ABSTRACT

GONZALEZ, EUGENIA. Characterization of isolates of *Glomerella cingulata* causal agent of Glomerella leaf spot and bitter rot of apples based on morphology and genetic, molecular, and pathogenicity tests (Under the direction of Dr. Turner B. Sutton).

Isolates of *Glomerella cingulata* obtained from leaves with Glomerella leaf spot (GLS) symptoms and isolates of *G. cingulata, Colletotrichum gloeosporioides* and *C. acutatum*, obtained from fruit with bitter rot symptoms collected from apple orchards in the US and Brazil, were characterized based on morphological characteristics, vegetative compatibility groups (VCGs), and mtDNA RFLP haplotypes. A subset of the isolates was further characterized by examining the sequence of a 200 bp intron of the glyceraldehyde 3-phosphate dehydrogenase (GDPH) gene. Another subset of the isolates was tested for pathogenicity on leaves of cultivars Gala and Golden Delicious on the green house, and on pathogenicity on fruit of cv. Gala in growth chambers. Growth rate, sensitivity of the isolates to benomyl, and optimum growth temperature were also determined for a subset of the isolates. The population structure of *G. cingulata* and *Colletotrichum* spp. associated with bitter rot of apples in two orchards of cv. Granny Smith was also studied. Isolates of *G. cingulata* and *C. gloeosporioides* were more variable than those of *C. acutatum*. Isolates of *G. cingulata* were separated into four morphological types, and six VCGs. Five morphological types and six VCGs were found within isolates of *C. gloeosporioides*. Three morphological types, and four VCGs differentiated isolates of *C. acutatum*. Eight different mtDNA RFLP haplotypes were observed within isolates of *G. cingulata,* two within isolates of *C. gloeosporioides*, and two within isolates of *C. acutatum*. Phylogenetic trees constructed based on Neighboring-Joining and Maximum Parsimony methods, using the intron sequence, produced similar topologies. Each species

was separated into distinct groups. All isolates tested were pathogenic on fruit. Only isolates with haplotypes G1, G1.1, G3, and G4 were capable of causing Glomerella leaf spot (GLS). *G. cingulata* was the predominant species associated with bitter rot in the two orchards of cv. Granny Smith. In these orchards, different morphological types and VCGs were observed among isolates of *G. cingulata* and *C. acutatum* but not among isolates of *C. gloeosporioides*. Isolates of *C. gloeosporioides* were found only in one of the orchards and represented the lowest proportion of the population. Only isolates of *G. cingulata* in VCG-1, VCG-4, and VCG-5 were pathogenic to leaves. Isolates of *G. cingulata* which were associated with bitter rot only were not found in these VCGs. VCG-1 included only isolates of *G. cingulata* from the US, whereas VCG-4 and VCG-5 included only isolates of *G. cingulata* from Brazil. Vegetative compatibility was a better indicator than molecular characters for distinguishing isolates of *G. cingulata* pathogenic on both leaves and fruit from the ones pathogenic only on fruit. Isolates of *G. cingulata* capable of causing both GLS and bitter rot were included in haplotypes and phylogenetic groups that also included isolates capable of causing bitter rot only. Additionally, isolates of *G. cingulata* from the US and Brazil which cause GLS were included in different haplotypes and phylogenetic groups. Therefore, our results suggest that isolates of *G. cingulata* from the US capable of causing both GLS and bitter rot arose independently of Brazilian isolates of *G. cingulata,* and may have arisen from isolates of *G. cingulata* from the US that originally were capable of causing bitter rot only. Slower growth, lower optimum growth temperature, and less sensitivity to benomyl distinguished isolates of *C. acutatum* from isolates of *G. cingulata* and *C. gloeosporioides*. These parameters were

not useful for distinguishing between isolates of *G. cingulata* and *C. gloeosporioides* or within mtDNA haplotypes or VCGs of each species.

Characterization of isolates of *Glomerella cingulata* causal agent of Glomerella leaf spot and bitter rot of apples based on morphology and genetic, molecular, and pathogenicity tests

by
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DEDICATION

To my wonderful family, who always supported me and never lost faith in me during this long and bumpy journey. Specially, to my loving and devoted mom, and my dearest brother Carlos, who I'm sure were always beside me in soul and spirit.

BIOGRAPHY

I obtained a five-year degree in Plant Science from the University of Costa Rica in 1997. In 1998 came to NC and started my Masters in Plant Pathology at NCSU working with Dr. Turner B. Sutton. In my project we looked at the effect of urea, *Trichoderma harzianum* T-22 and the shredding of leaf litter on the management of apple pathogens and species of arthropods that overwinter in apple leaf litter. After two years, I decided to switch over to the Ph.D. program and started working in a different project that involved the morphological, cultural, genetic and molecular characterization of isolates of *Glomerella cingulata* associated with Glomerella leaf spot of apples, and isolates of *Colletotrichum* species associated with bitter rot of apples.

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1. CHAPTER I

Morphological and genetic diversity among isolates of *Glomerella cingulata* associated with Glomerella leaf spot and isolates of *Colletotrichum* species associated with bitter rot of apples

1.1. ABSTRACT

Isolates of *Glomerella cingulata*, obtained from leaves with Glomerella leaf spot (GLS) symptoms, and isolates of *G. cingulata*, *Colletotrichum gloeosporioides* and *C. acutatum*, obtained from fruit with bitter rot symptoms collected from apple orchards in the US and Brazil, were characterized based on morphological characteristics (colony color, conidial shape, the ability to produce perithecia in culture, and the distribution of conidial masses and perithecia within the colonies) and vegetative compatibility groups (VCGs). The population structure of *G. cingulata* and *Colletotrichum* spp. associated with bitter rot of apples in two orchards of cv. Granny Smith was also studied. Isolates of *G. cingulata* and *C. gloeosporioides* were more variable than those of *C. acutatum*. A total of 238 and 225 isolates of *G. cingulata* were separated into four distinct morphological types (SP1, SP2, SP3, and CP), and six VCGs (VCG-1 to VCG-6), respectively. Five morphological types (SS1, SS2, SS3, SS4, and SS5) and six VCGs (VCG-7 to VCG-12) were identified among 74 and 36 isolates of *C. gloeosporioides*, respectively. Three morphological types [SSC, SSNC, and SSNC(O)], and four VCGs were identified among 74 and 23 isolates of *C. acutatum*, respectively. Only isolates of *G. cingulata* in VCG-1, VCG-4, and VCG-5 were pathogenic to leaves. Isolates of *G. cingulata* which were associated with bitter rot only were not found in these VCGs. VCG-1 included only isolates of from the US, whereas VCG-4 and VCG-5 included only isolates of *G. cingulata* from Brazil. *G. cingulata* was the predominant species associated with bitter rot in the two orchards of cv. Granny Smith. In these orchards, different

morphological types and VCGs were observed among isolates of *G. cingulata* and *C. acutatum*, but not among isolates of *C. gloeosporioides*. Isolates of *C. gloeosporioides* were found only in one of the orchards and represented the lowest proportion of the population.

1.2. INTRODUCTION

Colletotrichum species are associated with two different diseases of apples: bitter rot and Glomerella leaf spot. Bitter rot is a common fruit disease of apples in practically all countries were they are commercially grown. In moist and temperate growing regions, it is considered one of the most important diseases and it can cause crop losses as high as 50% (29). Three taxa, *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc., and *C. acutatum* J.H. Simmonds are associated with the bitter rot disease of apples (29). Monoconidial isolates of *C. gloeosporioides* from apple which produce fertile perithecia have been designated *G. cingulata* (27). Prior to 1965, *C. acutatum* was considered to be one of the many morphological variants synonymous with *C. gloeosporioides* (6). However, cultural and morphological characteristics of *C. acutatum*, including slower growth rate and predominantly ellipsoidal, fusiform conidia produced in bright orange masses or borne directly on the mycelium, have been useful in differentiating it from *C. gloeosporioides* (1,5,15,18,19,20,21,22,27,31). The presence of a pink to red pigment in the growing medium has also been frequently observed for some isolates of *C. acutatum* (15,18,22,27,37). Isolates of *C. acutatum* are less sensitive to benomyl than isolates of *C. gloeosporioides* (1,4,21,31), and this differential sensitivity to benomyl has also been used to differentiate these organisms. In addition, several researchers have described distinct molecular and genetic differences between *C. gloeosporioides* and *C. acutatum* (2,4,13,19,20,21,33,37). Therefore, *C. acutatum* is now recognized as one of the major aggregates in *Colletotrichum* and considered a cosmopolitan plant pathogen recovered from a wide range of hosts (6).

Struble and Keitt (28) recognized a considerable degree of diversity within isolates of *C. gloeosporioides* and *G. cingulata* recovered from apple fruit showing bitter rot symptoms, and described seven different types of *G. cingulata* based on colony color, distribution of perithecia in culture and relative abundance of conidia. Isolates of *C. gloeosporioides* were characterized as conidial and chromogenic (now known as *C. acutatum*) types. Isolates of *G. cingulata* (perithecial type-isolates) were differentiated from the conidial and chromogenic types by their ability to produce perithecia in culture. Plus and minus types, previously described by Edgerton (11), were observed within perithecial types and characterized based on colony color, distribution of perithecia and relative abundance of conidia. The self-fertility of plus-type isolates was the main characteristic that distinguished these isolates from the minus-type isolates. Later, in 1952, Wheeler and McGahen (36) proposed new designations for the plus and minus perithecial types of *G. cingulata* based on distribution of perithecia, and called them clumped-perithecial (CP) and scattered-perithecial (SP) types, respectively.

In the early 1980s Leite *et al.* (23) described a new apple leaf spot observed on the cultivars Gala and Golden Delicious in Paraná State in Brazil. The disease was named Glomerella leaf spot (GLS) and has been primarily associated with perithecial types of *C. gloeosporioides*. Under favorable conditions, GLS can cause 75% or more defoliation by harvest, which weakens apple trees and reduces yield (7,23,30). In the US, GLS was not reported until 1998 when it was observed causing a severe leaf spot in two orchards of cv. Gala in eastern Tennessee, resulting in extensive defoliation (17). Although GLS and bitter rot have been considered to be caused by the same fungus, differences in morphology and cultural characteristics between these pathogens have been observed (23,31). Taylor (32) and Leite *et al*. (23) also observed differences in the pathogenicity of the GLS and the bitter

rot pathogens. The morphological and pathogenic differences between the GLS and bitter rot fungi, coupled with the considerable genetic and molecular variability within isolates of *C. gloeosporioides* and *G. cingulata* obtained from fruit with bitter rot symptoms (3,28) and within populations of *C. gloeosporioides* and *G. cingulata* from different crops (9,12,14,20,33,35) suggest that the diversity among these pathogens is high.

Morphological and cultural characters remain useful for defining taxa in *Colletotrichum* at both the species and subspecies level; however, morphology alone is unlikely to resolve relationships below the species level (5,20). Vegetative incompatibility in *C. gloeosporioides* from apples has been used effectively to differentiate genetic groups of this pathogen (9). For example, isolates of *C. gloeosporioides* obtained from different apple orchards in New Zealand were separated into six different vegetative compatibility groups (VCGs) (3). Vegetative incompatibility in fungi is controlled by multiple *vic* genes, and incompatibility occurs when the mycelium of isolates that have different alleles at one or more *vic* loci fuse. Isolates that belong to the same VCG are often considered genetically similar (24). Nitrate non-utilizing mutants (*nit* mutants) have been successfully used for vegetative compatibility grouping (VCG) based on nutritional complementation of *nit* mutants and to study genetic variation and relationships within and among *Colletotrichum* species (9,16,25,34).

The objectives of this study were to characterize the variation in a collection of isolates of *C. acutatum*, *C. gloeosporioides* and *G. cingulata* obtained from apple fruit and leaves from different locations in the US and Brazil using genetic and morphological characters and to clarify the genetic relationship between isolates that cause GLS and bitter rot of apples. As part of our study, the population structure of *C. acutatum*, *C.*

gloeosporioides and *G. cingulata* in two orchards of cv. Granny Smith located in North Carolina was also characterized.

1.3. MATERIAL AND METHODS

1.3.1. Collection of monosporic isolates. A total of 486 isolates recovered from symptomatic fruit and leaves collected from apple orchards located in the US and Brazil were used in this study (Appendix 4.1). In the US, 12, 56, and 29 isolates were recovered from leaves in 1998, 2000, and 2002, respectively. Sixty-nine, 101, and 167 isolates were also recovered from fruit in orchards in the US in 2000, 2001, and 2002, respectively. Leaf isolates were obtained from four different orchards of cv. Gala where GLS has been observed, including two orchards in eastern Tennessee where the leaf spot was reported for the first time in the US (TN 1 and TN 2), one in Georgia (GA) and one orchard in North Carolina (NC 1). Fruit isolates were also obtained from the Gala orchards located in Georgia and North Carolina, and from an orchard of cv. Golden Delicious in Alabama (AL), an orchard of cv. Molly's Delicious in Ohio (OH), and from two orchards of cv. Granny Smith (NC 2 and NC 4), one of cv. Delicious (NC 3), and one of cv. Golden Delicious (NC 3) located in North Carolina. Isolates used were from the orchards of cv. Granny Smith in 2001 and 2002 represent a subsample of the isolates obtained from fruit collected for the population structure study described below. This subsample included cultures representing the different morphological types found in these orchards.

A total of 38 leaf isolates from Brazil were recovered from symptomatic leaves collected by Dr. Rosa Maria Sanhueza in 2001 in six different orchards of cv. Gala located in Rio Grande do Sul State (Brazil 3) and Santa Catarina State (Brazil 1, 4, 5, 6 and 7). Fourteen

cultures that belong to a collection of isolates maintained by Dr. Sanhueza at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil were also part of the collection of monosporic isolates examined in this study. The isolates were collected from fruit, leaves, and buds from orchards of cv. Gala, Golden Delicious, and Fuji located in Rio Grande do Sul State (Brazil 2) and Santa Catarina State (Brazil 8). Detailed information about the origin and location of the isolates collected in the US and Brazil is presented in Table 1.1.

1.3.2. Isolations and monosporic isolates. Fruit and leaf samples obtained in the field were stored at 4°C until they were processed. Fruit and leaves selected for the isolations were disinfested with 70% ethanol and 0.525% NaOCl for 30 sec, respectively, and allowed to dry in a laminar hood. Each fruit and leaf isolate was obtained from a small piece of tissue cut from an arbitrarily selected lesion on a fruit or leaf. Fruit isolates were placed in Petri dishes containing potato dextrose agar (PDA) and leaf isolates in Petri dishes containing PDA + streptomycin (200 μg/ml). Isolates were incubated in a growth chamber at 25°C and constant light for 24 h for 7-20 days. Monosporic isolates were obtained from each of the isolates. Perithecia or conidia from isolates growing on PDA or PDA + streptomycin were placed in a drop of deionized water on a microscope slide. Perithecia were covered with a cover slip and were crushed to release the ascospores by gently pressing down the cover slip. Then the crushed perithecia and ascospores were washed with a few drops of sterile deionized water into a Petri dish containing water agar (WA). Conidia were directly washed from slides into the WA dishes. WA dishes containing crushed perithecia or conidia were incubated at 25ºC and constant light for 10-15 h until the spores germinated. Single germinated spores were transferred to another Petri dish containing WA. Fungal colonies

emerging from WA isolates were hyphal-tip-transferred onto dishes of PDA. Monosporic isolates were stored desiccated on filter paper at 5ºC as described previously (9).

1.3.3. Morphological characterization. A preliminary morphological characterization of the isolates was conducted with 8 to 15-day-old cultures based on colony color, conidial shape, the ability to produce perithecia in culture, and the distribution of conidial masses and perithecia within the colonies. Before monosporic isolates were desiccated and stored, each 8 to15-day-old culture was characterized again based on the same morphological traits used in the preliminary characterization.

1.3.4. Vegetative compatibility tests. Nitrate non-utilizing mutants and testers were generated as previously described (8,26). *Nit* mutants were recovered by transferring fast-growing sectors of monosporic isolates growing on minimal agar medium containing potassium chlorate to a minimal agar medium without chlorate. For isolates of *G. cingulata* and *C. gloeosporioides*, the medium was amended with 1.5 to 3.0% potassium chlorate, and for isolates of *C. acutatum* with 4.5 to 6.0% potassium chlorate. Isolates that grew as thin expansive colonies with no aerial mycelium on minimal medium were considered *nit* mutants. One *nit* mutant per monosporic isolate was recovered and stored desiccated on filter paper at 5°C to use in the complementation tests. Sixteen pairs of complementing *nit* mutant testers were identified among the monosporic isolates. Each pair of testers was selected from 40-70 *nit* mutants from the same isolate. One of the nits that complemented with at least three other *nit* mutants was used as one of the testers. Monosporic isolates of each pair of testers were obtained as described above and then were stored desiccated on filter paper at

5°C. Testers were paired in all possible combinations to verify that each pair represented a different VCG. Vegetative compatibility of all *nit* mutants generated from the collection of 486 monosporic isolates was determined by pairing them on minimal medium with at least one of the testers. The formation of dense aerial wild-type mycelium where the *nit* mutants came in contact with each other indicated that the isolates were vegetatively compatible and belonged to the same VCG.

1.3.5. Population structure. Two orchards of cv. Granny Smith located in Wilkes Co. (NC 2) and Lincoln Co. (NC 4) in North Carolina were surveyed to study the structure of the population of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* associated with bitter rot of apples. Fruit samples were obtained from the orchard located in Wilkes Co. in 2001 and 2002, and from the orchard in Lincoln Co. in 2002. Samples were collected every 2 weeks from June through September, four times in 2001 and three times in 2002. In the Wilkes Co. orchard, samples of eight fruit showing typical symptoms of bitter rot were taken from each of 20 arbitrarily selected trees within every two rows in the orchard, for a total of 160 fruit per sample date. In order to eliminate biases in the selection of infected fruit, each tree was divided into four sampling quadrats and subsamples containing two fruit were collected from each quadrat. A predetermined height, depth and angle were used to establish the position in the tree canopy to collect the fruit. Each of the four quadrats within the 20 trees was tagged with numbers from 1 to 4, and different heights, depths and angles were randomly selected for each quadrat on each sample date. Sample measures for quadrats with same numbers were equal for all trees and were reselected every sample date. In the orchard located in Lincoln Co., eight-fruit samples were collected from an arbitrarily selected tree within each

of the 18 rows in the orchard, for a total of 144 fruit per sample date. Because bitter rot incidence was low in this orchard, fruit samples were collected arbitrarily from any part of the tree canopy to complete the eight-fruit sample.

Fungi were recovered from small pieces cut from an arbitrarily selected lesion on each fruit collected, as described above. Although the sampling protocol could have resulted in 160 isolates from the Wilkes Co. orchard and 144 isolates from the Lincoln Co. orchard for each sample date, some trees did not have enough symptomatic fruit to obtain the 8-fruit sample, and some cultures were contaminated with bacteria or fungi not associated with bitter rot. Consequently, in the Wilkes Co. orchard a total of 101, 88, 140, and 143 isolates were recovered in 2001 on the 4 sample dates, and a total of 129, 130, and 114 on the 3 sample dates in 2002. In the Lincoln Co. orchard, 26, 123, and 106 isolates were recovered on the 3 sample dates in 2002. All fruit isolates recovered in this experiment were differentiated based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture. However, Appendix 1.1 contains only the morphological characterization for the subsample of isolates selected for the vegetative compatibility test. A total of 72 and 52 isolates were selected from the Wilkes Co. orchard in 2001 and 2002, respectively. In 2002, 54 isolates were selected from the Lincoln Co. orchard. These subsamples included isolates from each of the different morphological types observed in the original sample.

1.4. RESULTS

1.4.1. Morphological characterization. Twelve morphological types were observed among the isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* examined (Table

1.2). Isolates of *G. cingulata* and *C. gloeosporioides* produced cylindrical conidia with rounded ends and were differentiated by the ability of the self-fertile isolates to produce perithecia in culture. Isolates of *G. cingulata* were separated into four morphological types (SP1, SP2, SP3, and CP) mainly distinguished by the distribution of perithecia in culture (Fig. 1.1). Although morphological characteristics of SP3 isolates were similar to those of SP1 isolates, perithecia produced by SP3 isolates were sterile. SP3 isolates were never recovered directly from fruit or leaves, and originated only from the segregation of CP monosporic isolates. All morphological types of *G. cingulata* produced dark colored acervuli. Isolates of *C. gloeosporioides* were separated into five morphological types (SS1, SS2, SS3, SS4, and SS5) mainly differentiated by the distribution of conidial masses in culture and colony color (Fig. 1.1). Three morphological types were found among isolates of *C. acutatum* [SSC, SSNC, and SSNC(O)]. These morphological types were distinguished by the presence of red pigment in culture and colony color (Fig 1.2). All morphological types of *C. acutatum* produced fusiform conidia with pointed ends and none formed perithecia in culture. Conidia were produced mostly within the mycelium and some in orange masses scattered within the colonies. Detailed characteristics of the morphological types found within *G. cingulata, C. gloeosporioides* and *C. acutatum* are presented in Table 1.2.

Isolates of *G. cingulata* were recovered from either fruit or leaves, or both, collected in all orchards surveyed, except the orchard in Alabama and Brazil 4. SP1 isolates were isolated from fruit and leaves collected in all the orchards of cv. Gala sampled, and in the orchard of cv. Molly's Delicious from Ohio. SP2 isolates were obtained from fruit collected in two orchards of cv. Granny Smith in North Carolina (NC 2 and NC 4). CP isolates were obtained from fruit from all orchards sampled in the US, except from the orchard of cv. Gala

located in Georgia and from the orchard of cv. Golden Delicious located in Alabama. CP cultures were also recovered from leaves from the orchards of cv. Gala in Tennessee. SP3 isolates originated from CP cultures, which were recovered from Granny Smith fruit collected in North Carolina from the NC 2 and NC 4 orchards.

SS1 isolates of *C. gloeosporioides* were recovered from fruit collected from orchards of cv. Granny Smith (NC 2) and Delicious (NC 3) located in North Carolina. SS2 and SS3 morphological types were found among isolates obtained from fruit from the orchard of cv. Golden Delicious located in Alabama and North Carolina (AL and NC 3) and from the orchard of cv. Delicious in North Carolina (NC 3). Isolates with morphological types SS4 and SS5 were recovered from fruit collected from the orchards of cv. Delicious and Golden Delicious located in North Carolina (NC 3).

SSNC(O) isolates of *C. acutatum* were recovered from fruit and leaf samples from three orchards of cv. Gala, Golden Delicious, and Fuji located in Brazil (Brazil 2, 4, and 8). SSC and SSNC isolates were obtained from fruit collected from the orchards of cv. Granny Smith (NC 2) and Gala (NC 1) located in North Carolina.

1.4.2. Vegetative compatibility groups and morphological types. Three-hundred and twenty-five isolates of *G. cingulata*, 36 isolates *C. gloeosporioides*, and 23 isolates of *C. acutatum* were characterized based on vegetative compatibility (Appendix 1.1). Among these isolates 384 isolates were separated into 16 VCGs. Six VCGs were found within isolates of *G. cingulata* (VCGs 1, 2, 3, 4, 5, and 6). VCGs 1, 2, 3, 4, and 5 had the same morphological type (SP1). Isolates within VCG-6 were either CP or SP3-type cultures. Some isolates within VCG-2 were also characterized as SP2-type cultures (Table 1.3). One

hundred and thirty-two of the 325 isolates of *G. cingulata* belonged to VCG-1. These isolates were recovered from fruit and leaves from all the orchards of cv. Gala sampled in the US (NC 1, GA, and TN 1), except TN 2. VCG-2 isolates were found among 11 and 64 isolates recovered from Gala (NC 1) and Granny Smith (NC 2 and NC 4) fruit, respectively, collected in North Carolina. Three isolates obtained from Molly's Delicious fruit collected in Ohio belonged in VCG-3. Twenty-four isolates recovered from Gala leaves from orchards in Brazil (Brazil 1, 2, 3, 5, 6, 7, and 8) were placed in VCG-4 and VCG-5. Eighty-five isolates of *G. cingulata* were found within VCG-6. These isolates were recovered from fruit collected from orchards of the cv. Granny Smith, Delicious, and Golden Delicious located in North Carolina (NC 1, NC 2, NC 3, and NC 4). A total of 51 isolates of *C. acutatum*, 38 isolates *C. gloeosporioides*, and 13 isolates of *G. cingulata* were not compatible with any of the VCGs identified.

Six VCGs were found among isolates of *C. gloeosporioides*. Eleven isolates from Alabama recovered from Golden Delicious fruit were separated into VCG-7 and VCG-8, which were differentiated into two morphological types, SS2 and SS3, respectively. Nineteen fruit isolates recovered from the orchard of cv. Granny Smith in North Carolina (NC 2 and NC 4) belonged in VCG-9 and VCG-10 and had the same morphological type (SS1). VCG-11 and VCG-12 included five fruit isolates recovered from the orchard of cv. Delicious located in North Carolina (NC 3). VCG-11 isolates were characterized as SS3-type cultures and VCG-12 isolates as SS5-type cultures.

Isolates of *C. acutatum* separated into four VCGs (VCGs 13, 14, 15, and 16). VCG-13 and VCG-14 isolates had the same morphological type, SSNC(O), and included 17 isolates from Brazil recovered from fruit and leaves collected from orchards of cv. Gala,

Golden Delicious, and Fuji (Brazil 4, 2, and 8). Six isolates of *C. acutatum* obtained from Granny Smith fruit from the NC 2 orchard in North Carolina were separated into two VCGs, VCG-15 and VCG-16, which were also differentiated into two morphological types, SSC and SSNC, respectively.

1.4.3. Population structure. Isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* were obtained in 2001 and 2002 from the orchard of cv. Granny Smith located in Wilkes Co., North Carolina (NC 3) (Fig. 1.3). However, only isolates of *G. cingulata* and *C. acutatum* were found in the orchard of cv. Granny Smith located in Lincoln Co., North Carolina (NC 4) (Fig. 1.4). In both orchards, isolates of *G. cingulata* were more abundant than isolates of *C. acutatum*. In the Wilkes Co., orchard isolates of *G. cingulata* and *C. acutatum* comprised 60 to 86% and 11 to 29% of the population, respectively, and 96 to 100% and 1 to 4%, in the Lincoln Co. orchard. Isolates of *C. gloeosporioides* were the least common isolates in the Wilkes Co. orchard, representing only 2 to 4% of the population (Figs. 1.3 and 1.4). Two morphological types were found within isolates of *G. cingulata* in the two orchards, SP2 and CP. Isolates of *C. acutatum* were also separated in two morphological types (SSC and SSNC), and isolates of *C. gloeosporioides* were characterized as SS1-type cultures. Differences in frequencies among the different morphological types found within the two orchards remained relatively similar through out the season, both in 2001 and 2002. However, in October of 2001 the number of SP2 isolates was proportionally higher than at other sample dates throughout the season. In October of 2002, CP isolates were more abundant than at any other time of the season; the other morphological types only

represented 4 to 8% of the population. In addition, more isolates of *G. cingulata* were found in 2002 than in 2001 in the Wilkes Co. orchard.

CP isolates were the most abundant isolates obtained from the Wilkes Co. orchard, with frequencies between 40 to 78%. In the Lincoln Co. orchard, CP isolates represented 26 to 46% of the population, but SP2 isolates had higher frequencies that ranged from 54 to 73%. SP2 isolates comprised 8 to 38% of the population in the Wilkes Co. orchard. SSNC isolates were only found in the Wilkes Co. orchard and represented 4 to 10% of the population. SSC isolates were more abundant than SSNC isolates, with frequencies between 6 to 29% in the Wilkes Co. orchard and 1 to 4% in the Lincoln Co. orchard.

CP and SP2 isolates from the Wilkes Co. orchard and the Lincoln Co. orchard belonged in VCG-6 and VCG-2, respectively (Appendix 1.1). SS1-type isolates obtained from the Wilkes Co. orchard belonged in VCG-9 and VCG-10. All isolates of *C. acutatum* examined for vegetative compatibility were obtained from the Wilkes Co. orchard, and most of them were not compatible with any of the VCGs that included isolates of C. *acutatum*. However, two SSC-type isolates and two SSNC-type isolates were separated into VCG-15 and VCG-1, respectively.

1.5. DISCUSSION

Morphological characterization and vegetative compatibility analysis successfully distinguished isolates of *G. cingulata* from apple from those of *C. gloeosporioides* and *C. acutatum*. Furthermore we were able to distinguish isolates of *G. cingulata* associated with GLS in the US and Brazil. Using these criteria we were also able to separate isolates within *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* into different morphological types and vegetative compatibility groups. We found the greatest morphological and genetic diversity

within isolates of *C. gloeosporioides*. Variability within isolates of *G. cingulata* was similar to that of isolates of *C. acutatum*.

Morphological characterization of isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* based on colony color, conidial shape, and the ability to produce perithecia in culture, which are morphological traits commonly used to differentiate these three taxa (2,4,18,19,20,27), clearly separated isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* from apple to the species level. The production of a red pigment in culture and light orange or gray-olive colonies, and fusiform conidia with pointed ends, were the main morphological characters that distinguished isolates of *C. acutatum* from isolates of *G. cingulata* and *C. gleosporioides* that produced light to dark gray colonies and cylindrical conidia with rounded ends. The ability of isolates of *G. cingulata* to produce perithecia in culture (self-fertility) separated them from isolates of *C. gloeosporioides.* Isolates of *C. acutatum* also lacked the ability to produce perithecia in culture. Shi *et al*. (27) observed similar morphological differences among isolates of *Colletotrichum* obtained from apple fruit with bitter rot symptom.

Colony color and the production of a red pigment in culture were also useful to distinguish three morphological types within *C. acutatum* and separate isolates of *C. acutatum* from the US and Brazil. Isolates from the US produced a red pigment in culture or gray-olive colonies, whereas those from Brazil produced light orange colonies. The chromogenic and non-chromogenic morphological types observed by Shi et al (27) within isolates of *C. acutatum* from apple fruit with bitter rot symptoms, coincide with our description of the morphological types of *C. acutatum* observed in the US. Isolates of *C. acutatum* obtained from rotted apples in New Zealand were also placed into two different

morphological groups with characteristics similar to those of the chromogenic and non-chromogenic isolates that we found in the US (22).

Colony color and mycelial growth were also useful characteristics for distinguishing different morphological types within isolates of *G. cingulata* and *C. gloeosporioides*. However, in order to distinguish all morphological types observed within isolates of *G. cingulata* and *C. gloeosporioides*, it was necessary to characterize the distribution of acervuli and perithecia within the colonies. In previous morphological studies, which included isolates of *C. gloeosporioides* from apple fruit with bitter rot symptoms, only colony color and conidial shape were considered, and all isolates were placed within the same morphological group (20,27). Struble and Keitt (28) distinguished two morphological types within isolates of *G. cingulata* from apple fruit with bitter rot symptoms that were mainly differentiated by colony color and the distribution of perithecia within the colony. The plus or clumped-perithecial (CP) type produced light colonies with perithecia in scattered clumped masses, and the minus or scattered-perithecial (SP) type produced dark colonies and perithecia singly or in groups of two or three perithecia over the entire colony. Additionally, they observed that SP-type isolates did not occur in isolates recovered from naturally infected fruit, but were derived from monosporic isolations of CP-type isolates. The characteristics of the CP-type isolates that we observed agree with those of CP-type isolates observed by Struble and Keitt (28). Although morphological characteristics of the SP-type isolates described by Struble and Keitt (28) are similar to those described in the present study for SP1 and SP3-type isolates, only SP3- isolates resulted from segregation of CP- isolates.

Sexual crosses between isolates of *C. gloeosporioides* should yield a large number of VCGs within field populations, because it is estimated that there are 3-7 *vic* loci within *C.*

gloeosporioides (9). Nevertheless, Correll *et al*. (9) found that only one or a few VCGs predominate in a given apple orchard. However, our study indicated that genetic diversity within *G. cingulata, C. gloeosporioides,* and *C. acutatum* from apples is relatively high because of the presence of multiple VCGs within *G. cingulata, C. gloeosporioides,* and *C. acutatum*, the cultivar specificity of some VCGs within the same species (more evident in *C. gloeosporioides*), and the fact that over one-half of the isolates of *C. gloeosporioides* and *C. acutatum* tested did not show compatibility with any of the VCGs. These observations provide additional evidence for sexual reproduction within populations of *C. gloeosporioides* and *C. acutatum* in apple orchards. Previous studies on vegetative compatibility within isolates of *C. gloeosporioides* from apple are consistent with our observations. Beever *et al* (3) observed six different VCGs within isolates of *C. gloeosporioides* recovered from apple fruit with bitter rot symptoms obtained from an orchard of cv. Granny Smith, and suggested that the existence of multiple VCGs within a cultivar indicated sexual reproduction between different VCGs, which will generate progeny with different combinations of *vic* genes. Furthermore, Correll *et al*. (9) stated that the high VCG diversity in populations of *C. gloeosporioides* from coffee and *C. acutatum* from apple might be evidence of sexual reproduction within individuals of these populations.

In this study, using morphological characterization and vegetative compatibility tests, we found that isolates of *G. cingulata* capable of causing GLS (Chapter 2) belonged to the morphological type SP1 and VCGs 1, 4, and 5, and were obtained only from fruit or leaves of cv. Gala. Although isolates of *C. acutatum* from Brazil were recovered from leaves they were not pathogenic to leaves and were likely growing saprophytically in necrotic tissue. Several types within isolates of *G. cingulata* could be distinguished using morphological

characteristics, but morphological characters were not sufficient to separate isolates capable of causing both GLS and bitter rot from isolates capable of causing bitter rot only. However, isolates of GLS were distinguished from isolates that caused bitter rot in the VCG analysis, placing the isolates of GLS in three different VCGs, which at the same time separated isolates of GLS from the US (VCG-1) from isolates of GLS from Brazil (VCG-4 and VCG-5). Isolates of *G. cingulata* from all locations in the US where GLS has been observed are included in VCG-1. Considering the ability of *G. cingulata* to sexually reproduce and the presence of GLS for over 20 years in Brazil, it was not surprising that isolates of GLS were observed in multiple VCGs. These results suggest that VCG-1, VCG-4, and VCG-5 probably represent clonal populations of *G. cingulata* within the US and Brazil, and are associated with GLS.

The VCG analysis suggests that there were genetic differences between strains of *G. cingulata* associated with GLS and bitter rot and those associated with bitter rot only, as well as within isolates of *G. cingulata* associated with both GLS and bitter rot. Therefore, VCGs cannot be used to determine the extent of the genetic differences between strains of *G. cingulata* that cause bitter rot and GLS. Consequently, additional studies involving molecular characterization of isolates of *G. cingulata* associated with GLS and bitter rot, described in Chapter 2, were conducted to clarify genetic and molecular relationships between GLS and bitter rot strains of *G. cingulata.*

The population structure of bitter rot fungi was more diverse in the Granny Smith orchard located in Wilkes Co. than in the Granny Smith orchard in Lincoln Co. *G. cingulata* was the predominant species in the two orchards. Two morphological types of *G. cingulata,* representing two different VCGs, were observed in the Wilkes Co. and Lincoln Co. orchards.

CP-type isolates of *G. cingulata* were predominant over SP2 isolates. The morphological characteristics of CP-type isolates coincide with descriptions of isolates of *G. cingulata* commonly associated with bitter rot (27,28). Additionally, in the morphological and genetic analysis, CP-type isolates that belonged to VCG-6 were observed in all orchards sampled in the US, regardless of the cultivar and location. *C. acutatum* was also represented by two morphological types that also belonged to two different VCGs. Chromogenic (SSC) isolates of *C. acutatum* predominated over non-chromogenic (SSNC) isolates. However, only the SSC type was present in the Lincoln Co. orchard. *C. gloeosporioides* was the least abundant species in the population in the Wilkes Co. orchard and it was not observed in the Lincoln Co. orchard. In a previous study, *C. acutatum* was the predominant bitter rot fungus present in most of the orchards sampled. Chromogenic and non-chromogenic isolates were observed within *C. acutatum*, and the chromogenic isolates predominated (27). *G. cingulata* and *C. gloeosporioides* were predominant species only in one and two orchards, respectively, of cv. Delicious.

The population structure of *Colletotrichum* species and *G. cingulata* associated with bitter rot of apples is influenced by environmental conditions, cultivar, sample date, sources of inoculum, management practices, and pesticide use (27). It is likely that a combination of these influences determine which species are initially introduced into an orchard and subsequently increase in numbers. For example, although some of the VCGs observed in the Granny Smith orchard in Wilkes Co. were also present in the Granny Smith orchard in Lincoln Co., those not present in the Lincoln Co. orchard were the same VCGs not present in an orchard of cv. Gala, located approximately 2 km away. Once the fungi are established in an orchard, the population structure appears to remain relatively stable from year to year as

well as throughout the growing season. Although we collected data for only 2 years, the relative frequencies of *G. cingulata, C. gloeosporioides,* and *C. acutatum* were similar from year to year, despite the fact that 2001 was a wet growing season and 2002 was very dry. Similarly, the population structure did not vary greatly among sample dates. In the Wilkes Co. orchard the relative frequencies of the three taxa was similar throughout both seasons. Thus, while environmental conditions influence the incidence and severity of bitter rot, they do not appear to have great influence on the population structure once it is initially established. The Wilkes Co. orchard has a history of high bitter rot incidence and typically is wetter than the Lincoln Co. orchard. It is possible that the fungicides used could affect the population structure, but there is no evidence that fungicides have any differential activity in the orchard. Additional studies are needed over more seasons and in more orchards to confirm our hypothesis that the population structure of *G. cingulata, C. gloeosporioides,* and *C. acutatum* remains relatively stable in an orchard once it is established.

1.6. LITERATURE CITED

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Table 1.1. Isolates of *C. acutatum, C. gloeosporioides*, and *G. cingulata* obtained from leaf and fruit samples collected from different apple orchards located in the US and Brazil

Species[y]	Geographical origin[z]	Source		Number of isolates recovered	Year collected
		Host tissue	Cultivar		
C. acutatum	Brazil 2	Leaf	Gala	2	n/a*
C. acutatum	Brazil 2	Fruit	Gala	2	n/a*
C. acutatum	Brazil 2	Fruit	Golden Delicious	1	n/a*
C. acutatum	Brazil 2	Fruit	Fuji	1	n/a*
C. acutatum	Brazil 4	Leaf	Gala	16	2001
C. acutatum	Brazil 8	Fruit	Gala	1	n/a*
C. acutatum	Brazil 8	Fruit	Fuji	1	n/a*
C. acutatum	Brazil 8	Fruit	Golden Delicious	2	n/a*
C. acutatum	NC 1	Fruit	Gala	1	2001
C. acutatum	NC 2	Fruit	Granny Smith	39	2001
C. acutatum	NC 2	Fruit	Granny Smith	7	2002
C. gloeosporioides	AL	Fruit	Golden Delicious	11	2001
C. gloeosporioides	NC 2	Fruit	Granny Smith	13	2001
C. gloeosporioides	NC 2	Fruit	Granny Smith	8	2002
C. gloeosporioides	NC 3	Fruit	Golden Delicious	14	2002
C. gloeosporioides	NC 3	Fruit	Delicious	28	2002
G. cingulata	Brazil 1	Leaf	Gala	7	2001
G. cingulata	Brazil 2	Leaf	Gala	1	n/a*
G. cingulata	Brazil 2	Fruit	Gala	1	n/a*
G. cingulata	Brazil 2	Bud	Gala	1	n/a*
G. cingulata	Brazil 3	Leaf	Gala	3	2001
G. cingulata	Brazil 5	Leaf	Gala	3	2001
G. cingulata	Brazil 6	Leaf	Gala	7	2001
G. cingulata	Brazil 7	Leaf	Gala	2	2001
G. cingulata	Brazil 8	Leaf	Gala	1	n/a*
G. cingulata	GA	Leaf	Gala	22	2000
G. cingulata	GA	Leaf	Gala	3	2002
G. cingulata	GA	Fruit	Gala	38	2000
G. cingulata	GA	Fruit	Gala	7	2002
G. cingulata	NC 1	Leaf	Gala	34	2000
G. cingulata	NC 1	Leaf	Gala	26	2002
G. cingulata	NC 1	Fruit	Gala	14	2001
G. cingulata	NC 1	Fruit	Gala	1	2002
G. cingulata	NC 2	Fruit	Granny Smith	27	2000
G. cingulata	NC 2	Fruit	Granny Smith	20	2001
G. cingulata	NC 2	Fruit	Granny Smith	35	2002

Table 1.1 Continued

Species[y]	Geographical origin[z]	Source		Number of isolates recovered	Year collected
		Host tissue	Cultivar		
G. cingulata	NC 3	Fruit	Golden Delicious	7	2002
G. cingulata	NC 3	Fruit	Delicious	4	2000
G. cingulata	NC 3	Fruit	Delicious	8	2002
G. cingulata	NC 4	Fruit	Granny Smith	51	2002
G. cingulata	NC 5	Fruit	Gala	1	2002
G. cingulata	OH	Fruit	Molly's Delicious	3	2001
G. cingulata	TN 1	Leaf	Gala	4	1998
G. cingulata	TN 2	Leaf	Gala	8	1998

[y] Species designation was assigned after morphological characterization.

[z] GA = Gala orchard located in Blue Ridge, Georgia; NC 1 = Gala orchard located in Lincoln Co., NC; NC 2 = Granny Smith orchard located in Wilkes Co., NC; NC 3 = Golden and Delicious orchards at the Central Crops Research Station Clayton, NC; NC 4 = Granny Smith orchard located in Lincoln Co., NC; NC 5 = Gala orchard located in Wilkes Co., NC; OH = Molly's Delicious orchard located in Ohio; Brazil 1,4,5,6,7,8 = Gala and Golden Delicious orchards located in Santa Catarina State in Brazil; Brazil 2,3 = Gala orchards located in Rio Grande do Sul State in Brazil; TN 1 = Gala orchard located Cleveland, TN; TN 2 = Gala orchard located in Buffalo Valley, TN; AL = Golden Delicious orchard located in Alabama.

n/a* = year collected not available. Isolates obtained from a collection of isolates maintained by Dr. Rosa Maria Sanhueza at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) Bento Gonçalves, RS, Brazil.

Table 1.2. Description of the morphological types of isolates of *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* based colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture [x]

Species	Morphological type	Perithecia		Acervuli and perithecia[y]	Colony	Conidial shape
		Fertility	Distribution			
G. cingulata	SP1	Self-fertile	Single or small groups over entire colony	*a*/*p*	Dark gray, sparse and appressed mycelium	Cylindrical with rounded ends
G. cingulata	SP2	Self-fertile	Scattered small groups	*a*/*p*	Gray and appressed mycelium	Cylindrical with rounded ends
G. cingulata	SP3	Sterile	Single or small groups over entire colony	*a*/*p*	Dark gray, sparse and appressed mycelium	Cylindrical with rounded ends
G. cingulata	CP	Self-fertile	Scattered clumps	*a*/*p*	Gray/white and abundant mycelium	Cylindrical with rounded ends
		Acervuli color	**Conidia distribution**			
C. gloeosporioides	SS1	Light	Small orange masses over entire colony	*a*	Light gray and appressed mycelium	Cylindrical with rounded ends
C. gloeosporioides	SS2	Dark	Small orange masses over entire colony	*a*	Light gray and abundant mycelium	Cylindrical with rounded ends
C. gloeosporioides	SS3	Dark	Large orange scattered masses	*a*	Light gray and abundant mycelium	Cylindrical with rounded ends
C. gloeosporioides	SS4	Dark	Small orange masses over entire colony	*a*	Dark gray and abundant mycelium	Cylindrical with rounded ends
C. gloeosporioides	SS5	Dark	Large orange scattered masses	*a*	Dark gray and abundant mycelium	Cylindrical with rounded ends
C. acutatum	SSNC(O)	n/a[z]	Mostly within mycelium	*a*	Light orange, no pigment	Fusiform with pointed ends
C. acutatum	SSC	n/a	Mostly within mycelium	*a*	Red pigment in culture	Fusiform with pointed ends
C. acutatum	SSNC	n/a	Mostly within mycelium	*a*	Gray-olive, no pigment	Fusiform with pointed ends

[x] Eight to 15 day-old isolates grown on PDA medium were characterized after incubation at 25°C with constant light.
[y] '*a*' indicates presence of acervuli in culture; '*p*' indicates presence of perithecia in culture.
[z] n/a = isolates did not produce acervuli in culture.

Table 1.3. Vegetative compatibility group, morphological type, origin, and source of the isolates of *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* examined for vegetative compatibility[x]

VCG	Morphological type[z]	Species	Number of isolates	Source		Geographical origin
				Cultivar	Host Tissue	
1	SP1	*G. cingulata*	86	Gala	Leaf	NC, GA, TN
1	SP1	*G. cingulata*	46	Gala	Fruit	NC, GA
2	SP1	*G. cingulata*	11	Gala	Fruit	NC
n/a[y]	SP1	*G. cingulata*	1	Gala	Fruit	Brazil
n/a	SP1	*G. cingulata*	1	Gala	Bud	Brazil
n/a	SP1	*G. cingulata*	1	Gala	Leaf	TN
2	SP2	*G. cingulata*	64	Granny Smith	Fruit	NC
3	SP1	*G. cingulata*	3	Molly's Delicious	Fruit	OH
4	SP1	*G. cingulata*	3	Gala	Leaf	Brazil
5	SP1	*G. cingulata*	21	Gala	Leaf	Brazil
6	CP	*G. cingulata*	85	Granny Smith, Red and Golden Delicious	Fruit	NC
n/a	CP	*G. cingulata*	10	Gala	Leaf	TN
6	SP3	*G. cingulata*	6	Granny Smith	Fruit	NC
7	SS2	*C. gloeosporioides*	5	Golden Delicious	Fruit	AL
8	SS3	*C. gloeosporioides*	6	Golden Delicious	Fruit	AL
9	SS1	*C. gloeosporioides*	18	Granny Smith	Fruit	NC
10	SS1	*C. gloeosporioides*	2	Granny Smith	Fruit	NC
n/a	SS1	*C. gloeosporioides*	3	Delicious	Fruit	NC
11	SS3	*C. gloeosporioides*	2	Delicious	Fruit	NC
n/a	SS3	*C. gloeosporioides*	10	Granny Smith, Red and Golden Delicious	Fruit	NC
n/a	SS4	*C. gloeosporioides*	8	Delicious	Fruit	NC
12	SS5	*C. gloeosporioides*	3	Delicious	Fruit	NC
n/a	SS5	*C. gloeosporioides*	17	Red, Golden Delicious	Fruit	NC
13	SSNC(O)	*C. acutatum*	8	Gala	Leaf	Brazil
14	SSNC(O)	*C. acutatum*	7	Gala	Leaf	Brazil
14	SSNC(O)	*C. acutatum*	2	Golden Delicious and Fuji	Fruit	Brazil
n/a	SSNC(O)	*C. acutatum*	3	Gala	Leaf	Brazil
n/a	SSNC(O)	*C. acutatum*	6	Gala, Golden Delicious and Fuji	Fruit	Brazil
15	SSC	*C. acutatum*	3	Granny Smith	Fruit	NC
n/a	SSC	*C. acutatum*	21	Granny Smith, Gala	Fruit	NC
16	SSNC	*C. acutatum*	3	Granny Smith	Fruit	NC
n/a	SSNC	*C. acutatum*	21	Granny Smith	Fruit	NC

[x] A subsample of 87 isolates of *G. cingulata* (42 from fruit and 45 from leaves), six fruit isolates of *C. gloeosporioides* and five isolates of *C. acutatum* (four from fruit and one from leaves), that were included in the VCG analysis were tested for fruit and leaf pathogenicity as described in Chapter 2. All isolates were pathogenic on fruit. Only 10 fruit isolates and 40 leaf isolates of *G. cingulata* that belong to VCGs 1, 3, and 4 were pathogenic on leaves.

[y] n/a = isolates with this morphological type, origin, and source were not compatible with any of the VCGs.

[z] Morphological types previously described in the results and Table 1.2 based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture.

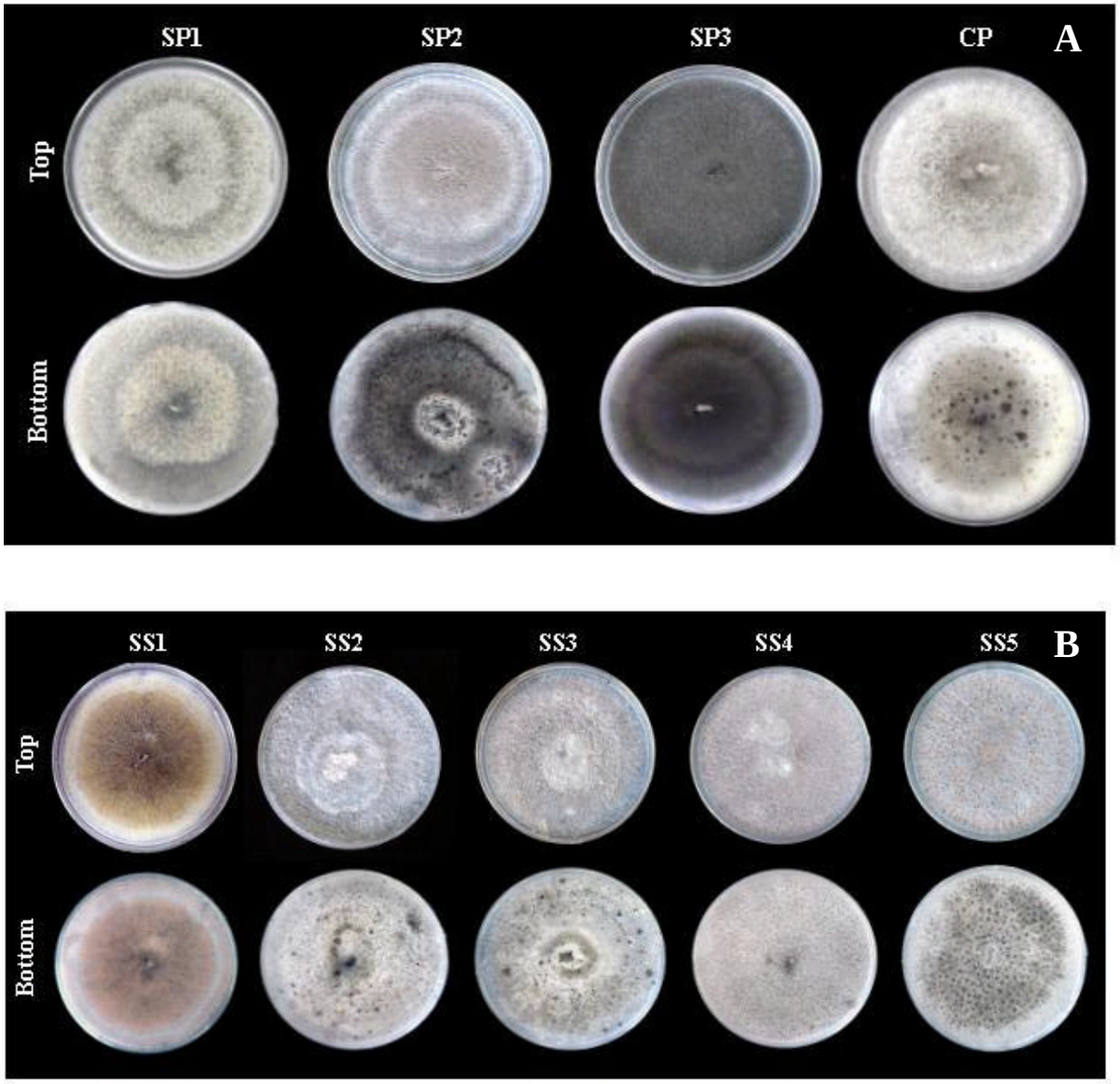

Figure 1.1. Morphological types found among isolates of *G. cingulata* (A) and *C. gloeosporioides* (B) obtained from symptomatic fruit and leaves collected in different orchards located in the US and Brazil. Characterization was based on colony color, conidial shape, the ability to produce perithecia in culture, and the distribution of conidial masses and perithecia within the colonies.

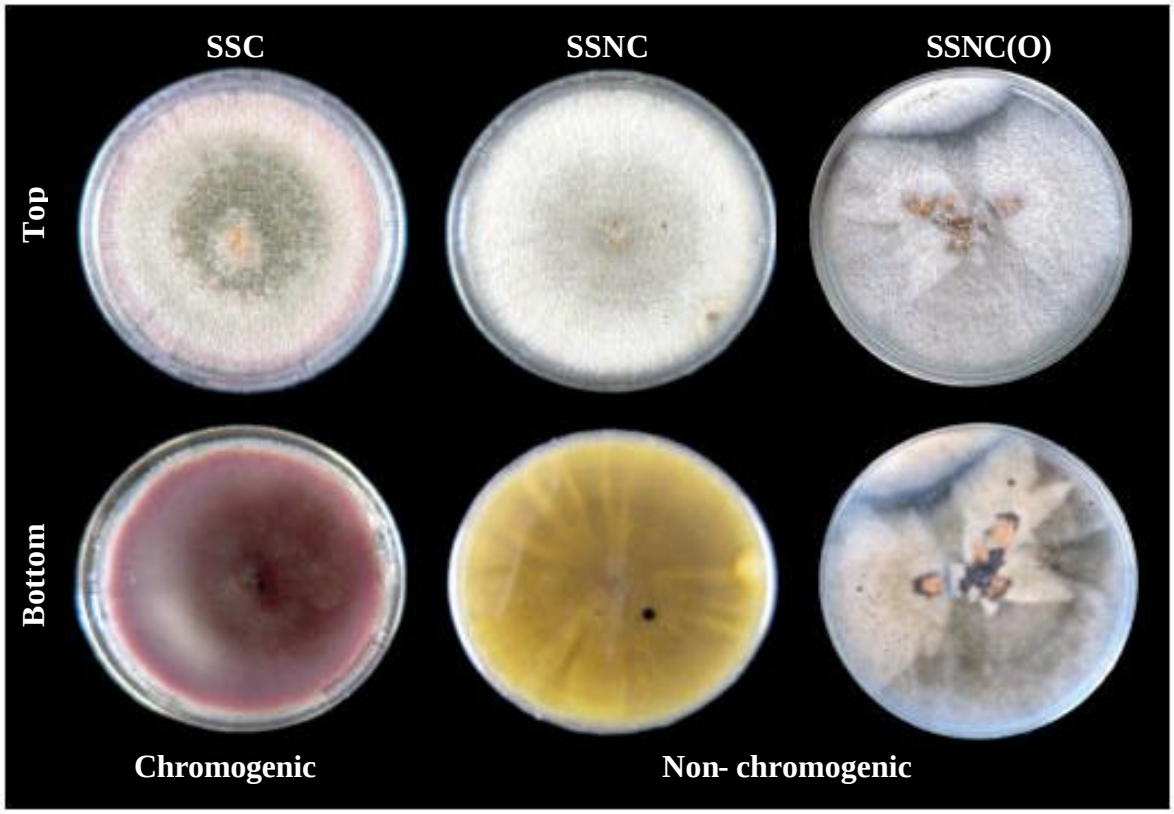

Figure 1.2. Chromogenic and non- chromogenic morphological types found among isolates of *C. acutatum* obtained from symptomatic fruit and leaves collected in different orchards located in the US and Brazil. Characterization was based on colony color, conidial shape, the ability to produce perithecia in culture, and the distribution of conidial masses and perithecia within the colonies.

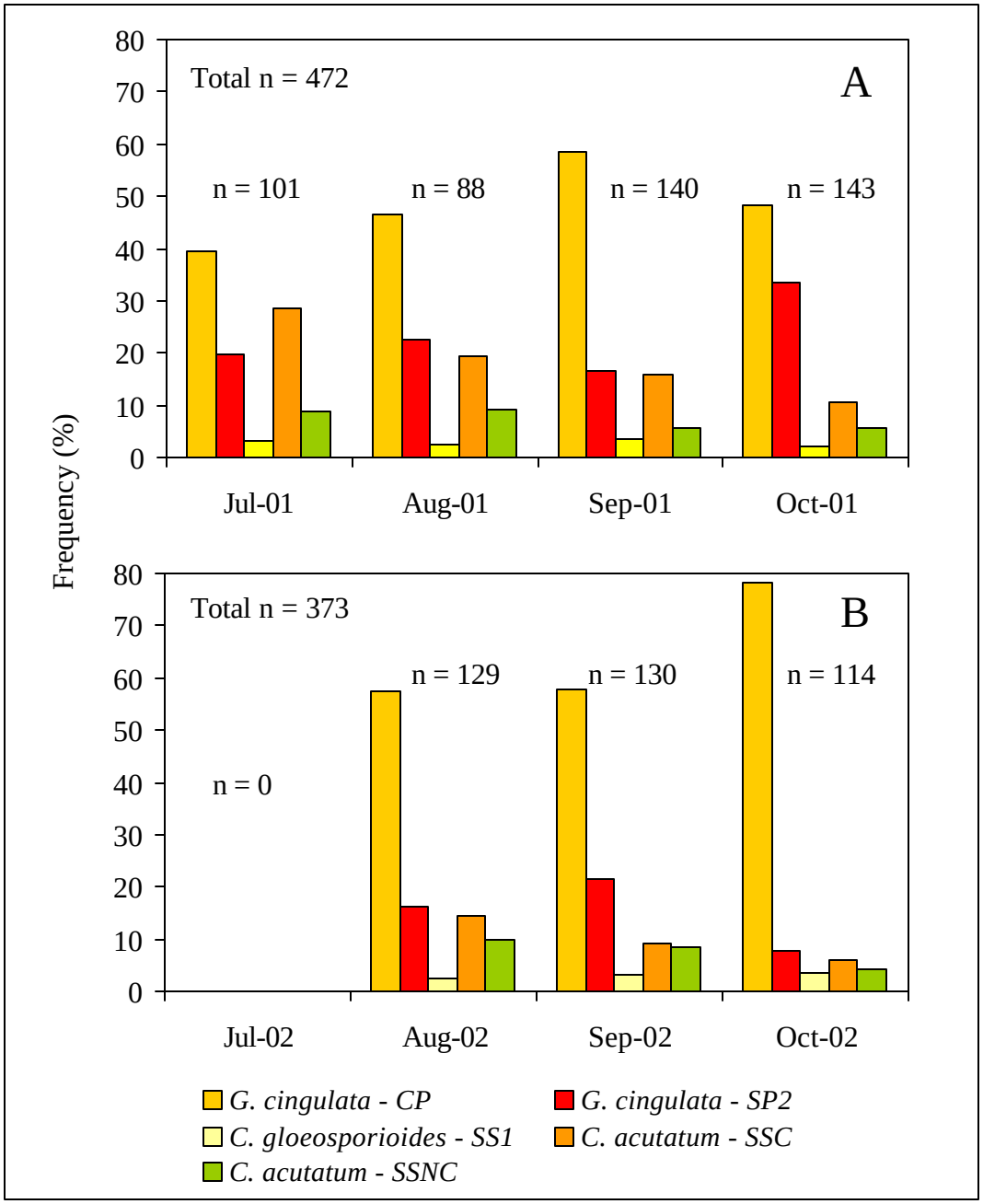

Figure 1.3. Frequencies of the morphological types of fruit isolates of *C. acutatum* (SSC and SSNC), *C. gloeosporioides* (SS1) and *G. cingulata* (CP and SP2) recovered from an orchard of cv. Granny Smith located in Wilkes Co., NC during 2001 and 2002. A. 2001. B. 2002. Fruit samples were collected monthly from 20 arbitrarily selected trees within the orchard. Morphological types were previously described in the results and Table 1.2 based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture. Frequency represents the percentage of isolates of a certain morphological type with respect to the total number of isolates recovered in each sampling date. Frequencies based on the total of isolates recovered for each sample date. Total n = total number of fruit collected; n = number of fruit collected per sample date.

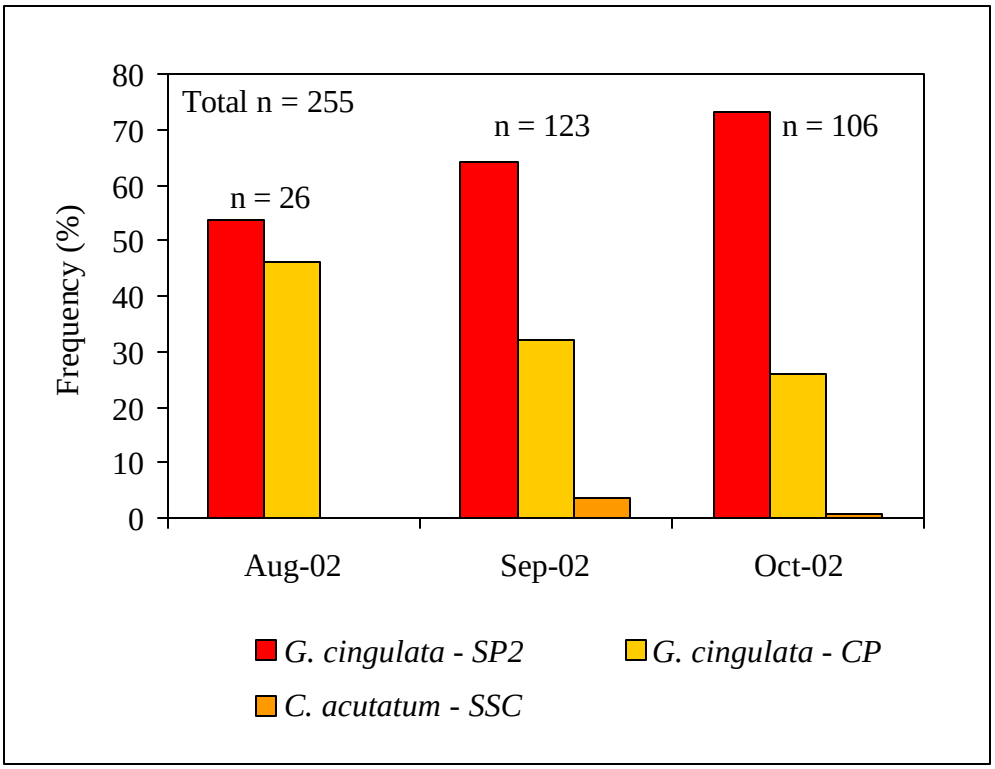

Figure 1.4. Frequencies of the morphological types of fruit isolates of *C. acutatum* (SSC) and *G. cingulata* (CP and SP2) recovered from an orchard of cv. Granny Smith located in Lincoln Co., NC during 2002. Fruit samples were collected monthly from 18 arbitrarily selected trees within the orchard. Morphological types were previously described in the results and Table 1.2 based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture. Frequency represents the percentage of isolates of a certain morphological type with respect to the total number of isolates recovered in each sampling date. Total n = total number of fruit collected; n = number of fruit collected per sample date.

2. CHAPTER II

Characterization of *Colletotrichum* spp causing Glomerella leaf spot and bitter rot of apples based on molecular, cultural, and pathogenicity tests

2.1. ABSTRACT

One hundred and fifty-five isolates of *Glomerella cingulata* (93 from fruit, 61 from leaves, and one from buds), 42 isolates of *Colletotrichum gloeosporioides* from fruit and 14 isolates of *C. acutatum* (10 from fruit and 4 from leaves), collected from orchards located in the US and Brazil, and previously characterized based on morphology and vegetative compatibility, were characterized based on mtDNA RFLP haplotypes. A subset of 24 isolates was further characterized by examining the sequence of a 200 bp intron of the glyceraldehyde 3-phosphate dehydrogenase (GDPH) gene. Ninety-eight isolates were also tested for pathogenicity on leaves of cultivars Gala and Golden Delicious in the greenhouse, and 24 isolates were tested for pathogenicity on fruit of cv. Gala in growth chambers. Growth rate, sensitivity to benomyl, and optimum growth temperature were determined for a subset of the isolates. Eight different mtDNA RFLP haplotypes were observed within isolates of *G. cingulata* (G1, G1.1, G2, G2.1, G3, G4, A3, and A3.1), two within isolates of *C. gloeosporioides* (B2 and B3), and two within isolates of *C. acutatum* (C1 and D1). Haplotypes G1 and A3 predominated within isolates of *G. cingulata* from the US. All isolates of *G. cingulata* from Brazil belonged to haplotypes G3 and G4. All isolates tested were pathogenic on fruit. Only isolates with haplotypes G1, G1.1, G3, and G4 were capable of causing Glomerella leaf spot (GLS). Phylogenetic trees constructed based on Neighboring-Joining and Maximum Parsimony methods, using the intron sequence, produced similar topologies. Each species was separated into distinct groups. Vegetative

compatibility was a better indicator than molecular characters for distinguishing isolates of *G. cingulata* pathogenic on both leaves and fruit from the ones pathogenic only on fruit. Isolates of *G. cingulata* capable of causing both GLS and bitter rot were included in haplotypes and phylogenetic groups that also included isolates capable of causing bitter rot only. Additionally, isolates of *G. cingulata* from the US and Brazil which cause GLS were included in different haplotypes and phylogenetic groups. Therefore, our results suggest that isolates of *G. cingulata* from the US capable of causing both GLS and bitter rot arose independently of Brazilian isolates of *G. cingulata,* and may have arisen from isolates of *G. cingulata* from the US that originally were capable of causing bitter rot only. Slower growth, lower optimum growth temperature, and less sensitivity to benomyl distinguished isolates of *C. acutatum* from isolates of *G. cingulata* and *C. gloeosporioides*. These parameters were not useful for distinguishing between isolates of *G. cingulata* and *C. gloeosporioides* or within mtDNA haplotypes or VCGs of each species.

2.2. INTRODUCTION

Bitter rot is a common disease in practically all countries where apples are commercially grown, and in warmer and moist growing regions crop loss can reach up to 50% (28). Bitter rot symptoms usually appear as circular lesions with concentric rings of reproductive structures on the fruit surface. The disease was first described by Berkeley in England in 1856, and was identified in the United States in 1867 (28). Three taxa, *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc., and *C. acutatum* J.H. Simmonds, can cause bitter rot (28).

In the early 1980s, Leite *et al.* (23) described a new apple leaf spot observed on the cultivars Gala and Golden Delicious in Paraná State in Brazil. The disease was named Glomerella leaf spot (GLS), and has been primarily associated with perithecial isolates of *C. gloeosporioides*. Since this disease was first reported, it has increased in severity and has become a great concern to Brazilian apple growers, because the most widely grown cultivars, Gala and Golden Delicious, are highly susceptible to the disease. Under favorable conditions GLS can exceed in 75% defoliation by harvest, weakening apple trees and reducing yield (7,23,29). Additionally, GLS has been observed on other cultivars of commercial importance grown in Brazil, such as Granny Smith and Pink Lady (Dr. Rosa Maria Sanhueza; *personal communication*, 2002).

GLS was first reported in the US in 1998 as a severe leaf spot on cv. Gala apples in two orchards in eastern Tennessee, which resulted in extensive defoliation (16). In 1999 the disease was also observed in two orchards of cv. Gala, one located in Georgia and one in North Carolina. However, experiments conducted in Georgia (31) with a strain of *G. cingulata* suggest that strains of the fungus capable of causing the leaf spot were present

before 1998. Taylor (31) reported that a strain of *G. cingulata* caused a leaf spot on leaves of Golden Delicious following inoculations in the greenhouse, and associated it with leaf blotch symptoms and defoliation observed in orchards of Golden Delicious throughout the US named necrotic leaf blotch. However, necrotic leaf blotch of Golden Delicious has been shown to be a physiological disorder, triggered by a combination of environmental factors including low light intensity and high soil moisture (29). Therefore, Sutton and Sanhueza (29) hypothesized that the leaf spot symptoms described by Taylor and caused by a strain of *G. cingulata* were similar to those of GLS in Brazil, and not the physiological disorder necrotic leaf blotch.

Although GLS and bitter rot are associated with the same species (23), differences in morphological and cultural characteristics and pathogenicity between isolates from the leaf spot and those from bitter rot apples have been observed. The GLS fungus produces perithecia, asci, and viable ascospores, almost invariably, whereas the bitter rot fungus does not. In addition, GLS isolates initially produce white and cottony colonies in culture, which later become light to medium-gray with white borders, while colonies of the bitter rot fungus usually produce a pinkish color in PDA (31). When leaves and fruit are inoculated with isolates of the GLS fungus, both a leaf spot and a fruit rot are produced, but if they are inoculated with isolates of the bitter rot fungus, symptoms are produced only on the fruit (23,31). The differences between the GLS and the bitter rot fungi and the considerable cultural, morphological, genetic and molecular variability that has been documented between and within populations of *C. gloeosporioides*, *C. acutatum* and *G. cingulata* (4,10,11,13,15,17,20,27,32,34), suggest that the diversity among these pathogens is high.

Molecular characteristics have been useful for separating isolates of *C. acutatum* from isolates of *C. gloeosporioides* (1,2,12,13,14,20,32), and isolates of *C. gloeosporioides* from isolates *G. cingulata* (17,18), and for determining the variability of isolates within *C. acutatum* (1,6,12,14,15,22,30), *C. gloeosporioides* (11,34) and *G. cingulata* (17,18). Using mtDNA RFLPs, Guerber *et al*. (18) distinguished five haplotypes among isolates of *C. acutatum*; two haplotypes among isolates of *C. gloeosporioides*; and four haplotypes among isolates of *G. cingulata.* All isolates were obtained from apple fruit collected in different locations. Additionally, they sequenced a 1 kb intron of the glutamine synthetase (GS) gene and a 200 bp intron of the glyceraldehyde 3-phosphate dehydrogenase (GDPH) gene, both found in 10 species of *Colletotrichum* including *C. gloeosporioides*, *C. acutatum* and *G. cingulata,* and found that isolates within the same species clustered, suggesting that sequence analysis of these two introns can provide a high level of resolution for determining inter and intra-specific diversity and phylogenetic relationships among species of *Colletotrichum*.

The purpose of this study was to clarify the genetic relationship among isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum* that cause bitter rot and/or GLS of apples using mtDNA RFLPs and sequence analysis of a 200 bp intron of the GDPH gene. The pathogenicity of a subset of the isolates associated with GLS and bitter rot on leaves and fruit was also studied.

2.3. MATERIALS AND METHODS

2.3.1. Origin of the isolates. Isolates used for the experiments conducted in this study were obtained from a collection of isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* recovered from symptomatic fruit and leaves collected from apple orchards located

in the US and Brazil during 1998, 2000, 2001, and 2002, which were previously characterized based on morphology and vegetative compatibility criteria (Table 2.1). In the US, leaf isolates of *G. cingulata* were obtained from four different orchards of cv. Gala, including two orchards in eastern Tennessee where the leaf spot was first reported in the US (TN 1 and TN 2), one in Georgia (GA) and one in North Carolina (NC 1). Fruit isolates of *G. cingulata* were also recovered from the same orchards located in Georgia and North Carolina. Isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* recovered from fruit were also obtained from an orchard of cv. Golden Delicious in Alabama (AL), an orchard of cv. Molly's Delicious in Ohio (OH), and from two orchards of cv. Granny Smith (NC 2 and NC 4), one of cv. Delicious (NC 3), and one of cv. Golden Delicious (NC 3) located in North Carolina. Isolates of *G. cingulata* and *C. acutatum* from Brazil were recovered from symptomatic leaves collected by Dr. Rosa Maria Sanhueza from different orchards of cv. Gala located in Rio Grande do Sul State (Brazil 3) and Santa Catarina State (Brazil 1, 4, 5, 6 and 7). These isolates included isolates obtained from fruit, leaves, and buds collected from orchards of cv. Gala, Golden Delicious, and Fuji located in Rio Grande do Sul State (Brazil 2) and Santa Catarina State (Brazil 8). Cultures of *G. cingulata* and *C. acutatum* that belong to a collection of isolates maintained by Dr. Sanhueza at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil, were also part of the collection of monosporic isolates examined in this study.

2.3.2. Isolations and monosporic isolates. Isolations and generation of monosporic isolates were conducted as previously described (Chapter 1). Isolations from fruit were made onto Petri dishes containing potato dextrose agar (PDA), and isolations from leaves onto

Petri dishes containing PDA + streptomycin (200 µg/ml). Petri dishes were incubated in a growth chamber at 25°C with continuous light for 7-20 days. Ascospores or conidia from colonies within the dishes were used to obtained monosporic isolates from each of the isolates by transferring single germinated conidia or ascospores from a Petri dish containing water agar (WA) to a new WA Petri dish. Fungal colonies emerging from isolates growing on WA were hyphal-tip-transferred onto dishes of PDA. Eight to 15-day-old monosporic isolates were previously characterized morphologically based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture (Chapter 1). Following morphological characterization, monosporic isolates were stored desiccated on filter paper at 5ºC as described before (8).

2.3.3. Pathogenicity tests. Eighty-seven isolates of *G. cingulata* (42 from fruit and 45 from leaves), six fruit isolates of *C. gloeosporioides* and 5 isolates of *C. acutatum* (4 from fruit and 1 from leaves) (Appendix 4.2) were selected from the collection of monosporic isolates to conduct leaf pathogenicity tests on apple trees of cv. Gala. The pathogenicity of 12 isolates of *G. cingulata* (seven from fruit and five from leaves and pathogenic on leaves of Gala), 6 isolates of *C. gloeosporioides* from fruit and six isolates of *C. acutatum* (three from fruit and three from leaves) were also tested on trees of cv. Golden Delicious (Table 2.4). Trees used for inoculations were maintained in green house conditions and were cut back to two buds approximately 4 weeks before inoculations in order to generate 1-2 new shoots with 14-40 leaves each. Trees were maintained for 24 h in humidity chambers at ambient temperature (~24°C) before inoculation. After the preconditioning period, trees were removed from the humidity chambers and sprayed until runoff with spore suspensions (1 X

10^5 spores/ml) of 7 to14-day-old monosporic isolates. One tree was inoculated with each of the isolates selected for this experiment. After inoculation, trees were returned to the humid chambers for 48 h at ambient temperature. Disease severity was assessed on all leaves of each shoot on each tree beginning 2 to 4 days after inoculation and then every 2 days for 6 days using a modified Horsfall-Barratt scale with values from 0-6, where 0 = no lesions; 1 = 0-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50%, where the percentages represent percent leaf area affected. Disease incidence was determined by calculating the percentage of affected leaves on each tree.

Twenty-four isolates of *G. cingulata* (13 from fruit and 11 from leaves), five fruit isolates of *C. gloeosporioides* and five isolates of *C. acutatum* (three from fruit and two from leaves) were selected from the collection of monosporic isolates to conduct fruit pathogenicity tests on apples of cv. Gala (Appendix 4.2). Pathogenicity of isolates on fruit was tested following a previously described procedure with some modifications (3). Squares of 2.5 X 2.5 cm laboratory towels were soaked with 0.3 ml of a spore suspension (1 X 10^5 spores/ml) and then were placed on an uninjured surface of the fruit and covered with Parafilm. Three fruit per isolate were inoculated with each isolate and fruit were kept in humidity chambers at 26°C until completion of the experiment. Disease incidence was determined 7-10 days after inoculation every 2 days for 6 days by scoring the total number of diseased fruit.

2.3.4. Data analyses. Means of the area under the disease progress curves (AUDPC) of disease severity and incidence within each geographic location and species were compared using an analysis of variance (SAS® Windows version, release 6.12) and means were

separated by the Waller-Duncan *k*-ratio *t* test. For the statistical analysis of the means of species and geographic location, trees inoculated with isolates of the same species (*G. cingulata, C. gloeosporioides,* and *C. acutatum*) and with isolates obtained from the same location (Georgia, North Carolina or Brazil) were considered repetitions, respectively. Only one isolate from Tennessee was pathogenic on leaves; therefore, this location was not included in the statistical analysis.

2.3.5. Molecular characterization. One hundred and fifty-five isolates of *G. cingulata* (92 from fruit and 61 from leaves), 42 fruit isolates of *C. gloeosporioides* and 14 isolates of *C. acutatum* (10 from fruit and 4 from leaves) from the collection of isolates from the US and Brazil, previously characterized based on morphology and vegetative compatibility (Chapter 1), were examined for mtDNA RFLPs (Appendix 4.3). One isolate of *C. acutatum* (A138), two isolates of *C. gloeosporioides* (NC131 and NC329), and four isolates of *G. cingulata* (960, A45, NC211 and NC246) were also obtained from the University of Arkansas from a collection of isolates maintained by Dr. James Correll. These isolates represented six different mtDNA RFLP haplotypes and were used as reference isolates for the analysis of mtDNA RFLPs.

2.3.6. Mycelium production and DNA extraction. Each of the monosporic isolates used for molecular characterization was grown in liquid culture. Spore suspensions with conidia, ascospores or both conidia and ascospores, obtained from 8-15 day-old monosporic cultures were used to inoculate 200 ml Erlenmeyer flasks containing 150 ml of potato dextrose broth (PDB). Cultures were incubated in a rotary shaker for 3-4 days at 26°C and

250 rpm. After this period, mycelium was harvested by filtration on Miracloth and placed in 50 ml conical tubes. The mycelium was frozen at -80°C for about 1 hr and then lyophilized for 3-4 days until dry. The mycelium was ground with a pestle in a mortar containing liquid nitrogen. Total DNA was extracted from the ground mycelium by the "mini-prep" procedure for DNA extraction as previously described (9). Genomic DNA was digested with the restriction enzyme *Msp*I according to manufacturer's recommendations (Promega Corporation, Madison, WI). Restricted DNA was separated electrophoretically on 0.8% agarose gels containing ethidium bromide for further visualization of the genomic DNA fragments with ultraviolet light. The fragments were transferred to positively charged nylon membranes. Southern blots were probed with two non-overlapping mtDNA clones (4u40 and 2u18) developed by Correll *et al*. (9), to determine the mtDNA RFLP haplotypes. An enhanced chemiluminescense DNA labeling kit (ECL, Amersham, Arlington Hight, IL) was used to label the clones. Isolates with the same mtDNA restriction fragment pattern were placed in the same mtDNA RFLP haplotype. A cluster analysis was conducted in NTSYS-pc Version 2.02j to determine the similarity of coefficients of the mtDNA RFLP haplotypes, using the method of unweighted pair grouping (UPGMA) with arithmetic averages.

2.3.7. Intron amplification and DNA sequencing. The forward primer GDF1 (5'-GCCGTCAACGACCCCTTCATTGA-'3) and the reverse primer GDR1 (5'-GGGTGGAGTCGTACTTGAGCATGT-'3), developed by Guerber *et al*. (18), were used to amplify the 200 bp intron of the GDPH gene from the genomic DNA of 14 isolates of *G. cingulata* (nine from fruit and five from leaves), eight fruit isolates of *C. gloeosporioides* and two isolates of *C. acutatum* (one from fruit and one from leaves), selected from the isolates

previously examined for mtDNA RFLPs and vegetative compatibility (Table 2.6). PCR amplification of the intron was performed in a P*x2* Thermal cycler (Thermo Hybaid, Franklin, MA) using the following protocol: 35 cycles of denaturation at 94°C and annealing at 60°C for 1 minute, with a final extension at 72°C for 3 minutes. Amplification of the intron was confirmed electrophoretically on a 2% agarose gel at 100 V for 3 h. PCR products containing the double-stranded intron were purified using the QIAquick® PCR Purification Kit (Qiagen, Inc., Valencia, CA). Purified 200 bp intron of the GDPH was sequenced following the procedure previously described by Guerber *et al*. (18). Sequencing reactions were performed directly from both strands using primers GDF1 and GDR1.

2.3.8. Sequence alignment and phylogenetic analysis. Sequences were combined using the DNA sequence editor Chromas (Version 1.45, School of Health Science, Griffith University, Queensland, Australia), and then aligned using The Biology WorkBench 3.2 (San Diego Supercomputer Center, University of California, San Diego). Phylogenetic analysis and basic statistics were performed using PAUP* 4.0 beta 10 as described by Guerber *et al* (18). Two methods of tree building were used: Neighbor-Joining (NJ) and Maximum-Parsimony (MP). Tree topologies were evaluated by statistical confidence in bootstrap values.

2.3.9. Cultural characterization. Twenty-eight isolates of *G. cingulata* (14 from fruit and 14 from leaves), 12 fruit isolates of *C. gloeosporioides*, and 7 isolates of *C. acutatum* (four from fruit and three from leaves) were selected from the collection of monosporic isolates, previously characterized based on morphology, vegetative compatibility

(Chapter 2), and mtDNA RFLP haplotypes, to determined growth rate, sensitivity of the isolates to benomyl, and optimum growth temperature (Appendix 4.4). The isolates represented nine different mtDNA haplotypes (G1, G2, G3, G4, A3, B2, B3, C1, and D1) and 10 VCGs (1, 2, 3, 4, 5, 6, 7, 8, 9, and 13). The growth rate (in mm/day) of isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* was determined by measuring the colony diameter of isolates every 24 h for 6 days at 25ºC with constant light. Petri dishes containing approximately 15 ml of PDA were inoculated with 5 mm-diameter plugs of each isolate, obtained from the margins of 4-day old cultures. Three Petri dishes were used for each isolate and the experiment was repeated once. This procedure was also used to study the sensitivity of the isolates to benomyl. Three Petri dishes per isolate containing 15 ml of PDA were amended with each of the following concentrations of benomyl: 0, 0.01, 0.1, 1 and 10 µg/ml. Two runs of the experiment were conducted.

To determine optimum growth temperature, 5 mm-diameter plugs from the margins of 4-day-old cultures of the isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* were placed in the center of Petri dishes containing 15 ml of PDA medium and incubated in the dark at 14, 18, 24, 26, and 30°C. Five mm-diameter plugs of each isolate were placed on three different PDA dishes, and colony diameter was measured after 2, 4, and 6 days.

2.3.10. Data analyses. The optimum temperature for the growth of each species was estimated by fitting a quadratic equation to the growth of each species at all temperatures tested [growth = b0 + b1(temp) + b2($temp^2$)] and solving the following equation: maximum growth = (-b1/2b2), where b1 and b2 are the coefficients for the linear and quadratic terms. The reduction in growth of isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* at

less then optimum temperatures was determined by calculating the percentage reduction in colony diameter compared to the colony diameter at the temperature where maximum growth occurred.

Mean growth within haplotypes and species in each experiment was compared with an analysis of variance using SAS® (Windows version, release 6.12). Means were separated by the Waller-Duncan *k*-ratio *t* test. The EC_{50} (effective concentration of benomyl to reduce growth by 50%) of each isolate to benomyl was calculated based on the mean of the colony diameter of isolates within each haplotype and species 6 days after incubation using the Proc Probit log10 program in SAS®.

2.4. RESULTS

2.4.1. Mitochondrial DNA RFLPs. Twelve different mtDNA RFLP haplotypes were found among the isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* examined (Figs. 2.1 and 2.2, and Table 2.2). The duster analysis of the mtDNA RFLP haplotypes separated the haplotypes of *C. acutatum* from haplotypes of *G. cingulata* and *C. gloeosporioides* into two main clusters that had 53% similarity (Fig. 2.2). One cluster included isolates of *C. acutatum* and the other cluster was a complex of isolates of *G. cingulata* included in groups 1, 2, and 3 and isolates of *C. gloeosporioides* in group 4.

The 156 isolates of *G. cingulata* from the US and Brazil were separated into eight different haplotypes (G1, G1.1, G2, G2.1, G3, G4, A3 and A3.1). The majority of the isolates of *G. cingulata* from the US belonged to haplotypes G1, G2, and A3, with 60, 16, and 43 isolates, respectively. Only 7, 2, and 6 isolates of *G. cingulata* were characterized as G1.1, G2.1 and A3.1 haplotypes, respectively. The 21 isolates of *G. cingulata* from Brazil were

included in haplotypes G3 and G4 (Table 2.2). The cluster analysis divided the isolates of *G. cingulata* into three groups with 68% of similarity between group 1 and groups 2 and 3, and 73% between 2 and 3 (Fig. 2.2). Group 4, with isolates of *C. gloeosporioides*, included haplotypes B2 and B3 with 97% of similarity. Of the 42 isolates of *C. gloeosporioides*, 17 belonged to haplotype B2, and 25 to haplotype B3 (Table 2.2). The 14 isolates of *C. acutatum* were represented by two haplotypes (C1 and D1) with a similarity of 75%. Eight isolates of *C. acutatum* were characterized as haplotype C1, and were recovered from orchards located in the US, and six were D1 isolates recovered from Brazil (Table 2.2).

G1, G1.1, G3, and G4 were the only haplotypes that included isolates pathogenic to leaves. All isolates characterized as G3 and G4 haplotypes were capable of causing the leaf spot, whereas G1 and G1.1 haplotypes also included isolates not pathogenic on leaves (Table 2.4).

2.4.2. Pathogenicity tests. Regardless of the origin and source of the 24 isolates of *G. cingulata*, 5 isolates of *C. gloeosporioides*, and 5 isolates of *C. acutatum* tested, all were pathogenic on fruit (Table 2.3 and Appendix 4.2), producing typical bitter rot symptoms. Of the 87 isolates of *G. cingulata* recovered either from fruit or leaves only 10 fruit isolates and 40 leaf isolates collected from orchards of cv. Gala located in Georgia, North Carolina, Brazil, and Tennessee, were capable of causing the leaf spot (Table 2.4). Thirty-two fruit isolates and five isolates from leaves did not cause a leaf spot in the pathogenicity tests. Isolates of *G. cingulata* obtained from the orchard of cv. Gala in Georgia that were pathogenic on leaves were obtained from either fruit or leaves. Isolates that were pathogenic on leaves from orchards of cv. Gala located in North Carolina and Brazil were obtained from

leaves only, and only one leaf isolate from the orchard of cv. Gala in Tennessee was pathogenic (Table 2.4).

Brazilian isolates were the least pathogenic and had the lowest AUDPC for both incidence and severity of the leaf spot, 457.3 and 12.7, respectively (Table 2.5). No significant differences in the AUDPC of disease incidence and severity were observed among isolates from North Carolina and Georgia (either from fruit or leaves), which were pathogenic to leaves. The narrow ranges in the AUDPC for disease incidence within isolates from the same location indicates that isolates have a similar ability to cause the leaf spot. All isolates of *G. cingulata* pathogenic on leaves were very aggressive, and disease incidence was approximately 100% 4 days after inoculation (data not shown). Isolates from the same location varied more in their aggressiveness as indicated by the wider range in AUDPC for severity than for incidence (Appendix 4.2). Only the isolates of *G. cingulata* that were pathogenic to leaves of the cv. Gala in the pathogenicity tests were capable of causing the leaf spot on trees of cv. Golden Delicious (Table 2.4).

2.4.3. Sequence and phylogenetic analysis of the 200 bp intron. The 200 bp intron of the GDPH gene was successfully amplified from isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum*. Both phylogenetic analyses, Neighbor-Joining (NJ) and Maximum-Parsinomy (MP), produced phylogenetic trees with similar topologies, and similar statistically supported groups for the sequence of the intron of the isolates (Figs. 2.3 and 2.4). Two main clusters were observed in each phylogenetic tree. One cluster included the *G. cingulata*/*C. gloeosporioides* complex and was divided into seven groups in the MP phylogenetic tree, and into six groups in the NJ phylogenetic tree. The other cluster included

only isolates of *C. acutatum*. The *G. cingulata*/*C. gloeosporioides* complex group 1 included four isolates of *G. cingulata* with haplotypes G1, G1.1, G2, and G2.1, and VCGs 1, 2 and 3, collected from orchards located in the US and pathogenic to either fruit or fruit and leaves (Tables 2.4 and 2.6). Group 3 also included three isolates of *G. cingulata* collected from orchards located in the US, but showed less molecular diversity than isolates of *G. cingulata* in group 1. These isolates belonged into haplotypes A3, and A3.1, and VCG-6, and were only pathogenic on fruit. Two isolates of *C. gloeosporioides* included in group 4 belonged to haplotype B2 and VCG-7 and VCG-12. They were pathogenic only on fruit and were collected from orchards in the US. Groups, 5, 6, and 7 in the MP phylogenetic tree and 5 and 6 in the NJ phylogenetic tree included six isolates of *C. gloeosporioides* recovered from orchards in the US, with haplotypes B2 and B3, and VCGs 8, 9, 10, and 11, which were pathogenic only on fruit. Group 2 included three Brazilian isolates of *G. cingulata* pathogenic on fruit and leaves with haplotypes G3 and G4 and VCG-4 and VCG-5. Isolates of *C. acutatum* included one isolate recovered from orchards in the US with haplotype C1 and VCG-15, and another isolate recovered from orchards in Brazil with haplotype D1 and VCG-14. These isolates were pathogenic on fruit only.

2.4.4. Cultural characterization. Overall, the 27 isolates of *G. cingulata* and the 12 isolates *C. gloeosporioides* grew faster and were more sensitive to benomyl than the 7 isolates of *C. acutatum* (Table 2.8 and Appendix 4.4). There were no significant differences in growth rate and benomyl sensitivity among isolates of *G. cingulata* and *C. gloeosporioides* (Table 2.8). A3 haplotype isolates grew faster (13.0 mm/day) but the growth rate was not significantly different from that of the B2 and G2 haplotype isolates. There were no

significant differences in growth rate among G2, G4, and B2 isolates, nor among G1, G3, and G4 isolates. The rate of growth among C1 and D1 haplotypes, which included isolates of *C. acutatum* collected from the US and Brazil, respectively, was significantly different, and C1 haplotype isolates grew slower than all of the haplotypes tested (8.5 mm/day). B3 haplotype isolates grew faster than C1 and D1 haplotypes, but slower than all other haplotypes of *G. cingulata* and *C. gloeosporioides.*

There were no significant differences in the sensitivity of G1, G2, and G3 isolates to benomyl. A3 and B3 isolates were the least sensitive isolates of *G. cingulata* and *C. gloeosporioides*, respectively, to benomyl. Nevertheless, the EC_{50}s of A3 and B3 isolates were not significantly different from isolates in the other haplotypes of *G. cingulata* (G1, G2, G3) and *C. gloeosporioides* (B2). C1 and D1 haplotypes which included isolates of *C. acutatum* had higher EC_{50}s than isolates of *G. cingulata* and *C. gloeosporioides*. D1 isolates were the least sensitive with an EC_{50} of 0.66 µg benomyl/ml (Table 2.8).

All isolates grew slowest at 14°C, except isolates of *C. acutatum* of haplotype C1, which grew slowest at 30°C (Fig. 2.5). The predicted optimum temperatures for the growth of isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* were 24.0, 24.2, and 22.0°C, respectively (Fig. 2.6). Fourteen degrees Celsius was the least favorable temperature for all species and growth at this temperature was reduced by 68-72% compared to the optimum. The greatest differences in growth among species occurred at 30°C. At 30°C, isolates of *C. acutatum* grew significantly less than isolates of *G. cingulata* and *C. gloeosporioides.*

2.5. DISCUSSION

Bitter rot of apples is caused by different VCGs of *G. cingulata, C. gloeosporioides*, and *C. acutatum*, which vary in number and proportion from orchard to orchard (Chapter 1). In this study, all haplotypes of each species, when tested for pathogenicity on apple fruit, were capable of causing typical bitter rot symptoms. However, only isolates of *G. cingulata* included in four haplotypes were capable of causing GLS. These isolates of *G. cingulata* were recovered from fruit or leaves from orchards of the cultivar Gala located in the US and Brazil. Isolates of *C. acutatum* from Brazil, also recovered from leaves from the cultivar Gala, were not pathogenic to leaves and were likely growing saprophytically in necrotic tissues.

The mtDNA RFLP analysis distinguished among isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum*, as well as among isolates of the same species. The sequence analysis of the 200 bp intron of the GDPH gene also separated isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum* and distinguished different groups within each species. Guerber and Correll (17) also observed variability in mtDNA haplotypes within isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum*, and haplotypes within each taxon were distinct for each species.

Mitochondrial DNA haplotypes and phylogenic groups that included isolates of *G. cingulata* pathogenic to leaves and fruit also included isolates of *G. cingulata* pathogenic to fruit only. Isolates of *G. cingulata* from Brazil capable of causing GLS and bitter rot belonged to haplotypes G3 and G4, while those from the US were included in haplotypes G1 and G1.1. Haplotypes G3 and G4 included only isolates of *G. cingulata* capable of causing GLS and bitter rot; however, haplotypes G1 and G1.1 also included isolates of *G. cingulata*

that were not pathogenic on leaves, but were capable of causing bitter rot. However, a VCG analysis including all the isolates of *G. cingulata* examined for mtDNA RFLP in the present study, separated leaf pathogenic and non-pathogenic isolates within haplotypes G1 and G1.1 into VCG-1 and VCG-2, respectively (Chapter 1). The phylogenetic analysis of the intron also included isolates of *G. cingulata* capable of causing GLS and bitter rot, and isolates of *G. cingulata* capable of causing bitter rot only in the same group. Only isolates of *G. cingulata* with haplotypes A3 and A3.1 that were capable of causing bitter rot only were distinguished in the mtDNA RFLP and phylogenetic analysis from isolates of *G. cingulata* capable of causing both GLS and bitter rot. Therefore, the VCG analysis was a better indicator than mtDNA RFLPs or DNA sequencing, for distinguishing isolates of *G. cingulata* capable of causing GLS and bitter rot, from isolates of *G. cingulata* only capable of causing bitter rot. Other studies involving vegetative compatibility among isolates of *C. orbiculare* and *C. coccodes*, and pathogenicity tests suggested a distinct correspondence between VCGs and pathogenic characteristics or virulence of phenotypes (24,33). Similar correlations were also observed between VCGs and *forma specialis* of the fungus *Fusarium oxysporum*, suggesting that vegetative compatibility may represent a fast and easy way to distinguish pathotypes of the pathogen (8,25). In order to distinguish leaf and fruit pathogenic isolates of *G. cingulata* from isolates pathogenic on fruit only, more detailed molecular studies, such as characterization of multiple genes, including pathogenicity genes of the GLS and the bitter rot fungi, are necessary.

Mitochondrial DNA RFLP analysis also distinguished isolates of *G. cingulata* and *C. acutatum* from the US from isolates of *G. cingulata* and *C. acutatum* from Brazil. Isolates of *G. cingulata* from the US belonged to haplotypes G1, G1.1, G2, G2.1, A3, and A3.1,

whereas isolates of *G. cingulata* from Brazil belonged to haplotypes G3 and G4. Isolates of *C. acutatum* from the US belonged to haplotype C1, and isolates of *C. acutatum* from Brazil were included into haplotype D1. Although, isolates of *G. cingulata* from the US and Brazil were included in the same cluster (*G. cingulata*/*C. gloeosporioides* complex) in the sequence analysis of the intron, they were separated in different groups within the cluster. Isolates from the US were included in groups 1 and 3, and isolates from Brazil belonged to group 2. Isolates of *C. acutatum* from the US and Brazil were included in the same phylogenetic group.

Slower growth, less sensitivity to benomyl and a lower optimum growth temperature also differentiated isolates of *C. acutatum* from isolates of *G. cingulata* and *C. gloeosporioides*. However, these cultural characteristics were not useful to distinguish between *G. cingulata* and *C. gloeosporioides*, or among different groups within each species. In previous studies, isolates of *G. cingulata* and *C. gloeosporioides* were distinguished by their faster growth rate (19,21,26,30) and greater sensitivity to benomyl (1,5,21,30) compared to isolates of *C. acutatum* obtained from apples, citrus, strawberry, peach, and other hosts. Additionally, Adaskaveg and Hartin (1) observed a lower optimum growth temperature for isolates of *C. acutatum* obtained from strawberry, almond, and peach compared to the optimum growth temperature for isolates of *C. gloeosporioides* obtained from citrus and papaya. However, Gunnell and Gubler (19) stated that isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* from strawberry had the same optimum growth temperature.

Although isolates of *G. cingulata* were separated into more mtDNA haplotypes than isolates of *C. gloeosporioides* and *C. acutatum*, the sequence analysis of the 200 bp intron of

the GDPH gene separated isolates of *G. cingulata* and *C. gloeosporioides*, into three and four groups in the MP phylogenetic tree, respectively, and three groups each in the NJ phylogenetic tree. These results indicate that there is high genetic diversity within *G. cingulata* and *C. gloeosporioides* on apples. This supports previous molecular and genetic studies where isolates of *C. acutatum* obtained from US apples were divided in only two mtDNA RFLP haplotypes, compared to isolates of *G. cingulata* and *C. gloeosporioides* obtained from US apples that were separated into four and eight mtDNA RFLP haplotypes, respectively (18). Considering that the only means of genetic recombination in *C. acutatum* is believed to be through the parasexual cycle and that its sexual state has not been observed in nature (10,17), it is not surprising that the molecular diversity of *G. cingulata* and *C. gloeosporioides* is greater than that of *C. acutatum*.

Previous observations and results from the VCG analysis, which separated US and Brazil isolates of *G. cingulata* (Chapter 1), suggest that isolates of *G. cingulata* from the US that were capable of causing GLS arose independently from GLS isolates from Brazil. Because isolates of *G. cingulata* from the US that were capable of causing both GLS and bitter rot were included in the same mtDNA haplotypes and phylogenetic groups that also included isolates of *G. cingulata* only capable of causing bitter rot, it is possible that the GLS fungus arose from isolates of *G. cingulata* that were originally only pathogenic on fruit. Sequence analysis of multiple genes from isolates of *G. cingulata* pathogenic to fruit and leaves and isolates of *G. cingulata* pathogenic to fruit only, may help to clarify the origin of the GLS fungus in the US.

2.6. LITERATURE CITED

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Table 2.1. Isolates of *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* obtained from leaf and fruit samples collected from different apple orchards located in the US and Brazil

Species[y]	Geographical origin[z]	Source		Number of isolates recovered	Year collected
		Host tissue	Cultivar		
C. acutatum	Brazil 2	Leaf	Gala	2	n/a*
C. acutatum	Brazil 2	Fruit	Gala	2	n/a*
C. acutatum	Brazil 2	Fruit	Golden Delicious	1	n/a*
C. acutatum	Brazil 2	Fruit	Fuji	1	n/a*
C. acutatum	Brazil 4	Leaf	Gala	16	2001
C. acutatum	Brazil 8	Fruit	Gala	1	n/a*
C. acutatum	Brazil 8	Fruit	Fuji	1	n/a*
C. acutatum	Brazil 8	Fruit	Golden Delicious	2	n/a*
C. acutatum	NC 1	Fruit	Gala	1	2001
C. acutatum	NC 2	Fruit	Granny Smith	39	2001
C. acutatum	NC 2	Fruit	Granny Smith	7	2002
C. gloeosporioides	AL	Fruit	Golden Delicious	11	2001
C. gloeosporioides	NC 2	Fruit	Granny Smith	13	2001
C. gloeosporioides	NC 2	Fruit	Granny Smith	8	2002
C. gloeosporioides	NC 3	Fruit	Golden Delicious	14	2002
C. gloeosporioides	NC 3	Fruit	Delicious	28	2002
G. cingulata	Brazil 1	Leaf	Gala	7	2001
G. cingulata	Brazil 2	Leaf	Gala	1	n/a*
G. cingulata	Brazil 2	Fruit	Gala	1	n/a*
G. cingulata	Brazil 2	Bud	Gala	1	n/a*
G. cingulata	Brazil 3	Leaf	Gala	3	2001
G. cingulata	Brazil 5	Leaf	Gala	3	2001
G. cingulata	Brazil 6	Leaf	Gala	7	2001
G. cingulata	Brazil 7	Leaf	Gala	2	2001
G. cingulata	Brazil 8	Leaf	Gala	1	n/a*
G. cingulata	GA	Leaf	Gala	22	2000
G. cingulata	GA	Leaf	Gala	3	2002
G. cingulata	GA	Fruit	Gala	38	2000
G. cingulata	GA	Fruit	Gala	7	2002
G. cingulata	NC 1	Leaf	Gala	34	2000
G. cingulata	NC 1	Leaf	Gala	26	2002
G. cingulata	NC 1	Fruit	Gala	14	2001
G. cingulata	NC 1	Fruit	Gala	1	2002
G. cingulata	NC 2	Fruit	Granny Smith	27	2000
G. cingulata	NC 2	Fruit	Granny Smith	20	2001
G. cingulata	NC 2	Fruit	Granny Smith	35	2002

Table 2.1. Continued

Species[y]	Geographical origin[z]	Source		Number of isolates recovered	Year collected
		Host tissue	Cultivar		
G. cingulata	NC 3	Fruit	Golden Delicious	7	2002
G. cingulata	NC 3	Fruit	Delicious	4	2000
G. cingulata	NC 3	Fruit	Delicious	8	2002
G. cingulata	NC 4	Fruit	Granny Smith	51	2002
G. cingulata	NC 5	Fruit	Gala	1	2002
G. cingulata	OH	Fruit	Molly's Delicious	3	2001
G. cingulata	TN 1	Leaf	Gala	4	1998
G. cingulata	TN 2	Leaf	Gala	8	1998

[y] Isolate species was determined after morphological characterization.

[z] GA = Gala orchard located in Blue Ridge, Georgia; NC 1 = Gala orchard located in Lincoln Co., NC; NC 2 = Granny Smith orchard located in Wilkes Co., NC; NC 3 = Golden and Delicious orchards at the Central Crops Research Station Clayton, NC; NC 4 = Granny Smith orchard located in Lincoln Co., NC; NC 5 = Gala orchard located in Wilkes Co., NC; OH = Molly's Delicious orchard located in Ohio; Brazil 1,4,5,6,7,8 = Gala and Golden Delicious orchards located in Santa Catarina State in Brazil; Brazil 2,3 = Gala orchards located in Rio Grande do Sul State in Brazil; TN 1 = Gala orchard located Cleveland, TN; TN 2 = Gala orchard located Buffalo Valley, TN; AL = Golden Delicious orchard located in Alabama.

n/a* = year collected not available. Isolates obtained from a collection of isolates maintained by Dr. Rosa Maria Sanhueza at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil.

Table 2.2. Mitochondrial DNA haplotype, origin and source of isolates of *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* examined for mtDNA RFLPs

Species	mtDNA haplotype (*Msp*I)[y]	Number of isolates (leaves/fruit or bud)	Source		Geographical origin
			Cultivar	Host Tissue	
G. cingulata	G1	31/29	Gala, Granny Smith, Molly's Delicious	Leaf/fruit	GA, NC, TN, OH
G. cingulata	G1.1	3/4	Gala, Granny Smith	Leaf/fruit	NC, TN
G. cingulata	G2	16	Gala, Granny Smith	Fruit	NC
G. cingulata	G2.1	2	Gala	Fruit	NC
G. cingulata	G3	10/2	Gala	Leaf/fruit	Brazil
G. cingulata	G4	8/1	Gala	Leaf/bud	Brazil
G. cingulata	A3	37/6	Granny Smith, Red and Golden Delicious, Gala	Leaf/fruit	NC, TN
G. cingulata	A3.1	3/3	Granny Smith, Gala	Leaf/fruit	NC, TN
C. gloeosporioides	B2	17	Golden Delicious, Delicious Granny Smith,	Fruit	NC
C. gloeosporioides	B3	25	Granny Smith, Red and Golden Delicious	Fruit	NC
C. acutatum	C1	8	Granny Smith	Fruit	NC
C. acutatum	D1	4/2	Gala	Leaf/fruit	Brazil

[y] mtDNA RFLP haplotypes of genomic DNA digested with *Msp*I.
[z] Morphological types described in Chapter 1, based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture.

Table 2.3. Number of isolates of *G. cingulata, C. gloeosporioides*, and *C. acutatum* pathogenic to fruit and leaves[x]

Species	Source (host tissue)	Pathogenicity[y]	
		Leaves	Fruit
G. cingulata	Leaf	40/45	13/13
	Fruit	10/42	11/11
C. gloeosporioides	Leaf	n/a[z]	n/a
	Fruit	0/6	5/5
C. acutatum	Leaf	0/1	2/2
	Fruit	0/4	3/3

[x] Pathogenicity was tested on trees of cv. Gala grown under greenhouse conditions.
[y] Number of pathogenic isolates/number of non-pathogenic isolates.
[z] n/a = *C. gloeosporioides* was not recovered from leaves.

Table 2.4. Pathogenicity of isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* on leaves of the cultivars Gala and Golden Delicious

Isolate designation	Species	mtDNA haplotype (*Msp*1)[y]	Geographical origin	Source		Pathogenicity[yz]	
				Host tissue	Cultivar	Gala	Golden Delicious
BR 1	*G. cingulata*	G4	Brazil	Leaf	Gala	+	n/a
BR 4	*G. cingulata*	G3	Brazil	Leaf	Gala	+	n/a
BR 7	*G. cingulata*	G3	Brazil	Leaf	Gala	+	n/a
BR 9	*G. cingulata*	G4	Brazil	Leaf	Gala	+	+
BR 10	*G. cingulata*	G3	Brazil	Leaf	Gala	+	+
BR 11	*G. cingulata*	G4	Brazil	Leaf	Gala	+	n/a
BR 12	*G. cingulata*	G4	Brazil	Leaf	Gala	+	n/a
BR 13	*G. cingulata*	G3	Brazil	Leaf	Gala	+	n/a
BR 16	*G. cingulata*	n/a	Brazil	Leaf	Gala	+	n/a
BR 20	*G. cingulata*	n/a	Brazil	Leaf	Gala	+	n/a
BR 21	*G. cingulata*	G4	Brazil	Leaf	Gala	+	+
CROTTS(L) 2	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 3	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 4	*G. cingulata*	G1.1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 5	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 6	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 8	*G. cingulata*	G1.1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 9	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 10	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 13	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 14	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 15	*G. cingulata*	G1	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 16	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 17	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 18	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 19	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	n/a
CROTTS(L) 22	*G. cingulata*	n/a	North Carolina	Leaf	Gala	+	+
GA(L) 1	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 2	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 4	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 5	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 7	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 8	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA(L) 9	*G. cingulata*	n/a	Georgia	Leaf	Gala	+	n/a
GA(L) 10	*G. cingulata*	n/a	Georgia	Leaf	Gala	+	n/a
GA(L) 11	*G. cingulata*	n/a	Georgia	Leaf	Gala	+	n/a
GA(L) 12	*G. cingulata*	n/a	Georgia	Leaf	Gala	+	+
GA(L) 14	*G. cingulata*	n/a	Georgia	Leaf	Gala	+	n/a

Table 2.4. Continued.

Isolate designation	Species	mtDNA haplotype (*Msp*1)[y]	Geographical origin	Source		Pathogenicity[yz]	
				Host tissue	Cultivar	Gala	Golden Delicious
GA(L) 16	*G. cingulata*	G1	Georgia	Leaf	Gala	+	n/a
GA 3	*G. cingulata*	n/a	Georgia	Fruit	Gala	+	n/a
GA 5	*G. cingulata*	n/a	Georgia	Fruit	Gala	+	n/a
GA 6	*G. cingulata*	n/a	Georgia	Fruit	Gala	+	n/a
GA 7	*G. cingulata*	n/a	Georgia	Fruit	Gala	+	n/a
GA 8	*G. cingulata*	n/a	Georgia	Fruit	Gala	+	n/a
GA 10	*G. cingulata*	G1	Georgia	Fruit	Gala	+	n/a
GA 12	*G. cingulata*	G1	Georgia	Fruit	Gala	+	n/a
GA 21	*G. cingulata*	G1	Georgia	Fruit	Gala	+	n/a
GA 22	*G. cingulata*	G1	Georgia	Fruit	Gala	+	n/a
GA 24	*G. cingulata*	G1	Georgia	Fruit	Gala	+	n/a
TN 7	*G. cingulata*	G1	Tennessee	Leaf	Gala	+	+
CROTTS 1	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	-
CROTTS 3	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	-
CROTTS 5	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	-
CROTTS 6	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	n/a
CROTTS 8	*G. cingulata*	G2.1	North Carolina	Fruit	Gala	-	n/a
CROTTS 9	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	n/a
CROTTS 10	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	n/a
CROTTS 11	*G. cingulata*	n/a	North Carolina	Fruit	Gala	-	n/a
CROTTS 12	*G. cingulata*	G2.1	North Carolina	Fruit	Gala	-	n/a
CROTTS 13	*G. cingulata*	G2	North Carolina	Fruit	Gala	-	n/a
RD 1	*G. cingulata*	A3	North Carolina	Fruit	Delicious	-	-
RD 3	*G. cingulata*	A3	North Carolina	Fruit	Delicious	-	n/a
LD 3	*G. cingulata*	A3.1	North Carolina	Fruit	Granny Smith	-	n/a
LD 5	*G. cingulata*	A3	North Carolina	Fruit	Granny Smith	-	n/a
LD 6	*G. cingulata*	A3	North Carolina	Fruit	Granny Smith	-	n/a
LD 7	*G. cingulata*	G1.1	North Carolina	Fruit	Granny Smith	-	n/a
LD 8	*G. cingulata*	A3	North Carolina	Fruit	Granny Smith	-	-
LD 10	*G. cingulata*	G1	North Carolina	Fruit	Granny Smith	-	n/a
LD 12	*G. cingulata*	A3	North Carolina	Fruit	Granny Smith	-	-
LD 13	*G. cingulata*	G1	North Carolina	Fruit	Granny Smith	-	n/a
LD 15	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	n/a
LD 16	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	-
LD 17	*G. cingulata*	G1.1	North Carolina	Fruit	Granny Smith	-	n/a
LD 23	*G. cingulata*	G1	North Carolina	Fruit	Granny Smith	-	n/a
LD 25	*G. cingulata*	G1	North Carolina	Fruit	Granny Smith	-	n/a
LD 30	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	n/a

Table 2.4. Continued.

Isolate designation	Species	mtDNA haplotype (*Msp*1)[y]	Geographical origin	Source		Pathogenicity[yz]	
				Host tissue	Cultivar	Gala	Golden Delicious
LD 31	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	n/a
LD 32	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	n/a
LD 41	*G. cingulata*	n/a	North Carolina	Fruit	Granny Smith	-	n/a
OH 1	*G. cingulata*	G1	Ohio	Fruit	Molly's Delicious	-	n/a
OH 2	*G. cingulata*	n/a	Ohio	Fruit	Molly's Delicious	-	n/a
OH 3	*G. cingulata*	G1	Ohio	Fruit	Molly's Delicious	-	n/a
TN 1	*G. cingulata*	G1.1	Tennessee	Leaf	Gala	-	n/a
TN 5	*G. cingulata*	A3.1	Tennessee	Leaf	Gala	-	n/a
TN 8	*G. cingulata*	A3	Tennessee	Leaf	Gala	-	n/a
TN 9	*G. cingulata*	A3	Tennessee	Leaf	Gala	-	n/a
TN 11	*G. cingulata*	A3	Tennessee	Leaf	Gala	-	n/a
AL 1	*C. gloeosporioides*	B3	Alabama	Fruit	Golden Delicious	-	-
AL 4	*C. gloeosporioides*	B3	Alabama	Fruit	Golden Delicious	-	-
AL 5	*C. gloeosporioides*	B2	Alabama	Fruit	Golden Delicious	-	-
AL 9	*C. gloeosporioides*	B2	Alabama	Fruit	Golden Delicious	-	-
LD Cg 1	*C. gloeosporioides*	B3	North Carolina	Fruit	Granny Smith	-	-
LD Cg 8	*C. gloeosporioides*	B3	North Carolina	Fruit	Granny Smith	-	-
LD Ca(b) 4	*C. acutatum*	C1	North Carolina	Fruit	Granny Smith	-	-
LD Ca(b) 6	*C. acutatum*	C1	North Carolina	Fruit	Granny Smith	-	n/a
LD Ca 5	*C. acutatum*	C1	North Carolina	Fruit	Granny Smith	-	-
LD Ca 10	*C. acutatum*	C1	North Carolina	Fruit	Granny Smith	-	-
BR Ca 3	*C. acutatum*	D1	Brazil	Leaf	Gala	n/a	-
BR Ca 4	*C. acutatum*	D1	Brazil	Leaf	Gala	-	-
BR Ca 6	*C. acutatum*	n/a	Brazil	Leaf	Gala	n/a	-

[y] n/a = isolates were not tested for either mtDNA RFLPs or pathogenicity.
[z] Pathogenicity of the isolates was tested on trees of cv. Gala and Golden Delicious grown under greenhouse conditions. '+' indicates that isolates were pathogenic; '-' indicates that isolates were not pathogenic.

Table 2.5. Incidence and severity of Glomerella leaf spot on trees of cv. Gala[w]

Geographical origin	Species	Number of isolates tested	Source		Incidence (AUDPC)[x]		Severity (AUDPC)[y]	
			Host tissue	Cultivar	Range	Mean[z]	Range	Mean[z]
North Carolina (NC 1)	*G. cingulata*	16	Leaf	Gala	437.5-500.0	488.5 a	13.2-23.6	16.6 a
Georgia	*G. cingulata*	12	Leaf	Gala	477.8-500.0	495.3 a	11.6-22.0	17.3 a
Georgia	*G. cingulata*	10	Fruit	Gala	388.6-500.0	479.4 a	12.1-22.4	16.4 a
Brazil	*G. cingulata*	11	Leaf	Gala	364.0-500.0	457.3 b	9.6-18.3	12.7 b

[w] Pathogenicity was tested on trees of cv. Gala grown under greenhouse conditions.
[x] Foliar incidence represents the mean of area under the disease progress curve (AUDPC) of the percentage of diseased leaves rated every 2 days over 6 days.
[y] Foliar severity represents the mean of the AUDPC of the percentage of leaf area affected rated every 2 days over 6 days. Severity was estimated using a modified Horsfall-Barratt disease rating scale with values from 0-6, where 0 = no lesions; 1 = 1-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50%.
[z] Means followed by the same letter within the same column are not significantly different at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test.

Table 2.6. Mitochondrial DNA haplotypes and vegetative compatibility of isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* used for the sequence analysis of the 200 bp intron of the GDPH gene

Isolate designation	mtDNA haplotypes (*Msp* I)[x]	VCG[y]	Species	Geographical origin	Source	
					Host tissue	Cultivar
GA(L) 1	G1	1	*G. cingulata*	Georgia	Leaf	Gala
LD 10	G1	2	*G. cingulata*	North Carolina	Fruit	Granny Smith
OH 1	G1	3	*G. cingulata*	Ohio	Fruit	Molly's Delicious
GA 17	G1.1	1	*G. cingulata*	Georgia	Fruit	Gala
LD 17	G1.1	2	*G. cingulata*	North Carolina	Fruit	Granny Smith
CROTTS 13	G2	2	*G. cingulata*	North Carolina	Fruit	Gala
CROTTS 8	G2.1	2	*G. cingulata*	North Carolina	Fruit	Gala
BR 8	G3	4	*G. cingulata*	Brazil	Leaf	Gala
BR 17	G3	5	*G. cingulata*	Brazil	Leaf	Gala
BR 21	G4	5	*G. cingulata*	Brazil	Leaf	Gala
LD 5	A3	6	*G. cingulata*	North Carolina	Fruit	Granny Smith
TN 9	A3	?	*G. cingulata*	Tennessee	Leaf	Gala
LD 5	A3	6	*G. cingulata*	North Carolina	Fruit	Granny Smith
LD 1	A3.1	6	*G. cingulata*	North Carolina	Fruit	Granny Smith
AL 7	B2	7	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious
LD 54	B2	10	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith
GD 8	B2	11	*C. gloeosporioides*	North Carolina	Fruit	Golden Delicious
RD 16	B2	12	*C. gloeosporioides*	North Carolina	Fruit	Delicious
GD 13	B2	17	*C. gloeosporioides*	North Carolina	Fruit	Golden Delicious
AL 1	B3	8	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious
LD Cg 12	B3	9	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith
RD 7	B3	18	*C. gloeosporioides*	North Carolina	Fruit	Delicious
LD Ca 21	C1	15	*C. acutatum*	North Carolina	Fruit	Granny Smith
BR Ca 17	D1	14	*C. acutatum*	Brazil	Leaf	Gala

[x] mtDNA RFLP haplotypes of genomic DNA digested with *Msp* I.
[y] Vegetative compatibility groups (VCG) from analysis previously conducted (Chapter 1). '?' = isolate was not compatible with any of the VCGs.
[z] Pathogenicity was tested on fruit and tress of the cultivar Gala grown under greenhouse conditions. '+' indicates that isolates were pathogenic. '-' indicates that isolates were not pathogenic.

Table 2.7. Pathogenicity of the *G. cingulata*/*C. gloeosporioides* complex and *C. acutatum* according to the sequence analysis groups, mtDNA haplotypes, and VCGs

Species	Sequence analysis[x]	mtDNA haplotype (*Msp*I)	VCG[y]	Geographic origin	Source		Pathogenicity[z]	
					Cultivar	Host tissue	Leaf	Fruit
G. cingulata/*C. gloeosporioides complex*	Group 1 (*G. cingulata*)	G1	1	GA, NC, TN	Gala	Leaf	+	+
			1	NC	Gala	Fruit	+	+
			2	NC	Gala, Granny Smith	Fruit	-	+
			3	OH	Molly's Delicious	Fruit	-	+
		G1.1	1	TN	Gala	Fruit	+	+
			1	NC	Granny Smith	Fruit	-	+
			2	NC	Granny Smith	Fruit	-	+
		G2	2	NC	Gala, Granny Smith	Fruit	-	+
		G2.1	2	NC	Gala	Fruit	-	+
	Group 2 (*G. cingulata*)	G3	4	Brazil	Gala	Leaf	+	+
			5	Brazil	Gala	Leaf	+	+
		G4	5	Brazil	Gala	Leaf	+	+
	Group 3 (*G. cingulata*)	A3	6	NC, TN	Granny Smith, Red and Golden Delicious, Gala	Fruit	-	+
		A3.1	6	NC, TN	Granny Smith, Gala	Fruit	-	+
	Group 4 (*C. gloeosporioides*)	B2	7	AL	Golden Delicious	Fruit	-	+
			12	NC	Delicious	Fruit	-	+
	Group 5 (*C. gloeosporioides*)	B2	n/a	NC	Golden Delicious	Fruit	-	+
		B3	8	AL	Golden Delicious	Fruit	-	+
			9	NC	Granny Smith	Fruit	-	+
	Group 6 (*C. gloeosporioides*)	B2	11	NC	Delicious	Fruit	-	+
		B3	11	NC	Delicious	Fruit	-	+
	Group 7 (*C. gloeosporioides*)	B2	10	NC	Granny Smith	Fruit	-	+
C. acutatum	---	C1	15	NC	Granny Smith	Fruit	-	+
		D1	14	Brazil	Gala	Leaf	-	+

[x] In the phylogenetic tree based on Neighbor-Joining (NJ) group 7 was included within group 5.
[y] n/a = isolates were not compatible with any of the VCGs.
[z] '+' indicates that isolates were pathogenic; '-' indicates that isolates were not pathogenic.

Table 2.8. Growth rate and benomyl sensitivity of isolates of *G. cingulata*, *C. gloeosporioides* and *C. acutatum*

Species	Number of isolates	mtDNA haplotype (*Msp*I)	VCG[w]	Growth (mm/day)[xz]		EC_{50}[yz]	
				Haplotype mean	Species mean	Haplotype mean	Species mean
G. cingulata	5	A3	6	13.0 a		0.19 c	
G. cingulata	7	G3	4,5	12.1 c		0.12 dc	
G. cingulata	4	G2	2	12.4 ba		0.10 dc	
G. cingulata	2	G4	5	12.3 bc		- **	
G. cingulata	9	G1	1,2,3	12.2 c	12.4 a	0.15 dc	0.14 b
C. gloeosporioides	4	B2	7	12.9 ba		0.16 dc	
C. gloeosporioides	8	B3	8	11.4 d	11.9 a	0.07 d	0.10 b
C. acutatum	4	C1	-*	9.2 e		0.37 b	
C. acutatum	3	D1	13	8.5 f	8.9 b	0.66 a	0.47 a

[w] Vegetative compatibility groups (VCG) described in Chapter 1. *Vegetative compatibility was not examined.

[x] Growth represents the colony diameter (mm/day) of the isolates at 26°C over 6 days.

[y] EC_{50}s were calculated using Proc Probit log 10 in SAS®, based on the colony diameter of the isolates at 0, 0.01, 0.1, 1 and 10 µg/ml of benomyl after 6 days of incubation at 26°C.

z Means followed by the same letter are not significantly different at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test. ** Haplotype was not examined.

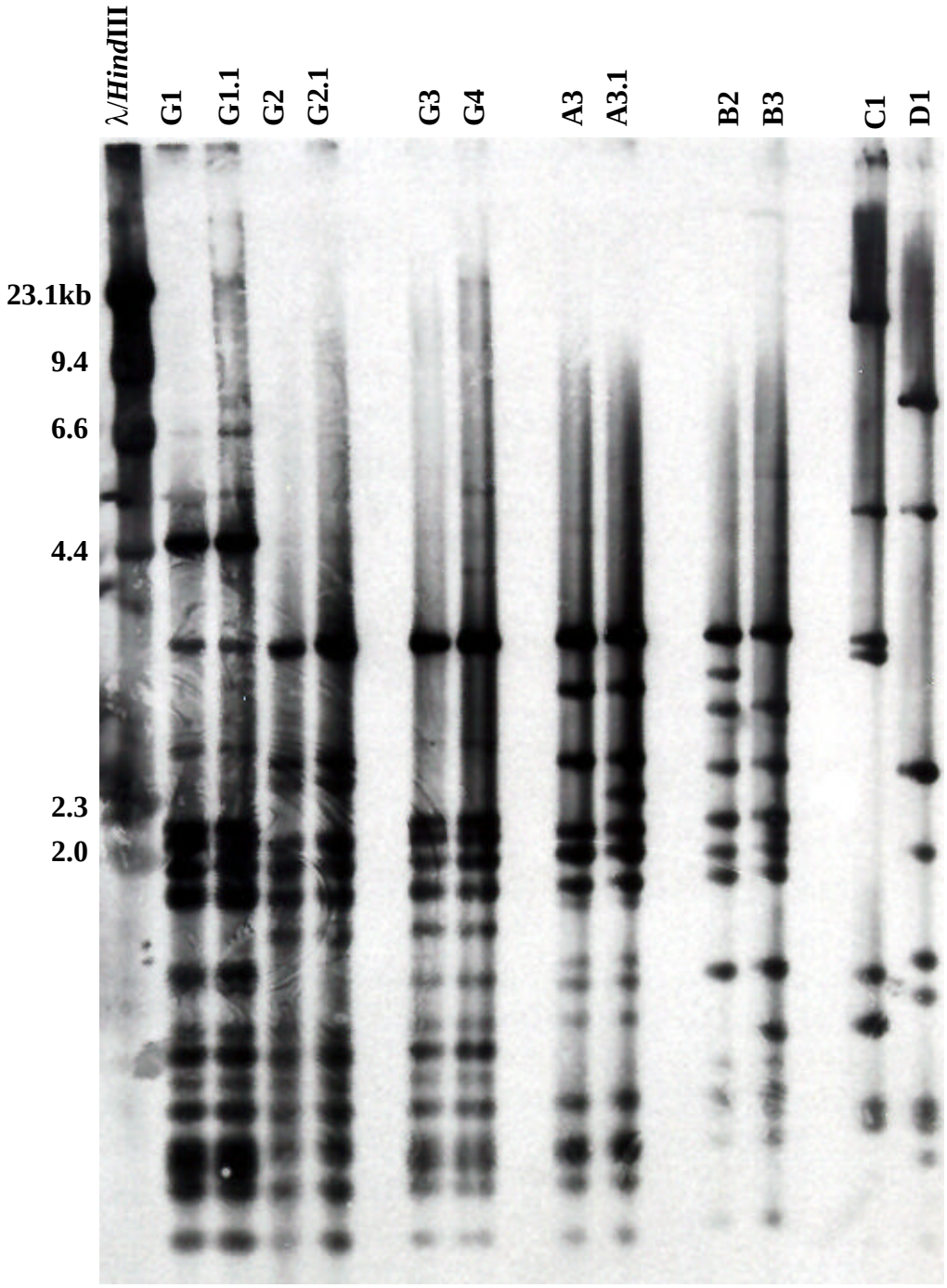

Figure 2.1. Mitochondrial DNA RFLP haplotypes found among isolates of *G. cingulata* (lanes 1-4, 6 and 7, 9 and 10), *C. gloeosporioides* (12 and 13), and *C. acutatum* (15 and 16) collected from fruit and leaves of apples. Genomic DNA was digested with the restriction enzyme *Msp*I and probed with two non-overlapping mtDNA clones (4u40 and 2u18). Haplotype designations appear at the top of each lane.

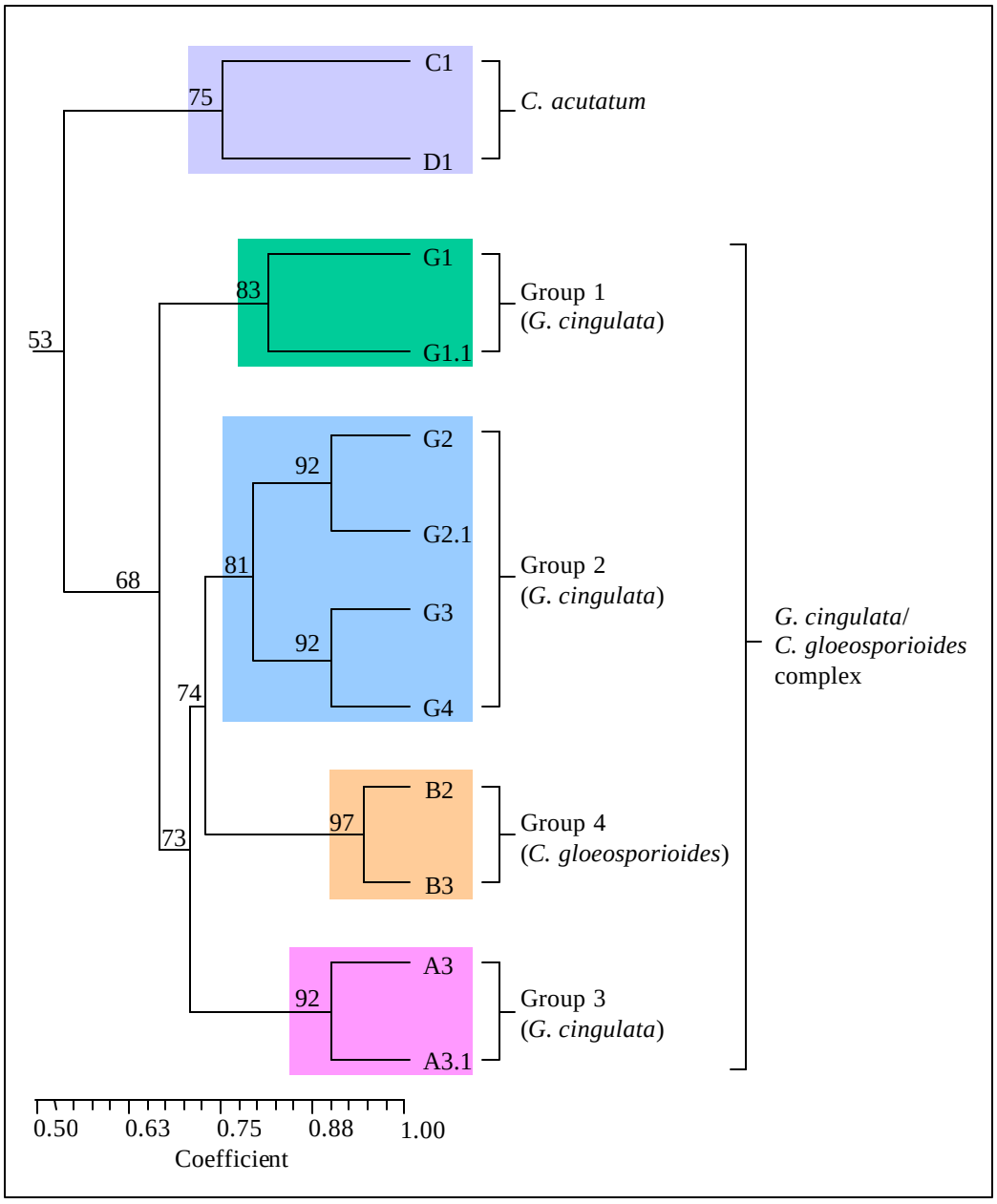

Figure 2.2. Similarity of mtDNA haplotypes obtained from the mtDNA RFLP analysis of genomic DNA digested with *Msp*1 of isolates of *C. acutatum*, *C. gloeosporioides* and *G. cingulata* from the US and Brazil. Analysis of similarity was done with the clustering method UPGMA using NTSYSpc.

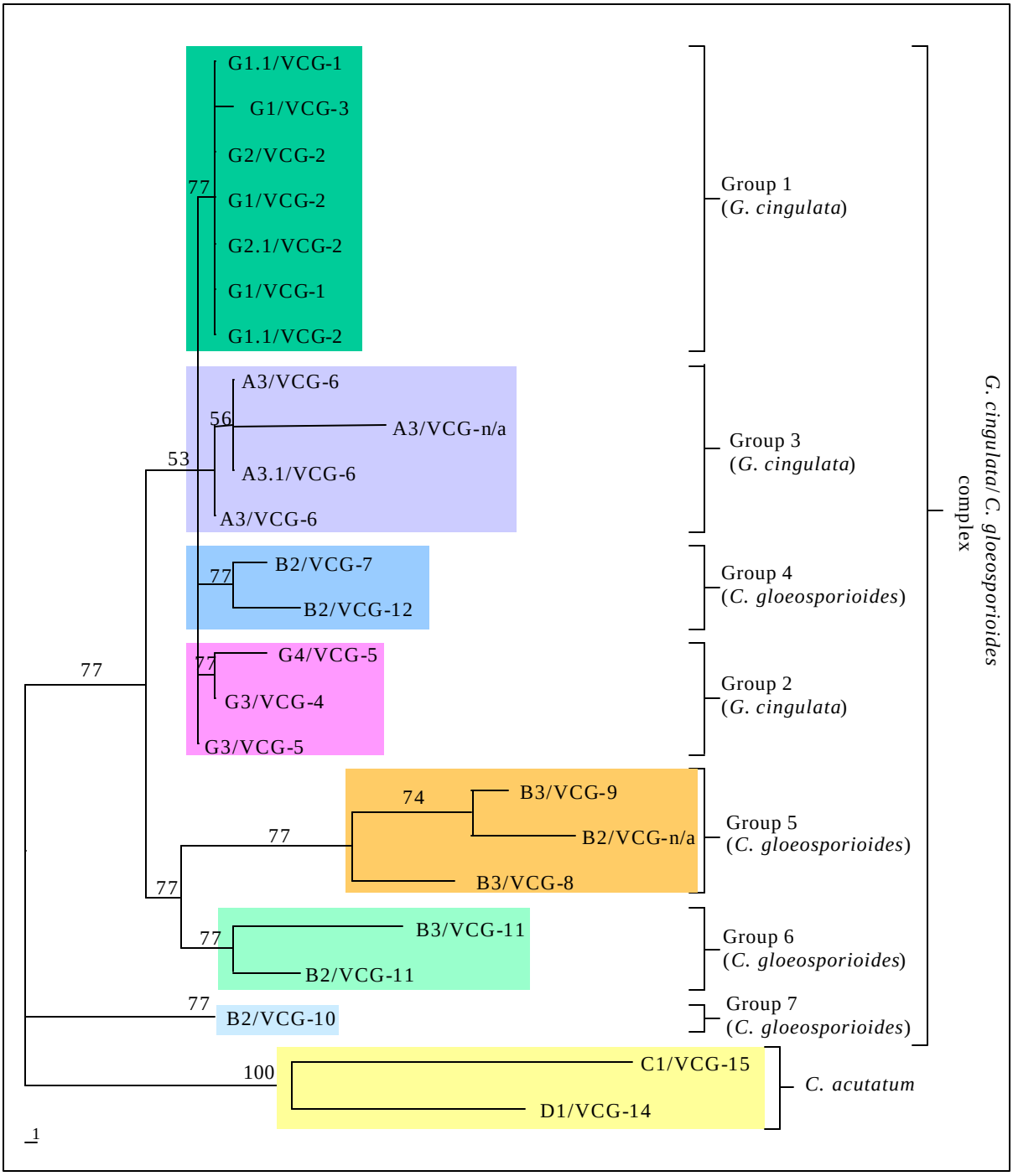

Figure 2.3. Maximum Parsimony (MP) phylogenetic tree based on the sequence of a 200 bp intron of the GDPH gene showing the relationship among and between isolates of *G. cingulata, C. gloeosporioides,* and *C. acutatum* obtained from a collection of isolates previously characterized based on morphology, vegetative compatibility, and mtDNA RFLPs. Values for bootstrap 50% majority-rule consensus are labeled on the branches of the tree. Number of bootstrap replicates was equal to 100. Scale bar represents the number of transformation from one character to another. Isolates are designated by their haplotype and VCG. n/a = isolates were not compatible with any of the VCGs.

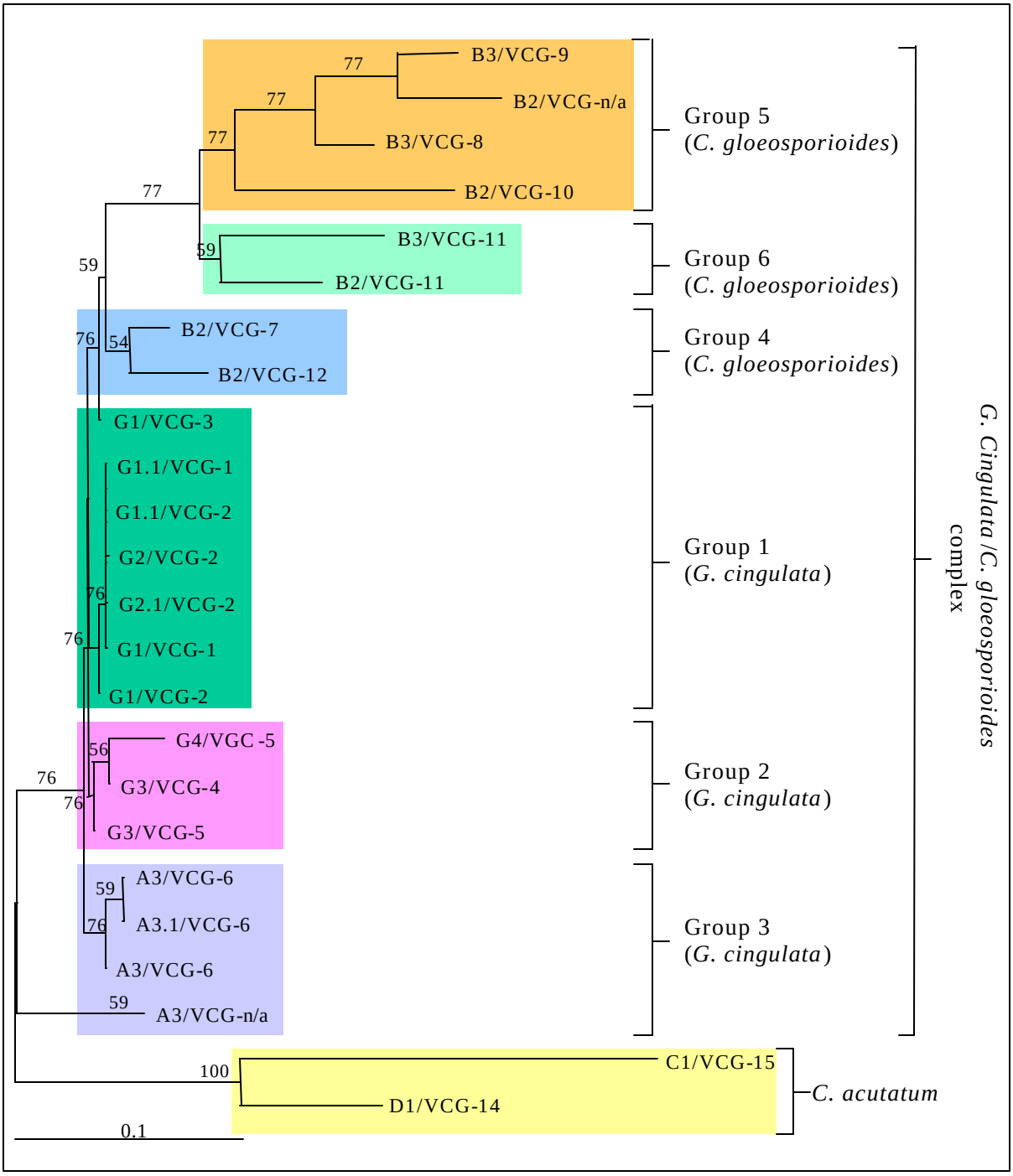

Figure 2.4. Phylogenetic tree based on Neighbor-Joining (NJ) analysis of the sequence of a 200 bp intron of the GDPH gene representing the relationship among and between isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* obtained from a collection of isolates previously characterized based on morphology, vegetative compatibility, and mtDNA RFLPs. Values for bootstrap 50% majority-rule consensus are labeled on the branches of the tree. Scale bar represents the average number substitutions per site over time. Isolates are designated by their haplotype and VCG. n/a = isolates were not compatible with any of the VCGs.

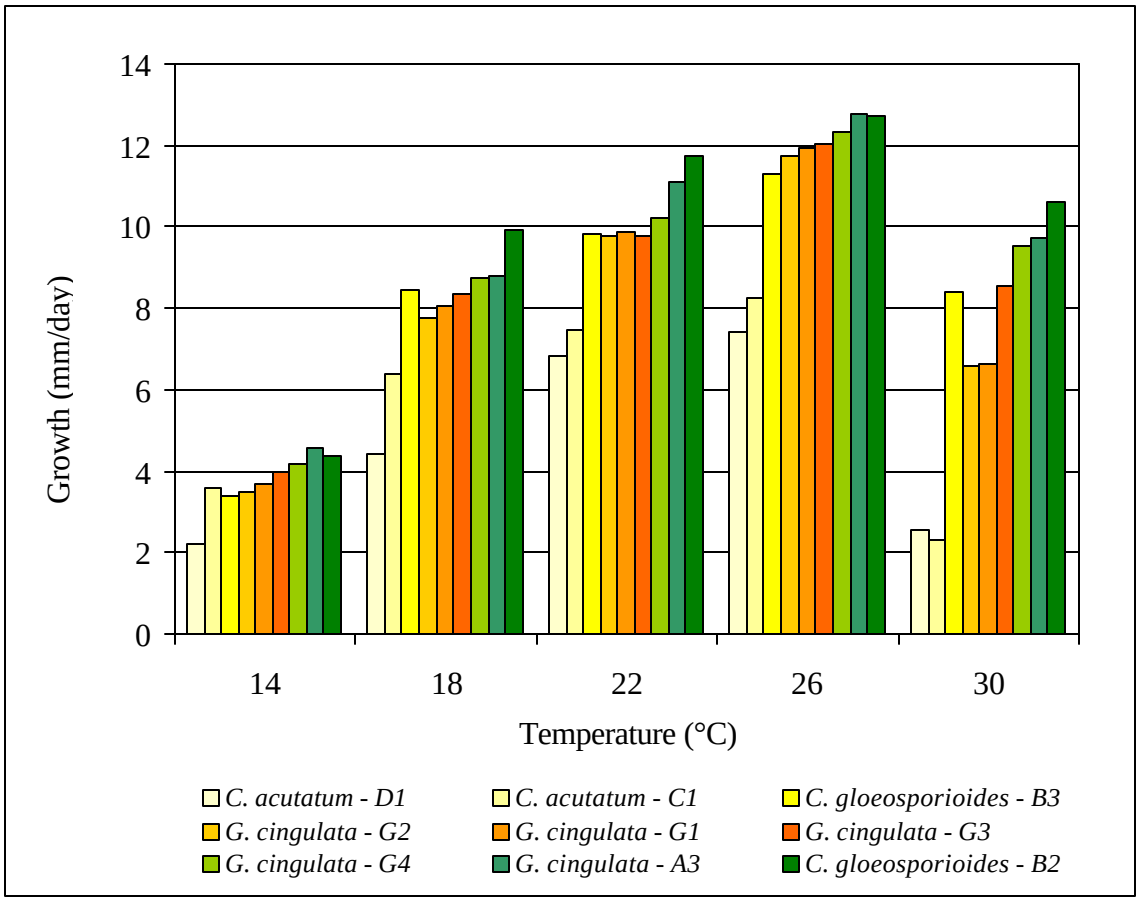

Figure 2.5. Growth of isolates of *G. cingulata, C. gloeosporioides* and *C. acutatum* at five different temperatures (14, 18, 22, 26, and 30°C). Growth represents the average over 6 days of the mean colony diameter (mm/day) of the isolates measured every 2 over 6 days. Isolates tested represent 9 haplotypes found in the mtDNA RFLP analysis (Chapter 2), and are the same isolates listed in Appendix 2.3.

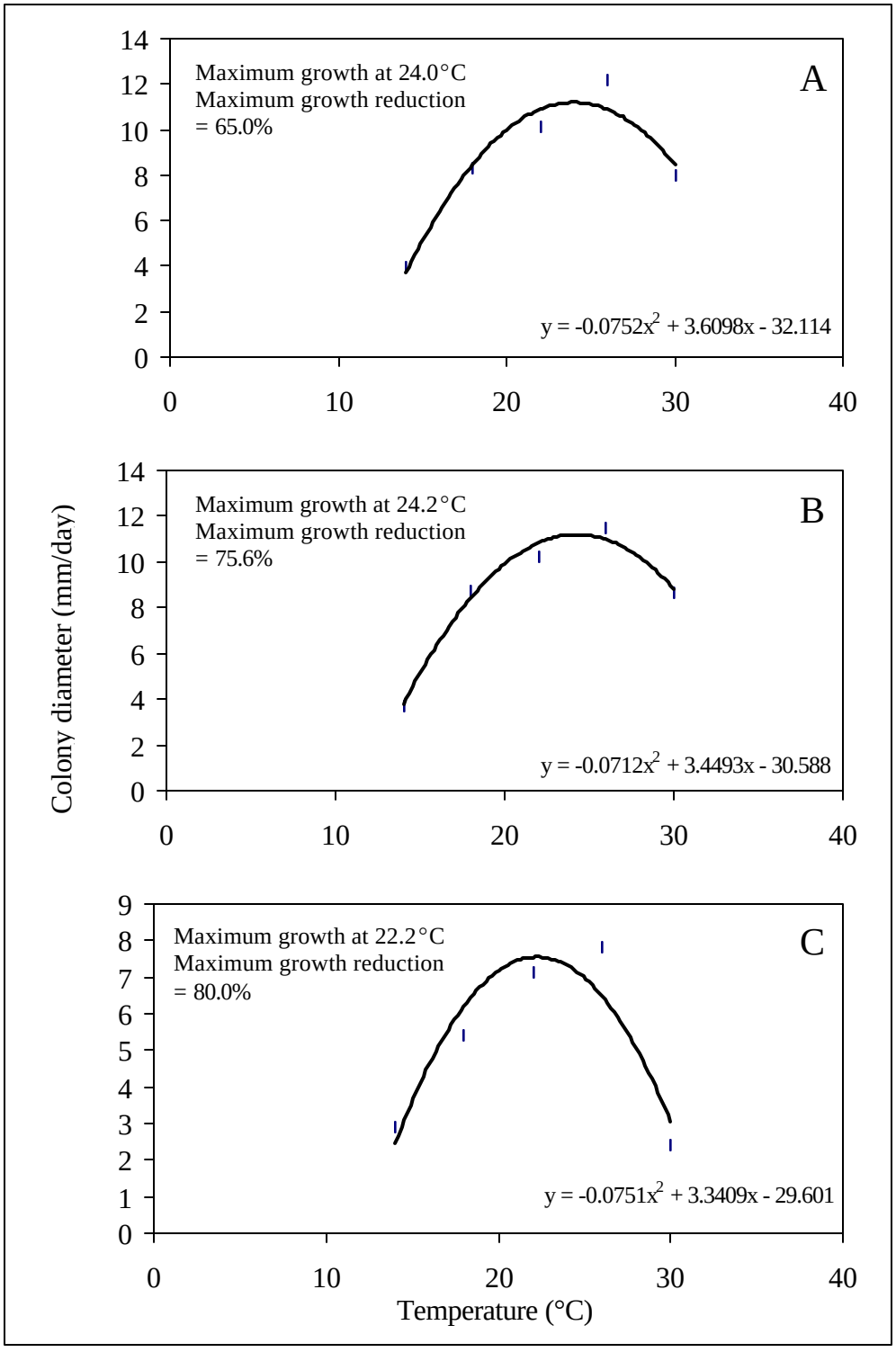

Figure 2.6. Effect of temperature on the growth of isolates of *G. cingulata* (A), *C. gloeosporioides* (B), and *C. acutatum* (C). Maximum growth = (-b1/2b2), where b1 and b2 are the coefficients for the linear and quadratic terms, respectively, in the quadratic equation [growth = b0 + b1(temp) + b2(temp^2)]. Maximum growth reduction was calculated at 14°C for the three species.

3. CHAPTER III

Effect of urea, *Trichoderma harzianum* T-22 and the shredding of leaf litter on the management of apple pathogens and species of arthropods that overwinter in apple leaf litter

3.1. ABSTRACT

The effect of pre-leaf drop applications of urea and *Trichoderma harzianum* T-22, followed by leaf litter shredding, on various pathogens and arthropods that overwinter in apple leaf litter were studied during 1999 and 2000. Treatments consisting of a pre-leaf drop application of 5% urea followed by leaf litter shredding in early December; pre-leaf drop application of T-22 followed by leaf litter shredding in early December; and leaf litter shredding only in early December, were applied to plots arranged in a randomized block design in an orchard of cv. Delicious located in Henderson Co., NC. Airborne ascospores of *V. inaequalis* were monitored using Burkard spore traps to determine ascospore concentrations during the main periods of ascospore discharge. Fruit and foliar incidence of apple scab were also determined. Incidence and severity of Alternaria blotch (*Alternaria alternata*), the population of the spotted tentiform leafminer (*Phyllonorycter blancardella*), and the population of the European red mite (*Panonychus ulmi*) were also monitored. Leaf litter shredding in the late winter significantly reduced the leaf litter the following spring. Urea applications and leaf litter shredding successfully reduced the concentration of ascospores of *V. inaequalis* in the air 1999 and 2000, but had no influence on the incidence of fruit and foliar scab. There were no differences among the treatments in the incidence and severity of Alternaria blotch or populations of the leafminer and mites. Fungicides, insecticides, and acaricides, superimposed upon all treatments may have masked the influence of the treatments on the incidence and severity of diseases and populations of arthropod pests.

3.2. INTRODUCTION

Control of diseases and arthropods in apple orchards relies heavily on pesticides. As a consequence, pesticide use and costs in apples is among the highest of any crop. In North Carolina, 12 to 14 fungicide and 6 to 8 insecticide/acaricide applications are typically made each season to protect apple fruit and foliage. This intensive use of pesticides represents important costs for growers and can have a negative impact on the environment (17). Another problem that the continued use of pesticides has created is the acquired resistance of pests to pesticides. Resistance of *Venturia inaequalis*, cause of apple scab, to the benzimidazole fungicides (benomyl and thiophanate methyl) and dodine has been observed for a number of years (12), and resistance to the sterol demethylation (DMIs) and strobilurin fungicides (12,14) is a concern. Additionally, several apple arthropod pests have developed resistance to a number of insecticides commonly used in apples (3,13,16,20,21,23,29,31). Some of these arthropods include the spotted tentiform leafminer (*Phyllonorycter blancardella*), the tufted apple bud moth (*Platynota idaeusalis*), the rosy apple aphid (*Dysaphis plantaginea*), and the European red mite (*Panonychus ulmi*).

Integrated pest management (IPM) has promoted alternative tactics to manage pathogens and arthropods in apples in order to reduce the number of pesticide applications. These programs have included cultural practices and biological control, complemented with scouting programs and predictive models (15,17). Apple scab is considered the most economically important disease of apples worldwide Therefore, the majority of IPM measures for management of apple pathogens have been aimed at this disease.

Sanitation practices have been used for the management of apple scab in order to reduce or eliminate the production, development, or availability of primary inoculum (15).

Because leaf litter is the overwintering source of ascosporic inoculum for *V. inaequalis*, sanitation practices have focused on removing or destroying scabbed leaves on the ground by tilling or shredding the leaf litter. Sutton *et al*. (26) found that shredding leaf litter in apple orchards in the fall or spring reduced the risk of apple scab by 80-90%. Urea has been utilized to accelerate leaf litter decomposition and to reduce pseudothecial formation. This practice has been shown to decrease primary infection and disease incidence (5,7,18,26,27). Several fungi including *Athelia bombacina*, *Coniothyrium* sp., *Phoma* sp., *Microsphaeropsis* sp. and *Trichoderma* sp., isolated from apple leaves, have been found to be antagonists of *V. inaequalis* and to reduce the number of pseudothecia and ascospores produced in leaf tissue (4,5,22). Other antagonists, such as *Chaetomium globosum* and *Trichoderma viride*, can also inhibit conidia production, which results in a reduction of disease development (1).

Practices that enhance leaf litter decomposition may also have potential to control other apple pathogens that overwinter in dead leaves in the orchard, such as *Alternaria alternata* f.sp. *mali* (=*A. mali* Roberts). This fungus causes the disease known as Alternaria blotch, which has become a serious disease of cv. Delicious apples in the southern United States (9). Severe infection can result in significant defoliation. In addition, control of Alternaria blotch has become a problem for apple growers, because fungicides registered to control other diseases have little effect on this disease (8).

The spotted tentiform leafminer (*Phyllonorycter blancardella*), an important indirect apple insect pest, overwinters inside leaves on the orchard floor. Although the influence of leaf litter reduction on insect populations in apple orchards has not yet been studied, this practice has been shown to reduce and even eliminate overwintering populations of insect pests in other crops (24,25,28). This suggests that leaf litter reduction may also have

potential to aid in the management of apple insects that overwinter in leaves. However, leaf litter reduction could also affect populations of beneficial organisms that overwinter inside leaves, such as the coccinelid *Stethorus punctum*, a mite predator commonly found in apple orchards that helps keep European red mite, *Panonychus ulmi*, populations below damaging levels (17).

The purpose of this study was to determine the effect of pre-leaf drop applications of urea, and a biological control, *T. harzianum* strain T-22 and leaf litter shredding on the incidence and severity of apple scab and Alternaria blotch, and the populations of the spotted tentiform leafminer and the European red mite. The influence of these practices on populations of the mite predator *Stethorus punctum* was also evaluated.

3.3. MATERIAL AND METHODS

3.3.1. Practices for leaf litter management. A 2.4-ha orchard of cv. Delicious located in Henderson Co., North Carolina, was selected for the study. There was a distance of 7.6 m between rows in the orchard, and every fifth row was a pollonizer of either Mutsu or Paula Red. The grower had used an IPM program in the orchard for the last 10 years.

Three different treatments were evaluated in the winter of 1998: a) pre-leaf drop airblast sprayer application of 5% urea (44.8 kg/ha), followed by leaf litter shredding in early December; b) pre-leaf drop application of *T. harzianum* strain T-22 at 3.4 kg/ha + 1% Latron B1956 (Rohm and Haas) with an airblast sprayer, followed by leaf litter shredding in early December; and c) control treatment that consisted of standard grower practices according to the Integrated Orchard Management Guide from Commercial Apples in the Southeast (30). *T. harzianum* strain T-22 was used because *Trichoderma* species have exhibited activity to *V. inaequalis* (1,4,5,22) and T-22 is commercially available (10). A fourth treatment consisted

of leaf litter shredding only in early December added in the winter of 1999. Also in early April 2000 a second application of T-22 at the same rate was made to the leaves on the orchard floor by directing the sprayer nuzzles toward the ground. Airblast sprayer applications were made using 935.3 litters of water/ha. All treatments were applied to four-row plots with 50-80 trees each. Plots were separated on two sides by a pollinizer row and on the other two sides by four non-treated border trees (Fig. 3.1). The plots were arranged in a randomized block design, with four replications, except the replications of the treatment with leaf litter shredding only that were located together in a section of the orchard that was not included in the study in the winter of 1998. A standard in-season pesticide program was superimposed on each treatment.

Leaf litter density was determined monthly from December through April in the plots using a modification of the point-intercept method (19). Using this procedure, the percentage of ground area covered by leaf litter in 50 x 50 cm squares along points every 1 m apart in transects that ran across adjacent rows was estimated. Two transects each were located in the middle of rows 1 and 2, rows 2 and 3, and rows 3 and 4 within each replication. Six different transects were evaluated in each plot (Fig. 3.1).

3.3.2. Influence of leaf litter management on pathogens and arthropods. The ascospore concentration of *V. inaequalis* in the air was monitored with Burkard spore traps from late March through early May in 1999 and 2000. Spore traps were located in the middle of plots (replications) in each of the three replications in 1999. In 2000 traps were located in the middle of three of the replications of the control and in two replications of the other treatments. Two leaf wetness and temperature loggers (Spectrum Technologies, Inc)

and a leaf wetness meter (IFG de Wit BV, The Netherlands), were used to monitor leaf wetness within the orchard. These data were used to track rain events that trigger *V. inaequalis* ascospore discharge. Aerial ascospore concentration was quantified during these rain events by counting the total number of ascospores trapped during each period, and adjusting the total according to the airflow of the Burkard trap during each wet period. Foliar and fruit incidence of apple scab were determined at the end of May and at harvest, respectively. Six experimental trees within each plot were arbitrarily selected to conduct these ratings. Foliar scab incidence was determined counting all scabbed leaves on the experimental trees within each plot during a 2-minute search on 31 May 1999 and 7 June 2000. Fruit scab incidence in 1999 was estimated by counting the number of fruit with scab lesions from a sample of 150 fruit per plot collected from the trees within each plot at harvest on 1 October, and in 2000 it was obtained on 28 June by counting scabbed fruit on the trees within each plot during a 2-minute search.

Four terminals were selected on each of six trees per plot and were rated for incidence and severity of Alternaria blotch. Disease incidence was estimated by calculating the percentage of diseased leaves on each terminal. Severity was assessed using a modified Horsfall-Barratt disease rating scale with values from 0-6, where 0 = no lesions; 1 = 1-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50%, where the percentages represent leaf area affected. Ratings were conducted every 2 weeks from mid-June through mid-August in 1999, and from mid-July to mid-August in 2000.

The first generation of spotted tentiform leafminer was monitored by arbitrarily selecting 20 clusters of spur leaves on six trees per plot and counting the total number of mines present in each cluster. For sequential generations (second, third and fourth), 20 new

clusters were selected on each tree, and the number of mines per cluster were differentiated as sap feeding, tissue feeding or emerged stages. Additionally, 10 leaves from each tree were arbitrarily selected to determine the number of mites per leaf. Mites were counted using 10X magnifying visor lens. In 1999, leafminer populations were monitored monthly from late May through late July, and mites were monitored every 2 weeks in June. The leafminer was not monitored in 2000 since populations were very low and only one rating of the mite population was conducted in mid-June.

3.3.3. Data analysis. Treatments were compared in SAS® (Windows version, release 6.12) using analysis of variance and means were separated by the Waller-Duncan *k*-ratio *t* test. Because some spore traps failed to operate during the time of the main periods of ascospore discharge, some of the treatment repetitions were lost and therefore the influence of the treatments on ascospore concentration of *V. inaequalis* was not statistically analyzed.

3.4. RESULTS

3.4.1. Effect of the treatments on leaf litter density. The amount of leaf litter was higher in the control treatment, compared with all other treatments in both 1999 and 2000, with densities of 38 and 24% in the winter, and declining to 15 and 9% in the spring, respectively (Fig. 3.2). Differences among the other treatments were not significant. Leaf litter densities were lower in 2000 than in 1999 in all treatments. In 1999 densities ranged from 38% in the control treatment to 3% in the T-22 plus leaf shredding treatment, and in 2000 from 24% in the control treatment to less than 1% in the treatment with leaf shredding only.

3.4.2. Effect of leaf litter management on pathogens and arthropods. Five and three main periods of ascospore discharge occurred in 1999 and 2000, respectively (Tables 3.1 and 3.2). Overall, ascospore concentrations were lower in 2000 than in 1999. However, in both years the highest number of ascospores were discharged on the same date, 15 April. A mean of 655.2 ascospores/period were trapped on this date in 1999 (third period), and a mean of 106.8 ascospores/period on this date in 2000 (second period). Compared to the control, all treatments reduced the number of ascospores. Percentage reduction ranged from 67-92% in 1999 and from 81-91% in 2000. The treatment with urea and leaf litter shredding had lower ascospore concentrations than the other treatments, with reductions ranging from 67-100%. However, in the first discharge period of 1999, more ascospores were trapped in the urea plus leaf shredding treatment than in the control (Tables 3.1 and 3.2).

Some differences in foliar and fruit scab incidence occurred among the treatments; however, they were neither consistent nor significant (Table 3.3). Although ascospore discharge during all wetting periods monitored was higher in the control treatment, disease incidence in this treatment was lower on leaves in 2000 and on fruit in 1999. In addition, fruit scab incidence in 2000 was higher in the treatments with urea plus leaf shredding and the T-22 plus leaf shredding than in the control. Only in 1999 was foliar incidence in the control plots higher than in the other treatments. In the urea plus leaf shredding plots, foliar and fruit scab incidence in 2000 was higher than in plots of the other treatments.

In 1999 and 2000, the incidence of Alternaria blotch was lower in the control plots and the plots with leaf shredding, respectively (Fig. 3.3 and 3.4). No significant differences in disease incidence occurred among the other treatments. Overall, disease incidence in all

treatments was higher in 1999 than in 2000. Disease severity was similar among treatments and ranged from 1 to 1.3, which represents only 1-3% of leaf area affected (Fig. 3.3 and 3.4).

European red mite and spotted tentiform leafminer populations were very low in both 1999 and 2000. In general, the plots treated with urea plus leaf litter shredding had a lower number of mites per leaf than the other treatments, but these differences were not significant (Table 3.4). Lower numbers of leafminer mines were found in the control plots on the last leafminer rating date (July, 1999), but there were no significant differences among the treatments (Table 3.5).

3.5. DISCUSSION

All treatments that included leaf litter shredding significantly reduced the leaf litter in spring. Pre-leaf drop applications of urea or T-22 followed by leaf litter shredding in early December and leaf litter shredding alone in early December substantially reduced the concentration of *V. inaequalis* ascospores in the air the following spring. Pre-leaf drop applications with urea and leaf shredding are recommended as practices that enhance leaf litter decomposition and reduce the primary inoculum of apple scab (5,7,18,26,27). Reduction of leaf area by shredding and softening of leaf tissue with urea applications, make leaves easily accessible and more palatable for leaf-degrading microorganisms (18). Additionally, applications of urea increase numbers and diversity of microorganisms involved in leaf litter decomposition (27). Some of these microorganisms can also act as antagonists to *V. inaequalis*, affecting growth development and production of pseudothecia, ascospores, and conidia (1,4,5,22). This may explain the greater reduction of ascospores in the plot sprayed with urea. Even though ascospore concentration of *V. inaequalis* in the air

was successfully reduced in all treatments, compared to the control, the effect of treatments was not reflected by significant reductions in the incidence of scab on the leaves or fruit. Because leaf litter is the primary overwintering site of *A. alternata* (9) we expected a reduction in the incidence and severity of Alternaria blotch. However, we also observed a lack of consistency in the influence of the treatments on incidence and severity of Alternaria blotch.

Heijne *et al*. (11) found that under field conditions, fungicide programs following a pre-leaf drop application of urea masked the effect of urea on apple scab. This might explain the lack of influence of the treatments on apple scab and Alternaria blotch, since standard grower practices, including fungicide programs were superimposed on the experimental plots. Furthermore, the lower incidence of Alternaria blotch in the control treatment suggests interplot contamination. Ascospores of *V. inaequalis* are released by rain and are airborne. However, a reduction of 99% in the concentration of airborne ascospores occurs within 5-6 m from the source of inoculum (6). In the present study trees used for the evaluation of apple scab and Alternaria blotch were separated from each other by at least 20 m and were located in the middle of plots to avoid contamination from the other sources. Thus, the plots should have been large enough to detect treatment effects on *V. inaequalis* ascospore inoculum. However, *Alternaria* is mainly an airborne pathogen and can be disseminated farther distances than *V. inaequalis*, and larger experimental plots than the ones used in this study may be necessary to detect treatments effects on Alternaria blotch. Additionally, the plots with only leaf litter shredding, which were included only in 2000 and showed the lowest incidence of Alternaria blotch that year, were not randomly arranged and more trees were missing within each plot.

Trichoderma sp. has been repeatedly found among populations of microorganisms isolated from leaves collected in apple orchards. This saprophytic fungus has also been reported as antagonist of *V. inaequalis* inhibiting ascospore, pseudothecia, and conidia production of the fungus (1,2,4,5,22). Philion *et al*. (22) found that among a series of antagonists of *V. inaequalis*, *Trichoderma* sp. (strain 1H22) inhibited ascospore production more than 93%. Based on these reports we expected to find greater ascospore reduction in the treatment with T-22 followed by leaf litter shredding than in the treatment with only leaf litter shredding. However, reductions in this treatment were either similar or lower than in the T-22 plus leaf litter shredding treatment. T-22 was selected among other strains of *T. harzianum* because it is highly rhizosphere competent and is recommended for control of root diseases in greenhouses and nurseries, and for the control of foliar diseases caused by *Botrytis* and powdery mildews (10). However, while T-22 is extremely persistent on root surfaces, it does not persist at biologically significant levels in the absence of roots (10) and repeated applications are necessary to achieve satisfactory control. Therefore, it is possible that when T-22 was applied before leaf drop, it did not survive on leaves through leaf drop. Additionally, it is possible that temperatures after T-22 applications, especially low temperatures during the winter, affected the survival of *Trichoderma* on leaf litter. We were unable to detect T-22 in the leaf litter in samples taken in January and February of 2000 (E. González, unpublished); consequently a second application of the T-22 was applied in the spring of 2000 to leaves on the orchard floor. However, we still did not observe a significant effect of T-22 application.

In a study conducted in the Netherlands, population densities of *Phyllonorycter plattani*, a lifeminer on *Platanus* that overwinters in the pupal stage in the mines of fallen

leaves, were almost completely eliminated by removing all fallen leaves from the ground (28). Because the practices used in the present study substantially reduced leaf litter, and the because spotted tentiform leafminer and the mite predator *Stethorus punctum* overwinter in the leaf litter of apple orchards, we expected that the reduction of leaf litter would reduce the populations of leafminers and possibly increase mite populations. The absence of treatment effects on these arthropods was probably due to the very low population densities of leafminers and mites; neither pest reached damaging levels even in the control treatment.

Our study demonstrated that leaf litter shredding in the late fall significantly reduced the leaf litter in the spring. Although we were not able to show differences among the treatments because of low pathogen and arthropod populations and interplot interference (Alternaria blotch), we believe that leaf litter shredding in the fall is a sound apple orchard IPM practice that has the potential to aid in the management of diseases and insect and arthropod pests. In addition to *V. inaequalis* and *A. alternata*, *Mycophaerella pomi*, cause of Brooks spot, also overwinters in apple leaves, and populations of this pathogen should also be reduced by leaf litter shredding. Additional studies are needed to determine if there are any negative effects of leaf shredding on beneficial arthropod species.

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Table 3.1. Main periods of ascospore discharge for *Venturia inaequalis* during 1999 in an orchard of cv. Delicious located in Henderson Co., NC[x]

Treatment	Ascospore discharge (ascospores/period)[yz]				
	1	2	3	4	5
Urea + leaf shredding	75.2	154.3 (67)	114.3 (83)	2.6 (90)	4.0 (89)
T-22 + leaf shredding	n/a	n/a	124.7 (81)	4.4 (82)	2.8 (92)
Control	62.2	463.4	655.2	24.7	36.0

[x] Main ascospore discharge periods correspond to wet periods from March through April. 1: 31 March 7 am to 8 pm; 2: 8 April 1 pm to 12 pm; 3: 15 April 11 am to 12 pm; 4: 27 April 7 pm - 28 April at noon; 5: 29 April 1 am - April 30 at noon.
[y] Total number of ascospores trapped with Burkard traps in each wet period at an airflow of 10 L of air/min.
[z] Numbers between parenthesis represent percentage reduction in ascospore discharge compared to the control.

Table 3.2. Main periods of ascospore discharge for *Venturia inaequalis* during 2000 in an orchard of cv. Delicious located in Henderson Co., NC[x]

Treatment	Ascospore discharge (ascospores/period)[yz]		
	1	2	3
Urea + leaf shredding	2.8 (91)	0 (100)	N/a
T-22 + leaf shredding	4.4 (86)	10.0 (91)	4.7 (90)
Leaf shredding	n/a	8.9 (92)	8.9 (81)
Control	32.2	106.8	45.8

[x] Main ascospore discharge periods correspond to wet periods in April. 1: 13 April 4 am to 10 pm; 2: 14 April 2 pm - 15 April at noon; 3: 15 April 2 pm - 16 April 10 am.
[y] Total number of ascospores trapped with Burkard traps in each wet period at an airflow of 10 L of air/min.
[z] Numbers between parenthesis represent percentage reduction in ascospore discharge compared to the control.

Table 3.3. Foliar and fruit incidence of Apple scab (*Venturia inaequalis*) in 1999 and 2000 in an orchard of cv. Delicious located in Henderson Co., NC

Treatment	Scab incidence[x]			
	Foliar[y]		Fruit[z]	
	June 1999	June 2000	October 1999	June 2000
Urea + leaf shredding	10.0 a	12.8 a	14.8 a	59.3 a
T-22 + leaf shredding	10.5 a	8.7 a	18.0 a	40.5 a
Leaf shredding	n/a	6.2 a	n/a	27.3 a
Control	13.1 a	5.3 a	15.5 a	31.0 a

[x] Means followed by the same letter within the same column are not significantly different at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test

[y] Foliar incidence represents the total number of leaves with apple scab lesions observed in a 2 min search

[z] Fruit incidence in 1999 represents the total number of fruit with apple scab lesions in a sample of 25 fruit/experimental tree/plot for a total of 150 fruit/treatment. Fruit incidence in 2000 represents the total number of fruit with apple scab lesion observed in a 2 min search plus the total number of fruit with apple scab lesions on the ground.

Table 3.4. Population of mites during 1999 and 2000 in an orchard of cv. Delicious located in Henderson Co., NC

Treatment	Number of mites/leaf[yz]				
	Summer 1999				Summer 2000
	Late April	Late May	Mid June	Late June	June
Urea + leaf shredding	0.01 a	0.00 a	0.38 a	0.38 a	5.80 a
T-22 + leaf shredding	0.01 a	0.02 a	0.43 a	0.68 a	5.91 a
Control	0.03 a	0.01 a	0.32 a	0.62 a	5.98 a

[y] Number of mites/leaf was determined by counting the number of mites present on 10 leaves/experimental tree

[z] Means followed by the same letter within the same column are not significantly different at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test.

Table 3.5. Population of leaf miners during 1999 in an orchard of cv. Delicious located in Henderson Co., NC

Treatment	Number of mines/20 leaf clusters[yz]		
	May	**June**	**July**
Urea + leaf shredding	0.00 a	0.06 a	0.13 a
T-22 + leaf shredding	0.01 a	0.01 a	0.13 a
Control	0.01 a	0.04 a	0.25 a

[y] Number of mines was determined by counting the total number of mines present in 20 clusters of spur leaves/experimental tree.

[z] Means followed by the same letter within the same column are not significantly different at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test.

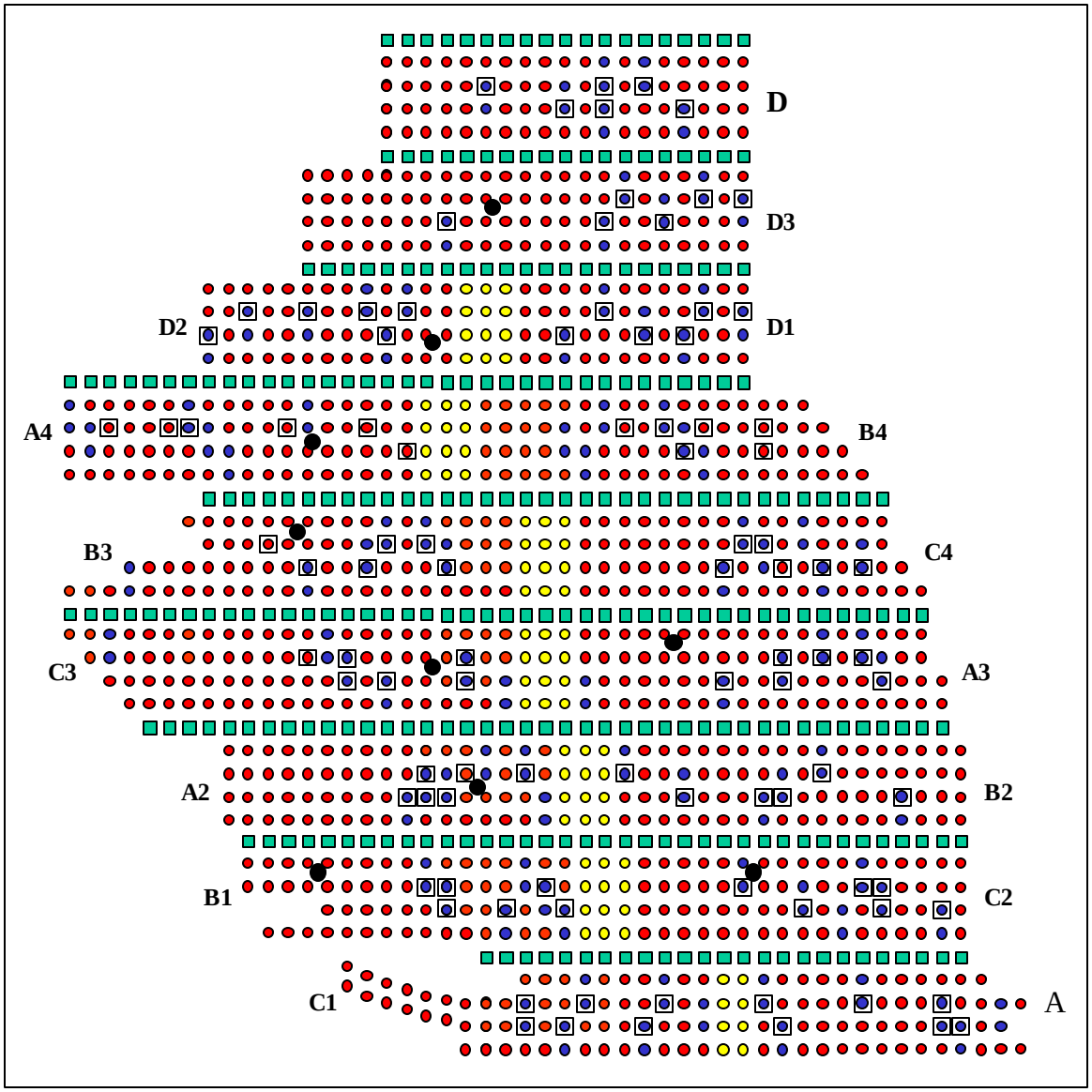

Figure 3.1. Layout of the treatments in the orchard of cv. Delicious located in Henderson Co., NC. A. Control treatment; B. Urea plus leaf litter shredding treatment; C. T-22 plus leaf litter shredding treatment; D. Leaf litter shredding only treatment. Delicious trees; transects in which the leaf litter density was estimated; Mutsu or Paula trees (pollinizers); border trees between plots; experimental trees within each plot used to collect the data; location of the Burkard spore traps in each plot.

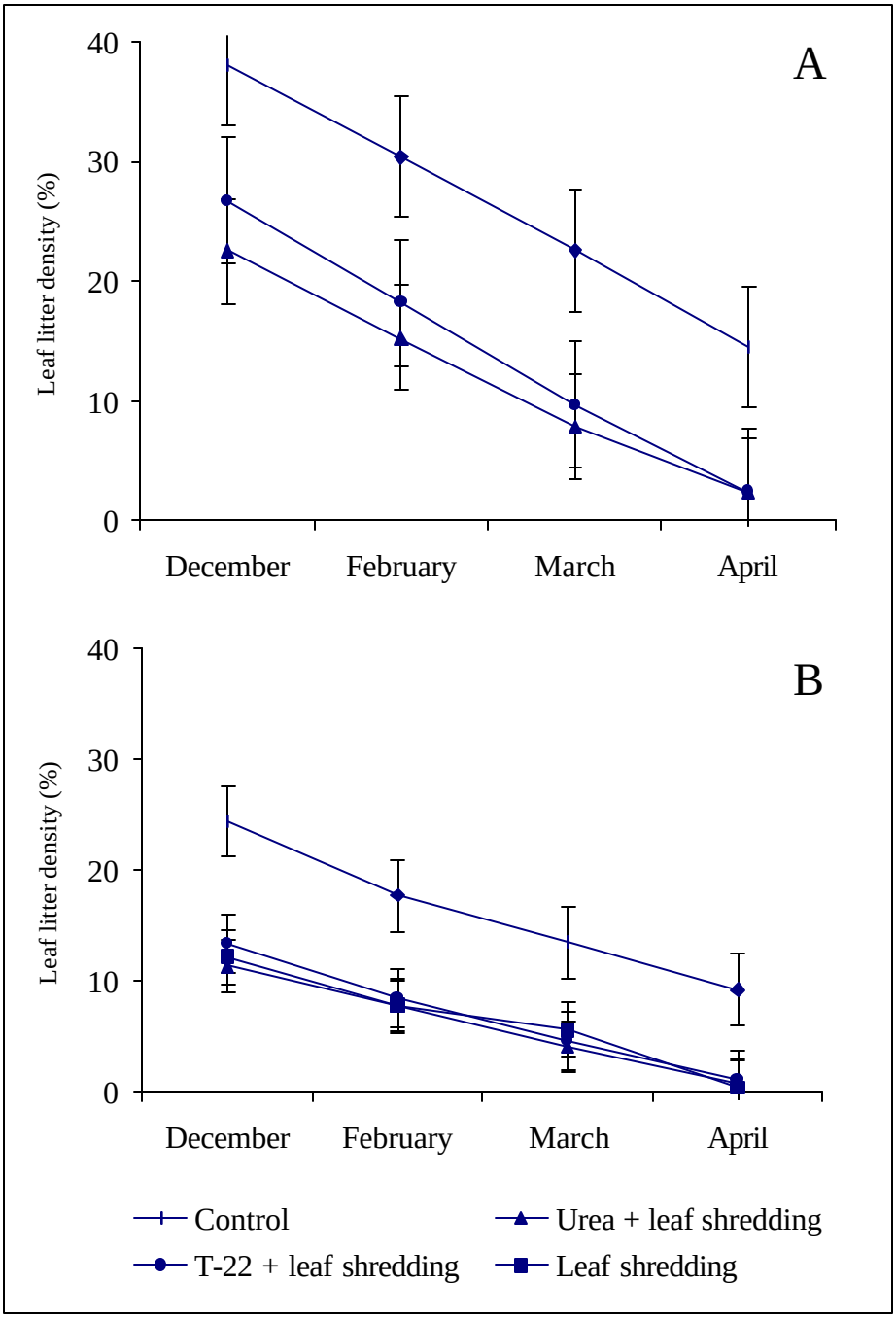

Figure 3.2. Leaf litter density in an orchard of cv. Delicious located in Henderson Co. NC. A. 1999. B. 2000. Density was determined by estimating the percentage of area covered by leaf litter in 50 x 50 cm squares along points every 1 m apart in transects that run across adjacent rows.

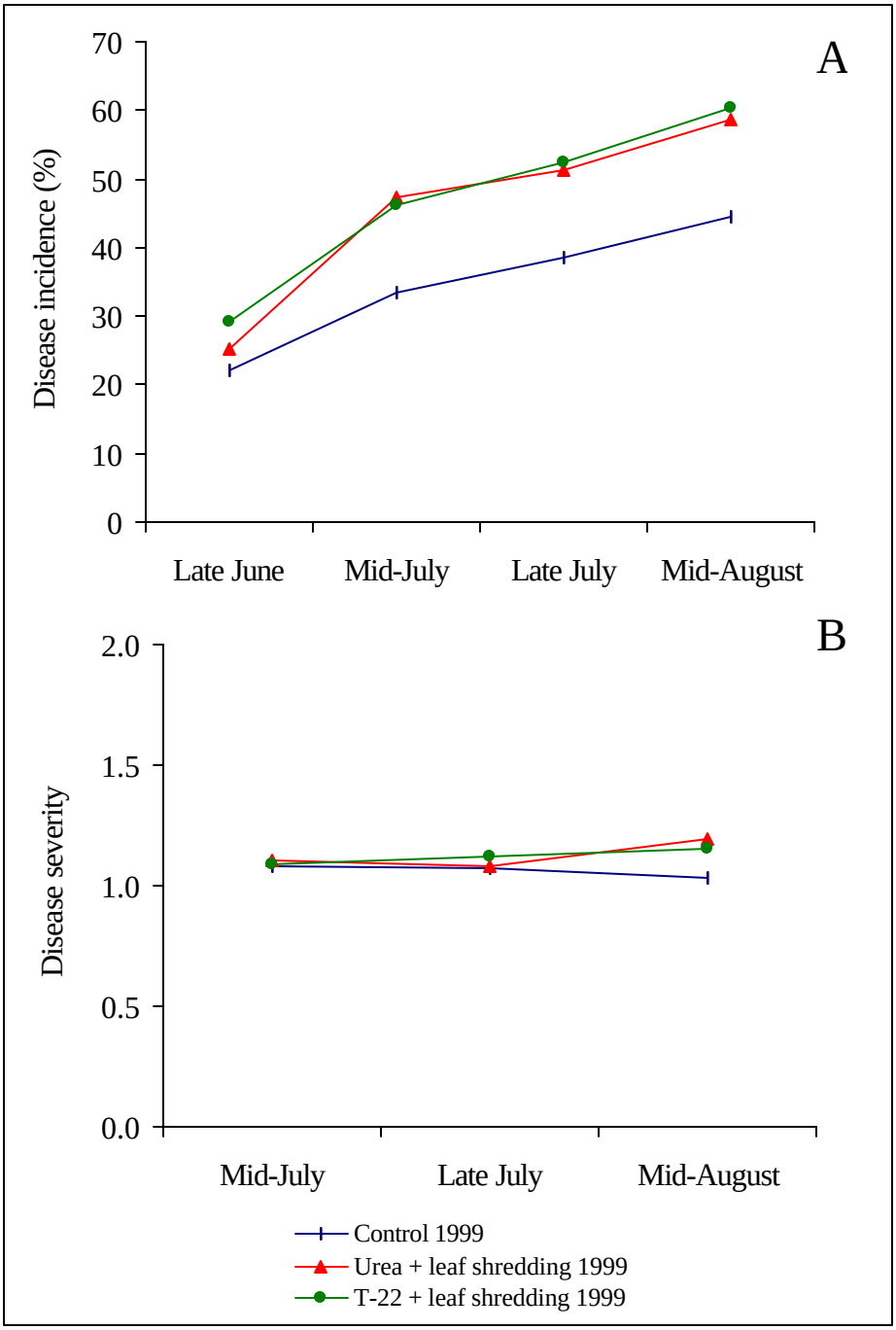

Figure 3.3. Disease progress curve of Alternaria blotch (*Alternaria alternata*) during 1999 in an orchard of cv. Delicious located in Henderson Co., NC. A. Disease incidence represents the percentage of diseased leaves. B. Disease severity represents the percentage of leaf area affected and was rated using a modified Horsfall-Barratt disease scale with values from 0-6, where 0 = no lesions; 1 = 1-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50%. Incidence and severity were determined in four terminals of six trees arbitrarily selected within each of the experimental plots.

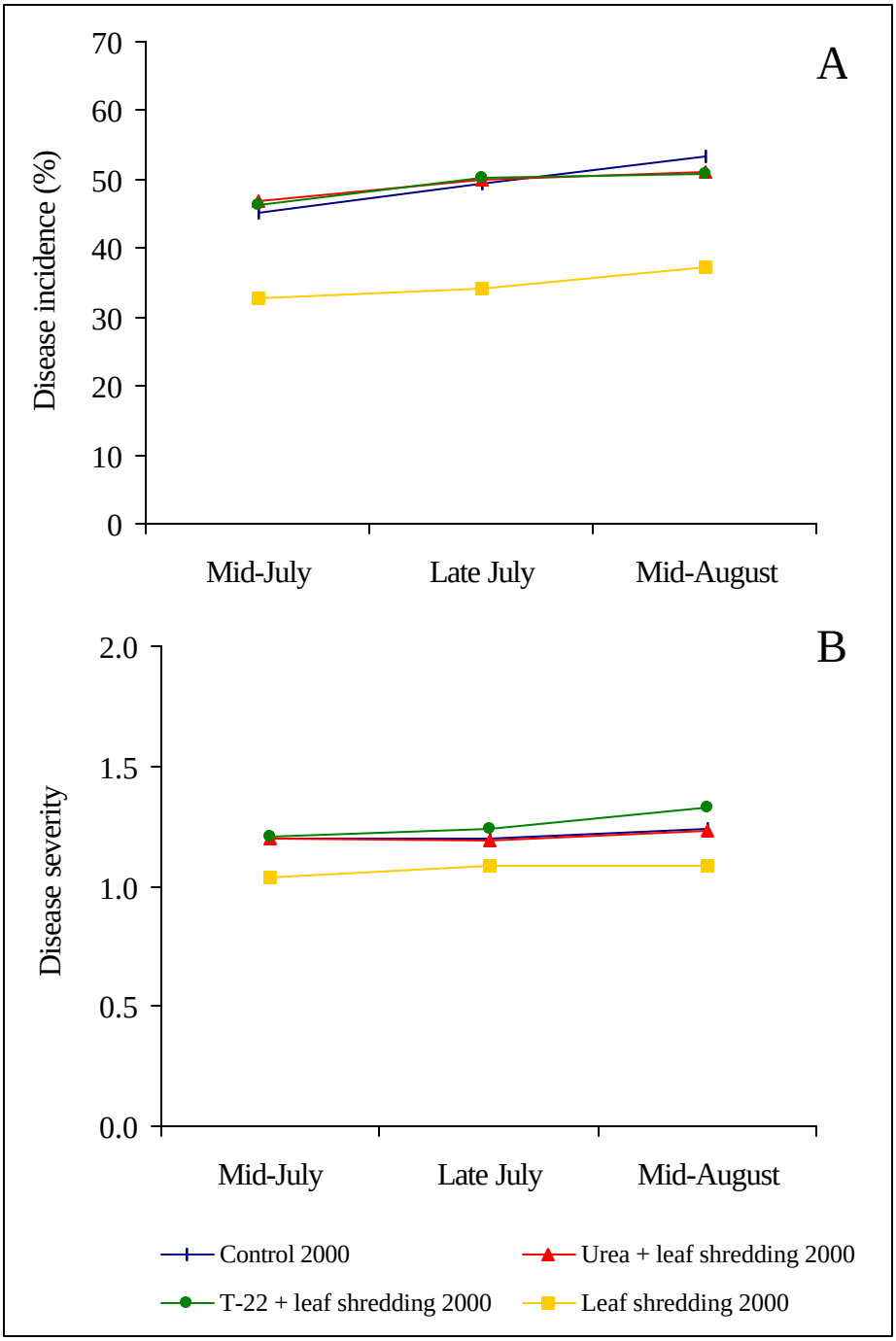

Figure 3.4. Disease progress curve of Alternaria blotch (*Alternaria alternata*) during 2000 in an orchard of cv. Delicious located in Henderson Co., NC. A. Disease incidence represents the percentage of diseased leaves. B. Disease severity represents the percentage of leaf area affected and was rated using a modified Horsfall-Barratt disease scale with values from 0-6, where 0 = no lesions; 1 = 1-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50%. Incidence and severity were determined in four terminals of six trees arbitrarily selected within each of the experimental plots.

4. APPENDICES

Appendix 4.1. Vegetative compatibility groups (VCG) and morphological types of the isolates of *C. acutatum* , *C. gloeosporioides*, and *G. cingulata* examined for vegetative compatibility and characterized morphologically

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
1	SP1	CROTTS 15	*G. cingulata*	NC 1	Fruit	Gala	2002
1	SP1	CROTTS(L) 1	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 2	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 3	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 4	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 5	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 6	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 7	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 8	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 9	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 10	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 11	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 12	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 13	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 14	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 15	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 16	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 18	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 19	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 20	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 21	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 22	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 23	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 24	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 25	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 26	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 27	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 28	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 29	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 30	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 31	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 32	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 33	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 34	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 35	*G. cingulata*	NC 1	Leaf	Gala	2000
1	SP1	CROTTS(L) 36	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 37	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 38	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 39	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 40	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 41	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 42	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 43	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 44	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 45	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 46	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 47	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 48	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 49	*G. cingulata*	NC 1	Leaf	Gala	2002

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
1	SP1	CROTTS(L) 50	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 51	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 52	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 53	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 54	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 55	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 56	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 57	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 58	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 59	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 60	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	CROTTS(L) 61	*G. cingulata*	NC 1	Leaf	Gala	2002
1	SP1	GA 1	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 2	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 3	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 4	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 5	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 6	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 7	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 8	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 9	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 10	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 11	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 12	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 13	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 14	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 15	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 16	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 17	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 18	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 19	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 20	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 21	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 22	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 23	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 24	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 25	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 26	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 27	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 28	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 29	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 30	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 31	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 32	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 33	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 34	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 35	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 36	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 37	*G. cingulata*	GA	Fruit	Gala	2000
1	SP1	GA 38	*G. cingulata*	GA	Fruit	Gala	2000

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
1	SP1	GA 40	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 41	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 42	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 43	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 44	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 45	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA 46	*G. cingulata*	GA	Fruit	Gala	2002
1	SP1	GA(L) 1	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 2	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 3	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 4	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 5	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 6	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 7	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 8	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 9	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 10	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 11	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 12	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 13	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 14	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 15	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 16	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 17	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 18	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 19	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 20	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 21	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 22	*G. cingulata*	GA	Leaf	Gala	2000
1	SP1	GA(L) 23	*G. cingulata*	GA	Leaf	Gala	2002
1	SP1	GA(L) 24	*G. cingulata*	GA	Leaf	Gala	2002
1	SP1	GA(L) 25	*G. cingulata*	GA	Leaf	Gala	2002
1	SP1	TN 7	*G. cingulata*	TN 1	Leaf	Gala	1998
2	SP1	CROTTS 1	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 2	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 3	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 5	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 6	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 8	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 9	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 10	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 11	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 12	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	CROTTS 13	*G. cingulata*	NC 1	Fruit	Gala	2001
2	SP1	LD 7	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 10	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 11	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 13	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 14	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 16	*G. cingulata*	NC 2	Fruit	Granny Smith	2000

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
2	SP1	LD 17	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 18	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 20	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 21	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 23	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 24	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 25	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 26	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
2	SP1	LD 30	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 31	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 32	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 33	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 40	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 45	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 46	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 47	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
2	SP1	LD 71	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 75	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 76	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 79	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 80	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 81	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 82	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 83	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 84	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 85	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 86	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP1	LD 87	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
2	SP2	GS 1	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 2	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 3	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 4	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 5	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 6	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 7	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 8	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 9	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 10	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 11	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 12	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 13	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 14	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 17	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 33	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 36	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 38	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 39	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 42	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 43	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 44	*G. cingulata*	NC 4	Fruit	Granny Smith	2002

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
2	SP2	GS 45	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 46	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 47	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 48	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 49	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 50	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 52	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
2	SP2	GS 53	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
3	SP1	OH 1	*G. cingulata*	OH	Fruit	Molly's Delicious	2001
3	SP1	OH 2	*G. cingulata*	OH	Fruit	Molly's Delicious	2001
3	SP1	OH 3	*G. cingulata*	OH	Fruit	Molly's Delicious	2001
4	SP1	BR 3	*G. cingulata*	Brazil 1	Leaf	Gala	2001
4	SP1	BR 8	*G. cingulata*	Brazil 3	Leaf	Gala	2001
4	SP1	BR 10	*G. cingulata*	Brazil 3	Leaf	Gala	2001
5	SP1	BR 1	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 2	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 4	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 5	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 7	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 7	*G. cingulata*	Brazil 1	Leaf	Gala	2001
5	SP1	BR 9	*G. cingulata*	Brazil 3	Leaf	Gala	2001
5	SP1	BR 11	*G. cingulata*	Brazil 5	Leaf	Gala	2001
5	SP1	BR 12	*G. cingulata*	Brazil 5	Leaf	Gala	2001
5	SP1	BR 13	*G. cingulata*	Brazil 5	Leaf	Gala	2001
5	SP1	BR 14	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 15	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 16	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 17	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 18	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 19	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 20	*G. cingulata*	Brazil 6	Leaf	Gala	2001
5	SP1	BR 21	*G. cingulata*	Brazil 7	Leaf	Gala	2001
5	SP1	BR 22	*G. cingulata*	Brazil 7	Leaf	Gala	2001
5	SP1	BR 24	*G. cingulata*	Brazil 2	Leaf	Gala	2002
5	SP1	BR 25	*G. cingulata*	Brazil 8	Leaf	Gala	n/a*
6	CP	CROTTS 4	*G. cingulata*	NC 1	Fruit	Gala	2001
6	CP	CROTTS 7	*G. cingulata*	NC 1	Fruit	Gala	2001
6	CP	CROTTS 14	*G. cingulata*	NC 1	Fruit	Gala	2001
6	CP	GD 3	*G. cingulata*	NC 3	Fruit	Golden Delicious	2002
6	CP	GD 4	*G. cingulata*	NC 3	Fruit	Golden Delicious	2002
6	CP	GD 5	*G. cingulata*	NC 3	Fruit	Golden Delicious	2002
6	CP	GD 6	*G. cingulata*	NC 3	Fruit	Golden Delicious	2002
6	CP	GD 7	*G. cingulata*	NC 3	Fruit	Golden Delicious	2002
6	CP	GS 15	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 16	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 18	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 19	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 20	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 21	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 22	*G. cingulata*	NC 4	Fruit	Granny Smith	2002

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
6	CP	GS 23	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 24	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 25	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 26	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 27	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 31	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 32	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 34	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 35	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 37	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 40	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 41	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 51	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	GS 54	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	CP	LD 1	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 2	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 3	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 4	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 5	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 6	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 8	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 9	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 12	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 15	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 19	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 22	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 28	*G. cingulata*	NC 2	Fruit	Granny Smith	2000
6	CP	LD 29	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 34	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 35	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 36	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 37	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 38	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 41	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 42	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 43	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 44	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 48	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	CP	LD 49	*G. cingulata*	NC 5	Fruit	Gala	2002
6	CP	LD 50	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 52	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 53	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 55	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 58	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 59	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 60	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 61	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 62	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 64	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 65	*G. cingulata*	NC 2	Fruit	Granny Smith	2002

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
6	CP	LD 66	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 67	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 69	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 70	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 72	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 73	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 74	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 77	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	LD 78	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	CP	RD 1	*G. cingulata*	NC 3	Fruit	Delicious	2000
6	CP	RD 2	*G. cingulata*	NC 3	Fruit	Delicious	2000
6	CP	RD 3	*G. cingulata*	NC 3	Fruit	Delicious	2000
6	CP	RD 4	*G. cingulata*	NC 3	Fruit	Delicious	2000
6	CP	RD 5	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 9	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 10	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 13	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 14	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 15	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	CP	RD 25	*G. cingulata*	NC 3	Fruit	Delicious	2002
6	SP3	GS 28	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	SP3	GS 30	*G. cingulata*	NC 4	Fruit	Granny Smith	2002
6	SP3	LD 39	*G. cingulata*	NC 2	Fruit	Granny Smith	2001
6	SP3	LD 51	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	SP3	LD 63	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
6	SP3	LD 68	*G. cingulata*	NC 2	Fruit	Granny Smith	2002
7	SS2	AL 5	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
7	SS2	AL 6	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
7	SS2	AL 7	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
7	SS2	AL 8	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
7	SS2	AL 9	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 1	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 2	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 3	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 4	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 10	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
8	SS3	AL 11	*C. gloeosporioides*	AL	Fruit	Golden Delicious	2001
9	SS1	LD Cg 1	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 2	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 3	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 4	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 5	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 6	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 7	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 8	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 9	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 10	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 11	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 12	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001
9	SS1	LD Cg 13	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2001

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
9	SS1	LD Cg 14	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
9	SS1	LD Cg 15	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
9	SS1	LD Cg 16	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
9	SS1	LD Cg 17	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
9	SS1	LD Cg 18	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
10	SS1	LD 54	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
10	SS1	LD 57	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
11	SS3	RD 7	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
11	SS3	RD 29	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
12	SS5	RD 16	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
12	SS5	RD 23	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
12	SS5	RD 24	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
13	SSNC(O)	BR Ca 1	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 4	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 6	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 7	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 8	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 9	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 10	*C. acutatum*	Brazil 4	Leaf	Gala	2001
13	SSNC(O)	BR Ca 11	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 2	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 12	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 13	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 14	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 15	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 16	*C. acutatum*	Brazil 4	Leaf	Gala	2001
14	SSNC(O)	BR Ca 17	*C. acutatum*	Brazil 2	Leaf	Gala	n/a*
14	SSNC(O)	BR Ca 22	*C. acutatum*	Brazil 8	Fruit	Fuji	n/a*
14	SSNC(O)	BR Ca 27	*C. acutatum*	Brazil 8	Fruit	Golden Delicous	n/a*
15	SSC	LD Ca 4	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
15	SSC	LD Ca 6	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
15	SSC	LD Ca 19	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
16	SSNC	LD Ca(b) 5	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
16	SSNC	LD Ca(b) 20	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
16	SSNC	LD Ca(b) 24	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SP1	BR 23	*G. cingulata*	Brazil 2	Bud	Gala	n/a*
n/a	SP1	BR 26	*G. cingulata*	Brazil 2	Fruit	Gala	n/a*
n/a	SP1	TN 1	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 2	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 3	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 4	*G. cingulata*	TN 1	Leaf	Gala	1998
n/a	CP	TN 5	*G. cingulata*	TN 1	Leaf	Gala	1998
n/a	CP	TN 6	*G. cingulata*	TN 1	Leaf	Gala	1998
n/a	CP	TN 8	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 9	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 10	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 11	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	CP	TN 12	*G. cingulata*	TN 2	Leaf	Gala	1998
n/a	SS1	RD 20	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS1	RD 22	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
n/a	SS1	RD 34	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS3	GD 2	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 9	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 10	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 11	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 12	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 13	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 15	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	GD 16	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS3	LD 56	*C. gloeosporioides*	NC 2	Fruit	Granny Smith	2002
n/a	SS3	RD 8	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 18	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 19	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 26	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 27	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 28	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 30	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 38	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS4	RD 40	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	GD 1	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	GD 8	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	GD 17	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	GD 18	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	GD 19	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	GD 20	*C. gloeosporioides*	NC 3	Fruit	Golden Delicious	2002
n/a	SS5	RD 11	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 12	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 17	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 21	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 31	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 32	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 33	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 35	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 36	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 37	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SS5	RD 39	*C. gloeosporioides*	NC 3	Fruit	Delicious	2002
n/a	SSNC(O)	BR Ca 3	*C. acutatum*	Brazil 4	Leaf	Gala	2001
n/a	SSNC(O)	BR Ca 5	*C. acutatum*	Brazil 4	Leaf	Gala	2001
n/a	SSNC(O)	BR Ca 18	*C. acutatum*	Brazil 2	Leaf	Gala	n/a*
n/a	SSNC(O)	BR Ca 19	*C. acutatum*	Brazil 8	Fruit	Golden Delicious	n/a*
n/a	SSNC(O)	BR Ca 21	*C. acutatum*	Brazil 2	Fruit	Gala	n/a*
n/a	SSNC(O)	BR Ca 23	*C. acutatum*	Brazil 8	Fruit	Gala	n/a*
n/a	SSNC(O)	BR Ca 24	*C. acutatum*	Brazil 2	Fruit	Golden Delicious	n/a*
n/a	SSNC(O)	BR Ca 25	*C. acutatum*	Brazil 2	Fruit	Fuji	n/a*
n/a	SSNC(O)	BR Ca 26	*C. acutatum*	Brazil 2	Fruit	Gala	n/a*
n/a	SSC	LD Ca 1	*C. acutatum*	NC 1	Fruit	Gala	2001
n/a	SSC	LD Ca 2	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 3	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 5	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 7	*C. acutatum*	NC 2	Fruit	Granny Smith	2001

Appendix 4.1. Continued

VCG[a]	Morphological type[b]	Isolate designation	Species	Geographical location[c]	Source		Year collected
					Host tissue	Cultivar	
n/a	SSC	LD Ca 8	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 9	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 10	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 11	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 12	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 13	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 14	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 15	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 16	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 17	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 18	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 20	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSC	LD Ca 21	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSC	LD Ca 22	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSC	LD Ca 23	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSC	LD Ca 24	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSNC	LD Ca(b) 1	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 2	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 3	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 4	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 6	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 7	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 8	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 9	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 10	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 11	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 12	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 13	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 14	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 15	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 16	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 17	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 18	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 19	*C. acutatum*	NC 2	Fruit	Granny Smith	2001
n/a	SSNC	LD Ca(b) 21	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSNC	LD Ca(b) 22	*C. acutatum*	NC 2	Fruit	Granny Smith	2002
n/a	SSNC	LD Ca(b) 23	*C. acutatum*	NC 2	Fruit	Granny Smith	2002

[a] n/a = isolate were not compatible with any of the VCGs.
[b] Morphological types previously described in the results and Table 1.2 based on colony color, conidial shape, the ability to produce perithecia in culture, and distribution of acervuli and perithecia in culture.
[c] GA = Gala orchard located in Georgia; NC 1 = Gala orchard located in Lincoln Co. in North Carolina; NC 2 = Granny Smith orchard located in Wilkes Co. in North Carolina; NC 3 = Golden and Delicious orchards at the Central Crops Research Station of NCSU located in Johnston Co. in North Carolina; NC 4 = Granny Smith orchard located in Lincoln Co. in North Carolina; NC 5 = Gala orchard located in Wilkes Co., NC; OH = Molly's Delicious orchard located in Ohio; Brazil 1,4,5,6,7,8 = Gala and Golden Delicious orchards located in Santa Catarina State in Brazil; Brazil 2,3 = Gala orchards located in Rio Grande do Sul State in Brazil; TN 1,2 = Gala orchards located in eastern Tennessee; AL = Golden Delicious orchard located in Alabama.
n/a* = year collected not available. Isolates obtained from a collection of isolates maintained by Dr. Sanhueza at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil.

Appendix 4.2. Area under the disease progress curve (AUDPC) of the leaf incidence and severity of isolates of *C. acutatum, C. gloeosporioides*, and *G. cingulata* tested for foliar pathogenicity

Isolate designation	Species	Geographical origin	Source		Incidence (AUDPC)[xz]	Severity (AUDPC)[yz]
			Host tissue	Cultivar		
BR 1	*G. cingulata*	Brazil	Leaf	Gala	485.7	14.6
BR 4	*G. cingulata*	Brazil	Leaf	Gala	452.0	10.4
BR 7	*G. cingulata*	Brazil	Leaf	Gala	481.0	14.7
BR 9	*G. cingulata*	Brazil	Leaf	Gala	364.3	8.4
BR 10	*G. cingulata*	Brazil	Leaf	Gala	500.0	14.9
BR 11	*G. cingulata*	Brazil	Leaf	Gala	450.0	9.6
BR 12	*G. cingulata*	Brazil	Leaf	Gala	450.0	10.9
BR 13	*G. cingulata*	Brazil	Leaf	Gala	492.3	13.1
BR 16	*G. cingulata*	Brazil	Leaf	Gala	500.0	18.3
BR 20	*G. cingulata*	Brazil	Leaf	Gala	491.4	15.6
BR 21	*G. cingulata*	Brazil	Leaf	Gala	364.0	9.6
CROTTS(L) 2	*G. cingulata*	North Carolina	Leaf	Gala	484.6	17.0
CROTTS(L) 3	*G. cingulata*	North Carolina	Leaf	Gala	500.0	16.6
CROTTS(L) 4	*G. cingulata*	North Carolina	Leaf	Gala	437.5	13.2
CROTTS(L) 5	*G. cingulata*	North Carolina	Leaf	Gala	463.0	15.0
CROTTS(L) 6	*G. cingulata*	North Carolina	Leaf	Gala	500.0	23.6
CROTTS(L) 8	*G. cingulata*	North Carolina	Leaf	Gala	500.0	14.7
CROTTS(L) 9	*G. cingulata*	North Carolina	Leaf	Gala	500.0	17.1
CROTTS(L) 10	*G. cingulata*	North Carolina	Leaf	Gala	492.6	16.8
CROTTS(L) 13	*G. cingulata*	North Carolina	Leaf	Gala	459.1	13.6
CROTTS(L) 14	*G. cingulata*	North Carolina	Leaf	Gala	500.0	16.4
CROTTS(L) 15	*G. cingulata*	North Carolina	Leaf	Gala	487.1	13.8
CROTTS(L) 16	*G. cingulata*	North Carolina	Leaf	Gala	500.0	19.5
CROTTS(L) 17	*G. cingulata*	North Carolina	Leaf	Gala	491.9	14.4
CROTTS(L) 18	*G. cingulata*	North Carolina	Leaf	Gala	500.0	19.6
CROTTS(L) 19	*G. cingulata*	North Carolina	Leaf	Gala	500.0	16.8
CROTTS(L) 22	*G. cingulata*	North Carolina	Leaf	Gala	500.0	17.7
GA(L) 1	*G. cingulata*	Georgia	Leaf	Gala	477.8	15.2
GA(L) 2	*G. cingulata*	Georgia	Leaf	Gala	480.0	15.3
GA(L) 4	*G. cingulata*	Georgia	Leaf	Gala	500.0	17.9
GA(L) 5	*G. cingulata*	Georgia	Leaf	Gala	500.0	22.0
GA(L) 7	*G. cingulata*	Georgia	Leaf	Gala	490.9	11.6
GA(L) 8	*G. cingulata*	Georgia	Leaf	Gala	500.0	20.4
GA(L) 9	*G. cingulata*	Georgia	Leaf	Gala	500.0	15.6
GA(L) 10	*G. cingulata*	Georgia	Leaf	Gala	500.0	18.9
GA(L) 11	*G. cingulata*	Georgia	Leaf	Gala	500.0	17.6
GA(L) 12	*G. cingulata*	Georgia	Leaf	Gala	500.0	18.5

Appendix 4.2. Continued

Isolate designation	Species	Geographical origin	Source		Incidence (AUDPC)[xz]	Severity (AUDPC)[yz]
			Host tissue	Cultivar		
GA(L) 14	*G. cingulata*	Georgia	Leaf	Gala	500.0	17.6
GA(L) 16	*G. cingulata*	Georgia	Leaf	Gala	500.0	18.8
GA 3	*G. cingulata*	Georgia	Fruit	Gala	500.0	13.5
GA 5	*G. cingulata*	Georgia	Fruit	Gala	500.0	18.8
GA 6	*G. cingulata*	Georgia	Fruit	Gala	500.0	22.4
GA 7	*G. cingulata*	Georgia	Fruit	Gala	500.0	18.4
GA 8	*G. cingulata*	Georgia	Fruit	Gala	500.0	16.4
GA 10	*G. cingulata*	Georgia	Fruit	Gala	420.0	12.1
GA 12	*G. cingulata*	Georgia	Fruit	Gala	388.6	12.9
GA 21	*G. cingulata*	Georgia	Fruit	Gala	486.7	14.6
GA 22	*G. cingulata*	Georgia	Fruit	Gala	500.0	19.8
GA 24	*G. cingulata*	Georgia	Fruit	Gala	477.8	12.3
TN 7	*G. cingulata*	Tennessee	Leaf	Gala	+	+
CROTTS 1	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 3	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 5	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 6	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 8	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 9	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 10	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 11	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 12	*G. cingulata*	North Carolina	Fruit	Gala	-	-
CROTTS 13	*G. cingulata*	North Carolina	Fruit	Gala	-	-
RD 1	*G. cingulata*	North Carolina	Fruit	Delicious	-	-
RD 3	*G. cingulata*	North Carolina	Fruit	Delicious	-	-
LD 3	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 5	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 6	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 7	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 8	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 10	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 12	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 13	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 15	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 16	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 17	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 23	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 25	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-

Appendix 4.2. Continued

Isolate designation	Species	Geographical origin	Source		Incidence (AUDPC)[xz]	Severity (AUDPC)[yz]
			Host tissue	Cultivar		
LD 30	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 31	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 32	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
LD 41	*G. cingulata*	North Carolina	Fruit	Granny Smith	-	-
OH 1	*G. cingulata*	Ohio	Fruit	Molly's Delicious	-	-
OH 2	*G. cingulata*	Ohio	Fruit	Molly's Delicious	-	-
OH 3	*G. cingulata*	Ohio	Fruit	Molly's Delicious	-	-
TN 1	*G. cingulata*	Tennessee	Leaf	Gala	-	-
TN 5	*G. cingulata*	Tennessee	Leaf	Gala	-	-
TN 8	*G. cingulata*	Tennessee	Leaf	Gala	-	-
TN 9	*G. cingulata*	Tennessee	Leaf	Gala	-	-
TN 11	*G. cingulata*	Tennessee	Leaf	Gala	-	-
AL 1	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	-	-
AL 4	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	-	-
AL 5	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	-	-
AL 9	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	-	-
LD Cg 1	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	-	-
LD Cg 8	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	-	-
LD Ca(b) 4	*C. acutatum*	North Carolina	Fruit	Granny Smith	-	-
LD Ca(b) 6	*C. acutatum*	North Carolina	Fruit	Granny Smith	-	-
LD Ca 5	*C. acutatum*	North Carolina	Fruit	Granny Smith	-	-
LD Ca 10	*C. acutatum*	North Carolina	Fruit	Granny Smith	-	-
BR Ca 4	*C. acutatum*	Brazil	Leaf	Gala	-	-

[x] Foliar incidence represents the AUDPC of the percentage of diseased leaves in each tree rated every 2 days over 6 days.
[y] Foliar severity represents the AUDPC of the percentage of leaf area affected in each tree rated every 2 days over 6 days. Severity was estimated using a modified Horsfall-Barratt disease rating scale with values from 0-6, where 0 = no lesions; 1 = 1-3%; 2 = 4-6%; 3 = 7-12%; 4 = 13-25%; 5 = 25-50%; and 6 = >50
[z] '-' = isolates were not pathogenic on leaves. '+' = pathogenic on leaves, but incidence and severity was not tested.

Appendix 4.3. Mitochondrial DNA haplotypes and pathogenicity of isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* examined for mtDNA RFLPs

mtDNA haplotypes (*Msp* I)[w]	Species	Geographical origin[x]	Isolate designation	Source		Pathogenicity tests[y]	
				Host tissue	Cultivar	Foliar	Fruit
G1	*G. cingulata*	GA	GA 1	Fruit	Gala	+	
G1	*G. cingulata*	GA	GA 4	Fruit	Gala		
G1	*G. cingulata*	GA	GA 10	Fruit	Gala	+	
G1	*G. cingulata*	GA	GA 12	Fruit	Gala	+	
G1	*G. cingulata*	GA	GA 16	Fruit	Gala	+	+
G1	*G. cingulata*	GA	GA 18	Fruit	Gala		
G1	*G. cingulata*	GA	GA 21	Fruit	Gala	+	
G1	*G. cingulata*	GA	GA 22	Fruit	Gala	+	
G1	*G. cingulata*	GA	GA 24	Fruit	Gala	+	+
G1	*G. cingulata*	GA	GA 40	Fruit	Gala		
G1	*G. cingulata*	GA	GA 41	Fruit	Gala		
G1	*G. cingulata*	GA	GA 42	Fruit	Gala		
G1	*G. cingulata*	GA	GA(L) 1	Leaf	Gala		
G1	*G. cingulata*	GA	GA(L) 2	Leaf	Gala	+	+
G1	*G. cingulata*	GA	GA(L) 3	Leaf	Gala	+	
G1	*G. cingulata*	GA	GA(L) 4	Leaf	Gala	+	+
G1	*G. cingulata*	GA	GA(L) 5	Leaf	Gala	+	+
G1	*G. cingulata*	GA	GA(L) 6	Leaf	Gala		
G1	*G. cingulata*	GA	GA(L) 7	Leaf	Gala	+	
G1	*G. cingulata*	GA	GA(L) 8	Leaf	Gala	+	
G1	*G. cingulata*	GA	GA(L) 13	Leaf	Gala	+	
G1	*G. cingulata*	GA	GA(L) 16	Leaf	Gala	+	+
G1	*G. cingulata*	GA	GA(L) 18	Leaf	Gala	+	+
G1	*G. cingulata*	GA	GA(L) 19	Leaf	Gala		+
G1	*G. cingulata*	GA	GA(L) 23	Leaf	Gala		
G1	*G. cingulata*	GA	GA(L) 24	Leaf	Gala		
G1	*G. cingulata*	GA	GA(L) 25	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 1	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 2	Leaf	Gala	+	+
G1	*G. cingulata*	NC 1	CROTTS(L) 3	Leaf	Gala	+	
G1	*G. cingulata*	NC 1	CROTTS(L) 5	Leaf	Gala	+	
G1	*G. cingulata*	NC 1	CROTTS(L) 9	Leaf	Gala	+	
G1	*G. cingulata*	NC 1	CROTTS(L) 10	Leaf	Gala	+	
G1	*G. cingulata*	NC 1	CROTTS(L) 11	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 15	Leaf	Gala	+	+
G1	*G. cingulata*	NC 1	CROTTS(L) 21	Leaf	Gala	+	
G1	*G. cingulata*	NC 1	CROTTS(L) 27	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 36	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 37	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 38	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 39	Leaf	Gala		
G1	*G. cingulata*	NC 1	CROTTS(L) 41	Leaf	Gala		
G1	*G. cingulata*	TN 1	TN 7	Leaf	Gala	+	
G1	*G. cingulata*	NC 2	LD 10	Fruit	Granny Smith	-	+
G1	*G. cingulata*	NC 2	LD 11	Fruit	Granny Smith		
G1	*G. cingulata*	NC 2	LD 13	Fruit	Granny Smith	-	
G1	*G. cingulata*	NC 2	LD 14	Fruit	Granny Smith		
G1	*G. cingulata*	NC 2	LD 16	Fruit	Granny Smith	-	
G1	*G. cingulata*	NC 2	LD 20	Fruit	Granny Smith		+
G1	*G. cingulata*	NC 2	LD 23	Fruit	Granny Smith	-	+

Appendix 4.3. Continued

mtDNA haplotypes (*Msp* I)[w]	Species	Geographical origin[x]	Isolate designation	Source		Pathogenicity tests[y]	
				Host tissue	Cultivar	Foliar	Fruit
G1	*G. cingulata*	NC 2	LD 25	Fruit	Granny Smith	-	
G1	*G. cingulata*	NC 2	LD 75	Fruit	Granny Smith		
G1	*G. cingulata*	NC 2	LD 79	Fruit	Granny Smith		
G1	*G. cingulata*	NC 2	LD 81	Fruit	Granny Smith		
G1	*G. cingulata*	NC 2	LD 82	Fruit	Granny Smith		
G1	*G. cingulata*	OH	OH 1	Fruit	Molly's Delicious	-	+
G1	*G. cingulata*	OH	OH 3	Fruit	Molly's Delicious	-	
G1	*G. cingulata*	NC 4	GS 2	Fruit	Granny Smith		
G1	*G. cingulata*	NC 4	GS 4	Fruit	Granny Smith		
G1	*G. cingulata*	NC 4	GS 5	Fruit	Granny Smith		
G1.1	*G. cingulata*	GA	GA 17	Fruit	Gala		
G1.1	*G. cingulata*	NC 1	CROTTS 15	Fruit	Gala		
G1.1	*G. cingulata*	NC 1	CROTTS(L) 4	Leaf	Gala	+	+
G1.1	*G. cingulata*	NC 1	CROTTS(L) 8	Leaf	Gala	+	
G1.1	*G. cingulata*	TN 2	TN 1	Leaf	Gala	-	
G1.1	*G. cingulata*	NC 2	LD 7	Fruit	Granny Smith	-	
G1.1	*G. cingulata*	NC 2	LD 17	Fruit	Granny Smith	-	
G2	*G. cingulata*	NC 1	CROTTS 1	Fruit	Gala	-	+
G2	*G. cingulata*	NC 1	CROTTS 2	Fruit	Gala		
G2	*G. cingulata*	NC 1	CROTTS 3	Fruit	Gala	-	+
G2	*G. cingulata*	NC 1	CROTTS 5	Fruit	Gala	-	+
G2	*G. cingulata*	NC 1	CROTTS 6	Fruit	Gala	-	
G2	*G. cingulata*	NC 1	CROTTS 9	Fruit	Gala	-	
G2	*G. cingulata*	NC 1	CROTTS 10	Fruit	Gala	-	+
G2	*G. cingulata*	NC 1	CROTTS 13	Fruit	Gala	-	
G2	*G. cingulata*	NC 2	LD 83	Fruit	Granny Smith		
G2	*G. cingulata*	NC 2	LD 84	Fruit	Granny Smith		
G2	*G. cingulata*	NC 2	LD 85	Fruit	Granny Smith		
G2	*G. cingulata*	NC 4	GS 1	Fruit	Granny Smith		
G2	*G. cingulata*	NC 4	GS 6	Fruit	Granny Smith		
G2	*G. cingulata*	NC 4	GS 7	Fruit	Granny Smith		
G2	*G. cingulata*	NC 4	GS 8	Fruit	Granny Smith		
G2	*G. cingulata*	NC 4	GS 9	Fruit	Granny Smith		
G2.1	*G. cingulata*	NC 1	CROTTS 8	Fruit	Gala		
G2.1	*G. cingulata*	NC 1	CROTTS 12	Fruit	Gala	-	+
G3	*G. cingulata*	Brazil 1	BR 2	Leaf	Gala		
G3	*G. cingulata*	Brazil 1	BR 3	Leaf	Gala		
G3	*G. cingulata*	Brazil 1	BR 4	Leaf	Gala	+	
G3	*G. cingulata*	Brazil 1	BR 6	Leaf	Gala		
G3	*G. cingulata*	Brazil 2	BR 24	Fruit	Gala		
G3	*G. cingulata*	Brazil 3	BR 10	Leaf	Gala	+	
G3	*G. cingulata*	Brazil 3	BR 8	Leaf	Gala		
G3	*G. cingulata*	Brazil 5	BR 13	Leaf	Gala	+	
G3	*G. cingulata*	Brazil 6	BR 17	Leaf	Gala		
G3	*G. cingulata*	Brazil 6	BR 19	Leaf	Gala		
G3	*G. cingulata*	Brazil 8	BR 25	Leaf	Gala		
G3	*G. cingulata*	Brazil 8	BR 26	Fruit	Gala		
G4	*G. cingulata*	Brazil 1	BR 1	Leaf	Gala		
G4	*G. cingulata*	Brazil 2	BR 23	Bud	Gala		
G4	*G. cingulata*	Brazil 3	BR 9	Leaf	Gala	+	
G4	*G. cingulata*	Brazil 5	BR 11	Leaf	Gala	+	

Appendix 4.3. Continued

mtDNA haplotypes (*Msp* I)[w]	Species	Geographical origin[x]	Isolate designation	Source		Pathogenicity tests[y]	
				Host tissue	Cultivar	Foliar	Fruit
G4	*G. cingulata*	Brazil 5	BR 12	Leaf	Gala	+	
G4	*G. cingulata*	Brazil 6	BR 14	Leaf	Gala	+	
G4	*G. cingulata*	Brazil 6	BR 15	Leaf	Gala		
G4	*G. cingulata*	Brazil 7	BR 21	Leaf	Gala	+	
G4	*G. cingulata*	Brazil 7	BR 22	Leaf	Gala		
A3	*G. cingulata*	NC 1	CROTTS 4	Fruit	Gala		+
A3	*G. cingulata*	NC 1	CROTTS 7	Fruit	Gala		
A3	*G. cingulata*	NC 1	CROTTS 14	Fruit	Gala		
A3	*G. cingulata*	NC 2	LD 2	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 5	Fruit	Granny Smith	-	
A3	*G. cingulata*	NC 2	LD 6	Fruit	Granny Smith	-	
A3	*G. cingulata*	NC 2	LD 8	Fruit	Granny Smith	-	
A3	*G. cingulata*	NC 2	LD 12	Fruit	Granny Smith	-	+
A3	*G. cingulata*	NC 2	LD 19	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 50	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 52	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 53	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 55	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 78	Fruit	Granny Smith		
A3	*G. cingulata*	NC 3	GD 3	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	GD 4	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	GD 5	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	GD 6	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	GD 7	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	GD 14	Fruit	Golden Delicious		
A3	*G. cingulata*	NC 3	RD 1	Fruit	Delicious	-	
A3	*G. cingulata*	NC 3	RD 2	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 3	Fruit	Delicious	-	
A3	*G. cingulata*	NC 3	RD 4	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 6	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 9	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 10	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 14	Fruit	Delicious		
A3	*G. cingulata*	NC 3	RD 15	Fruit	Delicious		
A3	*G. cingulata*	NC 4	GS 15	Fruit	Granny Smith		
A3	*G. cingulata*	NC 4	GS 16	Fruit	Granny Smith		
A3	*G. cingulata*	NC 4	GS 18	Fruit	Granny Smith		
A3	*G. cingulata*	NC 4	GS 21	Fruit	Granny Smith		
A3	*G. cingulata*	TN 2	TN 3	Leaf	Gala		
A3	*G. cingulata*	TN 2	TN 8	Leaf	Gala	-	
A3	*G. cingulata*	TN 2	TN 9	Leaf	Gala	-	
A3	*G. cingulata*	TN 2	TN 10	Leaf	Gala		
A3	*G. cingulata*	TN 2	TN 11	Leaf	Gala	-	+
A3	*G. cingulata*	TN 2	TN 12	Leaf	Gala		
A3	*G. cingulata*	NC 2	LD 4	Fruit	Granny Smith		
A3	*G. cingulata*	NC 4	GS 28	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 51	Fruit	Granny Smith		
A3	*G. cingulata*	NC 2	LD 63	Fruit	Granny Smith		
A3.1	*G. cingulata*	NC 2	LD 1	Fruit	Granny Smith		
A3.1	*G. cingulata*	NC 2	LD 3	Fruit	Granny Smith	-	
A3.1	*G. cingulata*	NC 2	LD 22	Fruit	Granny Smith		

Appendix 4.3. Continued

mtDNA haplotypes (*Msp* I)[w]	Species	Geographical origin[x]	Isolate designation	Source		Pathogenicity tests[y]	
				Host tissue	Cultivar	Foliar	Fruit
A3.1	*G. cingulata*	TN 1	TN 4	Leaf	Gala		
A3.1	*G. cingulata*	TN 1	TN 5	Leaf	Gala	-	+
A3.1	*G. cingulata*	TN 2	TN 2	Leaf	Gala		
B2	*C. gloeosporioides*	NC 2	LD 54	Fruit	Granny Smith		
B2	*C. gloeosporioides*	NC 3	RD 22	Fruit	Delicious		
B2	*C. gloeosporioides*	AL	AL 5	Fruit	Golden Delicious	-	+
B2	*C. gloeosporioides*	AL	AL 6	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	AL	AL 7	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	AL	AL 8	Fruit	Golden Delicious	-	+
B2	*C. gloeosporioides*	AL	AL 9	Fruit	Golden Delicious	-	
B2	*C. gloeosporioides*	NC 3	GD 10	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	GD 11	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	GD 12	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	GD 13	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	GD 1	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	GD 8	Fruit	Golden Delicious		
B2	*C. gloeosporioides*	NC 3	RD 11	Fruit	Delicious		
B2	*C. gloeosporioides*	NC 3	RD 16	Fruit	Delicious		
B2	*C. gloeosporioides*	NC 3	RD 17	Fruit	Delicious		
B2	*C. gloeosporioides*	NC 3	RD 21	Fruit	Delicious		
B3	*C. gloeosporioides*	NC 2	LD Cg 1	Fruit	Granny Smith	-	+
B3	*C. gloeosporioides*	NC 2	LD Cg 2	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 3	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 6	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 7	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 8	Fruit	Granny Smith	-	+
B3	*C. gloeosporioides*	NC 2	LD Cg 11	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 12	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 13	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 14	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 15	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 16	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 17	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 2	LD Cg 18	Fruit	Granny Smith		
B3	*C. gloeosporioides*	AL	AL 1	Fruit	Golden Delicious	-	+
B3	*C. gloeosporioides*	AL	AL 2	Fruit	Golden Delicious		
B3	*C. gloeosporioides*	AL	AL 3	Fruit	Golden Delicious		
B3	*C. gloeosporioides*	AL	AL 4	Fruit	Golden Delicious	-	
B3	*C. gloeosporioides*	AL	AL 10	Fruit	Golden Delicious		
B3	*C. gloeosporioides*	NC 3	GD 2	Fruit	Golden Delicious		
B3	*C. gloeosporioides*	NC 3	GD 9	Fruit	Golden Delicious		
B3	*C. gloeosporioides*	NC 2	LD 56	Fruit	Granny Smith		
B3	*C. gloeosporioides*	NC 3	RD 7	Fruit	Delicious		
B3	*C. gloeosporioides*	NC 3	RD 8	Fruit	Delicious		
B3	*C. gloeosporioides*	NC 3	RD 12	Fruit	Delicious		
C1	*C. acutatum*	NC 2	LD Ca 5	Fruit	Granny Smith	-	+
C1	*C. acutatum*	NC 2	LD Ca 10	Fruit	Granny Smith	-	+
C1	*C. acutatum*	NC 2	LD Ca 21	Fruit	Granny Smith		
C1	*C. acutatum*	NC 2	LD Ca 22	Fruit	Granny Smith		
C1	*C. acutatum*	NC 2	LD Ca(b) 4	Fruit	Granny Smith	-	+
C1	*C. acutatum*	NC 2	LD Ca(b) 6	Fruit	Granny Smith	-	

Appendix 4.3. Continued

mtDNA haplotypes (*Msp* I)[w]	Species	Geographical origin[x]	Isolate designation	Source		Pathogenicity tests[y]	
				Host tissue	Cultivar	Foliar	Fruit
C1	*C. acutatum*	NC 2	LD Ca(b) 21	Fruit	Granny Smith		
C1	*C. acutatum*	NC 2	LD Ca(b) 22	Fruit	Granny Smith		
D1	*C. acutatum*	Brazil 2	BR Ca 17	Leaf	Gala		
D1	*C. acutatum*	Brazil 2	BR Ca 18	Leaf	Gala		
D1	*C. acutatum*	Brazil 2	BR Ca 21	Fruit	Gala		
D1	*C. acutatum*	Brazil 4	BR Ca 3	Leaf	Gala		+
D1	*C. acutatum*	Brazil 4	BR Ca 4	Leaf	Gala	-	+
D1	*C. acutatum*	Brazil 8	BR Ca 19	Fruit	Golden Delicious		
Reference isolates[z]							
A1	*G. cingulata*	AR	A45	Fruit			
A2	*G. cingulata*	AR	960	Fruit			
A3	*G. cingulata*	NC	NC 246	Fruit			
A4	*G. cingulata*	AR	NC 211	Fruit			
B2	*C. gloeosporioides*	NC	NC 329	Fruit			
B3	*C. gloeosporioides*	NC	NC 131	Fruit			
C1	*C. acutatum*	AR	A38	Fruit			
D1	*C. acutatum*	AR	A138	Fruit			

[w] mtDNA RFLP haplotypes of genomic DNA digested with *Msp*I.
[x] GA = Gala orchard located in Georgia; NC 1 = Gala orchard located in Lincoln Co., NC; NC 2 = Granny Smith orchard located in Wilkes Co., NC; NC 3 = Golden and Delicious orchards at the Central Crops Research Station, Clayton, NC; NC 4 = Granny Smith orchard located in Lincoln Co., NC; OH = Molly's Delicious orchard located in Ohio; Brazil 1,4,5,6,7,8 = Gala and Golden Delicious orchards located in Santa Catarina State in Brazil; Brazil 2,3 = Gala orchards located in Rio Grande do Sul State in Brazil; TN 1 = Gala orchards located in Cleveland, TN; TN 2 = Gala orchards located in Buffalo Valley, TN; AL = Golden Delicious orchard located in Alabama.
[y] Pathogenicity was tested on fruit and tress of the cultivar Gala grown under greenhouse conditions. '+' indicates that isolates were pathogenic. '-' indicates that isolates were not pathogenic.
[z] Isolates obtained from the University of Arkansas.

Appendix 4.4. Isolates of *G. cingulata*, *C. gloeosporioides*, and *C. acutatum* examined for optimum growth rate and benomyl sensitivity

Isolate designation	Species	Geographical origin	Source		mtDNA haplotype (*Msp* I)[x]	VCG	Growth (mm/day)[y]	EC_{50}[z]
			Host tissue	Cultivar				
GA(L) 13	*G. cingulata*	Georgia	Leaf	Gala	G1	1	12.6	0.15
GA 16	*G. cingulata*	Georgia	Fruit	Gala	G1	1	12.5	
CROTTS(L) 27	*G. cingulata*	North Carolina	Leaf	Gala	G1	1	11.7	
TN 7	*G. cingulata*	Tennessee	Leaf	Gala	G1	1	13.2	0.17
LD 11	*G. cingulata*	North Carolina	Fruit	Granny Smith	G1	2	11.8	0.11
LD 16	*G. cingulata*	North Carolina	Fruit	Granny Smith	G1	2	12.3	
LD 25	*G. cingulata*	North Carolina	Fruit	Granny Smith	G1	2	11.8	
LD 20	*G. cingulata*	North Carolina	Fruit	Granny Smith	G1	2	11.8	
OH 3	*G. cingulata*	Ohio	Fruit	Molly's Delicious	G1	3	11.5	0.16
CROTTS 1	*G. cingulata*	North Carolina	Fruit	Gala	G2	2	11.9	0.09
CROTTS 2	*G. cingulata*	North Carolina	Fruit	Gala	G2	2	12.9	0.12
CROTTS 3	*G. cingulata*	North Carolina	Fruit	Gala	G2	2	12.7	0.11
CROTTS 5	*G. cingulata*	North Carolina	Fruit	Gala	G2	2	12.2	0.09
BR 2	*G. cingulata*	Brazil	Leaf	Gala	G3	5	12.5	0.09
BR 3	*G. cingulata*	Brazil	Leaf	Gala	G3	4	11.7	
BR 8	*G. cingulata*	Brazil	Leaf	Gala	G3	4	11.6	0.11
BR 10	*G. cingulata*	Brazil	Leaf	Gala	G3	4	12.2	
BR 13	*G. cingulata*	Brazil	Leaf	Gala	G3	5	11.8	
BR 17	*G. cingulata*	Brazil	Leaf	Gala	G3	5	12.4	0.12
BR 19	*G. cingulata*	Brazil	Leaf	Gala	G3	5	12.8	0.16
BR 9	*G. cingulata*	Brazil	Leaf	Gala	G4	5	11.9	
BR 21	*G. cingulata*	Brazil	Leaf	Gala	G4	5	12.7	
RD 1	*G. cingulata*	North Carolina	Fruit	Delicious	A3	6	12.1	0.20
RD 2	*G. cingulata*	North Carolina	Fruit	Delicious	A3	6	13.0	0.19
LD 2	*G. cingulata*	North Carolina	Fruit	Granny Smith	A3	6	13.2	0.18
LD 12	*G. cingulata*	North Carolina	Fruit	Granny Smith	A3	6	12.9	0.17
TN 8	*G. cingulata*	Tennessee	Leaf	Gala	A3	6	13.7	0.24
AL 5	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B2	7	13.1	0.15
AL 6	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B2	7	12.9	0.17
AL 8	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B2	7	12.6	
AL 9	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B2	7	13.0	
AL 1	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B3	8	11.4	0.09
AL 2	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B3	8	11.6	
AL 4	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B3	8	11.3	0.07
AL 10	*C. gloeosporioides*	Alabama	Fruit	Golden Delicious	B3	8	11.2	
LD Cg 1	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	B3	9	11.7	0.07
LD Cg 8	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	B3	9	12.6	0.06
LD Cg 11	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	B3	9	10.9	

Appendix 4.4. Continued

Isolate designation	Species	Geographical origin	Source		mtDNA haplotype (*Msp* I)[x]	VCG	Growth (mm/day)[y]	EC_{50}[z]
			Host tissue	Cultivar				
LD Cg 13	*C. gloeosporioides*	North Carolina	Fruit	Granny Smith	B3	9	10.7	
LD Ca 5	*C. acutatum*	North Carolina	Fruit	Granny Smith	C1		8.9	0.37
LD Ca 10	*C. acutatum*	North Carolina	Fruit	Granny Smith	C1		9.1	0.50
LD Ca(b) 4	*C. acutatum*	North Carolina	Fruit	Granny Smith	C1		9.0	0.33
LD Ca(b) 6	*C. acutatum*	North Carolina	Fruit	Granny Smith	C1		9.8	0.28
BR Ca 4	*C. acutatum*	Brazil	Leaf	Gala	D1	13	8.0	0.46
BR Ca 3	*C. acutatum*	Brazil	Leaf	Gala	D1		9.2	0.85
BR Ca 6	*C. acutatum*	Brazil	Leaf	Gala	D1	13	8.4	

[x] mtDNA RFLP haplotypes of genomic DNA digested with *Msp*1 described in Chapter 2

[y] Growth represents the average over 6 days of the mean of the colony diameter (mm/day) of the isolates at 26°C.

[z] EC_{50}s were calculated using proc probit log 10 in SAS®, based on the colony diameter of the isolates at 0, 0.01, 0.1, 1 and 10 µg/ml of benomyl after 6 days of incubation at 26°C.