ABSTRACT

ALANIZ, ALEJANDRO. Analysis of Local and Regional Impacts on the Population Structure of the Two-Spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae), in North Carolina Tomato Agroecosystems and their Implications on Acaricide Resistance Management Strategies. (Under the direction of Dr. James F. Walgenbach and Dr. George G. Kennedy).

The twospotted spidermite (TSSM), *tetranychus urticae*, is an important agricultural pest throughout the world. Due to its wide host distribution, many pesticides have been used for its management. Worldwide, TSSM is resistant to nearly 100 active ingredients. In the early 2010's, growers in North Carolina began to report control failures with abamectin and bifenazate, the two most common acaricides for TSSM control in staked tomato. TSSM populations were sampled between 2015-2019 from two regions where staked tomato production is common. Sampled populations were exposed to two diagnostic doses of each acaricide: the field rate (high) and 1/5 field rate (low). Mortality was relatively low in 2015 when fields were first sampled with a large variance between fields. There was a positive relationship between year and mortality for both abamectin and bifenazate. Mortality was lower for abamectin at low rates compared to other treatments across all years, but still had a positive trend with increasing mortality across years. This positive trend in mortality across years may have been due to the introduction of a novel acaricide in 2014 that provided an alternative mode of action that reduced selection pressure for resistance to abamectin and bifenazate.

Microsatellite markers were utilized to investigate the genetic diversity and population structure of TSSM populations within fields on a small scale and among fields on a much larger regional scale. In the first study, TSSM populations were sampled from three individual staked tomato fields over a two-week period in the summer 2016. Each field was sectioned into four quadrants. Within each quadrant, one area of high density TSSM was located and 15 females were sampled then again two weeks later. Six microsatellites were amplified for each individual.

There were no significant departures from Hardy-Weinberg portions (HWP) for 19 of 24 patches sampled, but when patches were pooled together for each field, only 1 field did not demonstrate significant departures from HWP with excess homozygosity. Reinforcing the often-reported significant departure with excess homozygosity is a biologically founded phenomena resulting from high levels of inbreeding and the Walhund effect rather than null alleles. Two of the fields had significant decreases in inbreed and increases in heterozygosity over the two-week period. In the final study, 30 fields across Central and Western North Carolina were sampled in summer 2018 to represent at least 3 fields from each of 7 growers. Eleven microsatellite loci were used to evaluate the sampled TSSM populations. We did not detect any differences between growers or relatedness among fields managed by the same grower. Across regions, there was significant isolation by distance. Within regions, there was only significant isolation by distance in the western region where fields are more isolated from each other compared to the Central region. These results suggest that there may be key benefits to areawide coordinated management strategies for growers in the central region whereas growers in the Western region may see greater benefits for resistance management even if others in the region do not participate.

Analysis of Local and Regional Impacts on the Population Structure of the Two-Spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae), in North Carolina Tomato Agroecosystems and their Implications on Acaricide Resistance Management Strategies

by
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DEDICATION

This document would not have been made possible without the unwavering support of my fiancée, Courtney T. Ross. Through all the stressful days and sleepless nights, she always reminded me that we were all the hard work was an investment into our future together. I love you, Sweetheart, and I cannot wait to marry you.

BIOGRAPHY

Alejandro Alaniz was born on March 30th, 1989 and is the son of Mr. Alejandro Alaniz and Ms. Diana Garza. He was raised in the southern most city in Texas, Brownsville. He is the eldest of three children. He has one brother, Matthew Nicholas Alaniz, and one sister, Amanda Alaniz. He grew up with a deep appreciation for animals and nature. Even at a young age, he was deeply curious about all things creepy or crawly. He could often be found flipping over logs and looking under logs to find all the little animals around him.

He graduated from Homer Hanna High School in 2007 with a focus on veterinary medicine. He pursued those interests by continuing his education at Texas A&M University in College Station, majoring in biomedical sciences. He purposefully chose electives focusing on arthropods for his first year and by his second year at Texas A&M because it was interesting to him. By the middle of his second year, he changed majors to focus on Entomology where he was able to thrive. On October 5th, 2010, he entered an entomology research lab for the first time for an internship, not knowing his career trajectory would change forever. He worked on a project where he learned about extracting DNA from mosquitoes and amplifying DNA, but more so, he learned that research was powerful vessel for fostering his curiosity about the world around him. From then on, he knew that he wanted to spend the rest of his life conducting research. He graduated from Texas A&M in May of 2011, earning a bachelor's degree in Entomology.

By August of 2011, he was enrolled in a Master's Degree program in Biology at The University of Texas, Pan-American, now known as The University of Texas, Rio Grande Valley, in Edinburg, Texas where he worked on a molecular biology research project focused on annotating the HOX genes transcriptome of *Tomocerus minor*, a springtail arthropod, under the supervision of Dr. Matthew Terry. While working on his master's degree, Alex took a wide

variety of jobs to support himself financially, but there were two that were most impactful to his future. He earned an internship with USDA-APHIS where he assisted in mass rearing biological control agents to reduce populations of an invasive species. The other was at Texas A&M AgriLife Research and Extension services where he assisted an extension agent in her duties inspecting crops for pests and working on agricultural research projects. Both these opportunities gave him insight to the applied area of research in agriculture where he learned that his efforts in research could directly impact people and help the world by improving the sustainability of agricultural practices. He graduated in December of 2014, earning a Master's Degree in Biology.

In January of 2015, he was enrolled in an Entomology Ph.D. program at North Carolina State University in Raleigh, North Carolina. There he worked on a research project focused on resistance management and population genetics for *Tetranychus urticae*, the two-spotted spider mite, under the supervision of his co-advisors Dr. James F. Walgenbach and Dr. George G. Kennedy. During his time in North Carolina, thanks to his research, he was able to visit all throughout the state where he collected spider mite samples for testing. Although in his heart he would forever be a Texan, he fell in love with the beauty and diversity of North Carolina and the people that live there.

In his second year at NC State, Alex met his future wife, Courtney T. Ross. Together they adopted two dogs, Kaiju and Yoshi. Together, his little family provided moral and emotional support that helped to guide him to the end of his journey in earning a Ph.D in Entomology.

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I would like to thank everyone that helped make this work possible. I would especially like to thank Dr. Jim Walgenbach, Dr. George Kennedy, Dr. Martha Burford Reiskind, and Dr. Fred Gould for all their suggestions and guidance in my research endeavors. I would also like to thank Paul Labadie, the lab manager without whom I do not think I would have been able to complete my research. He helped to guide, troubleshoot, fix, and optimize all the equipment and protocols I used to conduct my research. He also provided moral support and listening ear when the work was difficult. I would finally like to thank all the grower cooperators for allowing me to sample from their fields.

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INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is one of the most economically important agricultural pests in the world (Ilias et al. 2014; Van Leeuwen et al. 2015). TSSM has a worldwide distribution and has been documented feeding on over 1100 host plant species including many high value agricultural crops (Migeon and Dorkeld 2019). TSSM are also notorious for their ability to develop resistance to acaricides due to their high fecundity, short lifecycle, and arrhenotokous reproduction (Grbić et al. 2011). Over 100 acaricides in 14 different IRAC mode-of-action classes have been registered for use in management of TSSM in agricultural crops worldwide (Van Leeuwen et al. 2015; IRAC 2019). They have the highest incidence of acaricide resistance of any arthropod, with documented resistance to 96 active ingredients among various populations (Xu et al. 2018; Mota-Sanchez and Wise 2019). The first cases of resistance to a newly introduced acaricide are usually reported within a few years after commercial introduction (Dermauw et al. 2013). Consequently, one of the few reliable means of managing TSSM populations resistant to commonly used acaricides has been the introduction of acaricides with novel modes of action.

In North Carolina, TSSM are an important agricultural pest in staked tomato production. Most fields planted in staked tomato are infested with TSSM at some point during the growing season (Meck et al. 2009, 2012). TSSM feed on chloroplasts resulting in reduced photosynthesis, increased plant respiration, and chlorotic stippling on damaged foliage (Gorman et al. 2002; Zhang 2003). They can also feed on tomato fruit, causing a similar chlorotic pattern called gold fleck on ripe fruit, which makes the fruit unmarketable as fresh market produce due to cosmetic

damage (Meck et al. 2012). TSSM populations can grow rapidly if left unmanaged and when infestations are severe, marketable yields can be reduced by almost 50% (Meck et al. 2012, 2013). TSSM are managed with multiple applications of acaricides throughout the growing season to prevent populations from reaching economic thresholds (Meck et al. 2009, 2012).

For the past 10 to 15 years, the two most commonly used acaricides in North Carolina staked tomato production have been abamectin and bifenazate. Abamectin is a macrocyclic lactone compound in IRAC group 6 (glutamate-gated chloride channel (GluCl) allosteric modulators) (IRAC 2019). Abamectin acts in the nervous system by altering the normal function of GluCl leading to paralysis and death (Ilias et al. 2017). Abamectin has translaminar activity and resides in mesophyll cells where mites acquire it through feeding (Syngenta 2019). It was first introduced as an agricultural acaricide for the United States in 1985 (Dybas 1989). Bifenazate is a hydrazine carbazate in IRAC group 20D (Mitochondrial complex III electron transport inhibitors) (IRAC 2019). Bifenazate is thought to act by disrupting energy metabolism through interactions with mitochondrial complex III (Van Nieuwenhuyse et al. 2009). Bifenazate is a contact acaricide. It was first commercialized as an agricultural acaricide in the United States in 1999 (Dekeyser 2005). These two acaricides have completely different modes of action and there have been no reported instances of cross-resistance between them.

Abamectin has a 7-day pre harvest interval (PHI) (Syngenta 2019) versus bifenazate with a 3-day PHI(Chemtura 2019). This difference in PHI dictate the patterns of use for these acaricides in NC fresh market tomato production. Abamectin is often used on a calendar spray schedule in a preventative management strategy without regard for mite density when tomato plants are maturing, but before fruit harvest begins. Fruit are often harvested multiple times per week for four to six weeks. During this period, bifenazate is commonly used for mite

management due to its much shorter PHI. To limit interruptions to harvest schedules, bifenazate applications are usually made as a rescue treatment using economic thresholds (Meck et al. 2012).

The heavy use of these two acaricides has been accompanied by growers in North Carolina reporting control failures in their fields throughout the early 2010 period. Prior to these reports, there had been no documentation of resistance to either acaricide in North Carolina tomatoes. The purpose of this study was to document and characterize variation in mortality over time in response to diagnostic concentrations of both abamectin and bifenazate among field-collected populations of TSSM from North Carolina. The collection sites were in two areas of NC with a long history of staked tomato production. The results provide an assessment of variation in response of TSSM populations to these acaricides and a baseline against which changes in occurrence of resistance can be assessed. They will also assist in establishing more judicious recommendations for TSSM management for staked tomato production in North Carolina.

METHODS

Sample collection and rearing

TSSM populations were sampled from two regions of North Carolina, USA, where staked tomatoes have been grown commercially for >50 years (Fig. 1.1). The central region, located in the rolling hills of the Piedmont at an elevation of ~200 m above sea level, is characterized by flat plains with wide expanses of contiguous agricultural fields. The western region is 180 km west of the Central region in the Southern Appalachian Mountains at an elevation of ~650 m above sea level. This region is generally characterized by small clusters of fields nested within valleys isolated from other fields by hills and forests.

A total of 38 fields were sampled over 4 years (2015, 2016, 2018, and 2019). Of these, 25 fields were from the central region and 13 were from the western region. Tomato fields were inspected periodically to assess the density of TSSM populations. A leaflet from the topmost fully expanded leaf of 20 randomly sampled tomato plants at least 15 feet away from the edge of the field were sampled to evaluate the severity of TSSM infestation. When a field had an infestation level with an average of at least 3 mites per sampled leaflet (but usually >10 mites per leaflet), leaves with live TSSM were randomly collected from throughout the field to provide adequate representation of the entire field and placed in a plastic bag. Bags were kept in a cool chamber until later the same day when infested leaves were placed on top of 6-8-week-old potted tomato plants (*solanum lycopersicum* L., 'Mountain Fresh Plus') in isolated rooms. These colonies were kept at ~25 ℃, 50-75% relative humidity, with a light:dark cycle of 16h:8h and maintained for two to six weeks before used in bioassays. A colony of TSSM was also maintained in the lab on tomato plants in the same conditions. This colony was maintained for >10 years in the lab without exposure to acaricides and served as a susceptible standard tested in 2019.

Bioassays

Bean (*Phaseolus vulgaris* L. 'Top Crop') leaf discs (1.8 cm diameter) were placed abaxial side up on water-soaked cotton discs in 5.5 cm diameter petri dishes. The lids of the petri dishes had a 1 cm diameter hole in the center covered with a fine mesh screen to allow for ventilation. Ten adult female TSSM from a given colony were placed on each leaf disc. Each round of bioassays included the recommended field-rate ("high rate"), 1/5 of the field rate concentration ("low rate"), and a water control for each acaricide. The recommended field rates for abamectin (Agri-Mek® 0.15 EC) and bifenazate (Acramite® 4 SC) were 17 and 600 ppm AI

respectively, based on applying materials with 935 liters/ha of water. Stock solutions were made prior to each bioassay. The water used for serial dilutions and the water control consisted of 500 mL DI water with one drop of surfactant (Triton AG-44 M). Four to six replications of the high and low rates of both acaricides and a water control were tested for each population. Each replicate consisted of 10 adult mites on a leaf disc in a petri dish. Acaricides were applied to the abaxial surface of leaf discs using a stationary compressed-air powered airbrush held 15 cm above the spraying focal point of the petri dish and delivering 50 µL of solution at a pressure of 0.3 bars. This resulted in each leaf disk being covered with 2.67 ± 0.17 mg/cm^2 of test solution. After spraying, petri dishes were left uncovered for 30 minutes to allow solution to air dry. Once dry, mites were recounted to ensure none had escaped then petri dishes were covered with lids and placed in an incubator held at 25 °C, 50-75% relative humidity, with a 16:8 L:D photoperiod. Mortality was evaluated 48 hours post treatment.

Mites were scored as either alive or dead. Mites were considered alive if they could be seen moving across a leaf disc or, if stationary, they responded to gentle prodding. Mites were considered dead if they showed no response or only twitched after prodding. Mites that were missing, dead due to fungal growth, or drowned in the wet cotton were excluded from the counts.

Statistical Analyses

Observed mortality in treatments was corrected using the Schneider-Orelli formula for mortality measurements on uniform populations based on the mortality in the control for each population (Püntener 1992). Four populations sampled in 2018 had mortality >25% in the control and were thus excluded from analyses. Mortality was not normally distributed for either AI based on Kolmogorov-Smirnov tests of normality, therefore non-parametric tests were used

for statistical analyses. Wilcoxon signed-ranked tests were used to evaluate differences in the median mortality between the high and low rates for field populations for either AI.

For each AI-rate combination, field populations were grouped into quartiles based on percent mortality: Q1 ≤ 25%, Q2 26-50%, Q3 51-75%, and Q4 76-100%. Chi square analyses were used to compare the distribution of field populations in the mortality quartile groups. This evaluation assumed there would be no differences between the distribution of field populations in each mortality quartile if susceptibility to a given AI was pervasive throughout. When distributions differed, post-hoc Z-scores were used to evaluate how the distributions differed. A generalized linear mixed model was used to analyze mortality in field populations grouped by year, rate, and region and a generalized linear regression was used to evaluate overall mortality in field populations across years for both AI (SAS Version 9.4 2019).

RESULTS

Overall

An overall generalized linear mixed model of mortality with AI, rate, and region as main factors and year as a random factor, revealed that AI ($F_{(1,125)} = 4.62$; $P = 0.033$) and rate ($F_{(1,125)} = 7.54$; $P = 0.007$) were significant predictors of mortality, but region and interactions between main factors were not. There was significantly higher mortality in treatments with bifenazate (75.8% ± 25.7 SD) versus abamectin (66.7% ± 28.1). These AI were analyzed separately to evaluate the impact of rate on mortality across years. Region was not included in further analyses.

Abamectin

For abamectin treatments, the susceptible lab colony incurred 93.6% ± 9.5 mortality at the high-rate and 97.9% ± 4.8 mortality at the low-rate (Fig. 1.2A). The overall mean mortality

for field populations was much lower than the lab colony at both the high (74.7% ± 26.3) and low rate (58.6% ± 27.9). The median mortality was significantly higher at the high rate versus the low rate (Wilcoxon (32) = 264, Z = -3.86; P < 0.001) (Fig. 1.2A & 1.3).

There were no differences in the distribution of field populations in comparisons between the high and low rates at the >25% and 26-50% mortality quartiles (χ^2(1, N = 68) = 2.6; p = 0.11). Differences between the high and low rate arose from the distribution of field populations within the 51-75% and >75% mortality quartiles (Fig. 1.4). Three-quarters of field populations responded in the >75% mortality quartile at the high rate versus only one-third of field populations at the low rate (χ^2(3, N = 68) = 13.4; p = 0.004; Adj Z = 3.4; P < 0.001). At the high rate, only a single field responded in the 51-75% mortality quartile versus one quarter of fields at the low rate (χ^2(3, N = 68) = 13.4; p = 0.004; Adj Z = 2.7; P = 0.006).

Analysis with a generalized linear mixed model of mortality with rate and year as main factors demonstrated that rate (F (1,60) = 5.02; P = 0.029) and year (F (3,60) = 2.95; P = 0.04) were significant predictors of mortality, but their interaction was not (F (3,60) = 0.36; P = 0.782). A generalized linear regression of overall mortality across years demonstrated a significant positive linear relationship in overall mortality across years (F (1,66) = 7.55; P = 0.008; slope = 0.06; slope 95% CI = 0.016 to 0.098; R^2 = 0.103) (Fig. 1.5A).

Additionally, there was a large variance in mortality between fields within years at both the high and low rate (Fig. 1.5A). In 2016, when mean mortality was lowest at both the high and low rate, the variance in mortality between fields was the greatest with 13.4% at the high rate and 14.1% at the low rate. In 2019, the variance between fields dropped to 0.7% at the high rate and 1.85% at the low rate.

Bifenazate

The susceptible lab colony incurred 100% mortality at both rates (Fig. 1.2B). The overall mean mortality for field populations was lower than the lab colony at both high (79.6% ± 22.2) and low rates (71.9% ± 28.7). The median mortality was higher at the high rate versus the low rate (Wilcoxon (31) = 110, Z = -2.704; p = 0.003) (Fig. 1.3).

There were no differences between the high and low rates in the distribution of field populations within quartiles ($\chi^2(3, N = 68) = 7.4$; P = 0.06), though within the <25% mortality quartile, there were 6 field populations at the low rate versus a single field population at the high rate (Fig. 1.4).

Analysis with a generalized linear mixed model of mortality with rate and year as main factors demonstrated that year (F (3,60) = 8.09; P < 0.001) was a significant predictor of mortality, but rate (F (1,60) = 1.62; P = 0.208) and the interaction between rate and year (F (3,60) = 0.26; P = 0.856) were not. A generalized linear regression of overall mortality across years demonstrated a significant positive linear relationship in overall mortality across years (Fig. 1.5B).

The variance in mortality between fields within years for the high and low rate was greatest in 2016 (7.5% and 16.5% respectively), but the lowest mean mortality occurred in 2015 (Fig. 1.5B). In 2019, the variance in mortality between fields dropped to 1.1% at the high rate and 0.6% at the low rate.

Comparisons between Abamectin and Bifenazate

The median mortality for abamectin at the high rate did not differ from bifenazate at both the high (Mann Whitney U (68) = 493, Z = 1; P = 0.298) and low (Mann Whitney U (68) = 523, Z = 0.7; P = 0.503) rate (Fig. 1.3). Alternatively, the median mortality for abamectin at the low

rate was lower than bifenazate at both the high (Mann Whitney U (68) = 293.5, Z = 3.5; P < 0.001) and low (Mann Whitney U (68) = 406.5, Z = 2.1; P = 0.036) rate.

A generalized linear mixed model of mortality with AI, rate, and year as main factors demonstrated that AI (F (1,120) = 5.03; P = 0.027), rate (F (1,120) = 6.40; P = 0.013), and year (F (3,120) = 8.79; P < 0.001) were significant predictors of mortality, their interactions were not.

Both AI followed a similar pattern of variance in mortality between fields within years; the variance was greatest in 2016 then decreased in 2018 and 2019. The variance in mortality between fields was greater at high rate for abamectin in 2015 and 2016, but the variance was greater at the low rate for bifenazate.

DISCUSSION

These studies showed that TSSM field populations sampled in North Carolina responded similarly to a field dose and 1/5 field-dose rate of bifenazate. Mortality responses at both rates were much lower in field populations versus the susceptible lab colony. The relatively high mortality in field populations treated with bifenazate at both rates in 2019 may indicate an increase in field populations susceptible to bifenazate.

Conversely, mortality responses in field populations treated with abamectin were much more variable within and among fields compared to bifenazate. Overall mortality for populations treated with abamectin was 16% lower at the low rate versus the high rate (Fig. 1.3). This suggests that management with the recommended field rate (i.e. high rate) could provide adequate immediate population suppression upon application, but mortality due to residual abamectin exposure (i.e. low rate) in the field may be less effective. Abamectin has been demonstrated to engage in translaminar activity, but the quantity retained in leaf material does not seem to be sufficiently high enough to produce reliable mortality (Martin et al. 2015). If low

concentration exposure is maintained by translaminar activity, it may provide a potential mechanism for low rate exposure that may inadvertently increase the rate of resistance development by selecting for individuals that do not respond to sublethal concentrations (Guedes et al. 2017).

In comparisons between abamectin and bifenazate, the mortality for high rate abamectin treatments was comparable to both the high and low rate bifenazate treatments, while the mortality for low rate abamectin treatments was lower than both the high and low rate bifenazate treatments. This suggests that in general, high-rate treatments of either acaricide should provide comparable levels of TSSM mortality, but at lower rates, only bifenazate could provide adequate TSSM population suppression.

Initial assessments of mortality responses to abamectin and bifenazate in 2015 confirmed the reports of control failure with mean mortality only reaching up to 70% for the high rate for either acaricide and even lower at the low rate. Despite multiple applications of acaricides during each season, we observed a marked trend of increasing mortality across the years sampled for both AI (Fig. 1.5). One potential explanation for this positive trend in mortality across years may be related to the introduction of cyflumetofen as a commercial acaricide into the US in 2014. Cyflumetofen is a benzoyl acetonitrile compound in IRAC group 25A (mitochondrial complex II electron transport inhibitors) (IRAC 2019). It is a relatively new acaricide with a novel mode of action different from both bifenazate and abamectin (Hayashi et al. 2013; Chong and Hale 2017). The use of cyflumetofen has increased over time, largely because of its relatively short 3-day preharvest interval. The introduction of cyflumetofen may have provided a reprieve from selection pressures favoring resistance alleles for abamectin and bifenazate as evidenced by the

increased effectiveness of both AIs over the course of the four years when populations were sampled.

This positive trend in mortality was also evident even when fields were separated by region. We expected to detect differences in mortality between the Central and Western regions of North Carolina due to the marked differences in planting and harvesting time due to elevation and annual weather cycles as well as the agroecosystem landscape structures, but no such differences were observed. Despite these differences, both regions had similar trends regarding mortality at high and low rates for both AI. We suspect that the highly regimented production practices associated with high volume fresh market tomato production common to both regions had a more significant impact on the reduction of susceptible populations than the differences between regional attributes. Due to their short generation time, TSSM can quickly respond to acute selection pressures such as acaricide applications in a similar fashion in both regions despite differing environmental conditions. As the season ends, TSSM overwinter with few or no generations during which the differences between the regions are most pronounced, offering little chance for selection to lead to observable differences from a resistance perspective.

Abamectin resistance has been associated with several mutations at the *glutamate-gated chlorine channel* gene leading to target-site insensitivity (Kwon et al. 2010, 2015). The frequencies of these mutations have been demonstrated to be unstable in the absence of selection (Sato et al. 2005; Kwon et al. 2010). Bifenazate resistance, on the other hand, has been associated with several mutations in the mitochondrial genome at the *cytochrome b* gene (Van Leeuwen et al. 2006, 2008; Van Nieuwenhuyse et al. 2009). These mutations to the mitochondrial genome were thought to impart deleterious effects on fitness in the absence of bifenazate selection (Fotoukkiaii et al. 2020), but fitness costs could not be determined in lab

studies comparing resistant and susceptible populations. This suggests that the frequency of a bifenazate resistance mutation would remain stable even in the absence of selection (Van Leeuwen et al. 2008).

Due to these differences in stability, we expected that there would be greater variability in mortality among years for abamectin versus bifenazate. Although resistance allele frequencies were not evaluated directly, mortality would likely be inversely related to resistance allele frequency because as the frequency of resistance alleles increase, we would expect mortality to decrease.

The mean mortality at both rates for abamectin in 2015 and 2016 were similar, but the variation in mortality was much greater in 2016. Figure 1.5A demonstrates that in 2015, mortality responses were more evenly spread across fields, but in 2016, there was a large split between fields with mortality >90% and mortality <30%. The same pattern followed in 2018 with a majority of fields with mortality >80% and a small subset with mortality <40%. In 2019, all fields had mortality >60%. These patterns may suggest that widespread overuse of abamectin in early 2010 lead to region wide reduction in mortality for abamectin as exemplified by the spectrum of mortality responses in 2015. As previously stated, there were no significant effects due to grower, thus the specific fields where mortality remained low in 2016 and 2018 were likely the result of a small, but persistent population of TSSM with low susceptibility to abamectin establishing themselves before a susceptible population.

The mean mortality for Bifenazate was low only in 2015, when there were area wide reductions in susceptibility to both the high and low rate (Fig.1.5B). In the following years, mortality responses significantly increased at both the high and low rate. The variation in mortality across fields was greatest in 2016 due to two fields demonstrating a significant lack of

susceptibility at both rates. These fields were isolated in the western region of North Carolina where there was likely low immigration and emigration. These fields were not sampled again for this study, but will be revisited in future studies.

These differences in the variance of mortality between fields within years for abamectin and bifenazate may be due to differing use patterns for abamectin and bifenazate rather than due to the inherent stability of resistance alleles in the field. Abamectin is regularly sprayed preventatively before harvest due to its longer preharvest interval, often regardless of mite population intensity; this places selection pressure on smaller, more inbred populations. Alternatively, bifenazate is usually sprayed on a curative basis during the harvest period only if TSSM populations reach economic thresholds. If populations do not reach economic thresholds, no spray is needed and when a spray is needed, the selection pressure is exerted on larger populations. Alternatively, these fields are rotated every year, thus the population sampled each year are different than the year before as new individuals colonize a field with differing degrees of susceptibility to these AI. In the future, studies will be conducted to evaluate how a field with low susceptibility changes from year to year without rotation.

In summary, we have demonstrated that both abamectin and bifenazate did indeed have relatively low mortality in 2015 when field populations were first sampled with a large variance between fields sampled, but there was an overall increase in mortality in the following years with a few fields in particular retaining low susceptibility. There were no differences between abamectin and bifenazate at the high rate even as mortality increased across years. At the low rate, on the other hand, mortality for abamectin was significantly lower than bifenazate and much more variable. In the final year that fields were sampled, the high rate treatments for both AIs resulted in high mortality, but mortality at the low rate of abamectin remained significantly lower

and highly variable among fields. This reduced level of residual control with abamectin versus bifenazate could favor resistance development because it would be expected to allow more rapid recolonization by populations with low levels of resistance. We did not find any regional differences in mortality between central and western North Carolina likely because the similarity between intensive production practices in both regions. Across years in both regions, we saw a positive trend in mortality that we did not expect to find, which may be associated with the introduction of a novel acaricide into the market. As stated earlier, the introduction of acaricides with novel modes of action is often the only means growers have to manage TSSM populations resistant to the current products available. Diversifying the modes of action can slow or reverse the rate of resistance development. In this case of fresh market tomato production in North Carolina, it seems that the introduction of cyflumetofen with a new mode of action may have helped to reduce selection for resistance to both abamectin and bifenazate, demonstrating the importance of diversity of modes of action for acaricide resistance management.

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Figures

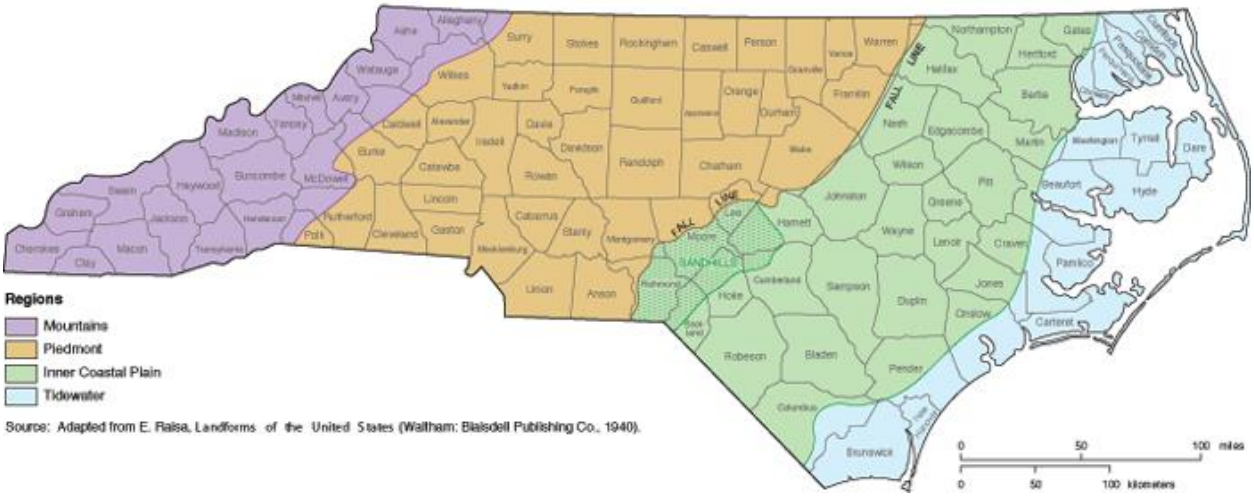

Figure 1.1 Map of North Carolina demarcating the regions where fields were sampled.

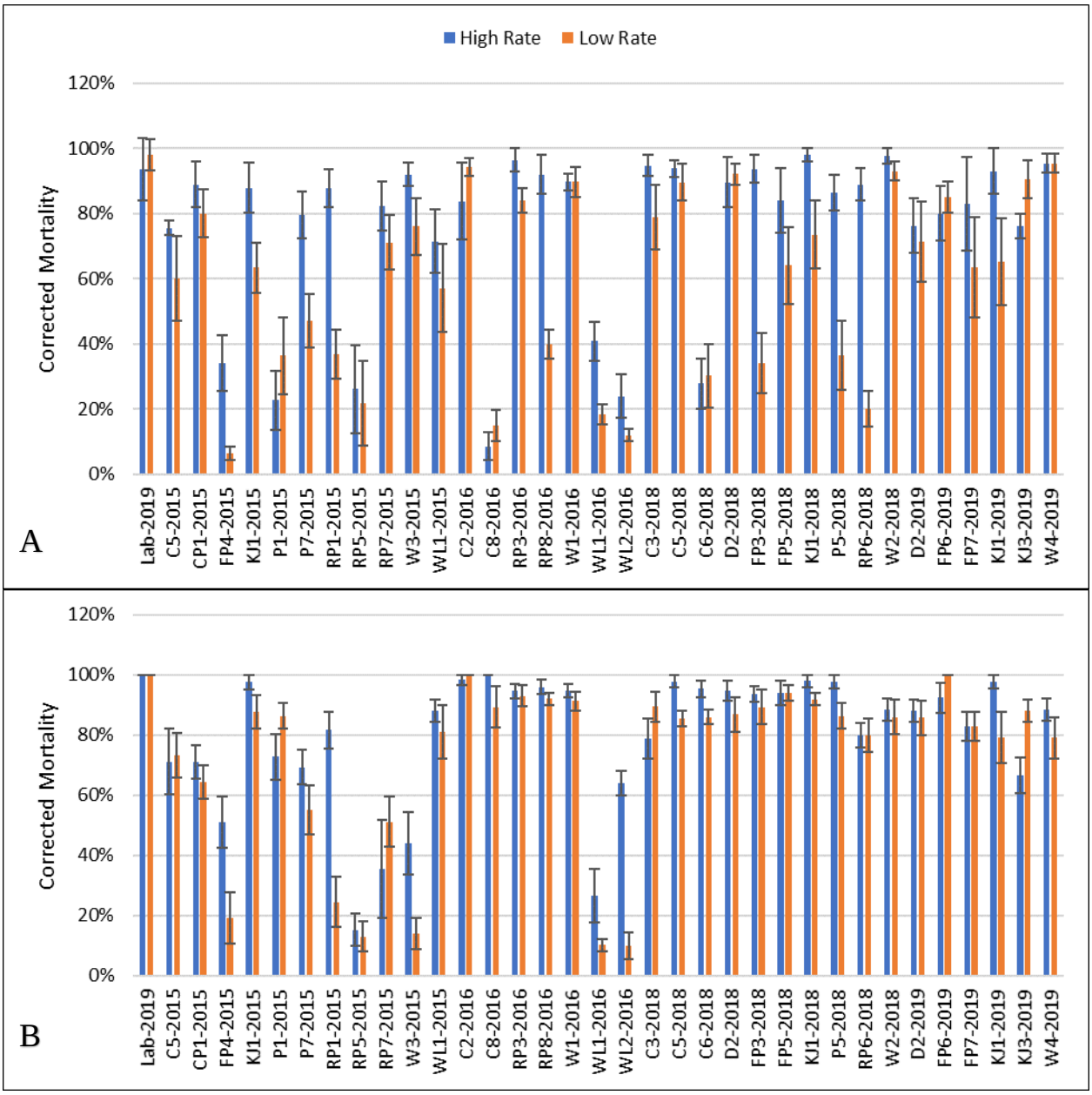

Figure 1.2 Bar chart of mean (±SEM) percentage mortality of spider mite populations by year to abamectin at field dose and 1/5 field dose of abamectin (A) and bifenazate (B).

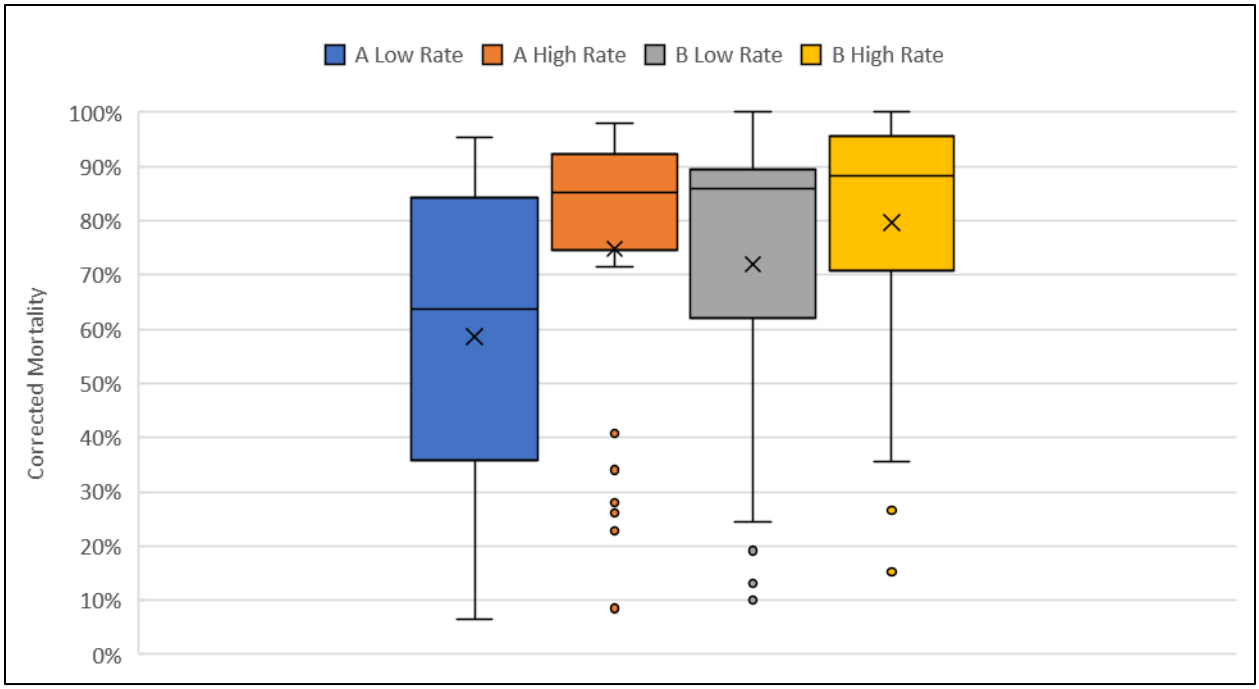

Figure 1.3 Box and whisker plot of percent corrected mortality of field populations to two rates each of abamectin and bifenazate.

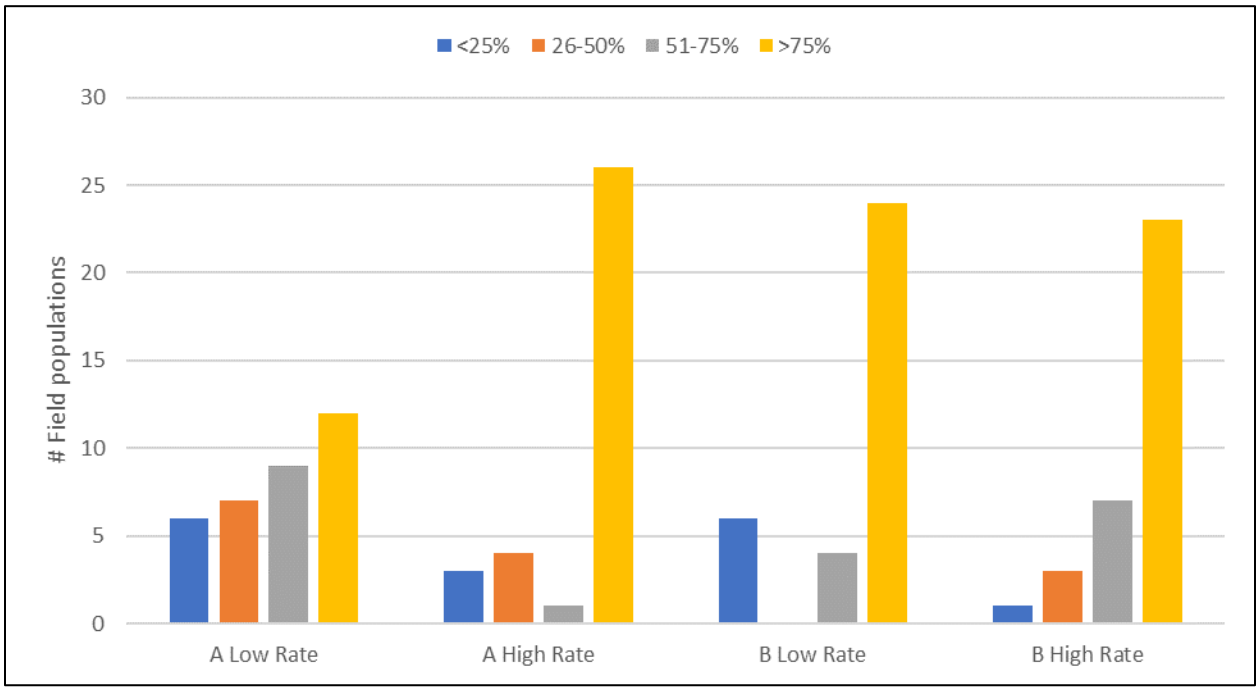

Figure 1.4 Frequency of mite populations with mortality falling into one of four quartiles following exposure to a high and low rate of abamectin (A) and bifenazate (B).

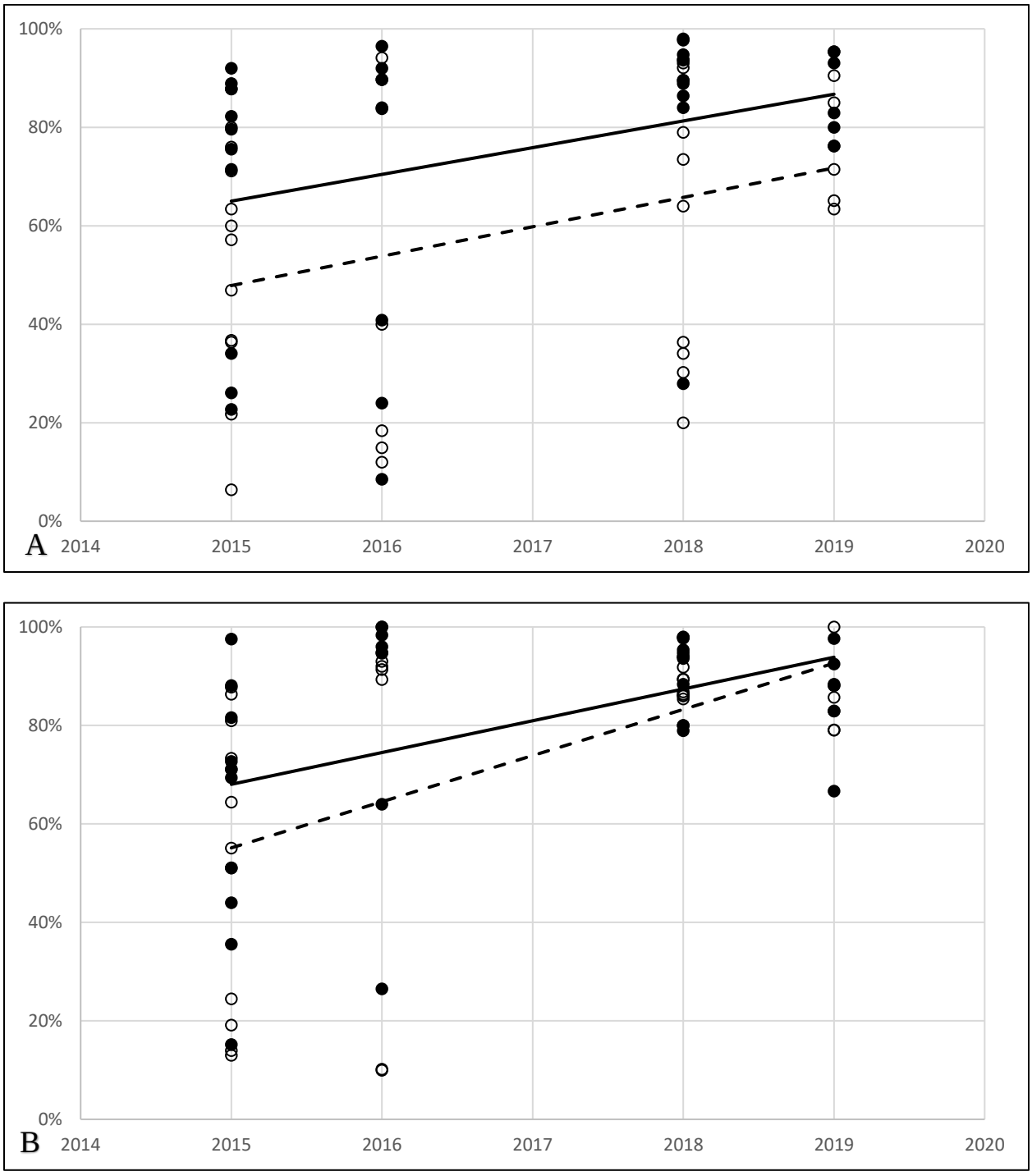

Figure 1.5 Mean percentage corrected mortality of field populations of spider mites over years to a high (solid circle and solid line) and low rate (open circle and dashed line) of abamectin (A) and bifenazate (B).

CHAPTER 2: Within-Field Changes in Patterns of Genetic Variation of *Tetranychus urticae* in Tomato Fields over a Single Growing Season

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is a major pest of multiple high value agricultural commodities (Migeon and Dorkeld 2019). Most TSSM population genetics studies have focused on populations collected from perennial plant hosts (Uesugi et al. 2009a, b; Pascual-Ruiz et al. 2014; Aguilar-Fenollosa et al. 2016; Hada et al. 2016). Fewer have focused on populations collected from annual, herbaceous hosts (Hinomoto and Takafuji 1994, 1995; Navajas et al. 2002), but most of these were conducted in greenhouses or outdoor from plants that were not representative of crops grown in conventional open-field settings.

In comparisons between TSSM populations sampled from both annual and perennial hosts, TSSM populations from perennial hosts demonstrated geographical genetic clustering while TSSM populations from annual hosts did not (Goka and Takafuji 1995). TSSM populations sampled from annual hosts have also demonstrated isolation by distance (IBD) while TSSM populations from perennial hosts in the same area did not (Sun et al. 2012). Although these are different metrics for assessing population genetics, these findings provide a contextual framework that suggests there are distinct differences in patterns of genetic diversity between TSSM populations living on perennial and annual hosts.

Annual agricultural crops, such as tomato, provide unstable habitats that are grown, harvested, and destroyed over the course of a few months, forcing TSSM populations to regularly disperse. After each dispersal event, individuals land on a new host where they are isolated from others. Isolated individuals will engage in higher levels of inbreeding with a small

number of individuals leading to increased rates of genetic drift and lower genotypic richness (Goka 1999; Nouvellet and Gourbière 2013). Conversely, perennial hosts generally provide more stable habitats because perennial hosts tend to be larger, have greater habitat complexity, and provide overwintering hibernacula. These factors may allow for long-term persistence of a native population. Immigrants arriving from other locations will immediately interact with individuals from the larger, established native population, reducing the likelihood of inbreeding as TSSM preferentially mate with unrelated individuals (Hussey and Parr 1963; Goka and Takafuji 1995; Goka 1999; Yoshioka and Yano 2014).

TSSM disperse from plant to plant and short distances over soil by crawling (Hussey and Parr 1963). They disperse long distances via aerial dispersal by climbing to the tops of a host plant where they orient themselves using a combination of wind and light and are carried aloft by an upward draft (Brandenburg and Kennedy 1982; Smitley and Kennedy 1985, 1988). Once in the air, they have no control over the direction of movement or landing location. If they land on a host that they are not adapted to, they can often adapt and establish populations on poor quality hosts (Agrawal 2000; Magalhães et al. 2007, 2009). As population densities increase and resources dwindle, newly emerged gravid females migrate towards the tops of host plants to disperse aerially to new food sources (Smitley and Kennedy 1985, 1988; Dunley and Croft 1992). Under low population densities, which are expected when new hosts are colonized, females deposit many eggs and the subsequent progeny do not disperse (Hussey and Parr 1963; So 1991). Females mate prior to dispersal (Helle and Overmeer 1973), thus even if individuals from multiple populations land in the same field, their first generation of progeny will not be outcrossed. Once they do land in a field, they exhibit a patchy distribution (Mitchell 1973; Rijal et al. 2016). Even if immigrant individuals arrive from the same field of origin, the founder

effect would likely cause differences in allele frequencies in each patch due to the initial high degree of inbreeding. Assuming a low density of individuals at colonization, individuals in the first generation will mate with each other, further facilitating high rates of inbreeding, which in turn leads to more rapid rates of allele fixation within the first few generations following colonization (Hinomoto and Takafuji 1994; Charlesworth 2003).

Globally, TSSM has the highest incidence of pesticide resistance of any arthropod, with documented resistance on to 96 registered active ingredients (Xu et al. 2018; Mota-Sanchez and Wise 2019). Decreased habitat complexity, isolation of newly colonizing individuals, and greater propensity for dispersal of TSSM in annual vs perennial hosts would be expected to result in differences in the onset and spread of acaricide resistance within landscapes. TSSM dispersing from a field with even a moderate frequency of pesticide resistance alleles could establish in the newly planted fields with much higher frequencies of resistant alleles due to the founder effect (Dunley and Croft 1992; Osakabe et al. 2009). Regular dispersal events from other fields may accelerate the development of pesticide resistance among fields independent of selection pressures within a field (Shi et al. 2019). To date, there have been no studies published that analyze how genetic variation and population structure for TSSM changes within a field during a growing season in an annual crop. An improved understanding of the genetic diversity and population structure of TSSM populations could aid in better understanding the development of acaricide resistance in annual cropping system.

In this study, we sought to investigate changes in genetic diversity and population structure of TSSM populations within individual tomato fields grown as annual crops during a single growing season. The results presented expand knowledge of how genetic diversity and population structure of TSSM changes spatially and temporally within an annual crop host and

identifies differences from the dynamics that have been reported in studies of perennial crops that thus far have been the focus of TSSM genetic studies in agroecosystems.

METHODS

Sampling

At the beginning of the growing season, TSSM were sampled from three commercial staked tomato fields in Rowan County, North Carolina, USA. Each field was divided into four equally sized quadrants. Within each quadrant, high-density patches of TSSM were located using an adaptive random sampling technique. First, the topmost fully expanded leaves were randomly sampled within a quadrant to inspect for presence or absence of TSSM. When a leaflet with at least one female TSSM was identified, a randomly sampled leaflet from 5 other plants within a 3-meter radius were also inspected. If at least 3 of the leaflets harbored TSSM, the area was considered sufficiently high enough to sample to serve as a patch of TSSM. The nearest tomato stake was marked as the center of a patch with a bright colored flag and an 8-meter radius around the flag was marked to demarcate the outer limits of a patch. The patches were selected such that their outer bounds did not reach within 5 meters of a quadrant margin to limit edge effects. The patch identification protocol was repeated in each of the four quadrants for each field. All fields were roughly square in shape, similar in size ranging in area from 22.5 km^2 to 26 km^2. No patches were closer than 50 meters to another patch where TSSM were collected for this study.

Once a patch was identified within each quadrant, 15 female TSSM from each patch were transferred from the foliage into 95% ethanol. Two weeks later, 15 more female TSSM were sampled from the same flagged location within each quadrant from each field. To ensure the greatest degree of genetic diversity within each patch, no more than one female was collected from any single plant sampled. All three fields were sampled at the beginning of the growing

season while tomatoes were still developing vegetative growth on May 29, 2016 and 15 days later on June 13, 2016. These dates provided the greatest separation in sampling dates without acaricide applications to reduce population density. Fields are annually sprayed with two acaricides. Abamectin is used during the vegetative growth period and sprayed again with bifenazate only when populations exceed threshold levels during the harvest period.

DNA Extraction

Adult female TSSM were transferred individually into wells on a 96-well plate and air dried for 30 minutes to allow the ethanol to evaporate. Mite were then crushed using a fine tip metal probe. Total DNA was isolated from each mite using the following IGEPAL DNA extraction protocol: 30 µL of an IGEPAL lysis buffer (final concentration: 1mM EDTA pH 8.0, 10mM Tris-HCl pH 8.4, 200 µg/mL proteinase K, and 1% IGEPAL suspended in mH_2O) was added to each well, thoroughly mixing the solution in each well to homogenize material in the solution. Plates were incubated at 95 °C for 15 minutes then immediately transferred to a -20 °C freezer. Prior to use in a PCR, DNA solutions were thoroughly mixed.

Microsatellite Amplification

Seventeen microsatellite markers were used to genotype individuals for this study (Table 2.1; Uesugi and Osakabe 2007; Sauné et al. 2015). Sequences were modified by the addition of an M13 tail (5'-CACGACGTTGTAAAACGAC-3') to the 5’ end of each forward primer for each primer pair for infrared visualization on an acrylamide gel. PCR amplifications were performed in a total volume of 5 µL consisting of 0.6 µL template DNA and 4.4 µL of a PCR master mix (final concentration: 1x NH_4, 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.17 pmol/µL forward primer, 0.17 pmol/µL reverse primer, 0.03 pmol/µL M13 IR dye, 0.04 units/µL Taq polymerase suspended in mH_2O). Cycling conditions for the PCR protocol were: initial denaturation for 5 minutes at 95

°C followed by 35 cycles of 30 seconds at 95 °C for denaturation, 50 seconds at 59 °C for annealing, 1 minute at 72 °C for extension, and a final 5 minute extension phase at 72 °C.

The amplified microsatellite products were then prepared for use on an acrylamide gel using the following: two µL of final product was transferred to a new 96-well plate. Eight µL formamide solution was added into each well and the plate incubated at 95 °C for 15 minutes before transferring it to a -20 °C freezer. The amplified microsatellite products were subsequently separated and visualized on an acrylamide gel using a Li-Cor 4200 DNA Analyzer Gene ReadIR (Li-Cor Biosciences, Lincoln, NE). Allele sizes were measured and scored using Gene Profiler v4.05 (Scanalytics Inc).

Microsatellite Analyses

Locus scoring errors due to stutter, allele dropout, and null alleles were evaluated using MICRO-CHECKER v 2.2.3 software (Van Oosterhout et al. 2004). After corrections, several datasets were generated to evaluate overall, within field, and across time comparisons. All data were transformed from the GENEPOP data format to FSTAT and GENETIX data formats using PGDSpider v 2.1.1.5 (http://www.cmpg.unibe.ch/software/PGDSpider/) (Lischer and Excoffier 2012). Genotypic linkage disequilibrium (LD) exact tests were performed using GENEPOP v 4.7.0 software (Rousset 2008). Significance probabilities for all exact tests were generated using MCMC parameters: 10,000 dememorization, 1,000 batches, 10,000 iterations per batch. Sequential Bonferroni corrections ($\alpha = 0.05$) were used for multiple comparisons to control for type I errors.

Hardy-Weinberg proportion (HWP) exact tests were performed using GENEPOP v 4.7.0 software (Rousset 2008). For populations significantly different from HWP, heterozygote deficiency and excess exact tests were performed globally and by locus. HWP were evaluated for

each patch at both sample dates then patches were pooled together for each field to evaluate HWP for each field at both time points. Observed heterozygosity (H_O), average unbiased expected heterozygosity (H_S), inbreeding coefficients (F_{IS}), and alleles per locus (A/L) were calculated using FSTAT v 2.9.3.2 (Goudet 2001) and GENETIX v 4.05.2 (Belkhir et al. 2004) software. Pairwise genetic distances (F_{ST}) based on Pairwise comparisons were calculated using GENEPOP v 4.7.0 software (Rousset 2008). Paired t-tests were used for comparisons of these metrics in each sample date for multiple levels of analysis. Patches were analyzed as individual units within a field and compared across sample dates for each field. All patches were maintained as individual units, but all fields were analyzed as a group across sample dates for an overall analysis. Finally, patches at each sample date were pooled together within each field to compare a field across each sample date.

RESULTS

Only five of the seventeen microsatellite loci were amplified consistently across all individuals. An additional locus, TuLS16, did not amplify for field 3, but did amplify for the other two fields, thus it was retained in the dataset but only used with the two fields where amplification was successful. These loci all retained a minimum of two alleles at each locus (Table 2.1). All other loci were excluded from the dataset. A total of 360 individuals were genotyped. Twenty-five individuals were genotyped for fewer than two loci and were excluded from the dataset. Due to the low number of individuals with successful amplification, 21 individuals that were genotyped for only two alleles were kept in the dataset. This provided for a total of 335 individuals genotyped across 6 microsatellite loci with a mean of 76.5% of individuals genotyped per locus ranging from 66.9-90.7%. No significant linkage disequilibrium (LD) was detected. All six loci were regarded as independent and used in the following analyses.

Hardy-Weinberg proportion (HWP) exact tests for patches overall revealed only 3 of 12 patches were significantly different from HWP at the first sample date (corrected sequential Bonferroni: n = 12, P < 0.005) and 2 of 12 were significantly different from HWP at the second sample date (corrected sequential Bonferroni: n = 12, P = 0.005). Further analyses of the three patches from the first sample date revealed all three had significant heterozygote deficiency (corrected sequential Bonferroni: n = 12, P < 0.0001) and none with significant heterozygote excess. The two patches significantly different from HWP at the second sample date had neither significant heterozygote deficit nor excess. HWP exact test for patches pooled together for each field at both sample dates revealed that fields 1 and 2 were significantly different from HWP at the first sample date (corrected sequential Bonferroni: n = 3, P < 0.001) and all three fields were significantly different from HWP at the second sample date (corrected sequential Bonferroni: n = 3, P < 0.05). Further analysis revealed that both fields at the first sample date had significant heterozygote deficiency (corrected sequential Bonferroni: n = 3, P < 0.01) and fields 1 and 3 in the second sample date had significant heterozygote deficiency (corrected sequential Bonferroni: n = 3, P < 0.005). None of the fields at either sample date had significant heterozygote excess. The disparity between HWP at the pooled field level and the individual patch level suggested that further analyses should not be conducted with patches pooled for each field.

In the overall patterns for patches across all fields, F_{IS} values were significantly lower in the second compared to the first sample date (mean difference = 0.29335; paired T test: t (11) = 4.5889, P = 0.0006; Table 2.2). H_O was significantly higher in the second compared to the first sample date (Mean difference = 0.1336; paired T test: t (11) = 3.8348, P = 0.0028). Pairwise F_{ST} comparisons between patches within their respective fields did not change significantly between sample dates (Mean difference = 0.0265; paired T test: t (17) = 1.6992 P = 0.1075; Table 2.3);

nor did H_S or *A/L* change significantly (Mean difference H_S = 0.0267; paired T test: t (11) = 1.0741 P = 0.3058; Mean difference *A/L* = 0.1917; paired T test: t (11) = 1.6483 P = 0.1275).

The pattern of changes in genetic diversity and structure between patches within each field at each sample date did not correlate directly with the overall patterns. In patches from field 1, F_{IS} was significantly lower in the second compared to the first sample date (mean difference = 0.4199; paired T test: t (3) = 8.4013, P = 0.0035). H_O was significantly higher in the second compared to the first sample date (mean difference = 0.1984; paired T test: t (3) = 3.1862, P = 0.0499. Pairwise F_{ST} comparison values between patches were significantly lower in the second compared to the first sample date (mean difference = 0.0623; paired T test: t (5) = 4.2951, P = 0.0078). In patches from field 2, F_{IS} was significantly lower in the second compared to the first sample date (mean difference = 0.3810; paired T test: t (3) = 5.8278, P = 0.0101). H_O was significantly higher in the second than the first sample date (mean difference = 0.1434; paired T test: t (3) = 3.6003, P = 0.0368). Pairwise F_{ST} comparison values did not significantly change between the two sample dates (mean difference = 0.0426; paired T test: t (5) = 1.9502, P = 0.1086). In contrast, the patches from field 3, neither F_{IS} nor H_O changed significantly (mean F_{IS} difference = 0.0792; paired T test: t (3) = 0.6467, P = 0.5639; mean H_O difference = 0.0592; paired T test: t (3) = 0.8716, P = 0.4476), but pairwise F_{ST} comparison values between patches were significantly lower in the second compared to the first sample date (mean difference = 0.0598; paired T test: t (5) = 3.1483, P = 0.0254). H_S and *A/L* did not significantly change for any of the fields.

DISCUSSION

Patterns of significant departure from Hardy-Weinberg proportions with significant heterozygote deficiency have been well documented in numerous TSSM microsatellite studies

(Navajas et al. 2002; Magalhães et al. 2007; Uesugi et al. 2009a, b; Li et al. 2009; Sun et al. 2012; Pascual-Ruiz et al. 2014; Aguilar-Fenollosa et al. 2016; Hada et al. 2016; Zhang et al. 2016; Shi et al. 2019). In general, significant heterozygote deficiency can be attributed to null alleles, which are alleles with a mutation that prevent amplification of a particular allele. A genotype for an individual with one null allele will give the false impression an individual is homozygous because the null allele did not amplify causing a disproportionate number of homozygotes to be recorded (Chapuis and Estoup 2007; Carlsson 2008). If these deficiencies in heterozygosity were confined to a small proportion of the microsatellite loci, null alleles would be the likely causative factor, but because the pattern of heterozygote deficiency is so prevalent across a majority of loci in TSSM population genetic studies, this phenomenon has been characterized as the result of biologically founded phenomena and the Wahlund effect (Navajas et al. 2002; Bailly et al. 2004; Carbonnelle et al. 2007; Zhang et al. 2016). The Wahlund effect describes an apparent deficit in heterozygotes caused by including samples of individuals from multiple subpopulations into one larger group (Garnier-Géré and Chikhi 2013). We see this scenario in this study where 19 of 24 patches analyzed did not differ significantly from HWP, but when patches were pooled together for each field, all but one of the fields were no longer in HWP with most demonstrating significant heterozygote deficiency. In haplodiploid species, significant deviation from HWP can occur even with random mating when there are differences in allele frequencies between haploid males and diploid females, but these differences lead to an excess of heterozygotes rather than the deficit of heterozygotes regularly reported (Hedrick and Parker 1997). This result provides more evidence that null alleles are not the sole cause of heterozygote deficiency regularly seen in TSSM population genetics studies, but rather, the Wahlund effect due to sampling multiple isolated groups to as representative of one area.

We expected to resolve several patterns of genetic differentiation and structure in this study. During the early colonization phase of a field, we expected that individuals were more likely to mate with closely related individuals in their patch this resulting in high F_{IS} as well as low H_O in at the first sample date. We also expected new colonizers to have multiple points of origin and would have small population sizes allowing for the founder effect and genetic drift to cause dramatic changes in allele frequencies for patches even if they originate from the same source. This would enable the detection of moderate levels of differences between patches based on pairwise F_{ST} values. After two weeks, which is roughly equivalent to two generations in the hottest summer months (Shih et al. 1976), populations were anticipated to grow rapidly and patch sizes to expand and overlap with one another. As individuals outcrossed with other patches, we expected a significant decrease in F_{IS} and a corresponding significant increase in H_O. The outcrossing between patches was also expected to lead to a significant decrease in pairwise F_{ST} values as patches homogenize. Additionally, we did not expect H_S and *A/L* to change significantly because they are metrics derived from allelic proportions rather than genotypic proportions. The introduction of new alleles by late colonizers would not be expected to significantly change the allelic proportions in a field where an established population already exists.

The overall pattern when analyzing all patches from all fields grouped together supported many of these hypotheses. The overall mean F_{IS} dropped from 0.2599 to -0.0335 and there was a corresponding increase in H_O from 0.3174 to 0.4510 over the two sample dates (Table 2.2). The H_S and *A/L* remained stable over this time period. The pattern of change in pairwise F_{ST} values did not follow the pattern we expected; there were no significant changes between the first and

second sample dates. The pattern of change that occurred on a field-to-field basis was much more varied.

In field 1, patches followed all of the expected patterns with F_{IS} and pairwise F_{ST} values significantly decreasing and H_O significantly increasing. Field 2 followed also had a significant decrease in F_{IS} and an increase in $H_{O,}$ but the average pairwise F_{ST} did not significantly change. In field 3 neither the F_{IS} nor H_O changed, but pairwise F_{ST} values between patches decreased significantly. In patches 1 and 2 for field 3, the F_{IS} values were negative in the first time point, suggesting an excess of heterozygotes. These negative F_{IS} values may indicate that these two areas have already gone through patch expansion where individuals would outcross with other patches. the other two patches in field 3 generally followed the expected pattern of a decrease in F_{IS} and an increase in H_O over these two time points. These data are curious because they suggest that there were either two separate colonization events or that the colonizing population was lower in one portion of the field, resulting in greater inbreeding than occurred in other portions of the field. The latter may be the more likely considering the average initial pairwise F_{ST} values among patches was 0.0822 and decreased significantly to 0.0224. These low values throughout suggest the individuals from four patches all originated from the same location. This may indicate that initial population density after dispersal can be variable enough to change the rate of inbreeding and expansion of each patch within a field.

The changes in average pairwise F_{ST} values between patches were significant for 2 of the 3 fields analyzed, but these changes only suggested marginal decreases in relationships, averaging a decrease of only 0.0265. Although significant, pairwise F_{ST} values between patches do not seem to be appropriate indicators of how alleles may behave within a field. Our initial assumption was that individuals from multiple sources would colonize a field in a random

distribution and the resulting new highly inbred patches of individuals would differ from each other. This does not appear to be the case. The overall mean pairwise F_{ST} within fields in the first time period was relatively low, 0.0474, suggesting that the individuals in each patch within a given field likely resulted from the same dispersal and colonization event. Due to the high initial degree of relatedness, mean pairwise F_{ST} values between patches should not change significantly due to high levels of inbreeding.

An important finding of this study is the pattern of a significant reduction in F_{IS} and corresponding increase in H_O over a two-week period. This may have direct implications on acaricide resistance management practices. In situations where females carrying resistance alleles disperse from other crop fields colonize a new field, significant inbreeding following colonization would lead to an increase in the proportion of individuals carrying resistance alleles in some patches. Proactive growers may seek to use chemical management to prevent TSSM populations from growing, but this response may further select for individuals carrying resistance alleles by selecting against inbred patches with high proportions of susceptible alleles. After two weeks, we saw significant decreases in in F_{IS} and increases in H_O, which may provide enough outcrossing to occur to dilute the number of homozygous resistant females as they outcross with susceptible males from other patches in a field. Although in such situations, early, proactive chemical management may delay the TSSM population growth curve, a second resurgence of the population will have a higher frequency of resistance alleles and the efficacy of chemical management will be reduced.

In this study, we collected two samples two weeks apart to avoid collection of samples after acaricide applications, which would have confounded results from samples collected afterwards. The sampling methods used to collect TSSM were only sufficient for detection of

mites after considerable population growth and outbreeding among founder colonies. In addition, low amplification of microsatellite loci as well as a low number of samples used reduced our ability to evaluate the patterns of changes in genetic diversity between sampled time points. We have found that in this short time, outcrossing may occur at such a rate that a decrease in F_{IS} was detected in 10 of 12 patches evaluated accounting for an overall decrease in F_{IS} for two out of three fields. This suggests that delaying acaricide applications for TSSM management could allow for homozygous resistant females to outcross with susceptible males as they reduce inbreeding to produce more heterozygous individuals that may be more susceptible to chemical management.

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Table 2.1 Microsatellite loci used for comparing twospotted spider mite populations within tomato fields; F, forward primer; R, reverse primer; N_A, # alleles where loci that were not used for analyses due to amplification failure are demarcated by X

Locus	Motif	Allele range	N_A	Primer Sequence (5'-3')	Accession number	Source
TuLS16	$(CAT)_{10}$	186-228	3	F: CACGACGTTGTAAAACGACAATTGCTTATCACCCACATC	KJ545960	Saune et al 2015
				R: TTAGTTGCTTGTTGAGCAGA		
TuLS17	$(ATG)_6$	192-207	2	F: CACGACGTTGTAAAACGACTCTTCGTTCGATAGCTTTTC	KJ545961	Saune et al 2015
				R: TCCTCAGGTATATCAGGTGG		
TuLS20	$(TTG)_6$	212-218	2	F: CACGACGTTGTAAAACGACAAGCTGGATTCATAGAAGCA	KJ545963	Saune et al 2015
				R: AAATTAATTCAGCCTCGTCA		
TuLS23	$(TAA)_6$	225-238	3	F: CACGACGTTGTAAAACGACTGGTAACTGCATCAACCATA	KJ545965	Saune et al 2015
				R: AAGATTCGGGAAGATTAAGG		
TuCT13	$(TC)_7$	163-168	2	F: CACGACGTTGTAAAACGACCAGATGGATTTGCTTTCCAC	AB263085	Uesugi and Osakabe 2007
				R: GATCCTAATCAACATGAGGGTC		
TuCT67	$(CT)_9$	95-107	8	F: CACGACGTTGTAAAACGACCCATCATCTTCATCATTCTTCACC	AB263090	Uesugi and Osakabe 2007
				R: TAGAACAGTCAAGCAAAAAGAGTC		
TuLS14	$(ATG)_7$	191-210	X	F: CACGACGTTGTAAAACGACGCAAATGAAGCTTACCAATTA	KJ545959	Saune et al 2015
				R: TAAAGGTTTGGCAGTTCAGT		
TuLS41	$(CAT)_6$	242-261	X	F: CACGACGTTGTAAAACGACGAATGAAGATTGGTGGGTTA	KJ545970	Saune et al 2015
				R: TCAAGATTTTGGAATCAGAGA		
Tu35b	$(TGA)_8$	91-113	X	F: CACGACGTTGTAAAACGACCTTCCCGAAGGCTGTTGATA	AJ419832	Saune et al 2015
				R: AATGGAATGAGTTATCGTTGGG		
TuCT04	$(CT)_8$	146-152	X	F: CACGACGTTGTAAAACGACCGTCATCATTGCCGTCATTTTAC	AB263083	Uesugi and Osakabe 2007
				R: GGAGCCGTTTCAAGAGAGTG		
TuCT09	$(CT)_{15}$	102-128	X	F: CACGACGTTGTAAAACGACGATCACTTTTTCATGTTATTCTG	AB263084	Uesugi and Osakabe 2007
				R: CTTGGAATGAACTTTAGCAC		
TuCT26	$(GA)_8$	150-156	X	F: CACGACGTTGTAAAACGACCGATGGAGCCGTTTCAAGAG	AB263088	Uesugi and Osakabe 2007
				R: TCGTCATCATTGCCGTCATTTTAC		
TuCT73	$(CA)_7$	111-113	X	F: CACGACGTTGTAAAACGACCGATGTGGGTGGTAAGCATG	AB263091	Uesugi and Osakabe 2007
				R: ACGATGATATTGATGATGAGCG		
TuCA02	$(CA)_2A$-$(CA)_7$	186-190	X	F: CACGACGTTGTAAAACGACGTCAAAGAGTTACCCAAAGAG	AB263077	Uesugi and Osakabe 2007
				R: TCATATCAAACCAGAAAAGGAG		
TuCA12	$(CA)_7$	263-275	X	F: CACGACGTTGTAAAACGACGATTTGTGGTCGTGGTTTTC	AB263078	Uesugi and Osakabe 2007
				R: GATCAACTCAAAAGGATAACGTTG		
TuCA25	$(TC)_{13}$	155-165	X	F: CACGACGTTGTAAAACGACAATGTGTTGGTTGTTTACGAAGTG	AB263079	Uesugi and Osakabe 2007
				R: TTGGTCAAAGCCGGTTACAG		
TuCA83	$(GT)_6$	206-208	X	F: CACGACGTTGTAAAACGACCAGGGTGAAACTTAGATACC	AB263081	Uesugi and Osakabe 2007
				R: CAATTTTCCCTCTACATCTC		

Table 2.2 Sample size (N), mean observed heterozygosity (H_O), mean expected heterozygosity (H_S), inbreeding coefficient (F_{IS}), and alleles per locus (A/L) for twospotted spider mite collected on two sample dates (T1 and T2) from patches of three different tomato fields.

Field	Patch	N	H_O	H_S	F_{IS}	A/L
	1A-T1	13	0.3068	0.4156	0.2854	2.667
	1B-T1	14	0.3774	0.5151	0.2764	2.833
	1C-T1	14	0.4067	0.4595	0.1261	2.5
	1D-T1	12	0.1109	0.2757	0.4576	1.8
1-T1		53	0.3398	0.4786	0.2961	3.333
	1A-T2	15	0.4496	0.375	-0.2256	2.667
	1B-T2	15	0.4975	0.4597	-0.096	3
	1C-T2	15	0.5522	0.4857	-0.1759	3.2
	1D-T2	15	0.4952	0.4926	-0.0366	3
1-T2		60	0.4356	0.3947	-0.1273	3
	2A-T1	14	0.2568	0.3773	0.3364	3
	2B-T1	15	0.3514	0.4655	0.2421	3.167
	2C-T1	12	0.1019	0.4345	0.6788	2.833
	2D-T1	13	0.3178	0.4271	0.2694	2.833
2-T1		54	0.2706	0.4199	0.3618	3.333
	2A-T2	15	0.427	0.4766	0.0883	3
	2B-T2	15	0.4222	0.3931	-0.0881	3
	2C-T2	15	0.3456	0.4605	0.1218	2.833
	2D-T2	15	0.4066	0.3792	-0.1191	2.833
2-T2		60	0.4095	0.4724	0.0474	3.333
	3A-T1	15	0.5333	0.4625	-0.1594	2.8
	3B-T1	14	0.4857	0.4349	-0.1218	2.6
	3C-T1	13	0.3684	0.4626	0.1945	2.8
	3D-T1	8	0.1917	0.3844	0.5333	2.6
3-T1		50	0.4290	0.4744	0.0960	3.2
	3A-T2	15	0.5067	0.4386	-0.1616	2.6
	3B-T2	13	0.4462	0.4443	-0.0043	2.6
	3C-T2	15	0.4168	0.5204	0.1997	3
	3D-T2	15	0.4462	0.5095	0.0960	3
3-T2		58	0.4550	0.4868	0.0580	3
Mean T1			0.3174	0.4262	0.2599	2.7028
Mean T2			0.4510	0.4529	-0.0335	2.8944

Table 2.3 Pairwise genetic distance (F_{ST}) values for twospotted spider mites populations sampled from four patches within each of four tomato fields on two sample dates (T1 and T2).

	1A-T1	1B-T1	1C-T1
1B-T1	0.0011		
1C-T1	0.0550	0.1223	
1D-T1	0.0642	0.1070	0.0831

	1A-T2	1B-T2	1C-T2
1B-T2	-0.0113		
1C-T2	0.0039	0.0104	
1D-T2	0.0259	0.0230	0.0073

	2A-T1	2B-T1	2C-T1
2B-T1	-0.0114		
2C-T1	-0.0023	-0.0115	
2D-T1	0.0129	-0.0382	-0.0226

	2A-T2	2B-T2	2C-T2
2B-T2	0.1200		
2C-T2	0.0134	0.0465	
2D-T2	0.0046	0.0267	-0.0287

	3A-T1	3B-T1	3C-T1
3B-T1	0.0348		
3C-T1	0.1390	0.1216	
3D-T1	0.0708	-0.0084	0.1358

	3A-T2	3B-T2	3C-T2
3B-T2	0.0069		
3C-T2	0.0614	0.0435	
3D-T2	-0.0183	0.0107	0.0306

CHAPTER 3: Variation in Genetic Structure of *Tetranychus urticae* in North Carolina (USA) Tomato Agroecosystems Using Microsatellite Markers

INTRODUCTION

The two-spotted spider mite (TSSM) *Tetranychus urticae* Koch is a major agricultural pest throughout the world. They are extremely polyphagous, having been documented feeding on over 1100 plant species, many of which are agricultural commodities (Migeon and Dorkeld 2019). They also have the highest incidence of pesticide resistance of any arthropod, with documented resistance to 96 registered active ingredients (Xu et al. 2018; Mota-Sanchez and Wise 2019). In order to reduce the rate of pesticide resistance development, it is essential to consider pesticide resistance management when developing an integrated pest management strategy. One of the key pillars of pesticide resistance management is the delay of pesticide resistance development by limiting gene flow of resistance alleles when possible. To better manage TSSM pesticide resistance, it is vital to understand factors that influence the genetic structure of TSSM populations such as dispersal, habitat fragmentation, and isolation by distance (Dunley and Croft 1992).

TSSM dispersal events occur in response to high population densities and declining host quality (Mitchell 1973; Smitley and Kennedy 1988). They disperse from field to field via aerial dispersal using a combination of wind and light to orient themselves at a proper angle to be carried aloft by an upward draft for undirected, long-distance aerial dispersal on air currents (Brandenburg and Kennedy 1982; Smitley and Kennedy 1985, 1988). Due to their extreme polyphagy, if they land on a poor quality host, they might still establish and reproduce, but their progeny may attempt to disperse again throughout the growing season (Brandenburg and Kennedy 1982; Agrawal 2000; Magalhães et al. 2009).

Many studies have applied microsatellite markers to evaluate patterns of genetic diversity and population differentiation in TSSM. A majority of these have focused on TSSM populations sampled from perennial crops (Carbonnelle et al. 2007; Uesugi et al. 2009b; Pascual-Ruiz et al. 2014; Hada et al. 2016). Perennial hosts reduce the propensity for dispersal because the habitat has greater temporal stability compared to annual crops that persist for a single cropping season or less. Many annual crops are only present for a portion of the growing season leading TSSM populations to disperse multiple times and colonize multiple hosts during a single growing season (Brandenburg and Kennedy 1982; Margolies 1995; Goka 1999). Further, at the end of the growing season when TSSM are exposed to short day lengths, they can enter winter diapause (Veerman 1977, 1991). Perennial crops provide the necessary protective hibernacula, whereas seasonal crops do not, forcing TSSM to disperse in search of overwintering sites (Margolies 1995; Raworth 2007; De Roissart et al. 2015; Aguilar-Fenollosa et al. 2016).

Fresh market tomato production is labor-intensive (Ivors 2010) and characterized by frequent movement of personnel and equipment among fields belonging to individual farms. The labor-intensive system may contribute grower-facilitated dispersal whereby TSSM are unintentionally transferred among fields by farm workers or machinery.

This study used microsatellite loci to analyze the genetic diversity and population structure of TSSM populations in two different tomato agroecosystems in North Carolina that differ in climatic conditions and topography. The field sites selected for sampling within each region were chosen with the intention of evaluating the impact that growers may have on genetic diversity and population structure through grower-facilitated dispersal of TSSM as well as the impact of landscape factors unique to each region. Analysis of how agroecosystem factors and

grower practices affect the genetic relationship of TSSM populations will play a valuable role in helping to develop acaricide resistance management programs for this key pest of tomatoes.

METHODS

Locations

TSSM were sampled from two regions of North Carolina, USA, where staked tomatoes have long been a component of the local agroecosystems. The Central region is located in the rolling hills of the Piedmont at an elevation of ~200 m above sea level. This region is characterized by flat plains with wide expanses of contiguous fields. The Western region is located 180 km west of the Central region in the southeastern area of the Blue Ridge Mountains at an elevation of ~650 m above sea level. This region is characterized by small pockets of fields nested within valleys isolated from other fields by lush forests. Temperatures in the Central region averaged 2.5 °C warmer than the Western region during the peak growing season (June-September) between 2012 and 2018 (Figure 3.2A; State Climate office of North Carolina). Based on a thermal model to predict initiation of oviposition after winter diapause (Raworth 2007), TSSM are estimated to break diapause and begin oviposition 6 weeks earlier in the Central region than the Western region (Figure 3.1B). The warmer climate in the Central region also allows tomato growers to plant crops 4 to 6 weeks earlier.

In Summer of 2018, TSSM were sampled from fields selected to represent growers that planted at least 3 fields while maximizing the distance between fields. Fifteen fields each were sampled in the Western and Central regions (30 total). Three growers were represented in the Western region (P, D, KJ) with 5 fields sampled from each grower. Four growers were represented in the Central region (RP, FP, C, W) with 4 fields sampled from three growers each

and 3 fields sampled from one grower. Due to differing degree days, fields in the Central region were sampled one month earlier than fields in the Western region.

Sampling and DNA Extraction

Leaflets were sampled at random from the topmost, fully expanded leaf of a tomato plant then visually inspected for presence or absence of multiple TSSM. Leaflets harboring multiple individuals were placed in a plastic bag. Leaflets were collected from plants no less than 5 feet away from any other plant where mites were sampled. A minimum of 60 leaflets were collected from each field. Plastic bags were kept in a cool, dry holding chamber until DNA extraction could take place later within1-2 days. Adult female TSSM were transferred individually into wells on a 96-well plate and crushed using a fine top metal probe. No more than one female was taken per leaflet.

Total DNA was isolated from each TSSM female using the following IGEPAL DNA extraction protocol: 30 µL of an IGEPAL lysis buffer (final concentration: 1mM EDTA pH 8.0, 10mM Tris-HCl pH 8.4, 200 µg/mL proteinase K, and 1% IGEPAL suspended in mH_2O) was added to each well, thoroughly mixing the solution in the well to homogenize material in the solution. Plates were incubated at 95 °C for 15 minutes then immediately transferred to a -20 °C freezer. Prior to placing in a thermocycler for PCR, DNA solutions were thoroughly mixed.

Microsatellite Amplification

Sixteen microsatellite markers were used to genotype Individuals for this study (Table 3.1; Uesugi and Osakabe 2007; Sauné et al. 2015). Forward Primers for each primer pair were labeled with either 6-FAM, VIC, NED, or PET fluorescent tags for use with ABI genotyping such that no PCR product overlapped with another within the same fluorescent group, which allowed for multiloading of all 16 microsatellite amplification products on the same run.

PCR amplifications were performed in a total volume of 5 µL containing 0.6 µL template DNA and 4.4 µL of a PCR master mix (final concentration: 1x NH_4, 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.17 pmol/µL forward primer, 0.17 pmol/µL reverse primer, 0.04 units/µL Taq polymerase suspended in mH_2O). Products were amplified using the following cycling conditions: initial denaturation for 5 minutes at 95 °C followed by 35 cycles of 30 seconds at 95 °C for denaturation, 50 seconds at 61 °C for annealing, 1 minute at 72 °C for extension, and a final 5 minute extension phase at 72 °C.

After all microsatellites were amplified for a set of individuals, the products were prepared for analysis using an Applied Biosystems 3730xl DNA Analyzer. Two µL of each product were pooled into a single well on a 96-well plate. The pooled products were thoroughly mixed, then 2 µL pooled product were transferred to a new plate and mixed with 0.5 µL 500 LIZ size-standard suspended in 7.5 µL Hi-Di formamide. The 96-well plate was covered with an adhesive foil and incubated at 95 °C for 15 minutes, then transferred to a -20 °C freezer. Samples were then analyzed at the Genomic Sciences Laboratory, North Carolina State University (USA). Fragment sizes were assigned using Geneious v 9.1.8 (https://www.geneious.com; Kearse et al. 2012).

Microsatellite Analyses

Data Curation

Locus scoring errors due to stutter, allele dropout, and null alleles were evaluated using MICRO-CHECKER v 2.2.3 software (Van Oosterhout et al. 2004). After corrections, several datasets were generated to evaluate populations overall, by region, by field, and by grower. All data were transformed from the GENEPOP data format to FSTAT, GENETIX, and STRUCTURE data formats using PGDSpider v 2.1.1.5

(http://www.cmpg.unibe.ch/software/PGDSpider/; Lischer and Excoffier 2012). Genotypic linkage disequilibrium (LD) exact tests were performed using GENEPOP v 4.7.0 software (Rousset 2008). Significance probabilities for all exact tests were generated using MCMC parameters: 10,000 dememorization, 1,000 batches, 10,000 iterations per batch. Sequential Bonferroni corrections ($\alpha = 0.05$) were used for multiple comparisons to control for type I errors.

Genetic Diversity

Hardy-Weinberg proportion (HWP) exact tests were performed using GENEPOP v 4.7.0 software (Rousset 2008). For populations significantly different from HWP, heterozygote deficiency and excess exact tests were performed globally and by locus. Observed heterozygosity (H_O), unbiased expected heterozygosity (H_S), inbreeding coefficients (F_{IS}), and allelic richness (*AR*) using the rarefaction approach were calculated using FSTAT v 2.9.3.2 (Goudet 2001) and GENETIX v 4.05.2 (Belkhir et al.) software.

Population Differentiation and Genetic Structure

Genetic distances (F_{ST}) based on Pairwise comparisons were calculated using GENEPOP v 4.7.0 software (Rousset 2008). Isolation by distance (IBD) was estimated by the correlation between the pairwise log-transformed geographic distances (km) and the linearized pairwise genetic distances ($F_{ST}/(1-F_{ST})$). Mantel tests were conducted to validate IBD between fields based on 10,000 permutations.

STRUCTURE v 2.3.4 software (Pritchard et al. 2000) was used to infer the optimal number clusters of individuals based on genotypes using Bayesian methods. Analyses were run with 50,000 burn-ins, 50,000 MCMC replicates, 10 iterations per K, and a K ranging from 1 to 30 for an analysis of all 26 populations and 1 to 20 when regions were analyzed separately. STRUCTURE Harvester Web v 0.6.94 (Earl and vonHoldt 2012) was used to collate results

generated by STRUCTURE and assess the mean likelihood values of K using the Evanno method (Evanno et al. 2005). CLUMPP v 1.1.2 (Jakobsson and Rosenberg 2007) software was used to align all the iterations of STRUCTURE for each value of K output by STRUCTURE harvester for each level of K to generate a final permutation that most closely matches the composite of the iterations.

A Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) was conducted using the ADEGENET package (Jombart 2008) for R v 3.5.3 (R Core Team 2019). DAPC is a multivariate, model-free method used to identify genetic clusters based on *a priori* group assignments that helps to validate the data generated in STRUCTURE. This analysis first performs a cross validation procedure to determine the optimal number of principal components in a principal component analysis (PCA) that give rise to a model with the highest predictive capacity to accurately assign a given individual to *a priori* group assignments. DAPC then performs a discriminant analysis on the optimal number of principal components to evaluate genetic structure. The cross-validation was run using 95 replicates with a 0.9 training set size to evaluate the genetic structure of the populations in each dataset. Scatterplots were then generated based on the suggested number of Principal Components.

Statistical Analyses

Comparisons of means and variances between the Western and Central region were conducted using independent T tests F tests respectively. Due to the small and varying sample sizes, comparisons between growers were conducted using a non-parametric Kruskal-Wallis one-way ANOVA using the PMCMR package (Pohlert 2016) using R v 3.5.3 (R Core Team 2019).

RESULTS

Data Curation

A total of 1448 individuals from 30 fields were genotyped at 16 microsatellite loci. Of these, four fields failed to yield population sizes of >5 individuals and were excluded from the dataset. Fragment analyses revealed a high number of amplification failures for three of the loci (TuCA96, TuCA25, TuCT26) and two of the loci (TuCT37, TuLS43) were monomorphic with the most frequent allele present at > 0.9. These five loci were also excluded from the dataset. In addition, we excluded 73 individuals that failed to yield at least five genotyped loci. These adjustments to the dataset provided a grand total 1185 individuals representing 26 fields genotyped across 11 microsatellite loci with a mean of 91.2% of individuals genotyped per locus ranging from 85.3-94.8%. The Western region was comprised of 531 individuals from 12 fields representing 3 growers. The Central region was comprised of 654 individuals from 14 fields representing 4 growers (Table 3.2).

We detected significant linkage disequilibria (LD) between 29 of 55 pairs of loci in the overall dataset (corrected sequential Bonferroni $\alpha = 0.0009$). In a more in-depth analysis, we evaluated the LD within each field. We detected significant LD in 71 of 1430 linkage by field comparisons, which revealed a pattern of linkage between loci TuCT13 and TuCA72 across 7 independent fields. This linkage was present in fields from both regions and multiple growers, thus it was likely the result of physical linkage rather than selection. Locus TuCT13 was removed from the dataset to ameliorate the LD. A follow up analysis of the 10 remaining loci revealed 24 of 45 pairs of loci with significant linkage disequilibrium (corrected sequential Bonferroni $\alpha = 0.0011$). We detected significant LD in 52 out of 1170 linkage by field

comparisons, but there were no evident patterns of linkage across multiple fields. The remaining 10 loci were regarded as independent and used for the subsequent analyses.

Patterns of Genetic Diversity

The overall average unbiased expected heterozygosity (H_S) was 0.475. The average *Hs* was significantly higher and had greater variance in the Western region than Central region (Two-tailed Independent T test: t (24) = 2.4082, P = 0.0241; F test F (11,13) = 0.2540, P = 0.0219). The overall average allelic richness (*AR*) was 2.847 based on a minimum sample of 10 individuals per population. The average *AR* in the Western region had significantly greater variance than the Central region (F test: F (11,13) = 0.3051, P = 0.0455). Both the highest and lowest values for *AR*, *Hs*, *Ho*, and F_{IS} occurred in the Western region (Table 3.2). Hardy-Weinberg exact tests revealed significant deviations from Hardy-Weinberg proportions were present for all loci in the overall dataset. Further investigation revealed that even at the field level, 23 of the 26 sampled fields displayed significant departures from HW proportions (Table 3.2). All fields deviating from HW proportions displayed significant heterozygote deficiencies. No significant heterozygote excess was detected in any of the populations.

Individuals from fields managed by the same grower were pooled together then the pooled groups were compared to each other both overall and within their own regions by *AR*, H_O, H_S, and F_{IS} using Kruskal-Wallis one-way ANOVA tests, but no significant differences were detected.

Genetic Differentiation and Structure

Overall

Overall average F_{ST} was 0.106 with pairwise F_{ST} values ranging from 0.381 to 0 (Table 3.4). Two pairwise comparisons had values of 0; both occurring between fields from within the

Central region. The pairwise F_{ST} values for fields in the Western region (mean F_{ST} 0.116) were significantly higher than the Central region (mean F_{ST} = 0.035) (Two-tailed Independent T test: t (155) = 8.2856, P < 0.00001). We found evidence of IBD in the overall dataset (Mantel test: P < 0.0001; R^2 = 0.1512; Figure 3.3A). Fields had a mean pairwise distance of 96.5 km. The STRUCTURE Harvester analyses revealed two genetic clusters (K = 2, ΔK = 68.974) divided along regional borders (Figure 3.3A). The clustering assignment divided 46.72% of the populations to cluster one and 53.28% of the populations to cluster two. The Western region was split with 72.55% of the populations assigned to cluster one and 27.45% of the populations assigned to cluster two. The Central region was roughly inverse with 25.74% of the population assigned to cluster one and 74.26% of the population assigned to cluster two. The Western and Central regions were significantly different from each other based on STRUCTURE cluster assignment (Two-tailed Independent T test: t (24) = 7.6310, P < 0.00001). When we classified individuals by region, the DAPC cluster analysis also supported this pattern with a 0.811 mean success using 18 PCA axes (RMSE = 0.192; Figure 3.4A). Analysis of the same dataset with individuals classified by field yielded a similar patter, but with a much weaker correlation with a 0.341 mean success using 24 PCA axes (RMSE = 0.660; Figure 3.5B). This weaker support from the DAPC analysis was likely due to the decreased ability of DAPC to successfully differentiate between multiple fields compared to only two regions. To better understand the differences between the two regions, we separated the datasets by region for independent analyses of the population structure within each region.

Western Region

The average F_{ST} for the Western region was 0.106 with pairwise F_{ST} values ranging from 0.336 to 0.011 (Table 3.4). We found evidence of IBD in the Western region (Mantel test: P =

0.013; R^2 = 0.0911; Figure 3.2B). There were two fields (P1 and P2) that were separated by only 0.01 km. In order to reduce bias towards correlation caused by these fields so close together, P2 was dropped to evaluate whether IBD was still present. Without field P2, there was still evidence of IBD in the western region (mantel test: P = 0.046; R^2 = 0.1213). The mean pairwise distance between fields was 4.96 km. The STRUCTURE Harvester analyses reported four genetic clusters (K = 4, ΔK = 17.638). There was less support for the adjacent number of genetic clusters, three (K = 3, ΔK = 0.400) or five (K = 5, ΔK = 3.954). Cluster assignment divided the populations with assigned 22.3% to cluster one, 23.71% to cluster 2, 27.04% to cluster 3, and 26.95% to cluster 4 (Figure 3.3B). We detected a pattern of genetic structure showing fields at the margins of the sampled area had the highest proportions of assignment (≥60%) to a single cluster. Populations P3, D3, D5, and P1 displayed skewed assignments greater than 60% to a single cluster. This was likely due to the increased isolation of the fields sampled as we moved further away from the urbanized central area in the western region. We classified individuals by field for the DAPC cluster analyses with 0.513 mean success using 24 PCA axes (RMSE = 0.489). The pattern reported by DAPC analyses showed moderate support for differentiation due to fields D3 and P1 extending further away from the generalized central cluster, though most fields had a large proportion of overlap (Figure 3.4C).

Central Region

The average F_{ST} for the Central region was 0.044 with pairwise F_{ST} values ranging from 0.141 to 0 (Table 3.4). We did not find evidence of IBD in the Central region (Mantel test: P = 0.25610; R^2 = 0.0017; Figure 3.2C). The mean pairwise distance between fields was 10.14 km. The STRUCTURE Harvester analysis reported two genetic clusters (K = 2, ΔK = 6.409). The clustering assignment divided the populations with 50.92% assigned to cluster one and 49.08%

assigned to cluster two (Figure 3.3C). Fields within the Central region were clustered in two geographically distinct regions within the Central region. Fields in the Northern area were separated by an average pairwise distance of 2.08 km and fields in the Southern area separated by an average pairwise distance of 1.41 km with the average pairwise distance between the Northern and Southern fields being 17.33 km. Although fields in the Central region were separated by a large distance, there were no significant differences between the northern and southern areas based on the cluster assignment by STRUCTURE (Two-tailed Independent T test: t (12) = -1.704, P = 0.1141). We classified individuals by field for the DAPC cluster analyses using 16 PCA axes (Mean success = 0.322, RMSE = 0.679). The pattern reported by DAPC analyses showed FP1 and C1 as the most distinct fields (Figure 3.5D), which correspond to the two fields with the highest proportion STRUCTURE cluster assignments to cluster two and cluster one respectively (Figure 3.3C).

Growers

The pairwise F_{ST} values for fields managed by the same grower were compared to pairwise F_{ST} values for fields between different growers within each region, but there were no significant differences (Central region two-tailed Independent T test: t (89) = 0.1441, P = 0.8857; Western region two-tailed Independent T test: t (64) = 0.5733, P = 0.5685). Datasets were created for each grower to evaluate IBD between fields managed by the same grower. There was evidence of significant IBD (Mantel test: P = 0.0325; r = 0.458) for only one grower in the Western region. We classified individuals by grower for the DAPC cluster analyses using 26 PCA axes (Mean success = 0.417, RMSE = 0.584). Although DAPC reported the same general pattern of spread between the Western and Central regions seen in Figures 3.4A and 3.4B, there

were no discernable patterns isolating any of the individual growers. Further, when we analyzed growers within their own regions, there was still no discernable pattern.

DISCUSSION

Departures from HWP

A pattern of a significant departure from HW proportions with significant heterozygote deficiency is a well-documented phenomena reported in numerous TSSM microsatellite studies (Navajas et al. 2002; Carbonnelle et al. 2007; Uesugi et al. 2009a, b; Li et al. 2009; Sun et al. 2012; Pascual-Ruiz et al. 2014; Aguilar-Fenollosa et al. 2016; Zhang et al. 2016; Hada et al. 2016; Shi et al. 2019). In general, this suggests the presence of null alleles. Although the presence of null alleles cannot be ruled out as a contributing factor to deficiencies in heterozygosity, the systematic lack of heterozygotes in multiple studies (Navajas et al. 2002; Bailly et al. 2004; Carbonnelle et al. 2007; Zhang et al. 2016) and specifically across all loci in this study suggests that this may be due to biologically founded phenomena such as significant inbreeding due to a combination of small, stochastic colonization events and arrhenotokous parthenogenesis.

Metapopulation Structure

In agricultural landscapes, many arthropod pest populations persist by virtue of metapopulation dynamics that promote regional population stability through a network of fragmented habitats where subpopulations disperse from mature crops as they are harvested to colonize newly planted crops throughout the growing season (Moilanen and Hanski 1998; Bailly et al. 2004). Metapopulation dynamics are inherently influenced by habitat fragmentation and the degree of connectivity between subpopulations, which in turn impact rate of increase in genetic differentiation between subpopulations and overall genetic diversity. Given the same number of

individuals, as subpopulations become isolated from each other due to isolation and habitat fragmentation, genetic diversity and population structure will be impacted as a function of the frequency of migration between the subpopulations (McCauley 1991; Gonzalez 1998; De Roissart et al. 2015).

When subpopulations are completely isolated, the effective population size of each subpopulation is relatively small, resulting in an increased level of genetic drift and a more rapid decrease in local heterozygosity. As each subpopulation trends to fixation of a given allele, the local heterozygosity moves towards zero, but if multiple fields become fixed for different alleles, the regional heterozygosity cannot further decrease. At the opposite extreme with complete panmixia, subpopulations have a relatively larger effective population size compared to a completely isolated scenario because individuals from other subpopulations increase the genetic diversity within each subpopulation. Larger effective population sizes reduce the effect of drift and reduce the regional rate of decline in heterozygosity, but because of the connectivity between subpopulations, drift can theoretically lead to regional heterozygosity reducing to zero over thousands of generations. At a moderate level of migration, subpopulations have some degree of connectivity with other subpopulations, but will still be influenced by drift to a greater degree than panmictic populations, but the rate of fixation may be offset by the introduction of new alleles through immigration from other subpopulations. The moderate isolation coupled with the introduction of new alleles through regular, low levels of immigration shields the overall regional heterozygosity from trending towards zero while allowing moderately isolated subpopulations to maintain some degree of genetic differentiation and increase the regional heterozygosity relative to both completely isolated and panmictic populations. The degree of migration necessary to prevent local fixation and maintain a given amount of overall

heterozygosity is dependent on a variety of factors such as effective population size, local recruitment, and habitat size (McCauley 1991; Allendorf et al. 2013).

Our study has revealed a strong pattern of differences between the Western and Central regions with the Western region being generally more genetically diverse and possessing more population structure compared to the Central region. These differences may be best explained with reference to the metapopulations and the impact of migration on population structure and genetic diversity. The overall dataset and the Western region had mean pairwise distances of 96.5 km and 4.96 km, respectively, and both demonstrated significant IBD while the Central region had a mean pairwise distance of 10.14 km and did not demonstrate IBD. This suggests that IBD evident in the Western region is not due to an inherent inability to disperse longer distances, because the Central region showed no IBD with fields sampled at twice mean pairwise distances. We believe that IBD in the Western region may be best explained by landscape factors that limit the rate of migration between fields, such as forests surrounding fields and valleys that create physical barriers to immigration as well as landscape resistance factors that impede air currents used by TSSM for aerial dispersal. This would also suggest that there was a higher degree of inbreeding within individual fields from the Western region compared to the Central region, but we were unable to detect any meaningful differences (One-tailed Independent T test $t(24) = 1.0605$, $P = 0.0607$). These landscape factors lead to increased population structure and higher genetic diversity compared to the Central region, where the overall population was much more uniform.

The STRUCTURE analysis for the overall dataset (Figure 3.3A) showed large, significant differences between the Western and Central regions, as expected; further validating IBD. This allowed for more fine scale analyses of each region and comparisons between the two

regions. The Central region STRUCTURE analysis (Figure 3.3C) showed two genetic clusters, but the low ΔK provides no confidence that these are true genetic clusters. In addition, DAPC cluster analysis (Figure 3.4D) failed to provide any meaningful separation between fields. We expected that sampling from the Northern and Southern areas that were separated by 17 km within the Central region would have produced signals of IBD leading to some degree of differentiation in STRUCTURE and DAPC, but differences between or within the two areas were not statistically significant. We therefore believe this region to be relatively panmictic in comparison to the Western region. The Western region STRUCTURE analysis (Figure 3.3B) showed four distinct genetic clusters, with the fields assigned >60% to any given cluster present only at the geographic margins of the sampling area. The DAPC cluster analysis for the Western region (Figure 3.4C) showed a pattern of greater spread with fields separating father away from the axes intersection in comparison to the DAPC cluster analysis for the Central region (Figure 3.4D). We believe these larger field to field differences in the Western region are indicative of reduced geneflow, causing populations to behave in accordance with metapopulations experiencing intermediate levels of migration.

As discussed earlier, regional populations with a moderate amount of dispersal between subpopulations in a fragmented landscape incur higher rates of local genetic drift within subpopulations, but can maintain higher levels of genetic diversity within the regional population as migrants from subpopulations reintroduce alleles to each other. Additionally, as populations become more isolated, they are more susceptible to local density dependent factors such as competition, predation, and disease leading to more genetic variance between fields (Gauffre et al. 2015). This was evident in comparisons between the two regions that showed significantly higher levels of average expected heterozygosity and genetic distances (F_{ST}) in the Western

region. Furthermore, there were larger variances in allelic richness and average expected heterozygosity within the Western region. The Western region had the highest and lowest values for allelic richness, expected heterozygosity, observed heterozygosity, and inbreeding coefficients (Table 3.2). In panmictic populations, local ecological factors have a reduced impact on the population leading to greater overall stability with less genetic variance between subpopulations.

Relevance to Pest Management

This study did not detect any effects relating to growers on genetic structure in either region. Comparisons of pairwise F_{ST} values between fields tended by the same grower against pairwise F_{ST} values of fields managed by different growers showed no significant differences suggesting that growers do not play a significant role in the dispersal of TSSM on a meaningful scale. Although we cannot rule out that the effect of individual grower practices as a contributing factor to the differences we observed when comparing fields, our data and observations have provided us with confidence in asserting that those differences were the result of landscape factors.

We expected to detect IBD at between the Western and Central regions and within the Central region where the mean pairwise distances were 96.5 km and 10.14 km respectively. Alternatively, in the Western region where IBD was significant, we hypothesized that growers were facilitating TSSM dispersal among fields via farm workers or machinery, which would result in IBD being less evident among fields farmed by the same grower. IBD was evident in the fields of only one grower from the Western region. This grower had the largest mean pairwise distances between fields (5.95 km) and these results suggest that the grower's activities were not contributing to significant dispersal of mites between fields. The other growers managed fields

with mean pairwise distances between 2.6 and 0.69 km. At these distances, it may be more difficult to discern whether dispersal between fields was an artefact of grower facilitation or natural dispersal behavior. The cluster assignment in STRUCTURE varied drastically between fields managed by the same grower with no discernable pattern, and DAPC scatterplots provided no meaningful separation between growers. These results suggest that the relative impact of growers on dispersal from field to field was minimal.

Concluding Remarks

This study sought to evaluate and characterize differences in *T. urticae* population genetic structure between and within two regions in North Carolina, USA, with drastically differing landscape features and population dynamics. We have shown that in the Western region where fields are more isolated and the growing season much shorter, TSSM populations develop stronger genetic clustering compared to Central region, where the landscape is more homogenous, the growing season much longer, and TSSM populations are comparatively more panmictic. This information can be used to better inform growers as they develop and optimize acaricide resistance management strategies to slow the onset and spread of resistance alleles in the environment. In the Central region where TSSM are more panmictic, an areawide resistance management approach may be appropriate because our results suggest that the actions of individuals growers have the potential of impacting populations 17 km away. This may incentivize growers to coordinate their management strategies. Alternatively, it may deter growers from participating if they conclude that their personal efforts may not make a significant impact on slowing the rate of resistance development if the actions of nearby growers can negate their actions. In the Western region where fields are more isolated, and the spread of resistance alleles among local populations is expected to occur at a much slower rate compared to the

Central region. Hence, decisions and actions of an individual grower would be expected to more directly impact the rate at which resistance develops within their fields.

In this study, fields were selected to evaluate if growers contributed to the dispersal of individuals within regions. Most growers owned or rented property within a small area and the impact of growers on the genetic structure was not apparent. In future long-term studies, fields should be selected with the intent of evaluating how landscape factors impact genetic structure more directly. While we did incorporate landscapes as a factor in explaining genetic structure, in the future we will use quantifiable methods to measure the impact of landscape resistance or facilitation of TSSM dispersal. This will help to better predict patterns of TSSM dispersal and spread of resistance alleles.

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Table 3.1 Microsatellites used to assess the genetic relationship of *T. urticae* populations collected from tomato fields in North Carolina. N_A Number of alleles. Fluorescent dyes are products for use with Applied Biosystems.

[a] Removed from final dataset due to Low PCR amplification

[b] Removed from final dataset because Loci was monomorphic

[c] Removed from final dataset due to linkage disequilibrium

Locus	Motif	Allele range	N_A	Fluorescent Dye	Primer Sequence (5'-3')	Accession number	Source
TuCT04	$(CT)_8$	146-152	3	6-FAM	F: CGTCATCATTGCCGTCATTTTAC R: GGAGCCGTTTCAAGAGAGTG	AB263083	Uesugi and Osakabe 2007
TuCA02	$(CA)_2A$-$(CA)_7$	186-190	5	6-FAM	F: GTCAAAGAGTTACCCAAAGAG R: TCATATCAAACCAGAAAAGGAG	AB263077	Uesugi and Osakabe 2007
TuLS20	$(TTG)_6$	212-218	2	6-FAM	F: AAGCTGGATTCATAGAAGCA R: AAATTAATTCAGCCTCGTCA	KJ545963	Saune et al 2015
TuLS43 [b]	$(GAT)_5$	262-277	1	6-FAM	F: AATGGAGGTATGGATGACG R: AAAGCTGCTGAAAGTCACTC	KJ545972	Saune et al 2015
TuCA96 [a]	$(TG)_7$	107-109	-	VIC	F: ATGGATTGTCACCGATTTCA R: CTGAAGTTTACTTGCTATAGTC	AB263082	Uesugi and Osakabe 2007
TuCA25 [a]	$(TC)_{13}$	155-165	-	VIC	F: AATGTGTTGGTTGTTTACGAAGTG R: TTGGTCAAAGCCGGTTACAG	AB263079	Uesugi and Osakabe 2007
TuLS17	$(ATG)_6$	192-207	3	VIC	F: TCTTCGTTCGATAGCTTTTC R: TCCTCAGGTATATCAGGTGG	KJ545961	Saune et al 2015
TuCA72	$(GT)_6$	264-270	3	VIC	F: ACTGTCTGGCGTCTTTTGTC R: GTTTACCAGTTTCCCTTTGACC	AB263080	Uesugi and Osakabe 2007
TuCT37 [b]	$(TC)_7C$-$(TC)_5$	121-127	8	NED	F: GTAATAATGGGTGTTGTTGC R: CGCAAAATATGAGTGAGAATG	AB263089	Uesugi and Osakabe 2007
TuCT67	$(CT)_9$	95-107	4	NED	F: CCATCATCTTCATCATTCTTCACC R: TAGAACAGTCAAGCAAAAAGAGTC	AB263090	Uesugi and Osakabe 2007
TuCT13 [c]	$(TC)_7$	163-168	5	NED	F: CAGATGGATTTGCTTTCCAC R: GATCCTAATCAACATGAGGGTC	AB263085	Uesugi and Osakabe 2007
TuLS41	$(CAT)_6$	242-261	3	NED	F: GAATGAAGATTGGTGGGTTA R: TCAAGATTTTGGAATCAGAGA	KJ545970	Saune et al 2015
Tu35b	$(TGA)_8$	91-113	4	PET	F: CTTCCCGAAGGCTGTTGATA R: AATGGAATGAGTTATCGTTGGG	AJ419832	Saune et al 2015
TuCT26 [a]	$(GA)_8$	150-156	-	PET	F: CGATGGAGCCGTTTCAAGAG R: TCGTCATCATTGCCGTCATTTTAC	AB263088	Uesugi and Osakabe 2007
TuLS14	$(ATG)_7$	191-210	3	PET	F: GCAAATGAAGCTTACCAATTA R: TAAAGGTTTGGCAGTTCAGT	KJ545959	Saune et al 2015
TuCA12	$(CA)_7$	263-275	4	PET	F: GATTTGTGGTCGTGGTTTTC R: GATCAACTCAAAAGGATAACGTTG	AB263078	Uesugi and Osakabe 2007

Table 3.2 Sample size (N), average alleles per locus (*A/L*), allelic richness based on a minimum of 10 individuals (*AR*), mean unbiased expected heterozygosity (H_S), observed heterozygosity bolded values signify significant heterozygote deficiency (P < 0.00111) (H_O), and inbreeding coefficient (F_{IS}) for twospotted spider mites collected from tomato fields.
[a] Highest value in the column
[b] Lowest value in the column

Field	Region	N	*A/L*	*AR*	*Hs*	*Ho*	F_{IS}
P1	W	36	3.3	3.0598	0.5347	**0.3111**	0.423
P2	W	48	3.3	2.9089	0.5171	**0.3236**	0.377
P3	W	45	3.2	2.8364	0.4581	0.3989	0.131
P4	W	48	2.9	2.7618	0.4696	**0.2358** [b]	0.5 [a]
P5	W	45	3.4	3.0755	0.5362	**0.322**	0.402
D2	W	47	3.3	2.8714	0.5245	**0.3513**	0.333
D3	W	40	3.4	2.9669	0.5204	**0.2962**	0.435
D5	W	48	2.7 [b]	1.9645 [b]	0.2881 [b]	0.2787	0.033 [b]
KJ1	W	32	3.1	2.8698	0.5354	**0.2945**	0.455
KJ2	W	48	3.3	2.9149	0.5553	**0.4238** [a]	0.239
KJ4	W	48	3.4	3.0385	0.5106	**0.3153**	0.385
KJ3	W	46	3.5 [a]	3.1914 [a]	0.5832 [a]	**0.4195**	0.283
	W Overall	531	3.23	2.87165	0.505	**0.331**	0.335
RP1	C	48	3.2	2.9854	0.4777	**0.3967**	0.171
RP3	C	46	3.2	2.8747	0.4637	**0.3444**	0.26
RP4	C	45	3.2	2.8127	0.463	**0.4058**	0.126
FP1	C	50	3.3	2.9392	0.4869	**0.3272**	0.33
FP2	C	44	3.3	2.9559	0.4891	**0.3389**	0.31
FP4	C	48	2.7 [b]	2.5111	0.4019	**0.3069**	0.243
FP3	C	45	3.4	2.9673	0.4517	0.386	0.147
C1	C	48	3	2.5371	0.3525	**0.2848**	0.194
C2	C	50	3.3	2.763	0.4193	**0.2672**	0.365
C3	C	48	3	2.8604	0.4529	**0.343**	0.245
C4	C	42	3.4	2.7971	0.4734	**0.3624**	0.237
W1	C	50	3.4	2.8593	0.4359	**0.2466**	0.437
W2	C	50	3.5 [a]	3.0877	0.475	**0.2889**	0.395
W3	C	40	3	2.6142	0.423	**0.3579**	0.157
	C Overall	654	3.21	2.826079	0.449	**0.333**	0.266
	Total	1185	3.22	2.84711	0.475	**0.332**	0.229

Table 3.3 Pairwise distances (km) between fields corresponding region where fields are located.

	Field	P1	P2	P3	P4	P5	D2	D3	D5	KJ1	KJ2	KJ3	KJ4	RP1	RP3	RP4	FP1	FP2	FP3	FP4	C1	C2	C3	C4	W1	W2
Field	Region	W	W	W	W	W	W	W	W	W	W	W	W	C	C	C	C	C	C	C	C	C	C	C	C	C
P2	W	0.1																								
P3	W	9.1	9.2																							
P4	W	8.2	8.3	3.2																						
P5	W	6.4	6.5	3.7	5.2																					
D2	W	8.2	8.2	9.2	11.0	5.8																				
D3	W	8.5	8.6	7.8	9.9	4.7	1.8																			
D5	W	6.1	6.2	6.1	7.6	2.5	3.4	2.7																		
KJ1	W	5.6	5.6	4.8	6.0	1.2	5.1	4.3	1.6																	
KJ2	W	5.9	6.0	3.9	5.1	0.5	5.9	4.9	2.5	0.9																
KJ3	W	5.5	5.6	4.0	4.7	1.2	6.4	5.5	3.0	1.3	0.7															
KJ4	W	5.8	5.8	3.4	3.8	1.7	7.3	6.3	3.8	2.2	1.4	0.9														
RP1	C	173.8	173.8	172.5	169.9	175.0	180.5	179.7	177.1	175.5	174.8	174.2	173.4													
RP3	C	173.4	173.5	172.2	169.5	174.6	180.2	179.4	176.8	175.2	174.4	173.8	173.0	0.5												
RP4	C	174.3	174.3	172.9	170.3	175.4	181.0	180.1	177.6	176.0	175.2	174.6	173.8	3.0	3.4											
FP1	C	175.2	175.2	173.7	171.1	172.6	181.8	181.0	178.4	176.8	176.0	175.5	174.6	3.1	3.6	0.8										
FP2	C	174.8	174.8	173.4	170.8	175.9	181.5	180.6	178.1	176.4	175.7	175.1	174.3	2.9	3.4	0.5	0.4									
FP3	C	175.6	175.7	174.4	171.7	176.8	182.4	181.5	179.0	177.4	176.6	176.0	175.2	1.9	2.3	3.0	2.7	2.7								
FP4	C	175.6	175.6	173.4	171.7	176.8	182.3	181.5	179.0	177.3	176.6	176.0	175.2	1.9	2.4	2.7	2.3	2.3	0.4							
C1	C	180.0	180.0	179.4	176.7	181.7	187.0	186.3	183.7	182.1	181.4	180.8	180.0	15.9	15.7	18.6	18.3	18.3	15.6	16.0						
C2	C	180.0	180.1	179.5	176.7	181.7	187.1	186.4	183.8	182.2	181.5	180.9	180.1	15.9	15.6	18.5	18.2	18.2	15.5	15.9	0.1					
C3	C	179.6	179.6	179.1	176.3	181.3	186.7	186.0	183.4	181.8	181.1	180.4	179.7	15.2	14.9	17.8	17.5	17.5	14.8	15.2	0.8	0.7				
C4	C	180.1	180.1	179.5	176.7	181.7	187.1	186.4	183.8	182.2	181.5	180.9	180.1	15.1	14.9	17.7	17.4	17.4	14.8	15.1	1.0	0.8	0.5			
W1	C	180.9	180.8	180.4	177.6	182.6	187.9	187.2	184.6	183.0	182.3	181.7	180.9	17.1	16.9	19.7	19.3	19.4	16.7	17.0	1.2	1.2	2.0	1.9		
W2	C	180.8	180.8	180.3	177.6	182.6	187.9	187.2	184.6	183.0	182.3	181.7	180.9	16.8	16.6	19.4	19.1	19.1	16.5	16.8	1.1	1.1	1.8	1.7	0.3	
W3	C	180.6	180.6	180.1	177.3	181.7	187.7	187.0	184.4	182.8	182.1	181.5	180.7	16.6	16.4	19.2	18.9	18.9	16.2	16.6	0.8	0.8	1.5	1.5	0.5	0.3

Table 3.4 Pairwise genetic distance (FST) values between twospotted spider mites collected from different fields

Field	P1	P2	P3	P4	P5	D2	D3	D5	KJ1	KJ2	KJ3	KJ4	RP1	RP3	RP4	FP1	FP2	FP3	FP4	C1	C2	C3	C4	W1	W2
P2	0.051																								
P3	0.162	0.170																							
P4	0.184	0.144	0.031																						
P5	0.128	0.098	0.052	0.026																					
D2	0.144	0.097	0.138	0.118	0.045																				
D3	0.184	0.143	0.231	0.188	0.126	0.142																			
D5	0.336	0.227	0.221	0.154	0.146	0.184	0.283																		
KJ1	0.100	0.071	0.052	0.030	0.011	0.056	0.118	0.156																	
KJ2	0.118	0.073	0.090	0.061	0.038	0.019	0.129	0.170	0.014																
KJ3	0.081	0.067	0.089	0.073	0.036	0.063	0.081	0.205	0.022	0.035															
KJ4	0.073	0.055	0.112	0.100	0.073	0.081	0.153	0.159	0.063	0.070	0.080														
RP1	0.156	0.171	0.020	0.064	0.081	0.172	0.181	0.255	0.071	0.111	0.082	0.126													
RP3	0.156	0.163	0.017	0.055	0.076	0.151	0.218	0.244	0.046	0.093	0.084	0.125	0.014												
RP4	0.143	0.143	0.021	0.034	0.065	0.150	0.226	0.197	0.051	0.097	0.077	0.100	0.031	0.025											
FP1	0.096	0.125	0.041	0.074	0.082	0.168	0.215	0.259	0.069	0.123	0.098	0.069	0.058	0.052	0.042										
FP2	0.134	0.135	0.007	0.038	0.046	0.127	0.177	0.222	0.035	0.083	0.060	0.104	0.005	0.007	0.008	0.040									
FP3	0.176	0.175	0.011	0.047	0.071	0.173	0.203	0.227	0.059	0.110	0.085	0.128	0	0.012	0.019	0.058	0.003								
FP4	0.230	0.233	0.057	0.105	0.111	0.193	0.244	0.233	0.100	0.160	0.112	0.167	0.037	0.047	0.062	0.108	0.038	0.025							
C1	0.228	0.252	0.070	0.163	0.188	0.248	0.337	0.381	0.175	0.183	0.169	0.222	0.071	0.073	0.098	0.141	0.075	0.078	0.136						
C2	0.156	0.160	0.015	0.055	0.068	0.169	0.229	0.225	0.074	0.129	0.106	0.112	0.032	0.031	0.045	0.040	0.019	0.026	0.067	0.095					
C3	0.182	0.182	0.010	0.058	0.086	0.149	0.245	0.224	0.065	0.093	0.109	0.128	0.013	0	0.029	0.061	0.015	0.012	0.054	0.057	0.036				
C4	0.171	0.167	0.011	0.050	0.065	0.135	0.225	0.258	0.054	0.082	0.085	0.133	0.023	0.000	0.032	0.066	0.011	0.018	0.061	0.061	0.033	0.002			
W1	0.188	0.204	0.027	0.094	0.105	0.187	0.238	0.276	0.098	0.139	0.121	0.141	0.025	0.034	0.025	0.053	0.021	0.018	0.054	0.074	0.054	0.011	0.041		
W2	0.174	0.184	0.029	0.060	0.086	0.180	0.181	0.236	0.083	0.125	0.084	0.120	0.008	0.030	0.044	0.058	0.026	0.015	0.036	0.103	0.032	0.027	0.041	0.038	
W3	0.167	0.195	0.055	0.139	0.112	0.212	0.275	0.371	0.119	0.172	0.128	0.173	0.067	0.063	0.057	0.052	0.046	0.062	0.116	0.083	0.050	0.039	0.067	0.022	0.091

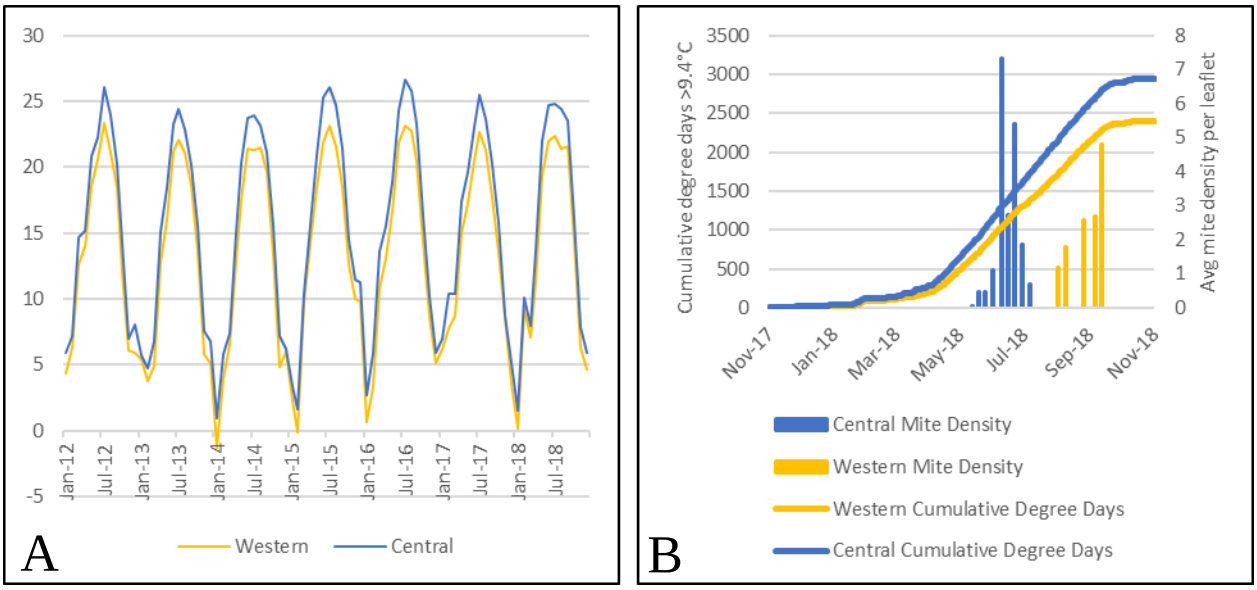

Figure 3.1 Average monthly temperature (A) degree-day accumulations (B) (base temperature of 9.4 °C) from Nov 2017-2018 and average mite density sampled in first fields with detectable presence of TSSM.

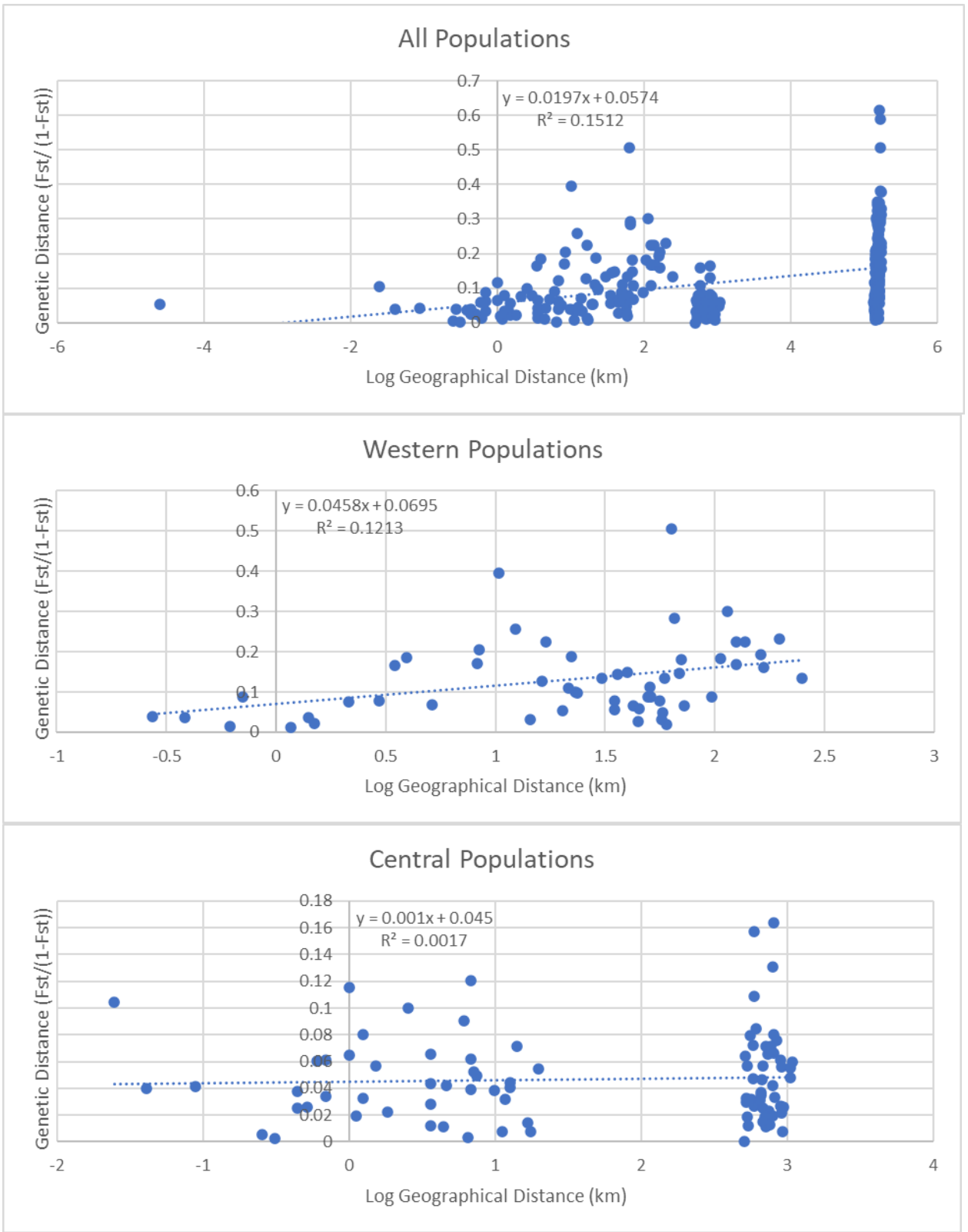

Figure 3.2 Isolation by Distance regression plots of the pairwise genetic distances (FST/(1-FST)) versus the corresponding pairwise log Euclidean distances for the overall data sets of twospotted spider mites collected from tomato fields in Western and Central NC.

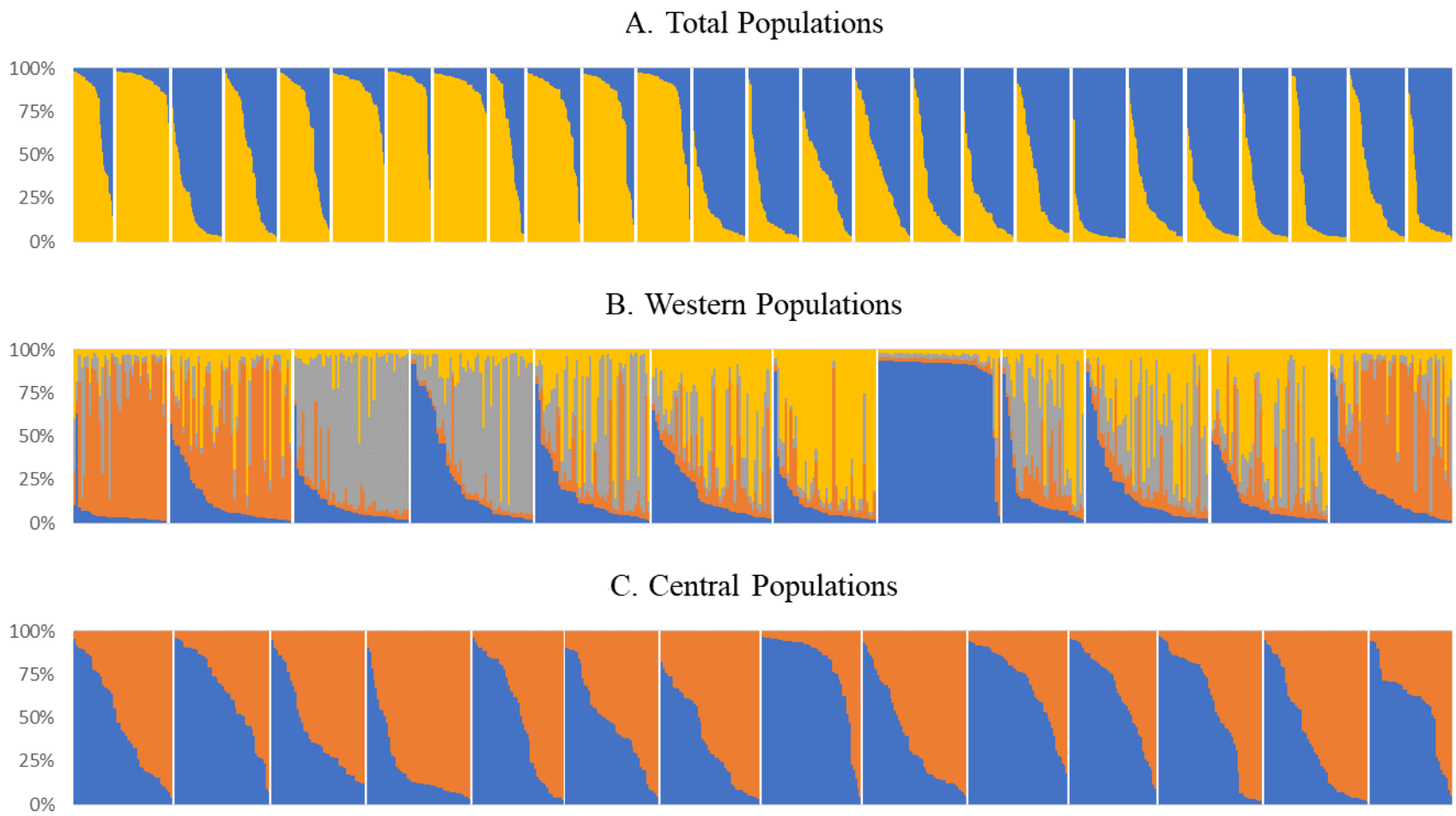

Figure 3.3 Structure results for (A) all populations sampled, (B) Western Region, and (C) Central region. Bar plots show proportions of individual multi-locus genotypes assigned to the most probable cluster based on optimal number of clusters. Fields are arranged from Western-most populations on the left to Eastern-most populations on the right

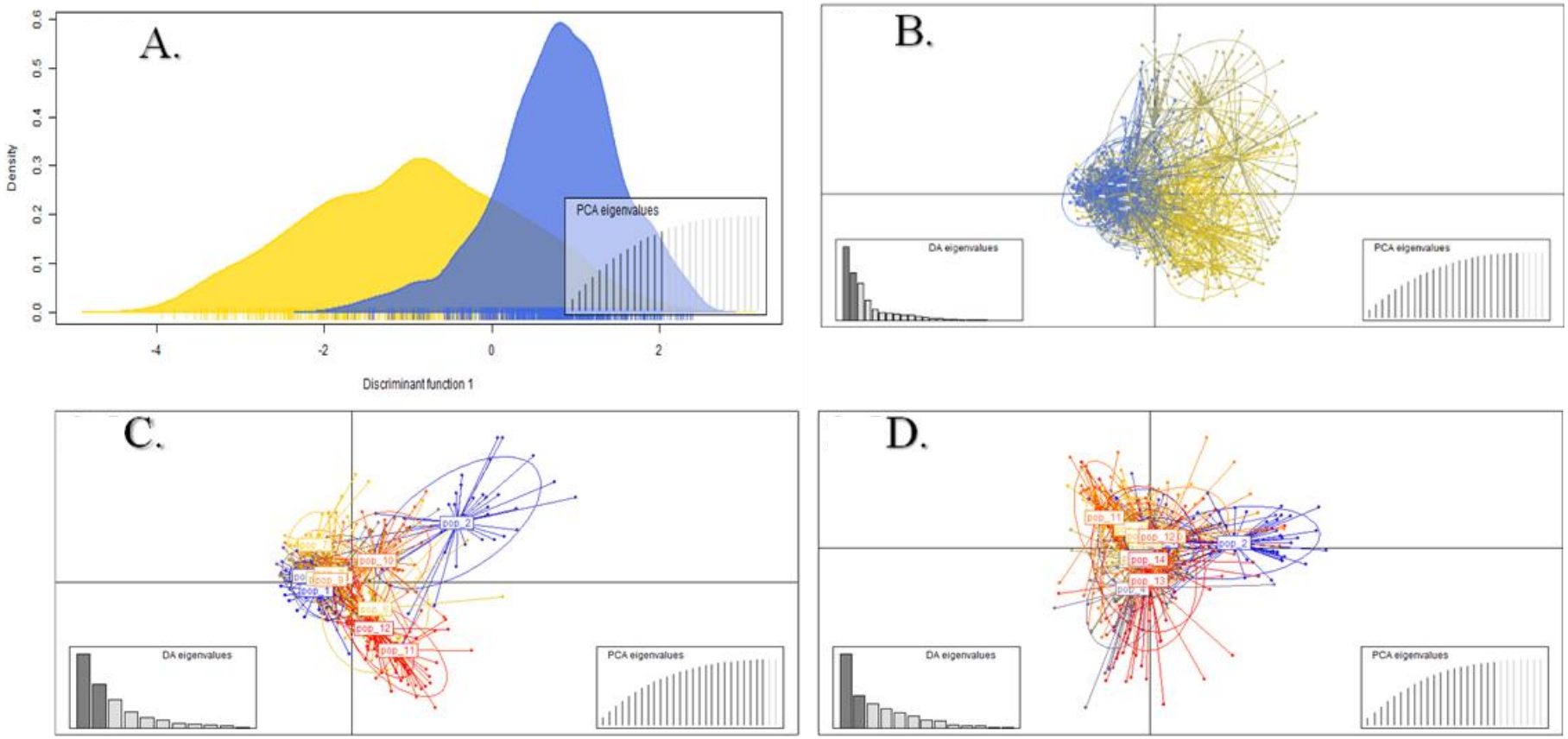

Figure 3.4 DAPC cluster analyses scatter plots. (A) All individuals assigned by region. Yellow represents the Western Region and Blue represents the Central region. (B) All individuals assigned by field. Yellow represents the Western Region and Blue represents the Central region. (C) Individuals from only the Western region assigned by field. (D) Individuals from only the Central region assigned by field