Selection for Pathogenicity to Strawberry in Populations of *Colletotrichum gloeosporioides* from Native Plants

S. J. MacKenzie, T. E. Seijo, D. E. Legard, L. W. Timmer, and N. A. Peres

First, second, third, and fifth authors: University of Florida, Gulf Coast Research and Education Center, Wimauma 33598, fourth author: University of Florida, Citrus Research and Education Center, Lake Alfred 33850.
Current address of D. E. Legard: California Strawberry Commission, Watsonville, CA 95077.
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ABSTRACT

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Colletotrichum gloeosporioides causes a serious crown rot of strawberry and some isolates from native plants are pathogenic to strawberry. *C. gloeosporioides* from lesions on wild grape and oak were sampled at two sites adjacent to commercial strawberry fields in Florida and two distant sites. Random amplified polymorphic DNA (RAPD) marker data and restriction enzyme digests of amplified rDNA were used to determine whether isolates were from the same *C. gloeosporioides* subgroup that infects strawberry. There were 17 to 24 native host isolates from each site that clustered with a group of strawberry crown isolates based on RAPD markers. Among strawberry isolates, there were two rDNA genotypes identified by restriction enzyme analysis. Both genotypes were present among native host isolates sampled from all four sites. There was some evidence that the different rDNA genotypes differentiated two closely related subpopulations, although the proportion of pathogenic isolates from native hosts among the two different genotypes was not different. The incidence of isolates pathogenic to strawberry was greater at sites close to strawberry fields relative to sites distant from strawberry fields for isolates with a *Bst*UI(–)/*Msp*I(+) rDNA genotype (44 versus 13%), a *Bst*UI(+)/*Msp*I(–) genotype (57 versus 16%), or when both genotypes were analyzed together (46 versus 15%). Based on these results, it appears that the *C. gloeosporioides* subgroup that causes crown rot on strawberry is widely distributed in Florida and that selection for pathogenicity on strawberry occurs in the area where this host is grown in abundance.

Additional keywords: evolution, *Glomerella cingulata*.

Three species of *Colletotrichum* produce crown rot symptoms on strawberry (*Fragaria* × *ananassa* Duch.): *C. gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk), *C. fragariae* A. N. Brooks, and *C. acutatum* J. H. Simmonds (7,13). Colletotrichum crown rot is a serious problem in the southeastern United States, because this region is relatively warm all year and has sufficient rainfall to spread the disease (13). All three *Colletotrichum* spp. that produce crown rot symptoms are present in Florida (13). However, in the production system currently used, Colletotrichum crown rot is caused primarily by *C. gloeosporioides* (30).

The taxonomic relationships among *Colletotrichum* spp. on strawberry have been investigated extensively (3,8–10,18,25,26). *C. gloeosporioides* and *C. fragariae* are more closely related to one another than either is to *C. acutatum* (26). Although they are closely related, *C. gloeosporioides* and *C. fragariae* can be distinguished using morphological criteria such as conidial shape and the ability of setae to function as phialides in the production of conidia (10). Sequence data from the nuclear ribosomal internal transcribed spacer (ITS) and mitochondrial DNA restriction fragment length polymorphisms indicate that there are two distinct populations on strawberry that are considered to be *C. gloeosporioides* (8,9,26). Isolates from one of these populations are homothallic and form the teleomorph, *Glomerella cingulata*, when grown singly in culture (10). A second population is not homothallic. Some isolates from this population produce fertile perithecia when paired with an isolate of the opposite mating type, indicating that isolates from this population are heterothallic (10,17), although the term nonhomothallic will be used to describe this population in the present study. A cross reference of isolates from previous research suggests that isolates described as having a Cgl-1 genotype are homothallic and those described as having a Cgl-2 genotype are nonhomothallic (8–10). Both genotypes have been found in Florida (8,9). The occurrence of homothallic isolates on strawberry fruit was reported in 1984 (12). Nonhomothallic isolates from strawberry were not described until later (10). These isolates previously were identified as *C. fragariae*, suggesting that the contribution of *C. gloeosporioides* to earlier crown rot epidemics in Florida may have escaped notice due to misidentification of the pathogen. At the present time, the vast majority of *Colletotrichum* spp. isolated from diseased strawberry crowns in Florida are from a nonhomothallic *C. gloeosporioides* population (35). This population is genetically diverse and recombination appears to occur at a relatively high frequency (30).

C. gloeosporioides has been isolated from foliar lesions of a broad range of introduced and native noncultivated plant species growing adjacent to strawberry fields in Florida (35). Wild strawberry species are not represented among these noncultivated species because the two wild strawberry species native to the eastern United States, *Fragaria vesca* and *F. virginiana*, are not found on the peninsula of Florida, where a subtropical climatic allows commercial winter strawberry production (5). *F. virginiana*, along

Corresponding author: S. J. MacKenzie; E-mail address: sjmac@ufl.edu

*The *e*-**X**tra logo stands for "electronic extra" and indicates that the online version contains supplemental material not included in the print edition. The online version contains two supplemental tables showing the frequencies of randomly amplified polymorphic DNA bands at each sampling site for *Colletotrichum gloeosporioides* subgroups, *C. fragariae*, and *C. gloeosporioides* isolates that clustered with crown isolates and possessed either the *Bst*UI(–)/*Msp*I(+) or *Bst*UI(+)/*Msp*I(–) rDNA genotype.

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with *F. chiloensis*, is one of the original progenitor species that gave rise to cultivated strawberry (5). Susceptibility to Colletotrichum crown rot is variable among individuals from each of these species (N. A. Peres, *personal communication*), although it is not clear whether *C. gloeosporioides* is a pathogen on either host in the wild. *C. gloeosporioides* also is found on strawberry petioles, where it forms quiescent infections (20). Isolates from noncultivated hosts and from strawberry petioles vary in their ability to cause crown rot, with some being aggressive pathogens on strawberry and others lacking the ability to produce crown rot symptoms (20,35). Genetic data also indicate that the majority of the pathogenic and nonpathogenic *C. gloeosporioides* isolates from noncultivated hosts are from the same population as the nonhomothallic *C. gloeosporioides* isolates from diseased strawberry crowns (35). Because *C. gloeosporioides* isolates from noncultivated hosts adjacent to strawberry fields are pathogenic to strawberry and are from the same population as those from infected strawberry plants, noncultivated hosts may provide inoculum for crown rot epidemics.

In Florida, commercial strawberry production is highly localized, with >90% of the area dedicated to strawberry production located in Hillsborough County (1). Although it appears that the *C. gloeosporioides* population on noncultivated hosts found close to strawberry fields and that on strawberry in this area is the same, there is no information presently available concerning the population of *C. gloeosporioides* on noncultivated hosts distant from strawberry production fields on the Florida peninsula, where commercial strawberry production and wild strawberry populations are absent. A genetic analysis of these populations could provide insight as to whether or not the *C. gloeosporioides* isolates obtained from noncultivated hosts close to strawberry fields are recent migrants from strawberry or if they represent an indigenous population of *C. gloeosporioides*. In addition, the *C. gloeosporioides*–strawberry pathosystem provides a system for examination of local selection for pathogenicity. If the *C. gloeosporioides* population on strawberry is derived from a widely dispersed population with a wide host range, the cultivation of strawberry in a particular area may influence the frequency of the pathogenic phenotype in the *C. gloeosporioides* population on noncultivated hosts. Studies examining mean host resistance and mean pathogen virulence have shown a positive correlation between these two traits in natural and agricultural pathosystems (19,29), suggesting directional selection in the pathogen population for increased infectivity. By analogy, the frequency of genes controlling pathogenicity on strawberry may increase in the broader *C. gloeosporioides* population at sites where strawberry is grown intensively, given that the determinants for pathogenicity on strawberry already occur in the population. If the genetic determinants for pathogenicity on strawberry recently evolved in response to the introduction of this species, pathogenic isolates may be completely absent in areas where strawberry is not cultivated.

In this study, we tested the hypothesis that the pathogenicity phenotype to strawberry is selected for in the broader *C. gloeosporioides* population on noncultivated hosts in areas where strawberry plants are grown intensively. To test the hypothesis, we sampled *C. gloeosporioides* from two native hosts, grape and oak, at two locations immediately adjacent to strawberry fields and two sites distant from commercial strawberry production. All isolates first were characterized genetically to confirm that isolates were from a population with the ability to cause crown rot, and then the incidence of isolates pathogenic to strawberry from this population at sites close to and distant from commercial strawberry production were compared.

MATERIALS AND METHODS

Sampling strategy and isolates. In Florida, strawberry plants are grown as an annual crop on plastic-mulched, raised beds. Plants are set in late September and harvested from November to late March. At the end of the harvest season, plants are chemically destroyed and the field tilled or an alternate crop planted on the mulched beds. Sampling of native host isolates was done in late August and early September 2002, 4 to 5 months after strawberry plants had been removed from fields, to avoid sampling migrants from strawberry fields that may have only transiently established themselves on native hosts. At least 20 isolates identified as *C. gloeosporioides* based on morphology (10) were obtained from necrotic lesions on oak (*Quercus* spp.) or wild grape (*Vitis* spp.) leaves at each of four sites (Fig. 1). Samples were taken from leaves of different trees and vines at each site to reduce the possibility of sampling clones. Two sites were adjacent to commercial strawberry fields in Dover, FL and two sites were distant from strawberry production fields. The two sites adjacent to commercial strawberry fields were 3.5 km apart within the strawberry production region in Hillsborough County. The native hosts sampled at these sites were 9 to 50 m from the edges of fields. The two distant sites were the University of Florida, Citrus Research and Education Center in Lake Alfred (Polk County) and a residential area in Sarasota (Sarasota County). Both sites contained natural stands of vegetation along edges of roads or groves and in preserved areas. The total area dedicated to commercial strawberry production in Polk and Sarasota Counties is <2% of that in Hillsborough County (2002 Census of Agriculture; United States Department of Agriculture, National Agricultural Statistics Service). The nearest commercial strawberry farm was ≈28 km from the Lake Alfred site and 15 km from the Sarasota site. No populations of wild strawberry are known to occur in any of the three counties sampled. Native host isolates from the two sites in Dover were designated D1-oak, D1-grape, D2-oak, and D2-grape followed by a number to identify specific isolates. *C. gloeosporioides* isolates were sampled from noncultivated hosts at the site designated D2 in 1996 and 1997 in a previously published study, and the site was designated NC1 in that study (35). None of the isolates from native plants from this previous sample were used in the current study. Isolates from Lake Alfred were designated LA-grape and LA-oak, and those from Sarasota SS-grape and SS-oak. Also included in the analysis were 20 *C. gloeosporioides* isolates from diseased strawberry crowns, 7 *C. gloeosporioides* isolates from citrus, and 1 *C. fragariae* isolate. *C. gloeosporioides* isolates from strawberry came from samples submitted by local growers to the diagnostic laboratory at the University of Florida, Gulf Coast Research and Education Center (GCREC) in Dover from 1995 to 2000 and were coded Straw-

Fig. 1. Map showing the three locations where *Colletotrichum gloeosporioides* isolates were sampled from lesions on oak and grape leaves. There were two separate sampling sites in Dover. Hillsborough County, the main strawberry-growing county in Florida, is shaded.

berry followed by a number to identify individual isolates. The strawberry isolates came from 14 different sites; none was homothallic on potato dextrose agar (PDA) media and, for a subset of isolates, AT-rich DNA banding patterns indicated that they were Cgl-2-type isolates (8). The single *C. fragariae* isolate was collected in 1988 and also came from the culture collection at the GCREC. Citrus isolates came from sweet orange (*Citrus sinensis*) or tangelo (*C. paradisi* × *C. reticulata*) fruit, twigs, or leaves from plantings near Lake Alfred, Avon Park, Vero Beach, or Frostproof, FL. These isolates were coded 'Citrus' followed by the isolate number. Some of the isolates from strawberry crowns and from citrus had been characterized previously and the isolate designations used in past studies are provided (Table 1). In the present study, these isolates were used to genetically characterize isolates from oak and grape hosts and were not evaluated for pathogenicity on strawberry. Isolates from diseased strawberry crowns, with few exceptions, are pathogenic to strawberry in greenhouse tests (30). *Colletotrichum gloeosporioides* isolates from citrus have been shown to be distinct from those on strawberry and there have been no examples of isolates from this population causing crown rot on strawberry (30,35).

Fungal isolation. Isolates from strawberry were obtained by placing tissue from necrotic crowns of wilted strawberry plants directly onto CIM media (12 g of potato dextrose broth, 17 g of

TABLE 1. Isolates of *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*) and *C. fragariae*

Isolate	RAPD group[a]	Location[b]	Host (tissue)	Alternate designations	References	Isolates field tested[c]
Native host isolates[d]						
SS-oak-(1 to 12)	*C.g.*-Sc	Sarasota, FL	Oak (leaf)	…	…	SS-oak-(1,2), SS-oak-(5,10)*
SS-grape-(1 to 11)	*C.g.*-Sc	Sarasota, FL	Grape (leaf)	…	…	SS-grape-2, SS-grape-7*
SS-grape-(12 and 13)	*G.c.*-Nh	Sarasota, FL	Grape (leaf)	…	…	…
LA-oak-(1 to 11)	*C.g.*-Sc	Lake Alfred, FL	Oak (leaf)	…	…	LA-oak-1, LA-oak-(2,11)*
LA-oak-12	*C.g.*-Cit	Lake Alfred, FL	Oak (leaf)	…	…	…
LA-oak-13	*C.f.*	Lake Alfred, FL	Oak (leaf)	02-135	16	…
LA-grape-(1 to 7)	*C.g.*-Sc	Lake Alfred, FL	Grape (leaf)	…	…	LA-grape-(1,3), LA-grape-2*
LA-grape-(8 and 9)	*C.g.*-Cit	Lake Alfred, FL	Grape (leaf)	…	…	…
D1-oak-(1 to 8)	*C.g.*-Sc	Dover, FL (site 1)	Oak (leaf)	…	…	D1-oak-1, D1-oak-5*
D1-oak-9	*G.c.*-Nh	Dover, FL (site 1)	Oak (leaf)	…	…	…
D1-grape-(1 to 9)	*C.g.*-Sc	Dover, FL (site 1)	Grape (leaf)	…	…	D1-grape-(1,2), D1-grape-(3,7)*
D2-oak-(1 to 13)	*C.g.*-Sc	Dover, FL (site 2)	Oak (leaf)	…	…	D2-oak-1, D2-oak-(6,9)*
D2-grape-(1 to 11)	*C.g.*-Sc	Dover, FL (site 2)	Grape (leaf)	…	…	D2-grape-1*, D2-grape-(2,3)
Cultivated host isolates[e]						
Strawberry-1	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	96-83R, S1[f]	16,35	…
Strawberry-2	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S2/95[f]	35	…
Strawberry-3	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	95-63A, S2/95[f]	16,35	…
Strawberry-4	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S2/97[f]	35	…
Strawberry-5	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S4[f]	35	…
Strawberry-6	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S5[f]	35	…
Strawberry-7	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	98-285, S6[f]	16,35	…
Strawberry-8	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S7[f]	35	…
Strawberry-9	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S9[f]	35	…
Strawberry-10	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S10[f]	35	…
Strawberry-11	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S11[f]	35	…
Strawberry-12	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	97-63, S11[f]	16,35	Yes
Strawberry-13	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S12[f]	35	…
Strawberry-14	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S14[f]	35	…
Strawberry-15	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S15[f]	35	…
Strawberry-16	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	S15[f]	35	…
Strawberry-(17 to 20)	*C.g.*-Sc	Dov/PC, FL	Strawberry (crown)	…	…	…
97-15a[g]	…	Dov/PC, FL	Strawberry (crown)	S2/97[f]	16,35	Yes
97-45a[g]	…	Dov/PC, FL	Strawberry (crown)	S14	16,35	Yes
00-117[g]	…	Dov/PC, FL	Strawberry (crown)	…	16	Yes
231[h]	…	USA, Florida	Strawberry (crown)	…	8,9,10,26	…
291[h]	…	USA, Florida	Strawberry (crown)	IMI345047	3,9,10,18,26	…
C. fragariae	*C.f.*	Dov/PC, FL	Strawberry (crown)	326-1	9,10	…
Citrus-1	*C.g.*-Cit	Lake Alfred, FL	Sweet orange (fruit)	LA-5	2	…
Citrus-2	*C.g.*-Cit	Lake Alfred, FL	Sweet orange (fruit)	…	…	…
Citrus-3	*C.g.*-Sc	Lake Alfred, FL	Sweet orange (twig)	…	…	…
Citrus-4	*C.g.*-Cit	Lake Alfred, FL	Minneola tangelo (leaf)	Min-ER3-M1A	23	…
Citrus-5	*C.g.*-Cit	Avon Park, FL	Sweet orange (fruit)	Dil-Ark-1	23	…
Citrus-6	*C.g.*-Cit	Vero Beach, FL	Sweet orange (fruit)	…	…	…
Citrus-7	*C.g.*-Cit	Frostproof, FL	Sweet orange (leaf)	SRL-FTP-9(L)	23	…

[a] Random amplified polymorphic DNA (RAPD) group refers to genetic groups identified in Figure 2. Abbreviations for *C. gloeosporioides* RAPD groups: *C. gloeosporioides*-strawberry crown = *C.g.*-Sc, *C. gloeosporioides*-citrus = *C.g.*-Cit, and *G. cingulata*-native host = *G.c.*-Nh. The abbreviation for the *C. fragariae* group is *C.f.*

[b] Dov/PC, FL = Dover/Plant City, FL.

[c] Isolates used in field study to validate greenhouse pathogenicity classifications. Isolates with asterisks were determined to be pathogenic using the greenhouse crown injection assay. Isolates were from SS = Sarasota, LA = Lake Alfred, and D1 and D2 = Dover sites 1 and 2, respectively.

[d] Only native host isolates belonging to the group that includes *C. gloeosporioides* from strawberry crown (*C.g.*-Sc) with an internal transcribed spacer (ITS) genotype matching isolates from the crown were used to examine selection for pathogenicity on strawberry.

[e] The pathogenicity of cultivated host isolates to strawberry was not determined in greenhouse tests in the current study.

[f] Isolate from strawberry *C. gloeosporioides* collection described by Xiao et al. (35). Designation refers to the sampling site.

[g] *C. gloeosporioides* isolates used as standards in field inoculation studies but not included in the phenogram presented in Figure 2.

[h] ITS sequence data from the European Molecular Biology Laboratory accession AJ536223 for nonhomothallic *C. gloeosporioides* isolate 291 and accessions Z32948 and Z32949 for homothallic *G. cingulata* isolate 231 were compared to ITS sequence data from fungi sampled in the current study. Isolate 291 is described as *C. fragariae* by Martinez Culebras et al. (18) in accession AJ536223, but is actually a Cgl-2-type *C. gloeosporioides* isolate (9).

agar, 100 mg of streptomycin, 250 mg of ampicillin, and 8 mg of iprodione per liter plus 0.02% tergitol). For isolations from native hosts, portions of oak and grape leaves with one or more circular necrotic lesions were surface sterilized with 0.525% sodium hypochlorite for 1 min, rinsed in sterile water, and placed on CIM media. Cultures were incubated under fluorescent light for 3 to 5 days and single-spore isolates produced from growing colonies. Cultures were stored at –85°C in 20% glycerol.

DNA extraction, DNA amplifications using a *C. gloeosporioides*/*C. fragariae*-specific primer and random amplification of polymorphic DNA. Mycelium was harvested from cultures grown in 50 ml of Emerson media (4 g of yeast extract, 15 g of soluble starch, 1 g of K_2HPO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter) and dried overnight in a centrifugal evaporator. DNA was isolated from 60 mg of the dried mycelia using a previously reported cetyltrimethylammonium bromide (CTAB) extraction procedure (35). The primer Cg/f Int1 (5′-GACCCTCCCGGCCTCCCGCC-3′), which amplifies from the ITS1 region of isolates from the *C. gloeosporioides* species aggregate and from *C. fragariae,* and the conserved universal primer ITS4 encoded within the large subunit rRNA gene (5′-TCCTCCGCTTATTGATATGC-3′), were used to confirm initial species designations based on morphology (30,34). Amplifications from 5 ng of template DNA were carried out under mineral oil in 20 µl containing 1× reaction buffer (10 mM Tris [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin), 200 µM dNTP, 1 unit of *Taq* polymerase, and 10 pmol of each primer/reaction. Cycling parameters consisted of a 5-min denaturing step at 94°C followed by 26 cycles at 94°C for 1 min, 60°C for 2 min, and 72°C for 2 min. Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) using four primers $(ACTG)_4$, $(GACA)_4$, $(TCC)_5$, and 5′-GTGAGGCGTC-3′ (OPC-2) (Operon Technologies, Alameda, CA) was used to differentiate *C. fragariae* from *C. gloeosporioides* and to identify populations within isolates identified as *C. gloeosporioides*. Reactions were carried out using 5 ng of template DNA under mineral oil in 20 µl containing 1× reaction buffer (50 mM Tris [pH 8.3], bovine serum albumin at 0.25 mg/ml, 2 mM $MgCl_2$, 0.5% Ficoll, and 1 mM Tartrazine) (Idaho Technology), 200 µM dNTP, 1 unit of *Taq* polymerase, and 20 pmol primer/reaction (primers $(ACTG)_4$, $(GACA)_4$, and $(TCC)_5$) or 8 pmol OPC-2/reaction. Cycling parameters consisted of a 5-min denaturing step at 95°C and 30 cycles of 1 min at 95°C, 1 min at 48°C, and 2 min at 72°C for primers $(ACTG)_4$ and $(GACA)_4$; 34 cycles of 1 min at 95°C, 1 min at 46°C, and 1.5 min at 72°C for primer $(TCC)_5$; or 38 cycles of 1 min at 95°C, 1 min at 35°C, and 2 min at 72°C for primer OPC-2. Amplified products were separated in 2% agarose gels made with 1× Tris-acetate EDTA. Samples were randomized before processing and two separate reactions performed for each primer–template combination. Bands were scored manually. Bands at distances in which weak or inconsistently amplified products were observed were excluded from the analyses.

Ribosomal DNA sequencing and restriction fragment length polymorphism analysis. DNA from ITS region 1, the 5.8S ribosomal RNA gene, and ITS region 2 was amplified using upstream primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and downstream primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (34). These primers anneal to conserved areas within the gene encoding the small subunit rRNA and large subunit rRNA, respectively. Template DNA (5 ng) was used for amplifications to produce products for sequencing. The amplifications were carried out in a 120-µl volume containing 1× reaction buffer (10 mM Tris [pH 9], 50 mM KCl, and 2 mM $MgCl_2$), 200 µM dNTP, 5 units of *Taq* polymerase, and 60 pmol of each primer/reaction. Cycling parameters consisted of a 4-min denaturing step at 94°C followed by 34 cycles at 94°C for 45 s, 52°C for 45 s, and 72°C for 1 min. Amplifications for restriction enzyme analysis were carried out using the same conditions except that only 2 ng of template DNA was used and the reactions were scaled down to a 35-µl volume. Prior to sequencing, DNA was concentrated using a Microcon YM-30 centrifugal filter (Millipore, Billerica, MA) to 20 ng/µl and 15-µl samples submitted to the University of Florida sequencing core laboratory in Gainesville. Sequence data were generated from fluorescent cycle sequencing reactions using an automated sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA). Sequence data was generated in forward and reverse directions and alignments were done using CLUSTAL W (28). Isolates chosen for ribosomal DNA sequencing were from each of the statistically validated clusters observed in a phylogram constructed using RAPD marker data. The isolates and GenBank accession numbers of deposited sequences are Strawberry-6, -11, -18, and -19 (EF177471, DQ868489, DQ868490, and DQ868491); D2-oak-6, -8, and -12 (DQ868492, DQ868494, and EF177472); D1-grape-6 (DQ868493); SS-grape-10, -12, and -13 (EF177474, DQ868495, and DQ868496); SS-oak-2 and -9 (EF177471 and EF177475); LA-grape-6 and -8 (EF177473 and DQ868500); LA-oak-6 and -13 (EF177476 and DQ868497); *C. fragariae* (DQ868498); and Citrus-7 (DQ868499). Sequences for previously characterized nonhomothallic *C. gloeosporioides* isolate 291 (AJ536223) (3,9,10,18,26) and homothallic *G. cingulata* isolate 231 (ITS1-Z32949 and ITS2-Z32948) (8–10,26,27) were obtained from the European Molecular Biology Laboratory (EMBL). Isolate 291, although reclassified as a *C. gloeosporioides* isolate in 1992 (10), also is described as *C. fragariae* in some publications (18,26) and is described as *C. fragariae* under the accession AJ536223. Eight isolates for which sequence data was generated also were deposited in the American Type Culture Collection (ATCC). The deposited isolates and ATCC accession numbers are Strawberry-6 and -18 (MYA-4130 and MYA-4131), D2-oak-6 and -12 (MYA-4132 and MYA-4133), LA-grape-8 (MYA-4135), LA-oak-13 (MYA-4137), SS-grape-12 (MYA-4136), and Citrus-7 (MYA-4134).

Sequence data revealed three distinct rDNA haplotypes among *C. gloeosporioides* isolates from the current study. One of these genotypes is described as either *C. gloeosporioides*-strawberry crown 1 or *C. cingulata*-native host. The others are *C. gloeosporioides*-strawberry crown 2 and *C. gloeosporioides*-citrus. These three haplotypes can be distinguished from one another using the restriction enzymes *Bst*UI and *Msp*I, which were used to screen PCR amplification products for the entire *C. gloeosporioides* population. Enzyme *Bst*UI identifies a polymorphism at site 69 within ITS region 1 by the occurrence of a 574- or 478-bp band following electrophoresis through a 2% agarose gel. *Msp*I identifies a site at position 73 within the ITS region 2 by the occurrence of a 336- or 292-bp band following electrophoresis through a 3% agarose gel.

Pathogenicity tests. A categorical assay was chosen to evaluate isolates in the present study because the vast majority of isolates from native plants are not pathogens on strawberry and, therefore, incremental differences in pathogenicity are not discernable. To assess the pathogenicity of isolates, conidia from cultures grown on PDA were adjusted to 10^6 conidia/ml and injected (≈0.1 ml) directly into the crown of three 'Camarosa' strawberry plants in a greenhouse. Using this technique, crowns of plants inoculated with isolates pathogenic to strawberry wilt when crown tissue becomes partially or completely necrotic, whereas there is no necrosis of crown tissue in plants inoculated with nonpathogenic isolates and they do not collapse. All native host isolates were evaluated for pathogenicity during the summer of 2003 by counting the number of collapsed plants after 4 weeks of incubation in a greenhouse. These data were used for statistical analysis. Under the warm environmental conditions (24 to 31°C), pathogenicity could be determined unequivocally because no isolate caused collapse of an intermediate number of plants. Isolates were categorized as pathogenic on strawberry if they caused collapse of all three plants and nonpathogenic if they failed to affect any of the plants. The pathogenicity of isolates also was evaluated in the

winter of 2002, in a second assay during the summer of 2003, or in the fall of 2004 to assess the repeatability of the assay conducted in 2003 when all isolates were evaluated simultaneously. The assay was highly repeatable even under variable environmental conditions, because only 1 of 89 isolates was classified differently (S. J. MacKenzie, *unpublished data*).

To validate that isolates identified as pathogens using the crown injection technique were also pathogenic to strawberry in the field, 1 ml of a 10^6/ml conidial suspension was applied topically to the crown of each plant in 10-plant plots of cv. Camarosa using a plastic pipette. In all, 12 plots were inoculated with oak or grape isolates determined to be pathogenic in greenhouse tests and 12 plots were treated with isolates determined to be nonpathogenic in greenhouse tests. As a positive control, four plots were inoculated with isolates from diseased strawberry crowns and four plots were inoculated with distilled water as a negative control. Isolates used for this experiment are listed in Table 1. Transplants were set on 24 October 2003 on plastic mulch-covered beds and inoculations conducted on 12 March 2004. Captan 80 WP (4.2 kg/ha) was applied to plants weekly, with applications suspended 2 weeks before plants were inoculated and resumed 2 weeks after inoculations to prevent natural infections. The proportion of plants collapsed was recorded for each plot 45 days after inoculation.

Statistical analyses. Cluster analysis was performed on Dice similarity coefficients using the unweighted pair group method with arithmetic averages (UPGMA) with NTSYS (PC version 2.0; Exeter Software, Setauket, NY). All scored bands were used for this analysis. Statistical support for phenogram branches was based on 1,000 replications using the Winboot bootstrap algorithm (21). Values of θ were estimated using the method of Weir and Cockerham (33), with confidence intervals generated by bootstrapping over loci using TFPGA (Utah State University, Logan). Statistical support for population subdivision was observed if the lower boundary of the 90% confidence interval generated by 10,000 bootstrap replications exceeded zero. The value θ measures the correlation of alleles of different individuals in the same population relative to all populations and is described by the equation $(Q - q)/(1 - q)$, where Q is the probability that two randomly sampled genes within a population are the same allele and q is the probability that genes randomly selected from different populations are the same allele (4). Bands absent or fixed in populations being compared were excluded from this analysis. Two-tailed Fisher's exact tests evaluating isolate frequencies in cells of two-by-two contingency tables were used to make pairwise comparisons of rDNA restriction fragment length polymorphism (RFLP) genotype frequencies between sites and between grape and oak hosts. The incidence of isolates pathogenic or nonpathogenic to strawberry at different sites, on different hosts, or among different rDNA RFLP genotypes also was evaluated using a two-tailed Fisher's exact test after organizing data into two-by-two contingency tables. The nonparametric Fisher's exact test was used for statistical comparisons due to the occurrence of relatively few isolates in cells of many of the contingency tables examined. Data from isolates that did not cluster with strawberry crown isolates using RAPD data or did not have one of the two rDNA RFLP genotypes observed for isolates from strawberry crown were not included in these analyses. The incidence of plant collapse in field plots inoculated with isolates determined to be pathogenic or nonpathogenic in greenhouse experiments were compared using one-way analysis of variance or unpaired *t* tests. Proportions were transformed using the arcsine-square root transformation prior to analysis. All pathogenicity data were analyzed with the SPSS 8.0 statistics package (SPSS Inc., Chicago).

RESULTS

Species identification and population structure. In all, 18 and 24 isolates collected from oak and grape lesions at Dover site 1 and 2, respectively, amplified a product with the *C. gloeosporioides/C. fragariae*-specific ITS1 PCR primer. For the two sites distant from strawberry production, 22 isolates from Lake Alfred and 25 isolates from Sarasota amplified a product with this primer. Cluster analysis using data from 38 RAPD bands grouped isolates from grape and oak into four distinct clusters (Fig. 2). One cluster, designated *C. gloeosporioides*-strawberry crown contained isolates similar to Cgl-2-type *C. gloeosporioides* isolates from strawberry crown, a second cluster designated *C. gloeosporioides*-citrus contained isolates similar to a group of isolates from citrus, a third cluster designated *C. fragariae* contained a single isolate from an oak leaf in Lake Alfred along with the standard *C. fragariae* isolate, and a fourth cluster designated *G. cingulata*-native host contained isolates distinct from those observed on any cultivated host. The cluster designated *G. cingulata*-native host was named in reference to the occurrence of the teleomorph *G. cingulata* among native host isolates that formed this cluster after single-spored cultures were incubated on PDA media for 14 days. Fertile perithecial production on PDA was not observed for isolates from any of the three other groups. The vast majority (82 of 89) of isolates from grape and oak clustered with 20 *C. gloeosporioides* isolates from diseased strawberry crowns. The cluster occurred in 89% of bootstrapped trees. One of the seven citrus isolates used as controls, Citrus-3, also clustered among isolates from strawberry crowns. There were three isolates that clustered among the remaining six citrus isolates. The three isolates all came from the Lake Alfred site situated close to citrus groves. The cluster occurred in 99% of the bootstrapped trees. The cluster that included the *C. fragariae* isolate and the oak isolate from Lake Alfred occurred in 100% of bootstrapped trees. The isolate from oak also produced tapered conidia, caused strawberry crown rot, and possessed setae that functioned as phialides, which are traits characteristic of *C. fragariae* (10). The cluster designated *G. cingulata*-native host contained two isolates from Sarasota and one from Dover site 1 and was observed in 99% of bootstrapped trees. Of the 38 RAPD bands scored, 9 were unique to the cluster defined by isolates of *C. gloeosporioides* from crown tissue, 6 were unique to the cluster defined by isolates from citrus, 4 were unique to the homothallic *G. cingulata* isolate cluster, and 6 were unique to the cluster containing *C. fragariae*.

For isolates that clustered with strawberry crown isolates, there was no evidence for population differentiation between those from oak and grape at any of the four sites based on RAPD marker frequencies (Table 2). In a hierarchal analysis in which isolates from all sites were used and sampling site was included in the analysis to delimit subpopulations, there also was no evidence for population subdivision based on host ($\theta = -0.028$, 90% confidence interval [CI] = −0.054 to 0.001). There was evidence for population subdivision between isolates taken from the Sarasota site and the other four samples (Lake Alfred, Dover1, Dover2, and diseased strawberry crowns) (Table 3). When the four samples that differed from the Sarasota population were compared, there was no evidence for population differentiation (Table 3).

Analysis of sequence data from the ITS1-5.8S rRNA-ITS2 region of the rDNA repeat for 19 isolates collected in the current study revealed five unique sequences that differed from one another by at least one base pair within either the ITS1 or ITS2 region (Fig. 3). There were no differences among isolates within the region encoding the 5.8S rRNA subunit. The *C. fragariae* reference isolate and isolate LA-oak-13 had two single-base-pair substitutions and an insertion of cytosine nucleotides within the ITS1 that distinguished them from all other sequences. The only difference between the sequences for these two isolates was the size of the cytosine insert. The ITS1-5.8S rRNA-ITS2 region for isolates Citrus-7 and LA-grape-8 were identical to one another and unique from all other sequences. Because these two isolates were from the RAPD cluster designated *C. gloeosporioides*-citrus, the

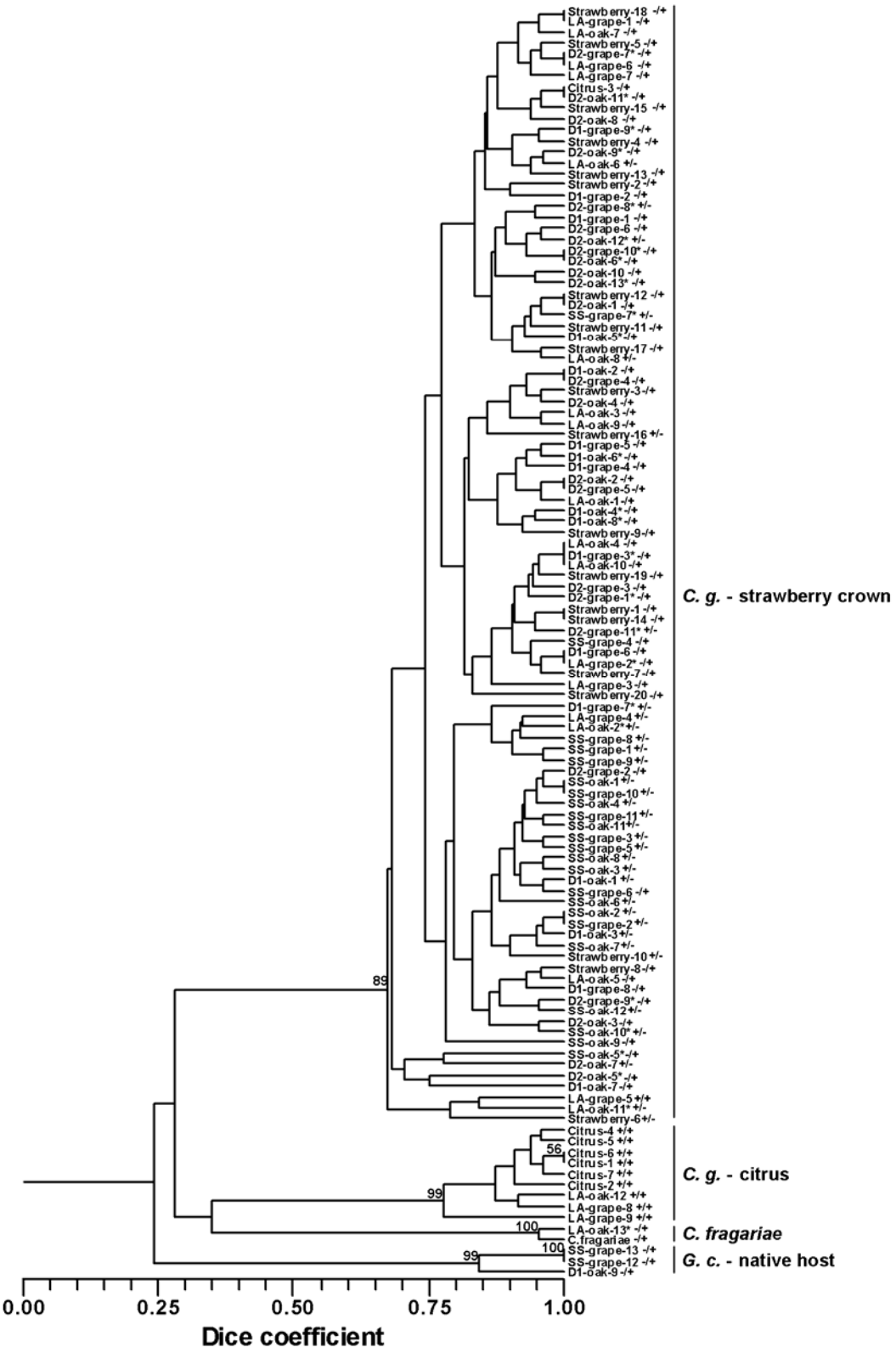

Fig. 2. Unweighted pair group method with arithmetic averages phenogram showing genotypic similarity between *Colletotrichum* isolates. *Colletotrichum gloeosporioides* (*C. g.*) isolates from oak and grape fell into three separate clusters based on high similarity among isolates within each cluster (similarity ≥0.65), low similarity between each cluster and other isolates (similarity ≤0.35), and high bootstrap support for clusters (≥50%). The three clusters consisted of isolates that were homothallic in culture (*Glomerella cingulata* [*G. c.*]), isolates similar to self-sterile strawberry crown isolates, and isolates similar to citrus isolates. A *C. fragariae* isolate and cluster is identified by name. Isolates coded with the prefix D1 and D2 are from the two sites in Dover, isolates coded LA are from Lake Alfred, and those coded SS from Sarasota. Oak and grape isolates with asterisks were pathogenic to strawberry in a greenhouse test. The symbols +/–, –/+ and +/+ indicate that the isolate had a *Bst*UI(+)/*Msp*I(–),*Bst*UI(–)/*Msp*I(+), or *Bst*UI(+)/*Msp*I(+) rDNA genotype, respectively. Numbers at branch points indicate the percent occurrence of the cluster to the right of the branch in 1,000 bootstrapped phenograms. Only branches occurring in at least 50% or more of bootstrapped phenograms are labeled.

sequence is also referred to as *C. gloeosporioides*-citrus in Figure 3.

Among isolates that clustered with strawberry using RAPD markers were two ITS1-5.8S rRNA-ITS2 sequences, designated *C. gloeosporioides*-strawberry crown 1 and *C. gloeosporioides*-strawberry crown 2, that differed by 3 bp (Fig. 3). The sequence designated *C. gloeosporioides*–strawberry crown 1 differed from the sequence for historic Cgl-2 genotype isolate 291 (9) by only a single base insertion in a cytosine repeat, whereas it differed from the sequence for historic Cgl-1 type isolate 231 (9) by 4 bp. There were three nucleotide differences between the *C. gloeosporioides*-strawberry crown 2 sequence and the sequence for both historic isolates. Both strawberry crown genotypes were present among strawberry isolates and isolates from all native host sampling sites. The ITS1-5.8S rRNA-ITS2 region for two isolates from the *G. cingulata*–native host cluster, SS-grape-12 and SS-grape-13, was identical to the sequence designated *C. gloeosporioides*-strawberry crown 1.

The restriction enzymes *Bst*UI and *Msp*I, which detect polymorphisms at position 69 in the ITS1 and position 73 in the ITS2 (Fig. 3), differentiate *C. gloeosporioides*-strawberry crown and *C. gloeosporioides*-citrus genotypes (strawberry-1 = *Bst*UI[–]/*Msp*I[+], strawberry-2 = *Bst*UI[+]/*Msp*I[–], citrus = *Bst*UI[+]/*Msp*I[+]). The six citrus isolates and three isolates from grape and oak that formed the citrus cluster using RAPD markers all had the *Bst*UI(+)/*Msp*I(+) genotype (Fig. 2). Of 103 isolates from the *C. gloeosporioides*–strawberry crown RAPD cluster, 102 were *Bst*UI(–)/*Msp*I(+) or *Bst*UI(+)/*Msp*I(–) (Fig. 2). A single Lake Alfred isolate was *Bst*UI(+)/*Msp*I(+). The single citrus isolate that grouped with strawberry isolates had a *Bst*UI(–)/*Msp*I(+) genotype. When the distribution of the *Bst*UI(–)/*Msp*I(+) and *Bst*UI(+)/*Msp*I(–) rDNA RFLPs from native hosts was examined, there was no evidence that the RFLP genotypes were distributed differently on grape and oak (Table 4). However, there was evidence that the *Bst*UI(+)/*Msp*I(–) genotype was more common at the Sarasota site (Table 4). When sites other than Sarasota were compared, there was no statistical support for differences in the distribution of ITS RFLP genotypes between sites. From strawberry crown tissue, three isolates had the *Bst*UI(+)/*Msp*I(–) genotype and 17 isolates had the *Bst*UI(–)/*Msp*I(+) genotype. There is statistical support ($P < 0.001$, Fisher's exact test) that the distribution of genotypes on strawberry is different from that observed at the Sarasota site, which had 19 isolates with the *Bst*UI(+)/*Msp*I(–) genotype and 4 isolates with the *Bst*UI(–)/*Msp*I(+) genotype. However, this distribution does not appear to be different from the distribution observed at the two Dover sites and Lake Alfred, which had 12 isolates with the *Bst*UI(+)/*Msp*I(–) genotype and 46 isolates with the *Bst*UI(–)/*Msp*I(+) genotype ($P = 0.75$, Fisher's exact test). To test for population differentiation between isolates with different ITS RFLPs, subsamples of each genotype were taken. This was done because the number of isolates with the least frequently occurring genotype at each site was too low to carry out a hierarchal analysis properly. The subsamples consisted of all isolates of the least frequent genotype at a site and an equal size random sample of the most frequent genotype. Isolates with the same genotype from all sites then were pooled to create two samples with 19 isolates. A test for population differentiation between these two samples using RAPD marker data suggests that there is some restricted gene flow between isolates with different ITS genotypes ($\theta = 0.122$, 90% CI = 0.044 to 0.189). A test for population differentiation comparing RAPD marker data for *Bst*UI(+)/*Msp*I(–) isolates from Sarasota to marker data from *Bst*UI(+)/*Msp*I(–) isolates from all other sites also was conducted and continued to support that there is restricted gene flow between the Sarasota site and the others examined ($\theta = 0.141$, 90% CI = 0.064 to 0.231).

Pathogenicity. The effects of site and host on isolate pathogenicity to strawberry were evaluated for both ITS genotypes combined and separately, because there was some evidence that these two groups were different. Based on a series of Fisher's exact tests (Table 5), there was no evidence that the incidence of pathogenicity at sites close to strawberry fields (Dover 1 and Dover 2) was different for isolates with the *Bst*UI(–)/*Msp*I(+) genotype ($P = 1.0$), the *Bst*UI(+)/*Msp*I(–) genotype ($P = 0.49$), or both genotypes combined ($P = 0.75$). There also was no evidence that the incidence of pathogenicity at sites distant to strawberry production (Lake Alfred and Sarasota) was different for isolates with either the *Bst*UI(–)/*Msp*I(+) genotype ($P = 0.45$), the *Bst*UI(+)/*Msp*I(–) genotype ($P = 0.18$), or both genotypes combined ($P = 1.0$). When isolates from sites close to strawberry production (Dover 1 + Dover 2) were compared with isolates from sites distant from strawberry production (Lake Alfred + Sarasota), there was statistical support for a higher incidence of pathogenicity among isolates from sites close to strawberry for each genotype when genotypes were analyzed separately ($P = 0.05$). When data from both genotypes were combined, the statistical support was much stronger ($P < 0.01$). The incidence of pathogenicity among isolates from grape and oak hosts does not appear to be different for those with either the *Bst*UI(–)/*Msp*I(+) genotype ($P = 0.56$), the *Bst*UI(+)/*Msp*I(–) genotype ($P = 0.69$), or both genotypes combined ($P = 1.0$). Tests for differences in the incidence of pathogenicity among isolates with different rDNA genotypes also were conducted for the sites near and close to strawberry. The sites were analyzed separately because genotypes were not equally represented across sites and there were differences in pathogenicity among sites close to and distant from strawberry. There was no statistical evidence that the frequency of pathogenic isolates among isolates with the *Bst*UI(–)/*Msp*I(+) genotype was different from isolates with the *Bst*UI(+)/*Msp*I(–) genotype at sites close to (Dover 1 + Dover 2) ($P = 0.68$) or distant from (Lake Alfred + Sarasota) ($P = 1.0$) strawberry fields.

TABLE 2. Estimates of θ for pairwise comparisons of *Colletotrichum gloeosporioides* isolates from oak and grape hosts at four sites

Site	θ[a]	90% CI θ[b]
Dover 1	–0.094	–0.114 to –0.069
Dover 2	0.031	–0.032 to 0.091
Lake Alfred	0.056	–0.032 to 0.157
Sarasota	–0.017	–0.057 to 0.040

[a] For individual pairwise comparisons, 13 to 15 bands were used to estimate θ.
[b] The 90% confidence interval (CI) was determined from 10,000 bootstrap replications. There was no statistical support for population differentiation for any pairwise comparison because the lower boundary of the 90% CI for θ never exceeded 0.

TABLE 3. Pairwise estimates of θ for *Colletotrichum gloeosporioides* populations at four sites and the estimated 90% confidence interval (CI) for θ[a]

	Dover 1	Dover 2	Lake Alfred	Sarasota	Strawberry crown
Dover 1	…	–0.021 (–0.032 to –0.010)	–0.031 (–0.039 to 0.129)	0.218* (0.116 to 0.310)	–0.005 (–0.032 to 0.028)
Dover 2	…	…	0.030 (–0.017 to 0.094)	0.275* (0.141 to 0.396)	–0.007 (–0.027 to 0.014)
Lake Alfred	…	…	…	0.168* (0.087 to 0.239)	–0.004 (–0.025 to 0.023)
Sarasota	…	…	…	…	0.281* (0.131 to 0.373)
Strawberry crown	…	…	…	…	…

[a] For individual pairwise comparisons, 15 to 17 bands were used to estimate θ. The lower boundary of the 90% CI for estimates followed by an asterisk is >0, indicating statistical support for population subdivision. The 90% CI was determined from 10,000 bootstrap replications.

Field plots topically inoculated with a collection of isolates determined to be pathogenic by crown injection had a higher incidence of crown rot than those that were determined to be nonpathogens (38.3 versus 3.3%, $P < 0.001$, unequal variances assumed, $n = 12$ per treatment). The relatively low incidence of crown rot in plots inoculated with isolates determined to be nonpathogenic using the greenhouse test was not different from plots sprayed with water (3.3 versus 2.5%, $P = 0.78$, $n = 12$ and 4). Incidence of plant collapse was not different in the field for pathogenic isolates from the four different sites ($F = 0.36$, $P = 0.78$, $n = 3$ per site), nor was there statistical support for a difference in the percentage of collapsed plants between plots treated with native host isolates and plots treated with isolates from diseased crown (38.3 versus 60%, $P = 0.17$, $n = 12$ and 4).

Isolate or RAPD group	Nucleotide Sequences
	ITS1
291	CTGAGTTTAC GCTCTACAAC CCTTTGTGAA CATACCTATA ACTGTTGCTT CGGCGGGTAG
C. g. - strawberry crown 1	---------- ---------- ---------- ---------- ---------- ----------
G. c. - native host	---------- ---------- ---------- ---------- ---------- ----------
C. g. - strawberry crown 2	---------- ---------- ---------- ---------- ---------- ----------
C. g. - citrus	---------- ---------- ---------- ---------- ---------- ----------
231	---------- ------T--- ---------- ---------- ---------- ----------
C.fragariae	---------- ---------- ---------- --------C- ---------- ----------
LA-oak-13	---------- ---------- ---------- --------C- ---------- ----------
	BstUI ITS1
291	GGTCTCCGTG ACCCTCCCGG CCTCCCGCCC CC***GGGCG GGTCGGCGCC CGCCGGAGGA
C. g. - strawberry crown 1	---------- ---------- ---------- ---------- ---------- ----------
G. c. - native host	---------- ---------- ---------- ---------- ---------- ----------
C. g. - strawberry crown 2	--------C- ---------- ---------T ---------- ---------- ----------
C. g. - citrus	--------C- ---------- ---------T ---------- ---------- ----------
231	--------C- ---------- ---------T ---------- ---------- ----------
C.fragariae	----C----- ---------- ---------- --CC------ ---------- ----------
LA-oak-13	----C----- ---------- ---------- --CCC----- ---------- ----------
	ITS1
291	TAACCAAACT CTGATTTAAC GACGTTTCTT CTGAGTGGTA CAAGCAAATA ATCA
C. g. - strawberry crown 1	---------- ---------- ---------- ---------- ---------- ----
G. c. - native host	---------- ---------- ---------- ---------- ---------- ----
C. g. - strawberry crown 2	---------- ---------- ---------- ---------- ---------- ----
C. g. - citrus	---------- ---------- ---------- ---------- ---------- ----
231	---------- ---------- ---------- ---------- ---------- ----
C.fragariae	---------- ---------- ---------- ---------- ---------- ----
LA-oak-13	---------- ---------- ---------- ---------- ---------- ----
	ITS2
291	CAACCCTCAA GCTCTGCTTG GTGTTGGGGC CCTACAGCTG ATGTAGGCCC TCAAAGGTAG
C. g. - strawberry crown 1	---------- ---------- ---------- ---------- ---------- ----------
G. c. - native host	---------- ---------- ---------- ---------- ---------- ----------
C. g. - strawberry crown 2	---------- ---------- ---------- ---------- ---------- ----------
C. g. - citrus	---------- ---------- ---------- --------C- ---------- ----------
231	---------- ---------- ---------- ---------- ---------- ----------
C.fragariae	---------- ---------- ---------- ---------- ---------- ----------
LA-oak-13	---------- ---------- ---------- ---------- ---------- ----------
	MspI ITS2
291	TGGCGGACCC TCCCGGAGCC TCCTTTGCGT AGTAACTTTA CGTCTCGCAC TGGGATCCGG
C. g. - strawberry crown 1	---------- ---------- ---------- ---------- ---------- ----------
G. c. - native host	---------- ---------- ---------- ---------- ---------- ----------
C. g. - strawberry crown 2	---------- --T------- ---------- ---------- ---------- ----------
C. g. - citrus	---------- ---------- ---------- ---------- ---------- ----------
231	---------- ---------- ---------- ---------- ---------- ----------
C.fragariae	---------- ---------- ---------- ---------- ---------- ----------
LA-oak-13	---------- ---------- ---------- ---------- ---------- ----------
	ITS2
291	AGGGACTCTT GCCGTAAAAC CCCCCAATTT TCC
C. g. - strawberry crown 1	---------- ---------- ----*----- ---
G. c. - native host	---------- ---------- ----*----- ---
C. g. - strawberry crown 2	---------- ---------- ----*----- ---
C. g. - citrus	---------- ---------- ---------- ---
231	---------- ---------- ---------- ---
C.fragariae	---------- ---------- ---------- ---
LA-oak-13	---------- ---------- ---------- ---

Fig. 3. Internal transcribed spacer 1 and 2 regions of the ribosomal DNA repeat for self-sterile *Colletotrichum gloeosporioides* (*C. g.*) isolate 291 from strawberry, homothallic *Glomerella cingulata* (*G. c.*) isolate 231 from strawberry, and isolates from each of the four random amplified polymorphic DNA (RAPD) clusters displayed in Figure 2. The sequence listed as "*C. gloeosporioides*-strawberry crown 1" was observed for isolates Strawberry-11, -18, and -19; SS-oak-9; LA-grape-6; D2-oak-6 and -8; and D1-grape-6. The sequence listed as "*G. cingulata*-native host" was observed for native host isolates SS-grape-12 and -13 from the *G. cingulata*-native host RAPD cluster and is identical to the sequence labeled *C. gloeosporioides*-strawberry crown 1. The sequence listed "*C. gloeosporioides*-strawberry crown 2" was observed for isolates Strawberry-6, D2-oak-12, LA-oak-6, SS-grape-10, and SS-oak-2. The sequence "*C. gloeosporioides*-citrus" was observed for isolates Citrus-7 and LA-grape-8. The sequences from isolates *C. fragariae* and LA-oak-13, that clustered together based on RAPD data, differed by one base. The location of polymorphic *Bst*UI and *Msp*I restriction sites are underlined. Sequences for isolates 291 and 231 were published previously (18,27). Asterisks represent alignment gaps and dashes (–) represent bases identical to those at the same site for isolate 291. GenBank and European Molecular Biology Laboratory accession numbers are provided in the text.

DISCUSSION

Among *C. gloeosporioides* isolates from native hosts closely related to isolates from strawberry, the proportion of pathogenic isolates was greater at sampling sites close to strawberry fields, supporting the hypothesis that local selection for pathogenicity on strawberry occurs where this host is grown in abundance. The selection response appears to be for an increase in the frequency of genes for pathogenicity to strawberry because pathogenic isolates were present at sites distant from strawberry fields. Unlike pathogens from the *Hordeum vulgare–Rhynchosporium secalis* and the wild *Linum marginale–Melampsora lini* pathosystems, where evolution of pathogenicity has been studied previously (14,15,19,29), the *C. gloeosporioides* population that infects strawberry is not host specific (35), and gene-for-gene interactions do not contribute to variation in cultivar susceptibility (16). *C. gloeosporioides* also forms infections in strawberry petioles that remain quiescent until infected tissue senesces (20). Because *C. gloeosporioides* has a broad host range and uses saprophytic and necrotrophic nutritional strategies, it would be difficult to identify a single host or tissue type imposing an overriding selection pressure on the pathogen in a natural environment. However, selection for individuals that can grow and are pathogenic on a large, genetically uniform, cultivated host population within an area might be discernible. Such selection would explain the greater incidence of pathogenicity to strawberry in the *C. gloeosporioides* population on the native hosts near commercial strawberry production and also is consistent with research that shows that the proportion of races immune to specific host resistance genes is greater in samples from areas where resistance genes are deployed (32).

The current study indicates that the population of *C. gloeosporioides* found on strawberry and on native host species adjacent to strawberry fields was not introduced into Florida along

TABLE 4. Distribution of internal transcribed spacer (ITS)1-5.8S rRNA-ITS2 genotypes on noncultivated hosts at four sites

	Number of isolates[a]			
	Grape		Oak	
Site	*Bst*UI(–)/*Msp*I(+)	*Bst*UI(+)/*Msp*I(–)	*Bst*UI(–)/*Msp*I(+)	*Bst*UI(+)/*Msp*I(–)
Dover 1	8	1	6	2
Dover 2	9	2	11	2
Lake Alfred	5	1	7	4
Sarasota[b]	2	9	2	10
All sites combined[c]	24	13	26	18

[a] Number of isolates at each site is categorized according to host (grape or oak) and occurrence of a *Bst*UI restriction site beginning at position 67 within the ITS1 and an *Msp*I site beginning at position 73 within the ITS2 of the rDNA repeat.
[b] Fisher's exact tests comparing distribution of rDNA restriction fragment length polymorphism (RFLP) genotypes in the Sarasota population to three other sites showed $P < 0.05$ for each host. Fisher's exact test showed $P > 0.05$ for all other pairwise comparisons.
[c] Fisher's exact tests comparing distribution of rDNA RFLP genotypes on grape to the distribution on oak at all sites combined showed $P = 0.65$.

TABLE 5. Pathogenicity to strawberry of *Colletotrichum gloeosporioides* isolates from the noncultivated hosts, grape and oak, at four sites with pathogenicity data organized into two-by-two contingency tables to examine the distribution of pathogenicity to strawberry for *Colletotrichum gloeosporioides* isolates from grape and oak at sites close to (Sarasota and Lake Alfred) and distant from (Dover 1 and Dover 2) commercial strawberry production

		Number of isolates[a]					
		*Bst*UI(–)/*Msp*I(+)		*Bst*UI(+)/*Msp*I(–)		Both genotypes combined	
Sites	Host	Pathogenic	Nonpathogenic	Pathogenic	Nonpathogenic	Pathogenic	Nonpathogenic
Dover 1	Grape	2	6	1	0	3	6
	Oak	4	2	0	2	4	4
Dover 2	Grape	4	5	2	0	6	5
	Oak	5	6	1	1	6	7
Lake Alfred	Grape	1	4	0	1	1	5
	Oak	0	7	2	2	2	9
Sarasota	Grape	0	2	1	8	1	10
	Oak	1	1	1	9	2	10
Dover 1	Grape + Oak	6	8	1	2	7	10
Dover 2	Grape + Oak	9	11	3	1	12	12
		$P = 1.0$[b]		$P = 0.49$		$P = 0.75$	
Lake Alfred	Grape + Oak	1	11	2	3	3	14
Sarasota	Grape + Oak	1	3	2	17	3	20
		$P = 0.45$		$P = 0.18$		$P = 1.0$	
Dover 1 + Dover 2	Grape + Oak	15	19	4	3	19	22
Lake Alfred + Sarasota	Grape + Oak	2	14	4	20	6	34
		$P = 0.05$		$P = 0.05$		$P = <0.01$	
All sites combined	Grape	7	17	4	9	11	26
	Oak	10	16	4	14	14	30
		$P = 0.56$		$P = 0.69$		$P = 1.0$	
Dover 1 + Dover 2	Grape + Oak	15	19	4	3	…	…
			$P = 0.68$			…	…
Lake Alfred+ Sarasota	Grape + Oak	2	14	4	20	…	…
			$P = 1.0$			…	…

[a] Isolates from each site–host combination are categorized according to pathogenicity on strawberry and the occurrence of restriction sites within the rDNA repeat beginning at position 67 of the internal transcribed spacer (ITS)1 region and at position 73 of the ITS2 region identified by the enzymes *Bst*UI and *Msp*I, respectively.
[b] Fisher's exact test results for two-by-two contingency table comprising the four cells in the two columns above the reported *P* value. For tests examining pathogenicity of isolates with different rDNA restriction fragment length polymorphisms within the Dover populations and Lake Alfred + Sarasota populations, the two-by-two contingency table consists of four cells in the row above the reported *P* value.

with cultivated strawberry plants. The vast majority of isolates far from strawberry production areas clustered with strawberry crown isolates using RAPD markers, and at least a portion of isolates far from strawberry fields are pathogenic to strawberry. Additionally, the two distinct ITS genotypes of isolates from strawberry crowns also were found at sites distant from strawberry and there was no evidence that RAPD band frequencies at the Lake Alfred site were different from the population on strawberry. However, it cannot be completely excluded that the *C. gloeosporioides* population found on native hosts initially came from strawberry and then spread throughout the state.

The RAPD band frequencies in the Sarasota population were not the same as those at the other three sites or among strawberry isolates. Frequency differences within this population probably are not due to the occurrence of a different species in the Sarasota area because there were no unique RAPD bands among Sarasota isolates and no evidence of a monophyletic group of isolates within this population. Although both ITS genotypes were observed in the Sarasota population, the frequencies of the two genotypes were not the same as those observed in other populations. This suggests that distinct subpopulations identifiable by ITS may explain the differences in RAPD band frequencies. Alternatively, differences in RAPD band frequencies might be due only to restricted gene flow. Based on results of pairwise comparisons, there was evidence that geographic distance plays a role in differentiating the populations and that ITS genotypes identify subpopulations with restricted gene flow between them.

Concerted evolution of repetitive rDNA units is responsible for the low intra-individual variation observed within ITS regions (6). The same molecular turnover mechanisms responsible for the concerted evolution of repetitive rDNA units are also responsible for homogenization of gene families within species. It is commonly held that individuals with different rDNA sequences are different species, although there are examples of more than one homogenized rDNA repeat family within the same interbreeding population (22,24). An explanation for this is that gene conversion events only occur between sister chromatids and not homologous chromosomes (24). As previously stated, RAPD marker frequencies provided some evidence that the ITS genotypes distinguished two different *C. gloeosporioides* subpopulations with the ability to infect strawberry crowns. However, numerous RAPD bands were polymorphic among isolates with both genotypes, isolates with different ITS genotypes did not form distinct clusters in the phenogram constructed using RAPD marker data, and isolates with both ITS genotypes had a common Cgl-2-type AT-rich DNA banding pattern (S. J. MacKenzie, *unpublished data*). These observations all indicate that there is exchange of genetic material between the two subpopulations and that the *C. gloeosporioides* population found on strawberry is another example of an interbreeding population with more than one homogenized rDNA repeat. To clearly determine whether this is true, a sample with more representative isolates of each genotype will have to be drawn from a single site to eliminate the confounding effects of geographic distance and ITS genotype frequency differences among sites observed in the present study.

Although the primary focus of the current study was to determine whether there is selection for pathogenicity in a *C. gloeosporioides* population, the genetic analysis of isolates that did not cluster with crown isolates also yielded noteworthy results. *C. fragariae* has been isolated from *Cassia obtusifolia* near a strawberry nursery in Florida (11) and from anthracnose lesions on cherimoya fruit in Mexico (31). The recovery of a *Colletotrichum fragariae* isolate from oak ≈28 km from commercial strawberry plants provides further support that the host range of this species is not restricted to strawberry. Isolates of *C. gloeosporioides* related to citrus isolates were recovered from grape and oak growing next to a citrus grove, where migration from citrus hosts would likely be high. Because only a small proportion of native host isolates from this site clustered with isolates from citrus, the population on citrus may be specific to this host. However, this finding also demonstrates that, if sufficient inoculum is present, alternate hosts can be colonized.

Combined ITS1 and ITS2 sequences were different for all of the RAPD clusters with the exception of the *G. cingulata*-native host isolate group. The combined sequence for these isolates was identical to the sequence for *C. gloeosporioides*-strawberry crown 1 isolates. Given that multiple RAPD bands were unique to each isolate cluster and homothallism was present only among *G. cingulata*-native host isolates, it appears that they are genetically isolated subgroups. These results also underscored the utility of RAPD markers for differentiating genetically isolated subgroups within the *C. gloeosporioides* species aggregate. The homothallic isolates from oak and grape were not pathogenic to strawberry and are not the same as homothallic isolates from strawberry based on the ITS sequences and RAPD marker data generated from two reference homothallic Cgl-1 genotype isolates from strawberry, 311-1, and 329-1 (8,10) (S. J. MacKenzie, *unpublished data*).

In summary, there was no evidence that *C. gloeosporioides* isolates from diseased strawberry crowns in Florida were genetically distinct from the *C. gloeosporioides* population broadly distributed on native hosts near and distant from commercial strawberry fields. Isolates pathogenic to strawberry also were broadly distributed on these native hosts, although they occurred at a higher frequency at sites close to strawberry fields. The high incidence of Colletotrichum crown rot in summer nurseries in Florida is one reason that transplants used for commercial fruit production in Florida are purchased from nurseries located in cooler areas. The current study suggests that it would be difficult to exclude *C. gloeosporioides* from summer nurseries in Florida even with clean material and isolation by distance from strawberry production fields.

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