Spore Production and Dispersal of Alternaria dauci

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ABSTRACT

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In greenhouse and growth chamber studies, conidia of *Alternaria dauci* were produced during night hours on necrotic areas of infected carrot leaf tissue. Relative humidities of 96-100% or free moisture were required for conidiophore and conidia production. Conidia were produced over the range of 8-28 C, but were not abundant when night temperatures were below 7-8 C. In field studies, conidia were dispersed by winds above 2 to 3 m/sec when relative humidity dropped soon after daylight. Conidia were not detected during night hours. The number of hours of relative humidity above 95% during the night preceding conidial release was not correlated with the relative abundance of conidia above infected carrot fields (r = 0.13), whereas hours of continuous foliar wetness during the same period were correlated with the observed abundance of conidia (r = 0.35).

Additional key words: *Daucus carota*, epidemiology.

Leaf blight of carrots caused by *Alternaria dauci* (Kühn) Groves and Skolko is often the most serious disease affecting carrots in the southern United States and causes serious losses in midwestern and eastern states and in other areas of the world (6, 7, 8, 14). Earlier workers have provided information on the etiology of this disease (6, 7) and the effects of light on sporulation (17). However, recent studies on closely related *Alternaria* spp., but not *A. dauci*, have provided detailed information on factors affecting spore production, release and dispersal (9, 10, 11, 12, 13) as well as in-depth studies of disease epidemics and methods for the simulation of epidemics (16). The objectives of this study were to develop information on factors affecting spore production and dissemination of *A. dauci*. This information would be useful in reducing numbers or better timing of fungicide applications for more effective control of Alternaria leaf blight.

MATERIALS AND METHODS

Field experiments.—The relative abundance of airborne *Alternaria dauci* spores above infected carrot fields was measured in six successive 1-acre carrot plots planted at approximately 6-wk intervals at Sanford, Florida, during the 1971-1972 growing season (September-June). Four of the plots were planted with Waltham Hicolor, and two of the plots were planted with Imperator 58. Both cultivars are susceptible to Alternaria leaf blight. Traps similar to those developed by Casselman and Berger (4) were placed near the centers of test plots. Spore intakes were 0.5 m above ground. Test plots averaged about 10-35% leaf area infected by *A. dauci* when traps were placed in them. Initially, traps were operated continuously but later were run only during daylight hours that corresponded to observed spore release periods. Temperature, relative humidity (RH) and rainfall were measured 1 m above ground with a recording hygrothermograph and rainfall recorder. Wind direction and velocity were recorded 4 m above ground. Leaf wetness was measured 0.1 m above ground with electrical resistance sensing grids and a recording system similar to the apparatus described by Davis and Hughes (5).

Controlled environment studies.—Carrot plants (cultivar Waltham Hicolor) used to produce infected tissue for controlled environment studies of sporulation were grown in a warm greenhouse (20-24 C) then inoculated by spraying with a spore suspension in water (10^3 conidia/ml) when plants were in the six to eight-leaf stage. Inoculated plants were kept at 20 C and 100% RH for 18-24 hr and then removed to a warm greenhouse (20-24 C). Symptoms were evident within 4 to 6 days, and lesions had sporulated within 8 to 10 days. Some infected leaves for humidity chamber studies were dried at room temperature and stored over $CaCl_2$ for use within 28 days. Spores for inoculations were obtained by washing them from 10- to 14-day-old cultures of *A. dauci* grown in petri dishes on V-8 juice agar (pH 6.0) at 20 C. Plates were placed 30 cm below two 40-W cool-white fluorescent lights (Sylvania FC 40) on an 18-hr day length cycle.

Humidity chamber studies.—To test the effect of RH on sporulation, necrotic lesions on infected living plants and on excised or dried carrot leaflets were freed of residual spores prior to use by directing a stream of compressed air to remove all conidia visible under a dissecting microscope. These tissues were then placed in small RH chambers 10 × 10 × 3 cm constructed of clear polystyrene. Aqueous solutions of glycerine were added to cover the bottom of the chambers to maintain 70, 80, 86, 92, 96, and 100% RH at 20 C (15). Tissues were placed above the glycerine solutions on small wire mesh screens to keep plant tissue from contact with the glycerine solution. Live, infected leaves were placed in similar humidity chambers modified with a 3-mm opening in one

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side to allow the insertion of the petioles. Cotton was packed around the petiole to reduce air exchange between the humidity chamber and the outside air. To test the effect of RH on germination, conidia were transferred from sporulating carrot tissue to 2 cm^2 pieces of dialysis membrane contained in humidity chambers, held in darkness at 24 C, and examined under the microscope at 1-hr intervals for germ tube growth. Conidia also were germinated on 2% water agar at 20 C to test for viability.

To test the effect of RH on sporulation, necrotic lesions on infected living plants as well as excised or dried carrot leaflets were freed of residual spores as described above then placed in RH chambers in the dark at 24 C and examined at 12-hr intervals for sporulation.

Growth chamber studies.—Dry, sterilized carrot petioles were used to measure sporulation under controlled environmental conditions. One-cm sections of fresh carrot petioles were sterilized with proplyene oxide for 24 hr and aerated for 2 hr before use. The sections were placed on water agar plates which 2 days previously had been flooded with a suspension of *A. dauci* conidia (1 ml per plate containing 10^3 conidia/ml). The ends of the petiole sections were inserted upright into the agar to a depth of 1 mm. After 24 hr, petiole sections were aseptically removed and inserted into sterile water agar in the same manner and kept in the dark at 24 C for an additional 24 hr. At this time, the petiole sections were colonized and covered with mycelium, but conidiophores had not yet formed. Water agar plates containing 20 petiole sections were placed in constant temperature growth chambers at 7, 10, 13, 16, and 19 C with 12 hr daylength at the beginning of the daylight cycle. A similar experiment was run using a constant daylight temperature of 19 C with night temperatures of 7, 10, 13, 16, and 19 C. At 12-hr intervals, 10 individual petiole sections from each treatment were removed and agitated vigorously in small vials containing 5 ml of a 1% detergent solution (Rohm & Haas 20134B) to dislodge and disperse conidia. Numbers of conidia per volume of liquid were determined from small aliquots using a microscopic counting chamber; the number of conidia per petiole section was calculated.

RESULTS

Results of field experiment.—The average weekly spore counts from two traps operated daily in six

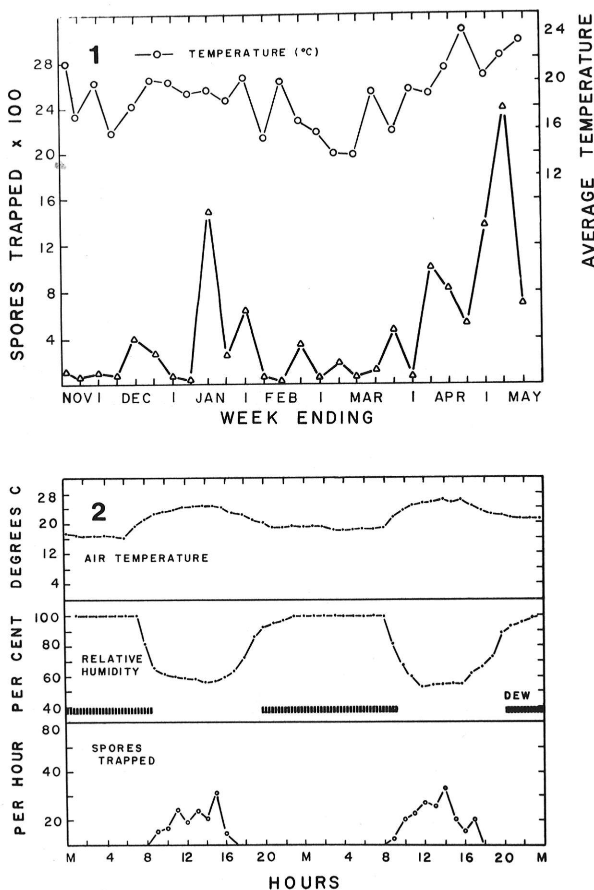


Fig. 1-2. 1) Weekly totals of *Alternaria dauci* spores trapped during the 1971-1972 carrot growing season at Sanford, Florida, and average weekly temperatures for the period. **2)** Spore catches for *Alternaria dauci* on two successive days as influenced by air temperature, relative humidity, and dew. Wind velocity (not shown) at time of spore release was above 4.4 m/sec.

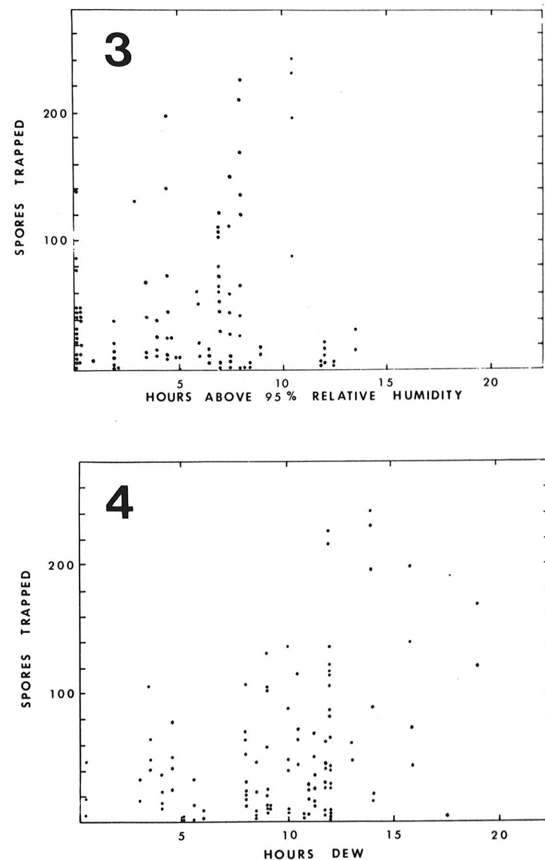
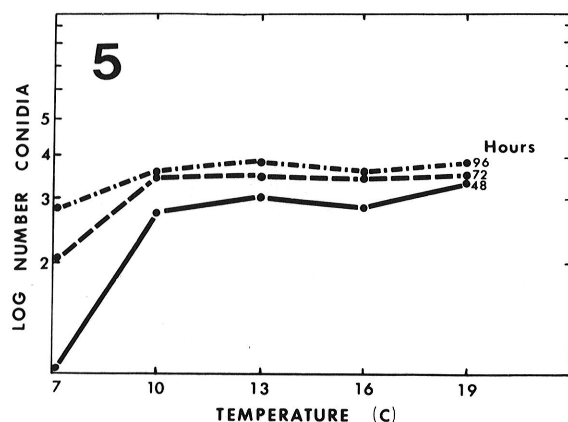


Fig. 3-4. Daily spore catches of *Alternaria dauci.* **3)** As influenced by hours of relative humidity above 95% during the night hours prior to spore release. Correlation coefficient 0.13. **4)** As influenced by recorded foliar wetness during the night hours prior to spore release. Correlation coefficient 0.39.

successive carrot plantings were plotted (Fig. 1). A 2-3 wk cycle of periods of spore abundance alternating with periods of spore scarcity was evident. Periods of relative spore abundance did not correspond with periods of high rainfall. However, greater numbers of spores were detected when average weekly temperatures increased in April and May (Fig. 1). Because of missing leaf wetness data for some days during the weekly intervals plotted in Fig. 1, no attempt to correlate hours of RH above 95% or hours of leaf wetness during the night preceding spore release on a weekly basis was made. However, analysis of daily spore counts for which leaf wetness data were available showed that abundant conidia were detected following nights when prolonged periods (8-12 hr) of 95-100% of RH or leaf wetness prevailed. After daylight, when RH dropped below about 80%, conidia were dislodged and carried by wind (Fig. 2). It was estimated that a velocity above 2 to 3 m/sec was required to cause large numbers of conidia to become airborne. Numbers of spores 2-5 times greater than those detected on windy, dry days were detected whenever harvesting operations or ground-operated farm equipment dislodged spores from infected plants in nearby carrot fields. Conidia were not trapped during hours of darkness or during daylight hours when rain or fog prevailed.



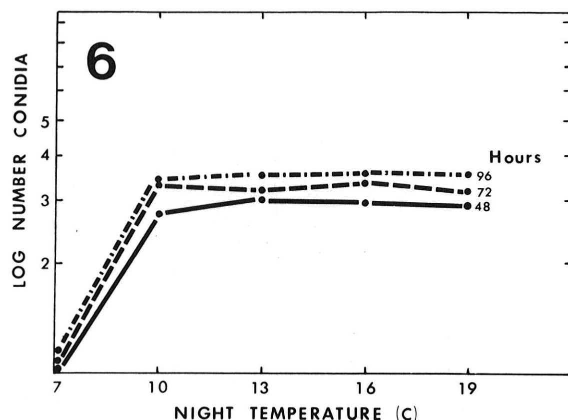


Fig. 5-6. Spore production by *Alternaria dauci* on sterilized carrot petioles. **5)** Under constant temperature conditions at 100% RH with 12-hr daylength. **6)** Under alternating day-night temperatures at 100% RH (19 C day temperature with 12-hr daylength).

Inspection of spore trap data indicated that heavy dew during the night preceding spore release often was an important factor in influencing spore abundance. Accordingly, hours of RH 95% or above (a value chosen because the hygrothermographs used were not accurate in distinguishing RH values above 95%) during the night preceding spore release were plotted against numbers of spores trapped during individual 1-day periods from the weekly total data plotted in Fig. 1. Hours of RH above 95% were not well correlated (correlation coefficient r = 0.13 was not significant) with observed spore abundance (Fig. 3). Hours of foliar wetness during the night preceding spore release estimated with electrical-resistance-sensing grids which simulated leaf surfaces were better correlated with the number of spores trapped (r = 0.35), a significant correlation at the $P = 0.01$ level (Fig. 4). Analysis of the same data considering hours of foliar wetness or RH above 95% for only those hours when temperatures were above 15 and 20 C did not produce significant r values.

Humidity chamber experiments.—When conidia produced on infected carrot leaf tissue at 24 C and 100% RH were removed and the tissue placed back in the same environment, conidiophores produced an additional crop of conidia each night. Conidia were produced on old conidiophores within 10 hr between 12-28 C at 100% RH. In relative humidity chambers maintained at 24 C which enclosed infected-living leaves or infected-excised leaves, new conidiophores as well as conidia from old conidiophores were produced only at relative humidities above 96% at 24 C. Most conidia produced were viable, and over 90% germinated within 2 hr on water agar at 2-28 C. Ninety % of the conidia germinated within 12 hr at 24 C on dialysis membranes at 100% RH, but less than 4% germinated on membranes held at 96% RH; none germinated on membranes at less than 96% RH.

Growth chamber experiments.—When inoculated petiole sections were placed in growth chambers at the beginning of an alternating 12 hr day/night cycle, conidiophores were produced within 24 hr, and conidia were produced within 48 hr between 10-19 C. Under conditions of constant temperature and a 12-hr day/night period, no conidia were produced after 48 hr at 7 C, but about equal numbers were produced between 10-19 C (Fig. 5). After 48 hr, no further increases in conidial production were measured at temperatures of 10 C or above, whereas at 7 C conidia were continuously produced, but at a lower rate for up to 96 hr when the experiments were terminated (Fig. 5).

In a similar series of experiments, 19 C day temperatures were alternated with 7, 10, 13, 16, and 19 C night temperatures (Fig. 6). Conidial production at night temperatures of 10-19 C did not differ greatly from a constant day/night temperature regime. However, at 7 C night temperatures, conidia were not produced within 48 hr and only sparingly produced even after 96 hr (Fig. 6).

DISCUSSION

The temperature and moisture requirements for spore production and dispersal of *A. dauci* conidia found in this study agreed closely with requirements reported for other

Alternaria sp. (9, 11, 12, 13, 16) and with those for *A. dauci* reported by Hooker (7). Requirements for periods of leaf wetness or high RH were similar to those observed for *Alternaria porri* f. *solani* in arid regions of Israel (11) and to the observation that rainy weather or dew were key factors in an epidemic of *A. porri* on onions in Nebraska (9).

Rotem (11) often detected spores of *A. porri* f. *solani* during night hours. However, spores of *A. dauci* were never trapped at night and only rarely in the late afternoon. Thus, trapping at night was discontinued. Spore counts of *A. dauci* reported on a weekly basis showed that spores were produced and dispersed over a wide range of temperature and moisture conditions throughout the carrot growing season and also demonstrated cyclic fluctuations in periods of relative spore abundance alternating with periods of spore scarcity. Similar fluctuations in populations of other organisms including crop pests are commonly observed (1), but their significance in this study was not explained.

Several disease forecasting programs have relied on the measurement of RH for predictive purposes often with excellent results (2, 3). Although monitoring RH could be used to predict the time of spore release and dispersal for *A. dauci*, it was not useful for forecasting spore abundance. Hours of leaf wetness were a better indicator of spore abundance. This observation is in good agreement with the report of Rotem and Reichert (12) who stressed the importance of dew for spore production by *Alternaria* sp. in arid regions and shows that dew can assume a similar importance in humid subtropical regions. Others have observed that severe disease outbreaks of *A. dauci* were associated with unusually humid or rainy seasons (6, 8, 13). Controlled environment studies reported here confirm the importance of free moisture for spore production and germination. Since *A. dauci* sporulated only on necrotic tissue which is usually very dry during daylight hours, moisture absorption from dew would be an important factor in spore production and disease increase. Strikingly similar data have been presented for *Alternaria solani* (16).

Zimmer and McKeen (17) found that *A. dauci* damage increased in late summer and fall and attributed it to the influence of daylength on sporulation. Others (6, 7) have made similar observations, but attributed the greater disease incidence in the fall to the increased susceptibility of older leaves. The duration of leaf wetness, which would be expected to increase during the cool, longer nights of late summer and fall was not considered. Doran and Guba (6) demonstrated that *A. dauci* spores germinated best in free water, and Hooker (7) found that free moisture is needed for leaf penetration as well. Thus, free moisture may be the limiting factor in disease incidence and severity.

In the present field studies, no clear relationship between temperature and spore production could be found. Others have shown that growth in vitro, sporulation and pathogenisis of *A. dauci* occur over a wide temperature range (6, 7). Controlled-environment and field studies presented here generally confirmed previous reports and show that night temperature may not be useful in predicting spore abundance because *A. dauci* is able to produce spores at almost all temperatures which prevail during the carrot-growing season. Rotem was unable to relate temperature to spore dispersal in his study of *A. porri* f. *solani* because spores were produced at all the temperatures he encountered during the growing season (11). In this study of *A. dauci*, low temperatures did not stop spore production but decreased the rate. Thus, various combinations of day and night temperatures may result in the production of significant numbers of conidia over a period of two or more nights (Fig. 6). The present study did show the importance of night temperature as well as fluctuating day/night temperatures for spore production at the lowest temperatures where sporulation occurred; i.e., 7 C. However, more work is needed to clarify the interactions of day and night temperatures especially since free moisture is usually present only during the night hours when necrotic tissues can absorb moisture and spore production can occur.

The daily abundance of *A. dauci* conidia observed in carrot fields in Florida is partly explained by the wide range of temperature conditions in which sporulation can occur and by the frequency of dew formation which occurs almost nightly.

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