

Evaluation of Elite Native Strawberry Germplasm for Resistance to Anthracnose Crown Rot Disease Caused by *Colletotrichum* Species

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ABSTRACT. Anthracnose crown rot of cultivated strawberry (*Fragaria ×ananassa* Duchesne ex Rozier) has been a major disease problem in the strawberry producing regions of the southeastern United States since the early 1970s. Chemical controls are often inadequate, but use of resistant cultivars is seen as a credible option for managing this disease. Only a small portion of *Fragaria* L. germplasm has been screened for resistance to anthracnose crown rot. A core subset of the *Fragaria* collection maintained at the U.S. Department of Agriculture National Clonal Repository in Corvallis, OR, has been constructed to contain an elite group of native *F. virginiana* Mill. and *F. chiloensis* (L.) Mill. This collection, referred to as the “core collection,” has been characterized for many horticultural traits, including reactions to several common foliar diseases, resistance to black root rot (causal organisms unknown), and resistance to northern root-knot nematode (*Meloidogyne hapla* Chitwood) and root-lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev & Shuurmans Stekhoven]. Our objective was to evaluate the core collection for resistance to a selection of isolates of three *Colletotrichum* Corda species known to cause strawberry anthracnose, *Colletotrichum fragariae* A.N. Brooks, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. [teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk], and *Colletotrichum acutatum* J.H. Simmonds (teleomorph *Glomerella acutata* J.C. Guerber & J.C. Correll). No *Fragaria* subspecies or geomorph was more resistant than any other; rather, individual genotypes within these groups were identified as sources from which resistance can be obtained. Collecting germplasm in areas of intense disease pressure may not be as beneficial as one might assume, at least where anthracnose crown rot disease is concerned.

Anthracnose crown rot and fruit rot caused by species of the fungal genus *Colletotrichum* produce significant losses in strawberry (*Fragaria ×ananassa*) production with crown rot, resulting in plant death and fruit rot, which in turn results in some percentage of unmarketable fruit (Maas, 1998). Anthracnose crown rot has been a major disease problem in the strawberry producing regions of the southeastern United States since the 1970s. Although initially considered a southeastern United States problem, anthracnose disease has increasingly

become a significant problem in other strawberry production regions in the United States and the world (Freeman et al., 1998; Howard et al., 1992; Maas, 1998).

Anthracnose is incited by the fungal species *Colletotrichum fragariae*, *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*) (Smith and Black, 1990), and *Colletotrichum acutatum* (teleomorph *Glomerella acutata*). *Colletotrichum fragariae* causes crown and fruit rot but is rarely found outside the southeastern United States, and its host range is limited (Smith, 1998a, 1998b). *Colletotrichum gloeosporioides* most commonly causes crown rot but can also cause fruit rot. *Colletotrichum acutatum* causes a destructive fruit rot (Howard et al., 1992; Smith, 1998a) in addition to runner and petiole lesions, and it can also cause crown rot (Smith, 1998b). Both *C. gloeosporioides* and *C. acutatum* have broad host ranges, including apple (*Malus ×domestica* Borkh.), key lime [*Citrus aurantiifolia* (Christm.) Swingle], mango (*Mangifera indica* L.), peach [*Prunus persica* (L.) Batsch], sweet orange [*Citrus sinensis* (L.) Osbeck], blueberry (*Vaccinium corymbosum* L.), and almond [*Prunus dulcis* (Mill.) D. A. Webb] (Farr et al., 1989; Freeman et al., 1998; Peres et al., 2005; Smith, 1998a, 1998b).

For control of various plant diseases, integrated pest management and sustainable agriculture strategies often

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incorporate the use of resistant or tolerant cultivars. However, very few cultivars resistant to anthracnose crown rot currently are available in North America. Only 'Pelican' is resistant to both anthracnose fruit and crown rot (Shuman, 2001; Smith et al., 1998). Though four parent clones (Galletta et al., 1993) are resistant to anthracnose crown rot, 'Gem Star' (Chang, 2002a) and 'Treasure' (Chang, 2002b) are the only cultivars other than 'Pelican' reported to be resistant to anthracnose crown rot.

Selection for additional cultivated strawberry genotypes resistant to anthracnose crown rot is a credible option for managing this disease (Ballington et al., 2002; Denoyes-Rothan and Guérin, 1996; Gupton and Smith, 1991; Hancock et al., 1991, 1996; Simpson et al., 1994; Smith et al., 1996). However, apparent "field resistance" (Olcott-Reid and Moore, 1995) may be due to genotype by environment interactions unfavorable to infection or spread, rather than to true genetic disease resistance (Smith and Black, 1987). Expression of resistance is strongly affected by interactions among environment, genotype, and isolate (Ballington and Milholland, 1993; Olcott-Reid and Moore, 1995; Smith and Black, 1987, 1990). In addition, individual isolates within and among the three *Colletotrichum* species vary in pathogenicity to *Fragaria* genotypes (Smith and Black, 1990).

A reliable assay has been used for 20 years to identify resistant strawberry genotypes (Smith and Black, 1987; Smith et al., 1990). Multiple isolates of *Colletotrichum* spp. may be used in this assay to screen for resistance to pathogenic strains collected from various hosts and geographic locations. Anthracnose-resistant genotypes (including US 70, US 159, US 292, US 438, and 'Pelican') selected using this assay have been grown in replicated field trials at the Small Fruit Research Station, Poplarville, MS, for 10 to 20 years under heavy anthracnose pressure without dying from anthracnose crown rot. Plants of commercial cultivars, including Chandler, grown in the same trials often died from anthracnose crown rot by late spring. While *C. fragariae* was the most common causal agent of anthracnose crown rot in these field trials, *C. acutatum* and *C. gloeosporioides* also were isolated from crowns of plants dying in the field. In addition, all three species have been isolated from symptomatic fruit on plants grown in these trials (B.J. Smith, unpublished data).

Only a small portion of *F. ×ananassa* germplasm has been screened for resistance to anthracnose crown rot (Smith and Black, 1990). The germplasm base of the cultivated strawberry is very narrow (Dale and Sjulín, 1990; Hancock and Luby, 1995; Sjulín and Dale, 1987); thus, there is not a large reservoir of germplasm among cultivars from which to draw resistance genes for developing new resistant cultivars. Hancock et al. (1993) suggested that the genetic diversity of *F. ×ananassa* should be expanded by introgressing genes from elite clones of wild native octoploid *Fragaria* species. Very little attention has been given to evaluation of wild octoploid *Fragaria* germplasm as potential sources of resistance to anthracnose. Moreover, the germplasm that has been evaluated has been screened with only a small number of *Colletotrichum* isolates that ultimately originated either from the southeastern United States or California. Only recently have some *Fragaria virginiana* clones from the southeastern U.S. been reported to be potential sources of genes for resistance to both *C. acutatum* and *C. fragariae* (J.R. Ballington, personal communication).

A core subset of the *Fragaria* collection maintained at the U.S. Department of Agriculture National Clonal Repository,

Corvallis, OR, has been constructed to contain an elite group of native *F. virginiana* and *Fragaria chiloensis*. This core subset is being characterized for many horticultural traits useful to breeders (Hancock et al., 2001a, 2001b), including reactions to several common foliar diseases, resistance to black root rot (causal organisms unknown) (Hancock et al., 2001b, 2002), and resistance to northern root-knot nematode (*Meloidogyne hapla*) and root-lesion nematode (*Pratylenchus penetrans*) (Pinkerton and Finn, 2005). Our objective in the present work was to evaluate many of the clones represented in the core collection for resistance to a selection of isolates of the three *Colletotrichum* species that can cause anthracnose crown rot.

Materials and Methods

PLANT MATERIAL. Young runner plants of the *F. ×ananassa* cultivars Pelican (resistant) and Chandler (generally not resistant), eight clones of *F. chiloensis*, and 12 clones of *F. virginiana* from the core collection (Table 1) were rooted under intermittent mist. 'Pelican' and 'Chandler' served as negative and positive treatment controls, respectively. After about 2 weeks, the rooted plants were transplanted to 10 × 10 cm² plastic pots containing a 1:1 (by volume) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand and grown for at least 6 weeks before inoculation in a greenhouse maintained at 28 °C day/18 °C night ± 6 °C with a 16-h photoperiod. Older leaves, runners, and flowers were removed 1–7 d before inoculation, and three or four young leaves remained on each plant at inoculation.

INOCULUM PRODUCTION AND INOCULATION. Isolates of *C. acutatum* (Goff and CA-1), *C. fragariae* (CF-63 and CF-75), and *C. gloeosporioides* (CG-162) (Smith and Black, 1990) have been used for 20 years to identify resistant strawberry genotypes (Smith and Black, 1987; Smith et al., 1990) and were selected for this experiment. Isolates were initiated from silica gel cultures maintained at USDA/ARS in Poplarville, MS. Cultures were grown on potato dextrose agar (PDA) under continuous fluorescent light at room temperature (20–28 °C). Conidial suspensions used for inoculations were prepared from 7- to 14-d-old cultures. Inoculum was prepared by flooding each PDA culture plate with sterile deionized water and gently scraping the agar surface with a glass rod to remove conidia. The resulting conidial suspension was filtered through cheesecloth, and the final conidial suspension was adjusted to 1.5 × 10⁶ conidia/mL.

Inoculations were conducted separately for each isolate in three independent experiments. Before inoculation, plants within each genotype were sorted by vigor and placed in blocks so that each of the *Colletotrichum* isolates would be tested on plants having the same range of vigor. Within each block, plants of each genotype were assigned randomly to an inoculant treatment. A hand pump sprayer was used to apply the inoculum, or water as a control treatment, as a mist uniformly over the foliage of the plants to the point of runoff so that inoculum or water accumulated in the crowns at the petiole bases. All genotypes were inoculated with *C. acutatum* isolate Goff, most were inoculated with *C. fragariae* isolate CF-63, and genotypes with sufficient available plants were inoculated with the other three isolates. In 2000, all inoculations were done on 24 Jan. To account for genotype by environment effects, subsequent inoculations were done at different times of the year. In 2003, plants were inoculated on 16, 23, 25, and 29

Table 1. Strawberry genotypes evaluated for reaction to infection by five isolates of three *Colletotrichum* species.^z

<i>Fragaria</i> taxa	PI number	Other name	Location	<i>Colletotrichum</i> isolate					Key horticultural traits ^y
				CA-1	GOFF	CG-162	CF-63	CF-75	
<i>F. × ananassa</i>	NA	'Chandler'	California	No data	MR ^x	MS ^x	S ^x	MS	
Duchesne ex Rozier	637960	'Pelican'	Mississippi	R ^x	R	R	R	R	
<i>F. chiloensis</i> (L.) Mill. ssp. <i>chiloensis</i> f. <i>chiloensis</i>	551736	CFRA 0372	Peru	MS	R	MS	MR	MS	Hermaphrodite, short-day, old land-race from Peru; large fruited with good color for South American <i>F. chiloensis</i> ; extremely long peduncles
<i>F. chiloensis</i> ssp. <i>pacifica</i> Staudt	551445	RCP37	California	MS	R	MS	S	S	Male, short-day; resistant to aphids, ^w two-spotted spider mite, red stele, leaf spot, powdery mildew, and root lesion nematode
	551453	CFRA 0042	Washington	MS	MR	S	S	S	Female; short-day; resistant to red stele and strawberry aphid
	551735	CFRA 0368	Alaska	R	MR	MR	MS	MS	Short-day; female; relatively large and high flavored; probably very winter hardy; free of all foliar diseases in native site
	612487	CFRA 0688	British Columbia	MR	MR	MR	S	S	Female; short-day; large fruit; winter hardy in Ontario but not Michigan; very resistant to leaf scorch and leaf spot
	612488	CFRA 1267	British Columbia	MR	MR	MS	S	S	Hermaphrodite, short-day; large orange fruit
	612489	HM1	Oregon	MS	MR	S	S	S	Female; short-day; unusually high fruit number
	612490	Scotts Creek	California	R	MR	MS	MS	MS	Short-day; male and female clones (female tested here); unusually large fruit size and superior color for a native North American <i>F. chiloensis</i> ; probably salt and drought tolerant
	<i>F. virginiana</i> Mill. ssp. <i>glauca</i> (S. Watson) Staudt	552275	CFRA 0982	Maine	MR	MR	S	MS	MS
	612494	BH2	South Dakota	MS	MR	S	MS	S	Female; cyclic flowering; probably winter hardy as found at 1550-m elevation
	612495	LH50-4	Montana	MR	MR	S	S	S	Cyclic flowering; hermaphrodite; extremely large, numerous fruit for <i>F. virginiana</i> ssp. <i>glauca</i>
<i>F. virginiana</i> ssp. <i>virginiana</i> (Northern)	612492	Eagle 14	Ontario	R	MR	S	S	MR	Weak cyclic flowering; partial hermaphrodite; resistant to powdery mildew and leaf scorch

continued next page

Table 1. Continued.

Fragaria taxa	PI number	Other name	Location	Colletotrichum isolate					Key horticultural traits ^y
				CA-1	GOFF	CG-162	CF-63	CF-75	
	612493	Frederick 9	Ontario	MR	MR	MS	MS	MR	Male; cyclic flowering; well colored fruit; resistant to powdery mildew and leaf scorch
	612497	Montreal River 10	Ontario	R	MR	MR	R	MS	Short-day; hermaphrodite; unusually large fruit; resistant to powdery mildew and leaf scorch
	612498	RH23	Minnesota	R	MS	S	S	MS	Partial hermaphrodite; cyclic flowering; large fruit; resistant to black root rot, leaf scorch and leaf spot
<i>F. virginiana</i> ssp. <i>virginiana</i> (Southern)	612323	NC96-35-2	Alabama	R	MR	R	S	R	Female; short-day; large fruit; resistant to leaf scorch and leaf blight; tolerant to powdery mildew
	612324	NC96-48-1	South Carolina	MR	R	MS	MS	No data	Short-day; female; high productivity; erect peduncles; resistant to powdery mildew; tolerant to leaf scorch and leaf blight
<i>F. virginiana</i> ssp. <i>grayana</i> (Vilm. ex J. Gay) Staudt	612486	NC95-19-1	Mississippi	No data	MR	MS	MR	MS	Very high fruit productivity; well-colored fruit; resistant to leaf scorch; tolerant to leaf blight and powdery mildew
	612570	JP95-1-1	Florida	MR	MR	R	MS	MS	Partial hermaphrodite; short-day
	616568	CFRA 1177	Kentucky	MR	MR	S	MS	MS	Extremely large fruited with excellent color

^zGenotypes are denoted by plant introduction (PI) number of the U.S. National Plant Germplasm System, cultivar name, accession or field collection name or number, and the state or country of original collection. *Colletotrichum* isolates were selected from three species: CA-1 and GOFF are isolates of *C. acutatum*; CG-162 is an isolate of *C. gloeosporioides*; and CF-63 and CF-75 are isolates of *C. fragariae*. Analyses of variance indicated significant differences between genotypes regarding response to the *C. fragariae* isolates and the *C. gloeosporioides* isolate but not with regard to response to either of the *C. acutatum* isolates.

^yHorticultural traits and taxonomic classification for wild strawberry genotypes are taken from Hancock et al. (2001a, 2001b, 2002).

^xDisease severity was rated on a standardized scale of 0 to 6 (0 = healthy plant with no visible symptoms; 1 = petiole lesions <3 mm long; 2 = petiole lesions 3–10 mm long; 3 = petiole lesions >10 to <20 mm long; 4 = petiole lesions >20 mm long; 5 = youngest leaf wilted; 6 = dead plant). Analyses of variance indicated significant genotype by year interaction effects. Therefore, a genotype with an average disease-severity rating ≤ 2 in any of the years tested was considered resistant (R), a genotype with an average rating ≤ 3 was considered moderately resistant (MR), a genotype with an average rating ≤ 4 was considered moderately susceptible (MS), and a genotype with an average rating >4 for any year was considered susceptible (S).

^wLeaf scorch [*Diplocarpon earlianum* (Ellis & Everh.) F.A. Wolf], leaf spot [*Mycosphaerella fragariae* (Tul.) Lindau], verticillium wilt (*Verticillium* C. G. D. Nees ex Wallroth.), two-spotted spider mite (*Tetranychus urticae* Koch.), red stele (*Phytophthora fragariae* Hickman), powdery mildew [*Sphaerotheca macularis* (Wallr.:Fr.) Jacz. f. sp. *fragariae* Peries], root lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven], root-knot nematode (*Meloidogyne hapla* Chitwood), black root rot [causal organism(s) not known], leaf blight [*Phomopsis obscurans* (Ellis & Everh.) Sutton], and strawberry aphid (*Chaetosiphon* Mordvilko).

Sept.; in 2005, plants were inoculated on 16, 21, 27 Mar., and 7 Apr.

Treated plants were immediately placed in a dew chamber at 100% relative humidity and 30 °C for 48 h and then into a greenhouse for 4 weeks with a temperature of 22 ± 7 °C. To prevent cross-contamination, plants were randomized within inoculant group within block when placed in the dew chamber for incubation and subsequently in the greenhouse. Plants

inoculated with the same isolate or the water control were placed on the same shelf in the dew chamber and grouped together in the greenhouse until they were rated.

Disease development based on petiole lesion length and crown rot symptoms was assessed 30 d after inoculation (Smith and Black, 1987). Disease severity was rated on a standardized scale of 0 to 6 (0 = healthy plant with no visible symptoms; 1 = petiole lesions <3 mm long; 2 = petiole lesions 3–10 mm long;

Table 2. Mixed-model analysis of the effects of *Colletotrichum* isolate and *Fragaria* subspecies (taxa) on the severity of anthracnose crown rot in greenhouse screenings of six subspecies of strawberry conducted in 2000, 2003, and 2005.^z

Source of variation	Numerator df	Test statistic ^y	P
Fixed effects		F	P > F
Isolate	4	6.07	0.0129
Taxa	5	2.26	0.1361
Isolate × taxa	20	2.47	0.0092
Random effects		χ ²	P > χ ²
Year	4	223.6	<0.0001
Block(year)	1	33.8	<0.0001
Year × isolate	2	30.5	<0.0001
Year × taxa	2	49.6	<0.0001
Year × isolate × taxa	1	1.7	0.1923
Residual		(1111.9) ^x	

^zData were combined over years and analyzed as a multiyear split-plot design treating year as a random effect. Sources of variation are sorted according to whether they were considered fixed effects or random effects.

^yF tests were performed on fixed effects with numerator df values shown in the table and denominator df values of 8.65, 9.0, and 35.2 for the effects of isolate, taxa, and its interaction, respectively; the denominator df values were calculated using the KENWARDROGER method (Kenward and Roger, 1997). Likelihood ratio χ² tests were performed on random effects with df values shown in table. Column shows the difference between the -2·log likelihoods of the full and reduced models.

^xThe -2·log likelihood of the full model is shown in parentheses.

3 = petiole lesions >10 but <20 mm long; 4 = petiole lesions >20 mm long; 5 = youngest leaf wilted; 6 = dead plant), as described previously (Smith and Black, 1987). According to this rating, a genotype with an average disease-severity rating of ≤2 is considered resistant, a genotype with an average rating of >2 to 3 is considered moderately resistant, a genotype with an average rating of >3 to <4 is considered moderately susceptible, and a genotype with a rating of ≥4 is considered susceptible.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES. The entire experiment was conducted three times, once in each of 3 years. The individual experiments were unbalanced within years because not all genotypes were included in all blocks. The experiments also were unbalanced across years because the number of blocks was unequal across experiments, and not all genotypes were inoculated with all of the isolates in each year because of limited plant material. Data were combined over years and analyzed as a multiyear split-plot design treating year as a random effect, inoculum source (i.e., *Colletotrichum* isolate) served as the whole-plot factor and either strawberry genotype or *Fragaria* subspecies (taxa) served as the split-plot factor. The split-plot factor was arranged in a randomized complete block design with four to eight replications (blocks) per experiment, depending upon the variability in plant vigor, with plants of similar vigor together in a block.

For all analyses, data were first transformed by adding 0.5 to the disease-severity rating and then calculating the square root. The data were analyzed as a generalized linear mixed model (GLMM) using the SAS routine PROC MIXED (version 9.1; SAS Institute, Cary, NC) with the default restricted maximum

likelihood (REML) method for parameter estimation. Strawberry genotype or *Fragaria* taxonomic group (taxa) and pathogen isolate were considered fixed effects; year, blocks, and interactions of year with fixed effects were considered random effects. F tests were used to determine if differences between means within the fixed effects (genotype, taxa, isolate) were significant. However, the variance component estimates for each of the random effects were tested (i.e., H₀: χ² = 0) using likelihood ratio χ² tests rather than relying on the Wald z test provided as part of the default output of PROC MIXED due to inadequate df for the test (Littell et al., 1996). To perform the likelihood ratio χ² tests, the difference between -2 times the log likelihood of the full model and the corresponding log

Table 3. Mixed-model analysis of the effects of *Colletotrichum* isolate and strawberry genotype on the severity of anthracnose crown rot in greenhouse screenings of 22 genotypes of strawberry conducted in 2000, 2003, and 2005.^z

Source of variation	Numerator df	Test statistic ^y	P
Fixed effects		F	P > F
Isolate	4	6.28	0.0155
Genotype	21	1.58	0.1180
Isolate × genotype	81	1.78	0.0024
Random effects		χ ²	P > χ ²
Year	4	293.3	<0.0001
Block(year)	1	50.4	<0.0001
Year × isolate	2	68.1	<0.0001
Year × genotype	2	122.3	<0.0001
Year × isolate × genotype	1	15.0	0.0001
Residual		(943.2) ^x	

^zData were combined over years and analyzed as a multiyear split-plot design treating year as a random effect. Sources of variation are grouped according to whether they were considered fixed effects or random effects in the analysis of variance.

^yF tests were performed on fixed effects with numerator df values shown in table and denominator df values of 7.56, 32.9, and 112 for the effects of isolate, genotype, and its interaction, respectively; the denominator df values were calculated using the KENWARDROGER method (Kenward and Roger, 1997). Likelihood ratio χ² tests were performed on random effects with df values shown in table. Column shows the difference between the -2·log likelihoods of the full and reduced models.

^xThe -2·log likelihood of the full model is shown in parentheses.

Table 4. F tests of the interaction of *Fragaria* taxonomic grouping (taxa) or genotype with *Colletotrichum* isolate to determine if differences between taxa or differences between individual genotypes were observed.

Isolate	Taxa			Genotype		
	df	F	P > F	df	F	P > F
GOFF	19.2	0.65	0.6653	79.3	0.43	0.9833
CA-1	27.5	0.31	0.9009	98.1	0.70	0.8151
CG-162	22.1	0.90	0.4955	93.5	1.88	0.0215
CF-63	19.1	4.86	0.0049	79.7	2.90	0.0003
CF-75	27.9	3.91	0.0082	110	2.39	0.0022

^yF tests were performed with numerator df value of 5 for comparisons of isolates within taxa and values of 21, 19, 21, 21, and 20 for GOFF, CA-1, CG-162, CF-63, and CF-75, respectively, for comparisons within genotype; the denominator df values were calculated using the KENWARDROGER method (Kenward and Roger, 1997).

likelihood calculated from the reduced model (the model with the random effect and its interactions removed from the full model) was calculated for each of the random effects in the full model. This difference has a χ^2 distribution with df equal to the difference in the number of covariance parameters between the full and reduced models.

The interaction terms, isolate by genotype and isolate by taxa, were significant in their respective analyses. Therefore, mean comparisons of strawberry genotypes or taxa were performed within isolate groups. This was accomplished using the SLICE option of PROC MIXED (Littell et al., 1996). To determine if different genotypes or taxa responded differently to the same isolate, pairwise differences were calculated from the LSMEANS and the PDIFF options of PROC MIXED and letter designations were assigned to significant groupings using the macro, %MULT (Piepho, 2005). Both the actual means and the estimated means were back-transformed by calculating the square and then subtracting 0.5 for presentation so they would correspond to the rating system used.

Results and Discussion

Eight core collection clones of *F. chiloensis* and 12 of *F. virginiana* from widely separated geographic origins were evaluated with two cultivars of *F. ×ananassa*—one generally resistant and the other partially susceptible—for resistance to anthracnose crown rot (Table 1). This was done by inoculation with two isolates of *C. acutatum* (Goff and CA-1), two of *C. fragariae* (CF-63 and CF-75), and one of *C. gloeosporioides* (CG-162) (Smith and Black, 1990). Although the selected isolates may not represent all the genetic variability present within these three *Colletotrichum* species, they have been used successfully for 20 years to identify resistant strawberry genotypes (Smith and Black, 1987; Smith et al., 1990).

When averaged across all isolates, disease-severity ratings were not significantly different among strawberry genotypes or taxa (Tables 2 and 3) (i.e., the main

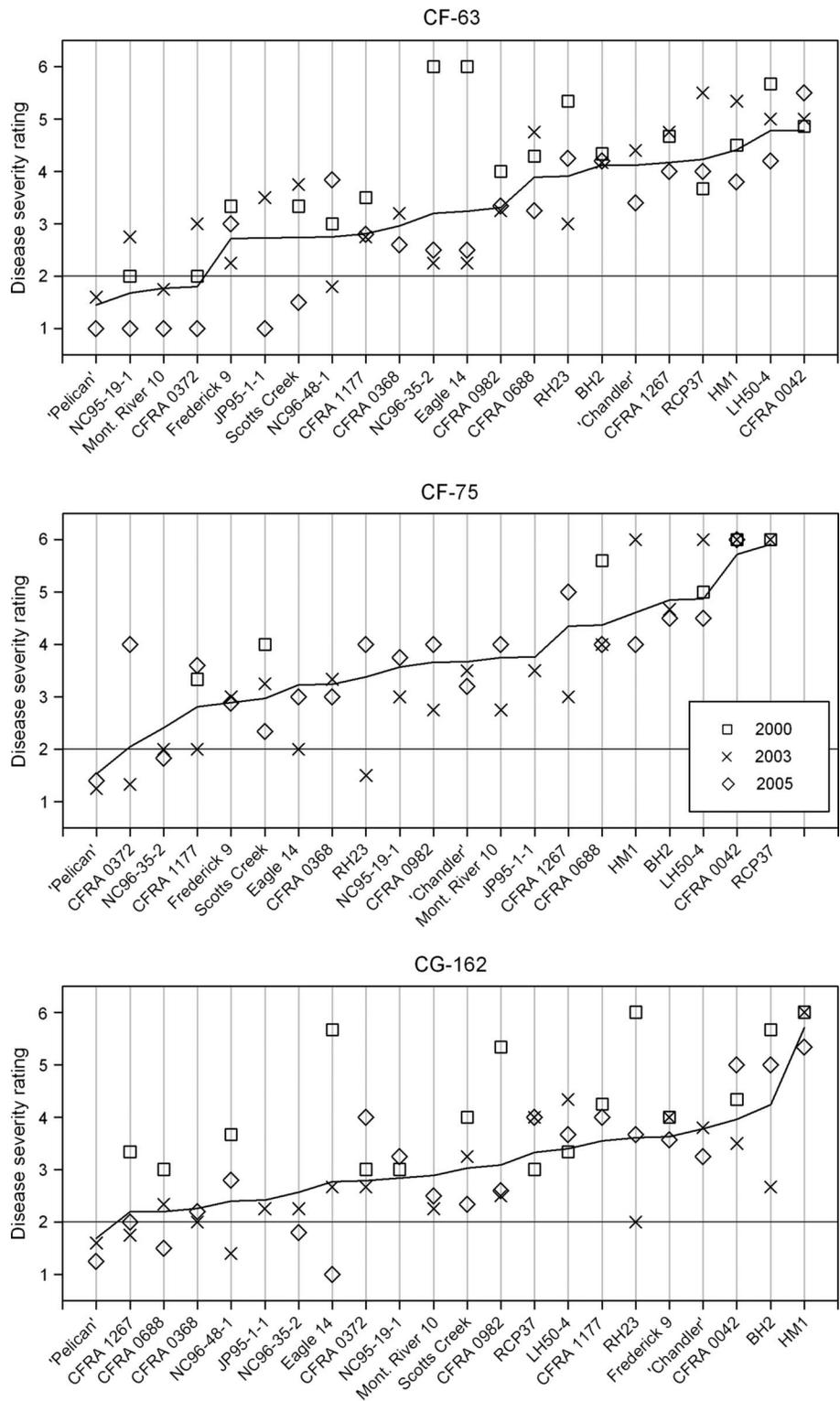


Fig. 1. Mean anthracnose crown rot severity scores for 22 genotypes of strawberry inoculated with one of five isolates of *Colletotrichum* in greenhouse screenings conducted in 2000, 2003, and 2005. The *Colletotrichum* isolates are from three species: *C. acutatum* (Goff and CA-1), *C. fragariae* (CF-63 and CF-75), and *C. gloeosporioides* (CG-162). Disease severity was rated for individual plants of genotypes on a standardized scale of 0 to 6 (0 = healthy plant with no visible symptoms; 1 = petiole lesions <3 mm long; 2 = petiole lesions 3–10 mm long; 3 = petiole lesions >10 to <20 mm long; 4 = petiole lesions >20 mm long; 5 = youngest leaf wilted; 6 = dead plant). Individual disease-severity ratings were averaged for each genotype within year. The solid line represents the least-square means for each genotype combined across years. A genotype with an average disease-severity rating ≤ 2 in any of the years tested was considered resistant, a genotype with an average rating ≤ 3 was considered moderately resistant, a genotype with an average rating ≤ 4 was considered moderately susceptible, and a genotype with an average rating >4 for any year was considered susceptible.

effects of genotypes and taxa were not significant). However, significant differences among strawberry genotypes or taxa could be detected within isolate groupings (Table 4; Fig. 1). When plants were inoculated with either *C. acutatum* isolate, no significant differences in disease-severity ratings were detected among strawberry genotypes or taxa. However, there were significant differences in disease-severity ratings among either strawberry genotypes or taxa when plants were inoculated with either of the two *C. fragariae* isolates (Table 4; Fig. 1). There also were significant effects among genotypes but not among strawberry taxa when plants were inoculated with *C. gloeosporioides* (Fig. 1; Table 4). The random effect of year and its interaction with strawberry genotypes or taxa was significant in both analyses. A significant year effect is not unusual given the differences that could be expected in planting material and environment from one year to the next (Gomez and Gomez, 1984).

No individual strawberry taxonomic group (taxa) stood out as being more likely to contain resistant genotypes across pathogen isolates. The analysis showed no significant differences between taxa when inoculated with *C. acutatum* or *C. gloeosporioides* isolates. Only when inoculated with the two *C. fragariae* isolates were significant differences among taxa detected (Table 4). The mean level of crown rot for each taxon when inoculated with these two isolates is presented in Table 5. Although *F. chiloensis* f. *chiloensis* ssp. *chiloensis* had the lowest level of crown rot when inoculated with the two isolates, this taxon was represented by only one genotype (PI 551736, CFRA 0372), and the level of infection was not significantly different from those of at least two other taxa. In addition, the level of crown rot on the geographically separated (northern and southern) clones (geomorphs) of *F. virginiana* ssp. *virginiana* was not statistically different (Table 5).

The susceptible control cultivar Chandler was not resistant to any of the isolates used, though it was rated moderately

resistant to *C. acutatum* isolate Goff. In fact, most genotypes were rated resistant or moderately resistant to the *C. acutatum* isolates, but there were no significant differences among genotypes when inoculated with either *C. acutatum* isolate (Table 4). This is not terribly surprising because *C. acutatum* is noted more for causing fruit rot than crown rot (Maas, 1998). The water-inoculated control plants all received a rating of resistant (data not shown).

The significant genotype by year interactions that were observed when the genotypes were inoculated with the two *C. fragariae* isolates and the *C. gloeosporioides* isolate (Fig. 1; Table 4) suggested that a genotype should be considered resistant to an isolate only if the mean disease-severity rating indicated it was resistant (≤ 2) each year tested. Likewise, a genotype should be considered moderately resistant to an isolate only if the mean disease-severity rating indicated it was either resistant (≤ 2) or moderately resistant (> 2 to 3) each year tested. ‘Pelican’ and Montreal River 10 were rated resistant to CF-63, while NC95-19-1 and CFRA 0372 were rated moderately resistant to CF-63 (Fig. 1). ‘Pelican’ and NC96-35-2 were rated resistant to CF-75, while Frederick 9 and Eagle 14 were rated moderately resistant to CF-75. ‘Pelican’ was rated resistant to CG-162 while CFRA 0688, CFRA 0368, JP95-1-1, NC96-35-2, and Montreal River 10 were rated moderately resistant, but JP95-1-1 was tested only 1 year. The variability of responses to this isolate was greater than to the two *C. fragariae* isolates. Although ‘Pelican’ was the only genotype that was rated resistant to all of the isolates, the other genotypes that were rated resistant or moderately resistant should still provide the basis for development of resistant cultivars. Resistance to multiple isolates is desirable to help enlarge the geographic area in which a new cultivar may be grown, but resistance to all available isolates of all *Colletotrichum* species may not be necessary for every cultivar development program. It would be desirable for new cultivars to be resistant to multiple *C. acutatum* and *C. gloeosporioides* isolates, as these species have wide host ranges and are distributed throughout the world, but *C. fragariae* is rarely found outside the southeastern United States, and its host range is limited to strawberry and a few weed hosts, so cultivars developed for regions outside the southeastern United States may not need to be resistant to any *C. fragariae* isolates.

New searches for additional genotypes resistant to different *Colletotrichum* isolates should not be limited to any particular *Fragaria* subspecies or geomorph. Individual genotypes from any of the subspecies or geomorphs can be potential sources of parental material for development of resistant cultivars. This is indicated by the significant interaction effects for *Colletotrichum* isolates and either strawberry genotypes or taxa (Table 4). It is more important to identify genotypes that will be most advantageous in a regional cultivar development program and then to evaluate them in order to identify those which also are resistant.

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Table 5. Least-square means of the crown rot severity ratings for *Fragaria* taxa inoculated with *Colletotrichum fragariae* isolate CF-63 or CF-75.^z

<i>Fragaria</i> taxa	<i>C. fragariae</i> isolate	
	CF-63	CF-75
<i>F. virginiana</i> ssp. <i>glauca</i>	4.05 ^a x	4.40 a
<i>F. chiloensis</i> ssp. <i>pacifica</i>	3.81 ab	4.36 a
<i>F. virginiana</i> ssp. <i>virginiana</i> (northern)	2.99 abc	3.29 ab
<i>F. xananassa</i>	2.64 bd	2.54 b
<i>F. virginiana</i> ssp. <i>virginiana</i> (southern)	2.56 cd	2.95 b
<i>F. chiloensis</i> f. <i>chiloensis</i> ssp. <i>chiloensis</i>	1.78 d	1.99 b

^aIndividual disease-severity ratings were averaged for each genotype, and the means presented are the back-transformed least-square means for each strawberry species.

^bDisease severity was rated for individual plants of genotypes on a standardized scale of 0 to 6 (0 = healthy plant with no visible symptoms; 1 = petiole lesions <3 mm long; 2 = petiole lesions 3–10 mm long; 3 = petiole lesions >10 to <20 mm long; 4 = petiole lesions >20 mm long; 5 = youngest leaf wilted; 6 = dead plant. A genotype with an average disease-severity rating ≤ 2 in any of the years tested was considered resistant, a genotype with an average rating ≤ 3 was considered moderately resistant, a genotype with an average rating ≤ 4 was considered moderately susceptible, and a genotype with an average rating > 4 for any year was considered susceptible.

^cMeans followed by the same letter are not significantly different from each other according to the PDIF option of SAS PROC MIXED ($P < 0.05$).

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