MOLECULAR PHYLOGENETICS AND POPULATION GENETICS OF *Fusarium oxysporum* f. sp. *radicis-lycopersici* AND ITS MANAGEMENT BY SILICON AMENDMENT

By

CHENG-HUA HUANG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

To my grandparents, parents, wife, and son for their support

ACKNOWLEDGMENTS

I would like to thank my major advisor, Dr. Pamela Roberts who provided the opportunity for me to conduct my doctoral studies and to complete my dissertation under her direction here at the University of Florida. I am grateful for her guidance, patience, and support in research. I would also like to thank my co-advisor, Dr. Lawrence Datnoff for his encouragement and suggestions in assisting me to pass through many difficulties during my study here. My sincere gratitude is extended to my supervisory committee members: Dr. John Scott for providing tomato seeds and inoculation approaches, Dr. Robert McGovern for sharing his isolates and inoculation experience, Dr. Wade Elmer for guiding my vegetative compatibility study, and Dr. Jeffrey Rollins for advising me in phylogenetics and molecular techniques.

I would also like to thank Drs. Kerry O'Donnell, H. Corby Kistler, James Correll, Raymond Schneider, and Jaacov Katan for providing isolates, Dr. Monica Ozores-Hampton, Dr. Rosa Muchovej, Brenda Rutherford, and Glades Crop Care, Inc. for collecting isolates, and Norma Flor, Ginger Clark, Drs. Monica Arakaki, Matt Gitzendanner, Doug Soltis, Pamela Soltis, Asha Brunings, Linley Dixon, and Liane Gale for guidance in laboratory techniques. I would also like to thank Eldon Philman and Herman Brown for their help in the greenhouse tasks.

I would like to express my sincere gratitude and appreciation to Steve Hardy at the Baptist Student Center in Gainesville, FL. His intelligent and insightful advice helped me to survive in the United States. I would especially like to thank my grandparents, parents, brother and sisters for providing me with love, encouragement, and support. Most importantly, I thank my wife and son for encouraging me and praying for me to complete my doctoral degree. Finally, I thank God for His mercy, righteousness and love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MOLECULAR PHYLOGENETICS AND POPULATION GENETICS OF *Fusarium oxysporum* f. sp. *radicis-lycopersici* AND ITS MANAGEMENT BY SILICON AMENDMENT

By

Cheng-Hua Huang

December 2009

Chair: Pamela D. Roberts
Major: Plant Pathology

Fusarium oxysporum f. sp. *radicis-lycopersici*, the causal agent of Fusarium crown and root rot of tomato, is an important soilborne pathogen. The objectives of this study were to investigate the population genetics of the pathogen, to conduct phylogenetic and mating-type analyses, and to evaluate the effect of silicon (Si) on disease severity. Twenty seven microsatellite loci were acquired from a bioinformatics approach and a microsatellite enrichment procedure. Ten of these 27 microsatellites along with vegetative compatibility group (VCG) assays revealed migration of the pathogen among three main tomato-growing regions in Florida. Hendry County had a higher overall average gene diversity than Manatee and Collier Counties. However, the highest mean number pairwise differences and average gene diversity of either VCG 0094 or 0098 were exhibited in Collier County, suggesting that these two VCGs might have migrated from Collier County or other regions to Manatee and Hendry Counties. VCG 0098 probably diverged from VCG 0094 according to VCG and phylogenetic analyses. Although VCG 0094 is still predominant in Florida, VCG 0098 possesses a higher virulence and increased frequency, suggesting that VCG 0098 may be helpful for screening resistant tomato lines. The complete region of intergenic spacer (IGS) provided more phylogenetic resolution than translation elongation factor (EF-1α) and FOL185, a noncoding microsatellite locus. At least two

evolutionary origins were revealed based on these three loci: VCGs 0094, 0098, and 0099 likely originated independent of the other VCGs. Each VCG carried a unique mating-type idiomorph but no perithecia were found after crossing isolates with opposite mating-type idiomorphs, suggesting that other factors may be required for sexual recombination. Si amendment significantly reduced disease severity of the stem due to delaying initial infection of roots and the basipetal movement of the pathogen from infected roots to stems. The increase in the Si content of roots correlated significantly with the reduction of disease severity. This study suggested restricting genotype flow of *F. oxysporum* f. sp. *radicis-lycopersici* for disease management, regularly monitoring the population structure of the pathogen, and further studying the application of Si fertilizers for controlling Fusarium crown and root rot in field-grown tomatoes.

CHAPTER 1
LITERATURE REVIEW

Taxonomy

Fusarium oxysporum (Sacc.) Snyder & Hans., a cosmopolitan soilborne haploid fungal plant pathogen, produces spores only through mitosis and causes vascular wilt or root rot in both agricultural crops and ornamental plants (120,121,132). Pathogenic isolates of *F. oxysporum* show a high level of host specificity and are classified into more than 120 *formae speciales* and races according to the plant species and cultivars in which they are able to cause disease (5). In contrast, those isolates unable to induce symptoms on a given plant species are considered non-pathogens of the given host species (120).

Fusarium crown and root rot of tomato**,** caused by *F. oxysporum* Schlechtend:Fr. f. sp. *radicis-lycopersici* W. R. Jarvis & R. A. Shoemaker, is an important disease on tomato worldwide (74) and the most serious soilborne disease limiting tomato production in southern Florida (27). The disease was first identified in Japan in 1969 (146) and thought to be a new race (J3) of *F. oxysporum* f. sp. *lycopersici*, the causal agent of Fusarium wilt on tomato (92,146). In Florida, Fusarium crown and root rot was first noted in 1975 in Palm Beach County but symptoms were thought to be caused by salt damage (159). Jarvis and Shoemaker (72) proposed that the causal agent of Fusarium crown and root rot is distinct from *F. oxysporum* f. sp. *lycopersici* based on symptoms, disease development, and physiological characteristics. Genetic difference has been suggested in that *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* are vegetatively incompatible, which means the inability to form heterokaryons between these two pathogens (128). Neither the divergence time and origin nor the existence of physiological races have been described for *F. oxysporum* f. sp. *radicis-lycopersici* (80).

Formae speciales and races of *F. oxysporum* cannot be identified according to morphological characteristics unless cumbersome pathogenicity tests are carried out (103). However, a particular *forma specialis* may be assigned preliminarily based on the host from which a pathogenic isolate of *F. oxysporum* was recovered (177). Likewise, without determining pathogenicity, pathogenic and nonpathogenic *F. oxysporum* species cannot be distinguished morphologically. Because nonpathogenic *F. oxysporum* species isolated from a given host may infect other plant species, these nonpathogenic isolates are defined as those that fail to cause disease on a given plant species (51). There is a need to develop rapid, precise molecular techniques for identification of a given *forma specialis* of *F. oxysporum*. Recently, specific primers for *F. oxysporum* f. sp. *radicis-lycopersici* were developed based on a comparison of the nucleotide diversity of *exo* polygalacturonase gene (*pgx4*) among *F. oxysporum* isolates (63). However, the specificity of the primers has not neen evaluated completely for geographically distinct isolates of *F. oxysporum* f. sp. *radicis-lycopersici*.

Vegetative Compatibility Group (VCG) and Population Structure

Vegetative compatibility refers to the ability of any two isolates to form stable heterokaryons, which are said to be vegetatively compatible and belong to the same vegetative compatibility group (VCG) (128). VCG tests may be used for studying population genetics of pathogenic fungi (95,96). It is suggested that VCG could be correlated with pathogenicity (79,104,128). However, the correlation may be strong, weak, or nonexistent depending on each *forma specialis* (95,96). For example, nonpathogenic isolates of *F. oxysporum* are not vegetatively compatible with pathogenic isolates of *F. oxysporum* f. sp. *cyclaminis* (176). In contrast, nonpathogenic isolates of *F. oxysporum* are vegetatively compatible with VCG 0031 but not with VCGs 0030 and 0032 (19). However, the correlation of VCG with pathogenicity in *F. oxysporum* f. sp. *radicis-lycopersici* has not been addressed.

Although *F. oxysporum* f. sp *radicis-lycopersici* was newly recognized in 1978 (72), considerable genetic variation has been shown to exist (80,139). A previous study (139) showed that VCG 0094 of *F. oxysporum* f. sp. *radicis-lycopersici* was predominant in Florida and that isolates of VCG 0094 in Europe probably migrated from Florida based on VCG and restriction fragment length polymorphism (RFLP) analyses. Thus, Florida is the probable center of origin of VCG 0094 although this VCG was first described in Belgium (139). A higher genetic diversity of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida suggests that rapid changes in population structure may occur while facing selection pressure such as the resistane gene *Frl* deployed in the field. The evolution of a local pathogen population may result from the selection for mutants, recombinants, or immigrants (107,108). The appearance of a new pathogenic race may be a result of either introduction or local selection (55). Given that the dominant resistance gene *Frl* has been deployed since 1983 (150), it is relevant to evaluate whether a physiological race has appeared in the field. VCGs 0094, 0098, and 0099 were reported in Florida in the 1990s (139) but the virulence of these three VCGs is not well known. Moreover, the frequency of these three VCGs may have changed in Florida due to selection processes. A survey of the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida seems to be pertinent following a previous study (139).

The genetic structure for a species is defined as the amount and distribution of genetic variation within and among populations of that species. Also, the genetic structure of a population is affected by the evolutionary history of that population (107,108). Knowledge of genetic structure is useful to understand the evolutionary process of pathogen populations in the past and also provides insight for their future evolutionary change. The genetic structure of a pathogen population is a consequence of the interactions among five evolutionary forces:

mutation, genetic drift, gene/genotype flow, reproduction/mating system, and selection. Understanding the evolutionary potential may provide a suitable strategy to manage plant disease (107,108).

Molecular markers such as RFLP (139) and random amplified polymorphic DNA (RAPD) (10), along with VCG, have been employed for studying the population genetics of *F. oxysporum* f. sp. *radicis-lycopersici*. However, highly polymorphic markers, such as microsatellites, have not been evaluated for their efficacy for understanding the population genetics of *F. oxysporum* f. sp. *radicis-lycopersici*. Microsatellites or simple sequence repeats (SSRs) are defined as at least five runs of tandemly repeated motifs that range from 1-6 bases found in both coding and noncoding regions and are usually characterized by extensive levels of length polymorphism (6,182). Due to the high variability and codominance, microsatellites have been used in investigating population genetics of plant pathogens, such as *Sclerotinia sclerotiorum* (152), *Phaeosphsaeria nodorum* (160), and *Phytophthora ramorum* (127). Since the genomic sequence of race 2 of *F. oxysporum* f. sp *lycopersici* was released in 2007 (available at http://www.broad.mit.edu), bioinformatics approaches may likely be useful to obtain microsatellite loci instead of *de novo* isolation of *F. oxysporum* f. sp. *radicis-lycopersici*.

Phylogenetics of *F. oxysporum* f. sp. *radicis-lycopersici*

Multiple gene genealogies are suggested for recognizing phylogenetic species in fungi (169). The rDNA internal transcribed spacer (ITS) region has been extensively used in species-level phylogenetics due to its higher evolutionary rate. However, many *Fusarium* species including the *F. oxysporum* species complex possess non-orthologous copies of the ITS2 due to interspecific hybridization or gene duplication prior to the evolutionary radiation (118). Therefore, ITS may lead to incorrect phylogenetic inferences for *Fusarium*. In contrast, non-orthologus copies of the translation elongation factor 1-α (EF-1α) have never been detected.

Also, EF-1α shows high polymorphism among closely related species (119), suggesting that it may serve as an excellent gene for phylogenetics and even as a single-locus identification marker in *Fusarium* (52). The intergenic spacer (IGS) of the rDNA has also been proven to resolve phylogenetically intraspecific relationships within *F. oxysporum* (4,19). Moreover, species-specific primers for *Fusarium* species have been developed based on the alignment of the IGS region (147), suggesting that this is a suitable candidate locus for phylogenetic analyses. Although mitochondrial small subunit (mtSSU) is not phylogenetically informative at the subspecies level, combined mtSSU and EF-1α may provide much better resolution among and within lineages (clades) (119). Recently, sequencing microsatellite loci has received a growing interest in phylogenetic species recognition of fungi due to their polymorphisms (29,30). However, this approach has not been examined for *F. oxysporum* f. sp *radicis-lycopersici*.

Phylogenetic analyses based on DNA sequences of mtSSU and EF-1α suggest that the *F. oxysporum* species complex is monophyletic but many *formae speciales* in the complex are polyphyletic. *Fusarium oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* are proposed to be paraphyletic (119), suggesting that they evolved from a common ancestor. Some VCGs between these two *formae speciales* may be more closely related than others within the same *forma specialis*. For example, Cai et al. (19) showed that VCG 0035 of *F. oxysporum* f. sp. *lycopersici* is more closely related to *F. oxysporum* f. sp. *radicis-lycopersici* than to other VCGs of *F. oxysporum* f. sp. *lycopersici* based on partial IGS sequences. However, Kawabe et al. (83) suggested that three isolates of *F. oxysporum* f. sp. *radicisi-lycopersici* without VCG information were phylogenetically closer to VCGs 0030 and 0032 of *F. oxysporum* f. sp. *lycopersici* than the others. Without including most known VCGs of *F. oxysporum* f. sp. *lycopersici* and *radicisi-lycopersici*, phylogenetic analyses may not uncover the real relationship among VCGs of these

two *formae speciales*. Moreover, multiple gene analyses have to be employed to construct genealogies and to recognize phylogenetic species (169).

To initiate sexual recombination, two isolates must be in the same biological species and carry distinct mating-type (MAT) idiomorphs, *MAT-1* or *MAT-2*, whereas vegetative incompatibility does not necessarily prevent the sexual cycle (97,181). These two mating types were found in *F. oxysporum* f. sp. *lycopersici*, correlated with phylogenetic lineage and VCG but not with race (83). Although *F. oxysporum* f. sp. *lycopersici* carries two mating type idiomorphs, successful crosses have never been observed (83). So far, little attention has been paid to determine the correlation between mating types and VCGs in *F. oxysporum* f. sp. *radicis-lycopersici*.

Silicon and Plant Disease

Silicon (Si) is the second most prevalent element in the earth's crust, comprising more than 25% of the earth's crust (158). Monosilicic acid, $Si(OH)_4$, is the main form of silicon in soils available to plants at a typical concentration of 0.1-0.6 mM (41), suggesting the low solubility of Si (60). Plants accumulating Si range from 1% to more than 10% of the dry biomass (41,42). Si accumulators are used to describe plants when a concentration of Si is greater than 1 % of the dry weight (43) and primarily accumulate Si in leaves as a result of the transpiration stream (7). Recently, the Low silicon rice 1 (*Lsi1*) gene encoding a Si uptake transporter has been isolated in rice, a typical Si-accumulating plant (101). In general, dicots such as tomato and soybean have a lower ability to accumulate Si than monocots (26). Si has not been considered as an essential element, although some plants absorb Si at levels equal to or greater than essential elements (26,42,43).

Beneficial effects of Si have been reported on the growth and development of plants and enhancing plant resistance to various biotic and abiotic stresses (20,26,41,42,43,48,135). Effects

of Si on plant disease are mainly observed in shoot organs due to the movement of Si through transpiration (7,60). The correlation between the content of Si in roots and soilborne diseases has not been well addressed. Interestingly, tomato, a Si excluder, accumulates more Si in roots than in shoots, and Si deposits mainly in the cell-wall-fraction (60). The reinforcement of cell walls has been suggested to reduce fungal penetration, whereas Si can also trigger defense responses (20,49). However, Diogo and Wydra (32) suggested that Si-mediated resistance in tomato against *Ralstonia solanacearum* was not located in roots but in stems as a result of reinforcing the pectic polysaccharide structure of stem cell walls and impeding the bacterial movement to stems. Surprisingly, a resistant cultivar, Hawaii 7998, treated with Si significantly reduced the bacterial population in both roots and stems compared to non-treated plants, suggesting that Si-mediated resistance might also locate in roots. No effect of Si was suggested in tomato against *Pythium aphanidermatum*, and the accumulation of Si in roots did not display a physical barrier for restricting the basipetal spread of the pathogen (61). It is not well known whether the efficacy of Si-mediated resistance in tomato depends on pathosystems investigated. Although Menzies et al. (112) suggested no effect of Si on Fusarium crown and root rot of tomato, the relationship between the Si effect and inoculum concentration and the effect of Si on disease progress have not been studied. It may be pertinent to further research whether Si can reduce disease severity of Fusarium crown and root rot of tomato as a result of either induced defense resistance and/or decrease in the root penetration by *F. oxysporum* f. sp. *radicis-lycopersici* that does not form appressoria prior to penetration but directly penetrate the junction of epidermal cells along roots or at the crown (178).

Hypotheses

This dissertation addressed evolutionary questions in regard to the population structure and phylogenetics of *F. oxysporum* f. sp. *radicis-lycopersici* and how this relates to disease

management. Moreover, incongruencies concerning the effect of silicon on Fusarium crown and root rot of tomato were evaluated. Chapters two, three, and four present research results that address these questions.

Chapter 2 determined the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* using VCGs and microsatellites. The first hypothesis was that more than one VCG coexist in the three main tomato-growing counties of Florida: Manatee, Hendry, and Collier Counties, but only one VCG predominated due to selective fit. Although no physiological race has been reported, the frequency of VCGs may help breeders to choose representative isolates for screening resistant tomato lines (125). The second hypothesis was that microsatellite primers acquired from the genome sequence of *F. oxysporum* f. sp. *lycopersici* can cross-amplify *F. oxysporum* f. sp. *radicis-lycopersici* since these two *formae speciales* are phylogenetically related (10,19). Bioinformatics approaches for developing microsatellite primers from the published genome sequence of closely related species may reduce the time needed to develop these primers for studying the population genetics of a given species. The final hypothesis of chapter 2 was that genotype flow shaped the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida. A pathogen with a high degree of genotype flow may have greater genetic diversity and pose a higher potential in breaking down resistance (107,108). If gene/genotype flow in *F. oxysporum* f. sp. *radicis-lycopersici* is high, disease management strategies should involve restricting genotype flow of the pathogen.

Chapter 3 investigated phylogenetics and mating types of *F. oxysporum* f. sp. *radicis-lycopersici* and their association with virulence. The first hypothesis was that the *forma specialis* is polyphyletic or of multiple evolutionary origins. Identifying the predominantly phylogenetic lineage may be helpful for breeding and for understanding the evolutionary history of the

pathogen. The second hypothesis was that each VCG carried only one mating-type idiomorph but the pathogen possesses two distinct idiomorphs. If two distinct idiomorphs are found, crossing isolates carrying opposite idiomorphs would be a direct approach to evaluate the possibility of sexual recombination in this pathogen. The third hypothesis was that *F. oxysporum* f. sp. *radicis-lycopersici* may have co-evolved with the domestication of its host due to selection by environmental and genetic uniformity of the agricultural ecosystem (161). The final hypothesis in chapter 3 was that virulence in various VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* is not identical even within the same phylogenetic lineage. Highly virulent isolates may be used for acquiring more consistent plant infection since the uncertainty of seedling infection by the pathogen has been reported (80).

Data presented in chapter 4 evaluated the effect of Si on the development of Fusarium crown and root rot of tomato. The first hypothesis was that Si can reduce disease severity due to a delay in initial infection and the basipetal movement of the pathogen. The effect of Si on disease severity can be specifically investigated through observing disease progress among Si treatments over time, resulting in an objective evaluation of the potential application of Si in the integrated pest management (IPM) of Fusarium crown and root rot of tomato. The second hypothesis was that the decrease in disease severity may be associated with increasing Si contents in roots. If the Si content of the roots influences disease development in tomato, an appropriate application of Si fertilizers may be recommended.

CHAPTER 2
POPULATION STRUCTURE OF *Fusarium oxysporum* f. sp. *radicis-lycopersici* IN FLORIDA INFERRED FROM VEGETATIVE COMPATIBILITY GROUPS AND MICROSATELLITES

Introduction

Fusarium oxysporum Schlechtend:Fr. f. sp. *radicis-lycopersici* W. R. Jarvis & R. A. Shoemaker, the causal agent of Fusarium crown and root rot of tomato**,** is an important disease on tomato worldwide (74) and limits tomato production in southern Florida (27). The pathogen is distinct from *F. oxysporum* f. sp. *lycopersici*, the causal agent of Fusarium wilt on tomato (92,146), based on symptoms, disease development, and physiological characteristics (72). *Fusarium oxysporum* f. sp. *radicis-lycopersici* is favored by cool weather (below 20°C) but Fusarium wilt of tomato is favored by temperatures around 27°C (110,141). Moreover, *F. oxysporum* f. sp. *radicis-lycopersici* has a wide range of hosts including species in Solanaceae, Leguminosae, Cucurbitaceae, and Chenopodiaceae, whereas *F. oxysporum* f. sp. *lycopersici* is host-specific to members of the genus *Lycopersicon* (110,141). Also, *F. oxysporum* f. sp. *radicis-lycopersici* causes cortical discoloration that usually extends no more than 25 cm above the crown, whereas vascular discoloration caused by *F. oxysporum* f. sp. *lycopersici* extends further. The pathogen can attack young tomato seedlings but infected plants usually do not wilt until the first full fruiting stage (72). Genetic differences have been revealed between these two *formae speciales* since *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* are vegetatively incompatible (128).

Vegetative compatibility, also known as heterokaryon compatibility or heterokaryon incompatibility (97), has been useful in characterizing genotypic diversity in *F. oxysporum* f. sp. *radicis-lycopersici* (80,82,125,139). Strains that can form stable heterokaryons are said to be vegetatively compatible and grouped into the same vegetative compatibility group (VCG)

(95,128). The VCG assay, although laborious, has helped to elucidate population structure and to differentiate *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* (128,139). Due to the absence of known physiological races for *F. oxysporum* f. sp. *radicis-lycopersici*, the frequency of VCG may help breeders to choose representative isolates of the pathogen for screening tomato lines for disease resistance (125). It has been suggested that Florida is the center of origin for the cosmopolitan VCG 0094 of *F. oxysporum* f. sp. *radicis-lycopersici* along with two recently found VCGs 0098 and 0099 (139). However, the distribution of these three VCGs in Florida has not been well evaluated since they were first reported in the 1990s. Because resistant cultivars carrying the *Frl* resistance gene have been released since the 1980s (50,148,150,151), it is pertinent to investigate the population structure of the pathogen for tomato breeding.

In addition to VCG, molecular markers such as restriction fragment length polymorphism (RFLP) (139) and random amplified polymorphic DNA (RAPD) (10) have been used for studying the population genetics of *F. oxysporum* f. sp. *radicis-lycopersici*. Microsatellites, also known as simple sequence repeats (SSR), consist of tandemly genetic loci of one to six base pairs and are found more abundantly in noncoding regions than in exons (58,167). They have been used to study population genetics of important plant pathogens and contribute to the knowledge of disease management due to their high polymorphism and rapid mutation rates (23,127,183). However, microsatellite primers for *F. oxysporum* f. sp. *radicis-lycopersici* have not been developed and used to study evolutionary forces. Such studies can help model the breakdown process of introduced resistance genes (107,108).

The main drawback of microsatellites is that they have to be isolated *de novo* from the species being examined if no genome sequence is available (182). Directly isolating microsatellites from *F. oxysporum* f. sp. *radicis-lycopersici* is likely laborious due to the

procedure of screening genomic libraries with appropriate probes (182). However, a recently released genome sequence of race 2 of *F. oxysporum* f. sp. *lycopersici* may help in the development of microsatellite primers by means of bioinformatics. The microsatellite primers designed from the genome sequence of *F. oxysporum* f. sp. *lycopersici* may cross-amplify *F. oxysporum* f. sp. *radicis-lycopersici* since these two *formae speciales* are phylogenetically related (19,119).

The genetic structure of a pathogen population is a result of the interactions among five evolutionary forces: mutation, genetic drift, gene/genotype flow, reproduction/mating system, and selection (107,108). Of these five forces, determining genotype flow of *F. oxysporum* f. sp. *radicis-lycopersici* may be most appropriate for investigating the genetic structure of the pathogen due to its extremely mitosporic reproduction (97). Pathogens exhibiting a high degree of gene/genotype flow have greater genetic diversity due to an increase in the effective population size, resulting in a greater risk in the breakdown of resistance (107,108). To effectively limit genotype flow of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida, commercial tomato-growing sites which donate and receive more migrants have to be identified.

The objectives of this study were (i) to investigate the frequency of VCGs of three main tomato-growing counties in Florida, (ii) to develop microsatellite primers for *F. oxysporum* f. sp. *radicis-lycopersici*, (iii) to evaluate genetic structure within and among populations, and (iv) to determine levels of migration between populations. Portions of this study were published previously as reports (66,67,69).

Materials and Methods

Fungal Collections

Diseased tomato plants with symptoms of Fusarium crown and root rot were sampled from three main Florida tomato-growing counties, Manatee, Hendry, and Collier Counties (Fig. 2-1).

Small pieces of stem and root tissues were sterilized in 5% sodium hypochlorite for 1 min, followed by rinsing in sterile deionized water. The pieces of the tissues were plated onto potato dextrose agar (PDA), water agar (WA), and Komada's agar (89). Morphological characteristics of *F. oxysporum* were examined based on the methodology of Nelson et al. (116). A monosporic isolate from each diseased plant was stored on sterile paper and in 15% glycerol for long-term storage.

Pathogenicity Tests

Pathogenicity tests were carried out as previously described (125,145). However, culturing *F. oxysporum* f. sp. *radicis-lycopersici* on rich media such as PDA may cause loss in its pathogenicity (94). Therefore, monosporic isolates were cultured on carnation leaf agar (CLA) at 25°C (12 h light photoperiod with photonflux of 40.8 μmol/m^2s) for 2 wk to obtain a massive production of conidia. Twenty tomato seeds (cv. Bonny Best) were dipped in a conidial suspension (10^6 mL^{-1}) for 3 min, placed on WA plates, incubated in the dark at 25°C for three days, and then moved to 25°C for additional four days. Isolates causing both dark-brown lesions on the root-stem transition region and more than 50% of the seedlings after inoculation were identified as *F. oxysporum* f. sp. *radicis-lycopersici* (125).

Molecular Differentiation of *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici*

Since *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici* are morphologically indistinguishable, molecular discriminations were conducted to further confirm that all isolates used in this study belonged to *F. oxysporum* f. sp. *radicis-lycopersici*.

For DNA extraction, single-spored isolates were grown on CLA before transferring mycelia plugs to PDA. Mycelia on PDA were harvested to extract DNA using DNeasy Plant Minikits (Qiagen, Inc., Valencia, CA). A 947 bp fragment specific for *F. oxysporum* f. sp.

radicis-lycopersici was amplified using primers sprlf (GATGGTGGAACGGTATGACC) and sprlr (CCATCACACAAGAACACAGGA), which targeted the *exo* polygalacturonase gene (*pgx4*) (63). Polymerase chain reaction (PCR) conditions were performed with 50 cycles of denaturation at 94°C (1min), annealing at 62°C (1 min), and elongation at 72°C (2 min), and finally 72°C for 10 min. *F. oxysporum* f. sp. *lycopersici* was identified using primer pairs P12-F2B (TATCCCTCCGGATTTGAGC) and P12-R1 (AATAGAGCCTGCAAAGCATG) to amply an ≈ 1 kb fragment of *SIX1*, a virulence locus (172). Amplifications were carried out using the following PCR conditions: 94°C for 2 min, 30 cycles at 94°C for 45 s, 64°C for 45 s, and 72°C for 45 s and a final elongation step at 72°C for 10 min (172).

Vegetative Compatibility Tests

Methods for media preparation, procedures for generating *nit* mutants and complementation tests were performed as previously described (24). Briefly, each isolate was cultured on minimal medium (MM) for 5-7 days, and then forty plugs were transferred to plates filled with either PDA or MM supplemented with 25 g of $KClO_4$ per liter (PDC and MMC media). After 7-14 days, rapid growing sectors were transferred to MM, nitrite agar with 0.5 g $NaNO_2$ L^{-1}, and hypoxanthine agar with 0.2 g $NaNO_2$ L^{-1}. Based on growth characteristics, three phenotypic classes of nitrate- non-utilizing (*nit*) mutants, *nit1*, *nit3* and NitM, were assigned. Two mutants each of *nit1*and *nit3* were selected in complementation tests on MM with NitM testers, representing previously reported VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* (Table 2-1). Plates were incubated at 25°C and evaluated daily for 2 wk. The presence of aerial mycelia at the point of hyphal contact was evidence of vegetative compatibility, and these isolates were assigned to the same VCG (128).

Development of Microsatellite Primers

Microsatellite loci were derived using a bioinformatics approach and a microsatellite enrichment procedure. For the bioinformatics approach, the genome sequence of *F. oxysporum* f. sp. *lycopersici* (strain 4287, race 2, VCG 0030) was downloaded from http://www.broad.mit.edu. Sequences with a microsatellite motif were identified using TANDEM REPEAT FINDER (13). Three alignment parameters, match, mismatch, and indel were set at 2, 7, and 7, respectively. The microsatellite enrichment protocol was based on the method of Edwards et al. (35) except a biotinylated $(GA)_{17}$ probe was used in this study. Briefly, genomic DNA of *F. oxysporum* f. sp. *radicis-lycopersici* (isolate HE-0631, VCG 0094) was extracted from mycelia using DNeasy Mini Kit (Qiagen, Valencia, CA), digested with *Sau*3AI, and ligated with *Sau*3AI linkers. Ligation was confirmed by PCR. The fragment library was enriched by hybridization with a biotinylated $(GA)_{17}$ probe and then captured by VECTREX Avidin D (Vector Laboratories, Burlingame, CA). The selected fragment was enriched by PCR using primers designed to the linker sequence. The enriched library was ligated into a TOPO TA pCR 4.0 vector, transformed into One Shot® *Escherichia coli* competent cells (Invitrogen, Carlsbad, CA), and grown overnight on Luria broth (LB) agar plates containing 50 μg mL^{-1} kanamycin. Transformed colonies were cultured in LB overnight and screened for microsatellite repeats using PCR with a $(GA)_{10}$ repeat primer and M13F/M13R. The appropriate sized amplicons (>200 bp) were screened on 2% agarose gels, amplified by rolling circle amplification (RCA), and sequenced with the T7 primer and BigDye Terminator Cycle Sequencing Chemistry (Applied Biosystems, Foster City, CA) at the Interdisciplinary Center for Biotechnology Research (ICBR) facility, University of Florida. Sequences were edited using SEQUENCHER 4.2 (Gene Codes Corp., Ann Arbor, MI). Primers were designed using PRIMER3 (142). An M13 tail

(CACGACGTTGTAAAACGAC) was added to the 5' end of the each forward primer for amplification with 6-FAM/VIC/NED/PET-labeled M13 primers (15).

Fourteen isolates of *F. oxysporum* f. sp. *lycopersici* and 33 isolates of *F. oxysporum* f. sp. *radicis-lycopersici* representing most known VCGs (Table 2-2) were used to verify designed microsatellite primers. PCR amplification was carried out in a 20 μL reaction mixture containing 0.5 U of GoTaq Flexi DNA polymerase (Promega Corp., Madison, WI) or *Taq* DNA polymerase (Bioline USA Inc. Taunton, MA), 1× Promega Colorless GoTaq Flexi Buffer or 1× Bioline KCl buffer, 2.5 mM $MgCl_2$, 0.01 μM of each forward primer labeled with the M13 tail, 0.15 μM of each reverse primer and 6-FAM/VIC/NED/PET-labeled M13, and 50 μM dNTP. Cycling conditions were: 94°C for 3 min, 35 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 45 s and a final elongation step at 72°C for 20 min (35). 6-FAM/VIC/NED/PET-labeled PCR products were mixed, diluted, and run on an ABI 3730x1DNA Analyser (Applied Biosystems) at ICBR by loading 1 μL of the diluted PCRs, 9.9 μL formamide, and 0.1 μL LIZ 600 size standard (Applied Biosystems). Fragment sizes were analyzed using PEAK SCANNER™ SOFTWARE version 1.0 (Applied Biosystems).

Allele numbers and gene diversity were analyzed using ARLEQUIN version 3.1 (45). A neighbor-joining (NJ) tree based on Nei's minimum genetic distance (115) was generated using POPULATIONS (Centre National de la Recherche Scientifique, Paris, France) to infer genetic distance among isolates. Two isolates of *F. commune* (Table 2-2) were also genotyped and utilized as outgroups.

Microsatellite Data Collection

Ten microsatellite loci (CH2-9, CH2-15, CH2-66, FOL20, FOL35, FOL99, FOL175, FOL185, FOL245, and FOL680) (Table 2-5) with high gene diversity were chosen to genotype

125 isolates (Table 2-3) of *F. oxysporum* f. sp. *radicis-lycopersici* identified using pathogenicity tests and the PCR-based discrimination. DNA extraction and PCR temperature cycling conditions were the same as above except that annealing temperature for FOL99 was increased to 58°C to avoid nonspecific amplification. A control isolate (HE-0601) with a reproducible fragment analysis with the ten loci was included in every run of 95 samples. Since DNA degradation, low DNA concentration, and primer-site mutation may resulted in genotyping error, MICRO-CHECKER was used to identify and correct microsatellite data (173).

Microsatellite Data Analysis

Isolates with the same microsatellite genotyping pattern within populations were removed and counted only once to generate clone-corrected microsatellite data (139). Depending on genetic analyses, VCG affiliation alone or VCG affiliation and geographic source was predefined as populations (139). Microsatellite haplotypes were identified using ARLEQUIN version 3.1 (45) and THE EXCELL MICROSATELLITE TOOLKIT (University College Dublin, Belfield, Ireland). The existence of identical microsatellite haplotypes shared between two populations was considered to be direct evidence of genotype flow (56). Formatted files were generated for the data analyses using THE EXCELL MICROSATELLITE TOOLKIT and CONVERT (54).

The mean number of pairwise differences and its standard deviation (SD) were determined according to Tajima (165). The average nucleotide diversity was calculated based on Nei (115) and Tajima (163). Fixation indices (*F* statistics) were used to examine qualifying differentiation between pairs of populations and the degree of population subdivision according to the sum of squared size differences for microsatellite data (156). If *P* was ≤ 0.05, genetic differentiation between two populations was considered significant. The distribution of variance within and among populations was based on using the analysis of molecular variance framework (AMOVA)

implemented in ARLEQUIN. A nonparametric approach with 1,023 permutations was performed (44).

Linkage equilibrium tests were used to determine whether recombination was probable for *F. oxysporum* f. sp. *radicis-lycopersici* in the field. Fisher's exact test implemented in GENEPOP version 3.4 (Insititut des Sciences de l'Evolution, Université Montpellier 2, Montpellier, France) was analyzed based on an Markov Chain Monte Carlo (MCMC) algorithm with 1,000 batches and 1,000 iterations/batch (17,131). A pair of loci were considered at linkage disequilibrium if its *P* value was $\leq$0.05. Two indices of multilous linkage disequilibrium, I_A and $\bar{r}_d$, were calculated using MULTILOCUS version 1.3 (Department of Biology, Imperial College, Silwood Park, UK) with 1000 randomizations for testing statistical significance (1). $\bar{r}_d$ is a modification of I_A and it is less sensitive to the number of loci used (1).

The population genetic structure of the microsatellite data was analyzed using the Bayesian model-based clustering program STRUCTURE version 2.2 (46,126). STRUCTURE infers population structure based on multilocus genotype data and calculates the membership coefficients to determine possible admixed/hybrid individuals. The number of populations (K) was computed from 1 to 3. Ten runs with the admixture model and a burn-in period of 10,000 generation and MCMC simulations of 100,000 iterations for each run. K for the best fit of the data was determined by estimates for the natural logarithm of the probability of the data (46,126). Graphic displays of STRUCTURE results were generated using DISTRUCT (138).

Historical migration between three Florida tomato-growing counties was evaluated using MIGRATE-N version 3.0.3 (11,12). Briefly, MIGRATE-N jointly calculated the mutation scaled population size Θ; that is, the effective population size *Ne* times the mutation rate μ per site and generation (2*Ne*μ for haploid *F. oxysporum* f. sp. *radicis-lycopersici*) and the mutation scaled

immigration rate M (m/μ) between pairs of populations based on the coalescence theory (85). Asymmetrical migration between population pairs was analyzed using the Bayesian inference module in MIGRATE-N. Ten replicates of one long chain and four chains of static heating scheme with four temperatures (1.0, 1.5, 3.0, and 10000) were used for running microsatellite data with Brownian motion, which assumed a stepwise mutation model. 50000 recorded steps in interval of 200 with 10 concurrent chains (100,000,000 visits) were performed, and 1,000,000 trees per chain were discarded as burn-in. Migrants per generation between two populations were derived from Θ multiplied by M.

The exponential growth rate (g) for evaluating the population size fluctuation of each population was calculated using Bayesian MCMC implemented in LAMARC version 2.1.3 (90). One long chain and four chains of static heating scheme with four temperatures (1.0, 1.5, 3.0, and 10,000) were used for running microsatellite data using the stepwise model. Twenty-five thousand recorded steps in intervals of 40 were performed and 100,000 samples were discarded as burn-in. Positive values of g suggested that the population size has been growing, whereas negative values indicate that the population size has been shrinking. Nevertheless, if the confidence intervals of g includes zero, the population size likely has little or no growth (90,91).

Results

Pathogenicity Tests

Of 148 isolates from Manatee, Hendry, and Collier Counties, 125 isolates caused disease symptoms on 50-100% of the tomato seeds 7 days after inoculation. A dark brown lesion on the root-stem transition region (crown) was observed, suggesting these isolates were the pathogenic *F. oxysporum* f. sp. *radicis-lycopersici* (145). These 125 isolates were further characterized using primers P12-F2B and P12-R1 to exclude *F. oxysporum* f. sp. *lycopersici*. The primers consistently amplified two ioaltes of *F. oxysporum* f. sp. *lycopersici* (MN24 and DA1). However,

no amplification was observed for these 125 isolates, and thus they were used for studying the population genetics of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida.

Molecular Identification of *F. oxysporum* f. sp. *radicis-lycopersici*

Primers sprlf and sprlr specific for *F. oxysporum* f. sp. *radicis-lycopersici* (63) were first tested with 24 isolates. Of these isolates, 15 isolates represent VCGs 0090, 0091, 0092, 0093, 0094, 0096, 0098, and 0099 of *F. oxysporum* f. sp. *radicis-lycopersici* and nine isolates belong to VCGs 0030, 0031, 0032, 0033, and 0035 of *F. oxysporum* f. sp. *lycopersici*. These two primers amplified the expected 947 bp fragment for isolates of *F. oxysporum* f. sp. *radicis-lycopersici* in VCGs 0090, 0091, 0092, and 0096, whereas no amplification was observed in the other VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* or in the isolates of *F. oxysporum* f. sp. *lycopersici* (Fig. 2-2). In contrast, *F. oxysporum* f. sp. *lycopersici* specific primers P12-F2B and P12-R1 correctly confirmed that the nine isolates belonged to *F. oxysporum* f. sp. *lycopersici* without further amplifying any isolates of *F. oxysporum* f. sp. *radicis-lycopersici*. Therefore, to identify *F. oxysporum* f. sp. *radicis-lycopersici*, pathogenicity tests along with primers P12-F2B and P12-R1 were used to exclude isolates of *F. oxysporum* f. sp. *lycopersici*.

Vegetative Compatibility Tests

One hundred and twenty five isolates collected from 2006 to 2008 were used for vegetative compatibility tests. Sixty-nine percent of these isolates could be assigned to one of three VCGs: 0094, 0098 and 0099. Thirty eight isolates could not be assigned to a known VCG. Frequencies of VCGs 0094, 0098 and 0099 were 38.6%, 24.4%, and 6.8% respectively, indicating that VCG 0094 was predominant among these isolates assessed (Table 2-4). Although 77% of VCG 0094 was mainly confirmed as subgroup I, some of them also formed heterokaryons with testers of subgroup II, III, or IV. Interestingly, VCG 0098 was previously reported in Collier County, but it has now been found in Manatee and Hendry Counties, whereas VCG 0099 seems restricted to

Collier County. Two isolates MN-0713 and MN-0724 from Manatee County were assigned to both 0094 and 0098 after repeating two single-spored isolations and confirming vegetative compatibility with testers of 0094I (01150-6 and 01152-31) and 0098 (CL-7/6, CL-75/4) (Table 2-1). Occasionally, all three VCGs 0094, 0098, and 0099 were recovered from a single sampling site in Collier County. On the other hand, VCGs 0094 and 0098 were found occasionally on single farms in both Manatee and Hendry Counties.

Development of Microsatellite Primers

A microsatellite enrichment procedure and a bioinformatics approach were used to develop microsatellite primers for studying the population genetics of *F. oxysporum* f. sp. *radicis-lycopersici*. Of 48 clones sequenced from the enrichment approach, 14 included microsatellites. For the bioinformatics approach, 38 microsatellite loci were acquired using TANDEM REPEAT FINDER. These 52 loci were used to design primers to initially amplify four representative isolates each of *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici*. Of these 52 original loci, 27 were amplified consistently and exhibited variation among the eight isolates. These 27 pairs of primers (Table 2-5) were further tested on another nine isolates of *F. oxysporum* f. sp. *lycopersici* and 27 isolates of *F. oxysporum* f. sp. *radicis-lycopersici* that included the most reported VCGs (Table 2-2).

These 27 loci were successfully genotyped in both *F. oxysporum* f. sp. *lycopersici* and *radicis-lycopersici*. Of these 27 loci, 13 did not encode any protein when queried by BLAST to GenBank. Allele numbers and gene diversity scores are shown on Table 2-5. The 27 loci had 1 to 14 alleles per locus (average of 6.7 alleles for *F. oxysporum* f. sp. *radicis-lycopersici* and 5 alleles for *F. oxysporum* f. sp. *lycopersici*). Loci FOL680 and FOL35 had the highest gene diversity for *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* respectively, whereas gene diversity of the 27 loci ranged from 0 to 0.95. A neighbor-joining tree

generated from the fragment analysis of the 27 microsatellite loci for these 47 isolates (Table 2-2) revealed four clusters: FORL clusters I and II, and FOL clusters I and II (Fig. 2-3). FORL cluster II and FOL cluster II represent predominant VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici*, respectively.

Molecular Diversity Indices

Ten of the 27 loci (CH2-9, CH2-15, CH2-66, FOL20, FOL35, FOL99, FOL175, FOL185, FOL245, and FOL680) were selected to genotype the 125 isolates of *F. oxysporum* f. sp. *radicis-lycopersici* collected from Manatee, Hendry, and Collier Counties for population genetics analyses.

Pairwise Fst values between isolates from these three counties suggested no genetic differentiation in either VCG 0094 or 0098 (Table 2-6 and Table 2-7). VCG 0098 was found in Collier County but not in the other two counties in the 1990s (139) and two isolates (MN-0713 and MN-0724) in this study were vegetatively compatible with testers of VCGs 0094 and 0098. Therefore, this study tested a hypothesis that these two VCGs originally migrated from Collier County to Manatee and Hendry Counties according to measures of intrapopulation diversity (mean number of pairwise differences and average gene diversity). The basic assumption was that a new population exhibits a lower level of molecular diversity as a result of the founder effect and less accumulation of mutations (107,108,139).

Mean numbers of pairwise differences and average gene diversity were compared for three Floridia populations (Table 2-8). Hendry County had a higher mean number of pairwise differences and average gene diversity when either whole microsatellite or clone-corrected data were used for analysis. However, to evaluate these two indices for each VCG, the clone-corrected data set was used to further classify each VCG and its affiliated geographical region (139). The two indices were the highest in Collier County for VCGs 0094 and 0098, suggesting

that these two VCGs might have migrated from Collier County to the other two counties. For isolates not assigned to a known VCG, the two indices were highest in Hendry County. Regarding individual VCGs, results of clone-corrected data suggested that VCG 0099 had a higher mean number of pairwise differences and average gene diversity than VCGs 0094 and 0098, whereas VCG 0094 was predominant in Florida.

Hierarchical Distribution of Total Gene Diversity

The AMOVA for *F. oxysporum* f. sp. *radicis-lycopersici* was used to determine the variance within and among populations. The results demonstrated that 84% of the variance occurred within populations and that a significant partition of variance, 16% (P<0.0001), was detected among populations. Φst was 0.159, suggesting a medium level of differentiation among populations (Table 2-9).

Linkage Disequilibrium

Fisher's exact test revealed that16.7% of pairwise loci in isolates from Manatee County and 62.3% in Collier County were at linkage equilibrium. However, two indices of multilocus linkage disequilibrium, I_A and $\bar{r}_d$, showed significant linkage disequilibrium, suggesting clonal reproduction for *F. oxysporum* f. sp. *radicis-lycopersici*. Despite the finding of up to 62.3% of pairwise loci of the Collier population at linkage equilibrium, $\bar{r}_d$, which is less sensitive to the number of loci sampled, for the population revealed significant deviation from linkage equilibrium (Table 2-10).

Population Admixture

In order to infer the best value of K clusters (real population groups), different runs of K=1 to 3 were performed using STUCTURE. Mean values of likelihood probability of data given K=1 to 3 were -665.8, -562.9, and -533.1, respectively. Therefore, K=3 was used to determine possible admixture in the three populations, suggesting that isolates sampled from the same

county could be defined as a population. Eighteen likely admixed individuals were identified: three in Manatee County (12% of the total clone-corrected individuals), 13 in Hendry County (50% of the total), and two in Collier County (5.89% of the total) (Fig. 2-4). Highly significant admixture found in Hendry County suggests that some individuals could be immigrants from the other two counties.

Historical Migration and Population Growth Rate

The population parameter theta (Θ) of the three counties was very close (Θ = 0.09738 to 0.09755). Asymmetrically historical migration was shown according to estimates of directional genotype flow (Table 2-11 and Fig. 2-1). Most migrants migrated from Manatee and Collier Counties to Hendry County ($2Nm_{\text{Manatee}\rightarrow\text{Hendry}}$ =2.75, $2Nm_{\text{Collier}\rightarrow\text{Hendry}}$ =2.15), whereas fewer migrants were found between Manatee and Collier Counties. The historical movement of migrants was much higher between Manatee and Hendry Counties than that between Collier and Hendry Counties no matter which direction of migration was compared.

Population growth rates, *g*, with 95% confidence intervals were 0.10+0.98/–22.5, -0.11+0.13/-0.14, and 0.10+1.27/–0.03 for Manatee, Hendry, and Collier Counties, respectively. This suggests that the population size of *F. oxysporum* f. sp. *radicis-lycopersici* in Manatee and Hendry Counties have shown little or no growth because the confidence levels include zero (90,91). In contrast, the population size in Collier County has been increasing.

Discussion

This study tested the hypothesis that migration played an important role in shaping the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* in the three currently major tomato-growing counties of Florida. This study reveals the current population structure of *F.*

oxysporum f. sp. *radicis-lycopersici* inferred from VCGs and microsatellites and supports the hypothesis.

Pathogenicity Tests

A laboratory pathogenicity bioassay has been used to identify *F. oxysporum* f. sp. *radicis-lycopersici* according to symptomology (71,145). Since the *F. oxysporum* f. sp. *radicis-lycopersici* specific primers were unable to identify predominant VCGs in Florida, the pathogenicity bioassay along with the *F. oxysporum* f. sp. *lycopersici* specific primers were used to confirm isolates of *F. oxysporum* f. sp. *radicis-lycopersici*. The isolates causing disease symptoms in greater than 50% of the assay and not amplified by *F. oxysporum* f. sp. *lycopersici* specific primers were defined as *F. oxysporum* f. sp. *radicis-lycopersici*. A subset of 12 isolates were also used for the standard root-dip inoculation method (80) and caused either typical dark-brown lesions on the root-stem transition region and/or cortical discoloration, indicating that the pathogenicity bioassay was reliable (125).

Molecular Identification

Molecular distinction of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* was originally evaluated to select isolates of *F. oxysporum* f. sp. *radicis-lycopersici* for studying population genetics. Species-specific primers for *F. oxysporum* f. sp. *radicis-lycopersici* targeting *exo* polygalacturonase (*pgx4*) (63) were first tested using most of the reported VCGs of *F. oxysporum* f. sp. *radicis-lycopersici*. However, these primers did not amplify VCGs 0093, 0094, 0098, and 0099, indicating high variation of *pgx4* in this pathogen. Moreover, the isolates originally used for developing the specific primers did not cover most known VCGs but only focused on Japanese isolates without VCG information (63). This is the probable reason for the lack of amplification in the majority of isolates from this study. In contrast, another set of primers, P12-F2B and P12-R1 correctly identified *F. oxysporum* f. sp.

lycopersici, but did not amplify isolates of *F. oxysporum* f. sp. *radicis-lycopersici* in this study. P12-F2B and P12-R1 targeted a virulence gene that is expressed in the xylem during colonization of *F. oxysporum* f. sp. *lycopersici* in tomato plants (172). Cell-wall-degrading enzymes (CWDEs) are secreted by *F. oxysporum* (31,77) and a comparison of their nucleotide diversity may be used to discriminate *formae speciales* of *F. oxysporum* (63). Regarding specificity of primers, however, virulence genes conferring a specific trait to a pathogen are likely ideal targets since they have subtle nucleotide difference within the same *formae speciales* (98). Based on the findings in the current study, there is a need to develop more specific primers for rapid diagnosis of *F. oxysporum* f. sp. *radicis-lycopersici*.

VCG Frequency

A VCG predominant in the population may be selectively more fit than others (95). Sixty-nine percent of the 125 isolates used in this study were assigned to three VCGs 0094, 0098, 0099, which were previously reported in the 1990s (139). While VCG 0094 is still predominant in Florida, its frequency has been reduced from 70.3 to 38.6% since the 1990s (139). On the other hand, VCG 0098, first reported in Florida, has increased in its distribution compared to a previous study (139). For example, VCG 0098 was not found in Manatee and Hendry Counties previously (139) but now is present in these two counties, probably due to migration. Interestingly, two isolates (MN-0713 and MN-0724) found in Manatee County were compatible with testers of both VCGs 0098 and 0094 I, suggesting some degree of genetic relatedness between these two VCGs (Fig. 2-3). Therefore, sympatric speciation may be another possibility to explain the appearance of VCG 0098 in Manatee County, which suggests VCG 0098 might have evolved from the local population of VCG 0094. It has been suggested that bridge isolates may be interpreted as a stage in a process of either convergence or divergence of VCGs (80). If a VCG is newly formed, it may exhibit low levels of molecular diversity due to the founder effect

and few accumulated mutations (139). Based on this assumption, VCG 0098 probably diverged from VCG 0094 as evidenced by low nucleotide diversity relative to VCG 0094. Further studies in molecular evolution of these two VCGs is necessary to clarify whether the ancestor of these two isolates originated from a native VCG 0094 of Manatee County or migrated from other regions.

Florida has been suggested as the probable center of origin of VCG 0094 and this VCG might have migrated from Florida to Europe according to restriction fragment length polymorphism (RFLP) analyses (139). The global distribution of this VCG is not well known (82). This study showed that the main VCG 0094 in Florida belonged to subgroup I (77%). Of these VCG 0094 I isolates, some could form heterokaryons with testers of subgroup II, III, and IV, suggesting variation in interisolate compatibility (139).

Although 31% of the isolates used could not be assigned to a known VCG, most of them had the same microsatellite haplotype as either VCG 0094 or 0098 (59%). Moreover, some interisolate pairings revealed several minor groups that compromised two to five vegetative compatible isolates. These finding suggested that these nonassigned isolates may not comprise new VCGs. More isolates of these minor groups need to be recovered from the tomato-growing region before VCG code numbers can be assigned to them (81,86). Mutations may occur in any of a number of genes controlling incompatibility, altering the VCG of an isolate (95). In addition, the selection of spontaneous mutants may also cause mutations that interfere with the heterokaryosis (97). These results may explain why some nonassigned isolates were not compatible with testers of VCGs 0094 and 0098 but had the same microsatellite haplotype as these two VCGs. On the other hand, increasing the frequency of unclassified isolates may also

suggest the emergence of another VCG within *F. oxysporum* f. sp. *radicis-lycopersici*. Regularly screening VCGs of the pathogen appears necessary to monitor its evolving genetic variation.

Microsatellites

Microsatellites are valuable for both taxonomic and population genetic studies (14). The microsatellite loci isolated from other *Fusarium* species may not be useful for *F. oxysporum* f. sp. *radicis-lycopersici* due to their high mutation rates (78). Since the genome sequence of *F. oxysporum* f. sp. *lycopersici* became available in 2007, a bioinformatics approach was used in this study to search microsatellite loci in addition to direct isolation from *F. oxysporum* f. sp. *radicis-lycopersici*. Surprisingly, 20 of 38 microsatellite loci selected from the genome sequence could consistently amplify known VCGs of both *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici*. Moreover, 7 of 14 microsatellite loci isolated *de novo* from *F. oxysporum* f. sp. *radicis-lycopersici* were cross-amplified between these two *formae speciales*. The cross amplification suggested that the two *formae speciales* are closely related. Genetic analyses of 27 microsatellite data for these two *formae speciales* showed that alleles per locus of *F. oxysporum* f. sp. *radicis-lycopersici* (6.7 alleles) were higher than those of *F. oxysporum* f. sp. *lycopersici* (5 alleles), indicating a higher level of genetic diversity in *F. oxysporum* f. sp. *radicis-lycopersici*. Also, the considerably larger number of VCGs reported in *F. oxysporum* f. sp. *radicis-lycopersici* may reflect its high genetic diversity (80,81).

Molecular markers, such as RFLP and RAPDs, have been used to investigate the evolutionary origins of VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* (10,38,139). To our knowledge, microsatellites have not been used to investigate genetic relatedness of these two *formae speciales*. A dendrogram generated from 27 microsatellite data exhibited four clusters: FORL clusters I and II, and FOL clusters I and II. In general, isolates within the same VCG are more closely related than others within different

VCGs, confirming previous results derived from other molecular markers (10,37,38). VCGs 0090, 0091, 0092, and 0093 of FORL cluster I were not found in this study, whereas VCGs 0090 and 0091 were previously reported in North America and VCGs 0092, 0093, 0096 were found in Israel and Europe (37,80). In contrast, isolates of *F. oxysporum* f. sp. *radicis-lycopersici* used for this study were grouped into FORL cluster II in which VCGs 0094, 0098, and 0099 are predominant. This change in population structure shows that the three VCGs replaced previous dominant VCGs 0090 and 0091 in Florida in agreement with a previous study by Rosewich et al. (139). According to the dendrogram analysis, FORL clusters I and II probably evolved from different progenitor populations. Regarding the evolution of *F. oxysporum* f. sp. *lycopersici*, VCGs 0030 and 0032 shared higher genetic similarity than VCGs 0031, 0033, and 0035. Like *F. oxysporum* f. sp. *radicis-lycopersici*, two probable ancestral progenitors were suggested in *F. oxysporum* f. sp. *lycopersici*, corresponding to previous studies (37,38). Interestingly, VCG 0033 of *F. oxysporum* f. sp. *lycopersici* and VCGs 0094, 0098, and 0099 of *F. oxysporum* f. sp. *radicis-lycopersici* showed a high degree of genetic similarity and were all found in this study.

Molecular Diversity Indices

The mean number of pairwise differences and average gene diversity were compared for three Florida populations (Table 2-8). Isolates from Hendry County had a higher mean number of pairwise differences and average gene diversity when either whole microsatellite data or clone-corrected data was used for analysis probably because it was the sink of migrants from the other two counties according to analysis of historical migration (Figs. 2-1 and 2-4). VCG 0098 was not previously reported in Hendry County before this study. Pairwise Fst values of VCGs 0098 between isolates from the three counties exhibited no genetic differentiation. Moreover, its nucleotide diversity in Hendry County was not higher than in the two other counties. Therefore, genotype flow from other tomato-growing regions may be the reason VCG 0098 appeared in

Hendry County. However, the possibility of VCG0098 arising from either another local VCG or nonpathogenic *F. oxysporum* cannot be completely ruled out as two isolates in this study were found to form heterokaryons with testers of VCGs 0094 and 0098. This cross vegetative compatibility indicated that both of these two VCGs belong to the same clonal lineage and may have diverged by somatic processes (38,55). Further analysis based on the coalescent theory (137) may provide more insights about the evolution of VCGs 0094 and 0098.

Pairwise Fst values of VCG 0094 or 0098 among these isolates from three counties suggested no genetic differentiation (Table 2-6 and Table 2-7). Measures of intrapopulation diversity (mean number of pairwise differences and average gene diversity) were used to infer the origin of these two VCGs according to a basic assumption that a new population exhibits a lower level of molecular diversity as a result of the founder effect and less accumulation of mutations (139). To evaluate these two indices for each VCG, the clone-corrected data set was used to further classify each VCG and its affiliated geographical region. The two indices were the highest in Collier County for VCGs 0094 and 0098, suggesting that these two VCG may have originated in Collier County and then migrated to Manetee and Hendry Counties. For those isolates not assigned to known VCGs, the two indices were highest in Hendry County. Regarding individual VCG, results of clone-corrected data showed that VCG 0099 had a higher mean number of pairwise differences and average gene diversity than VCGs 0094 and 0098, while VCG 0094 was predominant in Florida. VCG 0099 probably preexisted in Florida relative to VCGs 0094 and 0098; however, its frequency declined due to being less selectively fit in comparison to the others (139).

Hierarchical Distribution of Total Gene Diversity

Although no genetic differentiation was revealed for VCGs 0094 and 0098, The AMOVA for all isolates of *F. oxysporum* f. sp. *radicis-lycopersici* suggested a significant partition of

variance among counties. VCG 0099 in Collier County and other nonassigned VCG isolates in these three counties may have contributed to the significant genetic variation among counties. Moreover, most variance occurred within populations (84%), indicating that genotype flow was higher among the counties than within the counties.

Linkage Disequilibrium

The *F. oxysporum* species complex has been viewed as an extremely mitosporic species, whereas it comprises a broad diversity of *formae speciales* that are able to infect numerous hosts (55,97). Phylogenetic analyses showed that this species complex is monophyletic but most *formae speciales* are polyphyletic (119). It has been suggested that recombination is possible in this species complex in addition to clonal reproduction (168). Since Florida, where two new VCGs, 0098 and 0099, were first reported, has been considered as the probable center of origin of VCG 0094, this study tried to determine linkage disequilibrium for understanding the possibility of recombination in the field although its sexual stage has not been reported. Results showed that *F. oxysporum* f. sp. *radicis-lycopersici* sampled from these three counties exhibited linkage disequilibrium according to index of association (Table 2-10), but up to 62.3% of pairwise loci for isolates of Collier County were at linkage equilibrium, suggesting random association of alleles. Even in a mitosporic fungus, parasexual recombination and reassortment resulting from protoplast fusion can cause chromosome rearrangement (170), contributing to alleles at one locus to be randomly associated. However, other processes, such as selection, gene/genotype flow, drift, and linkage, can also cause linkage disequilibrium (113). Crossing isolates from Collier County carrying different mating-type idiomorphs may provide a direct confirmation of recombination.

Population Admixture and Historical Migration

The best value of K clusters (real populations) was three, suggesting that defining a county as a population was reasonable in this study. K = 3 was used to determine possible admixture in the three populations. Eighteen likely admixed individuals were identified, and most of them were found in Hendry County (50% of the total) (Fig. 2-4). Highly significant admixture found in Hendry County suggests that some alleles found in isolates were most likely drawn from Manatee and Collier Counties and that alleles were exchanged among populations. Moreover, historical migration revealed that more immigrants moved into Hendry County than into the other two counties (Table 2-11). Because high gene/genotype flow can enhance genetic diversity of a population as a result of increasing the size of genetic neighborhood (107,108), it may partially explain why Hendry County had a higher genetic diversity of *F. oxysporum* f. sp. *radicis-lycopersici*. Given that a population with high genetic diversity may have more alleles to overcome a resistance gene (107,108), lowering genotype flow of *F. oxysporum* f. sp. *radicis-lycopersici* among these three counties is necessary to reduce the break-down risk of a resistance gene. Moreover, *F. oxysporum* f. sp. *radicis-lycopersici* has been considered as a soilborne, airborne, and waterborne pathogen (59,74,133,140). Therefore, methods involved in reducing genotype flow would have to thoroughly evaluate unique epidemiological characteristics of this pathogen (82). Natural dispersal of pathogen propagules by wind, water, and insects is difficult to restrict. However, long distance dispersal by man may be limited by means of inspecting infected plant material, soil, or contaminated equipment (107,108).

Population Growth Rate

Since population growth rates, *g*, with 95% confidence intervals, of Manatee and Hendry Counties included zero, the population size of *F. oxysporum* f. sp. *radicis-lycopersici* in these two counties have experienced little or no growth (90,91). In contrast, *g* of Collier County did

not contain zero, suggesting a growing population. The genetic structure of a population is dynamic and determined by five evolutionary forces: mutation, genetic drift, gene/genotype flow, reproduction/mating system, and selection (107,108). A pathogen population with a high population size and high standing genetic diversity may have greater potential to break down a host resistant gene through mutation. Therefore, in addition to limiting genotype flow among these three counties, reducing the population size of *F. oxysporum* f. sp. *radicis-lycopersici* through disease management in these three counties is another important consideration to decrease the evolutionary potential of the pathogen. Utilizing regular crop rotations and avoiding extremely susceptible cultivars are two simple ways to minimize pathogen population size (107,108). Lettuce has been suggested for using in rotation and intercropping with tomato due to evidence of phenolic compounds from lettuce inhibiting the growth of *F. oxysporum* f. sp. *radicis-lycopersici* (73,74).

Table 2-1. Tester strains of vegetative compatibility groups of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* used in this study

VCG	Tester strains[z]	References
F. oxysporum f. sp. *radicis-lycopersici*		
0090 I	FRC-O-1090/1, FRC-O-1090/B	(80), (128)
0090 II	FORL-C-407/1, FORL-C415/3	(80)
0090 III	FORL-C696A/3, FORL-C710/A62	(82)
0091 II	FORL-C69E3	(80)
0094 I	01150-6, 01152-31	(82)
0094 II	D69/7, D69/19	(82)
0094 III	26787/6, 26787/10	This study
0094 IV	CL-2/36, CL-2/40	This study
0096	FORL-C624A/3, FORL-C622A/6	(80)
0098	CL-7/6, CL-75/4	(139)
0099	LE-20/11, LE-20/12	This study
F. oxysporum f. sp. *lycopersici*		
0033	DA-1/7	(139)

[z] Testers were provided by H. C. Kistler, except those of VCGs 0094 III, 0094 IV, and 0099 developed in this study.

Table 2-2. Isolates used for developing microsatellite primers

Isolate[x]	Species[y]	VCG	Race	Origin	Source[z]	Sampling year
NRRL 26771 (0-1090)	FORL	0090 I	-	Canada	e	1975
NRRL 26772 (FORL-C63F)	FORL	0090 I	-	Israel	e	1988
NRRL 26773 (CRNK-11C)	FORL	0090 II	-	Israel	e	1988
NRRL 26774 (FORL-C726)	FORL	0090 II	-	Israel	e	1990
NRRL 26775 (FORL-C696A)	FORL	0090 III	-	Israel	e	1990
NRRL 26776 (FORL-C710B)	FORL	0090 III	-	Israel	e	1990
NRRL 26777 (FORL-1)	FORL	0091 I	-	Israel	e	1986
NRRL 26778 (FORL-C75)	FORL	0091 I	-	Israel	e	1988
NRRL 26779 (FORL -C69E)	FORL	0091 II	-	Israel	e	1988
NRRL 26780 (FORL-FA2)	FORL	0091 II	-	France	e	1985
NRRL 26781 (CRNK-78)	FORL	0092	-	Israel	e	1988
NRRL 26782 (FORL - C201)	FORL	0093	-	Israel	e	1988
NRRL 26783 (FORL-C202)	FORL	0093	-	Israel	e	1988
NRRL 26884 (01152)	FORL	0094 I	-	Belgium	e	1987
HE-0616	FORL	0094 I	-	Florida	a	2006
HE-0631	FORL	0094 I	-	Florida	a	2006
HE-0611	FORL	0094 I	-	Florida	a	2006
NRRL 26786 (FORL-D69)	FORL	0094 II	-	UK	e	1990
NRRL 26787 (FORL-149/74R/89)	FORL	0094 III	-	UK	e	1988
CL-2	FORL	0094 IV	-	Florida	c	1995
MN-0713	FORL	0094I/0098	-	Florida	a	2007
MN-0724	FORL	0094I/0098	-	Florida	a	2007
NRRL 26788 (FORL-C 623)	FORL	0096	-	Israel	e	1990
NRRL 26789 (FORL-C 624A)	FORL	0096	-	Israel	e	1990
CL-0601	FORL	0098	-	Florida	a	2006
CL-06122	FORL	0098	-	Florida	a	2006
MN-0630	FORL	0098	-	Florida	a	2006
CL-0626	FORL	0099	-	Florida	a	2006
CL-06196	FORL	0099	-	Florida	a	2006

Table 2-2. Continued

Isolate[x]	Species[y]	VCG	Race	Origin	Source[z]	Sampling year
CL-06202	FORL	0099	-	Florida	a	2006
CL-06220	FORL	0099	-	Florida	a	2006
93-193	FORL	-	-	Florida	d	1993
JBF6	FOL	0030	2	Florida	b	-
BE1(5397)	FOL	0030	3	Florida	b	-
F189	FOL	0031	2	California	b	-
OSU451	FOL	0031	2	Ohio	b	-
MM59	FOL	0032	2	Arkansas	b	1993
MM61	FOL	0032	2	Arkansas	b	1993
MM62	FOL	0032	2	Arkansas	b	1993
MM64	FOL	0032	2	Arkansas	b	1993
MN-24	FOL	0033	3	Florida	c	1996
DA-1	FOL	0033	3	Florida	c	1996
MN-0619	FOL	0033	3	Florida	a	2006
MN-0805	FOL	0033	3	Florida	a	2008
DF0-23	FOL	0035	2	California	f	1989
NRRL 22903	*F. commune*	-	-	-	e	-
NRRL 28387	*F. commune*	-	-	-	e	-

[x] Previous isolate designation in parentheses (80,105).

[y] FORL = *Fusarium oxysporum* f. sp. *radicis-lycopersici*; FOL = *F. oxysporum* f. sp. *lycopersici*.

[z] a = this study; b = J. C. Correll; c = H. C. Kistler; d= R. J. McGovern; e = K. O'Donnell; f = R. W. Schneider.

Table 2-3. Isolates used for studying the population genetics of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Florida

Isolate	VCG	Tomato cultivar	County
MN-0630	0098	FL 91	Manatee
MN-0631	0094	FL 91	Manatee
MN-0632	0094	FL 91	Manatee
MN-0633	0098	FL 91	Manatee
MN-0634	0094	FL 91	Manatee
MN-0635	0098	FL 91	Manatee
MN-0701	0098	?[x]	Manatee
MN-0702	0094	?	Manatee
MN-0703	0094	?	Manatee
MN-0704	0098	?	Manatee
MN-0705	Nonassigned	?	Manatee
MN-0706	Nonassigned	?	Manatee
MN-0707	0098	?	Manatee
MN-0708	0098	?	Manatee
MN-0709	Nonassigned	?	Manatee
MN-0713	0094/0098	Marriana	Manatee
MN-0714	Nonassigned	Marriana	Manatee
MN-0715	0098	Marriana	Manatee
MN-0716	Nonassigned	?	Manatee
MN-0717	0098	?	Manatee
MN-0718	0098	?	Manatee
MN-0719	0094	?	Manatee
MN-0720	Nonassigned	?	Manatee
MN-0721	0094	?	Manatee
MN-0722	0098	?	Manatee
MN-0723	0098	?	Manatee
MN-0724	0094/0098	?	Manatee
MN-0729	0094	Beauty	Manatee
MN-0730	0094	Beauty	Manatee
MN-0731	Nonassigned	Beauty	Manatee
MN-0732	0094	Tygress	Manatee
MN-0733	0094	Tygress	Manatee
MN-0734	0094	BHN-745	Manatee
MN-0735	Nonassigned	BHN-745	Manatee
MN-0801	Nonassigned	FL47	Manatee
MN-0802	0094	FL47	Manatee
MN-0803	Nonassigned	FL47	Manatee
HE-0601	0094	Marriana	Hendry
HE-0602	0094	Marriana	Hendry
HE-0603	0098	Marriana	Hendry
HE-0604	0094	Marriana	Hendry
HE-0605	0094	Marriana	Hendry

Table 2-3. Continued

Isolate	VCG	Tomato cultivar	Origin
HE-0606	Nonassigned	Marriana	Hendry
HE-0607	Nonassigned	Marriana	Hendry
HE-0609	0094	Marriana	Hendry
HE-0610	0098	Marriana	Hendry
HE-0611	0094	Marriana	Hendry
HE-0612	0094	Marriana	Hendry
HE-0614	0098	Marriana	Hendry
HE-0615	Nonassigned	Marriana	Hendry
HE-0616	0094	Marriana	Hendry
HE-0619	0094	Marriana	Hendry
HE-0621	0094	Marriana	Hendry
HE-0622	Nonassigned	Marriana	Hendry
HE-0623	Nonassigned	Marriana	Hendry
HE-0624	0094	Marriana	Hendry
HE-0625	Nonassigned	Marriana	Hendry
HE-0626	0094	Marriana	Hendry
HE-0627	0094	Marriana	Hendry
HE-0628	0094	Marriana	Hendry
HE-0630	Nonassigned	Marriana	Hendry
HE-0631	0094	FL 47	Hendry
HE-0632	Nonassigned	FL 47	Hendry
HE-0633	0098	FL 47	Hendry
HE-0634	0094	FL 47	Hendry
HE-0635	0094	FL 47	Hendry
HE-0636	Nonassigned	FL 47	Hendry
HE-0637	0098	FL 47	Hendry
HE-0638	Nonassigned	FL 47	Hendry
HE-0639	Nonassigned	FL 47	Hendry
HE-0640	0094	FL 47	Hendry
HE-0641	0094	FL 47	Hendry
HE-0801	Nonassigned	FL 47	Hendry
HE-0802	Nonassigned	FL 47	Hendry
HE-0803	Nonassigned	FL 47	Hendry
HE-0804	Nonassigned	FL 47	Hendry
CL-0601	0098	Grape tomato	Collier
CL-0602	0098	Grape tomato	Collier
CL-0603	0094	Grape tomato	Collier
CL-0612	0098	Grape tomato	Collier
CL-0613	Nonassigned	Grape tomato	Collier
CL-0618	0094	FL 47	Collier
CL-0620	0098	FL 47	Collier
CL-0623	?	FL 47	Collier
CL-0626	0099	FL 47	Collier

Table 2-3. Continued

Isolate	VCG	Tomato cultivar	Origin
CL-0668	0098	Grape tomato	Collier
CL-0672	0098	Grape tomato	Collier
CL-0673	0094	Grape tomato	Collier
CL-0678	Nonassigned	Grape tomato	Collier
CL-0686	Nonassigned	Grape tomato	Collier
CL-0690	0094	Grape tomato	Collier
CL-0692	Nonassigned	Grape tomato	Collier
CL-0696	Nonassigned	Grape tomato	Collier
CL-0697	0099	Grape tomato	Collier
CL-06118	0098	?	Collier
CL-06120	Nonassigned	?	Collier
CL-06122	0098	?	Collier
CL-06124	0094	?	Collier
CL-06128	0098	?	Collier
CL-06135	0094	?	Collier
CL-06136	0094	?	Collier
CL-06137	0094	?	Collier
CL-06139	Nonassigned	?	Collier
CL-06140	0098	?	Collier
CL-06142	Nonassigned	?	Collier
CL-06152	0094	?	Collier
CL-06171	0094	FL 47	Collier
CL-06175	0094	FL 47	Collier
CL-06176	0099	FL 47	Collier
CL-06182	Nonassigned	FL 47	Collier
CL-06186	Nonassigned	?	Collier
CL-06190	Nonassigned	?	Collier
CL-06191	0099	?	Collier
CL-06196	0099	?	Collier
CL-06197	0099	?	Collier
CL-06201	Nonassigned	Grape tomato	Collier
CL-06202	0099	Grape tomato	Collier
CL-06203	0094	Grape tomato	Collier
CL-06210	0099	Grape tomato	Collier
CL-06212	0099	Grape tomato	Collier
CL-06214	0098	Grape tomato	Collier
CL-06220	0099	FL 47	Collier
CL-06222	0098	FL 47	Collier
CL-06224	0094	FL 47	Collier
CL-06230	Nonassigned	FL 47	Collier

[x] ? = unknown cultivar due to confidentiality of the tomato growers.

Table 2-4. Frequency of vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* sampled from 2006 to 2008 in Florida

		VCG (%)			
Population	Number of isolates	0094	0098	0099	Nonassigned
Manatee	37[z]	38.5	35.9	0	25.6
Hendry	39	48.7	12.8	0	38.5
Collier	49	28.6	24.5	20.4	26.5
Overall	125	38.6	24.4	6.8	30.2

[z] Isolates MN-0713 and MN-0724 were assigned to 0094 and 0098 after repeating two single-spored isolations and confirming vegetative compatibility with testers of 0094I (01150-6 and 01152-31) and 0098 (CL-7/6 and CL-75/4).

Table 2-5. Characteristics of 27 microsatellite loci derived from a bioinformatics approach and a microsatellite enrichment procedure for 13 isolates of *Fusarium oxysporum* f. sp. *lycopersici* and 32 isolates of *F. oxysporum* f. sp. *radicis-lycopersici*

					Number of alleles		Gene diversity[y]	
Locus[v]	Primer sequence (5′-3′)[w]	Repeat motif	GenBank accession no.	Size (bp)[x]	FOL	FORL	FOL	FORL
CH2-9	F: GGGCTTCAAGGTGCTGAGTA R: TAAACGAAGCTGGGAATGGA	$(GTGA)_7$	FJ882019	216	2	6	0.36	0.73
CH2-15	F: ATCTTCCTCACGGTTTTGGA R: TGTAGCGTAGCACAACAGTGG	$(CT)_8$	FJ882020	203	8	7	0.92	0.82
CH2-16	F: GGGCTTCAAGGTGCTGAGTA R: AGCTGGGAATGGAAATTTGA	$(TGAG)_8$	FJ882021	209	1	2	0	0.51
CH2-31	F: CGACAGGAGGCTGAGGAGTA R: CGTCAATTGAGAACCATCCA	$(CT)_5CG(CT)_5$	FJ882022	191	4	4	0.74	0.77
CH2-51	F: ATACGAGCACAAGGGACGAG R: CATCCATTCCGTCTCCATTT	$(AG)_8$	FJ882023	243	5	7	0.76	0.77
CH2-66	F: GAAGCGCTTACAGTGCCAAT R: CCCTTGACTCTCCACGAAAC	$(AG)_{14}$	FJ882024	236	4	10	0.76	0.88
CH2-71	F: TGTAGCGTAGCACAACAGTGG R: ATCTTCCTCACGGTTTTGGA	$(AG)_8G(GA)_3$	FJ882025	203	4	2	0.74	0.52
FOL15	F: TATGGACGGATCAGGAAAGG R: TCAACAACGCACTGAAGACC	$(AAG)_{17}$	-[z]	236	6	9	0.83	0.85
FOL20	F: CATTGAGGAAGAGCGGAAAG R: CACATTTGGCACAGCAATCT	$(AGCAC)_{19}$	-	269	9	9	0.88	0.79
FOL35	F: GTCGTTTTCAAGGACGCACT R: GGTGGCAGTTTCCTCCTTTT	$(GAA)_{19}$	-	269	7	14	0.88	0.91
FOL89	F: ATATCCCACCCTCCTTGCAT R: TGCTCAGTCTCGTCACAACC	$(TGTAT)_9$	-	263	4	5	0.78	0.71
FOL99	F: AGTTGAGGTTGTCGCTGGTT R: CTATTCCTCCCGCTGCAC	$(CTC)_{11}CCC(CTC)_3$	-	257	6	4	0.88	0.60
FOL104	F: GGAACCCGAAACCACCTTAT R: ATTGGCACTTGCTTTGCTTT	$(GTGCCT)_8$	-	242	2	4	0.53	0.68

Table 2-5. Continued

Locus[v]	Primer sequence (5′-3′)[w]	Repeat motif	GenBank accession no.	Size (bp)[x]	Number of alleles		Gene diversity[y]	
					FOL	FORL	FOL	FORL
FOL118	F: GAGGCCACAGAAATGAGAGC R: GGTTGGTTGGAAGGATTCAG	$(CAGGA)_9$	-[z]	196	3	3	0.67	0.17
FOL175	F: ACCAGCAAGCAGCTTCATTT R: ACGAGTCGCAGGGTATCAAC	$(GAAT)_{13}$	-	255	6	6	0.87	0.79
FOL185	F: TCCTTGTAGCGCCTTGCTAT R: AAATGCAACACCGCACTGTA	$(AGGT)_{15}$	-	267	4	6	0.74	0.81
FOL245	F: TGAAAGGCGCGCTATTTAGT R: GAGAGGCGGAGGAAGAAGA	$(CTT)_{14}$	-	258	5	10	0.80	0.87
FOL293	F: AAGACTCGGGCAAGTCAGAA R: ATTTTCGTAAACCCCATCCA	$(TTTCT)_{29}$	-	379	7	3	0.88	0.57
FOL296	F: CACTGAAGGAAATGCAGCAG R: TAGGCTCTGGAGATGCTTGG	$A_{23}(AAG)_{22}$	-	234	6	10	0.86	0.72
FOL338	F: GAACCCTTTCCCACGAGAC R: AACTCGCTGTTGGTGATGTG	$(TGATT)_{10}$	-	216	3	6	0.67	0.81
FOL356	F: CCTCCTGCTCTTCCTCATCTT R: CGGTATTGTTGGGGGTTTAG	$(CAA)_{14}$	-	261	6	9	0.87	0.87
FOL602	F: CTCGTCACTGCTGGAATCAA R:TGTCAAAGAATGGCCCATATTA	$(AGT)_{15}$	-	248	6	6	0.81	0.69
FOL624	F: CAAGAGGCCAGCGATAGTGT R: AGCTTTTGATACCCCATTCG	$(GTA)_{31}$	-	240	4	12	0.70	0.73
FOL638	F: GAAGCACTCGCTACGTGTCA R: CGGTTGTGCAGCTCAAATAA	$(TGAGA)_{11}$	-	244	4	5	0.78	0.73
FOL665	F: ACCCTGGGTACTCCGGTTAT R: GGCGCAGCTTCAAGACTAAT	$(GT)_{25}$	-	213	2	8	0.54	0.81

Table 2-5. Continued

Locus[v]	Primer sequence (5′-3′)[w]	Repeat motif	GenBank accession no.	Size (bp)[x]	Number of alleles		Gene diversity[y]	
					FOL	FORL	FOL	FORL
FOL680	F: CGCAGAATGGCTCTTCAAAT R: TGCAACATCATCGACCACTT	$(TTTA)_{11}$	-[z]	254	10	12	0.95	0.84
FOL803	F: GTGGTAGCGTGGAGTGGATT R: GTTCGACATTCGCTCGAGTT	$(AGACA)_{11}$	-	240	7	4	0.87	0.64

[v] Locus indicated by a CH2-number was isolated directly from isolate HE-0631 (VCG 0094) of *F. oxysporum* f. sp. *radicis-lycopersici* (FORL). Loci indicated by an FOL-contig number were identified by a search of the genome sequence of *F. oxysporum* f. sp. *lycopersici* (FOL) at http:// www.broad.mit.edu.

[w] M13 tag (CACGACGTTGTAAAACGAC) added to 5′ end of forward primers for amplification with fluorescently labeled M13.

[x] Based on the genome sequence of isolate HE-0631 and *F. oxysporum* f. sp. *lycopersici.*

[y] Based on Nei (115).

[z] - = microsatellite loci derived from searching the genome sequence of *F. oxysporum* f. sp. *lycopersici*.

Table 2-6. Pairwise Fst values (above diagonal) and their *P* values (below diagonal) between populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* VCG 0094 from three counties in Florida

	Manatee	Hendry	Collier
Manatee		-0.03	0.09
Hendry	0.55		0.06
Collier	0.12	0.11	

Table 2-7. Pairwise Fst values (above diagonal) and their *P* values (below diagonal) between populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* VCG 0098 from three counties in Florida

	Manatee	Hendry	Collier
Manatee		-0.10	0.18
Hendry	0.52		0.05
Collier	0.15	0.30	

Table 2-8. Mean number of pairwise difference and average nucleotide diversity of microsatellite haplotypes for isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from three counties in Florida

VCG[w]	Locality	Sample size	Polymorphic loci[x]	Mean number of pairwise differences and SD[y]	Nucleotide diversity and SD[z]
	Manatee	15	1	1.00 (0.79)	0.10 (0.10)
0094	Hendry	19	2	1.27 (0.92)	0.13 (0.09)
	Collier	14	4	2.20 (1.45)	0.22 (0.11)
	Total Florida clone-corrected	10	4	1.75 (1.11)	0.18 (0.10)
	Manatee	14	1	1.00 (0.89)	0.10 (0.10)
0098	Hendry	5	1	1.00 (1.00)	0.10 (0.10)
	Collier	12	2	1.33 (1.09)	0.13 (0.09)
	Total Florida clone-corrected	6	2	1.47 (1.03)	0.15 (0.10)
0099	Collier	10	3	2.00 (1.30)	0.20 (0.15)
	Total Florida clone-corrected	5	3	2.20 (1.45)	0.22 (0.12)
	Manatee	10	10	6.21 (3.30)	0.62 (0.03)
Nonassigned	Hendry	15	10	6.48 (3.30)	0.65 (0.06)
	Collier	13	5	3.00 (1.71)	0.30 (0.11)
	Manatee	37	10	2.12 (1.21)	0.21 (0.05)
Overall	Hendry	39	10	3.44 (1.80)	0.34 (0.06)
	Collier	49	6	1.99 (1.14)	0.19 (0.08)
Overall clone-corrected	Manatee	25	10	2.90 (1.58)	0.29 (0.06)
	Hendry	26	10	3.99 (2.06)	0.39 (0.07)
	Collier	34	6	2.45 (1.36)	0.24 (0.10.)

[w] Vegetative compatibility group

[x] Among a total of ten microsatellite loci.

[y] Mean number of pairwise differences and standard deviation (SD) (in parentheses) based on Tajima (165).

[z] Average nucleotide diversity and SD (in parentheses) according to Nei (115) and Tajima (163).

Table 2-9. Hierarchical distribution of gene diversity among populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida

Source of variance[y]	df	Sum of squares	Variance components	Percentage of variation	Fixation index	*P*[z]
Among populations	2	18378	276	16	Φ_{st}=0.159	<0.0001
Within populations	82	118887	1450	84		
Total	84	137266	1726			

[y] Molecular analysis of variance (AMOVA) was performed using ARLEQUIN version 3.1 (45). Distance method was according to the sum of squared size differences (*R*st) between two haplotypes for microsatellite data (156).

[z] Based on 1,023 permutations.

Table 2-10. Tests for random association of alleles within each locus and between pairs of loci in the population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from three counties in Florida

Population	I_A[x]	*P*	$\bar{r}_d$[xy]	*P*	GD[z]
Manatee	3.19	<0.001	0.36	<0.001	42/45
Hendry	3.56	<0.001	0.40	<0.001	45/45
Collier	1.26	<0.001	0.15	<0.001	17/45
Overall	2.40	<0.001	0.27	<0.001	45/45

[x] I_A and $\bar{r}_d$ are indices of multilous linkage disequilibrium (157).

[y] $\bar{r}_d$ is a modification of I_A and independent of sample numbers of loci (1).

[z] Pairs of loci at significant linkage disequilibrium according to Fisher's exact test implemented in GENEPOP version 3.4 (131).

Table 2-11. Pairwise numbers of migrants per generation inferred from Bayesian analyses implemented in MIGRATE-N

	Population receiving migrants[z]		
Population donating migrants	Manatee	Hendry	Collier
Manatee		2.75 (0-5.50)	1.33 (0-3.50)
Hendry	2.18 (0-4.50)		1.65 (0-3.50)
Collier	1.94 (0-4.50)	2.15 (0-4.00)	

[z] Values in parentheses indicate 95% confidence intervals.

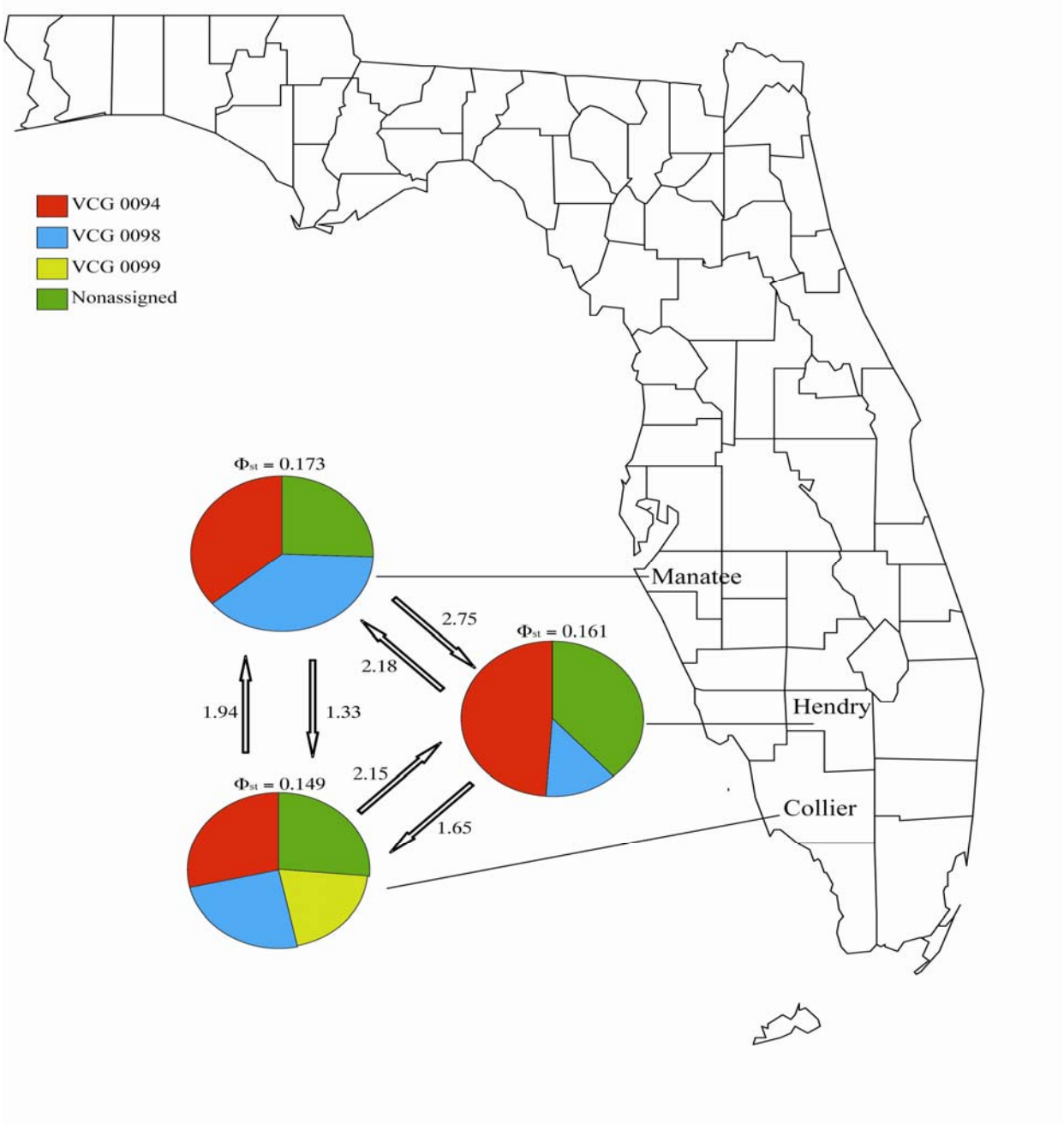

Figure 2-1. Map of three Florida populations of *Fusarium oxysporum* f. sp. *radicis-lycopersici* analyzed in this study and distribution of vegetative compatibility groups (VCGs). Width of section is proportional to the percentage of isolates assigned in each VCG. Population differentiation (Φst) is labeled on each population. Migrants per generation between populations are labeled next to arrows. Directions of arrows indicate migration between sink and source populations.

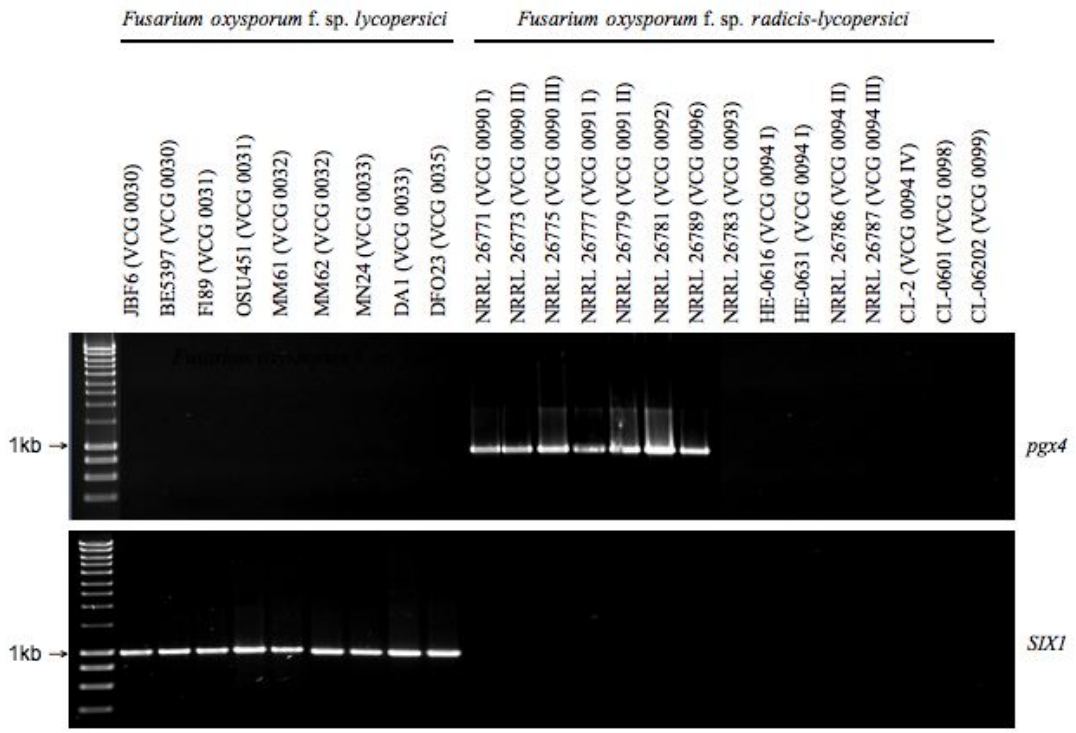

Figure 2-2. Identification of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) using FORL-specific primers sprlf and sprlr (63) (top panel) and FOL-specific primers P12-F2B and P12-R1(172) (bottom panel), repectively. Vegetative compatibility groups (VCGs) are shown in parentheses of isolates. Sprlf and sprlr amplify a fragment of FORL exo polygalacturonase gene (*pgx4*), and P12-F2B and P12-R1 target a virulence gene of FOL, *SIX1* (secreted in xylem 1).

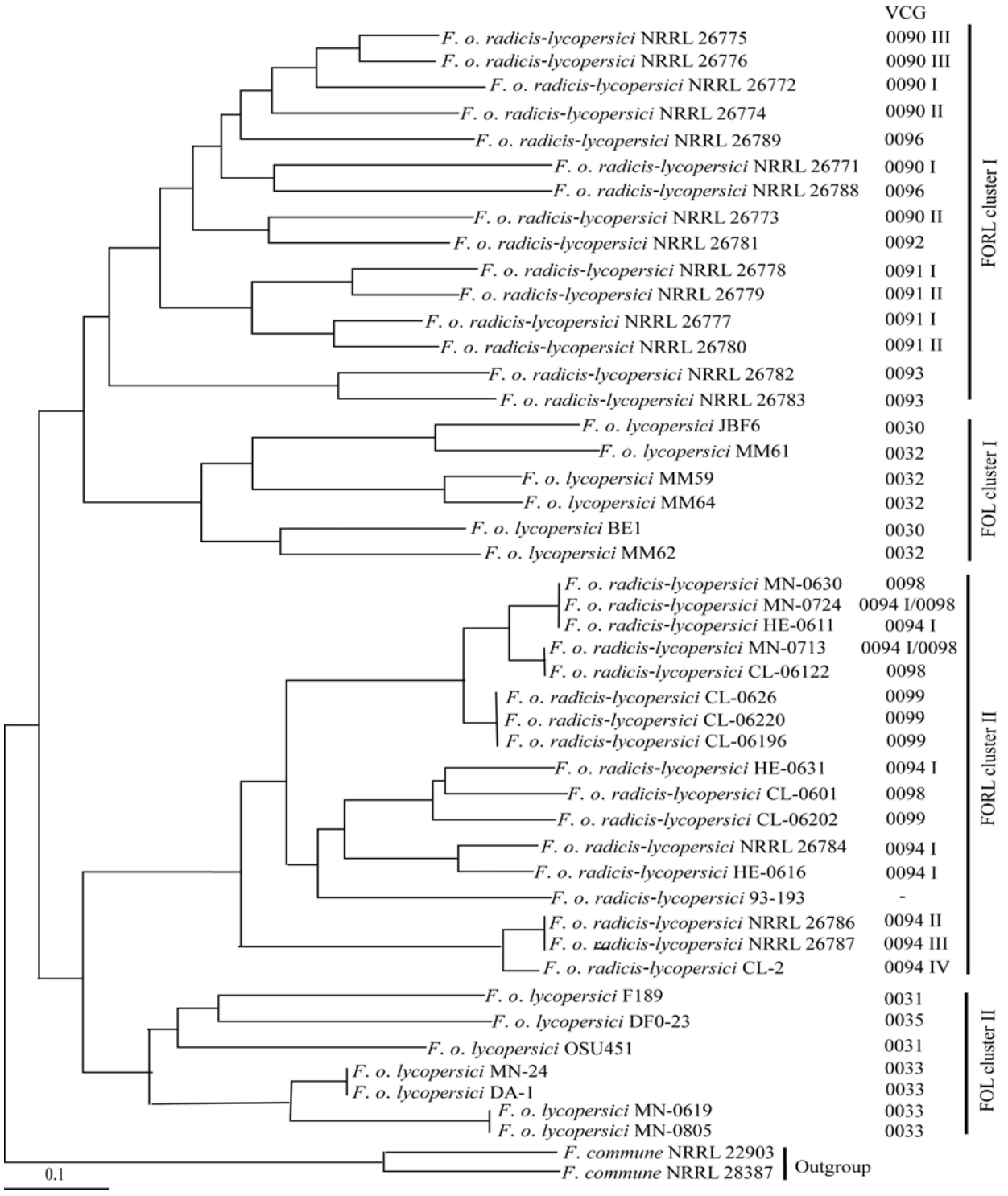

Figure 2-3. Neighbor-joining tree based on Nei's minimum genetic distance (115) between individuals. Four clusters are indicated including *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) clusters I and II, and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) clusters I and II.

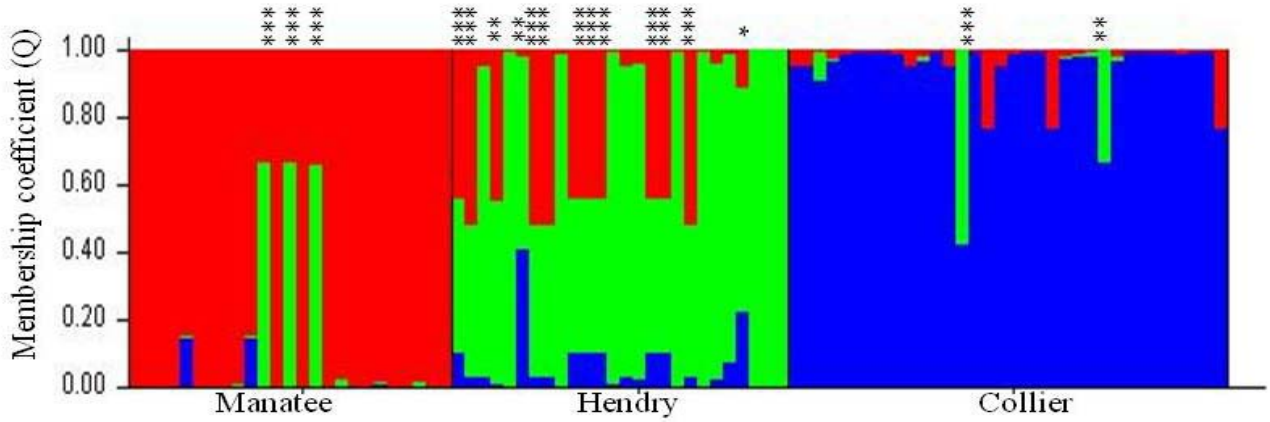

Figure 2-4. Estimated population structure inferred from multilocus microsatellite data of *Fusarium oxysporum* f. sp. *radicis-lycopersici* using STRUCTURE according to membership coefficient. Each individual is represented by a thin vertical line, which is partitioned into three colored segments from which alleles are most likely derived. Black lines separate individuals of different populations that are labeled below the figure. The bar length suggests its membership coefficient (Q) to variously colored populations. Individuals marked with an asterisk along the top are of statistically significant admixture.

CHAPTER 3
PHYLOGENETIC AND MATING-TYPE ANALYSES OF *Fusarium oxysporum* f. sp. *radicis-lycopersici* AND THEIR ASSOCIATION WITH VIRULENCE

Introduction

Fusarium oxysporum Schlechtend.: Fr. is a cosmopolitan soilborne plant pathogen infecting a wide range of economically important plant hosts (8,31,97). More than 100 host-specific *formae speciales* have been described in the *F. oxysporum* species complex (97). *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* are two important fungal plant pathogens of tomato and cause Fusarium crown and root rot and Fusarium wilt, respectively (72,74,174). Three races have been reported for *F. oxysporum* f. sp. *lycopersici*, whereas no physiological races are currently known for *F. oxysporum* f. sp. *radicis-lycopersici* (75,125).

Vegetative compatibility groups (VCGs) have been useful in characterizing the population structure of these two *formae speciales* (19,139). Compared to *F. oxysporum* f. sp. *lycopersici*, a number of VCGs have been described in *F. oxysporum* f. sp. *radicis-lycopersici* (36,80). It has been suggested that isolates within the same VCG belong to the same clonal lineage (55). A *forma specialis* is often assumed to be monophyletic in which all VCGs and races have been derived from the same common ancestor. However, multiple VCGs and races within a given *forma specialis* could evolve from multiple independent origins, which shows para- or poly-phyly (8,119). In other words, isolates of a *forma specialis* could be more closely related to members of other *formae speciales* of *F. oxysporum* than those of the same *forma specialis*. Previous studies showed that *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* are paraphyletic, suggesting that VCGs of these two *formae speciales* within the same phylogenetic clade (lineage) may have been derived from a common ancestor. These studies inferred the phylogeny of these two *formae speciales* according to partial regions of the gene encoding translation elongation factor 1α (EF-1α), mitochondrial small subunit (mtSSU) rDNA, and

itergenic spacer region (IGS) (19,119). It may be of interest to further investigate whether adding more characters, such as the entire IGS region and microsatellite loci, increases the phylogenetic resolution for these two *formae speciales*. Moreover, the inclusion of too few VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* in previous phylogenetic studies might have resulted in an incomplete inference in their phylogeny.

Phylogenetic analyses has revealed that the *F. oxysporum* species complex is closely related to the *Gibberella* clade, even though the teleomorph of the *F. oxysporum* species complex is unknown (117,118). Results of RT-PCR analysis revealed that mating type (MAT) genes of *F. oxysporum* are still expressed and processed correctly (181). Two isolates must be in the same biological species and carry distinct mating-type alleles, *MAT-1* or *MAT-2*, for a sexual cross to happen, whereas vegetative incompatibility does not necessarily prevent the sexual cycle (95,97). Mating types of various VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* have not been well investigated. Moreover, crossing *F. oxysporum* f. sp. *radicis-lycopersici* isolates with opposite mating types has not been done.

Pathogenicity is the qualitative ability of a parasite to infect and cause disease on a host. In contrast, virulence is defined as the degree of damage caused by a parasite to a host and it is suggested to be negatively correlated with host fitness (143). Some commercial tomato cultivars carry the *Frl* resistance gene against Fusarium crown and root rot, but physiological races have not been found for *F. oxysporum* f. sp. *radicis-lycopersici* (151). It is not clear how the pathogen adapts to different resistant tomato cultivars by means of adjusting its virulence. There is no universal relationship between parasite reproductive capacity and virulence (143), but a positive relationship has been observed in some pathosystems (65,114). Therefore, pathogens may increase virulence for accelerating their spread (65). Virulence tests and phylogenetic analyses

for reported VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* would shed insight into how virulence evolves among different phylogenetic lineages and within a phylogenetic lineage while under the selection pressure from the host. This study tested the hypothesis that virulence was significantly different among VCGs within the same phylogenetic lineage despite already being pathogenic on tomato.

Fusarium crown and root rot was first found in greenhouses of tomatoes in Japan in 1969. The causal agent was identified as a new race (J3) of *F. oxysporum* f. sp. *lycopersici* (146). However, according to symptomology, etiology and pathogenesis, it was renamed as a new *forma specialis* of *F. oxysporum* and designated as *F. oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis & Shoemarker (72). Although *F. oxysporum* f. sp. *radicis-lycopersici* is known as a relatively new pathogen, its divergence time has not been estimated based on sequence data. Moreover, its demographic history has not been well known since it was identified in 1969. Recently, a new approach to address these evolutionary questions has been proposed using Bayesian inference for microbial pathogens sampled at different dates (153). The approach might provide insights into the evolutionary history of *F. oxysporum* f. sp. *radicis-lycopersici*.

The purpose of this study was four-fold (i) to investigate classification of VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* according to multilocus DNA sequence data; (ii) to evaluate the occurrence of opposite mating types within VCGs and the possibility of sexual recombination based on crossing isolates carrying distinct mating-type idiomorphs; (iii) to compare association of virulence within VCGs with phylogenetic analyses; (iv) to determine the nucleotide substitution rate and population dynamics of *F. oxysporum* f. sp. *radicis-lycopersici*. A preliminary report of this work has been published (68).

Materials and Methods

Collection of Isolates

Thirty one isolates of *F. oxysporum* f. sp. *radicis-lycopersici* and 13 isolates of *F. oxysporum* f. sp. *lycopersici* that comprised most of the known VCGs of these two *formae speciales* (Table 2-2) were included in the molecular studies. In addition, three isolates of VCGs 0098, CL-0620, HE-0610, and HE-0620 were also included as ingroups. Six outgroups including *F. commune* NRRLs 22903 and 28387, *F. redolens* NRRL 31075, *F. hostae* NRRL 29889, *F. subglutinans* NRRL 22016, and *F. foetens* NRRL 31852 were used for phylogenetic analyses. Each isolate was single-spored and stored on sterile paper and/or at -80°C in 15% glycerol.

DNA Extraction and Polymerase Chain Reaction

Monosporic isolates from the culture collection were grown on carnation leaf agar (CLA) before transferring mycelia plugs to potato dextrose agar (PDA) for DNA extraction. About 200 mg mycelia were ground in liquid nitrogen before using DNeasy Plant Minikits (Qiagen, Inc., Valencia, CA) to extract DNA. Amplification of an approximate 690-bp fragment of the EF-1α gene was performed using primers ef1 and ef2, and polymerase chain reaction (PCR) as previously described (119). The nuclear ribosomal IGS region (≈3 kb) was amplified using primers NL11 and CNS1 (3). The sequence of a noncoding microsatellite locus FOL185 (Table 2-5) was used to redesign primers AGGTf (CCATCTTTCCGTCTCCACAT) and AGGTr (TTGCGCAAAGTTTGAATGAG). A fragment of 786 bp was amplified using the following PCR conditions: 94°C for 3 min, 34 cycles at 94°C for 30 s, 56.5°C for 30 s, and 72°C for 45 s and a final elongation step at 72°C for 10 min.

Direct Sequencing

Primers ef1 and ef2 were used to sequence the amplification products of the EF-1α gene. Four internal sequencing primers were used to sequence the IGS rDNA region: iNL11 (5'-AGGCTTCGGCTTAGCGTCTTAG-3'), NLa (5'-TCTAGGGTAGGCKRGTTTGTC-3'), iCNS1 (5'-TTTCGCAGTGAGGTCGGCAG-3'), and CNSa (5'-TCTCATRTACCCTCCGAGACC-3') (K. O'Donnell, *unpublished data*). Primers AGGTf and AGGTr were used for sequencing the microsatellite locus. Sequences were obtained using BigDye Terminator Cycle Sequencing Chemistry and ABI 3730 XL DNA Sequencer (Applied Biosystems, Foster City, CA) at the Interdisciplinary Center for Biotechnology Research (ICBR) facility, University of Florida, Gainesville.

Phylogenetic Analyses

Sequences were edited using Sequencher™ version 4.6 (Gene Codes Corporation, Ann Arbor, MI) and aligned using Clustal X version 2.0.6 (93). The alignment was adjusted by eye using Se-Al version 2.0a11 (University of Oxford, Oxford, UK). Gaps were considered missing data. Prior to phylogenetic analyses, sequence divergence was determined among isolates of *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *lycopersici*, and both *formae speciales* according to p-distance implemented in MEGA version 4 (166).

Phylogenetic analyses were first performed on DNA sequences of EF-1α, FOL185, and IGS as individuals. To test whether the three data sets could be combined, incongruence length difference (ILD) was assessed (47). ILD implemented as the partition homogeneity test in PAUP* 4.0b10 (162) was performed with 1000 data partitions using heuristic search with 1000 replications of random stepwise addition and branch swapping algorithm using tree bisection-

reconnection (TBR). If the null hypothesis of homogeneity is rejected, the data sets should not be combined without further justification (18).

Parsimony analyses were conducted using PAUP*. Heuristic searches for most parsimonious trees were performed with random stepwise addition (1000 replications) and TBR. Clade stability was evaluated using 1000 bootstrap replicates with arrangements limited to 1,000,000 per replicate.

For maximum likelihood (ML) and Bayesian analysis, MODELTEST version 3.7 (124) was used to determine appropriate models of nucleotide substitution. The best-fit model of sequence evolution was chosen based on the Akaike Information Criterion (70). ML was analyzed using GARLI version 0.96 (184) in which a stochastic genetic algorithm-like approach was used to simultaneously estimate the topology, branch lengths, and substitution model parameters that maximize the log-likelihood. A bootstrap analysis was performed using 1000 replicates.

Bayesian analysis was performed using MRBAYES version 3.1.2 (136). The Markov Chain Monte Carlo (MCMC) was run with four chains for 10,000,000 generations, sampling every 100 generations and starting with a random tree. Stationarity was reached at approximately generation 30,000; thus the first 300 trees were the "burn-in" of the chain, and phylogenetic inferences were based on these trees sampled after generation 30,000. The remaining 99,700 trees were imported to PAUP* 4.0b10 to generate a 90% majority-rule consensus tree rooted with outgroups.

Mating Type Determination

The mating type (MAT) of each isolate was determined as previously described (84). Isolates from which a fragment size of 200 bp was amplified with fusALPHAfor and

fusALPHArev were typed as *MAT-1*. Isolates were determined as *MAT-2* if a fragment of 260 bp was amplified with fusHMGfor and fusHMGrev.

Crossing

Sexual crosses were carried out as previously described (87,88). For all matings, each isolate was started from a single conidium. Two isolates, CL-06191 (VCG 0099) and CL-06196 (VCG 0099), carrying *MAT-2* acted as females and were cultured on carrot agar. Five isolates, CL- 0620 (VCG 0098), CL- 06122 (VCG 0098), CL-06124 (VCG 0094), CL-06125 (VCG 0094), and CL-06128 (VCG 0098), with *MAT-1* acting as males (the spermatizing agent) were cultured on CLA. Matings were conducted 10 days after the cultures were initiated. A conidial suspension was made from the male parent using sterile water and spread evenly over the surface of the female culture. After mating, these plates were incubated at 25°C with a 12-h-photoperiod (photonflux of 40.8 μmol/m^2s). Observation for perithecia by microsacope was carried out every week until three months after mating. All male parents were crossed with the female isolates at least two times.

Virulence Test

Seedlings of the cultivar 'Bonny Best' (susceptible to Fusarium crown and root rot) and Fla. 7781 (resistant to Fusarium crown and root rot) (149) were grown in 17 × 25 × 6 cm trays with a commercial potting mix (Metro Mix 300, $pH_{1:2} = 5.27 \pm 0.16$). Seedlings at the cotyledon stage were gently uprooted, washed, and then replanted into 15-cm-diameter pots with 1.5 kg sand (University of Florida Turfgrass Research Envirotron) for approximately 2 weeks or until the first two true leaves had emerged. Each pot contained two tomato plants. Since soil pH affects the development of Fusarium crown and root rot (76), a total of 600 ml Hoagland's solution (64) adjusted to pH 5 was applied to each pot before inoculation to favor infection.

Eleven isolates of *F. oxysporum* f. sp. *radicis-lycopersici* that represented VCGs 0090, 0094 and 0098, 0099 and nonassigned VCGs including a control of plants inoculated with sterile water were used for the virulence test (Fig. 3-4). Ten ml of a conidial and mycelial fragment suspension with 10^6 conidia mL^{-1}, recovered from 14-day-old cultures grown on CLA, was placed on crowns using a pipette with sterile tips. Ten seedlings were inoculated with each isolate. The inoculated seedlings were then placed in an incubator at 20°C with a 12 h photoperiod (photonflux of 70.7 μmol/m^2s).

Disease severity was assessed 35 days after inoculation. The inoculated plants were gently uprooted and washed with tap water. The length of discolored area in stem was measured, and it was divided by the total stem length to represent the percentage of disease severity. Disease severity in crowns was determined according to a 0-4 scale, where 0 = no symptom; 1 = 1-25 % discoloration; 2 = 26-50 %; 3 = 51-75%; and 4 = 76-100%. The percentage of infected root was also recorded.

The inoculation experiment, repeated twice, was arranged in a completely randomized design with five replicates (pots) per isolate per cultivar and two plants per pot. Statistical analyses were performed with PROC GLM implemented in SAS version 9.2 (SAS Institute, Cary, NC). The difference in virulence between isolates was compared using the least significant difference test (LSD) at p=0.05. The three disease severity ratings were used for cluster analysis according to Ward's minimum variance method (175) implemented in JMP version 7 (SAS Institute, Cary, NC).

Nucleotide Substitution Rates and Population Dynamics

To estimate rates of nucleotide substitution and population dynamics in *F. oxysporum* f. sp. *radicis-lycopersici*, a Bayesian MCMC approach was performed using BEAST version 1.4.8

(34). The method relied on the sampling date for each isolate of *F. oxysporum* f. sp. *radicis-lycopersici* and explored evolutionary models with tree topology, substitution rate, and population size changes. Sequence data of *F. oxysporum* f. sp. *radicis-lycopersici* were realigned, and nucleotide substitution models for IGS, EF-1α, and FOL185 were determined using MODELTEST. The uncorrelated relaxed lognormal clock implemented in BEAST was used since variation in the nucleotide substitution rate among branches was detected by BEAST in these three data sets as a result of the standard deviation in branch rates greater than the mean rate. The result suggested that these three data sets exhibit very substantial rate heterogeneity among lineages, rejecting the strict molecular clock (33). Bayesian MCMC was run for 1,000,000,000 generations with sampling every 100,000 generations. The first 1000 samples were discarded as burn-in. Default priors were used to analyze nucleotide substitution rates (μ) and most recent common ancestor (MRCA). At least three independent runs were analyzed to corroborate these results. TRACER version 1.4.1 (University of Oxford, Oxford, UK) was used to visually examine posterior probabilities for Markov chain stationarity and to summarize population parameters.

Bayesian skyline plots with ten population groups of unique sizes were used to investigate historical changes in effective population size (N_e) of *F. oxysporum* f. sp. *radicis-lycopersici*. Priors for substitution rates with the 95% high-probability density (HPD) were derived from above analyses. All other parameters of these BEAST runs were the same as above. TRACER was used to generate Bayesian skyline plots.

Results

Phylogenetic Analysis

Mean pairwise sequence divergences (uncorrected p-distances) were lower than 1% (ranging from 0.32-0.97%) among IGS, EF-1α, and FOL185 for *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici*. IGS was the fastest evolving marker and FOL185 was the slowest (Table 3-1).

Since the ILD test rejected the null hypothesis of homogeneity, the three data sets were not combined but analyzed separately (Table 3-2) (18). The three loci provided different levels of phylogenetic resolution among isolates used. However, *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* formed a strongly supported clade (96-100% bootstrap and 100% Bayesian posterior probability) in these three phylogenies (Figs. 3-1, 3-2, and 3-3). The IGS data set provided more resolution than either the EF-1α or FOL185 data set due to considerably more informative characters (Table 3-3).The IGS data set consisted of 2419 nucleotide characters, 314 of which were cladistically informative. In contrast, the EF-1α data set included 86 informative sites among 721 characters, and the FOL185 data set contained 86 informative sites among 798 characters. Maximum parsimonious trees derived from these three data sets showed very low homoplasy as a result of high consistency and retention indices (CI and RI).

The IGS phylogeny revealed four clades (Fig. 3-1). Clade 1 consisted of three predominant VCGs, 0094, 0098, and 0099, of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida, whereas in the EF-1α (Fig. 3-2) and FOL185 (Fig. 3-3) phylogenies these three VCGs were placed in a clade with VCGs 0031, 0033, and 0035 of *F. oxysporum* f. sp. *lycopersici*. Clade 2 contained VCGs 0030 and 0032 of *F. oxysporum* f. sp. *lycopersici* and VCGs 0090, 0091, 0092, 0093, and

0096 of *F. oxysporum* f. sp. *radicis-lycopersici*, which was mostly congruent with the EF-1α and FOL185 phylogenies except that VCG 0091 in the EF-1α phylogeny fell into an individual clade. A methodological incongruence was found in clade 2 of the IGS phylogeny as maximum likelihood analysis showed VCG 0093 as a distinct clade, contradicting with maximum parsimonious and Bayesian analyses. The IGS phylogeny showed that VCGs 0031 and 0035 were grouped in clade 3, and VCG 0033 was placed in clade 4. The stability of these two clades was strongly supported (bootstrap = 100%; Bayesian posterior probability = 100%), but these three VCGs were not well resolved in the EF-1α and the FOL185 phylogeny.

The evolution of races in *F. oxysporum* f. sp. *lycopersici* was not completely related to phylogenetic lineage. Clade 2 of the IGS phylogeny consisted of isolates of race 2 and BE1 which belongs to race 3 (105), whereas clades 3 and 4 seemed restricted to race 2 and 3, respectively (Table 2-2, Fig. 3-1).

Mating-Type Analyses and Mating

The mating-type analysis showed that each VCG carried a single unique *MAT* idiomorph (Fig. 3-1). Within the same clade of the IGS phylogeny, *MAT-1* and *MAT-2* were not found in *F. oxysporum* f. sp. *lycopersici* but in *F. oxysporum* f. sp. *radicis-lycopersici* clade 1 and 2 both mating types were found. However, no perithecia were observed after crossing isolates carry distinct mating-type idiomorphs in clade 1of the IGS phylogeny.

Virulence

No isolate of *F. oxysporum* f. sp. *radicis-lycopersici* was observed to infect the resistant cultivar, Fla. 7781. However, cluster analysis of virulence on the susceptible cultivar, Bonny Best, for VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* showed that VCG 0098 had a higher

virulence than the other two VCGs in Clade 1, and VCG 0090 in Clade 2 of the IGS phylogeny (Figs. 3-1 and 3-4).

Nucleotide Substitution Rates and Population Dynamics

Mean nucleotide substitution rate varied among IGS, EF-1α, and FOL185 data sets and ranged from 1.27×10^{-4} to 4.92×10^{-4}, with a 95% credible interval of 2.74×10^{-5} - 9.80×10^{-4}. The mean estimate of a most recent common ancestor (MRCA) of *F. oxysporum* f. sp. *radicis-lycopersici* ranged from 38 to 129 years ago (Table 3-4). However, the MRCA estimated from the IGS data set was older than from the other two data sets.

These rate estimates with 95% credible intervals from each data set were used as priors of the relaxed molecular clock rate to generate Bayesian skyline plots. The increase in the scaled population size (equivalent to the effective population size (N_e) multiplied by generation length (g)) were revealed by IGS, EF-1α, and FOL185 data sets, suggesting a population expansion of *F. oxysporum* f. sp. *radicis-lycopersici* in the last 15 years (1992-2007) (Fig. 3-5).

Discussion

Although the phylogeny of *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* have been previously studied based on sequence data, phylogenetic resolution for these two *formae speciales* was not well resolved and the evolution of VCGs is not well known. This study used more characters than previous studies for phylogenetic analyses (19,171). One of the goals of this study was to evaluate polymorphisms of IGS, EF-1α, and a noncoding microsatellite locus, FOL185 among VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* for developing diagnostic markers.

Of the three loci examined, EF-1α has been used most commonly for distinguishing *Fusarium* species (52). However, it did not provide good resolution for the two closely related

formae speciales in this study. In contrast, the complete region of IGS was phylogenetically informative due to its fast-evolving nature (Tables 3-1 and 3-3). Moreover, the phylogenetic resolution derived from the complete region of IGS for these two *formae speciales* is better than previous studies using the partial region (19,83,171), indicating that sequencing the complete IGS region may be useful for studying phylogenies of *formae speciales* of *F. oxysporum*. According to this study, IGS may be used to design primers for rapid diagnosis of predominant VCGs of *F. oxysporum* f. sp. *radicis-lycopersici*.

FOL185 was evaluated for constructing the phylogeny of *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* as it revealed high gene diversity for these two *formae speciales* (Table 2-5). However, FOL185 showed the lowest average pairwise sequence divergence (Table 3-1), suggesting a phylogenetically uninformative marker. Based on sequence alignment, insertion and deletion were frequent in the microsatellite motif, but transition and transversion rarely occurred in the flanking region of the microsatellite motif. This mutation type likely makes it more appropriate for studies of population genetics (39).

The ILD test implemented in PAUP* suggested that the evolutionary rate was not homogeneous among IGS, EF-1α, and FOL185 data sets. Therefore, combining data sets did not correctly improve the phylogenetic estimation. Instead, these three data sets were subjected to separate phylogenetic analyses (18). Phylogenetic congruence can be defined as two identical trees obtained from different data sets or different methodologies (57). Instead, phylogenetic incongruence was shown in this study as topologies derived from IGS, EF-1α, and FOL185 were different, suggesting unique evolutionary histories of these three loci. However, a species tree reveals most parts of the genetic history of a species and has a variance well represented by the diversity of trees derived from different genes (102). Therefore, the phylogenetic incongruence

revealed in this study is not uncommon. Other fast-evolving loci, if any, need to be developed for further elucidating the phylogeny and for designing diagnostic markers of these two *formae speciales*.

Fusarium oxysporum f. sp. *radicis-lycopersici* and *lycopersici* have multiple evolutionary origins (polyphylectic) but share a common ancestor (paraphyletic), whereas an incongruence was shown within topology of three phylogenies derived from IGS, EF-1α, and FOL185. These three phylogenies revealed that *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* formed a strongly supported clade, suggesting that they evolved from the same common ancestor. At least two evolutionary origins were shown for *F. oxysporum* f. sp. *radicis-lycopersici*. The evolutionary origin of VCGs 0094, 0098, and 0099 could be independent of other VCGs of *F. oxysporum* f. sp. *radicis-lycopersici*. VCG 0094 is cosmopolitan, and Florida has been suggested as its probable center of origin. Moreover, VCG 0094 in Europe might have migrated from Florida (139). Phylogenetic analyses showed that an isolate from Belgium was placed in a clade with Florida VCG 0094 isolates, suggesting that Florida and European VCG 0094 are closely related even though geographically separated. Further phylogenetic analyses involving more VCG 0094 isolates worldwide may uncover its phylogeographical distribution and probable center of origin. VCGs 0092, 0093, and 0096 were only found in Israel (82). Like VCGs 0094, 0098, and 0099 in Florida, they were phylogenetically close and fell into clade 2 of the IGS phylogeny, probably suggesting sympatric speciation, which describes two or more descendant species inhabiting the same geographic location and deriving from a single common ancestor species (161). VCGs 0090 and 0091 were widely distributed in North America and placed in clade 2 of the IGS phylogeny. However, they were not found in this study (Fig. 2-1)

but replaced by VCGs 0094, 0098, and 0099 of clade 1, suggesting another independent evolutionary event of *F. oxysporum* f. sp. *radicis-lycopersici*.

No perithecia were observed after mating isolates with different mating-type idiomorphs in clade 1of the IGS phylogeny. This finding suggests that other factors may be required for sexual recombination since RT-PCR analysis revealed that mating type genes of *F. oxysporum* are still expressed and processed correctly (181).

Virulence tests showed that VCG 0098 had a higher level of virulence than the other VCGs. VCG 0098 was recently found in Florida and phylogenetically close to VCG0094 according to sequence data and VCG assays, suggesting that the pathogen may have diverged toward higher virulence. However, Validov et al. (171) suggested that virulence is not associated with the phylogenetic clade of the IGS phylogeny. No VCG information was available and partial IGS was used in their study, as a consequence our data could not be directly compared with theirs. Further studies including all the VCGs in different clades will be necessary to better understand the evolution of virulence in *F. oxysporum* f. sp. *radicis-lycopersici*. However, based on this study, VCG 0098 may be useful for breeding against Fusarium crown and root rot of tomato due to its high virulence and evolutionary origin.

Estimates of mean most recent common ancestor (MRCA) derived from IGS, EF-1α, and FOL185 data sets ranged from 38 to 129years ago (1969-1878), suggesting that rate heterogeneity may be across regions of the genome and that *Fusarium oxysporum* f. sp. *radicis-lycopersici* is a relatively new pathogen compared to the invention of agriculture approximately 10,000 years ago (161). Based on the mean MRCA estimates, the pathogen might have diverged from its ancestor around the nineteenth or twentieth century. It has been suggested that tomatoes had undergone considerable domestication before being taken to Europe in the 15th century and a

much more intensive level of domestication occurred throughout Europe in the eighteenth and nineteenth century (9,155). Moreover, tomato only gained economic importance by the end of the nineteenth century or beginning of the twentieth century (123). These results suggest that *F. oxysporum* f. sp. *radicis-lycopersici* might have coevolved with domestication of its host and has been selected by the environmental and genetic uniformity of the agricultural ecosystem (161).

IGS, EF-1α, and FOL185 data sets revealed the population expansion in *F. oxysporum* f. sp. *radicis-lycopersici* in the last 15 years (1992-2007). Numerous resistant cultivars have been developed since 1983 (150), probably causing a considerable decrease in the population size of *F. oxysporum* f. sp. *radicis-lycopersici*. However, the population size has been increasing since the 1990s according to these three data sets (Fig. 3-5). It is not clear whether other factors, such as the phase-out of methyl bromide and the *Frl* resistance gene not introgressed into most commercial cultivars, may also have caused this increase in the population size. Using regular crop rotations and avoiding extremely susceptible cultivars are suggested to minimize the pathogen population size (107,108). Regularly monitoring the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* is also necessary for disease management.

Table 3-1. Mean pairwise nucleotide sequence differences for each locus using p-distances (in percentages) among isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *F. oxysporum* f. sp. *lycopersici* (FOL), and both *formae speciales*

Locus name	FORL	FOL	FORL+FOL
IGS	0.76	0.97	0.88
EF-1α	0.73	0.74	0.77
FOL185[z]	0.36	0.32	0.39

[z] A noncoding microsatellite locus obtained from contig number 185 of the genome sequence of *F. oxysporum* f. sp. *lycopersici* at http:// www.broad.mit.edu.

Table 3-2. Results of partition homogeneity test among three data sets of intergenic spacer (IGS), partial elongation factor 1-alpha (EF-1α) and a noncoding microsatellite locus, FOL185

	P values (1000 partitions)[y]	
Locus name	IGS	EF-1α
IGS		
EF-1α	0.001	
FOL185[z]	0.004	0.638

[y] Partition homogeneity test was performed using heuristic search with 1000 replications of random stepwise addition and branch swapping algorithm using tree bisection-reconnection (TBR) implemented in PAUP* 4.0b10 (162).

[z] A noncoding microsatellite locus obtained from contig number 185 of the genome sequence of *F. oxysporum* f. sp. *lycopersici* at http:// www.broad.mit.edu.

Table 3-3. Data set properties and nucleotide substitution models used in phylogenetic analyses

Data set	Substitution model	Characters	PI[w] sites	Tree length	CI[x]	RI[y]
IGS	TrN + G	2419	314	796	0.895	0.929
EF-1α	GTR + G	721	86	151	0.914	0.955
FOL185[z]	TrN + G	798	86	187	0.904	0.922

[w] Number of potentially parsimonious informative sites.

[x] Consistency index.

[y] Retention index.

[z] A noncoding microsatellite locus obtained from contig number 185 of the genome sequence of *F. oxysporum* f. sp. *lycopersici* at http:// www.broad.mit.edu.

Table 3-4. Bayesian Markov chain Monte Carlo (MCMC) estimates of evolutionary dynamics of *Fusarium oxysporum* f. sp. *radicis-lycopersici* using the uncorrelated relaxed lognormal clock model implemented in BEAST

Data set	Model selected	Characters	Isolation date range	Mean rate (subs/site/yr)	HPD[x] of rate (subs/site/yr)	Mean MRCA[y] (yr)	HPD of MRCA (yr)
IGS	HKY+I+Γ	2335	1975-2007	1.68×10^{-4}	2.84×10^{-5}, 3.46×10^{-4}	129	32, 310
EF-1α	HKY	699	1975-2007	1.27×10^{-4}	2.74×10^{-5}, 2.57×10^{-4}	81	32, 150
FOL185[z]	GTR+I+Γ	752	1975-2007	4.92×10^{-4}	8.44×10^{-5}, 9.80×10^{-4}	38	32, 56

[x] 95% high-probability density.

[y] Most recent common ancestor.

[z] A noncoding microsatellite locus obtained from contig number 185 of the genome sequence of *F. oxysporum* f. sp. *lycopersici* at http:// www.broad.mit.edu.

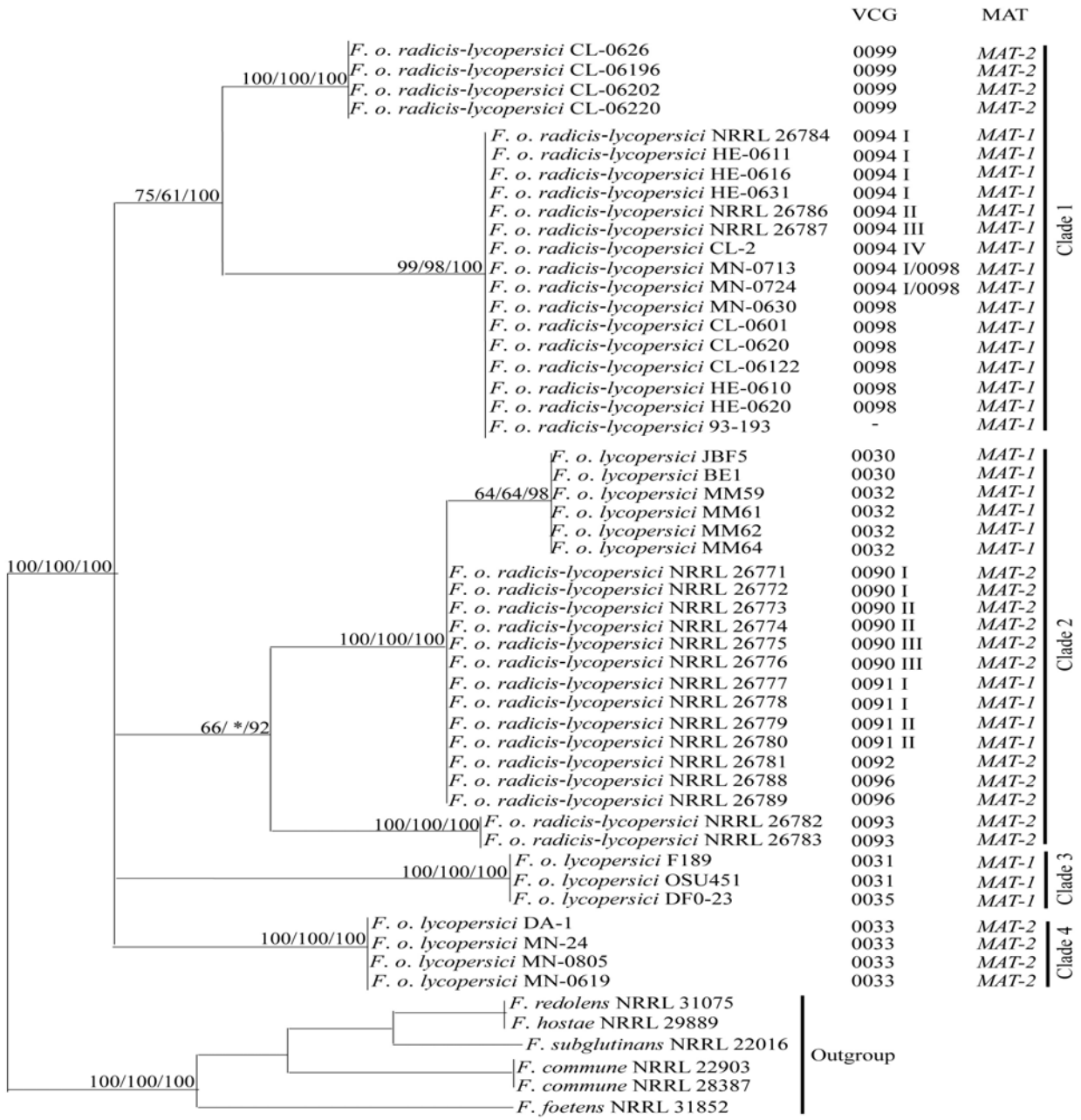

Figure 3-1. Phylogeny for *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* inferred from the intergenic spacer (IGS) region of nuclear ribosomal DNA. Numbers on nodes represent bootstrap support values for maximum parsimony (front), maximum likelihood (middle), and Bayesian posterior probabilities presented as percentage (back). Values represented by an "*" were less than 50% for bootstrap. Maximum likelihood analysis revealed VCG 0093 was an individual clade, which was the only incongruence compared to the other two analyses. The mating type (MAT) of each isolate was determined as previously described (84).

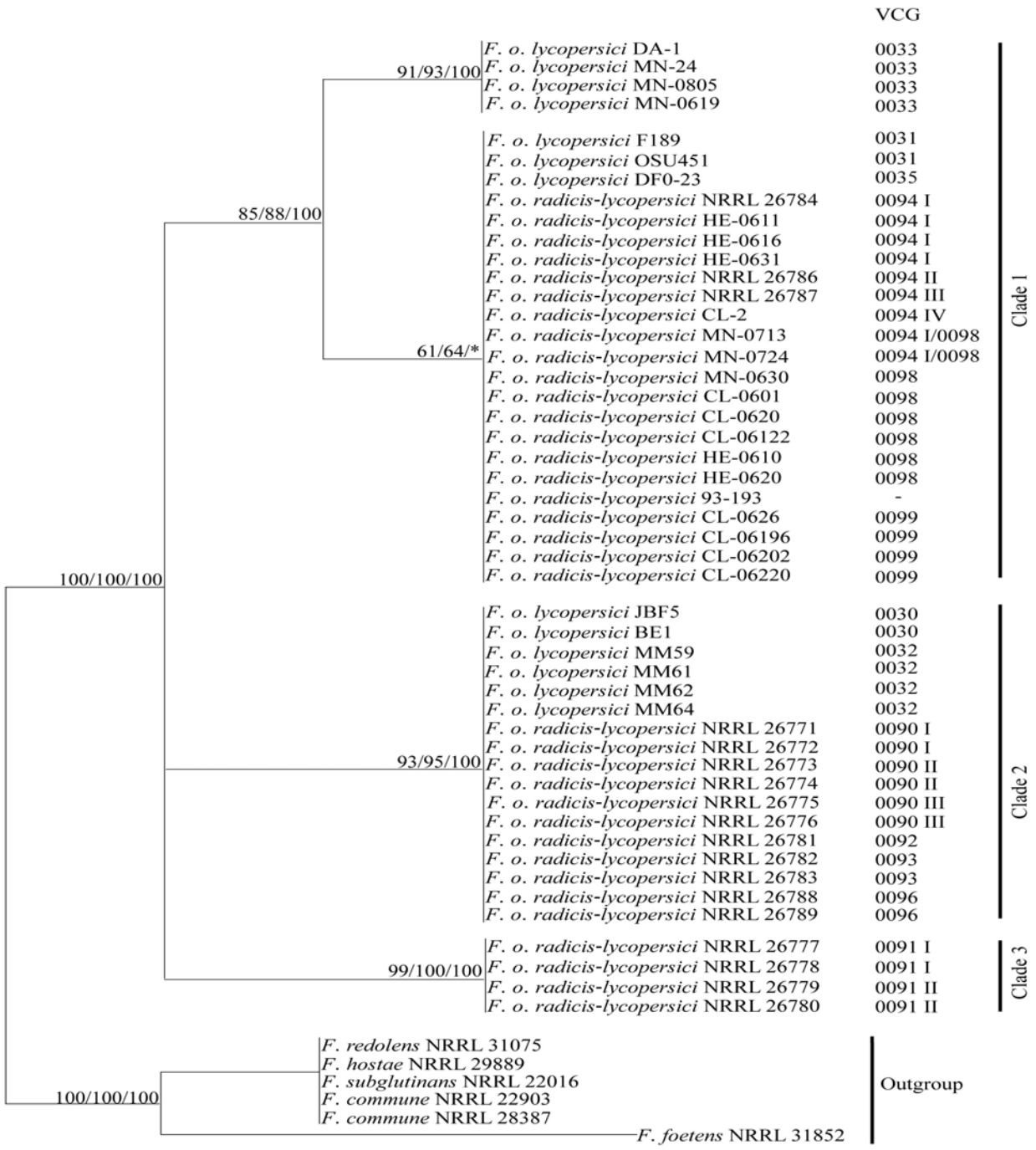

Figure 3-2. Phylogeny for *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* inferred from partial elongation factor 1-alpha (EF-1α). Numbers on nodes represent bootstrap support values for maximum parsimony (front), maximum likelihood (middle), and Bayesian posterior probabilities presented as percentage (back). Values represented by an "*" were less than 90% of Bayesian posterior probabilities.

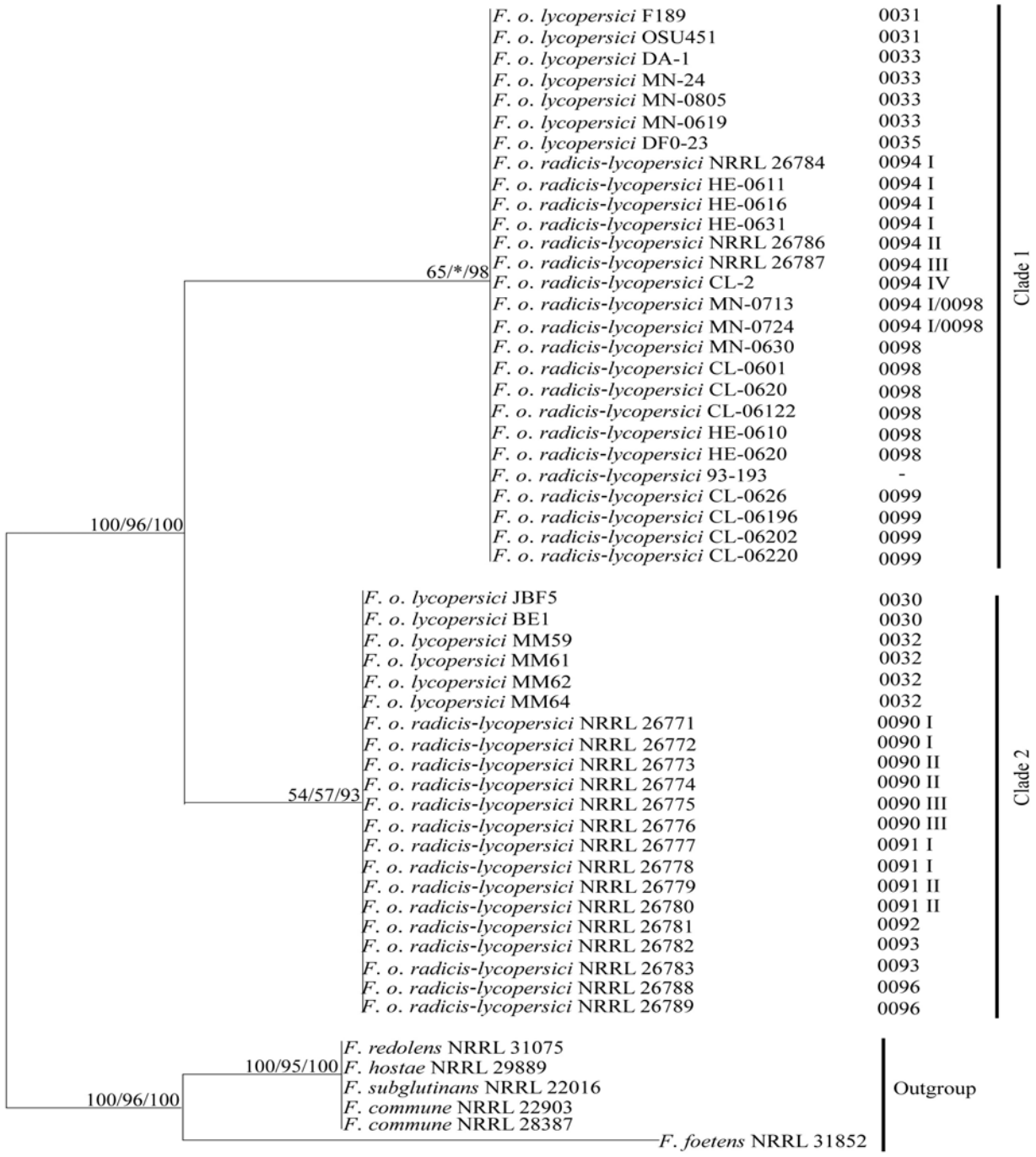

Figure 3-3. Phylogeny for *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* inferred from a noncoding microsatellite locus, FOL185. Numbers on nodes represent bootstrap support values for maximum parsimony (front), maximum likelihood (middle), and Bayesian posterior probabilities presented as percentage (back). Values represented by an "*" were less than 50% for bootstrap.

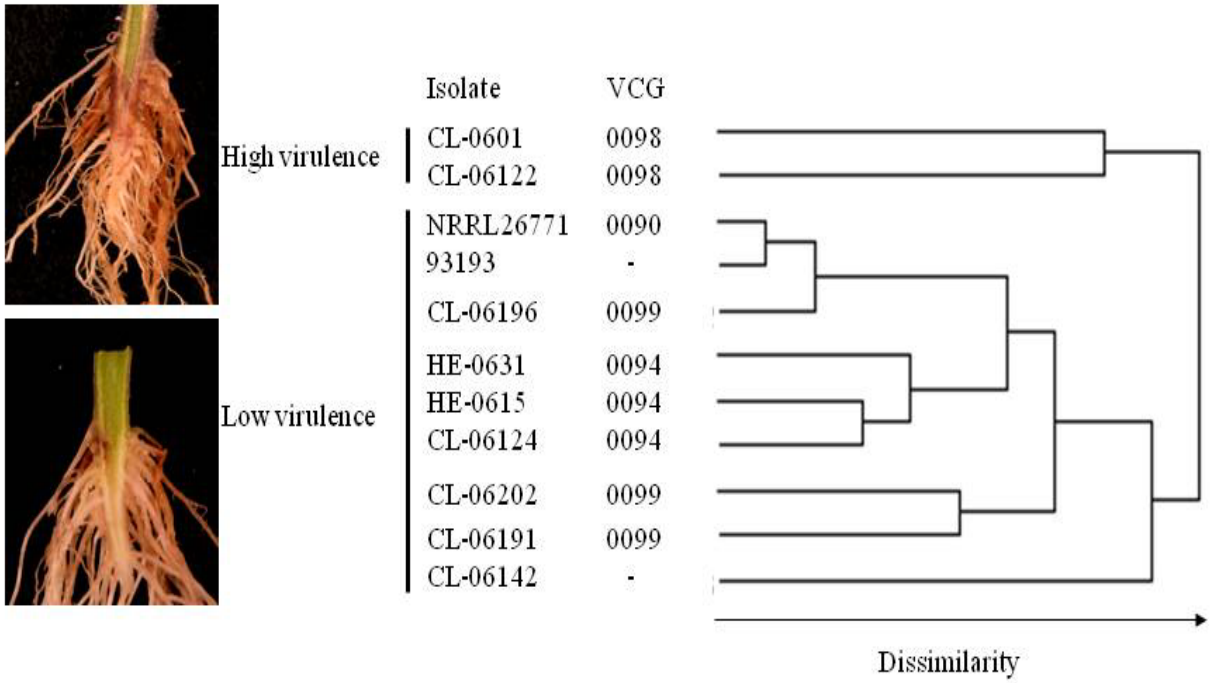

Figure 3-4. Cluster analysis of virulence for vegetative compatibility groups (VCGs) of *Fusairum oxysporum* f. sp. *radicis-lycopersici* according to Ward's minimum variance method (175) implemented in JMP. Three disease severity ratings were used including the percent of cortical, crown, and root discolorations. "-" represents nonassigned VCGs.

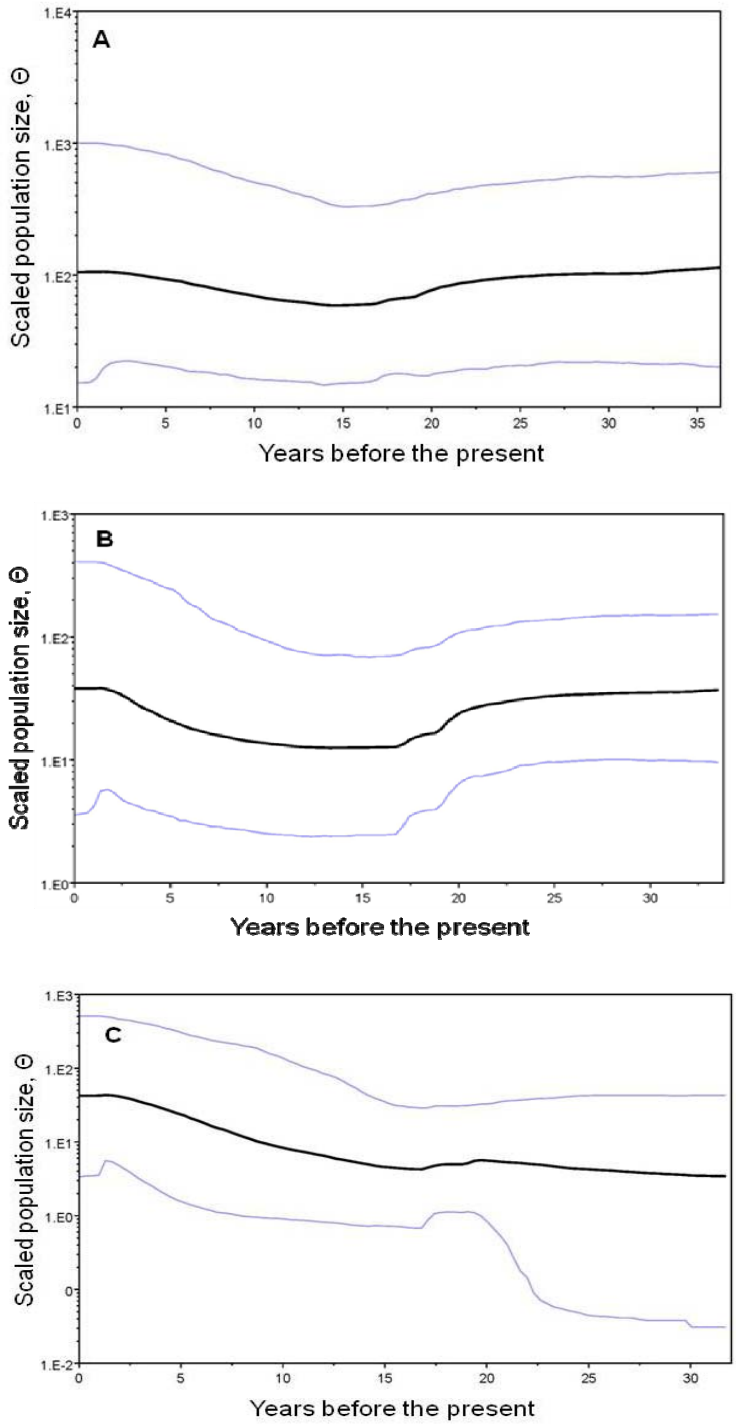

Figure 3-5. Bayesian skyline plots of *Fusarium oxysporum* f. sp. *radicis-lycopersici* derived from IGS (A), EF-1α (B), and FOL185 (C) data sets using BEAST version1.4.8. The x-axis is time as measured in years before present and the y-axis is the scaled population size (Θ, which is equivalent to the effective population size (*Ne*) multiplied by generation length (*g*)). Each curve is a plot of the median, with its 95% credible interval estimates indicated by the blue lines.

CHAPTER 4
EFFECT OF SILICON ON FUSARIUM CROWN AND ROOT ROT OF TOMATO CAUSED BY *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Introduction

Fusarium oxysporum f. sp. *radicis-lycopersici*, the causal agent of Fusarium crown and root rot, is an important soilborne pathogen of tomato (72,80). This pathogen has been found in many tomato-growing regions of the world although it is a relatively newly identified pathogen (80). No physiological races have been reported, but considerable genetic diversity is suggested by the existence of many vegetative compatibility groups (VCGs) (80,82). The pathogen can be introduced to new tomato-growing regions by means of infected seeds, transplants, soil and media (59,74,111). Once introduced, this polycyclic pathogen can be disseminated via root-to-root contact, dispersal of airborne conidia, water flow, and fungus gnats of the genus *Bradisya* (53,59,74, 133,140), making control of this disease more difficult. Soil fumigation has been investigated to manage this disease (62,106,109) but social and environmental concerns have caused the phaseout of certain chemicals such as methyl bromide. Further research in biological control needs to be conducted for application in the field (27). Thus, the use of resistance cultivars seems logical and effective for controlling the disease. However, resistance may be broken down due to the evolutionary potential of the pathogen and environmental conditions (107,108). Moreover, the *Frl* resistance gene to *F. oxysporum* f. sp. *radicis-lycopersici* is only deployed in some newer cultivars (151). Alternative and environment-friendly approaches for managing Fusarium crown and root rot of tomato need to be evaluated.

Silicon (Si) has been suggested to moderate biotic and abiotic stresses on tomato (2,25,32, 122), although tomato has been defined as being a Si excluder (99). Si accumulates in the cytoplasmic fraction of Si-accumulator plants but it is mainly deposited in the cell-wall fraction in roots of Si-excluder plants such as tomato (60). The reinforcement of root cell walls may

affect the penetration of *F. oxysporum* f. sp. *radicis-lycopersici* because the pathogen penetrates the epidermis of tomato roots directly and then produces intracellular and intercellular hyphae in the outer parenchyma of cortical tissues beneath the penetrated sites (16). Resistant cultivars can form a defensive barrier in the parenchyma cells and prevent the pathogen from spreading toward the central vascular bundle (16,178). Like the defensive barrier induced in the resistant cultivar, Si may influence the creation of a physical barrier and also induce other defense responses in the host (20,26). Further research in the relationship between the penetration caused by this pathogen and the structure of cell wall strengthened by Si needs to be conducted, whereas no effect of Si on Fusarium crown and root rot has been previously suggested (112).

Si-mediated resistance in tomato may not be located in the roots. For example, Si-mediated resistance in tomato against *Ralstonia solanacearum* was likely located in stems due to changes in the pectic polysaccharide structure of stem cell walls, restricting the bacterial movement to the stems (32). However, Si significantly decreased the bacterial population in roots and stems of a resistant cultivar, Hawaii 7998, compared to non-treated plants. These results suggested that Si-mediated resistance may also exist in tomato roots. It is not well known whether Si content of roots correlates with disease severity of Fusarium crown and root rot of tomato.

The objectives of this study were (i) to investigate effects of Si and inoculum concentrations on disease severity of Fusarium crown and root rot (ii) to evaluate the effect of Si on disease progress (iii) to determine whether there was an association of disease severity with Si content of roots and shoots.

Materials and Methods

Plant Growth and Silicon Amendment

Seeds of ‘Bonny Best’, susceptible to Fusarium crown and root rot, were soaked in 5% bleach for 2 min for surface sterilization and then washed several times with sterile water. The

sterilized seeds were sowed in a commercial growth medium (Metro Mix 300, Palmetto, FL). One week after sowing, seedlings at the cotyledon stage were transplanted to 15-cm-diameter plastic pots (Hummert Internatinal, Earth City, Missouri) filled with 1.5 kg sand provided by the University of Florida Turfgrass Research Envirotron. Each pot included two tomato plants. The seedlings were grown in a growth chamber held at 20°C (12 h light photoperiod with photonflux of 70.7 μmol/m^2s). Hoagland's nutrient solution (64) with (+ Si) or without (–Si) 100 mg Si L^{-1} (3.56 mM) as sodium metasilicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$) (42) was adjusted to pH 5 using 36 N sulfuric acid (H_2SO_4) before applying to tomato plants since lowering the pH of fine sand increases disease severity of Fusarium crown and root rot (76). The nutrient solution contained N 224 mg, P 62 mg, K 235 mg, S 32mg, B 0.5 mg, Mn 0.5, Zn 0.05, Cu 0.02, Mo 0.01 mg, and Fe 1.56 mg $L^{-1}$and was prepared using deionized water. Each pot was fertigated with a total of 600 ml of the nutrient solution with or without Si during 3 weeks after transplanting. Fifty mL of the nutrient solution was given to each pot every other day for the first 18 days after transplantation. After which, 50 mL of the solution was applied to each pot for 3 consecutive days. Deionized water was used to irrigate tomato plants as needed.

Inoculum Production and Inoculation Procedure

Isolate CL-0601, belonging to VCG 0098, of *F. oxysporum* f. sp. *radicis-lycopersici* was used for inoculation due to its high virulence revealed by earlier studies (Chapter 3). Depending on experiments, 10 ml of a conidial and mycelial fragment suspension with 10^5 and/or 10^6 conidia mL^{-1}, recovered from 14-day-old cultures grown on carnation leaf agar (CLA), was placed on crowns using a pipette with sterile tips 3 weeks after transplanting. The inoculated plants were placed in a completely randomized design in an incubator at 20°C with 12 h light photoperiod (photonflux of 70.7 μmol/m^2s).

Disease Assessments

Three disease severity ratings were made on roots, crowns, and stems. Root infection was rated visually as the percentage of roots showing discoloration. Brown discoloration in crowns was determined according to a 0-4 scale: 0 = no symptom; 1 = 1-25 %; 2 = 26-50 %; 3 = 51-75%; and 4 = 76-100%. Disease severity in stems was defined as the ratio of the lesion length divided by the stem length. The area under disease progress curve (AUDPC) was calculated using the method of Shaner and Finney (154) for studying the effect of silicon on disease progress over time. Diseased plants were sampled to confirm the presence of the causal agent, isolate CL-0601, identified using vegetative compatibility grouping (24).

Effects of Silicon and Inoculum Concentration

A factorial design of six treatments with five replicates (10 plants) was arranged using a completely randomized design in the incubator: (i) plants with silicon, inoculated with sterile water (+Si–FORL), (ii) plants with silicon, inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* at a concentration of 10^6 conidia/plant (+Si+FORL1), (iii) plants with silicon, inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* at a concentration of 10^7 conidia/plant (+Si+FORL2), (iv) plants without silicon, inoculated with sterile water (–Si–FORL), (v) plants without silicon, inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* at a concentration of 10^6 conidia/plant (–Si+FORL1), and (vi) plants without silicon, inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* at a concentration of 10^7 conidia/plant (–Si+FORL2). Four weeks after inoculation, all plants were harvested, washed and rated for disease severity and then divided into shoots and roots for silicon quantification.

Effect of Silicon on Disease Progress over Time

To further determine and confirm the effect of silicon on Fusarium crown and root rot of tomato, disease progress over time was determined using partial treatments of the above factorial design: +Si–FORL, +Si+FORL1, –Si–FORL, and –Si+FORL1. Ten plants of each treatment were evaluated for disease severity and divided into shoots and roots for silicon concentration analysis 2, 3, 4, and 6 weeks after inoculation.

Determination of Dry Root and Shoot Weight and Silicon Quantification

After rating for disease severity, plants were divided into shoots and roots, washed in deionized water, and dried separately in paper bags for 72 h at 80°C (Isotemp Oven, Fisher Scientific). Dry roots and shoots were weighed, ground using a Cyclotec™ 1093 sample mill (FOSS, Denmark), passed through a 40-mesh screen, and stored in 20 ml plastic scintillation vials (Fisher Scientific, Pittsburgh, PA). Si analysis was based on the method of Elliott and Snyder (40) using a colorimetric analysis with a modification of the digestion procedure of plant tissues. One hundred mg plant tissues were used for digestion in a 100 ml plastic high-speed polypropylene copolymer tube (Nalgene) using 2 ml of 30% H_2O_2 and 3 ml of 100% NaOH. The tube was then placed in a 100°C water bath for one hour to initiate the tissue digestion before autoclaving for 20 min. If necessary, additional amount of 30% H_2O_2 was added and the autoclave cycle was repeated until complete digestion.

Statistical Analysis

All data collected were subjected to analysis of a factorial experiment in SAS v. 9.2 (SAS Institute, Cary, NC) using PROC GLM to evaluate effects of Si and inoculum concentration and their interaction. Standard analysis of variance (ANOVA) was also performed. Treatment mean comparisons were analyzed using Fisher's Protected Least Significant Difference test (FLSD) at

$P \leq 0.05$. Regression analysis was performed to determine the relationship between silicon content and disease severity using SIGMAPLOT version 10.0 (Systat Software, Chicago, IL).

Results

Effects of Silicon and Inoculum Concentration

The application of Si significantly increased the concentration and uptake of Si in shoots and roots at the time of inoculation (3 weeks after transplanting) and at the time of harvest (4 weeks after inoculation) (Table 4-1). Si supply also significantly enhanced dry weight of roots by 20.8%, whereas no significant difference was shown for dry weight of shoot and total dry weight per plant between +Si and –Si treatments (Tables 4-1 and 4-2). The effect of inoculum concentration significantly affected dry weight, silicon uptake of roots and shoots, and Si concentration in shoots except the Si content in roots. However, no significant interaction was revealed between Si and inoculum effects for these plant components estimated except the Si uptake of the shoot (Table 4-2).

The application of Si significantly decreased disease severity of the stems 4 weeks after inoculation, although disease severity of the root and crown was not affected by Si supply (Tables 4-3 and 4-4). No significant interaction between Si application and inoculum concentration was detected, suggesting the response to inoculum concentration was consistent between +Si and –Si treatments. Four weeks after inoculation, disease severity of both the root and crown was not significantly different between two the inoculum concentrations, 10^6 and 10^7 conidia plant^{-1}, but there was significantly larger stem lesions with the higher concentration (Table 4-5).

Effect of Silicon on Disease Progress

Similar Si effects were observed as in the above experiment. The application of Si significantly increased dry weight of roots, and the concentration and uptake of Si in shoots and roots, whereas the +Si treatment did not significantly affect dry shoot weight (Figs. 4-1 and 4-2). Root discoloration appeared earlier for the –Si treatment than for the +Si treatment. Moreover, the +Si treatment significantly decreased root infection by 82.1% 3 weeks after inoculation (Fig. 4-3). No significant difference was detected between –Si and +Si treatments for disease severity of roots 4 and 6 weeks after inoculation. Crown discoloration was also shown to occur earlier in tomato plants without Si. Three and 4 weeks after inoculation, disease severity of the crown in tomato plants amended with Si was significantly lower than those without Si. Like the other two rating systems, stem discoloration was first observed in tomato plants without Si. The +Si treatment significantly decreased disease severity of the stems 4 weeks after inoculation but no significant difference was shown between –Si and +Si treatments 6 weeks after inoculation (Fig. 4-3).

Si supply significantly lowered the AUDPC for disease severity of stem by 52.5%, whereas the AUDPC for disease severity of root and crown was not significantly different between –Si and +Si treatments (Table 4-6).

Regression analysis was used to further investigate the effect of silicon on disease severity of Fusarium crown and root rot development. The results showed that a linear model best described the relationship between silicon content of roots and disease severity of root, crown, and stem (Fig. 4-4). Regression analysis also suggested that disease severity decreased consistently with increasing silicon content of roots. Interestingly, the linear model also best described the relationship between silicon content of shoots and disease severity of stem,

showing that disease severity of stem decreased consistently with increasing silicon content of shoots (Fig. 4-5).

Discussion

One of the goals of this study was to determine the effect of Si on Fusarium crown and root rot of tomato through studying disease progress and effects of inoculum concentration, since no effect of Si was previously suggested for this disease (112). This study also showed no significant difference between –Si and +Si treatments for root infection 4 weeks after inoculation. However, Si was able to significantly reduce the disease severity of stems 4 weeks after inoculation, whereas a significant decrease in the disease severity of crowns by Si supply was merely observed in the experiment on disease progress (Table 4-4 and Fig. 4-3). Possible causes in reducing disease severity by Si are discussed.

Inoculum concentration significantly affected the disease severity of roots, crowns, and stems, whereas no significant interaction was revealed between Si and inoculum levels (Table 4-3), suggesting that the response to inoculum concentrations was consistent between +Si and –Si treatments in this study. In other words, the effect of Si was not significantly impacted by the inoculum level 4 weeks after inoculation. When effects of Si and inoculum concentration were separated to analyze their impact on disease severity, the +Si treatment significantly reduced the disease severity of the stem but not the root or crown. This result suggested that disease progress already had reached the stem 4 weeks after inoculation and that the pathogen had proliferated in roots and crown overwhelmingly (Table 4-4). Levels of inoculum concentration also significantly affected disease progress. The disease severity of the stem in plants inoculated with 10^7 conidia plant^{-1} was significantly higher than in those inoculated with 10^6 conidia plant^{-1}, whereas no significant difference was shown for the disease severity of either roots or crowns

between these two inoculum concentrations (Table 4-5). These results suggested that the higher the inoculum concentration, the faster the disease progress of Fusarium crown and root rot.

Inoculum concentration of 10^6 conidia $plant^{-1}$ was selected for studying disease progress to corroborate the effect of Si on Fusarium crown and root rot of tomato since the inoculum density in the root zone would be able to cause disease (134). Further analysis of disease progress suggested that the decrease in disease severity by the +Si treatment was probably due to delaying initial infection in roots and the movement of the pathogen from roots to stems (Fig. 4-3). Tomato plants treated with Si did not show visible discoloration in roots, crowns, and stems but those without Si supply revealed some brown lesions 2 weeks after inoculation.The disease severity of roots showed a significant difference between –Si and +Si treatments 3 weeks after inoculation as a result of significant variance in Si content of roots (Figs. 4-2 and 4-3). These findings suggested that soilborne diseases may be reduced using Si fertilizers for Si non-accumulator plants with a limited capacity to accumulate Si in roots and inefficient translocation of this element to shoots.

The reduction in disease progress of Fusarium crown and root rot was positively correlated with an increase in the Si content of tomato roots (Fig. 4-4), suggesting a silicon-induced resistance and/or reduction of fungal colonization. Formation of a physical barrier has been proposed to explain Si-mediated resistance (20,26,179) because Si can accumulate and deposit beneath the cuticle to form a cuticle-Si double layer and thus prevent leaves from penetration of pathogens (26,144). Although physical barriers in roots may not be associated with silicon-induced resistance in tomato to *R. solanacearum*, silicon-induced changes in the pectic polysaccharide structure of tomato stem cell walls have been observed (32). Moreover, silicon-induced resistance in tomato to *R. solanacearum* may be associated with the capability of the

plant to restrict the bacterial movement to the stems due to a significant correlation between the bacterial population in stem and the resistance (32). Our study also showed that the +Si treatment likely limited the basipetal spread of *F. oxysporum* f. sp. *radicis-lycopersici* from infected roots (Fig. 4-3), whereas the reinforcement in cell walls of tomato roots was not evaluated. Also, a significant relationship between silicon content of shoots and disease severity of stems was exhibited (Fig. 4-5), suggesting that Si concentration of the stem may be associated with the movement of the pathogen in stems. Due to Si accumulation mainly in the cell-wall fraction of tomato roots (60) and unclear mechanisms of Si-mediated resistance in tomato, it is pertinent to further investigate physical barrier, biochemical, and molecular mechanisms involved in silicon-induced resistance (20,26) to *F. oxysporum* f. sp. *radicis-lycopersici*.

Si concentrations in roots were higher than those in shoots of tomato (Figs. 4-1 and 4-2) and this is typical for a Si non-accumulator plant in agreement with previous studies (25,60). Si amendment significantly increased Si contents in both roots and shoots, even though tomato is categorized as a Si non-accumulator (99). Infection by *F. oxysporum* f. sp. *radicis-lycopersici* did not increase the Si accumulation in tomato, whereas cucumber plants accumulated more Si around penetration sites (21). Tomato plants were pretreated with Si, but Si application was discontinued after inoculation in this study, resulting in a rapid decrease in Si contents of roots and shoots during incubation (Figs. 4-1 and 4-2). A rapid decline of Si-induced resistance to *Sphaerotheca fuliginea* was observed after transferring cucumber plants treated with Si to Si-free solution (144), and this was also found in the *Pythium aphanidermatum*/bitter gourd system (61). The availability of soluble silicic acid, but not the total Si concentration in roots, at the time of infection has been suggested as the main contributor to Si-mediated resistance (22). Although Si accumulates in the cell-wall fraction of roots in tomato (60), it is not clear whether the continual

application of Si after inoculation may increase the availability of soluble silicic acid and enhance Si-mediated resistance to Fusarium crown and root rot.

Silicon is not defined as an essential nutrient for plants, but it has been suggested as an essential element involved in the physiology of tomato growth through phytohormone synthesis (100). To maintain normal plant biology, 1 mM Na_2SiO_3 is recommended to be included in a modified Hoagland solution (42). This study utilized 3.56 mM Si in Hoagland's nutrient solution, suggesting that enough Si was utilized to support normal tomato growth. Si significantly increased dry weight of roots, whereas no significant difference was shown for dry weight of shoots between –Si and +Si treatments. In addition to Si-mediated resistance, dry weight of roots increased by Si supply, suggesting that Si application may also benefit tomato plants by maintaining normal physiology (43). Plant roots release exudates in the rhizosphere which microorganisms can use as a primary food source. This influences the population density and activity of these microorganisms and the outcome of the pathogen infection as a result of the interaction between pathogens and other microorganisms (130). Since Si has been reported to mitigate biotic and abiotic stresses on tomato (2,25,32,122), how to apply Si fertilizers for field-grown tomatoes is worth of being further investigated.

Table 4-1. Effect of silicon (Si) on Si concentration, dry weight, and Si uptake of tomato inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* at the time of inoculation (week 0) and the time of harvest (week 4)

Week 0							
	Shoot			Root			
Treatments	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Total dry weight (g)
Without Si	0.30	0.31	0.09	0.03	1.51	0.06	0.34
With Si	0.39	0.95	0.36	0.06	3.31	0.21	0.45
FLSD ($P \leq 0.05$)	0.16	0.25	0.11	0.02	0.43	0.09	0.17
Week 4							
	Shoot			Root			
Treatments	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Total dry weight (g)
Without Si	1.62	0.45	0.73	0.24	0.77	0.18	1.86
With Si	1.65	0.92	1.53	0.29	1.55	0.44	1.94
FLSD ($P \leq 0.05$)	0.14	0.11	0.17	0.04	0.13	0.06	0.17

Table 4-2. Analysis of variance for effects of silicon (Si) supply and inoculum concentration (IC) on plant components

		F values[y]						
		Shoot			Root			
Source of variation	df[z]	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Dry weight (g plant^{-1})	Si concentration (mg (g dw)$^{-1}$)	Si uptake (mg)	Total dry weight (g)
Si	1	0.15^{ns}	74.3^{***}	98.9^{***}	5.69^{*}	156^{***}	78.1^{***}	0.83^{ns}
IC	2	18.5^{***}	5.49^{*}	26.7^{***}	24.9^{***}	1.37^{ns}	15.5^{***}	23.7^{***}
Si × IC	2	0.84^{ns}	2.05^{ns}	6.75^{**}	0.48^{ns}	0.89^{ns}	2.14^{ns}	0.31^{ns}

[y] Levels of probability: ns = not significant, $^{*} \leq 0.05$, $^{**} \leq 0.01$, and $^{***} \leq 0.001$.

[z] df = degrees of freedom.

Table 4-3. Analysis of variance of effects of silicon (Si) supply and inoculum concentration (IC) on disease components

		F values[x]		
		Disease severity[y]		
Source of variation	df[z]	Root	Crown	Stem
Si	1	0.16^{ns}	0.01^{ns}	5.15^{*}
IC	2	55.6^{***}	146^{***}	215^{***}
Si × IC	2	0.10^{ns}	0.01^{ns}	2.26^{ns}

[x] Levels of probability: ns = not significant, $^{*} \leq 0.05$, $^{**} \leq 0.01$, and $^{***} \leq 0.001$.

[y] Disease severity was estimated 4 weeks after inoculation. Root infection was rated visually as the percentage of root system showing discoloration; disease severity of crown was evaluated using a 0-4 scale where 0 represents health and 4 means 100% discoloration; Stem discoloration was defined as the ratio of the lesion length in stem divided by the stem length.

[z] df = degrees of freedom.

Table 4-4. Disease severity of tomato plants amended with or without sodium metasilicate (Na_2SiO_3) and inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* 4 weeks after inoculation

	Disease severity[z]		
Treatments	Root	Crown	Stem
Without Si	44.7	1.80	27.7
With Si	42.3	1.80	23.7
FLSD ($P \leq 0.05$)	12.1	0.31	3.63

[z] Root infection was rated visually as the percentage of root system showing discoloration; disease severity of crown was evaluated using a 0-4 scale where 0 represents health and 4 means 100% discoloration; Stem discoloration was defined as the ratio of the lesion length in stem divided by the stem length.

Table 4-5. Effect of inoculum concentration on disease severity of tomato plants inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* 4 weeks after inoculation

	Disease severity[z]		
Inoculum concentration (conidia/plant)	Root	Crown	Stem
0	0	0	0
10^6	62.0	2.6	35.9
10^7	68.5	2.8	41.2
FLSD ($P \leq 0.05$)	14.8	0.38	4.46

[z] Root infection was rated visually as the percentage of root system showing discoloration; disease severity of crown was evaluated using a 0-4 scale where 0 represents health and 4 means 100% discoloration; Stem discoloration was defined as the ratio of the lesion length in stem divided by the stem length.

Table 4-6. Area under disease progress curve (AUDPC) of tomato plants amended with or without sodium metasilicate (Na_2SiO_3) and inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici*

	AUDPC[z]		
Treatments	Root	Crown	Stem
Without Si	66.2	8.76	157
With Si	39.2	5.10	74.6
FLSD ($P \leq 0.05$)	49.1	4.47	78.3

[z] Root infection was rated visually as the percentage of root system showing discoloration; disease severity of crown was evaluated using a 0-4 scale where 0 represents health and 4 means 100% discoloration; Stem discoloration was defined as the ration of the lesion length in stem divided by the stem length. An inoculum concentration at 10^6 conidia/plant was used.

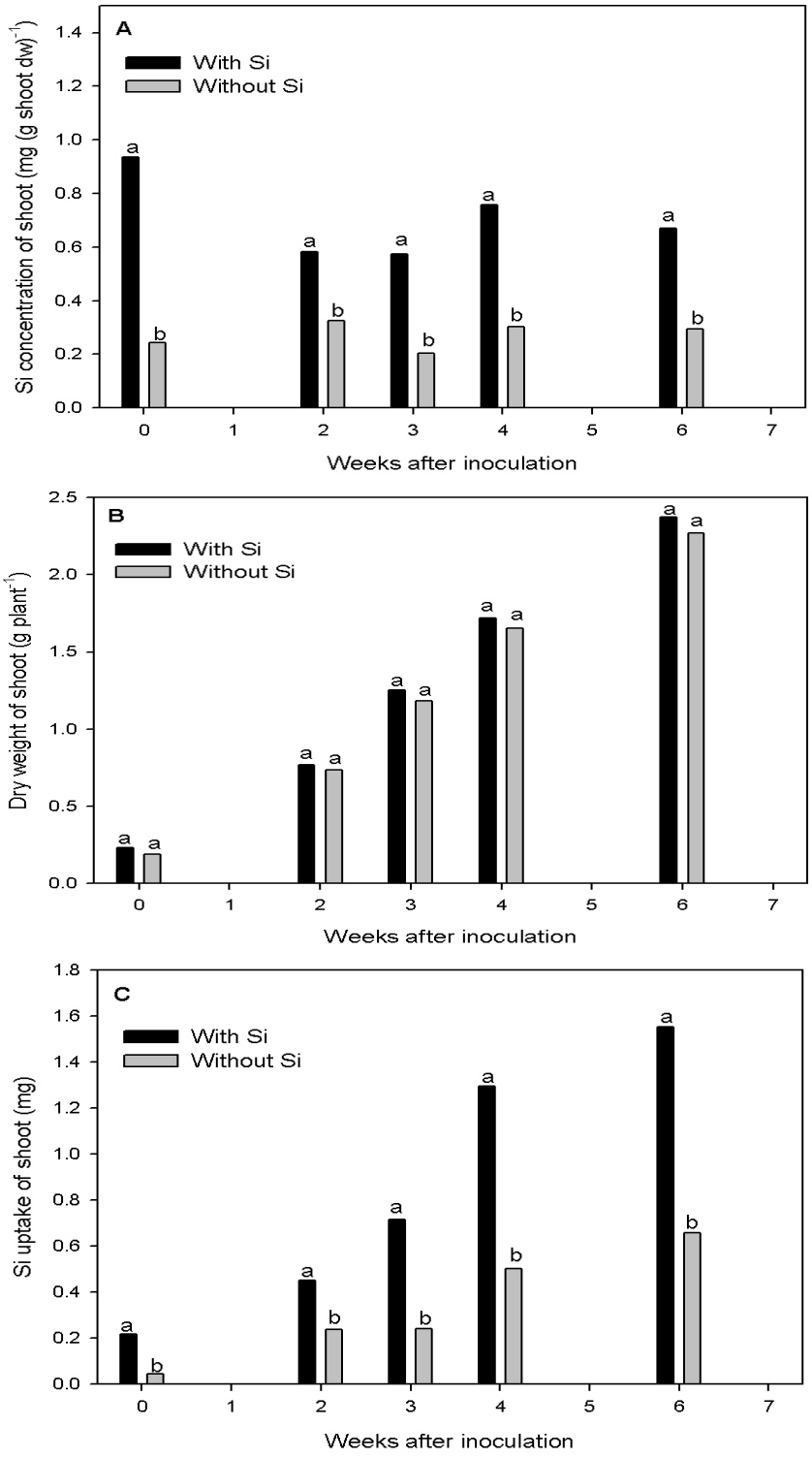

Figure 4-1. Effect of silicon (Si) on Si concentration (A), dry weight (B), and Si uptake (C) of the tomato shoot. Bars with the same letter at each time period do not differ significantly at $P \leq 0.05$ as determined by Fisher's protected least significant difference test.

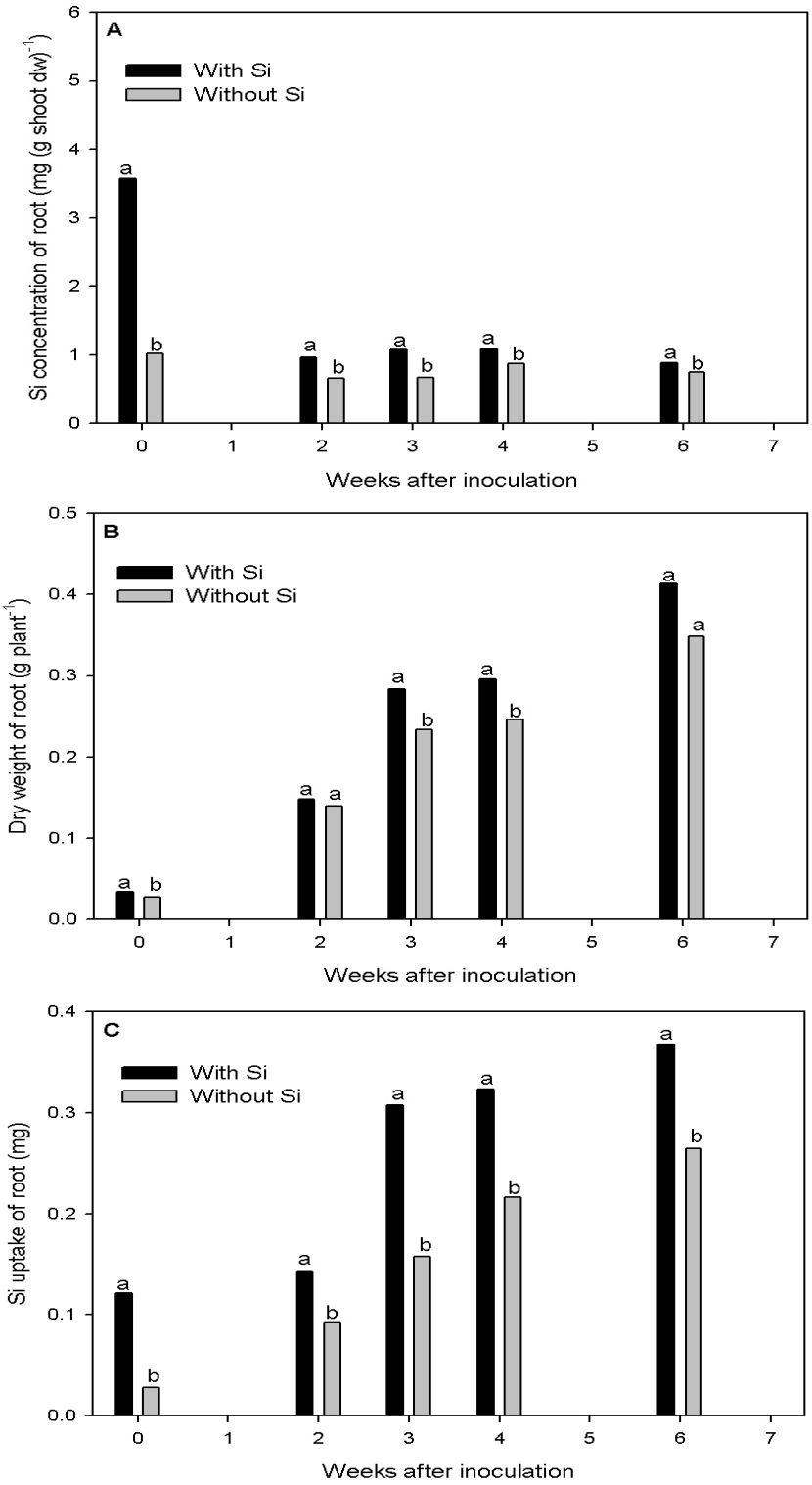

Figure 4-2. Effect of silicon (Si) on Si concentration (A), dry weight (B), and Si uptake (C) of the tomato root. Bars with the same letter at each time period do not differ significantly at $P \leq 0.05$ as determined by Fisher's protected least significant difference test.

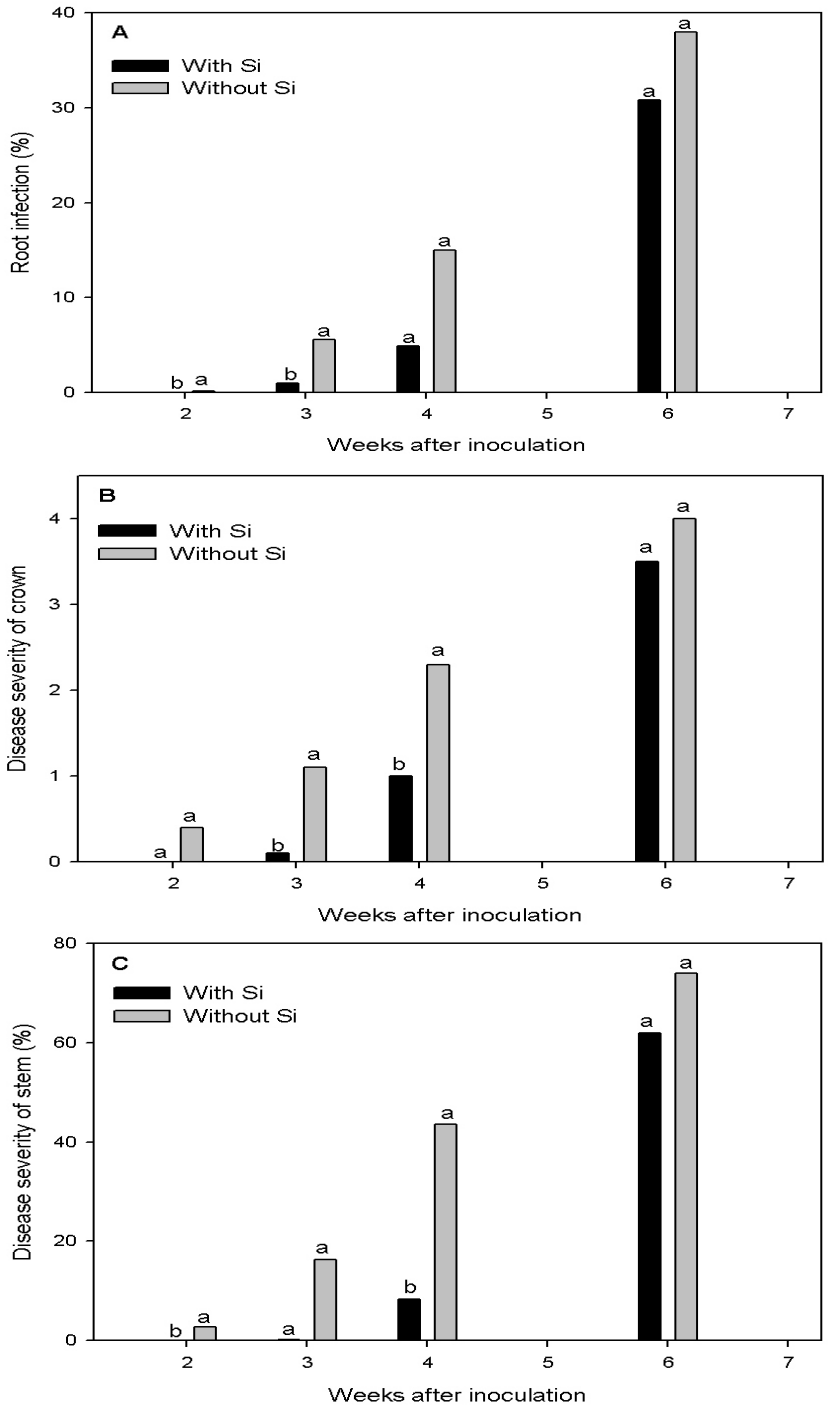

Figure 4-3. Effect of silicon on symptom development of Fusarium crown and root rot expressed as root infection (A), disease severity of crown (B) and of stem (C) on tomato cultivar Bonny Best over 6 weeks after inoculation. A 0-4 index was used according to the infection percentage of crown. Disease severity of stem is defined as the lesion length in stem divided by the stem length. Bars with the same letter at each time period do not differ significantly at $P \leq 0.05$ as determined by Fisher's protected least significant difference test.

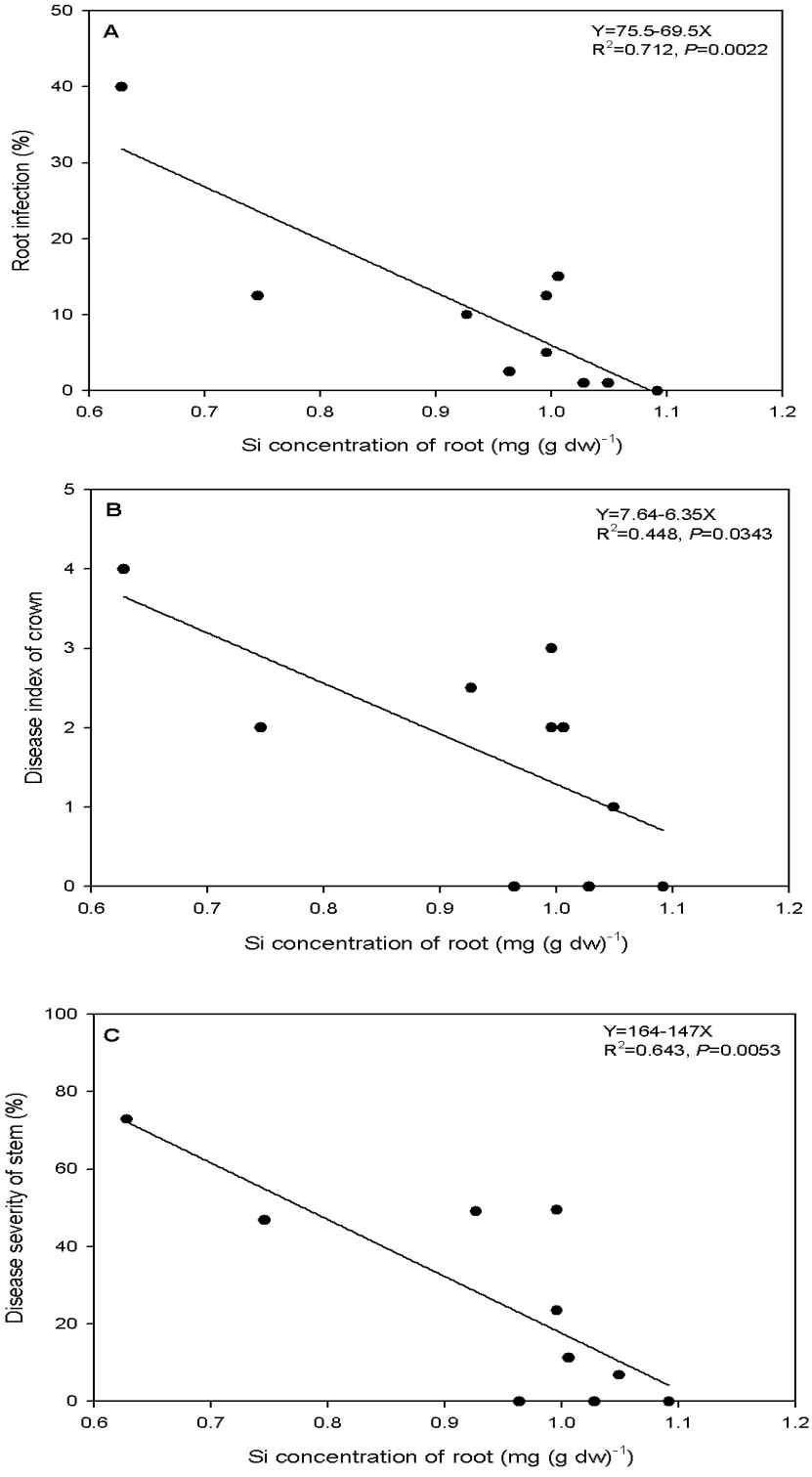

Figure 4-4. Relationship between root infection (A), disease severity of crown (B) and stem (C) and silicon concentration of tomato root 4 weeks after inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

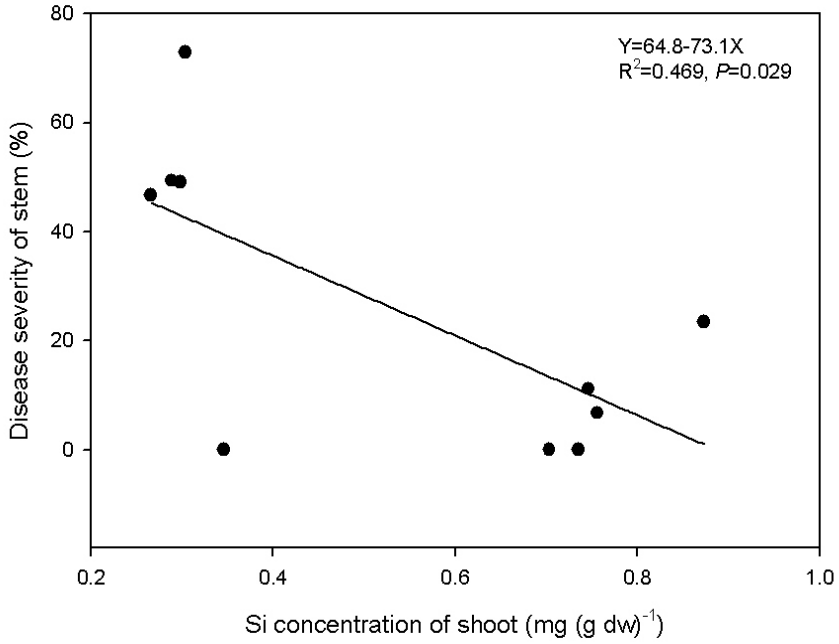

Figure 4-5. Relationship between disease severity of stem and silicon concentration of tomato shoot 4 weeks after inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

CHAPTER 5
CONCLUSION

Analyses of microsatellites and vegetative compatibility groups (VCGs) revealed the current population structure of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida which is the probable center of origin for the cosmopolitan VCG 0094 occurred along with two newly reported VCGs 0098 and 0099 (139). A bioinformatics method and a microsatellite enrichment approach used in this study successfully developed primers for 27 microsatellite loci amplifying both *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici*, suggesting that microsatellites may be derived from searching the published genome sequence of closely related species instead of *de novo* isolation from the species studied.

VCG 0094 is still predominant in Florida, but its frequency has decreased since the 1990s (139). In contrast, the distribution of VCG 0098 has increased, suggesting an increased selective fitness. Moreover, VCG 0098 may have migrated from Collier County or other tomato-growing regions to Manatee and Hendry Counties since a previous study did not find this VCG in these two counties (139). Two bridge isolates, vegetatively compatible with testers of both VCGs 0098 and 0094 I, were found in Manatee County, probably resulting from a process of either convergence or divergence (80). The frequency of VCG 0098 has increased and this VCG has a lower genetic diversity than VCG 0094. Moreover, VCG 0098 is phylogenetically related to VCG 0094 (Fig. 3-1). These findings suggest that VCG 0098 may have diverged from VCG 0094. Migration and sympatric speciation are two possible causes to explain how VCG 0098 appeared in Manatee County.

Although 38 isolates could not be assigned to a known VCG, most of them showed the same microsatellite haplotype as either VCG 0094 or 0098, suggesting that these nonassigned isolates might not comprise many new VCGs. Mutations in any of the genes controlling

incompatibility may change the VCG of an isolate (95). Therefore, nonassigned isolates with the same microsatellite haplotype as either of VCG 0094 or 0098 may be genetically related to either these two VCGs but not compatible with testers of VCGs 0094 and 0098. However, this study cannot totally rule out another possibility; that is, increasing the frequency of unclassified isolates may also suggest the emergence of a new VCG within *F. oxysporum* f. sp. *radicis-lycopersici*. Thus, regularly screening VCGs and virulence of the pathogen seems necessary to monitor its genetic variation.

Fusarium oxysporum f. sp. *radicis-lycopersici* specific primers need to be further developed as the primers targeting *exo* polygalacturonase (*pgx4*, 63) failed to amplify all known VCGs of the pathogen due to considerable genetic diversity within this pathogen. Florida VCGs 0094, 0098, and 0099 were not identified by the specific primers, suggesting that a comparison of their nucleotide diversity of cell-wall-degrading enzymes (CWDEs) may not be a reliable approach to discriminate *formae speciales* of *F. oxysporum*. Instead, virulence genes conferring a specific trait to a pathogen are more likely to distinguish closely related *formae speciales* since they may have subtle nucleotide differences within a *forma specialis* but show obvious dissimilarity among *formae speciales* (98). Before primers with a higher specificity for *F. oxysporum* f. sp. *radicis-lycopersici* are developed, both pathogenicity and VCG tests should be conducted for identifying the pathogen. The laboratory pathogenicity bioassay as previously described (71,145) may be appropriate to diagnose the pathogen according to symptomology, whereas inoculum preparation is critical for the success of seedling inoculation (80).

Microsatellites developed in this study have revealed the current population structure of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida. Hendry County had a higher mean number of pairwise differences and average gene diversity when either whole microsatellite data or clone-

corrected data were used for analysis. This may be because Hendry County was the sink of migrants from Manatee and Collier Counties based on the analysis of historical migration (Figs. 2-1 and 2-4). Considering the genetic structure of each VCG and its affiliated geographical region, pairwise Fst values of VCG 0094 or 0098 between these three tomato-growing regions suggested no genetic differentiation (Table 2-6 and Table 2-7). However, the mean number of pairwise differences and average gene diversity were higher for either VCG 0094 or 0098 in Collier County, suggesting that these two VCGs might have migrated from Collier County to the other two counties. The gene diversity of VCG 0094 was higher in Collier County contradicting an earlier study by Rosewich et al. (139) showing a higher gene diversity in Hendry County. This difference may be caused by sample size bias (180) , changes in population size (129), and other demographic events acting as selection (28,164). Our study used a more equal sample size of VCG 0094 from each tomato-growing region than the previous work (139) and suggested an increasing population size in Collier County, corroborating a higher gene diversity observed in this county. However, whether other selection factors affect the gene diversity of VCG 0094 in Florida needs to be investigated further. VCG 0099 had a higher mean number of pairwise differences and average gene diversity than either VCG 0094 or 0098, suggesting its pre-existence in Florida (139).

Migration of *F. oxysporum* f. sp. *radicis-lycopersici* among tomato-growing regions counties was exhibited by population admixture and historical migration analyses (Figs. 2-1 and 2-4). High gene/genotype flow can enhance genetic diversity of a pathogen population as a result of increasing the size of genetic neighborhood, resulting in more alleles to overcome a resistance gene in the host (107,108). Moreover, *F. oxysporum* f. sp. *radicis-lycopersici* has been considered as a soilborne, airborne, and waterborne pathogen (59,74,133,140). Therefore,

limiting gene/genotype flow of *F. oxysporum* f. sp. *radicis-lycopersici* within Florida and migration of the pathogen to other tomato-growing regions is vital to reduce the break-down risk of the resistance gene *Frl*.

Of IGS, EF-1α, and the noncoding microsatellite locus, FOL185, examined in this study, the complete region of IGS was phylogenetically informative for *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* due to its fast-evolving nature (Tables 3-1 and 3-3). IGS may be used to design primers for rapid diagnosis of predominant VCGs 0094, 0098, and 0099 of *F. oxysporum* f. sp. *radicis-lycopersici* since these VCGs formed a supported clade (Fig. 3-1). Although the evolutionary rate of IGS, EF-1α, and FOL185 was not homogeneous, phylogenetic analyses showed that *F. oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* have multiple evolutionary origins (polyphylectic) but share a common ancestor (paraphyletic). At least two evolutionary origins were shown for *F. oxysporum* f. sp. *radicis-lycopersici* in agreement with a dendrogram generated from 27 microsatellite data (Fig. 2-3). The evolutionary origin of VCGs 0094, 0098, and 0099 could be independent of other VCGs of *F. oxysporum* f. sp. *radicis-lycopersici*.

Mating isolates with distinct mating-type idiomorphs from Collier County in clade 1 of the IGS phylogeny was not successful since no perithecia were observed to form, whereas up to 62.3% of pairwise loci for isolates of this county were at linkage equilibrium. Even for a mitosporic fungus, parasexual recombination and reassortment resulting from protoplast fusion can cause chromosome rearrangement (170), resulting in alleles at one locus to be randomly associated. Other factors may be required for sexual recombination because RT-PCR analysis revealed that mating type genes of *F. oxysporum* are still expressed and processed correctly (181).

Restricting the migration of VCG 0098 to other tomato-growing regions in the United States is critical in disease management of *F. oxysporum* f. sp. *radicis-lycopersici* since this VCG was recently found in Florida (139) and showed a higher level of virulence than the other VCGs used in this study. Moreover, VCG 0098 is phylogenetically closer to VCG0094 according to sequence data and VCG assays, suggesting that the pathogen might have diverged toward higher virulence. Based on this study, VCG 0098 may be used in breeding against Fusarium crown and root rot of tomato due to its high virulence and evolutionary origin.

Fusarium oxysporum f. sp. *radicis-lycopersici* is a relatively new pathogen compared to the invention of agriculture approximately 10,000 years ago (161). The mean most recent common ancestor (MRCA) of this pathogen ranges from 38 to 129 years ago when estimated from IGS, EF-1α, and FOL185 data sets. Based on the mean MRCA derived from these three loci, the pathogen might have diverged from its ancestor around the 19th or 20th century. Interestingly, tomatoes acquired a much more intensive level of domestication that occurred throughout Europe in the 18th and 19th century (9,155), and were widely cultivated by the end of the 19th century or beginning of the 20th century (123). These findings suggest that *F. oxysporum* f. sp. *radicis-lycopersici* might have coevolved with domestication of its host and been selected by the environmental and genetic uniformity of the agricultural ecosystem (161). The global population size of *F. oxysporum* f. sp. *radicis-lycopersici* has been increasing since the 1990s as revealed by the IGS, EF-1α, and FOL185 data sets. Factors that caused this increase in the population size need to be further investigated. Regularly monitoring the population structure of *F. oxysporum* f. sp. *radicis-lycopersici* is essential for disease management since a plant pathogen with a higher population size has greater evolutionary potential to break down resistance (107,108).

Silicon (Si) significantly reduced disease severity of stems 4 weeks after inoculation (Tables 4-4 and 4-6), whereas no significant difference was revealed between –Si and +Si treatments in disease severity of crown and root. Inoculum concentration significantly affected disease severity of the root, crown, and stem, but no significant interaction was revealed between Si and inoculum concentration effects (Table 4-3), suggesting the response of Si consistent over inoculum levels. The analysis of disease progress suggested that the decrease in disease severity by Si amendment probably resulted from delaying initial infection in roots and the movement of the pathogen from roots to stems (Fig. 4-3). Si contents of roots and shoots were significantly higher in tomato plants supplied with Si than those without Si amendment. Moreover, the increase in the Si content of roots was significantly correlated with the reduction of disease severity of roots, crowns, and stems (Fig. 4-4), indicating a Si-induced resistance and/or reduction of fungal colonization. The +Si treatments likely limited the basipetal spread of *F. oxysporum* f. sp. *radicis-lycopersici* from infected roots to stems (Figs. 4-3, 4-4, and 4-5). Although laboratory experiments including this study have shown Si able to alleviate biotic and abiotic stresses on tomato (2,25,32,122), further research in applying Si fertilizers for field-grown tomatoes needs to be conducted to further elucidate the effects of Si on the development of Fusarium crown and root rot.

REFERENCES

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

2. Al-Aghabary, K., Zhu, Z., and Shi, Q. H. 2004. Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. J. Plant Nutr. 27:2101-2115.

3. Aoki, T., O'Donnell, K., Homma, Y., and Lattanzi, A. R. 2003. Sudden death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex : *F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia 95:660-684.

4. Appel, D. J., and Gordon, T. R. 1996. Relationships among pathogenic and nonpathogenic isolates of *Fusarium oxysporum* based on the partial sequence of the intergenic spacer region of the ribosomal DNA. Mol. Plant-Microbe Inter. 9:125-138.

5. Armstrong, G. M., and Armstrong, J. K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. Pages 391-399 in: *Fusarium*: diseases, biology, and taxonomy. P. E. Nelson, T. A. Tousson, and R. J. Cook, eds. Pensylvania State University Press, University Park, PA.

6. Ashley, M. V., and Dow, B. D. 1994. The use of microsatellite analysis in population biology: background, methods, and potential application. Pages 185-201 in: Molecular Ecology and Evolution: Approaches and Applications. B. Schierwater, B. Streit, G. P. Wanger, and R. DeSalle, eds. Birkháuser Verlag, Basel.

7. Aston, M. J., and Jones, M. M. 1976. A study of transpiration surfaces of *Avena sterlis* L. var. algerian leaves using monosilicic acid as a tracer for water movement. Planta 130:121-129.

8. Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L., Roebroeck, E. J. A., and Waalwijk, C. 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. Phytopathology 90:891-900.

9. Bai, Y. L., and Lindhout, P. 2007. Domestication and breeding of tomatoes: What have we gained and what can we gain in the future? Ann. Bot. 100:1085-1094.

10. Balmas, V., Scherm, B., Di Primo, P., Rau, D., Marcello, A., and Migheli, Q. 2005. Molecular characterisation of vegetative compatibility groups in *Fusarium oxysporum* f. sp. *radicis-lycopersici* and f. sp. *lycopersici* by random amplification of polymorphic DNA and microsatellite-primed PCR. Eur. J. Plant Pathol. 111:1-8.

11. Beerli, P. 2004. Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. Mol. Ecol. 13:827-836.

12. Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22:341-345.

13. Benson, G. 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucl. Acids Res. 27:573-580.

14. Bogale, M., Wingfield, B. D., Wingfield, M. J., and Steenkamp, E. T. 2005. Simple sequence repeat markers for species in the *Fusarium oxysporum* complex. Mol. Ecol. Notes 5:622-624.

15. Boutin-Ganache, I., Raposo, M., Raymond, M., and Deschepper, C. F. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:25-28.

16. Brammall, R. A., and Higgins, V. J. 1988. A histological comparison of fungal colonization in tomato seedlings susceptible or resistant to *Fusarium* crown and root rot disease. Can. J. Bot. 66:915-925.

17. Brown, A. H. D., Feldman, M. W., and Nevo, E. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. Genetics 96:523-536.

18. Bull, J. J., Huelsenbeck, J. P., Cunningham, C. W., Swofford, D. L., and Waddell, P. J. 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42:384-397.

19. Cai, G., Gale, L. R., Schneider, R. W., Kistler, H. C., Davis, R. M., Elias, K. S., and Miyao, E. M. 2003. Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. Phytopathology 93:1014-1022.

20. Cai, K., Gao, D., Chen, J., and Luo, S. 2009. Probing the mechanisms of silicon-mediated pathogen resistance. Plant Sig. Behav. 4:1-3.

21. Chérif, M., Benhamou, N., and Belanger, R. R. 1991. Ultrastructural and cytochemical studies of fungal development and host reactions in cucumber plants infected by *Pythium ultimum*. Phys. Mol. Plant Pathol. 39:353-375.

22. Chérif, M., and Belanger, R. R. 1992. Use of potassium silicate amendments in recirculating nutrient solutions to suppress *Pythium ultimum* on long English cucumber. Plant Dis. 76:1008-1011.

23. Ciampi, M. B., Meyer, M. C., Costa, M. J. N., Zala, M., McDonald, B. A., and Ceresini, P. C. 2008. Genetic structure of populations of *Rhizoctonia solani* anastomosis group-1 IA from soybean in Brazil. Phytopathology 98:932-941.

24. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.

25. Dannon, E. A., and Wydra, K. 2004. Interaction between silicon amendment, bacterial wilt development and phenotype of *Ralstonia solanacearum* in tomato genotypes. Phys. Mol. Plant Pathol. 64:233-243.

26. Datnoff, L. D., Rodrigues, F. Á., and Seebold, K. W. 2007. Silicon and plant disease. Pages 233-246 in: Mineral Nutrition and Plant Disease. L. E. Datnoff, W. H. Elmer, and D. M. Huber, eds. The American Phytopathological Society Press, St. Paul, MN.

27. Datnoff, L. E., Nemec, S., and Pernezny, K. 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Cont. 5:427-431.

28. Depaulis, F., Mousset, S., and Veuille, M. 2003. Power of neutrality tests to detect bottlenecks and hitchhiking. J. Mol. Evol. 57:S190-S200.

29. Dettman, J. R., Jacobson, D. J., and Taylor, J. W. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57:2703-2720.

30. Dettman, J. R., Jacobson, D. J., Turner, E., Pringle, A., and Taylor, J. W. 2003. Reproductive isolation and phylogenetic divergence in *Neurospora*: Comparing methods of species recognition in a model eukaryote. Evolution 57:2721-2741.

31. Di Pietro, A., Madrid, M. P., Caracuel, Z., Delgado-Jarana, J., and Roncero, M. I. G. 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. Mol. Plant Pathol. 4:315-325.

32. Diogo, R. V. C., and Wydra, K. 2007. Silicon-induced basal resistance in tomato against *Ralstonia solanacearum* is related to modification of pectic cell wall polysaccharide structure. Phys. Mol. Plant Pathol. 70:120-129.

33. Drummond, A. J., Ho, S. Y. W., Phillips, M. J., and Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:699-710.

34. Drummond, A. J., and Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

35. Edwards, C. E., Arakaki, M., Quintana-Ascencio, P. F., Soltis, D. E., and Soltis, P. S. 2007. Isolation and characterization of microsatellite loci from the endangered highlands scrub hypericum (*Hypericum cumulicola*). Mol. Ecol. Notes 7:1135-1137.

36. Elias, K. S., and Schneider, R. W. 1991. Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici*. Phytopathology 81:159-162.

37. Elias, K. S., and Schneider, R. W. 1992. Genetic diversity within and among races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici* as determined by isozyme analysis. Phytopathology 82:1421-1427.

38. Elias, K. S., Zamir, D., Lichtmanpleban, T., and Katan, T. 1993. Population structure of *Fusarium oxysporum* f. sp. l*ycopersici*: Restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. Mol. Plant-Microbe Inter. 6:565-572.

39. Ellegren, H. 2004. Microsatellites: Simple sequences with complex evolution. Nature Rev. Genetics 5:435-445.

40. Elliott, C. L., and Snyder, G. H. 1991. Autoclave-induced digestion for the colorimeteric determination of silicon in rice straw. J. Agric. Food Chem. 39:1118-1119.

41. Epistein, E., and Bloom, A. J. 2005. Mineral Nutrition of Plants: Principles and Perspectives. Sinauer Associations, Sunderland, MA.

42. Epstein, E. 1994. The anomaly of silicon in plant biology. PNAS 91:11-17.

43. Epstein, E. 1999. Silicon. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50:641-664.

44. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

45. Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1:47-50.

46. Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567-1587.

47. Farris, J. S., Kallersjo, M., Kluge, A. G., and Bult, C. 1995. Testing significance of incongruence. Cladistics 10:315-319.

48. Fauteux, F., Remus-Borel, W., Menzies, J. G., and Belanger, R. R. 2005. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol. Lett. 249:1-6.

49. Fauteux, F., Chain, F., Belzile, F., Menzies, J. G., and Belanger, R. R. 2006. The protective role of silicon in the Arabidopsis-powdery mildew pathosystem. PNAS 103:17554-17559.

50. Fazio, G., Stevens, M. R., and Scott, J. W. 1999. Identification of RAPD markers linked to fusarium crown and root rot resistance (*Frl*) in tomato. Euphytica 105:205-210.

51. Fravel, D., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. New Phytol. 157:493-502.

52. Geiser, D. M., Jimenez-Gasco, M. D., Kang, S. C., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A., and O'Donnell, K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. Eur. J. Plant Pathol. 110:473-479.

53. Gillespie, D. R., and Menzies, J. G. 1993. Fungus gnats vector *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Ann. Appl. Biol. 123:539-544.

54. Glaubitz, J. C. 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes 4:309-310.

55. Gordon, T. R., and Martyn, R. D. 1997. The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol. 35:111-128.

56. Grünig, C. R., Duò, A., and Sieber, T. N. 2006. Population genetic analysis of *Phialocephala fortinii* s.l. and *Acephala applanata* in two undisturbed forests in Switzerland and evidence for new cryptic species. Fungal Genet. Biol. 43:410-421.

57. Graur, D., and Li, W.-H. 2000. Fundamentals of molecular evolution. Sinauer Associates, Inc., Sunderland, MA.

58. Hancock, J. M. 1995. The contribution of slippage-like processes to genome evolution. J. Mol. Evol. 41:1038-1047.

59. Hartman, J. R., and Fletcher, J. T. 1991. Fusarium crown and root rot of tomatoes in the UK. Plant Pathol. 40:85-92.

60. Heine, G., Tikum, G., and Horst, W. J. 2005. Silicon nutrition of tomato and bitter gourd with special emphasis on silicon distribution in root fractions. J. Plant Nutr. Soil Sci. 168:600-606.

61. Heine, G., Tikum, G., and Horst, W. J. 2007. The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. J. Exp. Bot. 58:569-577.

62. Hibar, K., Daami-Remadi, M., Ayed, F., and El-Mahjoub, M. 2007. Fusarium crown and root rot of tomato and its chemical control. Int. J. Agric. Res. 2:687-695.

63. Hirano, Y., and Arie, T. 2006. PCR-based differentiation of *Fusarium oxysporum* ff. sp. *lycopersici* and *radicis-lycopersic*i and races of *F. oxysporum* f. sp. *lycopersici*. J. Gen. Plant Pathol. 72:273-283.

64. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Circ. 347. Univ. of Calif. Agric. Exp. Station, Berkley.

65. Hovmller, M. S., Yahyaoui, A. H., Milus, E. A., and Justesen, A. F. 2008. Rapid global spread of two aggressive strains of a wheat rust fungus. Mol. Ecol. 17:3818-3826.

66. Huang, C.-H., Datnoff, L. D., Gale, L. R., and Roberts, P. D. 2009. Development of microsatellite markers for *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Mol. Ecol. Res. (Accepted).

67. Huang, C., Roberts, P. D., and Datnoff, L. E. 2008. Development of microsatellite markers for *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp *radicis-lycopersici*. (Abstr.) Phytopathology 98:S69-S70.

68. Huang, C., Roberts, P. D., and Datnoff, L. E. 2009. Phylogenetic relationships between *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* inferred from IGS, EF1-α, and a SSR locus. (Abstr.) Phytopathology 99:S56.

69. Huang, C., Roberts, P. D., Gale, L. R., Elmer, W. H., and Datnoff, L. E. 2009. Population structure of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Florida inferred from vegetative compatibility groups and microsatellites. (Abstr.) Phytopathology 99:S55-56.

70. Huelsenbeck, J. P., and Rannala, B. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227-232.

71. Jarvis, W. R., and Thorpe, H. J. 1976. Susceptibility of *Lycopersicon* species and hybrids to the foot and root rot pathogen *Fusarium oxysporum*. Plant Dis. Rep. 60:1027-1031.

72. Jarvis, W. R., and Shoemaker, R. A. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679-1680.

73. Jarvis, W. R., and Thorpe, H. J. 1981. Control of fusarium foot and root rot of tomato by soil amendment with lettuce residues. Can. J. Plant Pathol. 3:159-162.

74. Jarvis, W. R. 1988. Fusarium crown and root rot of tomatoes. Phytoprotection 69:49-64.

75. Jones, J. B., Jones, J. P., Stall, J. P., and Zitter, T. A. 1991. Compendium of tomato diseases. American Phytopathological Society: St. Paul, MN.

76. Jones, J. P., Jones, J. B., Woltz, S. S., and Scott, J. W. 1993. Influence of soil pH, nitrogen source, and transplant drenches on development of crown rot of tomato. Proc. Fla. State Hort. Soc. 106:170-172.

77. Jorge, I., Navas-Cortes, J. A., Jimenez-Diaz, R. M., and Tena, M. 2006. Cell wall degrading enzymes in Fusarium wilt of chickpea: correlation between pectinase and xylanase activities and disease development in plants infected with two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*. Can. J. Bot. 84:1395-1404.

78. Karaoglu, H., Lee, C. M. Y., and Meyer, W. 2005. Survey of simple sequence repeats in completed fungal genomes. Mol. Biol. Evol. 22:639-649.

79. Katan, T., Hadar, E., and Katan, J. 1989. Vegetative compatibility of *Fusarium oxysporum* f. sp. *dianthi* from carnation in Israel. Plant Pathol. 38:376-381.

80. Katan, T., Zamir, D., Sarfatti, M., and Katan, J. 1991. Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Phytopathology 81:255-262.

81. Katan, T. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*. Phytoparasitica 27:51-64.

82. Katan, T., and Katan, J. 1999. Vegetative compatibility grouping in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. Plant Pathol. 48:541-549.

83. Kawabe, M., Kobayashi, Y., Okada, G., Yamaguchi, I., Teraoka, T., and Arie, T. 2005. Three evolutionary lineages of tomato wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici*, based on sequences of IGS, *MAT1*, and *pg1*, are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. J. Gen. Plant Pathol. 71:263-272.

84. Kerenyi, Z., Moretti, A., Waalwijk, C., Olah, B., and Hornok, L. 2004. Mating type sequences in asexually reproducing *Fusarium* species. Appl. Environ. Microbiol. 70:4419-4423.

85. Kingman, J. F. C. 2000. Origins of the coalescent: 1974-1982. Genetics 156:1461-1463.

86. Kistler, H. C., Alabouvette, C., Baayen, R. P., Bentley, S., Brayford, D., Coddington, A., Correll, J., Daboussi, M. J., Elias, K., Fernandez, D., Gordon, T. R., Katan, T., Kim, H. G., Leslie, J. F., Martyn, R. D., Migheli, Q., Moore, N. Y., O'Donnell, K., Ploetz, R. C., Rutherford, M. A., Summerell, B., Waalwijk, C., and Woo, S. 1998. Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 88:30-32.

87. Klaasen, J. A., and Nelson, P. E. 1996. Identification of a mating population, *Gibberella nygamai* sp. nov., within the *Fusarium nygamai* anamorph. Mycologia 88:965-969.

88. Klittich, C. J. R., and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417-423.

89. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.

90. Kuhner, M. K. 2006. LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768-770.

91. Kuhner, M. K., and Smith, L. P. 2007. Comparing likelihood and Bayesian coalescent estimation of population parameters. Genetics 175:155-165.

92. Kuniyasu, K., and Yamakawa, K. 1983. Control of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* race J3 by grafting to KNVF and KVF rootstocks of interspecific hybrids between *Lycopersicon esculentum* × *L. hisutum*. Ann. Phytopathol. Soc. Jpn. 49:581-586.

93. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948.

94. Leary, J. V., and Sanchez, L. E. 1976. Mutagenesis of *Fusarium oxysporum*: analysis of the fungal genome in relation to the expression and regulation of pathogenicity. Pages 12-15 in: Biochemistry and cytology of Plant-Parasite Interaction. K. Tomiyama, J. M. Daly, I. Uritani, H. Oku, and S. Ouchi, eds. Kodansha Ltd, Tokyo.

95. Leslie, J. F. 1993. Fungal vegetative compatibility. Ann. Rev. Phytopathol. 31:127-150.

96. Leslie, J. F. 1996. Fungal vegetative compatibility - promises and prospects. Phytoparasitica 24:3-6.

97. Leslie, J. F., and Summerell, B. A. 2006. The *Fusarium* laboratory manual. Blackwell Publishing, Ames, IA.

98. Lievens, B., Rep, M., and Thomma, B. 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. Pest Manag. Sci. 64:781-788.

99. Ma, J. F., Miyake, Y., and Takahashi, E. 2001. Silicon as a beneficial element for crop plants. Pages 17-39 in: Silicon in Agriculture: Studies in Plant Science, Vol. 8. L. E. Datnoff, G. H. Snyder, and G. H. Korndorfer, eds. Elsevier Science, Amsterdam, The Netherlands.

100. Ma, J. F., and Takahashi, E. 2002. Soil, Fertilizer, and Plant Silicon Research in Japan. Elsevier Science, Amsterdam.

101. Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., and Yano, M. 2006. A silicon transporter in rice. Nature 440:688-691.

102. Maddison, W. P. 1997. Gene trees in species trees. Syst. Biol. 46:523-536.

103. Manicom, B. Q., Bar-Joseph, M., and Kotze, J. M. 1990. Molecular methods of potential use in the identification and taxonomy of filamentous fungi, particularly *Fusarium oxysporum*. Phytophylactica 22:233-240.

104. Manicom, B. Q., Barjoseph, M., Kotze, J. M., and Becker, M. M. 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f. sp. *dianthi*. Phytopathology 80:336-339.

105. Marlatt, M. L., Correll, J. C., Kaufmann, P., and Cooper, P. E. 1996. Two genetically distinct populations of *Fusarium oxysporum* f. sp. *lycopersici* race 3 in the United States. Plant Dis. 80:1336-1342.

106. Marois, J. J., and Mitchell, D. J. 1981. Effects of fumigation and fungal antagonists on the relationships of inoculum density to infection incidence and disease severity in Fusarium crown rot of tomato. Phytopathology 71:167-170.

107. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Ann. Rev. Phytopathol. 40:349-379.

108. McDonald, B. A., and Linde, C. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124:163-180.

109. McGovern, R. J., Vavrina, C. S., Noling, J. W., Datnoff, L. D., and Yonce, H. D. 1998. Evaluation of different application methods of metam sodium for management of Fusarium crown and root rot in tomato in southwest Florida. Plant Dis. 82:919-923.

110. Menzies, J. G., Koch, C., and Seywerd, F. 1990. Additions to the host ranges of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Plant Dis. 74:569-572.

111. Menzies, J. G., and Jarvis, W. R. 1994. The infestation of tomato seed by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Plant Pathol. 43:378-386.

112. Menzies, J. G., Ehret, D. L., Chérif, M., and Bélanger, R. R. 2001. Plant-related silicon research in Canada. Pages 323-341 in: Silicon in Agriculture: Studies in Plant Science, Vol. 8. L. E. Datnoff, G. H. Snyder, and G. H. Körndorfer, eds. Elsevier Science, Amsterdam, The Netherlands.

113. Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Ann. Rev. Phytopathol. 34:457-477.

114. Montarry, J., Corbiere, R., Lesueur, S., Glais, I., and Andrivon, D. 2006. Does selection by resistant hosts trigger local adaptation in plant-pathogen systems? J. Evol. Biol. 19:522-531.

115. Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY.

116. Nelson, P. E., Tousson, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An illustrated Manual for Identification. Pennsylvania State University Press, PA.

117. Nirenberg, H. I., and O'Donnell, K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90:434-458.

118. O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phyl. Evol. 7:103-116.

119. O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. PNAS 95:2044-2049.

120. Olivain, C., and Alabouvette, C. 1997. Colonization of tomato root by a non-pathogenic strain of *Fusarium oxysporum*. New Phytol. 137:481-494.

121. Olivain, C., and Alabouvette, C. 1999. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. New Phytol. 141:497-510.

122. Peaslee, D. E., and Frink, C. R. 1969. Influence of silicic acid on uptake of Mn, Al, Zn, and Cu by tomatoes (*Lycopersicum esculentum*) grown an an acid soil. Soil Sci. Soc. Am. Proc. 33:569-571.

123. Peralta, I. E., and Spooner, D. M. 2007. History, origin and early cultivation of tomato (Solanaceae). Pages 1-24 in: Genetic improvement of solanaceous crops. Volume 2: tomato. M. K. Razdan and A. K. Mattoo, eds. Science Publishers, Enfield, NH.

124. Posada, D., and Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817-818.

125. Primo, P. D., Cartia, G., and Katan, T. 2001. Vegetative compatibility and heterokaryon stability in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Italy. Plant Pathol. 50:371-382.

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

127. Prospero, S., Grunwald, N. J., Winton, L. M., and Hansen, E. M. 2009. Migration Patterns of the Emerging Plant Pathogen *Phytophthora ramorum* on the West Coast of the United States of America. Phytopathology 99:739-749.

128. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

129. Pyhäjärvi, T., García-Gil, M. R., Knurr, T., Mikkonen, M., Wachowiak, W., and Savolainen, O. 2007. Demographic history has influenced nucleotide diversity in European *Pinus sylvestris* populations. Genetics 177:1713-1724.

130. Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., and Moenne-Loccoz, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341-361.

131. Raymond, M., and Rousset, F. 1995. GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.

132. Recorbet, G., Steinberg, C., Olivain, C., Edel, V., Trouvelot, S., Dumas-Gaudot, E., Gianinazzi, S., and Alabouvette, C. 2003. Wanted: pathogenesis-related marker molecules for *Fusarium oxysporum*. New Phytol. 159:73-92.

133. Rekah, Y., Shtienberg, D., and Katan, J. 1999. Spatial distribution and temporal development of Fusarium crown and root rot of tomato and pathogen dissemination in field soil. Phytopathology 89:831-839.

134. Rekah, Y., Shtienberg, D., and Katan, J. 2001. Population dynamics of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in relation to the onset of Fusarium crown and root rot of tomato. Eur. J. Plant Pathol. 107:367-375.

135. Richmond, K. E., and Sussman, M. 2003. Got silicon? The non-essential beneficial plant nutrient. Curr. Opin. Plant Biol. 6:268-272.

136. Ronquist, F., and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

137. Rosenberg, N. A., and Nordborg, M. 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nat. Rev. Genet. 3:380-390.

138. Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4:137-138.

139. Rosewich, U. L., Pettway, R. E., Katan, T., and Kistler, H. C. 1999. Population genetic analysis corroborates dispersal of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida to Europe. Phytopathology 89:623-630.

140. Rowe, R. C., Farley, J. D., and Coplin, D. L. 1977. Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. Phytopathology 67:1513-1517.

141. Rowe, R. C. 1980. Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and field -grown tomatoes in North America and Japan. Phytopathology 70:1143-1148.

142. Rozen, S., and Skaletsky, H. 2000. Primer 3 on the WWW for general users and for biologist programmers. Pages 365-386 in: Bioinformatics methods and protocols: methods in molecular biology. S. Krawetz and S. Misener, eds. Humana Press, Totowa, NJ.

143. Sacristan, S., and Garcia-Arenal, F. 2008. The evolution of virulence and pathogenicity in plant pathogen populations. Mol. Plant Pathol. 9:369-384.

144. Samuels, A. L., Glass, A. D. M., Ehret, D. L., and Menzies, J. G. 1991. Mobility and deposition of silicon in cucumber plants. Plant Cell Environ. 14:485-492.

145. Sanchez, L. E., Endo, R. M., and Leary, J. V. 1975. A rapid technique for identifying the clones of *Fusarium oxysporum* f. sp. *lycopersici* causing crown-and root-rot of tomato. Phytopathology 65:726-727.

146. Sato, R., and Araki, T. 1974. On the tomato root-rot disease occurring under vinyl-house conditions in southern Hokkaido. Annu. Rep. Soc. Plant Prot. North Jpn. 25:5-13.

147. Schweigkofler, W., O'Donnell, K., and Garbelotto, M. 2004. Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. Appl. Environ. Microbiol. 70:3512-3520.

148. Scott, J. W. 1999. University of Florida tomato breeding accomplishments and future directions. Soil Crop Sci. Soc. Fla. Proc. 58:8-11.

149. Scott, J. W., and Jones, J. P. 2000. Fla. 7775 and Fla. 7781: Tomato breeding lines resistant to Fusarium crown and root rot. Hortscience 35:1183-1184.

150. Scott, J. W., and Gardner, R. G. 2007. Breeding for resistance to fungal pathogens. Pages 421-456 in: Genetic improvement of solanaceous crops. Volume 2: tomato. M. K. Razdan and A. K. Mattoo, eds. Science Publishers, Enfield, NH.

151. Scott, J. W. 2008. Fresh market tomato breeding in the USA. Acta Horticult. 789:21-25.

152. Sexton, A. C., and Howlett, B. J. 2004. Microsatellite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian canola fields. Curr. Genet. 46:357-365.

153. Shackelton, L. A., Rambaut, A., Pybus, O. G., and Holmes, E. C. 2006. JC virus evolution and its association with human populations. J. Virol. 80:9928-9933.

154. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilizer on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

155. Sims, W. L. 1980. History of tomato production for industry around the world. Acta Horticul. 100:25-26.

156. Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

157. Smith, J. M., Smith, N. H., Orourke, M., and Spratt, B. G. 1993. How clonal are bacteria? PNAS 90:4384-4388.

158. Sommer, M., Kaczorek, D., Kuzyakov, Y., and Breuer, J. 2006. Silicon pools and fluxes in soils and landscapes - a review. J. Plant Nutr. Soil Sci. 169:310-329.

159. Sonoda, R. M. 1976. The occurrence of a Fusarium root rot of tomato in south Florida. Plant Dis. Rep. 60:271-274.

160. Stukenbrock, E. H., Banke, S., and McDonald, B. A. 2006. Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. Mol. Ecol. 15:2895-2904.

161. Stukenbrock, E. H., and McDonald, B. A. 2008. The origins of plant pathogens in agro-ecosystems. Ann. Rev. Phytopathol. 46:75-100.

162. Swofford, D. L. 2001. PAUP*. Phylogenetic Analysis Using Parasimony (*and Other Methods). v.4 ed. Sinauer Associates, Sunderland, MA.

163. Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437-460.

164. Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

165. Tajima, F. 1993. Measurment of DNA polymorphism. Pages 37-59 in: Mechanisms of Molecular Evolution: Introduction to Molecular Paleopopulation Biology. N. Takahata and A. G. Clark, eds. Sinauer Associates, Sunderland, MA.

166. Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.

167. Tautz, D., and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl. Acids Res. 12:4127-4138.

168. Taylor, J. W., Jacobson, D. J., and Fisher, M. C. 1999. The evolution of asexual fungi: Reproduction, speciation and classification. Ann. Rev. Phytopathol. 37:197-246.

169. Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31:21-32.

170. Teunissen, H. A. S., Verkooijen, J., Cornelissen, B. J. C., and Haring, M. A. 2002. Genetic exchange of avirulence determinants and extensive karyotype rearrangements in parasexual recombinants of *Fusarium oxysporum*. Mol. Gen. Genet. 268:298-310.

171. Validov, S. Z., Qil, S., Azarova, T., Kamilova, F., and Lugtenberg, B. J. J. 2006. Heterogeneity of *Fusarium oxysporum* strains isolated as formae specialis *radicis-lycopersici*. Bulletin OILB/SROP 29:151-155.

172. van der Does, H. C., Lievens, B., Claes, L., Houterman, P. M., Cornelissen, B. J. C., and Rep, M. 2008. The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. Environ. Microbiol. 10:1475-1485.

173. van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

174. Walker, J. C. 1971. Fusarium wilt of tomato. Monogr. 6. American Phytopathology Society, St. Paul, MN.

175. Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. J. American Stat. Assoc. 58:236-244.

176. Woudt, L. P., Neuvel, A., Sikkema, A., Vangrinsven, M., Demilliano, W. A. J., Campbell, C. L., and Leslie, J. F. 1995. Genetic variation in *Fusarium oxysporum* from cyclamen. Phytopathology 85:1348-1355.

177. Wright, G. K., Pascoc, I., van Heeswijck, R., Guest, D., and Wimalajeewa, S. 1992. Characterisation of isolates of *Fusarium oxysporum* from carnations in Victoria. Acta Horticul. 307:65-72.

178. Xu, L., Nonomura, T., Suzuki, S., Kitagawa, Y., Tajima, H., Okada, K., Kusakari, S., Matsuda, Y., and Toyoda, H. 2006. Symptomatic evidence for differential root invasion by Fusarium crown and root rot pathogens between common tomato *Lycopersicon esculentum* and its varieties. J. Phytopathol. 154:577-586.

179. Yoshida, S., Ohnishi, Y., and Kitagishi, K. 1962. Chemical forms, mobility and deposition of silicon in rice plant. Soil Sci. Plant Nutr. 8:107-113.

180. Yu, N., Jensen-Seaman, M. I., Chemnick, L., Ryder, O., and Li, W. H. 2004. Nucleotide diversity in gorillas. Genetics 166:1375-1383.

181. Yun, S. H., Arie, T., Kaneko, I., Yoder, O. C., and Turgeon, B. G. 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella*/*Fusarium* species. Fungal Gen. Biol. 31:7-20.

182. Zane, L., Bargelloni, L., and Patarnello, T. 2002. Strategies for microsatellite isolation: a review. Mol. Ecol. 11:1-16.

183. Zheng, Y., Zhang, G., Lin, F., Wang, Z., Jin, G., Yang, L., Wang, Y., Chen, X., Xu, Z., Zhao, X., Wang, H., Lu, J., Lu, G., and Wu, W. 2008. Development of microsatellite markers and construction of genetic map in rice blast pathogen *Magnaporthe grisea*. Fungal Gen. and Biol. 45:1340-1347.

184. Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. The University of Texas, Austin.

BIOGRAPHICAL SKETCH

Cheng-Hua Huang was born in Taiwan. He received his bachelor's and master's degrees in soil science in Taiwan. His thesis is entitled "Effects of Nitrogen Forms and Organic Acids on the Infection of Gray Mold on Gloxinia by *Botrytis cinerea*." From 2002 to 2005, he was employed at the MOA International Foundation of Nature Ecology in Taiwan, where he was an organic inspector interacting with hundreds of organic farmers. Meanwhile, he became aware of the importance of plant pathology, leading him to pursue an advanced degree in the United States. In 2005, his major advisor, Dr. Pamela D. Roberts, kindly provided an assistantship for his work on *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of Fusarium crown and root rot of tomato. His co-advisor, Dr. Lawrence E. Datnoff, enlightened him on the research of silicon effects on plant disease.

Cheng-Hua received his Ph.D. from the University of Florida in the fall of 2009. He plans to continue studying phylogenetics, population genetics, and bio-based control strategies of plant pathogens beginning in January 2010 as a post-doc with Dr. Gary E. Vallad at the Gulf Coast Research and Education Center, UF/IFAS.