Inheritance of Reactions to Gray Leaf Spot and Maize Dwarf Mosaic Virus in Maize and Their Associations With Physiological Traits

by

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Dissertation submitted to the Faculty of

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agronomy

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April, 1989

Blacksburg, Virginia

INHERITANCE OF REACTIONS TO GRAY LEAF SPOT AND MAIZE DWARF MOSAIC VIRUS IN MAIZE AND THEIR ASSOCIATIONS WITH PHYSIOLOGICAL TRAITS

by

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(ABSTRACT)

Gray leaf spot, caused by *Cercospora zeae-maydis*, can be a yield limiting factor in maize where continuous minimum tillage practices are followed. Commercial corn hybrids were evaluated for response to gray leaf spot for seven years at two Virginia locations (Shenandoah and Wythe Counties) and one year at a third location in Virginia (Montgomery County). Yield losses, when comparing resistant to susceptible classes, were approximately 2,000 kg ha^{-1} at Wythe County in 1982, 750 kg ha^{-1} at Shenandoah County in 1984, and 2,150 kg ha^{-1} at Montgomery County in 1988. The inheritance of reaction to gray leaf spot was studied using a 14 inbred diallel in Montgomery and Wythe Counties, Virginia in 1987 and 1988 planted in randomized complete block designs. Resistance was found to be highly heritable and controlled by additive gene action. Inbreds producing high yielding, resistant, and agronomically superior hybrids were identified (B68, NC250, Pa875, Va14, Va17 and Va85); and several hybrids between these lines had high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875). Currently available inbreds could be used to produce hybrids with higher levels of resistance than hybrids currently available to growers, and these could serve as a basis for gray leaf spot breeding programs. Lesion size measurements were not correlated with disease scores. Late-season photosynthesis rates were associated positively with resistance. The hybrids of

some inbreds were found to produce high levels of pigment (believed to be anthocyanins) around the gray leaf spot lesions. These did not limit the size of the individual lesion later in the season. Some pigment(s)-producing genotypes were found to be resistant when the pigment character was expressed. This type of resistance must prevent or inhibit infection of the leaf but not later colonization, once established. Maize dwarf mosaic virus (MDMV) also limits maize production in some areas where johnsongrass (*Sorghum halepense* L.) is a problem. Resistance to MDMV was found to be mainly additive and highly heritable. However, a strong specific combining ability component was found, indicating that the background of the material receiving resistance genes may have a strong effect on the expression of resistance. Inbreds capable of producig high-yielding, resistant, and agronomically acceptable hybrids are available (B68, NC250, A632, Pa875, Va17, and Va85); and several hybrids between these lines have high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875).

ACKNOWLEDGEMENTS

I express appreciation to all who assisted me in conducting the experiment and preparing this dissertation. Thanks to Dr. Erik Stromberg who served as my friend and advisor and who laid the groundwork for this dissertation. Thanks also to and who assisted me in several areas of my research, including harvesting of the plots. I would also like to thank who was a supportive friend and understanding supervisor. I would like to thank the other members of my committee; Dr. Thomas Starling, Dr. George Lacy, and Dr. David Parrish for assistance in editing the manuscript and for the many helpful suggestions they made. Special thanks go to Dr. Curtis Roane for his help on the manuscript. Without the above help the experiment could not have been conducted. I would like to also thank for running the statistical analysis program.

Finally I would like to thank my wife, and my sons, and who put up with me while I was working on my dissertation and course work. My ability to complete this degree was only possible because of their support. Two degrees should be given; one for the student and one for his family.

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INTRODUCTION AND OBJECTIVES

Gray leaf spot of maize (*Zea mays* L.), a disease caused by the fungus *Cercospora zeae-maydis* (Tehon and Daniels, 1925), has become increasingly important in Virginia in the last decade (Donahue *et al.*, 1985, 1986, 1987; Stromberg, 1984, 1985a, 1985b; Stromberg and Donahue, 1986). Its increasing frequency and severity have been associated with non-tillage methods of maize production (Beckman *et al.*, 1981; Beckman and Payne, 1982; Hilty *et al.*, 1979; Kingsland, 1963; Roane *et al.*, 1974; Roane, 1950; Rupe *et al.*, 1982; Stromberg, 1984, 1985a). It is estimated that between 10 and 25% of the yield potential can be lost annually in areas where gray leaf spot is a problem, and in certain years where disease conditions are optimum, losses can be much higher (Stromberg and Donahue,1986). Even in years where yield loss is not apparent, susceptible hybrids show a 10 to 20 fold increase in lodging, which can also be a cause of yield loss (Stromberg and Donahue, 1986; Ayers *et al.*, 1984).

Losses caused by gray leaf spot, but not associated with lodging, are related to the premature loss of photosynthetic area. In years of severe blighting, susceptible hybrids develop symptoms that mimic frost damage due to the necrosis of leaf area caused by the disease. The dominant sink of the post-anthesis maize plant is the ear. Because of loss of photosynthetic area by blighting, photosynthate is diverted from the stalk and roots at greater than normal levels, causing them to senesce prematurely. The result is a loss of yield, an increase in stalk lodging, and premature plant senescence (Dodd, 1980, 1983; Koehler *et al.*, 1925; Morris, 1931).

Ayers (1984) at Pennsylvania and Manh (1977) at Virginia Polytechnic Institute and State University (VPI&SU) made preliminary studies of gray leaf spot and concluded that gray leaf spot resistance was dominant, quantitatively inherited, and easily selected for in a nursery. Thompson *et al.* (1987) explored the inheritance of reaction to gray leaf spot in maize using a diallel design and found that gray leaf spot resistance was dominant and highly heritable. Huff *et al.* (1988) also investigated gray leaf spot resistance and also concluded that gray leaf spot resistance was quantitatively inherited, highly heritable, and dominant.

In this investigation, 14 lines were crossed in diallel (A632, B68, B73, H93, Mo17, NC250, KB1250, Pa91, Pa875, Va14, Va17, Va22, Va35, and Va85) to determine the mode of inheritance of resistance to gray leaf spot and maize dwarf mosaic virus (MDMV) and to study some physiological aspects of reaction to *C. zeae-maydis*. This group of maize inbreds includes some of the lines used in the production of the best commercially available, high-yielding hybrids and represents some of the best germplasm currently available to maize growers and breeders. In addition to studying the inheritance of reaction to these pathogens in the 14 lines, the association of reaction to *C. zeae-maydis* with the following traits was investigated:

1) Photosynthesis (O_2 evolution) of the leaves at anthesis and black-layer.

2) Lesion size (width and length).

3) Yield losses in commercial hybrids.

This dissertation will consist of a review of literature on gray leaf spot, followed by four separate chapters covering various aspects of the research. Chapter one will reports losses in commercial hybrids to gray leaf spot. Chapter two presents data from the diallel study regarding field reaction to gray leaf spot and its inheritance. Chapter three discusses the association of physiological traits with reaction to gray leaf spot. The inheritance of reaction to MDMV in this diallel will be reported in chapter four. The literature review for virus reaction will be included as part of the virus chapter.

GRAY LEAF SPOT LITERATURE REVIEW

Occurrence And Relation With Reduced-Tillage Methods of Production

Gray leaf spot was first described by Tehon and Daniels (1925) in Illinois. Hyre (1943) recorded several incidences of maize disease in eastern Tennessee and Kentucky with "an elongated narrow spot" on the leaves. *Cercospora zeae-maydis* was the most common pathogen isolated from these spots. During this time, gray leaf spot was more of a curiosity than a large threat to maize production. Roane (1950) saw gray leaf spot in Virginia, near Blacksburg, in 1949 and 1950. Some inbreds showed resistance, but none of the experimental four-way hybrids being evaluated expressed resistance. Roane concluded that gray leaf spot was a minor disease but severe damage could occur if the disease developed early in the season.

Gray leaf spot remained a minor disease until Kingsland (1963) observed an isolated severe occurrence in a non-tillage maize field in South Carolina in 1962. Since that time, gray leaf spot has been recognized as a serious problem on non-tillage areas in the eastern United States.

Leonard (1974) considered gray leaf spot to be the most destructive disease of maize in the mountainous regions of North Carolina, an area where no-till production is common. Concurrently, Roane *et al.* (1974) reported that gray leaf

spot had occurred annually in the mountain valleys of Virginia since it was first reported in 1949. From 1971 to 1973, however, they observed that gray leaf spot occurred earlier in the growing season and was more severe than before, presumably linked to non-tillage practices. All the commercial hybrids they tested appeared susceptible. Roane and Genter (1976) later found some tolerant genotypes but none that were highly resistant. Hilty *et al.* (1979) reported that gray leaf spot increased in occurrence and severity in Tennessee during the mid- to late 1970's. In all cases this was limited to minimum-tillage fields.

Turner (1982), addressing the American Seed Trade Association, noted that gray leaf spot also occurred in the Midwest, citing gray leaf spot outbreaks in Illinois, Missouri, and Iowa. Latterell and Rossi (1983) documented the movement of gray leaf spot progressively north from Virginia into Maryland and West Virginia.

With increasing use of non-tillage methods in the eastern United States, gray leaf spot has increased in severity, until in some areas it is a limiting factor in maize production (Ayers, 1984; Stromberg, 1984, 1985a, 1985b). With predictions that non-tillage and reduced-tillage acreages combined will reach as much as one-half of maize-production acreages (Worsham, 1980), gray leaf spot could become an important problem in other maize producing areas of the country.

Microclimate And Disease Development

Beckman *et al.* (1981) noted that microclimatic factors, such as high relative humidity and long periods of leaf wetness, contribute to infection. This may partially explain the increased occurrence of gray leaf spot after the plant canopy

closes. Beckman and Payne (1982, 1983) reported that *C. zeae-maydis* requires high moisture to penetrate and colonize maize leaves. There was no effect of plant age on efficiency of stomatal penetration. They also noted that lesion development occurred when inoculated plants were kept under high relative humidity conditions for 2 weeks or longer. This supports field observations that relative humidity and leaf wetness are important for disease development.

Rupe (1982) determined the influence of environment and maturity on disease development in Kentucky. Locations where gray leaf spot occurred had many days with 12 to 13 hours of high (>90%) relative humidity and 11 to 13 hours of leaf wetness. Payne and Waldron (1983) noted that microclimate is important to spore release. High humidity and long periods of leaf wetness were required for high numbers of spores to be released.

Disease Cycle / Life cycle of Pathogen

Leaf lesions of gray leaf spot are gray to brown, long (0.5 to 5 cm), narrow, and rectangular (Shurtleff, 1980). The lesions are restricted at first by the larger veins but will later coalesce under optimum disease conditions and the entire leaf can be killed. Total loss of the crop can occur if an early infection is followed by favorable disease conditions; i.e., prolonged levels of high temperature and humidity with long periods of leaf wetness.

Conidia of *C. zeae-maydis* are straight to slightly curved, hyaline, slender, and multiseptate, measuring 40 to 165 X 4 to 9 um with 6 to 12 septa. A *Mycosphaerella*

sp. is thought to be the perfect stage of *C. zeae-maydis* (Shurtleff, 1980; Latterell and Rossi, 1977, 1983).

Cercospora zeae-maydis, like many other foliar fungal pathogens, is a poor soil competitor and will survive only on infested maize debris (Stromberg and Donahue, 1986), which is the source of inoculum for the next year. Since the perfect stage of the fungus is not important to the disease cycle, air-borne condia are the only means by which the fungus can infect the new crop (Latterell and Rossi, 1977). Fungi within infested debris can release conidia as early as June in southwestern Virginia (Stromberg, unpublished).

Lower leaves are the most common site where lesions are first observed under field conditions. When conditions become favorable, these lesions produce conidia that can serve as a source of inoculum for the upper leaves. Under unfavorable conditions the fungus remains dormant but will become active when favorable conditions return (Latterell and Rossi, 1983).

Physiology of Disease: Role of Photosynthates

Dodd (1980, 1983) noted that photosynthetic leaf area loss is one of the major contributors to stalk rot. Morris (1931) also determined that leaf area loss and sugar levels in the stalk are correlated with stalk rot. Sugar levels are positively correlated with stalk rot resistance in maize; the higher the level of sugar in stalks, the less susceptible they are to stalk rot (Donahue, 1986). Since loss of photosynthetic area by blighting will result in lower amounts of sugar available to the various sinks in the maturing plant, it is not surprising that yield, lodging

resistance, and grain moisture are correlated with gray leaf spot reaction (Stromberg and Donahue, 1986).

Cercosporin

Cercosporin is a non-specific toxin produced by members of the genus *Cercospora*. It was first isolated in 1957 (Kuyama) from *Cercospora kikuchii* T. Matsu and Tomoyasu, a soybean pathogen, and has been isolated from other *Cercospora* species (Assante *et al.* ,1977; Balis and Payne, 1971; Fajola, 1978; Lu, 1983; Lynch and Geoghegan, 1977; Mumma *et al.*, 1973). Cercosporin has also been isolated from plants infected with other *Cercospora* spp. (Fajola, 1978; Kuyama, 1957).

When pure cercosporin is placed on a wide range of host species, it causes necrotic lesions. Cercosporin sensitizes plant cells to light (Daub, 1982). Cell photosensitizers in their native state are not toxic to cells, but they absorb light and are then converted to an excited triplet state (Foote, 1976; Spikes, 1977). This highly reactive intermediate produces superoxide ions or a singlet oxygen (Daub, 1987). Cercosporin produces both singlet oxygen and superoxide when irradiated with light (Daub and Hangarter, 1983; Dobrowlski and Foote, 1983). These compounds are toxic to living cells, oxidizing lipids, proteins, carbohydrates, and nucleic acids (Daub, 1987; Spikes, 1977).

Fajola (1978) noted that species of *Cercospora* characteristically produce a red, photodynamic toxin, cercosporin. Working with beets, Steinkamp *et al.* (1981) concluded that cercosporin seemed to play a role in spreading the fungus through

plant tissue. The fungus is not efficient in colonizing live tissue and requires the toxin to kill tissue in order to efficiently colonize it.

Cercosporin exhibits a general toxicity to many organisms. Most plants express a sensitivity to cercosporin, including those resistant to the various diseases caused by *Cercospora* spp. (Balis and Payne, 1971; Fajola, 1978). Daub noted that *Cercospora* spp. produce high concentrations of cercosporin in the light but are unaffected by it since they are tolerant to the generalized toxicity of the singlet oxygen and superoxide.

Two facts link cercosporin to the diseases caused by *Cercospora* spp. One is that light is required for the development of symptoms in *Cercospora* caused diseases (Calpouzos, 1966; Calpouzos and Corke, 1963; Calpouzos and Stalknecht, 1967). The other is that the treatment of sugarbeet leaves with cercosporin produces changes similar to those caused by infection with *C. beticola* Sacc. The changes are consistent with cercosporin's known mode of action (Steinkamp *et al.*, 1979, 1981).

In tobacco, Daub (1982a, 1982b) noted that cercosporin has a wide host range and is nonspecific. Daub (1984) studied the possibility of using cercosporin to select resistant genotypes in cell cultures but was unsuccessful in selecting cercosporin-tolerant tobacco and sugarbeet protoplasts in cell cultures following mutagenesis (Daub 1984, 1987).

Daub (1987) also studied the resistance mechanisms of *Cercospora* spp. to cercosporin. Since cercosporin resistance has not been found in plants, she sought to understand the mechanisms of resistance in order to develop new control

methods for *Cercospora* caused diseases. She tried to determine whether the resistance to cercosporin was specific to *Cercospora* spp. or if it could be found in other fungi. Oomycetes were very sensitive to cercosporin, but yeasts and some of the other mycelial-fungi were resistant. Despite these differences in resistance, none of the traits studied was found linked to resistance, leaving the mode of action of resistance unexplained.

Gwinn *et al.* (1987) investigated the relationship of cercosporin to gray leaf spot development. Cultivars of maize with different levels of resistance to *C. zeae-maydis* were studied in the greenhouse. Leaf disks from 1-, 2-, and 3-month-old plants were either soaked with 1.2 uM cercosporin or inoculated with a mycelial suspension of *C. zeae-maydis*. No varietal differences were detected for either sensitivity to cercosporin or susceptibility to *C. zeae-maydis*. Cercosporin treatment caused significantly less ion leakage from disks of older plants. Significantly more stomates of older plant tissue were penetrated by the fungus (Gwinn *et al.*, 1987). This indicates that a real age-dependent relationship to *C. zeae-maydis* establishment exists in maize and suggests that the mechanism of this resistance to disease development may be different from the varietal resistance seen in the field. This contrasts with the results of Beckman and Payne (1982) who found no relationship between leaf age and resistance to the pathogen. Beckman and Payne counted appressoria, while Gwinn *et al.* counted mycelia with or without appressoria present.

Gwinn *et al.* (1987) used three genotypes in their study, and one of these was an inbred line. While it is inconvenient to work with many genotypes, it is important to look at a range of genotypes that represent those available to the breeder or

maize grower. A conclusion that varietal differences did not exist for the traits studied may not have been warranted when only three genotypes were considered. An aspect of this study that may limit the relevance of results to field conditions was that the maize plants were grown in 15-cm pots in the glasshouse in the winter without supplemental light. Plants grown under such conditions are not entirely typical of those grown in the field during the normal growing season and may not react similarly to field grown plants.

Yield Losses

Ayers *et al.* (1984) presented a paper documenting yield losses from gray leaf spot and the inheritance of resistance to this disease. In the yield-loss experiment, three fungicides or fungicide combinations to control gray leaf spot were used on a 7-day or 14-day schedule with a non-sprayed check. The spray treatments consisted of zinc-maneb, chlorothalonil, and zinc-maneb with benomyl. Applications were made with a tractor-mounted boom sprayer when tips of tassels were showing. Gray leaf spot lesion development was less than 1% at the time of first spray. The 7-day interval treatments received six sprays, and the 14-day treatments received three sprays. Zinc-maneb applied every 7 days resulted in the highest yield, which was 20% more than the non-sprayed control. A part of the response to this treatment may have resulted from foliar feeding of zinc and manganese in the fungicide, tending to cast doubt on only the fungicidal effects.

Host Resistance

In recent years, many researchers have explored the inheritance of reaction to *C. zeae-maydis* and practices for control of gray leaf spot. Stromberg (1984, 1985a,

1985b) worked with control strategies for gray leaf spot in association with non-tillage and minimum-tillage systems. He noted gray leaf spot's association with non-tillage and formulated control strategies using tolerant hybrids, rotation, and plowing. In Virginia, gray leaf spot has become of such great interest to farmers that results from the gray leaf spot trials have been included in the maize hybrid trial results distributed by the state extension service (Donahue *et al.*, 1985, 1986, 1987).

Ayers *et al.* (1984) did not detect any strong resistance in the commercially available hybrids evaluated in Pennsylvania, but inbreds Va59 and Pa887p showed good resistance. A new inbred, Pa875, was released because of its performance in these gray leaf spot evaluation trials. They investigated the inheritance of reaction to this disease and found that resistance appeared to be multigenic and additive in nature. In a test with susceptible and resistant hybrids, pathogen isolate differences were noted but no hybrid X isolate interactions occurred, indicating that hybrids tend to keep the same rank regardless of the isolate used.

Thompson *et al.* (1987) studied the inheritance of reaction to gray leaf spot in maize using disease ratings from three experiments grown under naturally occurring field epiphytotics. Inheritance estimates were based on generation mean analysis of four populations, diallel analysis of all possible single crosses, and correlation comparisons between 16 inbreds *per se* and their crosses with a common parent. Plants were rated just prior to leaf death on a plot basis utilizing a scale of 1 (resistant) to 5 (susceptible). In the generation mean experiment the susceptible parents B73 and B73rhm were rated 4.1 to 4.3 and resistant parents H99 and NC250 were rated 1.4. For populations from susceptible X resistant parents, estimates of

pooled additive effects were large. Mean squares for general combining ability were significant, but those for specific combining ability were not. The 16 inbreds were correlated with their ratings in crosses at 0.69. They stated that resistance to gray leaf spot in the maize genotypes tested seemed to be conditioned mainly by additive effects, was not very complex, and could be evaluated effectively using inbreds *per se*. This indicates that resistance can be transferred easily by the usual backcrossing and selection techniques.

The most recent study involving the inheritance of reaction to gray leaf spot was conducted by Huff *et al.* (1988). In their diallel study, crosses were attempted with eight inbreds but only six were crossed in all combinations. They, like some other investigators, found that both general combining ability (GCA) and specific combining ability (SCA) effects were significant with the GCA effect being much larger. This indicates that inheritance of gray leaf spot resistance is mostly additive and moderately heritable.

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Hybrid Performance and Yield Losses Associated with Gray Leaf Spot Disease of Maize in Virginia

by

P.J. Donahue* and E.L. Stromberg

ABSTRACT

Commercial maize hybrids were evaluated under field disease pressure for response to gray leaf spot for seven years at two locations (Shenandoah and Wythe Counties, Virginia) and one year at a third location (Montgomery County, Virginia). Diseased plants were scored three times during each growing season. Percent lodging, percent grain moisture, and grain yield were regressed against a disease index to correlate losses with disease severity. Significant yield losses due to gray leaf spot pressure occurred in two of six years at the Shenandoah County site, one of six years at the Wythe County site, and in the one year at the Montgomery County site. Premature dry-down due to early senescence occurred in five of six years at Shenandoah County, six of six year at the Wythe county site, and at the Montgomery County site. Significant increases in lodging occurred in two of six years at the Shenandoah County site and five of six years at the Wythe County site. Yield losses (when comparing the resistant to susceptible classes) was approximately 2,000 kg ha^{-1} at Wythe County in 1982, 750 kg ha^{-1} at Shenandoah County in 1984, and 2,150 kg ha^{-1} at Montgomery County in 1988. Since these data were from a range of genotypes, the fact that these associations are significant indicates the strong effect gray leaf spot can have on a crop if environmental conditions are favorable.

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Gray leaf spot, caused by *Cercospora zeae-maydis* Tehon & Daniels, is a disease of maize (*Zea mays* L.). This disease, until recently, has been considered a minor, late season, foliar disease occurring predominantly in river valley fields of the Appalachian region of the United States (Kingsland, 1963; Latterell and Rossi, 1983; Roane, 1950). Since the early 1970's, the disease has been observed earlier in the growing season and with greater severity (Hilty *et al.*, 1979; Leonard, 1974; Roane *et al.*, 1974). It is no longer associated strictly with the river valley fields of the Appalachian region (Latterell and Rossi, 1983; Roane *et al.*, 1974). This increase in disease is associated with non-tillage maize production (Latterell and Rossi, 1983; Roane *et al.*, 1974) and, in particular, continuous non-tillage maize production (Stromberg, 1984, 1985b).

There have been reports of losses in yield attributable to gray leaf spot. Hilty *et al.* (1979) reported grain losses as high as 27.7% in paired fungicide and non-fungicide treated hybrids. The decreases corresponded to higher disease indices in the non-treated hybrids. In this study, however, micronutrients (Mn^{+2}, Zn^{+2}) included in the fungicide mancozeb may have favorably influenced the yields of the treated hybrids. Before 1982, hybrids had only been evaluated in one test for leaf reaction and silage yield (dry matter) (Roane *et al.*, 1974) and in another test for leaf reaction without grain yield (Hilty *et al.*, 1979).

Published information is not available on losses sustained by hybrids under field production conditions (without fungicide treatment). Although several researchers have observed hybrid performance, including yield, moisture, and lodging, under disease pressure (Donahue *et al.*, 1985, 1986, 1987; Ganoe *et al.*, 1985a, 1985b; Lovell, 1982, 1983, 1984; Stromberg, 1984, 1985a, 1985b; Stromberg *et al.*, 1982), no attempt has been made to correlate these agronomic characters to gray leaf spot

disease indices. Field observations (Stromberg, unpublished) indicate that hybrid performance varies under different levels of disease pressure; resistance or tolerance may be overwhelmed by heavy pressure.

Ayers *et al.* (1984) presented a paper documenting yield losses from gray leaf spot and the inheritance of resistance to gray leaf spot. In the yield-loss experiment, like the Hilty (1979) experiment, three fungicides or fungicide combinations to control gray leaf spot were used on a 7-day or 14-day schedule with a non-sprayed check. The spray treatments consisted of zinc-maneb, chlorothalonil, and zinc-maneb with benomyl applied with a tractor-mounted boom sprayer when tips of tassels were showing. Gray leaf spot lesion development was less than 1% at the time of first spray. The 7-day interval treatments received six sprays, and the 14-day treatments received three sprays. Zinc-maneb applied every 7 days resulted in the highest yield, which was 20% more than the non-sprayed control. As previously indicated, these treatments may have provided foliar feeding of zinc and manganese, which may have affected the fungicide effects.

A seven-year study was carried out in western Virginia to correlate grain yield by weight, percentage grain moisture, and percentage of stalk lodging for commercially available maize hybrids to gray leaf spot disease indices under field conditions at two locations having distinctly different disease pressures. This is the first study using regression analyses to examine the effect of gray leaf spot on these agronomic characters in relation to hybrid maturity classes, locations, and years. Results of this study could form the basis for breeders to assess their progress in selecting hybrids that better tolerate or resist gray leaf spot disease.

MATERIALS AND METHODS

Hybrids. In this study, hybrids were each evaluated for one to seven years. Disease ratings, lodging, grain moisture, and grain yield for each hybrid have been published and will not be repeated here (Donahue *et al.*, 1985, 1986, 1987; Stromberg, 1984, 1985a, 1985b; Stromberg *et al.*, 1982).

Location. Three fields were selected for their long history with gray leaf spot. Two fields had been in continuous non-tillage or reduced-tillage maize production for 15 to 20 years. One field was located in northwestern Virginia near Mt. Jackson, Shenandoah County and the second was in southwestern Virginia near Wytheville, Wythe County. The third site, Montgomery County, was located next to the New River at the Whitethorne Farm owned by Virginia Polytechnic Institute and State University. *Cercospora zeae-maydis* was introduced to the Montgomery County location by inoculation of susceptible spreader rows, and conidia from these infested rows were the source of inoculum for the experiment. It was felt that this method more closely resembled natural infection than direct inoculation. Since this location was near the river, it consistently had high humidity and fog early in the mornings during the growing season, which favored disease development. By visual observation, the Shenandoah County location consistently had greater disease pressure than the Wythe County site.

Plots. Twenty-four to 42 commercial hybrids were evaluated in each of seven years. They were planted by the first or second week of May each year. To provide a source of inoculum for each season's evaluations, infested stubble was left on the soil surface after harvest. No-tillage soil field preparation, herbicides, and nitrogen (112 kg/ha) were applied by the grower-cooperators following standard

management practices. Plot size was one of the following: four rows 6.1 m in length, with rows spaced 0.96 m apart; 4.4 m in length, spaced 0.76 m apart; or 5.3 m in length, spaced 0.76 m apart. Plots were planted to get a final population of 57,000 plants per hectare. Plots were arranged in randomized complete block designs with four replicates.

Disease index. Monthly, from July through September, all plants from the two middle rows of each plot were rated for disease on an index of 0 to 5 as described by Roane *et al.* (1974) and expanded by Hilty *et al.* (1979). For this index, 0 = no symptoms, 1 = trace of lesions below the ear but no lesions above the ear, 2 = many lesions below the ear but only a trace of lesions above the ear, 3 = severe lesion development below the ear and all the leaves above the ear have some lesions, 4 = all leaves show severe lesion development but green tissue is still visible, and 5 = all leaves are dry and dead. This rating system was selected for three reasons: i) lesions form progressively in an acropetal pattern on leaves and stalks and are, therefore, not amenable to Horsfall and Barratt (1945) percentile estimations; ii) it more accurately reflects the photosynthetic contribution of the upper leaves to grain filling (Evans, 1975; Vietor *et al.*, 1977), and iii) this scale has been used by others in describing leaf spot disease and, therefore, permits comparison of data from those studies with this report (Elliot and Jenkins, 1946; Donahue *et al.*, 1985, 1986, 1987; Stromberg, 1984, 1985a, 1985b; Stromberg *et al.*, 1982).

Lodging and moisture. The two middle rows of each plot were rated at harvest for percent stalk lodging (number of plants lodged below the ear divided by the total number of plants in two rows multiplied by 100). Because no consistent way was developed to decide which plants would be lost by machine harvest, ears from all

plants in the two middle rows, standing or lodged, were hand-harvested, shelled, weighed, and evaluated for moisture content (grain moisture meter, model TY9304, John Deere and Company, Conyers, GA 30208). Grain yield was standardized to kilograms per hectare at 15.5% moisture. Maize plants in the plot guard rows were harvested by combine and the infested debris was left on the soil surface to provide inoculum for the following season's evaluations.

Data analysis. The effect of gray leaf spot on lodging, grain moisture, and relative grain yield was estimated by the linear regression model:

$$Y = B_o + B_i X_1 + E_i$$

where Y is the response variable, B_o is the intercept, B_i is the slope of the regression line, X_1 is the regressor variable, and E_i is the unexplained variation.

RESULTS AND DISCUSSION

Disease index, lodging, moisture, and yield. Table 1 shows the total number of individual plots in the various gray leaf spot evaluation tests. The numbers of plots evaluated ranged from 76 to 158, with the number of genotypes tested being one quarter of these numbers.

Table 2 contains the correlation coefficients for maize yields regressed against gray leaf spot disease index. A significant negative correlation occurred between grain yield and gray leaf spot score in Shenandoah County in 1984 and 1985, Wythe County in 1982, and Montgomery County in 1988. While not significant, all other correlation values were negative.

Correlation coefficients between grain moisture at harvest and gray leaf spot index are shown in Table 3. In all locations and years, a significant negative correlation occurred between moisture and gray leaf spot score. This indicates that gray leaf spot had a significant effect by causing premature senescence shown by lower grain moisture if the hybrid was susceptible.

Table 4 contains data which indicate that lodging was significantly correlated with the gray leaf spot index for one year in Shenandoah County, for four years at the Wythe County site, and for one year at the Montgomery County site.

Figures 1 through 4 contain graphs of the regression of gray leaf spot index on grain yield, percent lodging, and percent grain moisture at harvest. Figure 1 contains the graphs for regression of gray leaf spot index on grain yield in kilograms per hectare. When the regression lines are used to estimate the yield losses associated with gray leaf spot reaction, they range from approximately 2,150 kg ha^{-1} (Montgomery County, 1988) between the most resistant class and most susceptible class to an approximate low of 750 kg ha^{-1} (Shenandoah County, 1985). Of the location-years where grain yields were significantly correlated with the gray leaf spot index, the average yield loss was approximately 1000 kg ha^{-1}, indicating that when conditions are favorable gray leaf spot can be a significant cause of yield loss.

Table 1. Number of plots from which data were correlated for various traits and gray leaf spot score.

	Year						
County	1982	1983	1984	1985	1986	1987	1988
Shenandoah	-	116	76	104	92	94	113
Wythe	120	-	76	100	96	87	158
Montgomery	-	-	-	-	-	-	154

- not tested

Table 2. Maize grain yields correlated with gray leaf spot score.

	Year						
County	1982	1983	1984	1985	1986	1987	1988
Shenandoah	-	-.10	-.31**	-.36**	-.15	-.19	-.19
Wythe	-.52**	-	-.07	-.01	-.09	-.03	-.05
Montgomery	-	-	-	-	-	-	-.34**

- not tested

**Significant at .01 level

Table 3. Grain Moisture correlated with gray leaf spot score.

	Year						
County	1982	1983	1984	1985	1986	1987	1988
Shenandoah	-	-	-.51**	-.51**	-.51**	-.37**	-.35**
Wythe	-.26**	-	-.23*	-.31**	-.38**	-.30**	-.40**
Montgomery	-	-	-	-	-	-	-.52**

- not tested

*Significant at .05 level

**Significant at .01 level

Table 4. Percent lodging correlated with gray leaf spot score.

	Year						
County	1982	1983	1984	1985	1986	1987	1988
Shenandoah	-	-.12	.17	.37**	.17	.14	.03
Wythe	.27**	-	.27**	.53**	.14	.10	.33**
Montgomery	-	-	-	-	-	-	.25**

- not tested

**Significant at .01 level

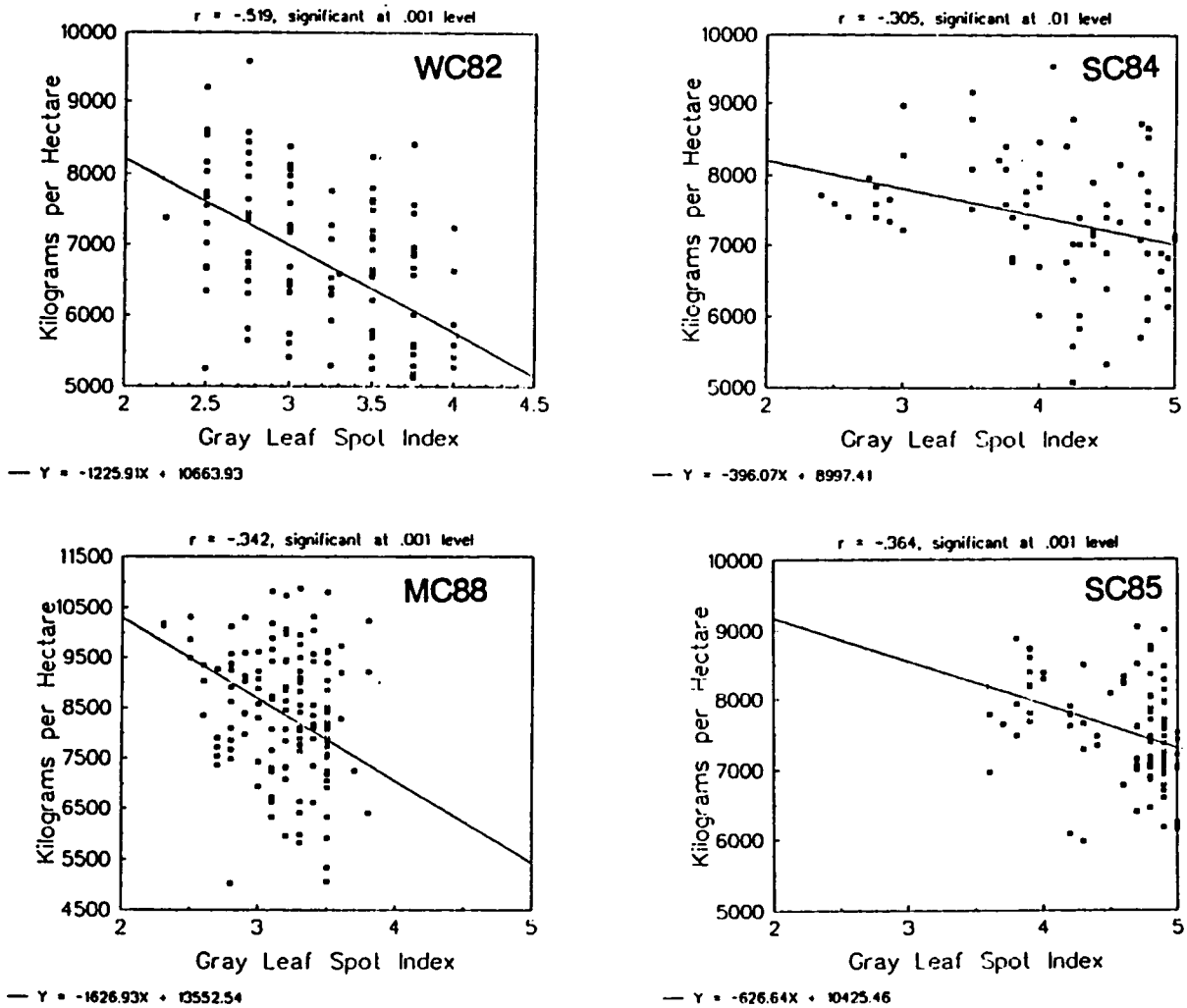

Figure 1. Grain Yield in kilograms per hectare versus gray leaf spot index. Selected graphs of values in Table 2, based on commercial hybrids raised in Montgomery, Shenandoah, and Wythe Counties in the years 1982 through 1988. WC82 - Wythe County, 1982; SC84 - Shenandoah County, 1984; MC88 - Montgomery County, 1988; SC85 - Shenandoah County, 1985.

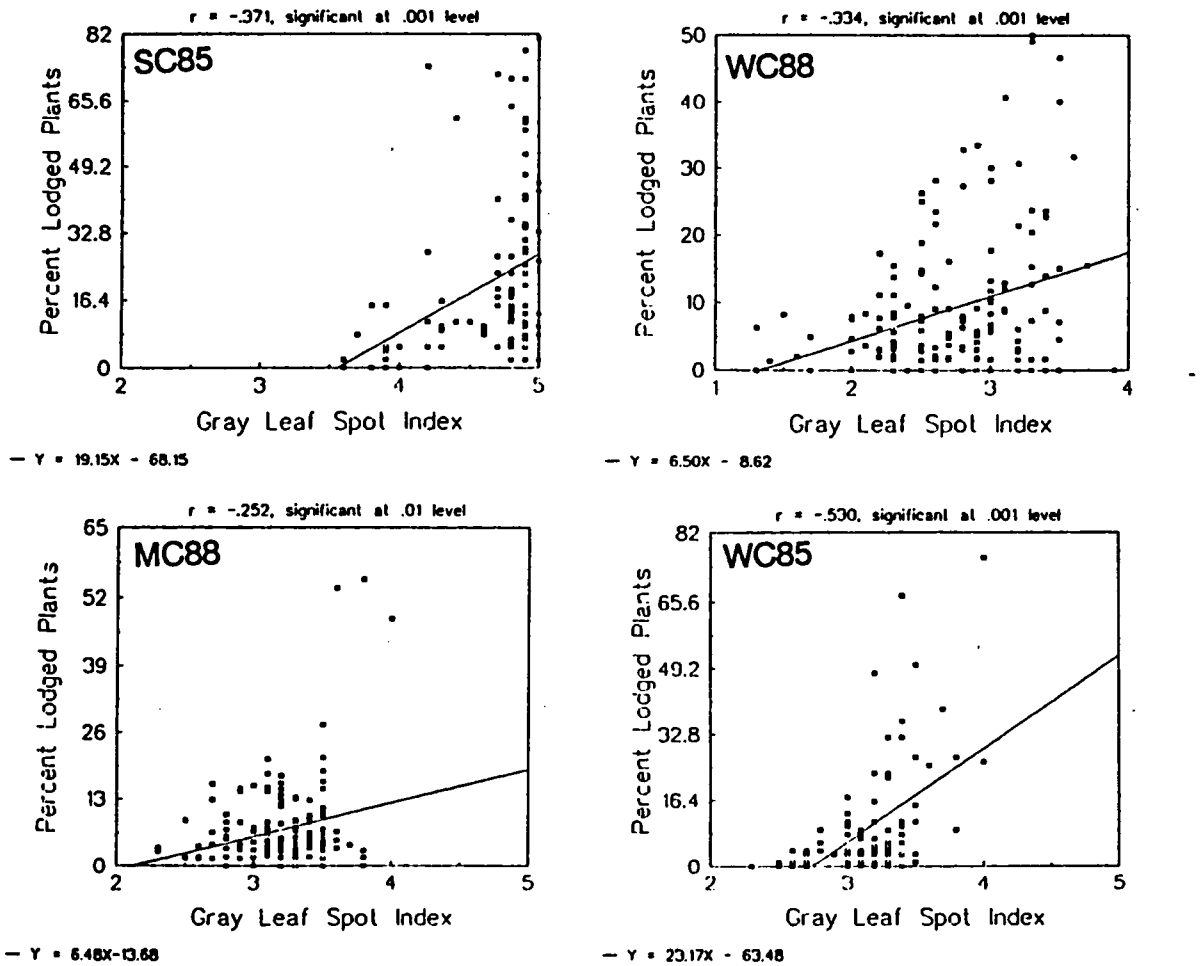

Figure 2. Percent lodged plants versus gray leaf spot index. Selected graphs of values in Table 3, based on commercial hybrids raised in Montgomery, Shenandoah, and Wythe Counties in the years 1982 through 1988. SC85 - Shenandoah County, 1985; WC88 - Wythe County, 1988; MC88 - Montgomery County, 1988; WC85 - Wythe County, 1985.

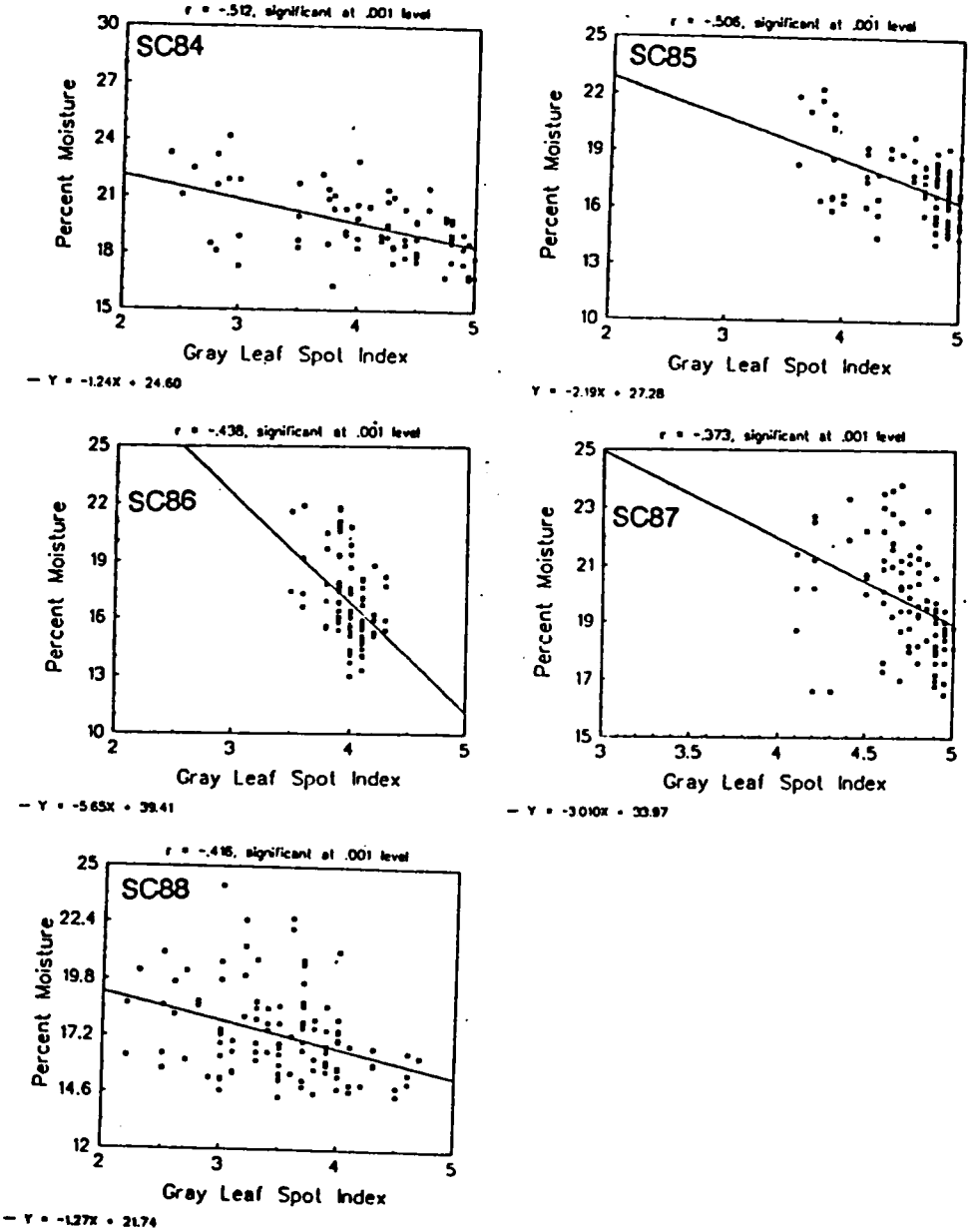

Figure 3. Percent grain moisture at harvest versus gray leaf spot index. Selected graphs of values in Table 4, based on a group of commercial hybrids raised in Montgomery, Shenandoah, and Wythe Counties in the years 1982 through 1988. SC84 - Shenandoah County, 1984; SC85 - Shenandoah County, 1985; SC86 - Shenandoah County, 1986; SC87 - Shenandoah County, 1987; SC88 - Shenandoah County, 1988.

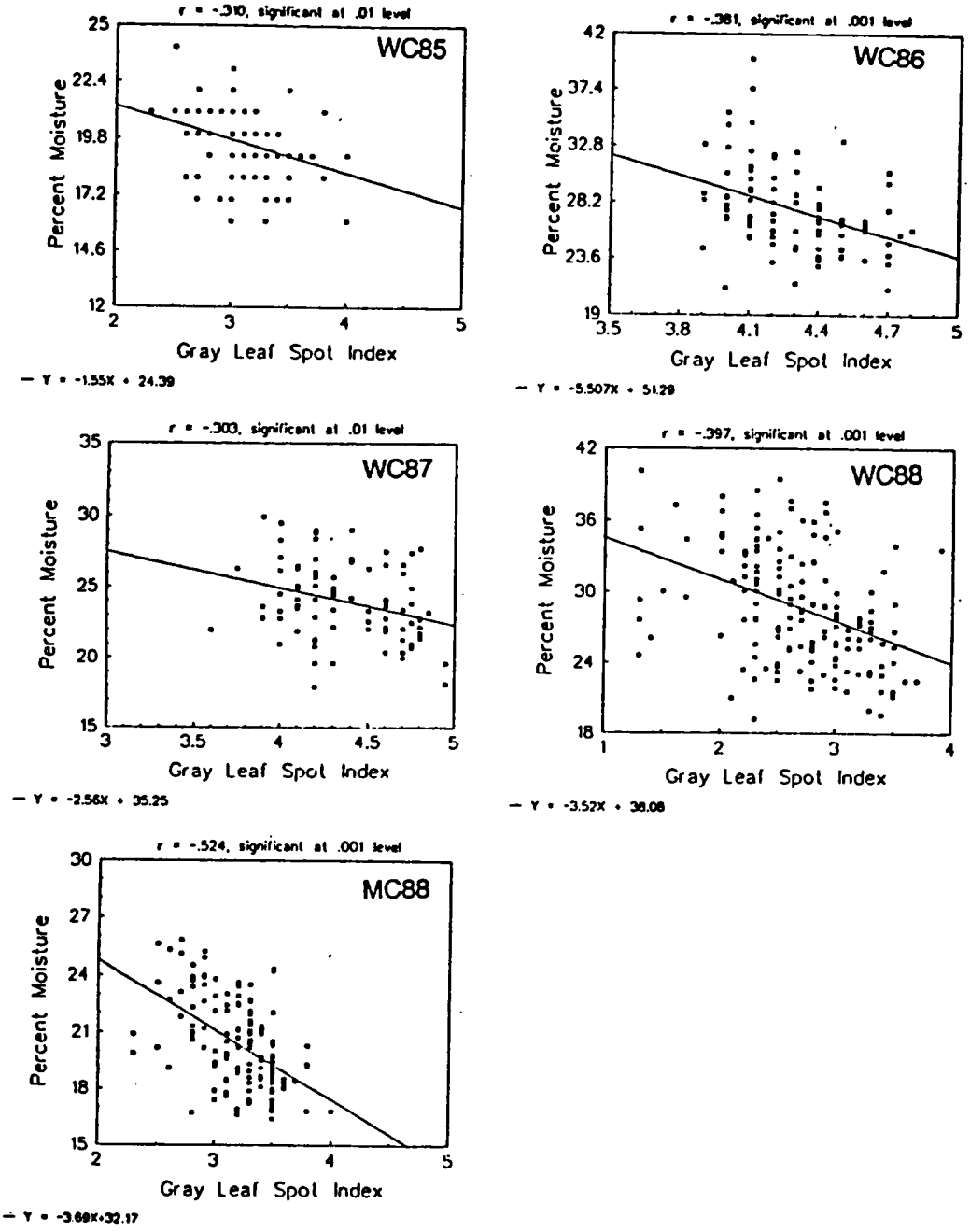

Figure 4. Percent grain moisture at harvest versus gray leaf spot index. Selected graphs from a group of commercial hybrids raised in Montgomery, Shenandoah, and Wythe Counties in the years 1982 through 1988. WC85 - Wythe County, 1985; WC86 - Wythe County, 1986; WC87 - Wythe County, 1987; WC88 - Wythe County, 1988; MC88 - Montgomery County, 1988.

Figure 2 contains graphs of the percent lodged plants versus gray leaf spot index in years and at locations where gray leaf spot had a significant effect on the percent lodged plants. When the regression lines are used to estimate the increase in lodging associated with gray leaf spot, the approximate increase in lodging ranged from 30 % (Shenandoah County, 1985) to 12 % (Montgomery County, 1988), with an average lodging of approximately 22 %. This suggests the strong effect gray leaf spot can have on lodging when conditions are favorable for the disease.

The graphs containing the data that show the relationship of percent grain moisture at harvest and gray leaf spot index are found in Figures 3 and 4. The approximate drop in grain moisture ranged from 3 % (Shenandoah County, 1984, 1985, 1986, 1987, 1988; Wythe County, 1985, and 1987) to 12 % (Wythe County, 1988). These figures indicate the early senescence (death) caused by the loss of photosynthetic area in the plants.

Gray leaf spot disease of maize had a negative effect on the agronomic characters evaluated. Disease pressure and the differences in genetic background of the commercial hybrids evaluated affected the extent of losses from the disease. Despite these differences, a significant correlation occurred between gray leaf spot and moisture in all years and location. Since lodging is linked with the availability of sugar in the plant and since anything that limits the availability of sugar to the developing plant can cause the redirection of sugar from the stalk to the developing ear (Dodd, 1980, 1983; Donahue 1986), it seems likely that lodging will continue to be a problem in areas where gray leaf spot is epidemic.

Since yield is also a function of photosynthate availability it seems likely that in years were gray leaf spot occurs early and the environment continues to favor

development and sporulation, losses to gray leaf spot can be great. Since the hybrids in this study are from a group of commercial hybrids from different seed companies, the yield potentials in this group of hybrids probably differ. Despite this, a measurable correlation occurred at four different sites. Since gray leaf spot is a quantitatively inherited trait (Huff, 1988; Thompson *et al.*, 1987), estimates of yield losses under field conditions will continue to be somewhat inexact. These data do show, however, that the negative effects are great in the agronomic characters evaluated, including yield. This suggests that development and use of gray leaf spot resistant germplasm is essential in areas were gray leaf spot is epidemic.

Gray leaf spot is an important yield-limiting disease in many portions of the mid-Atlantic region of the United States. Its association with reduced- or non-tillage maize production make it potentially damaging to other maize growing areas where these tillage forms have recently been adopted. Hybrids possessing greater resistance or tolerance to this disease are required by growers to assure more consistent grain and silage production.

ACKNOWLEDGMENTS

This research was supported in part by the Virginia Corn Board. We wish to thank J.A. Wright and W.E. Dolezal of Pioneer Hi-Bred International, Inc., Union City, TN and D.L. Dowden, Dekalb-Pfizer Genetics, Mt. Olive, NC for their kind assistance in planting plots. Additionally we wish to thank T. Fleming, Mt Jackson, VA and H. Leedy, Crockett, VA for the use of their maize farmland.

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Inheritance of Reaction to Gray Leaf Spot in a Diallel Cross of Fourteen Maize Inbreds

By
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ABSTRACT

Gray leaf spot, caused by *Cercospora zeae-maydis*, is a limiting factor in some areas where continuous minimum tillage practices are followed. The inheritance of reaction to this pathogen was studied using a diallel of 14 elite inbreds. The 91 single crosses were grown in field nurseries in Montgomery and Wythe counties, Virginia in 1987 and 1988 using randomized complete block designs. Data on gray leaf spot disease scores, lodging, yield, and moisture were taken during the growing season. Analyses of variance and combining ability analyses were made on these data. Resistance was found to be highly heritable and controlled by additive gene action. Many widely used inbreds (B73, Pa91, Mo17, and A632) were susceptible to gray leaf spot, explaining why many of the commercially available hybrids do not show high levels of resistance. Inbreds which provide high yielding, resistant, and agronomically acceptable hybrids were identified (B68, NC250, Pa875, Va14, Va17, and Va85) and several hybrids between these lines had high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875). This suggests that currently available inbreds could be used to produce agronomically acceptable hybrids with high levels of resistance for use in Virginia and that these inbreds could serve as a basis for breeding programs stressing gray leaf spot resistance.

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Gray leaf spot of maize (*Zea mays* L.), a disease caused by the fungus *Cercospora zeae-maydis* (Tehon and Daniels, 1925), has become increasingly important in Virginia in the last decade (Stromberg and Donahue, 1986; Donahue *et al.*, 1985, 1986, 1987; Stromberg, 1984, 1985a, 1985b). Its increasing frequency and severity have been associated with non-tillage methods of maize production (Beckman *et al.*, 1981; Beckman and Payne, 1982; Hilty *et al.*, 1979; Kingsland, 1963; Roane, 1950; Roane *et al.*, 1974; Rupe *et al.*, 1982; Stromberg, 1984, 1985a). It is estimated that between 10 and 25% of the yield potential can be lost annually in areas where gray leaf spot is a problem and in certain years where disease conditions are optimum, losses can be much higher. Even in years where yield loss is not apparent to the grower, susceptible hybrids often show a large increase in lodging, which can be a source of greater yield loss (Ayers *et al.*, 1984; Stromberg and Donahue, 1986).

Losses caused by gray leaf spot that are not associated with lodging are related to the premature loss of photosynthetic area. In years of severe blighting, susceptible hybrids develop symptoms that look like frost damage due to the necrosis of leaf area. The dominant sink of the post-anthesis maize plant is the ear. Because of loss of photosynthetic area by blighting, photosynthate is diverted from the stalk and roots at greater than normal levels causing them to senesce (expire) prematurely. The result is a loss of yield, an increase in stalk lodging, and death of the plant (Dodd, 1980, 1983; Koehler *et al.*, 1925; Morris, 1931).

Ayers *et al.* (1984) and Huff *et al.* (1988) in Pennsylvania and Manh (1977) in Virginia conducted preliminary studies on resistance to gray leaf spot. All found that while commonly used inbreds lacked resistance, resistant germplasm was

available and gray leaf spot resistance was a highly heritable, quantitative trait. Thompson *et al.* (1987) explored the inheritance of resistance to gray leaf spot in maize using a diallel design and also found resistance to be quantitatively inherited. They used eight inbreds, three of which were close variants of B73, a widely used, high yielding inbred from the Midwest.

We investigated the inheritance of reaction to gray leaf spot and related agronomic traits in a group of 14 maize inbreds (Table 5) representing some of the best commercially-available, high-yielding hybrids and germplasm that are available to maize growers and breeders. These inbreds either are used in hybrids *per se* or are used in maize line improvement programs.

MATERIALS AND METHODS

Fourteen inbred lines (Table 5) were crossed in all possible combinations in Virginia during the 1985 and 1986 growing seasons. Because of heat and drought stress, many of the pollinations were not successful. The crosses were repeated at the Purdue Agricultural Alumni winter nursery in Homestead, Florida in 1986. The reciprocal crosses were mixed together to make the 91 single cross hybrids in this experiment. Because of land limitations, we did not explore maternal effects since this would require 182 entries in the experiment. Because of inbreeding depression and the resulting low vigor we did not include the parents in the diallel design.

The diallel crosses were grown in randomized complete block designs with three replications at two locations for two years. These locations were Montgomery and Wythe Counties, Virginia, where gray leaf spot epiphytotics are common.

Table 5. Pedigree and origin of the fourteen maize inbred lines used in diallel cross representing elite Corn Belt and Eastern germplasm (Henderson, 1984).

Inbred line	Pedigree	Origin*
A632	(Mt42 x B14) B14	Minnesota
B68	(41.2504B x $B14^3$) Sel.	Iowa
B73	Iowa Stiff Stalk Syn. C_5	Iowa
H93	(37 x GE440)B37 BC_4 Ht	Indiana
Mo17	187-2 x C103	Missouri
NC250	(Nigeria Composite A-Rb x B37) x B37	N. Carolina
KB1250	NC250 x H93	Beck's Hybrids
Pa91	(WF9 x Oh40B) S_4 x (Ind 38-11 x L317) Ind 38-11 S_4	Pennsylvania
Pa875	PaWF9 Syn	Pennsylvania
Va14	(VaCBS Sel x Va17) Va17	Virginia
Va17	WF9 x T8	Virginia
Va22	Va17 x C103 BC	Virginia
Va35	(C103 x T8) T8	Virginia
Va85	Va. Long Ear Syn.	Virginia

*Lines having a state origin were released by that state's land grant institution.

Plots were four rows wide, planted at a density of 57,000 plants per hectare. Rows at the Montgomery County site were 81.3 cm wide and 7.6 m long, and at Wythe County the rows were 91.4 cm wide and 6.1 m long. This gave a plot of 6.2 m^2 in Montgomery County and 5.6 m^2 in Wythe County. Data on yield, moisture, disease score, number of plants, and number of lodged plants were taken during the growing season on the center two rows of each plot. Plots in Montgomery County also were scored for silking dates to estimate maturity.

Plots were scored for gray leaf spot using the same scoring methods that have been used for several years (Donahue *et al.*, 1985, 1986, 1987; Stromberg, 1984, 1985a, 1985b; Stromberg and Donahue, 1986). This scale is: 0 = no symptoms; 1 = trace of lesions below the ear; 2 = many lesions below the ear; 3 = large lesions below the ear and all leaves above the ear have some lesions; 4 = all leaves have large lesions but green leaf area remains; 5 = all leaves dead.

Data for yield, lodging, and disease score were analyzed using Model I analysis of Eisenhart (1947), along with Method 4 of Griffing (1956). We assumed in these analyses that all factors except error terms were fixed, since the lines used were not a random sample. Combining abilities were calculated as defined by Sprague and Tatum (1942). Simple correlations were calculated between disease score and yield, percent lodging, and moisture.

RESULTS AND DISCUSSION

Conditions for development of gray leaf spot disease were variable depending on locations (Montgomery and Wythe Counties, Virginia) over the two years (1987 and

1988). Conditions ranged from extremely dry with virtually no gray leaf spot (Montgomery County, 1987) to extremely high levels of gray leaf spot (Wythe County, 1987). Disease development in 1988 was between these two extremes. Because of the low levels of gray leaf spot in the 1987 Montgomery County test, it was not rated for gray leaf spot and was not included in the gray leaf spot analyses. For these reasons each year and site combination was analyzed as a location.

In Table 6 we list the analysis of variance (ANOVA) for gray leaf spot score. The analysis confirms the observation that locations were highly variable for the magnitude of gray leaf spot. The F value of 2146.67 was highly significant at the 0.01 level. This result reflects that gray leaf spot is an extremely environmentally sensitive disease requiring high humidities and extended leaf wetness. Because of this variability, breeding sites for gray leaf spot resistance should be chosen carefully. If a proper screen for germplasm under selection is to be obtained, irrigation of the plots may be required to create optimum disease conditions. The significant F value of 12.25 for the Rep(Location) effect, shows that even within a location levels of gray leaf spot were quite variable.

Hybrids in the test differed significantly for gray leaf spot score as shown by the significant F value in Table 6. When this hybrid variation is partitioned into general combining ability (GCA) and specific combining ability (SCA), both are significant with the GCA portion being approximately 30 times greater than the SCA portion. This indicates that resistant genotypes could easily be selected in a

segregating population and this corroborates the work of Thompson *et. al* (1987) and others (Ayers *et. al*, 1984; Huff *et. al*, 1988; Manh, 1977).

Table 7 contains the GCA effects for gray leaf spot score, grain yield in Kg ha^{-1}, and percent lodged. Lines Pa875 and Va14 had the lowest GCA effects for gray leaf spot score (-0.5 and -0.4 respectively), with B68, KB1250, NC250, Va17, and Va85 all being below zero. These lines were classed as resistant. Inbred lines B68, Pa875, and NC250 have been reported by others as being resistant (Thompson *et. al*, 1987; Huff *et. al*, 1988), but Va14, KB1250, Va17, and Va85 are all newly identified potential sources of resistance for breeding programs. Interestingly the lines B73 and Pa91 had the highest GCA effects of 0.6 and 0.5, respectively. These lines were widely grown as a hybrid (B73 X Pa91) in the southern maize growing regions. Inbred lines A632, H93, and Va22 were also strongly positive and were classed as susceptible. Lines Mo17 and Va35 were near zero in their GCA effects and were classed as intermediate. Since most of the susceptible lines, either *per se* or as their close relatives, have been widely used in hybrids, it is not surprising that levels of resistance in commercially available materials have been low.

In Table 8 the mean gray leaf spot ratings for hybrids in the diallel are listed. Several of the hybrids had very low mean gray leaf spot ratings. The cross Pa875 x Va17 had the lowest score of 1.6. Several other hybrids had a score of 2 or below. These were Va14 x B68, H93 x Pa875, KB1250 x Va14, KB1250 x Va85, NC250 x Va14, Pa875 x Va14, Pa875 x Va85, Va14 x Va85, and Va17 x Va85.

Table 6. ANOVA of gray leaf spot score from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	2	223.85	2146.67**
Reps(Location)	6	1.28	12.25**
Hybrids	90	2.22	21.31**
GCA	13	12.83	128.30**
SCA	77	0.43	4.30**
Locations x Hybrids	180	0.42	4.04**
Error	540	0.10	

R2 = .928

C.V. = 12.56

LSD(.05) = 0.29

**Significant at .01 level

Table 7. General combining ability effects (GCA) from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	GCA effects		
Inbred	GLS* score	Grain yield	Percent lodged
A632	0.3	-122.8	1.1
B68	-0.3	112.7	-3.6
B73	0.6	402.0	1.4
H93	0.3	154.6	-0.9
KB1250	-0.2	119.7	-0.8
Mo17	0.1	314.5	-1.9
NC250	-0.2	-62.7	0.9
Pa875	-0.5	236.0	-2.8
Pa91	0.5	-16.4	-1.4
Va14	-0.4	-929.4	-1.3
Va17	-0.2	-854.8	0.6
Va22	0.3	-100.0	0.1
Va35	0.0	140.5	2.1
Va85	-0.3	605.8	6.4

*Gray Leaf Spot

Table 8. Average gray leaf spot ratings* for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	2.2	3.5	3.7	2.8	3.0	2.7	2.4	3.6	2.3	2.6	3.0	2.9	2.5
B68		2.6	2.5	2.2	2.2	2.4	2.2	2.8	2.0	2.1	2.5	2.2	2.3
B73			3.7	3.3	3.6	3.0	2.3	3.5	2.5	2.9	3.4	3.2	2.7
H93				2.8	2.7	2.7	1.9	3.7	2.5	2.7	3.2	2.7	2.5
KB1250					2.4	2.3	2.1	2.6	2.0	2.2	2.5	2.3	2.0
Mo17						2.3	2.1	3.2	2.3	2.3	2.8	2.7	2.5
NC250							2.1	2.7	1.9	2.1	2.7	2.3	2.2
Pa875								2.7	1.7	1.6	2.2	2.1	1.7
Pa91									2.0	2.7	3.6	3.3	2.6
Va14										2.7	2.3	2.1	1.9
Va17											2.7	2.4	2.0
Va22												2.9	2.7
Va35													2.7

*This gray leaf spot scale is: 0 = no symptoms; 1 = trace of lesions below the ear; 2 = many lesions below the ear; 3 = large lesions below the ear and all leaves above the ear have some lesions; 4 = all leaves have large lesions, but green leaf area remains; 5 = all leaves dead.

All of these crosses are between resistant inbreds, further corroborating the additive nature of resistance.

The SCA effects are listed in Table 9. Twenty-five of the 91 hybrids in the diallel had a SCA effect of zero and 60 percent had an effect of 0.1 to -0.1. This confirms that most of the gene action for resistance is accounted for in the GCA effects. The hybrid with the greatest SCA effect was Va14 x Va17. This positive effect indicates that both of these lines may have the same resistance genes and are not able to take advantage of any additive gene action. Pedigrees listed on Table 5 confirm that Va14 is derived from Va17.

Table 10 contains the ANOVA for grain yield in kg ha^{-1} and shows that location effects are highly significant as expected due to the widely varying disease levels, weather patterns, and soils among the locations. Hybrids were also significantly different. When hybrid yields were partitioned into GCA and SCA effects, both were significant with the GCA effects being approximately twice the SCA effects. The GCA effects for yield for each inbred are shown in Table 7. B73 had a high GCA of 402.0, corroborating its good general combining ability that others have shown (Donahue, 1986; McClane, 1985; Perry, 1983). However, it is highly susceptible to gray leaf spot. The line with the best (indicated by a positive estimate) GCA is Va85, which had a GCA estimate of 605.8. This line also had good GCA effects for gray leaf spot (indicated by a negative estimate), suggesting that this would be a good line in a breeding program stressing high yield and gray leaf spot resistance. Three other lines in the diallel had good resistance to gray leaf spot (indicated by a negative GCA estimate) and good yield (indicated by a positive

Table 9. Specific combining ability effects for gray leaf spot score from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-0.4	0.0	0.5	0.1	0.1	0.0	0.0	0.2	-0.2	-0.1	-0.1	0.0	-0.1
B68		-0.3	-0.1	0.1	-0.2	0.3	0.4	0.0	0.1	0.0	-0.1	-0.1	0.3
B73			0.2	0.3	0.4	0.0	-0.3	-0.1	-0.2	0.0	0.0	0.0	-0.2
H93				0.1	-0.3	0.0	-0.5	0.3	0.0	0.0	0.0	-0.2	-0.1
KB1250					-0.1	0.1	0.2	-0.3	0.0	0.0	-0.2	-0.1	-0.2
Mo17						-0.2	0.0	0.1	0.1	-0.1	-0.1	0.0	0.1
NC250							0.2	-0.2	-0.1	-0.1	0.0	-0.1	0.1
Pa875								0.2	0.1	-0.2	-0.1	0.0	-0.1
Pa91									-0.6	-0.1	0.3	0.2	-0.2
Va14										0.8	-0.1	-0.1	0.0
Va17											0.1	0.0	-0.1
Va22												0.0	0.1
Va35													0.4

Table 10. ANOVA of yield data (kg ha^{-1}) from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	3	2664790656.00	3088.75**
Reps(Location)	8	5036059.35	5.84**
Hybrids	90	16559116.70	19.19**
GCA	13	28576488.34	33.12**
SCA	77	14530716.30	16.84**
Locations x Hybrid	270	3353523.56	3.89**
Error	720	862741.17	

R^2 = .944

C.V. = 16.18

LSD(.05) = 743

**Significant at .01 level

GCA estimate); they are B68, KB1250, and Pa875. These also would be good candidates for a breeding program stressing high yield and gray leaf spot resistance.

The grain yields of hybrids in kg ha^{-1} are given are Table 11. Seven of the hybrids in the diallel averaged over 7000 kg ha^{-1} over the four locations. Three of these hybrids (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875) were resistant x resistant crosses that also have good gray leaf spot scores. Two of these hybrids (H93 x Pa875 and Va35 x Va85) are resistant x susceptible crosses that have acceptable gray leaf spot scores. This compares with the hybrids B73 x Pa91 and B73 x Va22 which were and are widely sold *per se* or as their close relatives. These hybrids had yields of 7042 and 7073 kg ha^{-1}, respectively. This indicates that high yielding resistant germplasm is available to seed producers and growers. High yields of the susceptible checks also indicates that although separation of susceptible and resistant hybrids was possible, gray leaf spot disease levels were low early in the season and did not have a great impact on yield.

The SCA effects for grain yield are given in Table 12. The high positive values for all the hybrids having high yields underscores the importance of non-additive gene action in yield superior hybrids. Other researchers have noted this and it will not be discussed in detail here.

Tables 13, 14, and 15 contain the ANOVA for percent lodged, the mean lodging percentages for the hybrids, and the SCA effects for lodging, respectively. Lodging varied by location and replication in a fashion similar to yield. Several of the high yielding, gray leaf spot resistant hybrids in the diallel also had low mean lodging

Table 11. Grain yield in (kg ha^{-1}) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	3323	5053	6595	6121	6274	5964	5172	5513	6025	5635	6350	5417	6080
B68		5961	6287	7058	6651	6618	5860	6001	5924	6187	5958	6120	4400
B73			4717	5590	6234	5943	6795	7042	5543	6465	7073	6681	6723
H93				3334	6500	3282	7625	5948	5948	6514	6145	6465	7491
KB1250					6939	2375	7039	6152	6309	5792	6470	6594	6659
Mo17						6073	6540	4881	5170	5016	6307	5567	6618
NC250							7053	6022	5317	5225	6477	6977	6917
Pa875								6253	4561	4769	6263	5183	4714
Pa91									4079	4254	5382	6328	6944
Va14										1243	3412	4833	5479
Va17											2953	4042	6643
Va22												4942	6064
Va35													7533

Table 12. Specific combining ability effects for grain yield (kg ha^{-1}) from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-2436	-995	794	355	313	381	-710	-117	1308	844	804	-370	-172
B68		-323	251	1057	455	799	-258	136	972	1160	176	98	-2087
B73			-1609	-701	-251	-165	388	887	301	1149	1002	370	-54
H93				-2709	262	-2579	1466	41	954	1445	321	401	962
KB1250					736	-3451	914	280	1350	758	681	565	165
Mo17						52	221	-1186	16	-213	324	-657	-71
NC250							1111	332	540	374	871	1130	605
Pa875								265	-514	-381	358	-962	-1897
Pa91									-744	-644	-271	435	586
Va14										-2742	-1328	-147	34
Va17											-1861	-1013	1123
Va22												-867	-211
Va35													1018

Table 13. ANOVA for percent lodged for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	3	3904.79	77.67**
Reps(Location)	8	255.10	5.07**
Hybrids	90	294.83	5.86**
GCA	13	875.76	17.42**
SCA	77	196.75	3.91**
Locations x Hybrids	270	112.87	2.25**
Error	720	50.27	

R^2 = .662

C.V. = 68.89

LSD(.05) = 5.7

*Significant at .05 level

**Significant at .01 level

Table 14. Percent lodged for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	6.8	9.4	15.5	7.6	9.7	18.9	6.3	14.8	7.1	7.8	13.1	12.6	17.3
B68		6.7	6.2	4.8	4.8	4.2	5.3	5.3	6.0	6.1	9.3	8.3	16.5
B73			16.9	13.4	8.8	17.8	4.2	14.1	10.8	14.5	6.4	15.5	12.6
H93				4.5	9.1	3.8	1.0	8.8	3.9	18.9	11.7	9.5	13.5
KB1250					7.4	7.0	4.9	4.7	12.7	12.4	14.7	14.3	16.3
Mo17						13.6	6.1	8.0	9.3	13.1	5.7	4.6	11.3
NC250							5.7	11.1	10.3	13.3	11.3	15.0	12.8
Pa875								7.3	4.6	7.2	13.8	10.7	23.3
Pa91									8.2	7.2	6.4	6.3	15.5
Va14										8.0	7.3	15.8	14.2
Va17											13.4	9.5	9.5
Va22												6.1	16.2
Va35													31.5

Table 15. Specific combining ability effects for percent lodged from a 14 line diallel grown in Montgomery and Wythe Counties,VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-0.9	-3.4	5.0	-3.0	0.2	6.6	-2.3	4.8	-3.0	-4.2	1.6	-0.9	-0.5
B68		-1.4	0.4	-1.1	0.0	-3.4	1.4	0.0	0.6	-1.1	2.5	-0.5	3.5
B73			6.1	2.4	-1.1	5.2	-4.7	3.7	0.4	2.2	-5.5	1.6	-5.5
H93				-4.1	1.6	-6.5	-5.6	0.7	-4.2	8.9	2.2	-2.1	-2.3
KB1250					-0.3	-3.4	-1.8	-3.5	4.5	2.3	5.0	2.6	0.4
Mo17						4.3	0.5	0.9	2.2	4.1	-2.9	-6.0	-3.5
NC250							-2.7	1.2	0.4	1.5	0.0	1.6	-4.8
Pa875								1.1	-1.6	-0.9	6.2	1.0	9.4
Pa91									0.6	-2.3	-2.7	-4.8	0.2
Va14										-1.6	-1.8	4.7	-1.2
Va17											2.4	-3.5	-7.8
Va22												-6.5	-0.6
Va35													12.7

scores, showing again that agronomically acceptable hybrids resistant to gray leaf spot do exist and these can be put into use immediately if seed companies wish to do so.

CONCLUSIONS

From this study we conclude that high yielding, agronomically acceptable hybrids are available to seed growers and producers. While many of the widely used Midwestern inbreds are susceptible, a germplasm base does exist for breeding programs to develop high yielding, gray leaf spot resistant inbreds and hybrids. Our data shows that gray leaf spot resistance is highly heritable and not very complex, indicating that screening of advanced lines should be sufficient to identify gray leaf spot resistance in a breeding program. Susceptibility to gray leaf spot among many currently produced hybrids probably results from a lack of screening of midwestern materials. The current outbreak of gray leaf spot probably is due to susceptibility of currently used germplasm coupled with an increase of conservation tillage and is not a result of any increase in virulence by the pathogen.

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Physiological Associations With Gray Leaf Spot Resistance

By

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ABSTRACT

Gray leaf spot is a limiting factor in some areas where continuous minimum tillage practices are followed. Gray leaf spot reaction of maize was studied using all possible crosses among 14 elite inbreds. The diallel study was conducted in the field in Montgomery and Wythe Counties, Virginia in 1987 and 1988, to investigate any associations with some physiological traits. The experimental designs were randomized complete blocks. Data on gray leaf spot disease scores, lesion width, lesion length, lesion area, maize dwarf mosaic virus score, and days to silk were taken during the season. Analyses of variance and combining ability analyses were performed on all traits. Lesion size measurements were not correlated with disease scores. Late season photosynthesis rates were positively associated with resistance. The hybrids of some inbreds were found to produce high levels of pigment around the gray leaf spot lesions. However, this did not seem to limit the size of the lesion later in the season. Some high anthocyanin producing genotypes were observed to have fewer lesions when the character was expressed. Since disease scores were not associated with lesion size, selection of cercosporin resistant clones may not be successful in producing resistant genotypes.

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Gray leaf spot, a disease of maize (*Zea mays* L.) caused by the fungus *Cercospora zeae-maydis*, was first described by Tehon and Daniels (1925) in Illinois. Its increasing frequency and severity have been associated with non-tillage methods of maize production (Beckman *et al.*, 1981; Beckman and Payne, 1982; Hilty *et al.*, 1979; Kingsland, 1963; Roane, 1950; Roane *et al.*, 1974; Rupe *et al.*, 1982; Stromberg, 1984, 1985a). It is estimated that between 10 and 25% of the yield potential can be lost annually in areas where gray leaf spot is a problem; and, in certain years where disease conditions are optimum, losses can be much higher. Beckman *et al.* (1981) noted that microclimatic factors such as high relative humidity and long periods of leaf wetness contribute to infection.

Leaf lesions are gray to brown, long (0.5 to 5 cm), narrow, and rectangular (Shurtleff, 1980). The lesions are restricted at first by the larger veins but will later coalesce under optimum disease conditions and the entire leaf can be killed. There is no effect of plant age on efficiency of stomatal penetration (Beckman and Payne, 1982, 1983). Total loss of the crop can occur if an early infection is followed by ideal disease conditions.

Fajola (1978) noted that species of *Cercospora* characteristically produce cercosporin, a red, photodynamic toxin. Working with beets, Steinkamp *et al.* (1981) concluded that cercosporin seemed to play a role in spreading the fungus through the plant tissue.

Cercosporin is a non-specific toxin produced by members of the genus *Cercospora*. It was first isolated in 1957 (Kuyama) from *Cercospora kikuchii* T. Matsu and Tomoyasu, a soybean pathogen, and has been isolated from other

Cercospora species (Assante *et al.*, 1977; Balis and Payne, 1971; Fajola, 1978; Lu, 1983; Lynch and Geoghegan, 1977; Mumma *et al.*, 1973).

When cercosporin is placed on a wide range of host species, it causes the typical necrotic lesions. Cercosporin sensitizes plant cells to light (Daub, 1982). Cell photosensitizers in their native state are not toxic to cells, but they absorb light and are then converted to an excited triplet state (Foote, 1976; Spikes, 1977). This highly reactive intermediate produces superoxide ions or a singlet oxygen (Daub, 1987). Cercosporin produces both singlet oxygen and superoxide when irradiated with light (Daub and Hangarter, 1983; Dobrowlski and Foote, 1983). These compounds are toxic to living cells, oxidizing lipids, proteins, carbohydrates, and nucleic acids (Spikes, 1977; Daub, 1987). Daub (1984) studied the possibility of using cercosporin in selection of resistant genotypes in the cell culture. Daub (1987) was unable to select for cercosporin-resistant tobacco and sugarbeet protoplasts in cell cultures following mutagenesis.

Two facts link cercosporin to the diseases caused by *Cercospora* spp. One is that light is required for the development of symptoms in *Cercospora* diseases (Calpouzos, 1966; Calpouzos and Corke, 1963; Calpouzos and Stalknecht, 1967). The other is that the treatment of sugarbeet leaves with cercosporin produces changes similar to those infected with *Cercospora beticola* Sacc. The changes are consistent with cercosporin's known mode of action (Steinkamp *et al.*, 1979; 1981).

Gwinn *et al.* (1987) studied cultivars of maize with different levels of resistance to *Cercospora zeae-maydis* in the greenhouse. Leaf disks from 1- ,2-, and 3-month-old plants were either soaked with 1.2 uM cercosporin or inoculated with a mycelial

suspension of *C. zeae-maydis*. Cercosporin treatment caused significantly less ion leakage from disks of older plants.

The objective of this study was to investigate lesion characters, photosynthetic rates, and other physiological characters associated with gray leaf spot disease using a 14 line diallel of maize that had previously been scored for disease reaction.

METHODS AND MATERIALS

Fourteen inbred maize lines (Table 5) were crossed in all possible combinations in Virginia during the 1985 and 1986 growing season. Because of heat and drought stress, many of the pollinations were not successful. The crosses were repeated at the Purdue Agricultural Alumni winter nursery in Homestead, Florida in 1986. The reciprocal crosses were mixed together to make 91 hybrids in this experiment. Because of land limitations, we did not explore maternal effects, since this would require 182 experimental entries. Inbred parents were not included in the experiment due to the inbreeding depression and poor growth habits associated with using maize inbreds.

The diallel crosses were grown in randomized complete block designs with three replications at two locations for two years. These locations were in Montgomery and Wythe Counties, Virginia, where gray leaf spot epiphytotics are common. Plots were scored for gray leaf spot in mid-August using the same scoring methods that have been used for several years (Donahue *et al.*, 1985, 1986; Stromberg, 1984, 1985a, 1985b; Stromberg and Donahue, 1986). This scale is: 0 = no symptoms;

1 = trace of lesions below the ear; 2 = many lesions below the ear; 3 = large lesions below the ear and all leaves above the ear have some lesions; 4 = all leaves have large lesions, but green leaf area remains; 5 = all leaves dead. This scale was used with gray leaf spot by Roane *et al.* (1974) and expanded by Hilty (1979). Since lesions start from the bottom and progress up the stalk, and the upper leaves are more important photosynthetically, the Horsfall and Barratt (1945) estimation method for percentage of leaf area lost to disease was not used. However, as cited above, other researchers working with other maize pathogens have used this type of rating scale (Elliot and Jenkins, 1946).

Plots were four rows wide, planted at a density of 57,000 plants per hectare. The rows at Montgomery County were 81.3 cm wide and 7.6 m long and at Wythe County the rows were 91.4 cm wide and 6.1 m long. This gave a plot area of 6.19 m^2 in Montgomery County and 5.6 m^2 in Wythe County. Yield, moisture, disease score, number of plants, and number of lodged plants were taken during the growing season on the center two rows of each plot. Plots in Montgomery County also had the silking dates taken in order to estimate maturity.

Net photosynthesis was measured with a Licor^R photosynthesis unit. Photosynthesis was measured twice during the growing season on susceptible and resistant crosses from a common parent, A632. This was done to lessen any confounding from hybrid maturity. Photosynthesis was measured first at anthesis on six genotypes; the other photosynthesis measurement was taken on seven genotypes just before blacklayer. Photosynthesis was only measured on green tissue on the first leaf below the flag leaf and was not taken on necrotic tissue. Data were recorded as umol m^{-2} s^{-1}.

The leaf above the top ear leaf was removed from plants before senescence in the first two replications at Wythe County in 1987 and Montgomery County in 1988. These samples were dried and five lesion widths (mm) and five lesion lengths (mm) were measured on discrete lesions located in the center of the leaf. The mean of these measurements were used for analyses and calculation of the average lesion area (mm^2). Approximately 4,000 lesions were measured using only plot number, not treatment number, as an identifier. This was done in order to insure that investigator's prior knowledge of field resistance levels did not bias measurements.

Data for gray leaf spot score, lesion width, lesion length, and lesion area were statistically analyzed using Model I analysis of Eisenhart (1947) and Method 4 of Griffing (1956). Method 4 assumes that all factors except error terms are fixed since the lines used are not a random sample. Combining ability estimates were calculated using the method of Sprague and Tatum (1942). Data for lesion size were estimated for a missing cross (H93 X Va14) using the method of Hinkelman (1968). Simple correlations were calculated between disease score and grain yield, percent lodging, lesion size, and moisture of grain at harvest.

RESULTS AND DISCUSSION

Photosynthesis Experiment

The relationship between photosynthesis at anthesis and gray leaf spot disease score is shown in Figure 5. As shown by the non-significant low R^2 (0.019), there was no association between early season photosynthesis levels and reaction to gray leaf spot. Later in the season, there was an association (.665, significant at .05

level) as shown in Figure 6, with photosynthesis levels dropping as disease levels increased. This supports the observation of Stromberg and Donahue (1986) that while infection can take place before anthesis, lesion development does not take place until after anthesis.

The above evidence suggests that late season susceptibility is related to factors associated with leaf aging *per se*. If so, this is similar to the resistance shown by maize to stalk rot, wherein genotypes that maintain a higher metabolic level remain resistant to stalk rotting pathogens, while those genotypes that begin to senesce (die) "prematurely" become susceptible (Dodd, 1980). In the same fashion, this work suggests that those genotypes that begin to senesce early, as shown by their photosynthesis levels, also apparently become susceptible.

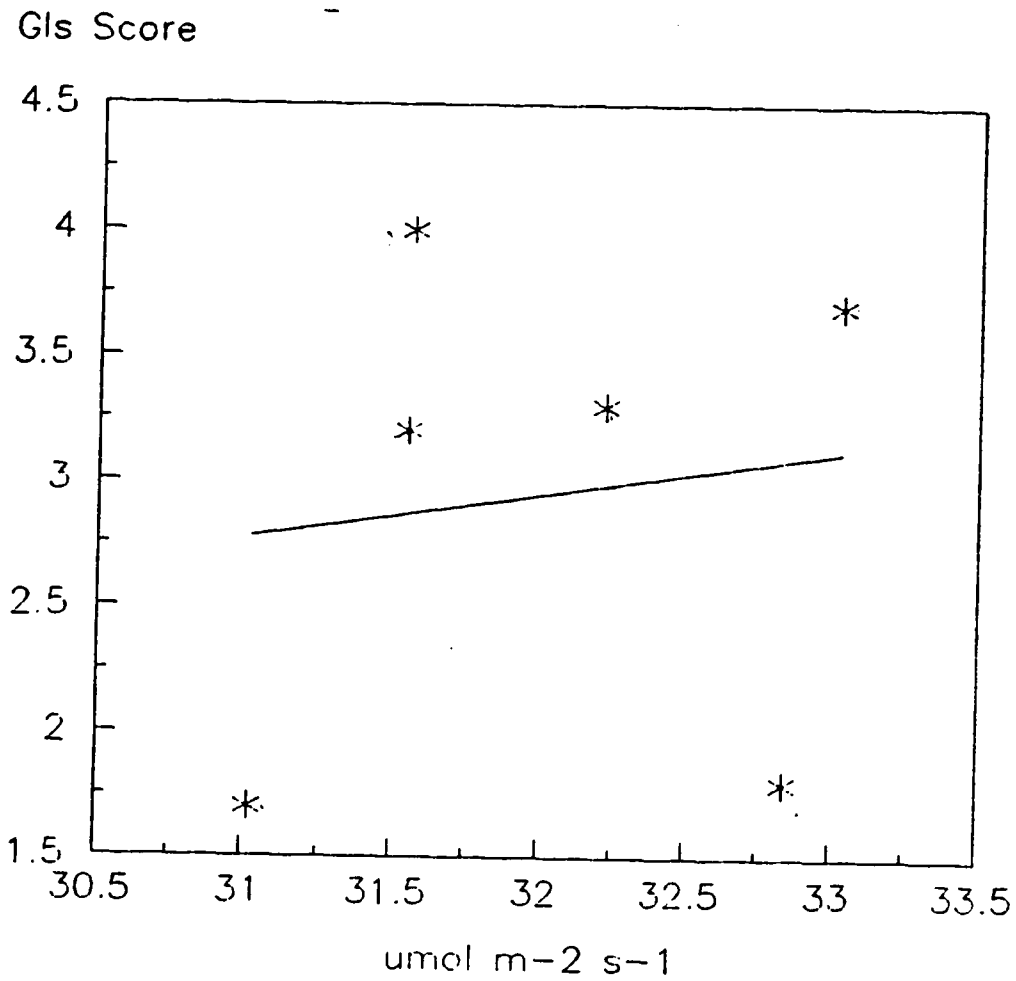

Figure 5. Early season net photosynthesis measurements from green tissue taken on resistant and susceptible A632 crosses versus gray leaf spot score (0-5) grown in Montgomery County, VA in 1988; $R^2 = 0.019$.

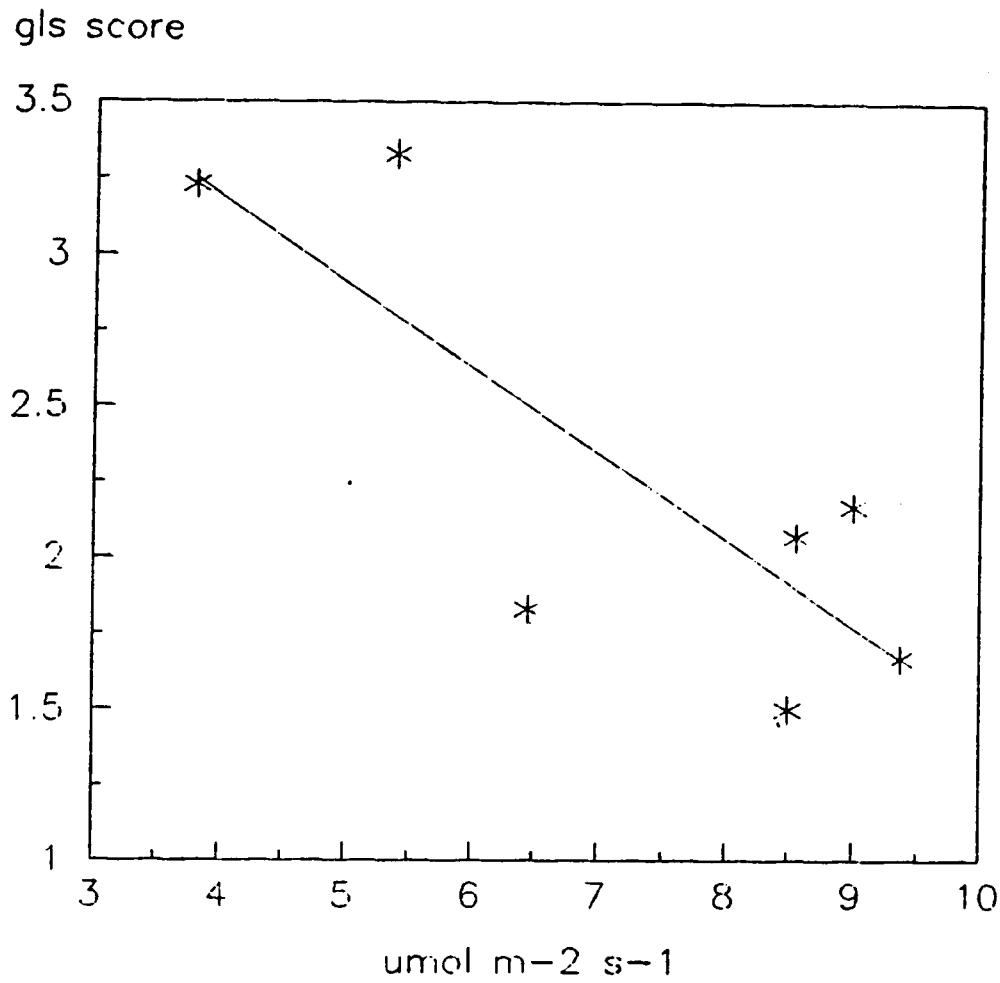

Figure 6. Late season net photosynthesis measurements from green tissue taken on resistant and susceptible A632 crosses versus gray leaf spot score (0-5) grown in Montgomery County, VA in 1988; $R^2 = 0.665$.

Lesion Measurements

Table 16 contains the ANOVA of lesion width values. Lesion width was significantly different at the two locations as shown by the large F value. This indicates that the environment had a strong effect on lesion size. Hybrids were also significantly different. The high R^2 value indicates that much of the variation in the test was accounted for by the model. Mean lesion widths are given in Table 17 and the LSD(.05) is given in Table 16 (0.62 mm). The hybrid B73 X Mo17 had one of the highest gray leaf spot scores (3.6), and it had a mean lesion width of 1.13 mm. This compares with a width of 2.62 mm for H93 X Pa875, which had a lower gray leaf spot score (1.9). This result did not match our field perceptions; the larger lesion size perceived in the field on susceptible genotypes must be due to lesions coalescing to form larger necrotic areas and not larger lesions *per se*.

Table 18 contains the GCA effects for lesion width and Table 19 contains the SCA effects. The GCA effects follow the trend seen in the individual hybrid data discussed above. The inbred with the best GCA for lesion width was B68 (-0.42). This inbred also had one of the best GCA values for gray leaf spot score (-0.3). The next best GCA for lesion width was B73 (-0.30), which had the worst GCA value for gray leaf spot score (0.6). This indicates that lesion width was not linked with gray leaf spot scores representing field reaction. When a correlation coefficient was calculated between the gray leaf spot field score and lesion width (Table 20) a non-significant correlation was obtained (0.070). This further confirms that lesion width is not correlated with field evaluated reaction.

Table 16. ANOVA for lesion width (mm) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	1	18.95	94.35**
Reps(Location)	2	0.07	0.35
Hybrids	89	0.47	2.34**
Locations x Hybrids	89	0.21	1.03
Error	155	0.20	

R^2 = .726

C.V. = 21.49

LSD(.05) = 0.62

**Significant at .01 level

Table 17. Mean lesion width in mm for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	1.44	1.90	1.84	2.30	1.61	1.99	2.28	2.06	1.78	2.55	2.32	1.61	2.14
B68		1.44	1.58	1.86	1.70	1.58	1.69	1.85	1.95	1.91	1.60	1.78	1.62
B73			1.79	1.13	1.60	1.94	1.27	1.84	2.30	2.05	1.98	1.48	2.70
H93				2.03	1.90	2.36	2.62	2.36	2.21	2.38	2.24	1.67	1.97
KB1250					2.01	2.65	1.96	2.16	2.33	2.42	2.41	1.99	1.78
Mo17						2.14	1.84	1.78	2.19	2.57	2.29	2.36	2.23
NC250							1.92	1.90	1.71	2.01	2.88	2.37	1.75
Pa875								1.72	2.02	2.11	2.75	2.32	1.97
Pa91									1.72	2.89	2.17	2.25	2.07
Va14										2.58	2.64	2.41	2.41
Va17											2.88	2.54	2.55
Va22												2.44	2.74
Va35													2.29

Table 18. General combining ability effects from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	GCA effects				
Inbred	GLS* score	Lesion width	Lesion length	Lesion area	Days to silk
A632	0.3	-0.10	-0.59	-4.98	-3.1
B68	-0.3	-0.42	-1.04	-9.56	0.4
B73	0.6	-0.30	-0.87	-6.88	-0.5
H93	0.3	-0.01	1.19	2.45	0.5
KB1250	-0.2	0.00	0.75	4.25	0.1
Mo17	0.1	-0.07	-3.36	-9.14	-0.7
NC250	-0.2	0.01	0.12	-0.48	-0.2
Pa875	-0.5	-0.05	-2.65	-5.97	0.7
Pa91	0.5	-0.02	-0.27	-2.22	0.8
Va14	-0.4	0.10	1.68	5.31	1.8
Va17	-0.2	0.37	1.29	10.46	1.2
Va22	0.3	0.36	0.34	7.78	0.8
Va35	0.0	0.04	2.04	5.48	-0.4
Va85	-0.3	0.10	1.36	3.52	-1.3

*Gray Leaf Spot Score

Table 19. Specific combining ability effects for mean lesion width from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-.12	.23	-.13	.32	-.29	.00	.35	.10	-.30	.21	-.01	-.41	.06
B68		.08	-.07	.20	.11	-.10	.08	.21	.19	-.11	-.42	.09	-.14
B73			.02	-.65	-.11	.15	-.46	.08	.42	-.09	-.15	-.34	.82
H93				-.04	-.10	.27	.60	.31	.04	-.06	-.19	-.44	-.20
KB1250					.00	.56	-.07	.10	.15	-.03	-.03	-.13	-.40
Mo17						.11	-.13	-.21	.08	.19	-.08	.31	.12
NC250							-.13	-.17	-.49	-.45	.43	.24	-.44
Pa875								-.29	-.12	-.29	.36	.25	-.16
Pa91									-.44	.47	-.25	.16	-.08
Va14										.03	.11	.19	.13
Va17											.07	.05	.01
Va22												-.04	.20
Va35													.07

The results for lesion length were similar to those obtained for lesion width. The ANOVA (Table 21) for lesion length, like the lesion width ANOVA, contains a significant location effect and a significant hybrid effect. The mean lesion lengths are given in Table 22 and the LSD(.05) is given in Table 21 (7.42 mm). The susceptible hybrid discussed above (B73 X Mo17) had a mean lesion length of 15.6 mm and the resistant hybrid discussed above (H93 X Pa875) had a lesion length of 25.9 mm. Like the lesion width data given in the above paragraph, this is contrary to perceptions.

Table 18 contains the GCA effects for lesion length and Table 23 contains the SCA effects. The GCA effects followed the trend seen in the individual hybrid data discussed above. The inbred with the best GCA for lesion length was Mo17 (-3.36). This inbred had intermediate GCA values for gray leaf spot score (0.1). The next best inbred for lesion length GCA was Pa875 (-0.30) which had the best GCA value's for gray leaf spot score (-0.5). This indicated that lesion length like lesion width was not very strongly linked with field evaluations of resistance. When a correlation coefficient was calculated between the gray leaf spot field score and lesion length (Table 20), a no significant correlation was obtained (0.022). This, like the lesion width data, confirms that lesion length is not correlated with field reaction.

Table 20. Correlation coefficients from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Character	% lodged	Days to silk	Virus score	GLS+	% Moisture
Yield	-0.286*	0.590*	-0.179*	0.472*	0.160
% lodged		-0.281*	-0.029	-0.166*	0.004
Days to silk			0.294*	-0.258*	0.043
Virus score				-0.099	-0.207*
GLS+					-0.306*
Lesion width				0.070	
Lesion length				0.022	
Lesion area				0.045	

*Significant at .05 level

+Gray Leaf Spot Score

Table 21. ANOVA of mean lesion length (mm) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	1	4074.98	142.05**
Reps(Location)	2	174.59	6.09**
Hybrids	89	55.78	1.94*
Locations x Hybrids	89	38.12	1.33
Error	155	28.69	

R^2 = .745

C.V. = 25.27

LSD(.05) = 7.42

*Significant at .05 level

**Significant at .01 level

Table 22. Mean gray leaf spot Lesion lengths (mm) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Inbred line	Inbred line												
	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	16.3	20.8	23.0	19.5	18.8	25.1	20.0	21.2	21.3	19.9	22.1	18.5	20.4
B68		18.8	21.5	22.5	19.4	18.8	15.3	19.6	20.7	22.5	18.7	22.9	24.5
B73			19.9	24.2	15.6	19.9	15.1	18.3	28.3	22.7	20.4	16.4	23.2
H93				19.2	12.6	25.9	25.9	26.3	24.1	29.0	23.1	17.1	20.5
KB1250					15.5	29.4	19.1	21.0	31.1	19.8	21.0	19.8	20.9
Mo17						18.6	16.7	12.9	21.4	16.7	14.9	26.1	24.6
NC250							20.3	16.6	17.5	18.3	19.2	25.7	20.2
Pa875								19.9	14.2	15.2	22.1	21.3	16.9
Pa91									21.5	24.7	21.1	24.3	23.2
Va14										25.7	25.2	27.5	15.6
Va17											24.4	26.8	23.8
Va22												20.7	25.1
Va35													25.1

Table 23. Specific combining ability effects for gray leaf spot lesion length (mm) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-3.2	1.2	1.3	-1.8	1.7	4.5	2.2	1.0	-0.9	-1.9	1.3	-4.0	-1.5
B68		-0.4	0.3	1.7	2.7	-1.4	-2.1	-0.1	-1.0	1.2	-1.7	0.8	3.1
B73			-1.5	3.3	-1.3	-0.4	-2.5	-1.6	6.4	1.2	-0.2	-5.9	1.6
H93				-3.8	-6.3	3.5	6.3	4.3	0.2	5.5	0.5	-7.2	-3.1
KB1250					-3.0	7.5	-0.1	-0.5	7.6	-3.3	-1.2	-4.1	-2.3
Mo17						0.8	1.7	-4.6	2.0	-2.4	-3.2	6.4	5.5
NC250							1.8	-4.4	-5.4	-4.2	-2.3	2.5	-2.4
Pa875								1.7	-5.9	-4.5	3.3	0.9	-2.9
Pa91									-0.9	2.6	0.0	1.4	1.1
Va14										1.6	2.1	2.7	-8.5
Va17											1.7	2.4	0.1
Va22												-2.8	2.4
Va35													6.9
Va85													

When mean lesion area (mm^2) was calculated, the results followed those of the length and width data. The ANOVA for lesion area is given in Table 24. These results paralleled the ANOVA tables for length and width with significant location and hybrid effects. The mean lesion area values (mm^2) are given in Table 25 (LSD(.05) = 25.95 mm^2). Like the above length and width data, the susceptible hybrid (B73 X Mo17) had one of the smallest lesion areas (25.0 mm^2) and the resistant hybrid (H93 X Pa875) had one of the largest lesion areas (73.6 mm^2).

The GCA effects for lesion area (mm^2) are given in Table 18 and the SCA effects for lesion area are given in Table 26. Following the above trends, the line that had the best GCA for lesion area was B68 (-9.56), followed by Mo17 (-9.14) and B73 (-6.88). B68 had a resistant field reaction (GCA for gray leaf spot score = -0.3), Mo17 had an intermediate field reaction (GCA for gray leaf spot score = 0.1), and B73 had a susceptible field reaction (GCA for gray leaf spot score = 0.6). All of this further confirms that lesion size characteristics are not correlated with gray leaf spot scores. When a correlation coefficient is calculated between the gray leaf spot field score and lesion area (Table 20), no significant correlation was obtained (0.045).

Table 24. ANOVA for mean lesion area (mm^2) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	1	47336.12	135.01**
Reps(Location)	2	711.20	2.03
Hybrids	89	747.91	2.13*
Locations x Hybrids	89	409.28	1.17
Error	155	350.61	

R^2 = .741

C.V. = 40.25

LSD(.05) = 25.95

*Significant at .05 level

**Significant at .01 level

Table 25. Mean gray leaf spot lesion area (mm^2) for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Inbred line	Inbred line												
	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	24.4	39.8	41.3	44.5	31.9	49.7	46.9	44.9	39.4	54.4	52.8	31.5	44.0
B68		29.8	33.8	73.8	34.4	30.7	27.5	37.7	41.4	44.9	30.3	42.2	39.5
B73			39.3	51.6	25.0	41.0	19.1	37.9	70.8	47.4	41.8	25.0	54.2
H93				38.9	24.7	62.7	73.6	63.3	53.9	74.9	55.4	29.8	43.2
KB1250					33.0	77.1	41.3	46.2	72.4	49.5	50.7	39.9	37.3
Mo17						39.8	33.6	22.6	49.5	45.0	36.7	64.7	54.7
NC250							41.3	31.8	30.0	36.9	55.7	68.1	34.5
Pa875								34.2	27.9	33.7	62.3	50.9	41.4
Pa91									37.3	71.4	47.9	55.4	48.0
Va14										66.5	67.1	68.2	44.6
Va17											74.3	69.2	62.7
Va22												53.1	70.4
Va35													73.1

Table 26. Specific combining ability effects from a gray leaf spot lesion area for a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	-7.6	5.1	-2.8	-1.3	-0.6	8.6	11.3	5.6	-7.4	2.3	3.5	-15.6	-1.1
B68		-0.4	-5.7	32.6	6.6	-5.8	-3.5	3.0	-0.9	-2.5	-14.5	-0.23	-1.0
B73			-2.8	7.7	-5.5	1.8	-14.6	0.5	25.8	-2.8	-5.7	-20.2	11.0
H93				-14.3	-15.2	14.1	30.6	16.5	-0.4	15.4	-1.4	-24.7	-9.3
KB1250					-8.7	26.8	-3.6	-2.4	16.3	-11.8	-7.8	-16.4	-17.0
Mo17						2.9	2.1	-12.6	6.8	-2.8	-8.5	21.8	13.8
NC250							1.2	-12.1	-21.4	-19.6	1.9	16.5	-15.1
Pa875								-4.2	-18.0	-17.4	13.9	4.9	-2.8
Pa91									-12.4	16.6	-4.2	5.6	0.1
Va14										4.2	7.5	10.8	-10.8
Va17											9.5	6.7	2.2
Va22												-6.7	12.5
Va35													17.5
Va85													

Table 27 contains the ANOVA for gray leaf spot field reaction and is included for comparison with the lesion data. Field results have been discussed in Chapter 2 and will not be discussed here. Lesion width, length, and area and gray leaf spot score for each hybrid are given in Table 28 to allow comparison between individual hybrid lesion characteristics and their gray leaf spot field reaction. As stated above, lesion characteristics did not relate to gray leaf spot field reaction. The fact that lesion width, length, and area did not affect the resistance level of the hybrid implies that resistance to the toxin (cercosporin) after infection may not be involved in lesion restriction and resistance to gray leaf spot.

Other Associations

Many of the genotypes which displayed resistance in 1987 and 1988 had a red halo around their lesions. This was especially strong in Va14, Va17, and Va85 hybrids. Anthocyanin mutants were also planted in these years as marker rows. These mutants are green before anthesis but darken after anthesis until completely red. It was noted that when these plants were green, the plants had numerous lesions. Following the reddening of the leaves the lesions were few. Since anthocyanins are quenchers of free radicals such as those generated by cercosporin and the production of these compounds occurred in resistant genotypes, it seems possible that these mutants may prove to be a source of some resistance.

Table 27. ANOVA for gray leaf spot scores from a 14 line diallel grown in Montgomery and Wythe Counties, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	2	223.85	2146.67**
Reps(Location)	6	1.28	12.25**
Hybrids	90	2.22	21.31**
GCA	13	12.83	128.30**
SCA	77	0.43	4.04**
Locations x Hybrids	180	0.42	4.04**
Error	540	0.10	

R^2 = .928

C.V. = 12.56

LSD(.05) = 0.29

**Significant at .01 level

Table 28. Lesion width (mm), length (mm), and area (mm^2), and gray leaf spot score (GLS) for a 14 line diallel. Lesion data are from two locations and two replications and GLS data are from three locations and three replications.

Hybrid	Lesion width	Lesion length	Lesion area	GLS score
A632 X B68	1.44	16.27	24.37	2.2
A632 X B73	1.90	20.78	39.77	3.5
A632 X H93	1.83	22.95	41.25	3.7
A632 X KB1250	2.29	19.49	44.52	2.8
A632 X Mo17	1.61	18.82	31.88	3.0
A632 X NC250	1.99	25.11	49.71	2.7
A632 X Pa875	2.28	20.04	46.85	2.4
A632 X Pa91	2.06	21.23	44.90	3.6
A632 X Va14	1.78	21.31	39.44	2.3
A632 X Va17	2.55	19.86	54.36	2.6
A632 X Va22	2.32	22.08	52.82	3.0
A632 X Va35	1.60	18.54	31.46	2.9
A632 X Va85	2.13	20.36	44.04	2.5
B68 X B73	1.44	18.79	29.75	2.6
B68 X H93	1.58	21.46	33.75	2.5
B68 X KB1250	1.86	22.49	43.77	2.2
B68 X Mo17	1.70	19.39	34.39	2.2
B68 X NC250	1.57	18.79	30.74	2.4
B68 X Pa875	1.69	15.30	27.53	2.2
B68 X Pa91	1.84	19.64	37.73	2.8
B68 X Va14	1.95	20.66	41.41	2.2
B68 X Va17	1.91	22.52	44.90	2.1
B68 X Va22	1.60	18.71	30.27	2.5
B68 X Va35	1.78	22.84	42.23	2.2
B68 X Va85	1.62	24.50	39.47	2.3
B73 X H93	1.79	19.94	39.33	3.7
B73 X KB1250	2.13	24.20	51.59	3.3
B73 X Mo17	1.60	15.56	25.00	3.6
B73 X NC250	1.94	19.89	40.99	3.0
B73 X Pa875	1.27	15.06	19.10	2.3
B73 X Pa91	1.83	18.33	37.92	3.5
B73 X Va14	2.30	28.29	70.78	2.5
B73 X Va17	2.05	22.70	47.35	2.9
B73 X Va22	1.98	20.40	41.78	3.4
B73 X Va35	1.48	16.37	24.97	3.2
B73 X Va85	2.00	23.19	54.21	2.7
H93 X KB1250	2.02	19.22	38.90	2.8
H93 X Mo17	1.90	12.59	24.69	2.7
H93 X NC250	2.35	25.92	62.65	2.7
H93 X Pa875	2.62	25.93	73.62	1.9

(continued)

Table 28(continued). Lesion width (mm), length (mm), and area (mm^2), and gray leaf spot score (GLS) for a 14 line diallel. Lesion data are from two locations and two replications and GLS data are from three locations and three replications.

Hybrid	Lesion width	Lesion length	Lesion area	GLS score
H93 X Pa91	2.36	26.28	63.27	3.7
H93 X Va17	2.38	29.01	74.86	2.7
H93 X Va22	2.24	23.12	55.37	3.2
H93 X Va35	1.67	17.09	29.76	2.7
H93 X Va85	1.97	20.53	43.17	2.5
KB1250 X Mo17	2.01	15.50	32.96	2.4
KB1250 X NC250	2.65	29.39	77.13	2.3
KB1250 X Pa875	1.96	19.06	41.25	2.1
KB1250 X Pa91	2.15	21.01	46.18	2.6
KB1250 X Va14	2.33	31.07	72.40	2.0
KB1250 X Va17	2.42	19.81	49.46	2.2
KB1250 X Va22	2.40	20.99	50.73	2.5
KB1250 X Va35	1.99	19.75	39.86	2.3
KB1250 X Va85	1.77	20.93	37.29	2.0
Mo17 X NC250	2.14	18.59	39.80	2.3
Mo17 X Pa875	1.84	16.70	33.56	2.1
Mo17 X Pa91	1.78	12.86	22.55	3.2
Mo17 X Va14	2.19	21.34	49.45	2.3
Mo17 X Va17	2.56	16.66	45.02	2.3
Mo17 X Va22	2.29	14.90	36.66	2.8
Mo17 X Va35	2.35	26.09	64.72	2.7
Mo17 X Va85	2.23	24.55	54.72	2.5
NC250 X Pa875	1.92	20.30	41.33	2.1
NC250 X Pa91	1.90	16.56	31.75	2.7
NC250 X Va14	1.71	17.48	30.00	1.9
NC250 X Va17	2.01	18.25	36.88	2.1
NC250 X Va22	2.88	19.20	55.74	2.7
NC250 X Va35	2.37	25.70	68.06	2.3
NC250 X Va85	1.75	20.15	34.46	2.2
Pa875 X Pa91	1.72	19.88	34.17	1.7
Pa875 X Va14	2.02	14.24	27.86	2.7
Pa875 X Va17	2.11	15.25	33.69	1.7
Pa875 X Va22	2.75	22.06	62.28	1.6
Pa875 X Va35	2.32	21.34	50.91	2.2
Pa875 X Va85	1.97	16.89	41.35	2.1
Pa91 X Va14	1.72	21.55	37.27	2.0
Pa91 X Va17	2.89	24.65	71.42	2.7
Pa91 X Va22	2.17	21.13	47.92	3.6
Pa91 X Va35	2.25	24.27	55.37	3.3

(continued)

Table 28(continued). Lesion width (mm), length (mm), and area (mm^2), and gray leaf spot score (GLS) for a 14 line diallel. Lesion data are from two locations and two replications and GLS data are from three locations and three replications.

Hybrid	Lesion width	Lesion length	Lesion area	GLS score
Pa91 X Va85	2.07	23.20	47.95	2.6
Va14 X Va17	2.58	25.67	66.48	2.7
Va14 X Va22	2.64	25.19	67.09	2.3
Va14 X Va35	2.41	27.50	68.17	2.1
Va14 X Va85	2.41	15.61	44.55	1.9
Va17 X Va22	2.88	24.43	74.28	2.7
Va17 X Va35	2.54	26.78	69.15	2.4
Va17 X Va85	2.55	23.77	62.71	2.0
Va22 X Va35	2.44	20.68	53.09	2.9
Va22 X Va85	2.74	25.12	70.36	2.7
Va35 X Va85	2.29	31.41	73.05	2.7

We also studied the relationship of gray leaf spot to maturity. Table 18 contains the GCA effects for gray leaf spot score and days to silk. A632 was the earliest inbred in the diallel with a GCA for days to silk of -3.1 and was also susceptible to gray leaf spot (GCA=0.3). Va14 was the latest line (GCA=1.8) in the diallel and was one of the most resistant of the inbreds (GCA=-0.4). Between these two lines, early and late lines varied randomly for their reaction to gray leaf spot. Table 20 contains the correlation coefficient for days to silk and gray leaf spot, which was a significant but not very predictive -0.258. This correlation was most likely caused by the fact that the earliest inbred was the most susceptible and the latest inbred the most resistant and not by any strong trend in the data.

If resistance to cercosporin in the leaf tissue were the only resistance mechanism, lesion size on the leaf would be expected to be strongly correlated with reaction to the pathogen. This was not the case; some of the most susceptible hybrids had the smallest lesion measurements and some of the largest lesion measurements occurred on the most resistant hybrids. This indicates that reaction to the pathogen occurs during infection of the leaf and that once infection takes place, resistance is not a function of the plant restricting further colonization of tissue and leaf. The resistance observed in the anthocyanin mutants seems to affect infection of the leaf and not colonization of the leaf.

This suggests that resistance to cercosporin is not the main mechanism of field resistance and that selection of cercosporin resistant clones by tissue culture may not produce genotypes with field resistance. Resistance must, therefore, be involved with the ability of resistant genotypes to prevent infection by hypersensitivity reactions or by the inability of the fungus to recognize certain

genotypes as being susceptible and thus not beginning to produce cercosporin quickly.

Resistance to gray leaf spot as shown by these data is largely a quantitative trait. From our data, no one trait seems to be associated with resistance; rather several traits appear to act together for final expression of field resistance. While maintenance of photosynthesis levels and anthocyanin production seem to have an effect on reaction to the pathogen and some resistant inbreds tend to have small lesions, no strong physiological association was found between these traits and the level of disease development.

Anthocyanin production may be important in delaying or stopping early colonization of the leaf but does not seem to be important in later restriction of lesion formation. Several of the most resistant types (Va14,Va17, and Va85) had red borders on lesions, but this production of pigment did not stop later lesion development and growth. Either there is a different pigment in these plants or anthocyanin production is not linked to reaction after colonization. These anthocyanins may slow or stop early colonization.

Our preliminary study on photosynthesis and resistance suggests indirectly that chloroplast vigor late in the season may be linked with resistance. This would explain why gray leaf spot does not occur before anthesis and why resistance work involving small greenhouse plants has been inconclusive. Chloroplast vigor late in the season may also be a possible mechanism for quantitative resistance to other leaf blights.

Lesion size had no association with gray leaf spot resistance. Resistance mechanisms must operate during initial colonization and establishment of the

fungus, and not later in the colonization process. This was a surprising, unexpected result and suggests that future studies on the physiological mechanisms of resistance need to concentrate on infection, and not later colonization and lesion development.

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Resistance to Maize Dwarf Mosaic Virus in a Diallel Cross of Maize Inbreds

By
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ABSTRACT

Maize dwarf mosaic virus (MDMV) limits maize production in some areas where johnsongrass (*Sorghum halepense* L.) is a problem. Resistance to MDMV was studied using all possible crosses of 14 elite inbreds. The diallel study was conducted in Virginia in 1987 and 1988. The experimental designs were randomized complete blocks. Data on virus scores, lodging, yield, and moisture were taken during the season. Analysis of variance and combining ability analyses were performed on all traits. Resistance was found to be mainly additive and highly heritable. However, a strong specific combining ability component was found indicating that the background of the material receiving the resistance genes may have a strong effect on the expression of resistance. This has important consequences for gene transfer techniques. Many of the inbreds that have been widely used (B73, Pa91, and Mo17) were found to be susceptible to this virus, explaining why many of the commercial hybrids do not show high levels of resistance. High yielding, resistant, and agronomically acceptable inbreds are available (B68, NC250, A632, Pa875, Va17, and Va85) and several hybrids between these lines have high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875). This suggests that for the short term, hybrids are available to seed producers and growers with a high level of resistance and that these lines could serve as a basis for a breeding programs stressing virus resistance.

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Maize dwarf mosaic (MDMV) and maize chlorotic dwarf (MCDV) viruses can be limiting factors in maize production in areas where johnsongrass (*Sorghum halepense* L.) is not controlled. Depending on when the vector(s) transfers the virus particles to the plant and the susceptibility of the host, yield losses can range from slight to complete loss of grain production. Using inbred lines Va85, Oh7B, Pa405, B68, and others, one of the genes controlling resistance to MDMV has been shown to occur on maize chromosome 6 and is linked to genes conditioning endosperm color (y1) and endosperm type (su2) (Roane *et al.*, 1989a, 1989b). Using a scale of 1 to 7 and by accepting a partitioned display of symptoms, a major gene controlling resistance was described by Roane *et al.* (1989a).

Many others have studied MDMV resistance and have estimated from 1 to 10 genes controlling it (Findley *et al.*, 1973, 1977; Mikel *et al.*, 1984; Rosenkranz and Scott; 1984). As might be expected from this range of results, the issue is less than clear on the number and function of genes controlling resistance to MDMV.

A 14-line diallel that was initially made for study of reaction to *Cercospora zeae-maydis* was planted in an area were the plants where exposed to natural MDMV inoculum and some maize chlorotic dwarf mosaic virus. Inoculation took place when surrounding johnsongrass was mowed when the plants were 7 to 9 inches tall, forcing the vectors to move to new live plant tissue. Two of the lines in Roane's studies (1989a, 1989b) were included in this diallel (B68 and Va85) and are thought to contain a single dominant gene giving a high level of resistance. This gave us the opportunity to study the effects of other genes working with the major resistance genes and to see how these lines performed from a quantitative aspect.

MATERIALS AND METHODS

Fourteen inbred lines (Table 5) were crossed in all possible combinations in Virginia during the 1985 and 1986 growing season. Because of heat and drought stress, many of the pollinations were not successful. The crosses were repeated in 1986 at the Purdue Agricultural Alumni winter nursery in Homestead, Florida. The reciprocal crosses were combined to make 91 hybrids in this experiment. Because of land limitations, we did not explore any possible maternal effects.

The diallel crosses were grown in randomized complete block designs with three replications in Montgomery County, VA for two years. Response to MDMV was scored on a scale of 1 to 9 (Dollinger *et al.*, 1970). The plots were scored on the overall appearance of the center two rows. While most of the damage to the plots was done by MDMV, some maize chlorotic dwarf virus was also present at low levels. Since MCDV is spread at a much lower frequency than MDMV, these scores represent mostly MDMV. Gray leaf spot scores were also taken on this same test as part of a separately reported gray leaf spot study and are given in Chapter 2.

Plots were four rows wide, planted at a density of 57,000 plants per hectare. The rows were 81.3 cm wide and 7.6 m long, making a plot size of 6.2 m^2. Data on grain yield and moisture percentage, reaction to MDMV and gray leaf spot, number of plants, and number of lodged plants were recorded during the growing season on the center two rows of each plot. Silking dates were recorded to estimate hybrid maturity.

Yield, lodging, and disease scores were analyzed using Model I analysis of Eisenhart (1947), along with Method 4 of Griffing (1956). These analyses assumes that all factors except error terms are fixed, since the lines used are not a random sample. Combining abilities were calculated as described by Sprague and Tatum (1942).

RESULTS AND DISCUSSION

Table 28 contains the analysis of variance (ANOVA) for virus scores. As shown by the significant F value, there were differing levels of disease in 1987 and 1988. The main cause of this likely was a difference in the time when the vector(s) moved into the plot as related to stage of growth. The earlier the plant is infected, the more severe the symptoms. In both years the plants were infected after adjoining land areas containing johnsongrass were mowed, causing the vector(s) to move into the plots. This proved to be a very effective form of inoculation, with susceptible plots being close to 100 % infected.

Significant differences also occurred between hybrids in the diallel as shown by the significant F value. Table 29 contains the mean virus scores for the diallel. The LSD(0.05) for these data was calculated to be 0.9, indicating that there was good separation of the genotype means. Scores ranged from 6.9 for a cross where both parents were susceptible (Va14 x Va17) to 1.0 for the resistant x resistant cross, A632 x Pa875, with other scores being distributed between these two values.

Table 28. Anova for virus score for a 14 line diallel grown in Montgomery County, VA in 1987 and 1988.

Source of variation	DF	Mean square	F value
Locations	1	23.22	36.10*
Reps(Location)	4	.62	0.97
Hybrids	90	13.99	12.75**
GCA	13	56.36	88.06**
SCA	77	10.69	16.70**
Locations x Hybrids	90	4.89	7.61**
Error	360	0.64	

R2 = .88

C.V. = 31.68

LSD(.05) = 0.9

*Significant at the .05 level

**Significant at the .01 level

Table 29. Mean virus scores for a 14 line diallel grown in Montgomery County, VA in 1987 and 1988.

	Inbred line												
Inbred line	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	1.8	1.5	1.4	1.4	1.1	1.9	1.0	1.8	1.8	1.9	1.6	1.9	1.3
B68		2.5	1.4	1.3	1.1	1.2	1.7	1.2	2.6	2.0	1.3	1.1	1.0
B73			3.4	3.7	2.4	3.6	1.7	2.5	4.3	1.5	1.7	1.9	2.5
H93				4.7	1.5	4.1	4.6	1.6	3.9	1.5	1.3	1.2	1.7
KB1250					3.0	5.9	2.0	4.6	5.0	4.1	1.8	1.7	1.7
Mo17						4.4	1.8	5.1	6.7	5.4	3.4	1.8	1.7
NC250							1.3	3.6	6.6	4.4	3.1	1.5	1.0
Pa875								1.2	2.1	1.4	1.4	1.6	2.8
Pa91									6.4	5.6	2.4	2.7	1.7
Va14										6.9	5.1	2.5	1.9
Va17											2.5	2.6	1.7
Va22												1.4	1.2
Va35													1.0

Hybrids made from lines previously identified as having a single dominant gene had a wide range of virus scores. If only a single dominant gene controlled resistance, this range of reaction to virus would not be expected. If the gene for resistance was completely dominant, then all of the crosses with B68 and Va85 should have been resistant. This raises the possibility of important interacting or modifying genes that have a large effect on the major gene.

In Table 28 the specific combining ability effects (SCA) and general combining ability effects (GCA) are partitioned from the hybrid effects. The GCA effects were significant and are greater than the SCA effects, but the SCA effects are also significant and large. While virus resistance is moderately heritable, the presence of these SCA interactions may mean that some complex gene interactions are taking place and suggests that larger population sizes should be used when breeding for virus resistance than would normally be used for a single dominant gene.

The GCA effects for virus score, yield, percent lodged, and days to silk are given in Table 30. Inbred lines A632 and B68 had the strongest resistance (indicated by a negative estimate) and Va14 was the most susceptible (indicated by a positive estimate). Pa875, Va22, and Va85 also had high levels of resistance as shown by the negative GCA estimates. Lines KB1250, Mo17, NC250, Pa91, and Va17 all had positive GCA estimates, reflecting their susceptibility. Interestingly, B73 and H93 had an intermediate GCA, indicating that they had some resistance genes but not a complete set. These lines have been considered completely susceptible by many researchers, but on the basis of these data, they seem to contain some resistance genes. When looking at the range of GCA effects and the range of MDMV scores in Table 29, one would not conclude that they fit a single gene model.

Table 30. General combining ability effects for a 14 line diallel grown in Montgomery County, VA in 1987 and 1988.

	GCA effects				
Inbred	Yield kg/ha	Virus score	GLS* score	Percent lodged	Days to silk
A632	-122.8	-1.1	0.3	1.1	-3.1
B68	112.7	-1.1	-0.3	-3.6	0.4
B73	402.0	0.0	0.6	1.4	-0.5
H93	154.6	-0.1	0.3	-0.9	0.5
KB1250	119.7	0.6	-0.2	-0.8	0.1
Mo17	314.5	0.5	0.1	-1.9	-0.7
NC250	-62.7	0.8	-0.2	0.9	-0.2
Pa875	236.0	-0.7	-0.5	-2.8	0.7
Pa91	-16.4	0.6	0.5	-1.4	0.8
Va14	-929.4	1.9	-0.4	-1.3	1.8
Va17	-854.8	0.7	-0.2	0.6	1.2
Va22	-100.0	-0.4	0.3	0.1	0.8
Va35	140.5	-0.9	0.0	2.1	-0.4
Va85	605.8	-1.0	-0.3	6.4	-1.3

*Gray Leaf Spot

The SCA effects in Table 31 also suggest that the gene action involved is not simply additive. Unlike the gray leaf spot data for this diallel, which contained a large number of SCA effects close to or at zero, the SCA effects here vary more and are much more similar to SCA effects normally associated with yield. This suggests that non-additive gene action may also be important in virus resistance.

It has been a common belief of many maize breeders that gray leaf spot resistance is linked to virus resistance (personal information). Table 30 contains the GCA effects for both gray leaf spot score and virus score. A comparison of the two sets of values indicates that this is not true. A632 and B68 have the best (most negative) GCA effects for MDMV. While B68 is resistant to gray leaf spot (most negative GCA), A632 is not. Also the most susceptible line to virus as shown by the GCA effects (1.9) was Va14, which was one of the most resistant lines to gray leaf spot (GCA=-0.4). This would indicate that the traits are in no way linked. Table 32 confirms this with a non-significant correlation coefficient of -0.099 between the gray leaf spot and virus scores.

B68 has been widely used in the south by maize breeders and commercial companies in both breeding nurseries and commercial hybrids (personal information). The fact that B68 is resistant to both pathogens has caused some breeders to theorize that the two traits are linked. Our data show that this is not true.

Table 32 contains correlation coefficients for the various traits evaluated in the experiment. The correlation between gray leaf spot score and MDMV score was of

Table 31. Specific combining ability effects for virus score for a 14-line diallel grown in Montgomery County, VA in 1987 and 1988.

	Inbred line												
Inbred	B68	B73	H93	KB1250	Mo17	NC250	Pa875	Pa91	Va14	Va17	Va22	Va35	Va85
A632	1.4	0.0	0.0	-0.7	-0.9	-0.4	0.2	-0.3	-1.6	-0.3	0.5	1.3	0.8
B68		1.0	0.0	-0.8	-0.9	-1.1	0.9	-0.9	-0.8	-0.2	0.2	0.5	0.5
B73			0.9	0.5	-0.7	0.3	-0.1	-0.7	-0.1	-1.8	-0.4	0.2	0.9
H93				1.6	-1.5	0.8	2.8	-1.5	-0.5	-1.7	-0.8	-0.4	0.2
KB1250					-0.7	1.9	-0.5	0.8	-0.1	0.2	-1.0	-0.6	-0.5
Mo17						0.5	-0.6	1.4	1.7	1.6	0.7	-0.4	-0.4
NC250							-1.3	-0.3	1.4	0.4	0.2	-1.0	-1.3
Pa875								-1.2	-1.6	-1.1	0.0	0.6	2.0
Pa91									1.4	1.7	-0.3	0.4	-0.5
Va14										1.8	1.1	-1.1	-1.5
Va17											-0.3	0.2	-0.6
Va22												0.1	0.1
Va35													0.3

Table 32. Correlation coefficients for all characters measured in a 14 line diallel grown in Montgomery County, VA in 1987 and 1988.

Character	% lodged	Days to silk	Virus score	GLS[+] score	Percent moisture
Yield	-0.286*	0.590*	-0.179*	0.472*	0.160
% lodged		-0.281*	-0.029	-0.166*	0.004
Days to silk			0.294*	-0.258*	0.043
Virus score				-0.099	-0.207*
GLS					-0.306*

*Significant at .05 level

[+]gray leaf spot

main interest. The others are included for comparison. Two of the strongest correlations are between days to silk and yield, indicating that the later hybrids tend to yield more. The other correlation of interest is that of gray leaf spot and yield, which is opposite of expectations. This may be an artifact resulting from the fact that many of the susceptible lines included in this study have been used for their high yield potential in the commercial seed industry and many of the resistant inbreds included in the study do not combine well for yield. The other significant correlation values are small and are not very predictive, indicating that gray leaf spot and virus reaction are not linked with the other traits.

These results, taken with the results of Roane (1989) and others, suggest that while a major gene exists for virus resistance, interacting and modifying genes are also important in expression of resistance. This has important consequences for biotechnologically based breeding programs. It is not enough to identify and move only the major gene(s) when breeding for resistance. As shown in this study, additive genes are also important. These data suggests that the background of the parents used for line improvement is as important as the major genes being transferred. This also means that field testing of resistance genes on a wide variety of genotypes is essential to make maximum use of resistance.

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SUMMARY AND DISCUSSION

The strong effect gray leaf spot can have when environmental conditions are favorable is shown by the fact that significant yield losses due to gray leaf spot pressure occurred in two of six years at the Shenandoah County site, one of six years at the Wythe County site, and in 1988 at the Montgomery County site and that premature dry-down due to early senescence occurred in five of six years at Shenandoah County, six of six years at the Wythe County site, and at the Montgomery County site. Yield losses from the resistant to susceptible class were approximately 2,000 kg ha^{-1} at Wythe County in 1982, 750 kg ha^{-1} at Shenandoah County in 1984, and 2,150 kg ha^{-1} at Montgomery County in 1988. Along with this, a significant increase in lodging associated with gray leaf spot occurred in two of six years at the Shenandoah County site and five of six years at the Wythe County site.

Since years favorable for gray leaf spot development are also those years with high moisture levels, these losses are not always apparent to the producer. Many times this early senescence is attributed to other factors, but the economic losses of this disease are now apparent. These losses can be as high as 30 percent, resulting in a loss as high as 185 to 370 dollars per hectare. The economic value of using resistant varieties is therefore great, and the development of more resistant varieties is important to Virginia's economic base.

Since resistance to gray leaf spot was found to be highly heritable and controlled by additive gene action, and high yielding, resistant, and agronomically acceptable inbreds were identified (B68, NC250, Pa875, Va14, Va17, and Va85), selection of adapted resistant germplasm is feasible in many public and private breeding programs. The need for this work is shown by the disclosure that many widely used inbreds (B73, Pa91, Mo17, and A632) are susceptible to highly susceptible to gray leaf spot. This demonstrates why many of the commercially available hybrids do not show high levels of resistance. For short term use, several hybrids between these lines had high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875). These lines could be used to produce resistant hybrids for use in Virginia.

Gray leaf spot lesion size measurements were not correlated with disease scores. This was a surprise that ran contrary to our perceptions. Clearly, retarding lesion number, not lesion size, is the primary mechanism of resistance in this set of germplasm. This would imply that selection of resistant germplasm in a breeding nursery needs to be made under field conditions since lesion type is not an indictor of resistance. A high inoculum density would be ideal for germplasm evaluation and would most likely be found in a plot located within a field with highly infested maize debris and with the high humidities required for development of this disease.

Late season photosynthesis rates were positively associated with resistance. This reveals that, like stalk rot and other diseases of senescence, plant vigor and specifically photosynthesis vigor is involved with resistance. Vigorous tissue with

strong photosynthesis levels may be able to resist initial infection and colonization much better than tissue that is beginning to senesce. It would be interesting to see if selection for resistant germplasm would be possible using a photosynthesis meter.

The hybrids of some inbreds were found to produce high levels of pigment (anthocyanins) around the gray leaf spot lesions. This, however, did not seem to limit the size of the lesion later in the season. Some high anthocyanin producing genotypes were found to be resistant when the anthocyanin character was expressed. Since this did not limit later lesion size, this type of resistance must stop early colonization or infection of the leaf and not later colonization. This suggests that the cercosporin toxin may be important for infection but not later colonization. Since disease scores were not associated with lesion size, selection of cercosporin resistant clones may not be successful in producing resistant genotypes.

Resistance to MDMV was found to be mainly additive and highly heritable. However, a strong specific combining ability component was found, indicating that the background of the material receiving the resistance genes may have a strong effect on the expression of resistance. This has important consequences for gene transfer techniques. Many of the inbreds that have been widely used (B73, Pa91, and Mo17) were found to be susceptible to virus, explaining why many of the commercially available hybrids do not show high levels of resistance. High yielding, resistant, and agronomically acceptable inbreds are available (B68, NC250, A632, Pa875, Va17, and Va85) and several hybrids between these lines have high levels of resistance, high yield, and good general agronomic characters (B68 x KB1250, KB1250 x Pa875, and NC250 x Pa875). This suggests that for the short term,

hybrids are available to seed producers and growers with a high level of resistance and that these lines could serve as a basis of a breeding program stressing MDMV resistance.

The gray leaf spot work reported herein will be an ideal platform for future biotechnology projects. Since gray leaf spot is a quantitative trait and since there is not a large non-additive portion to this resistance, it will be an ideal model for using restriction length fragment polymorphisms (RFLP) technology. Since it is a quantitative trait, selection of resistant types and classification of these types would be difficult on a phenotypic basis and requires large population sizes. To be able to identify the segments of DNA that carry resistance and to use this in selection of resistant germplasm would be a great help in a breeding program and would speed up the breeding of gray leaf spot resistant germplasm. This technology would also be helpful for selection in other quantitatively inherited traits like silking delay, drought resistance, and prolificacy, all of which may be important to sustainable maize production in Virginia.

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