Resistance to *Colletotrichum fragariae* in Strawberry Affected by Seedling Age and Inoculation Method

BARBARA J. SMITH, Research Plant Pathologist, U.S. Department of Agriculture, Agricultural Research Service, Small Fruit Research Station, Poplarville, MS 39470; L. L. BLACK, Professor, Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803; and G. J. GALLETTA, Research Geneticist, U.S. Department of Agriculture, Agricultural Research Service, Fruit Laboratory, Beltsville, MD 20705

ABSTRACT

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Two- to 4-wk-old strawberry seedlings (age after transplanting at the first true-leaf stage) were more susceptible to *Colletotrichum fragariae* than 14- to 18-wk-old seedlings when spray-inoculated with a conidial suspension. Comparisons were made among inoculation methods used to evaluate strawberry resistance to anthracnose crown rot caused by *C. fragariae*. Spray inoculation was the best method to determine the overall host reaction (i.e., the foliar and crown reaction of large populations of plants), but crown injection was more reliable to assess the crown response. In crown injection tests, more than half of 14- to 18-wk-old seedlings from crosses of anthracnose crown rot-resistant clones were resistant to the crown rot phase of this disease, while most cultivars and progenies of susceptible parents were very susceptible. Thus, it seems possible to screen for foliar and crown rot resistance.

Colletotrichum fragariae Brooks, the incitant of strawberry anthracnose crown rot (12), may infect the fruit, leaves, petioles, or crown of strawberry (*Fragaria* × *ananassa* Duch. [1,2,7–9]). When the fungus enters the crown, wilt and sudden death of the plant often follow (7,10). The disease is most devastating during warm, humid weather and frequently causes severe losses in summer nurseries in the southeastern United States (10). Resistance to *C. fragariae* has been reported in the cultivars Apollo, Dover, Florida Belle, Rosanne, and Sequoia (3,11). Since their release however, some of these cultivars have been found to be susceptible to certain isolates of *C. fragariae* in greenhouse tests (4,13).

The USDA-ARS Fruit Laboratory in Beltsville, Maryland, and the Small Fruit Research Station in Poplarville, Mississippi, are conducting a breeding program in cooperation with state agricultural experiment stations to develop anthracnose crown rot-resistant strawberry cultivars adapted to the southeastern states (14). A reliable greenhouse disease resistance screening technique is needed to identify and incorporate resistant germ plasm into commercial cultivars. Previous reports on greenhouse methods for evaluating resistance to *C. fragariae* have raised a question as to the most appropriate inoculation procedure. Delp and Milholland (3) reported that inoculum entering the crown during spray inoculations allowed the pathogen to overcome resistance in plants of strawberry lines with which they were working. Therefore, they recommended a distal petiole inoculation method and the use of petiole lesion size to evaluate strawberry plant resistance to *C. fragariae*. Smith and Spiers (14) identified strawberry plants that withstood spray inoculations with *C. fragariae* conidial suspensions suggesting that resistance to the crown rot phase of the disease exists in some of their strawberry lines.

The influence of age on strawberry seedling response to *C. fragariae* has not been examined. In most studies, strawberry runner plants of clonal lines have been evaluated for their reaction to *C. fragariae* (1,3,4,7). Horn et al (6) used seedlings in a study of anthracnose crown rot resistance, but they did not report the age of their seedlings at the time of inoculation. Smith and Spiers (14) inoculated 6- to 8-wk-old seedlings, but they did not compare the disease response of seedlings at various ages.

Part of the current study was designed to determine whether older plant resistance to *C. fragariae* is expressed in the juvenile stage and if not, at what age seedlings could be screened to assess mature plant resistance. Crown rot is the most destructive phase of the disease in the Gulf Coast states, therefore, a second part of this study was initiated to compare inoculation methods used to identify resistance to *C. fragariae* in strawberry seedlings, cultivars, and clones with particular emphasis on selection for resistance to the crown rot phase.

MATERIALS AND METHODS

Source and propagation of strawberry plants. "Seedlings" refers to unselected progenies grown from seed, "clone" to a seedling selection that was increased and maintained as a numbered clonal line for use as a breeding parent, and "cultivar" to a named clonal selection available to the public. Seed derived from 20 crosses made among 28 cultivars and clones at Beltsville and from cultivar open crosses (Table 1) were germinated on moist, finely ground spaghnum in a growth chamber (Controlled Environments, Model PGW36, Pembina, ND) at 24/18 ± 1C (day/night) with a 16-hr photoperiod. Strawberry seeds do not germinate uniformly; therefore, strawberry seedling age was designated as the time after transplanting at the first true-leaf stage into Jiffy-7 peat pellets (Jiffy Products, Ltd., Norway). The seedlings were grown in a greenhouse with supplemental lights (General Electric F400 daylight fluorescent) to achieve a 16-hr photoperiod with day/night temperatures of 22/18 ± 4C. Weekly applications of soluble fertilizer (10-30-20) at the rate of 0.3 g/l were made beginning 3 wk after transplanting. Older leaves and runners were removed from all seedlings 1–7 days before inoculation, leaving the two to four youngest leaves on each plant at the time of inoculation. Plants of cultivars were purchased as dormant crowns from a commercial nursery, and clonal plants were propagated from runners in the greenhouse. These plants were potted in 10 cm × 10 cm plastic

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pots in a 1:1 (v/v) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand and grown in the greenhouse for a minimum of 6 wk before inoculation.

Inoculum preparation. Cultures of *C. fragariae* isolates (Table 2) were grown at room temperature (approximately 25 C) on a 1:1 (v/v) mixture of Difco oatmeal agar and Difco potato-dextrose agar in petri dishes for 7–14 days under continuous fluorescent light. Conidia were washed from cultures with distilled water containing two drops of Tween 20 per liter. A final conidial suspension containing 1.5×10^6 conidia per milliliter was prepared with a hemacytometer. Mixed isolate inoculum was prepared by adjusting the conidial concentration of each isolate to 1.5×10^6, then mixing equal volumes of the conidial suspension from each isolate.

Seedling age and disease response. The effect of seedling age on disease severity following inoculation was evaluated in two experiments. In experiment 1, seedling age × progeny effects were tested with a mixture of *C. fragariae* isolates. Two-, 4-, 8-, and 14-week-old seedlings of five selected crosses and a susceptible open-pollinated (OP) cultivar, Tufts, were divided into 10 replications with seven or eight plants from each seedling population at each age. Seedlings were inoculated by spraying them with a mixed conidial suspension composed of equal numbers of conidia from isolates CF-1, CF-4, Fla-2, La-1, CF-75, and CF-card. In experiment 2, seedling age × progeny × isolate effects were tested. Conidial suspensions from two isolates, CF-1 and CG-164, were used separately to spray inoculate 3-, 14-, and 18-wk-old seedlings from five selected crosses and a susceptible open-pollinated cultivar, Tioga. Each seedling population at each age was divided into eight groups with five plants per group. Four groups (replications) were spray-inoculated with isolate CF-1 and four with isolate CG-164.

Inoculation methods and disease response. Five inoculation methods were evaluated for their influence on subsequent disease development. The methods were plant spray, distal petiole spray, plant spray plus crown drops, plant spray plus crown injection, and crown injection. For the plant spray method, inoculum was applied to all the above-ground plant parts to the point of runoff with a hand-pump sprayer (14). Plants inoculated with the distal petiole spray were held upside down, the distal half of each plant sprayed to the point of runoff with a hand-pump sprayer, and the excess inoculum was allowed to drip off before the plant was turned upright (3). The plants inoculated with plant spray plus crown drops were spray-inoculated, and then three drops of inoculum were placed at the leaf axils in the crown area with a Pasteur pipet without wounding the plant. The plant spray plus crown injection method was accomplished by injecting 0.2 ml of inoculum directly into the crown at the leaf axils with a 1-cc Tuberculin syringe followed by a plant spray inoculation. For the crown injection inoculation method, plants were inoculated the same as in the preceding method without the plant spray procedure.

Three experiments were conducted to evaluate inoculation methods. In experiment 1, two inoculation methods, plant spray and plant spray plus crown drops, were compared with each of five *C. fragariae* isolates for their effect on subsequent disease severity in mature strawberry plants. A group of four plants of each of 12 cultivars was inoculated separately by each inoculation method with each of the fungal isolates.

In experiment 2, isolate CG-164 was used to evaluate four inoculation methods on 8- to 14-wk-old progeny of seven crosses of resistant clones and progeny from a susceptible OP cultivar. Seedlings were divided into groups of six plants each from the eight populations. The methods compared were 1) distal petiole spray, 2) plant spray, 3) plant spray plus crown drops, and 4) plant spray plus crown injection. Control plants were treated by methods 1 and 2 with sterile distilled water. The experiment was replicated four times.

In experiment 3, the use of crown injection alone as a technique to evaluate mature strawberry plants for resistance

Table 1. Strawberry seedling populations and the reported anthracnose crown rot response to their parent lines

Clone/ cultivar	Parent lines	Anthracnose designation[a]
82-78	MSUS 27 × LA 7113A	$R_2^{b,c} \times R_2^{b,d}$
82-79	MSUS 31 × FLA 73-1872	$R_2^{b,c} \times R^e$
82-80	LA 7525A × US 78-1760AN	$R_2^{b,d} \times R_2^{b,e}$
82-84	FLA 76-577 × NC 3920	$R^e \times R^f$
82-85	US 78-1839AN × LA 7517A	$R_2^{b,c} \times R_2^{b,d}$
83-65	LA 883 × Dover	$R^d \times VR^g$
83-66	MSUS 27 × US 78-1760AN	$R_2^{b,c} \times R_2^{b,e}$
83-67	Prelude × Dover	$S^g \times VR^g$
83-68	Atlas × Florida Belle	$S^g \times R^g$
83-70	LA 883 × Olympus	$R^g \times U$
83-72	MSUS 42 × MDUS 5146	$R_2^{b,c} \times U$
83-73	LA 7922A × Douglas	$R_2^{b,d} \times VS^g$
83-76	Florida Belle × Pajaro	$R^g \times VS^g$
84-61	Cardinal × Fla 77-163	$U \times R^e$
84-65	Tangi × Fla 77-169	$S^g \times R^e$
84-66	Fla 76-577 × MDUS 3771	$R^e \times U$
84-67	Fla 77-163 × Allstar	$R^e \times S^g$
84-69	LA 883 × US 78-1760AN	$R^d \times R_2^{b,e}$
84-70	LA 7922A × Fla 76-802	$R^d \times R^e$
84-71	MSUS 31 × Dover	$R_2^{b,c} \times R^g$
Tioga OP	Tioga Open-Pollinated	$VS^g \times VS^g$
Tufts OP	Tufts Open-Pollinated	$VS^g \times VS^g$

[a] Reported anthracnose crown rot response of parent lines: VS = very susceptible, S = susceptible, R = resistant in field, R_2 = resistant in greenhouse screening and in field, VR = very resistant in field, and U = unknown.
[b] Selected as resistant in greenhouse screening at Poplarville, MS.
[c] Selected as resistant in field at Poplarville, MS.
[d] Selected as resistant in field at Baton Rouge, LA.
[e] Selected as resistant in field at Dover, FL.
[f] Selected as resistant in field at Clinton, NC.
[g] From J. L. Maas (11).

Table 2. Source of *Colletotrichum fragariae* isolates from strawberry plants with anthracnose crown rot symptoms

Code	Isolated by	Year isolated	Plants from
CF-1	N. Horn, Louisiana State University	1968	Louisiana
CF-4	R. Milholland, North Carolina State University	1978	North Carolina
CF-63	B. Smith, USDA, Mississippi	1981	Mississippi
CF-75	B. Smith, USDA, Mississippi	1981	Mississippi
CF-card	B. Smith, USDA, Mississippi	1980	North Carolina
CG-163	C. Howard, University of Florida	1982	Tennessee
CG-164	C. Howard, University of Florida	1982	North Carolina
Fla-1	C. Howard, University of Florida	1978	Florida
Fla-2	C. Howard, University of Florida	1978	Florida
LA-1	N. Horn, Louisiana State University	1979	Louisiana
MS-9	B. Smith, USDA, Mississippi	1978	Mississippi

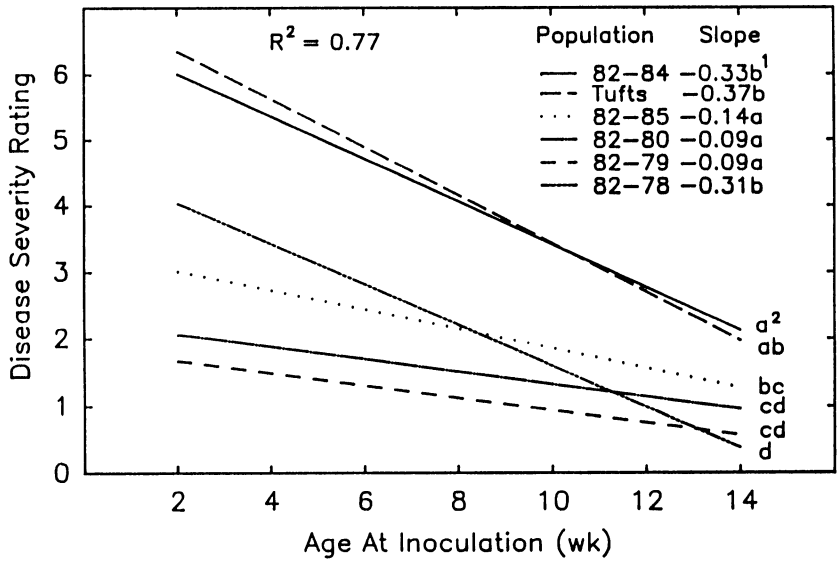

Fig. 1. Relation of disease severity rating to seedling age at inoculation for six strawberry seedling populations 5 wk after plant spray inoculation with a mixture of six *Colletotrichum fragariae* isolates, CF-1, CF-4, Fla-2, La-1, CF-75, and CF-card. Seedlings were inoculated at 2, 4, 8, or 14 wk after being transplanted at the first true-leaf stage. [1]Slopes of regression lines followed by same letter are homogenous, F test, $P = 0.05$. [2]Predicted DSRs at 14 wk followed by same letter are homogenous, F test, $P = 0.05$.

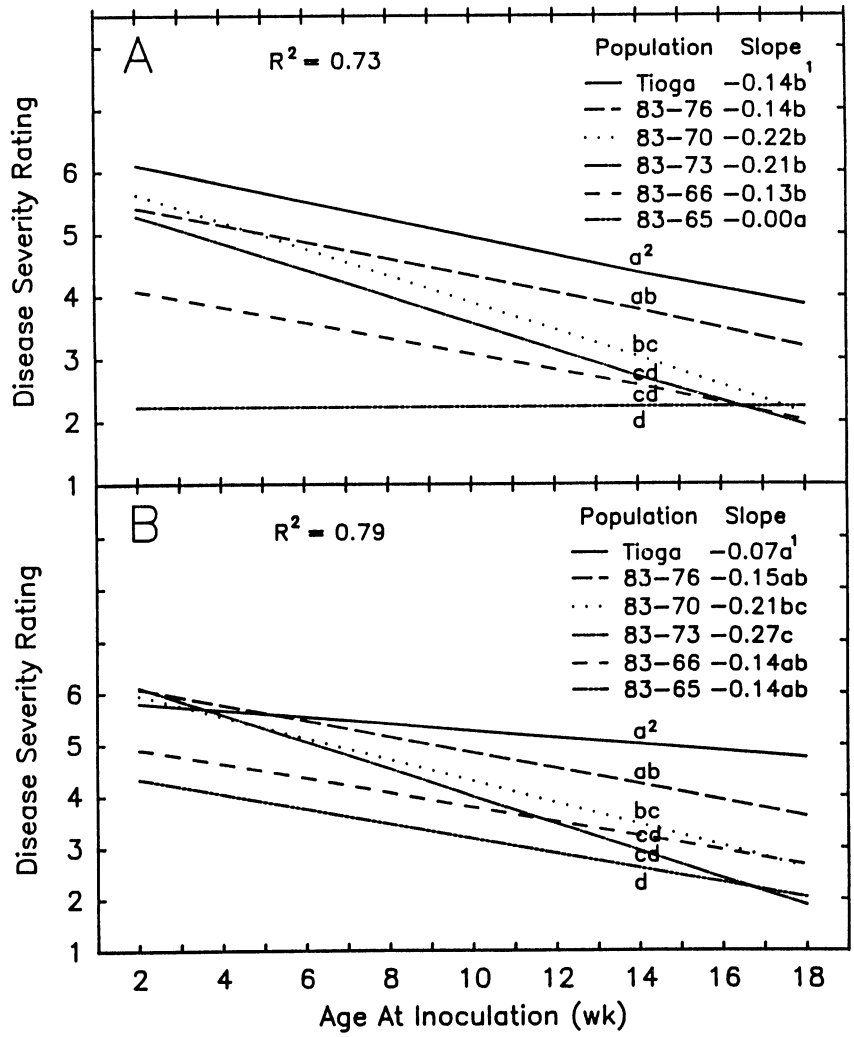

Fig. 2. Relation of disease severity rating to seedling age at inoculation for six strawberry seedling populations 30 days after plant spray inoculation with *Colletotrichum fragariae* isolate CF-1 **(A)** or CF-164 **(B)**. Seedlings were inoculated 3, 14, or 18 wk after being transplanted at the first true-leaf stage. [1]Slopes of regression lines followed by same letter are homogenous, F test, $P = 0.05$. [2]Predicted DSRs at 14 wk followed by same letter are homogenous, F test, $P = 0.05$.

to anthracnose crown rot was tested using six isolates to inoculate four plants each of four cultivars and three clones.

Immediately after inoculation, all plants were placed in a dew chamber for a 48-hr incubation period and then were transferred to a greenhouse. The temperature of the unlighted dew chamber was 32 ± 1 C and that of the greenhouse was about 28 C, except in experiment 3 in which the greenhouse temperature was about 32 C.

Disease severity rating scale. Disease development was assessed with a disease severity rating (DSR) scale ranging from 0 to 6 (12) where 0 = plant with no visible lesions, 1 = plant with petiole lesions <3 mm long, 2 = plant with petiole lesions 3–10 mm long, 3 = plant with petiole lesions >10–20 mm long, 4 = plant with petiole lesions >20 mm long, 5 = plant whose youngest leaf was wilted with or without petiole lesions, and 6 = dead plant with necrotic crown. Following inoculation by the various surface application methods, plants were grouped by their DSRs as follows: 0–2 = resistant, 2.1–3.9 = intermediate, and 4–6 = susceptible. When one of the crown injection methods of inoculation was used, the plants were grouped by DSRs as follows: 0–3.5 = resistant, 3.6–4.4 = intermediate, and 4.5–6 = susceptible.

Data analyses. The SAS statistical package (5) was used for analysis of variance tests. When an F test indicated significant treatment differences, mean separation was by least significant differences, the Waller-Duncan test, or least squares mean.

RESULTS

Seedling age. Younger seedlings generally were more susceptible than older seedlings in both studies (Figs. 1 and 2, Tables 3 and 4). In experiment 1, disease severity of all strawberry seedling populations decreased as age of seedlings at the time of inoculation increased. The slopes of the regression lines for the three populations 82-79, 82-80, and 82-85 were significantly less than those of the other three populations (Fig. 1). The predicted DSRs at 14 wk of age for populations 82-84 and Tufts OP were significantly greater than those of populations 82-78, 82-79, and 82-80. No seedling population had a mean DSR of ≤ 2 (resistant) when inoculated at the 2- or 4-wk-old stages, but three seedling populations had DSRs ≤ 2 when inoculated at 14 wk of age (Table 3).

In experiment 2, similar results were obtained, i.e., older inoculated seedlings were usually more resistant to each isolate of *C. fragariae* than younger seedlings (Table 4). The slopes of the regression lines for all populations inoculated with isolate CG-164 and all populations except 83-65 inoculated with isolate CF-1 were negative (Fig. 2A,B).

These results show that the seedling populations became more resistant with age up to 18 wk, the oldest seedling age at inoculation.

Inoculation methods and disease response. In experiment 1, there were no differences between the DSRs of plants within each cultivar inoculated with the same isolate by the plant spray method or the plant spray plus crown drops method (Table 5). Similar symptoms, including foliar and crown infection symptoms, were observed on both plants inoculated with plant spray and with plant spray plus crown drops. There were significant differences in the DSRs among cultivars and isolates (*data not shown*), but there was no difference in the mean DSR for any of the cultivars based on inoculation method. The overall average DSR for all cultivars inoculated by the plant spray method was 3.5, while that of plants inoculated by the plant spray plus crown drops method was 3.4.

In experiment 2, mean DSRs over all seedling populations tested were not significantly different 30 days after inoculation among the distal petiole spray, the plant spray, and the plant spray plus crown drops inoculation techniques (Table 6). However, the plant spray plus crown injection technique resulted in a significantly higher mean DSR than those obtained by the other inoculation techniques tested. In subsequent observations 50 days after inoculation, 54–100% of the seedlings in the seven selected seedling populations inoculated by the plant spray plus crown injection method were alive, while only 32% of the Tufts OP seedlings remained alive. Some of the control plants died in the more susceptible populations probably because of natural infection, which is difficult to eliminate over a long-term experiment within a hot, humid greenhouse. This natural infection most likely occurred near the end of the experiment because the DSR of these control plants was near zero at the 30-day rating.

Some resistance to crown infection was identified by crown injection in experiment 2; therefore, a crown injection inoculation without foliar inoculation was used to evaluate crown resistance in various strawberry breeding clones and cultivars in experiment 3. Thirty days after injection, most of the cultivars had a DSR of ≥ 4.5 (susceptible), but two of the clones, MSUS 42 and MSUS 70, had a DSR of ≤ 3.5 (resistant) to three or more of the isolates (Table 7). Fifty days after inoculation,

Table 3. Influence of plant age at time of inoculation[v] on disease severity rating[w] of six strawberry seedling populations spray-inoculated with *Colletotrichum fragariae* in experiment 1

Seedling population	Disease severity rating[x]				
	Seedling age (wk)[y]				
	2	4	8	14	Mean
82-79	2.4 abB[z]	3.3 aB	1.5 abC	1.2 bC	2.1 D
82-80	2.9 abB	3.3 aB	2.5 abC	1.1 bC	2.4 CD
82-85	4.0 aAB	4.2 aAB	3.7 aAB	1.4 bBC	3.3 BC
82-78	5.6 aA	4.9 aAB	3.3 abBC	2.1 bABC	4.0 B
82-84	5.9 aA	5.9 aA	5.3 abAB	3.8 bA	5.2 A
Tufts OP	5.9 aA	6.0 aA	5.5 aA	3.2 bAB	5.2 A
Mean	4.4 ab	4.6 a	3.6 b	2.1 c	

[v] Plants inoculated with suspensions of 1.5×10^6 conidia per milliliter composed of equal volumes of conidia from six *C. fragariae* isolates (CF-1, CF-4, Fla-2, LA-1, CF-75, and CF-card).
[w] Disease severity rating scale: 0 = no symptoms to 6 = plant dead.
[x] Average rating of seven or eight seedlings per replication and 10 replications per age group. Disease severity rated 35 days after inoculation.
[y] Age of seedlings at the time of inoculation based on time after transplanting at the first true-leaf stage.
[z] Mean separation in rows (a–c) and mean separation in columns (A–D) by least squares mean, $P = 0.05$.

Table 4. Influence of plant age at time of inoculation on disease severity rating[s] of six strawberry seedling populations spray-inoculated with *Colletotrichum fragariae* in experiment 2

Seedling population	Disease severity rating[t]			
	Seedling age (wk)[u]			
	3	14	18	Mean
Inoculated with isolate CF-1[v]				
83-65	2.2[w]	2.4	2.2	2.2 f[x]
83-66	4.1	2.3	2.3	2.9 de
83-70	5.4	3.0	2.2	3.5 c
83-73	5.1	2.6	1.9	3.3 cd
83-76	5.3	3.8	3.3	4.1 b
Tioga OP	5.9	4.6	3.7	4.8 a
Mean	4.7 a[x]	3.1 b	2.6 c	
Inoculated with isolate CG-164[y]				
83-65	4.3[z]	2.3	2.3	2.9 d[x]
83-66	5.1	2.2	3.5	3.6 c
83-70	5.9	3.0	2.9	3.9 c
83-73	5.9	2.8	2.0	3.6 c
83-76	6.0	4.1	3.7	4.6 b
Tioga OP	5.9	4.4	5.2	5.2 a
Mean	5.5 a[x]	3.1 b	3.3 b	

[s] Disease severity rating scale: 0 = no symptoms to 6 = plant dead.
[t] Average DSR of five plants per replication and four replications per age; rated for disease severity 30 days after inoculation.
[u] Age of seedlings at the time of inoculation based on time after transplanting at the first true-leaf stage.
[v] Plants inoculated with a suspension of 1.5×10^6 conidia per milliliter from *C. fragariae* isolate CF-1.
[w] LSD = 1.31 for each seedling population-age combination, $P = 0.05$.
[x] Mean separation in row and column by Waller-Duncan, $k = 100$.
[y] Plants inoculated with a suspension of 1.5×10^6 conidia per milliliter from *C. fragariae* isolate CG-164.
[z] LSD = 1.03 for each seedling population-age combination, $P = 0.05$.

Table 5. Comparison of the plant spray plus crown drops and the plant spray inoculation methods for their effect on anthracnose crown rot disease severity on strawberry cultivars

Strawberry cultivar	Mean disease severity rating[a]	
	Plant spray + crown drops[b]	Plant spray[c]
Tioga	4.5	4.7
Tangi	4.1	3.9
Albritton	4.0	4.0
Sequoia	3.6	3.9
Sunrise	3.9	3.6
Florida Belle	3.4	3.5
Titan	3.3	3.3
Tennessee Beauty	3.3	3.2
Florida Ninety	2.9	3.2
Prelude	2.8	3.3
Apollo	2.7	2.4
Cardinal	2.2	2.6
Mean	3.4 NS	3.5 NS

[a] Pooled mean of DSRs for each cultivar to five *Colletotrichum fragariae* isolates (CF-4, MS-9, CF-75, CF-1, and CG-164). Groups of four plants from each cultivar were inoculated separately with each isolate. Disease ratings made 30 days after inoculation on a scale of 0–6 where 0 = no symptoms and 6 = dead plant.
[b] Plants inoculated by a plant spray of inoculum (1.5×10^6 conidia per milliliter) followed by placing three drops of inoculum directly into crown of each plant.
[c] Plants inoculated by a plant spray of inoculum (1.5×10^6 conidia per milliliter).

only three of the MSUS 42 plants and nine of the MSUS 70 plants had died from a total of 24 plants of each clone injected. By comparison, 15 to 22 plants of each cultivar were dead 50 days after inoculation (Table 7). Crown-injected plants receiving DSRs of less than 5 had petiole rather than crown symptoms. These plants usually developed lesions on the petioles of leaves that had not emerged from the crown at the time of inoculation.

DISCUSSION

The disease responses of seedlings in these greenhouse studies cannot be compared directly with the reported responses of their parent lines because the parental response was usually based on field observations (Table 1). However, most seedling populations that had a parent that had been rated either very resistant (VR) or that was a selection from the anthracnose screening program (R_2) were found to be more resistant than progeny of parents rated resistant (R) or susceptible (S). These results suggest that a breeding program coupled with a good disease resistance screening program could lead to the development of strawberry cultivars resistant to anthracnose crown rot.

Most of the seedling populations derived from crosses among selected clones and cultivars were more resistant to isolates of *C. fragariae* than the Tioga OP or Tufts OP seedlings. Several of the seedling populations demonstrated a high level of resistance. For example, in the seedling age experiment 1, all populations except 82-84 had predicted DSRs ≤ 2 (resistant) following inoculation at 14 wk of age (Fig. 1). In the seedling age experiment 2 and in the inoculation method experiment 2, seedling populations 83-65, 83-67, 83-72, and 83-73 consistently had lower DSRs than other populations in these studies (Tables 4 and 6) and a high percentage (96, 96, 100, and 83%, respectively) of the plants survived crown injection inoculations. The slopes of the regression lines for three of the seedling populations (82-79, 82-80, and 83-65; Figs. 1 and 2) are close to zero which suggests that seedlings in these populations express both juvenile and older plant resistance.

The current study has shown age to be an important consideration when screening strawberry seedlings for resistance to *C. fragariae*. In general, seedlings became more resistant as they increased in age. Resistance in most seedling populations could be identified only after seedlings were 14 wk old. Within three populations, 2-wk-old seedlings expressed a distinguishable level of resistance; however, if resistance screening is done at this age some potentially valuable sources of resistance may be eliminated.

A previous report (3) stressed the necessity of preventing inoculum from entering the crown of plants being evaluated for resistance to anthracnose crown rot; however, three of the inoculation methods compared in this paper (distal petiole spray, plant spray, and

Table 6. Disease severity ratings[u] and percentage of strawberry seedlings that died following inoculation with *Colletotrichum fragariae* isolate CG-164 by various inoculation methods

	Inoculation method[v]											
									Controls[w]			
	Distal petiole spray		Plant spray		Plant spray + drop		Plant spray + inject		Plant spray		Plant spray + inject	
Seedling population	DSR[x]	Dead[y]	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead
83-65	1.6	0	2.1	0	2.1	0	2.8	4	0.0	0	0.1	0
83-66	2.7	4	2.5	0	2.5	4	3.5	13	0.0	0	0.5	0
83-67	2.8	0	1.8	4	2.7	0	2.9	4	0.3	0	1.2	0
83-68	2.8	25	3.0	29	2.3	8	3.8	46	0.3	13	0.5	8
83-72	2.1	0	1.9	0	2.0	0	2.8	0	0.0	0	0.0	0
83-73	1.8	0	1.9	0	2.6	0	3.8	17	0.5	0	0.8	4
83-76	2.3	4	2.4	0	2.2	17	3.8	37	0.1	13	0.9	13
Tufts OP	3.7	45	3.5	45	4.3	59	5.0	68	1.2	27	1.1	23
LSD = 0.90, $P = 0.05$												
Mean	2.5 b[z]		2.4 b		2.6 b		3.6 a		0.3 d		0.6 c	

[u] Disease severity rating scale: 0 = no symptoms to 6 = plant dead.
[v] Inoculated 8 to 14 wk after the first true-leaf stage with a suspension of 1.5×10^6 conidia per milliliter.
[w] Controls treated with sterile, distilled water.
[x] Average disease severity rating of four replications with six plants per replication for each seedling population-treatment combination; rated 30 days after inoculation.
[y] Percentage of plants that were dead 50 days after inoculation.
[z] Means followed by the same letter within a row are not significantly different according to Waller-Duncan, $k = 100$.

Table 7. Mean disease severity ratings[a] after 30 days and number of plants dead 50 days after crown injection[b] of four plants of seven strawberry cultivars and lines with conidial suspensions from six *Colletotrichum fragariae* isolates

	Strawberry cultivar or line													
	Rosanne		Tangi		MSUS 27		Tenn. Beauty		Cardinal		MSUS 70		MSUS 42	
Isolate	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead	DSR	Dead
CG-164	4.8[c]	2	6.0	4	6.0	4	6.0	4	3.3	1	4.0	3	4.3	2
CF-card	6.0	4	4.8	3	5.8	4	5.3	3	5.3	3	2.8	1	3.5	0
CG-163	5.3	4	5.5	4	5.3	3	4.0	2	5.3	4	4.8	2	3.3	0
Fla-2	6.0	4	5.5	3	5.5	3	4.3	3	4.0	2	2.8	0	4.0	1
CF-63	6.0	4	5.5	4	4.3	2	3.3	2	4.8	3	3.8	2	3.0	0
CF-1	6.0	4	6.0	4	3.5	1	5.0	3	4.0	2	3.5	1	2.5	0
Mean	5.7	3.7	5.5	3.7	5.1	2.8	4.6	2.8	4.4	2.5	3.6	1.5	3.4	0.5
Water[d]	0.0	0	0.8	0	0.3	0	0.8	0	2.0	0	0.0	0	0.0	0

[a] Disease severity rating (DSR) scale: 0 = no symptoms to 6 = plant dead.
[b] Inoculated by injecting 0.2 ml of a suspension of 1.5×10^6 conidia per milliliter into crown of mature plants.
[c] LSD ($P = 0.05$) of DSR within cultivar-isolate means = 1.98 for crown injected plants.
[d] Plants injected with sterile, distilled water.

plant spray plus crown drops) resulted in DSRs that were not significantly different. The distal petiole spray prevented inoculum from entering the crown while the plant spray plus crown drops deliberately introduced inoculum into the crown. The lack of differences in the DSRs of these two methods indicates that it is not necessary to prevent inoculum from entering the crown of test plants. While any of these three methods could be used to evaluate plant response to the petiole phase and, to a limited extent, to the crown rot phase of anthracnose crown rot, the plant spray method is the easiest to use and is as reliable as the other two.

One of our primary objectives was to identify plants resistant to the crown rot phase of anthracnose crown rot. Therefore, it appeared necessary to use an inoculation method that introduces the inoculum into the crown of the plant. To do this, the plant spray plus crown injection and the crown injection methods were used. The seedling populations and clones chosen were derived from clones previously designated anthracnose crown rot-resistant based on field observations and/or greenhouse spray-inoculation tests. A high level of resistance to the crown rot phase of the disease was demonstrated by the survival of many or all of the plants of several seedling populations and two clones following crown injection with conidia from several individual *C. fragariae* isolates (Tables 6 and 7). The crown-injected plants had DSRs higher than spray inoculated plants when the same fungal isolates were compared. But, when adjustments were made to the disease response ranges to reflect the severity of the inoculation methods, most cultivars and seedling populations responded similarly. The original and adjusted DSR ranges are as follows for after the plant spray inoculation: DSR ≤ 2 = resistant, 2.1–3.9 = intermediate, and ≥ 4 = susceptible. The DSR ranges for after crown injection are: DSR ≤ 3.5 = resistant, 3.6–4.4 = intermediate, ≥ 4.5 = susceptible.

The results of this research show that inoculum entering the strawberry crown even by injection does not overcome high levels of resistance to anthracnose crown rot. In fact, crown injection may be the best inoculation method to use when screening advanced resistant selections, because this rigorous method kills the less resistant plants. This method also guards against the potential selection of plants that may have petiole resistance but not crown resistance. Clones resistant to crown infection in this study also were resistant to the petiole phase. The DSR scale used in these studies is based primarily on petiole response and was used to provide a direct comparison of disease response of plants inoculated by the various methods. This DSR scale is not recommended for use in general practice to evaluate plants inoculated by crown injection. A simple "alive" or "dead" rating made 50 days after inoculation would be a better rating scale for crown-injected plants.

To identify strawberry plants resistant to the crown rot phase of anthracnose crown rot, a plant spray inoculation is recommended as a preliminary screening of large populations to determine the overall reaction. Individual plants found resistant by the plant spray inoculation should be further challenged by crown injection of inoculum. Plants still alive 50 days after crown injection may be considered resistant. Based on previous studies (12), plants should be incubated in a dew or moist chamber for 48 hr at 32–35 C and then maintained in a greenhouse at 32 C following inoculation by either method for best disease development. Seedling populations to be screened should be at least 14 wk old at the time of inoculation to reliably assess mature plant resistance.

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