Pathogenicity and Conditions for Infection of Chrysanthemum and Rose Flowers by Bipolaris (Helminthosporium) setariae

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ABSTRACT

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Inoculation with *Bipolaris (Helminthosporium) setariae* induced lesions on the flowers of chrysanthemum cultivars Doubloon, Southern Comfort, Tinsel, Yellow Knight, and Yellow Shasta but not on those of Blue Chip, Bronze Chip, Dolly, Iceberg, Yellow Iceberg, or Jackstraw. Flowers of the hybrid tea rose cultivars Improved Red American Beauty and Tropicana also were very susceptible. Reddish brown lesions, two to three times as long as broad and 1-2 mm in length developed on chrysanthemums. The lesions did not further increase in size. On roses, tan-colored spots up to 2 mm in diameter initially developed. With time, the spots increased in size and coalesced, resulting in large, tan, necrotic areas on the petals. Severely infected petals fell from the flowers. Severe disease developed at 29 and 24 C, with increasingly lower levels at 18, 13, 35, and 7 C, respectively. Seventeen percent of the petal tissue was necrotic (percent disease) on inoculated rose flowers covered with polyethylene bags for 3 hours (high humidity and free water on the petals) at 25 ± 1 C. Disease became progressively more severe as the duration of the incubation period in polyethylene bags increased to 6, 12, 24, and 30 hours. Sixty-two percent disease was present after 30 hours.

Additional key words: Botrytis.

Bipolaris (Helminthosporium) setariae (Saw.) Shoemaker was isolated in Florida from small, elongate (1-2 mm) reddish-brown lesions on the petals of a spider-type chrysanthemum. *Bipolaris setariae* commonly is a pathogen on cereals and grasses (1, 3) and rarely is found on petals of flowers. *Helminthosporium* sp. was reported to cause a flower spot on *Tagetes erecta* L. (2), the Aztec (African) marigold.

Objectives of this research were to determine the pathogenicity of *B. setariae* on chrysanthemum and rose flowers and to determine environmental factors that could allow this organism to infect chrysanthemum and rose.

MATERIALS AND METHODS

In a preliminary experiment to establish pathogenicity, a spore suspension of *B. setariae* of unknown concentration was atomized on cut flowers of the chrysanthemum cultivar Yellow Knight. The inoculum was obtained by growing a wild isolate obtained from lesions on petals of a chrysanthemum on potato-dextrose agar. The inoculated flowers and the controls were placed for 72 hours in a polyethylene-covered chamber equipped with a water vaporizer. A single-spore isolate was used in seven subsequent experiments reported herein.

Inoculum was prepared by growing a single-spore isolate of *B. setariae* in petri plates on Difco potato-dextrose agar. Cultures were grown in a laboratory incubator at 24 C with constant cool-white fluorescent light at 1,937 lx. When cultures were 13-24 days old, spores were harvested by adding distilled water to the petri plates and gently brushing the cultures with an artist's brush. Tween-20 (polyoxyethylene sorbitan monolaurate) at 250 μg/ml was used in the spore suspensions and in the controls (distilled water) included with each experiment. The spore concentrations of the suspensions used for the different tests varied from 125,000 to 300,000 spores per ml.

Flowers were inoculated by placing the flasks on a turntable that revolved at a constant speed. Mounted on the turntable were stands, each of which revolved one-third of a turn while the turntable completed one revolution. Flowers held in the flasks were atomized with a spore suspension through three revolutions of the turntable, thus uniformly exposing the flowers to the inoculum. The spore suspension was stirred constantly with a magnetic stirrer throughout the inoculation procedure. A DeVilbiss atomizer, mounted on a laboratory stand, was used to apply the spore suspension at one atmosphere (15 psi) of pressure. The petals were wetted to the point of run-off. No water soaking of the petal tissue occurred. Experiments were conducted using three replications consisting of three flowers each with stems in distilled water in an Erlenmeyer flask.

After the flowers were inoculated in the pathogenicity tests, they were handled gently so the inoculum was not shaken off. They were placed on the laboratory bench and covered with a polyethylene bag for 48 hours. Free water remained on the petals and leaves throughout this 48-hour period. Room temperature in the laboratory was

maintained at 25 ± 1 C, relative humidity (RH) at 55-65%, and continuous illumination at 2,140 lx was provided by cool-white fluorescent light. Pathogenicity tests were conducted on 11 chrysanthemum and two rose cultivars.

The effect of temperature on disease development was investigated by using the rose cultivar Improved Red American Beauty as the test plant. Inoculated rose flowers were covered with polyethylene bags and incubated at 7, 13, 18, 24, 29, and 35 C. The bags were removed after 48 hours and disease was rated after 72 hours. Effect of duration of the wet period was determined by incubating inoculated Improved Red American Beauty rose flowers in polyethylene bags for 3, 6, 12, 24, 30, 42, and 48 hours at 25 ± 1 C. Disease was rated 72 hours after the flowers were inoculated. Roses were used because they were very susceptible to the pathogen and disease could be rated easily by estimating the percent necrotic tissue on the petals.

RESULTS

Pathogenicity on chrysanthemum was established in a preliminary experiment on the spider-type cultivar Yellow Knight. Disease symptoms like those on the original host were reproduced as small reddish-brown lesions two to three times as long as broad and 1-2 mm in length. The lesions were more abundant on flower petals near the centers than on the margins of the inflorescence.

Chrysanthemum flowers were inoculated in five experiments. Symptoms developed on the petals of the cultivars Doubloon, Southern Comfort, Tinsel, Yellow Knight, and Yellow Shasta. More than 100 spots per petal developed on the petals of the daisy-type chrysanthemum Yellow Shasta. No symptoms developed on the cultivars Blue Chip, Bronze Chip, Dolly, Iceberg, Yellow Iceberg, or Jackstraw. Symptoms were visible in 3 days and disease was rated between the 4th and the 9th days after inoculation. After lesions developed on chrysanthemum petals, they did not continue to increase in size or cause a decay of the petal tissue. An occasional lesion on the flowers was not seen easily; however, when they were very abundant, as on the cultivar Yellow Shasta, not only were there many lesions on the petals, but the flowers had a "dirty" appearance which rendered them non-marketable.

In two replicated experiments with the chrysanthemum cultivar Yellow Shasta, the inoculated flowers averaged over 100 lesions per petal, whereas the controls (H_2O) had none. In a similar experiment, inoculated flowers of the cultivar Southern Comfort averaged 3.4 lesions per petal and the controls 0.5. In another experiment, inoculated flowers of the cultivar Tinsel averaged 18 petals and the control five petals with three or more lesions per petal. *Bipolaris setariae* was reisolated from inoculated flowers, but not from the controls. Flowers obtained from commercial growers sometimes develop spots when incubated in polyethylene bags. Thrips-damaged spots resemble those caused by *B. setariae*. An *Alternaria* sp. usually was isolated from the lesions on the controls.

Flowers of the hybrid tea rose cultivars Improved Red American Beauty and Tropicana were very susceptible to *B. setariae*. They were inoculated in the bud stage when the first petal was unfurling. Symptoms were very prominent within 24 hours. They developed only on tissue that was exposed to the spore suspension. In two replicated experiments with the cultivar Improved Red American Beauty, the average percent disease on the inoculated flowers was 46 and on the controls two. In two similar experiments with the rose cultivar Tropicana, the average percent disease on the inoculated flowers was 20, and on the controls it was one.

The initial symptoms on the petals of roses were tan-colored spots up to 2 mm in diameter. Nearly 100 spots developed on some petals. As severity of infection increased, the spots coalesced and formed large, tan, necrotic areas on a petal, especially near the outer margins. Severely infected petals curled inward and fell off.

Bipolaris setariae consistently was reisolated from lesions on inoculated plants, but not from control plants of both chrysanthemums and roses in each of the

TABLE 1. *Bipolaris setariae:* effect on incubation temperature on percent disease symptoms on flowers of Improved Red American Beauty rose

Temperature (C)	Petal tissue necrosis[a] Control (%)	Inoculated (%)
7	0	4
13	0	14
18	0	21
24	0	59
29	0	69
35	0	13
LSD ($P = 0.05$)		16

[a]Means of three replications per treatment, three flowers per replication. The flowers with stems were placed in an Erlenmeyer flask. They were inoculated by atomizing them with a spore suspension, and then covering them with polyethylene bags for 48 hours to maintain wetness. Disease was rated 72 hours after inoculation.

TABLE 2. *Bipolaris setariae:* effect of duration of free moisture period on percent disease symptoms on flowers of Improved Red American Beauty rose

Duration of free moisture at 25 C (hours)	Petal tissue necrosis[a] Control (%)	Inoculated (%)
0	. . .[b]	0
3	. . .	17
6	1	34
12	. . .	40
24	2	49
30	. . .	62
42	. . .	52
48	5	56
LSD ($P = 0.05$)		12

[a]Means of three replications per treatment, three flowers per replication. The flowers with stems were placed in an Erlenmeyer flask. They were inoculated by atomizing them with a spore suspension and then covering with a polyethylene bag to maintain free water on the plants. Disease was rated 72 hours after inoculation.

[b]Control treatments were used only where there are numbers.

experiments.

The highest levels of disease developed on rose flowers at 29 C with 69% of the petal tissue necrotic followed by 59% at 24 C. Significantly, less disease developed at 18 C (21%), 13 C (14%), 35 C (13%), and 7 C (4%) (Table 1). No disease developed on the controls.

Duration of the wet period (incubation in polyethylene bags) had a major effect on the level of disease. Seventeen percent disease developed when inoculated flowers were wet as little as 3 hours. No disease developed when inoculated flowers were not placed in bags. Disease increased progressively with an increase in duration of the wet period with 34% at 6 hours, 40% at 12 hours, 49% at 24 hours, and 62% at 30 hours. Fifty-two percent developed at 42 hours and 56% at 48 hours (Table 2). Generally, there was significantly more disease with a wet period of 30 or more hours than at 12 hours or less.

DISCUSSION

Symptoms of B. setariae on chrysanthemum petals were first observed on flowers in a commercial range. The small lesions on petals were not numerous so they did not constitute a serious problem. However, the potential for serious damage exists. In the experiments reported herein, inoculated Yellow Shasta developed so many petal spots the flowers were nonsalable. The small lesions may also serve as infection sites for secondary infections and in postharvest disease problems.

No logical genetic relationships existed among susceptible and resistant cultivars of chrysanthemum.

Rose flowers were highly susceptible and were severely damaged when inoculated with the pathogen. Also, on roses the symptoms were similar to those incited by *Botrytis cinerea* Pers. ex Fr. (A. W. Engelhard, *unpublished*). Thus the problems of misidentification and control procedures arise.

The significance in nature of *B. setariae* as a pathogen on chrysanthemum and rose flowers is not known. *Bipolaris setariae* is a new and unusual pathogen on these crops. Since the initial isolation, it has been isolated in this laboratory from random collections of flower specimens on only one other occasion. No systematic surveys to determine its prevalence have been made.

Bipolaris setariae has the potential for causing a postharvest problem. Seventeen percent disease developed on rose flowers incubated for only 3 hours in polyethylene bags (free moisture was maintained on the flowers) at 25 ± 1 C. Increasing the duration of the free moisture period in polyethylene bags increased disease. In addition, disease developed when rose flowers were incubated in polyethylene bags for 48 hours at 7 C and progressively more disease developed as the incubation temperatures were progressively increased to 13, 18, 24, and 29 C. Shipping conditions for commercial flowers, from the packing house to the cooler to the wholesale florist, include environmental conditions that duplicate the incubation and temperature ranges used in these experiments.

It is concluded that *B. setariae* has the potential for causing (i) a field disease problem on certain cultivars of chrysanthemum and rose, and (ii) a postharvest disease problem resulting from field infection that may occur during shipping.

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