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ORIGINAL ARTICLE

Genetic variability suggests that three populations of *Ceratocystis fimbriata* are responsible for the Ceratocystis wilt epidemic on kiwifruit in Brazil

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Abstract *Ceratocystis fimbriata* is a native, soilborne pathogen in South America that causes a lethal wilt disease on a broad range of economically important plants. Ceratocystis wilt on kiwifruit (*Actinidia* spp.) was first recognized in 2010 in the state of Rio Grande do Sul, Brazil. The genetic variation among kiwifruit isolates was analized to determine if a single introduced strain of the pathogen was responsible for the epidemic or if there was substantial genetic variation in the population, suggesting that the fungus was soilborne and indigenous to the region. We used 14 microsatellite (simple sequence repeat, SSR) markers to identify 18 genotypes of *C. fimbriata* among 76 isolates from eight kiwifruit farms. The 18 genotypes clustered into three groups based on UPGMA analysis of the microsatellite alleles. The largest group comprised 60 isolates of 11 closely-related microsatellite genotypes obtained from seven of the eight farms. These genotypes appeared to have originated from a single farm that had supplied cuttings for grafting to the other farms. The population of the pathogen from the farm that supplied the cuttings had the highest level of genotypic diversity and relatively high gene diversity, suggesting that this source population represented an indigenous, soilborne population. Phylogenetic analyses of the DNA sequences of the mating type locus (including portions of *MAT1-1-2* and *MAT1-2-1*) placed the isolates into three groups, corresponding to the three microsatellite groups. Most of the isolates, including all the tested isolates from the farm that supplied the cuttings, had mating type gene sequences that were distinct from other Brazilian populations of *C. fimbriata*. A second group comprised isolates from one farm that had mating type gene sequences typical of Mata Atlântica (Rio de Janeiro and São Paulo) populations of *C. fimbriata* on *Colocasia esculenta* and *Mangifera indica*. Three farms purchased kiwifruit plants or rootstocks from commercial nurseries in Brazil as well as scions from the source farm, and some of the isolates from these farms were genetically similar to *Eucalyptus* isolates of *C. fimbriata* from Bahia and Minas Gerais, Brazil. The kiwifruit epidemic in Rio Grande do Sul is the southern-most report of *C. fimbriata* in Brazil, and the primary pathogen population on kiwifruit appears to be indigenous and originated from a single farm that distributed the pathogen in grafting material. In addition, commercial nursery stock was also implicated as sources of *C. fimbriata* genotypes. The disease is a major limiting factor for kiwifruit production in southern Brazil, and the results suggest that clean planting stock will be important to successful production.

Keywords *Actinidia* spp · Genetic diversity · Kiwifruit disease · Latin American Clade · Microsatellite · Phylogeny

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Introduction

Ceratocystis fimbriata causes lethal, wilt-type diseases or cankers in important plantation crops in Brazil, such as mango (*Mangifera indica*), *Gmelina arborea*, rubbertree (*Hevea*

brasiliensis), *Eucalyptus* spp. and fig (*Ficus carica*) (Baker et al. 2003; CAB 2001; Ferreira et al. 2010; Valdetaro et al. 2015). Brazilian isolates of *C. fimbriata* may vary in aggressiveness to different hosts, but there does not appear to be strong host-specialization (Baker et al. 2003; Ferreira et al. 2010; Harrington et al. 2011). Although *C. fimbriata* has been recognized as a pathogen on other hosts in Brazil for more than 75 years (Ferreira et al. 2010), Ceratocystis wilt on kiwifruit (*Actinidia deliciosa*) was first recognized in 2010 in the state of Rio Grande do Sul, Brazil (Sônego et al. 2010; Piveta et al. 2016). Infected plants show wilting and death of leaves and reddish-brown staining in a radial pattern in the xylem. The disease reduces the number of harvestable fruit, and most affected plants die. Although there is potential for the expansion of the kiwifruit crop in southern Brazil, the planted area is currently small (about 200 ha) and the yield relatively low (15 t/ha) (Gervasio Silvestrin, personal communication, 2016). Kiwifruit production has required few chemical inputs because there are few pest or disease problems (Grellmann 2005), but Ceratocystis wilt has become a major limiting factor to kiwifruit cultivation in southern Brazil and threatens expansion of the crop (Piveta et al. 2016). A recent survey carried out in Rio Grande do Sul (Alfenas, unpublished data) found 25 – 30% annual mortality due to Ceratocystis wilt in some of the affected orchards.

Ceratocystis fimbriata and relatives in the Latin American Clade of the *C. fimbriata* complex are soilborne pathogens but can be readily introduced to new areas on contaminated tools and infected propagative material (Baker et al. 2003; CAB 2001; Engelbrecht et al. 2007; Ferreira et al. 2010, 2011; Harrington 2013). Introduced populations of *Ceratocystis* spp. typically show little or no genetic variation because of genetic bottlenecks (Engelbrecht and Harrington 2005; Engelbrecht et al. 2007; Ocasio-Morales et al. 2007; Ferreira et al. 2010, 2011; Oliveira et al. 2015; Li et al. 2016). In Brazil, *C. fimbriata* populations on *Eucalyptus* or mango planted on sites with natural, soilborne inoculum may have relatively high genetic diversity, while populations of *C. fimbriata* introduced in infected nursery stock of fig, mango or *Eucalyptus* tend to show very limited genetic diversity (Ferreira et al. 2010, 2011, 2013; Oliveira et al. 2015).

No serious losses due to Ceratocystis wilt had been recorded in the southernmost states of Brazil until the reports of *C. fimbriata* on kiwifruit in Rio Grande do Sul. Initially it was suspected that the pathogen was newly introduced to the region, but isolates from kiwifruit showed a surprising amount of variability in ITS rDNA sequences (Piveta et al. 2016). However, such variation in ITS rDNA sequences does not always correspond with variation in other genetic markers (Harrington et al. 2014). Piveta et al. (2016) identified five ITS rDNA haplotypes among isolates from kiwifruit, and these haplotypes were placed among other Brazilian isolates in the Latin American Clade of the *C. fimbriata* complex. Isolates of these ITS haplotypes were morphologically similar to isolates of *C. fimbriata* from other hosts in Brazil. Eight kiwifruit isolates representing four ITS haplotypes varied somewhat in aggressiveness to three kiwifruit cultivars. However, all isolates were pathogenic and all three cultivars were highly susceptible (Piveta et al. 2016). The first objective of this study was to use 14 polymorphic microsatellite loci and sequences of mating type genes to determine if the local epidemic of Ceratocystis wilt on kiwifruit was associated with a genetically diverse population of *C. fimbriata* or if the population was genetically uniform, suggestive of an introduced strain. Secondly, the relatedness of populations of the pathogen on different farms and across the Latin American Clade was determined to see if some kiwifruit isolates may have been derived from another region of Latin America.

Materials and methods

Ceratocystis fimbriata isolates

We analyzed isolates from symptomatic plants at eight kiwifruit farms near Farroupilha, Rio Grande do Sul state, with a known history of Ceratocystis wilt (Table 1, Fig. 1). This region is in a subtropical, high-rainfall area that would naturally be in the southern tip of the Serra do Mar coastal forests in the Mata Atlântica vegetation type. Rio Grande do Sul and Santa Catarina are the southernmost and the most temperate states in Brazil, and commercial kiwifruit production in Brazil is limited to these two states. Both states have seen serious losses to Ceratocystis wilt (Piveta et al. 2016). Among the earliest producers of kiwifruit in the region was the PM farm, which had obtained planting material from a producer in Chile and a commercial nursery in São Paulo. Six other surveyed farms had obtained scions from the PM farm, and the eighth farm (PP) received some grafted plants from PM to replace dead kiwifruit plants (Table 1). Besides the PM scions, farms PC, PB and PL, purchased scions for grafting from commercial nurseries in Rio Grande do Sul or São Paulo. The PA farm had purchased grafted plants or scions from a Chilean nursery in addition to the scions from PM.

A zigzag pattern of sampling was used to collect samples from symptomatic trees scattered throughout the plantings. Plants showing typical symptoms were selected for ease of isolation, and samples of discolored xylem were collected from the wilting plants (Piveta et al. 2016). The sampled cultivars included Monty, MG06, Yellow Queen, Golden King, Bruno, Elmwood, and Hayward. Isolates were baited from dead or dying vines by placing pieces of the discolored xylem tissue between two discs of carrot root (Moller and DeVay 1968) and incubated for 10 days at room temperature. Ascospore masses from perithecia forming on the carrot discs were transferred to a drop of oil and spread on agar media.

Table 1 Lineages and genotypes based on DNA sequences of mating type genes or microsatellite alleles, genotypic diversity and gene diversity of *Ceratocystis fimbriata* populations found in eight kiwifruit farms with differing origins of planting stock

Farm	Origin of planting stock[1]	Mating type lineages (no. isolates)[2]	Microsatellite data				
			No. of genotypes/ no. of isolates tested	Microsatellite genotypes (no. of isolates)[2]	Stoddart & Taylor's genotypic diversity (G)[3]	Nei's gene diversity (H) all isolates	Nei's gene diversity (H) clone-corrected
PM	Chile nursery and commercial nurseries	PM (4)	6/13	PM (13)	2.66	0.1361	0.1389
PA	Chile nursery and PM scions	PM (2)	2/13	PM (13)	1.23	0.0101	0.0357
PG	PM scions	NT	4/14	PM (14)	1.75	0.0437	0.1071
PCT	PM scions	PM (1)	3/9	PM (9)	1.58	0.0741	0.0952
PB	PM scions and commercial nursery	PM (1) and *Eucalyptus* (2)	3/10	PM (4) and *Eucalyptus* (6)	2.05	0.4129	0.4286
PC	PM scions and commercial nurseries	PM (1)	3/9	PM (7) and *Eucalyptus* (2)	1.90	0.3034	0.3810
PL	PM scions and commercial nurseries	PM(1) and *Eucalyptus* (1)	2/3	PM (2) and *Eucalyptus* (1)	2.00	0.3492	0.3929
PP	Chile nursery and PM plants[1]	PM (1) and Mata Atlântica (2)	3/7	Mata Atlântica (7)	2.15	0.0466	0.0537

[1] Sources of kiwifruit planting stock originated from Chile, São Paulo and Rio Grande do Sul nurseries, and whole plants or scions from PM farm

[2] Lineages based on mating type gene sequences or genotypes based on combination of microsatellite alleles were either typical of isolates from farm PM, *Eucalyptus* isolates from Minas Gerais and Bahia, or isolates typical of Mata Atlântica populations in Rio de Janeiro and São Paulo

[3] Stoddart & Taylor's genotypic diversity (G) with rarefaction, maximum value = 3

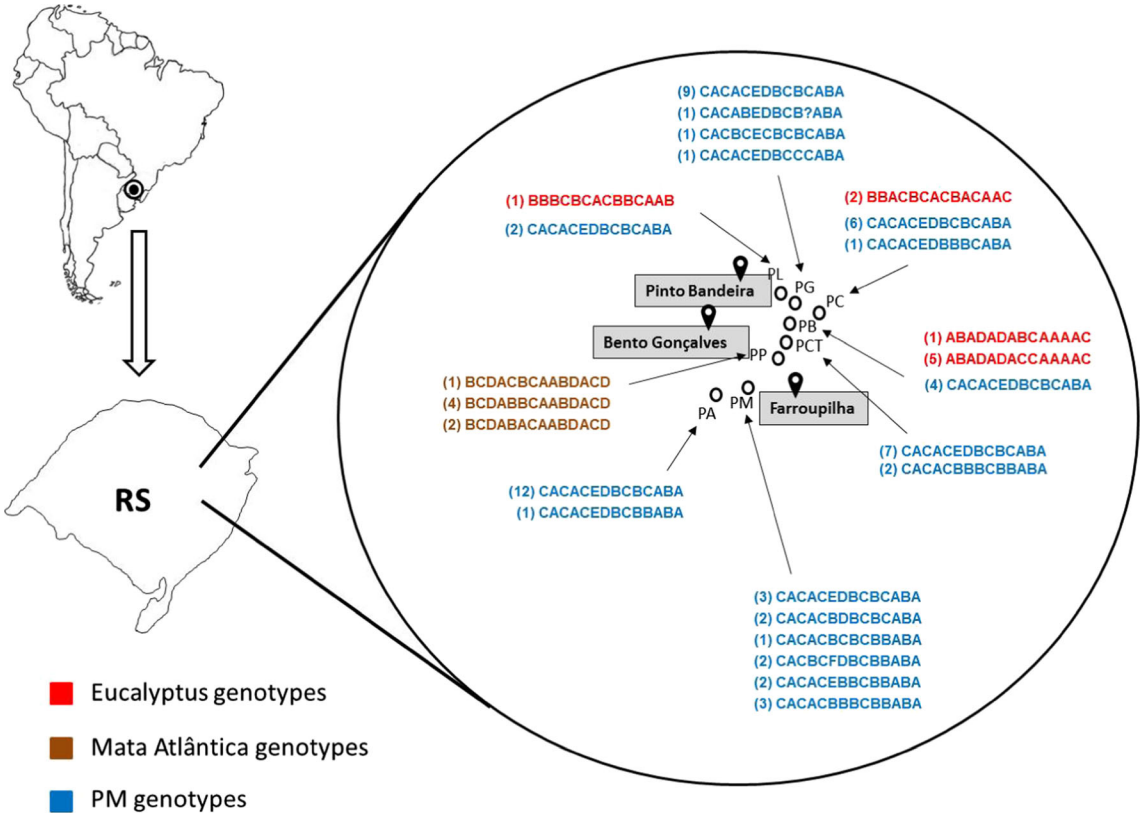

Fig. 1 Locations of *Ceratocystis fimbriata* genotypes obtained from kiwifruit at eight farms near Farroupilha, Rio Grande do Sul. The sampled farms are designated PA, PM, PG, PB, PC, PCT, PP and PL. The number of sampled isolates of each genotype at each farm is indicated in parentheses. Different microsatellite alleles are designated by different letters ("?" is missing data) for 14 respective loci: AAG8, AAG9, CAA9, CAA10, CAA15, CAA38, CAA80, CAT1, CAT1200, CAG5, CAG15, CAG900, GACA60 and GACA650. The colors red, orange, and blue represent microsatellite genotypes typical of *Eucalyptus* isolates from Minas Gerais and Bahia (*Eucalyptus* genotypes), genotypes typical of Mata Atlântica populations in Rio de Janeiro and São Paulo (Mata Atlântica genotypes), and genotypes typical of the farm PM, respectively

Single ascospore colonies were transferred to PDA for purification and then storage. Each isolate was from an individual, grafted plant, and only one isolate was used per plant. The cultures were stored at room temperature (Castellani 1939) before DNA extraction.

DNA extraction

A CTAB-based protocol (Murray and Thompson 1980) was used to obtain DNA from cultures for use as template in polymerase chain reaction (PCR). Isolates were grown in 25 ml of liquid medium (2% malt extract and 0.2% yeast extract) at room temperature for two weeks before DNA extraction.

DNA sequencing and phylogenetic analyses

Portions of the mating type locus, including portions of the *MAT1-1-2* and *MAT1-2-1* gene regions, were amplified and sequenced from self fertile isolates using the primers and protocols described by Harrington et al. (2014). Primers CFMAT1-F (5'-CAGCCTCGATTGAKGGTATGA-3') and CFMAT1-R (5'-GGCATTTTTACGCTGGTTAG-3') were used to amplify and sequence about 1000 bp of the MAT1 region that included most of the *MAT1-1-2* gene. The primers X9978a (5'-GCTAACCTTCACGCCAATTTTGCC-3') and CFM2-1 F (5'-AGTTACAAGTGTTCCCAAAAG-3') were used to amplify and sequence about 1150 bp that included most of the *MAT2* gene. The thermocycler settings for amplifying both MAT1 and MAT2 regions included: initial denaturation at 94 °C for 2 min, with 36 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were purified (Harrington et al. 2014) and sequenced with the PCR primers at the Iowa State University DNA Facility.

Sequences of the kiwifruit isolates and representative isolates of the Latin American Clade of the *C. fimbriata* complex from Brazil and elsewhere were compared using parsimony analysis (Swofford 1998). The sequences in the combined MAT1/MAT2 dataset were readily aligned manually, except for some minor ambiguity in alignment with the outgroup

taxon, *C. variospora*, which is a member of the North American Clade of the *C. fimbriata* complex (Johnson et al. 2005). The aligned dataset was analyzed by maximum parsimony using PAUP 4.0b10 (Sinauer Associates), with settings as described in Harrington et al. (2014). Bootstrap values using 1,000 replicates also were determined in PAUP. Posterior probability estimates were determined using Mr. Bayes 3.2.1 (Ronquist and Huelsenbeck 2003), with 1,000,000 generations, diagnosis frequency = 5000, sample frequency = 500, and burninfrac = 0.25.

Microsatellite markers

We analyzed 14 PCR-based microsatellite markers (AAG8, AAG9, CAA9, CAA10, CAA15, CAA38, CAA80, CAT1, CAT1200, CAG5, CAG15, CAG900, GACA60 and GACA650) developed from the genomic DNA of an isolate of *C. cacaofunesta* (Steimel et al. 2004) and previously used in earlier studies of *C. fimbriata* populations (Engelbrecht et al. 2004, 2007b; Ocasio-Morales et al. 2007; Ferreira et al. 2010; Oliveira et al. 2015; Li et al. 2016). These microsatellite (simple sequence repeat, SSR) loci have been well characterized (Steimel et al. 2004; Simpson et al. 2013), and most have tri-nucleotide repeats, except for the four-base repeats of GACA60 and GACA650. For each primer pair, one of the primers was fluorescently labeled. PCR amplifications of all microsatellite loci were performed using a 96-well thermal cycler (PTC-100, MJ Research) following the earlier described conditions (Ferreira et al. 2010). The PCR products were electrophoresed using a four-capillary ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). Band sizes of the products were determined using marker standards and ABI GeneScan Analysis Software v3.1.2 and Genotyper 2.0 software (Applied Biosystems). Each estimated product length was rounded to the nearest whole number, and alleles were assigned based on comparison to a large dataset of more than 1,300 isolates. The reproducibility of the PCR amplification and fragment analysis were confirmed by repeating 12% of the isolates in this study.

Data analysis

Based on the potentially large size ranges of microsatellite alleles within and among populations and species of *C. fimbriata* (Ferreira et al. 2010, 2011; Oliveira et al. 2015; Li et al. 2016), an infinite alleles model of mutation was assumed. Alleles of a locus that were similar in length, such as a 3-bp difference, were not considered more closely related to each other than alleles differing by 9 or 12 bp, and each allele of a locus was considered independent from the others in all analyses.

Multilocus genotypic diversity was estimated with the Stoddart and Taylor's *G* index (Stoddart and Taylor 1988) with rarefaction. To compare genotypic diversity values among populations with different sample sizes, *G* was scaled to the expected number of genotypes for the smallest sample size being compared based on rarefaction curves using the R-package v.2.6.1 and vegan package from CRAN (Grünwald et al. 2003; R Development CoreTeam 2007). Relationships among genotypes were illustrated using Microsoft Excel 2010/XLSTAT-Base (Version 2016 18.7.01, Addinsoft) and principal component analysis (XLSTAT procedure: PCA) of the microsatellite alleles, with the main variation described in the first two components. Nei's gene diversity (H) (Nei 1973) based on the microsatellite alleles for the populations at each farm and for each of the three groups of isolates (based on MAT1/MAT2 sequences) was calculated with clone-corrected data using PopGen 1.32 software (Yeh and Boyle 1997). Clone-corrected datasets were a subset of the population left after removing isolates that were genetically identical.

Results

Phylogenetic analysis

Analysis of the combined *MAT1-1-2* and *MAT1-2-1* DNA sequences of 16 isolates from seven of the eight farms (Table 1) placed the kiwifruit isolates into three lineages among other South American populations of *C. fimbriata* (Fig. 2). One of the three kiwifruit lineages was identified at seven of the eight farms, and this is referred to as the PM mating type lineage (Table 1), which was distinct from all other identified mating type lineages found in South America (Fig. 2). All four tested isolates from PM had this MAT1/MAT2 sequence, as did seven other isolates (Table 1). In addition to an isolate of the PM lineage, two out of three tested isolates from PP had a sequence similar to Mata Atlântica isolates (Fig. 2) from mango in eastern Rio de Janeiro and taro in São Paulo (Oliveira et al. 2015). In addition to representatives of the PM mating type lineage, two of the tested isolates from PB and one from PL had mating type sequences similar to those commonly found in *Eucalyptus* and mango isolates from Bahia and Minas Gerais, Brazil and in China (Li et al. 2016).

Microsatellite analysis

All 14 microsatellite loci were polymorphic, and a total of 18 genotypes were identified among the 76 kiwifruit isolates from the eight farms (Fig. 3, Table 2). The highest genotypic diversity value was found in the population from PM, the source of kiwifruit plants or scions for each of the other farms (Table 1). Six genotypes were found among the 13 isolates

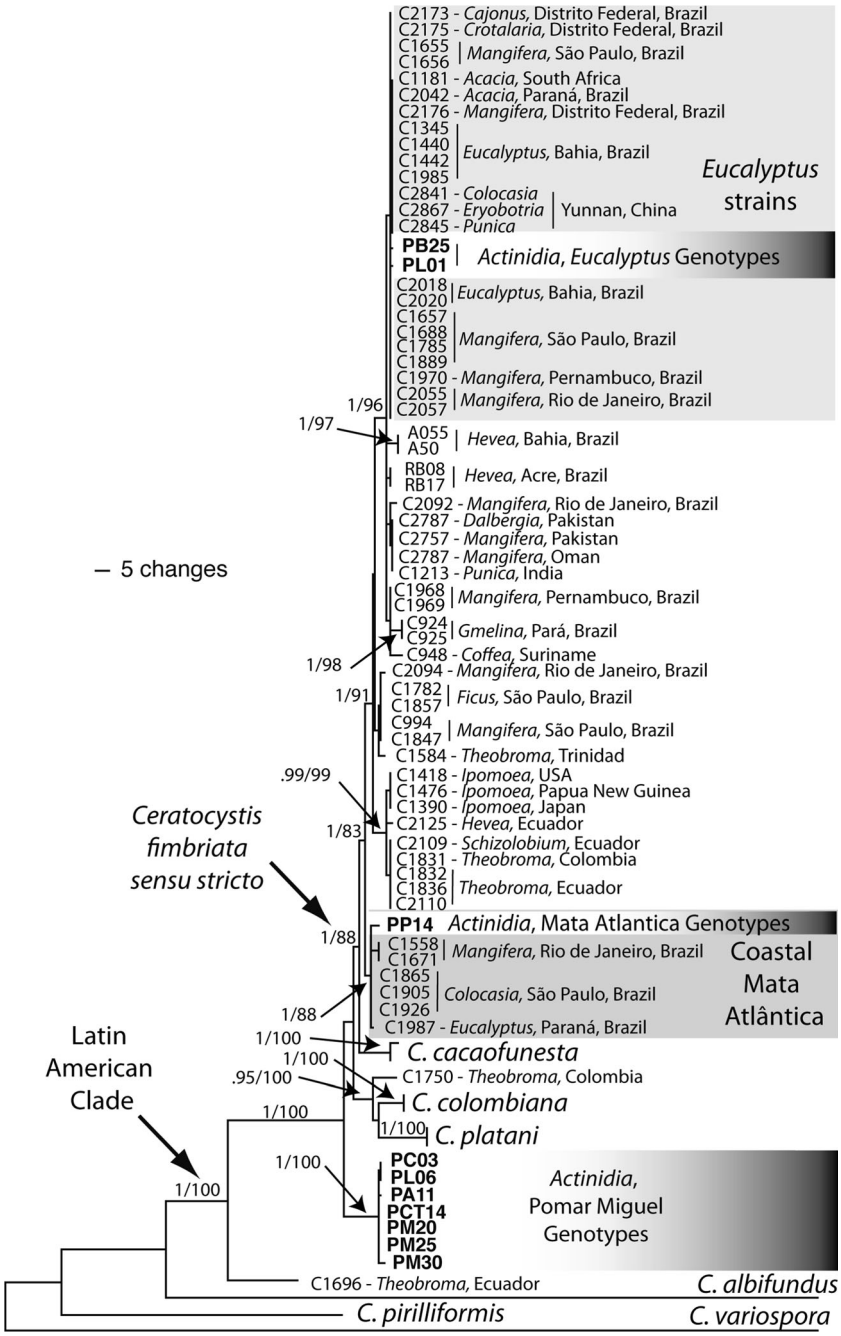

Fig. 2 The single most parsimonious tree of 893 steps based on 2164 aligned bases of the MAT1 and MAT2 regions of isolates of the Latin American Clade of the *Ceratocystis fimbriata* complex. The tree is rooted to *C. variospora* of the North American Clade and includes *C. pirilliformis* of the Asian Clade and *C. albifundus* of the African Clade. Representatives of kiwifruit isolates from Rio Grande do Sul are in shaded boxes, indicating DNA sequences typical of isolates from farm PM, *Eucalyptus* isolates from Minas Gerais and Bahia (*Eucalyptus* genotypes), or isolates typical of Mata Atlântica populations in Rio de Janeiro and São Paulo (Mata Atlântica genotypes). Values for branches with both high posterior probability (>0.95) and bootstrap (>80%) support are given

from PM farm (Table 1), and no single genotype was found in more than three isolates (Fig. 1). In contrast, half or more of the isolates collected at each of the other seven farms had the same single genotype (Fig. 1).

Gene diversity values for populations at the eight farms differed greatly. The isolates from PM had an intermediate level of gene diversity, with or without clone correction (Table 1). Farms PA, PCT and PG had grafted scions from PM, but these three farms did not purchase planting stock from other Brazilian nurseries, and they had very low levels of gene diversity, even with clone correction (Table 1). In contrast, farms PB, PC, and PL had grafted scions from PM as well as planting stock from commercial nurseries in Brazil, and the populations from those farms had high gene diversity values (Table 1), suggestive of introductions of genotypes from multiple sources.

The 18 microsatellite genotypes grouped into three apparent mega-populations or groups, which generally corresponded to the three groups based on mating type lineages (Table 1, Table 2, Fig. 3). In general, the three microsatellite groups showed moderate levels of genotypic diversity (Fig. 3), indicative of asexual reproduction, perhaps in

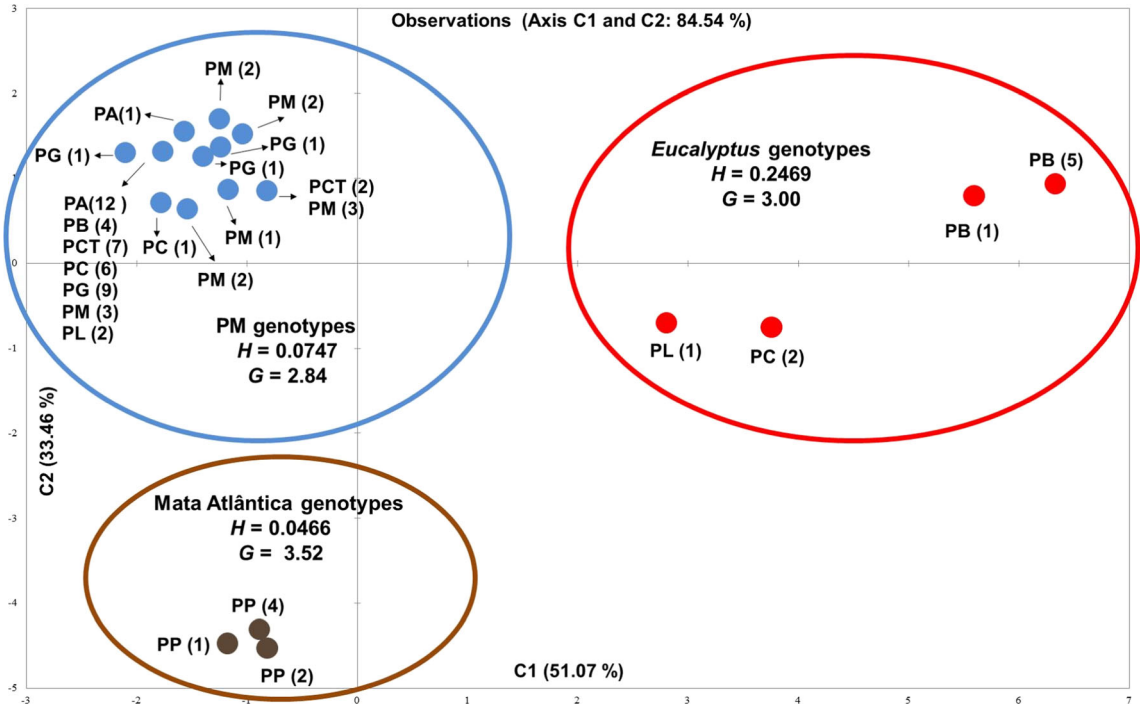

Fig. 3 Principal component analysis of *Ceratocystis fimbriata* genotypes on kiwifruit. Scores of component 1 (C1) and component 2 (C2) account for 84.54% of the total variation. Numbers in parentheses indicate number of isolates of that genotype identified from that farm. The red, brown, and blue circles group genotypes typical of *Eucalyptus* isolates from Minas Gerais and Bahia (*Eucalyptus* genotypes), isolates typical of Mata Atlântica populations in Rio de Janeiro and São Paulo (Mata Atlântica genotypes), and isolates typical of the PM farm, respectively. Values of gene diversity (*H*) and genotypic diversity (*G*) are given for each of the three populations

vegetative propagation material or spores dispersed on tools. The PM group included 60 isolates of 11 genotypes ($G = 2.84$ with rarefaction, maximum possible value = 7) from seven farms, and the overall gene diversity value of the combined group was only $H = 0.07$, suggesting a narrow genetic bottleneck. The most common and widespread genotype comprised 43 isolates from six farms, including PM (Fig. 3). The *Eucalyptus* microsatellite group included nine isolates and four genotypes from three farms, and the group had a value of $G = 3.00$ with rarefaction, maximum value possible = 7, but the overall gene diversity value of the group was relatively high ($H = 0.247$). The Mata Atlântica microsatellite group comprised seven isolates (all from PP) and four genotypes ($G = 3.52$ with rarefaction, maximum possible = 7), and the overall gene diversity value of the group was low (H = 0.047) (Fig. 3).

The grouping of isolates into three groups based on mating type gene sequences and microsatellite alleles was consistent for all but isolate PC03, which had a mating type sequence typical of the PM group (Fig. 2) but the microsatellite markers typical of the *Eucalyptus* genotypes (Fig. 3). All PP isolates tested for microsatellite markers had similar alleles (Table 2), but one of three tested isolates for mating type sequences had a PM mating type sequence (Table 1). However, this isolate was not tested for microsatellite alleles.

Discussion

All of the isolates obtained from kiwifruit in Rio Grande do Sul morphologically matched *C. fimbriata* and had *MAT1-1-2* and *MAT1-2-1* DNA sequences that placed them in the Latin America Clade of the complex, along with other Brazilian isolates from taro, *Eucalyptus* spp. and mango (Ferreira et al. 2010; Harrington et al. 2011, 2014; Oliveira et al. 2015). According to the phylogenetic analysis of the DNA sequences of the mating type genes, the kiwifruit isolates belong to three lineages within the LAC, and the microsatellite alleles likewise suggested three populations of *C. fimbriata* on kiwifruit.

Piveta et al. (2016) determined the ITS rDNA sequences of 39 isolates from kiwifruit, and they identified five ITS haplotypes that also formed three distinct groups in the LAC of *C. fimbriata*. Representatives of four of the five ITS haplotypes were each pathogenic when inoculated into kiwifruit (Piveta et al. 2016), suggesting that kiwifruit is highly susceptible to a diversity of isolates from the *C. fimbriata* complex. Because of the variation in ITS sequences, Piveta et al. (2016) concluded that the population on kiwifruit in Rio Grande do Sul was native to the region. An alternative interpretation of the genetic data is that there were three genetically distinct sources of isolates, and a mixture of these populations is

Table 2 Sizes of microsatellite alleles (bp) found in three populations of *Ceratocystis fimbriata* on kiwifruit

Populations	Size of alleles (bp)												
	AAG8	AAG9	CAA9	CAA10	CAA15	CAA38	CAA80	CAT1	CAT1200	CAGDL5	CAG15	CAG900	GACA60	GACA650
PM genotypes	186 (60) [1]	391(60)	217 (60)	125 (57) 128 (3)	327 (59) 324 (1)	299 (50) 309 (2) 225 (8)	317 (51) 314 (2) 308 (7)	249 (60)	376 (59) 373 (1)	325 (59) 328 (1)	277 (58) ?[2] (1)	194 (60)	195 (60)	200 (60)
Eucalyptus genotypes	180 (6) 183 (3)	397 (9)	172 (6) 211 (3)	134 (6) 131 (3)	318 (6) 324 (3)	246 (6) 237 (3)	302 (9)	249 (1) 261 (8)	376 (6) 373 (3)	317 (8) 325 (1)	270 (6) 280 (3)	194 (9)	187 (9)	215 (8) 213 (1)
Mata Atlântica genotypes	183 (7)	403 (7)	266 (7)	125(7)	327 (1) 324 (7)	201 (2) 225 (5)	314 (7)	247 (7)	370 (7)	325 (7)	284 (7)	194 (7)	200 (7)	219 (7)

[1] The numbers in parentheses are the number of isolates with that allele in that population. [2] (?) Missing data.

contributing to the high gene diversity found on kiwifruit in the region.

Most of the microsatellite genotypes identified on kiwifruit were found at the PM farm, and this farm had the highest level of genotypic diversity, comparable to that of other natural soilborne populations of *C. fimbriata* on *Eucalyptus* and mango (Ferreira et al. 2010, 2011; Oliveira et al. 2015). It is possible that the population on kiwifruit at the PM farm was introduced in nursery stock from Chilean or Brazilian nurseries. However, the moderate levels of gene and genotypic diversity in the population at PM suggest that the population is native and naturally soilborne at the PM farm, and the pathogen may have been present before forest clearing for agriculture (Ferreira et al. 2010; Oliveira et al. 2015).

The other seven farms that were sampled had either grafted scions from the PM farm or planted whole plants from the PM farm. One or more isolates from each of these seven farms had either mating type gene sequences and/or microsatellite alleles typical of the population at the PM farm. Thus, the majority of the isolates recovered from kiwifruit were distributed in scions or whole plants grown at the PM farm. Only PM genotypes were detected in three of these farms (PA, PG and PCT), which only obtained grafted material from PM, and these three farms had the lowest gene and genotypic diversity of the eight sampled farms. This suggests a severe genetic bottleneck as a small number of isolates were moved from a natural soilborne population to new farms in infected propagation material. This is similar to what was found in *Eucalyptus* plantings in Bahia planted with infected, rooted cuttings from commercial nurseries with *C. fimbriata* infestations (Ferreira et al. 2010, 2011, 2013).

The isolates from the PP farm were distinct from all other isolates in microsatellite markers, and two of the three tested PP isolates had the mating type gene sequences typical of the Mata Atlântica populations of *C. fimbriata* described earlier from taro, mango, *Annona squamosa* and other hosts from Rio de Janeiro, São Paulo, and Paraná (Baker et al. 2003; Silveira et al. 2006; Harrington et al. 2014; Oliveira et al. 2015). The PP population on kiwifruit may be native to this farm and may represent the southern extreme of the Mata Atlântica population of *C. fimbriata*. The low level of microsatellite gene diversity found in the PP population, however, might suggest that the population was not natural and may have been the product of a limited number of genotypes from a source other than farm PM. Some whole kiwifruit plants from PM were planted on the PP farm, and an isolate from PP collected after the microsatellite study had the mating type gene sequence typical of PM isolates, but it was not tested for microsatellite alleles.

The third group of microsatellite genotypes found on kiwifruit was similar to isolates from *Eucalyptus* in Bahia and

Minas Gerais and may have come in on nursery stock from São Paulo or from other commercial nurseries in Brazil (Oliveira et al. 2015). There were relatively few isolates of these microsatellite genotypes, and they had mating type gene sequences of isolates found further north in Brazil, and in introduced (non-native) strains in China (Harrington et al. 2014, 2015; Li et al. 2016). The *Eucalyptus* microsatellite genotypes were found at the PB, PC and PL farms, which also had microsatellite and mating type lineages typical of isolates from the PM farm, and these three farms each had grafted scions that originated from PM. Thus, the PB, PC and PL populations were apparently mixtures of *Eucalyptus* genotypes and PM genotypes, the mixture apparently contributing to artificially high levels of microsatellite gene diversity, by far the most diverse of the farm populations on kiwifruit. Thus, high gene diversity but low genotypic diversity in a *C. fimbriata* population may be indicative of a mixture of introduced strains rather than naturally diverse populations.

The first major planting of kiwifruit in the area was at farm PM, which appeared to have the gene and genotypic diversity typical of natural soilborne populations of *C. fimbriata* in Brazil (Ferreira et al. 2010, 2011; Oliveira et al. 2015). Unfortunately, many of the local farmers had obtained planting stock from this farm before Ceratocystis wilt was recognized. Typical of epidemics of Ceratocystis wilt on other crops (Ferreira et al. 2011; Oliveira et al. 2015; Li et al. 2016), the epidemic on kiwifruit in southern Brazil appears to be the result of a highly susceptible host and a variety of genotypes of *C. fimbriata* that have been moved in vegetatively propagated material, in this case, infected scions of kiwifruit. Greater care needs to be taken in dispersing *C. fimbriata* in symptomless propagation material (Ferreira et al. 2011; Harrington 2013, 2014; Oliveira et al. 2015).

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