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Population Biology

Population Genetic Characteristics and Mating Type Frequency of *Venturia effusa* from Pecan in South America

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Abstract

Scab, caused by the plant-pathogenic fungus *Venturia effusa*, is a major disease of pecan in South America, resulting in loss of quantity and quality of nut yield. Characteristics of the populations of *V. effusa* in South America are unknown. We used microsatellites to describe the genetic diversity and population structure of *V. effusa* in South America, and determined the mating type status of the pathogen. The four hierarchically sampled orchard populations from Argentina (AR), Brazil (BRC and BRS), and Uruguay (UR) had moderate to high genotypic and gene diversity. There was evidence of population differentiation ($F_{st} = 0.196$) but the correlation between geographic distance and genetic distance was not statistically significant. Genetic differentiation was minimal between the UR, BRC, and BRS populations, and these populations were more clearly differentiated from the AR population. The *MAT1-1* and *MAT1-2* mating types occurred in all four orchards and their frequencies did not deviate from the 1:1 ratio expected under random mating; however, multilocus linkage equilibrium was rejected in three of the four populations. The population genetics of South American populations of *V. effusa* has many similarities to the population genetics of *V. effusa* previously described in the United States. Characterizing the populations genetics and reproductive systems of *V. effusa* are important to establish the evolutionary potential of the pathogen and, thus, its adaptability—and can provide a basis for informed approaches to utilizing available host resistance and determining phytosanitary needs.

Keywords: ascomycete, exotic pathogen, genetic variability, *MAT1-1*, *MAT1-2*, mating type, population genetic diversity, population genetic structure

Pecan (*Carya illinoinensis* (Wangenh.) K. Koch) is a major specialty crop native to North America but now cultivated in several other regions of the world, including southern South America, South Africa, China, and Australia (Wells 2017). *Venturia effusa* (G. Winter) Rossman & W. C. Allen is a haploid, heterothallic ascomycete (Young et al. 2018) and the cause of scab on pecan. The disease can be devastating on susceptible cultivars (Gottwald and Bertrand 1983; Sanderlin 1995; Stevenson and Bertrand 2001). Symptoms on leaves, fruit, and shoots are typified by black spots <1 to 7 mm in diameter, and initially have a velvety appearance. On older leaves, lesions are dark grey or silvery and can crack and drop out of the leaf, resulting in a shot-holed appearance. Older lesions on fruit are dark brown and often have a cracked surface (Bock 2013). Yield loss manifests through reduced nut weight and premature nut drop, as well as loss in quality of nutmeats (Gottwald and Bertrand 1983; Stevenson and Bertrand 2001). Scab occurs on pecan in higher-rainfall areas of several of the pecan-growing regions of the world, including the southeast of the United States, several countries in South America, and parts of South Africa (Bock 2013). The pathogen produces asexual conidia that are dispersed in rain splash and wind (Gottwald and Bertrand 1982), with rainfall being the main driver of the epidemics (Sparks et al. 2009). The disease is polycyclic, with a relatively short latent period of as little as 7 to 8 days (Gottwald 1985; Turechek and Stevenson 1998), allowing epidemics to build rapidly. Recently, the sexual stage was demonstrated in vitro (Charlton et al. 2020) but its role in the epidemiology of the disease is yet to be established.

Pecan scab is challenging to control, in part because of the crop dimensions (Bock et al. 2016a). Wherever scab occurs on pecan, costly and potentially environmentally harmful fungicides must be applied (Standish et al. 2021). Fungicide resistance is a major issue with *V. effusa*, and the pathogen has developed resistance to several of the fungicides applied for its control (Littrell 1976; Reynolds et al. 1997; Standish et al. 2019, 2021). Furthermore,

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being a particularly tall tree crop, there are issues with ensuring adequate coverage for comprehensive control of the disease throughout the vertical profile of the tree (Bock et al. 2013, 2015). Host resistance is an alternative and, in some cases, has been effective over many decades (for example, cultivar Elliott). However, most agronomically or consumer-preferred cultivars tend to be susceptible to scab. Furthermore, several previously resistant cultivars have succumbed to the adaptability of *V. effusa* (Thompson and Conner 2012), demonstrating the threat to host resistance posed by *V. effusa* to this long-lived host. Understanding pathogen population biology and reproduction can provide insights to inform disease management and resistance breeding strategies to maximize resistance durability.

As noted, pecan is native to North America and has only relatively recently (approximately 100 years) been introduced to other regions of the world (Wells 2017). Along with pecan, *V. effusa* was likely cointroduced from the United States to the other locations where pecan is cultivated, and where the disease occurs. However, no research has been conducted to characterize populations of pecan scab beyond the United States (Bock et al. 2014, 2017). Pecan is widely cultivated in the southern regions of South America. The southeastern region of the South American continent has a climate conducive to scab (hot, wet summers), classified as "Temperate, no dry season, hot summer" (Cfa) type climate based on the Köppen climate classification system (Chen and Chen 2013). Reports of pecan culture in southeastern South America are available from Brazil (Bilharva et al. 2018), Argentina (Frusso and Lavado 2021), and Uruguay (Varela et al. 2015), and reports of pecan scab have been made from Paraguay (Kobayashi 1984), Uruguay (Leoni et al. 2019), Brazil (Ortiz and Camargo 2005), and Argentina (Mantz et al. 2009). Interestingly, Ortiz and Camargo (2005) commented that, in Brazil, there were no reports of scab until the introduction of the pecan cultivar Wichita, which is thought to have been imported to Brazil in the late 1960s by Geraldo Linck, with plantings at Cachoeira do Sul (E. R. Ortiz, *unpublished data*; L. Wells, University of Georgia, *personal communication*). Thus, considering the reports of pecan culture, import of living pecan material, and timing of the first observations of scab, it is possible that scab was introduced to southeastern South America with scion wood or whole plants of susceptible pecan cultivars, perhaps on multiple occasions.

Knowledge of the genetic diversity and reproductive mode of a pathogen is required for informed disease management, including breeding for host resistance and understanding the risks posed by the pathogen to different management strategies (McDonald 2015; McDonald and Linde 2002; McDonald and Mundt 2016). In addition to research on many other ascomycete pathogens of diverse crops, the population biology and genetic characteristics of pathogens closely related to *V. effusa* have been studied. For example, *V. inaequalis*, cause of apple scab (Broggini et al. 2011; Gladieux et al. 2008, 2010; Le Cam et al. 2019; Lemaire et al. 2016; Leroy et al. 2013; Shiller et al. 2015), has provided a basis to guide development of effective, long-term solutions to disease management through use of host resistance.

In haploid, heterothallic ascomycete fungi such as *V. effusa*, strains of opposite mating type are required for a compatible interaction (i.e., they are self-sterile) (Young et al. 2018). The mating type genes called *MAT1-1-1* and *MAT1-2-1* are located at a single locus named *MAT1-1* or *MAT1-2* (the *MAT* idiomorphs) (Turgeon 1998), and are at equilibrium frequencies in most populations of *V. effusa* in the United States (Bock et al. 2018; Young et al. 2018). Recently, the sexual stage was observed in vitro, confirming the ability of the fungus to undergo sexual recombination (Charlton et al. 2020). Although the sexual stage is yet to be found in the field, the described genetic diversity, population structure, and mating type equilibria are all indicative of a sexually recombining species across the pathogen's North American range (Bock et al. 2017; Young et al. 2018). A *MAT1-1*/*MAT1-2* ratio of 1:1 generally indicates regular sexual reproduction (Milgroom 1996; Zhan et al. 2002). Whether *V. effusa* undergoes a regular sexual cycle in other regions where it occurs is unknown but has ramifications for the adaptability of the pathogen, and the threat it poses to host resistance (McDonald 2015; McDonald and Linde 2002; McDonald and Mundt 2016). Furthermore, there are reports of a single mating type of a pathogen being transported to a new region, resulting in a clonally reproducing founder population, as described for the citrus black spot pathogen *Phyllosticta citricarpa* in Florida (Carstens et al. 2017; Wang et al. 2016). There are similar reports of loss of sexual reproduction for other introduced pathogens (Gladieux et al. 2015, 2018; Hessenauer et al. 2020; Stauber et al. 2021). If both mating types of *V. effusa* coexist in equilibrium in South America, the knowledge will provide a basis to understand the pathogen's reproductive biology, epidemiology, and potential threat posed to the pecan crop in the region. The knowledge will also provide the basis for informed disease management, and help guide phytosanitary regulation regarding transfer of host material between North and South America.

Although pecan has been grown in South America for over a century, and scab has been a production issue for several decades, there is no information on the pathogen population biology or presence of mating types from the region. Thus, we sought to address several questions regarding populations of *V. effusa* in South America. Are populations from geographically distinct orchards differentiated? Are both mating types of the pathogen present? Are the populations asexual, sexual, or mixed? And, how might the genetic diversity compare with that described for populations in the United States? To this end, we used previously developed microsatellite markers (Bock et al. 2016b, 2022) to (i) determine the genetic diversity of *V. effusa* in pecan orchards in South America, (ii) determine whether there was population genetic structure at different spatial scales, and (iii) gain insight into the reproductive mode of the fungus based on characteristics of the genetic diversity and frequency of the mating types.

Materials and Methods

Isolate source orchards, countries, sampling, culture, and DNA isolation

In conducting the study, we endeavored to adhere to recommended best practices for population genetic studies (Grunwald et al. 2017), including sampling protocols, marker selection, and methods of data analysis. In South America, one pecan orchard was located in each of Argentina and Uruguay, and two orchards were located in Brazil (Table 1; Fig. 1). The number of trees from which isolates of *V. effusa* were collected varied depending on orchard, with six and four trees sampled from the two orchards in Brazil (BRC and BRS, respectively), six trees sampled from the orchard in Argentina (AR), and four trees sampled from the orchard in Uruguay (UR). The number of trees sampled in a given orchard varied due to tree availability. The sampling plan was similar in each orchard. The AR orchard was planted to trees of the cultivar Stuart and the UR orchard was planted to trees of cultivar Shoshoni. The two Brazilian orchards comprised mixed plantings of cultivar Choctaw in cultivar Barton (BRC) or cultivar Shawnee in cultivar Barton (BRS). Barton is resistant to scab but was interplanted with rows of Shawnee and Choctaw that were used as pollinators, both of which are scab susceptible. The cultivars used for sampling are common in pecan orchards in South America and, thus, were selected. The sampling plan for trees within the orchards and samples of scabbed fruit within tree canopies were as previously described (Bock et al. 2017).

We followed previously published protocols for single-spore isolation, isolate culture on potato dextrose agar, and DNA extraction procedures using a Quick-DNA Fungal/Bacterial Miniprep Kit (ZymoResearch, Irvine, CA, U.S.A.) (Bock et al. 2014, 2017). The

samples of DNA, once extracted, were stored in Tris-EDTA buffer at −20°C until use. In total 336 isolates were isolated.

PCR and marker scoring

All isolates were screened against 15 previously identified polymorphic microsatellite markers (obtained from Eurofins MWG Operon, Huntsville, AL, U.S.A.) (Bock et al. 2016b, 2017, 2022). The 15 microsatellite markers were among 30 used previously to characterize more than 1,400 isolates of *V. effusa* from the United States (Bock et al. 2017, 2018). The primers were labelled with either FAM, NED, HEX, or PET dye (some were labelled with M13 tails), and reactions were multiplexed. The working microsatellite primer concentration was 10 µM, with a reaction volume of 5 µl, containing 1.5 µl of template DNA (1.5 to 2.0 ng), 0.25 µl of primer (total of forward and reverse primers), 0.75 µl of distilled H_2O, and 2.5 µl of master mix (Promega Corp., Madison, WI, U.S.A.). The conditions for the PCR were 94°C for 1 min; 94°C for 40 s, 58°C for 40 s, and 72°C for 20 s (repeated for 33 cycles); followed by 72°C for 30 min. Amplicons were subsequently denatured at 95°C for 3 min and were processed on a Hitachi 3500 Genetic Analyzer (Thermo-Fisher Scientific, Grand Island, NY, U.S.A.). Because *V. effusa* is haploid, a single amplicon at each locus was detected. The peaks were scored by base-pair size for each marker using GeneMarker (SoftGenetics, State College, PA, U.S.A.), and an internal size standard (Gene Scan 500 LIZ Dye Size Standard; Thermo-Fisher Scientific) was included to ensure accuracy. Approximately 20% of reactions were repeated to ensure reliability. The very few failed reactions were repeated to determine whether failure was due to issues with reagents in the specific reaction or due to a specific sample DNA–primer incompatibility.

Fig. 1. Map of southern South America showing the locations of the four pecan orchards where fruit with symptoms of scab were sampled for isolation of *Venturia effusa* in Brazil (BRC and BRS), Argentina (AR), and Uruguay (UR).

Mating types

The *MAT1-1* and *MAT1-2* mating types were identified using a previously described multiplex procedure (Young et al. 2018). Primers for *MAT1-1-1* (MTA-F: ATCACACTTGCCGCCAAGC GACC and MTA-R: TTGATGAGAGGGCAGACGAT [242-bp amplicon]) and *MAT1-2-1* (MTB-F1: AAGGTTCCTCGCCCAGC CAATG and MTB-R2: CTAAAGTTGGAAGAGAGGTTGG [775-bp amplicon]) were used; the *TUB2* gene was also amplified to confirm the reaction worked (tubB-F: AAGGTTCCTCGC CCAGCCAATG and tubB-R: CGGTGTAGTGTCCTTTGGCCCA [517 bp]). The PCR was performed in a total volume of 25 µl containing 3 ng of genomic DNA, 1.0 U of GoTaq DNA Polymerase (Promega Corp.), 1× Green GoTaq reaction buffer containing 1.5 mM $MgCl_2$, 0.2 mM each dNTP (Promega Corp.), and 1 µM target-specific primers. The cycling parameters were 94°C for 1 min; then, 30 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 45 s; followed by 72°C for 10 min. Reaction products were subject to gel electrophoresis in 1.5% agarose in a 1× Tris-boric-EDTA buffer and visualized with GelRed (Biotium, Inc., Fremont, CA, U.S.A.) by UV transillumination. Amplicon size was compared against an All-Purpose Hi-Lo DNA Marker (50 to 10,000 bp) (Bionexus, Oakland, CA, U.S.A.).

Data analysis: Diversity indices

Unless otherwise stated, analyses were performed using R software (R CoreTeam 2020) and different R packages. Orchard- and tree-level genetic population data based on haploid multilocus genotypes (MLGs) and allele presence and frequency were analyzed for genetic diversity using the R package *poppr* (Kamvar et al. 2014). Populations of *V. effusa* defined by mating type were analyzed similarly. The genetic indices included the number of MLGs, clone-corrected number of MLGs, the expected MLGs (eMLG) at the largest shared sample size based on rarefaction (and the standard error of eMLG), and genotypic diversity (the Shannon-Wiener index [H], and Evenness [*E.5*]. Nei's unbiased gene diversity was calculated (H_{exp}). All analyses were performed in R using *poppr*. To compare populations among orchards and among trees within

TABLE 1. Location, elevation, cultivar, and number of isolates of *Venturia effusa* collected from four pecan orchards in different countries in South America to assess population genetic diversity

Source location (town and country of population sample)	Abbr[a]	Latitude and longitude of site	Elevation (m)	Number of trees sampled	Number of isolates (number per tree)[b]	Month and year of sample collection	Trees age at sampling	Pecan cultivar	Cultivar parentage[c]
Abasto, La Plata, Argentina	AR	S 34°58′58.00″, W 58°03′57.00″	28	6	114 (11–25)	March 2020	13	Stuart	Unknown
Cachoeira do Sul, Brazil	BRC	S 29°59′48.58″, W 52°55′16.16″	108	6	93 (13–18)	May 2018	12	Choctaw	Success × Mahan
Cachoeira do Sul, Brazil	BRS	S 30°00′15.52″, W 52°53′25.34″	78	4	55 (11–19)	May 2018	8	Shawnee	Schley × Barton
San José, Uruguay	UR	S 34°17′18.55″, W 56°44′11.01″	53	4	74 (15–20)	March 2018	42	Shoshoni	Odom × Evers
Total	–	–	–	20	336	–	–	–	–

[a] Abbreviation used in the article for each of the four populations.
[b] Each isolate was from an individual lesion on a fruit, with only one isolate being taken per fruit.
[c] According to Sparks (1992).

orchards, we determined the mean allele richness (and standard error) after rarefaction (El Mousadik and Petit 1996; Petit et al. 1998) using the R program *popgenreport* (Adamack and Gruber 2014). After rarefaction, sample sizes were 51 individuals at the orchard level and 9 individuals at the tree level. Equivalent analyses were performed for populations defined by mating type (*MAT1-1* and *MAT1-2*).

Data analysis: Linkage disequilibrium

Clones were censored due to sensitivity of some analyses of genetic diversity and population structure to the presence of more than one MLG per population. Also, clone-corrected populations approximate in behavior to sexual populations. This is particularly important for pathogens with a mixed mating system (sexual and asexual), and conducting analyses using both clone-censored and noncensored data provides insight into the contributions of each reproductive mode to the genetic structure of the population (Grunwald et al. 2017). Thus, we conducted analyses based on both approaches. Linkage disequilibrium for each orchard population of *V. effusa* was explored using the Index of Association (I_A) (Brown et al. 1980) and the standardized Index of Association ($\bar{r}_d$). The advantage of $\bar{r}_d$ is independence of the number of loci in the analysis (Agapow and Burt 2001). Significance of I_A and $\bar{r}_d$ was tested by its deviation from the expected value under random mating (zero), with 1,000 randomizations performed for each population on all and clone-corrected data. A heat map was constructed to visualize $\bar{r}_d$ values for the clone-corrected data set for all pairwise combinations of markers (calculations were performed in R using *poppr*). The number of pairwise loci exhibiting linkage disequilibrium in each population was calculated using Arlequin V3.5.2 (Excoffier et al. 2005) with 100,000 permutations. In addition, linkage disequilibrium analyses were performed for populations defined by mating type (*MAT1-1* and *MAT1-2*) based on both clone-censored and noncensored data.

Data analysis: Population structure

Genetic structure of populations of *V. effusa* was explored using hierarchical analysis of molecular variance (AMOVA) for the four orchards at three spatial scales (between orchards, between trees within orchards, and within individual trees) based both on all data and on the clone-corrected data. The significance of the hierarchical AMOVAs was based on randomization test with 1,000 permutations to determine whether populations were significantly different at that level in the hierarchy (i.e., if $P \leq 0.05$) (analyses were performed in R using *poppr*). Differentiation between orchard and between tree populations was estimated based on fixation indices (F_{st}), calculated as $F_{st} = (H_S - H_T)/H_T$, where H_S is the average subpopulation variance in allele frequency and H_T is the total variance in allele frequency (Nei 1973). A discriminant analysis of principal components (DAPC) was conducted to characterize population subdivision. DAPC is a multivariate method designed to identify and describe clusters of genetically related individuals that was calculated in R using the *adegenet* package (Jombart 2008). A cross-validation was used to determine the number of principal components (PCs) to retain in each analysis, as described in the *adegenet* tutorial (Jombart and Collins 2017), and posterior membership probabilities calculated and presented for each of the isolates from the four populations. DAPC provides a basis to determine how well the data in the predefined population groups are explained based on the marker data and, in this case, we sought to assess the degree to which the populations of *V. effusa* in South America were differentiated. To further explore genetic structure, a dendrogram based on Nei's unbiased measure of pairwise genetic distance between populations was calculated for populations of *V. effusa* in trees in orchards and for orchard populations. AMOVA and DAPC analyses were performed for populations of *V. effusa* defined by mating type as well, as described above.

Using log Nei's measure of pairwise genetic distance (log D) and geographic distance (log km) between the four populations of *V. effusa*, a Mantel test was performed to determine isolation by distance in R using the *ade4* package (Thioulouse et al. 2018). The Mantel test was based on 10,000 permutations.

Data analysis: Mating types

Mating type frequencies were calculated for each orchard sample and the total sample to determine mating type equilibrium. Clone correction was performed based on the haploid MLGs identified using the markers. Analyses were conducted using SAS V9.4 (SAS Institute, Cary, NC, U.S.A.). The proportion of *MAT1-1* and *MAT1-2* isolates present was tested for equilibrium (the null hypothesis of a 1:1 ratio) using an exact binomial test (two-tailed) to determine whether observed mating type frequency deviated from the expected 1:1 ratio.

Results

Geographic population-based analyses

The number of markers depended on locus and ranged from 4 to 11. When based on individual populations, the UR population had four loci that were represented by only a single allele. Overall, the percentage of missing loci ranged from 0.14 to 0.48% although, based on individual loci, a single locus in population BRS had 7.27% loci missing. All loci that failed were subject to PCR a further two times, at which point amplification failure was determined to be due to causes other than faulty amplification conditions. To confirm reliability and repeatability, 20% of the isolates were subject to repeat amplifications. In all cases, results were consistent, and all loci were retained in the analyses.

Genetic diversity

The four orchard-level populations had 55 to 114 isolates collected in total, with 30 to 114 MLGs, depending on population (Table 2). The number of genotypes that would be expected at the smallest, shared sample size (eMLG), was 24.6, 44.4, 40.0, and 55.0 for the UR, BRC, BRS, and AR populations, respectively. Rarefaction indicated that genotypic richness did vary among populations, with UR having the fewest MLGs at the largest shared sample size, whereas the BRC, BRS, and AR populations tended to be more comparable. Shannon-Wiener measures of genotypic diversity depended on population, with the AR population consistently having the highest measures of genotypic diversity ($H = 4.74$) (Table 2). H ranged from 2.85 to 4.06 among the remaining populations. Evenness (*E.5*), which measures the distribution of MLG abundances, was 1.000 for the AR population and ranged from 0.550 to 0.775 for the remaining populations, whereas gene diversity (H_{exp}) was moderate (0.524) for the BRS population and ranged from a relatively low diversity of 0.178 (UR) to 0.500 (AR) for the remaining populations. Mean allele richness after rarefaction based on 51 individuals in each orchard ranged from 2.07 (UR) to 4.34 (BRS).

At the tree level within orchards, number of isolates, number of MLGs, and eMLGs were all reduced when compared with the orchard scale (Table 2). Measures of genotypic diversity were less in all individual tree populations compared with the orchard-level measures, although moderate to high genotypic diversity was still prevalent (H ranged from 1.71 [UR, tree 4] to 3.22 [AR, tree 2]). Similarly, evenness was moderate to high in all tree populations (*E.5* ranged from 0.623 to 1.000). Gene diversity (H_{exp}) was low in the trees in the UR orchard (0.161 to 0.214), and low to moderate in the tree populations in the remaining orchards (0.289 to 0.535). Mean allele richness at the tree level was based on nine individuals and ranged from 1.42 (UR, tree 2) to 2.71 (BRS, tree 4), and followed the same pattern as for the orchard-level allele richness.

Linkage disequilibrium

The total number of alleles in each population ranged from 33 (UR) to 66 (BRS) (Table 3). Based on the markers used in the study, and using the clone-corrected data, there were a proportion of loci that were not at equilibrium in each population (10.48, 11.43, 20.00, and 9.09% of pairwise comparisons for AR, BRC, BRS [all with 15 polymorphic loci], and UR [with 11 polymorphic loci], respectively). All populations exhibited linkage disequilibrium as measured by I_A or $\bar{r}_d$ when based on all data. Based on the clone-corrected data, the loci for isolates from population UR were at equilibrium but no other populations were at equilibrium based on the 15 loci (Supplementary Fig. S1). Consequently,

TABLE 2. Population genetic characteristics of *Venturia effusa* from pecan orchards in South America, and for each of the trees sampled within those four populations[a]

Scale, Pop[b]	Subpop[c]	N[d]	Richness[e]	MLG[f]	eMLG (SE)[g]	H[h]	$E.5$[i]	H_{exp}[j]
Orchard								
AR	–	114	3.40 (0.32)	114	55 (0)	4.74	1.000	0.500
BRC	–	93	3.77 (0.22)	69	44.4 (2.06)	4.06	0.751	0.423
BRS	–	55	4.34 (0.34)	40	40 (0)	3.52	0.775	0.524
UR	–	74	2.07 (0.25)	30	24.6 (1.68)	2.85	0.550	0.178
Total		336		244	48.6 (2.36)	5.21	0.558	0.494
Tree in orchard								
AR	Tree 1	20	2.68 (0.20)	20	11 (0)	3.00	1.000	0.531
	Tree 2	25	2.53 (0.21)	25	11 (0)	3.22	1.000	0.494
	Tree 3	11	2.37 (0.15)	11	11 (0)	2.40	1.000	0.492
	Tree 4	24	2.46 (0.23)	24	11 (0)	3.18	1.000	0.497
	Tree 5	14	2.32 (0.22)	14	11 (0)	2.64	1.000	0.445
	Tree 6	20	2.47 (0.16)	20	11 (0)	3.00	1.000	0.511
BRC	Tree 1	18	2.11 (0.19)	12	8.22 (0.984)	2.29	0.757	0.382
	Tree 2	18	2.28 (0.24)	12	8.37 (0.982)	2.34	0.852	0.384
	Tree 3	16	2.30 (0.19)	14	9.92 (0.709)	2.57	0.885	0.408
	Tree 4	13	2.10 (0.15)	12	10.29 (0.456)	2.46	0.961	0.289
	Tree 5	17	2.46 (0.16)	14	9.62 (0.808)	2.56	0.887	0.453
	Tree 6	11	2.14 (0.21)	11	11 (0)	2.40	1.000	0.319
BRS	Tree 1	12	2.47 (0.17)	9	8.5 (0.5)	2.14	0.935	0.480
	Tree 2	19	2.44 (0.13)	13	8.55 (1)	2.41	0.818	0.452
	Tree 3	13	2.39 (0.17)	9	7.77 (0.659)	1.95	0.687	0.405
	Tree 4	11	2.71 (0.18)	11	11 (0)	2.40	1.000	0.535
UR	Tree 1	19	1.50 (0.13)	12	8.23 (0.995)	2.33	0.838	0.161
	Tree 2	20	1.42 (0.15)	9	6.6 (0.966)	1.97	0.788	0.153
	Tree 3	20	1.63 (0.14)	13	8.47 (1.01)	2.42	0.836	0.214
	Tree 4	15	1.46 (0.12)	8	6.34 (0.855)	1.71	0.623	0.161
Total	–	336	–	244	10.66 (0.593)	5.21	0.558	0.494

[a] Data were obtained using 15 microsatellite markers.
[b] Pop: Populations: UR = Uruguay (Shoshoni), BRC = Brazil (Choctaw), BRS = Brazil (Shawnee), and AR = Argentina (Stuart).
[c] Subpop: the population of *V. effusa* in each tree sampled within the orchard.
[d] N = number of individuals in the population.
[e] Allelic richness was based on rarefactioned sample sizes of 51 and 9 individuals at the orchard and tree scales, respectively, and was calculated using *popgenreport* (Adamack and Gruber 2014).
[f] Number of multilocus genotypes (MLGs) in that population. MLG and the remaining statistics were calculated in *poppr* (Kamvar et al. 2014).
[g] Expected MLG (eMLG) is an approximation of the number of genotypes that would be expected at the smallest, shared sample size. In parentheses is the standard error (SE) based on eMLG.
[h] H = Shannon-Wiener Index of MLG diversity.
[i] $E.5$ = Evenness, a measure of the distribution of genotype abundances, wherein a population with equally abundant genotypes where $E.5 = 1$ and a population dominated by a single genotype approximately 0; thus, for clone-corrected population, $E.5 = 0$ (Grünwald et al. 2003).
[j] H_{exp} = Nei's unbiased estimate of average heterozygosity (or gene diversity) (Nei 1978).

TABLE 3. Measures depicting random mating among populations of *Venturia effusa* sampled from four pecan orchards in South America based on 15 microsatellite markers

Data, population[a]	Number of alleles	Pairs of loci exhibiting disequilibrium (%)[b]	I_A (P value)[c]	$\bar{r}_d$ (P value)
All data				
AR	58	–	**0.229 (0.001)**	**0.017 (0.001)**
BRC	63	–	**0.496 (0.001)**	**0.036 (0.001)**
BRS	66	–	**1.18 (0.001)**	**0.084 (0.001)**
UR	33	–	**0.751 (0.001)**	**0.079 (0.001)**
All	90	–	**1.063 (0.001)**	**0.076 (0.001)**
Clone corrected				
AR	58	13/105 (12.38)	**0.229 (0.001)**	**0.017 (0.001)**
BRC	63	16/105 (15.24)	**0.384 (0.001)**	**0.027 (0.001)**
BRS	66	21/105 (20.00)	**0.723 (0.001)**	**0.052 (0.001)**
UR	33	6/66 (9.09)	0.249 (0.054)	0.026 (0.054)
All	90	–	**0.675 (0.001)**	**0.048 (0.001)**

[a] Populations: UR = Uruguay (Shoshoni), BRC = Brazil (Choctaw), BRS = Brazil (Shawnee), and AR = Argentina (Stuart).
[b] Number of pairs of loci exhibiting disequilibrium of the total pairs of loci, and the percentage indicated in parentheses. Data shown only for the clone-corrected data set.
[c] The index of association (I_A) is a measure of linkage disequilibrium. The standardized index of association, $\bar{r}_d$, is a more robust measure, less sensitive to the number of loci (Agapow and Burt 2001; Brown et al. 1980; Maynard-Smith et al. 1993). Statistics were calculated based on 1,000 randomizations. Analyses were performed using *poppr* (Kamvar et al. 2014). Bold indicates populations with loci that deviate significantly from random recombination.

the null hypothesis of random mating could be rejected for the BRC, BRS, and AR populations but was accepted for the UR population.

Genetic structure and isolation by distance

The AMOVA based on all data and the clone-corrected data provided similar results; therefore, only the clone-corrected data are presented. Most of the variance (77.3%) was within trees in orchards but there was also evidence of significant ($P = 0.001$) structure between trees within orchards (3.1%) and between orchards (19.6%) (Table 4). F_{st} values affirmed detectable differentiation at the orchard level ($F_{st} = 0.196$) but provided little evidence of differentiation among trees within orchards ($F_{st} = 0.031$). The dendrograms based on Nei's genetic distance of populations of *V. effusa* on trees showed a preponderance of pecan trees clustering based on orchard (Fig. 2A). Although those trees from the UR orchard clustered as a group, they clustered most closely with two trees from the BRC orchard and BRS orchard (bootstrap support: 94.1%). All trees from the AR orchard populations clustered together (bootstrap support: 100%). Among the orchards, all populations were clearly differentiated, with bootstrap support ≥81.3% (Fig. 2B). The DAPC indicated a degree of population clustering, with isolates from each population tending to group together (Fig. 3). Isolates from UR and BRC tended to cluster together, whereas the isolates from AR and BRS tended to group separately from each other and other populations. The cluster membership probability of the posteriors for each of the isolates from the four populations based on the DAPC indicated that most isolates were reassigned to the original clusters with a moderate to high probability; however, some showed affinity for one of the other three clusters. The proportions of successful reassignments (based on the discriminant functions) of individuals to their original clusters was 1.000 for UR, 0.764 for BRC, 0.800 for BRS, and 0.947 for AR (where larger values indicate clear-cut clusters and low values indicate shared ancestry). Nei's unbiased genetic distance between *V. effusa* populations provided evidence that some pairs of populations were genetically distant; however, the data indicated that genetic distance was not always greater when geographic distance was greater (Supplementary Table S1). A Mantel test failed to support an effect of geographic distance on genetic distance ($r = 0.200$), with no support of isolation by distance based on the permutation analysis ($P = 0.3$). It should be noted that, although the AR and UR populations are geographically relatively close, the Rio de la Plata, one of the widest rivers in the world (at this point approximately 100 km wide on the line between the two orchards) could act as a natural barrier to dispersal between the two populations.

Mating type frequencies

One isolate from the UR population failed to amplify a mating type gene (UR tree 3, isolate 20). Thus, the isolate was not included in the mating type analyses. Based on the exact binomial analysis, the *MAT1-1* and *MAT1-2* mating types in each orchard were at equilibrium regardless of whether data were clone corrected or not (Table 5). Mating types in equilibrium are a compelling indicator of sexual reproduction in the sampled populations. Mating type frequencies at the tree level indicated two tree populations (one in BRS and one in AR) that were not in equilibrium based on all data (Supplementary Table S2). However, when clone corrected, only one tree population in the AR orchard was not at mating type equilibrium.

Genetic diversity of isolates grouped by mating types

All measures of genetic and gene diversity were similar in groups of isolates sharing the same mating type (*MAT1-1* and *MAT1-2*) (Table 6). MLG, eMLG, the Shannon-Weiner Index, and Nei's unbiased estimate of gene diversity were all similar for the two mating types. Evenness, however, did show some discrepancy between the two mating types, with *MAT1-1* having an *E.5* value of 0.566 and *MAT1-2* a value of 0.757. Genotypic richness was similar for both the *MAT1-1* and *MAT1-2* groups, although the *MAT1-2* group was marginally richer. There were clones present for both groups and, in four cases, the same MLG was represented by both *MAT1-1* and *MAT1-2* isolates.

TABLE 5. Frequencies of mating types (*MAT1-1* and *MAT1-2*) of isolates of *Venturia effusa* sampled from each of four pecan orchards in Uruguay, Brazil, and Argentina in South America

Analysis, sample	Sample size[a]	*MAT1-1*:*MAT1-2*	*P* value[b]
All data			
Argentina	114	50:64	0.2
Brazil C	93	46:47	1.0
Brazil S	55	33:22	0.7
Uruguay	73	41:32	0.7
Total	335	170:165	0.8
Clone-corrected data			
Argentina	114	50:64	0.2
Brazil C	69	35:34	1.0
Brazil S	40	24:16	0.3
Uruguay	30	14:16	0.9
Total	244	118:126	0.7

[a] All data and clone-corrected data are presented. The total sample size for all data is 335 (not 336), because the mating type for one isolate from tree 3 (Uruguay) could not be determined.
[b] Probability from an exact binomial analysis (two-tailed) to test whether mating type frequencies deviate significantly from a 1:1 ratio.

TABLE 4. Hierarchical analysis of molecular variance (AMOVA) of the population structure of *Venturia effusa* collected from pecan fruit in different trees in four orchards in different countries in South America, based on clone-corrected data[a]

Source	df[b]	Sum sq[c]	Mean sq[d]	σ[e]	Variation (%)[f]	ϕ[g]	*P* value[h]	F_{st} values[i]
Between orchard	3	342.2	114.1	1.63	19.6	0.227	0.01	0.196
Between trees within orchard	16	158.5	9.9	0.25	3.1	0.038	0.01	0.031
Within trees	253	1,626.7	6.4	6.43	77.3	0.196	0.01	–
Total	272	2,127.4	7.8	8.32	100.0	–	–	–

[a] AMOVA was performed using *poppr* (Kamvar et al. 2014).
[b] df = degrees of freedom.
[c] Sum sq = sum of squares.
[d] Mean sq = mean square.
[e] σ = Variance for each hierarchical level.
[f] Variation (%) = the proportion of σ in %.
[g] ϕ = Hierarchical population differentiation statistics.
[h] *P* value = probability of ϕ based on a randomization test with 1,000 permutations to determine whether populations were significantly different (i.e., significant population differentiation given that the observed ϕ does not fall within the distribution expected from the permutation).
[i] Fixation index (F_{st}) was calculated as $F_{st} = (H_S - H_T)/H_T$, where H_S is the average subpopulation variance in allele frequency, and H_T is the total variance in allele frequency.

Linkage disequilibrium associated with mating types

There were 81 and 84 alleles in the *MAT1-1* and *MAT1-2* groups, respectively. Measuring by I_A or $\bar{r}_d$ and based on all data or the clone-corrected data showed that both *MAT1-1* and *MAT1-2* groups deviated significantly from linkage equilibrium (Table 7). Based on the markers used in this study and using the clone-corrected data, there were a proportion of loci that were not at equilibrium in each population (44.76 and 50.48% of pairwise comparisons for the *MAT1-1* and *MAT1-2* populations, respectively) (Supplementary Fig. S2). Thus, based on these 15 loci, the null hypothesis of random mating could be rejected. Of all pairs of loci exhibiting linkage disequilibrium, 34 were shared by both mating types (with 13 unique to *MAT1-1* and 19 unique to *MAT1-2*).

TABLE 6. Statistics summarizing the genetic variation (genotypic and genic), grouping isolates of *Venturia effusa* by mating type (*MAT1-1* and *MAT1-2*)[a]

Group	N[b]	MLG[c]	eMLG (SE)[d]	H[e]	$E.5$[f]	H_{exp}[g]
MAT1-1	170	124	121 (1.06)	4.54	0.566	0.487
MAT1-2	165	129	129 (0.00)	4.70	0.757	0.500
Total	335	244	131 (4.00)	5.21	0.558	0.495

[a] Analyses were performed using *poppr* (Kamvar et al. 2014). All isolates were included in the analyses of genotypic and genic diversity.
[b] N = number of individuals in the population. The total sample size for all data is 335 (not 336), because the mating type for one isolate from tree 3 (Uruguay) could not be determined.
[c] Number of multilocus genotypes (MLGs) in that population.
[d] Expected MLG (eMLG) is an approximation of the number of genotypes that would be expected at the smallest, shared sample size. In parentheses is the standard error (SE) based on eMLG.
[e] H = Shannon-Wiener index of MLG diversity.
[f] Evenness ($E.5$) is a measure of the distribution of genotype abundances, wherein a population with equally abundant genotypes where $E.5 = 1$ and a population dominated by a single genotype approximately 0; thus, for clone-corrected population, $E.5 = 0$ (Grünwald et al. 2003). For the clone-corrected data, $E.5 = 1.0$ for all populations.
[g] H_{exp} = Nei's unbiased estimate of average heterozygosity (or gene diversity) (Nei 1978).

Genetic structure of mating types

The AMOVA based on the clone-corrected data indicated that 99.91% of the variance was found within groups of isolates sharing the same mating type; thus, there was no evidence for differentiation between them (Table 8). Furthermore, the F_{st} value (F_{st} = 0.001) provided no evidence for differentiation between mating populations. The DAPC based on 52 PCs after cross validation indicated very limited differentiation between *MAT1-1* and *MAT1-2* populations (Fig. 4A). The membership probability chart indicated admixture (Fig. 4B). Prior and posterior group size was *MAT1-1* = 170 and *MAT1-2* = 165. However, the proportions of successful reassignment (based on the discriminant functions) of individuals to their original clusters was relatively low for *MAT1-1* (0.724) and for *MAT1-2* (0.715), providing further evidence that the two mating type populations are not genetically differentiated.

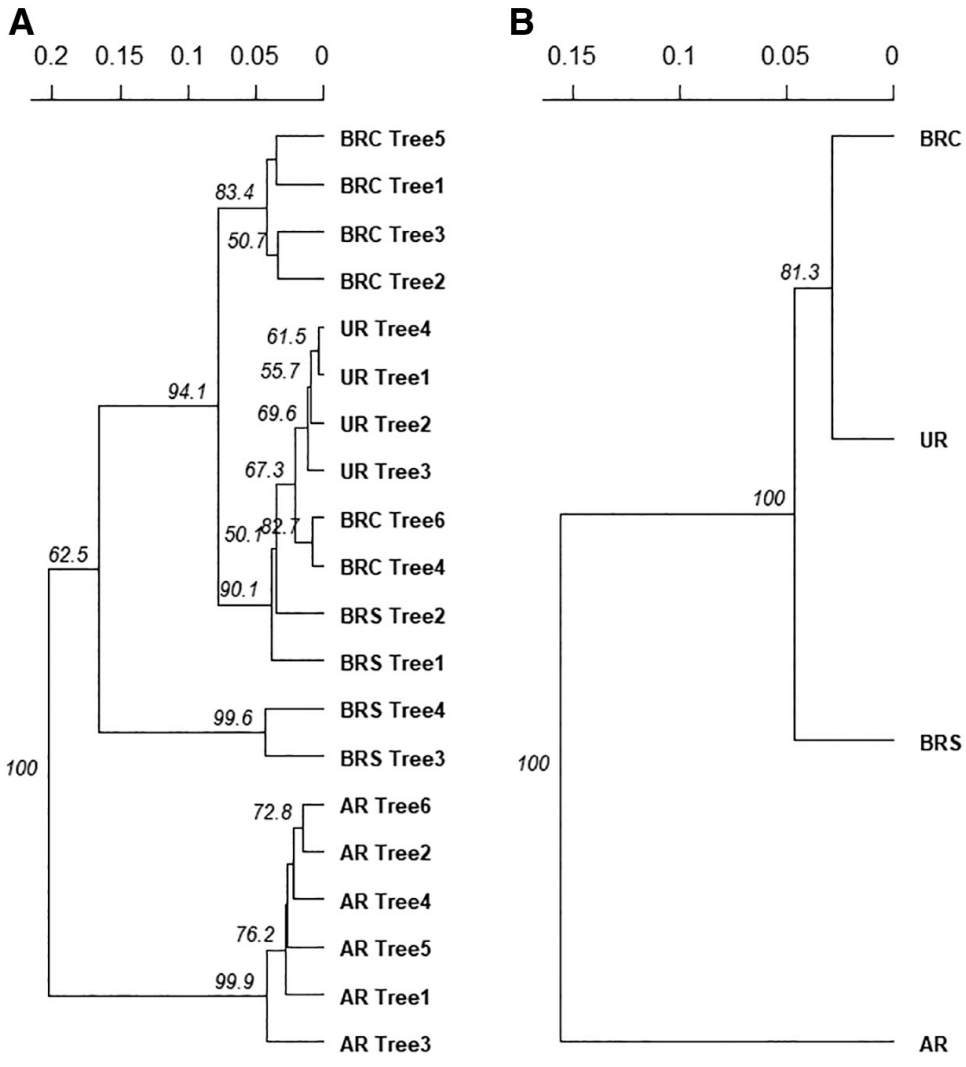

Fig. 2. Dendrogram based on the pairwise Nei's distances between isolates of *Venturia effusa* (336 isolates total) **A,** from different trees in each of **B,** four pecan orchards in South America based on 15 microsatellite markers. The four populations are: UR = Uruguay, BRC = Brazil (Choctaw), BRS = Brazil (Shoshone), and AR = Argentina. Tree numbers are indicated. Numbers at nodes indicate the percentage of times that branch occurred out of 1,000 bootstrapped permutations. Cut-off was set to 50%. The analysis was calculated in R using the *poppr* package (Kamvar et al. 2014).

Discussion

The populations of *V. effusa* sampled from all four orchards in South America were genetically diverse. Genotype diversity based on the Shannon-Weiner index ranged from 2.85 to 4.74, and was thus similar to a previous study, where genotype diversity of populations of *V. effusa* in the United States ranged from 3.49 to 4.59 (Bock et al. 2017). However, gene diversity measured by H_{exp} ranged from 0.513 to 0.713 in the United States and from 0.178 to 0.524 in South America, suggesting slightly less gene diversity among the South American populations. This suggests that, although the numbers of genotypes are similar in North and South American populations, the gene variability between genotypes is greater in the North American populations. When compared with the North American populations and corrected by rarefaction to the smallest population sample size of 30, the South American populations had a mean allele richness of 1.89 to 3.86 compared with that for the North American populations of 5.07 to 7.83 (C. Bock, *unpublished data*). Thus, although there appear to be some similarities in diversity, the populations in South America appear to have a paucity of alleles compared with those populations from the previous study in North America. The lower genetic diversity of the South American populations compared with that reported for populations of *V. effusa* in the United States suggests that the pathogen may have been introduced with pecan at some point in the last century. Indeed, in Brazil, there were no reports of scab prior to introduction of the cultivar Wichita in the late 1960s by Geraldo Linck (E. R. Ortiz, *unpublished data*; L. Wells, University of Georgia, *personal communication*).

There was some evidence of population structure in *V. effusa* sampled from the different orchards in South America. The AMOVA showed that 77.3% of the variance resided at the level of individual trees but 19.6% of variance was between orchards, indicating a significant and discernable differentiation between orchard-level populations. This is similar to the structure observed in the United States, where there was 81 and 16% of variation at the within- and between-orchard levels, respectively (Bock et al. 2017). Studies of the closely related apple scab (*V. inaequalis*) pathogen indicated high levels of variation within samples but slightly lower evidence of variation between populations (Gladieux et al. 2010; Koopman et al. 2017; Li et al. 2021; Xu et al. 2008). With populations of *V. carpophila* from peach in the southeastern United States, Bock et al. (2021) found 88.0% of variation within orchards and 12.0% between orchards. Similarly, studies assessing variation at equivalent spatial scales for other ascomycete fungal pathogens with a mixed sexual and asexual reproductive system have found comparable distribution of genetic variation within and between populations (Gout et al. 2006; Kimunye et al. 2021; Linde et al. 2002). The dendrogram and DAPC both provided further evidence of detectable

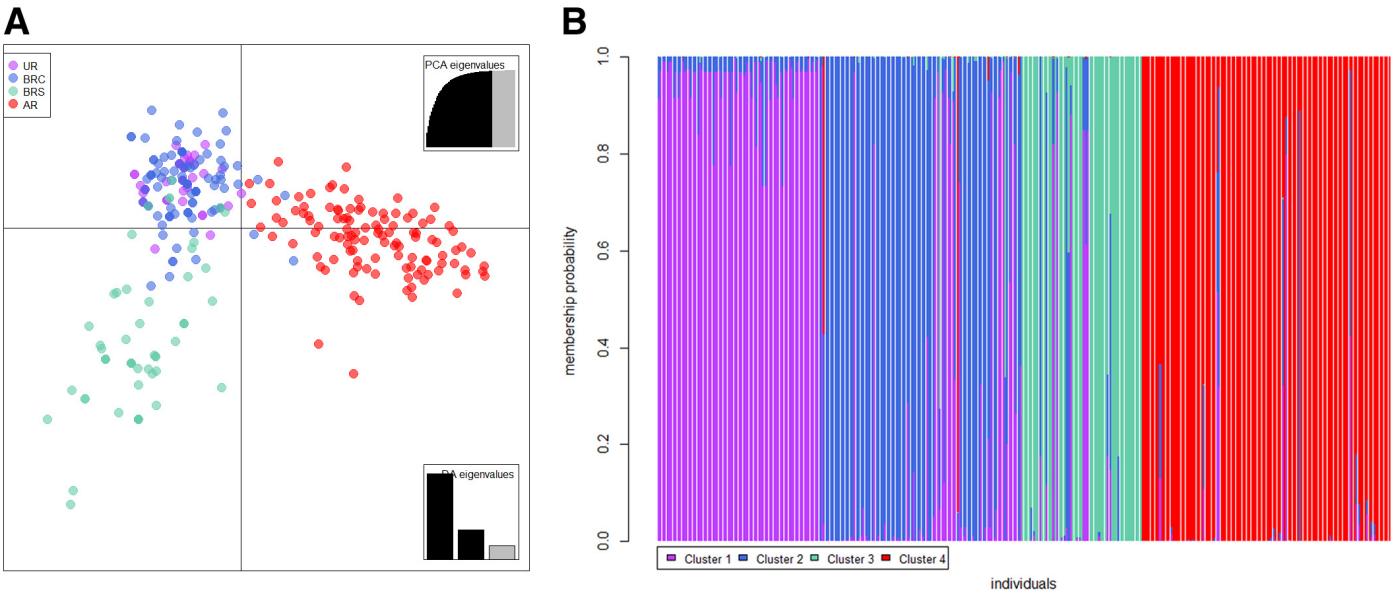

Fig. 3. Results of **A,** a discriminant analysis of principal components (DAPC) based on an analysis of the 336 isolates of *Venturia effusa* collected from four pecan orchards in South America and **B,** a membership probability chart for each of the isolates from the four populations based on the DAPC. The analysis was based on 15 microsatellite markers. Populations: UR = Uruguay (Shoshoni), BRC = Brazil (Choctaw), BRS = Brazil (Shawnee), and AR = Argentina (Stuart). There were 55 principal components retained for the analysis. Prior group size was UR = 74, BRC = 93, BRS = 55, and AR = 114 and postgroup size was UR = 100, BRC = 80, BRS = 46, and AR = 110. Proportions of successful reassignments (based on the discriminant functions) of individuals to their original clusters was 1.000 for UR, 0.764 for BRC, 0.800 for BRS, and 0.947 for AR (where larger values indicate clear-cut clusters and low values indicate admixed groups). Clusters 1, 2, 3, and 4 represent UR, BRC, BRS, and AR, respectively.

TABLE 7. Measures used to infer random mating in groups of *Venturia effusa* isolates sharing the same mating types, *MAT1-1* and *MAT1*-2, sampled from four pecan orchards in South America (data based on 15 microsatellite markers)

Data	Group	Number of alleles	Pairs of loci exhibiting disequilibrium (%)[a]	I_A (P value)[b]	$\bar{r}_d$ (P value)
All data	*MAT1-1*	81	–	1.142 (**0.001**)	0.082 (**0.001**)
	MAT1-2	84	–	1.009 (**0.001**)	0.072 (**0.001**)
Clone corrected	*MAT1-1*	81	47/105 (44.76)	0.604 (**0.001**)	0.043 (**0.001**)
	MAT1-2	84	53/105 (50.48)	0.735 (**0.001**)	0.053 (**0.001**)

[a] The number of pairs of loci exhibiting disequilibrium of the total pairs of loci, with the percentage indicated in parentheses. Data shown only for the clone-corrected data set.

[b] I_A is the index of association, which is a measure of linkage disequilibrium. The standardized index of association ($\bar{r}_d$) is a more robust measure, less sensitive to the number of loci (Agapow and Burt 2001; Brown et al. 1980; Maynard-Smith et al. 1993). Statistics were calculated based on 1,000 randomizations. Analyses were performed using *poppr* (Kamvar et al. 2014). Bold indicates populations with loci that deviate significantly from random recombination.

structure to the populations of *V. effusa* in South America. Studies with other pathogens, including *V. inaequalis* (Passey et al. 2016; Xu et al. 2013), indicate that host genotype can have a significant (although generally relatively small) effect on differences between populations—which may have contributed to some of the genetic variation observed among orchard populations of *V. effusa* in South America. However, a recent study comparing *V. effusa* populations on two genotypes of pecan did not discern genetic difference attributable to host genotype (Bock et al. 2022).

Although there was detectable differentiation between orchard-level populations, the Mantel test failed to demonstrate isolation by distance. In a previous study, isolation by distance was observed among populations of *V. effusa* in the United States, (Bock et al. 2017, 2022) but distances between population in the United States were greater and, whereas *V. effusa* is endemic to the United States, it might not have had a chance to fully establish population structure in South America, where it is likely introduced. Thus, among the four populations sampled, geographic distance between population pairs did not necessarily relate to genetic distance. In particular, the AR and UR populations are geographically moderately close (approximately 144 km) but the Rio de la Plata, one of the widest rivers in the world (at this point approximately 100 km wide on the line between the two orchards), could act as a barrier between the populations, at least for natural dispersal processes. Population differentiation (0.088 to 0.142) between the remaining three populations (UR, BRC, and BRS), all located north of the Rio de la Plata, suggested higher gene flow. Furthermore, considering that pecan was introduced to South America in the last approximately 100 years (Wells 2017), it is possible that there have been multiple introductions of the scab pathogen along with plant material in one or more areas, resulting in genetically contrasting founder populations, which could also explain some of the observations of genetic distance between populations.

With the exception of the UR population, all other South American populations deviated from linkage equilibrium, which is similar to North American populations (Bock et al. 2017, 2022). Studies on the closely related species *V. inaequalis* have indicated that linkage equilibrium predominates among populations (Gladieux et al. 2010). Deviations from linkage equilibrium can occur in sexually recombining populations for several reasons, including asexual reproduction, nonrandom mating, linkage, selection, population structure due to isolation and low gene migration, or genetic drift in small or founder populations (Crow and Kimura 1970; Taylor et al. 1999). Furthermore, some of the markers are

TABLE 8. Analysis of molecular variance (AMOVA) of the population structure by mating type (*MAT1-1* and *MAT1-2*) of *Venturia effusa* collected from pecan fruit from trees in four different orchards in different countries in South America[a]

Source	df[b]	Sum sq[c]	Mean sq[d]	σ[e]	Variation (%)[f]	ϕ[g]	*P* value[h]	F_{st} values[i]
Between mating types	1	9.0	9.0	0.008	0.095	0.001	0.310	0.001
Within mating types	251	2,008.7	8.0	8.003	99.905	–	–	–
Total	252	2,017.7	8.0	8.010	100.000	–	–	–

[a] AMOVA was performed using *poppr* (Kamvar et al. 2014). Results of the AMOVA based on all data and clone-corrected data are presented.
[b] df = degrees of freedom.
[c] Sum sq = sum of squares.
[d] Mean sq = mean square.
[e] Variance (σ) for each hierarchical level.
[f] Variation % = the proportion of σ in percent.
[g] Hierarchical population differentiation statistics (ϕ).
[h] Probability of ϕ based on a randomization test with 1,000 permutations to determine whether mating type populations were significantly different (i.e., with the clone-corrected data no significant population differentiation given that the observed ϕ does not fall within the distribution expected from the permutation).
[i] The fixation index (F_{st}) was calculated as $F_{st} = (H_S - H_T)/H_T$, where H_S is the average subpopulation variance in allele frequency and H_T is the total variance in allele frequency.

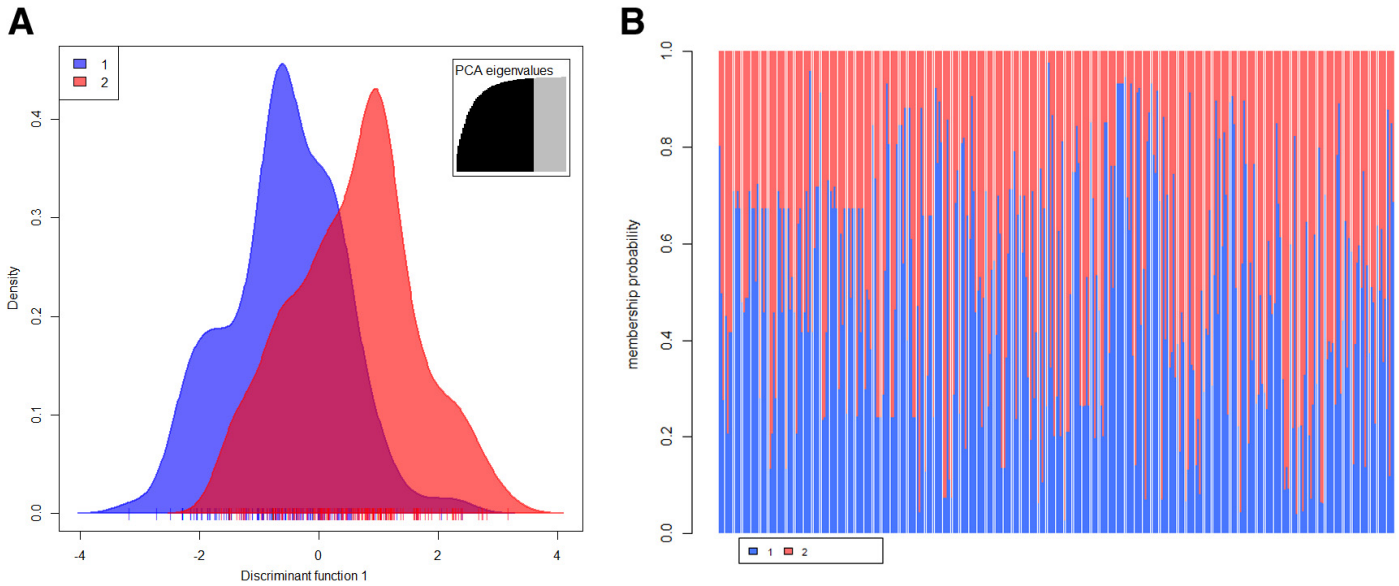

Fig. 4. Results of **A,** a discriminant analysis of principal components (DAPC) based on mating type (*MAT1-1* [1] and *MAT1-2* [2]) of the 335 isolates of *Venturia effusa* collected from four pecan orchards in South America, and **B,** a membership probability chart for each of the isolates for the two mating types based on the DAPC. The analysis based on 15 microsatellite markers. There were 52 principal components retained for the analysis. Prior and posterior group size was *MAT1-1* = 170 and *MAT1-2* = 165. Proportions of successful reassignment (based on the discriminant functions) of individuals to their original clusters was 0.724 for *MAT1-1* and 0.715 for *MAT1-2* (where values close to one indicate clear-cut clusters and lower values indicate shared ancestry).

physically close (Bock et al. 2022), and physical linkage is known to affect genetic linkage (Attanayake et al. 2014). To explore whether deviation from linkage equilibrium was dependent on proximity of specific markers, we removed two of the most proximal markers (P189 and P274) (Bock et al. 2022) and, although it reduced the I_A or $\bar{r}_d$, the characteristics of the populations remained the same, with deviations from linkage equilibrium predominating in three of the four populations, even after clone correction when only the UR population was at linkage equilibrium (data not shown). When analyzed based on seven microsatellite markers, each on a different chromosome and when clone corrected, all four populations were at linkage equilibrium (data not shown), although seven markers may be minimal to test for linkage equilibrium. The proportion of loci exhibiting linkage disequilibrium was low (9.09 to 20.00%), further indicating that the South American populations of *V. effusa* likely undergo recombination and exchange of genetic material.

We confirmed that both mating types of *V. effusa* occur commonly in orchards in southeastern South America. In situations where a fungal pathogen is likely introduced, there are cases where only one mating type has been reported, as with the citrus black spot pathogen in Florida (Wang et al. 2016) and other pathogens (Gladieux et al. 2015, 2018; Hessenauer et al. 2020; Stauber et al. 2021). Occurrence of a single mating type may have ramifications for the epidemiology of the disease, and ability of the pathogen to adapt. The overwhelming presence of the *MAT1-1* and *MAT1-2* mating types of *V. effusa* in roughly equal frequencies at both the orchard and tree scale provides evidence that the pathogen is undergoing sexual reproduction, as previously observed in the United States (Young et al. 2018). Indeed, the sexual stage of *V. effusa* was recently demonstrated in vitro (Charlton et al. 2020) but its role in the epidemiology of the disease in the field has not been described. Both mating types of *V. effusa* have previously been demonstrated to cooccur at the level of an individual leaf (Bock et al. 2018), the proximity providing opportunity for development of the sexual stage when conditions are conducive. The sexual stage of a closely related pathogen, *V. inaequalis* (cause of apple scab), has a mixed reproductive system and both ascospores and conidia are important components in the disease epidemiology (Bowen et al. 2011). Interestingly, although there is considerable knowledge regarding origins of *V. inaequalis* and pathogen population genetic diversity and structure (Gladieux et al. 2008, 2010; Leroy et al. 2013; Le Cam et al. 2019), there appears to be limited information available on the mating type idiomorph (Bock et al. 2021; Young et al. 2018). Nor does there appear to be information on the frequencies of the mating types in populations of *V. inaequalis*. The mating types of *V. inaequalis* do not appear to have been formally described, perhaps because the sexual stage and aspects of the population genetics are already so well characterized. There are many other ascomycete pathogens where the sexual stage is characterized, and the mating types described, and that are in 1:1 frequencies (Kimunye et al. 2021; Zhan et al. 2002). There are reports of other ascomycete pathogens for which no sexual stage has been found in the field but generally have populations with high genetic diversity, with mating types generally in equal frequencies, and yet deviate from linkage equilibrium; for example, *Cercospora beticola* (Knight et al. 2018; Vaghefi et al. 2017) and *Rhynchosporium commune* (Linde et al. 2009). Knowledge of the reproductive system and resulting population genetic structure of plant pathogens is important because it provides a basis for understanding pathogen adaptability; for example, gauging the risk the pathogen poses to host resistance or of developing fungicide resistance (McDonald and Linde 2002; McDonald and Mundt 2016).

As noted, although the sexual stage of *V. effusa* has been described in vitro (Charlton et al. 2020), its occurrence and role in the field has not been established. Thus, we explored the pathogen population genetics in relation to mating type for the South American populations to determine whether they conformed to an organism undergoing regular sexual recombination, as indicated by the roughly equal frequencies of *MAT1-1* and *MAT1-2* mating types. All measures of genotypic and genetic diversity were similar for both mating type populations. Interestingly, we detected MLGs that differed only in mating type. Both populations exhibited linkage disequilibrium when based on the 15 markers. As noted already, linkage disequilibrium can occur in sexually recombining populations for several reasons. The AMOVA (on clone-corrected data) showed there was no detectable population differentiation between isolates of opposite mating types, and the DAPC further indicated shared ancestry between the two mating type populations (with reassignment to original cluster being relatively low). The population genetic data for the two mating types of *V. effusa* are similar to those previously described for mating type populations of *V. carpohila* in the southeastern United States (Bock et al. 2021). Overall, the data suggest that exchange of genetic material between mating types in South American populations of *V. effusa* is relatively unimpeded.

Characterizing the genetic diversity, genetic structure, and reproductive systems are important to establish the evolutionary potential of a pathogen and, thus, its adaptability. It is already established that *V. effusa* can overcome host resistance in pecan (Thompson and Conner 2012), and rapidly develop resistance to fungicides (Standish et al. 2021). The considerable population genetic diversity and occurrence of both mating types (and, consequently, the likely mixed reproductive structure) suggests that *V. effusa* poses a serious threat to pecan production in the Americas, and that informed approaches are needed to develop effective use of available resistance in new cultivars. Recently, the first genomes of pecan have been published (Huang et al. 2019; Lovell et al. 2021) and, combined with those published for *V. effusa* (Bock et al. 2016c; Winter et al. 2020), constitute a first step to developing a knowledge-based approach to utilizing host resistance in relation to pathogen risk that can eventually help ensure durability of resistance in this valuable, long-lived nut crop.

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