Importance of *Alternaria carthami* and *A. alternata* in Causing Leaf Spot Diseases of Safflower

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ABSTRACT

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Isolations from leaf spots on field-grown safflower (*Carthamus tinctorius*) in Montana in 1976 in early stages of development yielded *Alternaria carthami* almost exclusively, whereas toward maturity, *A. alternata* was predominant. Under greenhouse conditions, *A. carthami* was pathogenic on safflower at all growth stages. *A. alternata* also infected healthy safflower plants but infections remained dormant until leaf senescence. Both fungi were isolated from safflower seeds. Four seed-treatment fungicides provided only partial control of these fungi in field and greenhouse tests. Seed produced in Arizona were nearly free of these fungi, whereas seed produced under wetter conditions in Montana were heavily infested with *A. carthami* and *A. alternata* and had inferior germination and seedling vigor.

Additional key words: conidia trapping

Leaf spot diseases of safflower (*Carthamus tinctorius* L.) severely reduce yields in Montana when prolonged moist periods occur during flowering (1,2,13). Leaf spot diseases on safflower have been reported from India (3), Israel, the USSR, East Africa (4), and Australia, where yield losses as high as 90% have been reported (5). The causal agent of this disease is reported to be *Alternaria carthami* Chowdhury (3), but other *Alternaria* spp. (2) and *Pseudomonas* spp. (6,8) have been isolated from leaf spots on safflower.

The objectives of these experiments were to determine the importance of *Alternaria* spp. in causing leaf spot diseases of safflower in Montana and to study disease development in the field. A preliminary abstract of this work has been published (10).

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MATERIALS AND METHODS

Conidia trapping, isolation, and culture techniques. Conidia trapping was done during the 1976 growing season with rotorod samplers (Model 60 A, Metronics Associates Inc., Palo Alto, CA 94304) in a safflower field (cultivar S208) and in a disease nursery in the lower Yellowstone River Valley, near Sidney, MT. The latter had been continuously cropped since 1961 to a composite of 555 safflower introductions from the 1960 USDA World Safflower Collection. Conidia trapping was conducted every Monday, Wednesday, and Friday from 0800 to 1600 hours from 12 July through 23 August 1976. *Alternaria* conidia were counted on two rods per exposure time and calculated as conidia per liter of air per hour. During the sampling period, isolations were made from leaf spots in the disease nursery and from different safflower cultivars and breeding lines in nearby field plots. The isolated fungi were identified as *A. carthami* and *A. alternata* (Fr.) Keissler after Ellis (4). The fungi were cultured on potato-carrot agar (PCA) prepared by macerating 20 g each of peeled white potatoes and carrots in a blender, then mixed with 30 g of commercial potato-dextrose agar in 1,000 ml water and autoclaved. This medium allowed good sporulation of *A. carthami*. To avoid bacterial contamination, 2.5 μg/ml of microfiltered oxytetracycline hydrochloride was added to the PCA after autoclaving.

Seed transmission of *Alternaria* spp. and seed treatment. Seeds of safflower cultivars US-10, S208, and Sidwill used in these experiments were harvested from plants grown at the Eastern Agricultural Research Center, Sidney, MT, except for one Sidwill seed sample obtained from increase plots grown at Yuma, AZ. Samples of commercially grown safflower cultivar S208 were supplied by Continental Grain Co., Culbertson, MT. Safflower plants used in greenhouse experiments were grown in autoclaved sandy loam topsoil mixed with peat moss (3:1) in 25-cm pots or metal trays (38 × 20 × 10 cm) on greenhouse benches illuminated by 1,000W metal halide lamps with a 16-hr light period.

To determine seed transmission of *Alternaria* spp. on safflower, seeds treated in 0.5% NaOCl for 10 min and untreated seeds of cultivars US-10, S208, and Sidwill from different origins were plated out on PCA, 96–100 seeds on 8–10 PCA plates per cultivar per origin. Fungal growth was examined under a light microscope at ×125. Forty-five to 50 seeds from each of the same cultivars and origin were also planted in metal trays under greenhouse conditions (9–10 replicates of 5 seeds per cultivar). After 12 days, emergence of seedlings with and without distinct *Alternaria* lesions on hypocotyl and cotyledons were counted (Table 1). Eighty-five isolations were made from distinct lesions to determine the organism causing them.

Four fungicides, thiram (50% a.i. Arasan 50-Red), chloroneb (65% a.i. Demosan T), mancozeb (80% a.i. Dithane M-45), and PMA (7% a.i. phenylmercury ammonium acetate) were tested as seed treatments to control seedborne *A. carthami* on commercially produced safflower cultivar S208 in field as well as greenhouse experiments.

Field trials were done at the Arthur H. Post Research Farm, Bozeman, MT, and at the Eastern Agricultural Research Center, Sidney, MT. The fungicides were chosen because they inhibited mycelial development of *A. carthami* when 0.05 ml of suspensions of thiram (0.5 mg a.i./ml), chloroneb (0.07 mg a.i./ml), mancozeb (0.8 mg a.i./ml), and PMA (7×10^{-5} ml a.i./ml) were placed in 5-mm wells made

in PCA plates with 3-day-old cultures of the fungus and incubated at 20 C. Each fungicide suspension was tested on two PCA plates at three concentrations (10 mg [PMA:0.01 ml] product/ml diluted 10 and 100 times) and a control of distilled water in four wells per plate. Seeds were treated at recommended rates and twice the recommended rates for each of the fungicides (Table 2). At each field location, treated seeds were planted in four 3-m-long rows (60 seeds per row), four replicates per treatment.

In field trials at Bozeman, the percentage of seedling emergence and plants showing leaf spot symptoms was determined 32 days after planting. Because of geographical distance, the field trials at Sidney were only visited once; therefore, emergence counts and leaf spot ratings were made 60 days after planting. Leaf spot ratings were done using a scale of 0–10, adapted from James (7), indicating the percentage of leaf area covered with leaf spots: 0 = no symptoms, 1 = 1–5, 2 = 6–15, 3 = 16–30, 4 = 31–45, 5 = 46–59, 6 = 60–69, 7 = 70–79, 8 = 80–89, 9 = 90–99, and 10 = plants totally wilted.

In greenhouse tests, 40 treated seeds in eight replicates per treatment were planted in autoclaved soil in metal trays (five seeds per treatment per tray). Emergence of seedlings with and without distinct Alternaria seedling blight symptoms were determined 12 days after planting.

Greenhouse inoculation technique. Inoculum of *A. carthami* was prepared by spreading 1 ml of a sterile water suspension of conidia and mycelial fragments of the fungus over the entire surface of a PCA plate and incubating it for 12–15 days at 22–24 C with a 12-hr cool-fluorescent (3,200 lux) light period. Conidia were harvested by pouring 20 ml of distilled water on the plate, scraping the surface gently with a glass rod, and straining the suspension through a single layer of cheesecloth. Inoculum concentration ($2–5 \times 10^4$ conidia and/or mycelial fragments per milliliter) was determined by counting conidia and mycelial fragments on a hemacytometer. The inoculum suspension (15 or 20 ml) was atomized onto four or five safflower plants until runoff with an airbrush (Paasche Airbrush, type HS No. 5, Paasche Airbrush Co., Chicago, IL 60614) operated by constant air pressure (207 kPa). The plants were placed in a mist chamber for 40 hr after inoculation, where they were kept wet without runoff with a cold-mist humidifier. To obtain good development of the leaf spots, inoculated plants were returned to the mist chamber at night and kept on greenhouse benches in the daytime for 10–12 days after inoculation. Temperature in the greenhouse and mist chamber ranged from 18 to 24 C.

Table 1. Natural infestation by *Alternaria* spp. of safflower seeds and seedlings from different cultivars and origin in 1977

Cultivar and origin of seed	Seeds tested (no.)	Seeds with *Alternaria* sp. growth on potato-carrot agar		Seeds planted[a] (no.)	Seedlings emerged (%)	Seedlings with Alternaria leaf spot symptoms (%)
		A. carthami (%)	*A. alternata* (%)			
Sidney, MT						
US-10	96	28.1	63.5	50	66.0	75.8
S208	100	18.0	27.0	45	66.7	20.0
Sidwill	96	24.0	64.6	50	78.0	51.3
Montana[b]						
S208	100	28.0	55.0	45	57.8	61.5
Arizona						
Sidwill	96	0	7.3	50	96.0	4.2

[a] Experiments done in greenhouse.
[b] Commercially grown.

RESULTS

The number of conidia trapped in the two safflower fields from 2 wk before flowering to 1 wk after flowering is shown in Figure 1. Few conidia were trapped during July, but from the beginning of August until 11 August, increasing numbers of conidia were trapped. Peaks of conidia trapped correlated well with precipitation (Fig. 1). For example, the number of conidia trapped increased after rainy and cloudy weather, but during dry periods (last 10 days of July), very few conidia were trapped. The low numbers of conidia recorded on 4 and 9 August were attributed to precipitation just before or during part of the exposure time. Severity of leaf spot development in both fields was light during July, increasing to moderate (ratings of 5–6) by mid-August. Lengths of *Alternaria* conidia trapped varied considerably from 18 to 60 μm; large conidia (60–160 μm long) with beaks, which are characteristic for *A. carthami*, were seldom observed. Thus, the conidia trapped were nearly all of the *A. alternata* type.

Leaf spots observed on safflower leaves

Table 2. Effect of seed treatment on seedling transmission of Alternaria leaf spot in safflower cultivar S208[w]

Treatments	Rates (a.i.)	Greenhouse tests[x]		Field trials[y]			
				Bozeman, MT		Sidney, MT	
		Emergence (%)	Emerged plants with Alternaria leaf spot symptoms (%)	Emergence (%)	Emerged plants with Alternaria leaf spot symptoms (%)	Emergence (%)	Leaf spot disease rating
PMA	0.07 ml/kg	80.0 ab[z]	34.4 a	54.2 a	15.4 a	60.4 a	2.8 a
	0.38 ml/kg	82.5 a	6.1 a	50.4 a	13.2	56.3	2.3 a
Thiram	1.55 g/kg	70.0 abc	32.1 a	47.5 a	20.2 a	62.9 a	2.3 a
	3.20 g/kg	70.0 abc	42.9 a	56.3 a	12.6 a	65.0 a	2.3 a
Chloroneb	2.02 g/kg	47.5 c	42.1 a	62.5 a	14.7 a	52.1 a	2.3 a
	4.16 g/kg	57.5 abc	52.2 a	59.2 a	9.9 a	54.6 a	2.0 a
Mancozeb	0.96 g/kg	60.0 abc	45.8 a	51.7 a	12.1 a	53.8 a	2.3 a
	1.92 g/kg	52.5 bc	42.9 a	52.1 a	10.4 a	58.3 a	2.8 a
Untreated		47.5 c	52.6 a	60.0 a	12.5 a	55.4 a	2.5 a

[w] Commercially grown in Montana.
[x] Read 12 days after planting.
[y] Read 32 days and 2 mo after planting at Bozeman and Sidney, respectively. Leafspot rating scale based on percentage of leaf area covered with symptoms: 0 = no symptoms, 1 = 1–5% of leaf area covered with leaf spots, 2 = 6–15, 3 = 16–30, 4 = 31–45, 5 = 46–59, 6 = 60–69, 7 = 70–79, 8 = 80–89, 9 = 90–99, and 10 = plants totally wilted.
[z] Numbers in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

in the fields during early flowering were small, irregular lesions (0.5–1 cm diameter) with necrotic centers and a distinct margin. Leaf spots mainly occurred on lower leaves. Isolations made from such lesions nearly always yielded *A. carthami*. Of 15 isolations made from 12 to 22 July, all except one were *A. carthami*. During the second half of the flowering period, when leaf lesions increased in size and often coalesced, *A. alternata* became more dominant. Of 12 isolations made on 4 August, eight were *A. carthami* and four were *A. alternata*. Of 17 isolations made on 9, 18, and 21 August, only three were *A. carthami* and the remaining 14 were *A. alternata*.

Both *A. carthami* and *A. alternata* were carried on seed (Table 1) as reported previously (5,11). Surface-sterilization of seeds tested on PCA did not eradicate *Alternaria* spp. but reduced it by 0–25%. *A. alternata* was reduced most; little reduction or occasionally a slight increase in number of seeds with *A. carthami* were found after surface-sterilization. Data in Table 2, combined from 50% surface-sterilized and 50% untreated seeds, showed that 17–28% of seeds produced in Montana carried *A. carthami*. Seeds of cultivar Sidwill produced in Arizona were free of *A. carthami* when tested on PCA and emergence was 96% in greenhouse tests. In comparison, 24% of seeds of the same cultivar produced in Montana yielded *A. carthami* and had 78% emergence (Table 1).

The Arizona-produced seed sample of Sidwill was less discolored than a sample produced in Montana (Fig. 2). Seedlings from the Arizona-produced seed in greenhouse tests were more vigorous and uniform than seedlings from Montana-produced seed. Symptom-free seedlings from Montana-produced seed were often nonuniform in appearance and varied from stunted to large and vigorous. Typical seedling symptoms appeared as a distinct lesion on the cotyledon, often with a necrotic streak downward on the hypocotyl. Later, infected cotyledons were completely wilted and a distinct lesion developed on the stem at the soil level. Often, this lesion would girdle the stem, resulting in death of the plant. Seventy-five of 85 isolations from such symptoms on cotyledons and hypocotyls from greenhouse-grown seedlings yielded *A. carthami* and none yielded *A. alternata*; however, isolations made from decaying preemerged cotyledons and less distinct lesions (water-soaked lesions and somewhat swollen cotyledons and hypocotyls with small necrotic spots or streaks) from severely stunted and yellowing seedlings frequently yielded *A. alternata* (35 of 73 isolations) but *A. carthami* was only isolated in a few cases (7 of 73 isolations).

Inoculation of safflower plants with suspensions of conidia and mycelial fragments proved pathogenicity of *A. carthami* on safflower in all growth stages. Symptoms were irregular leaf and stem lesions, as found under field conditions. Total wilting of leaves and stems resulted after heavy inoculation (more than 10^5 conidia and mycelial fragments per milliliter) under optimal conditions for the pathogen. A minimum of 18 hr in the mist chamber was required to obtain infection by *A. carthami*. A 40-hr mist period always resulted in heavy infection. Inoculation with *A. alternata* showed this fungus capable of infecting healthy safflower leaves, causing very small lesions barely visible without a microscope. *A. alternata* was isolated from these minute lesions 10–12 days after inoculation. *A. alternata* was able to develop further and sporulate when inoculated leaves, detached and placed in a moist petri dish, became senescent.

All fungicides used for seed treatment tests of commercially produced safflower seeds of cultivar S208 (Table 2) inhibited mycelial growth of *A. carthami* on PCA. In greenhouse tests, however, only seed treatment with PMA improved emergence significantly over that from untreated seeds. No significant difference in emergence or leaf spot development was observed between untreated and any of the seed treatments used in field tests at Bozeman and Sidney. Thirteen percent of the emerged plants, encompassing all

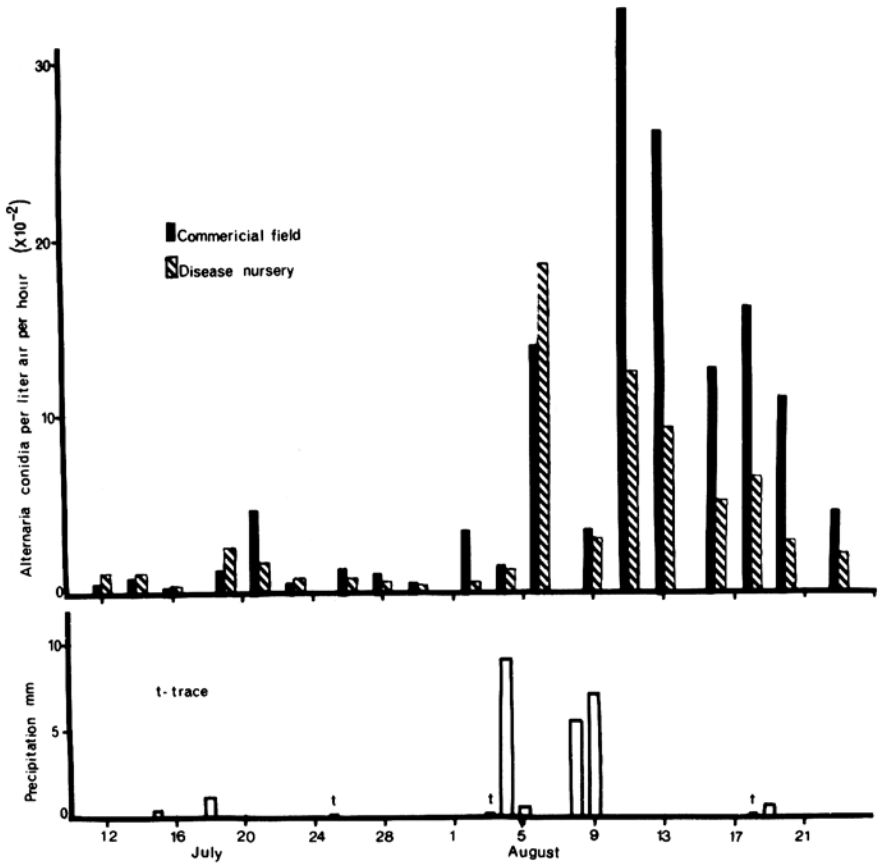

Fig. 1. Conidia of *Alternaria* collected in safflower with a rotorod spore sampler at two sites in Sidney, MT, in 1976 and precipitation for the same period.

Fig. 2. Seed samples of safflower cultivar Sidwill produced (left) at Sidney, MT, and (right) at Yuma, AZ. Yuma-produced seeds were less discolored and almost free of *A. carthami*.

treatments at Bozeman, showed Alternaria leaf spot symptoms 32 days after planting. At flowering, leaf spot disease severity was moderate (ratings of 4–5).

DISCUSSION

After a few days of rainy and cloudy weather in early August, the number of *Alternaria* conidia trapped in two safflower fields increased considerably (Fig. 1). During the same time period, leaf spot development increased accordingly. Our data support observations by Zimmer et al (13) that rainy and moist weather conditions enhanced severe leaf spot disease development in safflower fields in Montana and North Dakota in 1962. In this study, isolations made from leaf spots during the growing season showed that different species of *Alternaria* were involved in symptom development. In July, *A. carthami* was isolated almost exclusively, but there was a gradual increase in *A. alternata* as plants reached maturity in August. *A. alternata* is reported to be a saprophyte commonly found on a wide range of dead plant material (4).

Irwin (5) reported that *A. alternata* was frequently isolated from safflower seeds but never from developing leaf lesions; however, in our studies and in a study reported elsewhere (9), a low frequency of infections occurred after inoculation with *A. alternata* on safflower plants, but these infections did not result in large lesions until the leaf tissue started to senesce. It is possible that these dormant infections of *A. alternata* could accelerate senescence of the leaves, as was shown by Skidmore and Dickinson (12) for barley leaves (*Hordeum vulgare* L.) when inoculated with nonpathogenic species of *Alternaria* and *Stemphylium*. Thus, *A. alternata* could be a contributing factor to leaf spot development of safflower, especially when other pathogenic organisms or unfavorable conditions have reduced vigor of the plants.

The heavy infestation of *A. alternata* found on safflower seeds grown in Montana could contribute to damping-off of safflower seedlings under conditions unfavorable for seed germination. Another organism involved in damping-off and leaf spots in early plant stages of safflower is the bacterium *Pseudomonas syringae* van Hall (6,8); however, the major pathogen in leaf spot disease in Montana is *A. carthami* as reported previously (2).

Data obtained in this study confirm seed transmission of *Alternaria* spp. (5,11). Seed-treatment experiments in Australia (5) and in our work show that many commercially available fungicides cannot eradicate *A. carthami* infestations in safflower seeds, indicating that the fungus is carried internally. Further studies with systemic fungicides to control internal fungal infection are warranted. Furthermore, our observations indicate that natural infestation of *A. carthami* on seeds of safflower produced in Montana is sufficient to initiate severe leaf spot epidemics if extended periods of rainy and moist conditions occur during and after flowering. Because Arizona-produced seed of Sidwill proved to be virtually free of *A. carthami* and produced more vigorous and uniform seedlings than Sidwill seeds produced in Montana, perhaps other pathogenic organisms (ie, *Pseudomonas syringae*) can also be avoided in the seed produced in Arizona under drier conditions unfavorable for development of these pathogens.

Safflower cultivars or breeding lines are not highly resistant to *A. carthami* (9). Recent breeding lines, including Sidwill, have moderate resistance but are not immune to Alternaria leaf spot. Under environmental conditions favoring the pathogen, these lines are susceptible to Alternaria leaf spot but to a lesser extent than S208. If the primary inoculum can be eliminated or reduced by producing the seed in Arizona, yield losses caused by Alternaria leaf spot disease in Montana could be minimized.

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