Seed-Treatment Fungicides for Control of Seedborne *Alternaria helianthi* on Sunflower

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ABSTRACT

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Inoculation of sunflower seed with conidial suspensions (about 410, 4,100, and 41,000/ml) of *Alternaria helianthi* resulted in development of disease symptoms on seedlings. Growth of *A. helianthi* in vitro was highly inhibited by chlorothalonil and thiabendazole; inhibited moderately by benomyl, captan, mancozeb, and thiram; inhibited slightly by iprodione; and inhibited the least by triadimenol. Seedborne *A. helianthi* was controlled in the field by seed treatment with benomyl, captan, chlorothalonil, iprodione, mancozeb, or triadimenol.

In 1978, Zimmer and Hoes (8) indicated that *Alternaria helianthi* (Hansf.) Tubaki & Nishihara was potentially dangerous to sunflower (*Helianthus annuus* L.) production in North America because it is seedborne and could be introduced on imported seed. Sackston (5) also suggested that infested seed imported from other continents constituted a threat to the production of sunflower on this continent. These predictions became a reality in 1980, when the occurrence of *A. helianthi* on sunflower was reported in several states (3,4,6). Lipps and Herr (3) reported that the sunflower crop in Ohio was severely affected with leaf and stem spots caused by *A. helianthi* during the 1980 growing season. They (2) demonstrated that *A. helianthi* was present on seed and attributed the introduction of the pathogen into Ohio to the planting of infested seed.

The objective of this study was to determine if seedborne *A. helianthi* could be controlled by seed-treatment fungicides.

MATERIALS AND METHODS

Seed transmission. Conidial suspensions of *A. helianthi* were obtained from 4-wk-old petri plate cultures grown on fresh potato-dextrose agar (FPDA) (1) by adding 10 ml of sterile water to each plate, rubbing the colony surface, and straining the resulting suspension through two layers of cheesecloth. Carboxymethylcellulose (1%) was added to the conidial suspension as a spreader and adhesive. Seeds of sunflower cultivar Sun Hi-S304 were surface-sterilized in a 1:1 (v/v) mixture of 95% ethyl alcohol and 5.25% sodium hypochlorite solution for 1 min, then air-dried on paper towels. One hundred surface-sterilized seeds were placed in a plastic bag and inoculated by atomizing 1 ml of conidial suspension (about 410, 4,100, and 41,000/ml) onto the surface of the seeds while agitating them to ensure complete coverage. Inoculated seeds were then air-dried on paper towels. Four 15-cm pots containing sterile vermiculite were planted with 25 inoculated seeds for each of the three conidial concentrations, and four pots were planted with uninoculated seeds as controls. Treatments were completely randomized on a greenhouse bench, and plants were grown at about 25 C with supplemental fluorescent lighting (9,300 lux) for 12 hr per day. After 3 wk, plants were placed in a mist chamber at 27 C for 48 hr, then returned to the greenhouse. The number of plants with stem lesions and the number of plants with leaf lesions per pot were recorded 1 wk after removal from the mist chamber.

Paper-disk bioassay. A modified version of the paper-disk fungicide assay described by Sharvelle (7) was used to determine sensitivity of *A. helianthi* to eight fungicides: benomyl (Benlate), captan (Captan), chlorothalonil (Bravo 500), iprodione (Rovral), mancozeb (Manzate 200), thiram (Arasan 50 Red), thiabendazole (Mertect), and triadimenol (Baytan). Stock solutions were prepared and diluted to obtain four concentrations (200, 100, 50, and 25 μg a.i./ml) of each of the fungicides. Conidial suspensions (about 5×10^4 conidia per milliliter) were prepared from approximately 4-wk-old cultures of *A. helianthi* as described previously.

All equipment was autoclaved for 30 min at 121 C. Nine Schleicher and Schuell filter paper disks (12.7 mm in diameter) were placed on paper towels in a petri dish. The disks were impregnated with one of the fungicide concentrations by adding two drops (about 140 μl) of the stock dilution to each disk with a 10-ml pipette, then one drop (about 50 μl) of the conidial suspension was added to each disk with a 2-ml pipette. The nine treated and inoculated disks were transferred aseptically to three FPDA plates. Each plate, containing three disks, constituted a replicate and three plates were used per fungicide concentration. Paper disks treated with sterilized water plus the conidial suspension served as controls. Petri plates with the treated and inoculated disks were placed on a laboratory bench under 1,000-lux continuous fluorescent lighting at 24 ± 2 C. After 4 days, counts were taken of the number of disks with growth of *A. helianthi* and the percentage of the area colonized by *A. helianthi* on each disk was estimated. The experiment was then repeated.

Seed-treatment field tests. All field trials were conducted at Wooster, OH, on Wooster silt loam soil but at different field sites. Before planting, 112 kg/ha of nitrogen, 57 kg/ha of phosphorus, and 57 kg/ha of potassium were broadcast on all plots, and trifluralin (Treflan, 1.12 kg/ha) was incorporated before planting for weed control.

To determine the effect of seedborne *A. helianthi* and seed-applied fungicides on emergence of sunflower seedlings, a field trial was conducted using surface-sterilized, inoculated and uninoculated seed. Seed surface-sterilization and seed inoculation (5×10^4 conidia per milliliter) were performed as described previously. After inoculation, when seeds were dry, one of five fungicides (captan, chlorothalonil, iprodione, mancozeb, and thiram) was atomized onto 100 seeds shaken in a plastic bag using 1 ml of a 150-μg a.i. concentration of fungicide per milliliter. Controls consisted of surface-sterilized, inoculated and uninoculated seed atomized with 1 ml of sterile water. All treatments were arranged in a randomized block design with four replicates. Each

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plot consisted of 100 seeds planted by hand in 10.7-m-long rows spaced 75 cm apart. Established plants were counted 3 wk later.

Three field trials were conducted to determine the effectiveness of seed-applied fungicides for control of seedborne *A. helianthi*. Seeds for each trial were surface-sterilized, inoculated (5×10^4 conidia per milliliter for trial 1 and 2.5×10^4 conidia per milliliter for trials 2 and 3), and treated with fungicides (captan, chlorothalonil, iprodione, mancozeb, and thiram for trial 1 and benomyl, captan, chlorothalonil, iprodione, mancozeb, thiabendazole, thiram, and triadimenol for trials 2 and 3) using 1 ml of a 150-μg a.i. concentration of fungicide per milliliter per 100 seeds as described previously. Inoculated, surface-sterilized seed served as controls.

In trial 1 (planted in 1981) and trial 2 (planted in 1982), each plot consisted of 100 seeds planted in 10.7-m-long rows spaced 70 cm apart. In trial 3 (planted in 1982), each plot consisted of 50 seeds planted in 5.4-m-long rows spaced 70 cm apart. In all three trials, treatments were arranged in a randomized block design with four replicates. Established plants were counted about 3 wk after planting.

RESULTS

Seed transmission. Inoculation of sunflower seeds with conidial suspensions of *A. helianthi* resulted in the development of lesions on the stems and leaves (Fig. 1). In each treatment (about 410, 4,100, and 41,000 conidia per milliliter), leaf lesions developed on a significantly greater ($P = 0.05$) number of seedlings than did stem lesions. Stem lesions did not girdle stems or kill any of the plants; leaf lesions were confined mainly to the cotyledons and first pair of true leaves.

Paper-disk bioassay. There was a correlation ($r = 0.81$) between the number of disks with growth and the percentage of area on the disks covered with growth of *A. helianthi* for all fungicides tested. Because this correlation coefficient was highly significant ($P < 0.01$), only the data from the percentage area covered are presented (Fig. 2). Surfaces of all untreated control disks were covered with growth of *A. helianthi* 4 days after conidia were placed on them. Growth did not occur on inoculated paper disks treated with thiabendazole or chlorothalonil at any concentration tested. Triadimenol did not significantly inhibit growth of the fungus at any concentration and iprodione was less inhibitory than benomyl, captan, mancozeb, or thiram. At concentrations of 100 and 200 μg a.i./ml, captan, mancozeb, and thiram inhibited growth of the fungus as effectively as thiabendazole or chlorothalonil.

Seed-treatment field tests. In the field trial designed to determine the effect of fungicide seed treatments and seed inoculation on incidence of seedling blight caused by seedborne *A. helianthi*, the mean stand in the no-fungicide, inoculated seed plots (75%) was significantly less ($P = 0.05$) than stands in the no-fungicide, uninoculated seed plots (86%) (Table 1). Captan, chlorothalonil, iprodione, mancozeb, and thiram increased stands over the no-fungicide control when inoculated seeds were treated, but only chlorothalonil increased stands over the no-fungicide control when uninoculated seeds were treated. Of the fungicides tested in this experiment, only captan resulted in a significantly reduced stand when inoculated seeds (85%) were treated compared with when uninoculated seeds were treated (90%).

In the three field studies conducted to determine the effectiveness of fungicides for control of *A. helianthi* on surface-sterilized, inoculated seed, plots planted with chlorothalonil-treated seed had the greatest mean plant stands and the no-fungicide control had the lowest mean plant stands in all trials (Table 2). Although plots planted with chlorothalonil-treated seed had the greatest mean plant stand in all three trials, stands from seed treated with benomyl, mancozeb, or triadimenol were not statistically different from those of chlorothalonil-treated seed in two of three trials. Seed treated with thiabendazole or thiram did not produce stands significantly different from the no-fungicide control in any of the trials.

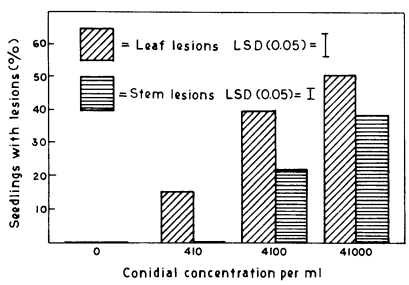

Fig. 1. Effect of inoculating seeds with three concentrations of conidia of *Alternaria helianthi* on the incidence of leaf and stem lesions on 4-wk-old sunflower plants grown in the greenhouse. Conidia were suspended in 1% carboxymethylcellulose and atomized onto seed surfaces before planting.

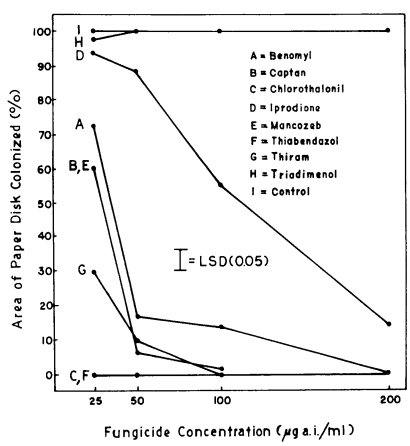

Fig. 2. Effects of eight fungicides at four concentrations on growth of *Alternaria helianthi* as determined by paper-disk bioassay. Means based on estimates of the percentage of colonization on nine 12.7-mm-diameter paper disks after 4 days at 24 ± 2 C. Data points are the means of two experiments.

DISCUSSION

This investigation verified that *A. helianthi* on seed could be a source of inoculum for leaf and stem lesions and seedling blight on sunflower seedlings.

Table 1. Effect of seed-treatment fungicides on stands from sunflower seeds inoculated or not inoculated with conidia of *Alternaria helianthi*[a]

Treatment[b]	Plant stand (%)[c]	
	Seed inoculated	Seed not inoculated
Captan	85[d]	90
Chlorothalonil	90	94
Iprodione	84	82
Mancozeb	83	80
Thiram	80	81
No fungicide	75	86

[a] One hundred seeds were surface-sterilized in 1:1 (v/v) 95% ethyl alcohol:5.25% sodium hypochlorite solution for 1 min and atomized with 1 ml of 5×10^4 conidia per milliliter in 1% carboxymethylcellulose (inoculated seed) or with 1 ml of sterile water (uninoculated seed).
[b] Fungicides were applied by atomizing 1 ml of a 150-μg a.i./ml concentration onto 100 seeds.
[c] Stand based on percentage of established plants 3 wk after planting 100 seeds per 10.7-m row.
[d] Interaction LSD ($P = 0.05$) = 4.4.

Table 2. Effectiveness of seed-treatment fungicides in controlling seedborne *Alternaria helianthi* and their effect on plant stands[a]

Treatment[b]	Plant stand (%)[c]		
	Trial 1	Trial 2	Trial 3
Benomyl	···	69	82
Captan	85	68	81
Chlorothalonil	91	74	88
Iprodione	84	69	81
Mancozeb	83	70	84
Thiabendazole	···	67	81
Thiram	80	68	79
Triadimenol	···	72	85
No fungicide	75	63	76
LSD (0.05)	5.2	5.6	5.4

[a] One hundred seeds were surface-sterilized in 1:1 (v/v) 95% ethyl alcohol:5.25% sodium hypochlorite solution for 1 min and atomized with 1 ml of 5×10^4 conidia per milliliter in 1% carboxymethylcellulose (inoculated seed) or with 1 ml of sterile water (uninoculated seed).
[b] Fungicides were applied by atomizing 1 ml of a 150-μg a.i./ml concentration onto 100 seeds.
[c] Stand based on percentage of established plants 3 wk after planting 100 seeds per 10.7-m row for trials 1 and 2 and 50 seeds per 5.4-m row for trial 3.

Lesions on seedlings in the greenhouse study were smaller than lesions that generally develop on plants in the field, where environmental conditions may be more favorable for lesion development and subsequent sporulation of the pathogen. Lesions developing on seedling leaves from seedborne inoculum could cause the pathogen to spread within and possibly between fields.

We did not attempt to count the diseased plants in the field trials because it was not possible to determine what proportion of the disease symptoms was due to seed inoculation with conidia of *A. helianthi*. Some lesions may have developed from windblown conidia from sources outside the test plot or from lesions developing on seedling leaves in seed-inoculated control plots. Evaluation of seed-treatment materials effective in reducing loss of stands from *A. helianthi* was considered valid because seedling blight has been reported as an important phase of this disease (6).

Because seed can be a source of inoculum, fungicide seed treatments may be an important method for limiting the introduction of *A. helianthi* into new areas. Since 1981, when *A. helianthi* caused severe defoliation in some Ohio sunflower fields (3), much of the commercial seed sold has been treated with a fungicide (captan). This treatment may help reduce the spread of *A. helianthi* within Ohio and to new sunflower-growing areas.

Of the eight fungicides tested in vitro, chlorothalonil and thiabendazole completely inhibited the growth of *A. helianthi*, whereas in field seed-treatment tests, only chlorothalonil provided consistent control of seedling blight as determined by stand counts (Table 2). Thiabendazole did not improve plant stands over the no-fungicide control, indicating that it may not have controlled other seedborne (2) or soilborne organisms pathogenic to sunflower seedlings. On the other hand, triademinol did not inhibit growth of *A. helianthi* at any concentration tested in vitro, but in field seed-treatment tests, it controlled seedling blight as effectively as chlorothalonil. Perhaps, in the field study, triademinol inhibited *A. helianthi* by some indirect means or the material was converted to some more toxic form. Additional work is needed to determine the effect of triademinol on seedborne *A. helianthi*. Considering all of the field trials conducted in this study (Tables 1 and 2), benomyl, captan, chlorothalonil, iprodione, mancozeb, and triadimenol may be effective in reducing stand losses to Alternaria seedling blight of sunflower.

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