Phytophthora capsici populations are structured by host, geography, and fluopicolide sensitivity

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ABSTRACT

Phytophthora capsici epidemics are propelled by warm temperatures and wet conditions. With temperatures and inland flooding in many locations worldwide expected to rise as a result of global climate change, understanding of population structure can help to inform management of *P. capsici* in the field and prevent devastating epidemics. Thus, we investigated the effect of host crop, geographical origin, fungicide sensitivity, and mating type on shaping the population structure of *P. capsici* in the eastern U.S. Our fungicide *in vitro* assays identified the emergence of insensitive isolates for fluopicolide and mefenoxam. A set of 12 microsatellite markers proved informative to assign 157 *P. capsici* isolates to five distinct genetic clusters. Implementation of Bayesian structure, population differentiation, genetic diversity statistics, and index of association analysis, allowed us to identify population structure by host with some correspondence with genetic clusters for cucumber and squash isolates. We found weak population structure by state for geographically close isolates. In this study, we discovered that North Carolina populations stratify by fluopicolide sensitivity with insensitive isolates experiencing nonrandom mating. Our findings highlight the need for careful monitoring of local field populations, improve selection of relevant isolates for breeding efforts, and hypervigilant surveillance of resistance to different fungicides.

INTRODUCTION

Vegetable produce occupy an important place in the world's food table, and have a crucial role in supporting rural economies. In the past decade, the United States (U.S.) fresh market and processing vegetable production doubled, reaching its highest peak in 2018 and remaining anchored at about 970,000 hectares harvested annually (USDA-NASS 2021). In 2019, production of processed and fresh vegetables valued at 14 billion dollars represented crucial components of the U.S. gross domestic product (Kassel and Morrison 2020). After California, U.S. states including Florida, Georgia, South Carolina, North Carolina, Tennessee, Virginia, Maryland, Delaware, New Jersey, New York, and Michigan produced 15% of the total U.S. vegetable fresh market for 2019 (USDA-NASS 2021). Snap beans, cucumbers, cantaloupe, honeydew, peppers, pumpkins, squash, and watermelon represent important specialty crops for many farmers in the U.S.. Altogether these farmers harvested up to 246,736 hectares in 2019 (USDA-NASS 2021). Annual growing seasons for these crops experience heavy rainfall, strong winds, and with the advent of climate change increased flooding events across the eastern and southern U.S. (USGCRP 2018). These climatic conditions propel outbreaks of diseases that in turn threaten production and economic viability of these crops (Ayala-Usma et al. 2019).

Every year the soilborne pathogen *Phytophthora capsici* threatens production of key specialty crops causing severe yield loss and increased expenses for disease control worldwide (Sánchez-Borges et al. 2015; Ali et al. 2017; Cara et al. 2017; Vélez-Olmedo et al. 2020; Zhao et al. 2020; Parada-Rojas et al. 2021a). The disease is known as Phytophthora blight as well as Phytophthora root, crown, or/and fruit rot according to its symptomatology (Parada-Rojas et al. 2021a). Since its first report in pepper fields in 1918, this oomycete pathogen has been reported on a wide range of crops including cucurbits, tomatoes, snap beans, and eggplants among others

(Leonian 1922; Kousik et al. 2015; Naegele et al. 2014; Parada-Rojas et al. 2021a). Plants infected with *P. capsici* experience symptoms that vary from foliar blighting, damping-off, wilting, and severe rotting of the roots, stem, and fruits (Hausbeck and Lamour 2004; Granke et al. 2012a). Flooding events, high humidity and temperatures above 19°C encourage disease development resulting in complete yield losses due to compromised root systems and advanced plant necrosis (Quesada-Ocampo et al. 2011a; Granke et al. 2012a; Bornt 2012; Vogel et al. 2020). The primary inoculum launching Phytophthora blight epidemics consists of sporangia produced copiously on the surface of infected plant material (Granke et al. 2009; Lamour et al. 2012b). Each sporangium harbors between 20 - 30 motile zoospores that disseminate through water in flooded soil. Zoospores exhibit chemoattraction to plant roots and adhere during the encystment process to ultimately produce germ tubes, effectively driving secondary outbreaks in the field (Erwin and Ribeiro 1996; Granke and Hausbeck 2009; Dunn and Smart 2015; Granke et al. 2011). Infested soil and water aid in the geographical dispersal of *P. capsici* from field to field (Gevens et al. 2007; Granke et al. 2009). *Phytophthora capsici* heterothallism presents an epidemiological advantage by allowing this oomycete pathogen to reproduce both asexually and sexually (Erwin and Ribeiro 1996). Isolates of *P. capsici* exist as mating types A1 and A2 which upon fusing of gametes (antheridia and oogonia) results in the production of oospores (Lamour et al. 2012a; Babadoost and Pavon 2013). Oospore production increases the evolutionary potential of *P. capsici* by generating genetic diversity through recombination and enabling the persistence of pathogen populations yearly during winter conditions (Pavón et al. 2007; Carlson et al. 2017; Vogel et al. 2020). The oospore thick cell walls made of cellulose and β-glucan confer the advantage to persist in the soils for several years and germinate to produce mycelium and

sporangia under wet conditions (Granke et al. 2012a; Babadoost and Pavon 2013; Parada-Rojas et al. 2021a).

Management of *P. capsici* epidemics is achieved through a combination of control strategies that range from crop rotation, avoidance of infested fields, monitoring irrigation sources, use of raised plant beds covered with plastic mulch, host resistance, frequent fungicide applications targeted at hot spots, destruction of infected plants, and early harvest (Lamour and Hausbeck 2003a, 2003b; Gevens et al. 2007; Foster and Hausbeck 2010; Quesada-Ocampo and Hausbeck 2010; Foster et al. 2012; Enzenbacher and Hausbeck 2012; Granke et al. 2012a; Parada-Rojas and Quesada-Ocampo 2019; Kousik et al. 2017; Quesada-Ocampo et al. 2016). Several fungicide classes, with prophylactic and corrective effects, are available to vegetable farmers in the U.S. for control of Phytophthora blight (Hausbeck and Lamour 2004; Granke et al. 2012a). The most frequently used fungicides against *P. capsici* include mefenoxam, fluopicolide, dimethomorph, mandipropamid, and oxathiapiprolin. Mefenoxam (Fungicide Resistance Action Committee 4), a commonly used fungicide, inhibits ribosomal RNA synthesis, specifically RNA polymerization (Randall et al. 2014). Fluopicolide (FRAC 43) removes spectrin-like proteins in the cytoskeleton (Toquin et al. 2007). The carboxylic acid amides dimethomorph (FRAC 40) and mandipropamid (FRAC 40) impede cellulose synthesis (Feng and Baudoin 2018). Oxathiapiprolin (FRAC 49), an oxysterol binding protein homologue inhibitor (OSBPI), halts lipid transport and storage (Pasteris et al. 2016). Despite extension specialists advocating for the use of these fungicides in an alternating program approach, *P. capsici* remains at high risk for developing resistance to many of these fungicides. In the early 2000s, resistance to mefenoxam was reported in many *Phytophthora* spp. including *P. capsici* (Lamour and Hausbeck 2003b). Most recently, fluopicolide and oxathiapiprolin resistance has been documented in *P. capsici*

115 populations, suggesting potential selection pressures and spread of resistance in U.S. populations
116 (Keinath and Kousik 2011; Lu et al. 2011; Wang and Ji 2020; Siegenthaler and Hansen 2021).
117 The emergence of resistance, limited affordability of chemical interventions, and potential
118 changes in thresholds of fungicide residue levels compel the development of host resistance as a
119 sustainable management strategy (Michelmore et al. 2017). However, introgression of resistance
120 to *P. capsici* into commercially available cultivars for pepper, tomato, eggplant and cucurbits
121 remains challenging (Parada-Rojas and Quesada-Ocampo 2019; Quesada-Ocampo and Hausbeck
122 2010; Foster et al. 2012; Enzenbacher and Hausbeck 2012). In addition, virulence differences
123 among *P. capsici* isolates highlight the potential for the pathogen populations to eventually
124 overcome such desired resistance (Granke et al. 2012b; Parada-Rojas and Quesada-Ocampo
125 2019).

126 Climate change threatens agriculture production with extreme events such as hurricanes,
127 heatwaves, droughts, and inland flooding that continue to intensify (Scheelbeek et al. 2018;
128 Velásquez et al. 2018). These conditions exacerbate Phytophthora blight epidemics and require
129 an integrated approach to monitor and control new outbreaks (Anderson and Garton 2000;
130 LaMondia et al. 2010; Sánchez-Borges et al. 2015; Vélez-Olmedo et al. 2020). Combating
131 Phytophthora blight epidemics requires insights into the biology and evolution of *P. capsici.*
132 Understanding of pathogen population emergence, dispersal, reproduction, and diversity can
133 inform deployment of management strategies in the field (McDonald and Linde 2002; McDonald
134 and Mundt 2016; McDonald and Stukenbrock 2016). Today, we know that geographical origin,
135 mating type, and mefenoxam sensitivity can shape *P. capsici* population structure in the U.S.
136 (Quesada-Ocampo et al. 2011b; Carlson et al. 2017; Vogel et al. 2020). Little is known if other
137 factors such as fungicide resistance or host specialization imposed on the U.S. *P. capsici*

populations can contribute to shaping local pathogen populations. Knowledge of factors influencing evolution of *P. capsici* populations in the U.S is vital to evaluate and improve management practices for farms experiencing outbreaks. In this study, we examine the population structure of *P. capsici* isolates from multiple U.S. states to draw baseline inferences of whether *P. capsici* is structured by origin, host, fungicide sensitivity, or mating type. To establish the role of these factors in the structuring of *P. capsici* populations, we analyzed 157 isolates with previously developed microsatellite markers (Parada-Rojas and Quesada-Ocampo 2018). We aimed to determine if *P. capsici* populations genetically structure by geographic location (North Carolina, Tennessee, South Carolina, New York, New Jersey, Georgia, Michigan, and Florida), host crop (cucumber, pepper, pumpkin, squash, watermelon, zucchini, muskmelon, winter squash, bitter gourd, sponge gourd, snap beans, tomato, and creek water), fungicide sensitivity (mefenoxam, fluopicolide, dimethomorph, and oxathiapiprolin) and mating type (A1 and A2).

MATERIALS AND METHODS

Sampling, isolations, culturing, and DNA extraction.

Phytophthora capsici isolates included in this study were sourced from multiple states in the eastern US including NC, SC, TN, GA, FL, NJ, NY, and MI (Supplementary Fig. S1; Table S1). For NC isolates, we utilized the NC extension network and reports of outbreaks from the Plant Disease Insect Clinic at NC State University, to collect symptomatic plants, fruits, and water samples in seven NC counties across the state. Sampled counties are located across the three NC main geographic regions including the mountains, piedmont, and coastal plains. All samples were collected in 2015 and 2016 from farmer fields or adjacent creeks. The greatest

number of samples came from cucumber, followed by peppers, squash, zucchini, creek water samples, muskmelon, snap beans, watermelon, and winter squash. Symptomatic stems and fruits were surface disinfected with 20% v/v sodium hypochlorite for 5 min, then 70% v/v ethanol for 2 min, and rinsed with abundant sterile distilled water. A small incision was made to remove the surface tissue and three small infected tissue plugs were plated in V8 isolation media (V8-PARPH, 12 g of agar, 60 mg of $CaCO_3$, 50 mg of Hymexazol, 100 mg of Pentachloronitrobenzene, 10 mg of Rifampicin, 250 mg of Ampicillin, 10 mg of Pimaricin, 40 ml of V8 juice, and 960 ml of distilled water) (Parada-Rojas et al. 2021a). Plates were incubated for 3 days transferring a single plug from the edge of the growing colony to new V8-PARPH media. These plates were stored at room temperature (21 ºC) for 2 days and used to transfer a single hyphal tip to new media. Water creek samples were baited for *P. capsici* by submerging in the water sample 4 split hemp seeds. Baiting was conducted under dark and room temperature conditions for 5 days. One week after baiting setup, infested hemp pieces were plated on V8-PARPH media and allowed to grow at room temperature for 2 days. A single hyphal tip was transferred to new V8-PARPH media. A total of 90 isolates were collected from NC. Isolates from SC, TN, GA, FL, NJ, NY, and MI, including one previously described (12889) (Quesada-Ocampo et al. 2009) and the isolate used for sequencing the *P. capsici* genome (Lamour et al. 2012a) were received from collaborators and transferred to V8-PARPH plates.

All isolates were deposited in long term storage screw-cap tubes containing 1 ml of sterile water and two surface sterilized hemp seeds as described by Parada-Rojas et al. (2021). For genomic DNA extraction one-week-old mycelia from each isolate was vacuum-harvested from UCV8 broth plates and freeze-dried for 48 h. Genomic DNA was extracted from ground freeze-dried mycelia as previously described (Parada-Rojas and Quesada-Ocampo 2018). All isolates in this

study were confirmed as *P. capsici* by performing ID-PCR using COX, NAD, ITS primers followed by Sanger sequencing (Quesada-Ocampo et al. 2011b) (Table S1).

Phenotypic characterization

Immediately after isolation or recovery from long term storage, mating type determination and fungicide sensitivity assays were conducted on all 157 *P. capsici* isolates (Table S1). Mating type assays were conducted as described previously (Parada-Rojas et al. 2021a). In brief, isolates were cocultured on unclarified V8 agar plates with MI isolates of known mating type (A1: 12898 and A2: SP98)(Quesada-Ocampo et al. 2011b). After incubating plates in the dark for 2 weeks, mating type membership was determined by verifying the presence or absence of oospores in the interface between isolates using a compound microscope. To characterize the extent of fungicide insensitivity associated with *P. capsici* isolates, a single discriminatory concentration for each of 4 commonly used fungicides (mefenoxam, fluopicolide, dimethomorph, and oxathiapiprolin) was selected to screen the entire population of isolates *in vitro.* For mefenoxam sensitivity plate assays, a standard concentration of 100 mg/L of mefenoxam was selected as previously described by Lamour and Hausbeck (2000) (Ridomil Gold EC; Syngenta AG, Basel, Switzerland). For fluopicolide, dimethomorph, and oxathiapiprolin, minimum inhibitory concentrations were calculated by exposing a diverse panel of 20 isolates to varying concentrations of UCV8 amended with fungicides. The concentrations tested for these three fungicide active ingridients (a.i.) ranged from 0, 0.5, 1.0, 1.5, 2.0, 3.0 mg a.i./L for fluopicolide (Presidio, Valent, California, U.S.), 0, 0.5, 1.0, 2.0, 3.0, 5.0 mg a.i./L for dimethomorph (Acrobat, BASF Corp., NC, U.S.), and 0, $1x10^{-4}$, $2.5x10^{-4}$, $5x10^{-4}$, 0.001, 0.005, 0.01 mg a.i./L for oxathiapiprolin (Orondis SC, Syngenta AG, Basel, Switzerland). A 5 mm plug

from the edge of an actively growing colony on UCV8 was transferred to UCV8 media amended with each of the tested concentrations. For each isolate, two plates were included for each fungicide concentration and two perpendicular colony diameters per plate were measured and averaged. The experiment was repeated once. Isolates were incubated for 4 days at 23°C in constant light conditions. Colony diameter was used to calculate percentage growth under each concentration. The lowest concentration in which 90 - 100% of isolates exhibited no growth on amended media was identified as the discriminatory concentration for either fluopicolide, dimethomorph, or oxathiapiprolin.

Upon determining the minimum inhibitory concentration for fluopicolide (2 mg/L) , dimethomorph (2 mg/L), and oxathiapiprolin (0.005mg/L), isolates sensitivity was calculated by transferring a 5 mm UCV8 agar plug to duplicate UCV8 plates amended with respective fungicide concentrations. Unamended UCV8 plates served as controls for each isolate tested. Inoculated plates were incubated at 23C for 4 days under light conditions and colony diameters were measured twice perpendicularly using a caliper (Traceable® Products, Texas, U.S.). Percent growth of each isolate on respective amended media was calculated using the equation (Lamour and Hausbeck 2003b) (1) :

$$\text{Percentage growth} = \frac{\underline{x}a - p}{\underline{x}c - p} \times 100 \quad \text{Equation (1)}$$

Where p, the transfer plug diameter (5 mm), is subtracted from the mean diameter of each colony and dividing the average diameter of the amended plates ($\underline{x}a$) by the average diameter of the unamended control plates ($\underline{x}c$). For each isolate and each fungicide, two replicates were used to measure twice perpendicular colony diameters per plate. The entire experiment was conducted twice. Isolates were assigned putative fungicide sensitivities based on the percentage growth compared to the control. Isolates scoring less than 30% of the control were classified as

sensitive, intermediately sensitive if growth was between 30 and 90% of the control, and insensitive if growth was greater than 90% of the control (Lamour and Hausbeck 2000).

Microsatellite genotyping.

To provide insight into the population structure of *P. capsici*, a previously characterized set of 12 polymorphic and codominant microsatellite markers were selected for genotyping (Table S2) (Parada-Rojas and Quesada-Ocampo 2018). Genomic DNA from each of 157 *P. capsici* isolates was amplified following the protocol described in Parada-Rojas and Quesada-Ocampo (2018). Amplicons labeled with different fluorescent dyes were diluted 100-fold and pool-plexed in 96-well plates. Fragment analysis was conducted in the 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) by adding HiDi Formamide and Liz600 size standard ladder to each well. Samples were genotyped at the Genomic Science Laboratory in North Carolina State University. A standardized peak calling and binning protocol was implemented on raw data to call allele sizes using the Geneious Microsatellite Plug-In (https://www.geneious.com/tutorials/microsatellites/) as previously described in Parada-Rojas and Quesada-Ocampo (2018). All genotyping data are available at https://figshare.com/s/968924dc4779eaa4a44b

Population structure and differentiation.

To assess broadscale population structure and determine the optimal number of clusters within *P. capsici* isolates, we used the Bayesian clustering and the admixture model with correlated allele frequencies. The Bayesian clustering method was implemented in the program STRUCTURE v. 2.3.4 (Pritchard et al. 2000), which we ran through Structure_threader (Pina-

Martins et al. 2017). The admixture model with no population information was run for 10 replicates on each K (K = 1 to 10) with 1'000,000 Monte Carlo Markov chain (MCMC) generations and an additional burn-in of 10'000,000 generations. We used the program Structure Harvester to assess the most optimal number of genetic clusters (K). Population structure figures sorted by the proportional membership (Q) of an isolate in a cluster were generated using the R packages *pophelper* and *ggplot2*. Figures allowed for the visualization of genetic cluster distribution in predefined categories of host, location, and fungicide sensitivity. To determine population differentiation across K, host, location, and fungicide sensitivity categories, we computed pairwise comparisons of F_{ST} values from populations using Arlequin v3.5.2 (Excoffier and Lischer 2010). To test the significance of calculated distances, we performed 1,000 permutations with a *P* value significant level set at 0.05. Genetic differentiation was interpreted to be low (<0.10), moderate (0.10 to 0.20), or high (>0.20) consistent with the guidelines proposed by Hartl and Clark (2007). The Arlequin F_{ST} pairwise output matrix was parsed and used to generate a matrix of pairwise genetic differentiation (F_{ST}) among *P. capsici* populations using the R packages *XML*, *corrplot*, *magrittr*, and *dplyr*.

Genotypic diversity and recombination analysis.

To compare genotypic diversity of *P. capsici* populations across K, host, location, and fungicide sensitivity categories, we examined the Shannon-Wiener index (H) and Nei's unbiased gene diversity index (Hexp) as calculated with the R packages *poppr* and *vegan* (Shannon and Weaver 1949; Nei 1978). Only clone-corrected populations and categories with at least eight individuals were included for analysis. To determine if populations are experiencing sexual recombination across K, host, location, and fungicide sensitivity, we employed the observed

index of association (rbarD) with 999 permutations as calculated by the R package *poppr* (Kamvar et al. 2014). The rbarD accounts for the number of loci tested and it is considered a more robust statistic (Agapow and Burt 2001). The observed index of association allowed us to test the null hypothesis that alleles observed at different loci are not linked therefore populations are expected to be under random mating (Kamvar et al. 2014).

RESULTS

Phenotypic characterization. Current *P. capsici* populations in the eastern U.S. span diverse geographical areas and environments across multiple states and latitudes. To understand the factors that drive *P. capsici* population structure, our analysis included a diverse collection of isolates sampled across 8 U.S. states that stretched 20 degrees of latitude and longitude from FL to MI (Supplementary Fig. S1). The 157 isolates of *P. capsici* were obtained from either laboratory culture collections (42%) or directly from field populations (58%) between 2015 to 2017 (Table S1). Isolates sourced from NC (N = 90) were highly represented in our collection followed by isolates obtained from NY, SC, TN, NJ, GA, MI, and FL. All except for three creek-baited isolates and two cross-field isolates derived from symptomatic tissues of diverse hosts that include 3 plant families (Table S1). Mating type A2 isolates (51%) comprised a slightly higher percentage of isolates compared to mating type A1 isolates (48%). To phenotypically characterize the degree of fungicide sensitivity among *P. capsici* isolates, we assessed the sensitivity to four commonly used fungicides (Fig. 1). A total of 23 and 10 *P. capsici* isolates registered insensitivity values >90 relative colony diameter (RCD) in fluopicolide and mefenoxam assays, respectively. Both fluopicolide and mefenoxam assays also revealed 9 and 19 isolates under intermediately sensitive RCD values (>30 RCD), respectively. Dimethomorph

299 and oxathiapiprolin sensitivity assays recorded more than 98% of the isolates as sensitive
300 without a single isolate exhibiting > 90 RCD for both fungicides (Fig. 1).

301 **Population structure and differentiation.** We genotyped all 157 isolates using 12
302 polymorphic microsatellite markers yielding allele tables for our population genetic analysis
303 (https://figshare.com/s/968924dc4779eaa4a44b). To assign *P. capsici* isolates to specific genetic
304 clusters, we performed a Bayesian clustering analysis implemented in STRUCTURE. A total of
305 K = 5 clusters yielded the strongest support based on the Evanno method (Supplementary Fig.
306 S2A and B). Visual inspection of admixture plots for K = 5 revealed 3 clusters with distinctly
307 supported membership (K2, K3, and K4), however, some isolates within these clusters shared
308 membership with other clusters, mainly K1 and K5 (Supplementary Fig. S2). We found that
309 cluster K1 and K5 showed admixed genotypes with contributions from all other clusters. To
310 characterize the level of genetic diversity accumulated in each of the 5 clusters, we referenced
311 both the Shannon-Wiener index (H) and Nei's unbiased gene diversity index (Hexp). Our genetic
312 diversity analysis showed a global H of 4.88 and Hexp of 0.57. Genetic diversity estimates for
313 isolates grouped by K indicated higher genetic diversity for K5 (H =3.47; Hexp = 0.53) and K1
314 (H= 3.69; Hexp=0.55). The lowest genetic diversity estimate corresponded to K3 (H = 2.56 ;
315 Hexp = 0.36) (Table 1). Our Bayesian and genetic diversity analysis prompted us to calculate
316 pairwise population differentiation statistics (F_{ST}) among clusters. Clusters K2, K3, and K4
317 showed the highest degree of pairwise genetic differentiation (Supplementary Table S3). In
318 general, F_{ST} values reflected the highest degree of divergence between K3 and all other clusters,
319 while the pairwise differentiation between K1 and K5 was the lowest with $F_{ST} = 0.04$ ($P < 0.001$)
320 (Supplementary Table S3). To corroborate the degree of genetic differentiation observed among
321 clusters and gain insight into their mating, we estimated the I_A and rbarD, and calculated

statistical significance for each cluster after clone correction. Isolates within K3 and K4 clusters showed the highest and significant values for I_A and rbarD indicating nonrandom mating with no evidence of recombination within these clusters (Table 1). In contrast, K1 ($P = 0.026$), K2 ($P = 0.253$), and K5 ($P = 0.011$) exhibited the lowest I_A and rbarD values suggesting that these clusters experience random mating (Table 1).

To understand how these 5 clusters relate to the biology of *P. capsici*, we examined the population structure by host, state, and fungicide sensitivity. We found that cucumber and squash isolates assigned clearly to K2 and K3 clusters, respectively, with very little contribution from other clusters (Fig. 2). Pepper, pumpkin, watermelon, and zucchini isolates consisted of admixed genotypes with varying contributions from all clusters (Fig. 2). In terms of genetic diversity, pepper and pumpkin isolates recorded the highest values of gene diversity (Hexp) and MLG diversity (H) (Supplementary Table S4). Cucumber and squash isolates showed the highest significant degree of pairwise genetic differentiation with $F_{ST} = 0.22$ ($P < 0.001$). In general, F_{ST} values were lower in pairwise comparisons among pepper, pumpkin, watermelon, and zucchini isolates (F_{ST} ranged from 0.02 to 0.07) (Supplementary Fig. S3; Supplementary Table S5). Regarding random mating, pepper, cucumber, pumpkin, zucchini, and squash isolates showed high and significant I_A and rbarD indicating nonrandom mating and low evidence of recombination within these isolates (Supplementary Table S4).

Our population structure analysis by state of origin revealed a weak population structure in most states, with all genetic clusters represented in most populations. Isolates from NC showed contributions from all clusters K1-K5 (Fig. 3). Isolates from GA, SC, and TN shared a similar pattern of cluster membership dominated by K1 and K5 clusters and little contribution from cluster K4. Northeastern (NY and NJ) isolates shared greater contributions from cluster K4

than GA, SC, and TN isolates (Fig. 3). After clone correction and by analyzing categories with at least eight individuals, NC isolates exhibited the highest value of MLG diversity (H = 4.32) followed by NY, TN and NJ. Isolates from GA and MI recorded lower MLG diversity among populations with N > 4 isolates (Supplementary Table S6). Analysis of pairwise population differentiation showed a high degree of divergence between NJ and SC isolates with F_{ST} = 0.21 (P < 0.001) (Supplementary Fig. S4). In contrast, the pairwise differentiation between NJ and NY was low with F_{ST} = 0.08 (P < 0.001) (Supplementary Fig. S4; Supplementary Table S7). When comparing F_{ST} values for populations from NC, TN, SC and GA, we observed low genetic differentiation with F_{ST} values ranging from 0.022 to 0.080 (Supplementary Fig. S4; Supplementary Table S7). In general, isolates from GA, NC, SC, NY, NJ, and MI exhibited high I_A and rbarD values indicating nonrandom mating and low evidence of recombination within these isolates (Supplementary Table S6). However, isolates from TN showed low I_A (0.025; P > 0.001) and rbarD (0.002; P > 0.001) values indicating that populations in TN experience random mating (Supplementary Table S6).

Our earlier finding of emerging insensitivity to fluopicolide among *P. capsici* isolates prompted us to investigate whether fluopicolide sensitivity can shape the population structure of *P. capsici*. A nested population structure analysis by fluopicolide sensitivity and state of origin showed that NC isolates can be found in all sensitivity categories (ie. I, IS, and S) (Supplementary Fig. S5). To test if fluopicolide is structuring field *P. capsici* populations, we subsampled NC isolates and analyzed whether they cluster by fluopicolide sensitivity. We found that the NC *P. capsici* population partitioned at K = 2 clusters (Fig. 4). Interestingly, fluopicolide insensitive isolates clustered together (red) with very little admixture from the other cluster (blue), which consisted of fluopicolide sensitive and intermediately sensitive isolates (Fig. 4).

We observed a reduction in diversity estimates for fluopicolide insensitive isolates (H = 2.48; Hexp = 0.421) as compared to sensitive isolates (H = 4.09; Hexp = 0.548) (Table 2). The insensitive and sensitive isolates showed the highest degree of pairwise genetic differentiation with F_{ST} = 0.12 ($P < 0.001$) (Fig. 5). In contrast, the pairwise differentiation between sensitive and intermediate sensitive isolates was lower at F_{ST} = 0.09 (Supplementary Table S8, Fig. 5). The I_A and rbarD indices recorded the lowest values for intermediate sensitive isolates suggesting random mating. However, sensitive and insensitive isolates exhibited significantly higher rbarD values indicating nonrandom mating and low evidence of recombination within these isolates (Table 2).

DISCUSSION

Our population structure analysis on a collection of 157 *P. capsici* isolates revealed unanticipated complexity with isolates clustered in five distinct genetic groups. These clusters point to a wide range of population diversity in the U.S. *P. capsici* populations and facilitate the identification of features that are important for structuring field populations. Genetic clusters in *P. capsici* populations often correspond closely to groups of geographically similar origins (Dunn et al. 2010; Quesada-Ocampo et al. 2011b; Parada-Rojas and Quesada-Ocampo 2018; Vogel et al. 2020). However, little is known about the effect of host and fungicide exposure as features that shape populations of *P. capsici*. Our study provides a general explanation for these five genetic groupings of *P. capsici* populations that infect diverse hosts, occupy geographically distinct regions, and express different fungicide sensitivities. When it comes to hosts as a population structuring feature, we found that clusters are assigned to different hosts with varying contributions. Bayesian structure, population differentiation and diversity analyses highlighted

how some hosts are predominantly represented by a particular cluster. For example, clusters K2 and K3 mainly contribute to cucumber and squash isolates, respectively. While pepper, pumpkin and watermelon isolates consisted of diverse contributions from all clusters. Diversity statistics and F_{ST} values pointed to greater diversity and low genetic differentiation accumulated in pepper, pumpkin, and watermelon isolates. General correspondence between host affiliation and genetic grouping has been reported for *P. capsici* isolates infecting Solanaceae and Cucurbitaceae hosts (Bowers et al. 2007; Quesada-Ocampo et al. 2011b). However, these studies grouped isolates together in a single clade or genetic cluster independent of host origin (Bowers et al. 2007). We identified correspondence between genetic cluster and host affiliation in cucumber and squash isolates but less distinctive assignments for pepper, pumpkin, and watermelon isolates. Continued gains of diversity in pepper, pumpkin, and watermelon *P. capsici* isolates will play a critical role in the durability and deployment of resistant cultivars. Current breeding efforts, for example, for watermelon resistance to *P. capsici* rely on a single isolate for screenings (Kousik et al. 2018). This finding adds to the growing body of evidence that *P. capsici* isolates vary in virulence and that excessive dependence on few isolates to breed for resistance can prove inefficient if field populations are highly diverse (Lee et al. 2001; Islam et al. 2005; Granke et al. 2012b; Parada-Rojas and Quesada-Ocampo 2019). In addition, rbarD evidence points to nonrandom mating occurring within cucumber and squash isolates. Thus, continued surveillance of local pathogen populations infecting cucumber and squash proves beneficial to refine phenotyping efforts and avoid confounding effects (Wallace et al. 2020). Although our sampling of zucchini was limited to 13 isolates, it appears that zucchini isolates are expanding among several genetic clusters with more isolates represented by cluster K3. However, more temporal sampling is needed to confirm that cluster K3 isolates are adapting to

zucchini preferentially and if they specialize on *Cucurbita pepo* varieties such as zucchini and squash (Enzenbacher and Hausbeck 2012; Krasnow et al. 2017; Xanthopoulou et al. 2019). *Phytophthora capsici* adaptation to single or multiple hosts should not be disregarded but falls outside the scope of this study.

In terms of state of origin, we observed weak population structure for assigningment of genetic clusters to geographical regions; with the exception of NJ and NY isolates that shared contributions from cluster K4 and SC isolates with membership to cluster K3. Our microsatellite markers captured the geographical separation between SC and NJ populations, but yielded undefined population structure for TN, GA, NC and counties within states. Most of the inferred population structure in studies surveying isolates from diverse geographical origins report limited gene flow among *P. capsici* isolates (Dunn et al. 2010; Carlson et al. 2017; Vogel et al. 2020). This aligns with our findings of NJ and SC populations but remains unclear for TN, NC, and GA isolates. According to the biology of *P. capsici*, dispersal is limited to human movement, severe flooding that extends for miles, or movement of infected plant material (Parada-Rojas et al. 2021b, 2021b, 2021a). Our microsatellite markers are limited in their ability to discern geographical population structure in middle latitudes or nearby states (ie. NC and TN) as well as population structure by mating types (ie. A1 and A2). Diversity statistics and Fst values indicate high genetic diversity and low population differentiation between isolates from NC and TN. However, to our knowledge, there is no evidence of gene flow between TN and NC. Our study corroborates the presence of high genetic diversity and active random mating in TN, in agreement with a recent study describing the ratios of mating type A1 and A2 in TN field populations of *P. capsici* (Siegenthaler et al. 2020; Siegenthaler and Hansen 2021). The fact that we found evidence of random mating in TN populations is consistent with the presence of both

437 mating types of *P. capsici* in a specific geographic region (Pavón et al. 2007; Granke et al.
438 2012a; Carlson et al. 2017; Parada-Rojas et al. 2021a). This finding has implications for TN
439 vegetable growers as a population undergoing random mating can ultimately result in actively
440 evolving and overwintering populations (Carlson et al. 2017).

441 Since 1918, *P. capsici* has walloped vegetable farms in the U.S. and today continues to
442 wreak havoc on farms that experience more frequent and severe flooding events (Leonian 1922;
443 Siegenthaler and Hansen 2021). At the moment, a handful of fungicides appear to be effective
444 against *P. capsici* field populations (Wang and Ji 2020). But farmers must be wary of the
445 development of resistance to available fungicides. We recorded a concerning number of isolates
446 exhibiting insensitivity to fluopicolide in our *in vitro* assays. Driven by this result, we examined
447 NC isolates and observed the presence of two strongly supported genetic clusters that correspond
448 with sensitive and insensitive isolates (Fig. 4). Our observations agree with a recent study by
449 Siegenthaler and Hansen (2021), who reported widespread resistance to fluopicolide and
450 cyazofamid across isolates sampled in TN vegetable farms. Our findings highlight the potential
451 for fixation to either insensitive or sensitive phenotypes, depending on the presence or absence of
452 selection pressure by a fungicide, as intermediate sensitive isolates appear to undergo random
453 mating, maintaining fungicide resistance alleles in the population. We also observed significant
454 population differentiation between sensitive and insensitive isolates indicating that fluopicolide
455 imposes a selection pressure for *P. capsici* in NC. State and regional management
456 recommendations should consider adjusting fungicide programs to develop solutions that prevent
457 further development of fungicide resistance. Isolates with insensitive phenotype in fluopicolide
458 assays originated from a squash field in NC that experienced severe flooding after hurricane
459 Anna in 2015. A possible explanation for increased abundance of insensitive isolates from this

field is that environmental conditions helped to establish this nonrandom mating population as indicated by our rbarD analysis. Thus, these clonal genotypes predominated and utilized their fitness advantage of resistance to fluopicolide to prevail in the field, in the presence of fungicide selection pressure. To test this hypothesis, future research should investigate the potential population shifts before and after flooding events as water dispersal is associated with movement of the inoculum (Vogel et al. 2020). Taken together, our findings suggest the presence of diverse *P. capsici* populations in the U.S. that can be structured by host, state, and fluopicolide sensitivity. No single approach to combat *P. capsici* populations will be effective under the current population dynamics that we describe in the eastern U.S.. Our findings highlight the need for careful monitoring of local field populations, improving selection of relevant isolates for breeding efforts, and continued surveillance of resistance to different fungicides.

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TABLES

Table 1. Genetic diversity and index of association (I_A) estimates for clone-corrected *Phytophthora capsici* isolates grouped by membership from K1 to K5 genetic clusters

Genetic cluster	N[a]	MLG[b]	eMLG[c]	H[d]	G[e]	Lambda[f]	Hexp[g]	I_A[h]	rbarD[i]
K5	32	32	13	3.47	32	0.969	0.53	0.2449	0.0225
K1	40	40	13	3.69	40	0.975	0.557	0.1551	0.01423
K2	31	31	13	3.43	31	0.968	0.466	0.0637	0.00597
K4	16	16	13	2.77	16	0.938	0.434	0.7474*	0.07013*
K3	13	13	13	2.56	13	0.923	0.365	1.7836*	0.20524*
Total	132	132	13	4.88	132	0.992	0.573	0.217	0.0198

[a]N: number of individuals after clone correction.
[b]MLG: number of multilocus genotypes.
[c]eMLG: number of expected MLG at the smallest sample size based on rarefaction.
[d]H: Shannon-Wiener index of MLG diversity (Shannon and Weaver 1949).
[e]G: Stoddart and Taylor's index of MLG diversity (Stoddart and Taylor 1988).
[f]Lambda: Simpson's index (Simpson 1949), corrected for population size.
[g]Hexp: Nei's unbiased gene diversity (Nei 1978)
[h]I_A: index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.
[i]rbarD: standardized index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.

Table 2. Genetic diversity and index of association (I_A) estimates for North Carolina clone-corrected *Phytophthora capsici* isolates grouped by sensitivity to fluopicolide (Sensitive (S), intermediate (IS) and Insensitive (I)).

Fluopicolide sensitivity	N[a]	MLG[b]	eMLG[c]	H[d]	G[e]	Lambda[f]	Hexp[g]	I_A[h]	rbarD[i]
S	60	60	10	4.09	60	0.983	0.548	0.252*	0.0232*
I	12	12	10	2.48	12	0.917	0.421	3.382*	0.3239*
IS	3	3	3	1.1	3	0.667	0.606	-0.889	-0.0916
Total	75	75	10	4.32	75	0.987	0.566	0.326	0.0299

[a]N: number of individuals after clone correction.
[b]MLG: number of multilocus genotypes.
[c]eMLG: number of expected MLG at the smallest sample size based on rarefaction.
[d]H: Shannon-Wiener index of MLG diversity (Shannon and Weaver 1949).
[e]G: Stoddart and Taylor's index of MLG diversity (Stoddart and Taylor 1988).
[f]Lambda: Simpson's index (Simpson 1949), corrected for population size.
[g]Hexp: Nei's unbiased gene diversity (Nei 1978)
[h]I_A: index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.
[i]rbarD: standardized index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.

Table S1. Isolate phenotypic information including origin, host, mating type, dimethomorph, mefenoxam, fluopicolide, and oxathiapiprolin sensitivity. All 157 isolates were confirmed as *Phytophthora capsici* using COX, NAD, and ITS sequencing (Quesada-Ocampo et al. 2011b).

Isolate name	State	County	Host	Mating Type	Dimethomorph	Mefenoxam	Fluopicolide	Oxathiapiprolin
LT1534N[a]	CFI	UK	CFI	A2	S	S	S	S
LT1534O[a]	CFI	UK	CFI	A2	S	S	S	S
GACP68 [b]	GA	Rabun	Pumpkin	A1	S	S	S	S
NC16_025 [b]	GA	Rabun	Pumpkin	A2	S	S	S	S
NC16_026 [b]	GA	Rabun	Pumpkin	A2	S	S	S	S
NC16_027 [b]	GA	Rabun	Pumpkin	A2	S	S	S	S
NC16_028 [b]	GA	Rabun	Pumpkin	A1	S	IS	S	S
I12889[a]	MI	UK	Pepper	A1	S	I	S	S
LT51[a]	MI	UK	Cucumber	A1	S	S	IS	S
OP97[a]	MI	UK	Cucumber	A1	S	S	IS	S
R1 [b]	NC	Sampson	Pepper	A1	S	S	S	S
SP98[a]	MI	UK	Pumpkin	A2	S	S	S	S
NC16_023 [b]	NC	McDowell	Pepper	A2	S	S	S	S
NC16_024 [b]	NC	McDowell	Pepper	A2	S	S	S	S
NC17257 [b]	NC	Wayne	Cucumber	A2	S	S	S	S
NC18800 [b]	NC	Sampson	Pepper	A2	S	IS	I	S
NC19385 [b]	NC	Henderson	Muskmelon	A1	S	S	IS	S
NC21064 [b]	NC	Sampson	Pepper	A1	S	S	S	S

NC21810 [b]	NC	Guilford	Zucchini	A2	S	IS	I	S
NC22256 [b]	NC	Henderson	Pepper	A2	S	S	S	S
NCCP03 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP04 [b]	NC	Guilford	Zucchini	A2	S	S	I	S
NCCP05 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP06 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP07 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP08 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP09 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP10 [b]	NC	Guilford	Squash	A1	S	S	S	S
NCCP11 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP12 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP13 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP14 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP15 [b]	NC	Guilford	Squash	A2	S	S	I	S
NCCP17 [b]	NC	Buncombe	Winter Squash	A2	S	S	I	S
NCCP18 [b]	NC	Wilson	Creek	A2	S	I	S	S
NCCP20 [b]	NC	Wilson	Creek	A1	S	I	S	S
NCCP21 [b]	NC	Wilson	Pepper	A2	S	S	I	S
NCCP22 [b]	NC	Guilford	Zucchini	A1	S	S	I	S
NCCP24 [b]	NC	Guilford	Zucchini	A2	S	S	S	S
NCCP25 [b]	NC	Guilford	Zucchini	A2	IS	IS	I	IS

NCCP26 [b]	NC	Guilford	Zucchini	A2	S	S	I	S
NCCP27 [b]	NC	Guilford	Zucchini	A2	S	IS	S	S
NCCP28 [b]	NC	Guilford	Zucchini	A1	S	S	I	S
NCCP29 [b]	NC	Guilford	Zucchini	A1	S	IS	S	S
NCCP30 [b]	NC	Guilford	Zucchini	A2	S	IS	I	S
NCCP31 [b]	NC	Guilford	Zucchini	A2	S	S	I	S
NCCP32 [b]	NC	Guilford	Creek	A1	S	S	I	S
NCCP33 [b]	NC	Wilson	Watermelon	A2	S	S	S	S
NCCP34 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP35 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP36 [b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP37 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP38 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP41 [b]	NC	Wilson	Cucumber	A2	S	IS	S	S
NCCP42 [b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP43 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP44 [b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP45 [b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP46 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP47 [b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP48 [b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP49 [b]	NC	Wilson	Cucumber	A1	S	S	S	S

NCCP50[b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP53[b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP54[b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP55[b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP56[b]	NC	Wilson	Cucumber	A1	S	S	S	S
NCCP57[b]	NC	Wilson	Cucumber	A2	S	S	S	S
NCCP60[b]	NC	McDowell	Snap Beans	A1	S	S	S	S
NCCP61[b]	NC	McDowell	Pepper	A1	S	S	S	S
NCCP62[b]	NC	McDowell	Pepper	A1	S	S	S	S
NCCP63[b]	NC	McDowell	Pepper	A2	S	S	S	S
NCCP64[b]	NC	McDowell	Pepper	A2	S	S	S	S
NCCP65[b]	NC	McDowell	Pepper	A1	S	IS	S	S
NCCP66[b]	NC	McDowell	Pepper	A1	S	S	S	S
NCCP67[b]	NC	McDowell	Pepper	A1	S	S	S	S
R2[b]	NC	Sampson	Pepper	A1	S	S	S	S
R255[b]	NC	UK	Pepper	A1	S	S	S	S
R293[a]	NC	UK	Pepper	A1	S	IS	S	S
R294[a]	NC	UK	Pepper	A2	S	S	S	S
R297[a]	NC	UK	Pepper	A2	S	S	S	S
R360[a]	NC	UK	Pepper	A1	S	IS	S	S
R364[a]	NC	UK	Pepper	A1	S	S	S	S
R371[a]	NC	UK	Pepper	A2	S	S	S	S

R372[a]	NC	UK	Pepper	A1	S	S	IS	S
R377[a]	NC	UK	Pepper	A2	S	S	S	S
R378[a]	NC	UK	Pepper	A2	S	S	S	S
R380[a]	NC	UK	Pepper	A2	S	S	S	S
R383[a]	NC	UK	Pepper	A1	S	S	S	S
R386[a]	NC	UK	Pepper	A1	S	S	S	S
R388[a]	NC	UK	Pepper	A1	S	S	S	S
R391[a]	NC	UK	Pepper	A2	S	S	S	S
R392[a]	NC	UK	Pepper	A1	S	S	S	S
R399[a]	NC	UK	Pepper	A1	S	S	S	S
R402[a]	NC	UK	Pepper	A1	S	IS	S	S
R410[a]	NC	UK	Pepper	A1	S	S	S	S
R411[a]	NC	UK	Pepper	A1	S	S	S	S
R412[a]	NC	UK	Pepper	A1	S	I	S	S
R413[a]	NC	UK	Pepper	A1	S	S	S	S
R415[a]	NC	UK	Pepper	A2	S	S	S	S
R419[a]	NC	UK	Pepper	A2	S	IS	IS	S
R313[a]	NJ	UK	Pepper	A2	S	S	S	S
R315[a]	NJ	UK	Pepper	A1	S	IS	S	S
R317[a]	NJ	UK	Pepper	A2	S	I	S	S
R319[a]	NJ	UK	Pepper	UK	S	S	S	S
R320[a]	NJ	UK	Pepper	A1	S	S	S	S

R322[a]	NJ	UK	Pepper	A1	S	I	S	S
R328[a]	NJ	UK	Pepper	A2	S	I	S	S
R331[a]	NJ	UK	Pepper	A1	S	S	S	S
LC4 [b]	SC	UK	Sponge Gourd	A2	S	S	S	S
LC5 [b]	SC	UK	Sponge Gourd	A2	S	S	S	S
LC6 [b]	SC	UK	Sponge Gourd	A2	S	S	S	S
MC1 [b]	SC	UK	Bitter Gourd	A2	S	S	S	S
MC2 [b]	SC	UK	Bitter Gourd	A2	S	S	S	S
RCZ_11[a]	SC	UK	Zucchini	A2	S	S	S	S
WLB_10 [b]	SC	Beaufort	Watermelon	A1	S	S	S	S
WLB_122 [b]	SC	Beaufort	Watermelon	A2	S	IS	S	S
WLB_150 [b]	SC	Beaufort	Watermelon	A2	S	S	S	S
WLB_155 [b]	SC	Beaufort	Watermelon	A2	S	S	S	S
WLB_230 [b]	SC	Beaufort	Watermelon	A1	S	S	S	S
WLB_231 [b]	SC	Beaufort	Watermelon	A1	S	S	S	S
WLB_232 [b]	SC	Beaufort	Watermelon	A1	S	S	S	S
WLB_33 [b]	SC	Beaufort	Watermelon	A1	S	S	S	S
WLB_8 [b]	SC	Beaufort	Watermelon	A1	S	S	IS	S
LT248[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT252[a]	TN	Grainger	Pumpkin	A2	S	S	S	S
LT261[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT263N[a]	TN	Grainger	Pumpkin	A2	S	S	S	S

LT263O[a]	TN	Grainger	Pumpkin	A2	S	S	S	S
LT265[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT266[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT267[a]	TN	Grainger	Pumpkin	A2	S	IS	S	S
LT269[a]	TN	Grainger	Pumpkin	A1	S	S	IS	S
C15 [b]	NY	Ontario	Pumpkin	A1	S	S	S	S
H5 [b]	NY	UK	Watermelon	A2	S	S	S	S
H8 [b]	NY	UK	Watermelon	A2	S	S	S	S
IMK_328 [b]	Fl	Collier	Watermelon	A2	S	S	S	S
WMG_43 [b]	GA	Tift	Watermelon	A1	S	S	S	S
LT245[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT249[a]	TN	Grainger	Pumpkin	A2	S	S	S	S
LT264[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT268[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT262[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
LT251[a]	TN	Grainger	Pumpkin	A1	S	S	S	S
I0664_1[a]	NY	Monroe	Pepper	A1	S	S	S	S
I6180[a]	NY	Ontario	Winter Squash	A2	S	I	IS	S
I0752_14[a]	NY	Herkimer	Zucchini	A1	S	IS	S	S
A2_6_2[a]	NY	Ontario	Winter Squash	A1	S	S	S	S
EH_21A[a]	NY	Ontario	Pumpkin	A1	S	S	S	S
EH_84A[a]	NY	Ontario	Pumpkin	A2	IS	S	IS	S

GP_5A[a]	NY	Suffolk	Pumpkin	A2	S	I	S	S
GT_1A[a]	NY	Schenectady	Tomato	A1	S	IS	S	S
MM2_6A[a]	NY	Suffolk	Pepper	A2	S	S	S	S
MMG_1A[a]	NY	Suffolk	Pumpkin	A1	S	IS	S	S
MML_03A[a]	NY	Suffolk	Pumpkin	A2	S	I	S	S
MMZ_46C[a]	NY	Suffolk	Pumpkin	A1	S	S	S	S
MMH_4A[a]	NY	Suffolk	Pumpkin	A1	S	S	S	S

(UK = unknown, CFI = Cross-field isolate, [a] = laboratory culture collection, [b] = field population)

Supplementary Table S2. Diversity statistics of 12 microsatellites used to analyze the population structure of *Phytophthora capsici* isolates. Na: Number of alleles, He: gene diversity, Evenness: a measure of the distribution of Multilocus Genotypes (MLGs) within the isolates.

SSR id	SSR motif	Primer sequence	Allelic size range	Na	He	Evenness
Phyca_SSR07	(GA)6	F: CTCTGGCATTGAAAGAGCGC R: CCCAAAGTTGCGCCATTTGA	352-358	3	0.62	0.91
Phyca_SSR11z	(CAG)4	F:CAGCAACAGCAACAGTCGTC R:TCCAAGTCGCTCGTCTGAAC	178-223	5	0.70	0.81
Phyca_SSR13	(CAG)5	F:GAACACATCCGATTCGCAGC R:TTGCTGCTCAGATCCACTGG	122-134	4	0.70	0.88
Phyca_SSR14	(AAG)6	F:CAGAAACACACGTCTCCGGA R:GTTCGAACTGCTCCTGCTCT	217-229	5	0.61	0.79
Phyca_SSR15	(AGC)4	F:TCGTCGTTTTCCTCTGTGCA R:TTGAACTTCATCGCAGCCCT	178-181	2	0.35	0.76
Phyca_SSR17	(AAG)6	F:TATCGGACGTTCTCGCCATG R:TGAGCGGTTTCTGCTCGAAT	126 -129	2	0.49	0.98
Phyca_SSR18	(AGC)6	F:GGACGATATCATGCAGCCGA R:CCGAGTCTGAACCCGAAGAG	271-280	3	0.51	0.86
Phyca_SSR20	(AAG)7	F:CACGGAAGCTCAACGCAAAA R:GAGGTTGTCAGTGCTGTCGA	246-258	5	0.61	0.8
Phyca_SSR30	(CCAG)6	F:CACAGCCTCTCGACCGGA R:CGTTTTCCAGCACACCCTTG	286-306	6	0.76	0.85
Phyca_SSR40	(TCCTC)3	F:CAAGTCCCTGTCGTCGTTCT R:CATGGCAGTCACCGTCTCTT	210-215	2	0.47	0.98
Phyca_SSR41	(CACGAC)5	F:GACTACGACGTCTACCGCTG R:GACGTCGTGGTGGTCGTAG	105-123	3	0.53	0.77
Phyca_SSR50	(ACTTCA)4	F:GGGGCAGAAACGTCTCTGAA R:GGTCGTCGTCTGAGTCTGAC	237-249	2	0.48	0.97

Supplementary Table S3. Pairwise genetic differentiation (F_{ST}) among five genetic clusters of *Phytophthora capsici* calculated using 12 polymorphic microsatellite markers and computed from populations using Arlequin v3.5.2. Cells under the gray diagonal contain F_{ST} values for corresponding populations, while cells above the gray diagonal include corresponding *P* values.

	K5	**K1**	**K2**	**K4**	**K3**
K5		0.0000	0.0000	0.0000	0.0000
K1	0.05		0.0000	0.0000	0.0000
K2	0.06	0.07		0.0000	0.0000
K4	0.11	0.09	0.13		0.0000
K3	0.11	0.12	0.21	0.23	

Supplementary Table S4. Genetic diversity and index of association (I_A) estimates for clone-corrected *Phytophthora capsici* isolates grouped by host of origin.

Host	N[a]	MLG[b]	eMLG[c]	H[d]	G[e]	Lambda[f]	Hexp[g]	I_A[h]	rbarD[i]
CFI	1	1	1	0	1	0	0.583	NA	NA
Pumpkin	26	26	10	3.258	26	0.962	0.558	0.519*	0.0477*
Pepper	48	48	10	3.871	48	0.979	0.578	0.332*	0.0304*
Cucumber	22	22	10	3.091	22	0.955	0.483	0.952*	0.0894*
Muskmelon	1	1	1	0	1	0	0.417	NA	NA
Zucchini	12	12	10	2.485	12	0.917	0.522	1.23*	0.1146*
Squash	5	5	5	1.609	5	0.8	0.359	4.631*	0.5649*
Winter Squash	3	3	3	1.099	3	0.667	0.411	NA	NA
Creek	3	3	3	1.099	3	0.667	0.572	NA	NA
Watermelon	11	11	10	2.398	11	0.909	0.558	0.644	0.0596
Snap Beans	1	1	1	0	1	0	0.333	NA	NA
Sponge Gourd	2	2	2	0.693	2	0.5	0.375	NA	NA
Bitter Gourd	2	2	2	0.693	2	0.5	0.375	NA	NA
Tomato	1	1	1	0	1	0	0.5	NA	NA
Total	138	132	9.97	4.867	127	0.992	0.573	0.242	0.0221

[a]N: number of individuals.
[b]MLG: number of multilocus genotypes.
[c]eMLG: number of expected MLG at the smallest sample size based on rarefaction.
[d]H: Shannon-Wiener index of MLG diversity (Shannon and Weaver 1949).
[e]G: Stoddart and Taylor's index of MLG diversity (Stoddart and Taylor 1988).
[f]Lambda: Simpson's index (Simpson 1949), corrected for population size.
[g]Hexp: Nei's unbiased gene diversity (Nei 1978)

[h]I_A: index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.
[i]rbarD: standardized index of association for all individuals and for clone-corrected data (Brown et al. 1980). An asterisk indicates statistical significance ($P \leq 0.001$) compared with 999 resamplings.

Supplementary Table S5. Pairwise genetic differentiation (F_{ST}) among hosts of *Phytophthora capsici* populations calculated using 12 polymorphic microsatellite markers, computed from populations using Arlequin v3.5.2. Cells under the gray diagonal contain F_{ST} values for corresponding populations, while cells above the gray diagonal include corresponding *P* values.

	CFI[a]	Pump.[b]	Pepp.[c]	Cucu.[d]	Musk.[e]	Zucc.[f]	Squa.[g]	WinS.[h]	Cree.[i]	Wate.[j]	Snap.[k]	SpoG.[l]	BittG.[m]	Toma.[n]
CFI		0.6250	0.8477	0.4092	0.9990	0.5742	0.3223	0.4551	0.7559	0.2324	0.9990	0.3555	0.3184	0.9990
Pump.	0.00		0.0010	0.0000	0.9990	0.0049	0.0000	0.0342	0.8350	0.0449	0.9990	0.0332	0.0293	0.9990
Pepp.	0.01	0.01		0.0000	0.9990	0.0000	0.0000	0.0586	0.7412	0.0986	0.9990	0.0420	0.0479	0.9990
Cucu.	0.04	0.04	0.04		0.9990	0.0000	0.0000	0.0147	0.2227	0.0000	0.9990	0.0098	0.0059	0.9990
Musk.	0.11	0.02	0.04	0.08		0.3565	0.3154	0.4648	0.7256	0.4072	0.9990	0.3477	0.3125	0.9990
Zucc.	0.01	0.03	0.05	0.08	0.08		0.1484	0.1065	0.5732	0.0000	0.9990	0.1504	0.1729	0.9990
Squa.	0.09	0.08	0.12	0.19	0.13	0.03		0.2344	0.1260	0.0020	0.9990	0.1875	0.1572	0.9990
WinS.	0.05	0.06	0.06	0.10	0.07	0.06	0.08		0.3965	0.0137	0.9990	0.3174	0.3906	0.9990
Cree.	0.01	0.00	0.00	0.02	0.00	0.00	0.07	0.05		0.2607	0.9990	0.2129	0.2109	0.9990
Wate.	0.04	0.01	0.01	0.06	0.03	0.05	0.10	0.08	0.01		0.9990	0.1807	0.0762	0.9990
Snap.	0.19	0.12	0.10	0.08	0.27	0.17	0.25	0.25	0.08	0.09		0.3086	0.3594	0.9990
SpoG.	0.15	0.08	0.08	0.16	0.07	0.07	0.10	0.06	0.07	0.04	0.25		0.3311	0.9990
BittG.	0.15	0.09	0.09	0.16	0.04	0.07	0.10	0.06	0.08	0.05	0.25	0.00		0.9990
Toma.	0.00	0.04	0.02	0.15	0.17	0.08	0.12	0.15	0.04	0.01	0.23	0.16	0.17	

[a]CFI: Cross field isolate
[b]Pump.: Pumpkin (*Cucurbita pepo*)
[c]Pepp.: Pepper (*Capsicum annuum*)
[d]Cucu.: Cucumber (*Cucumis sativus*)
[e]Musk.: Muskmelon (*Cucumis melo*)
[f]Zucc.: Zucchini (*Cucurbita pepo*)
[g]Squa.: Squash (*Cucurbita pepo*)

[h]WinS.: Winter Squash (*Cucurbita maxima*)
[i]Cree.: Water creek
[j]Wate.: Watermelon (*Citrullus lanatus*)
[k]Snap.: Snap bean (*Phaseolus vulgaris*)
[l]SpoG.: Sponge Gourd (*Luffa aegyptiaca*)
[m]BittG.: Bitter melon (*Momordica charantia*)
[n]Toma.: Tomato (*Solanum lycopersicum*)

Supplementary Table S6. Genetic diversity and index of association (I_A) estimates for clone-corrected *Phytophthora capsici* isolates grouped by state of origin.

State	N[a]	MLG[b]	eMLG[c]	H[d]	G[e]	Lambda[f]	Hexp[g]	I_A[h]	rbarD[i]
CFI	1	1	1	0	1	0	0.583	NA	NA
GA	6	6	6	1.79	6	0.833	0.554	1.644**	0.16115**
MI	4	4	4	1.39	4	0.75	0.539	2.6044**	0.25508**
NC	75	75	10	4.32	75	0.987	0.566	0.3264*	0.02988*
NJ	8	8	8	2.08	8	0.875	0.438	3.8193*	0.35543*
SC	10	10	10	2.3	10	0.9	0.49	2.1267	0.20184
TN	12	12	10	2.48	12	0.917	0.509	0.0259	0.00245
NY	16	16	10	2.77	16	0.938	0.592	0.6187*	0.05721*
FL	1	1	1	0	1	0	0.364	NA	NA
Total	133	132	9.99	4.88	131	0.992	0.575	0.2289	0.02089

[a]N: number of individuals.
[b]MLG: number of multilocus genotypes.
[c]eMLG: number of expected MLG at the smallest sample size based on rarefaction.
[d]H: Shannon-Wiener index of MLG diversity (Shannon and Weaver 1949).
[e]G: Stoddart and Taylor's index of MLG diversity (Stoddart and Taylor 1988).
[f]Lambda: Simpson's index (Simpson 1949), corrected for population size.
[g]Hexp: Nei's unbiased gene diversity (Nei 1978)
[h]I_A: index of association for all individuals and for clone-corrected data (Brown et al. 1980). Asterisks indicate statistical significance (*$P \leq 0.001$, **$P \leq 0.05$) compared with 999 resamplings.
[i]rbarD: standardized index of association for all individuals and for clone-corrected data (Brown et al. 1980). Asterisks indicate statistical significance (*$P \leq 0.001$, **$P \leq 0.05$) compared with 999 resamplings.

Supplementary Table S7. Pairwise genetic differentiation (F_{ST}) among *Phytophthora capsici* populations by state of origin as calculated using 12 polymorphic microsatellite markers, computed from populations using Arlequin v3.5.2. Cells under the gray diagonal contain F_{ST} values for corresponding populations, while cells above the gray diagonal include corresponding *P* values.

	CFI	GA	MI	NC	NJ	SC	TN	NY	FL
CFI		0.5693	0.7881	0.8838	0.3486	0.0977	0.4766	0.8926	0.9990
GA	0.00		0.4600	0.8027	0.0127	0.0469	0.1611	0.5283	0.9990
MI	0.02	0.01		0.6006	0.0606	0.0020	0.0186	0.2344	0.9990
NC	0.00	0.00	0.01		0.0000	0.0020	0.0078	0.0186	0.9990
NJ	0.11	0.12	0.09	0.10		0.0010	0.0000	0.0068	0.9990
SC	0.08	0.02	0.09	0.04	0.18		0.0000	0.0000	0.9990
TN	0.00	0.01	0.04	0.02	0.10	0.06		0.0117	0.9990
NY	0.01	0.01	0.02	0.01	0.06	0.05	0.03		0.9990
FL	0.22	0.07	0.14	0.06	0.16	0.07	0.07	0.03	

Supplementary Table S8. Pairwise genetic differentiation (F_{ST}) among *Phytophthora capsici* populations by fluopicolide sensitivity as calculated using 12 polymorphic microsatellite markers, computed from populations using Arlequin v3.5.2. Cells under the gray diagonal contain F_{ST} values for corresponding populations, while cells above the gray diagonal include corresponding *P* values. (Sensitive (S), intermediate (IS) and Insensitive (I)).

	S	**I**	**IS**
S		0.0000	0.8711
I	0.12		0.0117
IS	0.00	0.09	

FIGURE CAPTIONS

Fig. 1. Frequency of dimethomorph, fluopicolide, mefenoxam, and oxathiapiprolin sensitivity in 157 *Phytophthora capsici* isolates. S = sensitive, <30% growth of control ; IS = intermediate, 30 to 90% growth of control; I = insensitive, >90% growth of control; and N = number of isolates.

Fig. 2. Estimated population structure by Bayesian clustering of 157 *Phytophthora capsici* isolates from eastern United States grouped by host of origin. Each isolate is represented by a thin vertical line, which is partitioned into K colored segments that represent the isolate's estimated membership ratio in a given K cluster. Populations are labeled above or below the figure, with their host affiliation including Bitter Gourd, Cross Field Isolate, Creek, Cucumber, Muskmelon, Pepper, Pumpkin, Snap beans, Sponge Gourd, Squash, Tomato, Watermelon, Winter Squash, or Zucchini.

Fig. 3. Estimated population structure by Bayesian clustering of 157 *Phytophthora capsici* isolates from eastern United States grouped by state of origin. Each isolate is represented by a thin vertical line, which is partitioned into K colored segments that represent the isolate's estimated membership ratio in a given K cluster. Populations are labeled above or below the figure, with their state affiliation including from left to right, Tennessee (TN), South Carolina (SC), New York (NY), New Jersey (NJ), North Carolina (NC), Michigan (MI), Georgia (GA), Florida (FL), and Cross field isolates (CFI).

Fig. 4. Estimated population structure by Bayesian clustering of 90 *Phytophthora capsici* isolates from North Carolina grouped by fluopicolide sensitivity. Each isolate is represented by a thin vertical line, which is partitioned into K colored segments that represent the isolate's estimated membership ratio in a given K cluster. Populations are labeled below the figure, with their fluopicolide sensitivity affiliation including from left to right, S = sensitive, IS = intermediate sensitive, and I = insensitive. The number of isolates (N) per genetic cluster is labeled above the figure.

Fig. 5. Pairwise genetic differentiation (F_{ST}) between fluopicolide sensitivity (Sensitive (S), intermediate sensitive (IS) and Insensitive (I)) of 90 clone corrected *Phytophthora capsici* isolates calculated using 12 polymorphic microsatellite markers, computed from populations using Arlequin v3.5.2, and graphed in R studio. *Significant ($P < 0.05$), **Significant ($P < 0.01$), ***Significant ($P < 0.001$). Colored scale bar represents an approximation to the F_{ST} value, darker blue indicates larger F_{ST} values while lighter blue to white indicate low F_{ST} values.

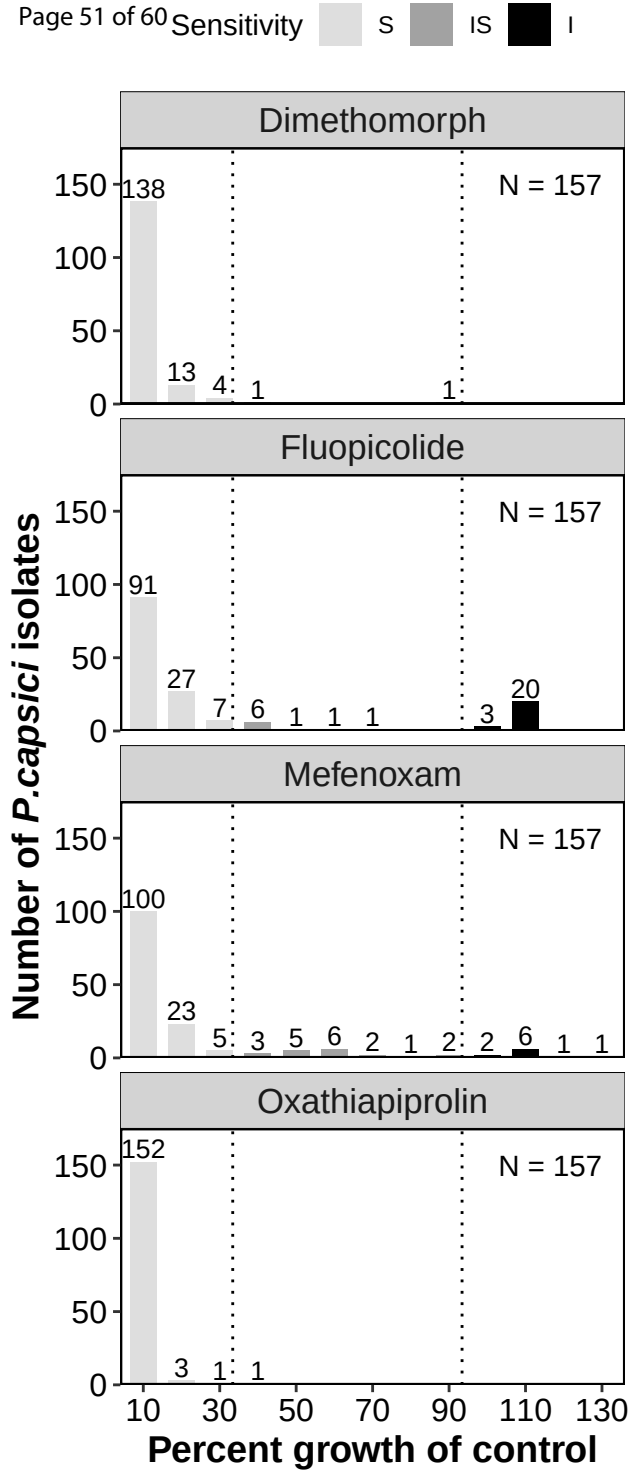
Supplementary Fig. S1. Spatial distribution of 157 *Phytophthora capsici* isolates utilized in this study as collected from the eastern of the United States. Points were randomly assigned to a location within the state where isolates were initially obtained. The number of isolates sampled from each state is represented by the size of the red dots in the map.

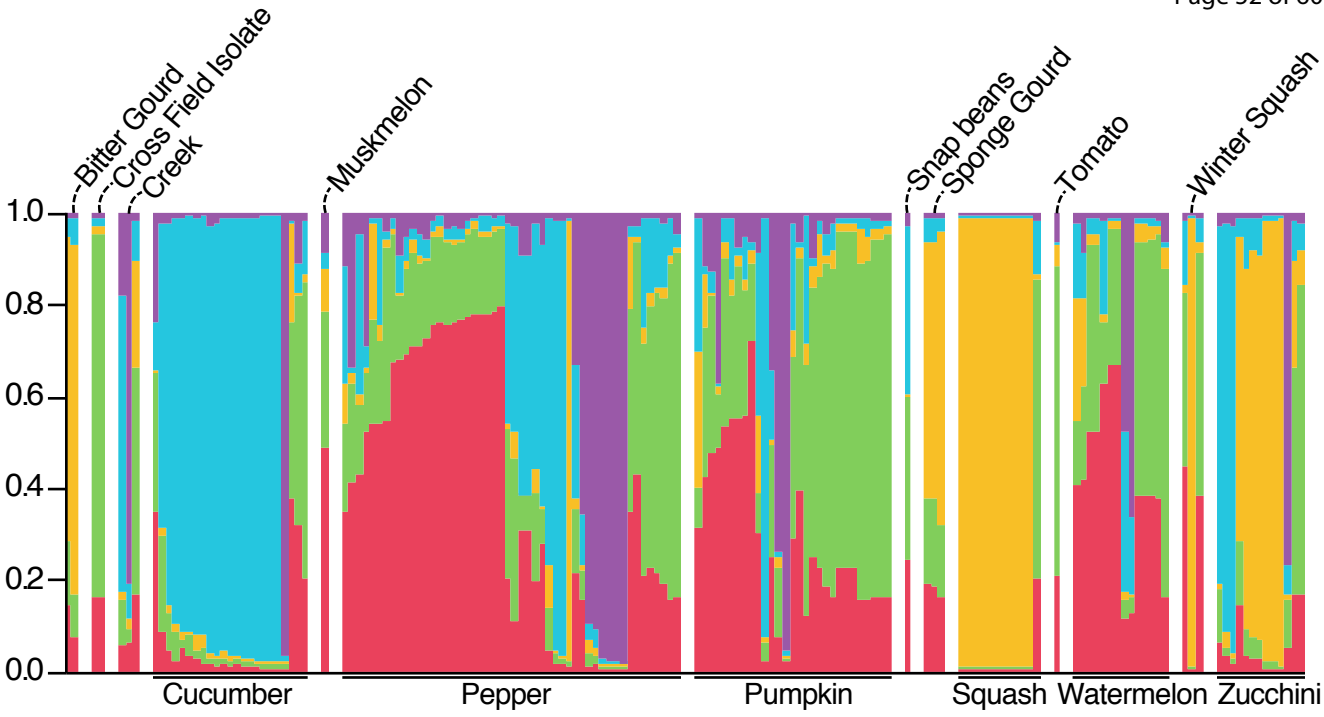
Supplementary Fig. S2. (A) Estimated population structure by Bayesian clustering of 157 *Phytophthora capsici* isolates from eastern United States. Each isolate is represented by a thin vertical line, which is partitioned into K colored segments that represent the isolate's estimated membership ratio in a given K cluster. Populations are labeled below the figure, with their cluster and state of origin affiliation from left to right, K1 to K5. The figure shown for a given K from 2 to 5 is based on the highest probability run at that K. **(B)** Most optimal number of clusters for 157 *P. capsici* isolates calculated by STRUCTURE HARVESTER. Delta K values plotted against the number of probable clusters (K).

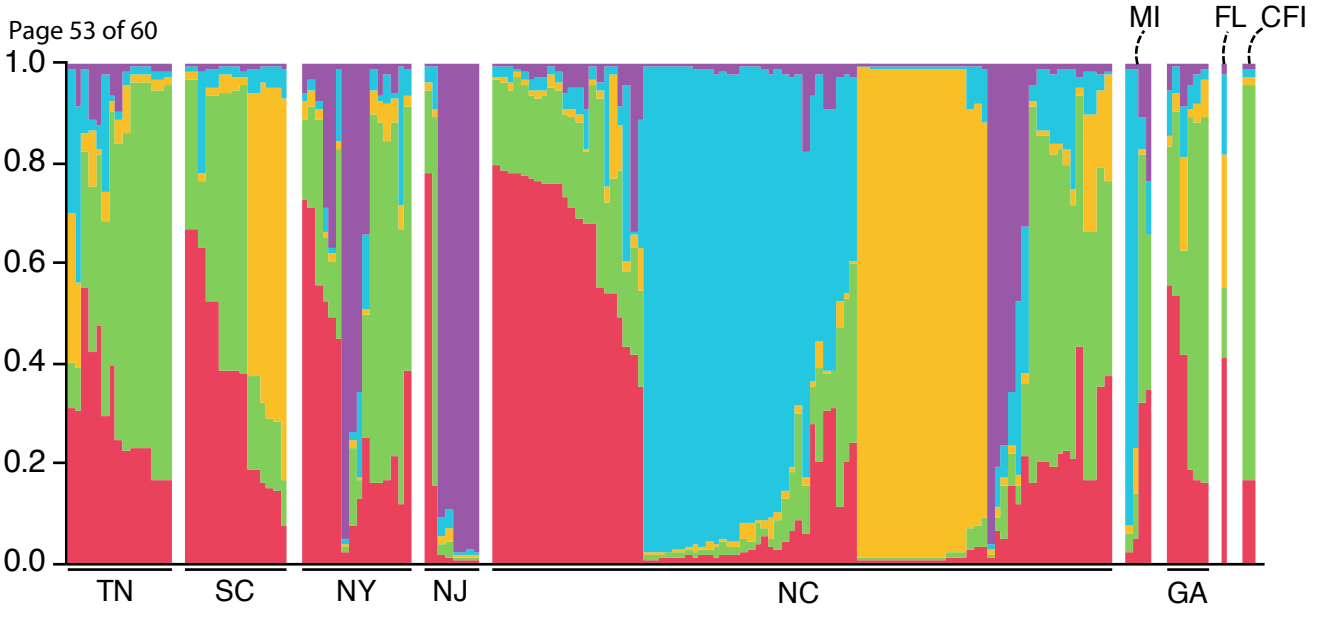
Supplementary Fig. S3. Pairwise genetic differentiation (F_{ST}) between plant hosts of clone corrected *Phytophthora capsici* isolates calculated using 12 polymorphic microsatellite markers and computed from populations using Arlequin v3.5.2 and graphed in R studio. *Significant ($P < 0.05$), **Significant ($P < 0.01$), ***Significant ($P < 0.001$). Colored scale bar represents an approximation to the F_{ST} value, darker blue indicates larger F_{ST} values while lighter blue to white indicate low F_{ST} values.

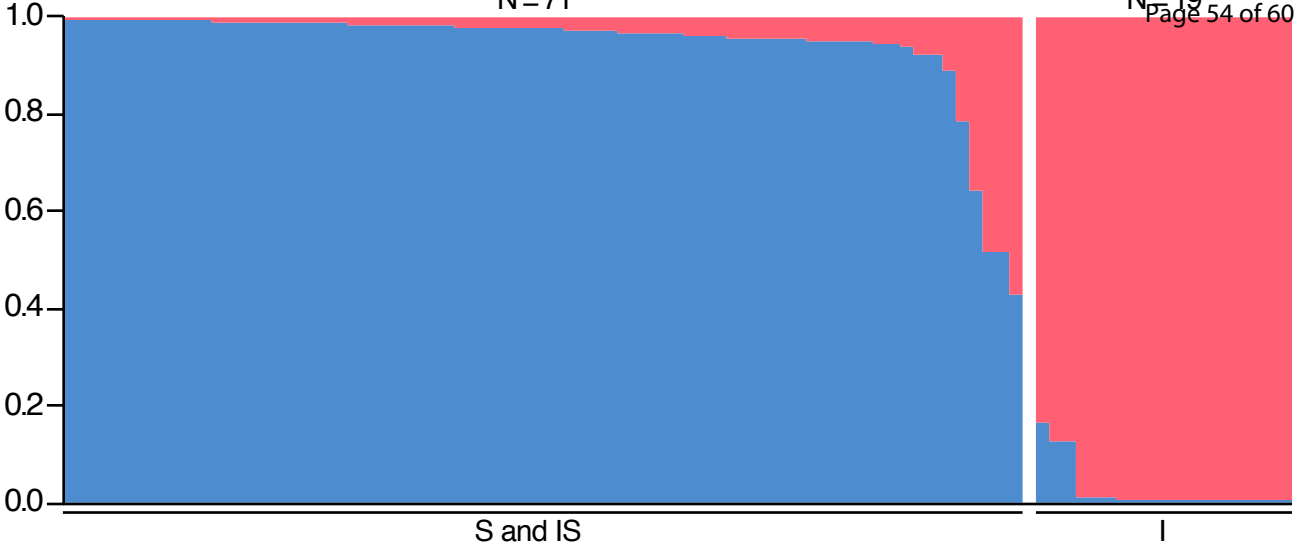
Supplementary Fig. S4. Pairwise genetic differentiation (F_{ST}) among state of origin for clone corrected *Phytophthora capsici* isolates calculated using 12 polymorphic microsatellite markers and computed from populations using Arlequin v3.5.2 and graphed in R studio. *Significant ($P < 0.05$), **Significant ($P < 0.01$), ***Significant ($P < 0.001$). Colored scale bar represents an approximation to the F_{ST} value, darker blue indicates larger F_{ST} values while lighter blue to white indicate low F_{ST} values.

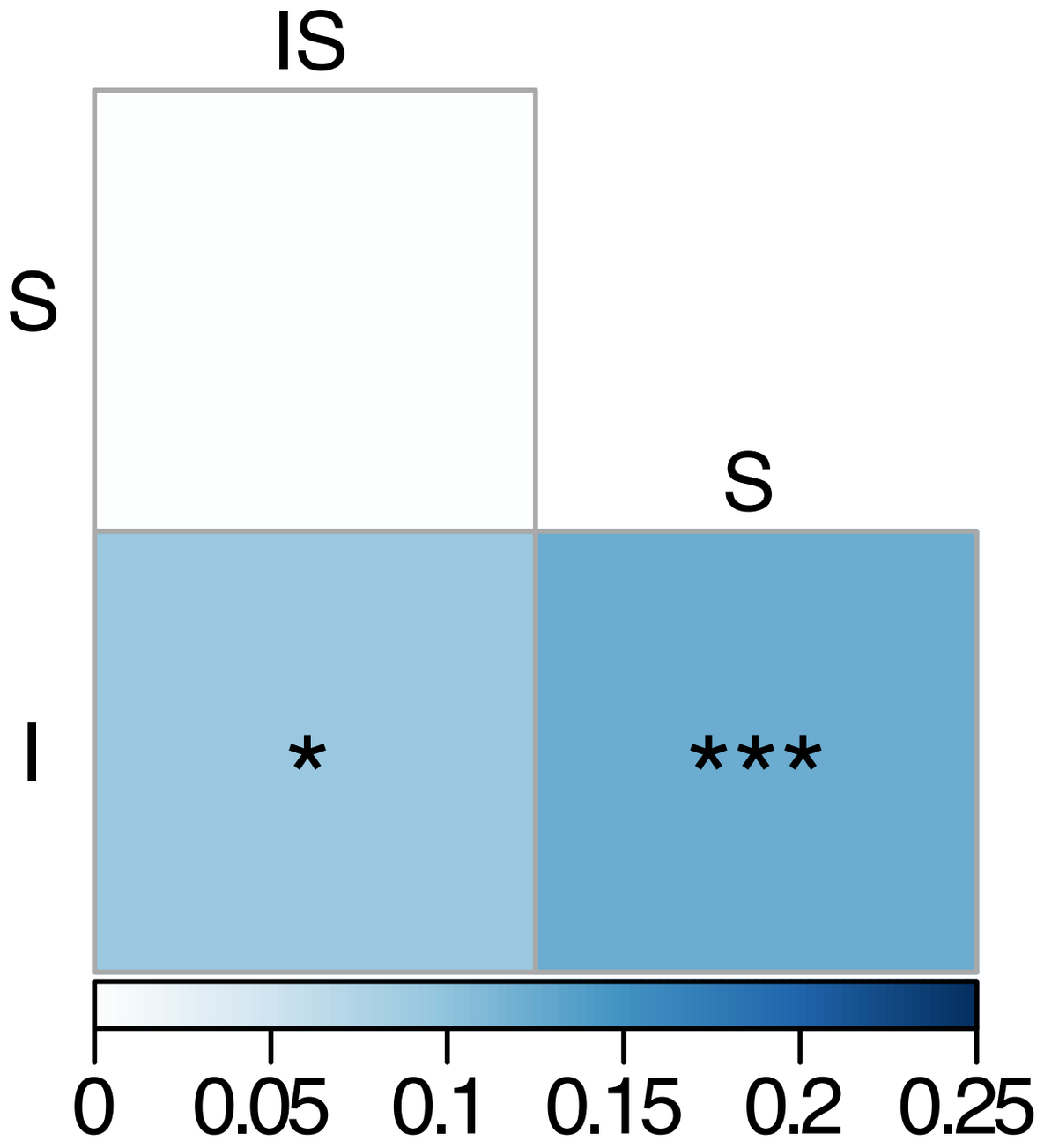
Supplementary Fig. S5. Estimated population structure by Bayesian clustering of 157 *Phytophthora capsici* isolates from North Carolina grouped by fluopicolide sensitivity. Each isolate is represented by a thin vertical line, which is partitioned into K colored segments that represent the isolate's estimated membership ratio in a given K cluster. Populations are labeled below the figure, with their fluopicolide sensitivity affiliation including from left to right, S = sensitive, IS = intermediate sensitive, and I = insensitive. Populations are also labelled above the figures with their corresponding state of origin.

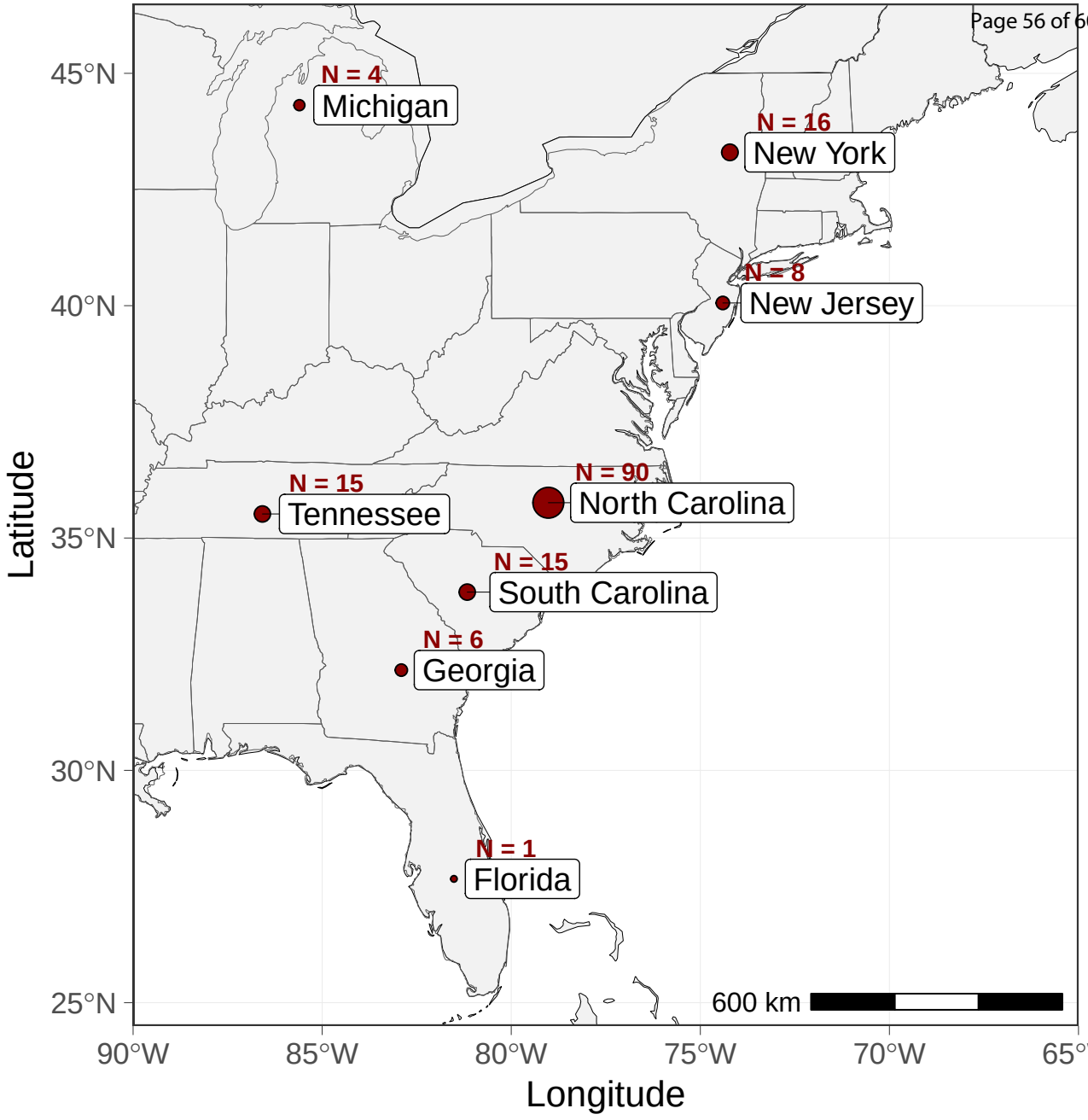

Sensitivity
S
IS
I
Dimethomorph
N = 157
138
13
4
1
1
Fluopicolide
N = 157
91
27
7
6
1
1
1
3
20
Mefenoxam
N = 157
100
23
5
3
5
6
2
1
2
2
6
1
1
Oxathiapiprolin
N = 157
152
3
1
1
0
50
100
150
10
30
50
70
90
110
130
Number of P.capsici isolates
Percent growth of control


Bitter Gourd
Cross Field Isolate
Creek
Muskmelon
Snap beans
Sponge Gourd
Tomato
Winter Squash
1.0
0.8
0.6
0.4
0.2
0.0
Cucumber
Pepper
Pumpkin
Squash
Watermelon
Zucchini


MI
FL
CFI
1.0
0.8
0.6
0.4
0.2
0.0
TN
SC
NY
NJ
NC
GA


N = 71
N = 19
1.0
0.8
0.6
0.4
0.2
0.0
S and IS
I


IS
S
S
I
*
***
0
0.05
0.1
0.15
0.2
0.25


N = 4
Michigan
N = 16
New York
N = 8
New Jersey
N = 90
North Carolina
N = 15
Tennessee
N = 15
South Carolina
N = 6
Georgia
N = 1
Florida
45°N
40°N
35°N
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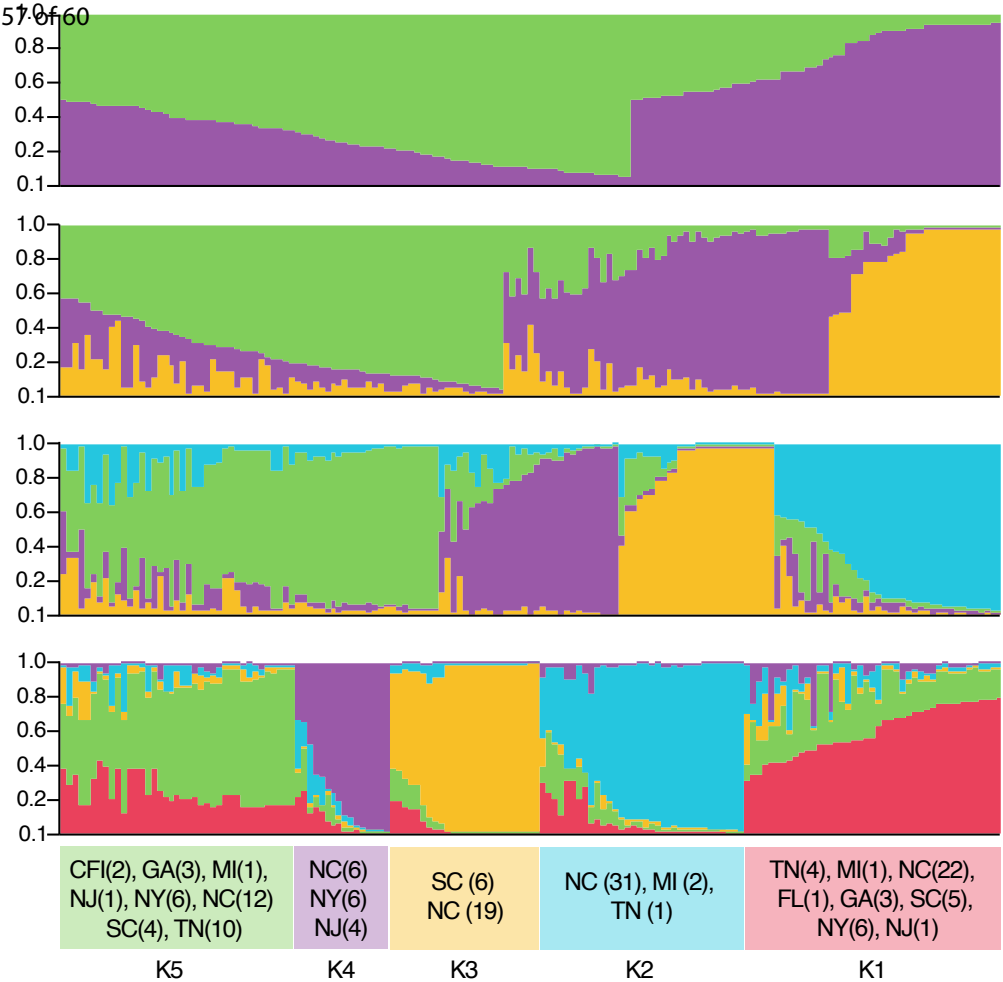
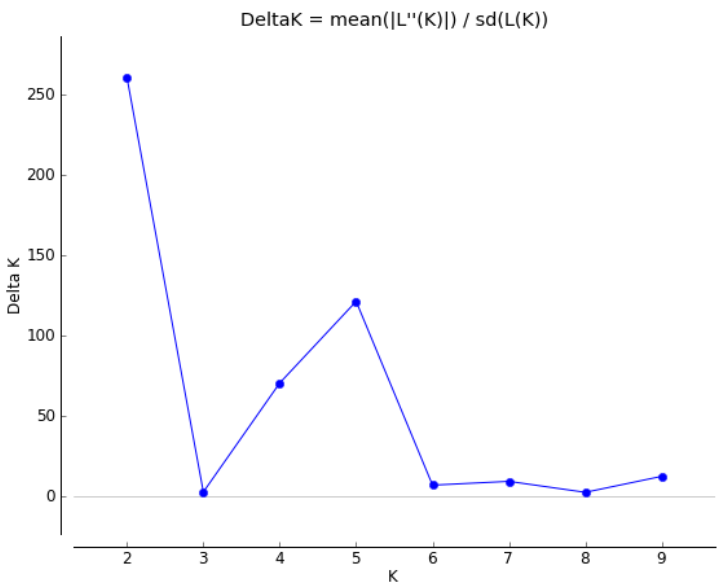
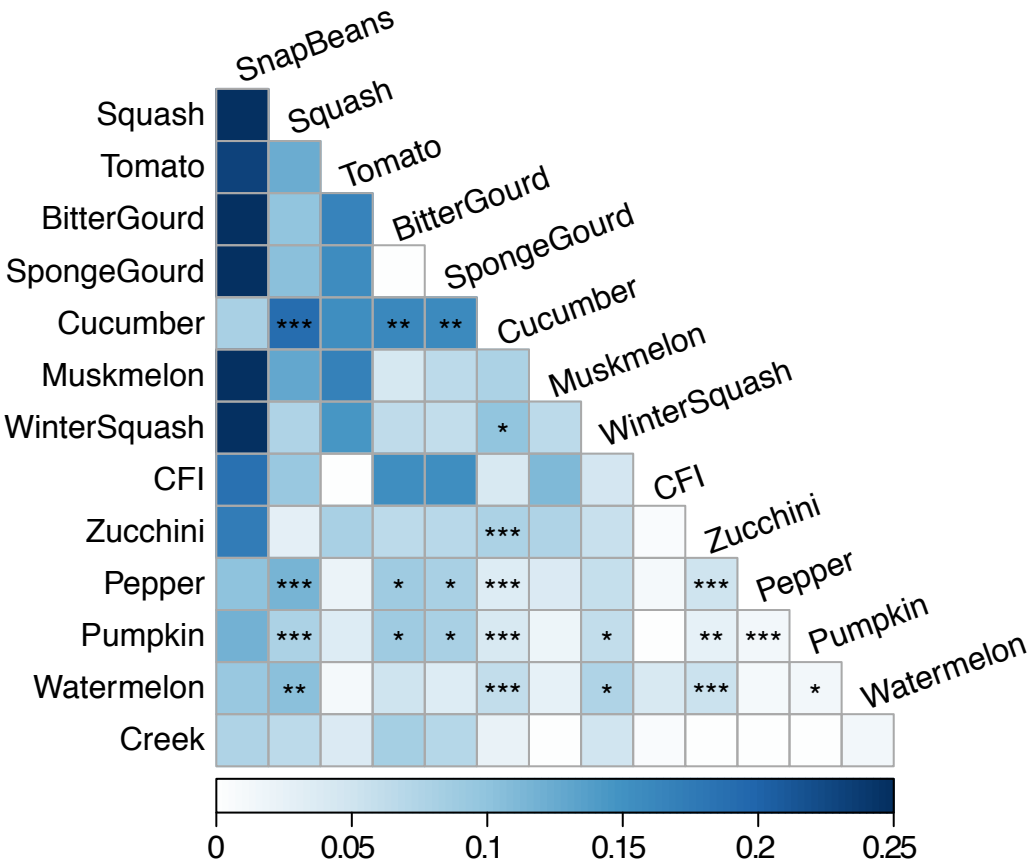
90°W
85°W
80°W
75°W
70°W
65°W
Latitude
Longitude
600 km

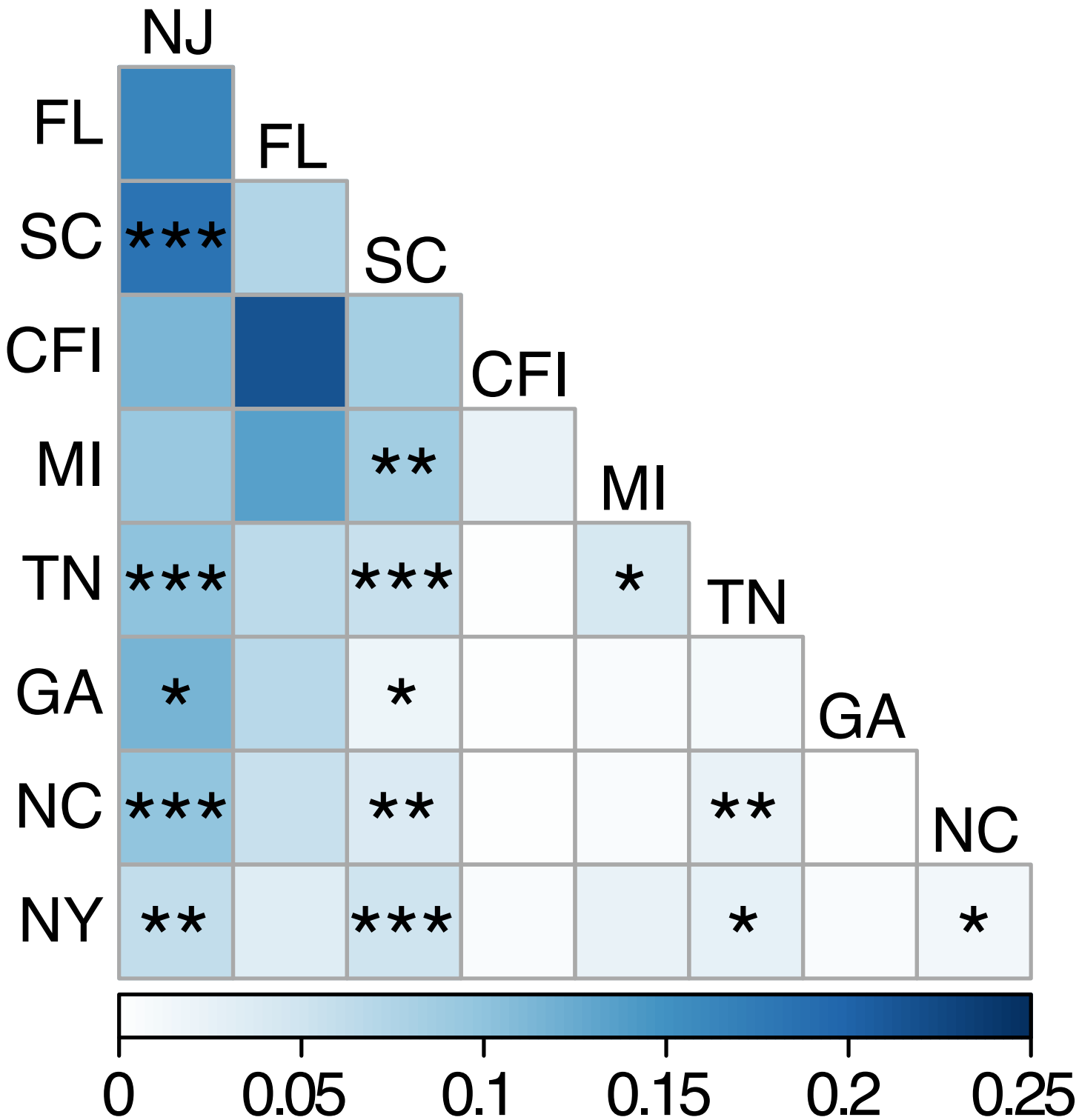
A

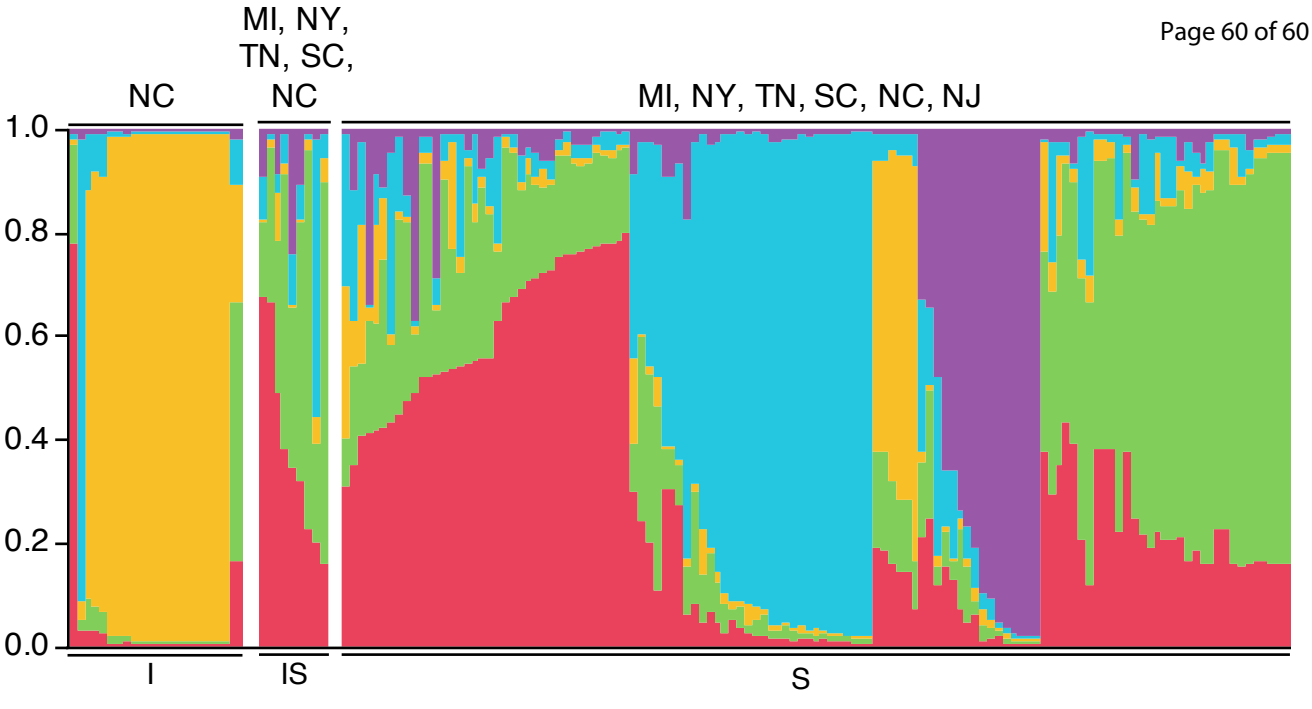

1.0
0.8
0.6
0.4
0.2
0.1
K = 2
K = 3
K = 4
K = 5
CFl(2), GA(3), MI(1), NJ(1), NY(6), NC(12) SC(4), TN(10)
NC(6) NY(6) NJ(4)
SC (6) NC (19)
NC (31), MI (2), TN (1)
TN(4), MI(1), NC(22), FL(1), GA(3), SC(5), NY(6), NJ(1)
K5
K4
K3
K2
K1

B


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
250
200
150
100
50
0
2
3
4
5
6
7
8
9
K


SnapBeans
Squash
Tomato
BitterGourd
SpongeGourd
Cucumber
Muskmelon
WinterSquash
CFI
Zucchini
Pepper
Pumpkin
Watermelon
Creek
0
0.05
0.1
0.15
0.2
0.25


NJ
FL
FL
SC
***
SC
CFI
CFI
MI
**
MI
TN
***
***
*
TN
GA
*
*
GA
NC
***
**
**
NC
NY
**
***
*
*
0
0.05
0.1
0.15
0.2
0.25


NC
MI, NY, TN, SC, NC
MI, NY, TN, SC, NC, NJ
1.0
0.8
0.6
0.4
0.2
0.0
I
IS
S