Resistance of Strawberry Cultivars to Crown Rot Caused by *Colletotrichum gloeosporioides* Isolates from Florida Is Nonspecific

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ABSTRACT

MacKenzie, S. J., Legard, D. E., Timmer, L. W., Chandler, C. K., and Peres, N. A. 2006. Resistance of strawberry cultivars to crown rot caused by *Colletotrichum gloeosporioides* isolates from Florida is nonspecific. Plant Dis. 90:1091-1097.

Isolates of *Colletotrichum gloeosporioides* from strawberry (*Fragaria* × *ananassa*) and native grape were tested for virulence on strawberry cultivars in field experiments for three seasons. Isolate aggressiveness and cultivar resistance were determined by the proportion of plants killed at a defined time. Each year, four to six isolates were inoculated on four to seven different cultivars, with a subset of isolates and cultivars evaluated again the next season. On the dates that disease was evaluated, incidence ranged from 10 to 84% for individual cultivars. Cultivar and isolate effects were significant in all three seasons, but there was no significant cultivar by isolate interaction in any season. Thus, resistance to *C. gloeosporioides* appears to be nonspecific. In the third season, one isolate of *Colletotrichum fragariae* from strawberry and one from oak were included. There was no significant cultivar by isolate interaction detected for this species, although there were significant differences among cultivars and isolates. When the resistance of cultivars to both species was compared, the rankings of cultivars were similar, but a modest cultivar by species interaction was evident. The cultivar Treasure was more resistant to crown rot caused by either species than any other cultivar tested.

Additional keywords: Colletotrichum crown rot, *Glomerella cingulata*

Colletotrichum crown rot of strawberry (*Fragaria* × *ananassa* Duchesne) can be caused by any of three fungi, *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*), *C. fragariae,* or *C. acutatum* (17,27). The closely related species *C. fragariae* and *C. gloeosporioides* produce a reddish-brown necrosis of crown tissue following infection that causes plants to wilt and collapse (27). Under greenhouse conditions or in summer nurseries, *C. fragariae* and *C. gloeosporioides* also may produce necroses on stolons (4), lesions on fruit (23,26), or black leaf spots (25). Although *C. acutatum* can also cause crown rot (17), disease symptoms in plants infected with this species are distinct from symptoms produced by *C. fragariae* and *C. gloeosporioides* (38). Also, where *C. acutatum* co-occurs with *C. gloeosporioides* in Florida, *C. acutatum* is infrequently isolated from diseased crowns (38). Crown rot is a serious disease in subtropical strawberry production regions such as those in the southeastern United States since infection by *C. fragariae* and *C. gloeosporioides* is favored by warm, moist conditions (30,33). Although crown rot is observed in fields during the winter production season, it is most severe in summer nurseries in the southeast and is one of the primary reasons that transplants are produced in temperate regions.

There is some uncertainty regarding the initial occurrence of the closely related pathogens *C. fragariae* and *C. gloeosporioides* in Florida and historically which of these species was responsible for epidemics in the region. *C. fragariae* was first reported in Florida nurseries by Brooks in 1935 (5). The first description of crown rot caused by *C. gloeosporioides* or its teleomorph *Glomerella cingulata* was reported in 1984 (26). The isolates in this report were homothallic and thus described as *G. cingulata*. Subsequently, a portion of the historical isolates from Florida previously described as *C. fragariae* were reclassified as *C. gloeosporioides* based on conidial and setal morphology (21). This suggests that *C. gloeosporioides* may have contributed to crown rot epidemics prior to the initial report in 1984, but was misidentified as *C. fragariae*. At least two of the reclassified isolates produced fertile perithecia when grown together in culture, indicating that they were heterothallic (21). Several of the reclassified isolates have been characterized using molecular methods, and in addition to being distinct from *C. fragariae* isolates, they also appear to be distinct from homothallic isolates of *G. cingulata* (18,19). Currently, *C. gloeosporioides* is the *Colletotrichum* sp. most frequently isolated from diseased crowns in Florida (38). The isolates of *C. gloeosporioides* from diseased crown tissue have diverse genotypes (38) and appear to be from a recombining population (38). They have the Cgl-2 genotype A+T-rich DNA banding pattern of *C. gloeosporioides* isolates originally identified as *C. fragariae* (18; S. J. MacKenzie, *unpublished*). The pathogen also appears to have a broad host range, since the population on noncultivated host species growing adjacent to strawberry fields is genetically indistinguishable from that on strawberry and contains isolates pathogenic to strawberry (40).

Previous studies examining variation in strawberry resistance to *C. fragariae* found a broad range of susceptibility among cultivars and aggressiveness among isolates (14,22,34). One study found a significant cultivar by isolate interaction (34). Host resistance studies using homothallic *G. cingulata* isolates were not as conclusive, as they included only two or three isolates (15,34). One study found significant variation in cultivar susceptibility and isolate aggressiveness, and a significant cultivar by isolate interaction (34). In a later study using a different set of isolates, only cultivar susceptibility varied (15). For *C. acutatum*, a specific interaction between strawberry cultivars and isolates was observed (15). A single dominant gene appears to be responsible for this interaction (16), with isolates having either an incompatible reaction with cultivars carrying this gene or an intermediate degree of compatibility (15). Assays used to evaluate resistance to *C. fragariae* and *C. acutatum* were based on symptom development on petioles or foliage and did not focus on the ability of plants to resist necrosis of crown tissue. Variation in cultivar susceptibility to *C. gloeosporioides* isolates from Florida has not been investigated.

In this study, we investigated the levels of resistance to crown rot caused by *C. gloeosporioides* in cultivars commonly grown in Florida as well as the patterns of virulence and aggressiveness among *C. gloeosporioides* isolates under field conditions. This study generated information

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useful to breeders in terms of the mechanism and sources of resistance to crown rot caused by *C. gloeosporioides*. This information is also valuable to growers deciding which cultivars to plant and from which nursery locations to purchase plants since losses due to crown rot would be especially severe for susceptible cultivars propagated in local Florida nurseries where the disease is most prevalent.

MATERIALS AND METHODS

Plant materials and cultivation. Four to seven cultivars were selected from those commercially available as leaf-on, bareroot transplants imported from Canada at the beginning of the 2001–2002, 2002–2003, and 2003–2004 seasons. The cultivars used included the standard cultivars in the industry, several new cultivars, and an advanced selection from the Florida Agricultural Experiment Station breeding program. The cultivars Aromas, Camarosa, Gaviota, and Camino Real were developed at the University of California; 'Carmine', 'Earlibrite', 'Strawberry Festival', 'Sweet Charlie', and FL 99-164 are cultivars or selections developed at the University of Florida. The cultivar Treasure is from J & P Research in Naples, FL. The cultivar Camarosa was previously shown to be susceptible to crown rot and was included as a positive control for all three seasons (38).

Plants were grown on raised, plastic mulch-covered beds 71 cm wide, 15 cm high at the edge, and 18 cm high in the center. Each bed contained two rows of strawberry plants with 30.5-cm spacing between rows and 38-cm spacing between plants within the rows. The distance between bed centers was 1.22 m. Before planting, the beds were fumigated with methyl bromide/chloropicrin (98:2) at 350 kg/ha. Leaf-on, bareroot transplants were set on 18 October for the 2001–2002 season, 16 October for the 2002–2003 season, and 29 October for the 2003–2004 season. Plants were overhead irrigated for 10 to 12 days to facilitate establishment. After establishment, water and fertilizer were provided through drip tape. Weekly applications of Captan 80WP (Micro Flo, Memphis, TN; *n*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) at 4.2 kg/ha were begun 2 weeks after plants were inoculated. This fungicide prevents new crown infections without inhibiting the progress of infections already established (J. C. Mertely, *unpublished*) and was used to prevent inoculum spread from collapsed crowns to adjacent plots. Freeze and frost protection was provided by overhead sprinklers when necessary.

Fungal isolates and inoculation procedure. Strawberry crown isolates were obtained from samples submitted to the diagnostic clinic at the University of Florida Gulf Coast Research and Education Center in Dover. During the 2002–2003 season, an isolate from a lesion on wild grape (*Vitis rotundifolia*) was included, and an ascospore isolate from a perithecium emerging from a strawberry petiole was included during the 2003–2004 season (Table 1). The isolates were identified as *C. gloeosporioides* using a species-specific internal transcribed spacer (ITS) region primer and random amplified polymorphic DNA (RAPD) analysis (38,40). Briefly, a species-specific ITS primer that produces a positive polymerase chain reaction (PCR) product from *C. gloeosporioides* or *C. fragariae* template DNA was used for initial species identification. Subsequently, bands from five RAPD primer amplification reactions were scored for each isolate. Based on RAPD banding patterns, *C. gloeosporioides* and *C. fragariae* isolates fell into two distinct clusters. All *C. gloeosporioides* isolates were in the same cluster, yet had distinct RAPD banding patterns from one another, indicating that they were not vegetatively produced from the same parent strain. Isolates were also obtained from seven cultivars to reduce the chance of over-sampling a race that might be selected for on a specific cultivar. Pathogenicity of isolates to strawberry was confirmed by injecting inoculum into crown tissue of greenhouse-grown strawberry plants prior to being used in field experiments. Four to six *C. gloeosporioides* isolates were used for inoculations each season. Two isolates of *C. fragariae*, one from a strawberry crown and the other from an oak (*Quercus* sp.) leaf lesion in Lake Alfred, FL, were included in the study during the 2003–2004 season.

Inoculum was prepared from 6- to 8-day-old cultures grown under continuous fluorescent light at 24°C on potato dextrose agar. Conidial suspensions used for inoculations were prepared in sterile deionized water, filtered through four layers of cheesecloth, and diluted to 5×10^5 conidia/ml. Inoculations were performed by spraying 2 ml of conidial suspension with a hand mister directly into the crown of plants 15 to 17 days after the plants were set in the field. The inoculation date was 2 November 2001 for the 2001–2002 season, 31 October 2002 for the 2002–2003 season, and 15 November 2003 for the 2003–2004 season.

Experimental design and statistical analysis. The experiment was designed as a two-factor complete block. There were four blocks in each experiment. Blocks consisted of a single 73-m bed for the 2001–2002 season or two parallel 73- to 76-m beds for the 2002–2003 and 2003–2004 seasons. All cultivar-isolate combinations were randomly assigned to 10-plant plots within each block along with one uninoculated plot of each cultivar. After inoculation, the number of collapsed plants within each bed was recorded weekly until the experiment was terminated at the end of the growing season. Experiments were terminated 133 days (15 March 2002) after inoculation for the 2001–2002 season, 148 days (28 March 2003) after inoculation for the 2002–2003 season, and 132 days (26 March 2004) after inoculation for the 2003–2004 season. Data used for statistical analysis was the proportion of plants collapsed in each plot on a specified date transformed to the arcsine square root. The formula for the transformation was $\arcsin\{\mathrm{sqrt}[(y + 3/8)/(n + 3/4)]\}$, where y = the number of collapsed plants per plot and n = the total number of plants per plot (1). Data for each season were analyzed separately using PROC MIXED of SAS (SAS institute, Cary, NC). In the analysis using

Table 1. Description of *Colletotrichum* isolates used to inoculate experiments conducted over three seasons in Dover, FL

Species	Isolate designation	Host – cultivar	Tissue	Collection site (FL) and year	Season used
C. gloeosporioides	95-63A	*Fragaria* × *ananassa* Duchesne – Oso Grande	Crown	Dover 1995	2001-2002
	96-15A	*Fragaria* × *ananassa* – Oso Grande	Crown	Dover 1996	2003-2004
	96-83H	*Vitis rotundifolia*	Leaf lesion	Dover 1996	2002-2003
	96-83R	*Fragaria* × *ananassa* – Selva	Crown	Dover 1996	2002-2003
	97-15A	*Fragaria* × *ananassa* – Sweet Charlie	Crown	Dover 1997	2001-2002
	97-45A	*Fragaria* × *ananassa* – Camarosa	Crown	Dover 1997	2001-2002, 2002-2003
	97-47C	*Fragaria* × *ananassa* – Camarosa	Crown	Dover 1997	2001-2002
	97-63	*Fragaria* × *ananassa* – Oso Grande	Crown	Dover 1997	2001-2002, 2002-2003
	98-285	*Fragaria* × *ananassa* – Sweet Charlie	Crown	Dover 1998	2001-2002, 2002-2003
	00-59	*Fragaria* × *ananassa* – Strawberry Festival	Crown	Dover 2000	2002-2003, 2003-2004
	00-117	*Fragaria* × *ananassa* – Rosa Linda	Ascospore – petiole	Dover 2000	2003-2004
	02-172	*Fragaria* × *ananassa* – Gaviota	Crown	Dover 2002	2003-2004
C. fragariae	C-16	*Fragaria* × *ananassa* – Camarosa	Crown	Dover 2002	2003-2004
	02-135	*Quercus* species	Leaf lesion	Lake Alfred 2002	2003-2004

C. gloeosporioides and *C. fragariae* data alone, "block" was considered a random effect and "cultivar" and "isolate" were considered fixed effects. In the analysis where *C. gloeosporioides* and *C. fragariae* data were combined, "species", "isolate", and "cultivar" were all considered fixed effects with "isolate" nested within "species". Block was considered a random effect. Uninoculated plots were not included in any reported analysis. The risk of type I error (α) was 0.05 for least square means *t* tests comparing transformed disease incidence between cultivars and was not adjusted for multiple comparisons.

RESULTS

Visually comparing graphs of plant mortality over time after inoculation with *C. gloeosporioides* revealed that the pattern of symptom development was different during the three seasons (Fig. 1A to C). During the 2001–2002 season, the majority of plants that developed crown rot symptoms did so in the first 55 days after inoculation. For the 2002–2003 season, rapid plant collapse within this time period only occurred for 'Gaviota' and 'Camarosa'. During this season, the rate at which plants developed symptoms slowed between day 50 and day 130 after inoculation. However, unlike the 2001–2002 season in which the progress of symptom development also slowed, there was a spike in plant death late in the season. For the 2003–2004 season, symptoms developed on plants throughout the season for all of the cultivars tested. At each time point, the rankings of plants with respect to susceptibility were approximately the same. However, some cultivars such as Earlibrite in the 2002–2003 season, Strawberry Festival in the 2003–2004 season, and FL 99-164 in the 2003–2004 season initially had relatively low disease incidence that increased at a faster rate relative to other cultivars toward the end of the season. Only 'Camarosa' was examined for susceptibility to *C. gloeosporioides* in all three seasons. At the end of each season, the incidence of plant collapse for this cultivar was consistently high, ranging from 62 to 84%. Plants were inoculated with *C. fragariae* only during the 2003–2004 season (Fig. 1D). During this season, the progress of symptom development on plants inoculated with *C. fragariae* showed a similar pattern to that observed for *C. gloeosporioides.* Spread of pathogens from sources outside the plots or among plots did not appear to affect results in any of the experiments since disease incidence at the end of the season in the uninoculated control plots ranged from 0% in 2002–2003 to 0.12% in 2003–2004.

Transformed disease incidence data used for statistical analysis were obtained at different time points after inoculation for each season. Data for analysis were taken on 4 January 2002, 28 March 2003, and 19 March 2004. These dates corresponded to 63, 148, and 125 days after inoculation, respectively. On these dates, the absolute value of the difference between the number of plots with 0% plant mortality and 100% plant mortality was minimized. This reduced compression bias toward one extreme value or the other (0 or 100% mortality). In addition, on these dates, the number of plots with 0 or 100% mortality was low and variance among treatment combinations was relatively high. Analysis of data for *C. gloeosporioides* revealed strong isolate and cultivar effects, but no significant cultivar by isolate interaction in each of the three seasons studied (Table 2; Fig. 2A to C). There was also a cultivar and isolate effect on disease incidence with *C. fragariae* during the 2003–2004 season, but no significant cultivar by isolate interaction (Table 3, Fig 2D). When the *C. gloeosporioides* and *C. fragariae* data from the 2003–2004 season were combined, in addition to a species, cultivar, and isolate effect on disease incidence, there was also a small but significant species by cultivar interaction (Table 3). This interaction resulted from a change in the rankings of 'Strawberry Festival' and 'Camino Real' across fungal species (Fig. 3). With the exception of this one rank change, disease reactions to isolates of the different *Colletotrichum* species were very similar. The variance component

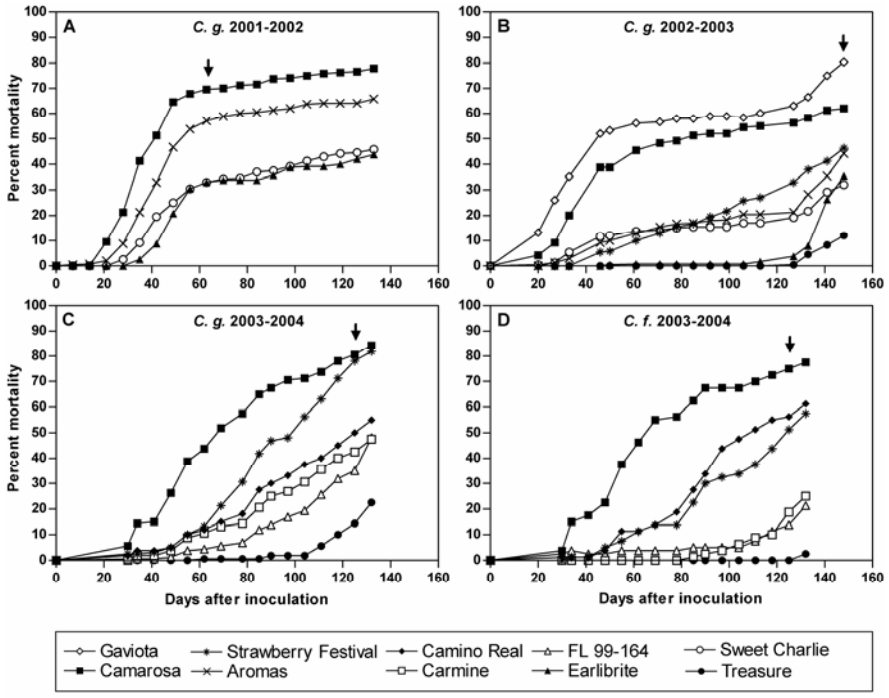

Fig. 1. Mean percent mortality for strawberry cultivars calculated at weekly intervals over the course of the growing season using data from all isolates. **A,** Plants inoculated with *Colletotrichum gloeosporioides* during the 2001–2002 season. **B,** Plants inoculated with *C. gloeosporioides* during the 2002–2003 season. **C,** Plants inoculated with *C. gloeosporioides* during the 2003–2004 season. **D,** Plants inoculated with *C. fragariae* during the 2003–2004 season. Arrows show the time points where disease incidence data were used for statistical analysis.

Table 2. Analysis of variance for three experiments evaluating incidence of crown rot in relation to strawberry cultivar and isolate of *Colletotrichum gloeosporioides*

Season	Source of variation	df	*F* value	*P* > *F*
2001-2002[x]	Cultivar	3	39.16	<0.001
	Isolate	5	45.04	<0.001
	Cultivar × isolate	15	1.52	0.123
2002-2003[y]	Cultivar	6	35.50	<0.001
	Isolate	5	31.63	<0.001
	Cultivar × isolate	30	1.34	0.134
2003-2004[z]	Cultivar	5	36.66	<0.001
	Isolate	3	21.08	<0.001
	Cultivar × isolate	15	0.65	0.823

[x] *F* values for 2001-2002 season were calculated using a residual variance estimate equal to 0.024 having 69 degrees of freedom.

[y] *F* values for 2002-2003 season were calculated using a residual variance estimate equal to 0.038 having 123 degrees of freedom.

[z] *F* values for 2003-2004 season were calculated using a residual variance estimate equal to 0.035 having 69 degrees of freedom.

"block" was not estimated to be greater than zero in any of the experiments ($P > 0.05$).

All cultivars except 'Gaviota', FL 99-164, 'Carmine', and 'Camino Real' were tested in at least two seasons. Eight of the *C. gloeosporioides* isolates were used for inoculations in one season and four isolates (97-45A, 97-63, 98-285, 00-59) were used in two. During the 2001–2002 season, 'Camarosa' was the most susceptible cultivar followed by 'Aromas'. 'Earlibrite' and 'Sweet Charlie' were more resistant and had essentially the same level of susceptibility (Table 4). The ranking of these cultivars for the 2002–2003 season was the same as for 2001–2002, although statistically 'Aromas' could not be separated from 'Earlibrite'. During the 2002–2003 season, 'Treasure', 'Strawberry Festival', and 'Gaviota' also were evaluated. 'Treasure' was more resistant and 'Gaviota' more susceptible than any of the other cultivars examined. 'Strawberry Festival' had an intermediate level of resistance, approximately the same as that of 'Aromas'. During the 2003–2004 season, rankings among cultivars carried over from the previous season remained the same. 'Treasure' was once again the most resistant cultivar and 'Camarosa' was more susceptible than 'Strawberry Festival', although disease ratings for 'Camarosa' and 'Strawberry Festival' were not statistically significantly different from one another during the 2003–2004 season as they were for the 2002–2003 season. During the 2003–2004 season, disease symptoms on 'Strawberry Festival' likely had more time to reach the ratings observed on 'Camarosa'. The three genotypes grown only during the 2003–2004 season, FL 99-164, 'Carmine', and 'Camino Real', were intermediate between the resistant 'Treasure' and the relatively susceptible 'Strawberry Festival' and 'Camarosa'.

During the 2001–2002 season, isolates fell into four groups based on average aggressiveness to the four cultivars tested (Table 5). Isolate 97-15A was the most aggressive and isolates 95-63A, 97-45A, and 97-47C had relatively high, comparable levels of aggressiveness. Isolates 98-285 and 97-63 were not as aggressive, with isolate 97-63 being even less aggressive than 98-285. Isolates 97-45A, 98-285, and 97-63 were re-evaluated in the 2002–2003 season. These three isolates along with 00-59, 96-83R, and 96-83H also produced four isolate clusters based on aggressiveness. The three isolates from the 2001–2002 season had intermediate levels of aggressiveness similar to the previous year. The rankings of these isolates with respect to one another also remained the same, although in the second season isolates 98-285 and 97-63 were not significantly different from each other. Isolates 00-59 and 96-83R were highly aggressive, and isolate 96-83H, a nonstrawberry isolate, was the least aggressive isolate examined. For the 2003–2004 season, only isolate 00-59 was re-evaluated. Once again it was a highly aggressive isolate. Other isolates examined fell into two groups, with isolate 02-172 being more aggressive than isolate 96-15A and ascospore isolate 00-117. In separate analyses comparing inoculated plots to uninoculated controls, all isolate treatments had significantly more crown rot than controls (data not shown).

Cultivar rankings for resistance to *C. fragariae* were very similar to those for *C. gloeosporioides* during the season that this species was included in the study (Table 4). 'Treasure' was highly resistant to *C. fragariae*; FL 99-164 and 'Carmine' displayed moderate levels of resistance, and 'Strawberry Festival' and 'Camarosa' were

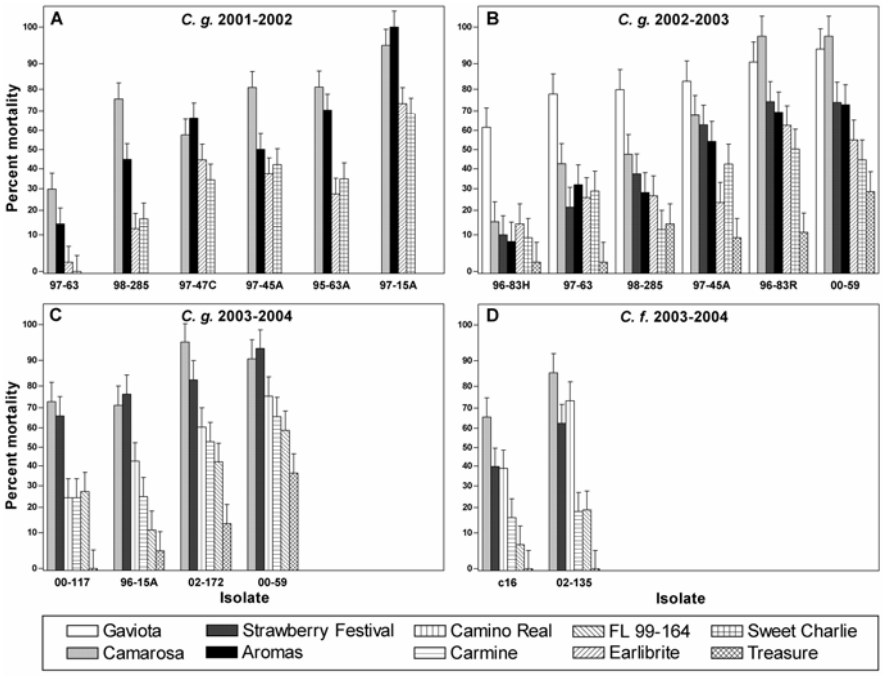

Fig. 2. Cultivar by isolate means for transformed disease incidence data calculated on a specific date during or at the end of the season. The y-axis shows the position of backtransformed incidences ranging from 0 to 100% at intervals of 10%. **A,** Disease incidences calculated for plots 63 days after inoculation with *Colletotrichum gloeosporioides* during the 2001–2002 growing season. **B,** 148 days after inoculation with *C. gloeosporioides* during the 2002–2003 growing season. **C,** 125 days after inoculation with *C. gloeosporioides* during the 2003–2004 growing season. **D,** 125 days after inoculation with *C. fragariae* during the 2003–2004 growing season.

Table 3. Analysis of variance for 2003-2004 experiment evaluating incidence of crown rot in relation to strawberry cultivar and isolate of *Colletotrichum fragariae* alone or in comparison with *C. gloeosporioides*

Species included	Source of variation	df	*F* value	*P* > *F*
C. fragariae[y]	Cultivar	5	24.66	<0.001
	Isolate	1	9.01	0.005
	Cultivar × isolate	5	0.91	0.485
C. fragariae and *C. gloeosporioides*[z]	Species	1	27.10	<0.001
	Cultivar	5	56.91	<0.001
	Isolate (species)	4	19.00	<0.001
	Species × cultivar	5	2.72	0.023

[y] *F* values for the analysis examining *C. fragariae* only were calculated using a residual variance estimate equal to 0.034 having 33 degrees of freedom.

[z] *F* values for the analysis examining both *C. fragariae* and *C. gloeosporioides* were calculated using a residual variance estimate equal to 0.033 having 125 degrees of freedom.

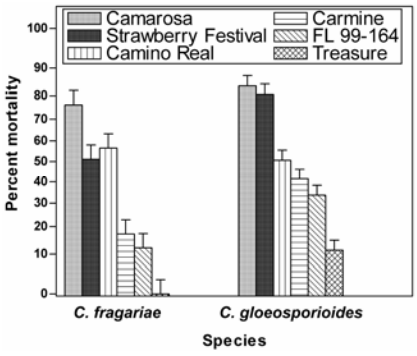

Fig. 3. Cultivar by species means for transformed disease incidences calculated 125 days after inoculation with *Colletotrichum fragariae* or *C. gloeosporioides* during the 2003–2004 season. The y-axis shows the position of backtransformed incidences ranging from 0 to 100% at intervals of 10%.

relatively susceptible. The rankings only differed in that 'Camino Real' appeared to be more resistant and 'Strawberry Festival' more susceptible to *C. gloeosporioides* than to *C. fragariae*, but not dramatically so. Only two *C. fragariae* isolates were included in the study. These isolates displayed different levels of aggressiveness: isolate 02-135, a *C. fragariae* isolate from a nonstrawberry host, was more aggressive than C-16, the isolate from strawberry (Table 5).

DISCUSSION

Pathogenicity trials conducted over 3 years using 12 distinct *C. gloeosporioides* isolates and 10 strawberry cultivars identified differences in disease resistance among cultivars and differences in aggressiveness among isolates, but failed to identify any cultivar by isolate interactions. A more limited study using two *C. fragariae* isolates inoculated on six strawberry cultivars conducted during the third year identified differences in disease resistance among cultivars and differences in aggressiveness among the isolates, but failed to identify a cultivar by isolate interaction. When cultivar resistance rankings were compared between the two pathogen species, there was a small but significant cultivar by species interaction.

Horizontal resistance is effective against all isolates of a pathogen, whereas vertical resistance is effective against a subset of isolates (39). These terms are synonymous with race-non-specific and race-specific resistance, respectively (3). In pathosystems where race-specific resistance occurs, an incompatible interaction between the host and pathogen often requires a dominant host resistance gene and a dominant pathogen avirulence gene. Such interactions are described by the gene-for-gene hypothesis (20). Race-non-specific resistance is less understood, but is believed to be governed by many host genes that incrementally contribute to the overall resistance of the plant (32,39). Vanderplank proposed using analysis of variance to determine the contribution of race-non-specific and/or race-specific resistance within a host population against a group of pathogen isolates (39). Absence of a cultivar by isolate interaction is evidence for race-non-specific resistance using this method. A cultivar by isolate interaction suggests that race-specific mechanisms contribute to the resistance observed within the host population, although deviations from additivity could be responsible for the statistical interaction (31). Deviations from additivity result from the scale used to measure resistance, whereas rank changes are consistent with physiological interdependency between the host and isolates. No cultivar by isolate interactions were detected in this study, suggesting that race-non-specific resistance mechanisms are responsible for the differences in resistance observed between cultivars. Using transformed percentages taken at a time point in which disease incidence was intermediate effectively reduced interactions attributable to deviations from additivity. This finding may be useful in examining resistance to other wilt diseases where use of arbitrary rating scales or measurements of area under the disease progress curve may produce results in which there are no transformations available to eliminate undesirable scale effects. A drawback of measuring resistance using the proportion of collapsed plants is that the rankings of cultivars could change at different time points after inoculation as resistance may occur at different levels of the infection process. Temporally distinct resistance mechanisms have been demonstrated for *Phytophthora palmivora* on cacao (28). The first level of resistance, referred to as penetration resistance, was attributed to morphological factors, and the second level of resistance, referred to as postpenetration resistance, impeded tissue invasion by the pathogen after colonization. In the current study, rank changes between cultivars over the course of the season were infrequent and occurred only between cultivars displaying similar levels of resistance, suggesting that sequential deployment of resistance mechanisms had little effect on the evaluation of cultivars. However, the relatively high levels of inoculum used could have saturated structural mechanisms that limit ingress into the host. Similarly, individual isolates within the pathogen population might differ in their ability to overcome plant defenses at different times during and after invasion of tissues. However, like the cultivar resistance rankings, pathogen aggressiveness rankings did not change over the course of the experiment (data not shown).

Table 4. Mean percent plant collapse of cultivars inoculated with *Colletotrichum gloeosporioides* or *C. fragariae* during three seasons in Dover, FL

	Disease incidence (%)[y]			
	C. gloeosporioides			*C. fragariae*
Cultivar	**2001-2002[z]**	**2002-2003**	**2003-2004**	**2003-2004**
Treasure		10.0 a	11.3 a	0.0 a
Sweet Charlie	30.3 a	29.9 b		
Earlibrite	30.7 a	33.9 bc		
FL 99-164			33.7 b	12.0 b
Carmine			41.5 bc	17.1 b
Camino Real			50.6 c	56.4 c
Aromas	59.6 b	43.1 cd		
Strawberry Festival		45.9 d	80.6 d	51.1 c
Camarosa	71.8 c	64.7 e	83.8 d	76.3 d
Gaviota		82.2 f		

[y] Statistical tests were conducted on mean transformed disease incidences. Reported values were calculated by backtransforming the mean arcsine square root disease incidences for all isolates inoculated on a cultivar.

[z] Statistical tests only compare means for plants inoculated with the same species within the same season. Means in each column followed by the same letter are not significantly different, least squares means *t* tests ($P = 0.05$).

Table 5. Mean percent plant collapse for *Colletotrichum gloeosporioides* or *C. fragariae* isolates used to inoculate strawberry cultivars during three seasons in Dover, FL

		Disease incidence (%)[y]		
Inoculate species	**Isolate**	**2001-2002[z]**	**2002-2003**	**2003-2004**
C. gloeosporioides	97-15A	86.8 a		
	00-59		69.4 a	71.8 a
	96-83R		66.9 a	
	02-172			59.1 b
	95-63A	53.8 b		
	97-45A	53.8 b	48.6 b	
	97-47C	50.7 b		
	96-15A			36.3 c
	98-285	36.0 c	34.3 c	
	00-117			33.4 c
	97-63	9.5 d	31.4 c	
	96-83H		15.3 d	
C. fragariae	02-135			40.9 a
	C-16			24.7 b

[y] Statistical tests were conducted on mean transformed disease incidences. Reported values were calculated by backtransforming the mean arcsine square root disease incidences for all cultivars inoculated with an isolate.

[z] Statistical tests only compare means for isolates of the same species within the same season. Means in each column followed by the same letter are not significantly different, least squares means *t* tests ($P = 0.05$).

During the 2001–2002 season, crown rot symptoms appeared to progress faster early in the season than they did in the 2002–2003 or 2003–2004 seasons. This was probably due to higher early-season temperatures since the average daily temperature in December was 18.3°C, 3.7°C higher than it was in the other seasons. The mean daily temperature of 11.6°C in January of the 2002–2003 season was 2.5°C lower than the mean temperature during January for the other two seasons and may have slowed down symptom development during this time period.

In a previous study examining resistance to *G. cingulata* and *C. fragariae* in strawberry, cultivar by isolate interactions were observed (34). The *G. cingulata* isolates used in that study were homothallic and therefore likely to have been from a genetically distinct subpopulation within the same taxonomic group as those used in the current study. Also, in that study, it is conceivable that scale effects produced by the severity rating system used to evaluate disease accounted for the interaction. However, the interactions may also have been due to use of cultivars not included in the current study. Commercial cultivars shipped from Canada were used to obtain the relatively large number of plants free of crown rot required for the experiment. A disadvantage of using these cultivars for studying mechanisms of resistance was that some of them were closely related, restricting the diversity of the germ plasm evaluated. For example, 'Earlibrite' (11) and 'Strawberry Festival' (12) share 'Rosa Linda' as a parent, and 'Strawberry Festival' (12) and 'Treasure' (U.S. Plant Patent 12,414) share 'Oso Grande' as a parent. Nevertheless, a great deal of variance in resistance was observed among them. It is also conceivable that strains with virulence patterns distinct from the isolates tested exist, but were not sampled due to their low frequency within the population. Use of isolates from different cultivars or hosts each with distinct RAPD banding patterns should have enhanced the probability of finding these strains; however, given the high variability of *C. gloeosporioides* (38) and the fact that only two *C. fragariae* isolates were used in the current study, we cannot conclude that a more extensive sample would not reveal isolates that react with cultivars differently.

A cultivar by isolate interaction for resistance has been demonstrated for *C. acutatum* (15). A single dominant gene (*Rca2*) appears to be responsible for this interaction (16) and distinguishes two groups of *C. acutatum*, those producing a noncompatible reaction with cultivars having *Rca2* (group 2) and those having an intermediate degree of compatibility with cultivars possessing the *Rca2* gene (group 1) (15). Although *C. acutatum* is more distantly related to *C. gloeosporioides* than *C. fragariae* (35) and produces different disease symptoms on strawberry (27), there is evidence that resistance to *C. acutatum*, *C. fragariae,* and *G. cingulata* is correlated (34). Cultivars at least moderately resistant to fruit rot caused by *C. acutatum* examined in the current study include 'Sweet Charlie' (8), 'Carmine' (10), and 'Earlibrite' (C. K. Chandler, *unpublished*). 'Sweet Charlie' is field immune to fruit rot, whereas 'Carmine' and 'Earlibrite' are moderately resistant. Although the mechanism of resistance to *C. acutatum* in these cultivars has not been examined, it is likely that these cultivars possess at least one copy of a dominant resistance gene to group 2 *C. acutatum* isolates. A dominant resistance gene to this group of isolates was found in 13 of 14 cultivars from the United States and Europe classified as being intermediately susceptible or resistant to anthracnose fruit rot, whereas it only occurred in one of seven cultivars classified as susceptible (16). Also, cultivars such as 'Dover', which is homozygous for the *Rca*2 gene (16), and 'Sequoia', which is heterozygous for the *Rca*2 gene (16), occur in the pedigrees of the three fruit rot resistant cultivars used in the current study (9–11). With the exception of 'Treasure', no cultivar displayed significantly greater resistance to crown rot than 'Sweet Charlie', 'Earlibrite', and 'Carmine', suggesting that major gene resistance to *C. acutatum* could also be effective against *C. gloeosporioides*. The lack of a cultivar by isolate interaction observed with isolates of *C. gloeosporioides* could be due to little variation in a gene product or products in this species homologous to those responsible for the interaction with *C. acutatum*. Also of importance is the fact that 'Treasure' was found to be highly susceptible to anthracnose fruit rot caused by *C. acutatum* in field trials conducted over 2 years (C. K. Chandler, *unpublished*), indicating that genes responsible for resistance to crown rot caused by *C. gloeosporioides* do not necessarily confer resistance to *C. acutatum.*

Race-specific resistance is found most frequently in biotrophic plant-microbe interactions where there is prolonged contact between the pathogen and the living host (20). *Colletotrichum* species use nutritional strategies ranging from necrotrophy to hemibiotrophy (29). *Colletotrichum* species such as *C. orbiculare*, *C. graminicola*, *C. sublineolum*, *C. destructivum*, *C. truncatum,* and *C. linicola* are all considered hemibiotrophs, as there is an asymptomatic biotrophic interaction between these species and host cells before the reaction becomes necrotrophic (29). The occurrence of a number of dominant race-specific resistance genes in bean to *C. lindemuthianum*, a member of the *C. orbiculare* species aggregate, suggests that gene-for-gene interactions play an important role in host resistance to these hemibiotrophic pathogens (41). No microscopic studies of strawberry crown invasion by *C. gloeosporioides* have been conducted, although invasion of subtropical fruits (2,6), northern jointvetch (36), and *Stylosanthes scabra* (37) by *C. gloeosporioides* has been investigated. On citrus and avocado fruit, *C. gloeosporioides* is a quiescent epiphyte that is able to become necrotrophic upon ripening of the fruit (2,6). On foliage of *S. scabra* and northern jointvetch, *C. gloeosporioides* has a brief biotrophic phase before entering an extended necrotrophic stage (36,37). The evidence for this biotrophic interaction is not nearly as clear as it is for interactions between *Colletotrichum* spp. commonly referred to as hemibiotrophs and is limited to the occurrence of a spherical vesicle inside an epidermal cell just beneath the appressorium from which infection hyphae emanate. A differential interaction between *S. scabra* cultivars and biotype A *C. gloeosporioides* isolates has also been demonstrated (37), although a great deal of the variation in resistance among cultivars is due to race-non-specific mechanisms (7). The histopathology of infections caused by the related pathogen, *C. fragariae,* on strawberry stolons showed that a brief biotrophic phase possibly occurred before the pathogen entered an extended necrotrophic phase (13). The biotrophic phase was less than 12 h, and it was considered a modification of necrotrophy rather than an example of hemibiotrophy. The lack of race-specific resistance observed in the current study suggests that biotrophic interaction between *C. gloeosporioides* or *C. fragariae* and strawberry is brief and limited. This finding is also consistent with evidence that both of these species infect hosts unrelated to strawberry (24,40).

An isolate of *C. gloeosporioides* from grape, a *C. gloeosporioides* ascospore isolate from a strawberry petiole, and an isolate of *C. fragariae* from oak were included in this study. The ascospore isolate has a different RAPD profile from other isolates obtained from the same perithecium from which it was isolated, and it only produces fertile perithecia in culture when paired with a second isolate, indicating that it is heterothallic (S. J. MacKenzie, *unpublished*). In an analysis of RAPD marker data, the ascospore and grape *C. gloeosporioides* isolates grouped with the *C. gloeosporioides* population from crown tissue and the *C. fragariae* oak isolate grouped with the *C. fragariae* population from strawberry (S. J. MacKenzie, *unpublished*). The ascospore isolate was one of the least aggressive isolates during 2003–2004, although it was as aggressive as one of the crown rot isolates. During the 2002–2003 season, the grape isolate was less aggressive than the isolates from strawberry crowns. This may result from more aggressive isolates being selected in the *C. gloeosporioides* population on

strawberry. The *C. fragariae* isolate from oak was more aggressive and had essentially the same virulence pattern as the isolate from strawberry. This is further evidence that *C. fragariae* from strawberry is derived from a population with a very broad host range, since the oak isolate was found 28 km from any strawberry production area. The only interaction detected was between the species of isolate and cultivar. The interaction was small, and only one rank change occurred between cultivars. Although further studies should be conducted to confirm this finding, it suggests that resistance to *C. gloeosporioides* and *C. fragariae* is positively correlated and that there are also differences in the mechanisms of resistance to these two species.

Information obtained from the current study could improve efficiency of germ plasm screens and aid progenitor selection. For future screens of strawberry plants for resistance to *C. gloeosporioides* isolates from Florida, the absence of a cultivar by isolate interaction suggests that use of numerous isolates is not necessary. Although virulence patterns of the isolate collection used in the current study were the same, there were differences in aggressiveness among isolates. A potentially useful strategy of isolate selection for future screens could employ a combination of isolates with different levels of aggressiveness. An advantage of this strategy is that less aggressive isolates could differentiate between genotypes with low or moderate resistance and aggressive isolates could be used to differentiate resistant cultivars. The study was not designed to determine if resistance in 'Treasure' is controlled by a major gene, however information from the pedigrees of 'Treasure' and 'Strawberry Festival' suggest that it may be. Both 'Treasure' and 'Strawberry Festival' share 'Oso Grande' as a parent (12; U.S. Plant Patent 12,414), yet have very different levels of resistance to crown rot. The occurrence of a major gene or genes for resistance to crown rot in 'Treasure' could account for this difference. Appropriate crosses would need to be conducted to confirm that this is the case. Also, because 'Treasure' is not resistant to fruit rot caused by *C. acutatum,* the resistance is probably unique from resistance conferred by the *Rca2* gene. That at least some disease was observed in all of the cultivars tested has important implications for disease control strategies. Given that *C. gloeosporioides* is broadly distributed on multiple hosts in Florida (40), at least a portion of plants of any cultivar are likely to become infected, with crowns infected by more aggressive isolates showing symptoms. Use of resistant cultivars would likely reduce spread among strawberry plants.

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