Efficacy of Fungicides Against Persistence of *Alternaria dauci* on Carrot Seed

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ABSTRACT

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Fungicide soaks or seed dressings did not completely eradicate *Alternaria dauci* from a naturally infested lot of carrot (*Daucus carota*) seed. Some treatments appeared effective when small numbers (100) of treated seeds were assayed. *A. dauci* could not be found on treated seeds tested by a simulated seed-germination blotter method or seedling assay. Larger seed samples tested in field isolation plots or in a controlled-environment seedling assay showed that *A. dauci* was not eradicated from all seeds. The most effective fungicides, thiram and iprodione, applied as a 24-hr soak, allowed *A. dauci* to persist on 0.4 and 0.01% of the treated seeds, respectively. The effectiveness of the iprodione soak was dependent on fungicide concentration, temperature, and treatment duration.

Additional key words: seedborne fungi

The seedborne phase of *Alternaria dauci* (Kühn) Groves & Skolko is important in the etiology of Alternaria leaf blight of carrot (*Daucus carota* L.) (2,4,7,8). *A. dauci* carried in or on the seed attacks young carrot seedlings, killing them before or soon after emergence, and causes typical damping-off symptoms (4,7,8). Conidia produced on the infected hypocotyl provide inoculum that, in turn, infects the foliage (4,7,8,10). Seed dressings such as thiram and captan can reduce losses from soil-inhabiting pathogens such as *Pythium* sp. but are apparently ineffective against seedborne *A. dauci*.

Maude (4,5) developed a thiram soak method for eradicating seedborne pathogens from a variety of crop seeds, including *A. dauci* from carrot seed. Iprodione has demonstrated activity against *Alternaria* sp. and offers potential for controlling *A. dauci* and eradicating it from seeds (3,6); thus, it was also evaluated.

In this study, some fungicides were evaluated as seed dressings or seed soaks to eradicate *A. dauci* from infested carrot seed. Methods commonly used to evaluate such treatments were compared with a seedling assay for seedborne *A. dauci*. Effects of treatment duration and temperature on the effectiveness of iprodione applied as a 24-hr soak were also investigated.

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MATERIALS AND METHODS

Seed treatment. A carrot seed lot (cultivar Imperator 58) naturally infested with *A. dauci* was used to evaluate fungicide treatments. Seeds were planted on Myakka fine sand at Sanford, FL, on 15 October 1980 in rows spaced 76 cm apart. Plants were later thinned to 5 cm apart. Phenamiphos (2.4 kg/ha) was applied at planting to control nematodes. The 0.25-ha field was managed for root production until early February 1981, when roots averaged 3–4 cm in diameter 1 cm below the crown. Fertilizer was applied at four intervals to supply 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. Fertilizer was discontinued and the crop was maintained with only hand weeding.

Flowering was irregular and began by late February. Seeds were harvested by 1 June. Seed was 1 yr old when the experiments were performed.

A lot of commercially produced carrot seed (cultivar Chantenay) assayed by the seed-germination blotter and seedling methods described later was found to be free of *A. dauci* and was employed as a control to test the effect of seed treatments on germination. Fungicide seed dressings were added to 2 g of carrot seeds in a small plastic bag (10 × 10 cm). Dry formulations were added at specified rates; liquid formulations were dispersed in 2 ml of water, then added. Plastic bags were expanded with air, sealed by twisting the opening shut to form a semirigid container, and the bags were vigorously shaken for 3 min to distribute the fungicide on the seeds. Small amounts of fungicide adhered to the bag. Seeds treated with liquid formulations were air-dried for 4 hr on trays before use.

Fungicide soaks were applied using a modification of the procedure outlined by Maude (4,5). Small lots (1 or 2 g) of seeds were placed in 120-ml glass prescription bottles containing specified rates of fungicide in 50 ml of water. Care was taken to immerse all seeds and they were not disturbed during treatment. Larger samples (5 or 10 g) were tied in loose-fitting cheesecloth bags and immersed in 400 ml of fungicide suspension. Bags containing seeds were occasionally agitated. Where treatment temperatures are specified, containers holding the fungicide preparation were immersed in a temperature-controlled water bath or kept in a temperature-controlled environment; otherwise, treatments were made at 22 C (ambient laboratory temperature). Seeds were removed by filtration and dried on paper towels before planting. Seeds were hand-counted for assay. For large numbers, the required number of seeds was weighed and additional samples of the required number were estimated by weight.

Fungicides used in treating carrot seeds included benomyl 50% (Benlate), chlorothalonil 40.4% (Bravo 500), iprodione 50% (Rovral), mancozeb 80% (Dithane M-45), and dichlone 50% (Quintar 5F).

Seed assay. Treated seeds were placed in petri dishes on filter paper moistened with water and kept at 22 C with a 10-hr day length (cool-white fluorescent light, 16 $\mu E/m^{-2}/sec^{-1}$). Seeds were examined daily for 5 days and again at 7 days for

conidia of *A. dauci*. Water was added to keep the filter paper moist. This test was designed to simulate the seed-germination blotter method used by others to assay seeds for *A. dauci* (1,4,7–9) that generally conforms to the International Seed Testing Association Guidelines (1,9).

A test complementary to the petri dish method, which was more time consuming but more sensitive in detecting seedborne *A. dauci*, was developed and used to assay some treated seeds. A plant-growth medium (TerraLite Vegetable Plug Mix, W. T. Grace and Co., Cambridge, MA) was loosely packed 5 cm deep in plastic bedding plant containers (12.5 × 12.5 × 6 cm). Eight of these containers were nested in a plastic master tray (27 × 54 × 6.5 cm) without drain holes (Com Pac D801 and F1020, respectively, T. O. Plastics, Minneapolis, MN). Carrot seeds were distributed on the surface of the growth medium (100 seeds per container) and covered with 1 cm of plant-growth medium. Trays containing the seeds were placed at 20 C with a 16-hr day length (cool-white fluorescent, 47 $\mu E/m^{-2}/sec^{-1}$) and watered as needed. Three times each week, plants were watered with half-strength Hoagland's solution (11). Large seed samples were seeded directly into the master trays (22 × 54 × 6.5 cm) (500 or 2,200 seeds per tray) and grown as described before. Ten days after planting and daily thereafter, seedlings were examined for symptoms of infection by *A. dauci*. Attack by the pathogen caused damping-off symptoms or collapse of seedlings. Suspected seedlings were removed from trays, placed on moist filter paper in petri dishes at room temperature (22 C), and examined after 24 hr and daily for 3 additional days for conidia and conidiophores of *A. dauci*; tests were terminated after 4 or 5 wk.

Treated seeds were also planted in the field in 1-m^2 plots separated by at least 200 m; there were no other carrot plants in the area chosen, and wild hosts (8) of *A. dauci* were not present. Wooden frames 1 m^2 × 19 cm high were buried in the soil (Myakka fine sand) near Sanford, FL. Ten centimeters of the frame protruded above the soil surface. Soil within the frame was packed lightly and about 5,000 carrot seeds (determined by weight) were evenly distributed on the soil surface and covered with 1.5 cm of additional soil. Aldicarb 15G (5.6 kg/ha a.i.) and 560 kg/ha of a 5-5-8 fertilizer were broadcast and plots were irrigated at planting and later as needed. Plants were examined weekly for 6 wk for foliar symptoms of *A. dauci*. Disease damage was rated on a subjective scale of 0–5, where 0 = no disease; 1 = few scattered lesions, less than 5% leaf area damaged; 2 = abundant lesions, 5–15% leaf area damaged; 3 = 20–40% leaf area damaged; 4 = 40–60% leaf area damaged; and 5 = severe defoliation, only new leaves remaining.

Table 1. Efficacy of some fungicide seed dressings in eradicating *Alternaria dauci* from infested carrot seed determined by simulated seed-germination blotter assay

Seed dressing	Rate of application (g/kg)	Germination[y] (%)	Seeds with *A. dauci*[y,z] (%)
Chlorothalonil	0.9	43.0 a	0.5 a
Thiram	2.2	33.5 b	0.0 a
Mancozeb	2.2	44.5 a	2.5 a
Dichlone	1.1	40.8 a	3.5 a
Control	None	53.3 a	23.5 b

[y] Values are averages for four replicates, 100 seeds per replicate. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Percent seeds bearing conidiophores and conidia of *A. dauci* 7 days after initiation of test.

Table 2. Effects of some 24-hr fungicide soak treatments on seed germination and survival of *Alternaria dauci* on infested carrot seed determined by simulated seed-germination blotter assay

Fungicide treatment	Fungicide concentration (%)	Percent germination[y]		Percent seeds with *A. dauci* after[y,z]	
		Healthy seed lot	*A. dauci*-infested seed lot	2 Days	5 Days
Mancozeb + benomyl	0.40 0.25	25.0 a	23.0 a	0 a	0 a
Thiram	0.20	78.0 b	56.8 b	0 a	0 a
Chlorothalonil	0.40	72.3 b	56.8 b	0 a	0 a
Water	···	74.8 b	61.3 b	0 a	4.3 b
No treatment	···	80.5 b	61.5 b	22.0 b	22.8 c

[y] Values are averages for four replicates, 100 seeds per replicate. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Percent infested seed remaining in an *A. dauci*-infested seed lot after fungicide treatment as determined by positive identification of conidia and conidiophores 2 and 5 days after initiation of test; 7-day values were unchanged from 5-day values.

Table 3. Comparison of some 24-hr fungicide soak treatments for eradication of *Alternaria dauci* from infested carrot seed determined by seedling assay

Fungicide soak treatment	Fungicide concentration (%)	Seedlings emerging[x,y] (%)	Seedlings infested[z] (%)
Thiram	0.20	62.2 a	0.28 a
Chlorothalonil	0.40	65.4 a	0.54 a
Iprodione	0.25	65.7 a	0.19 a
Iprodione	0.50	65.2 a	0.00 a
Water	None	42.8 b	71.80 b
No treatment	None	15.9 c	81.70 c

[x] Values are averages for four replicates, 500 seeds per replicate. Percentage values analyzed as arc sine values. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[y] Percent emerging seedlings compared with a concurrent germination test.
[z] Percent emerged seedlings showing typical damping-off symptoms and bearing conidiophores and conidia of *A. dauci*.

RESULTS AND DISCUSSION

Thiram applied as a seed dressing at the recommended rate (2.2 g/kg) prevented formation of *A. dauci* conidia on treated seeds when replicated samples consisting of 100 seeds were assayed by the simulated seed-germination blotter method (Table 1). Conidia were produced on seeds treated with chlorothalonil, mancozeb, and dichlone but not thiram (Table 1). Thus, thiram appeared to be effective when assayed by this method. When some of the fungicides used as seed dressings were employed as 24-hr seed soaks following the procedure of Maude (4,5), thiram, mancozeb plus benomyl, and chlorothalonil all appeared effective in eradicating *A. dauci* when treated seeds (100 seeds per replicate) were assayed by the seed-germination blotter method (Table 2). Dichlone and mancozeb alone were highly phytotoxic and severely reduced germination when applied as a seed soak; these data are not reported in Table 2. A 24-hr soak in water also reduced the apparent proportion of infested seeds (Table 2). This reduction in infested seeds by a water-soak occurred routinely during this study and may have resulted from removal of surfaceborne conidia common on *A. dauci*-infested seed (8; J. O. Strandberg, *unpublished*).

The thiram soak method appeared to work well when small samples (100 seeds per replicate) were assayed by a method that closely simulated the seed-germination blotter method often used to detect infested seeds (1,4,8,9). When larger samples (5,000) of thiram-treated seed were planted in replicated isolation plots in the field, occurrence of Alternaria leaf blight symptoms in these plots demonstrated that a significant but

unknown proportion of treated seeds were not freed of *A. dauci*. In isolation plots planted with untreated seeds and with seeds dressed with thiram (2.5 g/kg), all four replicates for both treatments contained plants with foliar damage from *A. dauci*; average disease damage ratings (0–5 subjective scale) were 2.2 and 3.3 at 6 wk after planting, respectively. In plots planted with thiram-soaked seed (0.2%, 24 hr) and seeds that were both thiram-soaked and thiram-dressed, three of four replicates contained diseased plants; disease damage ratings were 0.8 and 1 by 6 wk after planting, respectively. These disease damage ratings may reflect the level of original inoculum persisting on infested seeds after treatment. Disease damage ratings were lower in plots containing thiram-treated seeds. Maude (4) also found thiram dressings ineffective but he concluded that the thiram soak eradicated *A. dauci*. The apparent efficacy of treatments in eradicating *A. dauci* from carrot seed is dependent on the sensitivity of the assay method.

Seedling assay was more sensitive than the seed-germination blotter method in detecting *A. dauci*-infested seed (J. O. Strandberg, *unpublished*). Others have employed seedlings to test pathogenicity of *A. dauci* (1,4,7) but have not used them as an assay method. In the seedling test employed in this study, seedlings that collapse or show typical damping-off symptoms (1,4) can easily be checked for conidia of *A. dauci*.

There are problems associated with using the seedling test for the quantitative estimation of *A. dauci*-infested seeds. Seedlings killed before emergence are not scored; a concurrent seed-germination test must be run. Seedlings expected, but not observed, to emerge were usually killed by *A. dauci* before emergence. In addition, already emerged, uninfected seedlings were occasionally attacked and killed by *A. dauci* originating from a nearby infected seedling; the rate of this occurrence was low (<2%) at a spacing of 1 cm between seeds. These problems are recognized but judged not to severely affect the conclusions of this study.

A 24-hr seed soak in 0.5% iprodione at 30 C provided apparent eradication of *A. dauci* when medium-sized seed samples (500 seeds per replicate) were assayed with the seedling test (Table 3). Chlorothalonil (0.4%), iprodione (0.25%), or thiram (0.2%) did not completely eradicate the pathogen when treated seeds were assayed with the seedling test.

Effects of treatment time and iprodione concentration in the seed-soak method were examined in detail. The seedling-assay method was used to evaluate all treatments (100 seeds per replicate). At 30 C, iprodione concentrations lower than 0.5% did not eradicate the pathogen except for the 0.25% concentration, which required a 24-hr treatment to achieve apparent eradication (Fig. 1). At 0.5%, 12 hr was apparently sufficient to eradicate *A. dauci*. Analyzed as a 5 × 6 factorial, this experiment indicated significant interaction ($P = 0.05$) between treatment duration and iprodione concentration. There were no significant differences in seed germination and no interactions with seed germination and iprodione concentration or with treatment duration. Although 0.25% iprodione applied for 24 hr apparently eradicated *A. dauci* in this test, it did not do so in a previous experiment (Table 3), indicating variation in either treatment conditions or assay methods. Because controlled treatment conditions were employed and replicates of only 100 seeds were used, the assay methods were logically suspect.

Temperature during treatment was also important. A subeffective iprodione

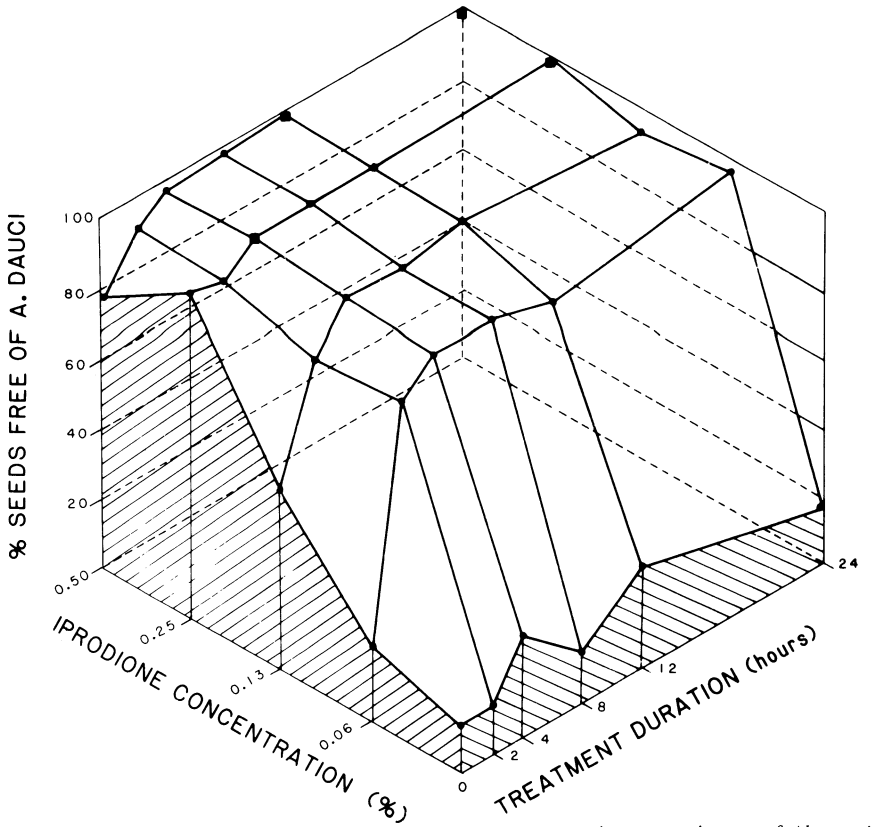

Fig. 1. Interaction of treatment duration and iprodione concentration on persistence of *Alternaria dauci* on carrot seeds. ■ = 100% of seeds free of pathogen.

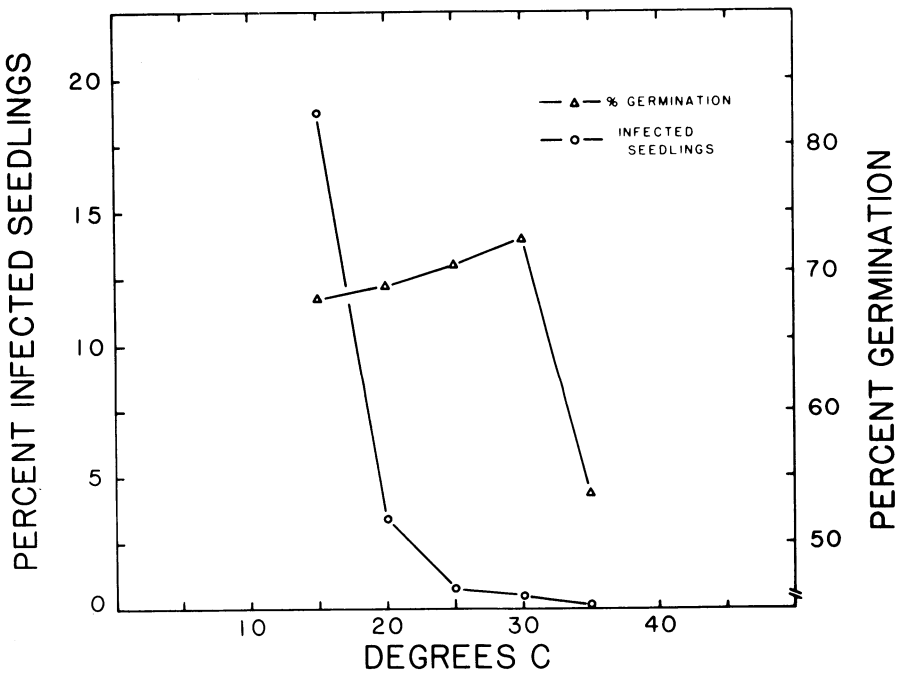

Fig. 2. Effect of temperature on persistence of *Alternaria dauci* on carrot seeds after an iprodione soak (24 hr, 125 ppm).

Table 4. Efficacy of thiram and iprodione seed soaks at 30 C in eradicating *Alternaria dauci* from infested carrot seed ascertained from large seed samples by seedling assay

Seed-soak treatment	Fungicide concentration (%)	No. seedlings emerging[x,y]	Seedlings infested[x,z] (%)
Water	None	393.8 a	36.33 a
Thiram	0.2	1,256.3 b	0.43 b
Iprodione	0.5	1,400.1 b	0.01 b

[x]Values are averages for six replicates, 2,200 seeds per replicate. Percentages analyzed as arc sine values. Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

[y]A value of 1,430 emerged seedlings is expected based on a concurrent germination test of the seed lot employed.

[z]Percent of emerged seedlings showing typical damping-off symptoms and bearing conidiophores and conidia of *A. dauci*.

concentration (0.125%) applied as a 24-hr soak at 15, 20, 25, 30, and 35 C demonstrated increasing effectiveness in eradicating *A. dauci* as treatment temperature increased to 35 C (Fig. 2). Seed damage occurred at temperatures higher than 30 C (Fig. 2). A separate test of 0.5% iprodione soaks applied to healthy carrot seed at 15, 20, 25, 30, 35, 40, and 45 C for 24 hr resulted in germinations of 77.7, 72.5, 70.3, 67.7, 38.2, 2.3, and 0.25%, respectively. Thus, unacceptable seed damage probably limits treatment temperature to 30 C or lower.

To confirm the efficacy of 0.5% iprodione applied as a 24-hr seed soak at 30 C, 2-g samples (about 2,200 seeds per sample, replicated six times) were treated and assayed in a seedling test suitably modified for large samples. Thiram applied as a comparison treatment at 0.2% for 24 hr at 30 C did not eradicate *A. dauci*; the pathogen persisted and attacked an average of 0.4% of the emerging seedlings (Table 4). Treatment with iprodione at 0.5% resulted in an average of 0.01% infected seedlings (Table 4); one infected seedling in one replicate was recorded that had typical symptoms and on which *A. dauci* conidia were produced after 24 hr. Iprodione significantly ($P = 0.05$) increased the number of emerging seedlings (Table 4). Based on a concurrently measured germination of 65% for the seed lot used, an average of 1,430 seedlings was expected to emerge. An average of 1,400 seedlings emerged, leaving 2% of the seedlings unaccounted for and possibly killed by *A. dauci* before emergence.

In carrot root production fields, from 2.1×10^6 to 3.1×10^6 seeds per hectare are commonly planted. Treatment of a large lot of carrot seed, such as the one employed in this experiment with iprodione, would allow an estimated 200–300 infected seedlings per hectare ($2.1–3.1 \times 10^6 \times 0.01\%$). Presumably, this could result in an epidemic of Alternaria leaf blight. The unusually high level of infestation in the seed lot employed in this study would seldom be encountered in commercial seed lots (4,8,9). Both thiram and iprodione soaks applied to less severely infested seed lots would be beneficial in reducing initial inoculum. Assumptions of eradiation of *A. dauci* or of pathogen-free carrot seed, however, clearly require both sensitive and rigorous methods of assessment.

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