Evaluating Techniques for Screening Strawberry Seedlings for Resistance to *Colletotrichum fragariae*

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ABSTRACT

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Large numbers of strawberry seedlings can be effectively screened in the greenhouse for resistance to *Colletotrichum fragariae* by spraying conidial suspensions of the fungus over the 2- to 3-mo-old plants. High humidity immediately after inoculation is necessary for infection and rapid symptom expression.

Additional key words: *Fragaria* × *ananassa*, strawberry anthracnose, strawberry crown rot

Strawberry anthracnose or crown rot, caused by *Colletotrichum fragariae* Brooks, was first reported on strawberry (*Fragaria* × *ananassa*) in Florida by Brooks (1) and has become severe in the southeastern United States in recent years (4,8,12). The fungicides benomyl (4,8) and captafol (2) have been reported to control anthracnose; however, benomyl has not remained effective in field use (2). Control with resistant cultivars has been difficult to attain because of varying levels of susceptibility among cultivars and breeding lines (3,11).

The U.S. Department of Agriculture, Agricultural Research Service, at Beltsville, Maryland, and Poplarville, Mississippi, in cooperation with several state experiment stations (Florida, Louisiana, North Carolina), is conducting a breeding program to develop anthracnose-resistant cultivars adapted to the strawberry-growing areas of the southeastern United States. This program requires evaluating large numbers of plants in a short time. An effective method of screening large populations of strawberry seedlings in the greenhouse for resistance to *C. fragariae* is necessary for quick identification of resistant seedlings.

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Published reports have used a variety of methods to artificially inoculate strawberry plants with *C. fragariae* and evaluate the disease levels obtained. In the initial report of strawberry anthracnose, Brooks (1) reported that high temperature and humidity favored disease development and that surface water was probably a means of distribution of spores in the field. He found that wounding was necessary during inoculation to obtain lesions on petioles or leaves but not on stolons. Brooks also reported that symptom expression was more rapid when plants were placed in a moist chamber after inoculation.

More recent researchers have inoculated plants by planting them in soil artificially infested with conidia (5,6), injecting conidia into the crowns (3,4,7), placing agar blocks of mycelium on the petiole (3), and spraying conidial suspensions on them with a garden sprayer (4), an atomizer (9,10), and an air brush (3). The concentration of conidial suspensions used has ranged from 800 (10) to 10^7 (3) conidia per milliliter. Temperature and humidity conditions have also varied. Disease development has been evaluated based on lesion development (1,9,10), percentage of infected plants (4), and percentage of plant loss or dead plants (6,7,8).

In 1980, a report (3) on methods of evaluating strawberry cultivars and breeding lines for resistance to *C. fragariae* involved spraying the top half of petioles with a conidial suspension and incubating plants at 100% relative humidity (RH) and 28–30 C for 48 hr. A disease index was used to evaluate disease development.

This study was initiated to determine the most effective way to screen large numbers of seedlings by artificial inoculation with *C. fragariae* in a greenhouse. Inoculation methods, incubation times, and methods of handling after inoculation are evaluated.

MATERIALS AND METHODS

Seedlings and inoculum. Seed from 15 crosses among 22 strawberry clones were germinated in ground sphagnum. The seedlings were transplanted to Jiffy-7 peat pellets when they had one true leaf and were grown for 6–8 wk in a greenhouse with 60–68% RH, 16-hr days, 22 ± 12 C day temperature, and 18 ± 8 C night temperature. Liquid fertilizer (10-13-20) was applied weekly beginning 3 wk after transplanting. Miticides were used as needed to control spider mites.

C. fragariae isolate CF-4 (obtained from R. D. Milholland, North Carolina State University) was cultured on oatmeal agar and incubated at 22 C under continuous fluorescent light for 10 days. Inoculum was prepared as a conidial suspension in distilled water. The concentration was adjusted to 9×10^5 conidia per milliliter ($\pm 1 \times 10^5$).

Length of time in humidity chamber. The humidity chamber was kept at 90–100% RH and 25–32 C (average 30 C), with a 16-hr day. Six- to 8-wk-old plants of eight crosses (132 or 264 plants per cross) were divided equally into a 2 × 2 factorial, with each cross being a replication. The treatments consisted of the length of time the plants were maintained in the humidity chamber before and after inoculation: 0 hr before and 0 hr after (0 + 0), 24 hr before and 0 hr after (24 + 0), 24 hr before and 72 hr after (24 + 72), and 0 hr before and 72 hr after (0 + 72). Plants were inoculated by brushing the inoculum on 5 mm of the petiole of the youngest fully expanded leaf of each plant with a small artist brush.

Susceptible seedlings were counted and removed from each flat 6 and 12 days after inoculation. Seedlings were considered susceptible if a red or black lesion at least 1 mm long developed on the inoculated petiole. Seedlings were rated

Table 1. Effect of duration of incubation in a humidity chamber on infection of strawberry seedlings by *Colletotrichum fragariae*

Time (hr) in humidity chamber		Susceptible plants[z]			
		After 6 days		After 12 days	
Before inoculation	After inoculation	Number	%	Number	%
0	0	0 c	0	8 c	3
24	0	0 c	0	0 c	0
24	72	131 b	44	146 b	49
0	72	237 a	80	263 a	89

[z]Total of 297 seedlings per treatment (eight crosses per treatment; 33 or 66 plants per cross). Values in a column followed by the same letter are not significantly different at the 5% level.

Table 2. Response of strawberry seedlings to six methods of inoculation with a conidial suspension (9×10^5 spores per milliliter) of *Colletotrichum fragariae*

Inoculation method[y]	Susceptible plants[z]			
	2 wk after inoculation		4 wk after inoculation	
	Number	%	Number	%
Brush	150 c	38	275 b	69
Spray	310 ab	78	331 b	84
Clip	396 a	100	396 a	100
Puncture	262 b	66	313 b	79
Bruise	389 a	98	396 a	100
Control	10 d	3	17 c	4

[y]In the brush method, inoculum was brushed on the petiole of the youngest fully expanded leaf. In the spray method, inoculum was sprayed over the flat of seedlings until it dripped from the leaves. In the clip method, plants were clipped to a height of 40 mm and immediately inoculated. In the puncture method, leaves were wounded with a device consisting of six needles and an inoculum pad. In the bruise method, petioles were wounded with pliers and immediately inoculated. Distilled water was used instead of conidial suspension in the control method.

[z]Total of 396 seedlings per treatment (six crosses per treatment, 66 plants per cross). Mean separation in columns by Duncan's multiple range test at the 5% level.

Table 3. Response of strawberry seedlings to three methods of handling after inoculation with *Colletotrichum fragariae*

Treatment[y]	Number of susceptible plants[z]		Number of dead plants[z]	
	21 days	28 days	21 days	8 wk
1	135 a	209 a	53 a	101 a
2	142 a	156 a	41 a	86 a
3	143 a	166 a	75 a	123 a

[y]In treatment 1, susceptible plants were counted and left in the same flat with resistant plants; in treatment 2, susceptible plants were counted, the infected petiole of each susceptible plant was cut off at least an inch below the lesion, and the plants were left in the same flat with resistant plants; in treatment 3, susceptible plants were removed from the flat of resistant plants and placed on another bench. All dead plants were discarded.

[z]A total of 396 seedlings were inoculated per treatment. Mean separation in columns by Duncan's multiple range test at the 5% level.

as resistant if no lesion or a lesion less than 1 mm long developed.

Method of inoculation. A total of 396 seedlings from each of six crosses (cross = replication) were randomly divided into six treatments of 66 plants each. The treatments were brush, spray, clip, puncture, bruise, and control. Inoculum was brushed onto the petiole of the youngest fully expanded leaf of each seedling in the brush treatment; care was taken not to allow any inoculum to run down the petiole into the crown. In the spray treatment, inoculum was sprayed over the top of all plants with a hand pump sprayer until it dripped from the leaves. Plants in the clip treatment were cut to a height of 40 mm above the crown (some of the plants had no leaf blades left on them) and immediately sprayed with inoculum. In the puncture treatment, the youngest leaf of each seedling was wounded with a device made by putting six size "0" insect needles through a cork and allowing their tips to extend about 2 mm. The needles were pushed through the leaf and into a pad soaked with inoculum, so that when the needles were removed, inoculum was pulled into the leaf. In the bruise treatment, a small sponge pad was taped to each side of a pair of needle nose pliers, the pads were dipped into the inoculum, and the petiole of the youngest leaf of each seedling was pressed firmly with the pliers. The plants in the control treatment were divided into six rows of 11 plants each. Each row was given one of the treatments listed except distilled water was used instead of inoculum, and one row was not treated at all.

All flats were placed into the humidity chamber 24 hr before inoculation, removed for treatment, and returned to the humidity chamber for 72 hr. The control and treatment flats were placed in the same chamber but were separated by about 60 cm. When the flats were removed from the humidity chamber, the control flats were placed on a separate bench in the greenhouse.

Handling after inoculation. An experiment was conducted to determine whether resistant seedlings were becoming infected by spores washing into their crowns from lesions on petioles of susceptible plants. We inoculated 198 plants from each of six crosses with the brush technique described above, placed them in the humidity chamber for 72 hr, and removed them to the greenhouse bench. Seven days after inoculation and every 7 days thereafter for 8 wk, we checked the plants for infection and handled them in one of the following ways: 1) susceptible plants were counted and left in the same flat with resistant plants; 2) the infected petiole of susceptible plants was cut off at least 2.5 cm below the lesion, and the plants were left in the same flat with resistant plants; 3) susceptible plants were removed from the flat of resistant plants and placed on another bench. All dead plants were discarded. The numbers of susceptible and dead plants were recorded at each examination.

RESULTS

Length of time in humidity chamber. The seedlings receiving the 0 + 0 treatment did not develop susceptible lesions within 6 days after inoculation (Table 1). Forty-four percent of seedlings receiving the 24 + 72 treatment and 80% of those in the 0 + 72 treatment developed susceptible lesions within 6 days. Lesion development was significantly greater in the 0 + 72 treatment than in the 24 + 72 treatment. There were no significant differences among crosses.

Method of inoculation. Wounding the plants before inoculation by cutting off the leaves or by bruising the petiole with pliers gave the highest percentage of susceptible plants (Table 2). All plants in these two treatments received a susceptible rating within 4 wk. Brushing the petioles with inoculum gave significantly fewer susceptible plants 2 wk after inoculation than other methods. Puncturing the leaf blades, spraying, and brushing the inoculum on gave the same percentage of susceptible plants after 4 wk. Crosses did not differ significantly in the percentage of susceptible plants.

Handling after inoculation. There were no significant differences among post-inoculation treatments in the number of susceptible or dead plants 21 or 28 days after inoculation, or in the number of dead plants 8 wk after inoculation (Table 3).

DISCUSSION

Inoculation methods involving severe wounding were too drastic, usually resulting in 100% susceptibility. Brooks (1) found it necessary to wound the petiole of leaves to get infection, but data from our study indicated that wounding of these tissues was not required. No differences in percentage susceptibility were evident between plants we inoculated by brushing inoculum on the petiole (preventing it from entering the crown) and those we inoculated by spraying inoculum over the leaf canopy (allowing it to enter the crown). This result differs from a previous report (3) indicating the necessity of avoiding screening techniques

that introduce inoculum into the crown. The technique reported in this previous report used a higher inoculum concentration (3×10^6 conidia per milliliter) than we used and an airbrush sprayer to spray the inoculum directly into the top of the crown. In our study, spores were not forced into the crown but were permitted to enter the crown area, which would approximate field conditions where spores may be spread by surface water (1). We found no significant differences in the percentage of susceptible plants between treatments in which infected plants were left in the flat with resistant plants and treatments where infected plants were removed every 6 days.

These results indicate that large populations of strawberry seedlings may be screened in the greenhouse for resistance to anthracnose in 4 mo or less from time of sowing seed to final evaluation. High humidity must be provided immediately after inoculation to get rapid symptom expression. Brushing inoculum on a young petiole of each seedling is very time-consuming when thousands of seedlings are being screened. Spraying the inoculum on the young leaves and petioles was the most practical method of inoculation. Susceptible seedlings may be identified as those that develop petiole lesions longer than 1 mm within 4 wk of inoculation.

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