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Epidemiology and predictive management of gray leaf spot of maize

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Epidemiology and predictive management of gray leaf spot of maize

by

Pierce Anderson Paul

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Plant Pathology
Program of Study Committee:
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Iowa State University
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2003

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This is to certify that the doctoral dissertation of

Pierce Anderson Paul

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

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For the Major Program
# TABLE OF CONTENTS

## CHAPTER 1. GENERAL INTRODUCTION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissertation Organization</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>History and distribution of gray leaf spot of maize</td>
<td>1</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>3</td>
</tr>
<tr>
<td>Impact of gray leaf spot on maize yield</td>
<td>11</td>
</tr>
<tr>
<td>Management strategies</td>
<td>12</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>20</td>
</tr>
</tbody>
</table>

## CHAPTER 2. A MODEL-BASED APPROACH TO PRE-PLANTING RISK ASSESSMENT FOR GRAY LEAF SPOT OF MAIZE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>29</td>
</tr>
<tr>
<td>Introduction</td>
<td>30</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>33</td>
</tr>
<tr>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>40</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>45</td>
</tr>
</tbody>
</table>

## CHAPTER 3. HYBRID REGRESSION-ARTIFICIAL NEURAL NETWORK MODELS FOR THE PREDICTION OF GRAY LEAF SPOT OF MAIZE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>56</td>
</tr>
<tr>
<td>Introduction</td>
<td>57</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>61</td>
</tr>
<tr>
<td>Results</td>
<td>69</td>
</tr>
<tr>
<td>Discussion</td>
<td>72</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>78</td>
</tr>
</tbody>
</table>

## CHAPTER 4. INFLUENCE OF TEMPERATURE ON THE RATE OF LESION EXPANSION IN GRAY LEAF SPOT OF MAIZE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>90</td>
</tr>
<tr>
<td>Introduction</td>
<td>91</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>93</td>
</tr>
<tr>
<td>Results</td>
<td>97</td>
</tr>
<tr>
<td>Discussion</td>
<td>99</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>103</td>
</tr>
</tbody>
</table>
CHAPTER 1
GENERAL INTRODUCTION

Dissertation organization

This dissertation consists of five chapters. The first chapter, a general introduction, gives an account of the history, epidemiology and management of gray leaf spot. In chapters two, three, four, and five, the findings of original research on the epidemiology and management of gray leaf spot are presented. Following these five chapters are a summary and general conclusions, and appendix.

Introduction

History and distribution of gray leaf spot of maize

The first report of gray leaf spot (GLS) of maize, caused by Cercospora zeae-maydis Tehon and Daniels, dates back to 1924 when the disease was observed in Alexander County, southern Illinois (58). By 1943, the disease was observed causing extensive leaf blighting in Tennessee and Kentucky, reaching severities as high as 94% (29). In 1949, the disease was observed in Blacksburg, Virginia with repeated and more severe occurrence in 1950 (46). During the summer of 1962, Kingsland (31) reported that the occurrence of gray leaf spot was restricted to the Blue Ridge mountain area in the western corner of South Carolina. The occurrence of the disease in that area was attributed to the existence of a specific set of microclimatic conditions. The author also speculated on the possible existence of
new pathogenic biotype of the fungus in that area. Gray leaf spot was subsequently reported as the most destructive disease of maize in the mountains of North Carolina in 1972 and 1973, causing extensive leaf death by mid-August (35). In Virginia, the disease progressed from river bottom fields where it was first observed to upland maize during the early 1970s, appearing progressively earlier during the growing season (47). Similar observations were made in Tennessee (27) in the mid 1970s when the disease occurred in counties west of the mountainous region where it was first reported, and symptoms were seen earlier in the season. For several years, gray leaf spot remained restricted to the mountainous regions of Virginia, Tennessee, Kentucky, and North Carolina (34). However, over the past 20 years the disease has become a concern in most maize-producing areas of the United States, spreading as far west as eastern Colorado, Kansas, and Nebraska and north into Wisconsin and Minnesota (36). Estimating the spread of gray leaf spot, Sparks (52) reported that from an area of approximately 1.09 million ha in 1924, the disease had spread to an area of 7.2 million ha by 1979. Between 1979 and 1997, an additional 7.70 million ha was affected by the disease, representing an increase at a rate of approximately 0.427 million ha/year. Outside of the United States, the disease has been reported in South and Central Africa (66), and South and Central America (7, 34, 66). After being first observed in the province of KwaZulu-Natal in South Africa in 1988, the disease was initially confined to an area of high relative humidity. Recently in Africa, however, the disease has spread in a way similar to the spread in the United States, reaching areas of relatively lower humidity and neighboring countries.
Epidemiology

**Survival of C. zeae-maydis** The increased prevalence and severity of gray leaf spot has been attributed to an increase in the use of minimum tillage (27, 34, 36, 39, 47, 63, 66); a practice which results in a considerable amount of the previous year's maize crop residue being left on the soil surface. Legislations and incentives aimed at promoting soil conservation, along with economic pressure have led to continuous cropping of maize and a great proportion of croplands in the midwest being under some form of minimum tillage, leaving more than 30% of the crop residue on the soil surface (39). Studying the direct influence of infested maize residue covering the soil on gray leaf spot epidemics in Ohio, de Nazareno et al (14) reported that when the weather conditions were favorable for the development of the disease, there was a significant positive relationship between the amount of maize residue cover and disease severity. Following the application of infested residue to the soil surface at rates of 0, 10, 35, and 85%, they observed that final disease severity, estimated as the number of lesions on the ear leaf and the third leaf above and below the ear leaf, increased as the amount of infested maize residue increased. Similar results were reported from studies conducted in North Carolina (44) and Maryland (51), demonstrating a positive association between gray leaf spot severity and the amount of surface maize residue. Payne et al. (44) and Ward et al. (63) reported that no-till planting in corn residue favored the early appearance of lesions of gray leaf spot, allowing for the occurrence of more secondary cycles and a higher final disease severity (44).
The significant positive relationship between the amount of surface residue and gray leaf spot severity is probably due to the fact the causal agent, *C. zeae-maydis*, is capable of surviving and sporulating on the previous season's maize residue left on the soil surface. Investigating the relationship between residue management and airborne conidia, Payne et al. (44) reported that significantly more conidia were trapped in plots with residue left on the soil surface than in plots that had the residue plowed under. These results were consistent with the findings made earlier by Payne and Waldron (43). In the latter study, the authors observed that *C. zeae-maydis* was better able to overwinter in maize residue left on the soil than in residue buried in the soil. Maize residue left on the surface in November still produced conidiophores in May of the following year. A study by de Nazareno et al. (13) also showed that the fungus survived well in residue left on the soil surface in Ohio and that infested residue produced viable spores throughout the winter and spring. Residue buried early in the winter decomposed and did not produce spores in the spring.

**Environment and host effects on the development of gray leaf spot.** Although surface maize residue has been shown to be important for the development of gray leaf spot epidemics, the conditions under which the crop is planted also are very important. These conditions affect the production and dissemination of inoculum from infested maize residue to new infection courts, and influence events leading to infection and subsequent disease development. Ward and coworkers (63) reported that in spite of the importance of surface residue, under environmental conditions favorable for the development of the disease and in areas
where the disease was endemic, maize residue played a lesser role in the development of the disease than weather. The findings of Smith (51) also supported the idea that the relative importance of surface maize residue depends on the prevailing weather conditions. Smith (51) and de Nazareno et al (14) suggested that in areas where the pathogen was endemic, the disease could reach high levels even in fields with low levels of surface maize residue, once the environmental conditions were favorable. de Nazareno et al (12) showed that in areas where no-till was predominant and the disease was endemic, disease gradients from a point source of inoculum were masked due to the ingress of spores from nearby fields.

Using spore traps to monitoring the release of spores from debris during the growing season in North Carolina, Payne and Waldron (43) observed that conidia were present in the air as early as 19 June; however, the spore concentration remained low until August when it increased steadily, peaking towards the end of September. Although spores were trapped early in the season, lesions were not observed and the rapid development of the disease did not occur until late in the season. They also reported that gray leaf spot severity was relatively lower in plots with poorly developed canopies. These results led the authors to conclude that plant canopy played an important role in creating a microclimate favorable for the development of the disease, and that the late-season development of the disease was due to environmental conditions and not to low levels of inoculum. After observing that no disease developed at the University of Kentucky's South Farm near Lexington even when large quantities of infested maize residue was left on the soil surface, Rupe and collaborators (48) hypothesized that extended periods of leaf
wetness and high relative humidity were the factors influencing disease development among locations with similar amounts of infested maize residue left on the soil.

Little work has been done under controlled conditions to provide conclusive evidence of the relationship between environmental conditions and gray leaf spot disease development; however, there is still enough evidence in the literature supporting the importance of moisture for the development of this disease. Moderate-to-high temperatures and prolonged periods of high relative humidity are generally accepted as being favorable for the development of gray leaf spot (35, 34, 48, 66). High gray leaf spot severity has also been associated with seasons and locations with high precipitation (34, 26, 45, 51). In a survey conducted in Maryland, Smith (51) observed that disease severity was highest in years and locations where the rainfall was greatest. Ringer and Grybauskas (45) studied disease components and gray leaf spot progress under field conditions. They concluded that rainfall and sporulation during early infection cycles had a significant effect on the development of the disease. Comparing the weather conditions at locations where gray leaf spot developed (Quicksand and Hazel Green, KY) with those at locations where the disease did not develop (South Farm and Spindletop, KY), Rupe et al (48) observed that significantly longer periods of leaf wetness and relative humidity above 90% occurred more frequently in areas where the disease was present that in areas where it was not.

Prolonged periods of high relative humidity seem to be especially important for infection by *C. zeae-maydis* to occur. Beckman and Payne (4) reported that the optimum conditions for gray leaf spot development under greenhouse conditions
were achieved when an intermittent misting system was used. Incubating inoculated plants under a system providing 14 hrs of mist per day (3 sec of mist every 4 min from 2000 hours to 1000 hours the following day) for two weeks, they reported that characteristic lesions of gray leaf spot developed within 11-25 days after inoculation. Gray leaf spot also developed in greenhouses without misting during the summer of that study; however, lesions were delayed 3-6 days when compared to the time of lesion appearance in greenhouses with misting. That summer was characterized by daily relative humidities in the greenhouse between 60 and 90% and nightly relative humidities above 96% for at least 10 hr. Lesions did not develop in greenhouses without misting during the winter, when the relative humidity was relatively lower, and fewer lesions developed when free water was present on the leaves for prolonged periods. Latterell and Rossi (34) reported that successful infection of C. zeae-maydis under greenhouse conditions only occurred when inoculated plants were incubated in a dew chamber for extended periods (up to 96 hours), followed by sequential incubations under periodic misting in a plastic tent and on a greenhouse bench.

Results of studies conducted to determine the effects of moisture on pre-penetration events supports the idea that prolonged periods of high relative humidity, but not free water, are necessary for gray leaf spot development. Thorson and Martinson (60) reported that germ tube elongation and appressorium formation were favored by extended periods of 95% relative humidity. Germ tubes were significantly longer at 95% relative humidity than at 90 and 80% relative humidity. Appressoria were formed at 95% relative humidity after 48 and 72 h of exposure, but none were
observed at relative humidities less than 95%. The number of appressoria formed per germ tube increased as exposure time increased. When compared to 95% relative humidity, fewer, but larger appressoria were formed in the presence of free water. Assessing the effect of intermittent periods of 95% relative humidity on germ tube growth and appressoria formation, Thorson and Martinson (60) concluded that germ tubes of *C. zeae-maydis* were capable of surviving extended periods of desiccation prior to continuing growth and penetration; however, survival was directly related to the relative humidity during the unfavorable periods and decreased as time spent at the unfavorable relative humidity increased. Beckman and Payne (3) reported similar results regarding the effects of free water on the formation of appressorium and the ability of germinated spores to survive for extended periods on the surface of maize leaves before penetration occurred. Fewer appressoria were formed and no penetration was observed on the upper surface of the leaves where free water was present. On the lower leaf surface, spores germinated after a 12-h moisture period with germ tube showing positive tropism towards stomata. Abundant appressoria were formed over stomata 4-5 days after inoculation. In the presence of free water on the upper surface of maize leaves, germ tubes grew extensively but did not show tropism towards stomata. These results have led researchers to speculate that free moisture may be inhibitory to infection and subsequent development of gray leaf spot of maize. This, however, conflicts with the findings of Rupe et al. (48) which suggested that extended periods of leaf wetness were necessary for gray leaf spot development. The apparent discordance among reports on the influence of leaf wetness on the development of gray leaf spot may be due in
part to differential effects of this factor on specific stages of the disease cycle, on the development of the fungus, and the interactions between the plant and the fungus. Lapaire and Dunkle (32, 33) reported the occurrence of microcycle conidiation in C. zeae-maydis on water droplets and on the trichomes of several plant species, including maize. However, this process did not occur on the surface of maize leaves and was inhibited by leaf washes. This suggests that in the presence of free water on surfaces other than the leaves of maize, the inoculum potential of C. zeae-maydis may substantially increase due to the production of secondary spores from primary spores. Assessing the effects of relative humidity on spore germination and microcycle conidiation, these researchers reported that germination occurred at relative humidities between 58 and 100%, but at relative humidities below 97%, germ tube growth was minimal, consequently, secondary conidiation did not occur. Although high relative humidity favors germination and conidiation, dryer conditions seem to favor spore detachment and dispersal. Results of wind simulation studies showed that dehydrated conidia of C. zeae-maydis were detached at wind speeds below average canopy wind speeds, while hydrated conidia were detached by greater wind speeds (33). These results corroborated the findings of Rupe et al. (48) which showed that spore release within the maize canopy was greatest in early afternoon when there is typically a rise in temperatures coincident with a drop in relative humidity. This suggests that fluctuating moisture conditions in the field may favor different stages of the disease cycle.

Relative to moisture, temperature seems to be a less limiting factor for the development of gray leaf spot. Once moisture requirements are met, the disease
seems to develop under a wide range of temperatures. Beckman and Payne (4) reported that once periods of sustained high relative humidity were provided, gray leaf spot lesions developed readily on plants kept in the greenhouse at 22-28°C. Studying the effect of temperature on spore germination and the elongation of germ tubes of C. zeae-maydis exposed to 12 h of high relative humidity, Beckman and Payne (4) observed that the optimum temperature was between 22 and 30°C and no germination occurred at 36°C. The findings of Garden and Hilty (22) were similar for the effects of temperature on sporulation and radial growth of C. zeae-maydis on potato dextrose agar. They observed that neither sporulation nor radial growth occurred at 32°C.

Lesions of gray leaf spot are generally observed first on the lower leaves of the maize plant, progressing upwards reaching leaves in the middle and upper canopy towards end of the growing season. Although symptoms may appear during the vegetative growth stages (V8), severe leaf blighting is most common after anthesis. This has led researchers to speculate on several possible causes for this pattern of disease development: the influence of the physiological age of the plant, the availability of inoculum, and the microclimate within the canopy. Rupe et al. (48) studied the effect of the environment and plant maturity on the development of gray leaf spot. They observed that regardless of the planting date, initial symptoms did not appear until plants were near anthesis. This led these researchers to the conclusion that plant maturity was an important factor in the late season development of gray leaf spot. Hilty et al (27) also observed that under field conditions, the onset of gray leaf spot epidemics coincided with silk emergence.
However, they concluded based on the fact that they were able to successfully inoculate 2-to-3 week-old seedlings that the disease was not associated with maize senescence. Similarly, Beckman and Payne (3) demonstrated that neither plant nor leaf age influenced the susceptibility to gray leaf spot under greenhouse conditions. They further reported that younger plants developed sporulating lesions 3-to-4 days earlier than mature plants. Based on the growth of hyphae across or through stomata of excised leaf disks, Gwinn et al. (25) reported a positive correlation between stomatal penetration by *C. zeae-maydis* and age of leaf tissue.

**Impact of gray leaf spot on maize yield**

Yield loss due to gray leaf spot may be direct or indirect. Direct yield losses occur as a result of the reduction of the photosynthetic area of the plant. The upper eight or nine leaves of the plant contribute 75 to 90% of the photosynthate for grainfill (1). Severe blighting substantially reduces the green leaf area of the plant, and consequently, the amount of photosynthate produced and distributed to the ears. To compensate for the loss of healthy leaf area, photosynthate is redistributed from the stalk and used for grainfill. This may predispose the plant to stalk rot and indirect yield losses due to lodging. Other components of yield affected by gray leaf spot are the size and number of kernels per ear (66).

The impact of gray leaf spot on maize yield depends on the time of onset of the disease relative to the growth stage of the plant, the level of disease severity, the weather conditions during grainfill, the susceptibility and tolerance of the genotype planted, and the severity of lodging due to stalk rot (39). Reports based on yield differences between fungicide-treated plots and nontreated controls indicate that
substantial yield losses may occur in both maize seed and grain production. Martinson et al. (41) reported that in seed production fields where gray leaf spot onset occurred prior to tasseling and the difference in ear leaf disease severity between sprayed plots and unsprayed control was 63%; yield losses were 21 to 27%. Research conducted in South Africa has shown that gray leaf spot may result in yield losses in grain production of as high as 30 to 60% during seasons of high disease severity. (63). In individual hybrid maize fields, yield losses ranging from 24 to 69% have been reported in Virginia (9, 53, 55-57), 11 to 44% in Iowa (30), 11% in Kentucky (62), 15 to 33% in Ohio (37, 38), and 50 to 65% in South Africa (66). According to Munkvold et al. (42), annual yield loss estimates in maize production in Iowa exceeded $100 million for several years during the 1990s.

Management strategies

Cultural practices. Given the strong relationship between infested surface residue and the development of gray leaf spot, management strategies aimed at reducing the amount of residue are among the most effective in preventing severe epidemics of this disease. *Cercospora zeae-maydis* is known to be pathogenic only to maize and according to Latterell and Rossi (34), does not survive in maize residue in the field beyond one year. One-to-two years of rotation away from maize is often enough to reduce the survival of the fungus (39). However, the effectiveness of crop rotation and tillage depends on their widespread adoption, since neighboring fields may serve as sources of inoculum. Other cultural practices that may be effective against gray leaf spot are planting date and genotype maturity. Both of these approaches aim at avoiding disease-favorable conditions and delaying the onset of
the disease. Late-maturing hybrids are at greater risk from gray leaf spot than early-maturing hybrids because they are exposed to the disease during a greater portion of the grainfill period (54).

**Chemical control.** Ward et al. (63) explored management options that would allow for the continued use of conservation tillage without increasing the impact of gray leaf spot in South Africa. They observed that conserved moisture under residue brought yield benefits that were sufficient to offset the detrimental effects of higher disease levels. Mean yield in plots with conventional tillage was 28 and 209 kg/ha lower than tillage plots with 82 and 26% surface maize residue, respectively. They reported that yield response to fungicide treatment ranged from 477 kg/ha in low-disease seasons to 3830 kg/ha in high-disease seasons. Ward et al. (65) evaluated the effects of frequency and timing of systemic fungicide applications on the development of gray leaf spot. They reported that during disease-favorable seasons, two- and three-spray application programs resulted in the best disease control and provided longer fungicide protection. Among single-spray treatments, the most effective control was achieved when fungicide was applied early in the season, when disease severity on the basal five leaves was between 2 and 3%. Martinson and Munkvold (40) reported that a single spray of propiconazole applied prior to silking (V7-V8) provided control comparable with two sprays of the same product in Iowa.

Even though fungicide applications have been shown to provide effective control, the resulting yield gain has not always been sufficient to offset the associated costs. According to Ward et al. (65), yield response to fungicide treatment may be a function of the time of initial application, the amount of disease
at the time of application, the duration of the protection offered by the fungicide, and control through physiological maturity. In South Africa, the gain in yield from fungicide treatment often exceeds the breakeven point needed to cover chemical and spray application costs, making chemical control economically feasible (63). One-, two- and three-spray application programs all provided cost-effective disease control, exceeding the breakeven increase in grain yield (64, 65). In the United States, chemical control of gray leaf spot in grain production is not always economically feasible. Munkvold et al. (42) estimated that the cost of a single application of propiconazole may provide economic benefit to farmers in Iowa when a susceptible hybrid is planted. A single fungicide application may be profitable for gray leaf spot management in maize grown for grain in Iowa; however, the probability of profitability is strongly dependent on the yield potential and susceptibility of the hybrid planted (42). Munkvold and coworkers (42) reported that the probability of achieving a net return with a single application ranged from 0.06 to more than 0.99, and that the probability was almost always higher using one application compared to the probability of receiving a net return when two fungicide applications were used. Due to the high value of the crop, fungicide application is often more profitable in maize seed production (41, 67).

**Host resistance.** When available, host resistance is probably the single most important management practice of any plant disease. Currently, there are no commercial maize hybrids that are highly resistant to gray leaf spot; however, several moderately-resistant hybrids are available. On these hybrids, the intensity of the disease may be reduced due to a reduction of the number and size of lesions, a
reduction in the apparent infection rate, sporulation capacity, and an increase in the
length of the latent period (20, 45). Freppon and Lipps (20) classified maize inbreds
and hybrids according to lesion types: restricted lesion with chlorosis; rectangular,
necrotic lesions; a mixture of chlorotic and rectangular, necrotic lesions; and
irregular, chlorotic flecks. Inbreds with a high level of resistance resulted in fleck-type
lesions, followed by chlorotic lesion response, while susceptible inbreds resulted in
necrotic, rectangular lesions. Crosses between inbreds with susceptible and
resistant lesions responses resulted in hybrids that exhibited resistant-type lesions
and low disease infection rates. Similarly, Coates and White (10, 11) and Donahue
et al. (17) demonstrated that when selected inbreds with resistance to gray leaf spot
were crossed with a susceptible inbred, the resulting hybrids showed resistance
comparable to that of the resistant parent.

Although sources of resistance to gray leaf spot are available in inbreds, the
task of moving genes to lines with the desired agronomic traits has not been easy
(52). Inheritance of resistance has been studied extensively and progress is being
made toward commercializing highly-resistant hybrids. In most of these reports,
resistance was found to be controlled by additive gene action and highly heritable
(11, 17, 23, 28, 59, 61). Elwinger and collaborators (18), however, suggested that
the additive model alone could not entirely explain the inheritance of resistance to
gray leaf spot and recommended that inbreds be screened prior to testing for
combining ability to eliminate those that are most susceptible. Working with inbreds
pre-selected on the basis of their performance to gray leaf spot, Coates and White
(11) found that inheritance of resistance was both additive and dominant. However,
they further reported that some crosses deviated from the additive-dominance model and that resistance was probably conditioned by plant maturity, since dominance was generally not significant for late-season disease ratings. Given the additive nature of resistance, Gevers et al. (23) and Thompson et al. (59) recommended backcrossing as the best approach for transferring resistance genes into desirable lines. According to Coates and White (11), difficulties in transferring resistance from the source into a line of interest may be due to the number of genes involved and difficulty in selecting the best genotypes. Saghai Maroof et al. (49) used molecular genetic markers (RFLP) to study the genetics of resistance to gray leaf spot in crosses between susceptible B73 and resistant Va14. They identified three major quantitative trait loci on chromosomes 1, 4, and 8 (QTL1, 4, and 8), which cumulatively explained 44 to 64% of the variation in the response to gray leaf spot in F₂ and F₂-derived F₃ generations. They reported that the results of their study were validated in a marker-assisted breeding program, resulting in hybrids with high resistance to gray leaf spot.

Tolerance is another characteristic that varies among hybrids. Stromberg and Donahue (54) observed that even though some hybrids were more prone to blighting during grainfill, they still yielded relatively more than hybrids that were relatively more resistant. However, both resistance and tolerance may be overwhelmed by heavy disease pressure (54). Even when moderately-resistant hybrids are planted substantial yield losses due to gray leaf spot may still occur (24).

In seed production, the use of genetic resistance is not always an option, even though inbreds with partial resistance to gray leaf spot are available. In order to
produce seed of a given maize hybrid, specific inbreds have to be planted, regardless of their level of resistance to gray leaf spot. Chemical control is often recommended for seed production (41).

Management through disease forecasting. Management decisions for gray leaf spot are often made without quantitative assessment of the risk posed by the disease. Currently, management decisions are made based on crop growth stage, susceptibility of the genotype planted, and disease severity thresholds. But disease thresholds do not provide an accurate prediction of the level of disease intensity that is likely to occur later in the season. Sound decision-making criteria based on risk assessment and disease prediction are necessary in order to achieve more effective and profitable management of gray leaf spot. Management decisions have to be made prior to planting (e.g. hybrid selection) or early during the growing season for fungicide recommendation. Martinson et al. (41) suggested that since detasseling removes the upper leaves in seed production, it is important to protect leaves below the point of detasseling to ensure satisfactory yield through the early application of fungicide. Accurate disease predictions would not only prevent unnecessary fungicide application, but fungicides would be used only when warranted, thus ensuring that gray leaf spot management could be more cost-effective.

The attributes of a plant disease that make forecasting economically worthwhile (8, 21) are satisfied by gray leaf spot of maize; its sporadic nature as affected by the environment, potential impact on yield, cost of control, and the value of the seed crop. Conventional approaches to developing disease forecasting models depend on an understanding of the mathematical relationships describing
the biology of the system. For gray leaf spot, these relationships are not thoroughly understood. This makes a mechanistic or process-based modeling approach difficult. Satisfactory results were obtained using an empirical approach to understand the relationship of environmental and cultural factors with gray leaf spot severity (5), laying the foundation for the development of a predictive model for this disease.

Artificial neural networks (ANN) (6) provide an alternative to regression approaches for model development. Like other modeling approaches, ANN can be thought of as a minimization technique in which the goal is to minimize the difference between model output and actual output. However, some of the proposed advantages of ANN over conventional methods are that it automatically allows for nonlinear relationships between predictor and response variables, and incorporates interaction between variables without requiring additional modeling as in the case of standard statistical approaches (50). Artificial neural networks models are fitted by adjusting weights which are analogous to coefficients/parameters in regression modeling. The predictors and responses are repeatedly presented to the network during a process called training and after each passage through the network, internal weights are adjusted so as to minimize the difference between network and actual outputs. In this iterative process, the network learns the relationship between the predictor and response variables so that when it is presented with a new set of inputs (validation), it is capable to predicting the outcome based on this relationship. There are several classes of ANN, each employing different types of architectures.
One of the most commonly used architectures is the multilayered network trained through error back-propagation (the back-propagation ANN, BPNN) (6).

The use of ANN in plant pathology-related research has increased over the past few years and has been shown to be superior to conventional modeling approaches in several cases. Batchelor et al. (2) used ANN to predict soybean rust epidemics and compared their results with those obtained using regression and simulation models for the same dataset. They found that their best ANN model resulted in higher coefficient of multiple determination than the regression and simulation models, even when validated on an independent data set. De Wolf and Francl (16) reported superior performance of BPNN over logistic regression for the classification of incidence of tan spot of wheat, and over stepwise logistic regression and multivariate discriminant analysis for the detection of infection periods for the same disease (15). Similarly, Yang and Batchelor (68) reported that BPNN's performed better than conventional modeling approaches in predicting wheat scab epidemics, while Francl and Panigrahi (19) showed the superiority of the same class of ANN's over discriminant analysis in predicting the wetness status of wheat leaves.

Gray leaf spot management requires more reliable decision-making criteria to guide hybrid recommendations and the need and timing of fungicide applications. Criteria are needed to quantitatively assess the need for resistant hybrids; to determine whether disease will be severe enough to warrant fungicide applications; and to predict the potential yield impact of the disease early in the season. In addition, more research is needed to elucidate the influence of the environment on specific disease components. The effects of temperature and relative humidity on
the expansion of lesions of gray leaf spot and the sporulation of *C. zeae-maydis* on diseased leaf tissue have not been studied. The objectives of this research were:

1. Develop risk assessment models for gray leaf spot based on pre-planting site and host factors.
2. Develop models to predict late-season gray leaf spot severity based on early- and mid-season site, genotype, and environmental information.
3. Determine the effects of temperature and relative humidity on the rate of lesion expansion and sporulation of *C. zeae-maydis* on diseased leaf tissue, and model the relationship between temperature and relative humidity, and these disease components.

**Literature Cited**


CHAPTER 2

A MODEL-BASED APPROACH TO PRE-PLANTING RISK ASSESSMENT FOR GRAY LEAF SPOT OF MAIZE

A paper to be submitted to Phytopathology

Pierce A. Paul¹ and Gary P. Munkvold²

Abstract

Management decisions for gray leaf spot, one of the most important foliar diseases of maize, are made without a quantitative assessment of the disease risk. This can lead to inappropriate hybrid selection and the inefficient use of costly fungicide applications. Pre-planting site and genotype data were collected in southern Iowa with the object of developing a model to estimate late-season gray leaf spot severity. Disease severity at the R4/R5 plant growth stage, categorized into five classes, was the response variable. The proportional odds (PO) and extended continuation ratio (ECR) ordinal logistic regression, and classification and regression tree (CART) modeling approaches were used to predict severity classes as a function of planting date, amount of surface residue, cropping sequence, genotype maturity, genotype gray leaf spot resistance ratings, and longitude. A total of 332 cases collected between 1998 and 2001 were used for model development, and 30 cases collected in 2002 were used to assess the predictive accuracy of the models.

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The logistic regression models correctly classified 66 to 73% of the validation cases, whereas the CART model correctly classified 56 to 73% of these cases. The majority of the cases misclassified by the CART model were due to overestimation, whereas the logistic models tended to misclassify cases by underestimation. Pre-planting data had a strong relationship with gray leaf spot severity assessed late in the growing season. Both the CART and logistic regression models have potential as decision-making tools for gray leaf spot management.

Introduction

The first report of gray leaf spot of maize, caused by *Cercospora zeae-maydis* Tehon and Daniels, dates back to 1925 when the disease was observed in Illinois (40). Since then, the disease has been reported in most of the maize-producing areas of the United States (16, 17, 18, 20, 22, 23, 34, 36) and, over the past 20 years, has become a major problem in both seed and grain maize production. The increased prevalence and severity of gray leaf spot has been attributed to an increase in the use of minimum tillage (17, 21, 36, 43), a practice that favors the survival of the pathogen in the previous year’s crop residue left on the soil surface (8, 32, 33, 42). Payne et al. (33) and Ward et al. (42) reported that no-tillage favored the early appearance of lesions of gray leaf spot and resulted in greater end-of-season disease severity than tillage.

Current management practices for gray leaf spot involve the use of moderately-resistant hybrids, crop rotation, tillage, timely planting, and foliar
application of fungicides (29, 43). Crop rotation and tillage can be difficult to fully implement because of economic considerations and soil conservation, respectively. Although foliar fungicide applications have been shown to be profitable in seed maize production (26), its use in grain maize production has been rare because the value of the yield response is often not sufficient to offset the cost of chemical control. A single fungicide application may be profitable for gray leaf spot management in maize grown for grain in Iowa; however, the probability of profitability is strongly dependent on the yield potential and susceptibility of the hybrid planted (29). This leaves hybrid selection as probably the single most important tool for effective and profitable management of gray leaf spot in grain maize production.

The decision to use a moderately-resistant hybrid for gray leaf spot management is often made based on the history of gray leaf spot at the location, cropping sequence, and the type of residue management practiced. However, these decision-making tools provide no quantitative measure of the likelihood and level of gray leaf spot severity, and none of these factors used alone is a good predictor of gray leaf spot disease severity. For example, although the amount of surface residue has been shown to have a significant positive relationship with gray leaf spot severity (5, 37), others factors complicate this relationship. Fields with little surface residue may still have higher levels of gray leaf spot than fields with more surface residue (37, 41). In addition, hybrids with the same gray leaf spot resistance rating planted under similar residue management conditions may differ in gray leaf spot severity among locations (5). Sound decision-making criteria based on accurate risk
assessment are needed to achieve more effective and profitable management of gray leaf spot. A pre-planting risk assessment model may provide valuable information regarding the likelihood and severity of gray leaf spot when a given set of management practices is used.

In human disease epidemiology, modeling approaches such as ordinal logistic regression and classification and regression tree (CART) have been used to classify patients into risk classes in order to guide the implementation of treatment (7, 13, 15, 25, 30). In addition to their use as data mining and variable selection tools, tree-based models are widely used to devise prediction rules for both classification and regression problems. These approaches provide advantages over linear and additive models in that they are non-parametric and relatively easy to implement and interpret. Nelson et al (29) concluded that in addition to assessing risk, CART models uncovered interactions among variables generally overlooked by more traditional modeling approaches. Several classes of logistic regression models may be used to model categorical response variables. If the response variable is dichotomous, a binary logistic regression model may be used. If the response is on an ordinal scale with 2 or more categories, an ordinal logistic regression model may be used. Unlike other approaches, such as discriminant analysis commonly used to model categorical data, logistic models make no assumption about the distribution of the predictors. Such methods can be applied to the field of plant pathology in order to guide management decisions. De Wolf et al. (10) used binary logistic regression models to assess the risk of wheat Fusarium head blight over location-years. They reported prediction accuracy of 62 to 85%. In this paper, we apply two classes of
ordinal logistic regression models and a CART model to assess the risk of gray leaf spot using data available prior to planting as predictor variables.

Materials and Methods

Site selection and data collection

Site history and agronomic information were collected at the beginning of the 1998, 1999, 2000, 2001, and 2002 growing seasons from several commercial seed production fields, hybrid strip trials, and research plots located in 17 counties in the southern half of the state of Iowa (between latitudes 40° 42' 04"N and 42° 12' 60"N). Data were collected from 13 sites in 1998, 11 in 1999, 10 in 2000, 11 in 2001, and five in 2002 (Figure A1-A3). These sites were chosen to represent areas with different histories of gray leaf spot, varying cropping practices, and a range of gray leaf spot-favorable weather conditions. At each location, three-to-eight maize genotypes (inbreds or hybrids) with gray leaf spot resistance ratings ranging from 2 (most susceptible) to 7 (most resistant) and maize maturity ranging from 98 to 119 days comparative relative maturity (CRM) were planted in 2- to 18-row plots. Plots were planted between 22 April (112 day of year) and 01 July (182 day of year).

At each location, the latitude and longitude were recorded using a hand-held battery-operated Magellan GPS 4000 global positioning unit (Magellan Systems Corporation, San Dimas, CA). Maize surface residue cover was estimated using a line transect method (28) as described previously (4, 5) and the previous crop (maize or soybean) was recorded. The number 1 was assigned to fields where maize was the previous crop, while 0 was assigned to fields where soybeans were
the previous crop. Planting dates (in day of the year), genotype maturity and gray leaf spot resistance ratings were also recorded for each genotype at each location.

Each location was visited at 14-day intervals and disease severity was assessed by visually estimating the percentage of the ear leaf covered with gray leaf spot lesions. At each assessment, ten plants from each genotype were arbitrarily selected and gray leaf spot severity on the ear leaf was determined by using a standard area diagram as a reference (31). Gray leaf spot severity on the ear leaf at the R4/R5 plant growth stage (34) was used as the response variable for model development. This leaf position and growth stage were chosen because gray leaf spot severity at approximately this stage was reported as providing the best relationship with yield loss (19). Disease severity was categorized into five classes: 1 - less than 20%; 2 - greater than or equal to 20% and less than 40%; 3 - greater than or equal to 40% and less than 60%; 4 - greater than or equal to 60% and less than 80%; and 5 - greater than or equal to 80%.

Model development

Factors previously reported as having strong linear relationships with gray leaf spot severity (5) and which could be determined prior to planting were used as input variables. These included maize surface residue (SR), planting date (PD), and genotype resistance (GLSR). Genotype maturity (MAT) and previous crop (PC) were also used. Since gray leaf spot severity was shown to vary considerably among locations in Iowa planted with the same genotype and having similar cropping practices, and because hours of gray leaf spot-favorable weather conditions were
observed to have a strong east to west variation across the state (5), longitude (LON) was also used as an input variable for model development.

Two classes of ordinal logistic regression and a classification and regression tree (CART) (6) modeling approaches were used to develop risk assessment models. A total of 332 cases collected between 1998 and 2001 were used for model development. Once fitted, the bootstrap validation method was used to validate the models and 30 independent cases collected in 2002 were used to assess their predictive accuracy. Misclassification rates were used as a measure of model performance on the independent set of data.

**Ordinal logistic regression.** Logistic regression models may be considered direct probability models, since they are stated in terms of the probability of the occurrence of an event (Y) under a given set of conditions (X), Prob{Y=y|X}. In this study, two classes of ordinal logistic regression models - the proportional odds (PO) (27) and the continuation ratio (CR) (1) - were used to model the relationship between selected pre-planting variables and gray leaf spot severity. The PO model is based on cumulative probabilities. For a dependent variable having 0, 1, 2, ..., k levels, the model is stated as follows:

\[
Pr[Y \geq j \mid X] = \frac{1}{1 + \exp[-(\alpha_j + X\beta)]},
\]

where \( j \), the cutoff level of \( Y \), = 1, 2, ..., \( k \), \( \alpha_j \) = intercept and \( \beta \) = regression coefficient. There are \( k \) intercepts. For any given \( j \), the model is an ordinal logistic model for \( Y \geq j \) and is read as the probability of \( Y \geq j \) given \( X \). The CR model is based on conditional probabilities and is stated as follows for \( Y = 0, ..., k \)
Pr[\(Y = j \mid Y \geq j, X\)] = \frac{1}{1 + \exp[-(\theta_j + X\gamma)]}, \quad (2)

in which \(j\) = the cutoff level of \(Y\), \(\theta_j\) = intercept, and \(\gamma\) = regression coefficient.

Both the PO and the CR models were fitted using the \textit{lrm} function of the \textit{Design} library (12) in S-plus 2000 (MathSoft Inc., Seattle, WA). The modeling approach and model diagnostics were performed as described by Harrell (13), Harrell et al. (15), and Bender and Benner (3). Since the objective was to estimate the odds of having high disease severity relative to low severity and not the opposite as the forward CR model (equation 2) seems to suggest, the backward CR model (3), \(P(Y = j \mid Y \leq j, X)\), was fitted instead. Prior to fitting the models, plots of the means of the predictor variables stratified by levels of the response variable and overlaid with the expected values for the PO and CR models were used to check the ordinality assumption of equal slope, and the PO and CR assumptions (Figure A4) (3, 13). Since the equal slope and CR assumptions were violated for some of the predictor variables, an extended continuation ratio (ECR) model was fitted instead of the CR model (13). The bootstrap validation technique (10) using 1000 replications was used to assess the predictive ability of the PO and the ECR models. Somers’ \(D_{xy}\) rank correlation (39) between predicted probabilities and observed responses was the index used to assess the performance of the models. \(D_{xy}\) was calculated using the following equation:

\[D_{xy} = 2 * (c - 0.5),\quad (3)\]

Where \(D_{xy}\) = difference between concordance and discordance probabilities and \(c\) = probability of concordance between predicted probability and response. \(D_{xy}\) ranges
from 0 to 1, with 0 indicating that the model is making random prediction and 1 indicating that the model is making perfect discriminations between classes.

**CART.** In this modeling approach, a classification tree is built by using a binary partitioning algorithm to recursively split the data in each node into increasingly homogeneous subsets until the response data is pure, that is, all the cases belong to the same class. Several non-negative functions (6) are used to determine the purity of the nodes. In S-plus, the deviance function is used. The S methodology implements classification trees as a probability model (41).

In this study, the model was developed using the *tree* function in S-plus 2000. The default recursive partitioning technique described in the S-plus User's Guide (MathSoft Inc., Seattle, WA 1999) was used to generate the dichotomous split of the predictor variables at each node of the tree. After building a tree with 23 terminal nodes (Figure A5), the tree was then simplified by pruning (removal of the least important splits) to fewer terminal nodes based on the relationship between the number of terminal nodes and the residual deviance (Figure A6). The residual mean deviance and misclassification error rates were used as a measure of goodness-of-fit of the pruned version of tree relative to the original tree (Table A1). The final tree had 14 terminal nodes.

**Results**

Eighty percent of the cases collected between 1998 and 2001 were in class 1, 10 percent in class 2, and approximately 3 percent each in classes 3, 4, and 5.
Gray leaf spot was most severe in 1998 and least severe in 2001. In 2001, there were no cases in classes 4 and 5. Of the four years, 1999 and 1998 had the highest percentage of cases in the over 80% severity class, seven and six percent, respectively.

**Logistic regression and CART**

The results of the PO and ECR models regarding the significant effects of the predictor variables were similar. In both cases, LON, SR, GLSR, and PD were highly significant as predictors of disease severity classes, whereas PC and MAT were not significant (Table 2.1). In both models LON, MAT, and GLSR had negative coefficients, whereas the coefficients for PD, PC, and SR were positive. The ECR model generated slightly smaller standard errors for all of the predictors than the PO model. There was strong evidence of an association with the disease severity for both models (Total $P < 0.0001$). The $D_{xy}$ value, an index used to measure the predictive strength of the models, was similar for both models and the likelihood ratio $\chi^2$ was higher for the ECR model.

From the CART model, the relationship between the input and output and the interaction between predictor variables can be perceived by starting at the top of the tree and moving down along the branches until reaching the terminal/output node. The relative position of the predictors in the tree was indicative of the fact that LON, SR, GLSR, and PD were again the most important variables in predicting disease severity classes using the CART modeling approach (Figure 2.2). PC was unimportant. The highest disease severity (>80%) occurred when the most susceptible hybrids (with resistance ratings less that 3) were planted at sites located
between longitudes 91.29W and 91.41W, or at sites located east of longitude 91.29W and had surface residue cover greater than 60%. For all locations west of longitude 91.41W, disease severity was between 0 and 20%.

**Bootstrap validation and misclassification rate**

The bootstrap method was used to perform an internal validation of the PO and ECR models and to obtain bias-corrected estimates of predictive accuracy as described by Harrell and coworkers (14). Bias may result from overfitting the models. For both models, 1000 bootstrap replications were used to estimate and correct for optimism in various statistical indices (Table 2.2). The bias-corrected indices were similar for both models. For the original $D_{xy}$ values the optimism from overfitting was estimated to be 0.02 for both models, resulting in bias-corrected estimates of predictive discrimination of 0.80 and 0.82 for the PO and ECR models, respectively. The intercept and slope were closer to zero and one, respectively, for the ECR model than for the PO model, and the maximum calibration error ($E_{\text{max}}$) was slightly smaller for the ECR model (Table 2.2).

In using the 30 new cases to assess the performance of the models on an independent dataset, the highest probability was used to assign each case to a class. When the models were used to determine the exact severity class of the new cases, CART correctly classified 17 of the 30 validation cases. Nine of erroneous classifications were due to overestimation and four to underestimation of disease severity class. When used to estimate the probability of having disease severity greater than 20% (class >1), 73.3% of the cases were correctly classified (Table 2.3), whereas four cases each were misclassified as being in class 1 and in a class
other than 1. The PO model correctly classified 66.7% of the cases when used to predict the exact class to which a case belonged, and 73.3% of the cases when predicting the probability of being in a class higher than 1. In both instances, most of the misclassified cases were predicted as being in a class lower than their actual class, eight and six, respectively, and two cases were misclassified as being in a higher class. Similarly, the ECR model correctly classified 18 of the 30 cases when used to estimate the exact class and 21 when used to assign cases to a class above class 1. Most of the misclassification was due to underestimation.

Discussion

The importance of surface residue (SR), planting date (PD), and genotype resistance (GLSR) for gray leaf spot development has been well documented in the literature (5, 9, 24, 32, 33, 37, 42, 43). All of the models used in this study identified these variables as significant predictor of gray leaf spot severity, corroborating these reports, and indicating their usefulness in assessing the risk of this disease. The late season development of gray leaf spot and its reported dependence on plant age under field conditions (37) seem to suggest that a late-maturing genotype may have higher levels of gray leaf spot severity at a given developmental stage than an early-maturing genotype with the same level of disease resistance. However, the results of this study indicated that genotype maturity (MAT) was not an important predictor of gray leaf spot severity, confirming previously published results (4). This may have been because the 21-day difference between the earliest- and latest-maturing
genotypes was not sufficient to capture the effect of this variable. In addition, for any
given location, the genotypes used in this study were planted on the same date,
regardless of their maturity. This may have resulted in coincidence of anthesis -
reportedly critical stage for gray leaf spot onset and development (37) - between
early- and late-maturing genotypes across locations, reducing the influence of plant
maturity. PD probably captured the influence of favorable late-season weather
conditions better that MAT. In late-planted maize, kernel development occurs during
the period when *C. zeae-maydis* activity is most favored. Coupled with infection-
favorable weather conditions, spore concentration in the air increases as the season
progresses (32), allowing for more secondary cycles. There was a 70-day difference
between the earliest and latest planting dates.

Previous crop (PC) was the least important predictor. This may be attributed
to the fact that it was used as an indirect measure of the survival of the fungus from
one season to another; a characteristic better reflected by the variable SR. For most
of the locations with a soybean-maize cropping sequence, maize was rotated with
soybeans for only one season. Even following a maize-soybean cropping sequence,
a substantial amount of maize residue still remained on the soil surface, favoring the
survival of the pathogen (8, 32, 33). In areas where conservation tillage is practiced,
with 30% or more residue left on the soil surface, a one year rotation away from
maize is not enough to reduce the survival of *C. zeae-maydis* (24).

The previously reported east to west variation in the weather conditions
across the state of Iowa (5) supports the importance of longitude (LON) as an
important predictor. LON was highly significant with a negative coefficient. Bhatia
and Munkvold (5) reported that the number of hours of RH greater than 95% was generally higher in eastern than in western Iowa, and that locations with high disease severity had correspondingly greater number of hours of RH over 95%. Prolonged periods of high relative humidity are known to favor the development of gray leaf spot (2, 21, 22, 37, 43).

Logistic regression

Both the PO and ECR models provided strong evidence of associations with disease severity (Total $P < 0.0001$) (Table 2.1). In addition to providing a quantitative method of assessing the utility of these models for estimating disease severity on new cases and for checking for overfitting or lack-of-fit, bootstrap validation was used to compare the two types of ordinal logistic regression models. In this approach, the original datasets were treated as if they were a population and 1000 samples were taken with replacement. The indices were calculated for each sample and the average index was used to assess model performance. The bootstrap validation produced similar indices of predictive accuracy for both the PO and ECR models. The original $D_{xy}$ values were 0.82 and 0.84, respectively, for the two models. Better estimates of how well the models will perform on future cases were obtained after subtracting the optimism due to overfitting from the original $D_{xy}$ values, yielding bias-corrected $D_{xy}$ values of 0.80 and 0.82 for the PO and ECR models, respectively. According to Harrell and coworkers (14), bias-corrected $D_{xy}$ values give a better estimate of the likely validation accuracy on new cases than the original values. The maximum absolute error in predicted probability, $E_{max}$, is a measure of unreliability. This index is small for both models. The slope and intercept (needed to
recalibrate the models to a 45° line) were close to the ideal values of zero and one for both models. Along with $E_{\text{max}}$ and $D_{xy}$, the latter two indices indicate that the PO and ECR models fit the data well.

**CART**

The CART modeling approach provided an insightful diagrammatic representation of the relationship between the predictors and the outcome. The tree (Figure 2.2) indicated that several different combinations of predictor variables may result in similar disease severity classes. However, it clearly indicated the strong influences of LON, GLSR and SR on late-season gray leaf spot severity. In general, the highest levels of disease occurred when the most susceptible genotypes (GLSR < 3) were planted east of LON 91.41 and when SR was high. West of LON 91.41, the other predictors appeared to have a weaker relationship with final disease severity class. These results suggest that even if a susceptible genotype is planted in an area with high surface residue (>82%), gray leaf spot severity rarely exceeds 20% (class 1) in western Iowa. As discussed previously (5), this is probably due to weather conditions in the western part of the state that are less favorable to disease development. The pruned (14 terminal nodes) tree performed better than the full tree (23 terminal nodes) in predicting the outcome of the validation cases (not shown). This is consistent with the idea that a simplified version of the tree is less likely to overfit the data, thus, generalizing better than the full tree (7, 30).

**Misclassification rate**

Although resampling is considered an excellent validation method, the performance of a model on an independent dataset is often considered a true
assessment of its predictive accuracy. The latter approach, called external validation, provides a more stringent assessment of a model and the entire data collection system (14). Misclassification rate is a commonly used method of assessing the performance of a model on an independent data set. It entails choosing a cutoff point for class assignment; comparing model classification with the actual class assignment; and then determining the proportion of correctly and incorrectly classified cases. Harrell (13), however, advised against the use of misclassification alone as a measure of predictive accuracy, stating that it is highly subject to the analyst's choice of cutoff point and that classification error is an insensitive and statistically inefficient measure. If the response is binary, a cutoff of 0.5 is often used, and if it is ordinal, the class with the highest probability is used. All models correctly classified 70% or more of the cases when used to predict the probability of being in a class greater than 1 (severity greater than or equal to 20%). This is of particular importance because above 20% severity, yield losses due to gray leaf spot occur. Jenco (19) reported that moderate to severe epidemics of gray leaf spot (severity in the middle third of the plant between 30 and 100%) resulted in large yield reductions. When using the CART model to predict the exact class of the 30 independent cases, there were occasions when a given case had equal probabilities (0.33) of being assigned to two adjacent classes (3 and 4). When this happened, the model arbitrarily selected one of the two classes. This probably explains the relatively inferior performance of CART in predicting the correct class of the validation cases. The overall misclassification resulting from the use of the three modeling approaches may have been due, in part, to differences in weather
conditions among locations and years during the growing season. Even though gray leaf spot severity was strongly related to pre-planting data, weather conditions after planting undoubtedly influenced the final level of severity observed at R4/R5, and these effects were not captured by the models.

**Model application**

Given the fact the gray leaf spot is a polycyclic disease, a predictive accuracy of over 65% using only pre-planting data is remarkable. All three modeling approaches performed creditably in predicting late season gray leaf spot severity class based on information available prior to planting. This augurs well for the future use of these models as decision-making tools for gray leaf spot management. They may be used for genotype selection and to assess the consequences of using a given combination of management practices. However, given the importance of LON as a predictor variable, these models would have to be refitted and revalidated prior to being applied outside of the state of Iowa. Further validation of these models may improve their predictive accuracy, and coupled with yield loss models, they may be used to make economically sound management decisions for gray leaf spot of maize.

**Literature Cited**


Figure 2.1. Percentage of cases in each maize gray leaf spot severity class for each year and for the total of the four years used to develop risk assessment models. Disease severity, percentage of the ear leaf covered with gray leaf spot lesions, was assessed at the R4/R5 plant growth stage on several genotypes selected from location in southern Iowa.
Figure 2.2. Classification tree used to estimate gray leaf spot severity classes (in rectangular boxes) as a function of pre-planting site and genotype information collected in Iowa between 1998 and 2001. The tree was pruned to 14 terminal nodes from the original 23-node tree. LON = longitude, GLSR = gray leaf spot resistance ratings (1 = most susceptible to 9 = most resistant), SR = percentage surface residue, PD = planting date in day of year, and MAT = genotype maturity rating in comparative relative maturity (CRM).
Table 2.1 - Summary of estimates for the proportional odds and extended continuation ratio ordinal logistic regression models used to assess the risk to gray leaf spot of maize using pre-planting site and genotype data collected in Iowa between 1998 and 2001

<table>
<thead>
<tr>
<th>Factor</th>
<th>Proportional odds</th>
<th></th>
<th></th>
<th>Extended continuation ratio</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>P</td>
<td>Coefficient</td>
<td>SE</td>
<td>P</td>
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<tr>
<td>LON</td>
<td>-1.031</td>
<td>0.244</td>
<td>0.0000</td>
<td>-0.767</td>
<td>0.185</td>
<td>0.0000</td>
</tr>
<tr>
<td>MAT</td>
<td>-0.028</td>
<td>0.038</td>
<td>0.4602</td>
<td>-0.023</td>
<td>0.032</td>
<td>0.4719</td>
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<tr>
<td>PD</td>
<td>0.044</td>
<td>0.013</td>
<td>0.0013</td>
<td>0.033</td>
<td>0.010</td>
<td>0.0014</td>
</tr>
<tr>
<td>SR</td>
<td>0.033</td>
<td>0.010</td>
<td>0.0007</td>
<td>0.024</td>
<td>0.008</td>
<td>0.0015</td>
</tr>
<tr>
<td>GLSR</td>
<td>-1.011</td>
<td>0.163</td>
<td>0.0000</td>
<td>-0.826</td>
<td>0.134</td>
<td>0.0000</td>
</tr>
<tr>
<td>PC</td>
<td>0.257</td>
<td>0.663</td>
<td>0.6977</td>
<td>0.518</td>
<td>0.570</td>
<td>0.3633</td>
</tr>
<tr>
<td>Overall</td>
<td>--</td>
<td>--</td>
<td>0.0000</td>
<td>--</td>
<td>--</td>
<td>0.0000</td>
</tr>
<tr>
<td>$D_{xy}$&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>LR$\chi^2$&lt;sup&gt;d&lt;/sup&gt;</td>
<td>162.78</td>
<td></td>
<td></td>
<td></td>
<td>179.46</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>LON = longitude, GLSR = gray leaf spot resistance ratings (1 = most susceptible to 9 = most resistant), SR = percentage surface residue, PD = planting date in day of year, PC = previous crop, and MAT = genotype maturity rating in comparative relative maturity (CRM).

<sup>b</sup>Standard error.

<sup>c</sup>Somers' rank correlation between predicted probabilities and observed responses.

<sup>d</sup>Likelihood ratio $\chi^2$. 
Table 2.2 - Summary of the bootstrap validation of the proportional odds and extended continuation ratio logistic regression models used to predict maize gray leaf spot severity classes as a function of pre-planting site and genotype data collected in Iowa between 1998 and 2001; severity was estimated as proportion of the ear leaf diseases at growth stage R4/R5

<table>
<thead>
<tr>
<th>Index</th>
<th>Original</th>
<th>Training</th>
<th>Testing</th>
<th>Optimism</th>
<th>Corrected Index</th>
</tr>
</thead>
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<tr>
<td></td>
<td>PO</td>
<td>ECR</td>
<td>PO</td>
<td>ECR</td>
<td>PO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Training</td>
<td>Testing</td>
<td>Optimism</td>
<td>Corrected Index</td>
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<td>0.00</td>
<td>-0.07</td>
<td>-0.04</td>
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<td>1.00</td>
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<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(D_{xy}^d\): Somers' rank correlation between predicted probabilities and observed responses.

\(E_{max}^e\): Maximum absolute error in predicted probability.

\(PO\): Proportional odds logistic regression model.

\(ECR\): Extended continuation ratio logistic regression model.

\(N\): 1000
Table 2.3 - Misclassification rates for the proportional odds and extended continuation ratio logistic regression models, and the classification and regression tree model developed using pre-planting data collected in Iowa between 1998 and 2001 and used to assign validation cases collected in 2002 to maize gray leaf spot severity classes; disease severity was estimated on the ear leaf at the R4/R5 plant growth stage

<table>
<thead>
<tr>
<th>Classification Rate (%)(^a)</th>
<th>Exact class(^b)</th>
<th>Severity (\geq 20%)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PO(^d)</td>
<td>ECR(^e)</td>
</tr>
<tr>
<td>Correct</td>
<td>66.67</td>
<td>60.00</td>
</tr>
<tr>
<td>Over</td>
<td>6.67</td>
<td>13.33</td>
</tr>
</tbody>
</table>

\(^a\) Percentage of the 30 validation cases correctly classified or incorrectly classified as a result of over or underestimation using risk assessment models.

\(^b\) Assignment of validation cases to one of five gray leaf spot severity classes (1 - < 20%; 2 - \(\geq 20\%\) and < 40%; 3 - \(\geq 40\%\) and < 60%; 4 - \(\geq 60\%\) and < 80%; and 5 - \(\geq 80\%\)).

\(^c\) Assignment of validation cases to a class having gray leaf spot severity greater than or equal to 20% (2, 3, 4, or 5).

\(^d\) Proportional odds logistic regression model.

\(^e\) Extended continuation ratio logistic regression model.
CHAPTER 3

HYBRID REGRESSION-ARTIFICIAL NEURAL NETWORK MODELS
FOR THE PREDICTION OF GRAY LEAF SPOT OF MAIZE

A paper to be submitted to Phytopathology

Pierce A. Paul¹ and Gary P. Munkvold²

Abstract

Regression and artificial neural regression (ANN) modeling approaches were combined to develop models to predict the severity of gray leaf spot of maize. Regression models were used as a preliminary step to select potentially useful variables to be used in ANN model development. A total of 329 cases were used for model development. These consisted of environmental, cultural, and location-specific variables collected from 17 counties in Iowa between 1998 and 2002. All-subsets regression was performed, generating different models from different combinations of 11 input variables. The best nine of 80 preliminary models were selected based on Mallow's Cp criteria, and the variables selected in these models were used to develop ANN models. A three-layer, feed-forward, back-propagation network with three hidden nodes was used to model the data. A random sample of 60% of the cases was used to train the network, and 20% each for testing and

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validation. The networks with the highest predictive accuracies corresponded well to the best subsets of variables selected by the regression models. The predictive accuracy of the top four networks ranged from 70 to 75%, with mean squared errors ranging from 174.7 to 202.8. Networks with seven and eight inputs generally performed better than those with nine inputs. The best predictors of gray leaf spot severity were longitude, surface residue, planting date, cumulative hours of daily temperatures between 22 and 30°C and nightly RH ≥ 90% between growth stages V4 and V12, mean nightly air temperature between V12 and R2, and gray leaf spot resistance rating. Using regression to select predictors prior to fitting ANN models resulted in faster convergence of networks to a solution when the best subsets of input variables were used. Four subsets of variables with good predictive accuracies were identified, allowing for greater flexibility in the choice of variables to be used to predict gray leaf severity.

Introduction

Gray leaf spot of maize (Zea mays L.) is caused by the fungus Cercospora zeae-maydis Tehon & Daniels. It was first reported in 1925 in Alexander County, in southern Illinois (49). For years, significant outbreaks were restricted to the mountainous regions of Virginia, Tennessee, Kentucky, and North Carolina (20). Over the past 20 years, the disease has become a concern in most maize-producing areas of the United States, spreading as far west as eastern Colorado, Kansas, and Nebraska and north into Wisconsin and Minnesota (23). In addition to the United
States, gray leaf spot has also been reported in Southern and Central America (6, 20), and Southern and Central Africa (54).

The increased prevalence of gray leaf spot coincides with the widespread adoption of conservation tillage (23), a practice which leaves a substantial amount of maize residue on the soil surface. *Cercospora zeae-maydis* overwinters in infested maize residue on the soil surface (37, 11). In Ohio, de Nazareno et al (12) found that under conditions favorable for disease development, the relationship between the amount of surface maize residue and disease severity was positive. Similar observations were made from studies conducted in North Carolina (38), Maryland (43), and Iowa (4). Payne et al. (38) and Ward et al. (52) reported that no-till planting in maize residue favored the early appearance of lesions of gray leaf spot and resulted in greater end-of-season disease severity than tillage. Payne et al. (38) concluded that the early appearance of gray leaf spot lesion in no-till plots allowed for the occurrence of more secondary cycles and, as a result, greater end-of-season disease severity than in tilled plots.

Gray leaf spot significantly impacts maize yield. Martinson and collaborators (30) reported that in seed production fields where gray leaf spot onset occurred prior to tasseling and the difference in ear leaf disease severity between sprayed plot and unsprayed control was 63%, yield losses ranged from 21 to 27%. In individual hybrid fields, yield losses ranging from 24 to 69% have been reported in Virginia (7, 45-48), 11 to 44% in Iowa (19), 11% in Kentucky (51), 15 to 33% in Ohio (24, 25), and 50 to 65% in South Africa (54). According to Munkvold et al. (32), annual yield loss
estimates in maize production in Iowa exceeded $100 million for several years during the 1990s.

Yield losses due to gray leaf spot may be minimized when appropriate management practices are deployed, including crop residue management. Incentives aimed at promoting soil conservation and economic pressures have led to continuous cropping of maize and a great proportion of the croplands in the midwest being under some form of minimum tillage, leaving more than 30% of the crop residue on the soil surface (26).

Coates and White (8) reported that many sources of resistance are available for use to improve maize hybrids in the midwestern United States. Currently, several moderately-resistant hybrids can be used to achieve satisfactory disease control, and steady progress is being made towards breeding for highly-resistant hybrids (44). Even when moderately-resistant hybrids are planted, however, substantial yield losses due to gray leaf spot may still occur (18). In seed production, the use of genetic resistance is not always an option even though inbreds with moderate resistance to gray leaf spot are available. In order to produce seed of a given maize hybrid, specific inbreds have to be planted, regardless of their level of resistance. Therefore, fungicide sprays are often recommended for seed production (30).

Fungicide applications have been shown to be effective at controlling gray leaf spot and reducing yield losses. Reports from South Africa (53) and the United States (29) have shown that chemical control may be profitable in grain production. However, in the United States, the profit margin is usually very small and the yield gain may not be enough to offset the cost of chemical control. A single fungicide
application may be profitable for gray leaf spot management in maize grown for
grain in Iowa, however, the probability of profitability is strongly dependent on the
yield potential and susceptibility of the hybrid planted (32). Due to the high value of
the crop, fungicide application is often profitable in maize seed production (30, 55).
However, sound fungicide application criteria are needed in order to achieve
adequate gray leaf spot control. Currently, decisions are made based on crop growth
stage, the susceptibility of the seed parent, and disease severity thresholds. But
disease thresholds do not accurately predict subsequent disease severity. To be
effective, fungicide applications must be made early in the season before the
epidemic accelerates (30). Martinson et al (30) suggested that since detasseling
removes the upper leaves that would have otherwise contributed to grain fill, it is
important to protect the remaining lower leaves to ensure satisfactory yield. In order
to make a management decision early in the growing season, one needs to be able
to make a projection as to the level of disease severity that is likely to occur during
the season, based on the relationship between the conditions at the time of
management and disease severity. If accurate predictions of gray leaf spot severity
could be made early in the season, unnecessary fungicide application could be
avoided and fungicides would be used only when warranted, making gray leaf spot
management more cost-effective.

In this paper, we report the results of a 5-year project to develop models that
predict late-season gray leaf spot severity based on early- and mid-season data in
order to determine the need for fungicide application. Preliminary results were
achieved in previous attempts to model the relationships between gray leaf spot
severity, and environmental and cultural factors using stepwise multiple regression (4). Since then, additional data have been collected; different modeling approaches and forms of representing weather data as input variables have been used; and gray leaf spot prediction models have been developed. Interim results of this study have been published (35).

Materials and Methods

Site selection and data collection

In order to generate epidemics of differing severity and to represent as many of the various combinations of variables likely to influence the development of gray leaf spot as possible, several locations were selected in Iowa between 1998 and 2002. These locations were chosen from regions with different histories of GLS, varying cropping practices, and a range of gray leaf spot-favorable weather conditions. A total of 50 locations in 17 counties predominantly in the southern half of the state (between latitudes 40° 42' 04"N and 42° 12' 60"N) were selected, including 13 in 1998, 11 in 1999, 10 in 2000, 11 in 2001, and five in 2002 (Figure A1-A3). Data were collected from commercial seed production fields, hybrid strip trials, or research plots planted with three to eight maize genotypes (inbreds or hybrids) with gray leaf spot resistance ratings ranging from 2 (most susceptible) to 7 (most resistant) and maturity ranging from 98 to 119 days CRM (comparative relative maturity). Genotypes varied among locations and years, but a few hybrids were common for most locations. At each location genotypes with a range of gray leaf spot resistance and physiological maturity were planted. Some fields were used for a
single growing season whereas others were used for several or all five growing seasons. Plantings were done between April 22\textsuperscript{nd} (112 day of year) and July 1\textsuperscript{st} (182 day of year), and plots were 12 to several hundred meters long and 2 to 18 rows wide. In general, plant population varied from 64,000 to 79,000 plants/ha.

At the Iowa State University Southeast Research and Demonstration Farm near Crawfordsville, Iowa, a location used in all five growing seasons, plots were established in a 2 x 2 x 3 factorial arrangement in a split-split-plot design, with tillage practice, planting date, and hybrid resistance representing the treatment factors. Two planting dates, early and late, were used as the main-plot factor; two types of tillage practices, till and no-till, were used as the sub-plot factor; and three hybrids, Pioneer Brand hybrids 3394, 3489, and 3335 with gray leaf spot resistance ratings 2, 4, and 5, respectively, as the sub-sub-plot factor. Twelve rows of each hybrid were planted at two planting dates in 25-m by 110-m plots in adjacent tilled (fall chisel/spring disk) and non-tilled strips. Planting dates for early and late plantings were 11 May (day 131) and 27 May (day 147), in 1998; 3 May (day 123) and 19 May (day 139), in 1999; 28 April (day 119) and 15 May (day 136), in 2000; 1 May (day 121) and 10 June (day 161), in 2001; and 24 April (day 114) and 8 May (day 128), in 2002.

In 2001 and 2002, similar plots were established at the Iowa State University Muscatine Island Research and Demonstration Farm in Fruitland, Iowa. At this location, a single planting date was used and 12 to 18 30-m rows of Pioneer Brand hybrids 3394, 3489, and 3335 were planted in tilled (moldboard plowed and disked) and non-tilled blocks. Overhead irrigation was done periodically at this location.
At each location, global coordinates, surface residue management, cropping sequence, planting date, and genotype susceptibility and maturity data were recorded. Latitude and longitude were recorded using a hand-held Magellan GPS 4000 global positioning unit (Magellan Systems Corporation, San Dimas, CA). Percentage maize residue cover was recorded using the line transect method (31). Three counts were made in each field and the average count was used. Cropping sequence was determined by recording whether the crop planted the previous growing season was maize or soybean. The number 1 was assigned to fields where maize was the previous crop, while 0 was assigned to fields where soybeans were the previous crop. Planting dates (in day of the year), and genotype maturity and gray leaf spot resistance ratings (provided by the seed suppliers) were also recorded for each genotype at each location.

On-site weather stations consisting of self-contained dataloggers and sensors were established approximately 10-15 m away from the edge of each field in an unobstructed area. At each location, all dataloggers were mounted on the same pole in a grass strip at a height of approximately 1.5 m above the ground. SPECWARE dataloggers (Spectrum Technologies, Inc., Plainfield, IL) were used to record temperature (°C) and surface wetness (0 to 15 scale) at 30-min intervals. The wetness sensor grid was painted with three coats of a proprietary latex paint (Bob Olson, Savannah, GA; 21) in order to enhance sensitivity to dew periods. The sensor faced north at an angle of 45°. Relative humidity at 1.5-m height was recorded at 15-min intervals using HOBO dataloggers (Model RH Stowaway, Spectrum Technologies, in 1998 and Model H8 Pro Series, Onset Computer
Corporation, Bourne, MA, in the other four years) placed inside radiation shields (Spectrum Technologies).

Each location was visited at 14-day intervals and weather data were downloaded from the dataloggers, plant growth stage was recorded, and disease severity was assessed by visually estimating the percentage of the ear leaf covered with gray leaf spot lesions. At each assessment, ten plants of each genotype were arbitrarily selected from the center of each plot and gray leaf spot severity on the ear leaf was estimated by using standard area diagrams as visual references (34). Gray leaf spot severity (%) on the ear leaf at the R4/R5 plant growth stage (40) was used as the response variable for model development. This stage was chosen because gray leaf spot severity in the middle third of the plant (ear leaf region) at approximately the R4/R5 growth stage was shown to provide the best relationship with yield loss (19).

Data organization and input variable selection

Weather data were edited to eliminate erroneous data points, delete or substitute missing data, and generate input variables. Duration of daily periods of favorable temperature (22 to 30°C) and RH (≥ 90 or 95%) were calculated (2, 3, 41, 50) and used as input variables. Duration of surface wetness also was calculated and used as an input variable. Leaves were considered wet when the datalogger output was > 0. Variables recorded at 30-min intervals were considered to represent 0.5 h per observation. The number of cases with missing temperature or RH data was small. However, due to occasional failure of the wetness sensors, data were occasionally lost. A binary logistic regression model was therefore developed to
estimate leaf wetness status as a function of temperature and RH (36). This model predicted leaf wetness with an accuracy of 87%. Less than 1% of the total leaf wetness data was estimated using this model.

In order to generate variables potentially more likely to represent conditions favorable for the development of gray leaf spot, weather data were summarized for four periods during the growing season. Predictive models developed for other Cercospora diseases use index values based on cumulative hours of RH above a critical value while temperature is within a critical range (9, 56). Based on these models, similar indices were derived for gray leaf spot. Temperature, relative humidity, and leaf wetness were examined for the following periods: 1) 45 days before R1 until 15 days after R1; 2) 15 days before until 15 days after R1; 3) 30 days before R1 until R1; and 4) 45 days before R1 until 15 days before to R1 (Figure 3.1B). These periods were chosen because they correspond to critical primary infection periods and are relevant to the timing of fungicide application decisions. For each period, cumulative hours of leaf wetness, temperatures between 22 and 30°C, and RH ≥ 90 and ≥ 95% were generated. Since previous reports on the relationship between temperature and RH within these ranges and gray leaf spot severity showed that individually these variables did not tend to have a significant effect on disease severity (4), different forms of representing these variables were explored and their relationships with gray leaf spot severity were reassessed. Cumulative hours of daily (600 to 1800 h) and nightly (1800 to 600 h), and mean daily and night temperature and RH within the abovementioned ranges were generated for each of the four periods. Cumulative daily and nightly time-duration values (TDV) (9),
defined as the number of hours having both temperatures between 22 and 30°C and RH ≥ 90%, were also derived for each period. Bhatia and Munkvold (4) showed that the strength of the relationship between the environment and gray leaf spot severity improved when TDV was used as an input variable in regression models instead of temperature and RH as individual variables. A total of 76 weather-related variables were created and analyzed for their usefulness as input variables for model development.

Several environment, location, and cultural factors were selected for model development. To eliminate unnecessary predictors and to avoid highly correlated predictors, a preliminary variable selection was performed based on correlation analysis. The 76 weather variables were analyzed and the temperature, RH, and leaf wetness variables with the highest correlations with gray leaf spot severity were selected. In general, variables summarized for period 4 (45 days R1 to 15 days before R1) showed the best correlation with gray leaf spot severity (Figure 3.1A). Within this period, cumulative hours of leaf wetness (CLW4), cumulative hours of daily temperature (CDT4), and cumulative hours of nightly RH ≥ 90% (NRH904) had the highest correlation coefficients. For period 2, mean nightly temperature (ANT2) had the highest correlation coefficient. The latter variable was selected since it was thought to provide information not provided by CDT4. In addition to weather-related variables, planting date (PD), percent maize surface residue cover (SR), previous crop (PC), genotype maturity (MAT), genotype gray leaf spot resistance (GLSR), latitude (LAT) and longitude (LON) were also used as input variables.
Model development and validation

Regression and artificial neural network (ANN) (5, 39) were used as complementary approaches to model the relationship between gray leaf spot severity and the predictors. As a preliminary step, Mallow’s Cp (28) variable selection criterion was used to determine the best subset of the 11 predictor variables (selected based on correlation coefficient) to be used in the input layer of the ANN. Variable selection prior to model development is useful for removing redundant predictors from the model, reducing noise in the data set due to unnecessary predictors, and avoiding problems of collinearity caused by having too many variables fulfilling the same function in the model (15). To identify the best subset of potentially useful predictors, all-subset regressions by leaps and bounds (17) was performed using the leaps function in S-plus 6.1 (Academic Site Edition, Insightful, Corp. Seattle, WA). Using this modeling approach, different numbers and combinations of input variables were used to develop regression models, and the best model was selected based on Mallow’s Cp criteria defined as:

\[ Cp = \frac{RSS_p}{\hat{\sigma}^2} + 2p - n \]

where \( RSS \) is the residual sums of squares from the model with \( p \) predictor variables; \( \hat{\sigma}^2 \) is the residual mean square from the model with all the predictors; and \( n \) is the sample size. The model with the smallest \( Cp \) value was selected as the best model. Ideally, a model should have a \( Cp \) value equal to or less than the number of predictors (\( p \)) used. A total of 80 models were developed from which the best nine...
(Table 3.1) were chosen. The variables selected by these models were used to develop ANN models.

In second step of the model development process, ANN was used to detect relationships between the predictors and outcome. Such relationships include nonlinear and interactions effects. Like many other modeling approaches, ANN can be thought of as a minimization technique in which the goal is to minimize the difference between model output and actual output. However, some of the proposed advantages of ANN over conventional methods are that it automatically allows for nonlinear relationships between predictor and response variables, and incorporates interaction between variables without requiring additional modeling as in the case of standard statistical approaches (42). ANN models are fitted by adjusting weights which are analogous to coefficients/parameters in regression modeling. The predictors and responses are repeatedly presented to the network during a process called training and after each passage through the network, internal weights are adjusted so as to minimize the difference between network and actual outputs. In this iterative process, the network learns the relationship between the predictor and response variable so that when it is presented with a new set of input (validation) it is capable to predicting the outcome based on this relationship. There are several types of ANN employing different types of architecture. One of the most commonly used architecture is the multilayered perceptron trained through error back-propagation, the back-propagation ANN (BPNN) (5).

A three-layer, feed-forward BPNN with fully connected layers was used to model the relationship between the predictors selected by the regression model and
gray leaf spot severity using NeuroShell2 (Wards Systems Group, Inc., Frederick, MD). A total of 329 cases were used. A case was defined as an observation which differed from another in the value of one or more variables. Sixty percent of the 329 cases were used for training the network, 20% for testing, and 20% for validation. Separate sets of models were developed for each set of input variables selected by the regression model. Before training, the input data was scaled from -1 to 1. The network was presented with the training cases using 200,000 iterations. After every 200 iterations, the network was presented with the test set which was used for calibration. Linear and logistic activation functions were used in the input and output layers, respectively, while different combinations of activation functions were tested in the nodes of the hidden layers. In addition, the number of nodes in the hidden layer and the initial weights and momentum were determined by trial and error until the best model was found on the basis of the coefficient of multiple determination ($R^2$) and mean squared error (MSE) of the training and test sets (Table A2). The network was saved and training was stopped on the best test set, that is, each time the error factor reached a new low for the test set. The validation cases were then used to assess the performance of the models on an independent data set. $R^2$, correlation coefficient, and MSE were used as measures of predictive accuracy.

**Results**

Following preliminary variable selection, the 76 weather-related variables were reduced to four based on their correlation coefficients; two temperature
variables and one variable each for leaf wetness and RH. With one exception, variables from period 4 showed the strongest correlation with gray leaf spot severity (Figure 3.1A). Among the variables for leaf wetness, cumulative hours of leaf wetness for period 4 (CLW4) had the highest coefficient (0.32). Cumulative hours of nightly RH greater than 90% for period 4 (NRH904) had the highest coefficient value of all the weather-related variables. Two temperature variables, from two growing-season stages and times during a 24-h period, were selected. Cumulative daily temperature for period 4, with correlation coefficient 0.20, and average nightly temperature for period 2 with the highest coefficient of all temperature variables, -0.29, were selected. Among location- and genotype-related variables, longitude (LON) had the highest correlation coefficient (-0.43) followed by surface residue (SR) (0.39), gray leaf spot resistance ratings (GLSR) (-0.37), previous crop (PC) (0.35), planting date (PD) (0.32), and genotype maturity (MAT) (-0.13). Latitude showed relatively weak correlation (LAT) with disease severity.

Final variable selection based on all-subsets regressions generated 80 models, of which the best 9 were selected on the basis of their relatively low Mallow's Cp values (Table 3.1). Seven to nine of the 11 initial variables were selected as potentially important predictors. All the selected models identified LON, GLSR, SR, PD, AVNT2, and NRH904 as important predictors, and all but one model incorporated CDT4 as a predictor. CLW4, PC, and MAT were the least selected of the predictor variables. Despite its relatively low correlation coefficient (-0.04), LAT was included in four of the nine models, including one of the best three. Based on
the $C_p$ values, models 1, 2, and 3 had the best combinations of input variables (Table 3.1).

For each subset of variables selected by the regression models, a series of BPNN models was developed. Several architectures with different activation functions, learning rates, momentum, initial weights, and numbers of hidden nodes were tested. The best architecture was a three-layer, feed-forward network with three hidden nodes, each having a different activation function. A combination of hyperbolic tangent, Gaussian, and Gaussian complement activation functions in the hidden nodes resulted in superior networks relative to other combinations of functions. The same architecture was used for all the networks and their predictive accuracy was assessed on the same set of validation cases. In general, the networks with the highest $R^2$ and Pearson's correlation coefficients, and smallest MSE (Table 3.2) coincided closely with the subset of variables with the smallest Mallow's $C_p$ values (Table 3.1). The best three subsets resulted in networks with the highest predictive accuracy. Seven- and eight-input networks generally performed better than 9-input networks. Network A2 with seven input variables, namely LON, GLSR, SR, PD, CDT4, ANT2, and NRH904 was the most superior, with a predictive accuracy of 75%. This network also had the smallest MSE and the strongest positive relationship between actual and predicted gray leaf spot severity (Figure 3.2) as confirmed by the correlation coefficient (Table 3.2). The addition of PC to network A2 resulted in model A3 with similar predictive accuracy (74%), whereas the use of LAT in network A1, resulted in slightly inferior predictive accuracy (71%). The substitution of LAT in network A5 for CDT4 in network A2 also resulted in inferior predicted
accuracy (70%). Model A2 was modified by removing LON to develop a tenth network (A10) with only 6 input variables. This model had a predictive accuracy of 68%, being superior to all of the 9-input models and two of the 8-input models. The best six models underestimated disease severity at high levels and overestimated at low levels (Figure 3.2). Generally, networks using MAT and CLW4 as input variables were among the most inferior. Overall, ANN models developed using the best subsets of predictor variables (according to Mallow’s Cp) (1, 2 and 3; Table 3.1) took less time to converge than those developed using other combinations of inputs.

Discussion

It is the general consensus that the buildup of infected maize surface residue on the soil surface has been responsible for the increase in gray leaf spot incidence and severity over the past 20 years (20, 54), and the importance of planting date (37, 41), and genotype resistance for the development of this disease has been well documented. Payne and Waldron (37) reported that although lesions first appeared in early-planted maize, final disease severity was greater on late-planted maize. The importance of prolonged periods of high relative humidity is also well known (20, 41, 54). So, the strength of the correlations between GLSR, SR, PD, and NRH904, and gray leaf spot severity serve to corroborate earlier findings. These variables were selected within the best subset of variables used for model development. The specific contribution of weather factors during periods of interest in terms of making management decisions is unknown. There is conflicting evidence regarding the
importance of leaf wetness for the development of gray leaf spot. Some reports suggest that free water was inhibitory to penetration of leaf tissue (2, 50), while other reports associate the development of the disease with locations with prolonged periods of leaf wetness (41). The fact that CLW4 did not improve the predictive accuracy of any of the models suggests that another moisture-related variable, possibly NRH904, may have been more important than leaf wetness. The correlation coefficient between NRH904 and CLW4 was 0.65.

The inability to successfully produce disease under controlled conditions has left many questions unanswered regarding the importance of temperature and RH during specific stages of the infection cycle. Thus, it was necessary to use empirical approaches in an effort to understand the dynamics of the disease in the field and to determine the role played by environmental variables. In a previous report, the importance of the joint role of temperature and RH for the development of gray leaf spot was made evident (4). Individually, these variables were reported to be less important. In this study we further assessed the importance of temperature and RH within a 24-hour period and during specific stages of the growing season. Cumulative hours of daily (600h to 1800h) temperatures between 22 and 30 °C and nightly RH ≥ 90% early in the growing season (between growth stages V4 and V15), and mean nightly temperatures (ANT2) between V12 and R2 were highly correlated with gray leaf spot severity assessed at R4/R5. This may be due to the fact that relatively warm, humid conditions during early spring may be necessary for the production of inoculum in infected residue on the soil. Studies on the effects of temperature and relative humidity on the production of spores on diseased tissue
supports this idea (Paul and Munkvold, unpublished). Spores were produced at temperatures between 20 and 30°C once RH was above 90%. The strong negative correlation between ANT2 and the strong positive correlation between NRH902, and gray leaf spot severity suggest that relatively cool, humid nights may be needed for infection to occur between V12 and R2. Relatively cool night temperature along with high relative humidity may have been the conditions prevalent in the mountainous regions and river bottom fields where gray leaf spot remained endemic for decades (20).

There are several documented reported of the use of ANN in plant pathology-related research (1, 13, 14, 16, 42, 56). Most of these focused on the comparison among different classes of ANN models, among different architecture of the same class of model, or among ANN and conventional modeling approaches. Comparative investigation of the performance of ANN relative to conventional modeling approaches often reveal that ANN models may perform just a well or better than conventional approaches (13, 14, 16, 33, 56). De Wolf and Francl (14) reported superior performance of BPNN over logistic regression for the classification of incidence of tan spot of wheat, and over stepwise logistic regression and multivariate discriminant analysis for the detection of infection periods for the same disease (13). Similarly, Yang and Batchelor (56) reported that BPNN’s performed better than conventional modeling approaches at predicting wheat scab epidemics, while Francl and Panigrahi (16) showed the superiority of the same class of ANN over discriminant analysis at predicting the wetness status of wheat leaves.
As is the case with conventional modeling approaches, ANN has strengths and weaknesses (42, 27). One of the major weaknesses of ANN modeling is the fact that it is a computationally intensive process (14, 42, 56). The flexibility allowed by this modeling approach in terms of choice of activation function, number of nodes, number and combination of inputs, learning rate, momentum, and initial may lead to several hours being spent before arriving at a single appropriate model. Yang and Batchelor (56) reported that as the number of hidden nodes of a BPNN model used to predict the epidemic of wheat scab increased, so did the number of events to conversion to a solution. In addition to increasing the complexity of the model, the use of several input variables may make the subsequent application of the model subject to the availability of all of the input variables. It is not always easy to interpret the relationship between the inputs and output based on an ANN model (42). Lee and coworkers (22) demonstrated that hybrid models could be used to explore the advantages of ANN and conventional modeling approaches. They proposed the combination of mechanistic and ANN models in a way that the ANN model accounted for the unknown and nonlinear part of the mechanistic model. Using a hybrid network to model a wastewater treatment process, they concluded that a parallel hybrid model involving principal component analysis and ANN resulted in accurate and cost effective modeling.

Even though ANN has been lauded for its ability to learn complex relationships, detect complex patterns, and solve complex problems, the performance of models developed using this technique are still dependent on the choice of the appropriate set of predictor variables. Yang and Batchelor (56)
emphasized the importance of biologically important predictors in fitting ANN models. They justified the superior performance of the ANN models used to predict appressorium formation in rice blast, seasonal progress of soybean rust, and wheat scab epidemic, stating that the predictive accuracy was very high (as high as 98.88%) because they were able to choose the right set of predictor variables due to the fact that the diseases had been widely studied. Choosing of the right combination of variables may enhance the predictive accuracy of a model, save time in model development, and results in models that are easier to interpret in term of the biological importance of the predictors. Comparing ANN and regression modeling approaches, Sargent (42) stated that regression modeling have the advantage of allowing the user to sequentially select predictors, eliminating those that do not contribute to the fit of the model. In this study, we explored this advantage of regression modeling, using all-subsets regression approach to select subset of variables to be used in developing ANN models. The best ANN models coincided with the best subset of predictor variables selected by the regression model. The best models had predictive accuracy ranging from 70 to 75% and used different combinations of variables. A small set of variables was identified as the most important, and comparison of the models led to an understanding of the importance of each variable in the model. Seven- and eight-input models were superior to nine-input models. Elimination of the trial and error step in the selection of predictors reduced the time taken to arrive at best model and resulted in models that converged to a solution faster. In addition, more that one model of acceptable predictive accuracy was developed, allowing for some flexibility in the choice of
predictors needed for future application of these models. The results of the study also led to a better understanding of the relationship between the weather and gray leaf spot severity. Relatively high daily temperature and cumulative hours of nightly RH \( \geq 90 \) during the beginning of the growing season was highly correlated with disease severity. Later in the season relatively cooler nights seemed to be most favorable. This probably reflects the different effects of these variables on different stages of the disease cycle.

Using early- and mid-season data, we were able to develop models to predict late-season gray leaf spot severity fairly accurately. With predictions made early during the growing season, these models may be used to make decisions regarding fungicide application. This would allow for more timely applications when warranted. Given the importance of temperature and relative humidity as predictors, the future application of these models would depend on how accurately these variables are measured and whether they are summarized for the correct periods during the growing season. The development of more than one model using different subsets of predictors allows for some flexibility in terms of the variables needed in order to make predictions. In spite of the importance of LON as a predictor we were able to develop a model (A10) with predictive accuracy of 68\% without using this variable. This augurs well for the future use of this model outside of the state of Iowa. However, further research is needed prior to the application of these models in other maize-growing regions, since these regions may have inherent characteristics not accounted for by the variables used in model development.
A management program for gray leaf spot of maize could be developed using the disease prediction models from this study in combination with pre-planting risk assessment models and yield loss models. For example, a risk assessment model could be used to recommend a hybrid prior to planting, then, a prediction model based on in-season weather data could be used to reassess the risk of the disease in order to recommend fungicide application.

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A

Temperature

Relative humidity

TDV

Period 1

Period 2

Period 3

Period 4

CT
CST
CTP
AT
GDT

30/V8

15/R2

Days/approximate growth stage before and after silking (R1)

B

P1

P2

P3

P4

45/V4

30/V8

15/V12

15/R2
Figure 3.2. Relationship between actual and back-propagation artificial neural network predicted severity of gray leaf spot of maize for the validation scenario from models A1 (A), A2 (B), A3 (C), A5 (D), A6 (E) and A10 (F) developed using different subsets of predictor variables.
Table 3.1. Models consisting of different combinations of variables selected using all-subsets regressions from 11 potentially important predictor of gray leaf spot of maize.

<table>
<thead>
<tr>
<th>Model</th>
<th>Input variables</th>
<th>Mallow's Cp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LAT, LON, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>7.11</td>
</tr>
<tr>
<td>2</td>
<td>LON, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>7.27</td>
</tr>
<tr>
<td>3</td>
<td>LON, PC, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>8.48</td>
</tr>
<tr>
<td>4</td>
<td>LAT, LON, PC, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>8.50</td>
</tr>
<tr>
<td>5</td>
<td>LAT, LON, GLSR, SR, PD, ANT2, NRH904</td>
<td>8.53</td>
</tr>
<tr>
<td>6</td>
<td>LAT, LON, GLSR, SR, PD, CLW4, CDT4, ANT2, NRH904</td>
<td>8.66</td>
</tr>
<tr>
<td>7</td>
<td>LAT, LON, MAT, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>9.03</td>
</tr>
<tr>
<td>8</td>
<td>LON, MAT, GLSR, SR, PD, CDT4, ANT2, NRH904</td>
<td>9.07</td>
</tr>
<tr>
<td>9</td>
<td>LON, GLSR, SR, PD, CLW4, CDT4, ANT2, NRH904</td>
<td>9.21</td>
</tr>
</tbody>
</table>

\( ^a \) Best nine of 80 models selected using different combinations of 11 predictors consisting of location and weather variables collected in Iowa between 1998 and 2002.

\( ^b \) Mallow's Cp value used to determine the best subset of predictors; a small Cp value is indicative of a good subset/model.

\( ^c \) LAT = latitude, LON = longitude, MAT = maturity, GLSR = gray leaf spot resistance, SR = surface residue, PD = planting date, CDT4 = cumulative daily temperature between 22 and 30°C for period 4 (growth stage V4 to R1), ANT2 = average nightly temperature for period 2 (V12 to R2), and NRH904 = cumulative nightly RH > 90% for period 4.
Table 3.2. Back-propagation artificial neural network models developed for gray leaf spot of maize using different combinations of input variables selected based on all-subsets regressions; the predictive accuracy of the models on the validation data set was based on coefficient of multiple determination, mean squared error, and correlation coefficient.

<table>
<thead>
<tr>
<th>ANN Model(^{a})</th>
<th>No. of input nodes</th>
<th>(R^2) (%)(^{b})</th>
<th>MSE(^{c})</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>8</td>
<td>71</td>
<td>202.77</td>
<td>0.84</td>
</tr>
<tr>
<td>A2</td>
<td>7</td>
<td>75</td>
<td>174.75</td>
<td>0.87</td>
</tr>
<tr>
<td>A3</td>
<td>8</td>
<td>74</td>
<td>176.84</td>
<td>0.87</td>
</tr>
<tr>
<td>A4</td>
<td>9</td>
<td>64</td>
<td>247.83</td>
<td>0.81</td>
</tr>
<tr>
<td>A5</td>
<td>7</td>
<td>70</td>
<td>206.42</td>
<td>0.84</td>
</tr>
<tr>
<td>A6</td>
<td>9</td>
<td>66</td>
<td>237.43</td>
<td>0.83</td>
</tr>
<tr>
<td>A7</td>
<td>9</td>
<td>61</td>
<td>267.81</td>
<td>0.80</td>
</tr>
<tr>
<td>A8</td>
<td>8</td>
<td>57</td>
<td>298.27</td>
<td>0.76</td>
</tr>
<tr>
<td>A9</td>
<td>8</td>
<td>64</td>
<td>247.32</td>
<td>0.81</td>
</tr>
<tr>
<td>A10</td>
<td>6</td>
<td>68</td>
<td>219.83</td>
<td>0.83</td>
</tr>
</tbody>
</table>

\(^{a}\) Back-propagation artificial neural network models developed using data collected in Iowa between 1998 and 2002. The models A1 to A9 were developed using the nine subsets of variables described in Table 3.1, while model A10 was developed following the omission of longitude from the subset of variables used in model A2.

\(^{b}\) Coefficient of multiple determination

\(^{c}\) Mean squared error
CHAPTER 4

INFLUENCE OF TEMPERATURE ON THE RATE OF LESION EXPANSION IN GRAY LEAF SPOT OF MAIZE

A paper to be submitted to Phytopathology

Pierce A. Paul\(^1\) and Gary P. Munkvold\(^2\)

Abstract

Understanding of the effects of the environment on epidemic components of gray leaf spot of maize, caused by *Cercospora zeae-maydis*, has been hampered by the inability to consistently produce disease under controlled conditions. A simple, inexpensive inoculation technique was used to study the effect of temperature on lesion expansion. Plants were spray inoculated at the V6 growth stage, bagged, and incubated at 25-28°C and 100% RH for 36-40 hr. Diseased plants were transferred to growth chambers and exposed to constant temperatures of 25, 30, and 35°C. Lesion area (length x width) was measuring at 4-day intervals and plotted against time. Linear regression was used to determine the rates of lesion expansion over time, and the relationship between temperature and rate of lesion expansion was modeled using a second-order polynomial. Temperature had a significant effect on the rate of lesion expansion \((P < 0.05)\). At 25 and 30°C, the rate of lesion expansion was constant and significantly higher than at 35°C \((P < 0.05)\). In general, maximum

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values of rate of lesion expansion were observed at 30°C; however, the mean value at this temperature was not significantly different from the mean at 25°C. The quadratic model accounted for 73, 93, and 80% of the variation in the rate of lesion expansion with variation in temperature for experiments 1, 2, and 3, respectively. A model of the relationship between temperature and the rate of expansion of lesions of gray leaf spot may provide a better understanding of the dynamics of the disease in the field and may be useful for the development of a simulator for this disease.

**Introduction**

Although gray leaf spot of maize, caused by *Cercospora zeae-maydis* Tehon & Daniels, is a polycyclic disease, relatively few secondary cycles may occur during a growing season. This may be due to the long latent period characteristic of gray leaf spot (4, 27). Ringer and Grybauskas (27) suggested that due to the long latent period for gray leaf spot (LP50 = 14-19 days) and limited number of infection cycles, the amount of inoculum generated during the primary infection cycle might be more important than the number of secondary cycles in determining the level of disease. Based on the observed association between rainfall patterns, disease progress curve, and infection cycle components, these researchers concluded that when levels of initial inoculum or precipitation were low during early infection cycles, high levels of gray leaf spot did not occur until late in the season due to the long latent period and low number of infection cycles. The strong relationship between gray leaf spot severity and the amount of infected residue on the soil (10, 12, 20, 25, 31, 37) reemphasizes the importance of initial inoculum and consequently the primary
infection cycle for the development of this disease. Based on a study conducted by de Nazareno et al. (13) which showed that the rate of disease increase on individual plants decreased with distance from a point source of inoculum (residue), Lipps (22) concluded that residue was the most important source of inoculum in determining the final amount of disease present on plants at the end of the season. If the level of initial inoculum is high and conditions are favorable for primary infection, final disease severity may still be high even though few secondary cycles may occur. Under such conditions, the expansion of the existing lesions may play an important role in determining the final disease severity measured as diseased leaf area.

Analyzing the importance of lesion expansion as an epidemic component, Berger and collaborators (9) identified three major consequences of lesion expansion: intensification of disease even when susceptible host tissue is no longer available for infection; increase in diseased leaf area even when conditions are unfavorable for infection; and increase in the area available for the production of inoculum. Newly colonized tissues become infectious almost immediately since they do not having to await the passage of a latent period as do new infections originating from the dispersal of inoculum (9). Even when new infections occur, lesion expansion is still an important component since it affects the final amount of disease and the shape of the disease progress curve (8, 19, 30). Lesion expansion may compromise the effectiveness of management strategies geared at preventing new infections since fewer lesions may be compensated for by the expansion of existing lesions (6, 7, 19). Lesion expansion may be used for the quantification of disease intensity. Berger and Jones (8) noted that under certain conditions, the areas of
healthy and diseased tissue may be increasing at exponential rates even though disease intensity measured as a proportion of the total leaf area (severity) remain constant. Thus, the use of disease severity alone as a measure of disease intensity may result in an underestimation of disease progress.

Modeling the effects of temperature on the expansion of lesions of gray leaf spot may enhance understanding of the dynamics of this disease in the field. For several pathosystems, lesion expansion as a function of temperature has been used as an important component of simulation models (1, 2, 11, 29, 35, 36). Berger et al. (9) demonstrated through simulation modeling that a radial lesion expansion rate of 0.1 mm/day resulted in >70% of total diseased area being due to lesion expansion. They speculated that since many plant pathogens have rates of radial expansion greater than 0.1 mm/day, more than 95% of the total diseased area could result from lesion expansion.

The objective of the present study was to determine the rate of expansion of lesions of gray leaf spot at different temperatures and to model the relationship between the rate of lesion expansion and temperature.

Materials and Methods

Seedling and inoculum preparation

Seeds of inbred B73 were planted, three per pot, in 20-cm-diameter pots in a steam-sterilized potting mixture of peat, perlite, and soil (1:2:1). Inbred B73 was chosen because it is susceptible to gray leaf spot and typically produced rectangular
necrotic lesions without chlorosis from infection to sporulation (15), making it easy to measure the length and width throughout the experiment. After germination, the seedlings were maintained in a greenhouse or growth chamber at temperatures between 25 and 30°C before being transferred to the growth chamber (Intellus Controller, Percival Scientific, Inc., Perry, IA) where they were inoculated. Starting one month after emergence, a nutrient solution (21:5:20) was applied to each pot on a weekly basis for the duration of the experiment.

Fresh cultures of *Cercospora zeae-maydis* were prepared using an isolate collected from a naturally infected cornfield in Iowa. The cultures were prepared and maintained as described by Thorson and Martinson (33). Spores were transferred to petri dishes containing V8-juice agar (5) amended with streptomycin sulfate, and the dishes were then incubated at room temperature (22-25°C) for 7 days under a 12-hour photoperiod. Spore plugs or sections were taken from stock cultures, transferred to a Waring blender cup containing 10% skim milk, and homogenized for 10 to 12 seconds. Approximately 2 ml of this mixture were poured onto freshly prepared V8-juice agar, and the excess was decanted. Dishes were then incubated. After incubation, the cultures were either used immediately for inoculation or stored in a refrigerator (usually for seven to 10 days) until the plants were ready to be inoculated.

**Inoculation and assessment of lesion expansion**

Conidia were harvested by flooding the petri dishes with a solution of Tween 20 and distilled water (one drop/500ml) and dislodging the spores with a small paintbrush. The resulting suspension was filtered through two layers of cheesecloth
and brought to the final volume with distilled water. The conidia concentration was then estimated using a hemacytometer. A conidial suspension containing approximately 4 to 7 x 10^4 conidia ml⁻¹ was atomized onto both surfaces of the leaves of maize plants at the V5-V6 growth stage. After inoculation, the plants were placed in transparent plastic bags and incubated in a growth chamber at 25 to 28°C under a 14-hour photoperiod, which was supplied by fluorescent and incandescent lights producing an intensity of 316 μmol m⁻² s⁻¹ approximately 100 cm from the source. After 36 to 40h, the plants were removed from the bags and kept in the same growth chamber until symptoms were seen. As soon as the first characteristic lesions of gray leaf spot were observed (10-21 days after inoculation), the plants were transferred to similar growth chambers maintained at 25, 30, or 35°C and exposed to a 14-hour photoperiod for the duration of the experiment. Six to seven pots, each containing two to three plants, were randomly assigned to each growth chamber.

Plants were selected from each temperature treatment and a total of 10 lesions were used to assess lesion expansion. At the time of the first assessment, 10 lesions were selected from 7 to 10 numbered plants from each growth chamber. The positions of these lesions relative to the leaf blade, base, tip, and midrib were recorded. A record was also made of the position of the leaf on the plant (third, fourth, or occasionally fifth leaf counting from the bottom). Measurements were made at 4-day intervals for 17 days (until the leaves senesced or the plants outgrew the growth chambers) and the same lesions were measured at each assessment. Lesion dimensions were measured in two perpendicular directions and used to
determine the lesion area (length x width). Changes in lesion area data were then plotted over time to determine the rate of lesion expansion at each temperature.

**Experimental design and data analysis**

In this experiment, a randomized complete block design was used. Each growth chamber constituted an experimental unit replicated over time, with temperature being the treatment randomly assigned to each unit. The experiment was conducted three times. Throughout this study, temperature and relative humidity within each growth chambers were monitored using HOBO dataloggers (Model H8 Pro Series; Onset Computer Corporation, Bourne, MA) recording at 5-min intervals.

Linear regression analyses were performed using SAS Proc GLM (SAS Institute, Cary, NC) to determine the relationship between lesion area and time under each temperature regime, and to estimate the rate of lesion expansion for each replicate. Analysis of variance and orthogonal contrasts were then used to compare the effects of temperature on the rate of lesion expansion. Levene's Test for homogeneity of variance was performed using Proc GLM to determine whether the data from the three experiments could be pooled. To determine the relationship between rate of lesion expansion and temperature, the rates were regressed against temperature by fitting a second-order polynomial:

\[ r_{LE} = b_0 + b_1 \cdot T + b_2 \cdot T^2 \]

in which \( b_0, b_1, \) and \( b_2 \) are regression coefficients. Separate regressions were performed for each experiment (full model) and for the pooled data (reduced model), and the regression coefficients were tested for significance using standard \( t \) tests. A total of 9 observations (three temperatures x three replicates) were used for each of
the full models and 27 observations (three temperatures x three replicates x three experiments) for the reduced model.

Results

Lesion development

Throughout this study, successful infection by *C. zeae-maydis* occurred at temperatures between 25 and 28°C (mean 26°C) and continuous 100% RH during the first 36-40 h after inoculation. For the remainder of the incubation period, the RH in the growth chamber ranged from 75 to 100% (mean 94%). Typical gray leaf spot lesions were first observed between 10 and 21 days after inoculation. The initial lesions were rectangular with a light brown appearance and averaged between 2 and 6 mm². At 25 and 30°C, the lesions expanded at a constant rate over a 17-day period, reaching a mean final area of 20 and 23 mm², respectively, compared to a mean final area of 8.7 mm² reached at 35°C(Figure 4.1). For the first four days after the initial lesions appeared, lesion area increased relatively rapidly at 35°C (similar to 25 and 30°C), but thereafter, the rate of expansion decreased and remained low for the duration of the experiment, resulting in smaller lesions. The largest lesion observed at 25°C was 47 mm²; at 30°C, 51 mm²; and at 35°C, 20 mm².

Effect of temperature on the rate of lesion expansion

In all three experiments, temperature had a significant effect on the rate of lesion expansion (*P* < 0.05) (Tables A3-A5). In 2 of 3 experiments, the highest rates of lesion expansion were observed at 30°C (Figure 4.2). In general, the rates at 25
and 30°C were not significantly different ($P > 0.05$). The exception was in experiment 2 (Figure 4.2) when the mean rates were significantly different between these two treatments (Table 4.1). The lowest rates occurred at 35°C. In all cases, the mean rates at 35°C were significantly lower than at 25 and 30°C. Lesions expanded at 25 and 30°C at rates between 0.44 and 1.00, and 0.73 and 1.31 mm²/day, respectively, faster than at 35°C (Table 4.1). The highest and lowest mean rates observed at 25, 30, and 35°C were 1.72 and 0.59; 2.26 and 0.81; and 0.41 and 0.19, respectively.

In two of the three experiments, the $F$-test for the significance of linear and quadratic terms in the model of the relationship between temperature and the rate of lesion expansion was significant at $P < 0.05$, with the quadratic term contributing a greater part of the total sums of squares. On the basis of this test and based on the shape of the raw data plots, the quadratic model was considered appropriate for the description of the relationship between temperature and the rate of lesion expansion. When the rate of lesion expansion was regressed against temperature using the quadratic equation, the models fitted to each experiment had relatively high coefficients of determination ($R^2$). All the fitted models had highly significant $F$-values (Tables 4.2 and A6). The regression coefficients for experiments 1 and 2 were significant ($P \leq 0.05$), while for experiment 3 they were not. Levene's Test for homogeneity of variance of rate of lesion expansion was not significant yielding a $F$-value of 2.75 with $P = 0.084$, allowing the data to be pooled. The fitted model of the pooled data produced parameters that were statistically significant. Using the equation for the pooled model, the predicted rate of lesion expansion reached a maximum value of 1.29 mm²/day at 28.24°C (Figure 4.3).
Discussion

The conditions under which successful infections by *C. zeae-maydis* occurred further supports the idea that moderate temperatures and prolonged periods of high relative humidity are favorable for the development of this disease (20, 28, 38). Latterell and Rossi (20) reported the successful infection of *C. zeae-maydis* under greenhouse conditions only occurred when inoculated plants were incubated in a dew chamber for extended periods (up to 96 hours), followed by incubation under periodic misting in a plastic tent. Similar observations were made by Beckman and Payne (5). They reported that the optimum conditions for gray leaf spot development under greenhouse conditions were achieved when an intermittent misting system was used. Incubating artificially inoculated plants under a system providing 14 hrs of mist per day (3 sec of mist every 4 min from 2000 hours to 1000 hours the following day) for two weeks, they reported that characteristic lesions of gray leaf spot developed within 11-25 days after inoculation at 22 to 28°C. These results were consistent with those observed in this study, where typical gray leaf spot lesions appearing 10-21 days after inoculation following a period of continuous 100% RH during the first 36 to 40h after inoculation. Prolonged periods of high RH in the absence of free water favor germination, appressorium formation, and penetration (4, 33).

Lesion expansion is an indirect measure of the growth and colonization of *C. zeae-maydis* within the tissue of the host. The effect of temperature on this process was comparable with the effect of this factor on other developmental processes of this fungus. Studying the effect of temperature on the germination of spores and the
elongation of germ tube of *C. zeae-maydis* exposed to 12h of high RH, Beckman and Payne (5) observed that the optimum temperature was between 22 and 30°C and no germination occurred at 36°C. Paul and Munkvold (24) reported that once the RH requirements were met, the fungus sporulated well on diseased leaf tissue at a wide range of temperatures. Temperatures between 25 and 30°C seem to favor the development of *C. zeae-maydis*, probably resulting in greater growth and colonization of healthy tissue from the adjacent diseased tissue, thus leading to greater lesion expansion. At 35°C, restricted lesions with distinct chlorotic borders developed. This type of lesion development was reported as being typical of a resistant reaction, contrary to what is known to occur on inbred B73 (15). This seems to suggest that at 35°C the fungus is less able to grow and colonize the plant tissue or the reaction of the plant to the invading fungus is one that resembles a resistant response. The findings of Garden and Hilty (16) on the effects of temperature on radial growth of *C. zeae-maydis* on potato dextrose agar support the former hypothesis. They observed that neither sporulation nor radial growth occurred at 32°C.

The influence of temperature on the expansion of lesions has been well documented for several other pathosystems (3, 18, 21, 26, 32, 34), with some of the results being consistent with those observed in this study. Kato and Kozaka (18) studied the effects of temperature on the enlargement of lesions of rice blast, caused by *Pyricularia oryzae*. They observed that at constant temperatures of 25 and 32°C lesions enlarged rapidly for the first 8-10 days; however, at the latter temperature lesions ceased to enlarge shortly thereafter. At 25°C, lesion enlargement continued,
taking an additional 12 days before leveling off at a maximum size. They concluded on the bases of the dissimilarity between the effect of high temperature on the growth of the fungus in culture and the expansion of lesions that high temperature restricts lesion size indirectly by affecting the development of the host. Leonard and Thompson (21) reported that the enlargement of lesions of *Colletotrichum graminicola* on corn was linear with time at 20°C, and the optimum temperature for lesion enlargement was 30°C. Lesions were smaller at 32 than at 30°C. Subbarao and Michailides (32) found that the optimum rate of expansion of lesions caused by *Fusarium moniliforme* and *Aspergillus niger* on figs occurred at 30 and 35°C, respectively, 5°C above the optimal temperature for mycelial growth.

At all temperatures, the expansion of gray leaf spot lesions was found to be a linear function of time and the rate of lesion expansion varied between treatments. Significantly higher rates of lesion expansion were observed at temperatures between 25 and 30°C than at 35°C. According to the fitted quadratic model (Figure 4.3), the maximum rate of lesion expansion occurred at approximately 28°C. Even though the effects of block and experiments were not significant, there still was considerable variation among replications and experiments (Figure 4.2 and 4.3). Among the factors that may affect lesion expansion are the position of the leaves and age of the plant (2, 14, 20), lesion age (11, 36), relative humidity (1), host resistance (11, 14, 23, 26, 30), density and proximity of lesions on the leaf (30), isolate/race of the pathogen (23, 32), and age of the culture used for inoculation. Characterizing the lesion response by maize to *C. zeae-maydis*, Freppon and coworkers (15) reported three distinct types of lesions based on the resistance of the
genotype studied: restricted lesions with chlorosis, typical of a resistant reaction; rectangular necrotic lesions, characteristic of a susceptible reaction; and irregular chlorotic flecks. They further reported that lesion size reduction was associated with chlorotic lesions on resistant inbreds. Since the same isolate of C. zeae-maydis was used to inoculate the same susceptible inbred (B73) at the same growth stage (V5-V6) in all replications, several of these confounding effects were eliminated. However, the fact that lesions appeared as late as 21 days after inoculation in some replicates, the effect of plant/leaf age may have played a role. In addition, repeated transfers of the isolate used in this study may have had an effect on the lesion development. Relatively fresh cultures were used in experiment 1 and 3, probably accounting for the generally higher rates of lesion expansion than in experiment 2 (Figure 4.2). The cultures used in experiment 2 were obtained from repeated transfers of the original culture used in the first replicate of experiment 1. The fungus was reisolated from diseased leaves and used to inoculate plants in experiment 3.

The lesion expansion function developed in this study may be used as a component of a simulator or predictor for gray leaf spot. For several pathosystems, temperature-driven lesion expansion functions have been incorporated into simulators for disease epidemics (1, 2, 11, 29, 35, 36), and in some models, lesion expansion was reported as being the most sensitive parameter (9, 17) affecting the final amount of disease. The effects of temperature on the expansion of lesions of gray leaf spot serves to further explain the dynamics of the disease in the field and may be useful for the management of this disease through simulation or prediction modeling. This polycyclic disease which seems to depend heavily on the initial
inoculum for its development and to have relatively few secondary cycles during the growing season may still reach high levels of severity. The expansion of existing lesions due to favorable mid- and late-season temperatures may partially explain the rapid increase in gray leaf spot severity typically observed during the mid and latter part of the growing season. Even with very few infection cycles, with a high level of infection in the primary cycle, final gray leaf spot severity may still be high once temperatures favor the rapid expansion of initial lesions.

Literature Cited


Table 4.1. Estimated difference in the mean rate of gray leaf spot lesion expansion between pairs of temperature treatments following artificial inoculation of leaves of maize inbred B73 with *Cercospora zeae-maydis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate*</td>
<td>P - value</td>
<td>Estimate</td>
<td>P - value</td>
<td>Estimate</td>
</tr>
<tr>
<td>30 - 25°C</td>
<td>0.39</td>
<td>0.131</td>
<td>0.28</td>
<td>0.046</td>
<td>-0.21</td>
</tr>
<tr>
<td>25 - 35°C</td>
<td>0.92</td>
<td>0.012</td>
<td>0.44</td>
<td>0.011</td>
<td>1.00</td>
</tr>
<tr>
<td>30 - 35°C</td>
<td>1.31</td>
<td>0.003</td>
<td>0.73</td>
<td>0.002</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* Difference in rate of lesion expansion in mm²/day between pairs of treatments.
Table 4.2. Summary of quadratic regression analysis of temperature effects on the rate of gray leaf spot lesion expansion on leaves of maize inbred B73 artificially inoculated with *Cercospora zeae-maydis*. After initial lesion formation was observed, plants were incubated for 17 days at 25 to 35°C.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>$R^2$</th>
<th>P-value</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td>6</td>
<td>1.018</td>
<td>0.73</td>
<td>0.020</td>
<td>0.000</td>
<td>-26.28</td>
<td>1.95/0.03</td>
<td>-0.034/0.03</td>
</tr>
<tr>
<td>Exp 2</td>
<td>6</td>
<td>0.059</td>
<td>0.93</td>
<td>0.000</td>
<td>0.000</td>
<td>-15.84</td>
<td>1.18/0.00</td>
<td>-0.020/0.00</td>
</tr>
<tr>
<td>Exp 3</td>
<td>6</td>
<td>0.408</td>
<td>0.80</td>
<td>0.008</td>
<td>0.000</td>
<td>-6.35</td>
<td>0.59/0.23</td>
<td>-0.012/0.17</td>
</tr>
<tr>
<td>Pooled</td>
<td>24</td>
<td>2.764</td>
<td>0.62</td>
<td>0.000</td>
<td>0.000</td>
<td>-16.16</td>
<td>1.24/0.001</td>
<td>-0.021/0.00</td>
</tr>
</tbody>
</table>

*Models fitted to data from each experiment (Exp) and to the pooled data.*
Figure 4.1. Mean lesion area for gray leaf spot on leaves of maize inbred B73 during a 17-days incubation at 25 to 35°C following artificial inoculation with *Cercospora zeae-maydis*. Temperature treatments were imposed after initial lesion formation was observed. Each point represents the mean of 90 lesions (3 experiments x 3 replications x 10 lesions/replication), and the vertical bars indicate the standard error of the mean at each time.
Figure 4.2. Effect of temperature on the rate of lesion expansion for gray leaf spot on maize inbred B73 artificially inoculation with *Cercospora zeae-maydis*. Each bar represents the mean of three replicates of 10 lesions each, and the vertical lines indicate the standard error of the mean for each temperature. For each experiment, bars labeled with the same letter did not differ significantly ($P > 0.05$) based on Duncan's multiple range test.
Figure 4.3. The relationship between rate of gray leaf spot lesion expansion and temperature for pooled data of three growth chamber experiments with maize inbred B73 artificially inoculated with *Cercospora zeae-maydis*. Each point represents the mean of nine observations (3 experiments x 3 replicates/experiment) and the vertical bars indicate the standard error of the mean at each temperature.
CHAPTER 5

INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON SPORULATION OF CERCOSPORA ZEAEMAYDIS ON DISEASED MAIZE LEAVES

A paper to be submitted to Phytopathology

Pierce A. Paul\(^1\) and Gary P. Munkvold\(^2\)

Abstract

The effect of temperature and relative humidity (RH) on the production of Cercospora zeae-maydis conidia on diseased leaves of maize was quantified and a model of the relationship between these variables was developed. Diseased leaf blades were collected from cornfields, surface sterilized, and gray leaf spot lesions were excised, measured, and incubated at 20, 25, or 30°C under 70, 80, 90 or 100% RH for 72h. An additional treatment combination of 35°C and 100% RH was included. Sporulation was estimated as the number of conidia produced per cm\(^2\) of diseased leaf tissue and then log-transformed. A quadratic function was used to model the relationship between the transformed data and temperature at 100% RH, and \textit{loess} nonparametric regression was used to describe sporulation as a function of temperature and RH. The interaction between temperature and RH was

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significant \((P \leq 0.05)\). At 100% RH, the effect of temperature on conidiation was significant \((P \leq 0.05)\), with maximum spore production occurring at 25 and 30°C. The quadratic model explained as much as 80% of the variation in log sporulation at 100% with variation in temperature. The \textit{loess} nonparametric model described 84% of the variation in log spore production/cm\(^2\) of diseased leaf tissue. The back-transformed predictions from this model showed that maximum spore production (approximately 18,200 spores/cm\(^2\)) occurred at 100% RH and 25.9°C.

**Introduction**

Although many aspects of gray leaf spot of maize have been studied over the past 20 years, the relationship between environmental factors and specific components of the disease cycle are still not fully understood. Most of the research work has been conducted in the field, and moderate to high temperatures and prolonged periods of high relative humidity (RH) are generally accepted as being favorable for the development of this disease \((15, 16, 21, 24)\). However, the effects of these conditions on the specific stages of the disease cycle are not clear. Thorson and Martinson \((23)\) reported that germ tube elongation and appressorium formation were favored by extended periods of 95% RH, but that appressorium formation was inhibited by RH > 95% and free water. Beckman and Payne \((2)\) reported similar results, leading to the assumption that free moisture may inhibit penetration and subsequent development of gray leaf spot of maize. However, Rupe at al. \((21)\) suggested that gray leaf spot development was favored by both high RH and
prolonged periods of leaf wetness. Similarly, increased gray leaf spot severity has been associated with seasons of high rainfall (8, 13, 15, 20).

The apparent discordance among reports on the influence of moisture on the development of gray leaf spot may be due in part to differential effects of this factor on specific stages of the disease cycle, on the development of the fungus, and the interaction between the plant and the fungus. Lapaire and Dunkle (14) reported the occurrence of microcycle conidiation in Cercospora zeae-maydis Tehon and Daniels on water droplets and trichomes of several plant species including maize. However, this process did not occur on the surface of maize leaves and was inhibited by leaf washes. This suggests that in the presence of free water on surfaces other than the maize leaf, the inoculum potential of C. zeae-maydis may be increased due to the production of secondary spores from primary spores. Assessing the effects of RH on spore germination and microcycle conidiation, Lapaire and Dunkle (14) reported that germination occurred at RH between 58 and 100%, but below 97% RH germ tube growth was minimal; consequently, secondary conidiation did not occur. Although high RH favors germination and conidiation, dryer conditions seem to favor spore detachment and dispersal. Results of wind simulation studies showed that dehydrated conidia of C. zeae-maydis were detached at wind speeds below average canopy wind speeds, while hydrated conidia were detached by greater wind speeds (14). These results were in support of findings made earlier by Rupe et al. (21) where spore release within the maize canopy was greatest in early afternoon, when there was a rise in temperatures, a drop in relative humidity, and drying of the leaves. These results suggest that fluctuating moisture conditions in the field may
favor different stages of the disease such as production and liberation of secondary spores.

The importance of maize residue on the soil surface as a potential source of primary inoculum for the development of gray leaf spot has been well documented (7, 18, 19), but the role of diseased leaf tissue as a source of secondary inoculum during the growing season is not clear. For most polycyclic diseases, sporulating lesions are the largest source of inoculum for the temporal and spatial spread of disease during the growing season. The specific set of conditions favoring the production of secondary inoculum of C. zeae-maydis is not known. The work of Lapaire and Dunkle (14) provided some information about the effect of varying RH on conidiation, but, the focus of their research was on the production of secondary conidia from primary conidia and the RH effects were assessed on spores mounted on glass slides. The effects of temperature and RH of the sporulation of diseased leaf tissue have not been addressed. Here we report the results of a study aimed at understanding the relationships among temperature, relative humidity, and sporulation, and propose models that may be used to estimate spore production per unit diseased leaf area as a function of these two environmental variables.

**Materials and Methods**

**Treatments and Experimental design**

Diseased leaves of Pioneer Brand hybrid 3394 were collected from naturally infected cornfields in Iowa and stored at 4°C until they were processed within 7 to 10
days after being collected. Leaves were washed in running tap water and blotted dry. Typical, well-developed gray leaf spot lesions were excised, measured using a standard ruler, surface disinfested in a 10% solution of sodium hypochlorite for 2 min, rinsed in sterile distilled water, and blotted and air dried. Approximately 3 cm² of diseased leaf tissue were randomly assigned to each experimental unit.

A split-plot design was used with five replicates of treatments consisting of different combinations of temperature and relative humidity. Temperature was used as the main-plot effect while RH was used as the sub-plot effect. Growth chambers (Intellus Controller, Percival Scientific, Inc., Perry, IA), and distilled water and saturated salts solutions (9) were used to control temperature and RH. The following salt solutions were used: NaCl + KCl (70% RH), (NH₄)₂SO₄ (80% RH), Mg SO₄ (90% RH at 20 and 25°C) and KNO₃ (90% RH at 30°C). The salt solutions were prepared as described by Winston and Bates (25) and Dhingra and Sinclair (9). Each salt was added to boiling distilled water to saturation. After cooling, the solutions were poured into 10 x 10 x 5 cm transparent plastic boxes (Show Box, Althor Products, Bethel, CT) and more salt was added to ensure that some undissolved crystals remained. Prior to the experiment, RH at each salt solution/temperature combination was monitored using HOBO dataloggers (Model RH Stowaway, Spectrum Technologies Inc., Plainfield, IL) sealed inside each box. Once the desired temperature and RH were achieved, each box was then fitted with a shelf (suspended over the salt solution) upon which an open petri dish containing the excised leaf tissues was placed. An RH indicator card (Sud-Chemie Performance Packaging, Colton, CA) was affixed to the inside of the lid of each box to monitor the RH during the
experiment. The boxes were then sealed airtight and randomly assigned (five boxes per RH treatment) to each of three growth chambers set at constant temperatures of 20, 25 and 30°C, under a 12-hr photoperiod supplied by fluorescent and incandescent lights producing an intensity of 316 μmol m⁻² s⁻¹ at approximately 100 cm from the source. Alternating light and dark regimes were reported as being most favorable for sporulation in vitro relative to continuous light and continuous darkness (3, 15). Five additional sets of lesions were placed in separate boxes containing distilled water (100% RH), and incubated at 35°C. The experiment consisted of 13 treatment combinations (three temperatures x four RH + one 35°C x 100% RH treatment combination) and a total of 65 observations. The experiment was conducted three times.

**Assessment of sporulation and data analysis**

After 3 days of incubation, the leaf tissue was removed from the boxes, placed into small vials containing a 5-ml solution of distilled water plus Tween 20 (one drop/500ml), and vortexed for one minute to dislodge the spores. After agitation, a hemacytometer was used to estimate the conidial concentration in each replicate. The mean number of conidia per milliliter was multiplied by five (volume of distilled water/Tween 20 solution used to dislodge spores) and then divided by the corresponding lesion area to determine sporulation per unit leaf area.

The spore production was then analyzed using PROC MIXED and PROC GLM (SAS Institute, Cary, NC) to assess the main and interaction effects of temperature and RH. Since the residual plots from the preliminary analysis of the original sporulation data indicated that the assumption of homogeneous variance
was violated, the date was log-transformed \((\log(\text{actual spore count} + 1))\). ANOVA was performed on the transformed data and contrasts were used to compare specific treatment combinations. A standard \(F\)-test was used to determine whether the results of the three experiments could be pooled.

Quadratic regression analysis was performed using SigmaPlot 2000 (SPSS, Chicago, IL) to model the relationship between temperature and spore production at 100% RH. Log sporulation at 100% RH was regressed against temperature for each experiment and for the pooled data. A total of 20 observations (four temperatures \(\times\) five replicates) were used to fit the models to the data from each experiment, while in fitting the model to the pooled data, 60 observations were used (three experiments \(\times\) four temperatures \(\times\) five replicates). S-Plus 6.1 (Academic Site Edition, Insightful, Corp. Seattle, WA) was used to perform \(loess\) nonparametric regression analyses of the pooled data to model the relationship among temperature, RH, and spore production. The fitted model was then used to estimate spore production and estimated values for each RH were plotted against temperature. Coefficient of determination \((R^2)\) and \(t\)-test of significance of the regression coefficients were used as measures of goodness-of-fit of the quadratic models, while multiple \(R^2\), residual plots, and correlation between predicted and actual sporulation were used as measures of goodness-of-fit of the \(loess\) nonparametric regression model.
Results

*Cercospora zeae-maydis* produced spores under all of the temperature and RH conditions used in this study. However, spore production was strongly influenced by both factors and depended on the interaction between them. The interaction between temperature and RH was highly significant (*P* ≤ 0.05) (Table A7). At RH between 70 and 90%, sporulation was equally low at all temperatures. At 100% RH, however, significantly more spores were produced at 25 and 30°C than at 20°C (Table 5.1). In general, spore production was most abundant at 25°C and least abundant at 20 and 35°C. The only exception was in experiment 3 when sporulation was significantly greater at 30°C than at 25°C. Significantly more spores were produced at 25 than at 30°C in experiment 1; however, in experiment 2 the mean difference between these two treatments was not significant (Table 5.1).

Since the *F*-test of the effects of experiment on log sporulation and the interaction between experiment and temperature were not significant (*F*-value = 2.07; *P* = 0.241 and *F*-value = 2.36; *P* = 0.055, respectively) (Table A7), the data from the three experiments were pooled. Quadratic regression was performed on the pooled data as well as the data from the individual experiments to model the relationship between temperature and log sporulation at 100% RH. In general, the quadratic model fitted well to all four of the datasets. All of the fitted models had relatively high coefficients of determination (*R*²) and highly significant *F*-values (Tables 5.2 and A8). The intercept parameter, *b₀*, for all the models was significantly different from zero (*P* ≤ 0.05) (Table 5.2). The model of the pooled data explained 63% of the variation in sporulation with variation in temperature. Using the equation
from this to predict log sporulation at 100% RH as a function of temperature, sporulation increased with temperature, reaching a maximum at approximately 27°C then decreased, reaching a minimum at 35°C (Figure 5.1). The model slightly underestimated sporulation at 25 and overestimated at 30°C.

The loess nonparametric regression model fitted to the pooled data described 84% of the variation in log sporulation as a function of temperature and RH. The residual standard error was 0.22. Diagnostic plots of the residuals against each predictor showed that the model was appropriate to explain sporulation as a function of temperature and RH. No lack of fit was evident from the plots. The three-dimensional plot of predicted log sporulation clearly indicated a strong interaction between temperature and RH (Figure 5.2). The predicted values from the model were back-transformed and sporulation at each RH was plotted against temperature (Figure 5.3). A maximum sporulation of 18,259 spores per cm² of gray leaf spot tissue occurred at 100% RH and 25.9°C. Optimum temperature for spore production at 95% RH was also between 25 and 26°C. Below 95% RH spore production was low regardless of the temperature. The correlation between back-transformed predictions and actual spore production was high (0.98), however, the model slightly underestimated sporulation at 25 and 30°C.

Discussion

The importance of temperature for the production of Cercospora zeae-maydis conidia on maize leaves was observed to be dependent on relative humidity. Rupe
and coworkers (21) reported that temperatures were similar at sites with and without gray leaf spot; however, there were more days with 12-13 h of RH>90% and 11-13 h of leaf wetness at sites with gray leaf spot than at sites without the disease. Once the moisture requirements are met, gray leaf spot develops well at temperatures between 22 and 30°C (8, 3). Beckman and Payne (3) reported that once periods of sustained high RH were provided, GLS lesions developed readily on plants kept in the greenhouse at 22-28C. Paul and Munkvold (17) studied the effect of temperature on the expansion of lesions of GLS. They concluded that the highest rates of lesion expansion occurred at 25 and 30°C, and lowest rates at 35°C. Similarly, studying the effect of temperature on the germination of spores and the elongation of germ tube of *C. zeae-maydis* exposed to 12h of high RH, Beckman and Payne (3) observed that the optimum temperature was between 22 and 30°C and no germination occurred at 36°C.

From the above-mentioned reports, it is implicit that the importance of temperature for the development for gray leaf spot and its effect on the development of *C. zeae-maydis* depends on prevailing moisture conditions. The results of this study were consistent with those reports. Sporulation peaked at temperatures between 25 and 30°C then decreased to a minimum at 35°C. Similar results were reported for the effects of temperature on sporulation on potato dextrose agar (PDA) (11) and V-8 juice agar (15); spore germination and germ tube growth on glass slides (3); and rate of lesion expansion (17). Garden et al. (11) reported that sporulation of *C. zeae-maydis* on PDA was greatest at 28°C; neither sporulation nor radial growth occurred at 32°C; and minimal sporulation occurred at 16°C. We
observed that although sporulation occurred at temperatures between 20 and 30°C, at 20°C, the RH effect was not significant. Similarly, at RH below 100%, the temperature effect was not significant (P ≤ 0.05). On no occasion did the pair-wise comparisons between RH treatments at 20°C and the pair-wise comparison between RH treatments below 100% (averaged across all temperatures) indicate significant differences (P ≤ 0.05) (data not shown). The effects of temperature were evident only at 100% RH (Table 5.3; Figure 5.2). Sporulation was equally low at 70, 80, and 90% RH, regardless of the temperature (Figure 5.2 and 5.3). Assessing the effects of RH (at 23 to 25°C) on spore germination and the production of secondary spores from primary spores (microcycle conidiation) in C. zeae-maydis, Lapaire and Dunkle (14) reported that germination occurred at a wide range of RH, but below 97% RH germ tube elongation and secondary conidiation ceased and resumed only when RH was raised above 97% again.

Others species of Cercospora were reported to have sporulation patterns similar to those observed for C. zeae-maydis in this study. On peanut leaves incubated at 100% RH, sporulation (conidia per mm² of diseased tissue) of C. arachidicola was greatest at 24 and 28°C, intermediate at 20°C, and least at 16 and 32°C (1). Similar results were reported by Gobina and Melouk (12). Alderman and Beute (1) observed that sporulation of C. arachidicola declined with decreasing water potential of lesions from -0.05 to -6.0 MPa, and increased in cyclic wet (100% RH)/dry (75% RH) regimes as the number of hours of wetness increased. Sporulation of C. carotae on carrot leaves increased with increasing temperature up to the optimum of 28°C then decreased as temperature increased to 32°C; however,
no sporulation was observed at 16 and 32°C when RH was 96% (4). Optimum temperature for sporulation of *C. kikuchi* (5), *C. cruenta* (10) and *C. asparagi* (6) in culture also were between 25 to 28°C. For all three species, these temperatures coincided with optimum temperatures for radial growth.

Although distinct and consistent trends in the relationship among sporulation of *C. zeae-maydis*, temperature, and RH were observed in this study, the variability within treatment and between experiments was still very high. This was probability due to the fact the lesions of different maturities were used. The confounding effect of this factor might have accounted for differences in sporulation under a given set of temperature and RH conditions. Studying the effects of hybrid resistance on the sporulation capacity of *C. zeae-maydis*, Ringer and Grybauskas (20) also attributed the high variability they observed in sporulation between samples to differences in sporulation among lesions of different maturity. Likewise, from this study of the survival of *C. zeae-maydis* in infected debris, de Nazareno et al (7) concluded that the high variability in conidial production was partly due to differences in lesion age. Beckman and Payne (2) reported that necrosis of gray leaf spot lesions progressed gradually from the center towards the borders, taking about three days for the entire lesion to become tan to brown in color. They observed that conidiophores bearing conidia of *C. zeae-maydis* were limited to the necrotic area of the lesion and arose 1-3 days after lesions became necrotic. The age of the leaves from which the lesions were excised also might have affected spore production. Older leaves generally have more stomata per cm² than younger leaves (22) and since conidiophores emerge through stomatal openings (15), more spores are like to be produced per
unit area of lesion originating from older than younger leaves. Differences in stomatal density were reportedly responsible for significant differences in spore production between lesions from leaf blades and sheaths (7).

In this study we characterized and described specific sets of temperature and RH conditions affecting the sporulation of C. zeae-maydis on diseased leaves of maize. Regression models that may be used to estimate sporulation, and thus, inoculum potential of developing lesions were also developed. This information complements previous reports and serves to enhance our understanding of the effects of the environment on the development of C. zeae-maydis and gray leaf spot of maize.

**Literature Cited**


Table 5.1. Estimated difference in mean sporulation of *Cercospora zeae-maydis* per cm² of gray leaf spot lesion excised from naturally infected leaves of Pioneer Brand hybrid 3394; lesions were submitted to temperatures between 20 and 30°C at 100% RH for 72h

<table>
<thead>
<tr>
<th>Mean Comparison</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate^a</td>
<td>P - value</td>
<td>Estimate</td>
</tr>
<tr>
<td>25 – 20°C</td>
<td>0.77</td>
<td>&lt;0.0001</td>
<td>1.04</td>
</tr>
<tr>
<td>30 – 20°C</td>
<td>0.28</td>
<td>0.0805</td>
<td>0.79</td>
</tr>
<tr>
<td>25 – 30°C</td>
<td>0.49</td>
<td>0.0034</td>
<td>0.25</td>
</tr>
</tbody>
</table>

^a Difference in log sporulation (log(spore + 1)) per cm² of diseased leaf area between temperature treatments.
Table 5.2. Summary of quadratic regression analysis of the effect of temperature on sporulation (log spores per cm²) by *Cercospora zeae-maydis* on naturally occurring maize leaf lesions (Pioneer Brand hybrid 3394). Lesions were incubated at 100% relative humidity for 72h at 20 to 35°C.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>SS</th>
<th>$F$</th>
<th>$R^2$</th>
<th>$P$-value</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td>17</td>
<td>1.803</td>
<td>0.49</td>
<td>0.003</td>
<td>0.63/0.002</td>
<td>-7.26/0.004</td>
<td>0.61/0.002</td>
<td>-0.01/0.001</td>
</tr>
<tr>
<td>Exp 2</td>
<td>17</td>
<td>1.394</td>
<td>0.80</td>
<td>&lt;0.001</td>
<td>1.11/0.000</td>
<td>-13.53/0.000</td>
<td>1.11/0.000</td>
<td>-0.02/0.000</td>
</tr>
<tr>
<td>Exp 3</td>
<td>17</td>
<td>1.938</td>
<td>0.76</td>
<td>&lt;0.0001</td>
<td>1.24/0.000</td>
<td>-15.68/0.000</td>
<td>1.24/0.000</td>
<td>-0.02/0.000</td>
</tr>
<tr>
<td>Pooled</td>
<td>57</td>
<td>7.25</td>
<td>0.63</td>
<td>&lt;0.0001</td>
<td>0.98/0.000</td>
<td>-12.16/0.000</td>
<td>0.98/0.000</td>
<td>-0.02/0.000</td>
</tr>
</tbody>
</table>

* Models were fitted to the log-transformed (log(spores + 1)) sporulation data for each experiment (Exp) and the pooled data.
Figure 5.1. Quadratic relationship between log-transformed spore production (log(spores + 1)) per cm² of gray leaf spot lesion and temperature. Spores were counted on lesions excised from leaves of Pioneer Brand hybrid 3394 naturally infected with *Cercospora zeae-maydis* and incubated for 72 h at 100% RH and 20 to 35°C. The numbers 1, 2, and 3 represent treatment means from three experiments, while the solid line represents the pooled data of the three experiments. Each numbered point represents the mean of five replicates, while each dot represents the mean of 15 observations (three experiments x five replicates). The vertical bars represent the standard error of the mean at each temperature for the pooled data.
Figure 5.2. Response surface of log sporulation (log(spores/1000 + 1)) per cm² of gray leaf spot lesion as a function of temperature and relative humidity. Spores were counted on lesions excised from leaves of Pioneer Brand hybrid 3394 naturally infected with *Cercospora zeae-maydis* and incubated for 72 h at 70 to 100% RH and 20 to 30°C. The plot was generated using values predicted from the fitted *loess* nonparametric regression model. The model was fitted using 36 observations (three experiments x 12 treatment combinations).
Figure 5.3. Prediction of sporulation of *Cercospora zeae-mayis* per cm² of gray leaf spot lesion as a function of temperature and relative humidity. Each line represents predictions at a different relative humidity obtained through back-transformation of values predicted using the fitted *loess* nonparametric regression model.
GENERAL SUMMARY AND CONCLUSION

In the first part of this dissertation (chapters 2 and 3) the findings of research aimed at developing risk assessment and disease prediction models for gray leaf spot of maize were presented. Site, genotype, and disease severity data were collected from several locations across the state of Iowa between 1998 and 2002. A total of 50 locations from 17 counties were used and 332 observations were collected. Classification and regression tree (CART), and ordinal logistic regression models were used to predict disease severity classes at the R4/R5 plant growth stage using data collected prior to planting as predictors. The most important predictors were longitude, surface residue, planting date, and gray leaf spot resistance ratings. Both CART and logistic regression models performed creditably correctly predicting more than 65% of the disease severity classes.

A more comprehensive approach was used to develop models to predict late season gray leaf spot severity based on early- and mid-season information. All-subsets regression and artificial neural networks (ANN) approaches were combined and several prediction models were developed. In addition to the pre-planting data used in the development of the risk assessment models, several weather-related variables were generated and used as predictors. All-subsets regression was used to select the most important predictors and feed-forward, back-propagation ANN was used to model the relationship between these predictors and gray leaf spot severity at the R4/R5 growth stage. We learned that cumulative daily temperature between 22 and 30°C and cumulative nightly RH ≥ 90 for the period between V4 and
V12, and average nightly temperature for the period between V12 and R2 were the most important weather-related predictors. These were used along planting date, surface residue, gray leaf spot resistance ratings, and longitude to develop ANN models. A total of 329 cases were used of which 20% were extracted for model validation. Ten networks were built using different subsets of predictors. Seven- and eight-input networks generally outperformed nine-input networks in terms of predictive accuracy on the validation cases. The top four networks had predictive accuracy equal to or greater than 70%. The tenth network was built without longitude as a predictor. This network had a predictive accuracy of 68%.

The risk assessment and prediction models developed in this study may be used to guide management decisions for gray leaf spot of maize. However, the application of these models should be limited to the region in which they were developed. Prior to being used in other maize-growing regions, the models should be refitted and revalidated in order to capture characteristics that may be inherent to each new region. One suggested application of these models would be to use them jointly in a management program in conjunction yield loss models. Firstly, the risk assessment models could be used for hybrid selection, then, the prediction models could be used to reassess the risk of the disease prior to recommending the application of fungicide. Ultimately, management decisions should be made based on the projected yield impact of the disease.

In the second part of this dissertation (chapter 4 and 5), the effects of the environment on the expansion of lesions of gray leaf spot and the sporulation of *Cercospora zeae-maydis* on diseased leaf tissues were studied and models were
developed to describe the relationships between the environment and these epidemic components. Maize seedlings (V5-V6) of inbred B73 were inoculated and incubated under continuous 100% RH for 36 to 40 h at 25 to 28°C. Temperature treatments were imposed after initial lesion formation was observed. Lesion area (mm²) was regressed against time and the rate of lesion expansion was estimated (slope of the regression line). The optimum rate of lesion expansion was between 25 and 30°C. The quadratic model best described the relationship between temperature and the rate of lesion expansion, explaining as much as 93% of the variation in the rate of lesion expansion with variation in temperature.

For the sporulation study, diseased leaves of Pioneer Brand hybrid 3394 were brought in from the field and gray leaf spot lesions were excised, surface disinfested, and incubated under different combinations of temperature and relative humidity (RH) for 72 h. Spores were counted using a hemacytometer, and the relationship between spore production per cm² of lesion and the environment was modeled using quadratic and loess nonparametric regression. The quadratic model explained as much as 80% of the variation in log sporulation at 100% RH with variation in temperature, while the loess nonparametric model described 84% of the variation in log sporulation/cm² of diseased leaf tissue with variation in temperature and relative humidity. At RH below 95%, sporulation was equally low at all temperatures; however, at 100% RH maximum sporulation occurred at temperatures between 25 and 30°C.

The results of this study serve to better our understanding of the effect of the environment on the development on C. zeae-maydis and gray leaf spot of maize.
Based on these results, we conclude that the rate of disease development in terms of lesion expansion is greatest at temperatures between 25 and 30°C and, once RH is above 95%, abundant spores may be produced on these lesions potentially contributing to secondary infections. The models presented herein may be used as components of simulators developed to understand the dynamics of gray leaf spot and to predict the severity of this disease.
APPENDIX
Table A1. Comparison between a full 23-node tree and trees pruned to 20, 14 and 10 terminal nodes used to estimate gray leaf spot severity classes as a function of pre-planting site and genotype information collected in Iowa between 1998 and 2001

<table>
<thead>
<tr>
<th>Index</th>
<th>Number of terminal nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RMD</td>
<td>0.60</td>
</tr>
<tr>
<td>MER</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<sup>a</sup>Indices used to assess the performance of the pruned version of classification trees relative to the original tree; RMD = residual mean deviance and MER = misclassification error rate.

<sup>b</sup>Original 23-node tree fitted to the original 332 cases.

<sup>c</sup>Pruned 14-node tree used to validate the model on 30 independent cases collected in 2002.
Table A2. Coefficient of multiple determination, mean squared error, and correlation coefficient for the training and testing scenarios for back-propagation artificial neural network models developed for gray leaf spot of maize using different combinations of input variables selected based on all-subsets regressions

| ANN Model<sup>a</sup> | No. of input nodes | Training |  | Testing |  |
|---------------------|--------------------|----------|-------------------|-------------------|
|                     |                    | $R^2$ | MSE<sup>c</sup> | $r$ | $R^2$ | MSE | $r$ |
| A1                  | 8                  | 73    | 131               | 0.86             | 65   | 161 | 0.81 |
| A2                  | 7                  | 80    | 100               | 0.90             | 71   | 128 | 0.85 |
| A3                  | 8                  | 84    | 79                | 0.92             | 75   | 119 | 0.87 |
| A4                  | 9                  | 76    | 114               | 0.88             | 65   | 173 | 0.81 |
| A5                  | 7                  | 81    | 92                | 0.90             | 69   | 148 | 0.84 |
| A6                  | 9                  | 80    | 94                | 0.90             | 70   | 147 | 0.84 |
| A7                  | 9                  | 76    | 115               | 0.87             | 69   | 153 | 0.84 |
| A8                  | 8                  | 74    | 123               | 0.86             | 59   | 198 | 0.77 |
| A9                  | 8                  | 85    | 71                | 0.92             | 63   | 180 | 0.80 |
| A10                 | 6                  | 85    | 69                | 0.93             | 61   | 186 | 0.79 |

<sup>a</sup> Back-propagation artificial neural network models developed using data collected in Iowa between 1998 and 2002. The models A1 to A9 were developed using the nine subsets of variables described in Table 3.1, while model A10 was developed following the omission of longitude from the subset of variables used in model A2.

<sup>b</sup> Coefficient of multiple determination

<sup>c</sup> Mean squared error

<sup>d</sup> Correlation coefficient
Table A3. Analysis of variance for the effects of temperature on the rate of gray leaf spot lesion expansion on leaves of maize inbred B73 artificially inoculated with *Cercospora zeae-maydис* in experiment 1. After initial lesion formation was observed, plants were incubated for 17 days at 25 to 35°C

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
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<td>0.759</td>
<td>0.379</td>
<td>5.860</td>
<td>0.065</td>
</tr>
<tr>
<td>Temperature</td>
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<td>2.706</td>
<td>1.353</td>
<td>20.910</td>
<td>0.008</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.259</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>3.724</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A4. Analysis of variance for the effects of temperature on the rate of gray leaf spot lesion expansion on leaves of maize inbred B73 artificially inoculated with *Cercospora zeae-maydис* in experiment 2. After initial lesion formation was observed, plants were incubated for 17 days at 25 to 35°C

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
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<td>0.984</td>
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<td>0.402</td>
<td>27.35</td>
<td>0.005</td>
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<tr>
<td>Error</td>
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<td>0.058</td>
<td>0.015</td>
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<tr>
<td>Total</td>
<td>8</td>
<td>0.864</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A5. Analysis of variance for the effects of temperature on the rate of gray leaf spot lesion expansion on leaves of maize inbred B73 artificially inoculated with *Cercospora zeae-maydis* in experiment 3. After initial lesion formation was observed, plants were incubated for 17 days at 25 to 35°C.

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>Mean square</th>
<th>$F$ value</th>
<th>$Pr &gt; F$</th>
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<tbody>
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<td>0.006</td>
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<td>Error</td>
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<td>Total</td>
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<td>2.084</td>
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</table>
Table A6. Quadratic regression analysis of variance for the effects of temperature on the rate of gray leaf spot lesion expansion on leaves of maize inbred B73 artificially inoculated with *Cercospora zeae-maydis* for experiments 1, 2, and 3, and the pooled data. After initial lesion formation was observed, plants were incubated for 17 days at 25 to 35°C

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Regression</td>
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<td>2.71</td>
<td>1.35</td>
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<tr>
<td>Total</td>
<td>8</td>
<td>3.72</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
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<tr>
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<td>0.40</td>
<td>40.70</td>
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<td>Residual</td>
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<tr>
<td>Total</td>
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<td>0.86</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
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</tr>
<tr>
<td>Regression</td>
<td>2</td>
<td>1.68</td>
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<td>12.33</td>
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<td>0.07</td>
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<td>0.26</td>
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<tr>
<td>Pooled</td>
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<tr>
<td>Regression</td>
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<td>2.30</td>
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<td>24</td>
<td>2.76</td>
<td>0.12</td>
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<tr>
<td>Total</td>
<td>26</td>
<td>7.36</td>
<td>0.28</td>
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</table>
Table A7. Split-plot analysis of variance for the effects of temperature and relative humidity on log sporulation (log(spores + 1)/cm²) by *Cercospora zeae-maydis* on naturally occurring maize leaf lesions (Pioneer Brand hybrid 3394) following incubation at 70 to 100% RH and 20 to 30°C for 72 h

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>Mean square</th>
<th>F value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block/Experiment</td>
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<td>1.86</td>
<td>2.07</td>
<td>0.241</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>2</td>
<td>4.91</td>
<td>2.45</td>
<td>2.74</td>
<td>0.178</td>
</tr>
<tr>
<td>Error A*</td>
<td>4</td>
<td>3.59</td>
<td>0.90</td>
<td>2.36</td>
<td>0.055</td>
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<tr>
<td>Relative humidity (RH)</td>
<td>3</td>
<td>90.43</td>
<td>30.14</td>
<td>79.44</td>
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</tr>
<tr>
<td>T x RH</td>
<td>6</td>
<td>37.50</td>
<td>6.24</td>
<td>16.47</td>
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<tr>
<td>Error B</td>
<td>162</td>
<td>61.47</td>
<td>0.38</td>
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<tr>
<td>Total</td>
<td>179</td>
<td>201.62</td>
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<td></td>
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</table>

* Error A = Interaction between temperature and block/experiment used to test the main effect of temperature.
Table A8. Quadratic regression analysis of variance for the effects of temperature on log sporulation (log(spores + 1)/cm$^2$) by *Cercospora zeae-maydis* on naturally occurring maize leaf lesions (Pioneer Brand hybrid 3394) following incubation at 100% RH and 20 to 35°C for 72 h for experiments 1, 2, and 3, and the pooled data.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Regression</td>
<td>2</td>
<td>1.73</td>
<td>0.86</td>
<td>8.14</td>
<td>0.003</td>
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<td>Residual</td>
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<td>1.80</td>
<td>0.11</td>
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<tr>
<td>Total</td>
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<td>3.53</td>
<td>0.19</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>2</td>
<td>5.47</td>
<td>2.74</td>
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<td>&lt;0.0001</td>
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<tr>
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<td>1.39</td>
<td>0.08</td>
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<tr>
<td>Total</td>
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<td>6.87</td>
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<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Regression</td>
<td>2</td>
<td>6.27</td>
<td>3.14</td>
<td>27.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
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<td>1.94</td>
<td>0.11</td>
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<tr>
<td>Total</td>
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<td>8.21</td>
<td>0.43</td>
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<tr>
<td><strong>Pooled</strong></td>
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<tr>
<td>Regression</td>
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<td>Total</td>
<td>59</td>
<td>19.59</td>
<td>0.33</td>
<td></td>
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</tr>
</tbody>
</table>
Figure A1. Maps of Iowa showing locations from which data were collected in 1998 (A) and 1999 (B).
Figure A2. Maps of Iowa showing locations from which data were collected in 2000 (A) and 2001 (B).
Figure A3. Map of Iowa showing locations from which data were collected in 2002
Figure A4. Plots used to check the ordinality of the response variable (D) for each predictor (X) by assessing if different D distinguishes the mean X and if the trend is monotonic. Solid lines = simple stratified means, dashed lines = expected value of $X|D = j$ (level of D) given that proportional odds (PO) holds. Expected values from the continuation ratio (CR) model are marked with c's.
Figure A5. The full 23-node classification tree used to estimate gray leaf spot severity classes as a function of pre-planting site and genotype information collected in Iowa between 1998 and 2001. LON = longitude, GLSR = gray leaf spot resistance ratings (1 = most susceptible to 9 = most resistant), SR = percentage surface residue, PD = planting date in day of year, and MAT = genotype maturity rating in comparative relative maturity (CRM).
Figure A6. Relationship between residual deviance and size (number of terminal nodes) of classification tree used to estimate gray leaf spot severity classes as a function of pre-planting site and genotype information collected in Iowa between 1998 and 2001. Since the first 14 nodes explained a significant part of the reduction in deviance, the original 23-node tree was pruned to 14 nodes.
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My colleagues John, Miralba, and Paige for their help in the laboratory.

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