COEXISTENCE OF SIBLING SPECIES OF CERCOSPORA CAUSING GRAY LEAF SPOT ON MAIZE IN SOUTHERN NEW YORK STATE

A Thesis

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ABSTRACT

Two *Cercospora* sibling species, *C. zeae-maydis* (*Czm*) and *C. zeina* (*Cz*), are recognized as the causal agents of gray leaf spot (GLS) of maize (*Zea mays*). A report from 1998 indicated the presence of the two species in South New York State. An intensive survey was conducted in central and southern New York State in the maize growing season in 2006. A total of 689 *Cercospora* isolates were obtained from 108 leaf lesions associated with GLS from 25 fields at 54 sampling sites across seven watersheds. Of the *Cercospora* isolates, 648 (94%) were recovered from 81 lesions collected from the Chemung, Tioga, and Owego-Wappasening watersheds. The isolates could be differentiated to two groups based on morphological traits. Two species-specific histone H3 primers were used to characterize 112 isolates representative of the two groups, confirming 32 and 77 isolates to be *Czm* and *Cz*, respectively. Among the 25 fields in which GLS pathogens were detected, only one species was detected in 16 fields, while both species were found in nine of the fields. Both *Czm* and *Cz* were isolated from three of the 81 lesions for which multiple isolates were analyzed. After adjusting for the detection probabilities, the occurrence of *Czm* and *Cz* were modeled by logistic regression based on the presence / absence binary data corresponding to various geographic variables. The results revealed that latitude was the most significant (P=0.0262) predictive variable for *Czm* occurrence, with a statistically significant positive coefficient of 0.91 and an odds ratio of 6.2 for latitude zone in a range from 42°00'N to 42°15'N. This indicated that *Czm* was more likely to be detected in southern than central part of New York State. Logistic regression model fitted to the observed data of *Cz* occurrence revealed that watershed was the only significant predictor of the probability of *Cz* occurrence. Based on the parameter estimates (β) and odds ratio (OR) for Chemung (β=2.52, OR=16.5) and Seneca

(β= -2.59, OR=0.1) watersheds in final logistic regression model, it was suggested that *Cz* was more likely to be detected in Chemung, while it was less likely to be detected in Seneca. These results might be explained by the differences in climatic conditions and weather trends observed between Chemung and Aurora. Additionally, there was a positive and moderate interspecific association between *Czm* and *Cz* occurrence across the 54 sampling sites. These results suggested that the *Cz* population, considered a new-comer, has acclimated to the new environment and responded similarly to *Czm* to host and environmental variations. However, the higher frequency of *Cz* detection in the Southern Tier of New York State, especially in Chemung watershed, implied that, in addition to temperature and latitude factors, there is a specific environmental or geographical condition around this region more favorable for the colonization of *Cz* than *Czm*.

BIOGRAPHICAL SKETCH

Lin-Si Hsieh graduated from National Pingtung University of Science & Technology in 1987 with a B.S. in plant protection. In 1994, he earned the M.S. degree in plant pathology from the National Taiwan University. During his studying and working at National Taiwan University from 1989 to 1997, his research interest mainly lay in the general area of taxonomy of fungi, more specially, the identification and taxonomic study of the entomopathogenic fungi based on morphological characteristics and isozyme profiles.

After four years as a post-master's fellow at the National Taiwan University and publishing a monograph on the entomopathogenic fungi of Taiwan in 1997, Hsieh joined a NGO and was involved in promoting natural resources/diversity conservation in Taiwan. From 2002 to 2003, he worked as a graduate research assistant at the National Chiayi University, conducting a project to survey and document Taiwan's aboriginal handicrafts. Hsieh's work climaxed by the publication of a book entitled *Introduction of Contemporary Taiwanese Aboriginal Craftsmen*, as an archival source to conserve and advance indigenous people's traditional art and culture.

Later in 2005, Hsieh was accepted by Cornell for graduate program in plant pathology and plant-microbe biology. Since then his interests have turned to the study of population biology of *Cercospora* fungi. He has currently completed the research project on the coexistence of two sibling species of *Cercospora* causing gray leaf spot of maize in the Southern Tier of New York State.

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CHAPTER 1

COMPARATIVE BIOLOGY AND TAXONOMY OF CERCOSPORA SPECIES INFECTING MAIZE

1.1 Overview of gray leaf spot disease on maize

1.1.1 Symptoms

Gray leaf spot (GLS), a foliar disease of maize (*Zea mays* L.), was first discovered by Tehon and Daniels (1925) in Illinois, USA, in 1924. It can be easily identified by a few diagnostic symptoms on the leaves in susceptible hybrids, such as the presence of light tan, vein-limited, rectangular necrotic lesions, a yellow halo surrounding the lesion, and the appearance of extensive, grayish leaf blighting with silver-gray cast as the disease progress under favorable conditions (Figure 1-1). Abundant sporulation can usually be observed on necrotic leaf lesions. Many small dark gray spots are typically arranged in rows, parallel to the long leaf axis between veins (Figure 1-2A). These dark gray spots are aggregated conidiophores bearing fusiform conidia that emerge through stomatal pores in the plant epidermis (Figure 1-2B).

1.1.2 Epidemiology

The disease causes loss in photosynthetic leaf area and thus reduction in carbohydrate availability for grain filling, resulting in the reduction in ear and kernel size. In addition, heavily diseased maize plants are subject to saprophytic stalk rot fungi, leading to severe lodging and excessive yield losses (Stromberg, 1986; Ward & Nowell, 1998). Further, GLS epidemics are usually more serious in low-lying areas and river bottoms where there are more favorable conditions for the development and spread of the disease (Lipps & Mills, 2001; De Wolf, 2002).

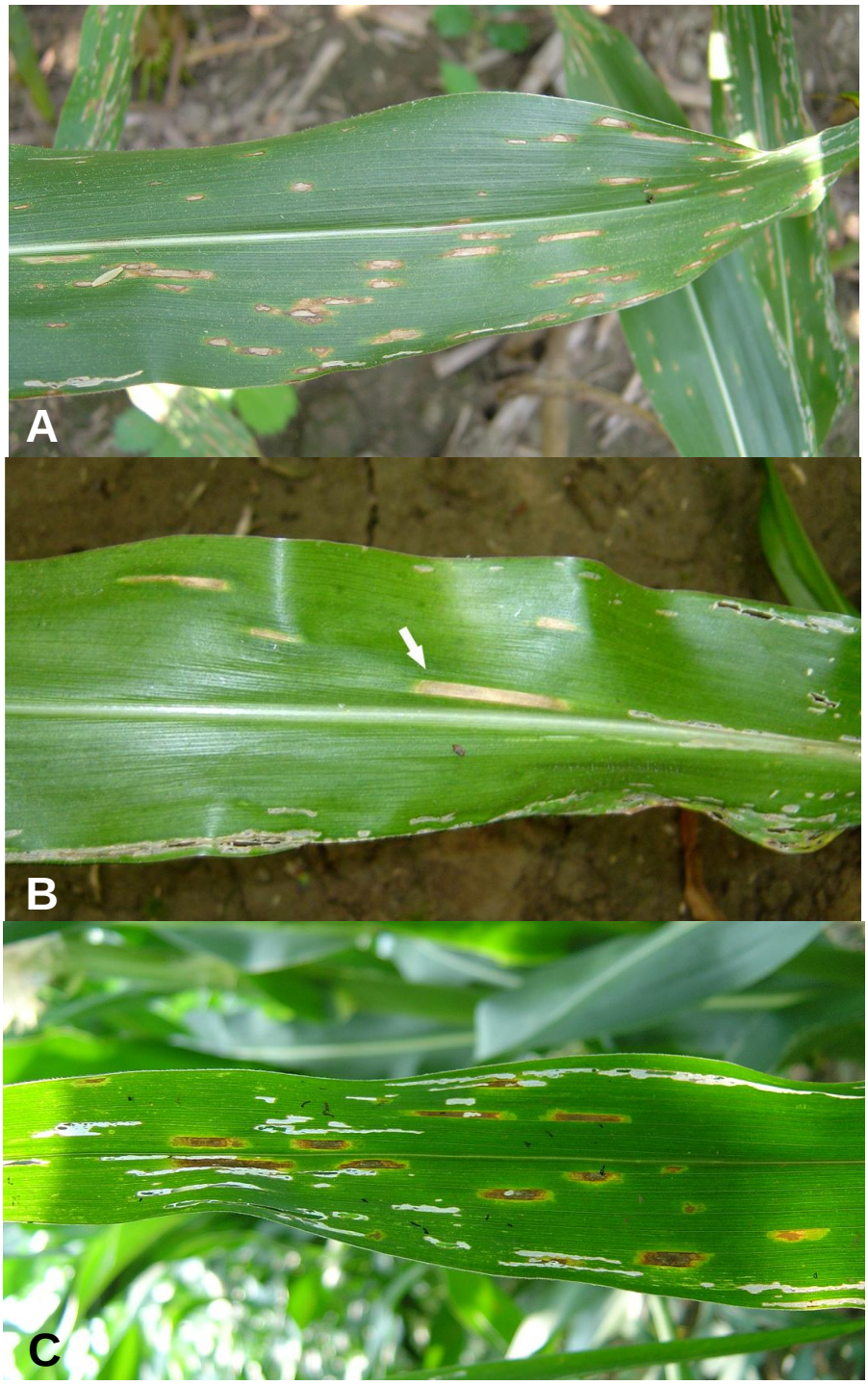

Figure 1-1. Gray leaf spot lesions on the lower corn leaf. A. Brownish tan spots on leaf surface with decreased leaf lesion size. B. Close-up of typical rectangular lesion (arrow), which is limited by leaf veins. C. The margins of leaf spots are surrounded by chlorotic (yellow) halos on leaf underside when the leaf is held up to the light.

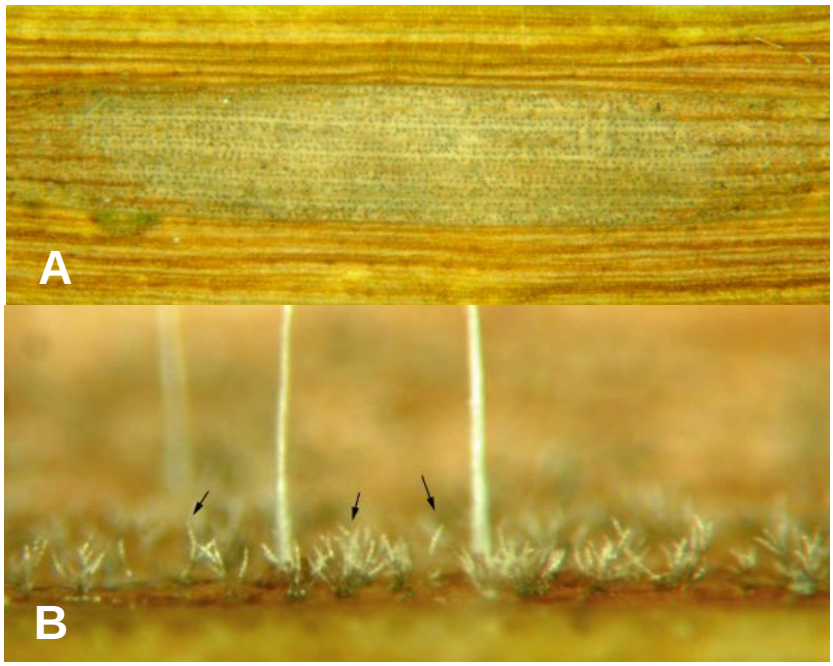

Figure 1-2. Characteristics of fungal sporulation on gray leaf spot lesion. A. Numerous dark gray spots arrange in rows parallel to the long axis of leaf between veins. B. Close-up of gray leaf spot lesion showing clusters of conidiophores and sporulation. Long, fusiform conidia are produced from aggregated conidiophores fasciles (arrows).

For almost one-half century, this disease was not a problem to maize production, though it had indeed been sporadically reported in several countries across Asia, Africa, North America, and Central and South America (Latterell & Rossi, 1983; Lipps, 1998; Ward *et al*., 1999; Ayodele *et al*., 2006). By the mid 1970's, however, because the minimum- and no-tillage practices were extensively adopted, along with cultivation of susceptible hybrids, the prevalence and severity of the disease had dramatically increased. Over the past 30 years, GLS has been reported in temperate, subtropical, and mid-altitude maize-growing areas worldwide and become a yield-limiting disease especially in the U.S. Corn Belt and Africa. Though foliar fungicides, risk assessment models (Paul & Munkvold, 2004), and moderately resistant hybrids (Menkir & Ayodele, 2005) have been continuously developed and applied for integrated management of GLS, it is now still recognized as one of the most

significant diseases of maize worldwide. It poses a serious threat to commercial maize productions in China, sub-Saharan Africa, and eastern USA, as well as to sustainable food security in developing countries (Li *et al*., 2005; Coates & White, 1995; Ward *et al*., 1999).

1.2 Taxonomic position of the genus Cercospora

The taxonomy of the genus *Cercospora* is a shifting system. *Cercospora* species are generally characterized by the acicular, hyaline, and septate conidia with a conspicuous hilum produced on pigmented, unbranched, septated, smooth conidiophores (Chupp, 1953). Based on morphological characteristic, *C. zeae-maydis* and *C. zeina* are classified to division Ascomycota, subdivision Pezizomycotina, class Dothideomycetes, order Capnodiales, family mitosporic Mycosphaerellaceae, genus *Cercospora*.

1.3 Comparative biology of C. zeae-maydis and C. zeina

1.3.1 Background

The genus *Cercospora* Fresenius is one of the largest genera of hyphomycetes with at least 659 species and 281 species regarded as synonyms of *C. apii* (Crous & Braun, 2003). *Cercospora* species are generally pathogenic to a wide spectrum of plant species. They affect many economically important crops, such as maize, banana, rice, coffee, sugarbeet, soybean and tobacco (Daub & Ehrenshaft, 2000; Daub *et al*., 2005), eliciting leaf spot or leaf blight symptoms. Though displaying comparatively homogeneous symptoms on their respective hosts, *Cercospora* species are actually highly heterogeneous in phenotypic and genetic characteristics, causing taxonomic ambiguity and uncertainty.

C. zeae-maydis was first reported by Tehon and Daniels in 1924 (Tehon & Daniels, 1925; Chupp, 1953; Ward *et al*, 1999) as a new species and a polycyclic facultative pathogen (Chupp, 1953; Stromberg & Donahue, 1986) in the genus *Cercospora* and a member of the family Mycosphaerellaceae. For a long time, *C. zeae-maydis* was mainly considered the culprit responsible for the worldwide occurrence of GLS, while the related sorghum pathogens, *C. sorghi* complex (*C. sorghi* Ellis & Everh and *C. sorghi* var. *maydis* Ellis & Everh) (Ellis & Everh., 1887; Ward & Nowell, 1998) and an unnamed *Cercospora* sp. related to *C. apii* complex (Crous *et al*., 2006) were also suspected of playing a role due to their association with GLS (Wang & Dunkle, 1998; Crous *et al*., 2006). However, the populations of *C. zeae-maydi*s have been under represented in traditional morphological taxonomy. Intraspecific variability in cultural, structural, and metabolic characteristics among isolates of *C. zeae-maydi*s has been reported previously (Latterell & Rossi, 1974, 1983; Carson *et al*., 1997). It was only in 2006 that the taxonomic position of *C. zeae-maydi*s was firmly established (Crous *et al*., 2006). The causal organisms of GLS are indeed two morphologically similar but physiologically and phylogenetically different fungal species, *Cercospora zeae-maydis* Tehon & Daniels and *C. zeina* Crous & Braun (Wang *et a*l, 1998; Dunkle & Levy, 2000; Goodwin *et al*, 2001; Crous *et al*, 2006).

Like most of the species in the genus *Cercospora*, *C. zeae-maydis* and *C. zeina* have no confirmed sexual stage in nature or *in vitro* pairings and are treated as clonal species. However, based on phylogenetic and mating type gene analyses of a variety of *Cercospora* species (Goodwin *et al*., 2001; Groenewald *et al*, 2006) and an earlier unconfirmed report of a field collection (Latterell & Rossi, 1977), both species are related to the teleomorphic genus *Mycosphaerella*.

1.3.2 Phylogeny, biogeography and population biology

The first evidence differentiating the *Cercospora* species of interest emerged from analyses of genetic variability of U.S. and African *C. zeae-maydis* isolates as well as *C. sorghi* by comparing their AFLPs profiles and partial sequences of ITS-5.8S rDNA (Wang *et al*., 1998). U.S. *C. zeae-maydis* isolates could not only be easily distinguished from *C. sorghi*, but were further separated into two divergent monophyletic groups, designated groups I and II, based on the inter-group differences in rDNA sequence and significantly higher intra-group genetic similarity (Wang *et al*., 1998). Furthermore, the African *C. zeae-maydis* population and the U.S. *C. zeae-maydis* group II population shared a high level of AFLP profile similarity (97.6%) and carried no private alleles based on the analysis of 85 AFLP loci. Thus, both populations were regarded as conspecific and considered to have been derived from a very recent common origin (Dunkle & Levy, 2000). These findings were further corroborated by a similar result obtained from an intensive comparison of complete ITS (ITS1-5.8S-ITS2 rDNA) sequences from the two groups. This study demonstrated that the two groups differed from each other by seven nucleotides, greater than an average of 5.3 nucleotides between species among 12 taxa of *Cercospora* (Goodwin *et al*, 2001). More recently, Crous *et al*. (2006) analyzed the DNA sequences of ITS1-5.8S-ITS2, elongation factor 1-α, histone H3, actin, and calmodulin, showing that *C. zeae-maydis* groups I and II isolates formed two distinct phylogenetic clades. Based on this evidence, associated with the development of a species-specific, PCR-based diagnostic test and new descriptions of cultural and microscopic characteristics, they concluded that the Group I and II of *C. zeae-maydis* are, in fact, two distinct species, and replaced *C. zeae-maydis* Group II with *C. zeina*, while *C. zeae-maydis* Group I was *C. zeae-maydis sensu stricto*. Later, phylogenetic analysis of mating type gene

sequences also revealed the dissimilarity of the two species, further supporting the distinction of the two species (Groenewald *et al*., 2006).

1.3.3 Genotypic and phenotypic features

In addition to this substantial body of molecular evidence, many other genotypic and phenotypic features provide insight into the biological differences between the two species. Though both species cause similar symptoms on maize and form olivaceous-grey colonies and spermatogonia on artificial media, comparisons of cultural characteristics and microscopic structures can distinguish *C. zeae-maydis* from *C. zeina*. Typically, when compared to *C. zeina*, *C. zeae-maydis* grows faster in culture. *C. zeae-maydis* has longer conidiophores. Unlike *C. zeina*, the conida of *C. zeae-maydis* are broadly obclavate-subcylindrical. Only *C. zeae-maydis* has the ability to produce a photoactivated phytotoxin, cercosporin, leading to the accumulation of light pink to reddish-purple pigment on PDA (Crous *et al*., 2006).

Another difference pertains to asexual reproductive structures. The formation of secondary conidia through microcycle conidiation has been described in *C. zeae-maydis* but not *C. zeina* (Lapaire & Dunkle, 2003). Microcycle conidiation is recognized as a survival mechanism for *C. zeae-maydis*, allowing it to maintain inoculum potential during adverse conditions. This observation has been further bolstered by an investigation of a collection of both species from Brazil. A substantial proportion (26%) of Brazilian *C. zeae-maydis* isolates could produce secondary conidia (microconidia) on V8 medium, whereas none of *C. zeina* isolates showed this character (Brunelli, 2004).

Little information is currently available to explain the evolutionary relationships and the mechanism of divergence of the two related species. A genomic analysis of the two species identified that *C. zeae-maydis* contains a single copy of species-specific

transposable element, designated Malazy, which was not discovered in *C. zeina*, suggesting that the two species diverged after the introduction of the transposable element into the genome of *C. zeae-maydis*. However, because Malazy is a degenerate and inactivated element, it is not thought to contribute to speciation or genetic variability between *C. zeae-maydis* and *C. zeina* (Shim & Dunkle, 2005). The primary mechanism of divergence is presumably geographic isolation, as described below.

1.3.4 Global distributions and geographic origin

Differences in global distribution of the two species are another indication of their differentiation. *C. zeae-maydis* populations are more prevalent in the U.S. and are distributed worldwide except in Africa. In contrast, African GLS is caused solely or predominantly by *C. zeina* populations, while both species have been reported in central Colombia (Vangegas *et al*., 2002a,b), central-south Brazil (Brunelli, 2004) and in the eastern third of the U.S. A web-published report from the International Institute of Tropical Agriculture (ITTA) has provided what is so far the only evidence suggesting the coexistence of both species in West Africa (Ayodele *et al*., 2006). Okori *et al*., (2004) also identified a single *Cercospora* isolate, designated Ug4, from Kapchorwa, Uganda. This isolate was previously recognized as *C. zeina* in accordance with the AFLP fingerprinting (Okori *et al*., 2003), but it grouped with an U.S. *C. zeae-maydis* isolate into a cluster distinct from the other East African *C. zeina* isolates based on RFLP profiling (Okori *et al*., 2004). This appeared to be another case implication of presence of another case of *C. zeae-maydis* in Africa.

Accordingly, the two species are differentially distributed throughout the world, with some spatial overlap in North and South America and possibly Africa. This is believed to have resulted from the relatively recent migration of one or both species. Still, the chronology and pathway(s) of the 20th century inter- and intra-continental

dissemination and colonization of the two species remain poorly understood. Several theories about the spatial and temporal origins and the intercontinental movement of the two species had been proposed.

I. North American origin

As mentioned above, GLS was first reported in 1924 in the U.S. (Tehon & Daniels, 1925). The causal fungus was named as *C. zeae-maydis*, and was described as a new *Cercospora* species and the first pathogenic to maize. Later reports tracking the nationwide spread of the disease in the U.S. showed accordance with the original taxonomic description of symptoms and fungal morphology, indicating that *C. zeae-maydis* was initially the only causal agent associated with GLS occurrence in the U.S. (Chupp, 1953; Latterell & Rossi, 1983). *C. zeae-maydis* was also found to be associated with GLS in Central and South America (Chupp, 1953; Boothroyd, 1964). According to the history of GLS world-wide reports, *C. zeae-maydis* may thus be presumed to be native to the U.S. and to have a North American origin. The possibilities remain that *C. zeae-maydis* may have migrated from elsewhere, or moved from another host in North America. Additional data on the comparative genetic diversity of different geographic populations of *C. zeae-maydis* could resolve this.

The first explicit report of a second form of GLS-inducing *Cercospora* was published in 1998 (Wang *et al*., 1998), based on molecular fingerprinting evidence. AFLP- and RFLP-based molecular profiling revealed two very genetically distinct and divergent forms of *C. zeae-maydis* (Wang *et al*., 1998). The term "sibling species" was used in the title of the 1998 publication, but the second species was not named. The terms "Group I and Group II" of *C. zeae-maydis* were used in the text and generally adopted (Dunkle & Levy, 2000; Goodwin *et al*., 2001; Carson *et al*., 2002; Okori, 2004; Okori *et al*., 2003, 2004; Kinyua, 2004). The two groups were shown to be

widespread throughout the maize-growing regions in the U.S. and generally distributed over the area where the fungus was first identified (Wang *et al*., 1998).

It is not clear when *C. zeina* first appeared in the U.S. (Wang *et al*., 1998). However, the phenotypic and genetic variation among the U.S. *C. zeae-maydis* isolates had been described as early as the 1970s. Latterell and Rossi (1974) reported that, in addition to cultural and structural variability, variation in metabolic characteristics (isolates producing or not producing the brilliant red crystals on PDA was observed among ten *C. zeae-maydis* isolates. The visible red crystals actually result from the accumulation of water-insoluble cercosporin in culture (Daub *et al*., 2005). Therefore, the variability of metabolic by-products present among *C. zeae-maydis* isolates seems to be the earliest implication that a cercosporin non-producing strain or species associated with GLS had existed in the U.S. for some time already. Additionally, Carson *et al*. (1997) reported that three groupings of *C. zeae-maydis* isolates from North Carolina were observable based on rDNA-RFLP and -RAPD profiles. These were later confirmed to be *C. zeae-maydis, C. zein*a, and *C. sorghi* var. *maydis* (Carson *et al*., 2002) Particularly, a group of isolates from Laurel Springs, North Carolina, was genetically distinct from isolates in the other groups with respect to the growth rate, aggressiveness and cercosporin production, suggesting that it may belong to a distinct species.

Prior to the recognition of two sibling species in 1998, Ward (1996) suggested that the earliest source of inoculum causing GLS in Africa may have come from the U.S. According to this hypothesis, the original inoculum arrived in Africa with maize imported from the U.S. during the drought years of the first half of 1980s in South Africa. This inference seemed to be plausible and compatible with the first observation of GLS in Africa in 1988 in KwaZulu-Natal, South Africa (Ward *et al*., 1997). It is, however, difficult to reconcile with the observation that *C. zeae-maydis* rather than *C.*

zeina is exclusively present or predominant in the maize-producing regions in the U.S. (Wang *et al*., 1998). According to Dunkle and Levy (2000), the region within the U.S. from which the maize was sourced for exportation to Africa would likely have had *C. zeae-maydis* rather than *C. zeina*.

II. Africa origin

A different argument was raised based on an AFLP comparison of the African and U.S populations of *C. zeina* (Dunkle & Levy, 2000). In principle, the "source" population should be older and more diverse than the population arising from a migration event. Using a small set of isolates from the U.S. and Africa, Dunkle and Levy (2000) argued that the African *C. zeina* population showed a higher level of genetic diversity than the U.S. populations. Among the nine isolates from six U.S. states, there were two unique haplotypes among the six haplotypes. For the African sample (30 isolates from Uganda and Zimbabwe), there were more 13 unique AFLP haplotypes among the 17 haplotypes. Considering this interpopulation genetic difference and the predominance of *C. zeina* in Africa, Dunkle and Levy proposed that the U.S. population of *C. zeina* most likely originated and migrated from Africa, possibly as a result of the human-assisted introduction via infested ear husks and leaf sheaths associated with intercontinental movement of seed.

This study showed convincingly that, as mentioned above, African and U.S. populations of *C. zeina* are closely related, as also shown by other authors (Okori *et al*., 2003; Kinyua, 2004; Okori, 2004). However, because of limited sampling of the two pathogen populations and their respective genomes (this study was conducted with a single AFLP primer - enzyme combination), their conclusion about the direction of migration may not be definitive. The relatively low and unbalanced number of *C. zeina* isolates from the U.S. (n=9) and East Africa (n=30), it may be difficult to

formulate definitive conclusions on genetic diversity of the two continental *C. zeina* populations. For comparative study of fungal diversity, the low number of samples used for AFLP analysis can compromise the confidence in diversity estimates, leading to biased and provisional conclusions. This had been reported in several studies of different fungi, such as *Claviceps africana* (Tooley *et al*., 2002), *Ophiosphaerella korrae* (Iriarte et al., 2004) and *Schoenoplectus* species (Fay et al., 2003). Analysis of a larger number of *C. zeina* isolates from the U.S. with more primer combinations should clarify the comparative genetic diversity between the two *C. zeina* populations and provide a chance to explore the evolutionary relationship between the two geographical populations.

Once the close genetic relatedness of U.S. and African *C. zeina* populations was revealed, several studies were conducted using molecular marker-based approaches to investigate the intra- and inter-specific genetic differentiation within and between *C. zeina* and *C. zeae-maydis*, and to understand the origin and distribution of their respective populations. Okori *et al*. (2003) examined 75 east and southern African *C. zeina* isolates obtained from Kenya, Uganda, Rwanda and Zimbabwe using AFLP and RFLP markers. This study showed that the 75 African *C. zeina* isolates had a gene diversity (0.16), while the sample of five U.S. *C. zeina* isolates had a gene diversity of 0.13 and four U.S. *C. zeae-maydis* isolates had a gene diversity of 0.03. There was no significant genetic differentiation between the African and U.S. *C. zeina* populations (the pairwise F_{ST} values ranged from 0.002 of Uganda-US populations to 0.069 of Rwanda-US populations). Most of the molecular variation in *C. zeina* was attributable to within-population variation rather than between-population variation. Among the African *C. zeina* populations from four geographical origins, no population differentiation was detected (F_{ST}= 0.01) and a high level of migration was inferred by indirect estimation (N_m = 49.5). These observations suggested that the African *C. zeina*

population had experienced extensive gene flow, resulting in a relatively even spatial distribution of a limited degree of genetic diversity.

Given the lack of differentiation observed across the large area of eastern and southern Africa, Okori *et al*. (2003) speculated that a human-mediated pathway for the intra- or inter-continental dispersal of *C*. *zeina* might account for the high level of gene flow estimated suggested. Presumably, this would refer to the cross-border seed trade and plant material movement. However, the conservation tillage has been increasingly adopted and the long-season GLS susceptible cultivars have been extensive grown for a long time in Africa (Ward *et al*., 1999). These agricultural practices provide a constantly favorable condition for disease spread and impose less selective pressure on pathogens. Therefore, the possibility still remains that the wind- and air current-dispersed conidia or microconidia (Lapaire & Dunkle, 2003; Brunelli, 2004) play an important role, accounting for the long-distance, intracontinental movement of *C*. *zeina*. Moreover, considering the large distance between the sampling sites (>700 km), it is possible that the nearby *C*. *zeina* populations within the unsampled areas can provide important genetic and epiphytotic information for further evaluating the gene flow and the dispersal of *C*. *zeina*.

Low genetic variability was also found within the Kenyan *C*. *zeina* population. By comparing the AFLP fingerprint profiles of 85 *C*. *zeina* isolates collected from a wide range of rural areas in Kenya, Kinyua (2004) determined that they clustered with the *C*. *zeina* isolates from Zimbabwe (n=2), South Africa (n=2) and the U.S. (n=1). The Kenyan isolates exhibiting limited genetic variability with a genetic similarity of greater than 95%. No well-defined subclusters were identified among the isolates. However, some degree of intraspecific variation was observed with respect to the slightly different growth characteristics and the separation of a couple of isolates that originated from the same leaf in different subclusters. The author suggested that the

Kenyan *C. zeina* population might have well co-evolved with maize for a period of time, leading to the establishment of a high genetic compatibility and stability with maize. In other systems, however, a lack of genetic diversity has been considered to reflect a bottleneck, typically as a consequence of a recent migration or shift in host (e.g., *Phytophthora infestans* outside of Mexico). In this light, the minor genetic variation detected within the population might be interpreted to indicate that the Kenyan *C. zeina* population was recently introduced to East Africa.

Overall, although a series of discoveries in Africa has revealed a lack of differentiation within and between African and U.S. populations of *C. zeina*, the origin of the pathogen is not clear. Elucidation of the geographic origin of African *C. zeina* populations still requires more sampling and genetic analysis.

III. South American origin

In addition to the previous hypothetical migratory route of *C. zeina* from Africa to the U.S., Dunkle and Levy (2000) also proposed an alternative possibility that both continental populations of *C. zeina* were recently introduced from other geographic regions where GLS occurred. Later, this hypothesis was supported by Brunelli (2004), who revealed that Brazil, in addition to the U.S., is another geographic area where both species coexist. Based on ITS-rDNA RFLP, 41 *C. zeae-maydis* and 28 *C. zeina* were identified in a sampling area across 1,500 km over the maize-growing region of central-south Brazil, with the exception of the State of Goias, where only *C. zeina* was found. When U.S. isolates of *C. zeae-maydis* (n=2) and *C. zeina* (n=2) and African isolates of *C. zeina* (n=2) were used as reference strains for AFLP analyses, limited genetic variability within each species was detected. The intraspecific similarity of *C. zeae-maydis* and *C. zeina* were 93.5% and 92%, respectively. The average similarity

of Brazil-U.S. populations of *C. zeae-maydis* was 90%. The Brazil-U.S. and Brazil-Africa similarities for *C. zeina* were 90.2% and 89%, respectively.

Because both species are sympatric in central-south Brazil, and their universal distribution pattern is distinct from that of Africa and the U.S., the author suggested that there is a greater genetic diversity in Brazilian *Cercospora* populations than in those of Africa and the U.S. Ultimately, the author speculated that *C. zeina* more likely originated and radiated, neither in the U.S. nor in Africa, but in Brazil, South America. This AFLP-based study, following the primer combinations and analytical methods applied by Wang *et al*, (1998), lacked rigorous comparisons of AFLP profiles, haplotypes and allele frequencies between and within populations of either in *C. zeina* or *C. zeae-maydis* for statistical evaluation of gene or genotypic diversity. The limited number of reference isolates from the U.S. and Africa also was insufficient for representing the different continental populations to further understand the respective population structure. Without comparable samples of North American, South American and African *Cercospora* populations, it is not possible to draw firm conclusions about the relative population diversities in a way that would bear upon discussions of the pathogens' evolutionary history. Hence, the argument that Brazil is the center of diversity and origin of *C. zeina* remains uncertain. Nevertheless, this study, coupled with two earlier reports showing that the two species were identified in the Departments of Valle del Cauca and Risaralda in central Colombia (Vangegas *et al*., 2002a,b), ascertained the presence of both species in South America, providing new insights into the origin and biogeography of both species.

IV. Relationships between related *Cercospora* species associated with GLS

Observing the limited intraspecific genetic variation in *C. zeina*, Dunkle and Levy (2000) suggested that the ancestral *C. zeina* population may have experienced a

genetic bottleneck prior to its relatively recent migration from its center of origin to other parts of the world. Based on this evolutionary inference, two biological possibilities for the ancient origin of the African *C. zeina* lineage were proposed (Dunkle & Levy, 2000; Crous, *et al*., 2006).

The first scenario assumed that the ancestor of *C. zeina* was a pathogen of maize and emerged in a maize agroecosystem. Considering that maize is believed to have come from south-central Mexico, the progenitor of *C. zeina* would be most likely to have originated in Mesoamerica or South America in association with the domestication and spread of maize. Underlying this scenario is a coevolutionary reasoning that the emergence and expansion of the pathogen would follow or coincide with the domestication of the host plant and the ensuing development of a specialized agroecosystem for the host. As a result, the center of origin of the pathogen would be correlated with the center of origin of cultivated host crop. This coevolutionary mechanism for the emergence of pathogens in agroecosystems (Stukenbrock & McDonald, 2008) has been described in several pathosystems, including the *Ustilago maydis*-maize (Munkacsi *et al*., 2007), *Phytophthora infestans*-potato (Fry and Goodwin, 1997), and *Mycosphaerella graminicola*-wheat (Stukenbrock *et al*., 2007). In the *C. zeina*-maize pathosystem, a link between the appearance of *C. zeina* and the geographical origin of maize, however, has yet to be identified. Under this scenario, the lack of detection of *C. zeina* in Mexico would be attributed to the low intensity of sampling (Dunkle & Levy, 2000) and failure of the pathogen species to spread widely prior to its migration to Africa. Alternatively, the pathogen might have become extinct in its region of origin.

The second, and probably more plausible, scenario was that *C. zeina* is indeed indigenous to Africa. In this scenario, the progenitor of *C. zeina* was not originally pathogenic to maize, but jumped from another grass species of African origin, such as

sorghum, to maize. Accordingly, the new species emerged in the wake of the adaption to the new host. In this light, a *Cercospora* species, *C. sorghi* (Ellis & Everhart, 1887), has been of interest as a putative progenitor of GLS pathogens due to its ability to infect numerous *Sorghum* species and the recurrent association of *C. sorghi* var. *maydis* with maize. This species appeared to be a plausible candidate able to bridge the host and nonhost and to coincide with the host jumps model for the emergence of new pathogen(s) (Stukenbrock & McDonald, 2008).

C. sorghi and *C. sorghi* var. *maydis* are taxonomically similar and recognized as conspecific (Ellis & Everhart, 1887). Although morphologically identical, these two related taxa have been considered to be separate species on the basis of their quite distinct host specificities (Chupp, 1953). *C. sorghi* can infect a wide range of cultivated, wild and weedy species within the genus *Sorghum*, such as sorghum (*S. bicolor*), Johnsongrass (*S. halepense*), Sudan grass (*S. vulgare* var. *sudanense*) and broom corn (*S. vulgare* var. *technicum*) (Ellis & Everhart, 1887; Chupp, 1953; Holliday, 1995; Okori *et al*., 2004). However, the capacity of *C. sorghi* to infect maize has not been substantiated (Chupp, 1954; Holliday, 1995; Carson & Goodman, 2006). *C. sorghi* var. *maydis*, on the other hand, has been found in association with GLS lesions, but it has not been shown to be pathogenic on sorghum or maize, nor has it been shown to be the cause of GLS based on limited inoculation tests and field trials (Carson & Goodman, 2006). Though the role of *C. sorghi* var. *maydis* in causing GLS still needs to be determined, Carson and Goodman (2006) considered that this fungus is probably a leaf saprophyte. It showed relatively rapid growth and earlier sporulation on leaf lesions than the other coexistent GLS-related *Cercospora* species under moist chamber conditions. In addition, it was found associated with the lesions of the other fungal foliar diseases of maize and with the necrotic leaf tissue caused by injuries. This interesting and peculiar condition, in which different species coexist in a single

lesion, appears to provide the opportunity for genetic exchange among related species, which could contribute to changes in host specificity and the occurrence of speciation events.

A number of molecular marker-based phylogenetic studies, mostly based on the analyses of ITS rDNA sequences, revealed that *C. sorghi* and *C. sorghi* var. *maydis* were closely related but genetically distinct (Goodwin *et al*., 2001; Kinyua, 2004; Okori, 2004; Crous *et al*., 2006; Carson & Goodman, 2006). These studies also demonstrated that these two species had close relationships with the two GLS pathogens, *C. zeae-maydis* and *C. zeina*.

The four GLS-associated *Cercospora* species were well identified and phylogenetically characterized by molecular methods and plausible speculations about their evolutionary history have been proposed. However, their evolution and phylogenetic relationship have so far been more difficult to define with certainty. Goodwin *et al*. (2001) considered that *C. sorghi* and *C. sorghi* var. *maydis* shared a relatively recent common ancestor with the other cercosporin-producing *Cercospora* species including *C. zeae-maydis*, as well as with *C. zeina*. Crous *et al*. (2006) also noted that *C. sorghi* appeared to be more similar to *C. zeina* than to *C. zeae-maydis* on the basis of comparative analysis of their ITS sequences. This finding led the authors to propose an ancestor-descendant relationship between *C. sorghi* and *C. zeina*, most likely through the process of interspecific host jump. This putative relationship was in support of the previous suggestion that Africa was the origin of *C. zeina* (Dunkel & Levy, 2000).

Still, few large-scale multilocus phylogenetic-based comparative studies of the four species have been conducted so far. In the absence of more substantive data, it is not possible to conclude which species is the progenitor and to infer the order of evolutionary events. In addition, interspecific recombination may occur in nature

between allopatrically or sympatrically divergent species, with the potential for ensuing speciation and host specialization (Ioos *et al*., 2006; Brasier, 2001; Man in ‘t Veld *et al*., 2007). With the small size of population samples and limited molecular markers that have been applied to date, it is impossible to clearly trace back the ancestry and the parental species that may have given rise to a new species or a derivative hybrid through interspecific genetic exchange (Stukenbrock & McDonald, 2008).

Inferences regarding the evolutionary history of the four species have been complicated by divergent results of phylogenetic analyses. A few reports have shown that, when compared with continent-wide samples of *C. zeae-maydis* and *C. zeina* populations, *C. sorghi* or *C. sorghi* var. *maydis* demonstrated relatively high levels of DNA polymorphism and genetic variability between and within the U.S. and Kenyan isolate collections (Carson *et al*., 2002; Kinyua, 2004). This led to the claim that *C. sorghi* and *C. sorghi* var. *maydis* may have a higher potential for evolution. This is, however, inconsistent with another study (Okori *et al*., 2004), which revealed that, similar to the African *C. zeina* population, the *C. sorghi* population in Uganda was genetically highly uniform with little population structure and high levels of gene flow, regardless of its origin and whether it was from wild or cultivated sorghum hosts. Such a discrepancy between these AFLP-based phylogenetic studies may result from the differences in sampling size, AFLP primer combinations, and clustering algorithms. This variance could be also derived from the diverse and different climate conditions in agroecological zones of Kenya and Uganda (Wood, 2008). This selective pressure may have resulted in significantly distinct genetic diversity and different adaption of the regional pathogen population to respective agroecosystem. Otherwise, it is surprising that there was such a high genetic differentiation between the *C. sorghi* populations from these two neighboring countries in East Africa, considered that *C.*

sorghi is an air-borne fungus with a potential for dispersal through seed (Reddy *et al*., 2005).

In order to reconcile these inconsistencies and to unravel the phylogeny of these closely related *Cercospora* species, more appropriate markers applications, sequence analyses, and extensive and ecologically explicit samplings from more diverse areas and hosts are required. It remains unknown whether mutation and/or asexual genetic exchange have occurred within and between *C. sorghi* and *C. sorghi* var. *maydis*, resulting in an interspecific hybrid as previously described by Carson and Goodman (2006), or leading to a new species with host specificity distinct from parental lineages. Therefore, if *C. sorghi* is the possible progenitor of *C. zeina*, it appears to be necessary to clarify the origin of *C. sorghi* and to accurately identify the genetic relationships between *C. sorghi* and *C. sorghi* var. *maydis* as part of gaining an understanding of the origin and global migratory route of *C. zeina*. Using appropriate combinations of nuclear and mitochondrial markers has been proved useful to facilitate parentage determination in *Phytophthora* subspecies (Ioos *et al*., 2006) and *Ophiostoma* species (Bates *et al*., 1993; Brasier, 2001). In addition, a broad pathogen collection, can also decrease the statistical bias and the impact of sampling size on the gene frequency of the population, to enhance the credibility of conclusions drawn.

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CHAPTER 2

COEXISTENCE OF SIBLING SPECIES OF *CERCOSPORA* CAUSING GRAY LEAF SPOT ON MAIZE IN SOUTHERN NEW YORK STATE

2.1 Introduction

Gray leaf spot (GLS) is a recently emergent constraint to maize production world-wide, especially in eastern U.S., sub-Saharan Africa, and China (Latterell and Rossi, 1983; Coates, 1995; Lipps *et al*., 1998; Ward *et al*., 1999; Li, 2005; Meisel *et al*., 2009). GLS is caused by two morphologically similar but physiologically and phylogenetically distinct species, *Cercospora zeae-maydis* Tehon & Daniels (*Czm*) and *C. zeina* Crous & Braun (*Cz*). The two species, initially designated as Group I and II of *C. zeae-maydis*, cause identical symptoms on maize but differ in their production of the phytototoxin cercosporin in culture: *Czm* produces the pigmented toxin while *Cz* does not. In planta, direct evidence has not been presented regarding the secretion of cercosporin in either species (Wang *et al*., 1998; Goodwin *et al*., 2001; Carson and Goodman, 2006; Crous *et al*., 2006), though indirect evidence suggests that cercosporin contributes to virulence in *Czm*. While no sexual stage or parasexual recombination has been confirmed for either species, the approximately equal frequencies of mating types detected in the populations of the two species suggested the potential for sexual reproduction (Groenewald *et al*., 2006). ITS sequencing data also suggested that they share a common ancestor with the other cercosporin-producing *Cercospora* species, and have close phylogenetic relationships with the teleomorphic genus *Mycosphaerealla* (Goodwin *et al*., 2001).

Czm was first identified in the U.S. (Tehon & Daniels, 1925) and has been reported as the causal agent of GLS epidemics worldwide since the 1970s, except in Africa. *Czm* is thus presumed to be of North American origin. *Cz*, on the other hand, remained undetected in the U.S. until 1998 (Wang *et al*., 1998) and was recently

recognized as a distinct species (Crous *et al*., 2006). *Cz* is solely or predominantly responsible for the widespread occurrence of GLS in sub-Saharan Africa (Meisel *et al*., 2009), and coexists with *Czm* in parts of the U.S. (Wang *et al*., 1998), Colombia (Vanegas *et al*., 2002a,b), Brazil (Brunelli, 2004), and possibly Nigeria (Ayodele *et al*., 2006). The original source of *Cz* is debated. It has been speculated that *Cz* originated in Africa (Dunkle and Levy, 2000; Crous *et al*., 2006), South America (Brunelli, 2004), or less plausibly, the U.S. (Ward *et al*., 1996). However, due to the relatively recent recognition of *Cz* and limited comparative analyses of regional and intercontinental populations of the two species, it is difficult to draw definitive conclusions on the geographic origin and evolutionary relationship of the two species. Some authors have speculated that *Czm* and/or *Cz* are derived from *C. sorghi* and/or *C. sorghi* var. *maydis*, two closely related *Cercospora* taxa frequently associated with the GLS lesions on sorghum and maize, respectively (Dunkle and Levy, 2000; Crous *et al*., 2006). Neither the taxonomic relationship between *C. sorghi* and *C. sorghi* var. *maydis*, nor their relationship to *Czm* and *Cz*, have been fully resolved. It is clear, however, that *Czm* and *Cz* are very distinct, representing allopatric members of a species complex (Dunkle and Levy, 2000; Crous *et al*., 2006; Groenewald *et al*., 2006).

As noted above, *Czm* and *Cz* have been shown to be sympatric in a few study areas in North and South America. While most studies have documented this co-existence at the state-wide level, some cases in Colombia and the eastern U.S. hinted at coexistence on a within-field scale (Vanegas *et al*., 2002a,b; Wang *et al*., 1998). The frequencies of *Czm* and *Cz* appeared to be nearly equal in the limited coexistent cases in the eastern U.S. For example, in two fields in Ohio and one in the state of New York, pairs of isolates collected from the same field were of the two species (Wang *et al*., 1998). In Colombia, in contrast, *Cz* was more prevalent than *Czm*, with a ratio of 9:1 (Vanegas *et al*., 2002a). The two species were found together in only a

single plot. These reports thus establish the sympatric distribution of the two species, but do not provide substantial evidence regarding the extent of species coexistence or its stability over time. If the coexistence of *Czm* and *Cz* is widespread and stable in agroecosystem over time, this cryptic species complex must be considered in population and epidemiological studies, resistance breeding, and seasonal disease management efforts.

Numerous economically important phytopathogenic fungi exist as sympatric species complexes (Brasier, 1987; Gudelj *et al*., 2004; Fitt *et al*., 2006a,b; Eyal, 1999; Shoemaker *et al*., 2001). In a recent review, Fitt *et al*. (2006b) concluded that niche differentiation may be involved in the sympatric coexistence of related fungal species. For instance, the interaction of slight phenotypic and genetic differences between the sibling species may interact with biotic and abiotic variables to permit the sibling species to segregate in space or time by occupying different target tissues, occurring at slightly different times, and/or by otherwise exhibiting differences in pathogenic strategies. Little work has been done, however, to examine the potential need for differential management of sibling species. If coexistence of the sibling *Cercospora* species were documented as a widespread (or potentially widespread) phenomenon in maize, it would be important to better understand the management of the species complex, given the importance of both the host and the disease, and the notable evolutionary and physiological differences in the sibling species.

The present study was undertaken to determine the composition, distribution and relative abundance of *Czm* and *Cz* populations in southern New York state. Information on the GLS pathogens was desired for two reasons. Firstly, the disease is expected to become an increasing threat to maize production in the state as projected trends in summer climate conditions (Frumhoff *et al*., 2007) are likely to favor the disease. It is thus important to understand the nature of the pathogen and to identify

suitable management options, such as resistant maize varieties. Secondly, we wished to shed light on the stability of the species complex in light of the weather trends over the past two decades. Pre-existing data on *Cercospora* populations in New York were extremely limited but suggested that the two species coexisted in the mid-1990s - as noted above, both species were represented among the two isolates collected from Chemung in 1994 by Dr. Gary Bergstrom (Wang *et al*., 1998).

Three potential outcomes were considered: (1) fixation of one species; (2) patchy coexistence on a regional or field basis; and (3) coexistence on a fine (within-field) scale. The idea underlying the hypothesis of fixation is that drift and/or selection would favor one species over the other. Selection could be related to host or environment. With regard to the environment, climatic factors could be relevant to a northward pathogen migration. As the disease spreads north, and/or encounters the changing climate over time in NY, temperature and humidity differences could affect fitness and relative fitness. Host-related conditions of maize variety and management practices could also influence pathogen population structure. As a start, we elected to obtain data on the climate trends for the study region as relevant data on host and management was not accessible.

The hypotheses of coexistence could be based on the idea that changing environmental conditions dictated by weather, crop variety and cultural practices impose selection pressure on the two species differently in different years and locations. If relative fitness of the species were determined by factors influenced by different topographies and relative distance to water bodies (wind, humidity), distributions of the species complex might correspond to topographical features such as watersheds, altitude and landscapes. If local weather, management or cultivars play a key role in determining the structure of the pathogen population, the effect of population patchiness could be expected to occur in the maize field due to the

compartmentalization of maize varieties and the differential dispersal of pathogen populations. Thus, an alternative hypothesis is the population structure of pathogens might show patchiness by plot, such that each maize field were dominated by a single species. Within-field coexistence could suggest identical fitness of the two species, could reflect fluctuating differential fitness, or could imply differences in the micro-niche of the two species.

2.2 Materials and methods

2.2.1 Study region and sampling strategy

In order to investigate the occurrence of GLS and to examine if the populations of the *Czm* and *Cz* are geographically differentiated, a sampling of GLS disease in Southern Tier of New York State was carried out during the maize growing season of 2006 (July to September). Three watersheds, the Chemung, Tioga and Owego-Wappasening watersheds associated with Chemung river in Southern Tier of New York State, were identified for intensive sampling. The profiles and topographic maps of the watersheds were collected from the websites of the United States Geological Survey (USGS; http://www.usgs.gov/), U.S. Environmental Protection Agency (EPA; http://cfpub.epa.gov/surf/locate/index.cfm) and New York State Dept. of Environmental Conservation (NYSDEC; http://www.dec.ny.gov/lands/47997.html)

Maize fields were randomly selected for sampling in areas with higher planting density, high relative humidity and higher incidence of GLS. One to ten fields in the valley bottoms or low-lying areas were chosen within a watershed, with a minimum distance of 1 km between fields. By walking a Z-shaped or W-shaped route across the field, two to five sampling sites (at least 20 m apart) per field were randomly selected dependent on the incidence level of the disease. Two to five leaves, each showing at least one rectangular necrotic GLS lesion, were collected per site.

2.2.2 Fungal pathogen collection and characterization

Isolation of fungi was modified from the methods described by Wang *et al*. (1998). One to ten single conidia of each leaf lesion collected from different plants were isolated and were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit) and V8-juice agar for 14 days under cool white light (fluorescent light, 2200 lux) in a 12 hr light/ 12 hr dark cycle at 25˚C. One isolate per lesion was used for data analysis. Morphological characters were analyzed based on the criteria described by Tehon & Daniels (1925), Chupp (1953), Latterell & Rossi (1983) and Crous *et al*. (2006). A set of 112 *Cercospora* isolates confirmed by morphological and physiological taxonomy were selected for molecular identification by application of the PCR-based diagnostic test and species-specific primer sets (CzeaeHIST and CzeinaHIST) developed by Crous *et al*. (2006).

2.2.3 Estimate of the weather trends in the southern and central New York State

Chemung and Aurora were two representatives of the locations of interest for GLS sampling in the southern and northern parts of the sampling area, respectively. The two localities were hence chosen to collect the weather data. The time series weather data corresponding to the maize growing seasons (June through September) from 1986 to 2006 were obtained from the National Climatic Data Center (NCDC) provided by the weather station at Chemung, Chemung County (COOP Station Number 301413, 42.00°N, 76.63°W) and Aurora Research Farm, Cayuga County (COOP Station Number NY3003313, 42.44°N, 76.39°W). The original monthly and seasonal records of climatological observations, including maximum and minimum temperatures and total rainfall, were compiled and used to estimate the local weather trends over the entire period that might be related to the fluctuation of the *Czm* and *Cz*

populations in this area. The differences in climatic conditions between the two locations were also compared.

2.2.4 Spatial occurrence of Czm and Cz in Southern Tier of New York State

The frequencies of occurrence and coexistence of the two species populations in Southern Tier of New York State were examined. The frequency with which each species was detected in each sampling category (location; watershed; elevation), and the relative frequency of detection of each, gave an insight into which species was dominant in that category. For comparing the frequency of *Czm* and *Cz* detected in a given area or a category, the chi-square test for goodness of fit was used to test a null hypothesis of a 1:1 frequency of *Czm* to *Cz* based on the finding reported by Wang *et al* (1998).

2.2.5 Consideration of sample sizes and imperfect detection probabilities

Selecting an adequate sample size (replication) is crucial for decreasing the probability of a false absence of a target species from a sampling unit. It is highly unlikely that a relatively minor species will be detected during a visit, we thus used the number of lesions per field as a spatial replication to correct the imperfect detection for *Czm* and *Cz* for each sampling field. The priori sample size calculation could vary greatly depending upon the estimate of the proportion of the species of interest. In order to obtain a confidence level of 95%, the sample size could be greater than 58 lesions/field, if the proportion of the relatively minor species of interest in the sampling area was 5%, or it could only be needed for 4 lesions/field if the proportion of the two species was essentially even (Figure 2-1). However, the proportion of *Czm* /

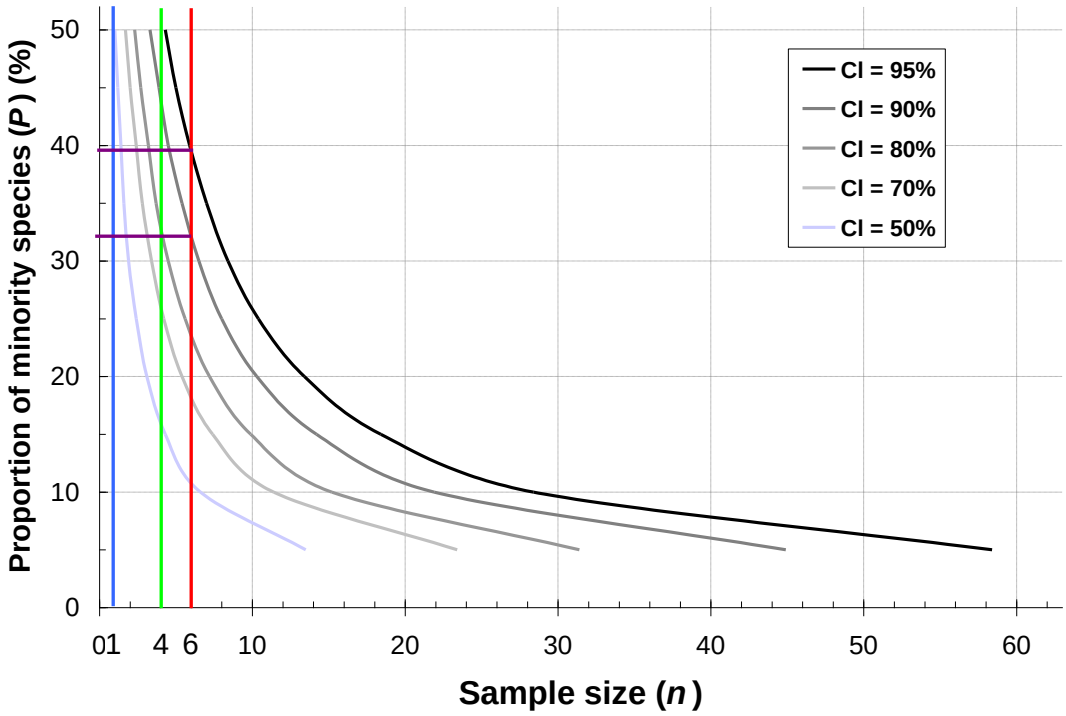


Figure 2-1. Estimated sample sizes required for different proportions of a minority species in a sampling unit at different confidence levels. Based on our sampling intensity of n=4 (full line in green) and n=6 (full line in red) lesions/field, we could obtain a confidence level between 80% to 95%, if the proportion of the relatively minor species population in a sampling field was between greater than 33% and greater than 39% (purple lines), respectively.

Cz populations in the Southern Tier of New York State was unknown when we initiated the study. The low disease intensities during the sampling year further constrained sample sizes and were highly likely to affect the detectability of target species. These unavailabilities made it difficult to collect adequate leaf lesions from each sampling field, and possibly led to the increases of the uncertainty between non-detection and true absence of the species of interest.

In light of these conditions, we used our primary sample collection based on the sampling intensity of 1-6 lesions per field to first estimate the overall proportion of the two species in the sampling area. The confidence levels and sampling power with 100% test sensitivity and specificity based on detected proportion of *Czm* / *Cz* was

evaluated as $n = \log (1-C) / \log (1-P)$ (DiGiacomo & Koepsell, 1986), where n is the sample size or the number of lesions for each field, C is the confidence level or probability (1-α level of significance) of detecting the species of interest, and P is the proportion of the species of interest desired to be detected, given the species is present. Based on the confidence levels (or power for a sampling unit), the appropriate sample size was determined for statistical analyses of the occurrence and geographic distribution of the two species.

2.2.6 Univariate tests for association and multiple logistic regression

Once the detection-non detection data of *Czm* and *Cz* were adjusted for the detection probability, the presence/absence data were used as the binary dependent variables. Chi-square test was applied for preliminary analysis to examine the univariate relationships between the species occurrence and each geographic variable, ie. watershed, elevation, latitude and longitude. Phi (φ) or Cramer's V (Cramér, 1999), the chi-square based correlation statistic, was followed to quantify the strength of association between pairwise categorical variables previously showed significant chi-square value ($P < 0.05$). The value of coefficient is the chi-square adjusted for sample size and the number of levels of each categorical variable. It ranges between 0 and 1, with the higher the number the stronger the relationship.

Any independent variable that was significantly [using a significance level of 0.25 as a screening cutoff point, suggested by Mickey and Greenland (1989)] associated with the occurrence of *Czm* or *Cz* in univariate tests was included in the multiple logistic regression analysis. Logistic regression (Hosmer & Lemeshow, 1989) was used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the presence/absence of *Czm* and *Cz* in response to geographic variables, and to develop models to predict the probability of *Czm* and *Cz* occurrence in the sampling

area based on the watershed. The Wald chi-square was used to test the significance of individual independent variables estimate. After a logistic regression model was fitted, likelihood ratio chi-square test was computed on the basis of the Hosmer-Lemeshow goodness-of-fit test, with an expected P-value >0.05 (Hosmer & Lemeshow, 1989), to access the adequacy of the resulting regression model. The predictive ability of the final model was also assessed by use of the area under the receiver operating characteristic (ROC) curve, which an area under the curve is close to 1 implying a good discriminative power for the model. All the statistical analyses were conducted using JMP v. 7.0 (SAS Institute Inc., Cary, NC). A *P*-value of less than 0.05 was considered statistically significant.

2.2.7 Interspecific association between Czm and Cz

The counts of presence or absence for *Czm* and *Cz* at the 41 sampling fields across four watersheds were summarized in a 2 x 2 contingency table, as shown below:

		Cercospora zeina		
		Present	Absent	Total
Cercospora zeae-maydis	Present	a	b	a+b
	Absent	c	d	c+d
	Total	a+c	b+d	a+b+c+d=41

Based on the two way contingency table, the significance of the independence between the two species was tested by Pearson's chi-square and Yates' correction for continuity. The strength of the association was mainly measured using Hurlbert's modification (1969) of Cole's coefficient of interspecific association (C_8) (Cole, 1949), with a range of values from -1 to +1 for complete negative and complete positive associations. Three indices of association and similarity, the Ochiai (OI), Dice (DI), and Jaccard (JI) (Janson & Vegelius, 1981), used to measure ecological coexistence

and the degree of association between pairs of species were also included for comparisons. These coefficients range from 0 to 1, with 0 indicating no association and 1 indicating the maximum association.

2.3 Results

2.3.1 Disease sampling and fungal isolation

A total of 54 maize fields along river valleys distributed in seven watersheds in southern and central New York were investigated and sampled from July 20, 2006 to September 1, 2006 (Figure 2-2). The incidence and severity of GLS were relatively low during the growing season in 2006, leading to a low number of diseased leaf samples and leaf lesions. One to three rectangular lesions on each leaf were most often observed, and these were mostly small with little coalescence of lesions. No symptoms were observed on leaf sheaths and stalks. Although all the leaf samples were associated with the presence of diagnostic symptoms on the leaves in the field investigation, the presence of *Cercospora* sp. was confirmed only in 25 fields after identification. Out of the 25 fields for which *Cercospora* isolates were recovered, 22 were located in the Chemung, Tioga and Owego-Wappasening watersheds.

In total, 689 single-conidia isolates were obtained from 83 leaf lesions associated with GLS symptoms (Table 2-1). Of the *Cercospora* isolates, 648 (94%) were obtained from the Chemung, Tioga and Owego-Wappasening watersheds in the Southern Tier of New York State, where a higher GLS incidence was observed. The other of 48 (6%) isolates were recovered from Seneca watershed in the Central New York State.

Figure 2-2. Map of sampling locations. Chemung County served as the center of a sampling semicircle of about 100 miles diameter in the Southern Tier of New York State. Among the 54 sampling fields, *Cercospora* sp. were identified in 26 fields (solid circle) and not in the others (open circle). CH: Chemung Watershed; CHE: Chenango Watershed; IN: Irondequoit-Ninemile Watershed; OW: Owego-Wappasening Watershed; SE: Seneca Watershed; TI: Tioga Watershed; US: Upper Susquehanna Watershed.

Table 2-1. Results of GLS sampling and *Cercospora* isolation.

Watershed	No. of fields sampled (% of fields yielding *Cercospora* isolates)	Number of *Cercospora* isolates (% of total collection)	Number of lesions (% of lesions that yielded isolates)
Chemung	13 (93%)	376 (55%)	40 (90%)
Tioga	6 (67%)	101 (15%)	11 (85%)
Owego-Wappasening	10 (60%)	171 (25%)	24 (73%)
Seneca	18 (22%)	41 (5.9%)	8 (100%)
Irondequoit-Ninemile	4 (0%)	0	-
Chenango	2 (0%)	0	-
Upper Susquehanna	1 (0%)	0	-
Total	54 (48%)	689 (100%)	83 (86%)

2.3.2 Cultural characteristics

The *Cercospora* isolates were separated into two groups based on cultural and morphological features (Table 2-2; Figure 2-3). The first group showed faster growth rate and less compact texture on PDA after 25 days than did the second group. The colony colors of both groups were similar, displaying olivaceous gray to dark olivaceous gray. However, a diffusible reddish-purple pigment (inferred to be cercosporin) was observed only on the medium for the first group of isolates, with some variations in pigment accumulation. The characteristics of conidiophores and conidiogenous cells in the two groups were similar. The isolates in both groups formed fasciculate, unbranched, septate conidiophores, mostly in clusters of 5-12, arising from stromata the basal mycelium. Conidiogenous cells were subcylindrical and geniculate, with 1-3 slightly thickened conidiogenous loci.

Although similar in microscopic structures, the two groups could be differentiated based on the size of the conidia. Detailed measurements were made for 15 isolates of the first and and 26 isolates of the second groups (Table 2-2). No distinct differences were observed between the two groups for conidial shape, color and septation. However, the size of conidia in the two groups were significantly (t-test: $P<0.0001$) different, with mean dimensions of $70.6 \pm 5.8 \times 8.2 \pm 0.3$ μm (range 56-99 × 6-9 μm; n=120) for the first group and with mean dimensions of $45.7 \pm 5.4 \times 6.8 \pm 0.3$ μm (range 31-74 × 6-8 μm; n=260) for the second group. The characteristics of the first and the second groups were consistent with taxonomic descriptions and previous reports for *C. zeae-maydis* and *C. zeina*, respectively (Tehon & Daniels, 1925; Chupp, 1953; Latterell & Rossi, 1983; Wang *et al*., 1998; Crous *et al*., 2006).

Table 2-2. Cultural characteristics of the two groups[a] of *Cercospora* fungi.

Characteristics	1st Group[1] (*Cercospora zeae-maydis*)	2nd Group[1] (*Cercospora zeina*)
Colony morphology		
Growth rate	moderately fast, reaching an average of 23-36 mm in diameter	relatively slow, reaching an average of 10-17 mm in diameter
Color	olivaceous gray to dark olivaceous gray	pale green, olivaceous gray to dark olivaceous gray
Texture	floccose, occasionally zonate, usually covered with irregular patches of woolly or fluffy whitsih aerial mycelium	compact, floccose-velutinous, slightly raised, occasionally zonate, sometimes covered with irregular patches of cottony or fluffy whitish aerial mycelium
Margin	irregular, submerged or floccose	narrow, irregular
Spermatogonia	global, scattered, abundant	global, scattered, moderately abundant
Pigmentation	reddish purple	absent
Microscopic structures		
Conidiophores	5-12, fasciculate, 1-4 septate, 45-168 × 4-6 μm, unbranched, straight to geniculate-sinuous, pale olivaceous to pale brown	5-12, fasciculate, 1-4 septate, 45-150 × 4-6 μm, unbranched, straight to geniculate-sinuous, pale olivaceous to pale brown
Conidiogenous cells	subcylindrical, geniculate, with 1-3 slightly thickened conidiogenous loci as small as 2-3 μm wide.	subcylindrical, geniculate, with 1-3 conspicuous conidiogenous loci as small as 2-3 μm wide.
Conidia[b]		
Shape	hyaline, obclavate to subcylindrical, usually slightly curved, with a thickened hilum	hyaline, broadly fusiform to obclavate-cylindrical, usually slightly curved,with a thickened hilum
Wall	thin and smooth	thin and smooth
Septum	3-9	2-6
Dimension (mean ± SE)	70.6±5.8×8.2±0.3 μm	45.7±5.4×6.8±0.3 μm

a Cultures were grown on PDA for 25 days at 25˚C under cool white light (fluorescent light, 2200 lux) in a 12 hr light/ 12 hr dark cycle.
b The number of measurement were 120 and 260 for the first and second group, respectively.

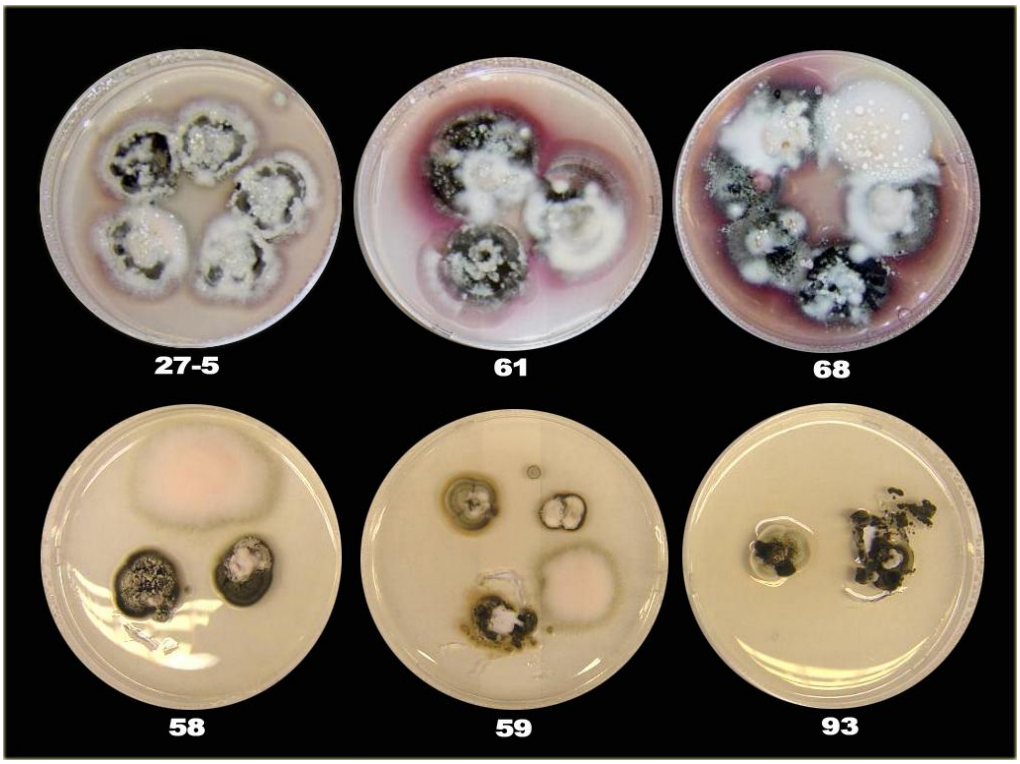


Figure 2-3. Colonies of *C. zeae-maydis* (*Czm*) (top) and *C. zeina* (*Cz*) (bottom) isolates grown on PDA at 28˚C for 21 days. *C. zeae-maydis* isolates can produce cercosporin resulting in the formation of reddish purple pigment in medium.

2.3.3 Identification using species-specific primers

A set of 84 *Cercospora* isolates, representing sub-sets putatively identified as *Czm* and *Cz*, were chosen for PCR assays. These isolates were derived from 57 lesions (1 or 2 single conidium per lesion) collected from 23 fields (Table 2-3). Two species-specific histone H3 primer pairs were used. The species-specific primers developed by Crous *et al*. (2006) were used to confirm species identity. The 284 bp fragment was present (Figure 2-4) when the PCR reaction mix comprised a combination of *Czm* DNA and primer CzeaeHIST (Lane 1) or *Cz* DNA and primer CzeinaHIST (Lane 2) respectively. Among all of the tested isolates, 23 and 58 *Cercospora* isolates were identified as *Czm* and *Cz*, respectively. This coincided with the preliminary identification relied on morphology and physiological characteristics. However, three

Table 2-3. Representative *Cercospora* isolates analyzed using species-specific primers. Numbers in parentheses indicate the designation of field / lesion. Isolates derived from the same leaf lesion are shown in bold.

Watershed	*Cercospora zeina*	*Cercospora zeae-maydis*	ND
Chemung	CH-2 (2-3) , CH-42 (2-3) CH-6-1 (2-2), CH-7-4 (2-2) **CH-8 (1-3), CH-12 (1-3)** CH-13 (1-1), CH-43 (1-1) CH-14-1 (1-2), CH-22-2 (1-2) CH-17-1 (6-1) CH-19-1 (6-3), CH-19-3 (6-3) **CH-20 (6-2)** CH-21-1 (6-4) CH-15-1 (7-2) CH-16-1 (7-1) CH-23-4 (4-4) CH-24-3 (4-5), CH-24-5 (4-5) CH-25-3 (4-3) CH-26-1 (4-1) CH-28-4 (4-2) CH-29-1 (5-2), CH-30-4 (5-2) CH-31-5 (3-7) CH-33-1 (3-4) CH-36-1 (8-4), CH-37-1 (8-4) CH-38-1 (8-5) CH-41-2 (8-1) ST-18-2 (46-1)	**CH-9 (1-3)** CH-10 (1-5) **CH-18 (6-2)** CH-32-1 (3-6) CH-34-1 (8-6), CH-35-2 (8-6)	CH-27-3 (4-1) CH-39-1 (8-3) CH-40-1 (8-3)
Tioga	**ST-5-1 (52-1), ST-10-1 (52-1)** ST-6-1 (52-4) ST-8-2 (53-2), ST-9-1 (53-2) ST-17-1 (49-1)	**ST-4-1 (52-1)** ST-7-1 (52-2) ST-12-2 (54-3), ST-13-1 (54-3) ST-14-4 (54-4) ST-15-1 (54-2) ST-16-3 (49-2)	
Owego-Wappasening	TI-1-1 (10-6), TI-1-6 (10-6) TI-2-4 (17-4), TI-3-1 (17-4) TI-4-2 (17-1) TI-10-2 (12-1) TI-11-2 (12-3) TI-15-4 (15-1) TI-16-1 (13-3), TI-16-4 (13-3) TI-17-2 (13-2), TI-17-5 (13-2) TI-7-6 (11-2), TI-8 (11-2) TI-18-1 (11-5), TI-19-1 (11-3), TI-19-5 (11-3)	TI-5-1 (15-7), TI-14-1 (15-7) TI-12-1 (12-4) TI-13-1 (12-5) TI-6-2 (11-1) TI-9-1 (11-4)	
Seneca	SC-1-1 (44-1), SC-2-2 (44-1) SC-3-1 (44-3)	TO-1-4 (24-1), TO-2-1 (24-1) TO-3-1 (23-2) CA-1-1 (27-3)	
Total	58	23	3

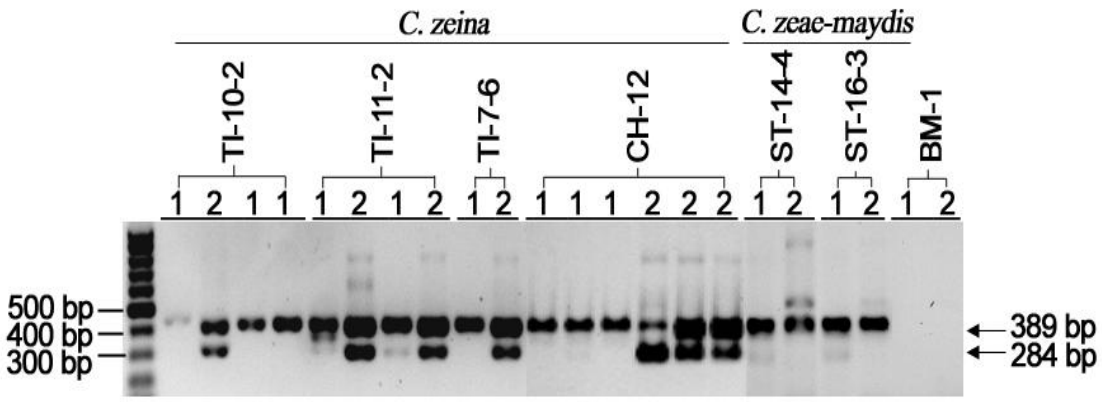


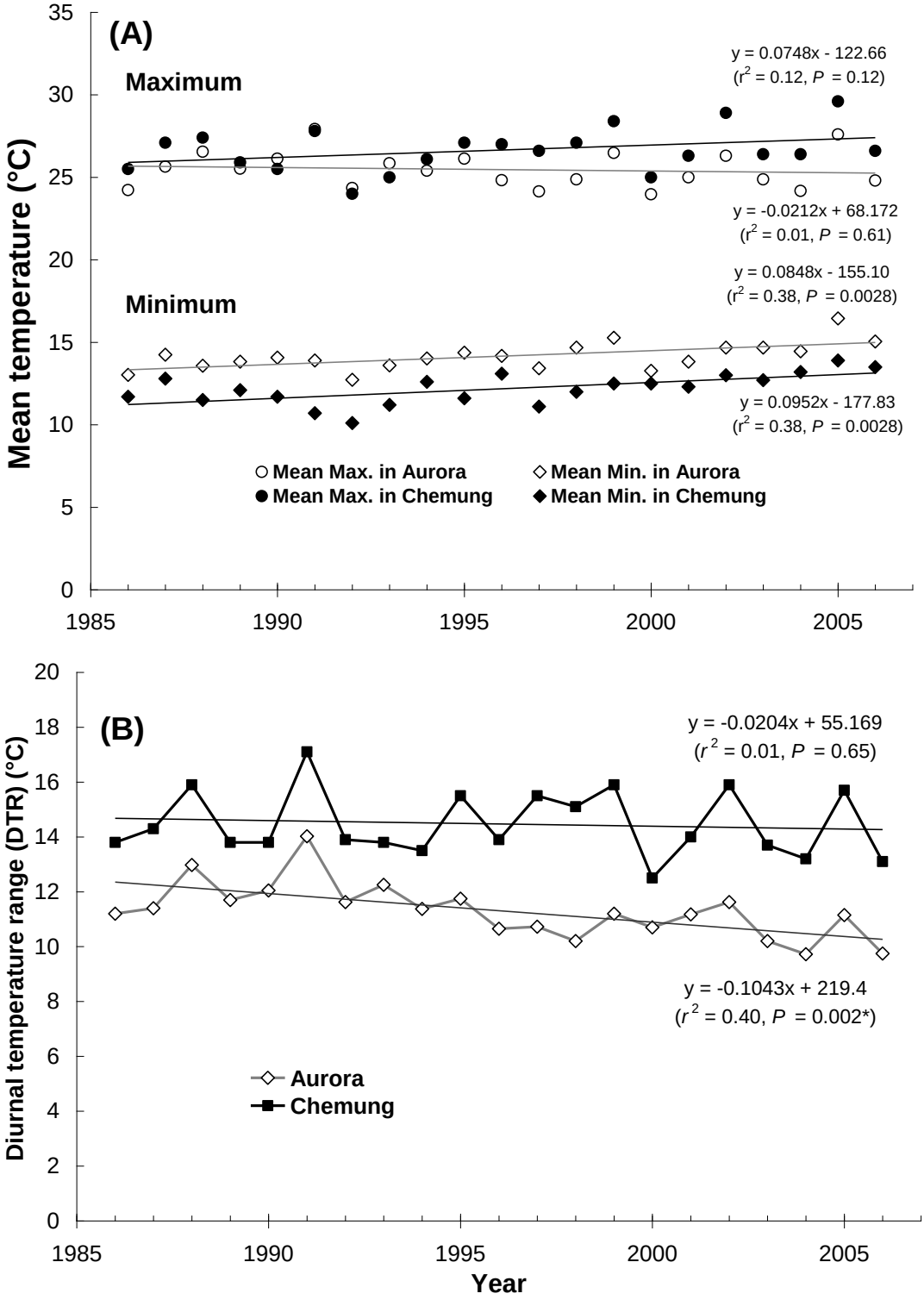
Figure 2-4. Four and two *Cercospora* isolates were identified as *C. zeina* and *C. zeae-maydis* respectively following the PCR assay using species-specific histone H3 primer pairs. TI-10-2, TI-11-2, TI-7-6, and CH-12 were confirmed as *C. zeina* based on the presence of the 284 bp fragment in Lane 2 but not in Lane 1. ST-14-4 and ST-16-3 were identified as *C. zeae-maydis* due to the presence of the 284 bp fragment in Lane 1 but not in Lane 2. The 284 bp and 389 bp fragments are absent in *Bipolaris maydis* isolate BM-1. The 389 bp fragment was used in the amplification as a reaction control present for all isolates.

isolates, previously recognized as *Cz*, could not be successfully identified. Additionally, among all of the detected lesions, three lesions, two collected from the same field in Elmira, Chemung County and one collected from Painted Post, Steuben County, were confirmed to have the sympatric coexistence of *Czm* and *Cz* (Table 2-3).

2.3.4 Weather Trends in Chemung and Aurora

To shed light on the possibility that changes in weather led to changes in pathogen population structure and that differences in climatic environments associated with differences in geographic distribution of pathogens, the weather trends in Chemung and Aurora were examined. A consistent and significant night-time warming trend for the maize growing season (June to September) was detected in the two localities over the 21-year period from 1986 to 2006 (Figure 2-5A). Linear regression of the seasonal mean minimum temperatures in the two localities showed a

Figure 2-5. Time series plots of seasonal mean maximum and minimum temperatures (A) and seasonal mean diurnal temperature range (DTR) (B), with linear regressions, for the maize growing season (June to September) over a 21-year period of 1986-2006 at the weather stations in Aurora (gray) and Chemung (black), New York. The increasing trends (slopes) for the mean minimum temperatures in the two localities and the decreasing trend in the DTR in Aurora, are statistically significant at the $P < 0.05$ probability level.


(A)
Maximum
y = 0.0748x - 122.66
(r^2 = 0.12, P = 0.12)
y = -0.0212x + 68.172
(r^2 = 0.01, P = 0.61)
Minimum
y = 0.0848x - 155.10
(r^2 = 0.38, P = 0.0028)
y = 0.0952x - 177.83
(r^2 = 0.38, P = 0.0028)
Mean temperature (°C)
Mean Max. in Aurora
Mean Min. in Aurora
Mean Max. in Chemung
Mean Min. in Chemung
(B)
y = -0.0204x + 55.169
(r^2 = 0.01, P = 0.65)
y = -0.1043x + 219.4
(r^2 = 0.40, P = 0.002*)
Diurnal temperature range (DTR) (°C)
Aurora
Chemung
Year

significant (P=0.0028 for both stations) increase by 1.7°C and 1.9°C in Aurora and Chemung, respectively, with a similar rate of steady increase of 0.1°C/year over that period. The mean maximum temperatures, however, did not show such significant increases (P>0.05) in the two localities. An insignificant decreasing (cooling) trend was even observed in Aurora.

The seasonal mean temperatures were significantly different between the two localities. Chemung had an average mean maximum temperature of 26.7°C, which was 1.2°C higher than 25.5°C in Aurora (Student's t test, P=0.003), while Chemung had an average mean minimum temperature of 12.2°C, 2°C lower than 14.2°C in Aurora (P<0.0001). The season diurnal temperature range (DTR) (difference between daytime maximum and nighttime minimum temperatures) was substantially larger (Student's t test, P<0.0001) in Chemung than in Aurora (14.5°C vs 11.3°C) (Figure 2-5B). However, no significant variation of the DTR was found in Chemung, while a significant (P=0.002) decline in the DTR was observed in Aurora.

The average seasonal rainfall in the two localities showed great fluctuation over the two decades but did not reveal a significant (P>0.1) decreasing or increasing tendency (Figure 2-6A). Nevertheless, the total rainfall from June to September for the two localities in 2006 actually exceeded 20-year average (1986-2005) for Aurora (375 mm) and Chemung (384 mm) by 136 mm and 158 mm, respectively (Table 2-4). The monthly rainfall for the 2006 growing season in the two localities was most intense in June (Figure 2-7), setting a June record of 216 mm in Chemung and the second most rain record of 159 mm in Aurora over the past two decades (Figure 2-6B).

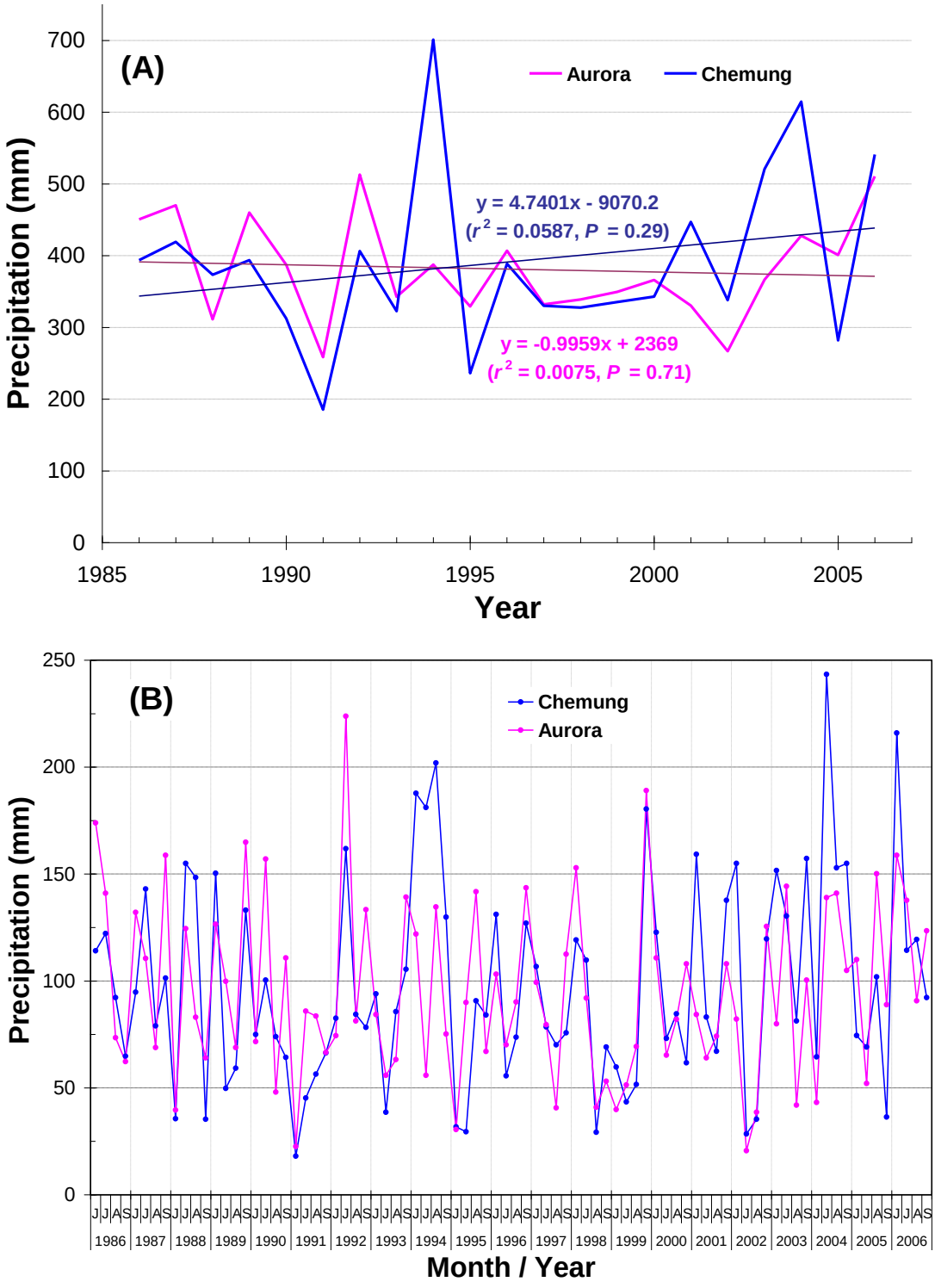


Figure 2-6. Time series annual seasonal rainfall and linear trend (A) and monthly rainfall (B) in Aurora and Chemung, New York, during the maize growing season (June to September) over a 21-year period from 1986 to 2006.

Table 2-4. Seasonal (June-September) mean temperature and total precipitation during 1986 to 2006 in Aurora and Chemung.

Year	Mean temperature (June - September) (°C)				Total precipitation (June - September) (mm)	
	Maximum (°C)		Minimum (°C)			
	Aurora	Chemung	Aurora	Chemung	Aurora	Chemung
1986	24.2	25.5	13.0	11.7	450.5	393.7
1987	25.7	27.1	14.3	12.8	470.1	419.1
1988	26.6	27.4	13.6	11.5	311.1	373.4
1989	25.5	25.9	13.8	12.1	460.0	393.7
1990	26.1	25.5	14.1	11.7	387.3	312.4
1991	27.9	27.8	13.9	10.7	258.6	185.4
1992	24.4	24.0	12.7	10.1	512.8	406.4
1993	25.9	25.0	13.6	11.2	342.6	322.6
1994	25.4	26.1	14.0	12.6	387.6	701.0
1995	26.1	27.1	14.4	11.6	329.2	236.2
1996	24.8	27.0	14.2	13.1	406.9	388.6
1997	24.2	26.6	13.4	11.1	332.0	330.2
1998	24.9	27.1	14.7	12.0	338.8	327.7
1999	26.5	28.4	15.3	12.5	349.5	335.3
2000	24.0	25.0	13.3	12.5	366.0	342.9
2001	25.0	26.3	13.8	12.3	330.4	447.0
2002	26.3	28.9	14.7	13.0	266.8	337.8
2003	24.9	26.4	14.7	12.7	366.5	520.7
2004	24.2	26.4	14.5	13.2	428.0	614.7
2005	27.6	29.6	16.5	13.9	401.1	281.9
2006	24.8	26.6	15.1	13.5	510.5	541.0
P value for trend	0.6078	0.1227	0.0028*	0.0028*	0.7089	0.2902
1986-2005[a]	25.6	26.7	14.2	12.1	374.8	383.5

a Average mean temperature and rainfall for the maize growing season over that timespan.
* Significant at a p = 0.05 level

2.3.5 Computing the minimum sample size

Given the low GLS incidence during the sampling year, we were concerned about incorrectly declaring a species to be absent from a given sampling field when the number of leaf lesions was small. Insufficient sample size could lead to incomplete information and a false declaration of absence of the species of interest when it was actually present. At a sampling density of 1-6 independent lesions per field, we

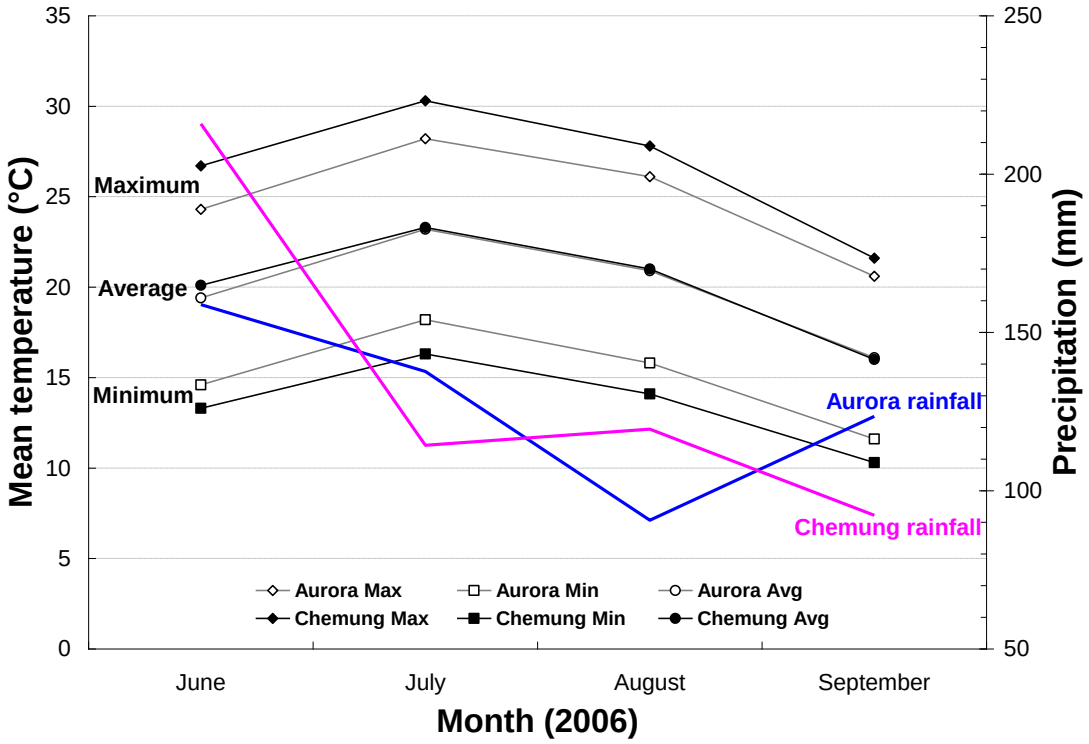


Figure 2-7. Monthly mean maximum, average, and minimum temperatures and total rainfall for the maize growing season (June to September) in Aurora and Chemung, New York, in 2006.

detected that the estimated proportion of *Czm* to *Cz* populations over the sampling areas was approximate 0.566 (1:1.8), with 37% of *Czm* (30 out of 81 lesions) and 65 % of *Cz* (53 out of 81 lesions) (Table 2-5). This overall estimate gave us a confidence level of 84% to 94% if the sample sizes were between 4 to 6 lesions per field (Figure 2-8), which were accounted for 61 % (49/81) of the total tested lesions and 40% (10/25) of the total fields with detected GLS incidence. Among the 25 locations, *Czm* and *Cz* were detected at least once at 15 (0.6) and 19 (0.76) locations, respectively.

The true absence of the *Czm* or *Cz* in a sampling field was probably inconclusive based on the sample size and confidence described, because we could not obtain a desired confidence (the conventional 95%), if the proportion or occupancy of either *Czm* or *Cz* was less than between 24% to 33% in a sampling field. However, it did

Table 2-5. (A) Detected proportions of *Czm* and *Cz* in all the lesion samples based on our sampling intensity of 1-6 lesions/field. (B) Conditional probability of detecting *Czm* and *Cz* at different estimated proportions of the two species if GLS present.

Sample size (No. lesions/field)	(A) No. sampling sites with detected occurrence of *Czm* and *Cz* according to lesion counts					(B) Conditional probability (%) of detection rate at various estimates of proportion *Czm*/*Cz*				
	Total (no. site)	% lesions		One species present (no. site)	Both species present (no. site)	50/50	40/60	30/70	20/80	10/90
		Czm	*Cz*							
1	3	1/3 (33%)	2/3 (66%)	3 (100%)	0 (0%)	50	40	30	20	10
2	7	5/14 (36%)	9/14 (64%)	6 (86%)	1 (14%)	75	64	51	36	19
3	5	8/15 (57%)	8/15 (57%)	4 (80%)	1 (20%)	88	78	66	49	27
4	**4**	**6/16 (38%)**	**10/16 (63%)**	**2 (50%)**	**2 (50%)**	**94**	**87**	**76**	**59**	**34**
5	**3**	**7/15 (47%)**	**9/15 (60%)**	**0 (0%)**	**3 (100%)**	**97**	**92**	**83**	**67**	**41**
6	**3**	**3/18 (17%)**	**15/18 (83%)**	**1 (33%)**	**2 (67%)**	**98**	**95**	**88**	**74**	**47**
	25	30/81 (37%)	53/81 (65%)	16 (64%)	9 (36%)					

gave us a power of greater than 84% to distinguish true absence from non-detection given the proportion of the target species was greater than 37%. Additionally, regardless of the absence data, we indeed found that the two species coexisted at 9 fields out of 25, and that the two species were even sympatrically present on the same lesions. We hence used at least four lesions/field as the cutoff value of sample size for following statistical analyses of the geographic distribution of the two species.

The frequency of presence/detection for the two species in each watershed were different, evaluated as the ratio of lesions on which the target species were detected to the total lesions sampled. The frequency for *Czm* was from 0.75 (6/8) in Seneca and

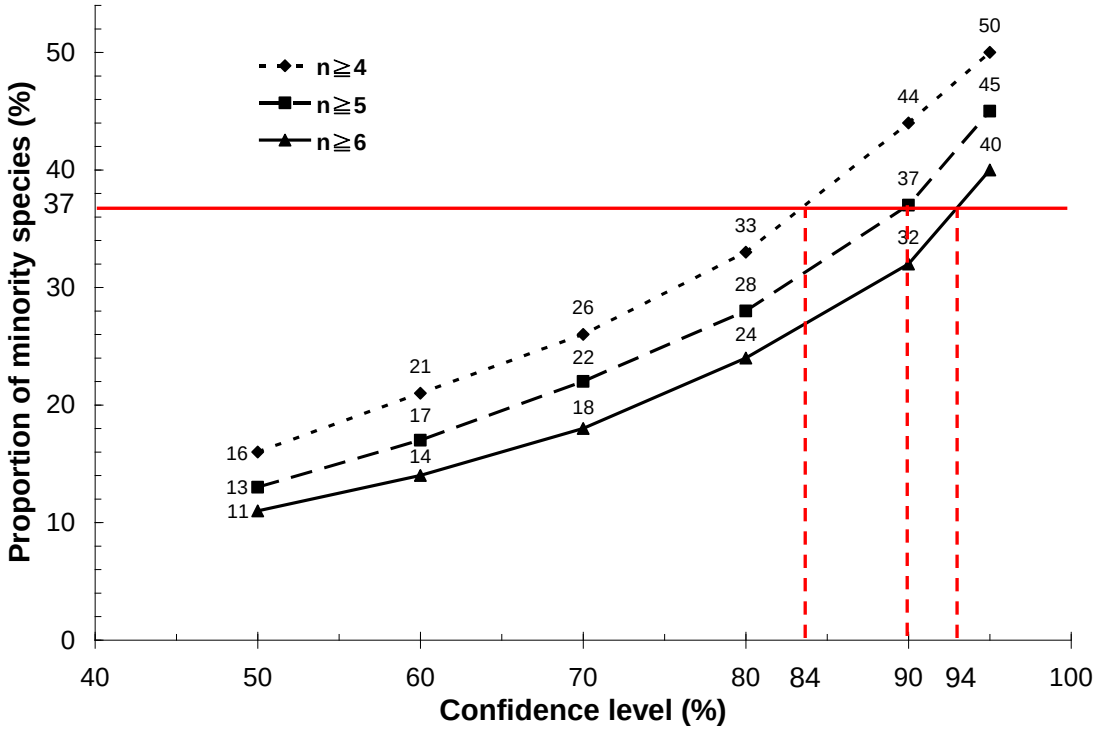


Figure 2-8. Detected overall proportions (37%) of the relatively minor species (*Czm*) and corresponding confidence level of 84%, 90%, and 94% (dashed lines in red) at sample sizes of 4, 5, and 6 lesions/field.

0.73 (8/11) in Tioga reduced to 0.42 (10/24) in Owego-Wappasening and 0.16 (6/38) in Chemung. In contrast, the frequency for *Cz* was higher in Chemung (0.9, 34/38) and in Owego-Wappasening (0.58, 14/24), but lower in Tioga (0.27, 3/11) and in Seneca (0.25, 2/8).

2.3.6 Frequencies of Czm and *Cz detections*

Based on the presence-only data, the distributions and frequencies of the two species populations detected in the Southern Tier and Central New York State were preliminarily examined (Figure 2-9 & Table 2-6). Of the 25 fields in which GLS pathogens were detected and recovered from one to six lesions per field, nine fields were detected the coexistence of *Czm* and *Cz*, with a tendency to a more frequent presence of *Cz*. The rest of the fields were found with only one species (six with only *Czm* and ten with only *Cz*). Overall, most of the fields that the GLS were found are located along the Chemung river and its tributaries in the Southern Tier of New York State. The detected frequencies of the two species in the sampling areas corresponding to various geographical categories were examined as below:

Watershed. Both *Czm* and *Cz* were commonly present across four watersheds, but their proportions varied among the watersheds. *Cz* was dominant (significantly different from the assuming 1:1 ratio at $P < 0.0001$) in Chemung with 73% relative frequency of occurrence across all the fields sampled (Figure 2-10 & Table 2-7a). *Czm*, however, was prevalent in Tioga and Seneca, with 67% and 75% relative frequencies, respectively. The in-field coexistence of the two species was usually observed across the corn production area.

Figure 2-9. Distribution of *Czm* and *Cz* in the Southern Tier and Central New York State based on the presence-only data. Black solid circle (●) and triangle (▲) represent the fields where only *Czm* and only *Cz* were respectively detected. Black square (■) represents the field where both species were found.

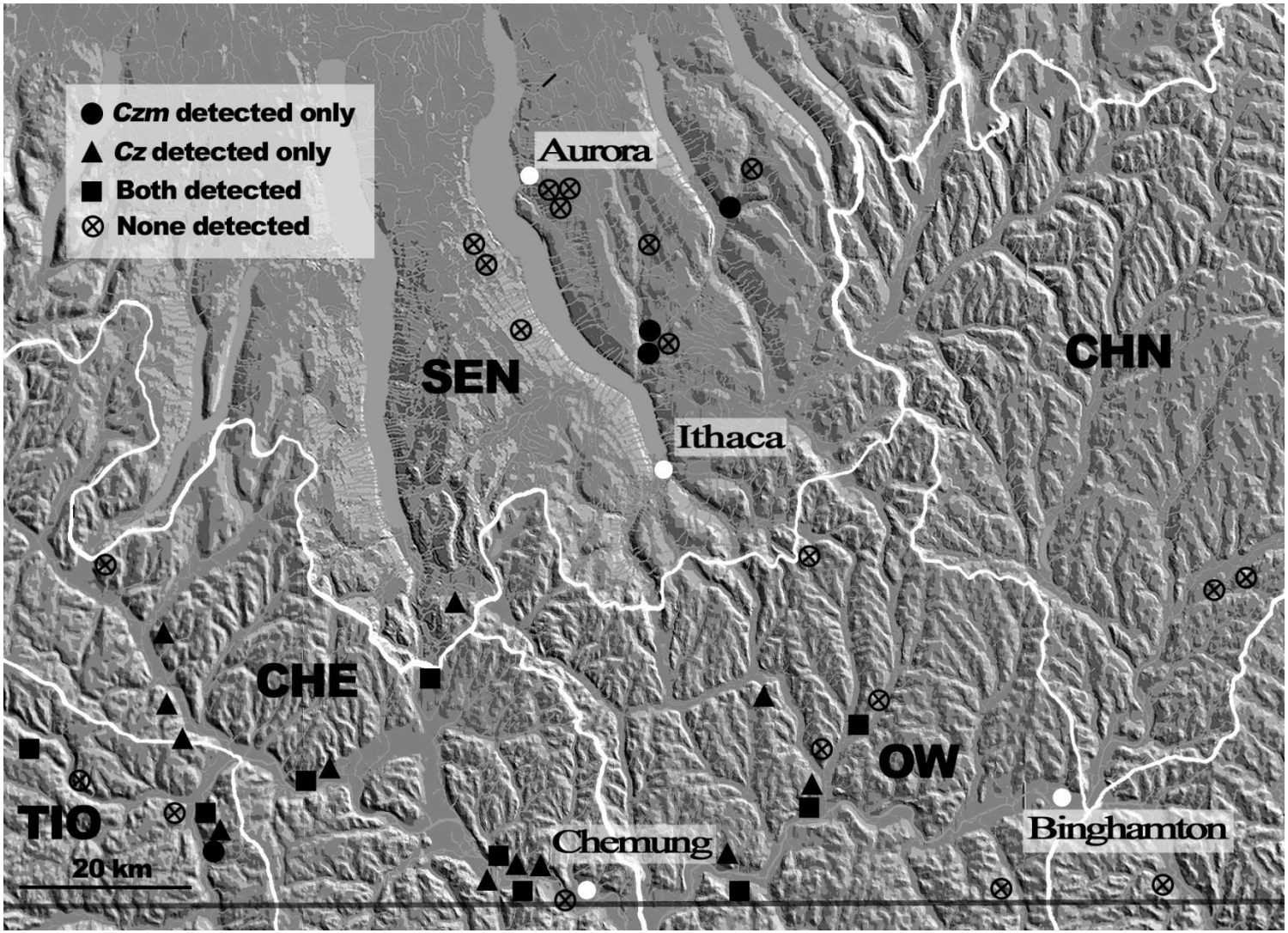

● Czm detected only
▲ Cz detected only
■ Both detected
⊗ None detected
Aurora
Ithaca
Chemung
Binghamton
SEN
CHN
CHE
OW
TIO
20 km

Table 2-6. Geographical distribution of *Czm* and *Cz* across 47 sampling fields in the Southern Tier and Central New York State.

Site	Geographic Origin		Number of *Cercospora* isolates (% of total collection)					Elev. (m)	Lat. (°N)	Long. (°W)
	County	Watershed[a]	Total	*Czm*[b]	*Cz*[c]	ND	No. lesions			
1	Big Flats, Chemung	CH	54	4 (7)	50(93)	-	5	268	42°07’	76°55’
2	Big Flats, Chemung	CH	38	0 (0)	38 (100)	-	3	277	42°08’	76°55’
3	Lowman, Chemung	CH	40	9 (23)	31 (77)	-	6	293	42°03’	76°43’
4	Lowman, Chemung	CH	104	0 (0)	104 (100)	-	6	244	42°01’	76°39’
5	Lowman, Chemung	CH	23	0 (0)	23 (100)	-	2	244	42°01’	76°38’
6	Chemung, Chemung	CH	38	1 (3)	31 (81)	6 (16)	4	448	42°00’	76°38’
7	Wellsburg, Chemung	CH	13	0 (0)	13 (100)	-	2	247	42°01’	76°43’
8	Horseheads, Chemung	CH	44	17 (39)	27 (61)	-	6	405	42°14’	76°47’
9	Sayre, PA[d]	CH	10	-	-	10 (100)	2	241	41°59’	76°37’
10	Barton, Tioga	OW	13	13 (100)	0 (0)	-	3	255	42°01’	76°22’
11	Nichols, Tioga	OW	42	22 (52)	20 (48)	-	5	253	42°00’	76°21’
12	Goodrich, Tioga	OW	42	24 (57)	18 (43)	-	4	250	42°06’	76°17’
13	Owego, Tioga	OW	30	0 (0)	30 (100)	-	4	259	42°08’	76°17’
14	Flemingville,Tioga[d]	OW	-	-	-	-	-	264	42°09’	76°15’
15	Flemingville, Tioga	OW	25	18 (72)	7 (18)	-	5	274	42°10’	76°14’
16	Newark Valley, Tioga[d]	OW	-	-	-	-	-	290	42°12’	76°11’
17	Candor, Tioga	OW	19	0 (0)	19 (100)	-	3	271	42°13’	76°20’
18	Vestal, Broome[d]	OW	-	-	-	-	-	329	42°00’	76°00’
22	Caroline, Tompkins[d]	OW	-	-	-	-	-	390	42°22’	76°17’
23	Ludlowville, Tompkins	SE	12	12 (100)	0 (0)	-	3	168	42°34’	76°31’
24	Ludlowville, Tompkins	SE	9	9 (100)	0 (0)	-	2	182	42°35’	76°32’
25	Groton, Tompkins[d]	SE	-	-	-	-	-	274	42°36’	76°31’
26	Genoa , Cayuga[d]	SE	-	-	-	-	-	370	42°40’	76°30’
27	Moravia, Cayuga	SE	2	2 (100)	0 (0)	-	1	331	42°44’	76°23’
28	Moravia, Cayuga[d]	SE	-	-	-	-	-	480	42°45’	76°21’
29	Aurora, Cayuga[d]	SE	-	-	-	-	-	257	42°44’	76°39’
30	Aurora, Cayuga[d]	SE	-	-	-	-	-	227	42°45’	76°40’
31	Aurora, Cayuga[d]	SE	-	-	-	-	-	278	42°45’	76°38’
32	Cayuga, Cayuga[d]	SE	-	-	-	-	-	147	42°57’	76°41’
33	Auburn, Cayuga[d]	SE	-	-	-	-	-	180	42°58’	76°33’
34	Auburn, Cayuga[d]	SE	-	-	-	-	-	133	42°59’	76°40’
35	Weedsport, Cayuga[d]	SE	-	-	-	-	-	120	43°03’	76°33’
36	Weedsport, Cayuga[d]	SE	-	-	-	-	-	118	43°04’	76°33’
41	Ovid, Seneca[d]	SE	-	-	-	-	-	240	42°40’	76°45’
42	Interlaken, Seneca[d]	SE	-	-	-	-	-	201	42°39’	76°43’
43	Trumansburg, Seneca[d]	SE	-	-	-	-	-	298	42°33’	76°40’
44	Alpine, Schuyler	SE	18	0 (0)	18 (100)	-	2	352	42°18’	76°46’
45	Bath, Steuben[d]	SE	-	-	-	-	-	349	42°22’	77°17’
46	Savona, Steuben	CH	4	0 (0)	4 (100)	-	1	319	42°16’	77°13’
47	Curtis, Steuben	CH	4	0 (0)	4 (100)	-	1	300	42°12’	77°09’

Table 2-6. (Continued)

Site	Geographic Origin		Number of *Cercospora* isolates (% of total collection)					Elev. (m)	Lat. (°N)	Long. (°W)
	County	Watershed[a]	Total	*Czm*[b]	*Cz*[c]	ND	No. lesions			
48	Coopers Plains, Steuben	CH	4	0 (0)	4 (100)	-	2	297	42°10’	77°09’
49	Cameron, Steuben	TI	18	11 (61)	7 (39)	-	2	316	42°11’	77°23’
50	Rathbone, Steuben[d]	TI	-	-	-	-	-	304	42°08’	77°19’
51	Erwin, Steuben[d]	TI	-	-	-	-	-	295	42°06’	77°10’
52	Erwin, Steuben	TI	35	22 (63)	13 (37)	-	3	291	42°06’	77°08’
53	Erwin, Steuben	TI	12	12 (100)	0 (0)	-	2	291	42°05’	77°08’
54	Presho, Steuben	TI	36	27 (75)	0 (0)	9 (25)	4	293	42°04’	77°08’
	Total	4	689	203 (30)	461 (67)	25 (3)	83			

[a] Watersheds CH: Chemung; OW: Owego-Wappasening; SE: Seneca; TI: Tioga

[b] *Cercospora zeae-maydis*; [c] *Cercospora zeina*

[d] The field was not found the typical GLS symptoms on maize during sampling and was considered as absence of *Czm* and *Cz* populations during the sampling period.

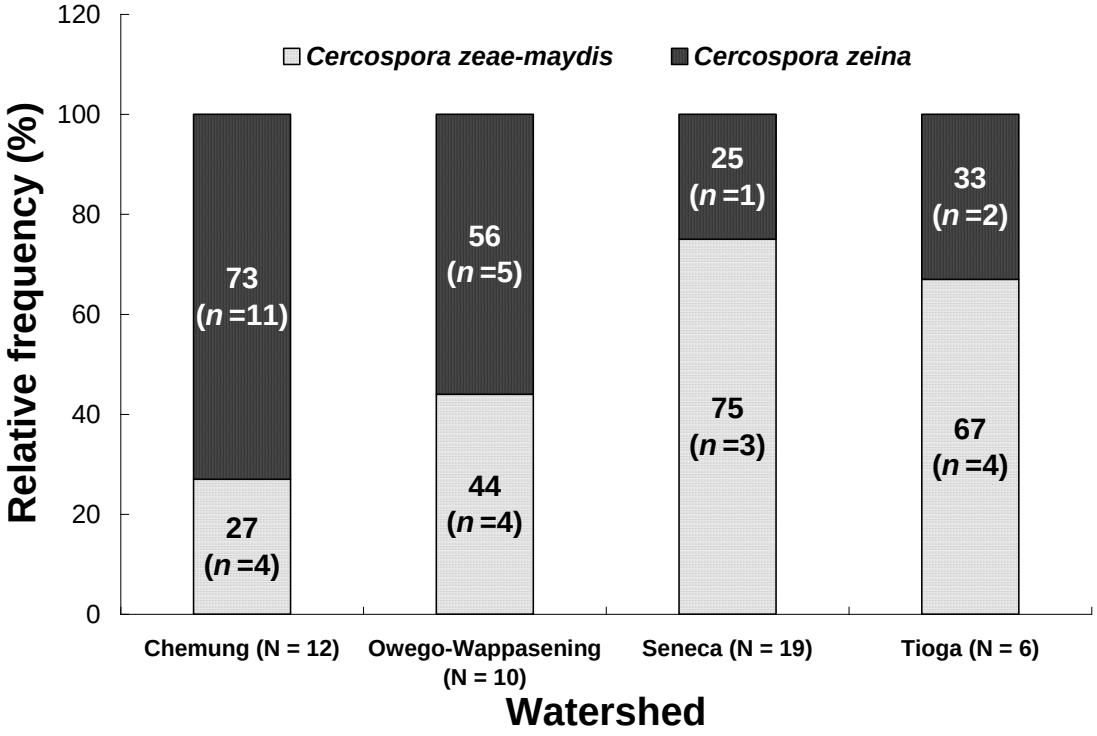


Figure 2-10. Relative frequency of *Czm* and *Cz* occurrence in 47 sampling fields across four watersheds in the Southern Tier and Central New York State in 2006, based on presence-only data (uncorrected for detection probability). The number in parenthesis represents the number of fields *Czm* or *Cz* detected within the respective watershed.

Altitude. GLS incidences and *Czm* and *Cz* populations seemed to be more frequently detected in the fields located at lower altitudes (< 365m) than higher altitudes (>365m) (Figure 2-11A & Table 2-7b). However, there was no significant difference from expected 1:1 ratio of detection frequency for the two species in the two altitude ranges (χ^2 = 1.96, P = 0.16 for < 365m; χ^2 = 1.0, P = 1.00 for > 365m).

Latitude. Most of the fields detected *Czm* and/or *Cz* were located in the southern latitudinal zones between 42°00' to 42°15'N, within the Southern Tier of New York State. *Czm* and *Cz* were present in 12 and 17 fields respectively, along with an insignificantly (χ^2 = 3.24, P = 0.07) different frequency of detection between the two

species (Figure 2-11B & Table 2-7c). Only three out of 21 fields were detected the GLS in northern latitudes approximately 42°16’ to 43°10’N, along with the identification of *Czm* and *Cz* in two and one fields, respectively.

Table 2-7. Frequencies of *Czm* and *Cz* occurrence in 47 sampling fields by watershed, altitude, latitude, and longitude, based on detection-non detection data (uncorrected for detection probability).

Category	Frequency of occurrence (fields) (%)					
	Cercospora zeae-maydis			*Cercospora zeina*		
	Detected (n=15)	Undetected (n=32)	Total (N=47)	Detected (n=19)	Undetected (n=28)	Total (N=47)
(a) Watershed						
Chemung	4 (33%)	8 (67%)	12	11 (92%)	1 (8%)	12
Owego-Wappasening	4 (40%)	6 (60%)	10	5 (50%)	5 (50%)	10
Seneca	3 (16%)	16 (84%)	19	1 (5%)	18 (95%)	19
Tioga	4 (67%)	2 (33%)	6	2 (33%)	4 (67%)	6
(b) Altitude (m)						
<365	13 (31%)	29 (69%)	42	17 (40%)	25 (60%)	42
>365	2 (40%)	3 (60%)	5	2 (40%)	3 (60%)	5
(c) Latitude (°N)						
42°00’-42°15’(South)	12 (46%)	14 (54%)	26	17 (65%)	9 (35%)	26
42°16’-43°10’(North)	3 (14%)	18 (86%)	21	2 (10%)	19 (90%)	21
(d) Longitude (°W)						
76°00’-76°45’ (East)	9 (26%)	25 (74%)	34	10 (29%)	24 (71%)	34
76°46’-77°30’(West)	6 (46%)	7 (54%)	13	9 (69%)	4 (31%)	13

Longitude. The detected GLS incidence between the two longitudinal ranges was not statistically different ($P = 0.82$) (Figure 2-11C & Table 2-7d). Likewise, the frequencies of *Czm* and *Cz* detection were not significantly different ($P > 0.05$) in the eastern half of the sampling area (76°00' to 76°45'W). However, *Cz* was significantly ($P = 0.046$) more frequently detected than *Czm* in the western half of the sampling area (76°46' to 77°30'W).

2.3.7 Modeling of the probability of Czm and Cz occurrences using logistic regression

After adjusting for the imperfect detection probabilities, the presence/absence data over 39 fields for *Czm* and 42 fields for *Cz* were used for examining the association of the *Czm* and *Cz* occurrence and various geographic variables as well as for modeling the probability of *Czm* and *Cz* occurrence.

***C. zeae-maydis*.** Chi-square analyses showed that, among all the pairs of response and explanatory variables, latitude (P = 0.005) and longitude (P = 0.047) were the two geographic variables that were significantly associated with the presence or absence of *Czm*, as the two were treated as categorical variables (Table 2-8). The value of Cramer's *V* for latitude and longitude were 0.454 and 0.317, respectively, indicating a moderate relationship between the frequency of *Czm* occurrence and latitude and longitude zones. Although unable to reject the null hypothesis, there was a marginal significance of 0.0507 for the association between watershed and the occurrence of *Czm*, with a Cramer's *V* of 0.447.

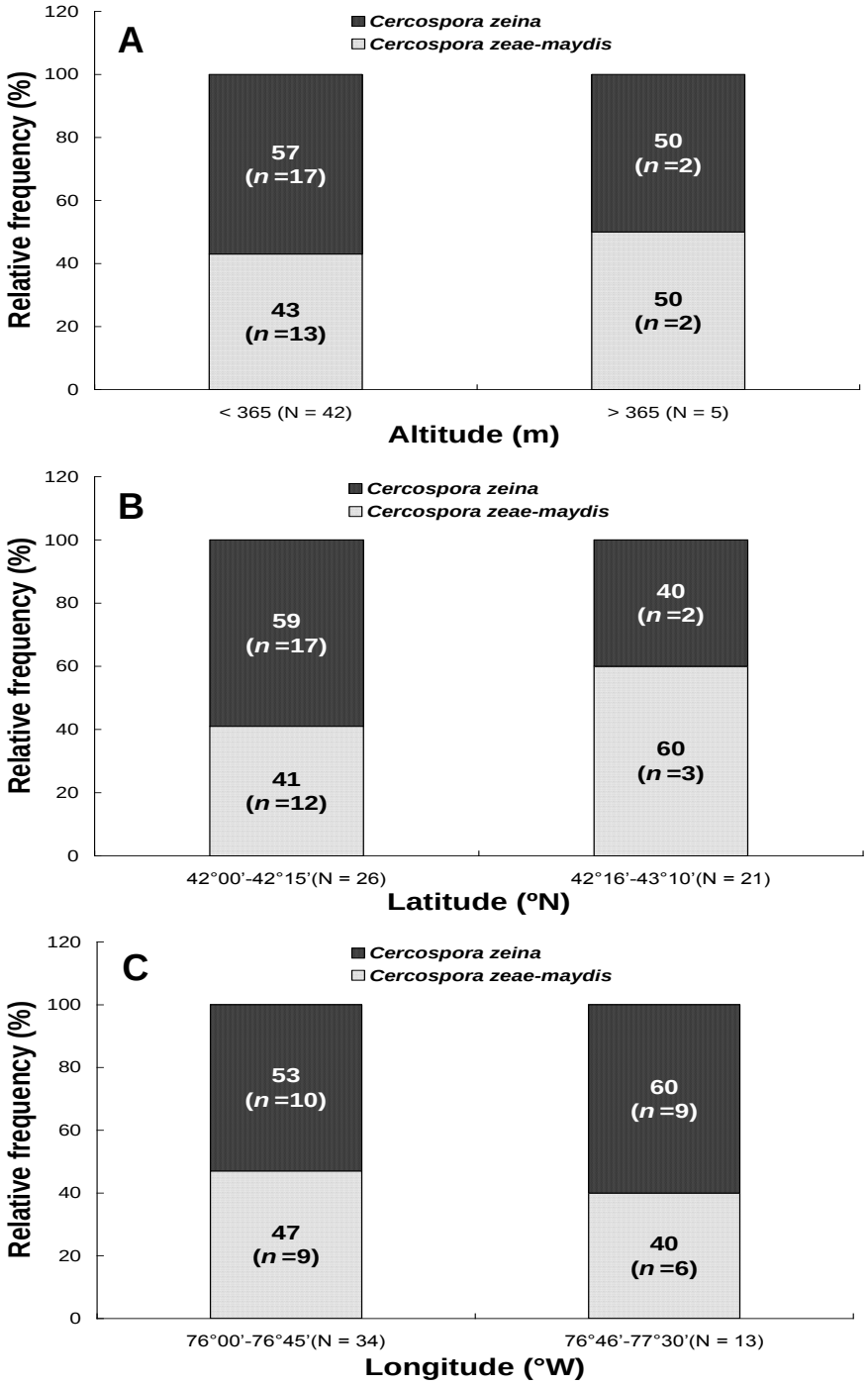


Figure 2-11. Relative frequency of *Czm* and *Cz* occurrence in 47 sampling fields by altitude (A), latitude (B), and longitude (C), based on presence-only data. The number in parenthesis represents the number of fields *Czm* or *Cz* detected under the respective category.

Table 2-8. Frequencies of *Czm* and *Cz* occurrence by watershed, altitude, latitude, and longitude, based on the detection-non detection data adjusted for detection probability.

Variable	Frequency of occurrence (%)					
	Cercospora zeae-maydis			*Cercospora zeina*		
	Present (n=15)	Absent (n=24)	Total (N=39)	Present (n=19)	Absent (n=23)	Total (N=42)
(a) Watershed						
Chemung	4 (67%)	2 (33%)	6	11 (92%)	1 (8%)	12
Owego-Wappasening	4 (44%)	5 (56%)	9	5 (56%)	4 (44%)	9
Seneca	3 (17%)	15 (83%)	18	1 (6%)	15 (94%)	16
Tioga	4 (67%)	2 (33%)	6	2 (40%)	3 (60%)	5
Chi-square	χ^2 = 7.782, *P* = 0.0507			**χ^2 = 20.701, *P* < 0.0001**		
Cramer's *V*	0.447			**0.702**		
(b) Altitude (m)						
Categorical						
<365	13 (38%)	21 (62%)	34	17 (46%)	20 (54%)	37
>365	2 (40%)	3 (60%)	5	2 (40%)	3 (60%)	5
Chi-square	χ^2 =0.006, *P* = 0.9396			χ^2 = 0.063, *P* = 0.8020		
Cramer's *V*	0.012			0.039		
Continuous	χ^2 =0.92, *P* = 0.3377			χ^2 =1.64, *P* = 0.2009		
(c) Latitude (°N)						
Categorical						
42°00'-42°15'(South)	12 (60%)	8 (40%)	20	17 (72%)	7 (28%)	24
42°16'-43°10'(North)	3 (16%)	16(84%)	19	2 (6%)	16(945%)	18
Chi-square	**χ^2 =8.046, *P* = 0.0046 (0.0079)[a]**			**χ^2 = 17.856, *P* < 0.0001 (0.0001)[a]**		
Cramer's *V*	**0.454**			**0.652**		
Continuous	**χ^2 = 4.48, *P* = 0.0343**			**χ^2 = 7.38, *P* = 0.0066**		
(d) Longitude (°W)						
Categorical						
76°00'-76°45' (East)	9 (30%)	21 (70%)	30	10 (34%)	19 (66%)	29
76°46'-77°30'(West)	6 (67%)	3 (33%)	9	9 (70%)	4 (30%)	13
Chi-square	**χ^2 =3.933, *P* = 0.0474**			**χ^2 = 4.375, *P* = 0.0365 (0.0494)[a]**		
Cramer's *V*	**0.317**			**0.323**		
Continuous	χ^2 = 1.04, *P* = 0.3076			χ^2 = 0.88, *P* = 0.3481		

Categorical variables in each population were compared using Pearson's chi-square test.
Statistically significant ($p < 0.05$) association between species and categorical variable is shown in bold.
[a] The *p*-value for two-tailed Fisher's exact test.

When the two significant geographic variables, longitude and latitude, which are correlated with each other, were simultaneously included in a multiple logistic regression model, only latitude had a significant effect on the presence or absence of *Czm* (Wald χ^2=4.94, P=0.0262) (Table 2-9a). Latitude was the only significant factor able to predict the probability of the *Czm* occurrence. There were a statistically significant positive coefficient of 0.91 with an odds ratio of 6.2 for latitude zone in a range from 42°00'-42°15'N. This indicated that *Czm* was more likely detected in south part of New York State, and suggested that the probability of *Czm* occurrence in south part was approximately 6 times greater than that in north part. The area under the ROC curve was 0.7625 (Figure 2-12), which was significantly greater than 0.5, indicating sufficient discriminating power for this model to predict or detect the occurrence of *Czm*.

Table 2-9. Results of multiple logistic regression. Parameter estimates of the logistic regression relating the probability of *Czm* and *Cz* occurrence evaluated with a Wald's chi-square test.

Variables	Coefficient (β)	Standard error	Wald χ^2	OR (e^{β})	*p*-value
(a) ***C. zeae-maydis***					
Intercept	-0.41	0.47	0.76		0.3835
Latitude (42°00'-42°15')	0.91	0.41	4.94	6.2	0.0262*
Longitude (76°00'-76°45')	-0.41	0.45	0.84	0.4	0.3605
(b) ***C. zeina***					
Intercept	-0.12	0.46	0.07		0.7906
Watershed (TI)	Ref.				
Watershed (CH)	2.52	0.87	8.36	16.5	0.0038*
Watershed (OW)	0.35	0.66	0.27	1.875	0.6017
Watershed (SE)	-2.59	0.87	8.93	0.1	0.0028*

* Significant at 0.05 level

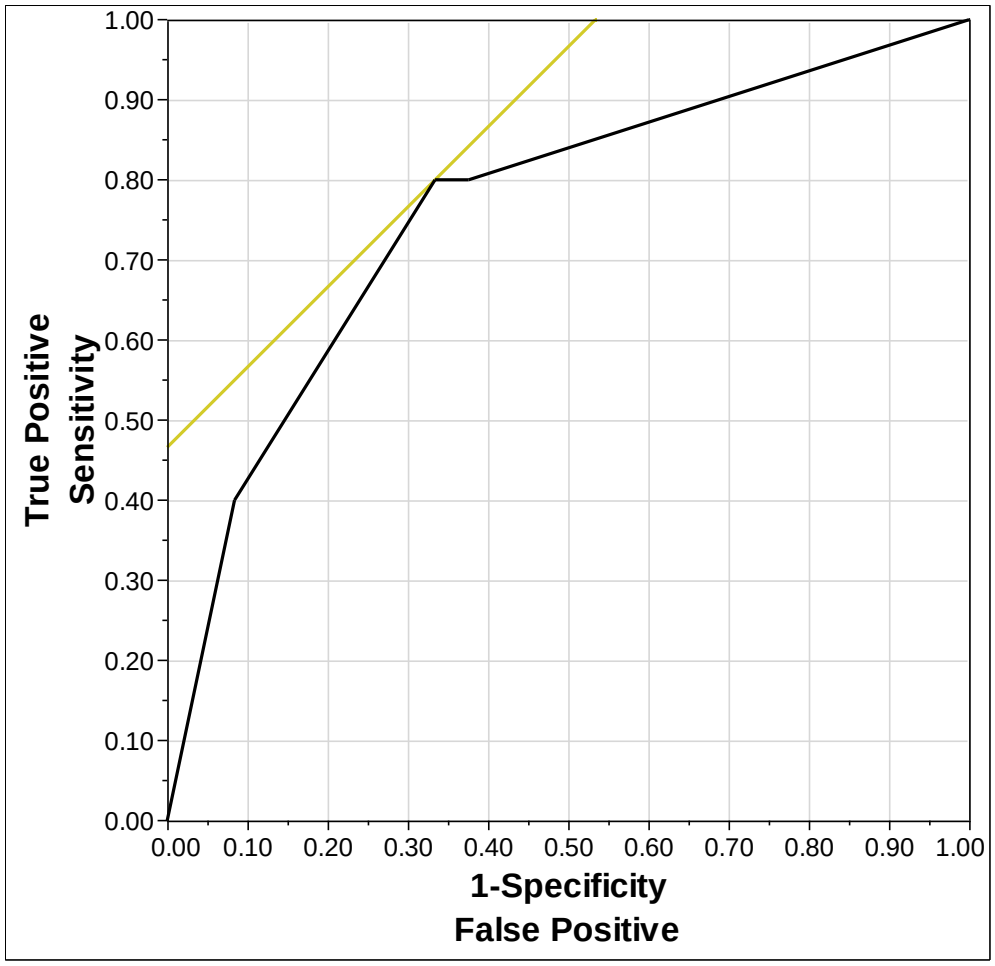


Figure 2-12. Receiver Operating Characteristic (ROC) curve for the multiple regression analysis of factors predicting the probability of *Czm* occurrence in the Southern Tier New York State. The area under the ROC curve is 0.7625, indicating a sufficient discriminative ability. (Using the presence of *Czm* =1 as the positive level).

C. zeina. The univariate chi-square analysis showed significant association between the frequency of *Cz* occurrence and watershed (χ^2 = 20.701, P < 0.0001), latitude zone (χ^2 = 17.856, P < 0.0001) as well as longitude (χ^2 = 4.375, P = 0.0365) (Table 2-8). The Cramer's *V* was 0.702, 0.652, and 0.323 for watershed, latitude, and longitude, respectively, indicating a strong correlation between the frequency of *Cz* occurrence and watershed and latitude.

A logistic regression model fitted to the binary presence or absence data of *Cz*, using watershed, latitude, and longitude as potential predictors was built. The final logistic regression model (Table 2-9b) revealed that watersheds, including Chemung and Seneca, was the two significant predictors of the probability of *Cz* occurrence in the Southern Tier and Central New York State. There were a statistically significant positive coefficient of 2.52 (P = 0.0038) with an odds ratio of 16.5 for Chemung watershed and a negative coefficient of -2.59 (P=0.0422) with an odds ratio of 0.07 for Seneca watershed. This indicated that *Cz* was more likely detected in Chemung, while it was less likely detected in Seneca. The high odds ratio of 16.5 for Chemung and a quite low odds ratio of 0.07 for Seneca (using Tioga as reference) suggested that the probability of *Cz* occurrence in Chemung watershed was approximately 16 times greater than in Tioga watershed. In contrast, the probability of *Cz* occurrence in Seneca was 0.07 times than that in Tioga watershed. The chi-square goodness-of-fit test showed that the final model was a good fit to the observed data (P = 0.5673). The area under the ROC curve was 0.8879 (Figure 2-13), significantly greater than 0.5, indicating sufficient discriminating power for this model to predict or detect the occurrence of *Cz*.

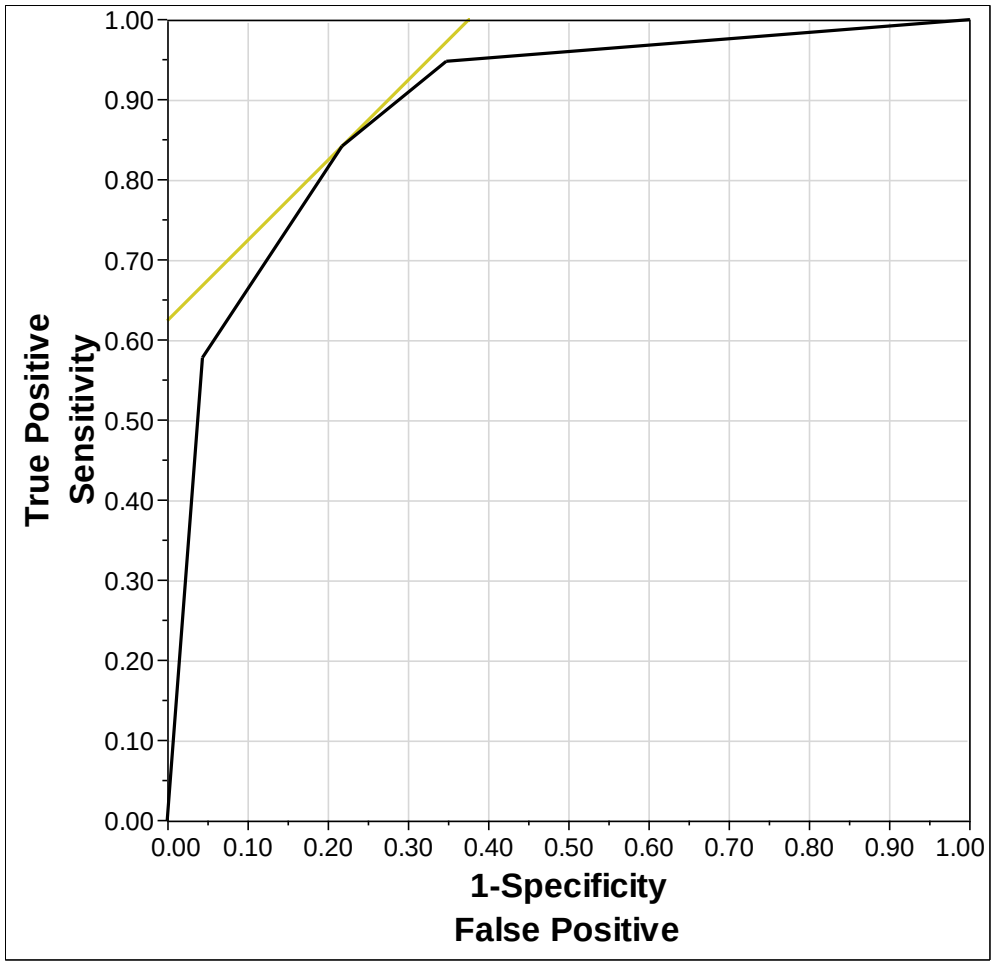


Figure 2-13. Receiver Operating Characteristic (ROC) curve for the multiple regression analysis of factors predicting the probability of *Cz* occurrence in the Southern Tier New York State. The area under the ROC curve is 0.8879, indicating a sufficient discriminative ability. (Using the presence of *Cz* =1 as the positive level).

2.3.8 Interspecific association of the two species

Based on the frequencies of the two species occurrences across the 54 sampling sites in the contingency table (Table 2-10a), a chi-square and Yates's correction chi-square test of independence confirmed that there was a statistically significant ($p < 0.05$) evidence to conclude that the relationship between the two species was not independent (Table 2-10b). The association coefficients C_8, *R*, and V were 0.3764,

0.3067 and 0.3223, respectively, indicating that the strength of association was moderate.

Table 2-10. (a) A 2 x 2 contingency table for the *Czm* and *Cz* relationship based on the binary presence/absence of observed counts from the 54 independent sampling sites across seven watersheds in the southern, central and northern New York State. (b) Chi-square analysis and strength of interspecific association between *Czm* and *Cz* at a p-value of 0.05 with degree of freedom = 1.

(a)

Cercospora zeae-maydis	*Cercospora zeina* Present	Absent	Total
Present	9	6	15
Absent	10	29	39
Total	19	35	54

(b)

	Chi-square		Yates's Chi-square		Coefficients of association				
	χ^2	*p*	χ^2	*p*	Hurlbert (C_8)	Pearson (*R*)	Ochiai (*OI*)	Dice (*DI*)	Jaccard (*JI*)
Czm x *Cz*	5.608	0.0179*	4.2026	0.0403*	0.3764	0.3067	0.5331	0.5294	0.36

* Significant at a P = 0.05

2.4 Discussion

The coexistence of *C. zeae-maydis* (Group I) and *C. zeina* (Group II) in southern New York State was first suggested by Wang *et al*. (1998), based on a sample of two isolates collected from Chemung in 1994. Our present study revealed that a decade later, *C. zeae-maydis* and *C. zeina* were commonly present in maize fields in central-south New York State. The two species either existed alone in separate fields or coexisted in the same field but not on the same lesion, which was in accordance with previous reports in the U.S. (Wang *et al*., 1998), Colombia (Vanegas *et al*., 2002a,b) and Brazil (Brunelli, 2004).

The isolates of two *Cercospora* species were recovered mostly from the leaf samples found in maize growing areas along the low-lying areas and river valleys where, presumably, there are more favorable climatic conditions for the development and spread of the disease (Lipps & Mills, 2001; De Wolf, 2002). No obvious difference in symptoms caused by the two species was observed. However, *Cz* produced significantly smaller conidia than *Czm*, which was coincided with the previous studies (Brewster & Carson, 2001; Crous *et al*., 2006).

The GLS incidence and the lesion levels were moderate in Southern Tier of New York State and quite low in Central New York State, during the survey in 2006. Still, the presence of disease was uncommon in sampling area because of its rather low frequency of detection in past years (M. Smith, Cornell University, personal communication). Some epidemiological possibilities involved with the higher disease level and the interactions between crop host, pathogen and environment were explored.

(1) The 2006 growing season tended to be wet in the Southern Tier and the Central New York State. The total rainfall from June to September in Chemung (541 mm) was 41% higher than the 1986-2005 average (383.5 mm), along with a new local rainfall record for June. We have no basis to assume that the relatively abundant

rainfalls would definitely contribute to high relative humidity and extended period of leaf wetness within the maize canopy during the 2006 growing season. Several studies (Latterel & Rossi, 1983; Ward & Nowell, 1998) had also indicated that high level of rainfall is not always essential for early infection and disease increase. A web-published report (Raley, 1999) further noted that rainfall sometimes might be adverse to the spread of the conidia. However, the heavy amount of rainfalls for early growing season (May and June) and above-average total seasonal rainfalls seemed to create a very conducive environment for primary infection of pathogen and GLS development (Ringer & Grybauskas, 1995). Additionally, the monthly mean temperatures in summer (from June to August) in Chemung and Elmira (COOP 302610) were 20 ºC, 22.8 ºC, 20.9 ºC and 18.6 ºC, 22 ºC, 20 ºC, respectively, which were close to normal or above average. It also contributed to a higher incidence of GLS in 2006 than the past years.

(2) Extensive samplings were carried out from the mid of July through the end of August as local maize populations were at or sometime after anthesis or tasselling, which is a typical time period for the emergence of initial GLS symptoms in the U.S. (Ward *et al*., 1999; Vincelli & Hershman, 1995). In light, the rectangular necrotic lesions were mostly observed in the lower canopy below the ear leaf during the field trips, indicating the early development of the disease. When the characteristic symptoms appeared, which was indeed preceded by a long incubation period varying from two to four weeks (Beckman & Payne, 1982, 1983; Latterell & Rossi, 1983; Ringer & Grybauskas, 1995), signifying that the disease had presumably been prevalent in the field. With subsequent sporulating lesions triggering the secondary infection, the persistent fungal inoculum might cause more disease later in the season under favorable conditions.

(3) It had been reported in several pathosystems, such as in the *Phytophthora*-potato system (Koh *et al*., 1994), that the introduction and establishment of a new species or a mating type to a region is likely to change the epidemiology of plant disease. Despite current lack of the substantial evidence of the recent *Cz* migration, it had been suggested that *Cz* is a relatively recent newcomer to North America when compared to *Czm* (Dunkle and Levy, 2000). This could be partially a contributing factor to the variation of the disease level in Southern Tier of New York State in time and space.

Climate change would lead to profound changes in the composition of ecosystems and the ability of pathogens to invade new regions (Welch, 2005; Garrett *et al*., 2006; Evans *et al*., 2008). The change of temperature for the maize growing season over the 21-year period of 1986-2006 in Aurora and Chemung was significantly higher for mean minimum (night time) temperature than mean maximum (day time) temperature, with a significant warming trend in the mean minimum temperature for both localities. There was a decreasing trend in the diurnal temperature range (DTR) in Aurora, which is in accordance with model projections and many observations across the United States (Easterling *et al*.,1997; Dai *et al*., 2001). Several studies has reported that increased night time temperature or changes in DTR caused by global warming would lead to different responses of yield in cereal crops in different growing regions (Lobell, *et al*., 2005; Peng *et al*., 2004; Lobell, 2007). However, whether the change in DTR would change the population structures of the pathogens or change the responses of pathogen populations to crop hosts and environments still remained unknown. As a result, whether the change of DTR would have influenced the maize-pathogen interactions, and leading to the differences of the frequency of *Czm* and *Cz* occurrence and the species abundance as well as the prevalence and severity of the disease are still needed further study.

There was a significant correlation between the *Czm* occurrence and two explanatory variables, latitude and longitude, in univariate analyses of association. After fitting the logistic regression model, it turned out that latitude was the only significant geographic variable able to predict the *Czm* occurrence, along with a greater possibility of detecting *Czm* in southern than in northern latitudinal zone. For *Cz* population, the watersheds, including the Chemung and Seneca, were the most significant factors in predicting the *Cz* occurrence. Furthermore, based on the logistic regression model, the probability of detecting *Cz* in Chemung is 165 times (OR = 165) greater than in Seneca. The results could be explained by the differences in climatic conditions and long term weather trends between Chemung and Aurora, the two regions in different watersheds within different latitudinal ranges. Chemung region is characterized by a higher seasonal mean maximum temperature and a lower mean minimum temperature than Aurora. The larger diurnal temperature range in Chemung was suggested creating the conditions conducive to dew formation at night with periods of prolonged leaf wetness favorable for the weather-dependent GLS development and the infection and colonization of *Czm* and *Cz*. In contrast, Aurora had lower mean daytime temperature and a smaller DTR, with an insignificant decreasing (cooling) trend. Consequently, more GLS incidences were observed and more *Czm* and *Cz* occurrence were detected in Chemung and the neighboring regions in the Southern Tier of New York State than in Aurora and the Central New York State.

Our results showed the moderate positive association between *Czm* and *Cz*, indicating that the two species have been steadily coexisted in the southern New York State. No negative interspecific association was detected between *Czm* and *Cz*, suggesting that the two species have overlapping niches but they do not avoid or directly compete against each other. Among the geographic variables, watershed was

the most significant predictor contributing to the probability of *Cz* occurrence in South New York State. We suggested that the distribution and dispersion of *Cz* is more geographic, weather and/or microclimate-directed. Compared with *Czm* population, *Cz* might have more strict requirement for colonization and spread. For a possible a relatively new immigrant, this may explain *Cz* has been restrictedly distributed in the northeastern area of the U.S. and South America due to its adaptation to the new environment. In addition, since the unique topographic characteristics enable to harbor specific climate conditions and vegetation areas, and create specific agroecosystems within the boundaries, watershed could be used as a sampling unit for studying the epidemiology of plant disease, and could be of great utility for species level population studies.

Cultural characteristics and species-specific primers were efficient in differentiating *Czm* from *Cz* and for distinguishing the two species from other fungal pathogens causing leaf diseases on maize, such as *Bipolaris maydis* (southern corn leaf blight) and *Exserohilum turcicum* (northern corn leaf blight). Cultural characteristics, such as the growth rate, colony morphology and cercosporin production were widely varied. However it was still reliable for identification as the isolates were first recovered from disease lesions. The in-vitro production of cercosoporin was particularly useful for identifying *Czm*; we did not find any isolates that failed to produce the diffusible purple pigment in medium were later identified as *Czm* through molecular method, or *vice versa*.

Due to the moderate to low disease intensity during the sampling year, it was difficult to collect adequate leaf lesions in each field required to obtain a desired confidence level of 0.95. It must be noted that the sample density for each field in this study we used to detected the presence or absence was small and this must be taken into account when interpreting the results and suggesting whether the species was true

absence in a given sampling field. Although the detection probabilities of *Czm* and *Cz* for each sampling field has been estimated and corrected by spatial replication, giving us a overall greater than 84% confidence to detect a minor species in a sampling field with a visit, given its proportion was greater than 37%. It was still not confident enough (lower than 80% confidence level) in separating true absence from non-detection if the actual occupancy of either *Czm* or *Cz* was less than between 24% to 33% in a sampling field. Nevertheless, we did find the two species coexisting in several fields, and in three cases that the two species were even found sympatrically coexisting on the same lesions. This result also demonstrated that using watershed or river basin as a sampling unit, coupled with the latitude as variable, seemed to be feasible and convenient for the study of the populations of GLS pathogen and for the prediction of the probability of *Czm* and *Cz* occurrence in regional agro-ecosystems. However, a more detailed survey of the occurrence of the two species and a predicting model based on the presence or absence data and various geographic and climatic variables are needed to be carried out.

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