease symptom ratings: 0 = no symptoms; 1 = slight necrosis; 2 = moderate necrosis; and 3 = severe necrosis, wilting, and mortality.
[b] Mean disease severity ± standard error of mean.
[c] Control treatments included sterilized distilled water only.

should be used for production of *A. helianthi* conidia for biocontrol of common cocklebur. Further studies also are needed to determine the effectiveness of *A. helianthi* in biological control of cocklebur and other weeds under field conditions.

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