Evaluating Strawberry Plants for Resistance to *Colletotrichum fragariae*

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ABSTRACT

DELP, B. R., and R. D. MILHOLLAND. 1980. Evaluating strawberry plants for resistance to *Colletotrichum fragariae*. Plant Disease 64:1071-1073.

A reliable technique for evaluating strawberry plants (*Fragaria* × *ananassa*) for resistance to *Colletotrichum fragariae* involves spraying the distal half of attached petioles with a 10^6 conidia per milliliter suspension, incubating at 100% RH and 28–30 C for 48 hr and then maintaining the plants near 25 C. A disease index was developed based on petiole reactions; flecking was considered resistant and girdling to petiole death was susceptible. Because plants inoculated in the crown died, this method was discontinued. The age of inoculum did not affect the amount of infection. Optimum inoculum density for resistance evaluation was 10^6 conidia per milliliter. Optimum time in the moist chamber was 48 hr; all plants became susceptible after 72 hr. Optimum temperature was 25 C; resistant cultivars became susceptible at 30 C.

Strawberry anthracnose caused by *Colletotrichum fragariae* Brooks, is a serious problem in Louisiana and North Carolina and has severely limited strawberry plant production in Florida since 1968 (1,7,11,12). The fungus attacks stolons, petioles, and crowns of strawberry plants (*Fragaria* × *ananassa*), often resulting in plant death (1,3,5,8). It has been reported to cause a fruit rot in Florida (10).

Several means of disease control have been investigated including sanitation practices, fungicide sprays, and resistant cultivars (2,4,6,9). The latter is promising if resistant plants can be identified.

This article describes an inoculation and evaluation technique for screening strawberry plants for resistance to *C. fragariae*. Tissue susceptibility and the effects of inoculum age, inoculum density, moisture period, and temperature on disease development are reported.

MATERIALS AND METHODS

Plant material. All plants, except two Louisiana selections, were obtained from a registered planting at the Sandhills Research Station, Jackson Springs, NC, where anthracnose has not been observed. Strawberry selections L-2556 and L-6632 were obtained from P. L. Hawthorne, Louisiana State University.

Plants were set in 10-cm pots containing Metromix (Grace Horticultural and Agricultural Products) and methyl bromide-treated soil (1:2, v/v) and allowed to grow 6–8 wk to confirm that they were free of anthracnose. All old petioles were removed before inoculation, leaving three to five healthy petioles. The cultivars and selections used in these tests included Surecrop and Sumner (susceptible); Tennessee Beauty (moderately susceptible); Florida-90 and Titan (moderately resistant); L-6632, L-2556, Apollo, and Sequoia (resistant).

Table 1. Percent strawberry plants dead 21 days after inoculation with *Colletotrichum fragariae*

Cultivar or selection	Field reaction[a]	Control	Inoculation		
			Petiole spray	Mycelium in crown	Crown spray
Surecrop	S	0	100	100	100
L-2556	R	0	25	50	75
L-6632	R	0	0	75	100

[a] S = susceptible, R = resistant.

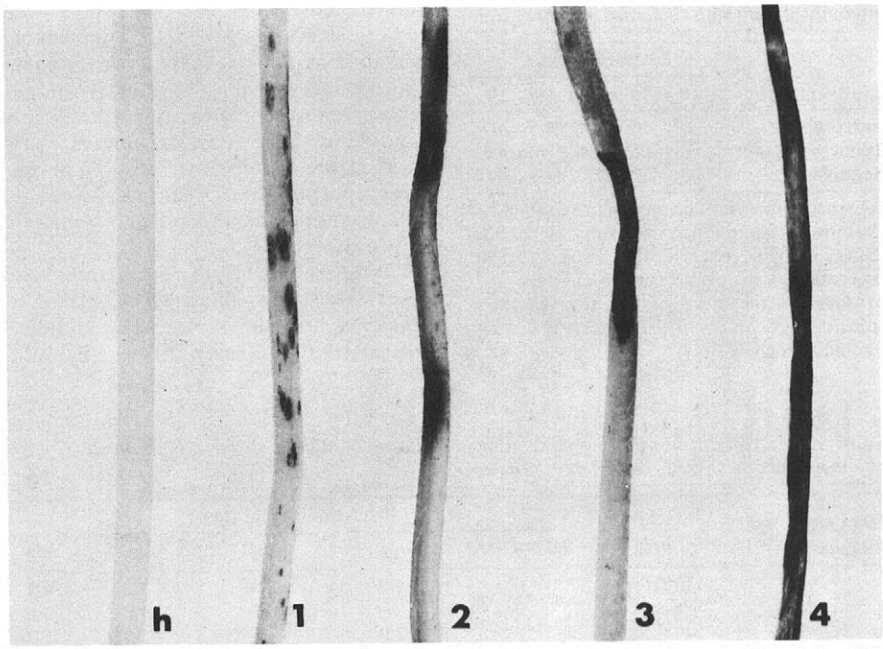

Fig. 1. Petiole reactions in strawberry anthracnose disease index: h = healthy petiole; 1 = lesions <3 mm long; 2 = lesions 2–10 mm long; 3 = lesions 10–20 mm long, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead (not shown).

Paper 6382 of the Journal Series of the North Carolina Agriculture Research Service, Raleigh.

Use of trade names implies neither endorsement of this product by the North Carolina Agriculture Research Service nor criticism of similar ones not mentioned.

Portions of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree, North Carolina State University.

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0191-2917/80/12107103/$03.00/0

Inoculum. Inoculum was prepared from *C. fragariae* grown on oatmeal agar in petri plates for 10 days at 25 C. Conidial suspensions were obtained by flooding the plates with 20 ml of distilled water, scraping the surface with a razor blade, and filtering the suspension through cheesecloth. Concentrations were adjusted with a hemacytometer to $1–3 \times 10^6$ conidia per milliliter, unless otherwise noted. Tween 20 was added to the suspension at 0.1%, v/v.

Tissue susceptibility. Approximately 0.2 ml of a conidial suspension of *C. fragariae* isolates CF-1, CF-4, CF-10, or CF-14 was injected into the crowns of three plants each of cultivars Surecrop, Sequoia, Titan, Apollo, and Florida-90. Control and inoculated plants were placed in a 95–100% RH moisture chamber at 28–30 C for 72 hr and then transferred to a greenhouse bench. The number of plants killed was determined 7 days after inoculation. The test was repeated with isolates of CF-1, CF-4, and CF-10.

A second test compared the susceptibility of crowns and petiole tissue. Four plants of the cultivars Surecrop, L-2556, and L-6632 were inoculated per treatment. One set of plants was inoculated with an airbrush sprayer by carefully spraying enough suspension of isolate CF-4 to wet only the distal half of the petioles, without allowing runoff. A second set was inoculated by spraying the suspension directly into the top of the crown. A third set was inoculated by carefully placing a 4-mm square of mycelium from a plate of oatmeal agar next to the youngest petiole as far into the crown as possible without wounding the tissue. Uninoculated plants served as controls.

Plants were placed in a moisture chamber for 48 hr and then transferred to a greenhouse bench. Plant death was determined after 21 days.

In all subsequent tests, plants were inoculated by carefully spraying the distal half of the petioles with a 3×10^6 conidia per milliliter suspension and were placed in a moisture chamber at 100% RH and 28–30 C for 48 hr, unless otherwise noted.

Disease index. A disease index based on petiole reactions (Fig. 1) was developed and used to evaluate disease development in all subsequent tests. In this 0–5 rating of lesion length and extent, 1 = <3 mm; 2 = 3–10 mm; 3 = 10–20 mm, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead. Plants with a rating greater than 2 were considered susceptible.

Inoculum age. Isolates of CF-1 and CF-4 were grown for 6, 10, 17, 24, 31, and 38 days. Conidial suspensions were prepared from each age and isolate and were used to inoculate four plants of the cultivars Surecrop and Sequoia. The test was repeated with inoculum of each age and isolate and five plants each of cultivars Surecrop, Florida-90, and Sumner. The number of plants with visible lesions and the average number of lesions per petiole were determined 7 days after inoculation.

Inoculum density. Conidial suspensions of isolate CF-4 were prepared and concentrations adjusted, with a hemacytometer, to 0, 10^4, 10^5, 10^6, and 10^7 conidia per milliliter. Three plants each of cultivars Surecrop, Tennessee Beauty, and Sequoia were inoculated with each inoculum density. The test was repeated with Surecrop and Sequoia plants.

Moisture period. Surecrop plants were inoculated with isolate CF-9 and placed in a moisture chamber with uninoculated plants. Four inoculated and two uninoculated plants were removed and transferred to a greenhouse bench after 0, 12, 24, 48, and 72 hr. Disease was evaluated 7, 14, and 21 days after inoculation. The test was repeated with Surecrop and Sequoia plants with 0, 24, 48, and 72 hr moisture periods.

Temperature. Surecrop plants were inoculated with isolate CF-4, placed in the moisture chamber for 48 hr, and then transferred to Sherer-Gillett CEL-7HL growth chambers maintained at 15, 20, 25, or 30 C. Plants received 16 hr of incandescent and fluorescent light in 24 hr. Disease was evaluated on 12 plants at each temperature 4, 7, and 14 days after inoculation. The test was repeated with three plants at each temperature, and disease was evaluated 4, 7, 14, and 21 days after inoculation.

A second test consisted of inoculating Surecrop and Sequoia plants with isolate CF-4. Three inoculated and two uninoculated plants of each cultivar were maintained at 15, 20, 25, and 30 C after a 48-hr moist period. Disease was evaluated 7, 14, and 21 days after inoculation. The test was repeated with Surecrop.

RESULTS AND DISCUSSION

Tissue susceptibility. A spore suspension of *C. fragariae* injected into the crown killed 99 of 105 plants inoculated after 7 days. No more than one plant survived in a treatment. This technique bypassed resistance mechanisms and illustrates that once the crown becomes infected even plants that are resistant in the field (Apollo and Sequoia) are susceptible.

In the second test, all of the inoculated Surecrop plants were dead after 21 days (Table 1); therefore, the three inoculation techniques induced infection. Preliminary tests indicated that the Louisiana selections L-2556 and L-6632 were very resistant to anthracnose; however, 75 and 100% of the L-2556 and L-6632 plants, respectively, were killed 21 days after a conidial suspension was sprayed into the crown. Placing mycelium at the base of the youngest petiole resulted in 50 and 75% plant death of L-2556 and L-6632, respectively. When the petioles of these selections were sprayed with the suspension, only small flecks appeared on the petioles after 21 days. Only one of the L-2556 plants and none of the L-6632 or control plants died. This indicates that there is a resistance mechanism of strawberries to infection by *C. fragariae* in the petioles but not in the crown. Inoculum introduced into the crown bypasses this resistance and the plant dies. The death of the single L-2556 plant that was inoculated by spraying the

Table 2. Effect of inoculum density on disease development on strawberry plants 14 days after inoculation with *Colletotrichum fragariae*

Cultivar	Conidia/ml				
	0	10^4	10^5	10^6	10^7
Surecrop	0[a,b]	1.8	4.2	4.5	4.7
Tennessee Beauty	0	0.3	1.0	5.0	4.3
Sequoia	0	1.5	2.0	1.8	4.0

[a] Disease index: 1 = lesions <3 mm long; 2 = lesions 3–10 mm long; 3 = lesions 10–20 mm long, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead.
[b] Values are the mean of two tests of three plants per treatment; Tennessee Beauty includes one test only.

Table 3. Effect of duration of moisture after inoculation on disease development on strawberry plants inoculated with *Colletotrichum fragariae*

Days after inoculation	Cultivar	Moisture period (hr)				
		0	12	24	48	72
7	Surecrop	0[a]	1.2	2.8	4.2	4.8
	Sequoia	0	···	0.5	1.0	2.5
21	Surecrop	0	2.0	4.8	5.0	5.0
	Sequoia	0	···	0.5	1.0	4.5

[a] Disease index: 1 = lesions <3 mm long; 2 = lesions 3–10 mm long; 3 = lesions 10–20 mm long, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead.

Table 4. Effect of temperature on disease development on Surecrop strawberry plants inoculated with *Colletotrichum fragariae*

Days after inoculation	Temperature (C)			
	15	20	25	30
4	0.2[a,b]	1.1	4.0	4.0
7	1.1	1.7	4.7	5.0
14	1.7	2.9	5.0	5.0
21	3.0	4.0	5.0	5.0

[a] Disease index: 1 = lesions <3 mm long; 2 = lesions 3–10 mm long; 3 = lesions 10–20 mm long, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead.
[b] Values are the mean of 15 plants except at 21 days where they are the mean of three plants.

Table 5. Effect of temperature on disease development on strawberry plants inoculated with *Colletotrichum fragariae*

Days after inoculation	Cultivar	Temperature (C)			
		15	20	25	30
7	Surecrop	1.9[a]	2.1	3.9	4.2
	Sequoia	1.0	1.0	1.0	1.0
21	Surecrop	3.0	3.7	4.9	5.0
	Sequoia	1.0	1.0	1.7	3.7

[a] Disease index: 1 = lesions <3 mm long; 2 = lesions 3–10 mm long; 3 = lesions 10–20 mm long, girdling petiole; 4 = entire petiole necrotic; 5 = crown necrotic, plant dead.

petioles may be explained by a drop of inoculum running down the petiole into the crown. It is crucial to avoid a technique that introduces inoculum into the crown either directly or by subsequent watering.

Inoculum age. The number of plants with visible lesions after 7 days did not differ with inoculum of various ages. The number of lesions per petiole varied among cultivars but not among inoculum ages.

Inoculum density. Disease development on plants inoculated with 10^4 conidia per milliliter was inconsistent. Only 80% of the inoculated plants became infected, and disease indices ranged from 1 to 4 within a cultivar. Resistant fleck-type lesions were observed on 67% of the susceptible Surecrop plants 14 days after inoculation. When Surecrop plants were inoculated with 10^5 to 10^7 conidia per milliliter, the expected susceptible reaction was observed (Table 2). The moderately susceptible Tennessee Beauty plants exhibited resistant reactions when inoculated with 10^5 conidia per milliliter, but all plants inoculated with 10^6 conidia per milliliter were dead after 14 days. The resistant cultivar Sequoia showed resistant reactions when inoculated with all inoculum densities up to 10^6 conidia per milliliter; 33% of the Sequoia plants inoculated with 10^7 conidia per milliliter were dead after 14 days, indicating that resistance was overcome. An inoculum density of 10^6 conidia per milliliter was therefore considered optimal for selecting strawberry plants with a high degree of resistance to anthracnose.

Moisture period. None of the plants placed on a greenhouse bench immediately after inoculation became infected (Table 3). Disease developed slowly on Surecrop plants receiving 12 hr of moisture, and the susceptible plants exhibited resistant reactions 21 days after inoculation. Surecrop plants receiving a 24-hr moisture period exhibited susceptible reactions after 21 days; however, 50% of the Sequoia plants were asymptomatic.

The most consistent reaction of susceptible and resistant cultivars occurred when plants were maintained in the moisture chamber for 48 hr. Disease developed rapidly on Surecrop plants. All plants exhibited susceptible reactions after 7 days and were dead after 21 days. Sequoia plants rapidly developed type 1 lesions, which did not develop further after 21 days. The resistance of Sequoia was broken in plants receiving a 72-hr moisture period; 50% of the plants were dead after 21 days.

Temperature. Low temperatures retarded disease development. Petioles on Surecrop plants maintained at 25 and 30 C became completely necrotic 4 days after inoculation (Table 4). Necrosis did not occur on plants maintained at 20 C until 21 days after inoculation. Petioles on plants maintained at 15 C were girdled after 21 days, but none became completely necrotic.

The inhibition of disease development by low temperature was also evident in the second test with the cultivars Surecrop and Sequoia. Lesion development on Surecrop plants maintained at 15 and 20 C was inconsistent, ranging from small flecks to necrosis of entire petiole after 21 days. Consistent reactions were obtained by maintaining Surecrop plants at 25 C. Disease developed rapidly, killing all but one plant after 21 days (Table 5). Sequoia plants showed resistant symptoms after 7 days at 25 C, with little further development at 21 days. Resistance was broken in Sequoia plants maintained at 30 C, resulting in a susceptible disease index of 3.7 after 21 days. The in vitro optimum temperatures for growth and sporulation of *C. fragariae* is 30 C (8).

In summary, a reliable inoculation technique for evaluating strawberry plants for resistance to *C. fragariae* consists of inoculating only the distal half of well-developed petioles by spray application of a 10^6 conidia per milliliter suspension, placing inoculated plants in a 100% RH moisture chamber at 28–30 C for 48 hr, and then maintaining the plants near 25 C. Plants inoculated and maintained in this manner can be evaluated 14–21 days after inoculation. The results correlate well with field observations.

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