Isolation, Storage, and Inoculum Production Methods for *Alternaria dauci*

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ABSTRACT

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Alternaria dauci was isolated from diseased carrot leaves, seedlings, or infested seeds by simple isolation methods. Pathogenicity or viability of the isolates was not related to sources of the fungus. Isolates stored for 6 mo on carrot leaf agar showed little change in pathogenicity, sporulation, or colony type. Conidia produced on cellulose disks impregnated with carrot leaf extract and then dried and stored at low relative humidity maintained viability and pathogenicity for at least 18 mo. On agar media, a pH from 6.0 to 6.5 was optimum for mycelial production; a pH near 7 was optimum for conidial production. Growth rate was proportional to temperature from 12 C to 28 C; significant growth was observed as low as 12 C. Minimum daylength of 4 hr was required for abundant production of conidia. A medium made from dried carrot leaves allowed reliable culture, storage, and production of conidia of *A. dauci*. Conidia to inoculate plants were efficiently recovered by washing them from agar plates with water containing a surfactant.

Recent identification of carrot (*Daucus carota* L.) germ plasm with useful levels of resistance to leaf blight caused by *Alternaria dauci* (Kühn) Groves and Skolko (10) has stimulated interest in evaluating cultivars and breeding lines for response to this pathogen. Carrots have been evaluated successfully in field plots where weather favorable for Alternaria leaf blight prevails (3,5,9), but a more efficient and reliable evaluation in a controlled environment is often desirable.

A. dauci sporulates erratically in culture. Pathogenicity, ability to sporulate, and colony growth characteristics often change during culture and storage (2,6). Information on growth and sporulation of *A. dauci* has been published (2,4,5,9,11,12), but reliable production of conidia in numbers needed to establish disease in the field or in a controlled environment has remained difficult.

This study established reliable methods for isolation and storage of the pathogen, provided improved methods for production of conidia in numbers suitable for establishment of disease, and identified some factors that affect the success of these procedures.

MATERIALS AND METHODS

Preparation of media. Potato-dextrose agar (PDA) was prepared from a dehydrated product (Difco Laboratories, Detroit, MI). V-8 juice agar (V-8A) was prepared from 200 ml of V-8 juice, 15 g of agar, and 3 g of $CaCO_3$ per liter (11). Carrot leaf infusion agar (CLA) was developed to enhance conidial production by *A. dauci*. Carrot plants (cultivar Chantenay) were grown in pots in a peat moss-vermiculite medium in a warm greenhouse and watered with half-strength Hoagland's solution (11) three times weekly. Leaves were harvested at the 6- to 10-leaf stage and dried for 4 days on trays in a greenhouse and then for 1 day in a forced-air drying room at 50 C. Dried leaves were finely chopped in a food processor, placed in plastic bags, and stored at −10 C until used. Twenty-five grams of dried carrot leaves was added to 1 L of distilled water and stirred for 1 hr. Leaf debris was removed by filtration through two layers of cheesecloth. Water was added to bring the filtrate volume to 1 L, and 15 g of agar was added before sterilization. This medium also was used at half strength (12.5 g of carrot leaves per liter) for storage of cultures on agar slants (WCLA). The pH of CLA was 5.8 after sterilization. Methods similar to those for carrot were used to prepare celery leaf agar (CeLA) by substituting celery leaves (*Apium graveolens* L. var. *dulce* DC. cultivar Florida 2-14).

Isolation from diseased plants. Infected leaves from carrot plants grown in pots outdoors were collected early in the morning, and lesions were examined at ×60 for conidia. Conidia were isolated by touching them lightly with a sterile inoculating needle, loop, or glass rod and streaking them on agar plates. Single conidia were isolated with a fine steel needle that was flamed and then moistened with sterile water. Conidia were placed on agar plates containing specified media, and the plates were incubated 4 days at 24 C and in a 16-hr daylength (cool white fluorescent light [General Electric Corp.] producing 50 or 150 $\mu E\ sec^{-1}\ m^{-2}$). The colonies were examined at ×60 for contamination and identified by the morphology of the conidia. Selected isolates were subcultured on fresh media.

Conidia were isolated also on CLA from samples collected from carrot fields in Florida. These samples included infected leaves from mature plants and hypocotyls of infected carrot seedlings collected within 3 wk after emergence. Infected seedlings grown in the greenhouse were used also as sources of conidia. The seedlings were grown from naturally infested seeds obtained from the United States, Europe, and Australia. The purpose of these isolations was to determine whether the source of the pathogen affected survival or growth in culture. After 4 days, colonies were transferred to PDA slants and held at ambient laboratory conditions. Subcultures of the same colonies were also transferred to WCLA slants and cellulose disks for storage (methods described below).

Subculturing and storage of cultures. Ten new isolates of *A. dauci* were selected at random and transferred at daily intervals to examine the effects of delaying subculturing of new isolates on survival, growth characteristics, and sporulation. The original plates were established by point inoculation of CLA plates with conidia obtained from the original colony within 4 days after isolation. These plates were kept at 24 C in an 18-hr daylength (fluorescent and incandescent lights, 150 $\mu E\ sec^{-1}\ m^{-2}$). Three days after the first subculture and then daily for 14 additional days, transfers were made from the original subculture of each of the 10 isolates by picking up conidia with an inoculating needle from an area a few millimeters behind the colony edge. Isolated conidia were placed on each of three sterile, 8-mm-diameter disks that had been punched from 47-mm-diameter cellulose filter pads (Gelman Sciences, Ann Arbor, MI), sterilized, and placed on the surface of CLA plates. The purpose of the disks, which were moist when the conidia were applied, was to promote sporulation. Three plates were made from each isolate each day. Plates containing the disks

were kept at 24 C in an 18-hr daylength (same light source as above) and examined 5 days after transfer for colony diameter, appearance (growth types), abundance of conidia (subjective rating scale of 0 = no conidia produced to 5 = abundant conidia on entire colony surface), and for normal or abnormal conidia. Following the final subculture (day 14), conidia produced on these plates as well as conidia from the original colony were tested for pathogenicity. In a similar experiment that sought to examine effects of frequent subculturing, three new isolates of *A. dauci* were serially subcultured 10 times at 4- to 10-day intervals to CLA plates by point inoculation. One milliliter of a suspension containing about 10^3 conidia per milliliter was spread on the agar surface, or a sporulating colony was rubbed with a 4-mm-diameter inoculating loop and the adhering conidia were streaked on the agar surface. Growth, colony type, and abundance and morphology of conidia were examined for each method of transfer at 5 days after inoculation.

For pathogenicity tests, conidia were washed from the surface of agar plates with a stream of water containing 50 ppm of a surfactant (Triton AG-98; Rohm & Haas Co., Philadelphia, PA) from a wash bottle; 50 ml of water was used to wash conidia from each plate. Water and suspended conidia were collected, and the concentration of conidia was adjusted to about 30,000/ml by appropriate dilution. Suspensions of conidia were sprayed on carrot plants (cultivar Chantenay) growing in pots in a greenhouse. Inoculated plants were placed in a moist chamber (100% relative humidity) in the dark for 24 hr at 24 C, then moved to a humid greenhouse (temperature range 25–35 C) and checked for disease symptoms at 7 and 14 days after inoculation.

Long-term storage of the fungus was also examined. Small disks 8 mm in diameter were punched from 47-mm-diameter cellulose filter pads, sterilized, then placed on sterile 47-mm-diameter cellulose pads in 47-mm-diameter plastic petri dishes with tight-fitting lids. Each pad was moistened with 2 ml of sterile carrot leaf extract (CLA without agar) or Czapek's solution applied with a sterile pipette (11). The plates containing the 8-mm-diameter disks were kept at 24 C in a 16-hr daylength (fluorescent and incandescent, 150 μE sec^{-1} m^{-2}) for 5 days to promote abundant sporulation by the pathogen. The disks were then removed and placed on a wire screen over $CaCl_2$ desiccant in a closed plastic container and stored at ambient laboratory conditions. Periodically, dried conidia were removed and tested for germination by transferring them to CLA plates. When the conidia were viable, new conidia produced on the CLA plates were tested for pathogenicity by inoculating carrot plants with methods already described.

Growth on culture media. Conidia obtained from a 7-day-old field isolate of *A. dauci* were used to inoculate 250-ml flasks containing 50 ml of Czapek's medium that had been adjusted to pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 with 1 *N* KOH or HCl before sterilization. The pH of sampled media at pH 4.5, 6.0, and 7.5 increased approximately 0.2 pH units during sterilization. There were four replicates for each pH value. The cultures were grown without agitation at 24 C in a 12-hr daylength (fluorescent light, 50 μE sec^{-1} m^{-2}). After 10 days of growth, mycelia were collected by filtration, washed once with water, dried for 24 hr at 100 C, and weighed.

About five to eight conidia collected from a 7-day-old field isolate of *A. dauci* were placed in the center of plates that were poured with V-8 agar that had been adjusted to pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 with 1 *N* KOH or HCl before sterilization. The pH of unadjusted V-8A was 6.2 before and 6.4 after sterilization. The pH of adjusted media was not measured after sterilization. Plates (five for each pH value) were held at 24 C in a 12-hr daylength (fluorescent light, 50 μE sec^{-1} m^{-2}). Colony diameter was measured after 4, 6, and 11 days. For temperature studies, V-8A plates adjusted to pH 6.5 with 1 *N* KOH before sterilization were prepared and seeded with conidia as described above. Plates were placed at 12, 16, 20, 24, and 28 C with a 12-hr daylength (fluorescent light, 50 μE sec^{-1} m^{-2}). Ten plates were used for each temperature, and colony diameters were measured at 4, 6, and 11 days after inoculation.

Effect of light, temperature, growth medium, and pH on production of conidia. Culture flasks containing 100 ml of Czapek's medium were seeded with the conidia of *A. dauci* adhering to a 4-mm-diameter inoculating loop that had been moistened and brushed across a 4-day-old sporulating culture growing on a CLA plate. Cultures were grown with slow shaking in darkness at 22 C. After 4 days of growth, 1 ml of the mycelial suspension was removed from the flasks and distributed on each CLA plate. Plates were paced at 20 C in daylengths ranging from 0 to 24 hr (fluorescent and incandescent light, 150 μE sec^{-1} m^{-2}), beginning with a light period where applicable. After 72 hr, 14-mm-diameter disks were cut from the agar with a cork borer and placed in vials containing water plus a surfactant (Triton AG-98, 50 ppm) and five glass beads. Vials were shaken vigorously for 30 sec to disperse and dislodge conidia, and the conidia in 20-μl samples were counted at ×100. Five plates were used for each daylength, and four disks were sampled from each plate. Numbers of conidia were determined by counting the conidia in each of 10-μl subsamples from each vial.

When *A. dauci* was grown on small cellulose disks placed on the surface of agar plates, sporulation was enhanced. Thus, the effects of daylength on the production of conidia were also examined with the cellulose disk method. About 5–10 conidia from a 7-day-old field isolate of *A. dauci* were deposited on sterile 8-mm-diameter cellulose disks that had been placed on CLA plates. The disks absorbed moisture from the medium and were moist when inoculated. The plates were kept at 24 C in daylengths of 4, 8, 12, 16, 20, and 24 hr. Five days after inoculation, the cellulose disks were removed and transferred to vials containing a surfactant solution and glass beads. Four plates containing three disks per plate were used for each daylength. Conidia were dislodged by shaking the vials for 30 sec, and the number of conidia per milliliter was determined by counting the conidia in each of 10 20-μl subsamples per vial.

The agar plate methods used in the daylength experiments described above were also used to examine the effect of temperature on production of conidia. Methods of inoculum production and inoculation were identical. Inoculated CLA plates were placed at 10, 15, 20, 25, or 30 C with 16-hr daylength (fluorescent and incandescent light, 150 μE sec^{-1} m^{-2}) at the beginning of a light period. After 72 hr, agar disk samples were removed with a cork borer and placed in a vial with a surfactant solution and glass beads; conidia in 10 20-μl subsamples per vial were then counted.

Since cellulose disks placed on CLA promoted sporulation, they were also used to examine the effect of temperature on production of conidia. Methods of inoculation were identical to those described for the daylength experiments. CLA plates bearing inoculated cellulose disks were placed at 10, 15, 20, 25, and 30 C in a 16-hr daylength (fluorescent and incandescent light, 150 μE sec^{-1} m^{-2}). After 5 days, disks were removed and conidia were dislodged by shaking in a detergent solution with glass beads. Number of conidia per milliliter was determined by counting the conidia in each of 10 20-μl subsamples per vial.

For a study of the production of conidia on different media, inoculum was prepared in liquid shake cultures as described above. One-milliliter portions were spread on plates containing PDA, V-8A, CLA, and CeLA. The plates were held at 24 C with 16-hr daylength (fluorescent and incandescent light, 150 μE sec^{-1} m^{-2}) for 4 days, and conidia produced per unit area of the different media were measured by removing 14-mm-diameter agar disks, shaking them in a vial with detergent solution and glass beads, and counting the number of conidia in each of 10 20-μl subsamples per vial.

The effect of medium pH on production of conidia was examined with V-8A plates by adjusting the pH of V-8 medium with 1 *N* KOH or HCl to pH values of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 before sterilization. Plates were seeded by spreading 1 ml of a suspension of conidia (about 100 conidia per milliliter) on each plate. Plates were placed at 24 C with a 16-hr daylength (fluorescent light, 50 μE sec^{-1} m^{-2}). After 7 days, 14-mm-diameter disks were cut from the agar, placed in vials, and shaken in

detergent with glass beads; numbers of conidia produced per unit area were determined by counting conidia in 10 20-μl subsamples per vial.

RESULTS AND DISCUSSION

Isolation of the pathogen. *A. dauci* was easily isolated from infected leaves and seedlings or from infested seeds by transferring one or more conidia onto agar plates. The type of medium did not affect the success of isolation. After 3 days of growth on CLA, PDA, or V-8A, *A. dauci* could be identified by the morphology of conidia, checked for contaminants, and then transferred to fresh media or stored. Colonies developing on the first subculture following isolation from plant material produced abundant conidia on all media tested; however, many conidia produced on PDA were atypical in form (Fig. 1A and B). Among the limited number of isolates tested, the source of the isolates did not affect pathogenicity, colony type, growth, or viability in storage. However, the medium used to subculture isolates did affect growth and sporulation.

Subculturing and storage of cultures. When subcultures of the original isolates were made on fresh plates of the same medium, colonies subcultured on PDA or V-8A usually produced more abundant aerial mycelia and smaller numbers of conidia than the original isolate. After three or four subcultures on PDA or V-8A, sporulation was very sparse and conidia were often deformed. Sporulation or growth characteristics did not change noticeably when repeated subcultures of the original isolates were grown on CLA or CeLA.

Early mortality (within 14 days of isolation) was frequently observed for subcultures maintained on PDA and was associated with isolates that produced little or no dark-colored pigment in the medium. In two experiments, 55 and 100% of the less pigmented isolates survived less than 14 days on PDA. Isolates that produced abundant brown-black pigment on PDA survived longer on PDA, but 50% of these isolates did not survive 6 mo on PDA at 4 C. All of the original isolates tested survived in storage at least 6 mo on V-8A, CLA, or WCLA slants at 4 C.

Colonies derived from repeated daily subcultures on CLA of 10 new field isolates showed no noticeable changes in colony diameter, appearance of colonies, or abundance of conidia when these characteristics were evaluated 5 days after transfer. Conidia were the normal form (Fig. 1A). Conidia from the original isolate and from the 14th subculture produced typical and abundant lesions on carrot leaves within 5–10 days after inoculation. These results showed that delays up to 14 days in subculturing new isolates were not important. In a similar experiment, 10 serial subcultures of a new isolate made on CLA plates at 4- to 10-day intervals produced colonies that were similar in all characteristics. Growth measurements were not made, but there were no apparent differences in colony size and appearance, abundance of conidia, or form of conidia when the colonies were examined 5–6 days after transfer. The use of CLA permitted frequent subculturing of colonies with little change in the colony characteristics examined.

Most isolates of the fungus remained viable and pathogenic for at least 6 mo when stored on CLA slants at 4–5 C; however, about one-third of the colonies recovered after 6 mo of storage on CLA did not sporulate. Conidia produced and stored at low humidity on cellulose disks remained viable for at least 18 mo. Colonies from the stored conidia produced pathogenic inoculum after 18 mo of storage. Methods used did not allow a quantitative comparison, but more colonies were obtained from conidia stored on disks impregnated with carrot leaf extract than with Czapek's solution. Conidia produced and stored on cellulose disks produced about the same ratio (1/3) of typical to poorly sporulating or nonsporulating colonies as the colonies recovered from cultures stored 6 mo on CLA slants. Many of the conidia produced on poorly sporulating colonies were misshapen and atypical in form (Fig. 1A and B).

Growth on culture media. *A. dauci* produced abundant mycelia in liquid culture (Czapek's medium) between pH 4.5 and 7.5. Maximum growth occurred at pH 6.0 (Fig. 2), but substantial growth was produced over the entire range of pH values tested. Radial growth rates on pH-adjusted V-8A also demonstrated a broad pH tolerance by the fungus (Fig. 3). Maximum growth on V-8A occurred between pH 5.5 and 7.0, but substantial growth occurred at pH 4.5 and 5.0. A pH range of 6.0–6.5 is adequate for future studies on agar media. Radial growth on V-8A at pH 6.5 increased proportionally with temperature over the range of 12–28 C (Fig. 4). Rates of colony growth satisfactory for convenient production of conidia (6–7 mm/day) occurred between 20 and 28 C.

Effect of light, temperature, growth medium, and pH on production of conidia. *A. dauci* produced abundant conidia on CLA (pH 6.3) between 15 and 30 C; few were produced at 10 C. Numbers of conidia produced per unit area were proportional to temperature over the range of 15–30 C (Table 1). Results with the agar disk method were similar but more variable than with the cellulose disk method. Abundant aerial mycelia produced on agar above 25 C made conidia difficult to recover and count with the agar disk method. Some conidia produced above 25 C were deformed or less pigmented than those produced at lower temperatures (Fig. 1B). A pH of 7.0 was optimum for production

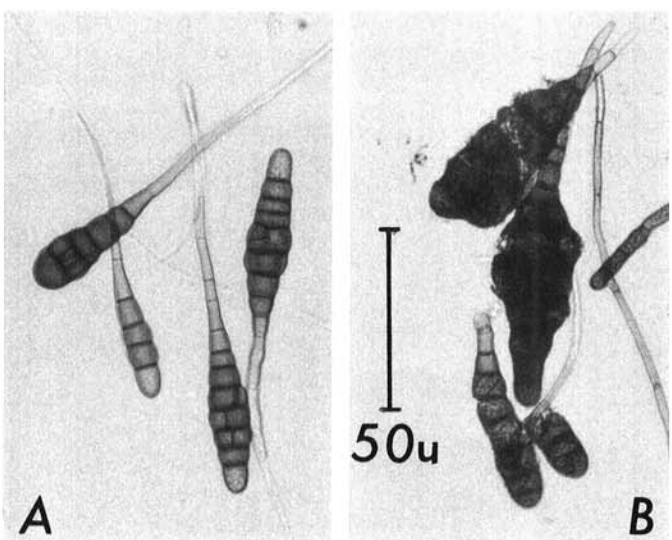

Fig. 1. Conidia of *Alternaria dauci* (×200). **A,** Conidia produced on carrot leaf agar are similar to those produced on lesions on carrot leaves. **B,** Atypical, deformed conidia produced in abundance on potato-dextrose agar and on carrot leaf agar in absence of light.

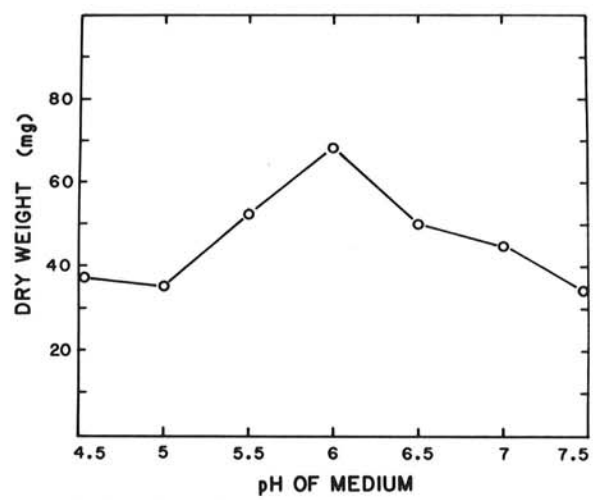

Fig. 2. Production of mycelial mass by *Alternaria dauci* in liquid culture (Czapek's medium) at 24 C. The pH values are at time of inoculation.

of conidia on V-8A, but large numbers of conidia were produced over the range of pH 5.5–7.5 (Fig. 5).

Only a few conidia were produced on CLA or on cellulose disks on CLA agar in the dark, and these conidia were atypical (Fig. 1B). Four hours of light were required to produce substantial numbers of conidia (Table 2), but there were no significant increases in numbers of conidia produced when daylength was increased above 4 hr. The small numbers of conidia produced in the absence of light were ignored in regression analysis of the data (Table 2). The slope of the regression line for daylength and number of conidia was not significantly different from zero. Results obtained with the agar disk method were similar but more variable than results presented in Table 2.

Colony diameters on CLA, V-8A, and PDA at 23 C were not significantly different after 5 days, but growth on CeLA was significantly slower than on other media (Table 3). Numbers of conidia produced per unit area differed greatly between media. Three to four times more conidia per square millimeter were produced on CLA and CeLA than on V-8A or PDA (Table 3).

This study identified some important considerations for producing conidia of *A. dauci* in culture. Strict requirements for pH, temperature, and daylength were not apparent from the results obtained. Unexplained observations in this study were loss of pathogenicity in culture, occasional poor or erratic sporulation that increased the variation in some of the data, and mortality in short-term storage by some (but not all) isolates and on some (but not all) media. Others have reported similar problems with the culture and storage of *A. dauci* (2). Possible explanations for these results include the effects of heterokaryosis (6) in *A. dauci*. *A. solani* (1,7,8), which has been more thoroughly investigated, appears to be similar to *A. dauci* in respect to erratic sporulation,

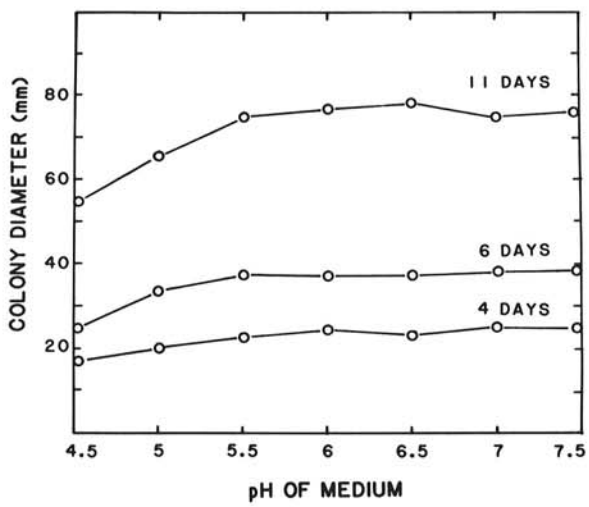

Fig. 3. Radial growth of *Alternaria dauci* on pH-adjusted V-8 agar.

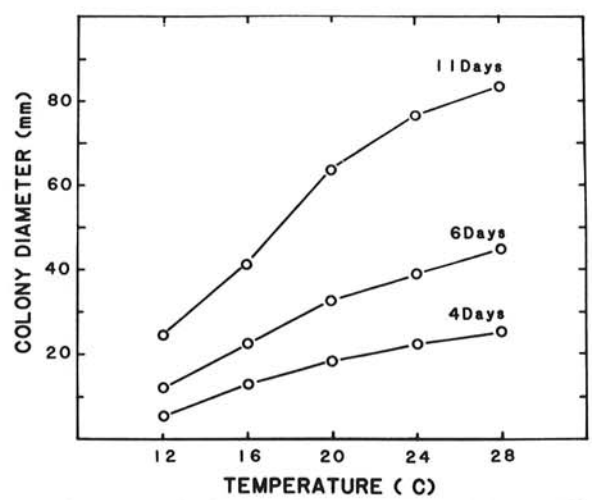

Fig. 4. Radial growth of *Alternaria dauci* on V-8 agar (pH 6.5) at different temperatures.

TABLE 1. Production of conidia by *Alternaria dauci* on cellulose disks in carrot leaf agar plates at different growth temperatures

Growth temperature (C)[b]	Colony diameter (mm)[c]	Conidia[a] recovered (per mm^2)[d]
10	7.5	0
15	14	87.2
20	24	336.7
25	33	555.6
30	29	594.3

[a] Regression equation for colony diameter and growth temperature: $Y = -0.363 + 0.125X$, $r^2 = 0.832$. Regression equation for conidia per millimeter and growth temperature: $Y = 12.2 + 0.139X$, $r^2 = 0.819$.
[b] Sixteen-hour daylength.
[c] Value is average for 12 replicates after 5 days of growth.
[d] Value is average for four samples from each of 12 replicates.

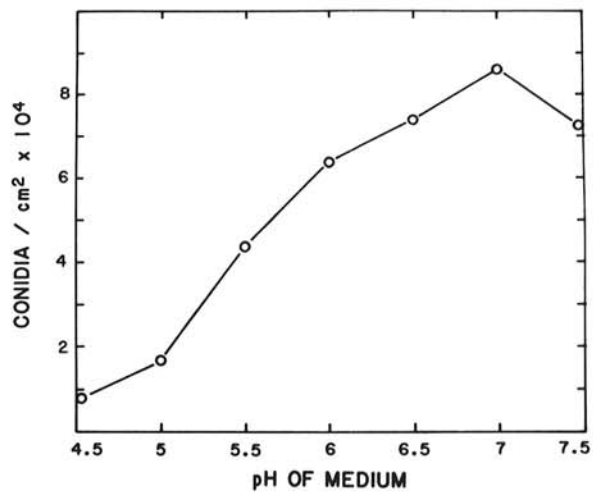

Fig. 5. Production of conidia by *Alternaria dauci* on pH-adjusted V-8 agar at 24 C, 16-hr daylength.

TABLE 2. Production of conidia by *Alternaria dauci* on colonies initiated on cellulose disks and placed on carrot leaf agar under different daylengths at 24 C[a]

Daylength (hr/24 hr)	Colony diameter (mm^2)[b] Total	Colony diameter (mm^2)[b] Sporulating area[c]	Conidia recovered/mm^2 of cellulose disk[d]
0	33.1	8.3	11.5
4	35.3	25.3	720.2
8	34.3	25.0	1,454.6
12	35.1	25.5	932.6
16	37.0	27.3	851.4
20	38.1	25.4	1,091.7
24	36.9	26.9	1,206.4

[a] Regression equation for total colony diameter was $y = 34.02 + 0.154X$, $r^2 = 0.21$; for sporulating area, $y = 24.77 + 0.786X$, $r^2 = 0.08$. Regression equation for conidia recovered per millimeter was $y = 895.9 + 8.92X$, $r^2 = 0.02$ ($F = 0.959$, ns). Zero daylength (continuous darkness) values omitted from regression analysis.
[b] Value is average for eight replicates and includes the 8-mm-diameter cellulose disk and surrounding growth on carrot leaf agar.
[c] Determined from dark-colored area of colonies and observation of conidia in this zone at ×60.
[d] Value is average for eight replicates with four subsamples per replicate.

variability, and loss of pathogenicity in culture (1,7,8).

Early mortality (within 14 days of isolation) on PDA seemed related to isolates that produced little or no pigment. Rotem (7) noted variability in pigment production on PDA by *A. solani*. He did not mention mortality in storage but observed that pigment production did not affect pathogenicity in *A. solani* (7).

Conidia produced on cellulose disks impregnated with Czapek's solution or carrot leaf extract were successfully stored for at least 18 mo at low relative humidity in an air-conditioned laboratory. The fungus could be stored also for at least 6 mo on WCLA. Ehr (2) found storage of conidia in sterile water or cultures on V-8A with or without oil immersion to be adequate. Ehr (2) did not store cultures more than 6 mo.

In the present study, strict requirements for light and temperature for growth and production of conidia were not found. However, erratic sporulation was observed more often at growth temperatures above 24 C. Ehr (2) reported that growth of *A. dauci* decreased abruptly above 28 C and that production of conidia decreased abruptly above 25 C. Observations of reduced growth above 28 C and erratic sporulation above 24 C suggest a growth temperature of 20–24 C for production of conidia. Although complex interactions of light and temperature on sporulation of *A. dauci* have been identified (2,12), results reported here were difficult to reconcile with published information. For example, in the present study, abundant conidia were produced in continuous light on CLA. Zimmer and McKeen (12) did not observe sporulation in continuous light. Ehr (2) found that abundant conidia were produced in continuous light but that they were atypical in appearance; he concluded that a 12-hr daylength was best. Both Ehr (2) and Zimmer and McKeen (12) used single-spore isolates that were stored on V-8 agar. Different isolates, storage methods, light quality, or unknown factors supplied by CLA could account for different results in the three studies. However, a daylength and temperature supported by the results of all three studies were chosen for routine production of inoculum (growth at 22–24 C in a 16-hr daylength with plates approximately 0.3 m from four FC-40W cool white fluorescent tubes and eight 40-W incandescent bulbs to provide 150 μE sec^{-1} m^{-2}). These conditions have provided reliable results over a 3-yr period.

TABLE 3. Production of conidia by *Alternaria dauci* on different agar media at 24 C[a]

Growth medium	Colony diameter (mm)	Conidia recovered (per mm^2)
Carrot leaf agar	47.0 a[b]	76.9 a
Celery leaf agar	40.5 b	61.5 b
V-8 agar	49.8 a	18.3 c
Potato-dextrose agar	48.5 a	18.0 c
	$F = 13.54$**	$F = 37.45$**

[a] Values are average for five replicates after 5 days of growth, 16 hr daylength.

[b] Means followed by the same letter are not significantly different using Duncan's new multiple range test ($P = 0.01$ level).

Atypical conidia that were produced in the dark on CLA or in all daylengths on PDA, as well as those produced on atypical or poorly sporulating colonies recovered from stored cultures, were all similar in appearance (Fig. 1B). Atypical conidia were deformed by excessive cell division in the zone where transverse cell walls are normally produced; rigid cell walls did not develop. Cells of atypical conidia often burst and conidia often disintegrated when placed in distilled water on microscope slides. Atypical conidia produced germ tubes when placed on CLA, but it is not known whether they were pathogenic.

Both CLA and CeLA provided good growth and abundant conidia. These media supply factors that greatly reduce the frequency of atypical conidia. It is possible that they also supply factors that make requirements for light and temperature less stringent. *A. solani* also grows and sporulates well on CLA and CeLA (*unpublished*).

The following procedure has been used for the routine production of large numbers of pathogenic conidia. Several conidia are spread evenly on the surface of CLA plates that are placed at 22–24 C with a 16-hr light period, about 0.3 m from four FC-40W cool white fluorescent lights plus eight 40-W incandescent bulbs (output measured at 150 μE sec^{-1} m^{-2}). After 4–7 days, the conidia are washed from the plate with a stream of water containing Triton AG 98 at 100 ppm from a wash bottle and collected for use. Fifty milliliters of washing solution per plate has routinely recovered about 10^4–10^5 conidia per milliliter.

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