Understanding the genotypic and phenotypic structure and impact of climate on *Phytophthora nicotianae* outbreaks on potato and tomato in the eastern US

Amanda C. Saville[1], Margaret T. McGrath[2], Chris Jones[3], John Polo[3], Jean B. Ristaino[1,4]

[1] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27606

[2] Long Island Horticultural Research and Extension Center, Cornell University, Riverhead, NY 11901

[3]Center for Geospatial Analytics, NC State University, Raleigh, NC 27695

[4] Emerging Plant Disease and Global Food Security Cluster, NC State University, Raleigh, NC 27606

Corresponding author: Jean Beagle Ristaino, E-mail address: jean_ristaino@ncsu.edu

ABSTRACT

Samples from potato fields with late blight-like symptoms were collected from eastern North Carolina in 2017 and the causal agent was identified as *Phytophthora nicotianae.* We have identified *P. nicotianae* in potato and tomato from North Carolina, Virginia, Maryland, Pennsylvania, and New York. Ninety-two field samples were collected from 46 fields and characterized for mefenoxam sensitivity, mating type, and SSR genotype using microsatellites. Thirty two percent of isolates were the A1 mating type, while 53% were A2 mating type. In six cases, both A1 and A2 mating type were detected in the same field in the same year. All isolates tested were sensitive to mefenoxam. Two genetic groups were discerned based on STRUCTURE analysis: one included samples from North Carolina and Maryland, and one included samples from all five states. The data suggest two different sources of inoculum from the field sites sampled. Multiple haplotypes within a field and the detection of both mating types in close proximity suggests that *P. nicotianae* may be reproducing sexually in North Carolina. There was a decrease in the average number of days with weather suitable for late blight, from 2012-2016 to 2017-2021 in all of the NC counties where *P. nicotianae* was reported. *Phytophthora nicotianae* is more thermotolerant than *P. infestans* and grows at higher temperatures (25-35°C) than *P. infestans* (18-22°C). Late blight outbreaks have decreased in recent years and first reports of disease are later, suggesting that the thermotolerant *P. nicotianae* may cause more disease as temperatures rise due to climate change.

INTRODUCTION

The oomycete *Phytophthora nicotianae* Breda de Haan 1896 is a cosmopolitan plant pathogen known to infect more than 255 genera of plants within 90 families (Cline et al. 2008). On tomatoes, it causes *Phytophthora* root and crown rot, as well as buckeye rot on fruit (Ristaino et al. 1988). The pathogen is soilborne and common in irrigated processing tomato fields in California (Ristaino et al. 1988). On potato, it causes a foliar blight and can infect tubers and cause a pink tuber rot (Taylor et al. 2008).

Foliar blight on potatoes caused by *P. nicotianae* can occur under warm, wet conditions and can be easily mistaken for foliar leaf infections caused by the late blight pathogen *P. infestans*, because the symptoms are similar (Taylor et al. 2008). However, there are several differences between the two *Phytophthora* species beyond symptoms. *Phytophthora nicotianae* can grow in vitro and infect potato and tomato at much higher temperatures of 25-35°C than *P. infestans,* which commonly grows and infects at 18-22°C (Taylor et al. 2008). In addition, differences in the disease cycle, mefenoxam sensitivity (Saville et al. 2015), and host range differ between the two *Phytophthora* species (**Table 1**).

The two *Phytophthora* species exhibit differences in their reproductive cycle. *Phytophthora infestans* predominately reproduces asexually with a series of clonal lineages occurring, and does not produce overwintering oospores in the US (Ristaino et al, 2018). In contrast, *P. nicotianae* reproduces sexually and can persist from season to season through the development of oospores and asexual chlamydospores (Gallup et al. 2017). *Phytophthora infestans* also sporulates abundantly on potato leaves, with sporangia visible on leaf surfaces (Fry et al. 2015). Sporangia are typically dispersed through aerial dispersal within fields and over kilometers. By contrast, *P. nicotianae* is normally soilborne and spreads via surface water, is locally splash dispersed, and sporulation occurs rarely on leaf lesions (Ristaino et al. 1988; Taylor et al. 2008). Subsequently, treatment methods for these two pathogens vary. *P. infestans* is typically treated through aerial sprays of fungicides, while treatment for *P. nicotianae* involves applications of fungicides via irrigation. Mefenoxam, a FRAC group 4 phenylamide fungicide, is a frequent chemical treatment for both pathogens, and mefenoxam resistant isolates have been identified for both species in the United States (Hu et al. 2008; Olson et al. 2013; Saville et al. 2015).

In 2017, we received reports from eastern North Carolina of potatoes exhibiting late blight-like symptoms, including large, water-soaked lesions on leaves and stems, and rapid decline (**Fig. 1**). However, after PCR testing DNA from field samples, we determined that disease in these samples was caused by *P. nicotianae*. Since 2017, multiple *P. nicotianae* outbreaks have been reported annually both from NC and other states along the eastern US, in regions that historically have reported late blight outbreaks. Many samples have been sent to our lab and concern has been raised among stakeholders about the disease. The climate has been wetter and hotter, and standing water in fields after tropical storm events in eastern NC has occurred. Prior to this, *P. nicotianae* had only been reported on potato from a limited number of states: Florida, Texas, Mississippi, and Delaware, and Nebraska (Taylor et al. 2008).

Coinciding with the increase in *P. nicotianae* reports, the number of reports of *P. infestans* in the eastern US has been declining. In 2017, 75 reports of *P. infestans* were recorded on the late blight tracking website USABlight (www.usablight.org), with outbreaks focused primarily in the northeastern US. By 2021, this number had dropped to 7. The increase in *P. nicotianae* reporting could also be partially attributed to climate change. For example, in 2018, no late blight was reported in Maine, one of the major seed potato production areas of the eastern

US. The lack of late blight was attributed to unusually dry weather (Steve Johnston, personal communication). The same year, the southeast experienced one of the warmest and wettest years on record, exacerbating *P. nicotianae* outbreaks (NOAA National Centers for Environmental Information 2019).

There is concern that increase in the prevalence of *P. nicotianae* and potential survival of the pathogen in soil may make it a recurring and important *Phytophthora* species on potato and tomato crops in the eastern US. Increased temperature and rainfall over the past few years may have resulted in conditions being less favorable for *P. infestans* than *P. nicotianae*, which is more heat tolerant. Increased rainfall means more opportunity for local splash dispersal of the pathogen from soil to stems and foliage of Solanaceous crops, thus allowing the normally soilborne sporangia of *P. nicotianae* to cause late blight-like foliar lesions.

We collected samples of the pathogen as outbreaks occurred from the eastern US and then tested isolates recovered for mating type, fungicide sensitivity and genotyped them using SSR markers in order to better understand the genetic structure of populations of *P. nicotianae* on potato, to determine the risk for recurring disease in the same field, and to inform management decisions. The objectives of this study were to: 1) Characterize a subset of isolates of *P. nicotianae* collected from potato and tomato in the eastern US for mefenoxam sensitivity and mating type; 2) Examine the population structure of *P. nicotianae* from multiple states and fields where disease occurred in the eastern US using SSR genotyping; 3) Infer potential sources of inoculum to better inform management decisions; and 4) Quantify number of disease conducive days for *P. nicotianae* and *P. infestans* in 2 NC counties based on temperature.

MATERIALS AND METHODS

Sampling and Isolation

A total of 92 samples of *P. nicotianae* were collected from five states between 2017 and 2021: North Carolina, Virginia, Maryland, Pennsylvania, and New York (**Supplemental Table 1, Fig. 2**). Samples were collected from both potato and tomato and were confirmed to be *P. nicotianae* via PCR (see below). Pieces of infected leaf, stem, or fruit material were surface sterilized using 10% bleach for approximately 30 seconds, followed by two rinses using sterile distilled water for 30 seconds each, then dried on sterile paper towels. Sterilized plant material was plated under PARP media (17g corn meal agar; 10mg pimaricin; 250mg ampicillin; 10mg rifampicin; 100mg pentachloronitrobenzene) and incubated at room temperature (~23°C) for at least three days before plates were examined for colony formation and transfer to lima bean agar (1L lima bean broth; 1g dextrose; 18g granulated agar).

DNA Extraction and Verification

DNA was extracted from tissue using a modification of a quick sodium hydroxide extraction (Wang et al. 1993). In brief, a piece of infected leaf tissue was ground in 90µl 0.5N sodium hydroxide, after which 3µl of the mixture was added to 300µl of 100mM Tris-HCl buffer, pH 8.0. For SSR genotyping, extractions were made from pure mycelium grown in pea broth using a hexadecyltrimethylammonium bromide (CTAB) extraction (May and Ristaino 2004).

Samples were first verified as *P. nicotianae* using restriction digests of PCR amplicons in comparison to a known *P. nicotianae* or *P. infestans* isolate (Drenth et al. 2006). These tests used DNA amplified from the internal transcribed spacer (ITS) region which was then digested using the restriction enzyme *MspI*. In 2017, a subset of DNA from the undigested PCR product samples was also sequenced to verify *P. nicotianae*. After 2018, isolates were identified using *P. nicotianae* specific primers and a subset were sequenced to confirm identity (**Supplemental**

Table 2)(Érsek et al. 1994). Master mixes were prepared at a 25 μL volume for each sample. Each reaction contained 2.5 μL of 10X PCR buffer (Genesee, San Diego, CA), 1.25 μL dNTP buffer (2mM per nucleotide), 1 μL each 10 μM forward and reverse primer, 0.9 μL $MgCl_2$ (50mg/mL), 0.125 μL BSA (20mg/mL), and 0.1 μL Taq (5 U/μL)(Genesee, San Diego, CA) and 2μl of genomic DNA. Thermal cycling conditions were 94˚C (2 min); then 35 cycles of 94˚ (15 sec), 66˚C (15 sec), 72˚ (15 sec); and a final extension of 72˚ (5 min). Results were visualized on a 1-2% agarose gel.

Isolate Characterization

A subset of isolates was characterized for mefenoxam sensitivity and mating type. A single mycelial disk of each isolate was placed on a lima bean agar plate amended with 0, 5, or 100ppm mefenoxam (Ridomil Gold SL, 45.3% mefenoxam). Plates were then incubated for a week at room temperature, after which colony growth was measured with two perpendicular diameter measurements. Three plates per concentration of mefenoxam were used for each isolate. Average colony growth at 5 and 100ppm was compared to colony growth at 0ppm (Hu et al. 2012). Isolates that showed less than 40% growth of the control at 5 and 100ppm were characterized as sensitive. Isolates that showed more than 40% growth of the control at 5ppm but less than 40% at 100ppm were characterized as having intermediate sensitivity. Isolates that showed more than 40% growth of the control at both 5 and 100ppm were characterized as insensitive to mefenoxam.

To determine mating type, agar plates were prepared with a clarified V8 agar (100mL clarified V8 juice; 900mL dH_2O; 0.05g β-sitosterol; 17g granulated agar). A single mycelial disk of the isolate to be evaluated was placed on the plate. A second mycelial disk from either a known A1 or A2 isolate of *P. nicotianae* was then placed on the same plate. A second plate was made in the same manner using the opposite known mating type. Plates were then sealed and incubated in the dark at room temperature for at least one week. Cultures were then evaluated for the presence of oospores. If oospores were observed with the A1 tester isolate, then the unknown isolate was designated A2. If oospores were observed with the A2 tester isolate, then the unknown isolate was designated A1. If oospores were observed on both the known A1 and A2 plates, the unknown isolate was designated as a mixed A1/A2 isolate. This was observed in only one case (**Supplemental Table 1**). For the initial tests in 2017, an isolate of *P. capsici* was used as the A1 mating type tester due to the unavailability of a *P. nicotianae* A1 mating type tester isolate and the ability for *P. capsici* to hybridize with *P. nicotianae*. In all other years, a *P. nicotianae* isolate identified as A1 from 2017 was used (**Supplemental Table 1**).

SSR Genotyping

A total of 70 samples were genotyped using microsatellite primers (Biasi et al. 2016). Nine primer pairs were used and these were multiplexed into pairs with the exception of P1509 which was not multiplexed (**Supplemental Table 3**). Master mixes were prepared at a 25 µl volume for each sample. Each reaction contained 2.5 µL of 10X PCR buffer (Genesee, San Diego, CA), 2.5 µL dNTP buffer (2mM per nucleotide), 0.25 µL each 10 µM forward and reverse primer for each half of the primer pair, 1 µL $MgCl_2$ (50mg/mL), and 0.1 µL Taq (5 U/µL)(Genesee, San Diego, CA) and 2µl of genomic DNA. Thermal cycling conditions were 94˚C (3 min); then 35 cycles of 94˚ (30 sec), 59˚C (30 sec), 72˚ (45 sec); and a final extension of 72˚ (10 min). For fragment analysis, 1-2µL of PCR product was added to a 10.3µL reaction mix consisting of 10µL highly deionized formamide and 0.3µL LIZ500 size standard (Applied Biosystems, Foster City, CA). Fragments were analyzed on an Applied Biosystems 3730xl DNA analyzer at the Genomic Sciences Laboratory at North Carolina State University. Alleles were scored using Geneious and a binning scheme developed using allele calls by Biasi et al. (2016) (**Supplemental Table 3**). Samples that did not amplify at the P1509 locus were assumed to not have the appropriate binding site and were classified as null alleles for the P1509 locus.

Data Analysis

Data were initially evaluated using the R library *poppr* v. 2.9.3 (Kamvar et al. 2014) and R v. 4.2.0(R Core Team 2022). A multilocus genotype (MLG) histogram was generated to examine the level of diversity of MLGs. The following population statistics were calculated from the SSR genotype dataset using *poppr*: number of samples (N), number of multilocus genotypes (MLG), number of expected MLGs at the smallest sample size of at least 10 (eMLG), Shannon Weiner Index of MLG diversity(Wang et al. 2017), evenness, and Nei's unbiased gene diversity (Hexp). The index of association (Ia), and the standardized index of association ($\underline{r}_d$)(Agapow and Burt 2001) were additionally calculated using the function *ia* and tested for significance using 999 permutations of the data. Population statistics were calculated for the overall dataset, as well as for subpopulations based on state and year collected.

The broad structure of the populations was evaluated via model-based Bayesian clustering using the program STRUCTURE v. 2.3.3.(Pritchard et al. 2000). Before analysis by STRUCTURE, the data were clone corrected (clones were removed such that each population contains only one representative of each haplotype) using *poppr*. Data were clone corrected by designating the state where they were collected as their population assignment. The data were run using a 20,000 repeat burn-in and 1,000,000 MCMC repeats under an admixture model. Independent runs of the model used *K* values from 1 to 10 with 10 replicate runs at each value of *K*. The optimal *K* was estimated using the second order rate of change (the "Evanno method") and the data visualized in the web tool CLUMPAK (Kopelman et al. 2015). The index of association and the standardized index of association were additionally calculated for the optimal number of genetic groups (K=2) as determined through STRUCTURE analysis.

Minimum spanning networks (MSN) were generated and examined for location, host, and year collected. In addition, a neighbor joining (NJ) tree based on Bruvo's distance was constructed using *poppr* and a combination (genome addition and genome loss) model. The dataset was bootstrapped using 1000 replicates. The NJ tree was generated with all samples that did not contain missing data, because the algorithm used cannot account for missing data.

Weather Examination

Weather data were retrieved from the Daymet weather and climate collection produced by U.S. Department of Energy's Oak Ridge National Laboratory (Thornton et al. 2021). Daymet provides several weather and climate estimates across the U.S. at 1 km^2 spatial resolution and daily time step based on data from weather stations across the country. The spatial resolution is calculated from an algorithm that relies on interpolation and extrapolation of data from weather stations and weights that are derived from the grid and weather station locations.

We used Google Earth Engine (GEE) to retrieve the following Daymet estimates: maximum temperature (tmax), minimum temperature (tmin), day length (dayl), and vapor pressure (vp) (Gorelick et al. 2017). Then we used GEE to calculate hourly mean temperature (tmean) and relative humidity (RH) for all days for the months April – August for 2012 to 2021. The hourly calculations relied on empirically derived formulas that depend on latitude and day of year and physical relationships between temperature and water vapor (Goudriaan and van Laar 1994). Our formulas can be found in this GitHub repository, https://github.com/ncsu-landscape-dynamics/Manuscript--Understanding-the-genotypic-and-phenotypic-structure-of-Phytophthora-nicotianae-outbreak. Hourly measurements of temperature and RH are important to determining the sporulation stage of late blight (Skelsey 2020). The calculated tmean and RH were then filtered in GEE to determine the number of hours in each day that met thresholds for *P. infestans* survival and sporulation and *P. nicotianae* survival. The rasters created by those filters were exported and we used R with the *terra* (Hijmans 2022), *tidyverse* (Wickham et al. 2019), and *lubridate* (Grolemund and Wickham 2011) packages to do further processing.

First we considered periods when there were two or more days that the tmean was between 18 and 22 C and RH > 90% for more than six hours, conditions conducive for infection by *P. infestans*. Starting with the second day that those two conditions were met, we counted every day that followed until either the tmean or RH failed to meet those conditions. We used a similar process to find days conducive to infection by *P. nicotianae*, however, in this case, we used tmax between 25 – 35 C and RH > 65% and just a minimum of one hour for those conditions (Kaur et al. 2021). The summary of each year considered the cumulative number of days between April and August in that year that met the necessary temperature or relative humidity conditions for each pathogen.

RESULTS

Samples Collected

A total of 92 isolates of *P. nicotianae* were collected between 2017 and 2021 from five states: NC, VA, NY, PA, and MD (**Fig. 2, Supplemental Table 1**). Most samples were from outbreaks from potato fields in eastern North Carolina (n=60). *Phytophthora nicotianae* outbreaks occurred in 49 fields and some of the same farms in Pasquotank Co, NC, had the disease more than once in subsequent years (n=4) (**Supplemental Table 1**). The remaining isolates were collected from tomato fields. The majority of samples collected from outside North Carolina came from the Long Island region of New York (n=18).

Mating type

Seventy-nine of the 92 isolates were tested to determine mating type (**Supplemental Table 1**). Both A1 and A2 mating types were detected from isolates collected in all states except Pennsylvania. Within the entire sample set, 37% of isolates were A1 mating type, while 62% were A2 mating type. One isolate from 2021 could not be determined to be A1 or A2 and was designated as mixed A1/A2. Most likely, this isolate was a mixture of two separate mating types isolated from the same leaf. The A2 mating type was detected more frequently than the A1 mating type from 2018 to 2020 (**Supplemental Fig 1.**). No A1 mating type isolates were detected in 2019. A higher number of A1 mating type isolates were detected in 2017 and 2021 than other years.

The A2 mating type was detected in most of the fields in NY and all fields in PA. At least one field in Pasquotank Co., NC had both A1 and A2 present in the same field in all years except 2019 (**Supplemental Table 1, Fig 2**). Other instances were observed where A1 and A2 were in found in nearby fields to one another, primarily in Pasquotank and Camden counties. A1 and A2 mating types were also detected in nearby fields in Suffolk Co., NY, in 2020 and in Dorchester Co., MD, in 2021. Six instances were observed where both A1 and A2 mating types were collected from plants from the same field (**Supplemental Table 1**).

Mefenoxam sensitivity

We tested for mefenoxam sensitivity among 69 isolates from 2017 to 2021. All isolates tested grew on mefenoxam amended media at less than 40% of the control at both 5 and 100ppm of mefenoxam. All of the tested isolates were sensitive to mefenoxam (**Supplemental Table 1**).

Population Diversity

Seventy isolates were genotyped using microsatellites for this study (**Supplemental Table 1, Supplemental Table 4, Table 2**). Of these, 49 were collected from North Carolina, 10 from New York, 4 from Pennsylvania, 5 from Virginia, and 2 from Maryland. We identified 51 multilocus genotypes (MLGs) among these isolates (**Table 2**). North Carolina had the highest number of MLGs detected (n= 40) and had the highest diversity values for the Shannon Weiner Index (3.588) followed by New York. Most of the samples were collected from NC fields. The highest number of MLGs were collected in 2021 when 20 MLGs were detected. Isolates collected in 2021 also had the highest diversity index values for Shannon Weiner (2.93). The next highest diversity index calculated by the Shannon Weiner Index was for 2018 (2.69). Samples collected in 2017 had the lowest diversity index value for the Shannon Weiner Index , and samples from that year had the lowest number of MLGs detected (n= 4). Fewer numbers of samples were taken in 2017 as fewer outbreaks were reported.

Population Structure

Cluster analysis of SSR genotypes from *P. nicotianae* populations revealed no clear structuring of any single group of samples identified by location or year of collection (**Supplemental Fig. 2**). One clade contained all the samples from 2019 in Virginia as well as all but two of the samples collected from New York with significant (>70%) bootstrap support. This clade was nested within a larger well-supported clade that contained all samples collected in New York, Pennsylvania, and Virginia, as well as several samples collected in North Carolina. The only isolate included in the tree from Maryland was nested within a clade consisting of samples from North Carolina.

A similar structure was observed from the minimum spanning network (MSN) (**Fig. 3**). The populations largely oriented North Carolina samples on the outer branches of the MSN, with samples collected from all other states oriented more centrally on the network. Two main branches with samples from North Carolina were observed. One consisted primarily of samples collected in 2021, with two samples collected in 2020 and one from 2018. The other included samples collected across the sampling period, and included one central node of a single haplotype with four individuals, from which multiple branches emerged. Two nodes were noted to include samples from more than one state. One node consisted of one sample from NC and one from MD, both collected in 2021. The other consisted of five samples from NY (collected in 2018, 2019, and 2020) and one from VA (collected in 2019). Four samples collected from tomato were included in the dataset, but the haplotypes from these samples did not cluster together.

Results from the STRUCTURE analysis revealed a similar pattern to the cluster analysis and MSN (**Fig. 4**).The optimal number of groups (*K*) was inferred to be 2 based on the second order rate of change across ten proposed *K* values (1-10). Under 2 groups, the clone corrected sample set was divided largely into samples collected in North Carolina, and samples collected from North Carolina and elsewhere (**Fig. 4**). One sample from Maryland grouped with the NC-exclusive group. Samples collected from North Carolina that grouped within the NC-non-exclusive group were collected in every year except 2017. Adding an additional *K* value (K=3) resulted in a subset of the NC-non-exclusive group breaking into two groups: one shared members from NC, NY, and PA, while the other included representatives from all states (NC, NY, PA, VA, and MD).

Ia and $\underline{r}_d$ were calculated as 1.368 and 0.177, respectively, for a clone corrected version of the dataset using 999 permutations of the data (**Supplemental Table 5**). Both of these values had a P of <0.001, indicating the null hypothesis of no linkage between alleles should be rejected and suggest a clonal population. Among clone corrected datasets of populations sorted by state, only VA had an Ia and $\underline{r}_d$ with a P >0.01, which would suggest a sexual population. Ia and $\underline{r}_d$ for MD and PA could not be calculated as clone correction resulted in the presence of only two haplotypes. When the samples were analyzed based on genetic similarity as assigned by STRUCTURE, the group consisting of samples from NC, NY, PA, VA, and MD had both an Ia and $\underline{r}_d$ with a P<0.001, suggesting a clonal population. However, the group consisting of samples from NC and one sample from MD had an Ia and $\underline{r}_d$ with a P >0.01, suggesting a sexual population. In addition, our mating type analysis indicated that opposite mating types were present in at least 6 sample sites, so the likelihood of overwintering of the pathogen and sexual reproduction is possible. Within these six sites, three had samples that were placed in the NC-MD genetic group, two had samples that were placed in the NC-NY-PA-VA-MD group, and one that was not included in the STRUCTURE analysis.

Weather Data

To identify conducive weather conditions important for infection by either *P. infestans* or *P nicotianae* , we split the weather data into two periods, 2012-2016, the "first period", and 2017-2021, the "second period", among four counties in North Carolina where Phytophthora blight had been reported over the study period. Those counties were Camden, Guilford, Pasquotank, and Wake., There was a drop in the average number of days with weather conditions suitable for infection by *P. infestans* from the first period to the second period in all of the counties (**Fig. 5 A**). For *P. nicotianae*, there was a very slight increase in the mean number of days conducive to disease development in Camden between the two periods. The other counties saw slight decreases in the mean number of days conducive for Phytophthora blight from the first to the second period (**Fig. 5 B**).

DISCUSSION

Phytophthora nicotianae has not historically been considered a major pathogen of potato, but reports of outbreaks going as far back as 2005 suggest that *P. nicotianae* is becoming an emerging threat (Taylor et al. 2015). Before the outbreaks in 2017, *P. nicotianae* had been previously reported in potato from five states: FL, TX, MS, DE, and NE (Taylor et al. 2008). Our study documents outbreaks that occurred in new parts of the US, especially in the southeast. Many of these outbreaks were reported from potato fields in North Carolina, where increasingly warm and wet summers, exacerbated by regular tropical storms and hurricanes, promotes infection by *P. nicotianae.*

In this study we characterized isolates of *P. nicotianae* collected in several parts of the eastern US for mefenoxam sensitivity. Results from our study were similar to previous studies of *P. nicotianae* on potato and tobacco (Taylor et al. 2008; Taylor et al. 2012). We documented mefenoxam sensitive isolates of *P. nicotianae* indicating the compound is still an effective chemical option in these areas. However, mefenoxam resistance has been documented in *P. nicotianae* from other hosts in VA and NC, primarily herbaceous annuals (Hu et al. 2008; Olson et al. 2013). Therefore, mefenoxam resistance should be monitored in *P. nicotianae* from Solanaceous hosts for signs of resistance development.

We also characterized the mating type of the isolates collected from various fields and counties. Both mating types were observed in several fields in our study. Most notably, there were six instances where both mating types were present in the same field during the same year. Some of those fields were on farms in eastern NC where the disease occurred in numerous years. This suggests the pathogen may be overwintering and surviving in soil from potato fields in some locations. Since *P. nicotianae* produces both chlamydospores and oospores, rotation out of potato to a nonsusceptible host and/or the use of soil applied oomycete targeted fungicides is warranted. Ridomil Gold SL (mefenoxam) and Orondis Gold (oxathiapiprolin and mefenoxam) are labeled for soil applications to manage other oomycete pathogens in potato and tomato in the US.

Genotyping of isolates using SSR markers revealed diverse multilocus genotypes throughout the fields sampled, with multiple haplotypes present in close proximity to each other. Examination of haplotypes using Bruvo's distance did not reveal strong patterns of genetic similarity based on state or year collected that could be resolved into an exclusive group, suggesting the population is largely panmictic. However, analysis of the data using STRUCTURE suggested the presence of two genetically distinct groups, one consisting almost exclusively of isolates collected in North Carolina along with a single field site in Maryland, and one that included samples from North Carolina, but also included all isolates from New York, Pennsylvania, and Virginia as well as a second Maryland field site.

The presence of two genetically distinct groups of *P. nicotianae* suggests that outbreaks in these areas are indicative of at least two different sources of inoculum introduction. It is likely that these outbreak sources originated in seed tubers brought in from seed production areas of Canada as well as Maine and South Dakota, all of which are regions where NC growers obtain seed potatoes (Taylor et al. 2008; Taylor et al. 2012) . Seed tubers planted on Long Island, NY, are mostly from Maine. Once introduced, the pathogen spreads locally via irrigation and heavy rainfall events that leave standing water in fields. A broader genotyping of *P. nicotianae* populations found in seed tubers from seed producing regions could reveal potential sources. The NC-MD genetic group 1 revealed via STRUCTURE appeared in 2017 before the broader NC-NY-PA-VA-MD group 2, which first appeared in 2018, suggesting that the NC-MD genetic group may have been introduced first in North Carolina based on the age of the oldest samples collected. Our data also indicated that both groups have persisted over the five years of our study in NC fields.

It is also possible that these outbreaks could have come from non-potato hosts. *P. nicotianae* has shown some ability to pass to potato from other species, such as tobacco or ornamentals (Taylor et al. 2012). However, most of the flu cured tobacco production in NC and ornamental production on Long Island does not occur in fields where potatoes are grown in rotation so this is a less likely scenario. Similarly, large scale ornamental production does not occur in eastern NC where most of the outbreaks occurred, so this is not a likely source of the pathogen introduction.

An increase in *P. nicotianae* outbreaks poses new challenges for growers. Both A1 and A2 mating types of *P. nicotianae* are present and established in the US. In this study, we found the A2 mating type to be dominant, which has been observed in other parts of the country (Taylor et al. 2008; Taylor et al. 2012). However, we identified at least six instances where both A1 and A2 were detected in a single field, suggesting the presence of a sexual population. Calculations of Ia and $\underline{r}_d$ based on state of disease occurrence suggested that the populations present are more clonal in nature. If *P. nicotianae* is being regularly introduced from outside sources via seed potatoes, particularly from the same sources, this could result in a population that appears more clonal. However, analysis of Ia and $\underline{r}_d$ based on the 2 genetic groups identified by STRUCTURE indicated that the group 1consisting of samples from North Carolina and Maryland constitutes a sexually reproducing population.

A broader, more systematic survey of *P. nicotianae* populations in the area could provide more information on whether sexual reproduction is occurring. While the ability to produce oospores is theorized to have less of an impact on the development of new genotypes than currently thought due to natural populations showing biased proportions of one mating type over the other (Panabiéres et al. 2016), the close proximity of A1 and A2 mating types should continue to be monitored for the possibility of development of novel genotypes that could develop fungicide resistance. In addition, *P. nicotianae* can produce asexual chlamydospores, enabling the pathogen to persist from year to year in soil warranting soil directed fungicide treatments.

Management of *P. nicotianae* differs from that of *P. infestans* in important ways. Due to *P. nicotianae*'s broad host range, developing an effective crop rotation is more difficult (Panabiéres et al. 2016). The ability to produce overwintering structures also greatly increases *P. nicotianae*'s ability to persist in the soil, limiting the utility of crop rotation. Chlamydospores of *P. nicotianae* can persist in soil for at least six months, and other oospore-forming species, such as *P. fragariae*, have been noted to persist in soil for up to three years (Duncan and Cowan 1980; Sneh and Katz 1988). *Phytophthora nicotianae* also differs from *P. infestans* in that applying fungicides to soil is more important than foliar sprays. Rapid species identification is needed to help target fungicide management decisions, in particular whether focus should be on applying fungicides to soil early in production or to foliage. We are in the process of developing fieldready LAMP assays to distinguish *P. nicotianae* from *P. infestans* (Ristaino et al. 2020a; Paul et al. 2021).

Changing weather patterns influenced by climate change may be shifting *Phytophthora* species that cause disease on potato as the weather becomes more conducive to *P. nicotianae* and less optimal for *P. infestans* (**Table 1**) (Ristaino and Saville, 2018). Our calculations for the number of days with suitable weather for *P. infestans* show that the number of days of suitable conditions have decreased for *P. infestans* and not changed or increased slightly for *P. nicotianae.* It has been speculated that increasing temperatures would favor *P. nicotianae* infections due to the pathogen's higher optimal temperature for growth and infection compared to *P. infestans* (Kamoun et al. 2015). We examined the number of days with suitable conditions for disease emergence from four counties where *P. nicotianae* was detected and compared them to data collected from the previous five years from the same counties. Across the time studied, the number of growing season days when conditions were conducive for *P. nicotianae* infection were consistently higher than the number of days conducive for *P. infestans* infection, suggesting climate change may provide *P. nicotianae* more of an opportunity to cause disease. This difference has increased in the most recent five-year period compared to the prior five-year period suggesting that as climate changes with warmer temperatures and more variable precipitation events Phytophthora blight caused by *P. nicotianae* may increase while late blight caused by *P. infestans* could decrease. Since *P. nicotianae* is primarily spread by local splash dispersal and in flowing water, increases in *P. nicotianae* outbreaks may also be exacerbated by more frequent and intense precipitation events. *P. nicotianae* releases zoospores in as a little as 10 minutes after a short flooding event, which would allow the pathogen to rapidly establish in a field experiencing frequent rainfall events (Thomson and Allen 1976).

Grower’s mange late blight more aggressively than Phytophthora blight on both hosts (Fry et al, 2013, Ristaino et al, 2020b). Management of potential inoculum sources such as contaminated seed potatoes, cull piles with infected tubers, and volunteer potato plants in a field where late blight occurred the previous year is often done. Educating the public about late blight has also recognized as important since the pandemic of 2009 that started in vegetable gardens in the northeast (Fry at al. 2013). This has helped reduce occurrences of disease. Further education about soilborne and tuberborne sources of *P. nicotianae* is now warranted.

Phytophthora nicotianae has become more prevalent in other pathosystems as temperatures rise. In South Africa, *P. nicotianae* is becoming more frequent than *P. cinnamomi* in eucalyptus (Nagel et al. 2013). In India, *P. nicotianae* was newly reported on black pepper in 2009 and was reported as the most commonly recovered *Phytophthora* species in India (Guha Roy et al. 2009). As a cosmopolitan, broad-host, heat-tolerant pathogen, impacts of *P. nicotianae* on agriculture are likely to increase over time. Monitoring the genetic structure of populations and improved diagnostics will be essential for tracking spread of the pathogen and adjusting fungicide treatment applications of affected crops accordingly.

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Duncan McSorley for assisting with the isolation, genotyping, and phenotyping of isolates used in this study.

FIGURES

Fig. 1. Symptoms of infection on potato and tomato caused by *Phytophthora nicotianae* **A**: Potato leaf lesion; B: Potato stem lesion; **C**: Potato tuber symptoms (image from Panabières et al. 2016); **D**: tomato leaf lesion (image courtesy of Inga Meadows); **E**: tomato stem lesion (Image courtesy of Inga Meadows); **F**: tomato fruit lesion.

Fig. 2. A map of counties in the eastern US where *Phytophthora nicotianae* outbreaks on potato and tomato occurred. Sites are identified by host, year, and mating type

Fig. 3. Minimum spanning network of populations of *Phytophthora nicotianae* collected between 2017 and 2021. Nodes indicate individual haplotypes, with the size of the node corresponding to the number of individuals sharing the haplotype. The color and thickness of lines between nodes indicates relative distance. Nodes are color coded by state of collection. Nodes with a 'T' under their year of collection were collected from tomatoes. All other samples were collected from potatoes.

Fig. 4. STRUCTURE analysis of populations of *Phytophthora nicotianae* collected between 2017 and 2021 based on 9-plex SSRs based on Bruvo's distance. The optimal number of groups (*K*) was determined to be 2 based on the second order rate of change across ten putative *K* values (1-10). Samples were clone corrected by location before analysis.

Fig. 5. Box and whisker plots of the number of days in April to August for two five year intervals that had weather conducive for infection by *P. infestans* (a) and *P. nicotianae* (b) in four counties

in North Carolina.The line in middle of the box is the median value, the upper box is the 75th percentile, the whisker above that extends 1.5 x the 75th percentile, the lower box is the 25th percentile, and the line extends past that 1.5 x 25th percentile. Suitable weather for *P. infestans* is relative humidity greater than 90% for six hours occurring during at least two days with temperatures of 18 - 22 C. Suitable weather for *P. nicotianae* is relative humidity greater than 65% with temperatures of 25 - 35 C.

LITERATURE CITED

Agapow, P.-M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1:101-102.

Biasi, A., Martin, F. N., Cacciola, S. O., di San Lio, G. M., Grünwald, N. J., and Schena, L. 2016. Genetic analysis of *Phytophthora nicotianae* populations from different hosts Using microsatellite markers. Phytopathology 106:1006-1014.

Cline, E. T., Farr, D. F., and Rossman, A. Y. 2008. A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. Plant Health Progress 9:32.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O’Dwyer, C., Irvine, G., and Irwin, J. A. G. 2006. Development of a DNA-based method for detection and identification of *Phytophthora* species. Australasian Plant Pathology 35:147-159.

Duncan, J. M., and Cowan, J. B. 1980. Effect of temperature and soil moisture content on persistence of infectivity of *Phytophthora fragariae* in naturally infested field soil. Transactions of the British Mycological Society 75:133-139.

Érsek, T., Schoelz, J. E., and English, J. T. 1994. PCR amplification of species-specific DNA sequences can distinguish among *Phytophthora* species. Appl Environ Microbiol 60:2616-2621.

Fry, W. E., Birch, P. R. J., Judelson, H. S., Grünwald, N. J., Danies, G., Everts, K. L., Gevens, A. J., Gugino, B. K., Johnson, D. A., Johnson, S. B., McGrath, M. T., Myers, K. L., Ristaino, J. B., Roberts, P. D., Secor, G., and Smart, C. D. 2015. Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology 105:966-981.

Gallup, C. A., McCorkle, K. L., Ivors, K. L., and Shew, D. 2017. Characterization of the black shank pathogen, *Phytophthora nicotianae*, across North Carolina tobacco production areas. Plant Disease 102:1108-1114.

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., and Moore, R. 2017. Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sensing of Environment 202:18-27.

Goudriaan, J., and van Laar, H. H. 1994. Modelling potential crop growth processes : textbook with exercises. Kluwer Academic Publishers.

Grolemund, G., and Wickham, H. 2011. Dates and times made easy with lubridate. Journal of Statistical Software 40:1 - 25.

Guha Roy, S., Bhattacharyya, S., Mukherjee, S. K., and Khatua, D. C. 2009. Molecular identification of *Phytophthora spp.* affecting some economically important crops in eastern India through ITS-RFLP and sequencing of the ITS region. Journal of Phytopathology 157:666-674.

Hijmans, R. 2022. *terra*: Spatial data analysis. R package version 1.6-7.

Hu, C. H., Perez, F. G., Donahoo, R., McLeod, A., Myers, K., Ivors, K., Secor, G., Roberts, P. D., Deahl, K. L., Fry, W. E., and Ristaino, J. B. 2012. Recent genotypes of *Phytophthora infestans* in the eastern United States reveal clonal populations and reappearance of mefenoxam sensitivity. Plant Disease 96:1323-1330.

Hu, J. H., Hong, C. X., Stromberg, E. L., and Moorman, G. W. 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. Plant Pathology 57:728-736.

Kamoun, S., Furzer, O., Jones, J. D. G., Judelson, H. S., Ali, G. S., Dalio, R. J. D., Roy, S. G., Schena, L., Zambounis, A., Panabières, F., Cahill, D., Ruocco, M., Figueiredo, A., Chen, X.-R., Hulvey, J., Stam, R., Lamour, K., Gijzen, M., Tyler, B. M., Grünwald, N. J., Mukhtar, M. S., Tomé, D. F. A., Tör, M., Van Den Ackerveken, G., McDowell, J., Daayf, F., Fry, W. E., Lindqvist-Kreuze, H., Meijer, H. J. G., Petre, B., Ristaino, J., Yoshida, K., Birch, P. R. J., and Govers, F. 2015. The Top 10 oomycete pathogens in molecular plant pathology. Molecular Plant Pathology 16:413-434.

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Kaur, Y., Thind, S. K., and Arora, A. 2021. Survival of *Phytophthora nicotianae* in citrus rhizosphere. Journal of Plant Pathology 103:1307-1313.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15:1179-1191.

May, K. J., and Ristaino, J. B. 2004. Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish potato famine. Mycological Research 108:471-479.

Nagel, J., Gryzenhout, M., Slippers, B., and Wingfield, M. 2013. The occurrence and impact of *Phytophthora* on the African continent. CABI International.

NOAA National Centers for Environmental Information. 2019. State of the Climate: Monthly National Climate Report for Annual 2018.

Olson, H. A., Jeffers, S. N., Ivors, K. L., Steddom, K. C., Williams-Woodward, J. L., Mmbaga, M. T., Benson, D. M., and Hong, C. X. 2013. Diversity and mefenoxam sensitivity of *Phytophthora spp.* associated with the ornamental horticulture industry in the southeastern United States. Plant Disease 97:86-92.

Panabiéres, F., Ali, G. S., Allagui, M. B., Dalio, R. J. D., Gudmestad, N. C., Kuhn, M.-L., Guha Roy, S., Schena, L., and Zampounis, A. 2016. *Phytophthora nicotianae* diseases worldwide: new knowledge of a long-recognised pathogen. Phytopathologia Mediterranea 55:20-40.

Paul, R., Ostermann, E., Chen, Y., Saville, A. C., Yang, Y., Gu, Z., Whitfield, A. E. Ristaino, J. B., and Wei. Q. 2021. Integrated Microneedle-Smartphone Nucleic Acid Amplification Platform for In-Field Diagnosis of Plant Diseases. Biosensors and Bioelectronics 187:113312.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ristaino, J., Duniway, J., and Marois, J. 1988. Influence of frequency and duration of furrow irrigation on the development of *Phytophthora* root rot and yield in processing tomatoes. Phytopathology 78:1701-1706.

Ristaino, J. B. and Saville, A. 2018. *Phytophthora infestans* look-alike causing disease pressure in eastern North Carolina. The Potato Grower 421:30-31.

Ristaino, J. B., Saville, A. C., Paul, R., Cooper, D. and Wei, Q. 2020a. Comparison of LAMP, real-time and Digital PCR for detection of *Phytophthora infestans*. Plant Dis. 104:708-716.

Ristaino, J. B., Cooke, D.E.L., Acuña, I., Muñoz, M. 2020b. The Threat of Late Blight to Global Food Security. Pages 101-133 in: In Emerging Plant Disease and Global Food Security. A. Records and J. B. Ristaino, eds. American Phytopathological Society Press, St. Paul.

Saville, A., Graham, K., Grünwald, N. J., Kevin, M., Fry, W. E., and Ristaino, J. B. 2015. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Disease 99:659-666.

Skelsey, P. 2020. Forecasting risk of crop disease with anomaly detection algorithms. Phytopathology 111:321-332.

Sneh, B., and Katz, D. 1988. Behaviour of *Phytophthora citrophthora* and *P. nicotianae* var. in soil, and differences in their tolerance to antimicrobial components of selective media used for isolation of *Phytophthora spp*. Journal of Phytopathology 122:208-221.

Taylor, R. J., Pasche, J. S., and Gudmestad, N. C. 2015. Etiology of a tuber rot and foliar blight of potato caused by *Phytophthora nicotianae*. Plant Disease 99:474-481.

Taylor, R. J., Pasche, J. S., Gallup, C. A., Shew, H. D., and Gudmestad, N. C. 2008. A foliar blight and tuber rot of potato caused by *Phytophthora nicotianae*: New occurrences and characterization of isolates. Plant Dis 92:492-503.

Taylor, R. J., Pasche, J. S., Shew, H. D., Lannon, K. R., and Gudmestad, N. C. 2012. Tuber rot of potato caused by *Phytophthora nicotianae*: Isolate aggressiveness and cultivar susceptibility. Plant Dis 96:693-704.

Thomson, S. V., and Allen, R. M. 1976. Mechanisms of survival of zoospores of *Phytophthora parasitica* in irrigation water. Phytopathology 66:1198.

Thornton, M. M., Shrestha, R., Thornton, P. E., Kao, S., Wei, Y., and Wilson, B. E. 2021. Daymet version 4 monthly latency: Daily surface weather data.

Wang, H., Qi, M. Q., and Cutler, A. J. 1993. A simple method of preparing plant samples for PCR. Nucleic Acids Research 21:4153-4154.

Wang, J., Fernández-Pavía, S. P., Larsen, M. M., Garay-Serrano, E., Gregorio-Cipriano, R., Rodríguez-Alvarado, G., Grünwald, N. J., and Goss, E. M. 2017. High levels of diversity and population structure in the potato late blight pathogen at the Mexico centre of origin. Molecular Ecology 26:1091-1107.

Wickham, H., Averick, M., Bryan, J., Chang, W., D'Agostino McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Lin Pedersen, T., Miller, E., Milton Bache, S., Müller, K., Ooms, J., Robinson, D., Paige Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., and Yutani, H. 2019. Welcome to the Tidyverse. Journal of Open Source Software 4.

Table 1. Important differences in symptoms, morphological traits and temperature thresholds between *Phytophthora infestans* and *Phytophthora nicotianae*

Trait	*P. infestans*	*P. nicotianae*
Optimum temperature	18 – 22°C	25 – 35°C
Mefenoxam sensitivity	Dominant lineage US-23 (sensitive to mefenoxam)	Mefenoxam resistance reported
Disease cycle	No soilborne phase	Soilborne oospores and chlamydospores
Mating types	A1 (US-23) dominant	A1 and A2 common
Oospores (Sexual overwintering structures)	Heterothallic but oospores uncommon in US, common in Mexico, Netherlands, and Scandinavia	Heterothallic and oospores common
Symptoms on potato	Tubers, stems, and foliage	All parts of plant including roots
Symptoms on tomato	Stems, foliage and fruit	All parts of plant including roots
Asexual survival stages	None	Chlamydospores (asexual overwintering structures)
Host range	Narrow: potato, tomato, petunia	Wide: potato, tomato, citrus, tobacco, ornamentals

Table 2. Diversity statistics sorted by state and by year of collection for populations of *Phytophthora nicotianae*.

Population	*N*[a]	MLG	eMLG(SE)	H	Evenness	Hexp
State						
North Carolina	49	40	9.50(0.672)	3.588	0.859	0.579
New York	10	6	6(0)	1.498	0.672	0.461
Pennsylvania	4	2	2(0)	0.693	1.000	0.508
Virginia	5	3	3(0)	1.055	0.950	0.383
Maryland	2	2	2(0)	0.693	1.000	0.537
Year						
2017	4	4	4(0)	1.39	1.000	0.448
2018	20	16	8.92(0.817)	2.69	0.896	0.592
2019	11	5	4.91(0.287)	1.52	0.893	0.438
2020	11	11	10(0)	2.40	1.000	0.570
2021	24	20	9.24(0.746)	2.93	0.904	0.573
All	70	51	9.42(0.727)	3.78	0.804	0.590

[a]n: number of individuals ; MLG: number of multilocus genotypes (MLG); eMLG: expected number of MLGs at smallest size of at least ten; SE: Standard error; H: Shannon Weiner index of MLG diversity ; Hexp: Nei's 1978 gene diversity.

Fig. 1. Symptoms of infection on potato and tomato caused by *Phytophthora nicotianae* **A**: Potato leaf lesion; **B**: Potato stem lesion; **C**: Potato tuber symptoms (image from Panabières et al. 2016); **D**: tomato leaf lesion (Image via Inga Meadows from grower); **E**: tomato stem lesion (Image via Inga Meadows from grower); **F**: tomato fruit lesion.

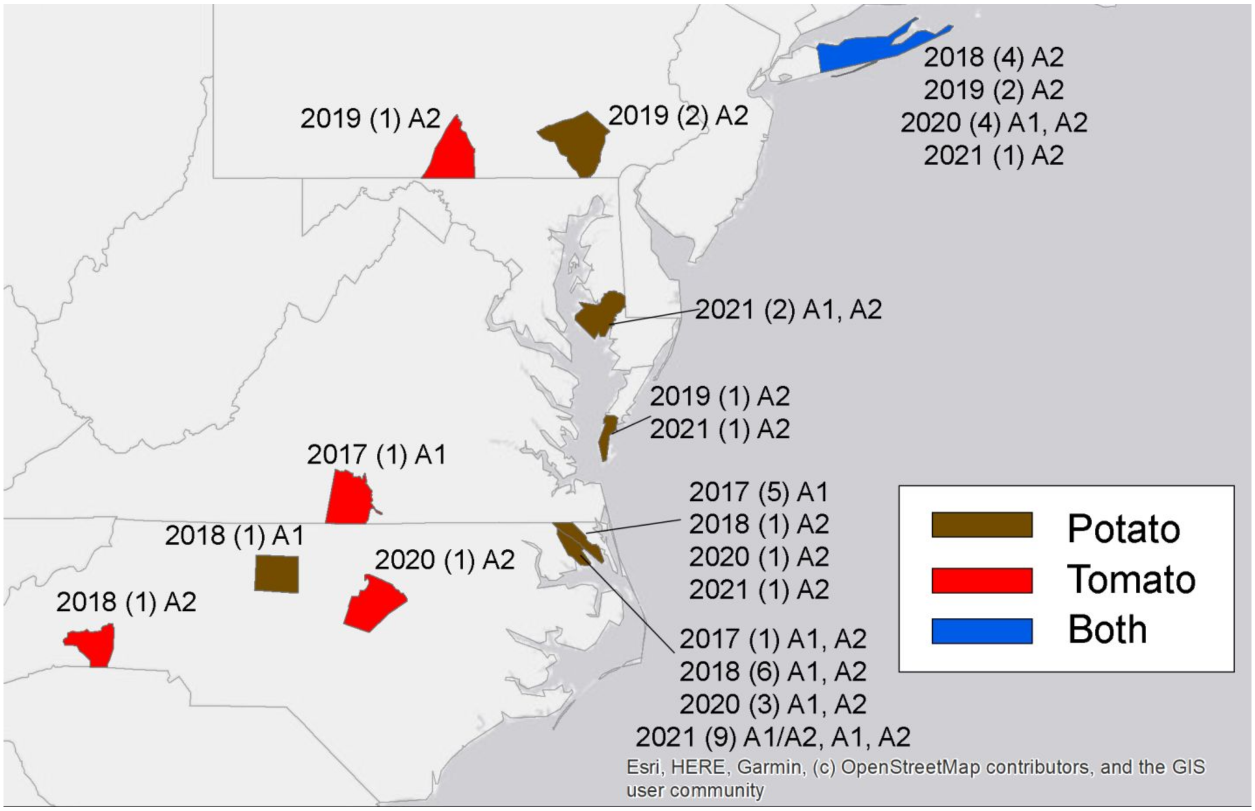


Fig. 2. A map of counties in the eastern US where *Phytophthora nicotianae* outbreaks on potato and tomato occurred. Sites are identified by host, year, and mating type

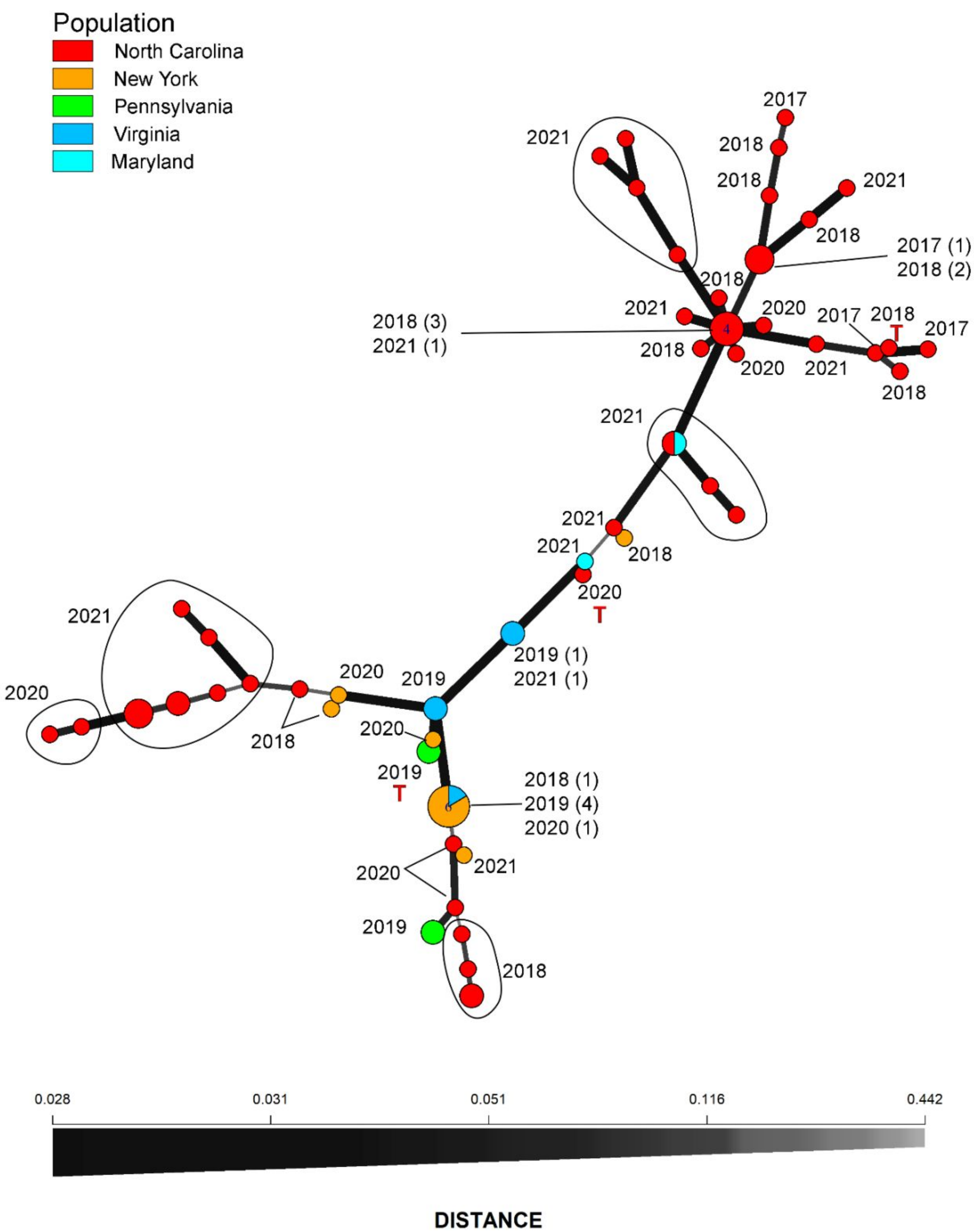
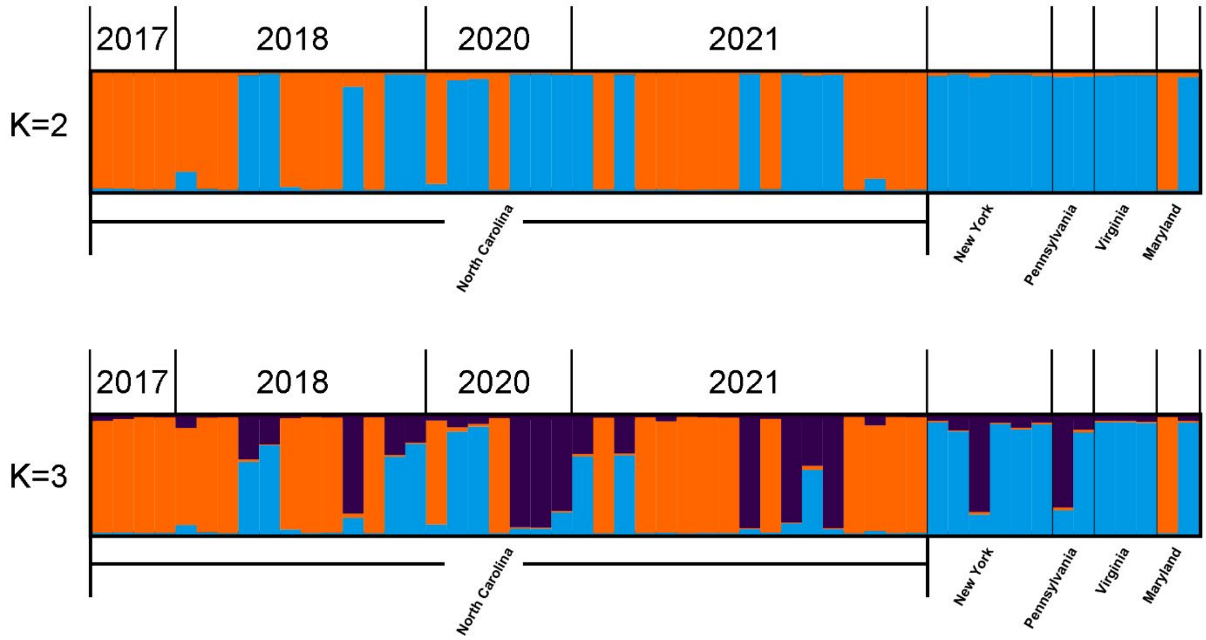


Fig. 3. Minimum spanning network of populations of *Phytophthora nicotianae* collected between 2017 and 2021. Nodes indicate individual haplotypes, with the size of the node corresponding to the number of individuals sharing the haplotype. Relative distance is indicated by the color and thickness of lines between nodes. Nodes are color coded by state of collection. Nodes with a 'T' under their year of collection were collected from tomatoes. All other samples were collected from potatoes.



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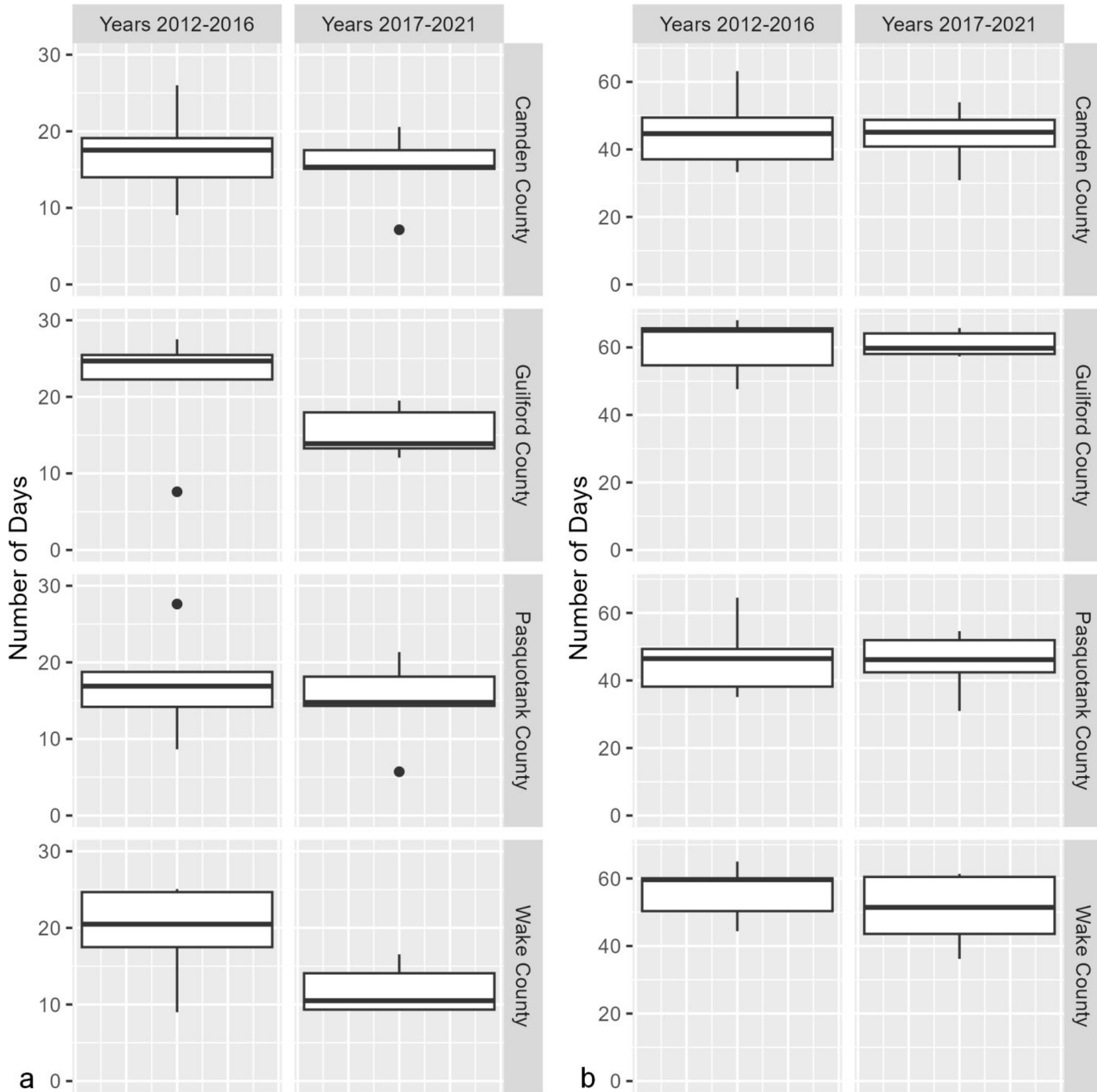

Fig. 5. Box and whisker plots of the number of days in April to August for two five year intervals that had weather conducive for infection by *P. infestans* (a) and *P. nicotianae* (b) in four counties in North Carolina.The line in middle of the box is the median value, the upper box is the 75th percentile, the whisker above that extends 1.5 x the 75th percentile, the lower box is the 25th percentile, and the line extends past that 1.5 x 25th percentile. Suitable weather for *P. infestans* is relative humidity greater than 90% for six hours occurring during at least two days with temperatures of 18 - 22 C. Suitable weather for *P. nicotianae* is relative humidity greater than 65% with temperatures of 25 - 35 C.

Supplemental Table 1. Samples collected and phenotyped for mating type and mefenoxam sensitivity are listed by state, county, field number, and host. Field numbers correspond only to the year collected and do not carry over to subsequent years.

Isolate	State	County	Field	Host	Mating Type	Mefenoxam Sensitivity
2017 (n = 11)						
6BF1 NC2017	NC	Camden	1	Potato	A1	Sensitive
6BF2 NC2017	NC	Camden	2	Potato	A1	Sensitive
6BHB2 NC2017*[a]	NC	Camden	3	Potato	A1	Sensitive
6BJB1 NC2017*	NC	Camden	4	Potato	A1	Sensitive
Camden-P2-C1	NC	Camden	5	Potato	A1	NA[c]
Pasquotank-P1-C1	NC	Pasquotank	6	Potato	A2	Sensitive
Pasquotank-P2-C1	NC	Pasquotank	6	Potato	A2	Sensitive
Pasquotank-P3-C1*	NC	Pasquotank	6	Potato	A2	Sensitive
Pasquotank-P4-C1	NC	Pasquotank	6	Potato	A2 [b]	Sensitive
Pasquotank-P4-C2*	NC	Pasquotank	6	Potato	A1 [b]	NA
27185	VA	Halifax	7	Tomato	A1	Sensitive
2018 (n = 27)						
NC18-1-L1*	NC	Pasquotank	1	Potato	NA	Sensitive
NC18-1-L2*	NC	Pasquotank	1	Potato	NA	Sensitive
NC18-1-L3*	NC	Pasquotank	1	Potato	A2	NA
NC18-2-L2*	NC	Camden	2	Potato	A2	Sensitive
NC18-2-S2*	NC	Camden	2	Potato	A2	NA
NC18-3*	NC	Pasquotank	3	Potato	A2	NA
NC18-4-L1*	NC	Pasquotank	4	Potato	NA	NA
NC18-4-L2*	NC	Pasquotank	4	Potato	NA	Sensitive
NC18-4-L3	NC	Pasquotank	4	Potato	A2	NA
NC18-7-L1*	NC	Pasquotank	5	Potato	A2 [b]	NA
NC18-7-L2*	NC	Pasquotank	5	Potato	A1 [b]	Sensitive
NC18-8-L1*	NC	Pasquotank	6	Potato	A1 [b]	NA
NC18-8-L2	NC	Pasquotank	6	Potato	A2 [b]	Sensitive
NC18-8-L3*	NC	Pasquotank	6	Potato	NA	Sensitive
NC18-9 (18-027a)	NC	Rutherford	7	Tomato	NA	Sensitive
NC18-9 (18-027b)	NC	Rutherford	7	Tomato	NA	Sensitive
NC18-9 (18-027c)	NC	Rutherford	7	Tomato	A2	NA
NC18-9 (18-027d)*	NC	Rutherford	7	Tomato	A2	Sensitive
NC18-11*	NC	Pasquotank	8	Potato	A2	Sensitive
NC18-12-L1*	NC	Guilford	9	Potato	A1	NA
NC18-12-S1*	NC	Guilford	9	Potato	NA	Sensitive
NC18-12-S2*	NC	Guilford	9	Potato	NA	NA
NY18-1*	NY	Suffolk	10	Potato	A2	NA
NY18-2	NY	Suffolk	11	Potato	A2	NA
NY18-3*	NY	Suffolk	12	Potato	A2	Sensitive
NY18-4*	NY	Suffolk	13	Potato	A2	Sensitive
NY18-4-L2	NY	Suffolk	13	Potato	A2	NA

2019 (n = 16)						
NY19-1*	NY	Suffolk	1	Potato	A2	Sensitive
NY19-1-L2*	NY	Suffolk	1	Potato	A2	Sensitive
NY19-1-L3	NY	Suffolk	1	Potato	A2	Sensitive
NY19-1-L4	NY	Suffolk	1	Potato	A2	Sensitive
NY19-2-L1	NY	Suffolk	2	Potato	A2	Sensitive
NY19-2-L2*	NY	Suffolk	2	Potato	A2	Sensitive
PA19-1*	PA	Franklin	3	Tomato	A2	Sensitive
PA19-1-L2*	PA	Franklin	3	Tomato	A2	Sensitive
PA19-1-F1	PA	Franklin	3	Tomato	A2	Sensitive
PA19-2-L1	PA	Lancaster	4	Potato	A2	NA
PA19-2-L2*	PA	Lancaster	4	Potato	A2	Sensitive
PA19-3-L2*	PA	Lancaster	5	Potato	A2	Sensitive
VA19-1-L1*	VA	Northampton	6	Potato	NA	Sensitive
VA19-1-L2*	VA	Northampton	6	Potato	A2	Sensitive
VA19-1-S1*	VA	Northampton	6	Potato	A2	Sensitive
VA19-1-S2*	VA	Northampton	6	Potato	A2	Sensitive
2020 (n = 14)						
NC20-1-1*	NC	Pasquotank	1	Potato	A1	Sensitive
NC20-2-1*	NC	Camden	2	Potato	A2	Sensitive
NC20-2-2*	NC	Camden	2	Potato	NA	NA
NC20-3-L1-1*	NC	Pasquotank	3	Potato	A1	Sensitive
NC20-3-S1-1*	NC	Pasquotank	3	Potato	A1	Sensitive
NC20-4-L2-1*	NC	Pasquotank	4	Potato	A2 [b]	NA
NC20-4-S1-1*	NC	Pasquotank	4	Potato	A1 [b]	Sensitive
NC20-5*	NC	Wake	5	Tomato	A2	Sensitive
NY20-1-L1*	NY	Suffolk	6	Potato	A2	Sensitive
NY20-1-L2	NY	Suffolk	6	Potato	NA	Sensitive
NY20-2-L1*	NY	Suffolk	7	Tomato	A2	Sensitive
NY20-2-L2*	NY	Suffolk	7	Tomato	A2	Sensitive
NY20-3-L1	NY	Suffolk	8	Tomato	NA	Sensitive
NY20-4-L1-1	NY	Suffolk	9	Tomato	A1	Sensitive
2021 (n = 24)						
NC21-1-L1*	NC	Pasquotank	1	Potato	A1	Sensitive
NC21-1-L2*	NC	Pasquotank	1	Potato	A1	Sensitive
NC21-2-L1*	NC	Pasquotank	2	Potato	A2	Sensitive
NC21-2-L2*	NC	Pasquotank	2	Potato	A2	Sensitive
NC21-3-L1*	NC	Pasquotank	3	Potato	A1 [b]	Sensitive
NC21-3-L2*	NC	Pasquotank	3	Potato	A2 [b]	Sensitive
NC21-4-L1*	NC	Pasquotank	4	Potato	A1	Sensitive
NC21-4-L2*	NC	Pasquotank	4	Potato	A1	NA
NC21-5-L1*	NC	Pasquotank	5	Potato	A1	Sensitive
NC21-5-L2*	NC	Pasquotank	5	Potato	A1	NA
NC21-6-L1*	NC	Camden	6	Potato	A2	Sensitive
NC21-6-L2*	NC	Camden	6	Potato	A2	Sensitive
NC21-7-L1*	NC	Pasquotank	7	Potato	A1/A2 [b]	Sensitive

NC21-7-L2*	NC	Pasquotank	7	Potato	A2 [b]	Sensitive
NC21-8-L1*	NC	Pasquotank	8	Potato	A1	Sensitive
NC21-8-L2*	NC	Pasquotank	8	Potato	A1	NA
NC21-9-L1*	NC	Pasquotank	9	Potato	A1	Sensitive
NC21-9-L2*	NC	Pasquotank	9	Potato	A1	NA
NC21-10-L1*	NC	Pasquotank	10	Potato	A1	Sensitive
NC21-10-L2*	NC	Pasquotank	10	Potato	A1	NA
MD21-2*	MD	Dorchester	11	Potato	A1	Sensitive
MD21-3*	MD	Dorchester	12	Potato	A2	Sensitive
NY21-3-S1*	NY	Suffolk	13	Potato	A2	Sensitive
VA21-1-L2*	VA	Northampton	14	Potato	A2	Sensitive
Mating Type [d]Testers						
51 (*P. capsici*)	NC	Sampson		Squash	A1	
P21 (*P. nicotianae*)	CA	ATCC 15408		Citrus	A2	

[a]Starred (*) indicates these isolates were used for SSRs genotyping by the methods of Biasi et al., 2016.
[b] Opposite mating types found in same field.
[c] NA – not applicable, mefenoxam sensitivity and/or mating type not tested
[d]A1 tester isolates (6BF2 NC2017, 6BHB2 NC2017, or Pasquotank-P4-C2) and A2 tester isolates (Pasquotank-P1-C1, NC18-8-L2, or NY19-2-L2) used in subsequent years.
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Supplemental Table 2. PCR primers used to identify species and SSR primers used to genotype isolates of *Phytophthora nicotianae i*n this study

Primer Name	Primer Sequence (5'- 3')	Source
PCR restriction Digest		Drenth et al. 2006
A2	5' – ACTTTCCACGTGAACCGTTTCAA – 3'	
I2	5' – GATATCAGGTCCAATTGAGATGC – 3'	
PCR verification		Érsek et al. 1994
PnicID_F	CTGACGATCCAGATCCTCTGCACG	
PnicID_R	CTTGCGAGGCTTGACCGCTTCCTA	
Microsatellites[a]		Biasi et al. 2016
P5 (1)	NED-CAAGCCCGCTGAGGTTGAA	
	GTTTCTCCGAGGTCCAAATGTGAT	
P15 (2)	FAM-AGCTTCTGCAGTAACGGTAA	
	GTTTCGATCAAAGATTACTGCAACT	
P17 (1)	FAM-GTCCTCAGGGATCAGCACAT	
	GTTTTGGATATCGTTCCCGTTGTT	
P643 (3)	NED-TTTCAATCGTTTGACCATGC	
	GTTTCAAGTCCAAACCGTCCTGTC	
P788 (3)	FAM-GATGGCAAACCGCCCGACTT	
	GTTTCGAGAAGCAGCAGAAGAAGC	
P1129 (4)	NED-CAGCCTCCAGATATGTTCAT	
	GTTTTGTTAGGGGTCTCCAACTGC	
P1509 (5)	FAM-CTAAGCCTAGCCAATCCAAAC	
	GTTTCCAGCTTGACGCCGGGATTA	
P2039 (4)	FAM-GCAGTCGGTTGGATTGATCA	
	GTTTTGAACCTTGTCCAGATTATTG	
P2040 (2)	NED-ACGAGTTTGGGCATCGTTTA	
	GTTTATTTTCGCACGGARGAGAT	

[a] Numbers in parenthesis indicate duplexing pair for each primer set. Forward primers were labeled with fluorescent dye (NED or FAM).

Supplemental Table 3. Allele names and bin ranges for nine microsatellite loci used to genotype *Phytophthora nicotianae* in this study.

Locus	Allele Name	[a]Bin Start	Bin End
P1509	136	135.2	137
	146	146.3	147.9
	150	149.2	151
	152	151.2	153
	154	153.2	155
	156	155.2	157
	158	157.2	159
	160	159.2	161
	162	161.2	163
	164	163.2	165
	166	165.2	167
	168	167.2	169
	170	169.2	170.9
	172	171.2	173
	174	173.2	175
P5	188	186.5	190.1
	192	190.5	194.1
	196	194.5	198.1
	200	198.5	202.1
	204	202.4	206.1
	208	206.5	210.1
	212	210.5	214.1
	216	214.5	218.1
	220	218.5	222.1
	224	222.5	226.1
	228	226.5	230.1
	236	234.5	238.1
	244	242.6	246.1
P17	102	100.6	103.4
	105	103.6	106.4
	108	106.6	109.4
	114	112.6	115.4
	116	115.6	118.4
	126	124.6	127.4
	129	127.6	130.3
	132	130.5	133.4
	138	136.6	139.4
	141	139.6	142.4
	144	142.6	145.4
	147	145.6	148.4
	156	154.6	157.4
P15	69	67.2	70
	72	70.2	73.1

	75	73.2	76
	78	76.2	79.1
	81	79.2	82
	84	82.2	85
	87	85.2	88.1
	90	88.2	91.1
	93	91.2	94.1
	96	94.2	97.1
	99	97.2	100
	102	100.2	103.1
	105	103.2	106.1
	111	109.2	112.1
	114	112.2	115.1
P2040	152	151.9	154.7
	155	154.9	157.7
	158	157.9	160.7
	161	160.9	163.7
	164	163.9	166.7
	167	166.9	169.7
P643	160	159.1	160.9
	162	161.1	162.9
	164	163.1	164.9
	166	165.1	166.9
	168	167.1	168.9
	170	169.1	170.9
	172	171.1	172.9
	174	173.1	174.9
	176	175.1	176.9
	178	177.1	178.9
P788	129	128.1	129.9
	131	130.1	131.9
	133	132.1	133.9
	135	134.1	135.9
	137	136.1	137.9
	139	138.1	139.9
	141	140.1	141.9
P1129	136	134.6	137.4
	139	137.6	140.4
	142	140.6	143.4
	145	143.6	146.4
	148	146.6	149.4
P2039	102	100.6	103.4
	111	109.6	112.449
	114	112.6	115.4
	123	121.6	124.4

[a]Data from Biasi et al. (2016) was used as a starting point for determining bins.

Supplemental Table 4. Microsatellite allele calls for seventy isolates examined in this study.

Sample	State	Date	Host	Population[a]	P1509	P5	P17	P15	P2040	P643	P788	P2039	P1129
6BHB2_NC2017	NC	2017	*S_tuberosum*	1	172/172/0	196/220/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
6BJB1_NC2017	NC	2017	*S_tuberosum*	1	0/0/0	196/220/0	108/144/0	96/96/0	158/158/0	166/174/0	139/141/0	102/123/0	142/145/0
Pasquotank_P3_C1	NC	2017	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	96/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pasquotank_P4_C2	NC	2017	*S_tuberosum*	1	0/0/0	196/220/0	108/144/0	84/96/0	158/158/0	166/176/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_1_L1	NC	2018	*S_tuberosum*	1	156/168/0	196/220/0	108/144/0	96/96/0	158/158/0	166/178/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_1_L2	NC	2018	*S_tuberosum*	1	0/0/0	196/220/0	108/144/0	96/96/0	158/158/0	166/178/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_1_L3	NC	2018	*S_tuberosum*	1	158/158/0	196/196/0	108/144/0	96/96/0	158/158/0	166/178/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_2_L2	NC	2018	*S_tuberosum*	2	156/156/0	196/228/0	108/132/0	78/96/0	158/158/0	166/166/0	137/139/0	102/144/0	142/145/0
Pnic_NC18_2_S2	NC	2018	*S_tuberosum*	2	0/0/0	220/224/0	108/132/0	78/96/0	158/164/0	166/166/0	137/137/0	114/114/0	142/142/0
Pnic_NC18_3	NC	2018	*S_tuberosum*	NA	0/0/0	196/196/0	108/144/0	96/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_4_L1	NC	2018	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	96/96/0	158/161/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_4_L2	NC	2018	*S_tuberosum*	NA	0/0/0	196/196/0	108/144/0	96/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_7_L1	NC	2018	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_7_L2	NC	2018	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	166/170/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_8_L1	NC	2018	*S_tuberosum*	NA	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_8_L3	NC	2018	*S_tuberosum*	NA	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_9	NC	2018	*S_lycopersicum*	2	160/172/0	192/216/0	129/147/0	72/84/0	158/158/0	168/168/0	131/139/0	102/123/0	142/145/0
Pnic_NC18_11	NC	2018	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	170/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC18_12_L1	NC	2018	*S_tuberosum*	NA	0/0/0	220/224/0	108/132/0	78/96/0	158/164/0	166/166/0	137/137/0	114/114/0	142/142/0
Pnic_NC18_12_S1	NC	2018	*S_tuberosum*	2	160/160/0	220/224/0	108/132/0	78/96/0	158/164/0	166/166/0	137/137/0	114/114/0	142/142/0
Pnic_NC18_12_S2	NC	2018	*S_tuberosum*	2	0/0/0	220/224/0	108/132/0	78/96/0	158/164/0	166/166/0	137/137/0	102/102/0	142/142/0
Pnic_NY18_1	NY	2018	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY18_3	NY	2018	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	111/111/0	139/142/0
Pnic_NY18_4	NY	2018	*S_tuberosum*	2	0/0/0	192/204/0	132/147/0	81/96/0	158/161/0	166/168/0	137/139/0	102/114/0	142/142/0
Pnic_NY19_1	NY	2019	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY19_1_L2	NY	2019	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY19_2_L2	NY	2019	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_PA19_1	PA	2019	*S_lycopersicum*	2	166/166/0	192/216/0	132/144/0	78/102/0	158/167/0	166/170/0	137/137/0	114/123/0	142/145/0
Pnic_PA19_1_L2	PA	2019	*S_lycopersicum*	NA	166/166/0	192/216/0	132/144/0	78/102/0	158/167/0	166/170/0	137/137/0	114/123/0	142/145/0
Pnic_PA19_2_L2	PA	2019	*S_tuberosum*	2	0/0/0	196/224/0	108/108/0	78/96/0	158/158/0	164/166/0	137/137/0	114/123/0	142/145/0
Pnic_PA19_3_L2	PA	2019	*S_tuberosum*	NA	0/0/0	196/224/0	108/108/0	78/96/0	158/158/0	164/166/0	137/137/0	114/123/0	142/145/0
Pnic_VA19_1_L1	VA	2019	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_VA19_1_L2	VA	2019	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	78/96/0	158/161/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_VA19_1_S1	VA	2019	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	78/96/0	158/161/0	166/166/0	137/137/0	102/123/0	145/148/0

Pnic_VA19_1_S2	VA	2019	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	75/96/0	158/161/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NC20_1_1	NC	2020	*S_tuberosum*	1	0/0/0	196/200/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	145/148/0
Pnic_NC20_2_1	NC	2020	*S_tuberosum*	2	0/0/0	196/220/0	108/108/0	78/96/0	158/158/0	166/166/0	137/137/0	114/123/0	142/145/0
Pnic_NC20_2_2	NC	2020	*S_tuberosum*	2	154/154/0	196/196/0	108/108/0	78/96/0	158/158/0	166/166/0	137/137/0	114/123/0	142/145/0
Pnic_NC20_3_L1_1	NC	2020	*S_tuberosum*	1	152/152/0	196/196/0	108/144/0	84/96/0	158/158/0	166/176/0	137/139/0	102/123/0	142/145/0
Pnic_NC20_3_S1_1	NC	2020	*S_tuberosum*	NA	0/0/0	196/196/0	108/144/0	84/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC20_4_L2_1	NC	2020	*S_tuberosum*	2	166/166/0	196/196/0	129/132/0	78/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC20_4_S1_1	NC	2020	*S_tuberosum*	2	166/166/0	196/220/0	129/132/0	78/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC20_5	NC	2020	*S_lycopersicum*	2	0/0/0	188/192/0	116/132/0	96/111/0	155/158/0	NA	129/139/0	123/123/0	142/148/0
Pnic_NY20_1_L1	NY	2020	*S_tuberosum*	2	156/156/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY20_2_L1	NY	2020	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY20_2_L2	NY	2020	*S_tuberosum*	2	166/166/0	192/196/0	108/132/0	78/96/0	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NC21_1_L1	NC	2021	*S_tuberosum*	2	156/156/0	196/220/0	108/132/0	75/96/0	158/158/0	166/166/0	137/139/0	102/114/0	142/145/0
Pnic_NC21_1_L2	NC	2021	*S_tuberosum*	1	158/168/0	196/196/0	108/144/0	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_2_L1	NC	2021	*S_tuberosum*	2	156/156/0	196/224/0	108/132/0	75/96/0	158/158/0	166/166/0	137/139/0	102/114/0	142/145/0
Pnic_NC21_2_L2	NC	2021	*S_tuberosum*	1	160/160/0	196/196/0	108/144/0	96/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_3_L1	NC	2021	*S_tuberosum*	1	174/174/0	196/196/0	108/144/0	84/99/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_3_L2	NC	2021	*S_tuberosum*	1	164/164/0	196/196/0	108/144/0	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_4_L1	NC	2021	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	81/96/0	158/158/0	166/172/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_4_L2	NC	2021	*S_tuberosum*	1	160/160/0	196/196/0	108/144/0	81/96/0	158/158/0	166/172/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_5_L1	NC	2021	*S_tuberosum*	2	166/166/0	196/220/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_5_L2	NC	2021	*S_tuberosum*	1	150/164/0	NA	NA	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	145/145/0
Pnic_NC21_6_L1	NC	2021	*S_tuberosum*	2	156/156/0	196/224/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_6_L2	NC	2021	*S_tuberosum*	2	156/156/0	196/220/0	108/132/0	75/96/0	158/158/0	166/170/0	137/139/0	102/114/0	142/145/0
Pnic_NC21_7_L1	NC	2021	*S_tuberosum*	2	168/168/0	196/224/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_7_L2	NC	2021	*S_tuberosum*	NA	168/168/0	196/224/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_8_L1	NC	2021	*S_tuberosum*	1	164/164/0	196/196/0	108/144/0	84/96/0	158/158/0	166/176/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_8_L2	NC	2021	*S_tuberosum*	1	166/166/0	196/196/0	108/144/0	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_9_L1	NC	2021	*S_tuberosum*	NA	166/166/0	196/220/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_9_L2	NC	2021	*S_tuberosum*	NA	166/166/0	196/220/0	129/132/0	75/96/0	158/158/0	160/166/0	129/139/0	102/114/0	142/142/0
Pnic_NC21_10_L1	NC	2021	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_NC21_10_L2	NC	2021	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	NA	NA	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_MD21_2	MD	2021	*S_tuberosum*	1	0/0/0	196/196/0	108/144/0	81/96/0	158/158/0	166/174/0	137/139/0	102/123/0	142/145/0
Pnic_MD21_3	MD	2021	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	NA	158/158/0	166/166/0	137/137/0	102/123/0	145/148/0
Pnic_NY21_3_S1	NY	2021	*S_tuberosum*	2	0/0/0	192/196/0	108/132/0	NA	NA	166/166/0	135/137/0	102/123/0	145/148/0

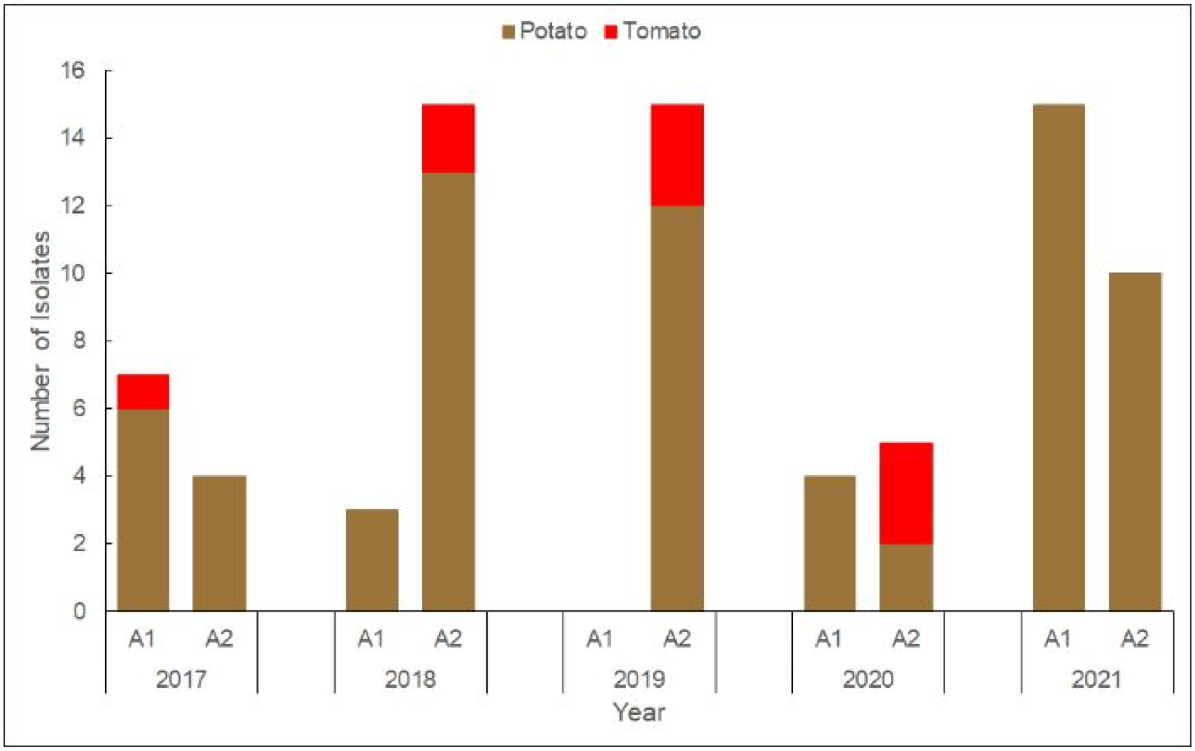
Pnic_VA21_1_L2	VA	2021	*S_tuberosum*	NA	0/0/0	192/196/0	108/132/0	75/96/0	158/161/0	166/166/0	137/137/0	102/123/0	145/148/0

[a] Population assignment as designated by STRUCTURE: 1: NC-MD; 2: NC-NY-PA-VA-MD; NA: Sample was not analyzed due to removal via clone correction.

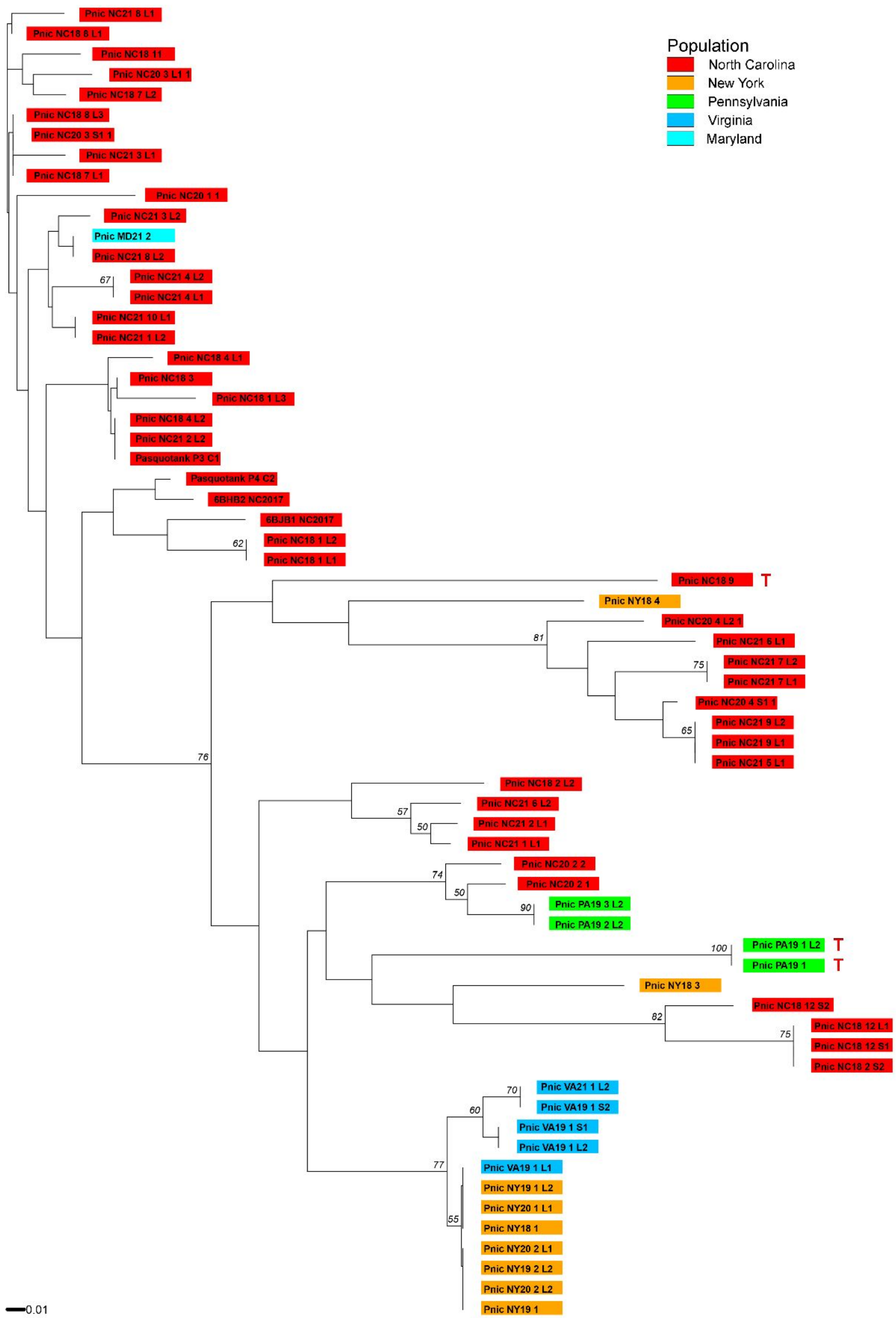
Supplemental Table 5 Index of association (Ia), and the standardized index of association ($\bar{r}_d$) calculated using the *poppr* function *ia* on a clone corrected dataset of *P. nicotianae*. The significance of each index was calculated using 999 permutations of the data.

	Ia[a]	$\bar{r}_d$
All	1.368***	0.177***
State		
North Carolina	1.892***	0.246***
New York	1.727**	0.248***
Virginia	-0.500(NS)	-0.500(NS)
STRUCTURE		
NC-MD	-0.121(NS)	-0.024(NS)
NC-NY-PA-VA-MD	1.291***	0.166***

[a]: NS: not significant; *: n<0.01; **: 0.001<n>0.01; ***: n<0.001



Supplemental Fig. 1. Frequency of mating types of *Phytophthora nicotianae* sorted by year and host from 2017 to 2021.



Supplemental Fig 2. Neighbor joining tree of populations of *Phytophthora nicotianae* collected between 2017 and 2021 from six states. Samples are color coded by state. Samples with a red "T" were collected from tomato while the rest were collected from potato.