Infection and Colonization of Inflorescences and Mericarps of Carrot by *Alternaria dauci*

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ABSTRACT

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A 0.25-ha field of carrots (*Daucus carota*) infested with *Alternaria dauci* was managed as an experimental seed production field. Extensive leaf blight in the field caused by *A. dauci* enhanced the probability of attack of flowers and fruit. Components of the carrot inflorescence were examined at intervals for infection by *A. dauci*; all were susceptible. Fruits (mericarps) were vulnerable to infection from early development to maturity. Mericarps infected early in their development contained seed structures completely colonized by the fungus. Tissues and spiny prickles of the vallecular ridges of fully developed mericarps were the most common sites of infection, colonization, and sporulation by *A. dauci*. The fungus was confined to the outer surface and tissues of dried pericarps and did not penetrate the seed coat and endosperm.

Alternaria dauci (Kühn) Groves & Skolko incites an important foliage blight of carrot (*Daucus carota* L.) that is severe in almost all areas where carrots are grown (1,5,7,8,11–13). This pathogen can be seedborne, which may account for its wide distribution (5,8,10,11). There have been two studies of incidence of the pathogen within commercial lots of carrot seed (11,12), and Maude (8,9) has reported eradication of *A. dauci* from infested seed. Netzer and Kenneth (11) demonstrated the location of *A. dauci* in and on carrot seed and found that weed hosts were possible sources of inoculum. Application of protective fungicides to the root-production crop remains the most common disease control practice; however, the role of infested seeds as a primary inoculum source and the impact of these seeds on both seed and crop production must be considered in developing disease management programs. A need for additional basic information on how and when carrot seeds become infected and on the specific location of the pathogen in or on infested seeds prompted this study.

MATERIALS AND METHODS

For convenience, the harvested, dried mericarps, which constitute commercial carrot "seeds" are referred to as seeds, but for specific references to developing fruits and for anatomical structures related to infection studies, botanical nomenclature is used.

Carrot seeds of cultivar Imperator 58 were planted on Myakka fine sand at Sanford, FL, on 15 October 1980 in rows spaced 76 cm apart. Plants were thinned later to 5 cm apart within rows. Phenamiphos (2.4 kg/ha) was applied in the row at planting to control nematodes. The 0.25-ha field was managed for root production until early February 1981, when the root diameters averaged 3–4 cm 1 cm below the crown. Fertilizer was applied at four intervals to provide 138, 180, and 184 kg/ha of N, P_2O_5, and K_2O, respectively. The 1980–1981 winter season was sufficiently cold to induce flowering. Thus, fertilizer was discontinued and the crop was maintained with only hand weeding. Flowering was irregular and began in late February. Seeds were harvested by 1 June. *A. dauci* was established in the field by December 1981 and moved rapidly to new leaves and seed stalks produced after February.

Throughout the flowering period (March through May), component parts of carrot inflorescences from the primary umbels were examined periodically and symptoms associated with *A. dauci* recorded. Infection and colonization by *A. dauci* was verified by examining lesions on plant parts collected early in the morning for the presence of conidiophores and conidia of *A. dauci* at ×60. Where conidiophores but not conidia were found or symptoms indicated recent infection, plant parts were incubated at 22 C in 100% relative humidity with a 10-hr photoperiod (20 $\mu E\ m^{-2}\ sec^{-2}$) for as long as 3 days after collection and examined each day for conidia.

To associate infection and symptom development with flower and fruit development, the following arbitrary growth stages were assigned to the primary umbel, which was the sampling unit employed: 1 = flowers just opening, abundant insect activity on umbel; 2 = flowers large, little or no insect visitation; 3 = petals drying and falling; 4 = petals dropped, small green fruits (schizocarps) prominent; 5 = fruits approaching ultimate size, remaining bright green; 6 = fruits full-sized, turning pale green; 7 = fruits turning brown (mericarp beginning to dry); and 8 = schizocarps and pedicels totally brown, ready for harvest. Time intervals required for each developmental stage were observed and recorded.

On 5 and 13 April, 10 primary umbels representing each of the eight growth stages of carrot inflorescences were collected and examined for disease symptoms and presence of *A. dauci* conidia as described before. Components of the inflorescence were examined for necrotic or discolored tissues or for small, necrotic water-soaked flecks, which were known to be early symptoms of infection by *A. dauci*. When colonization was verified by observing conidia representative of *A. dauci*, samples of infected tissue were fixed in formalin-acetic acid fixative, embedded in paraffin, sectioned at 20 μm, and stained with a fast green-safranin procedure (6).

Incidence of schizocarp infection at different umbel growth stages was examined by collecting five umbels of growth stages 4–8. These were selected at random. Twenty-five schizocarps representing the prevailing stage of schizocarp development within the umbel were excised and placed on filter paper in petri dishes, incubated as described before, and examined for conidiophores and conidia of *A. dauci*. Conidia of *A. alternata* (Fr.) Keissler were commonly observed on all plant parts.

Umbels bearing plump, dry schizocarps containing light brown mericarps were collected in late May, dried for 7 days in an air-conditioned laboratory, and threshed free of branches and stems by hand. Threshing also divided the dried schizocarps into the two component mericarps containing carrot seeds (2). Debris and aborted schizocarps, which were small and shriveled, were removed by hand sieving on a 1-mm screen. Harvested seeds were assayed for germination and incidence of *A. dauci* by placing them on distilled water-moistened filter paper in petri dishes kept at 22 C

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with a 10-hr photoperiod (20 μE m^{-2} sec^{-2}). Mericarps were checked daily for conidia of *A. dauci* for 1 wk. Germination was evaluated after 7 days. Seed milling (to remove prickles and associated wing tissues) was simulated by vigorously hand rubbing 2-g seed samples between the palms for 30 sec, then separating them from the residue by sieving them on a 1-mm sieve. Rubbing and sieving were repeated five times.

Conidia adhering to the surface of harvested and dried mericarps containing seed were tested for viability by vigorously stirring 1-g seed samples in 25 ml of water on a magnetic stirrer for 10 min, decanting the water after the seeds had settled for 30 sec, and filtering 5-ml aliquots through a metricel GA-1 filter (5 μm, 25 mm diam.) (Gelman Instruments Co., Ann Arbor, MI). The filter disks were incubated at 100% relative humidity in darkness for 16 hr at 22 C, dried at room temperature for 1 hr, cleared with 1-methyl, 2 pyrrolidinone, and conidia examined for germination at ×200 with a compound microscope.

RESULTS AND DISCUSSION

Abundant inoculum produced on the blighted foliage of the carrot root crop along with favorable weather promoted the rapid spread of *A. dauci* to new leaves and all parts of the inflorescences and seed stalks as these structures developed. Components of the inflorescences and approximate growth stage at which symptoms appeared on them were recorded (Table 1). Symptoms on foliage, seed stalks, involucre, and involucels were similar to those on leaves. *A. dauci* penetrated foliage through stomates. Early symptoms on petals consisted of necrotic flecks surrounded by a small translucent zone. Tissues became water-soaked, then necrotic, in advance of invasion by mycelia.

Occasionally, functional petals apparently infected in the bud stage displayed lesions bearing conidia, but more commonly, conidia were produced on petals that were drying and ready to fall. Symptoms on stamens were obscure but basically similar to lesions on other tissues. Sporulation occurred infrequently on stamens 5–10 days after anthesis. It was not possible to trace lesions on petals, stamens, and mericarps to the penetration site. Lesions on flower buds were frequently observed. Buds turned pale green, then dark brown, and finally became dried and shriveled. Infected buds occurred singly or in groups; often, areas 1–3 cm in diameter of discolored and damaged flower buds occurred within the umbel.

Infection of mericarps probably occurred soon after flowers opened because sporulating lesions were found on schizocarps before petal fall. Latent periods were estimated to be 5–7 days. New disease damage continued to appear until schizocarps turned brown and approached maturity; when schizocarps turned brown, it was not possible to easily identify new infections. The saprophyte *A. alternata* was abundant on all schizocarps and mericarps examined, but conidiophores and conidia were easily distinguishable from those of *A. dauci* by their relative size and morphology (10).

Infected schizocarps that were shriveled and discolored (greenish brown) were completely invaded by the fungus; the embryo and endosperm were extensively colonized by *A. dauci*. These seeds were not viable. Schizocarps that were normal in size and appearance and taken from umbels approaching maturity (growth stages 7–8) were also commonly infected with *A. dauci*, but the fungus was confined to pericarp tissue. In no instance were apparently viable seeds (as evidenced by plump appearance and abundant and advanced endosperm and embryo development) invaded by fungal mycelia. In 20-μm serial sections, mycelia were observed to approach but not penetrate the seed coat. No histological evidence of fungal structures within the endosperm or embryo tissue of 30 seeds of this type was found. These results are in good agreement with those of Netzer and Kenneth (11), who also concluded that *A. dauci* probably did not penetrate the seed coat and provided some histological and other evidence to support this conclusion.

As schizocarps matured and turned brown, it was not possible without histological examination to distinguish infected and colonized schizocarps from those only infested with surfaceborne conidia. The assay method employed only detected the presence of the pathogen. This was also true for mericarps assayed after harvest. Because either infection or infestation results in *A. dauci* being seedborne, it is convenient to refer to mericarps bearing the pathogen as infested.

On umbels damaged by *A. dauci*, the proportion of infested schizocarps greatly increased within umbels of later growth stages (Table 2). Older schizocarps were exposed to inoculum longer than those on younger umbels, thereby increasing the probability of infection.

An average of 28% of mericarps harvested on three occasions in May (weighing 2–5 kg/lot) were infested by *A. dauci*. Commercial seed lots commonly show much lower levels of infestations (3,4,11) although Netzer and Kenneth (11) found one lot with 75% infested seeds. Seed harvest and separation of debris removes many small shriveled seeds, and in affected crops, many of these seeds may be infested.

Mycelia, conidiophores, and conidia were most common on and in prickles and tissues of the winged vallecular ridges (the prominent winged ridge bearing the barbed prickles of mericarps), but *A. dauci* also invaded bristles and tissues of the primary ridge, cells surrounding oil ducts, interridge mericarp, and stylar tissues. Sampled schizocarps as well as harvested mericarps were often observed to carry conidia, which adhered tightly to the surface wings and prickles of the mericarps. About 90% of these conidia

Table 1. Periods of apparent susceptibility of components of the carrot inflorescence to infection and colonization of *Alternaria dauci* related to stages of umbel development

	Stage of umbel development							
Structure	Flowers open	Anthesis	Petals drying	Small fruit	Fruits enlarged	Fruits pale green	Fruits turning brown	Fruits dry and mature
Seed stalk					X	X	X[a]	
Involucre			X	X	X	X	X	X
Peduncle					X	X	X	
Involucel	X	X	X	X	X	X	X	X
Pedicels						X	X	
Flowers								
Petals	X	X	X					
Stamens			X					
Pistil			X					
Fruit								
Schizocarp				X	X	X	X	X
Style				X	X	X	X	
Development time (days)[b]	0	2–4	6–10	8–12	10–15	14–23	16–30	25–40

[a] X = stage of development when infection was observed.
[b] Times are approximate; not all flowers on each umbel developed at the same time. Flowers were open about 8–10 days after young buds were distinguishable. Latent infection period for *A. dauci* is 5–7 days.

Table 2. Percent infection of carrot schizocarps at various stages of development by *Alternaria dauci*

Stage of schizocarp development	Percent[a] infested by *A. dauci*
Small fruits at petal fall	0
Large green fruits	5
Large fruits turning pale green	10
Large fruits turning brown	90
Fruits dried, ready for harvest	100

[a] Values are averages for five replicates of 25 schizocarps.

Table 3. Proportion of carrot seeds infested by *Alternaria dauci* before and after simulated milling

Seed sample	Avg. sample wt[a] (g)	Percent[a] of sample wt	Percent germination[a]	Percent infected[a] with *A. dauci*
Field-collected	2.043	100.0	62.0 NS[b]	26.4
After milling	1.618	83.9	61.6 NS	4.8*[b]
Residue[c]	0.329	16.1	...	Not tested

[a] Values are averages for five replicates.
[b] * = Value significantly different at $P = 0.01$ (LSD, 9 df); NS = not significant.
[c] Prickles, wing tissue, and residue were removed from seeds by vigorous hand rubbing and separated by sieving with a 1-mm sieve (repeated five times).

were viable after 9 mo of seed storage at ambient laboratory conditions. Netzer and Kenneth (11) obtained similar results and it is concluded that seedborne conidia can also serve as a long-term source of primary inoculum.

Removal of 16% of the total seed sample weight as wings, prickles, and other debris (possibly surfaceborne conidia as well) by simulated seed milling reduced the portion of infested seeds from 26.4 to 4.8% without affecting germinability (Table 3). Commercial milling and cleaning is a much more rigorous process (1); removal of prickles, surface abrasion, and separation of small and shriveled seeds and other debris may significantly reduce the proportion of infested seeds in commercial lots.

Maude (8,9) has reported eradication of *A. dauci* from infested carrot seeds by soaking them in 0.2% thiram for 24 hr. The effectiveness of this treatment may reflect the limited penetration of carrot mericarps by this pathogen.

A. dauci apparently can attack all components of the inflorescence of carrot with equal facility and the mericarps remain vulnerable throughout their exposure in the field. Protective fungicide control programs for seed crops should be designed accordingly.

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