Pathogenicity of *Alternaria tagetica* on *Tagetes*

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ABSTRACT

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Blight of marigold (*Tagetes erecta* and *T. patula*) caused by *Alternaria tagetica* is characterized by dark lesions on leaves, stems, and petals. The pathogen did not cause symptoms on six other species of Asteraceae. Symptom severity increased with longer periods of high moisture after inoculation. The pathogen was isolated from commercial marigold seed. *A. tagetica* formed abundant mycelial growth on carrot-juice agar, potato-dextrose agar, and oatmeal agar. The number of spores produced by the pathogen was increased when oxythioquinox (100 mg a.i./L) was added to the medium and incubated under alternating periods of light and dark at 25 C.

Marigolds (*Tagetes* spp.) are popular annuals grown from seed for both private and commercial use. In 1979, necrotic spots on leaves, stems, and petals of African marigold (*T. erecta*) plants were observed in a garden in South Carolina. Symptoms first appeared on older leaves as circular, brown lesions that later enlarged, coalesced, and turned dark brown to black. Severely infected plants became black, appeared scorched, and eventually died. The pathogen was identified as *Alternaria tagetica* Shome & Mustafee.

Symptoms similar to those on plants in South Carolina were caused by two *Alternaria* spp. on marigold plants in India (3,7). *A. zinniae* Pape caused irregular brown spots on lower leaves. As the disease became severe, spots covered the leaf lamina and also infected flower heads (3). Disease symptoms produced by *A. tagetica* appeared first on leaves as brownish spots, and later, spots developed on stems. When flowering, the sepals and petals became severely infected and turned dark brown to black (7). *A. tagetica* was consistently isolated from spots on leaves, stems, and flowers of marigolds in South Carolina.

This paper reports results of pathogenicity tests with *A. tagetica* on *T. patula, T. erecta, Callistephus chinensis* Nees, *Calendula officinalis* L., *Cosmos bipinnatus* Cav., *Gazania longiscapa* DC., *Chrysanthemum maximum* Ramond., and *Zinnia elegans* Jacq. The effect of duration of intermittent mist after inoculation on appearance and severity of disease symptoms on *T. patula* and *T. erecta* is reported. Commercial marigold seed were examined to determine if the pathogen is seedborne. Results of several tests to determine growth and reproduction requirements of *A. tagetica* in vitro are given. Growth of the pathogen on carrot-juice agar (CJA) to which fungicides were incorporated is reported.

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MATERIALS AND METHODS

Isolation, identification, and inoculation methods. Leaf tissue from diseased plants was surface-sterilized for 2 min in 1% NaOCl, rinsed for 2 min in sterile distilled water, placed on CJA, and incubated at 25 C under a 12-hr photoperiod. Three single-spore isolates were grown on CJA for 7 days at 25 C under a 12-hr photoperiod. Spores were gently scraped from plates, blended in 1 L water, and standardized at 80 Klett units on a Klett-Summerson photoelectric colorimeter. Pathogenicity tests were conducted on seedlings of 8-wk-old Doubloon marigold (*T. erecta*) grown in the greenhouse. They were inoculated by shaking the spore suspension through a container with a perforated lid until runoff. After inoculation, seedlings were placed immediately under mist at 26–30 C for 24 hr, then moved to a greenhouse bench at the same temperature and observed for symptoms. The pathogen was reisolated from diseased tissues as described previously. Three isolates of the pathogen grown on CJA and incubated at 25 C were examined microscopically and 100 conidia were measured for identification. Final species determination was made by E. G. Simmons, Department of Botany, University of Massachusetts, Amherst.

Pathogenicity tests. After completion of initial pathogenicity tests, four pots containing three plants each of 10-, 11-, 12-, and 13-wk-old plants of 16 marigold cultivars were inoculated as described previously and examined 12, 22, and 44 days later for symptom differences among cultivars and among ages within a cultivar. A 0–5 average disease severity index was used based on percentage leaf area affected, where 0 = no visible symptoms, 1 = 0–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, and 5 = 41–50% leaf area affected. Four 11-wk-old plants each of aster (*Callistephus chinensis*), calendula (*Calendula officinalis*), cosmos (*Cosmos bipinnatus*), gazania (*G. longiscapa*), shasta daisy (*Chrysanthemum maximum*), and zinnia (*Z. elegans*) were also inoculated as described and observed for symptom development.

Foliar moisture effects. Effects of moisture on appearance and severity of disease symptoms were studied on inoculated 5-wk-old Fantastic Orange (hybrid) marigold plants placed under intermittent mist for 0, 2, 4, 8, 24, 48, and 72 hr in the greenhouse at 26 C. Plants placed under mist were not allowed to dry. Duration of mist was set at 30 sec/30 min for November through March and 30 sec/15 min for April through October. Four plants were used per treatment. All plants received 24 hr of mist before inoculation except one treatment, which received no mist. Inoculations were performed as described previously. Plants were removed from mist after the specified treatment time, then placed on a greenhouse bench at 26 C and observed for symptom development.

Seed infestation. Commercial marigold seed were examined to determine if the pathogen was seedborne. One hundred seeds from each of 10 marigold cultivars were surface-sterilized by soaking for 2 min in 1% NaOCl and rinsed in sterile water. Seed were then plated on moistened filter paper in sterile petri plates and fungal growth was examined microscopically after 5 days.

Fungal cultures isolated from seed were grown on CJA for 7 days at 25 C and inoculum was prepared as described. Four plants each of 12-wk-old Janie (*T. patula*), Doubloon (*T. erecta*), and Fantastic Orange (*T. erecta*) seedlings were inoculated as before and observed for symptoms. The pathogen was reisolated from diseased tissue.

Culture studies. *Alternaria* spp. grow rapidly on a variety of media, including potato-dextrose, bean, oatmeal, malt, carrot-juice, and prune agar (2,4–6). Vegetative growth was studied on CJA

(16 g agar, 360 ml carrot juice, and 640 ml water), Difco oatmeal agar (OMA), Difco potato-dextrose agar (PDA), and Difco cornmeal agar (CMA). Eight petri plates of each medium (30 ml/plate) were seeded with 5-mm mycelial disks obtained from the margins of 7-day-old cultures of *A. tagetica* and incubated at 25 C under a 12-hr photoperiod in a random-block design. Colony diameters were measured after 7 days.

The fungus was grown at 7.5, 10, 15, 20, 25, 30, and 32.5 C on 10 plates each of CJA. Plates were seeded as before and placed in a completely random design under a 12-hr photoperiod at each temperature. Colony diameters were measured after 7 days of growth.

Ellers and Baxter (4) increased sporulation of *A. zinniae* by adding oxythioquinox to either CJA or CMA (100 mg a.i./L) when the fungus was grown under a 12-hr photoperiod. Five plates of CJA with oxythioquinox (100 mg a.i./L) and five plates of CJA without oxythioquinox were seeded with 4-mm disks obtained from the margins of 7-day-old cultures of *A. tagetica* and incubated at 25 C under a 12-hr photoperiod. The fungus was gently scraped from each plate, mixed with 50 ml water, and strained through two layers of cheesecloth. Spores in 10 samples of the suspension for each plate were taken with a Levy Ultra Plane hemacytometer after 21 days to measure sporulation.

The fungus was grown in constant light, alternating light and darkness (12 hr each), or constant darkness for 7 days. Five plates each of CJA with oxythioquinox (100 mg a.i./L) were seeded as described and incubated for 21 days in a completely random design under each light regime at 25 C. Plates were placed 24 cm below a combination of fluorescent and incandescent lights (1,615 lux or 150 ft-c). Sporulation was measured as described.

Chemical study. Chemical sensitivity tests were conducted in the laboratory with the fungicides chlorothalonil, iprodione, mancozeb, and etaconazole (CGA-64251), which were added at 1, 10, 100, and 1,000 mg a.i./L to CJA after autoclaving. Eight plates of each treatment were seeded with 5-mm mycelial disks obtained from the margins of 7-day-old cultures and incubated at 25 C under a 12-hr photoperiod in a randomized complete block design. Colony diameters were measured after 7 days and percent growth inhibition was determined.

RESULTS

Isolation, identification, and inoculation methods. After inoculation of Doubloon marigolds and subsequent reisolation, the pathogen was identified as *A. tagetica*. The fungus has long-beaked conidia measuring 30 × 157 μm. The muriform, deep-brown conidia have a wide central part tapering at both ends, with a rounded base and a long rostrum on the apical end. Conidia were rarely catenulate. Hyphae were smooth, septate, profusely branched, and light to dark brown.

Pathogenicity tests. Time of symptom appearance was not affected by cultivar or age. Symptoms were first observed on all marigold plants 3 days after inoculation. Fantastic Orange (hybrid) was more susceptible than other cultivars (Table 1). Age did not affect severity of symptoms. Plants tested in the Asteraceae family other than *Tagetes* spp. were not susceptible.

Leaf spots were not observed on inoculated plants receiving no mist, on those receiving 24 hr of mist before inoculation only, or on those receiving 2 and 4 hr of mist. Moisture treatments did not affect time of symptom appearance but did affect severity of symptom expression. A few small scattered lesions were observed on plants receving 8 hr of mist after inoculation, but on plants receiving 24, 48, and 72 hr of mist, more and larger lesions were observed. The average diameters of lesions 7 days after inoculation were 3–5 mm after 48- and 72-hr treatments, whereas lesions in the 24-hr treatment were 1–3 mm.

Table 1. Disease rating of 16 marigold cultivars 44 days after inoculation in the greenhouse with *Alternaria tagetica*

Cultivar	DSI[a]
French	
Bolero	1.00[b]
Firelight	1.00
Janie	2.25
King Tut	1.00
Lemondrop	1.75
Panther	1.00
Red Wheels	1.75
African	
Doubloon	1.25
Happy Face	1.75
Pumpkin Crush	1.25
Sovereign	2.00
Toreador	1.25
Hybrids	
Fantastic Orange	3.75
Gingersnap	1.00
Orange Jubilee	1.50
Tiger	1.00

[a] Disease severity index based on percentage leaf area affected: 0 = no visible symptoms, 1 = 0–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, and 5 = 41–50% leaf area infected.
[b] Means of four replicates of plants 10 wk old at inoculation.

Seed infection. *A. tagetica* was isolated from two of 100 seeds of Fantastic Orange but not from seed of nine other cultivars examined. Germination of infected seed was not inhibited. Typical disease symptoms were produced 3 days after inoculation with the seed isolate on all marigold cultivars and the fungus was reisolated from diseased tissue.

Culture studies. The fungus grew well on CJA, PDA, and OMA as measured by colony diameters (Table 2). The optimum temperature for vegetative growth was 25 C, with growth occurring from 10 to 30 C. Growth did not occur at either 7.5 or 32.5 C.

Sporulation of *A. tagetica* was doubled in CJA amended with oxythioquinox (1.83×10^5 spores per milliliter) compared with CJA without oxythioquinox (7.65×10^4 spores per milliliter). Sporulation of the fungus on CJA with oxythioquinox was better when grown under a 12-hr photoperiod (1.83×10^5 spores per milliliter) than with constant light (9.12×10^4 spores per milliliter). Sporulation was not observed on plates grown in constant darkness.

Chemical study. In chemical sensitivity tests (Table 3), greatest inhibition of fungal growth was observed on CJA amended with either etaconazole or iprodione. Etaconazole significantly reduced fungal growth to a greater degree than iprodione at 1 and 10 mg a.i./L but not at 100 and 1,000 mg a.i./L. At 100 mg a.i./L, chlorothalonil gave a higher percent growth reduction than mancozeb, but there was no significant difference observed between these two at 1,000 mg a.i./L.

Table 2. Diameters of *Alternaria tagetica* colonies grown 7 days at 25 C under a 12-hr photoperiod

Medium	Colony diameter[y] (mm)
Carrot-juice agar	53.5 a[z]
Potato-dextrose agar	50.5 b
Oatmeal agar	50.0 b
Cornmeal agar	34.2 c

[y] Means of eight replicates.
[z] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Percent growth reduction of *Alternaria tagetica* on carrot-juice agar amended with various concentrations of chemicals after 7 days at 25 C

	Percent growth reduction			
Chemical	1 mg a.i./L	10 mg a.i./L	100 mg a.i./L	1,000 mg a.i./L
Etaconazole	61 a[z]	81 a	81 a	81 a
Iprodione	9 b	56 b	83 a	83 a
Chlorothalonil	1 c	6 c	40 b	60 b
Mancozeb	1 c	2 d	14 c	61 b

[y] Means of eight replicates.
[z] In each column, values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Percentages were arcsine transformed before analysis.

DISCUSSION

This is the first description of an *Alternaria* sp. that infects marigolds in the United States. An *Alternaria* sp. was reported from New Jersey as "? secondary" in 1960 (9). In this study, symptoms were produced on all cultivars of *T. erecta, T. patula,* and *Tagetes* hybrids inoculated with the pathogen, whereas closely related genera known to be susceptible to other *Alternaria* spp. did not develop disease symptoms after inoculation with *A. tagetica*. Strider (8) described a direct relationship between symptom expression on carnations and length of time foliage was kept moist after inoculation with *A. dianthi*. Severity of disease in our study was directly correlated with duration of high moisture. Moisture periods longer than 4 hr appeared to be necessary for disease development after inoculation in the greenhouse. More than 8 hr was required for severe infection. The disease spread to new leaflets among plants left under mist more than 24 hr after inoculation. Thus, this disease may become severe after extended periods of high moisture.

Survival and spread of *A. zinniae* appears to be through seed and has been reported to be either superficial or deep-seated (6). Because commercial marigold seed were found infested with *A. tagetica,* dissemination of the pathogen might be accomplished in this manner. Use of seed treatments or growth of seed in fungus-restrictive climates, as described for *A. zinniae* by Baker (1), should be tested on marigold seed infested with *A. tagetica*. Chemical sensitivity tests in the laboratory indicate that chemical control of *A. tagetica* by foliar application may be possible. Some fungicides reduced growth of *A. tagetica* in vitro, but field studies are needed to test for efficacy of these chemicals.

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LITERATURE CITED

1. Baker, K. 1956. Development and production of pathogen-free seed of three ornamental plants. Plant Dis. Rep. (Suppl.) 238:69-70.
2. Dimock, A. W., and Osborn, J. H. 1943. An Alternaria disease of zinnia. Phytopathology 33:372-381.
3. Edward, J. C. 1957. Leaf and inflorescence blight of *Tagetes erecta* (marigold) caused by *Alternaria zinniae* Pape. Sci. Cult. 22:683-684.
4. Ellers, K. L., and Baxter, L. W., Jr. 1974. Induced conidial formation in *Alternaria zinniae* grown on media amended with Morestan. (Abstr.) Proc. Am. Phytopathol. Soc. 1:159.
5. Forsberg, J. L., and Crane, J. L. 1970. A disease of ageratum new to the United States. Plant Dis. Rep. 54:1095-1096.
6. Gambogi, P., Trioli, P., and Vannacci, G. 1976. Experiments on the behavior of the seedborne fungus *Alternaria zinniae*. Seed Sci. Technol. 4:333-340.
7. Shome, S. K., and Mustafee, T. P. 1966. *Alternaria tagetica* sp. nov. causing blight of marigold (*Tagetes* sp.). Curr. Sci. 35:370-371.
8. Strider, D. L. 1978. Alternaria blight of carnation in the greenhouse and its control. Plant Dis. Rep. 62:24-28.
9. U.S. Department of Agriculture. 1960. Index of Plant Diseases in the United States. U.S. Dep. Agric. Agric. Handb. 165. 531 pp.