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Spatial and temporal genetic analyses of *Phyllosticta citricarpa* in two lemon orchards in South Africa reveal a role of asexual reproduction within sexually reproducing populations

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ABSTRACT

Elma Carstens, Celeste C. Linde, Paul H. Fourie, Aletta E. Bester-van der Merwe, Shaun D. Langenhoven and Adéle McLeod. 2020. Spatial and temporal genetic analyses of *Phyllosticta citricarpa* in two lemon orchards in South Africa reveal a role of asexual reproduction within sexually reproducing populations. Phytopathology.

Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa,* is a disease that affects citrus worldwide. In different regions of the world where both mating types occur, reports differ as to whether asexually produced pycnidiospores play an important role in the epidemiology of CBS and fruit infections. We, therefore, investigated the potential role of pycnidiospores in two lemon orchards in South Africa using microsatellite-based analysis of fruit populations over time (two seasons) and space (distance). The two orchards were situated in the semi-arid North West province (NW) and subtropical Mpumalanga province (MP). Each population contained both mating types in 1:1 ratios, and linkage disequilibrium analysis indicated a random mating population. A total of 109 and 94 multilocus genotypes (MLGs) were detected across the two seasons in the NW and MP orchards respectively. P_{sex} analyses indicated that most MLGs probably resulted from sexual reproduction, but there were six predominant MLGs in each orchard that were probably replicated via asexual reproduction. Each of the predominant MLGs was monomorphic for mating type. In the NW, five predominant and widespread MLGs caused 46% and 44% of the fruit infections in the two seasons, whereas in MP, three MLGs caused 34% and 48% of the infections. Asexual reproduction in both orchards was supported by relatively low MLG evenness values in all populations. In both orchards,

distance was not a reliable predictor of population genetic substructuring, nor season. Populations of *P. citricarpa* in the MP and NW orchards were significantly genetically differentiated from each other.

KEYWORDS

citricarpa, fruit infections, asexual, epidemiology

The Citrus Black Spot (CBS) fungal pathogen, *Phyllosticta citricarpa* (McAlpine) Aa, which is an economically important pathogen of citrus worldwide, produces two types of spores, namely waterborne conidia (pycnidiospores) and aerially dispersed ascospores. Different spore types are found on citrus fruit, leaves, branches and twigs. Fruit lesions are economically the most important, and often only become visible after fruit color break. The lesions contain pycnidia and pycnidiospores which are products of asexual reproduction. These asexual structures have also been observed on leaves that are still attached to the trees as well as abundantly in leaf litter on the orchard floor (Kotzé, 1981). Lesions on leaves attached to the tree and in the leaf litter are rare and have only been found on leaves on lemon trees (*Citrus limon*) and infrequently on Valencia orange trees (*Citrus sinensis*) (FAO, 2015; Kiely, 1948; Kotzé, 2000). Dead twigs may also contain lesions producing pycnidia, but these are not common (Kiely, 1948; Kotzé, 1981; 2000). Living old branches (brown bark without fruits or flower buttons) have been reported as a potential reservoir of the pathogen, although the incidence of the pathogen in these was low (0.8%) (De Oliveira Silva et al., 2017). It has been hypothesized that once infected branches die, pycnidia will be produced on the surface since artificially

inoculated autoclaved branches were a suitable substrate for pycnidial production (De Oliveira Silva et al., 2017). The sexually produced ascospores, formed within pseudothecia, are only produced in fallen leaves (leaf litter). *Phyllosticta citricarpa* is a heterothallic fungus and for sexual reproduction to take place two mating types (*MAT 1-1* and *MAT 1-2*) are required to be in direct physical contact (Kiely, 1948; Kotzé, 1981, Wang et al., 2016; Tran et al., 2017).

Ascospores and pycnidiospores have been reported to play different roles in the spatial distribution of CBS within orchards and trees (Kiely, 1948; Kotzé, 1981; Spósito et al., 2007, 2008, 2011). Pycnidiospores, which are exuded in a gelatinous mass, are dispersed over short distances and their contribution to pathogen spread is generally regarded as within tree distribution through a water wash down action and to a lesser extent laterally (Hendricks et al., 2017; Kiely, 1948; Kotzé, 1981; 2000; McOnie, 1964a; Spósito et al., 2011). Ascospores are forcibly discharged and dispersed over distances of up to 25 m (Spósito et al., 2007) and are considered to be the primary source of between-tree pathogen dispersal within and between orchards (Kiely, 1948; Kotzé, 1981; McOnie, 1964a; Spósito et al., 2007, 2008, 2011).

The importance of pycnidiospores and ascospores as an inoculum source for fruit infections varies in different regions of the world. In South Africa, pycnidiospores have not been considered as playing an important role in fruit infections, unless out-of-season, late hanging infected fruit or overlapping fruit crops are present in orchards. Late hanging fruit do not occur in well-managed orchards in South Africa, whereas overlapping fruit crops can sometimes occur in lemon orchards. Ascospores, on the other hand, have been considered as being by far the most important source of fruit infections (Kotzé, 1981;

McOnie, 1965). A similar situation occurs in Australia (Kiely, 1948). It should be noted, however, that the older studies (Kiely, 1948; McOnie, 1965; Kotzé, 1981; Fourie et al., 2013) did not identity the *Phyllosticta* species of ascospores obtained from spore traps, which might have included the endophytic *Phyllosticta* species that can co-occur with *P. citricarpa* in the same orchard (Baayen et al., 2002). Recently, Tran et al. (2020) was able to use qPCR to distinguish between *P. citricarpa* and endophytic *Phyllosticta* ascospores, and found that *P. citricarpa* predominated. However, neither trapped ascospore numbers nor pycnidiospore numbers from leaf litter correlated with fruit infection data in their study, which suggested that inoculum in tree canopies might contribute to fruit infection (Tran et al., 2020). Fungicide spray programs in South Africa and Australia have been aimed at protecting fruit against ascospore infections during the fruit susceptibility period, *i.e.* the 4 to 5 months after petal drop (Kiely, 1948; Kotzé, 1981; McOnie, 1964a). In Brazil, pycnidiospores are considered an important inoculum source for fruit infections due to the different climate (higher rainfall) and cultural management practices (overlapping fruit crops and lack of pruning) prevailing in this region in comparison to South Africa and Australia (Spósito et al., 2007, 2008, 2011). Spray programs are thus applied over longer periods in Brazil than in South Africa to effectively control CBS (Lanza et al., 2018). In Florida, pycnidiospores are the only source of fruit infection since only one mating type occurs, precluding sexual reproduction (Wang et al., 2016).

Assessing the distribution of genetic diversity in pathogen populations provides an alternative approach to spore trap studies and spatial mapping of fruit infections to investigate the reproductive system of the pathogen, and the role of ascospores and

112 pycnidiospores in CBS epidemics. Only one study, based on microsatellites, has
113 previously made inferences about the reproductive system of *P. citricarpa* (Carstens et
114 al., 2017). This study was performed at a global spatial scale and included populations
115 isolated from fruit in Australia, China, Brazil, USA and South Africa. The South African
116 populations (two lemon, two Valencia and one grapefruit population), as well as those
117 from Australia (one population each from lemon, soft citrus and navels), Brazil (one
118 lemon) and China (two soft citrus), were shown to reproduce sexually, based on linkage
119 disequilibrium analyses and the co-occurrence of mating types at equal ratios. In the
120 South African and Brazilian populations, a low level of genotype evenness was observed
121 along with some populations showing significant linkage disequilibrium (Carstens et al.,
122 2017), suggesting at least some clonal reproduction. However, in the latter study
123 populations were obtained through a random sampling strategy, as opposed to a
124 structured sampling, which precludes strong inferences being made about the role of
125 sexual versus asexual reproduction. Furthermore, linkage disequilibrium in itself does not
126 provide conclusive evidence for sexual reproduction (Milgroom, 2017). Nonetheless, the
127 study, including the recent findings by Tran et al. (2020), hint at pycnidiospores playing a
128 more important role in fruit infections and in the epidemiology of the pathogen in South
129 Africa and Australia than previously reported (Kiely, 1948; Kotze, 1981; McOnie, 1964a).
130 In South Africa, CBS is found in the citrus-producing regions of the KwaZulu-Natal,
131 Limpopo, Mpumalanga, North West and Eastern Cape provinces, but is absent from the
132 citrus- producing regions of the Western Cape and Northern Cape provinces (Carstens
133 et al., 2012; Paul et al., 2005; Yonow et al., 2013). The regions where CBS does occur
134 differ in the prevalence/severity of the disease due to the differences in climatic

conditions. For example, the subtropical Mpumalanga province is known as a region that is more conducive to CBS development than the North West province, which has a semi-arid climate (Magarey et al., 2015; Paul et al., 2005; Yonow et al., 2013). The localized regional occurrence of the pathogen and variability in the prevalence/severity of the disease is due to warm, humid climates with higher summer rainfall being more conducive to CBS. These climatic conditions provide opportunities for the pathogen to complete its sexual cycle, and for infection to occur during the fruit susceptibility period (Carstens et al., 2012; Fourie et al., 2013; Guarnaccia et al., 2017; Magarey et al., 2015; Paul et al., 2005; Yonow et al., 2013).

The main aim of the present study was to determine whether asexual reproduction in addition to sexual reproduction plays a role in the epidemiology of CBS and lemon fruit infections in South Africa, by utilizing a population genetics approach. *Phyllosticta citricarpa* populations in two lemon orchards [one in the Mpumalanga province (subtropical) and one in the North West province (semi-arid) in South Africa] were investigated using temporal analyses over two seasons as well as spatial analysis (populations separated by 50 to 400 m within an orchard). Genetic differentiation between the two orchard populations, obtained from fruit, was investigated as well as the evolutionary relationship among multilocus genotypes (MLG), and whether MLGs were shared between the orchards. The co-occurrence of MLGs was also investigated at the spatial and temporal scale within each orchard. Since a previous study reported that the microsatellite markers used in our study might not be polymorphic enough to discriminate genotypes (Carstens et al., 2017), P_{sex} analysis and mating type data were used to assess the true clonal nature of multimember MLGs. A better understanding of the epidemiology

158 of CBS and the contribution of pycnidiospores to fruit infections may lead to improved
159 control strategies in orchards.

160 **MATERIALS AND METHODS**

161 **Orchard locations and CBS fruit sampling.** Two lemon orchards, situated in the
162 Mpumalanga and North West provinces (Fig. 1), with different climates, were included in
163 the study. The North West province orchard contained 15-year-old Eureka lemon (*Citrus*
164 *limon*) trees and was located near Brits, with a BSh Köppen-Geiger climate classification
165 (semi-arid: low relative humidity, warm summers and mild winters) (StepSA, 2016; CGA,
166 2017). The Mpumalanga orchard contained 9-year-old Eureka lemon (*Citrus limon*) trees
167 and was located near Mbombela (Nelspruit), with a Cwa Köppen-Geiger classification
168 (humid, subtropical: hot summers and dry winters). The orchards have a known history
169 of CBS, but were established with plant material of unknown origin and *P. citricarpa* health
170 status. The two orchards were separated by approximately 400 km. CBS was not
171 managed intensively in the orchards, which allowed for the development of overlapping
172 fruit crops and sufficient CBS symptomatic fruit for conducting a structured sampling.

173 *Phyllosticta citricarpa* isolates were collected from fruit lesions using a structured
174 sampling strategy over two consecutive production seasons (2011/12 and 2012/13). Fruit
175 were collected in 2012 in the 2011/12 season and in 2013 in the 2012/13 season. In the
176 North West orchard, there were four sampling sites (subpopulations). Sequential sites
177 were separated by 50 m (subpopulations 1 vs 2, 2 vs 3 and 3 vs 4) (Fig. 2A). Sampling
178 sites 1 and 3 were separated by 100 m, as well as sites 2 and 4. Sites 1 and 4 were
179 separated by 150 m (34 tree rows). In the Mpumalanga orchard, there were three
180 sampling sites (subpopulations) (Fig. 2B). Sampling sites 1 and 2 were separated by 200

m (35 tree rows), 2 and 3 by 300 m, and 1 and 3 by 400 m (48 tree rows). Each sampling site consisted of a total of five trees located in three different rows; three in the middle row, and one tree in each of the adjacent rows (cross figure). At each sampling site, ten CBS symptomatic fruit were randomly collected from each of the five trees.

***Phyllosticta citricarpa* isolations and microsatellite genotyping.** Isolation of *P. citricarpa* from CBS lesions on lemon fruit (containing mycelia and/or conidia), verification of species identity using species specific-primers (Meyer et al., 2006; Peres et al*.,* 2007) and DNA extraction were performed as described by Carstens et al. (2017). Fifteen polymorphic microsatellite or simple sequence repeat (SSR) markers (Pc117, Pc179.1, Pc236, Pc440, Pc849, Pc1007, Pc2073, Pc3011, Pc12, Pc19, Pc20, Pc32, Pc37, Pc6, Pc7) were used to genotype *P. citricarpa* populations (Carstens et al., 2017; Wang et al., 2016). Primer labeling, as well as Polymerase Chain Reaction (PCR) and amplification conditions, were as previously described (Carstens et al., 2017). Electrophoresis was performed using a 3730XL Genetic Analyzer (Life Technologies, Carlsbad, California) and the SSR alleles were scored using GeneMapper software version 4 (Life Technologies).

SSR data analyses. *Phyllosticta citricarpa* populations were grouped into populations and subpopulations for the various data analyses. There were two regional populations (North West and Mpumalanga), four seasonal populations (North West/2012, North West/2013, Mpumalanga/2012 and Mpumalanga/2013) and several spatially separated subpopulations (four in North West and three in Mpumalanga) in each orchard (Table 1; Fig. 2).

Basic estimates of genetic diversity analyses were calculated using GenAlEx version 6.5 (Peakall and Smouse, 2012). The estimates included the number of alleles (Na), effective number of alleles (*Ne*) and Nei's measure of gene diversity (H_e) (Nei, 1973), for which clone-corrected datasets were used (Balloux et al. 2003). Non-clone corrected data sets were used to determine the number of MLGs, the expected number of MLGs after rarefaction (*eMLG*) (to account for different sample sizes), and the genotype evenness index (*E5*), using *Poppr* in the R package (R Core Team, 2020; Kamvar et al. 2014). The *eMLG*s were calculated across all subpopulations rarefactioned to the smallest sample size of 32. To compare seasonal and orchard populations, *eMLG*s were also calculated for the four populations (North West 2012 and 2013, and Mpumalanga 2012 and 2013), rarefactioned to the smallest sample size, 96 (*Poppr* in the R package (R Core Team, 2020; Kamvar et al. 2014). The equitability index *E5* is an indication of the evenness of MLGs present in subpopulations and populations (seasonal and regional), and varies from zero (no evenness) to one (all MLGs have equal abundance) (Grünwald et al., 2003; Shannon and Weaver, 1949). T-tests using an online GraphPadQuickCalcs software (https://www.graphpad.com/quickcalcs/ttest1/?Format=SEM) were performed to compare the means of H_e and *eMLGs* between pairwise comparisons of populations.

P_{sex} analysis. Multimember MLGs are usually assumed to result from asexual reproduction, but some such MLGs may appear incorrectly to be clonal/identical because of insufficient locus discriminatory power, or low allele diversity in the population. Therefore, the probability that repeated MLGs comprised a true clone, and were not the product of random mating, was analyzed with MLGsim v2.0 (Stenberg et al., 2003). The probability (p_{sex}) that isolates with identical combinations of alleles were derived

independently from distinct sexual events in a binomial expression (Arnaud-Haond et al., 2007; Berbegal et al., 2013) was investigated in each orchard where we combined the two seasonal populations. Here, the probability p_{sex} that $p_{sex} > p_{gen}$ (p_{gen} = the probability that an identical genotype is derived by chance as a product of the number of loci and allele frequencies), was estimated by 1000 random simulations. A low probability score ($P < 0.01$) for p_{sex} supports a clonal origin for genetically identical isolates (Arnaud-Haond et al., 2007).

Evolutionary relationships among MLGs. Relationships among MLGs from both orchards were visualized from the SSR data using a minimum spanning network based on Bruvo's distance (Bruvo et al., 2004). This analysis was conducted using *Poppr* in the R package (Kamvar et al., 2015) and the network was visualized using the package *igraph* (Csardi and Nepusz, 2006). The edge grey scale adjustment was weighted towards separating out larger values of Bruvo's distance. The network was visualized using the kamada.kawai layout option in the R package *magrittr* to display connections in 2D with minimal overlap of edges in the graph. Edge length is arbitrary.

Mode of reproduction. *Mating type distribution.* PCR analyses with mating type-specific primers (Wang et al., 2016) were used to determine the presence of the *MAT1-1-1* and *MAT1-2-1* alleles in 203 isolates that represented a clone corrected SSR dataset of each seasonal population in each orchard (109 North West and 94 Mpumalanga isolates; Table 2), as previously described (Carstens et al., 2017). A chi-square test was used to determine whether the populations deviated from the null hypothesis of a 1:1 ratio of the mating types (Fisher and Yates, 1963).

Considering that P_{sex} analysis revealed that members of some MLGs may have been derived sexually (see Results section), it was important to determine the mating type for all isolates belonging to MLGs that were predicted to have a clonal origin within each orchard (Table 3). This could serve as an additional marker to further assess clonality. Therefore, in addition to analyzing the clone corrected set of isolates for mating type, genotyping was also attempted for all isolates belonging to the 12 MLGs that p_{sex} predicted to have a clonal origin in each orchard (Table 3).

Linkage disequilibrium analyses

To infer the reproduction strategy (asexual or sexual) of *P. citricarpa* in different populations, linkage disequilibrium analyses were performed using non-clone corrected as well as per population clone corrected SSR datasets. Most fungi can reproduce both sexually and asexually. Thus, the presence of clones indicates asexual reproduction, but may mask the signal of sexual reproduction. Therefore, both non-clone corrected and clone corrected datasets were analyzed to test the null hypotheses of random mating in populations. The clone corrected data sets consisted of one isolate from each of the identified MLGs. Clones of the same MLG were considered as those isolates having identical SSR alleles at all loci. Not considering isolates of such MLGs as clones if P_{sex} analysis predicted it to be derived sexually, would have incorrectly increased linkage disequilibrium and thus the prevalence of asexual reproduction.

Linkage disequilibrium analyses were performed using *Poppr* in the R package (R Core Team, 2020; Kamvar et al. 2014) by calculating the index of association I_A and the standardized version of the index of association $\bar{r}_d$. *P* values were obtained after 999 permutations (Agapow and Burt, 2001). The I_A and $\bar{r}_d$ indices indicate the degree of

271 association of alleles at different loci, within and among populations compared to that
272 observed in a permutated dataset. A value of zero is expected for physically unlinked loci
273 under random mating, i.e. linkage equilibrium. A value significantly larger than zero is an
274 indication of linkage disequilibrium, which is generated when no or infrequent sexual
275 reproduction occurs.

276 **Genetic differentiation.** A hierarchical Analysis of Molecular Variance (AMOVA) was
277 performed on a per population clone corrected SSR data set in GenAlEx version 6.5
278 (Peakall and Smouse, 2012) to estimate the degree of genetic differentiation and to
279 partition genetic variation within and among populations of defined groups. These groups
280 included (i) seasonal populations; 2012 and 2013, (ii) regional populations; Mpumalanga
281 and North West, and (iii) subpopulations within all orchards. For the AMOVA analysis, the
282 relative contributions of within population genetic variation *PhiPT*, between populations
283 within regions *PhiPR*, and between regions *PhiRT* were calculated using GenAlEx version
284 6.5. The statistical significance for each AMOVA analysis was tested using 999
285 permutations. Genetic differentiation was also evaluated with pairwise *PhiPT* analyses
286 for sub- and seasonal populations in GenAlEx version 6.5 (Peakall and Smouse, 2012).
287 Finally, to determine the inter-relationship between the subpopulations within orchards, a
288 discriminant analysis of the principal components (DAPC) was performed in the R
289 package *adegenet* (Jombart, 2008). In the North West, the number of axes retained was
290 16 and 2 for the discriminant analysis. In Mpumalanga, the number of axes retained for
291 the principal component analysis was 24 and 2 for the discriminant analysis.

292 **Weather data.** Weather data, provided by the Soil, Climate and Water Business Unit,
293 Agricultural Research Council, South Africa, were collected from 2011 to 2013. In the

North West orchard, the weather station was approximately 35 km south-west of the orchard and in the Mpumalanga orchard, the weather station was approximately 29 km north-west of the orchard.

The prevailing weather data (rainfall, wind speed, humidity and temperatures) were evaluated in the two orchards during the fruit susceptibility period and ascospore release period in the 2011/12 and 2012/13 seasons. Lemon fruit in the North West and Mpumalanga provinces are susceptible to *P. citricarpa* infections between the beginning of October and the end of February and ascospore release typically overlaps this period of fruit susceptibility (Fourie et al., 2013; Kotze, 1981; McOnie, 1964b).

RESULTS

***Phyllosticta citricarpa* isolations.** In total, 599 *P. citricarpa* isolates were collected for SSR analyses over two years from the two orchards (Table 1). Of these, 373 isolates were obtained from the North West orchard (200 in 2012 and 173 in 2013) and 226 isolates from the Mpumalanga orchard (130 in 2012 and 96 in 2013). PCR analyses using species-specific primers confirmed that all of the isolates were *P. citricarpa*.

SSR genetic diversity. The effective number of alleles (*Ne*) across the 15 loci was low in all subpopulations in the North West (1.29 to 1.42) and Mpumalanga (1.34 to 1.41) orchards (Table 2). The seasonal populations also had a low effective number of alleles in the North West (1.37 and 1.40) and Mpumalanga (1.37 and 1.38) orchards (Table 2). This was due to the low overall allele diversity of the clone corrected data set. Four loci (Pc20, Pc32, Pc 2073 and Pc 3011) were monomorphic in all subpopulations in both orchards in both years and had the same alleles in both orchards. Only three of the loci (Pc117, Pc440 and Pc6) had more than 3 alleles in most of the subpopulations, with the

317 maximum number of alleles being six at locus Pc117 (Table 1). Considering all loci, the
318 2012 and 2013 North West populations contained a total of 31 and 29 alleles respectively.
319 In the Mpumalanga orchard, all of the loci yielded a total of 38 and 31 alleles in the 2012
320 and 2013 populations, respectively (Table 1).

321 The Mpumalanga orchard, but not the North West orchard, contained some seasonal
322 and subpopulations that differed significantly in *eMLGs* (Table 2). In the Mpumalanga
323 orchard, there was a significantly higher ($P < 0.05$) number of *eMLGs* in 2012 (45.1
324 *eMLGs*) than in 2013 (39 *eMLGs*). Two Mpumalanga subpopulations (1 and 3) in 2012
325 had significantly ($P < 0.05$) higher *eMLGs* (22.9 and 20.1) than the 2013 subpopulations
326 (16 *eMLGs*).

327 Evenness values (*E5*) are an indication of the relative abundance of different
328 genotypes in populations (1 indicates that all MLGs have an equal abundance). These
329 values for the seasonal populations were low in the North West orchard (0.587 and 0.638)
330 and in the Mpumalanga orchard (0.533 and 0.425) (Table 2). This indicated that a large
331 number of MLGs were abundant in both of the seasons. Evenness values were also low
332 for the subpopulations in each of the two orchards (Table 2).

333 Gene diversity (*He*) for the four subpopulations within each season ranged from 0.180
334 to 0.227 in the North West orchard and 0.178 to 0.234 in the Mpumalanga orchard.
335 According to the *t*-test, the *He* values did not differ significantly across subpopulations or
336 between the two orchards (Table 2).

337 **Probable origin and distribution of the multimember MLGs within each orchard.**
338 In the Northwest and Mpumalanga orchards, a total of 109 and 94 multimember MLGs
339 were identified, respectively, considering the populations from both seasons in each

orchard. In each orchard, most multimember MLGs had only a few members and had a non-trivial p_{sex} probability ($P > 0.01$) of being the result of sexual recombination (Table 3); we, therefore, did not consider them to be derived from an asexual event. However, in each orchard six MLGs had very significant p_{sex} probabilities ($P < 0.01$), indicating that it was very unlikely that most members of the same MLG were derived independently via sexual recombination. Thus it seemed likely that they are clonal, resulting from asexual reproduction. As a further assessment of the clonality of individuals from these 12 multimember MLGs (six within each orchard), the mating type of these individuals were assessed. However, since this analysis was conducted retrospectively, only a subset of these isolates could be successfully genotyped. From a total of 335 multimember MLG isolates, only 278 (61 North West and 41 Mpumalanga) isolates could be genotyped successfully (Table 4). Each orchard contained individuals of each mating type (Supplementary Table 1), but in the subset that was successfully genotyped, there was no diversity for mating type within any of the multimember MLGs from either orchard (Table 4). This result further supports an asexual origin of these multimember MLGs.

In the North West orchard, the six MLGs that were likely clonal (MLGs 3, 6, 7, 25, 26 and 28) occurred in both sampling years and in most subpopulations (Table 5). Five of these MLGs (MLGs 6, 7, 25, 26 and 28) pre-dominated in the North West population. MLG-26 and MLG-7 had the highest occurrences and were similarly abundant in both years; MLG-26 represented 12% of the 2012 and the 2013 populations, and MLG-7 constituted 11.5% and 12.7% of the 2012 and 2013 season populations, respectively. Both MLGs occurred in all four subpopulations in both seasons, representing 2.0 - 20.8% of each subpopulation (Table 5). The three other predominant MLGs (6, 25 and 28) each

363 comprised 6.4-8.5% of the population in both years and were found in all four
364 subpopulations. Thus, clonal populations accounted for more than 40% (46 and 44% in
365 the 2012 and 2013 seasons respectively) of infections on fruit sampled in the North West
366 orchard. MLG-3, which did not have a very high occurrence in the population, was mostly
367 detected at site 1 in both seasons (Table 5).

368 Of the six MLGs (17, 21, 23, 34, 37 and 55) in the Mpumalanga orchard, three (MLGs
369 17, 23 and 37) were predominant (Table 5). The two most common MLGs (17 and 37)
370 together represented 27% and 41.7% of the 2012 and 2013 populations, respectively.
371 MLG-17 was the more dominant of the two MLGs and represented 17% and 30.2% of the
372 2012 and 2013 populations, respectively (Table 5). Both MLG-17 and MLG-37 were
373 detected in each subpopulation in each year. The third most dominant MLG was MLG-
374 23, which was detected in both years (6.9 and 6.3% in 2012 and 2013 respectively) and
375 in five of the six subpopulations. All individuals belonging to MLG-21 and MLG-55 were
376 obtained from the same location, whereas isolates from MLG-34 were also almost all
377 found at site 3 (Table 5). In the Mpumalanga orchard clonal populations of the three
378 predominant MLGs (17, 37 and 23) accounted, considering both years, for approximately
379 40% the fruit infections (33.9 and 48% in 2012 and 2013 respectively).

380 **Evolutionary relationships among MLGs.** Evolutionary relationships among MLGs
381 were examined with a minimum spanning network (Fig. 3). Only relationships within each
382 orchard are considered, and not those between the two orchards due to the high
383 probability of homoplasy occurring when investigating MLG relationships between
384 orchards (see Discussion section). Within each of the orchards, the predominant MLGs
385 from the orchard tended to cluster toward the tips of the network branches (Fig. 3). All

edge widths (Bruvo's distance) between genotypes were similar. In Mpumalanga, MLG-17 and MLG-37, were closely related and were separated by five MLGs, whereas MLG-34 and MLG-37 were separated by one MLG. In the North West orchard, the two most dominant MLGs (7 and 26) were also separated by five MLGs, whereas the two second-most dominant MLGs, MLG-6 and MLG-25, were separated by one MLG.

Mode of reproduction. *Mating type distribution.* Amplification of the mating type loci yielded unambiguous results for each isolate. Only one of the two primer pairs amplified a product indicating that the isolate was either *MAT1-1* or *MAT1-2*. The mating type frequencies were found to not deviate significantly from a 1:1 ratio in either orchard (North West orchard: χ^2 = 1.00 - 1.07; P = 0.30; Mpumalanga orchard: χ^2 = 0.02 - 1.27; P = 0.90; P = 0.20) (Supplementary Table 1). These results are consistent with sexual reproduction.

Linkage disequilibrium analyses

Linkage disequilibrium analyses for the clone corrected data set of each of the subpopulations in the North West and Mpumalanga orchards indicated that the index of association (I_A) and standardized index of association ($\bar{r}_d$) did not differ significantly (P > 0.05) from zero in the subpopulations in the two seasons (Table 6). This is an indication that the loci are not linked and that the hypothesis of random mating occurring could not be rejected.

In the non-clone corrected dataset, only the 2013 seasonal Mpumalanga population, and only subpopulation 3 in the orchard, had an index of association (I_A) and standardized index of association ($\bar{r}_d$) that differed significantly (P < 0.05) from zero (Table 6).

Genetic differentiation at the spatial (distance), temporal (season) and orchard (regional) level. AMOVA showed that the two orchards' populations (North West and

Mpumalanga) were significantly different from each other with a high percentage of variation existing between the two populations (68%, *PhiRT* = 0.682; *P* = 0.001) (Table 7A). This was also true when the four seasonal populations (two from each orchard) were defined in the AMOVA (62% variation, genetic differentiation among orchards (*PhiRT* = 0.624; *P* = 0.001) (Table 7B). In each orchard, the two seasonal populations were similar since a very low percentage (0 to 3%) of the genetic differentiation could be attributed to genetic variation between these populations (*PhiPT* = 0.000 to 0.027; *P* = 0.013 to 0.732; Supplementary Table 2). In both orchards, there was a high diversity among individuals in each orchard, which was the main source of variation in the analysis. This was evident from the AMOVA showing that the total genetic variance attributable to differences among isolates within the subpopulations was high (93-99%; *PhiPT* = 0.013 to 0.068; *P* = 0.021 to 0.163) (Supplementary Table 3), as well as for the seasonal populations where 36% of the variation (*PhiPT* = 0.638; *P* = 0.001) was attributed to differences among isolates within populations (Table 7A).

In the North West orchard, pairwise *PhiPT* analyses involving all subpopulations indicated that most subpopulations were not significantly different from each other (Table 8). The only exceptions were the 2012 season subpopulation 1 which differed significantly from the 2012 subpopulation 4 (*PhiPT* = 0.113; *P* = 0.009). Subpopulation 1 in 2012 was also significantly differentiated from the 2013 season subpopulation 2 (*PhiPT* = 0.075, *P* = 0.034) (Table 8). Subpopulations 1 and 4 represent populations that were separated by the largest distance (150 m) in the North West orchard (Fig. 2A).

In the Mpumalanga orchard, almost all subpopulations showed significant pairwise *PhiPT* differences in both seasons. The exceptions were subpopulations 2 and 3, which

were not significantly differentiated from each other in either 2012 or 2013 (Table 8). These two subpopulations were separated by 300 m, which was not the longest (400 m) distance by which some of the subpopulations were separated within the orchard (Fig 2B).

DAPC plots, in general, supported the pairwise *PhiPT* analyses. In the North West orchard, subpopulation 1 and 4 clustered separately in the 2012 season, but in the next season, they overlapped and were thus not differentiated (Fig. 4). In the Mpumalanga orchard, DAPC revealed more overlap between subpopulations 2 and 3 for both seasons, than for the rest of the subpopulations. In general, for all subpopulations there was less overlap in 2013 than in 2012 (Fig. 5). The eigenvalues chose for both orchards and both seasons in the orchards represented 80% of the total variation.

Comparison of weather data between the North West orchard and Mpumalanga orchard. The monthly weather parameters (minimum and maximum temperatures, relative humidity, wind speed and rainfall) differed in the two orchards during the fruit susceptibility period (October through to February) in the 2011/12 and 2012/13 seasons (Supplementary Table 4). The Mpumalanga orchard had a higher total rainfall in both seasons (2012 season 765 mm; 2013 season: 951 mm) than the North West orchard (2012 season: 408 mm; 2013 season: 477 mm). In both seasons, the average monthly maximum temperatures were higher in the North West orchard than in the Mpumalanga orchard; 1.56 to 3.84°C warmer in the 2011/12 season, and 2.23 to 4.79°C warmer in the 2012/13 season. In contrast, the average monthly minimum temperatures for Mpumalanga were higher (1.36 to 2.79°C) in both seasons than in the North West. The

454 average maximum relative humidity was somewhat higher in Mpumalanga during these
455 periods than in the North West.
456 During the ascospore maturation period (July to September), the Mpumalanga
457 orchard had more rain in both seasons (73.40 mm and 125.48 mm) than the North West
458 orchard (1.52 mm and 39.62 mm). The average monthly minimum temperatures were
459 also higher in Mpumalanga (5.98 to 12.49 ˚C) than in the North West orchard (-0.26 to
460 8.09 ˚C) (Supplementary Table 4).
461 The maximum wind speed in North West and Mpumalanga were comparable, with the
462 highest weed speeds (2.97 and 3.42 m/s) in each region occurring in August 2013. The
463 maximum wind speeds were in general low (< 2 m/s) in both regions during the fruit
464 susceptibility period (October to end of February) (Supplementary Table 5).
465
466 **DISCUSSION**
467 Our study, using a population genetics approach, contributes towards a better
468 understanding of the epidemiology of *P. citricarpa* and the role of pycnidiospores and
469 clonal populations in causing fruit infections in two lemon orchards (sub-tropical and semi-
470 arid climatic region) in South Africa. Analyses of temporal and spatially separated
471 populations obtained from fruit lesions in both orchards supported the occurrence of
472 sexual and asexual reproduction, and thus a role of ascospores and pycnidiospores in
473 disease development. It was, however, not possible to determine the extent to which
474 ascospores contributed to fruit infections over space and time. The non-trivial role of
475 pycnidiospores and clonal populations in causing fruit infections was evident from the

476 widespread occurrence of several multimember MLGs that caused a large proportion (34-
477 48%) of the fruit infections in each season.

478 An important aspect that required investigation in our study was whether the SSR
479 markers used were informative enough to identify multimember MLGs for which asexual
480 reproduction was a probable contributing factor. This investigation was required because
481 a previous study reported that a genotype accumulation curve of our markers did not
482 reach a plateau, suggesting that the markers might not be sufficiently discriminatory
483 (Carstens et al., 2017). Thus we applied the P_{sex} analysis, which did indeed predict that
484 most of the multimember MLGs were likely derived through sexual reproduction rather
485 than asexual reproduction. However, P_{sex} analysis did predict that six MLGs in each
486 orchard were likely clonal. Importantly, these included the three and five predominant
487 MLGs in the Mpumalanga and North West orchards, respectively. Further support for the
488 clonal nature of the MLGs predicted by P_{sex} analysis to be derived asexually was provided
489 by mating type analysis of a subset of isolates indicating that each such MLG was
490 comprised of a single mating type. Members of three of the less frequently occurring
491 MLGs that were likely clonal, were almost all detected at the same location within each
492 orchard. Although the possibility exists that the members of the MLGs not included in the
493 mating type analysis could belong to a different mating type, the overall evidence
494 supported the clonal nature of these MLGs. It is likely that founder populations in South
495 Africa, and other regions outside of the pathogen's center of origin, inherently are
496 bottlenecked and have low genetic diversity. The center of origin of *P. citricarpa* is most
497 likely linked to that of *Citrus*, which is most probably Asia (Guarnaccia et al., 2019; Wu et
498 al., 2018). In the current study, the effective number of alleles was low in all seasonal

499 subpopulations (1.37 to 1.40) since 14 of the 15 loci only had one to three alleles. A similar
500 finding was reported for other South African populations and for Australian and Brazilian
501 populations, where the effective number of alleles ranged from 1.17 to 1.48. Two Chinese
502 populations only had a slightly higher effective number of alleles (1.60 and 1.79)
503 (Carstens et al., 2017).

504 Asexual reproduction and therefore the contribution of clonal pycnidiospore
505 populations to fruit infections were evident in both orchards. In the North West orchard,
506 asexual reproduction occurred and five dominant clonal MLGs persisted in fruit infections
507 between the two seasons, as well as in almost all of the spatially analyzed
508 subpopulations. These five clones together caused 46% and 44% of fruit infections in the
509 2012 and 2013 seasons, respectively. As a result, because some MLGs occurred at a
510 high frequency, evenness values were relatively low in all populations (*E5* = 0.587 to
511 0.832), supporting asexual reproduction. In the Mpumalanga orchard, three clones
512 together caused 34% and 48% of fruit infections in the 2012 and 2013 seasons,
513 respectively. The increased clonal reproduction in the 2013 versus 2012 population was
514 also evident from lower evenness values in 2013 (*E5* = 0.425) than in 2012 (*E5* = 0.533).
515 Linkage equilibrium analysis of non-clone corrected datasets in the Mpumalanga orchard
516 also supported asexual reproduction, since two non-clone corrected populations (one
517 spatial subpopulation in 2013 and the 2013 seasonal population) were in disequilibrium.
518 Greater asexual reproduction in the Mpumalanga orchard in 2013 compared to 2012 may
519 have been favored by the higher rainfall in the 2013 season during the fruit susceptibility
520 period (951 mm in 2013 vs. 765 mm in the 2012 season). In Brazil, asexual reproduction

521 has been reported as being more prominent under high rainfall conditions (Spósito et al.,
522 2007, 2008, 2011).

523 The more prominent role of pycnidiospores and clonal populations in the epidemiology
524 of CBS and fruit infections as identified in the current study, might be specific to this citrus
525 type and orchards that are not well managed. In our study orchards, CBS was not
526 managed and infected dead twigs could thus have been present, which led to unusually
527 high inoculum levels. Overlapping fruit crops were also present in these two lemon
528 orchards. In lemon orchards, unlike other citrus types, overlapping fruit crops commonly
529 occur as a result of two to three flowering periods, which can result in symptomatic fruit
530 remaining on the trees during fruit set of the next crop, thus promoting disease
531 development (Kotzé, 1981; McOnie, 1964a). In South Africa, lemon fruit from commercial
532 orchards designated for export are managed to prevent the occurrence of overlapping
533 crops. Lemons are furthermore more exposed to pycnidiospore infections than other
534 citrus types since CBS lesions containing pycnidia are more prevalent in lemon leaves
535 attached to trees than in other citrus types, where infections mostly remain latent while
536 leaves are still attached to the tree (Kiely, 1948; Kotzé, 1981). The pycnidiospore
537 inoculum causing fruit infections in the two investigated lemon orchards might thus have
538 been from the previous season's lemon crop, leaf lesions, dead twigs and infected living
539 branches on which pycnidia can form once they die.

540 The widespread occurrence of *P. citricarpa* clones over large distances (150 to 400
541 m) within each of the citrus orchards was unexpected given the reported short-distance
542 dispersal of pycnidiospores. The most likely cause for this phenomenon is that the clones
543 were introduced on the nursery plants during orchard establishment. This seems feasible

since Kiely (1948) reported that green leaves of nursery trees in Australia contained abundant latent *P. citricarpa* infections. No records could be obtained regarding the origin or health status of the trees used to establish the two investigated lemon orchards. Another explanation for the wide-spread occurrence of the predominant MLGs within each orchard could have been that the clones dispersed slowly in a step-wise manner over several years. This, however, seems less likely considering that *P. citricarpa* pycnidiospores are dispersed over relatively short distances (Kiely, 1948; Spósito et al., 2011). In Brazil, pycnidiospores from dead twigs or infected fruits placed in a disease-free orchard were dispersed at distances less than 80 cm, mainly downward and to a lesser extent laterally (Spósito et al., 2011). In Australia, pycnidiospores were also reported to have a short dispersal distance since spores were rarely trapped between trees and not at all around the border of the orchard (Kiely, 1948). However, in regions with frequent storms such as Florida, pycnidiospores have been reported to contribute to the dispersal of CBS across tree rows (6.7 m), most likely through wind-blown twigs and branches (with or without fruit) (Hendricks et al., 2017). The disease was, furthermore, estimated to expand at a rate of only 50 m over three seasons in one of the investigated Floridian orchards (Hendricks et al., 2017). In Florida, only one clonal mating type is present and the disease thus has to persist and spread as pycnidiospores (Wang et al., 2016). In our two study orchards, strong winds, in general, did not occur as is evident from the low maximum wind speeds (0.90 to 3.41 m/s) recorded during the 2-year study. High wind speeds of 10 to 20 m/s are predicted to only occur in these regions every 50 years (Kruger et al., 2013). However, even if such strong winds did occur once during the life span of our study orchards, step-wise dispersal of the dominant MLGs in

the orchards at distances of 150 and 400 m over the life-span of the North West (15-years old) and Mpumalanga (9-years olds) orchards respectively, seems unlikely.

The spanning network analysis suggested that some of the predominant MLGs were related to each other within an orchard. In each orchard, at least two of the predominant MLGs within an orchard might have evolved from each other because they are separated by only five closely related MLGs. These predominant clones apparently have fitness attributes that allow them to adapt well to local conditions, thus enabling survival and persistence over space and time. The relatedness of these MLGs will have to be confirmed through whole genome dataset analysis or some other across genome genotyping because similar SSR MLGs may have arisen independently (homoplasy) due to the occurrence of sexual recombination and low allele diversity in the populations. It is furthermore also likely that some of our SSR markers might be highly conserved and misrepresent some diversity across the genome. Consequently, comparisons between the relatedness and sharing of MLGs between the two orchards will have to be made with caution.

MLG-17 was interesting since it was the only MLG that had large numbers in each orchard (11 isolates in North West and 51 isolates in Mpumalanga). All other multi-member MLGs that were shared between the orchards dominated in only one orchard. There are several hypotheses to explain the sharing of the same MLG between the two orchards. First, each could have arisen independently through sexual reproduction in each orchard followed by clonal reproduction through conidial sporulation. Second, each could have come from a common source population, such as nursery material, and were then replicated asexually in each orchard. Third, there could be some type of gene flow

between the two orchards although this seems unlikely to have occurred given the distance between the orchards and no other apparent links.

Similar to previous studies on *P. citricarpa* populations in Brazil, South Africa, Australia and China, where both mating types occur (Carstens et al., 2017), our study also found that the pathogen is likely reproducing sexually. In both orchards, sexual reproduction was supported by equal ratios of mating types. Linkage disequilibrium analyses (I_A and $\bar{r}_d$) of clone corrected data sets further supported the occurrence of random mating in all of the populations and subpopulations. Future studies should also investigate the likelihood of sexual reproduction occurring by determining the frequency and quantity of *P. citricarpa* ascospores released from leaf litter and how these relate to the severity of fruit infections, as was recently conducted in Australia (Tran et al., 2020).

Phyllosticta citricarpa populations in lemon orchards in South Africa thus have several of the hallmarks of a pathogen that has a high evolutionary potential. These include a mixed reproduction system, a potential for genotype flow (latently infected nursery trees, planting of infected reset trees, or top-working with infected scion material) and a large effective population size (MacDonald and Linde, 2002). The latter is achieved through the production of pseudothecia in leaf litter as well as pycnidia in various plant materials (old branches, leaves, twigs and fruit). The extent of pycnidia production in orchards is difficult to assess since the pathogen can occur latently as an endophyte in most of these materials (De Oliveria Silva et al., 2017; Kiely, 1948; Kotze, 1981).

We found that neither distance within an orchard nor season was a reliable predictor of population sub-structuring. In the North West orchard, where subpopulations were separated by relatively short distances of 50 to 150 m, subpopulations were almost

613 always not significantly genetically differentiated (*PhiPt* = 0.000 to 0.058; *P* = 0.052 to
614 0.462). The only exception was for the two subpopulations in 2012 (*PhiPt* = 0.009), but
615 not 2013, that were separated by 150 m. In the Mpumalanga orchard, where
616 subpopulations were separated by larger distances (200 m to 400 m), populations were
617 mostly significantly differentiated (*PhiPT* = 0.117; *P* = 0.018), but sometimes also non-
618 significantly (*PhiPT* = 0.000; *P* = 0.338 and 0.403) irrespective of distance. We had
619 expected that a distance of more than 50 m would result in population sub-structuring
620 since wind-born ascospores do not disperse further than 25 m (Spósito et al., 2011).
621 Ascospores of *Venturia inaequalis,* an ascomycete pathogen causing apple scab that also
622 produces ascospores in leaf litter, also disperses its ascospores over a relatively short
623 effective distance of up to 33 m from the source (Holb et al., 2004). The finding that
624 distance was not a reliable predictor of population sub-structuring could be due to the
625 occurrence of the same multimember MLGs at different spatial locations within an orchard
626 or gene flow. Gene flow, however, as previously discussed is less likely to have occurred
627 at a spatial level. At the seasonal level, the persistence of several MLGs between seasons
628 within each of the orchards (28 and 11 MLGs in the North West and Mpumalanga
629 orchards respectively), and gene flow, likely contributed towards genetically
630 undifferentiated seasonal populations. However, it should be noted that most of these
631 MLGs were predicted to be derived from sexual reproduction. Therefore, the genetic
632 differentiation analysis results (spatial and seasonal) may have been influenced by the
633 fact that many multimember MLGs were predicted to be derived via sexual reproduction
634 (based on P_{sex} analysis), yet the genetic differentiation analyses assumed them to be
635 clonal.

In summary, the current study, using a spatial and temporal genetic analysis of *Phyllosticta citricarpa* in two lemon orchards in South Africa, demonstrated that asexual reproduction and clonal populations, within sexually reproducing populations, play a role in CBS disease development and fruit infections. Since CBS epidemiology is expected to be different in lemon orchards, further studies are required to gain a better understanding of the role that pycnidiospores play in the epidemiology of CBS in orchards of other citrus types in South Africa. Our data suggesting that most of the MLGs likely resulted from sexual events supported the fact that sexual reproduction and ascospores likely played a role in disease development, but the extent and frequency at which this occurred could not be deduced. The known important role of ascospores in CBS development (Kotze, 1981) relative to the role of pycnidiospores should, therefore, be determined since it could be changing as a result of climate change effects on the formation and production of pseudothecia and asci. Future studies should focus on determining the extent and frequency of sexual and asexual reproduction within orchards by collecting *P. citricarpa* ascospore trap data combined with a structured sampling of fruit lesions for population genetic analysis. It will also be important to conduct investigations into lemon orchards that are well managed and that do not have overlapping fruit crops, which are more typical of commercial orchards where fruit are destined for the export market. The findings of these studies may have implications for management strategies. Current management practices are largely focused on ascospore dispersal during the fruit susceptibility periods and only on weather conditions conducive for their formation, release and infection. Consequently, fungicide applications are applied during the period of fruit susceptibility, but are mainly targeted to when ascospores will be released from leaf litter. Should

659 pycnidiospores be a significant source of inoculum, additional fungicide sprays might be
660 required to improve the control of fruit infections.
661
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669
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TABLE 1. Gene diversity and number of alleles of 15 microsatellite loci across time (between 2012 and 2013 seasons) and space (within orchard, between subpopulations) between clone corrected *Phyllosticta citricarpa* populations in two lemon orchards in the North West and Mpumalanga provinces of South Africa.

		# Alleles														
Province	**Nr. Isolates**	***Pc* 117**	***Pc* 179.1**	***Pc* 236**	***Pc* 440**	***Pc* 849**	***Pc* 1007**	***Pc* 2073**	***Pc* 3011**	***Pc* 12**	***Pc* 19**	***Pc* 20**	***Pc* 32**	***Pc* 37**	***Pc* 6**	***Pc* 7**
North West																
2012																
Subpopulation 1	52	4	2	1	4	1	2	2	2	1	2	1	1	2	3	2
Subpopulation 2	50	3	2	1	3	1	2	2	2	1	2	1	1	2	3	2
Subpopulation 3	50	4	1	1	2	1	3	2	2	1	2	1	1	2	3	2
Subpopulation 4	48	3	1	1	2	1	2	2	2	1	1	1	1	1	3	2
Seasonal	**200**	**5**	**2**	**1**	**3**	**1**	**3**	**2**	**2**	**1**	**2**	**1**	**1**	**2**	**3**	**2**
2013																
Subpopulation 1	41	3	2	1	2	1	2	2	2	1	2	1	1	1	3	2
Subpopulation 2	46	4	1	1	2	1	2	2	2	1	2	1	1	1	3	2
Subpopulation 3	46	3	1	1	3	1	2	2	2	1	2	1	1	1	3	2
Subpopulation 4	40	2	1	1	2	1	2	2	2	1	2	1	1	1	2	2
Seasonal	**173**	**5**	**2**	**1**	**3**	**1**	**2**	**2**	**2**	**1**	**2**	**1**	**1**	**1**	**3**	**2**
Mpumalanga																
2012																
Subpopulation 1	41	5	1	1	3	1	2	2	2	1	2	1	1	2	3	1
Subpopulation 2	45	4	2	2	2	1	2	2	2	2	1	1	1	2	3	2
Subpopulation 3	44	5	3	2	3	2	2	2	2	1	2	1	1	2	3	2
Seasonal	**130**	**8**	**3**	**3**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**1**	**1**	**2**	**3**	**2**
2013																
Subpopulation 1	32	6	2	1	3	1	2	2	2	1	2	1	1	2	2	1
Subpopulation 2	32	5	2	1	3	1	2	2	2	1	1	1	1	2	2	1
Subpopulation 3	32	3	2	1	2	1	2	2	2	2	2	1	1	2	2	1
Seasonal	**96**	**7**	**2**	**1**	**3**	**1**	**2**	**2**	**2**	**2**	**2**	**1**	**1**	**2**	**2**	**1**

TABLE 2. Genetic diversity indices for populations of *Phyllosticta citricarpa* in two lemon orchards in the North West and Mpumalanga provinces of South Africa.

Province/Year	Nr. Isolates	Nr. MLGs[a]	*eMLG*[b]	SE[b]	*E5*[c]	*Ne*[d]	H_e[e]	*SE*[e]
North West								
2012								
Subpopulation 1	52	26	19.4	1.587	0.768	1.34	0.200	0.053
Subpopulation 2	50	25	19.3	1.524	0.832	1.37	0.212	0.055
Subpopulation 3	50	24	17.9	1.578	0.682	1.42	0.227	0.058
Subpopulation 4	48	22	17.4	1.443	0.751	1.36	0.183	0.060
Seasonal	**200**	**60**	**39.5**	**2.813**	**0.587**	**1.40**	**0.221**	**0.027**
2013								
Subpopulation 1	41	22	19.1	1.205	0.786	1.36	0.201	0.055
Subpopulation 2	46	23	17.9	1.486	0.718	1.38	0.185	0.062
Subpopulation 3	46	23	18.5	1.433	0.789	1.33	0.183	0.055
Subpopulation 4	40	20	17.5	1.167	0.768	1.29	0.180	0.049
Seasonal	**173**	**49**	**35.6**	**2.481**	**0.638**	**1.37**	**0.203**	**0.027**
Mpumalanga								
2012								
Subpopulation 1	41	28	22.9	1.295	0.733	1.41	0.223	0.060
Subpopulation 2	45	20	16.2	1.364	0.677	1.34	0.216	0.044
Subpopulation 3	44	25	20.1	1.414	0.728	1.36	0.227	0.041
Seasonal	**130**	**55**	**45.1**	**2.230**	**0.533**	**1.37**	**0.238**	**0.028**
2013								
Subpopulation 1	32	16	16.0	0.000	0.721	1.38	0.178	0.059
Subpopulation 2	32	17	17.0	0.000	0.558	1.36	0.188	0.061
Subpopulation 3	32	16	16.0	0.000	0.579	1.39	0.234	0.053
Seasonal	**96**	**39**	**39.0**	**0.000**	**0.425**	**1.38**	**0.212**	**0.033**

[a] MLGs = Multilocus genotypes
[b] *eMLG* = expected number of MLGs after rarefaction, SE = Standard error based on *eMLG*
[c] *E5* = Evenness
[d] *Ne* = effective number of alleles
[e] H_e = Nei's gene diversity, (Nei, 1973)
SE = standard error of H_e

TABLE 3. Probabilities of multilocus genotypes (MLGs) of *Phyllosticta citricarpa* (p_{gen}) and their reoccurrence resulting from distinct sexual events (p_{sex}) within each of two lemon orchard in the North West and Mpumalanga provinces of South Africa.

North West orchard						Mpumalanga orchard					
Repeated MLGs[a]	Nr. Isolates[a] 2012	2013	p_{gen}[c]	p_{sex}[d]	*P* value for p_{sex}[e]	Repeated MLGs[a]	Nr. Isolates[a] 2012	2013	p_{gen}[c]	p_{sex}[d]	*P*-value for p_{sex}[e]
MLG-2	0	2	0.0023	0.2131	0.4283	MLG-1	2	0	0.0056	0.0547	0.2267
MLG-3	4	5	0.0034	7.43E-06	**0.0001**	MLG-5	0	5	0.0098	0.0731	0.2573
MLG-6	17	11	0.0247	3.02E-07	**0.0002**	MLG-6	1	1	0.0331	0.9956	0.9988
MLG-7	23	22	0.0367	1.69E-11	**0.0000**	MLG-7	2	0	0.0021	0.0835	0.2880
MLG-8	5	6	0.0180	0.0771	0.2063	MLG-16	2	0	0.0092	0.6183	0.8468
MLG-9	9	6	0.0267	0.0784	0.2066	**MLG-17**	22	29	0.0546	0.000	**0.0000**
MLG-10	1	1	0.0034	0.3581	0.6122	MLG-20	2	1	0.0090	0.3307	0.6342
MLG-11	2	1	0.0050	0.5573	0.7705	**MLG-21**	2	0	0.0001	0.0003	**0.0070**
MLG-12	2	1	0.0073	0.5148	0.7382	**MLG-23**	9	6	0.0124	2.05E-07	**0.0000**
MLG-13	1	1	0.0109	0.9136	0.9624	MLG-24	3	3	0.0068	0.0050	0.04519
MLG-14	3	1	0.0079	0.3415	0.5922	MLG-26	3	0	0.0013	0.0036	0.0353
MLG-15	2	0	0.0015	0.0195	0.0735	**MLG-34**	7	0	0.0058	0.0004	**0.0080**
MLG-17	5	6	0.0156	0.0349	0.1134	MLG-36	2	0	0.0102	0.6706	0.8882
MLG-18	5	5	0.0232	0.3675	0.6283	**MLG-37**	13	11	0.0343	1.20E-06	**3.25E-05**
MLG-19	7	3	0.0114	0.1114	0.0485	MLG-41	2	1	0.0056	0.1340	0.3852
MLG-22	3	0	0.0013	0.0815	0.2126	MLG-43	2	0	0.0006	0.0097	0.07243
MLG-25	15	11	0.0273	1.65E-05	**0.0002**	MLG-44	2	1	0.0032	0.0377	0.1785
MLG-26	24	21	0.0405	1.09E-10	**0.0000**	MLG-45	0	0	0.0013	0.0360	0.1739
MLG-27	1	7	0.0198	0.4602	0.6886	MLG-47	0	3	0.0120	0.5132	0.7973
MLG-28	13	14	0.0294	0.0004	**0.0029**	MLG-49	3	2	0.0148	0.2436	0.5302
MLG-29	0	3	0.0055	0.3391	0.5880	MLG-52	2	0	0.0076	0.5103	07838
MLG-30	2	0	0.0010	0.0552	0.1621	**MLG-55**	3	0	0.0002	1.24E-05	**0.0005**
MLG-31	1	1	0.0022	0.2051	0.4201	MLG-56	9	3	0.0236	0.0079	0.0641
MLG-32	0	2	0.0016	0.1251	0.2980	MLG-58	0	2	0.0022	0.0907	0.3031
MLG-33	2	0	0.0119	0.9386	0.9770	MLG-59	3	2	0.0053	0.0077	0.0616
MLG-35	0	2	0.0024	0.2243	0.4437						
MLG-37	2	1	0.0172	0.9559	0.9836						
MLG-38	2	9	0.0256	0.3600	0.6161						
MLG-39	2	4	0.0126	0.3273	0.5761						
MLG-40	0	2	0.0186	0.9927	0.9973						
MLG-42	2	1	0.0035	0.1431	0.3391						
MLG-46	2	4	0.0063	0.0321	0.1070						
MLG-48	3	1	0.0047	0.0992	0.2492						
MLG-50	3	3	0.0069	0.0475	0.1419						
MLG-51	3	2	0.0050	0.0422	0.1310						
MLG-53	3	0	0.0044	0.2260	0.4464						
MLG-54	1	1	0.0032	0.3340	0.5821						
MLG-57	3	1	0.0026	0.0174	0.6699						

[a] Fifteen microsatellite markers were used to determine a unique multilocus genotype (MLG). Members or clones of the same MLG were considered as those having identical alleles at 15 microsatellite loci. MLGs indicated in bold had a significant *P*-value (< 0.01) for p_{sex}, thus indicating an asexual and clonal origin.

[b] Number of isolates belonging to each MLG in two different seasons (2012 and 2013).

[c] Probability of the MLG occurring in the population and is calculated as the product of the allele frequencies.

[d] Probability of obtaining the observed numbers of identical genotypes in the population as a result of random mating.

TABLE 4. Mating type composition of *Phyllosticta citricarpa* multilocus genotypes (MLGs) that were identified as being derived asexually based on P_{sex} analysis.

	North West orchard		Mpumalanga orchard		
MLG number [a]	**Mating type**	**Total number of isolates belonging to each MLG (number of isolates tested for mating type)[b]**	**MLG number [a]**	**Mating type**	**Total number of isolates belonging to each MLG (number of isolates tested for mating type) [b]**
MLG-3	*MAT1-2*	9 (2)	MLG-17	*MAT1-1*	51 (23)
MLG-6	*MAT1-2*	28 (7)	MLG-21	*MAT1-1*	2 (1)
MLG-7	*MAT1-2*	44 (5)	MLG-23	*MAT1-1*	15 (7)
MLG-25	*MAT1-2*	26 (13)	MLG-34	*MAT1-2*	7 (2)
MLG-26	*MAT1-1*	45 (22)	MLG-37	*MAT1-1*	24 (7)
MLG-28	*MAT1-2*	24 (12)	MLG-55	*MAT-1-2*	3 (1)
Total		**176 (61)**			**102 (41)**

[a] Members or clones of the same MLG were considered as those having identical alleles at 15 microsatellite loci.

[b] For each MLG, a subset of isolates belonging to a MLG was analysed for mating type since only some of the isolates, or their DNA, were still viable or amplifiable at the time of re-testing for mating type.

TABLE 5. Occurrence of *Phyllosticta citricarpa* multilocus genotypes (MLGs) with a likely clonal origin at different sampling sites within two lemon orchards in the North-West and Mpumalanga provinces in South Africa during the 2012 and 2013 seasons.

Orchard, site and season[a]	**Nr. of isolates within each MLG (% isolates)**[b]					
North West	**MLG-3**	**MLG-6***	**MLG-7***	**MLG-25***	**MLG-26***	**MLG-28***
Subpopulation 1						
2012 (52)	3 (5.8%)	3 (5.8%)	7 (3.5%)	5 (9.6%)	7 (13.5%)	2 (3.8%)
2013 (41)	3 (7.3%)	0 (0.0%)	3 (7.3%)	3 (7.3%)	7 (17.1%)	2 (4.9%)
Subpopulation 2						
2012 (50)	1 (2.0%)	5 (10.0%)	1 (2.0%)	5 (10.0%)	4 (8.0%)	4 (8.0%)
2013 (46)	0 (0.0%)	6 (13.0%)	8 (17.4%)	2 (4.3%)	3 (6.5%)	5 (10.9%)
Subpopulation 3						
2012 (48)	1 (2.0%)	3 (6.0%)	10 (20.0%)	3 (6.0%)	5 (10.0%)	5 (10.0%)
2013 (40)	0 (0.0%)	4 (10.0%)	7 (17.5%)	1 (2.5%)	4 (10.0%)	2 (5.0%)
Subpopulation 4						
2012 (48)	0 (0.0%)	6 (12.5%)	5 (10.4%)	2 (4.2%)	8 (16.7%)	2 (4.2%)
2013 (40)	1 (2.5%)	1 (2.5%)	4 (10.0%)	5 (12.5%)	7 (17.5%)	2 (5.0%)
Total 2012 (200)	**5 (2.5%)**	**17 (8.5%)**	**23 (11.5%)**	**15 (7.5%)**	**24 (12.0%)**	**13 (6.5%)**
Total 2013 (173)	**4 (2.3%)**	**11 (6.4%)**	**22 (12.7%)**	**11 (6.4%)**	**21 (12.1%)**	**11 (6.4%)**
Total 2012 + 2013 (372)	**9 (2.4%)**	**28 (7.5%)**	**45 (12.1%)**	**26 (7.0%)**	**45 (12.1%)**	**24 (6.4%)**
Mpumalanga	**MLG-17***	**MLG-21**	**MLG-23***	**MLG-34**	**MLG-37***	**MLG-55**
Subpopulation 1						
2012 (41)	10 (24.0%)	2 (4.9%)	3 (7.3%)	1 (2.4%)	3 (7.3%)	0 (0.0%)
2013 (32)	11 (34.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (6.3%)	0 (0.0%)
Subpopulation 2						
2012 (45)	5 (11.1%)	0 (0.0%)	3 (6.7%)	0 (0.0%)	8 (17.8%)	0 (0.0%)
2013 (32)	7 (21.9%)	0 (0.0%)	3 (9.4%)	0 (0.0%)	6 (18.8%)	0 (0.0%)
Subpopulation 3						
2012 (44)	7 (15.9%)	0 (0.0%)	3 (6.8%)	6 (13.6%)	2 (4.5%)	3 (6.8%)
2013 (32)	11 (34.4%)	0 (0.0%)	3 (9.4%)	0 (0.0%)	3 (9.4%)	0 (0.0%)
Total 2012 (130)	**22 (17.0%)**	**2 (1.5%)**	**9 (6.9%)**	**7 (5.4%)**	**13 (10.0%)**	**3 (2.3%)**
Total 2013 (96)	**29 (30.2%)**	**0 (0.0%)**	**6 (6.3%)**	**0 (0.0%)**	**11 (11.5%)**	**0 (0.0%)**
Total 2012 + 2013 (226)	**51 (23.0%)**	**2 (0.9%)**	**15 (6.6%)**	**7 (3.1%)**	**24 (10.6%)**	**3 (1.3%)**

[a] The total number of isolates genotyped for each subpopulation is shown in brackets. For the two seasonal populations and total population (212+2013) the total number of isolates genotyped in each orchard is also shown in brackets.
[b] MLGs in the table were predicted to be the product of asexual reproduction based on P_{sex} analysis when populations from each orchard were analysed independently. The number of isolates from each MLG that were identified at each sampling sites is shown, followed in brackets for the subpopulations, the percentage that the MLG contributed to each specific subpopulation. For each seasonal population, and the total orchard population (2012+2013) the total number of isolates for each MLG is indicated followed by the percentage of isolates that the MLG contributed to the seasonal or total population. MLGs that were considered as being the predominant genotype in each orchard are indicated by an asterisk. The location of sampling sites within each orchard are shown in Figure 2.

TABLE 6. Linkage disequilibrium analyses for populations of *Phyllosticta citricarpa* across space and time within two lemon orchards in South Africa with corresponding significance level (*P*-value).

Province/Year	Non-clone-corrected populations				Clone-corrected populations			
	I_A	*P* value	$\bar{r}_d$	*P* value	I_A	*P* value	$\bar{r}_d$	*P* value
North West/2012								
Subpopulation 1	0.001	0.454	0.000	0.454	-0.349	0.982	-0.045	0.978
Subpopulation 2	0.038	0.318	0.005	0.317	-0.104	0.749	-0.012	0.749
Subpopulation 3	0.130	0.088	0.017	0.088	-0.329	0.983	-0.042	0.982
Subpopulation 4	0.015	0.387	0.003	0.388	-0.086	0.610	-0.015	0.609
Seasonal	**0.019**	**0.330**	**0.002**	**0.328**	**-0.214**	**0.999**	**-0.025**	**0.999**
North West/2013								
Subpopulation 1	-0.045	0.660	-0.006	0.662	-0.300	0.960	-0.038	0.957
Subpopulation 2	0.043	0.267	0.007	0.264	0.041	0.364	0.007	0.365
Subpopulation 3	-0.060	0.743	-0.009	0.745	-0.349	0.988	-0.051	0.987
Subpopulation 4	-0.109	0.872	-0.017	0.874	-0.248	0.795	-0.035	0.788
Seasonal	**-0.056**	**0.880**	**-0.008**	**0.884**	**-0.268**	**1.000**	**-0.035**	**1.000**
Mpumalanga/2012								
Subpopulation 1	0.150	0.069	0.019	0.068	-0.109	0.789	-0.016	0.789
Subpopulation 2	0.147	0.124	0.014	0.123	-0.255	0.922	-0.026	0.911
Subpopulation 3	-0.068	0.708	-0.007	0.709	-0.315	0.979	-0.029	0.978
Seasonal	**0.108**	**0.060**	**0.010**	**0.058**	**-0.162**	**0.974**	**-0.015**	**0.975**
Mpumalanga/2013								
Subpopulation 1	0.219	0.074	0.029	0.076	0.283	0.091	0.036	0.094
Subpopulation 2	0.210	0.062	0.032	0.062	-0.188	0.845	-0.027	0.838
Subpopulation 3	0.325	0.022	0.038	0.022	-0.199	0.808	-0.022	0.805
Seasonal	**0.233**	**0.007**	**0.028**	**0.007**	**-0.137**	**0.914**	**-0.016**	**0.914**

I_A = Index of association; $\bar{r}_d$ = Standardized index of association. Clones of the same MLG were considered as those isolates having identical SSR alleles at all loci. For isolates of MLGs that were identified as likely being the product of sexual reproduction, only one isolate from each MLG was included in the linkage disequilibrium.

TABLE 7. Analysis of molecular variance (AMOVA) partitioning *Phyllosticta citricarpa* SSR data (A) among and within seasonal (2012 and 2013) and orchard (North West and Mpumalanga) populations (i.e. total SSR data set) and (B) among orchards, using 15 simple sequence repeat loci.

Source	**df**	**SS**	**MS**	**Estimated variance**	**Percentage variance**	**AMOVA statistics**	***P***
(A) Orchards							
Among orchards	1	488.759	488.759	4.783	68%	*PhiRT* = 0.682	0.001
Among populations within orchards	12	66.665	5.555	0.250	4%	*PhiPR* = 0.112	0.001
Within populations	189	373.852	1.978	1.978	28%	*PhiPT* = 0.718	0.001
(B) Total SSR data set							
Among orchard**s** and seasonal populations	3	525.001	175.000	3.414	62%	*PhiRT* = 0.624	0.001
Among populations within orchards and seasons	10	30.424	3.042	0.075	1%	*PhiPR* = 0.037	0.039
Within populations	189	373.852	1.978	1.978	36%	*PhiPT* = 0.638	0.001
Within populations	189	373.852	1.978	1.978	28%	*PhiPT* = 0.718	0.001

P-value estimates are based on 999 permutations. df = degrees of freedom, SS = sum of squares, MS = mean squared deviations.

TABLE 8. Estimates of pairwise *PhiPT* values (below the diagonal) averaged over 15 simple sequence repeat loci of *Phyllosticta citricarpa* subpopulations from the North West (NW) and Mpumalanga (MP) provinces, for 2012 and 2013. Significance values indicated above the diagonal. [a]

Populations[b]	NW12_1	NW12_2	NW12_3	NW12_4	NW13_1	NW13_2	NW13_3	NW13_4	MP12_2	MP12_1	MP12_3	MP13_2	MP13_1	MP13_3
NW12_1		0.462	0.154	**0.009**	0.435	**0.034**	0.075	0.115	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW12_2	0.000		0.435	0.072	0.462	0.062	0.432	0.446	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW12_3	0.032	0.000		0.443	0.403	0.432	0.451	0.402	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW12_4	0.113	0.057	0.005		0.161	0.429	0.241	0.220	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW13_1	0.000	0.000	0.000	0.036		0.089	0.430	0.480	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW13_2	0.075	0.048	0.000	0.000	0.050		0.052	0.107	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW13_3	0.047	0.000	0.000	0.029	0.000	0.058		0.437	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
NW13_4	0.058	0.000	0.000	0.025	0.000	0.070	0.000		**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
MP12_2	0.717	0.725	0.709	0.716	0.720	0.723	0.733	0.713		**0.035**	0.388	**0.001**	**0.001**	**0.001**
MP12_1	0.721	0.726	0.714	0.718	0.723	0.724	0.734	0.718	0.068		**0.006**	**0.001**	**0.001**	**0.001**
MP12_3	0.697	0.708	0.692	0.695	0.700	0.702	0.712	0.692	0.000	0.090		**0.001**	**0.001**	**0.001**
MP13_2	0.704	0.711	0.692	0.699	0.703	0.709	0.717	0.684	0.278	0.208	0.285		**0.018**	0.403
MP13_1	0.725	0.731	0.715	0.726	0.726	0.733	0.738	0.717	0.308	0.295	0.296	0.117		**0.045**
MP13_3	0.703	0.711	0.690	0.698	0.702	0.709	0.717	0.682	0.177	0.194	0.194	0.000	0.082	

[a] *P*-values indicated in bold were significant ($P \leq 0.05$).

[a] Population names consists of the abbreviation of the province, followed by the year of sampling (e.g. 12 for 2012 and 13 for 2013), followed by the subpopulation number after the underscore.

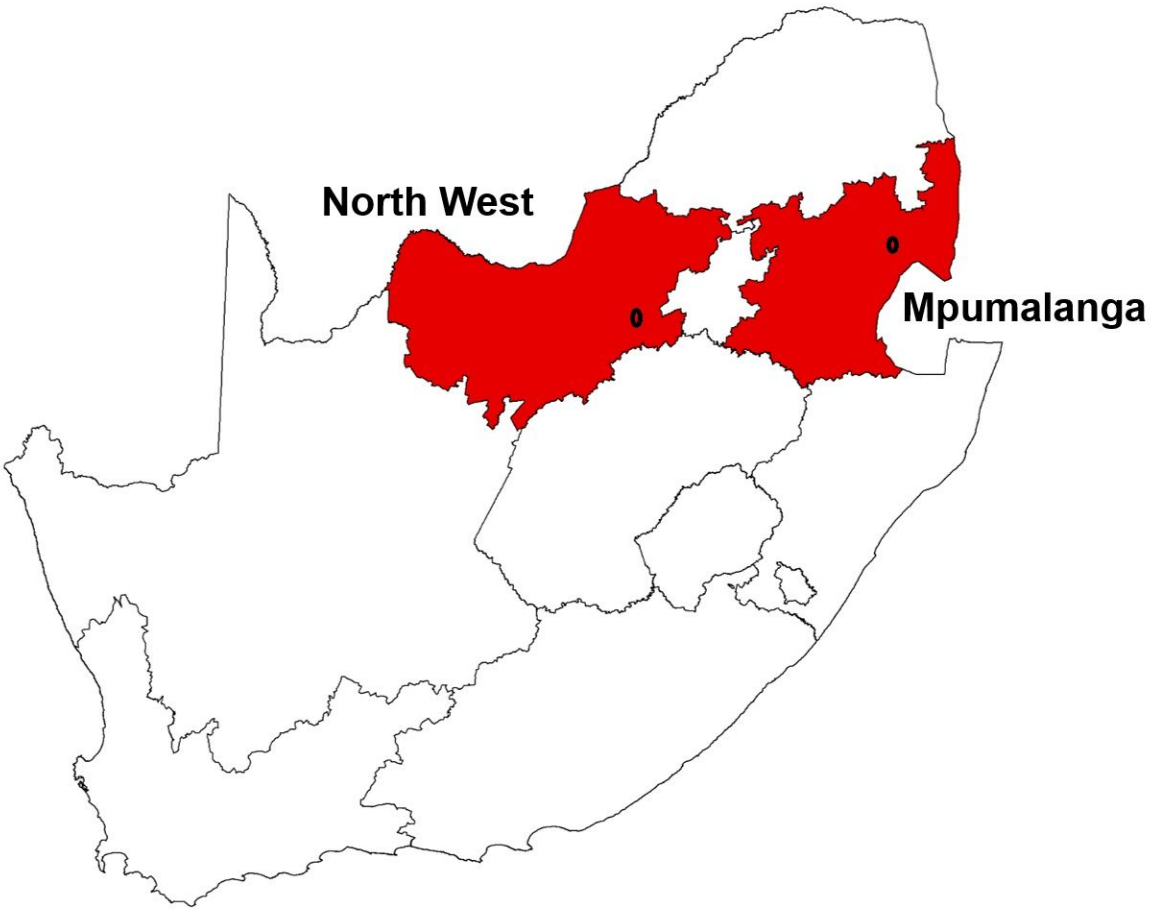


Fig. 1. Location of two orchards (black dots) in the Mpumalanga and North West provinces in South Africa where the population structure of *Phyllosticta citricarpa* was studied at the orchard spatial- and temporal scales.

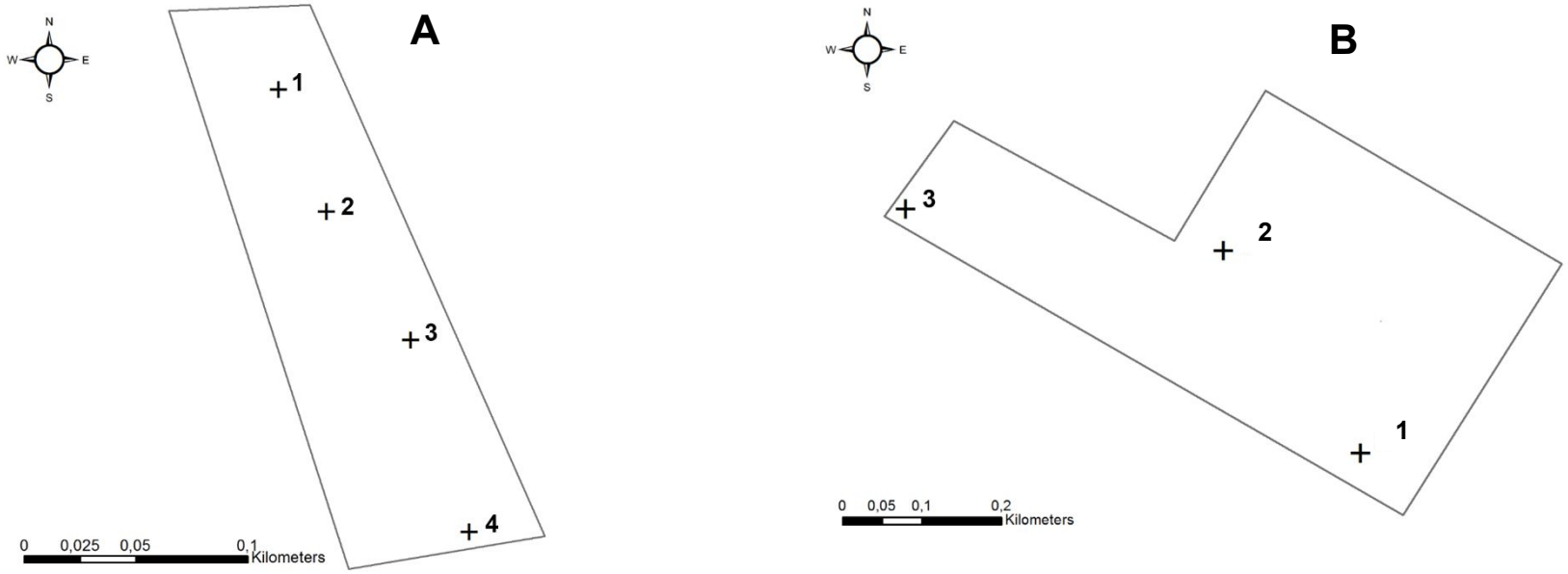


Fig. 2. Sampling strategy used for studying the population structure of *Phyllosticta citricarpa* at the spatial- and temporal scales in two lemon orchards situated in the (A) North West and (B) Mpumalanga provinces in South Africa. In each orchard, the selected sampling sites are indicated by "+" along with the *P. citricarpa* subpopulation numbers. At each of the sampling sites, five trees located in a cross over three rows were selected, from which 10 citrus black spot fruits were randomly sampled for subsequent *P. citricarpa* isolation and genotyping.

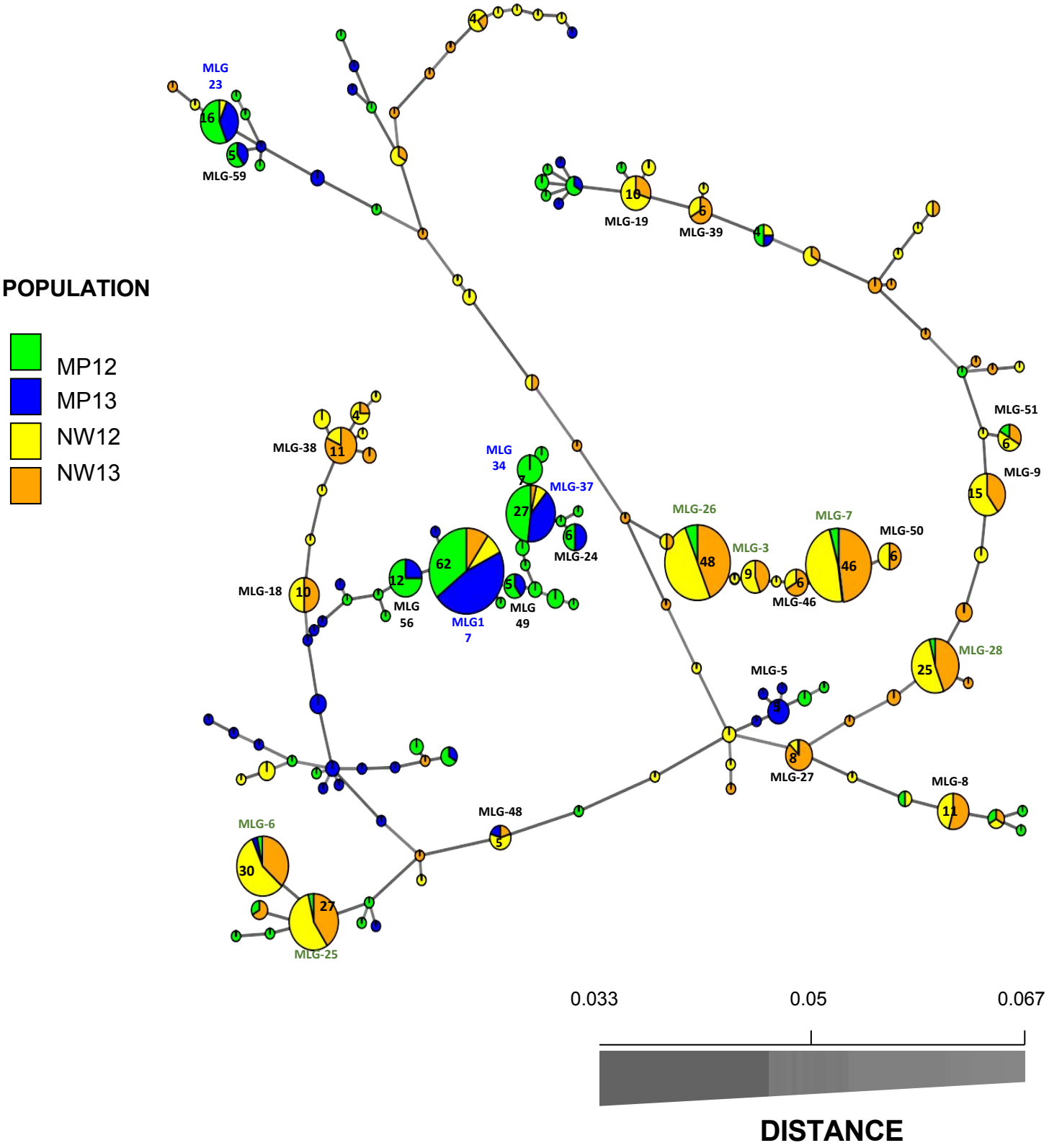
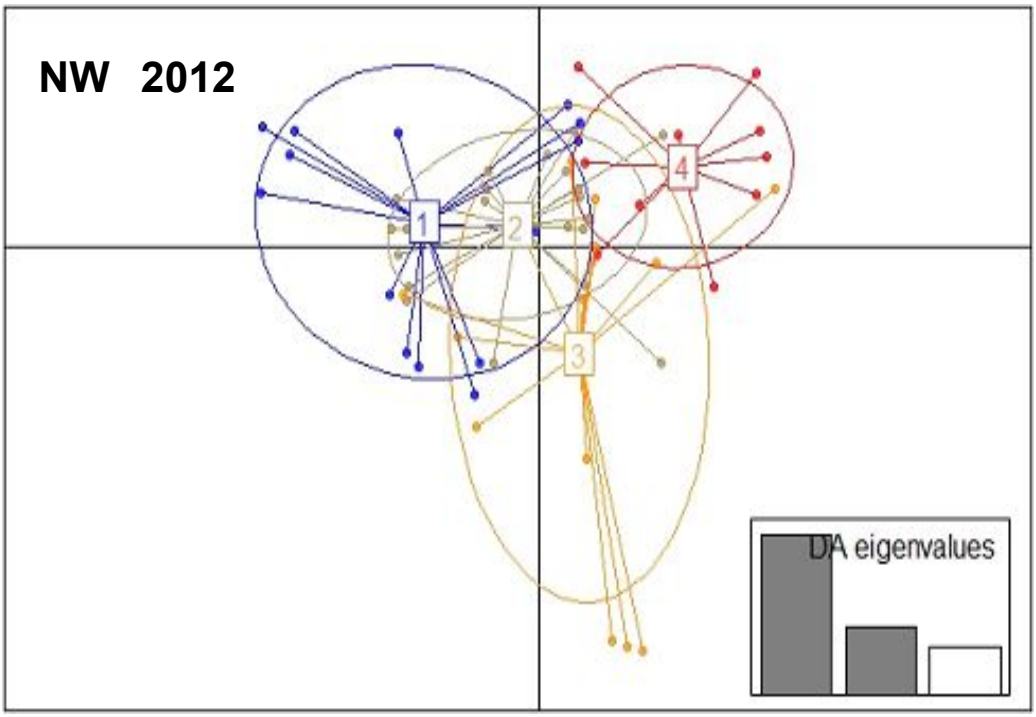


Fig. 3. Minimum spanning network showing the relationship among individual simple sequence repeat multilocus genotypes (MLGs) of *Phyllosticta citricarpa* observed in isolates collected from two citrus orchards (Mpumalanga [MP]) and North West [NW]) in South Africa over two years (2012 and 2013). Each node represents a different MLG. Node sizes and colours correspond to the number of individuals within a MLG and membership, respectively. MLG names (MLG-3 to MLG-51) are indicated for MLGs that occurred five or more times. MLG names in blue and orange were likely replicated asexually (clonal) based on P_{sex} analysis in the MP and NW orchards respectively (Table 3). Edge shading is proportional to Bruvo's distance, with the shading scale displayed in the inset.



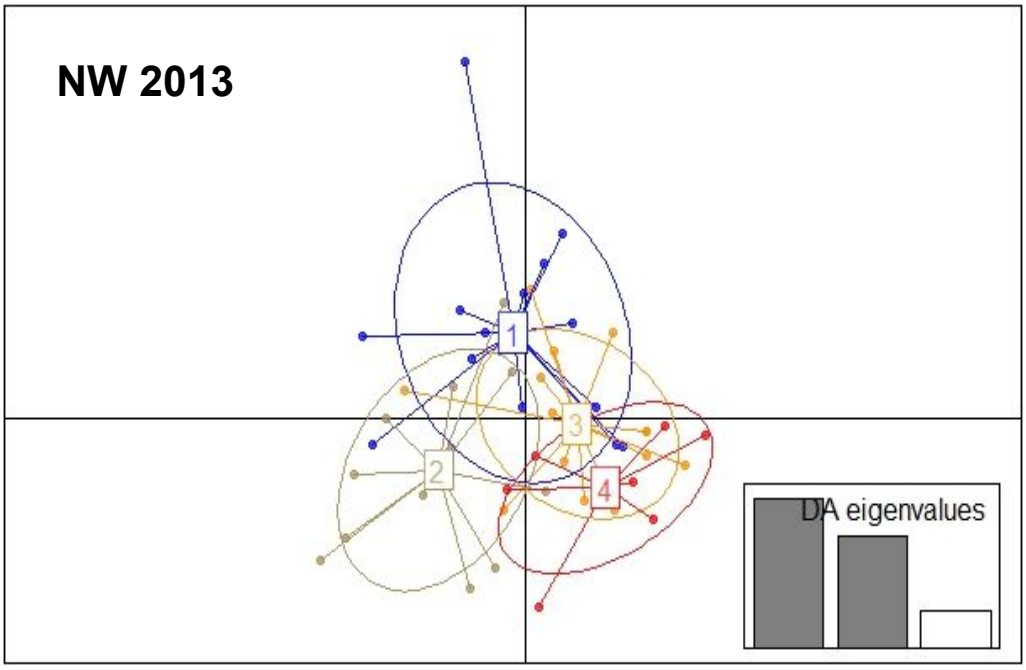


Fig. 4. Discriminant analysis of principal components (DAPC) of *Phyllosticta citricarpa* subpopulations (clone corrected) sampled from a lemon orchard in the North West province in 2012 and 2013. Subpopulations are indicted by numbers and different colours. The eigenvalues represented 80% of the variation in both orchards and seasons.

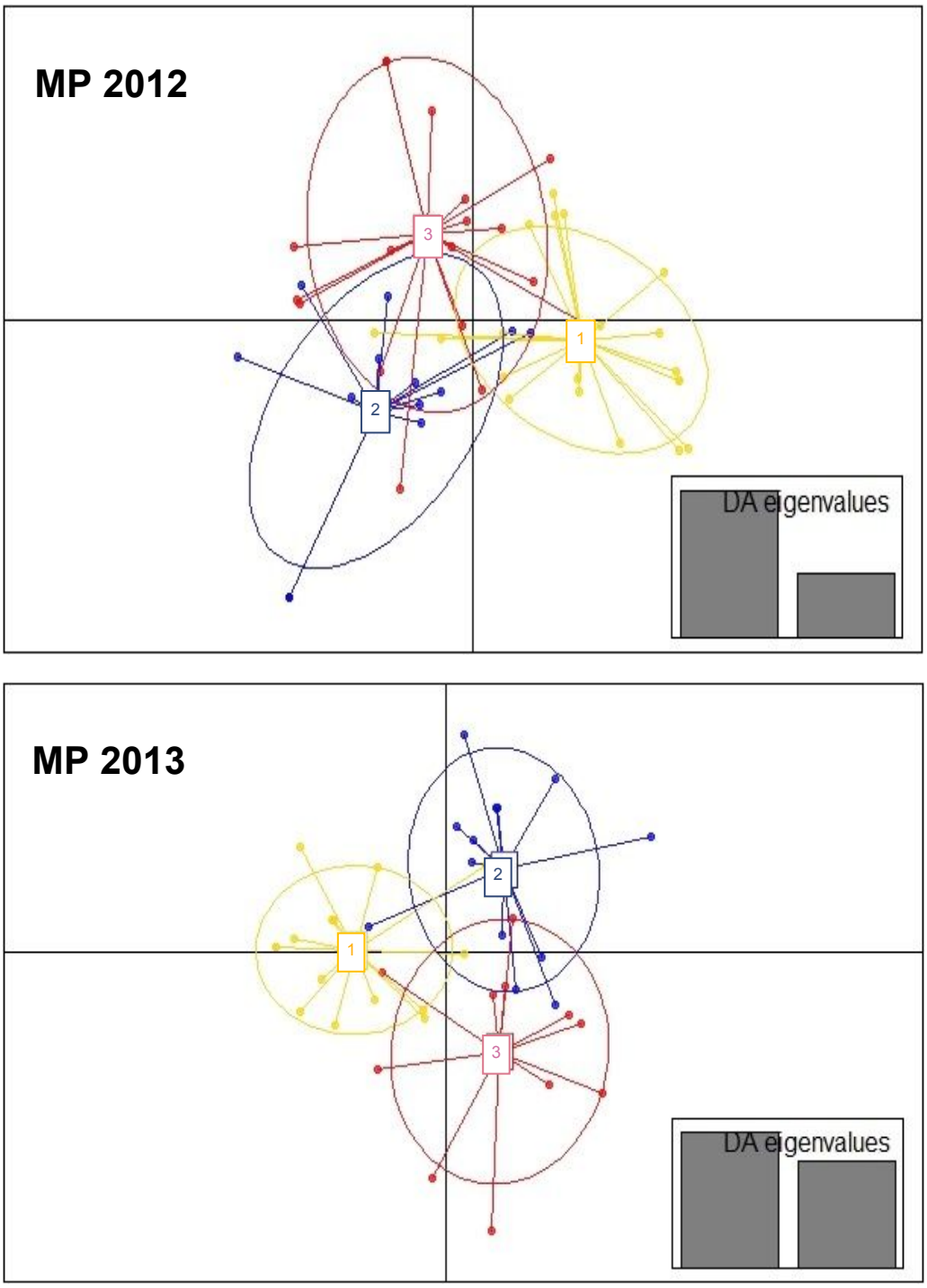


Fig. 5. Discriminant analysis of principal components (DAPC) of *Phyllosticta citricarpa* subpopulations (clone corrected) sampled from a lemon orchard in the Mpumalanga province in 2012 and 2013. Subpopulations are indicted by numbers and different colours. The eigenvalues represented 80% of the variation in both orchards and seasons.

Supplementary Table 1. Mating type composition and χ^2 test for expected 1:1 mating-type ratios in clone corrected *Phyllosticta citricarpa* populations collected in the 2012 and 2013 seasons from a lemon orchard in the North West and Mpumalanga provinces of South Africa.

Province and Year	Number of A1 mating type isolates	Number of A2 mating type isolates	Number of MLGs[a]	χ^2 value	*P*-value
North West					
2012	26	34	60	1.07	0.300
2013	21	28	49	1.00	0.300
Mpumalanga					
2012	27	28	55	0.02	0.900
2013	23	16	39	1.27	0.200

[a] The total number of multilocus genotypes (MLG) per population in clone corrected *Phyllosticta citricarpa* populations.

Supplementary Table 2. Analysis of molecular variance (AMOVA) of *Phyllosticta citricarpa* populations between two seasons (2011/12 and 2012/13) for each of the two lemon orchards (North West and Mpumalanga) using 15 simple sequence repeat loci.

Source	d.f.	SS	% Variation	AMOVA statistics	*P*
North West					
Among populations	1	1.149	0		
Within populations	107	173.860	100	*PhiPT* = 0.000	0.732
Total	**108**	**175.009**	**100**		
Mpumalanga					
Among populations	1	3.959	3		
Within populations	92	159.967	97	*PhiPT* = 0.027	0.013
Total	**93**	**163.926**	**100**		

SUPPLEMENTARY TABLE 3. Analysis of molecular variance (AMOVA) of *Phyllosticta citricarpa* subpopulations per season in each of the two orchards (North West and Mpumalanga) using 15 simple sequence repeat loci.

Source	d.f.	SS	% Variation	AMOVA statistics	*P*
North West 2012					
Among subpopulations	3	6.519	2		
Within sub populations	56	93.614	98	*PhiPT* = 0.020	0.163
Total	**59**	**110.133**	**100**		
North West 2013					
Among subpopulations	3	5.339	1		
Within subpopulations	45	69.171	99	*PhiPT* = 0.013	0.284
Total	**48**	**74.510**	**100**		
Mpumalanga 2012					
Among subpopulations	2	8.597	5		
Within subpopulations	52	118.676	95	*PhiPT* = 0.047	0.021
Total	**54**	**127.273**	**100**		
Mpumalanga 2013					
Among subpopulations	2	9.969	7		
Within subpopulations	36	92.390	93	*PhiPT* = 0.068	0.059
Total	**38**	**102.359**	**100**		

SUPPLEMENTARY TABLE 4. Monthly weather conditions prevailing in two regions (North West and Mpumalanga) where *Phyllosticta citricarpa* populations were studied. Weather conditions are summarized according to the ascospore maturation period (July to September) and fruit infection period (October to February).

Weather parameter	Month	Ascospore maturation period (July to end September)				Susceptible fruit infection period (October to end Feb)			
		North West	North West	Mpumalanga	Mpumalanga	North West	North West	Mpumalanga	Mpumalanga
		2011/12	2012/13	2011/12	2012/13	2011/12	2012/13	2011/12	2012/13
Total Rainfall (mm)	July	1.52	0	9.14	0				
	August	0	0	9.14	0.76				
	September	0	39.62	55.12	124.72				
	October					61.98	126.75	117.35	168.89
	November					67.56	76.71	59.95	134.11
	December					129.29	145.8	217.16	201.17
	January					96.52	83.06	264.67	272.3
	February					52.58	44.2	105.91	174.49
Total		**1.52**	**39.62**	**73.40**	**125.48**	**407.93**	**476.52**	**765.04**	**950.96**
Average Maximum humidity (%)	July	90.24	83.37	86.38	84.99				
	August	85.77	75.51	88.08	79.02				
	September	79.45	84.68	83.18	89.66				
	October					84.29	88.3	90.91	90.23
	November					84.57	87.49	90.22	90.68
	December					91.18	94.23	90.78	91.32
	January					92.76	90.77	92.23	92.08
	February					90.31	92.24	91.3	92.51
Average maximum temperature (°C)	July	21.9	23.78	22.22	24.96				
	August	25.2	26.28	24.4	27.86				
	September	30.59	28	29.3	25.51				
	October					31.33	30.19	27.5	26.67
	November					32.58	31.77	28.91	26.98
	December					30.47	30.37	28.91	28.14
	January					32.67	32.46	28.83	28.95
	February					33.51	34.04	31.31	29.21
Average minimum temperature (°C)	July	-0.26	1.46	5.98	6.59				
	August	2.48	3.99	6.98	7.44				
	September	7.23	8.09	11.18	12.49				
	October					11.6	12.42	14.5	15.21
	November					14.39	14.44	16.75	16.12
	December					16.4	16.18	18.4	18.44

Weather parameter	Month	Ascospore maturation period (July to end September)				Susceptible fruit infection period (October to end Feb)			
		North West	North West	Mpumalanga	Mpumalanga	North West	North West	Mpumalanga	Mpumalanga
		2011/12	2012/13	2011/12	2012/13	2011/12	2012/13	2011/12	2012/13
	January					16.73	17.43	18.52	18.79
	February					17.02	15.87	19.59	18.54
Average wind speed (ms)	July	0.84	0.91	1.14	0.44				
	August	0.93	1.12	1.06	1.1				
	September	1.06	1.28	1.07	0.83				
	October					0.95	1.06	0.9	0.85
	November					0.94	1.01	0.83	0.96
	December					0.86	0.84	0.58	0.85
	January					0.93	0.84	0.69	0.8
	February					0.79	0.85	0.79	0.8

SUPPLEMENTARY TABLE 5**.** Maximum wind speed recorded in two regions (North West and Mpumalanga) where *Phyllosticta citricarpa* populations were studied.

Month	North West		Mpumalanga	
	2011/12	**2012/13**	**2011/12**	**2012/13**
July	2.66	3.06	1.63	0.93
August	1.96	3.41	1.60	2.97
September	2.17	2.44	1.67	1.90
October	1.96	2.04	1.33	1.31
November	1.64	1.53	1.74	1.44
December	1.59	1.55	0.90	1.79
January	1.83	1.52	1.10	1.83
February	1.16	1.20	1.67	1.43
March	1.97	1.30	1.40	1.36
April	1.16	1.87	1.63	1.46
May	1.15	1.33	1.16	1.25
June	2.79	1.71	1.39	1.33

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