HOST RANGE AND PHYLOGENETIC DIVERSITY OF *CORYNESPORA CASSIICOLA*, CAUSE OF TARGET SPOT OF COTTON IN THE SOUTHEASTERN U.S.

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Abstract

Corynespora cassiicola is a ubiquitous fungus causing emerging diseases on crops worldwide including target spot of cotton, which has been rapidly increasing in incidence and severity throughout the southeastern U.S. since 2005. This increase in target spot has also been evident in tomato and soybean within the same region. Our aim is to understand the emergence of target spot by comparing the phylogenetic relationships of isolates from cotton with other hosts and determining the host range of these isolates. Isolates were collected from cotton, soybean, tomato, pepper, cucumber, *Hydrangea*, and *Mandevilla*. A total of 1709 nucleotides from four gene regions among the 39 isolates were sequenced to determine genetic relationships. Across the four gene regions, *C. cassiicola* from the southeastern U.S. clustered based on host of origin, regardless of geographic location. No genetic diversity among isolates from cotton in the southeastern U.S. was found and showed them to be genetically distinct from isolates of different hosts of origin. Thirty-two isolates from seven host species were tested for pathogenicity on cotton, soybean, tomato, and cucumber cultivars. Greenhouse experiments revealed significant differences among isolates. Isolates originally isolated from cotton were more aggressive on cotton than isolates from other hosts. Soybean, tomato, and cucumber isolates were only aggressive toward the same host from which they originated, indicating evidence of host specialization among these isolates. These results suggest that emerging epidemics of target spot are caused by the introduction of host-specialized isolates or the evolution of more aggressive strains.

Introduction

Emerging diseases are a constant threat to agriculture. *Corynespora cassiicola* is a ubiquitous fungal plant pathogen causing emerging diseases on economically important crops globally. Since 2005, target spot of cotton caused by *C. cassiicola*, has been significantly increasing in severity and incidence throughout the southeastern U.S. (Fulmer et al., 2012). The disease induces distinct symptoms including a target-like appearance, as well as premature defoliations that could reach of up to 70%. In the presence of the described symptoms, the disease can cause significant reductions in the yield of up to 224+ kilograms of lint per hectare (kg lint/ha) resulting in its economic importance (Hagan, 2014). The disease was previously reported on cotton in Mississippi in 1961 and identified *C. cassiicola* as the causal pathogen (Jones, 1961). Unlike the current emergence, *C. cassiicola* had not yet caused a major epidemic and thus, the most effective control measures have not been fully established. Of additional concern is that the pathogen has recently been shown to be the cause of an increasing disease intensity on tomato and soybean within the same region (Koenning et al., 2006).

Understanding the biology of this pathogen is critical to the development of management strategies. Few studies have addressed the population biology of *C. cassiicola*. The most comprehensive to date conducted by Dixon et al. (2009) sequenced 4 genes from 143 isolates across host plant species, and tropical and subtropical regions of the world. The resulting phylogenetic tree revealed six well-supported clades, also known as phylogenetic lineages (PL), geographically distributed throughout the world, but correlating with host of origin and pathogenicity. Despite the number of isolates used, those from cotton were not included, and all soybean were from outside the U.S. These data would be critical to knowing the relationships between isolates from differing hosts, and more specifically how the cotton populations are related to other populations of *C. cassiicola* and to which PL they belong. The characterization of additional isolates from the southeastern U.S. from many hosts would also help determine if this same population is causing emerging diseases on economically important crops, especially cotton, in the southeastern U.S.

Studying the population biology of *C. cassiicola* would provide information regarding the cause of disease emergence, whether through an introduction, the evolution of a more aggressive strain, change in host susceptibility, or an alteration of the environment. Moreover, genetic diversity can be identified which would help breeders in the

development of resistant cultivars of cotton, as well as chemical fungicides to apply based on pathogen population diversity for the emerging diseases. Host range of isolates would also be determined, which could help farmers decide which crops should and should not be rotated with cotton.

This study aims to understand the emergence of target spot of cotton by determining the evolutionary relationships of *C. cassiicola* on cotton with other populations. This will be accomplished by comparing the phylogenetic relationship of isolates from cotton with tomato, soybean, and other host plants. We will then characterize genetic diversity within each of the identified populations. Subsequently, host range will be determined by testing the pathogenicity and virulence of *C. cassiicola* populations from cotton, soybean, and tomato on crops grown in the southeastern U.S.

Materials and Methods

Determining the phylogenetic relationships of isolates of *C. cassiicola* from cotton with isolates from other hosts

A total of 39 isolates were collected from leaves and fruits with target spot symptoms from different cultivars of cotton (15), soybean (10), tomato (6), pepper (3), cucumber (1), *Hydrangea* (2), and *Mandevilla* (2) throughout FL, GA, TN, and VA. Deoxyribonucleic acid (DNA) was extracted using a DNeasy Plant Kit (Qiagen) following the manufacturer's protocol. It was then stored at -20°C until needed.

Four gene regions were sequenced from each isolate and compared to determine genetic relationships. These included: *actin*, the internal transcribed spacer region (ITS), and two hypervariable loci (*caa5* and *ga4*) (Dixon et al., 2009). Genes were amplified using the polymerase chain reaction (PCR) and amplicons were sequenced at the Georgia Genomics Facility (GGF) (University of Georgia, Athens, GA). The generated sequences were then visually edited in Geneious v.6 (Biomatters) and aligned for comparison using ClustalW (Kearse et al., 2012).In addition to the sequences obtained from isolates in this study, 23 isolates representative of the previously determined major lineages (PL) as well as isolates from Brazil were acquired from the publicly available database GenBank for comparison (Déon et al., 2014; Dixon et al., 2009). *Corynespora olivacea* was used as an outgroup to help organize the tree. Phylogenetic relationships were determined using the program MEGA5 (Tamura et al., 2011). Statistical support for each cluster was determined by bootstrap analysis (500 replicates).

Characterizing the genetic diversity of *C. cassiicola* from cotton

Genetic diversity and relationships within clusters were determined by constructing a haplotype network using the program TCS (Clement et al., 2000). The network shows how different isolates are related to each other based on sequence similarity.

Determining the host range of *C. cassiicola* isolates from cotton and pathogenicity of isolates from other hosts to cotton in the southeastern U.S.

Thirty-two isolates were tested for host range on cotton (DP1252, Phy367, Phy427, and Phy499), soybean (ASGROW1024631, ASGROW1024638, and PIONEER95Y70), tomato (Bonnie best, FL47, and FL91), and cucumber (Straight8) cultivars. The isolates represented diverse host origins and geographic locations. Seedlings of the different cultivars were grown in the South Milledge Greenhouse (University of Georgia, Athens, GA). For each cultivar there were three replicates with three seedlings per pot. In total, 3,267 seedlings were inoculated with a fungal spore suspension (20,000 conidia/mL) or a water control until runoff at the two to four true-leaf stage, then evaluated seven days after inoculation. The disease rating score of 0-3 developed by Onesirosan et al. (1975) was used, where: 0 = symptomless, no lesions on leaves or stems; 1 = weakly virulent or hypersensitive response, a few to many non-expanding pinpoint lesions; 2 = moderately virulent, many expanding lesions some coalescing but not resulting in a blighting effect; and 3 = highly virulent, lesions spreading to form large areas of dead tissue resulting in a blighting effect. Disease severity was calculated as the average disease rating for all replicates in a treatment. Disease incidence was recorded as the number of plants showing symptoms per treatment. The experiment was repeated twice. Statistically significant differences among treatments were determined by analysis of variance (ANOVA). Mean separations were performed in JMP Pro 11 (SAS Institute, Inc.) using Tukey's HSD to determine how treatments, if significant, differed from each other.

Results and Discussion

Across the four gene regions, no detectable diversity was found among the cotton isolates from the southeastern U.S. (Figure 1). The cotton isolate from Brazil was different from the U.S. isolate and grouped with soybean isolates from Brazil and the U.S. All cotton and soybean isolates from the southeastern U.S. and Brazil group within PL1, yet a soybean isolate from Guam belonged to PL3. Cotton and soybean isolates were distinct from tomato, pepper, and *Mandevilla* from the southeastern U.S., which were identical to each other for the four gene regions and clustered within PL4. Isolates from *Hydrangea* in the southeastern U.S. were not identical to any other isolates and belonged to two different clades.

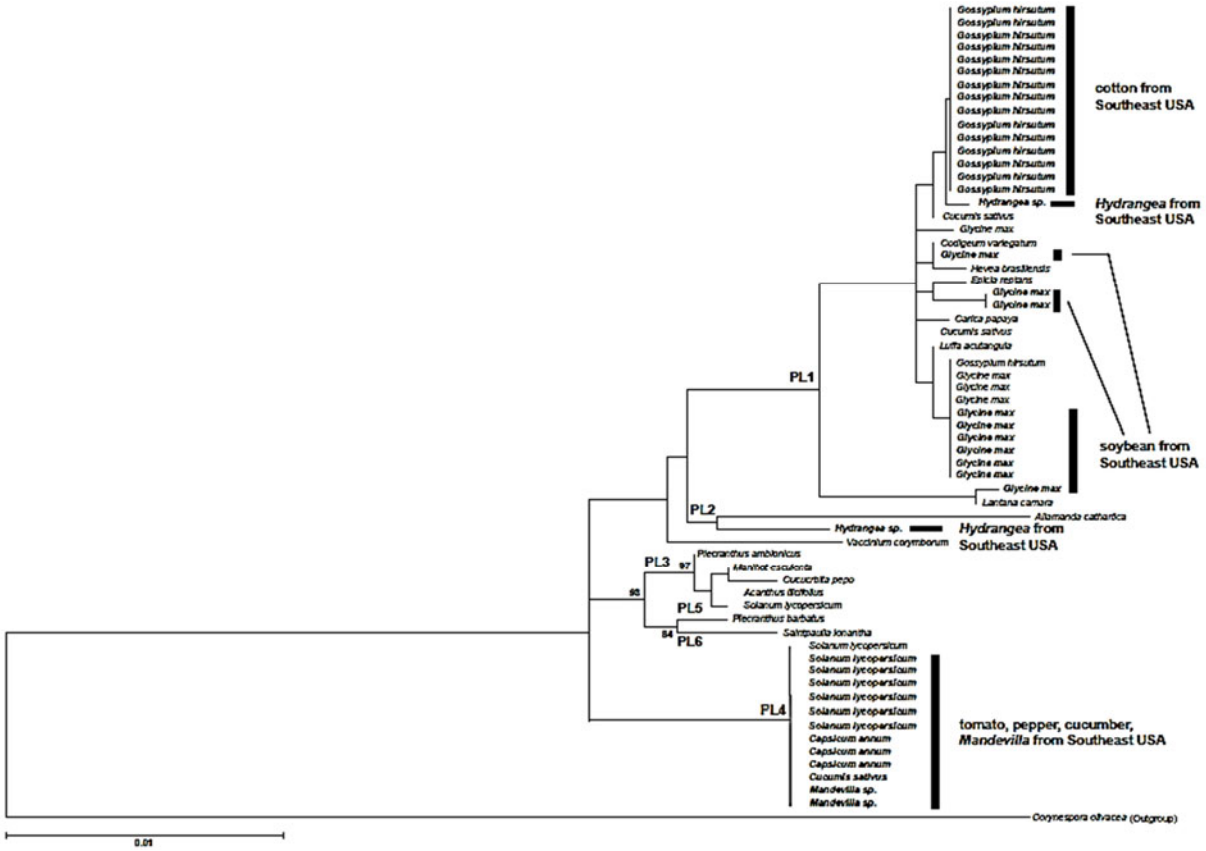

Figure 1. Genetic tree of *C. cassiicola* based on *actin,* ITS, *caa5*, and *ga4* showing genetic relationships. Original host and sampling location of isolates and statistical support for branches ≥70% are indicated. Isolates collected and sequenced by this project are in bold, while phylogenetic lineages (PL) from Dixon et al. (2009) are noted on initial branches of each lineage.

To further identify the relationships among populations of *C. cassiicola*, a haplotype network was constructed using the same sequence data from the phylogenetic tree (Figure 2). A haplotype is a unique sequence at a given genetic locus, meaning that individuals, in this case isolates, sharing this sequence have an identical haplotype. The network shows how isolates are related based on sequence, or haplotype similarity. In this study, populations of *C. cassiicola* from cotton, tomato, and cucumber from the southeastern U.S. were each represented by single haplotypes while both soybean and *Hydrangea* isolates were more diverse. Evidence of recombination suggested by loops in the network, was found within PL1. This suggests that these populations are exchanging genes and mating.

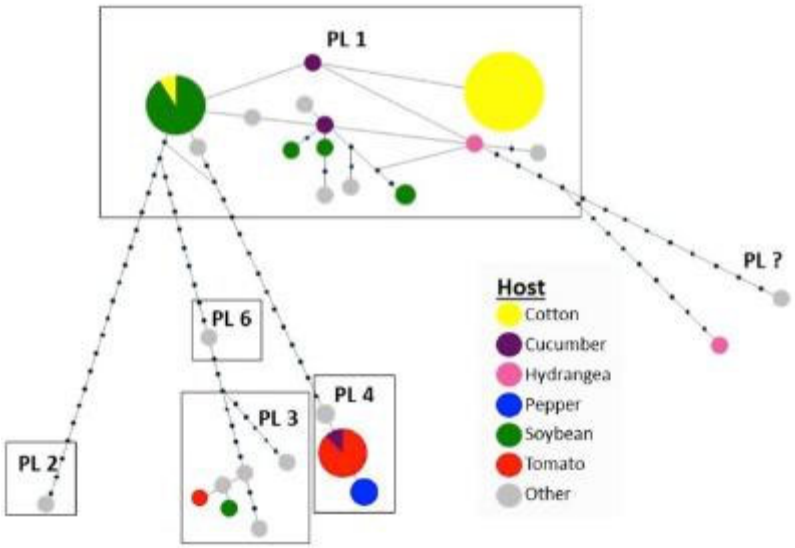

Figure 2. Haplotype network within *C. cassiicola* populations. Each large circle represents a unique haplotype with size being proportional to frequency. The color shown for each haplotype is based on the host of origin (see key). Small dots indicate a single change, or mutation, in the gene sequence. Each loop in the network indicates recombination.

Statistical analyses (ANOVA) showed significant differences in the severity and incidence of target spot by isolates from different hosts of origin on inoculated plant hosts (Figure 3). Mean comparison analyses (Tukey's HSD at α = 0.05) revealed that severity and incidence of *C. cassiicola* isolates were different on each of the inoculated hosts. Among the inoculated hosts, both cotton and tomato are most susceptible to *C. cassiicola* infections, regardless of the host of origin. However, *C. cassiicola* was most aggressive when inoculated on the same host as the host of origin. Isolates from cotton were most aggressive on cotton but less aggressive on other hosts.

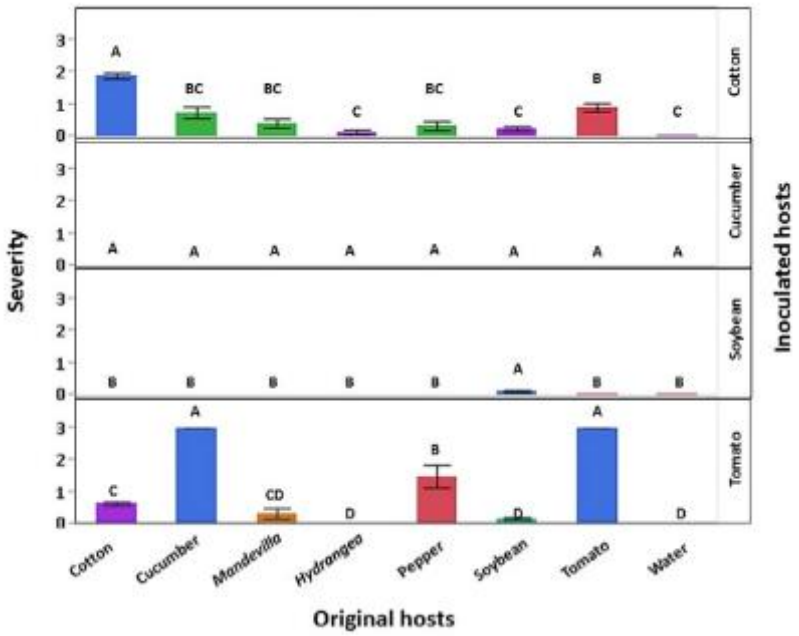

Figure 3. Mean disease severity of *C. cassiicola* infection by isolates from various plant hosts onto three replicates of the different host crops. Treatments with bars of the same color and letter above the bar were not statistically different (Tukey's HSD at α = 0.05).

Summary

C. cassiicola from cotton in the southeastern U.S. is not genetically diverse and is distinct from populations from other hosts. Yet, some genetic diversity was exhibited among soybean isolates from the U.S. Cotton isolates were distinct from soybean isolates; however, additional sampling of soybean isolates may reveal some similarity to cotton isolates. Our preliminary results suggest that the emerging cotton epidemics are likely caused by a recent introduction or the evolution of an aggressive strain. Additional markers with greater polymorphism are needed to identify if any variation exists among cotton isolates. In addition, the *C. cassiicola* populations causing emerging diseases in the southeastern U.S. tend to be host specific; therefore, populations on soybean, tomato, and cotton are likely to only cause severe diseases on the same host of origin.

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