BIPOLARIS ZEICOLA: PHYSIOLOGICAL RACES, MORPHOLOGY AND RESISTANCE ON MAIZE

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(ABSTRACT)

Conidial length, width, septation, curvature and pigmentation of 39 isolates of *Bipolaris zeicola* (Stout) Shoemaker from different geographical areas and representing distinct physiological races were examined. Wide variability in conidial morphology was found among isolates of each race. No association was found between races or groups of isolates producing the same shape of lesions and conidial morphology.

Morphology and expansion of lesions induced by three races of *B. zeicola* producing different shapes of lesion on susceptible maize (*Zea mays* L.) inbred lines were studied. Resistant type lesions induced by three races of *B. zeicola* were indistinguishable based on their shape or size. However, lesion

size correlated with the lesion type induced by each race, indicating that it may be useful to evaluate resistance to different races of *B. zeicola*.

Inheritance of reaction to one isolate of the proposed pathotype of *B. zeicola* was studied. Resistance was controlled by a single dominant gene in the cross Pr x B37.

Forty-nine isolates of *B. zeicola* from different geographical areas including all the previously described races and pathotype were characterized by their disease reaction, severity and symptoms incited on 14 maize inbred lines. Eleven physiological races were distinguished based on their differential reaction on the 14 inbred lines; however, 6 inbred lines were adequate to differentiate the races. A system based on binary notation is proposed to designate races of *B. zeicola* on maize. Two races induced typical symptoms of *Helminthosporium carbonum*-toxin (HC-toxin) production on Pr. Seven races produced oval to irregular lesions on susceptible hosts and did not produce typical lesions of HC-toxin production on Pr. One race induced predominantly long, linear lesions, and another was avirulent on all 14 maize inbred lines.

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LITERATURE REVIEW

Helminthosporium leaf spot of maize (*Zea mays* L.) is caused by *Bipolaris zeicola* (Stout) Shoemaker (teleomorph: *Cochliobolus carbonum* Nelson).

The imperfect stage of the fungus was described first from nodes of maize stalks in Illinois in 1930 as *Helminthosporium zeicola* Stout (Stout, 1930) which was recognized on maize leaves in Indiana in 1938 (Ullstrup, 1941a). Three races and one pathotype of *B. zeicola* pathogenic on maize have been described to date (Ullstrup, 1941a; Ullstrup, 1944; Nelson *et al.*, 1973; Dodd and Hooker, 1990); in addition, a non-pathogenic race on maize was reported (Welz and Leonard, 1988).

The disease is widespread in the U. S., Canada, Argentina, Colombia, Costa Rica, El Salvador, Guatemala, central and southern Africa, Southeast Europe, India, Cambodia, China, Australia and New Caledonia (Ellis and Holliday, 1972).

Economic importance

Maize is the third most important grain crop after wheat and rice in the world production. The world production of maize was 483 mil. metric tons in

1991. The U. S. produced 190 mil. metric tons of maize in 1991, which represented 39.4% of the world production (U. S. Bureau of the Census, 1992). The state of Virginia produced 930,000 metric tons of maize in 215,000 hectares in 1990 (Center for Public Service, 1992). Diseases affect maize production by reducing the stand, photosynthetic area, feeding quality of the grain or causing lodging. Leaf diseases reduce the photosynthetic tissue and may cause yield losses according to the time when a severe level of disease is reached (Ullstrup, 1977).

Helminthosporium leaf spot of maize was considered of minor economic importance because few inbred lines were susceptible to the first race detected (Ullstrup, 1944). However, yield losses of 20% in hybrids of susceptible parents to race 1 have been reported (Ullstrup and Miles, 1957). In recent years, a greater disease severity has been observed in the northern corn belt (Dodd and Hooker, 1990; Hooker, 1974) and the northeast United States (Hamid *et al.*, 1982a). Inbred lines are more affected by this disease than hybrids. Losses of up to 50% have been reported for some inbred lines in seed production fields (Hooker, 1974). Race 3 was estimated to caused 25 to 30% of yield losses on susceptible maize hybrids (personal communication of Ayers in Halseth *et al.*, 1991). Incidence of *B. zeicola* infecting maize leaves was between 11 and 95% in 10 maize fields in North Carolina in 1985 (Leonard *et al.*, 1988).

The pathogen

Cochliobolus carbonum Nelson is an ascomyceteous fungus belonging to the subclass Loculoascomycetidae, order Pleosporales and family Pleosporacea sensu Alexopoulos and Mims. Its anamorph stage is *B. zeicola*

(Stout) Shoemaker (Syn. *Helminthosporium zeicola* Stout = *H. carbonum* Ullstrup = *Drechslera zeicola* (Stout) Subram. & Jain) which belongs to the form-order Moniliales and form-family Dematiaceae sensu Alexopoulos and Mims.

The teleomorph, *C. carbonum*, can be produced under laboratory conditions (Nelson, 1959). There are no reports of its existence under natural conditions. It is a heterothallic fungus that produces pseudothecia when compatible isolates are paired on suitable substrates (Nelson, 1959). Pseudothecia are black, ellipsoidal to globose, setose, ostiolated, 355-450 × 320-430 µm. Asci are cylindrical or clavate with a short stalk and apparently unitunicate measuring 162-257 × 18.2 -27 µm. They arise from the base of the locule among pseudoparaphyses. Asci contains 1-8 ascospores in a helical arrangement. Ascospores are filiform, hyaline, 5-9 septated and measure 182-306.4 × 6.4-9.6 µm (Nelson, 1959).

The anamorph, *B. zeicola*, produces elongated, dark brown or dark olivaceous conidia that measure 25-115 × 7-18 µm. They are straight or slightly curved with 2-12 (usually 7-8) pseudosepta. Conidia are broader in the middle and taper slightly toward rounded ends. Conidiophores arise singly or in small groups and bear one to several conidia. They are dark brown or dark olivaceous, and measure 90-250 × 5-8 µm (Stout, 1930; Ullstrup, 1944; Ellis and Holliday, 1972).

Nelson *et al.* (1973) described the conidia of race 3 with a more pronounced curvature than conidia of the previously described races, although conidial length and width were in agreement with the original description of this species (Stout, 1930). Since there were slight differences in conidial

morphology, isolates of race 3 were mated with isolates of races 1 and 2, and fertile ascocarps were obtained. Consummation of fertility between the new isolate and races 1 and 2 was used as the basis to conclude that the isolates of the new race belonged to the same species. However, mating capacity should not be used as a sole factor to determine species since many species are interspecifically fertile (Alcorn, 1988).

The symptoms

Bipolaris zeicola infects mainly the leaf blade, but also leaf sheath, husk, ear and stalk. This pathogen presents the peculiarity of producing different foliar symptoms according to its race.

Race 1 induces oval to circular, tan colored lesions on leaves with dry light centers with broad yellowed zones and definite zonate pattern on necrotic areas on Pr, a susceptible inbred line (Ullstrup, 1944; Ullstrup, 1977). Lesions are abundant and range in size from hardly visible to 15 × 25 mm and often coalesce. Resistant inbred lines or hybrids develop yellowish-green flecks from 0.5 to 1 mm in diameter within 7 days after inoculation (Ullstrup, 1941a).

Race 2 produces elongated to irregular, chocolate brown lesions without distinct zonation, ranging from flecks to lesions measuring 3 × 20 mm (Ullstrup, 1941a, Ullstrup, 1944). Lesions are less abundant than for race 1 and coalesce, producing extensive areas of dead tissue. Ullstrup (1941a) described lesions on Pr, although similar lesions are produced on seedlings of other inbred lines. However, Nelson and Ullstrup (1961) stated that race 2 induces "only chlorotic-necrotic flecks" on Pr. This discrepancy could account for differences in time of evaluation. Disease reaction was evaluated one week

after inoculation by Ullstrup (1941a), but incubation time was not reported by Nelson and Ullstrup (1961).

Ears infected by race 1 or 2 have a black charred appearance (Ullstrup, 1944). Both races are indistinguishable based on ear symptoms according to Ullstrup (1944), however, infection of race 2 is more restricted to the tip of the ear (C. W. Roane, personal communication). Race 2 infects ears more frequently than race 1.

Race 3 usually produces long, narrow, linear, grayish-tan lesions, surrounded by a light to darkly pigmented margin, but smaller lesions are occasionally oval (Nelson *et al.*, 1973). In addition, some host-isolate combinations produce oval lesions (Nelson *et al.*, 1973; Castor *et al.*, 1976). Lesions are up to 20 mm in length and range from 0.2-2 mm in width. They linearly elongate between the leaf veins and reach a maximum size 7-10 days after inoculation. Lesion shape and color vary with the genotype of the host and the isolate.

The pathotype was described as causing oval to circular lesions, often with a zonate pattern, 5-10 mm in diameter, and was associated with early and sudden leaf death (Dodd and Hooker, 1990).

Bipolaris zeicola has also been found causing apparently little damage on stalks (Stout, 1930, Anderson and White, 1987); however, the race involved was not determined.

The disease

Bipolaris zeicola survives as a saprophyte on maize debris. Conidia become air-borne and are capable of infecting maize plants in all stages of development (Leonard *et al.*, 1988, Ellis and Holliday, 1972).

Moderate temperature and high relative humidity favor onset of the disease (Ullstrup, 1977). Race 1 sporulates abundantly on leaf blades and sheaths, husks and stalk nodes (Ullstrup, 1941a). Sporulation occurs on senescing or dead tissue under high humidity (O. E. Nelson and Ullstrup, 1964; Lodge and Leonard, 1984). It begins in lower leaves, on the leaf margins and tips, and where lesions coalesce, producing necrosis (Lodge and Leonard, 1984). Ear formation may be suppressed when plants susceptible to race 1 are infected early in the season and the disease becomes severe (O. E. Nelson and Ullstrup, 1964). Races 1 and 2 infect ears through tips, shanks, or directly through husks causing a black felt growth over the kernels which may infect them internally (Ullstrup, 1944).

Maize is the only known host crop for *B. zeicola* under natural conditions, although successful infection has been obtained on other cereals and grasses with artificial inoculation (Robert, 1962; Nelson and Kline, 1962, 1969, 1971; Ullstrup, 1977; Xiao *et al.* 1990).

Pathogenesis

Conidia of races 1, 2 and 3 germinate 2-4 hr after inoculation (Jennings and Ullstrup, 1957; Xiao *et al.*, 1992), and form appressoria over junctions of epidermal cells (Murray and Maxwell, 1975). The fungus penetrates susceptible or resistant leaf tissue directly, or occasionally through a stomata (Jennings and Ullstrup, 1957; Comstock and Scheffer, 1973; Murray and

Maxwell, 1975). Most hyphae of races 1 and 2 penetrate the subcuticular layer within 8 hr after inoculation. After penetration of the cuticle, hyphae of race 1 fill the epidermal cells and grow intra- and inter-cellularly into the chlorenchyma in susceptible hosts. Infected cells collapse, the fungus proliferates in necrotic tissue and invades tissue at the edge of the lesion (Jennings and Ullstrup, 1957; Comstock and Scheffer, 1973). Although a host-specific toxin, i. e., *Helminthosporium carbonum*-toxin (HC-toxin), is required for colonization of susceptible tissue by race 1, the presence of dead or seriously damaged tissue is not a requirement for colonization (Comstock and Scheffer, 1973). Hyphae of race 1 branch very poorly after penetration of resistant hosts. Unlike the compatible interaction, only one or two epidermal cells are invaded per infection site within 16-20 hr after inoculation, and there is little or no growth into the chlorenchyma. Nevertheless, the fungus remains viable for at least two months in chlorotic or necrotic flecks (Jennings and Ullstrup, 1957; Comstock and Scheffer, 1973).

Penetration of the subcuticular layer by hyphae of race 2 may occur up to 48 hr after inoculation (Murray and Maxwell, 1975). No reports have been published about the subsequent events of colonization by race 2. Penetration by race 3 is evident 12 hr after inoculation, and the fungus grows inter- and intra-cellularly within 24 hr after inoculation (Xiao *et al.*, 1992).

A host-specific toxic metabolite which affects only susceptible genotypes was detected in filtrates of *B. zeicola* race 1 (Scheffer & Ullstrup, 1965). Two toxic substances were isolated from cultures of race 1; one was a host-specific toxin called HC-toxin, and the other, carbotoxinine, was non-specific with low toxic properties to both susceptible and resistant hosts (Pringle and Scheffer,

1967). The HC-toxin is a cyclic tetrapeptide with a molecular weight of 436, an empirical formula of $C_{21}H_{32}N_4O_6$ and a sequence *cyclo*-[propylalanylalanyl-2-amino-8-oxo-9,10-epoxydecanoyl] (Pope *et al.*, 1983).

Races 2 and 3 lack the ability to produce HC-toxin (Scheffer *et al.* 1967; Xiao *et al.*, 1990). Race 3 produces a host-non-specific toxin complex, namely *Bipolaris zeicola* race 3-toxin (BZR-toxin), in spore germination fluids and culture. Although the toxin complex itself does not produce symptoms on maize, it does when inoculated in combination with non-pathogenic fungi, suggesting that the toxin plays an important role in the initial infection (Xiao *et al.* 1991). The BZR-toxin consists of four constituents, BZR-cotoxin I, II, III and IV. The major component of this complex is BZR-cotoxin II which is a cyclic nonadepsipeptide (Ueda *et al.*, 1992). Each component separately shows little biological activity, at least three cotoxins need to be combined for toxigenic activity (Xiao *et al.*, 1991). No toxin has been associated with race 2 to date.

Races

Helminthosporium leaf spot was originally reported to be caused by the asexual stage of *Cochliobolus heterostrophus* Drechsler (anamorph: *Bipolaris maydis* (Nisik.) Shoemaker = *Helminthosporium maydis* Nisik. = *Drechslera maydis* (Nisik.) Subram. & Jain), causal agent of southern leaf blight of maize. Two races were identified causing leaf and ear infection. Race 1 was first found in 1938 and race 2 in 1939, both in Indiana (Ullstrup, 1941a). In 1944, Ullstrup (1944) recognized that the pathogen with two physiological races was erroneously identified as *H. maydis* and he changed the name of the new species to *H. carbonum*.

Both physiological races were indistinguishable based on morphology, cultural behavior or symptoms induced on maize ears (Ullstrup, 1941a; Ullstrup, 1944), but distinguishable on the basis of host specialization, symptoms produced one week after inoculation and relative virulence. Race 1 produces a host-specific toxin which is the determinant of specialization on Pr and a few other inbred lines, i. e., K61, K44, Mo21a and N31 (Pringle and Scheffer, 1967; Ullstrup, 1977). It is highly virulent and capable of total destruction of susceptible hosts. Race 2 is non-specific on inbred lines of maize and mildly virulent. It induces disease in many lines under field and greenhouse conditions. Although differences in susceptibility were detected among inbred lines inoculated with race 2, none of the lines had the high resistance as shown by race 1 (Ullstrup, 1941a). Two near-isogenic lines, Pr and Pr 1, differ in the *Hm* gene for resistance to race 1; thus, Pr is susceptible and Pr1 is resistant to race 1. Both Pr and Pr1 are moderately susceptible to race 2 (Ullstrup, 1944).

In 1973 a new Helminthosporium leaf blight was reported in the northern corn belt (Hooker *et al.*, 1973; Wallin & Loonan, 1973). Conidial length, curvature and number of septa were intermediate between those of *B. zeicola* and those of *B. maydis*. The pathogenicity pattern on seedlings was similar to race 2, and no pathotoxin production was detected. Hybrids Pr × K61 and Pr1 × K61, which are susceptible and resistant to race 1, respectively, reacted similarly when inoculated with isolates of this pathogen. The identity of this pathogen was never resolved.

Nelson *et al.* (1973) reported a third race of *B. zeicola* in Pennsylvania in 1973; however, it was the predominant race in western Virginia since the early 1950's (C. W. Roane, personal communication). Even though conidial

curvature was more pronounced than in races 1 and 2 of *B. zeicola* and symptoms were different, the population in Pennsylvania was considered the same species and designated as race 3. Race 3 was reported to produce mainly linear lesions on several inbred lines, including Pr. Lesion shape, but not lesion type, was described, and no attempt was made to establish a set of differentials to distinguish the three races. Race 3 was suggested to be non-specific, which induced similar symptoms on many maize inbred lines (Blanco *et al.*, 1974; Castor *et al.*, 1976). Differences in parasitic fitness attributes, such as disease efficiency, sporulation capacity and lesion length have been found among isolates of race 3 and host × isolate interaction. These differences indicated that genotypes of the host reacted differently to different isolates of race 3 (Hamid *et al.*, 1982b; Gregory *et al.*, 1984). A comparison between the disease reaction of inbred lines indicated that differences existed between race 3 and the organism described by Hooker *et al.* (1973).

Welz and Leonard (1988) reported the occurrence of race 0 of *B. zeicola* which was avirulent on maize genotypes susceptible to races 1, 2 and 3; however, the maize genotypes that were challenged with this race were not reported.

A new pathotype was reported to cause unusual amounts of damage to maize inbred lines in seed production fields in Illinois in 1989. The conidia were morphologically indistinguishable from the conidia of races previously described, but it induced distinct symptoms and no ear infection was detected. Most of the susceptible inbred lines were B73 or those with a B73 background (Dodd and Hooker, 1990). The disease reaction of this pathotype was not compared with the established races.

Racial composition of isolates of *B. zeicola* collected from Pennsylvania, southeast and midwest U. S. was determined (Blanco *et al.*, 1974; Leonard, 1978; Lodge and Leonard, 1984; Leonard and Leath, 1990). Blanco *et al.* (1974) determined that 88% of the isolates collected in Pennsylvania in 1973 were race 2, 11% race 3, and less than 1% race 1. Interestingly, race identification was made on only one maize genotype, Pr × K61, 10 days after inoculation and used as the basis for race determination, i. e., race 1 induced zonate lesions, race 2 chlorotic-necrotic flecks and race 3 linear lesions. No susceptible host was used to distinguish race 2. Leonard (1978) determined that race 2 was the only race found in the midwest and the predominant race in the coastal plain and piedmont of North and South Carolina. Race 3 was predominant in the mountains of the Southeast, and expanded into the piedmont. Race 1 was only isolated from experimental inbred lines in the coastal plain region. Races were identified by lesion shape produced one week after inoculation on inbred line N31, which is susceptible to race 1 and produces round to oval or linear lesions when inoculated with races 2 and 3, respectively (Leonard, 1978). Lodge and Leonard (1984) and Leonard and Leath (1990) only studied the racial composition and cline of races 2 and 3. Race identification was based on lesion shape produced on a single host genotype, one of the following hybrids: DeKalb XL 394, Seneca Chief, Pioneer Brand 3368A (Lodge and Leonard, 1984) or Pioneer Brand 3369A (Leonard and Leath, 1990). However, the hybrids used by Lodge and Leonard (1984) did not distinguish between races 1 and 2. All isolates that caused oval lesions were considered to be race 2, because Leonard (1978) previously determined that race 1 was rare.

Resistance

Ullstrup (1941b), Ullstrup and Brunson (1947) and O. E. Nelson and Ullstrup (1964) studied the inheritance of resistance to *B. zeicola* race 1. A single completely dominant gene for resistance was first reported (Ullstrup, 1941b) and designated *Hm* because *B. maydis* (= *H. maydis*) was thought to be the causal agent of the disease. Segregation of F_2, F_3 and backcrosses to the susceptible parents of 3 crosses involving the susceptible inbred line Pr was studied. The same gene for susceptibility was detected in K61 and K44 (Ullstrup, 1944).

Ullstrup and Brunson (1947) determined the linkage relationships of the *Hm* gene by studying segregation of the crosses of two susceptible inbred lines, Pr and K61, to a series of translocation stocks with sugary endosperm. The *Hm* gene was located in chromosome 1 between the *P* (pericarp color) and *br* (brachytic). Roman and Ullstrup (1951) found that the *Hm* locus is approximately 22 recombination units from the centromere in the distal seven-eighths of the long arm of chromosome 1.

O. E. Nelson and Ullstrup (1964) reported that intermediate reactions of some stocks are due to the existence of a major gene, Hm_2, located in chromosome 9, and two additional alleles at the *Hm* locus, i. e., Hm^A and Hm^B. Plants with at least one dominant *Hm* allele are resistant regardless of the Hm_2 locus. However, when alleles at the *Hm* locus are recessive, the reaction is affected by the Hm_2 locus. When alleles at the Hm_2 locus are recessive, plants are fully susceptible; however, if they are heterozygous or dominant, plants show intermediate reactions at the seedling stage and become more resistant

as they grow older. Additionally the Hm^A allele confers intermediate resistance at all growth stages and the Hm^B allele conditions full resistance when homozygous; but susceptible in post-flowering stages if heterozygous.

Several maize inbred lines were evaluated for resistance to race 3 under field conditions and adequate resistance was detected (Castor *et al.*, 1976). The inheritance of resistance to *B. zeicola* race 3 has been studied by Hamid *et al.* (1982a) and Halseth *et al.* (1991). Hamid *et al.* (1982a) used a diallel cross to study the inheritance of three resistance parameters, i. e., disease efficiency, lesion length and sporulation capacity. They concluded that additive gene action was the most important component in the inheritance of these 3 traits in the diallel cross tested with one isolate of race 3. Halseth *et al.* (1991) studied the inheritance of resistance to race 3 by a diallel and a generation mean analysis. Inoculations were performed with a mixture of 5 isolates of race 3 which gave isolate × inbred line interactions in preliminary work, and evaluated severity as a measure of resistance. They concluded that the additive component was the most important factor, but they also found occasionally significant dominant interaction.

Studies to determine the nature of inheritance of resistance to race 2 are lacking, although it has been suggested that host resistance is under polygenic control (Ullstrup, 1954).

A mechanism of general resistance appears to be activated when a maize leaf is in contact with a potential pathogen. When a susceptible host to race 1 was first inoculated with race 2 followed by race 1, infection was prevented. However, the resistance induced by race 2 was overcome by addition of the HC-toxin (Cantone and Dunkle, 1990a). An inhibitory compound

released into the infection court is associated with this induced resistance that inhibits conidial germination and germ tube elongation, but this inhibitor is not formed in leaves giving a susceptible response (Cantone and Dunkle, 1990b; Cantone and Dunkle, 1991).

Genetics of the pathogen

Previously, researchers in genetic studies of *C. carbonum* used naturally occurring markers, i. e., virulence, mating type, ability to form pseudothecia, lesion length and fungicide tolerance; and morphological mutants, i. e., albino mutants. Molecular markers are currently being employed in studies of *C. carbonum* using isozymes (Simcox *et al.*, 1992; Gaul and Balducchi, 1992) and polymerase chain reaction (PCR) amplification (Jones and Dunkle, 1992).

Heterothallism of *C. carbonum* is conditioned by a single major gene, designated *A* (Nelson, 1960). However, the genetic control of its reproductive mechanism is not simple. Some isolates of *C. carbonum* inhibit pseudothecium formation (Nelson, 1964). A single gene, *i*, was identified to be responsible for blocking the formation of protopseudothecia if both paired isolates carried the gene in the inhibitory state. This trait was inherited independently of the gene for compatibility. Another single gene designated *S,* and inherited independently of the compatibility locus, inhibited the formation of asci in some isolates of *C. carbonum* (Dalmacio & Nelson, 1976b). Modifying genes influencing the expression of the major gene for compatibility could account for qualitative and quantitative variation in the mating capacity of isolates of *C. carbonum* (Nelson, 1970a). Semi-incompatibility occurs in some isolates of *C.*

carbonum and is controlled by the same single gene that inhibits pseudothecium formation (Dalmacio & Nelson, 1976a).

The inheritance of pathogenicity of *C. carbonum* was studied in crosses of races 1 and 2 (Nelson and Ullstrup, 1961). A single gene independently inherited from the compatibility gene controls pathogenicity in these races of *C. carbonum*. However, studies of inheritance of pathogenicity of race 3 have been inconclusive. The inheritance of the lesion length induced by the progeny of a cross of races 2 and 3 appeared to be controlled by 3-5 genes (Dalmacio *et al.*, 1979).

Segregation for the ability to produce HC-toxin in crosses of races 1 and 2 indicates that the trait is controlled by a single gene. However, the segregation pattern obtained in some crosses is significantly different from the expected 1:1 ratio for ability or lack of ability to produce HC-toxin suggesting that at least 2 genes are required for HC-toxin production in some isolates. The amount of HC-toxin produced appeared to be quantitatively inherited; crosses between low and high yielding HC-toxin isolates resulted in progeny that yielded intermediate, higher and lower toxin titer than the parental isolates (Scheffer *et al.*, 1967). A 22-kb DNA region which encodes the genes for enzymes involved in the biosynthesis of HC-toxin has been cloned (Panaccione *et al.*, 1990). This putative *Tox* locus is only found in isolates of race 1.

Nelson and Kline (1962, 1969, 1971) studied the ability of isolates of *B. zeicola* to infect grasses and cereals. A marked variation in pathogenicity among isolates was found. Segregation of crosses between isolates with different host range detected 33 genes for pathogenicity to 23 gramineous hosts (Nelson and Kline, 1969; Kline and Nelson, 1969; Nelson, 1970b). The

host range of a number of the progeny was wider than that of the parental isolates, and in some cases progenies were capable of causing lesions on hosts to which neither of the parents were pathogenic.

The teleomorph of *B. zeicola* has not been found under natural conditions. Differences in genetic polymorphisms for traits such as cycloheximide, carboxin and cadmium tolerance, differences in frequencies of mating types and absence of isolates with intermediate lesion length are indicative of lack or little exchange between races 2 and 3 (Leonard, 1978, Lodge and Leonard, 1984, Leonard and Leath, 1990). These polymorphic traits indicate that race 2 is genetically more variable than race 3 (Leonard and Leath, 1990).

The use of molecular markers in genetic studies of *B. zeicola* is more suitable because they are more likely to be selectively neutral (Leonard and Leath, 1990). Isozyme polymorphisms have been detected among isolates of *B. zeicola* and appeared to be useful in race identification (Simcox et al, 1992; Gaul and Balducchi, 1992). Eleven electrophoretic phenotypes of polymorphisms of 5 isozymes were detected. One, 7 and 3 electrophoretic phenotypes were associated with races 1, 2 and 3, respectively. PCR amplification of DNA permit differentiation between races 1, 2, 3 and 0, but fingerprints of race 2 were indistinguishable from fingerprints of the new pathotype (Jones and Dunkle, 1992).

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CHAPTER 2

CONIDIAL MORPHOLOGY OF RACES OF *Bipolaris zeicola*

Introduction

Bipolaris zeicola (Stout) Shoemaker causes Helminthosporium leaf spot on maize. The asexual stage was first described in 1930 on maize stalks (Stout, 1930), but the disease it causes was first recognized in 1938 (Ullstrup, 1941). Its sexual stage, *Cochliobolus carbonum* Nelson, has not been found under natural conditions; although, it can be produced in the laboratory by pairing compatible isolates (Nelson, 1959).

Three races and one pathotype of *B. zeicola* have been described causing disease on maize, in addition to a non-pathogenic race to maize (Ullstrup, 1944, Dodd and Hooker, 1990, Nelson et al., 1973, Welz and Leonard, 1988). Races 1 and 2 and the new pathotype were described according to the differential reaction they produced on selected inbreds, but race 3 was described according to the lesion shape it induces. Conidial morphology of races 1 and 2 and the new pathotype are indistinguishable; however, race 3 was described as producing conidia with a more pronounced curvature than the previously described races.

The objective of this research was to examine the conidial morphology of the different races of *B. zeicola* and determine if it can be used as an aid in race identification.

Materials and Methods

Thirty-nine isolates of *B. zeicola*, collected in different locations of the U. S., were examined (Table 2.1). Single conidial isolates were obtained from each original isolate by the separate-locate-isolate technique (Tuite, 1969), and grown on lactose casein hydrolysate (LCH) agar under constant cool-white fluorescent light at 23±1 C. Each isolate was inoculated on a set of 14 maize inbreds to determine its race (Chapter 5). Conidial suspensions were sprayed on all leaves of each plant, and incubated under greenhouse conditions. Fourteen days after inoculation leaf tissue infected with each isolate was collected, dried between towel papers at room temperature and stored at 4 C.

Infected leaf tissue was placed in a moist chamber and incubated at 23±1 C under diurnal illumination provided by a cool-white fluorescent light (3,600 lux) during 7 days.

A water mounted preparation of each isolate was used for microscopic examination. Length, width and number of pseudosepta were measured on 50 conidia randomly selected from each isolate at 1,000× using a light compound microscope.

Conidia were classified into three categories according to their curvature, i. e., straight, slightly curved and curved; and their pigmentation, i. e., dark brown, olivaceous brown, and very light brown. Isolates were grouped into races using two criteria, i. e., partially on differential reaction and partially on

lesion shapes (Ullstrup, 1944, Dodd and Hooker, 1990, Nelson *et al.*, 1973), and entirely on differential reaction (Chapter 5). All variables under study were statistically analyzed by analysis of variance, or chi-square test according to the nature of the variable.

Results

Highly significant differences among isolates were found for all variables studied, i. e., conidial length, width, number of pseudosepta and proportions of conidia classified according to their curvature and pigmentation. When isolates were grouped into races using either criterion, partially on differential reaction and partially on lesion shapes, or entirely on differential reaction, there were still significant differences among isolates belonging to each race.

Conidial length ranged from 22-139 µm and averaged 67.8µm (Table 2.2). Conidia ranged in width from 7-20µm and averaged 12.6µm. Number of pseudosepta varied from 3-16 with a mean of 7.5 pseudosepta per conidium. Sixty-six percent of the conidia from all isolates were straight, 30% were slightly curved, and 4% were curved. The proportion of conidia according to its curvature varied widely, although most of the differences were found in the proportions of straight and slightly curved conidia, since the proportion of curved conidia was very low for all isolates (0-14%). Eighty per cent of the conidia of race 3 were straight, 19% slightly curved, and 2% curved (Table 2.3). All isolates of race 3 showed a high proportion of straight conidia (76-92%), except isolate 84-3306 which had only 36% straight conidia (Table 2.2). The number of conidia classified with curvature in race 3 was similar to race 1, and

in race 2 was similar to the new pathotype. One isolate of race 0 examined in this research was intermediate.

Pigmentation of conidia was also variable, 50% of the conidia were dark brown, 44% olivaceous brown and 6% clear brown. Although, there were differences in pigmentation within each race, overall, race 3 showed a higher proportion of olivaceous brown conidia than the other races; however one isolate of race 3, i. e., WT-1, showed 76% of dark brown conidia and 24% olivaceous brown conidia (Table 2.2).

Means of the variables under study for the races identified by differential reaction are listed in Table 2.4. Isolates within each race were variable for all morphological characters.

Discussion

Isolates of *B. zeicola* are variable, not only in specificity (Chapter 5), but also in conidial morphology. Conidial length, width and number of pseudosepta were between the ranges described for *B. zeicola* (Stout, 1930, Ullstrup, 1944, Nelson et al., 1973) in 30 of the 39 isolates. Maximum conidial length of 4 isolates was slightly higher and minimum length of 1 isolate slightly lower than described before. Maximum width of 3 isolates was slightly higher and the maximum number of pseudosepta of 3 other isolates was also higher. According to Ellis and Holliday (1972), most conidia are between 60-80 μm long, however, one of the isolates of race 3 showed an average length of 93.5 μm and another, 42.7μm.

Most isolates of race 3 produced straight conidia, which disagrees with the original description of race 3 (Nelson *et al.* 1973); however, one of the

isolates of race 3 used in this research, produced a high proportion of slightly curved conidia. Even though isolates with a higher proportion of curved conidia were widely spread among the race 3 population; there are isolates producing similar symptoms whose conidial curvature do not fit that description. Therefore, curvature can not be used to distinguish race 3 from the others.

Pigmentation of conidia has been suggested to be another factor in race distinction; however, it was variable within each race. Most isolates of race 3 produced a high proportion of olivaceous brown conidia, but one isolate produced a high proportion of dark brown conidia, and another isolate, a high proportion of clear brown conidia. Therefore, pigmentation should not be used as a factor in race identification of *B. zeicola.*

None of the variables studied are reliable characters that can be used to differentiate races of *B. zeicola.* A set of differentials has been suggested and provides a more stable system for identifying races of *B. zeicola* (Chapter 5). One or two host limits the range of variability that can be expressed, and should be discouraged.

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Table 2.1: Isolates of *Bipolaris zeicola* used for conidial measurement and state of collection.

Isolate	State
80-1910	IL
80-3666	MO
81-1843	NY
81-5173	CO
82-1026	MI
82-4427	VA
84-1676	MN
84-1926	IL
84-2533	NE
84-3033	IA
84-3277	PA
84-3306	PA
84-4842	GA
85-1954	OH
85-2427	IN
85-2600	PA
85-3326	TN
86-1074	MI
Bz 23-55	NC
Bz 23-56	NC
Bz10-59	NC
Bz2-6	NC
D-0	IL
D-2	IA
D-4	NE
D-5	IA
D-9	IN
D-11	IL
D-13	IN
D-15	IA
D-17	IL
D-20	IN
G3-4	VA
G47-29	VA
M-2	IA
Purdue	IN
WT-1	VA
WT-2	VA
WT-3	VA

Table 2.2: Length, width, number of septa, and proportion of conidia showing curvature and of *Bipolaris zeicola*[a].

Race[b]	Race[c]	Isolate	Length			Width			No. pseudosepta			Curvature			Pigmentation		
			Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Straight	Slightly curved	Curved	Dark brown	Olivaceous	Clear brown
0	0	84-2533	68.1	91	39	12.7	15	11	8.4	12	4	0.66	0.28	0.06	0.82	0.18	0.00
1	1	M-2	66.2	102	41	12.4	16	9	7.3	12	4	0.50	0.42	0.08	0.70	0.30	0.00
1	17	Purdue	61.6	101	39	12.9	16	10	6.5	11	4	0.80	0.20	0.00	0.54	0.44	0.02
1	17	84-1676	64.2	108	32	13.1	17	11	7.0	11	3	0.92	0.08	0.00	0.60	0.26	0.14
1	17	84-1926	58.6	98	40	12.5	15	11	6.6	11	3	0.78	0.22	0.00	0.46	0.50	0.04
1	17	84-3277	52.8	98	29	11.7	14	9	5.7	9	3	0.98	0.02	0.00	0.78	0.22	0.00
1	17	85-2427	62.1	90	37	11.8	13	10	7.1	10	4	0.69	0.31	0.00	0.33	0.63	0.04
1	17	D-2	67.5	99	30	13.1	16	10	7.2	10	3	0.88	0.12	0.00	0.65	0.33	0.02
1	17	D-15	64.4	92	32	12.9	15	11	6.9	10	3	0.78	0.22	0.00	0.80	0.16	0.04
2	32	80-3666	74.1	120	47	12.6	16	10	8.6	13	5	0.36	0.58	0.06	0.56	0.44	0.00
2	32	81-5173	77.0	103	42	12.8	15	10	9.0	12	5	0.32	0.58	0.10	0.42	0.56	0.02
2	32	84-3033	72.7	106	36	13.0	17	9	7.9	11	4	0.60	0.34	0.06	0.92	0.08	0.00
2	36	84-4842	72.4	119	40	13.0	15	10	7.7	12	3	0.52	0.36	0.12	0.38	0.60	0.02
2	36	WT-3	42.9	82	22	10.2	13	7	5.3	9	3	0.64	0.36	0.00	0.10	0.82	0.08
2	36	WT-2	66.3	106	40	12.4	17	11	7.6	12	4	0.84	0.14	0.02	0.06	0.68	0.26
2	37	G3-4	73.7	104	38	13.3	16	11	8.3	13	3	0.52	0.36	0.12	0.42	0.32	0.26
2	44	80-1910	72.8	108	35	13.1	16	10	8.5	12	3	0.44	0.54	0.02	0.58	0.42	0.00
2	44	82-1026	72.9	119	40	12.6	15	10	7.8	11	4	0.52	0.36	0.12	0.52	0.42	0.06
2	44	85-2600	56.6	84	35	12.1	16	10	6.7	9	3	0.52	0.46	0.02	0.92	0.08	0.00
2	44	85-3326	63.1	100	42	12.4	15	11	6.7	12	4	0.60	0.40	0.00	0.80	0.18	0.02
2	*	Bz 23-55	74.6	102	44	11.2	13	9	8.1	11	3	0.63	0.33	0.04	0.37	0.61	0.02
2	*	Bz 23-56	78.7	106	45	11.5	14	9	8.2	11	4	0.65	0.33	0.02	0.43	0.53	0.04
3	0	86-1074	78.2	113	58	13.2	16	11	8.8	12	7	0.90	0.04	0.06	0.12	0.70	0.18
3	46	81-1843	61.7	79	46	12.5	15	10	7.0	9	5	0.82	0.18	0.00	0.20	0.72	0.08
3	46	82-4427	69.7	96	47	12.4	14	10	8.1	11	5	0.84	0.16	0.00	0.34	0.56	0.10
3	46	84-3306	93.5	139	55	12.1	14	10	10.0	16	5	0.36	0.58	0.06	0.14	0.76	0.10
3	46	85-1954	63.1	101	38	14.1	20	10	6.3	10	3	0.91	0.09	0.00	0.45	0.16	0.39
3	46	Bz10-59	74.7	96	48	12.6	14	11	7.8	11	4	0.88	0.12	0.00	0.24	0.66	0.10
3	46	G47-29	42.7	72	32	11.5	13	9	4.8	8	3	0.92	0.08	0.00	0.08	0.92	0.00
3	46	Bz2-6	79.7	107	44	13.0	17	10	9.1	12	4	0.76	0.22	0.02	0.16	0.80	0.04
3	46	WT-1	76.7	107	46	13.0	15	11	8.3	11	5	0.78	0.22	0.00	0.76	0.24	0.00
NP	4	D-20	69.9	96	36	13.4	16	11	6.8	10	4	0.82	0.18	0.00	0.66	0.26	0.08
NP	11	D-0	61.9	87	36	14.0	19	11	7.0	10	3	0.51	0.35	0.14	0.73	0.18	0.08
NP	11	D-5	67.0	100	35	12.4	15	10	8.0	11	5	0.50	0.40	0.10	0.64	0.36	0.00
NP	36	D-13	74.8	107	46	13.8	20	11	7.9	11	5	0.58	0.38	0.04	0.62	0.38	0.00
NP	37	D-11	55.4	71	26	11.7	13	9	6.7	9	4	0.62	0.38	0.00	0.90	0.08	0.02
NP	38	D-9	70.9	104	39	12.0	14	9	8.5	12	4	0.34	0.52	0.14	0.46	0.54	0.00
NP	38	D-17	79.2	116	47	14.4	17	11	8.8	12	6	0.36	0.56	0.08	0.66	0.34	0.00
NP	*	D-4	63.5	85	29	12.4	16	10	7.6	10	5	0.68	0.32	0.00	0.04	0.76	0.20

a Means, maximum and minimum values and proportions obtained from the measurement of 50 conidia.

b Classification based partially on differential reaction and partially in lesion shape (old classification).

c Classification based on differential reaction (new classification proposed in Chapter 5).

* Unclassified isolates.

Table 2.3: Means of length, width, number of septa and proportion of curved and pigmentation of conidia of *Bipolaris zeicola* according to the race identified with the old system[a].

Race	No. isolates	Length			Width			No. pseudosepta			Curvature			Pigmentation		
		Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Straight	Slightly curved	Curved	Dark brown	Oliva-ceous	Clear brown
0	1	68.1	91	39	12.7	15	11	8.4	12	4	0.66	0.28	0.06	0.82	0.18	0.00
1	8	62.2	108	29	12.5	16	9	6.8	12	3	0.79	0.20	0.01	0.61	0.36	0.04
2	13	69.1	120	22	12.3	17	7	7.7	13	3	0.55	0.39	0.05	0.50	0.44	0.06
3	9	71.1	139	32	12.7	20	9	7.8	16	3	0.80	0.19	0.02	0.28	0.61	0.11
NP	8	67.8	116	26	13.0	20	9	7.7	12	3	0.55	0.39	0.06	0.59	0.36	0.05
Overall	39	67.8	139	22	12.6	20	7	7.5	16	3	0.66	0.30	0.04	0.50	0.44	0.06

a Means and proportions on measurements of 50 conidia per isolate.

Table 2.4: Means of length, width, number of septa and proportion of curved and pigmentation of conidia of *Bipolaris zeicola* according to the race identified with the new system[a].

Race	No. isolates	Length			Width			No. pseudosepta			Curvature			Pigmentation		
		Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Straight	Slightly curved	Curved	Dark brown	Olivaceous	Clear brown
0	2	73.1	113	39	13.0	16	11	8.6	12	4	0.78	0.16	0.06	0.47	0.44	0.09
1	1	66.2	102	41	12.4	16	9	7.3	12	4	0.50	0.42	0.08	0.70	0.30	0.00
4	1	69.9	96	36	13.4	16	11	6.8	10	4	0.82	0.18	0.00	0.66	0.26	0.08
11	2	64.5	100	35	13.2	19	10	7.5	11	3	0.51	0.37	0.12	0.69	0.27	0.04
17	7	61.6	108	29	12.6	17	9	6.7	12	3	0.83	0.17	0.00	0.59	0.36	0.04
32	3	74.6	120	36	12.8	17	9	8.5	13	4	0.43	0.50	0.07	0.63	0.36	0.01
36	4	64.1	119	22	12.4	20	7	7.1	12	3	0.65	0.31	0.05	0.29	0.62	0.09
37	2	64.5	104	26	12.5	16	9	7.5	13	3	0.57	0.37	0.06	0.66	0.20	0.14
38	2	75.1	116	39	13.2	17	9	8.6	12	4	0.35	0.54	0.11	0.56	0.44	0.00
44	4	66.4	119	35	12.5	16	10	7.4	12	3	0.52	0.44	0.04	0.71	0.28	0.02
46	8	70.2	139	32	12.7	20	9	7.7	16	3	0.78	0.21	0.01	0.30	0.60	0.10

a Means and proportions on measurements of 50 conidia per isolate.

CHAPTER 3

EXPANSION OF LESIONS INDUCED BY RACES 1, 2 AND 3 OF *Bipolaris zeicola*

Introduction

Helminthosporium leaf spot of maize (*Zea mays* L.) caused by *Bipolaris zeicola* (Stout) Shoemaker (Syn. *Helminthosporium carbonum* Ullstrup), has become increasingly important in recent years, not only in seed production fields, but also in commercial production (Dodd and Hooker, 1990, Hooker, 1974, Hamid *et al.*, 1982a).

Three races and one pathotype of *B. zeicola* have been reported on maize (Ullstrup, 1941; Nelson *et al.*, 1973; Dodd and Hooker, 1990). Races 1 and 2, and the new pathotype were described according to differential reaction on selected inbred lines, i. e., Pr, Pr1 and B73. However, race 3 was reported as causing lesions of different shape, i. e., long, linear lesions, from the races previously described, and no differential response was shown (Nelson *et al.*, 1973).

Lesion shape induced by each race of *B. zeicola* have been described (Ullstrup, 1941; Ullstrup, 1944; Nelson *et al.*, 1973; Dodd and Hooker, 1990); however some discrepancies exist. Race 2 was originally described as producing elongated to irregular lesions (Ullstrup, 1941; Ullstrup, 1944), and later as inducing chlorotic or necrotic flecks on the same inbred line (Nelson

and Ullstrup, 1961). Linear lesions are characteristic of race 3; however, certain isolate-inbred line combinations give oval lesions (Nelson *et al.*, 1973). Evaluation of lesion shape on one hybrid has been used extensively as the criterion for race identification (Blanco *et al.*, 1974; Lodge and Leonard, 1984; Leonard and Leath, 1990).

Lesion size is one of the components of susceptibility of a host to a fungus disease (Populer, 1978). Larger lesions and rapid rate of expansion of lesion are expression of higher susceptibility. Lesion length has been used to estimate the resistance of several maize inbred lines to different isolates of race 3 (Hamid *et al.*, 1982b), and to study the inheritance of their resistance to one isolate of race 3 (Hamid *et al.*, 1982a). The main component in the inheritance of lesion length induced by race 3 was additive gene action.

This study was undertaken to compare the morphology and expansion of lesions induced by isolates of races 1, 2 and 3, and to assess the usefulness of lesion size in evaluating resistance to different races of *B. zeicola*.

Materials and Methods

Plant Material

Eight inbred lines were selected to study the expansion of lesions based on their response to races 1, 2 and 3. The same 5 inbred lines were used to study lesion expansion induced by races 2 and 3, i. e., B73, H95, Pr, W64A and Mo17; however, to study lesion expansion induced by race 1, inbred lines Pr, Mo21a and sel. (K41 × K44) were selected as susceptible hosts, and B37 and W64A as resistant hosts which produced some lesions in previous experiments.

Four seeds per pot were planted in plastic pots 15-cm in diameter and thinned after emergence to 3 seedlings per pot. The inbred lines to be tested with 4 isolates of each race were arranged in a completely randomized design with three replicates and each experiment was repeated twice. Inbred line Mo17 was tested with race 2 in only one repetition. The plants were grown in the greenhouse at 26±3 C.

Inoculum

Four single conidial isolates of races 1, 2 and 3 were used in this experiment (Table 3.1). Conidial suspensions were prepared from 7-9 day-old cultures grown on lactose casein hydrolysate (LCH) agar, and adjusted to 5×10^3 conidia per milliliter.

Plants were inoculated at the V4-V5 growth stage by spraying the conidial suspension on all leaves of each plant with a Devilbiss atomizer at a pressure of 0.83 bar. Immediately after inoculation, plants were placed in a mist chamber and maintained in darkness for 15-16 hours.

Lesion expansion

After the incubation period, plants were placed in a mist chamber for 15-16 hours per day in darkness until 8 days after inoculation to enhance lesion development and transferred to a greenhouse bench. Three lesions per plant were measured to the nearest 0.5 mm on the third leaf. Lesion length and width were measured 2, 3, 4 and 6 days after inoculation with race 1; 2, 4, 6 and 8 days after inoculation with race 2; and 2, 4 and 6 days after inoculation with race 3. Lesion area was estimated by approximating the lesion shape to

geometric figures, i. e., for races 1 and 2, the area was calculated with the ellipse equation, $\pi ab/4$, and race 3 with the rectangle equation, ab, where a is length and b is width.

Since lesions were marked and measured early after inoculation, some lesions remained as chlorotic or necrotic flecks for the duration of the experiment. Only the size of lesions that enlarged were analyzed for susceptible hosts. The proportion of lesions (y) that did not expand was estimated for each inbred line-isolate combination, and transformed to arcsin of the square root of y for statistical analysis. Lesion length, width, area and proportion of lesions that did not expand were subjected to analysis of variance, and differences among treatments were tested by the Tukey-Kramer (HSD) test at a significance level of 0.05.

Results

Lesions induced by race 1 on susceptible lines were oval to ellipsoidal with a tan center and a darker water-soaked margin; after the lesion enlarged a brownish to grayish zonate pattern was observed depending on the inbred line. A thin halo surrounded lesions on Mo21a and sel. (K41 $\times$ K44), but very seldom on Pr. Lesions on resistant lines remained as small, tan infection points, sometimes surrounded by a chlorotic area, other infection points developed into small necrotic, oval to circular lesions.

Race 2 produced tan to chocolate brown oval to irregular lesions on the leaf blade of all susceptible inbred lines, and irregular lesion on the leaf margin that coalesced 8 days after inoculation on some inbred lines, i. e., B73, Mo17. W64A produced the largest lesions when it was inoculated with race 2 and they

reached 6 × 1.5 mm, 8 days after inoculation. Lesions induced by race 2 on susceptible inbred lines showed neither chlorotic halo nor a concentric pattern. Lesions induced on resistant lines were circular to oval, chlorotic or necrotic, and expansion was none or limited from 2 to 8 days after inoculation.

All isolates of race 3 on the 5 inbred lines used in this experiment produced lesions with linear appearance. Even lesions induced on resistant lines that were chlorotic 2 days after inoculation, increased in length, and many remained chlorotic. Lesions developed on susceptible lines were long and narrow, tan to grayish with a darker margin and a chlorotic halo surrounding them. They expanded longitudinally from a tan infection point toward one or both sides. Actively expanding areas of the lesions were water soaked and wider, and located at one or both ends of the lesions, giving rise to a zonate pattern in the longitudinal axis which was more evident in Mo17 than in other inbred lines.

Lesion expansion showed the same tendency in both repetitions of each race. Significant differences among inbred lines were detected for all three variables, i. e., length, width and area, for each race, with the only exception that the lesion width, 2 days after inoculation with race 2 showed no difference among inbred lines. Lesion expansion in inbred lines inoculated with race 2 was slower than with the other races.

Differences among isolates of the same race were detected for lesion length 2 days after inoculation with race 1. The Purdue isolate induced significantly shorter lesions (1.2 mm) than isolates D-2 (1.5 mm), 85-2427 (1.4 mm) and D-15 (1.3 mm) over all inbred lines, although this difference is probably not biologically significant.

No isolate × inbred line interaction was detected in lesion length and area; however, lesion width showed significant interactions 3 days after inoculation with race 1, and 2 days after inoculation with race 3. The four isolates within each race were genetically similar in terms of specificity and could not be further differentiated with the inbred lines used in this experiment.

Inbred lines susceptible to race 1, Pr, Mo21a and the sel. (K41 × K44), exhibited longer, wider and larger lesions than B37 and W64A, resistant inbred lines (Figs. 3.1A, 3.2A and 3.3A). Lesions on Pr were the largest with mean length of 2 mm (range of 1-4 mm) and 11.4 mm (range 7-18 mm), 2 and 6 days after inoculation, respectively. Of the three susceptible inbred lines, Mo21a produced the smallest lesions ranging from 0.5-4 mm (mean 1.6 mm) and 0.5-19 mm (mean 9.3 mm), 2 and 6 days after inoculation, respectively. Sel. (K41 × K44) showed intermediate lesion size between Pr and Mo21a. Inbred lines resistant to race 1 induced significantly smaller lesions than susceptible inbred lines. Lesions on resistant inbred lines increased in size slightly from 2 to 6 days after inoculation. Lesions on W64A were slightly larger than those on B37. The mean length of lesions produced on W64A were 0.9 mm (range 0.5-2.5) and 1.1 mm (range 0.5-3 mm), 2 and 6 days after inoculation, respectively. The mean length of lesions produced on B37 were 0.7 mm (range 0.5-1.5 mm) and 0.9 mm (range 0.5-3.5 mm), 2 and 6 days after inoculation, respectively.

The width of lesion produced by race 1 were different between susceptible and resistant inbred lines (Figure 3.2A). Sel. (K41 × K44) exhibited the widest lesions 2 days after inoculation, 1.1 mm with a range of 0.5-2 mm, although Pr was not statistically different from sel. (K41 × K44). Lesions on Pr were wider than sel. (K41 × K44) after the 2-day evaluation period. The mean

width of lesions induced on Pr was 3.8 mm (range 2-7 mm), 6 days after inoculation. Mo21a produced narrower lesions than Pr and sel. (K41 × K44). Lesion width of resistant inbred lines, i. e., B37 and W64A, were similar at all times.

Pr produced lesions with a mean area of 1.71 and 34.54 mm^2, 2 and 6 days after inoculation, respectively (Figure 3.3A). The mean areas of lesions produced by Mo21a were 1.53 and 22.77 mm^2, 2 and 6 days after inoculation, respectively. Sel. (K41 × K44) developed lesions intermediate in size to Pr and Mo21a. W64A and B37 produced small lesions, with mean areas of 0.59 and 0.38 mm^2, 6 days after inoculation, respectively.

Pr gave a moderately resistant reaction to isolates of race 2; the mean length of lesions was 0.1, 0.1 and 0.2 mm longer than those on W64A, B73 and H95, respectively, 2 days after inoculation (Figure 3.1B) and slightly increased up to 1.2 mm (range 0.5-6 mm) 8 days after inoculation. However, the mean length of lesions on W64A and B73 extended to 2.3 mm (range 0.5-6 mm) and 1.9 mm (range 0.5-8 mm), respectively, 8 days after inoculation. H95 was the most resistant inbred line to this race, means of lesion length increased from 0.6 to 0.9 mm between 2 and 8 days after inoculation, respectively.

No differences in lesion width between inbred lines were found 2 days after inoculation with race 2 (Figure 3.2B). Lesions on W64A were wider than the rest of the inbred lines with means of 0.6 and 0.9 mm at 4 and 8 days after inoculation, respectively. The means of lesion width on H95 remained at 0.5 mm for the duration of the test.

Pr produced slightly larger lesions (mean 0.32 mm^2) than the susceptible lines B73 and W64A, 2 days after inoculation with race 2, but lesions increased

slowly compared to susceptible inbred lines. Lesion area of W64A increased from 0.28 to 1.74 mm^2 between 2 and 8 days after inoculation, whereas, lesion area of H95 increased from 0.22 to 0.42 mm^2 between 2 and 8 days after inoculation (Figure 3.3B).

B73 and W64A were the most susceptible inbred lines to race 3; Mo17, moderately susceptible; Pr, moderately resistant; and H95, resistant. Mo17 had longer lesions with a mean of 1.3 mm (range 0.5-4 mm) than other inbred lines, 2 days after inoculation, and Pr, shorter lesions with a mean of 1.0 mm (range 0.5-3 mm) (Figure 3.1C). However, B73 developed the longest lesions with a mean of 7.2 mm (range 0.5-15.5 mm), followed by Mo17 and W64A with a mean of 6.6 mm (range 0.5-19 mm) and 6.0 mm (range 0.5-20 mm), respectively, 6 days after inoculation. The mean length of lesions which enlarged on H95 were 1.0 mm and 2 mm, 2 and 6 days after inoculation, respectively.

Mo17 had the widest lesions, 4 and 6 days after inoculation with race 3 with means of 0.7 and 0.9 mm, respectively (Figure 3.2C). H95 and Pr developed the narrowest lesions with means of 0.5 and 0.6 mm, 4 and 6 days after inoculation, respectively.

Mo17 developed larger lesions than the rest of the inbred lines with means of 0.70, 2.79 and 6.30 mm^2, 2, 4 and 6 days after inoculation with race 3, respectively (Figure 3.3C). H95 developed the smallest lesions, with mean areas of 0.50, 0.85 and 1.27 mm^2, 2, 4 and 6 days after inoculation, respectively.

Each inbred line, W64A, B73, Mo17, Pr and H95, gave the same disease reaction, susceptible, susceptible, moderately susceptible, moderately resistant

and resistant, respectively, when inoculated with race 2 or 3. Lesion width induced by races 2 and 3 were similar 6 days after inoculation; however, lesion length induced by race 3 on both, susceptible and resistant lines, were always longer than those induced by race 2. Six days after inoculation, the length of lesions produced by race 3 was between 7 to 10 times and 3 to 5 times wider on susceptible and resistant lines, respectively. Six days after inoculation the length of lesions induced by race 2 were 2 to 3 times wider and 1 to 2 times on susceptible and resistant inbred lines, respectively. Size of lesions induced by race 1 on resistant inbred lines, W64A and B37, was similar to the size of lesions exhibited by H95 and Pr, resistant inbred lines to race 2.

With race 1 all lesions on Pr that were marked 2 days after inoculation enlarged (Table 3.2). Ninety-two and 98% of the marked lesions at 2 days after inoculation with race 1 enlarged on Mo21a and sel. (K41 × K44), respectively. However, 73 and 57% of the lesions on B37 and W64A, respectively, detected 2 days after inoculation did not increase in size. Significant differences in the proportion of non-developed lesions were found between both experiments with race 1, and a significant interaction of experiment × inbred line was detected. A higher proportion of non-developing lesions was detected on W64A in the second experiment (73%) than in the first one (40%). Despite differences between experiments, the resistant inbred lines to race 1, induced a higher proportion of lesions that did not increase in size, than did the susceptible lines.

Twelve days after inoculation with race 2, W64A showed the lowest proportion of non-developed lesions (13%) and H95, the highest (59.7%)(Table 3.2). However, 40.3% of the lesions did not enlarge on B73, which is susceptible to race 2.

H95 showed the highest proportion of non-developed lesions (28.7%) after inoculation with race 3 (Table 3.2). Mo17 showed the lowest proportion (7.9%) followed by B73 (9.7%) and W64A (12.1%).

Discussion

Lesion shape induced by races 1, 2 and 3 of *B. zeicola* on susceptible inbred lines were different (Ullstrup, 1941; Ullstrup, 1944; Nelson *et al.*, 1973). Race 1 produces very distinctive lesions on susceptible hosts; they are oval to ellipsoidal, measuring 11.4 × 3.8 mm on Pr, 6 days after inoculation. Lesions induced by race 2 were mainly chlorotic or necrotic flecks 2 days after inoculation, and by day 6 oval to irregular lesions developed, which measured 1.9 × 0.7 mm on W64A, a susceptible inbred line. Ullstrup (1941, 1944) first described lesion of race 2 as elongated one week after inoculation, and, later, Nelson and Ullstrup (1961) described lesions induced by race 2 on the same inbred line as chlorotic to necrotic flecks, although the incubation time was not established. This discrepancy in the description of the lesion shape induced by race 2 could be accounted for by evaluation of germplasm at different times. It is shown here that changes in morphology occurred between 2 and 8 days after inoculation.

Lesions induced by races 1 and 2 on resistant lines were indistinguishable based on their shape or size. Lesion shape on one hybrid has been used as the criterion to identify races of this pathogen, i. e., 1, 2 and 3 (Blanco *et al.*, 1974), or 2 and 3 (Lodge and Leonard, 1984; Leonard and Leath, 1990). The use of one hybrid for identification of a race could lead to artifacts if the hybrid is resistant to one race.

All isolates of race 3 used in this experiment induced linear lesions; however, race 3 lesions that ceased to develop after day 2 could not be distinguished from non-developed lesions produced by races 1 or 2. Although these isolates produced only linear lesions, other isolates induced predominantly linear lesions mixed with some elongated lesions, i. e., wider and shorter than the typical linear lesions. Linear and oval lesions of race 3 have been reported (Nelson *et al.*, 1973). Lesion shape is not useful as the primary criterion to identify races of *B. zeicola*, not only because races are erroneously identified, but because it is conceptually misleading. A set of differential lines may provide a more stable system for identifying races of this pathogen.

Lines both resistant and susceptible to races 1, 2 and 3 produced lesions that expanded beyond the infection point. Race 1 has been reported to remain viable in chlorotic flecks on resistant hosts for at least 60 days after inoculation (Jennings and Ullstrup, 1957). Susceptible lines produced longer, wider and, therefore, larger lesions than resistant or moderately resistant lines, independently of the race. Race 1 induced the largest lesions in the shortest time on susceptible lines, and race 2, the smallest lesions.

The occurrence of lesions that did not develop mixed with lesions that did, could be an expression of the pathogen or host. The fungus could be heterocaryotic and conidia originated from the same monosporous isolate could carry nuclei with different genetic composition producing different types of response on the same host. Another explanation is that conidia may germinate at different times and those that germinate late could be restricted by the host response producing lesions that did not enlarge. The host may also have a limited number of recognition sites beyond which the pathogen can penetrate

but not colonize the host tissue. Resistant lines displayed a higher proportion of lesions that did not increase in size between 2 and 6 days after inoculation than susceptible lines. B73 is susceptible to isolates of race 2 tested in this experiment and exhibited a high proportion of lesions that did not enlarge; however, developed lesions were longer, wider and larger than lesions induced on resistant lines. Disease efficiency is another component of disease resistance that should be considered since a higher number of lesions per unit of inoculum could have accounted for this discrepancy. Nevertheless lesion size could be used to aid in the identification of resistant genotypes to these races of *B. zeicola*.

In a previous study, Hamid *et al.* (1982b) found significant host × isolate interaction when they measured lesion length produced by 6 isolates of race 3 on 9 inbred lines. In the current study, 4 isolates of race 3 were inoculated on 5 maize lines and no host × isolate interaction for lesion length was detected. Variation in specificity among isolates that induce linear lesions is limited.

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Table 3.1: Isolate, state of collection, race designation and source of *Bipolaris zeicola*.

Isolate	State	Race	Source
D-2	IA	1	J. Dodd
D-15	IA	1	J. Dodd
85-2427	IN	1	D. R. Smith
Purdue	IN	1	H. L. Warren
80-1910	IL	2	D. R. Smith
82-1026	MI	2	D. R. Smith
85-2600	PA	2	D. R. Smith
85-3326	TN	2	D. R. Smith
Bz 2-6	NC	3	M. L. Carson
Bz10-59	NC	3	M. L. Carson
85-1954	OH	3	D. R. Smith
G47-29	VA	3	E. Traut

Table 3.2: Percentage of non-developed lesions after inoculation with *Bipolaris zeicola* races 1, 2 and 3.

Inbred line	Race 1	2		3	
W64A	56.5	13.0 [a]	c	12.1	b
B73	-	40.3	b	9.7	b
Mo17	-	27.8	bc	7.9	b
Pr	0.0	38.4	b	25.5	a
H95	-	59.7	a	28.7	a
Mo21a	7.5	-		-	
Sel. (K41 × K44)	1.9	-		-	
B37	72.7	-		-	

[a] Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

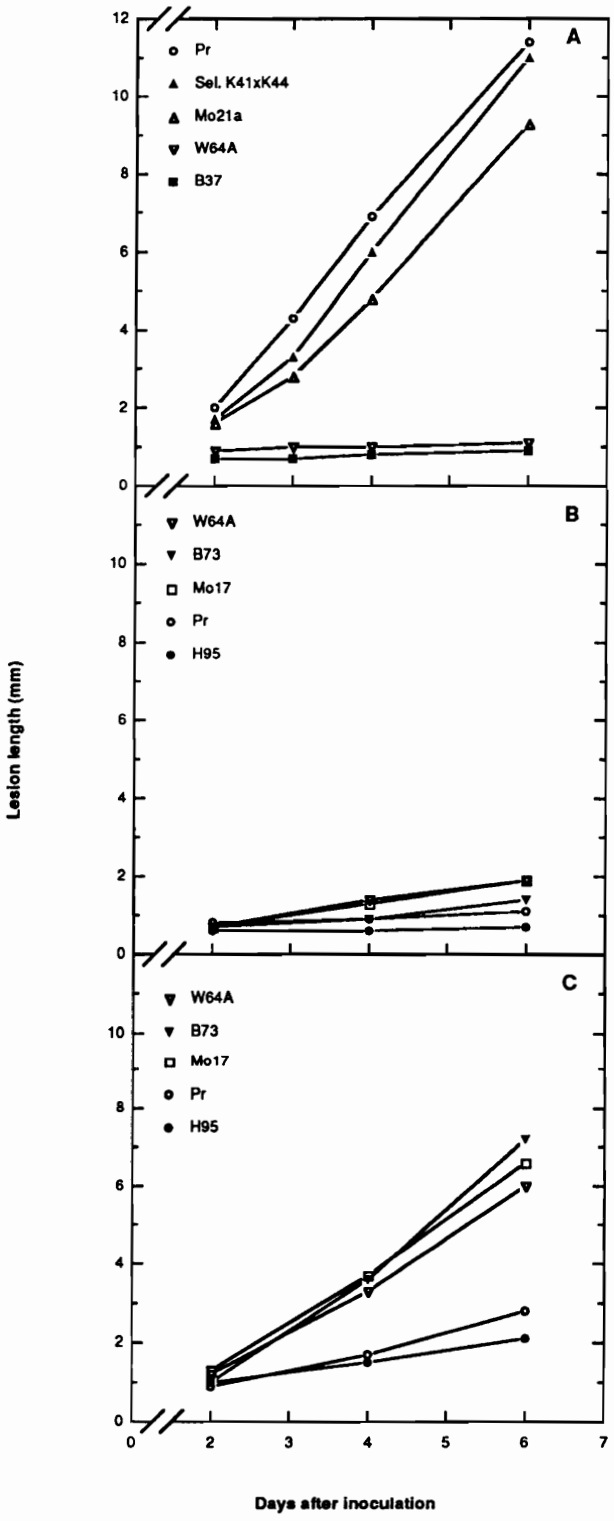

Figure 3.1: Progress of lesion length (mm) on maize inbred lines after inoculation with *Bipolaris zeicola* races (A) 1, (B) 2 and (C) 3.

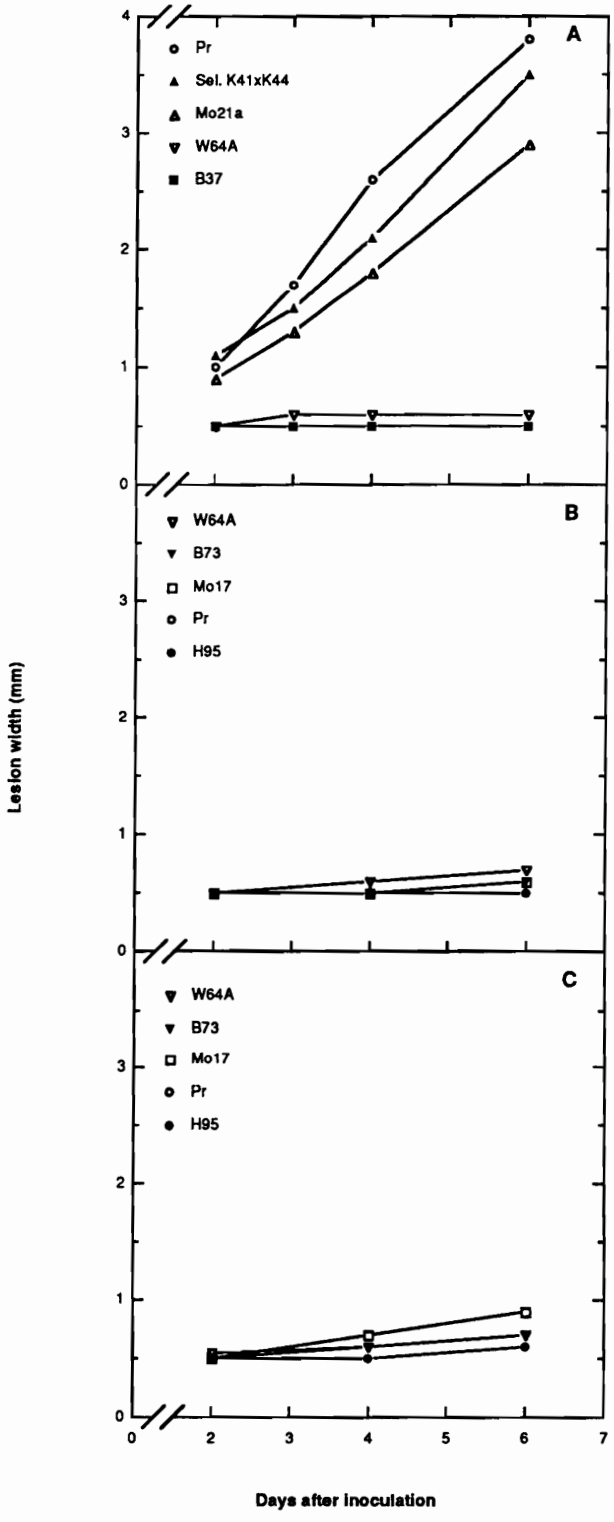

Figure 3.2: Progress of lesion width (mm) on maize inbred lines after inoculation with *Bipolaris zeicola* races (A) 1, (B) 2 and (C) 3.

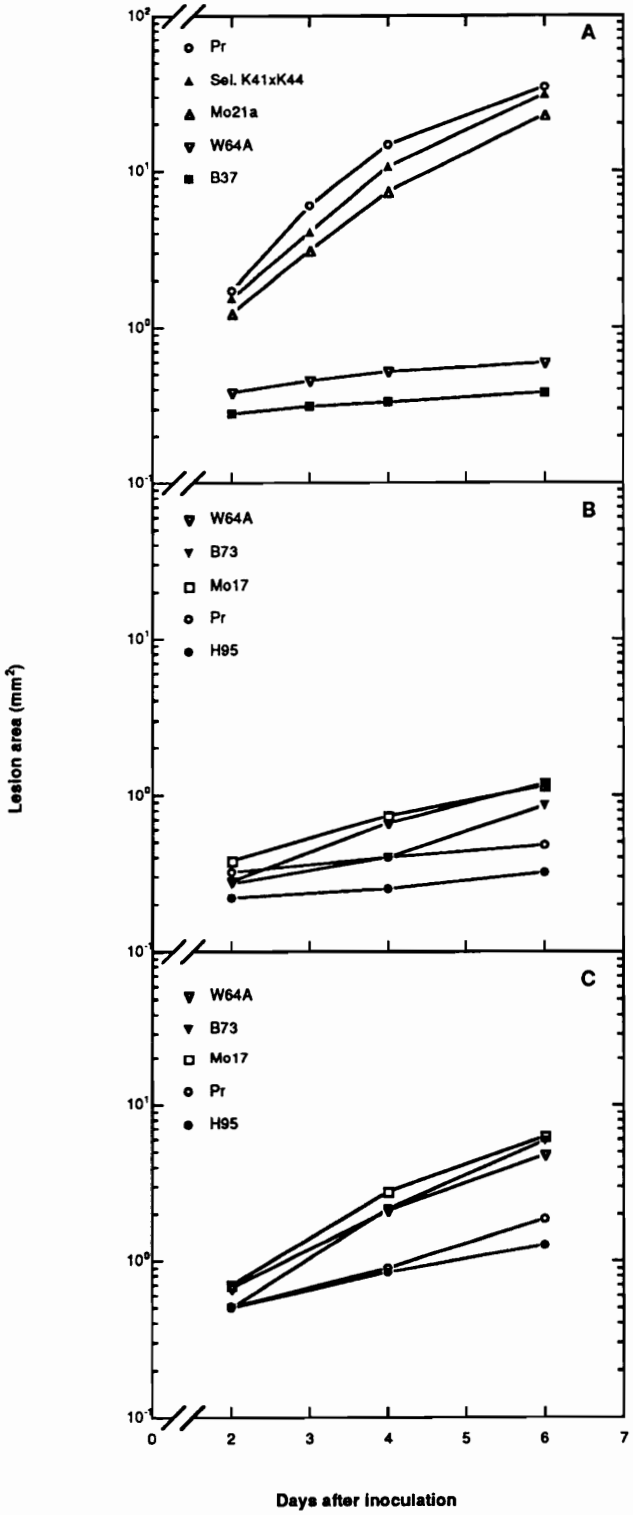

Figure 3.3: Progress of lesion area (mm^2) on maize inbred lines after inoculation with *Bipolaris zeicola* races (A) 1, (B) 2 and (C) 3.

CHAPTER 4

INHERITANCE OF REACTION IN MAIZE TO THE "NEW PATHOTYPE" OF *Bipolaris zeicola*

Introduction

Three races and one pathotype of *Bipolaris zeicola* (Stout) Shoemaker which cause Helminthosporium leaf spot of maize have been reported (Ullstrup, 1941b, 1944; Nelson *et al.*, 1973; Dodd and Hooker, 1990). This disease has become increasingly important, especially in seed production fields where losses of up to 50% have been reported (Dodd and Hooker, 1990; Hooker, 1974).

Resistance to race 1 is inherited by a single dominant gene, *Hm* (Ullstrup, 1941a, Ullstrup and Brunson, 1947), which could be modified by a second major gene, *Hm2*, when the first one is in the double recessive (*hmhm*) state (Nelson and Ullstrup, 1964). Additive gene action was the main component in studies of inheritance of reaction to *B. zeicola* race 3 in different diallel crosses (Hamid *et al.*, 1982; Halseth *et al.*, 1991). No studies have been published about the nature of the inheritance of reaction to race 2 and the new pathotype; however, it has been suggested that resistance to race 2 is polygenically inherited (Ullstrup, 1954).

The objectives of this study were to evaluate the reaction of different inbred lines to two isolates of the new pathotype to identify sources of resistance and determine the inheritance of reaction to this pathogen.

Materials and Methods

Inbred line screen

Fourteen maize inbred lines were used to test two isolates of the new pathotype under greenhouse conditions, i. e., A632, B14, B37, B73, C103, H84, H95, H111, Mo17, Oh43, Pr, Pr1, Va26 and W64A, arranged in a completely randomized design with 3 replicates and repeated twice. Four seeds per pot were planted in plastic pots 15-cm in diameter and thinned to three seedlings per pot after emergence.

Inoculum

Leaf tissue infected with *B. zeicola* was collected from Eldora, Iowa (D-5) and Brookston, Indiana (D-20) and identified by Dr. J. L. Dodd as the new pathotype based on foliar symptoms. The fungus was isolated by using standard techniques, single-spored and grown on lactose casein hydrolysate (LCH) agar under constant, cool-white fluorescent light (3,600 lux) at 23±1 C. Single conidial isolates were transferred to sterilized sorghum kernels and stored at 4 C. Maize tissue infected with each single conidial isolate was air dried and maintained at 4 C.

Conidial suspensions were prepared from 7-9 day-old cultures grown on LCH agar and adjusted to 5×10^3 conidia per ml.

Plants were inoculated at the V4-V5 growth stage by spraying the conidial suspension on all leaves of each plant at a pressure of 0.83 bar. Inoculated plants were incubated in a mist chamber in darkness for 15 to 16 hours.

Evaluation

Plants were evaluated for disease reaction and severity on the third and forth leaves, 12 days after inoculation. Disease reaction was classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) according to the predominant types of lesions in each plant. Resistant-type lesions were chlorotic or necrotic flecks to small lesions with a chlorotic halo, and susceptible-type lesions were medium to large lesions with water soaked margins. Resistant plants exhibited no lesions or had chlorotic or necrotic flecks; moderately resistant plants exhibited small lesions with a chlorotic halo. Moderately susceptible plants showed susceptible-type lesions mixed with chlorotic or necrotic flecks. Susceptible plants exhibited predominantly oval to irregular lesions with water soaked margin mixed with few resistant-type lesions. Disease severity was assessed in a scale of 1-5 with increments of 0.5, where 1 = lesion absent, 2 = lesions covering 25% of leaf area, 3 = lesions covering 50% of leaf area, 4 = lesions covering 75% of leaf area and 5 = lesions covering 100% of leaf area or dead plants.

Analysis of variance of disease severity was conducted for each isolate and means of disease severity for each inbred line were separated by the Tukey-Kramer HSD test.

Inheritance Studies

The genetic basis of reaction in maize to two isolates of the new pathotype of *B. zeicola* was evaluated by crossing resistant with susceptible inbred lines. F_1s were produced during 1990, and the F_2s and backcrosses to their parents during 1991 at the Whitethorne Farm, Blacksburg, Va. The following generations of germplasm for evaluation were obtained: P_R (resistant parent), P_S (susceptible parent), F_1, F_2, BC_R (backcross of F_1 to the resistant parent) and BC_S (backcross of F_1 to the susceptible parent). Seeds from each parent and each F_1 were bulked and those from F_2 and backcrosses were maintained and tested separately from each plant. Forty-eight to 50 seeds per ear of three ears of each F_2 and backcrosses were tested for segregation. Plants were evaluated for lesion types and disease reaction 12 days after inoculation. Both repetitions in time and progenies from each cross were tested for homogeneity and pooled whenever possible. Chi square tests were conducted to study the segregation ratios.

Results

Inbred line screen

Differences in specificity between both isolates of the new pathotype were found (Table 4.1). Isolate D-5 produced a susceptible reaction on A632 and B14, a resistant reaction on H95, Oh43, Va26 and C103. B73, Pr and Pr1 were moderately susceptible to this isolate and the remaining lines were moderately resistant. A632 and B14 showed the highest disease severity 12 days after inoculation, 3.58 and 3.39, respectively, and H95, the lowest, 1.19.

The reaction types correlates well with disease severity, i. e., plants exhibiting resistance reaction had the lowest disease severity. Isolate D-20 incited a moderately susceptible reaction on B37 with a severity rating of 2.83, 12 days after inoculation and the remaining lines were moderately resistant or resistant to this isolate.

Inheritance of reaction

B14 and Oh43 were selected to study the inheritance of reaction to isolate D-5, and B37 and Pr, to isolate D-20. Both sets of crosses showed neither significant differences in segregation of lesions types between the two repetitions, nor differences among plants of the same generation. Therefore, the data was pooled from both repetitions and all plants of the same generation.

Crosses with parents of Pr and B37 showed a good fit between observed and expected frequencies of resistant and susceptible plants. The F_2 and BC_S after inoculation with *B. zeicola* D-20 indicated a single dominant gene conditioning resistance (Table 4.2). Crosses involving B14 and Oh43 showed a significant deviation from the 1:1 expected ratio for a single dominant gene in the backcross of the F_1 by the susceptible parent (Table 4.3).

Discussion

The isolates of the new pathotype tested on 14 maize inbred lines exhibited differences in specificity and are considered two different races, i. e., 11 (D-5) and 4 (D-20) (Chapter 5). Isolate D-20 gave a moderately susceptible reaction on B73 which agrees with the original description of the new pathotype (Dodd and Hooker, 1990). However, isolate D-20 gave a resistant reaction on

B73. Unfortunately, there is no type culture for the pathotype described by Dodd and Hooker (1990). It is uncertain if their description was based on one or more isolates since their primarily identification was under field conditions with limited testing under control conditions. These two isolates induce oval to irregular lesions, which place them in the previously described race 2, since no set of differentials has been used to distinguish races of this pathogen nor has any studies been conducted on host specificity and genetic variability of the race 2 pathogen.

Isolate D-20 showed specificity on only one line, B37. The inheritance of the resistance to this isolate in the cross Pr × B37 is controlled by a single dominant gene. Isolate D-5 was less specific, it produced susceptible reaction on A632 and B14, and although there were resistant inbred lines, most of them gave an intermediate reaction. Inheritance of reaction to race 1 is controlled by one or two genes (Ullstrup, 1941a, Ullstrup and Brunson, 1947). No inheritance studies of resistance to race 2 have been reported, although it was suggested that it is under polygenic control (Ullstrup, 1954). These results indicate that an increase in the population of isolate D-20 may be controlled by introducing a single gene.

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Table 4.1: Disease reaction (DR) and severity on maize seedlings 12 days after inoculation with *Bipolaris zeicola*.

Inbred line	Isolate			
	D-5		D-20	
	DR	Severity[a]	DR	Severity
A632	S	3.58 a	R	1.67 bcd
B14	S	3.39 ab	R	1.67 bcd
B37	MR	1.89 cd	MS	2.83 a
B73	MS	1.67 d	R	1.36 cd
C103	R	1.42 d	R	1.29 cd
H84	MR	1.47 d	MR	1.92 abcd
H95	R	1.19 d	R	1.24 d
H111	MR	1.50 d	MR	1.75 bcd
Mo17	MR	2.00 bcd	MR	1.79 bcd
Oh43	R	1.25 d	MR	2.26 abc
Pr	MS	3.22 abc	R	1.50 cd
Pr1	MS	2.47 abcd	R	1.50 cd
Va26	R	1.22 d	MR	2.14 abcd
W64A	MR	2.11 bcd	MR	2.53 ab

[a] Means of three replications repeated twice. Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

Table 4.2: Expected ratio, observed number of plants and chi-square tests for lesion type on Pr, B37 and their F_1, F_2 and backcrosses after inoculation with *Bipolaris zeicola* isolate D-20.

Family	Expected ratio	Observed Number	χ^2
	R S	R S	
P_R (Pr)	1 : 0	24 : 0	...
P_S (B37)	0 : 1	0 : 24	...
F_1	1 : 0	48 : 0	...
F_2	3 : 1	103 : 41	0.9
BC_R	1 : 0	144 : 0	...
BC_S	1 : 1	72 : 72	0.0

Table 4.3: Expected ratio, observed number of plants and chi-square tests for lesion type on Pr, B37 and their F_1, F_2 and backcrosses after inoculation with *Bipolaris zeicola* isolate D-5.

Family	Expected ratio	Observed Number	χ^2
	R S	R S	
P_R (Oh43)	1 : 0	20 : 0	...
P_S (B14)	0 : 1	0 : 23	...
F_1	1 : 0	45 : 0	...
F_2	3 : 1	106 : 31	0.4
BC_R	1 : 0	143 : 0	...
BC_S	1 : 1	85 : 39	17.0**

** Indicates significant at P=0.01.

CHAPTER 5

PHYSIOLOGICAL RACES OF *Bipolaris zeicola* PATHOGENIC TO MAIZE

Introduction

Helminthosporium leaf spot of maize (*Zea mays* L.), caused by *Bipolaris zeicola* (Stout) Shoemaker (teleomorph: *Cochliobolus carbonum* Nelson) was first recognized in Indiana (Ullstrup, 1941). Although it was considered a minor disease, an increase in disease severity has been reported from seed and commercial production fields in recent years (Dodd and Hooker, 1990; Hooker, 1974; Halseth *et al.*, 1991). The pathogen mainly infects the leaf blade, but also leaf sheath, husk, ear and stalk.

Three races and one pathotype of *B. zeicola* have been reported to date, with the peculiarity that races 1 and 2 (Ullstrup, 1941; Ullstrup, 1944) and one pathotype (Dodd and Hooker, 1990) were described by their differential pathogenicity on selected inbred lines; whereas race 3 was defined according to the lesion shape it produces (Nelson *et al.*, 1973). Race 1 produces a host specific toxin, HC-toxin (Scheffer and Ullstrup, 1965), and race 3 produces a non-specific toxin, BZR-toxin (Xiao *et al.*, 1991).

Differences in the shape of lesions on leaves that each race induced were reported and used as the basis for race distinction. Race 1 incites oval to circular lesions with light brown centers and darker brown margins on

susceptible inbred lines, i. e., Pr, K44, K61, Mo21a and N31 (Ullstrup, 1944; Ullstrup, 1977). Race 2 produces elongated oval to irregular lesions on susceptible inbred lines (Ullstrup, 1944). Lesions caused by race 3 are narrow and linear, grayish-tan, surrounded by a light to dark pigmented border and elongate between veins of the leaf (Nelson *et al.*, 1973). However, some isolates of race 3 produce predominantly oval lesions on some inbred lines. A new pathotype was described as causing oval to circular lesions, often with concentric rings, mainly in inbred lines with a B73 background (Dodd and Hooker, 1990).

Race identification has been based on the shape of lesion induced in a single host genotype, one of the following: N31, Pr × K61, DeKalb XL 394, Seneca Chief, Pioneer Brand 3368A or Pioneer Brand 3369A (Blanco *et al.*, 1974; Leonard, 1978; Lodge and Leonard, 1984; Leonard and Leath, 1990). However, chlorotic or necrotic flecks have been associated with races 1 (Ullstrup, 1944) and 2 (Nelson and Ullstrup, 1961). Oval lesions are produced by races 1 and 2 (Ullstrup, 1944), the new pathotype (Dodd and Hooker, 1990) and some host-isolate combinations of race 3 (Nelson *et al.*, 1973). Most importantly, the concept of physiological races is based on differential reactions of host genotypes to different pathogen genotypes and the use of lesion shapes induced on one host genotype to distinguish races of the pathogen is conceptually misleading and also overlooks variability within the pathogen.

This research was undertaken to (1) find a set of differentials to distinguish the described races and the new pathotype, (2) determine whether there was intraracial variability of *B. zeicola* and (3) study the relationship of

lesion shapes with physiological races of the pathogen. A new system for race identification based on the disease reaction on a set of differentials is proposed.

Materials and Methods

Sources of Isolates

Forty-nine isolates of *B. zeicola* were obtained from different geographical areas of the United States (Table 5.1). Single conidial isolates were prepared from each original isolate by the separate-locate-isolate technique (Tuite, 1969) and grown on lactose casein hydrolysate (LCH) medium under constant, cool-white fluorescent light (3600 lux) at 23 ± 1 C. A conidial suspension of each single conidial isolate was inoculated into sterilized sorghum kernels, incubated for one week and stored at 4 C. After inoculation, leaf tissue infected with each isolate was dried between paper towels and stored at 4 C. All isolates were inoculated on a set of fourteen maize inbred lines to determine their race.

Plant Material

Fourteen maize inbred lines were selected for this study: A632, B14, B37, B73, C103, H84, H95, H111, Mo17, Oh43, Pr, Pr1, Va26 and W64A (Table 5.2). Two near-isogenic lines, Pr and Pr1, were included in this study because of their differential reaction to races 1 and 2 of *B. zeicola* (Ullstrup, 1944). Pr1 is a race 1 resistant derivative of Pr that differs by a single gene for resistance.

Four seeds from each of the 14 inbred lines were treated with Captan, planted in plastic pots 15-cm in diameter containing a soil mix (1 part soil, 2 parts web-lite), and fertilized with a 14-14-14 controlled release fertilizer (Osmocote) and a micronutrient fertilizer (Micromax) at rates of 6 g/l each. The fourteen maize inbred lines were arranged in a completely randomized design with three replicates within each isolate. Each isolate was repeated at least twice for most isolates (Table 5.1). Plants were grown in the greenhouse at 26 ± 3 C. After emergence, seedlings were thinned to three plants per pot.

Preparation of Inoculum

Conidial suspensions were prepared by flooding 7 to 9 day-old cultures grown on lactose casein hydrolysate (LCH) agar of each isolate of *B. zeicola* with distilled water. The conidia were dislodged with a rubber spatula and the suspension was filtered through three layers of cheesecloth to separate large mycelial fragments from conidia. Conidial concentration was measured with an improved Neubauer hemacytometer and adjusted to 5×10^3 conidia per milliliter. One drop of Tween 20 (polyoxyethylene sorbitan monolaurate) per 100 ml of conidial suspension was added as a wetting agent.

Inoculation

Plants were inoculated in the greenhouse at the V4 - V5 growth stage (Ritchie *et al.*, 1986) by spraying a conidial suspension of each isolate on all leaves of each plant with a Devilbiss atomizer (Somerset, PA) attached to an air pump at a pressure of 0.83 bar. Inoculations were performed late in the afternoon. Immediately after inoculation plants were placed in a mist chamber

where the humidity was maintained near saturation in darkness for 15 to 16 hours. The plants were transferred to greenhouse benches to allow disease development.

Lesion Type and Disease Severity

All plants of the fourteen inbred lines were observed daily for symptom development. Evaluations were done on the third and fourth leaves of each plant which were fully exposed to the inoculum. Lesion type, disease reaction of the whole plant and disease severity were assessed in the first repetition, 3, 5, 7 or 9, 12 and 14 (in most experiments) days after inoculation, and in the second repetition, 3, 5, 7, 9, 12 and 14 days after inoculation.

Lesion types were classified as resistant- or susceptible-types. Resistant-type lesions were restricted by the host response resulting in chlorotic or necrotic flecks to small lesions with a chlorotic halo (Figures 5.1C, 5.2D, 5.3C, 5.4D). Susceptible-type lesions had water soaked margins and expanded resulting in medium to large lesions (Figures 5.1A, 5.2A, 5.3A, 5.4A).

Disease reaction of each plant at each evaluation time was classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Resistant plants exhibited resistant-type lesions, mainly chlorotic or necrotic flecks (Figures 5.1C, 5.2D, 5.3C, 5.4D). Moderately resistant plants showed resistant-type lesions that enlarged slightly resulting in small necrotic lesions, frequently with extensive chlorosis (Figures 5.2C, 5.4C). Moderately susceptible plants exhibited susceptible-type lesions mixed with resistant-type lesions (Figures 5.1B, 5.2B, 5.3B, 5.4B). Susceptible plants showed predominantly susceptible-type lesions mixed with few or no resistant-

type lesions (Figures 5.1A, 5.2A, 5.3A, 5.4A). Disease reaction was transformed to a numerical scale for statistical purposes, where R = 1, MR = 2, MS =3 and S = 4.

In one or two repetitions, an additional score, lesion type index (number of susceptible-type lesions in its relation to the total number of lesions) was estimated using a scale of 1-4, where 1 = absent or resistant-type lesions, 2 = predominantly resistant-type lesions, 3 = predominantly susceptible-type lesions, and 4 = only susceptible-type lesions. This lesion type index (S) was transformed to a decimal scale (s) for statistical purposes, i. e., $s = (S-1) \times 0.33$.

Disease severity, Y (percentage of leaf area covered with lesions) was estimated with an index scale of 1-5 with increments of 0.5, where 1 = no lesion, 2 = lesions covering 25% of leaf area, 3 = lesions covering 50% of leaf area, 4 = lesions covering 75% of leaf area, and 5 = lesions covering 100% of leaf area or dead plants. This index was transformed to severity in decimal form (y) for statistical purposes, i. e., $y = (Y-1) \times 0.25$.

Area under the disease progress curve (AUDPC) was calculated for each treatment for the period of 5 to 12 days after inoculation by using the following equation:

$$AUDPC = \sum_{i=1}^{n} [(Y_i + Y_{i+1}) / 2] [x_{i+1} - x_i]$$

where Y_i equals the percentage of diseased tissue at time i, and x_i equals time expressed as days at the ith observation and n equals the number of ratings taken (Shaner & Finney, 1977).

Disease severity was transformed using the simple interest model, i. e., ln 1/(1-y) (Van der Plank, 1963). This transformation was preferred because of the lack of secondary spread under greenhouse conditions. Rates were estimated by regressing the transformed disease severity proportion (y) by time in days for each combination of inbred line and isolate. Slopes or linear regression coefficients (b) of these lines were used as estimates of the rate of disease increase.

Disease severity, lesion type index, AUDPC and rate of disease progress of isolates repeated at least twice were subjected to analysis of variance. Differences among treatments were tested for statistical significance using the Tukey-Kramer honestly significant difference (HSD) test at a significance level of 0.05.

Statistical Methods

An analysis of variance for factorial experiments was conducted to determine main and interactive effects for each variable under study. After determining a highly significant isolate × line interaction as expected when dealing with races, isolates were grouped and analyzed according to the following criteria: (1) race originally determined; (2) lesion shape induced on susceptible host; and (3) disease reaction on the 14 maize inbred lines.

Set of Differentials

Correlation coefficients were calculated for the disease reaction of the 14 inbred lines to all races of the pathogen evaluated 12 days after inoculation. The frequencies of disease reactions, i. e., R, MR, MS and S, were calculated

for each inbred line among all races of the pathogen. When two inbred lines showed a high correlation, they were compared among all races and unclassified isolates to determine if there was a differential reaction between both. If there was a differential reaction not detected by other inbred lines with lower correlation coefficients, both inbred lines were kept. If there was no differential reaction or detected by other inbred lines with lower correlation coefficients, the inbred line with a higher frequency for intermediate reactions (MR plus MS) was removed.

Results

Set of Differentials

Eleven races of *B. zeicola* were distinguished among 49 isolates based on their differential reaction on a set of 14 maize inbred lines (Table 5.3). Additionally 3 isolates that were tested once in the greenhouse, 23-55, 23-56 and 81-5032, and 2 isolates of the new pathotype that lost their pathogenicity after the first inoculation produced differential reactions (Table 5.5).

The coefficients of correlation for the disease reaction of each pair of the 14 maize inbred lines and the frequency of the reaction type of each inbred line to the 11 races and the 5 unclassified isolates are shown in Tables 5.6 and 5.7, respectively. A632 and B14, two related inbred lines, displayed the highest correlation coefficient ($r = 0.98$) (Table 5.6) and similar reaction to all races (Table 5.4). A632 was removed because of its higher frequency of intermediate reactions than B14 (Table 5.7). Subsequently, C103, H84, W64A, H111, Oh43, Va26 and Pr1 were removed in that order by using the same criterion.

The disease reaction of B14 and B73 were highly correlated (r = 0.83), but they reacted differentially to races 44 and 38 and isolate 23-55. A set of 6 inbred lines, B14, B37, B73, H95, Mo17 and Pr, distinguished the 11 races and the 5 unclassified isolates of *B. zeicola*. Potentially, the 6 inbred lines could distinguish 64 races.

Pr and Pr1 differ in a single gene for resistance to the originally described race 1 of *B. zeicola*, and, therefore, showed differential reaction to the races that induced symptoms of HC-toxin production. Pr1 has the *Hm* gene for resistance to the originally described race 1; however, other inbred lines showed resistance to races 1 and 17, B14, B37, B73 and Mo17.

Nomenclature of physiological races

The 11 physiological races of *B. zeicola* were designated races 0, 1, 4, 11, 17, 32, 36, 37, 38, 44 and 46 applying the binary notation (Habgood, 1970). The binary system requires to separate disease reaction of differential hosts to each race into two categories, i. e., resistant or susceptible. Therefore, intermediate reactions were considered resistant or susceptible according to the predominant lesion type. The differential hosts are arranged in a fixed order and the reaction of each inbred line to each race was given the value of 0 for resistance and 1 for susceptibility. This resulted in a binary code which was converted to decimal notation and used as the new race designation.

The set of 6 differential inbred lines was arranged in the following order: Pr, B14, B37, B73, H95 and Mo17. The binary code for each race, the new race designation in decimal notation and its correspondence with the previous race designation are shown in Table 5.8.

Lesion Types

Lesions types were mixed in most inbred line-isolate combinations; both resistant- and susceptible-type lesions were present in the same leaf. In addition, lesions evolved with time resulting in an increase, decrease or fluctuation in the lesion type index (Figure 5.5, 5.6 and 5.7). The lesion type index induced by each race 12 days after inoculation is shown in Table 5.9.

Two races were separated based on their differential reaction among the isolates inducing typical symptoms of HC-toxin production on Pr, 1 and 17. Both races produced a susceptible reaction on Pr; in addition, race 17 induced a moderately susceptible reaction on H95. Isolates Hc128, WT-4 and M-2 were classified as race 1, and isolates 84-1676, 84-1926, 84-3277, 85-2427, Purdue, D-2 and D-15, as race 17 (Table 5.3). All isolates of races 1 and 17 were originally identified as race 1, except D-2 and D-15 which were collected as the new pathotype and identified by symptoms on the leaf in the field.

All lesions incited by races 1 and 17 on Pr were susceptible-type 2 days following inoculation (Figure 5.5); they were oval to circular with light brown centers and darker brown margins. At the same time, the near isogenic line, Pr1, only showed small chlorotic pin point flecks. Lesions on Pr continually expanded until coalescence occurred, resulting in death of all infected tissue by 5-7 days after inoculation. The remaining inbred lines developed oval to irregular lesions 5 days after inoculation resulting in a low disease severity. Race 17 produced grayish, irregular, susceptible-type lesions on H95, 5 to 14 days after inoculation (Figure 5.5).

Twenty-seven isolates produced oval to irregular, brown to brownish tan lesions with water-soaked margins in compatible interactions. Isolates that

induced only oval to irregular lesions and did not produce typical lesions of HC-toxin production on Pr composed the most diverse group. This group included isolates originally identified as race 2, most isolates of the new pathotype, isolates originally identified as race 3, which consistently produced only oval lesions on the 14 inbred lines, and 2 isolates originally classified as race 1 which did not give symptoms of HC-toxin production on Pr or Mo21a. Seven races were distinguished among these isolates with differential reaction, namely 4, 11, 32, 36, 37, 38 and 44 (Table 5.4). In addition, five isolates tested once in the greenhouse gave different reactions from the 11 races (Table 5.5).

The progress in the proportion of susceptible-type lesions on 4 inbred lines after inoculation with races 4, 36 and 37 is illustrated in Figure 5.6. W64A exhibited a susceptible reaction with races 36 and 37, and a moderately resistant with race 4. The proportion of susceptible- to resistant-type lesions was similar for all 3 races 5 days after inoculation (Figure 5.6A). The number of susceptible-type lesions increased with races 36 and 37. Many resistant-type, pin point lesions, chlorotic and necrotic flecks became susceptible-type with time. After 5 days the proportion of susceptible- to resistant-type lesions incited by race 4 declined because some susceptible-type lesions were restricted at 7 days after inoculation and later resumed an active growth stage (susceptible-type lesion type) (Figure 5.6A).

B37 gave a moderately susceptible reaction with races 4, 36 and 37; the lesion type index increased with races 4 and 36, and fluctuated with race 37. This fluctuation was caused by an inhibition of lesion size and later an active development of lesions which resumed growth as the tissues grew older (Figure 5.6B).

Pr exhibited a resistant and moderately resistant reaction to races 4 and 36, respectively, which only developed a low proportion of active lesions (Figure 5.6C). However, Pr gave a susceptible reaction to race 37; the proportion of susceptible- to resistant-type lesions decreased between 5 and 7 days after inoculation because new pin point lesions developed, and, thereafter, the number of susceptible-type lesions increased (Figure 5.6C).

H95 displayed a resistant reaction to races 4, 36 and 37. Although, some susceptible-type lesions developed, they were restricted by the host resulting in a low proportion of susceptible-type lesions at all times (Figure 5.6D).

Only isolates originally identified as race 3 that induced predominantly linear lesions on the 14 maize inbred lines showed similar differential reaction, except isolate 86-1074. This race was renamed race 46. Susceptible-type lesions were narrow and linear, grayish-tan in color surrounded by a light to darkly pigmented border. Resistant-type lesions were chlorotic flecks to chlorotic linear lesions. In addition to linear lesions, some isolates, i. e., 85-1954, WT-1 and ATCC26840, also produced oblong lesions, long but also wide, approximately 6 × 2 mm, mixed among typical linear lesions on most inbred lines. Isolate 86-1074 produced resistant or moderately resistant disease reaction with all 14 inbred lines, and, therefore, it was included with the avirulent race 0.

Race 46 induced a susceptible reaction on B73 and W64A, a moderately resistant reaction on Pr, and resistant reaction on H95 (Table 5.4). The evolution of the proportion of susceptible- and resistant-type lesions produced by one isolate of race 46, i. e., 85-1954 on B73, H95, Pr and W64A is illustrated

in Figure 5.7. The number of susceptible-type lesions increased rapidly on W64A 5 to 7 days after inoculation. On the other hand, B73 maintained a similar proportion of susceptible- and resistant-type lesions between 5 and 7 days after inoculation; thereafter, the proportion of susceptible- to resistant-type lesions increased. Pr showed the same lesion type index during the experiment. H95 produced a low number of susceptible-type lesions that declined until 9 days after inoculation, and the number of susceptible-type lesions increased towards the end of the experiment (Figure 5.7).

Three isolates, D-6, 84-2533 and 86-1074 produced incompatible interactions with the 14 inbred lines and were considered as race 0; however, only isolate D-6 gave a resistant reaction on all 14 inbred lines. A moderately resistant reaction was induced on W64A by isolate 84-2533. B37, Mo17, Va26 and W64A exhibited a moderately resistant reaction when inoculated with isolate 86-1074. Isolates D-6 and 84-2533 induced chlorotic flecks and small necrotic oval lesions, whereas isolate 86-1074 produced resistant-type chlorotic or necrotic linear lesions.

Isolates of the new pathotype reacted differently among themselves (Table 5.3). Isolates D-2 and D-15 produced symptoms of HC-toxin production on Pr, and was classified as race 17. Isolate D-13 was included in race 36 among isolates originally identified as race 2. Races 4, 11 and 38 were exclusively formed by isolates of the new pathotype. Race 37 was composed of one isolate of the new pathotype, D-11, and isolate G3-4, which produced oval lesions. Isolate G3-4 was isolated from a maize field predominantly infected with race 46 in Blacksburg, Virginia. Isolate D-6 was avirulent on the 14 maize inbred lines and classified as race 0. Four isolates that were originally

identified as the new pathotype, i. e., D-0, D-4, D-11 and D-16, lost their pathogenicity in culture, and consequently only the first repetition was statistically analyzed. Four of the 12 isolates originally identified as the new pathotype gave a compatible reaction on B73. Isolates D-4 and D-16 produced a susceptible reaction, isolates of race 11, i. e., D-0 and D-5, induced a moderately susceptible reaction, and the remaining isolates of the new pathotype induced a resistant or moderately resistant reaction on B73.

Differential reactions were expressed at different times for each race. Isolates that produced oval lesions and the HC-toxin could be distinguished by a compatible interaction on Pr from the non-HC-toxin producing isolates 2 days after inoculation (Figure 5.5 and 5.6). Both HC-toxin producing races, 1 and 17, induced susceptible-type lesions on hosts other than Pr 7 days after inoculation. Races 1 and 17 could be separated as distinct races based on their reaction on H95, 7 days after inoculation (Figure 5.5). The time of symptom expression was variable in races that developed oval lesions, however, 12 days after inoculation symptoms could be readily distinguished. The proportion of susceptible- to resistant-type lesions was low on most inbred lines up to 7 or 9 days after inoculation with races inducing oval lesions, except races 11 and 37, which induced a higher proportion of susceptible- to resistant-type lesions at all times compared to other races that produced oval lesions (Figure 5.6). Isolates that induced linear lesions showed their differential reaction 9 days after inoculation. B73, a susceptible host to race 46, was not distinguished from resistant and moderately resistant inbred lines until 9 days after inoculation (Figure 5.7).

Disease Severity

Analysis of variance for disease severity at 12 days after inoculation and AUDPC showed no significant isolate × line interaction for each race, except races 0, 1 and 17. Rate of disease progress of each race that induced oval lesions showed no significant isolate × line interaction. Disease severity 12 days after inoculation, AUDPC and rate of disease progress for the 11 races are shown in tables 5.10, 5.11 and 5.12.

Pr exhibited the highest disease severity in the shortest time when it was inoculated with *B. zeicola* races 1 and 17 (Table 5.10) than with all other races. Consequently, these interactions showed the highest disease progress rates and the largest AUDPCs (Tables 5.11 and 5.12). The maximum severity was reached between 5 and 7 days after inoculation when lesions coalesced producing the death of all infected tissue. Although race 17 induced a moderately susceptible reaction on H95, the disease severity was low. The disease severity on Pr and H95 induced by one isolate each of race 1 (Hc128) and race 17 (84-1926) is illustrated in Figure 5.8.

There was a wide variation in the expression of the disease severity among the oval lesion producing races. Most inbred line-race combinations, however, gave intermediate levels of disease severity (Table 5.9). Races 11, 37 and 38 showed higher severity over all inbred lines than races 4, 32, 36 and 44. The disease progress of races 32, 36 , 37 and 44 on W64A, B73, Pr, Pr1, Mo17 and H95 is illustrated in Figure 5.9. W64A exhibited the highest disease severity for each race at each rating period, and H95 the lowest. W64A was susceptible to races 36, 37 and 44, and moderately susceptible to race 32, and Mo17 was susceptible to races 36 and 37 and moderately susceptible to races

32 and 44. Disease severity increased over time at a higher rate among susceptible inbred lines than in inbred lines exhibiting moderately susceptible disease reactions (Figure 5.9A-B). Pr and Pr1 were susceptible to race 37 and moderately resistant to races 32, 36 and 44, and exhibited a higher rate of disease progress in the compatible interaction (Figure 5.9C-D). B73 gave a susceptible disease reaction with race 44, moderately resistant with race 37 and resistant with races 36 and 32, and displayed a disease progress rate of 0.120, 0.038, 0.019 and 0.005 per day, respectively (Table 5.11) (Figure 5.9E). H95 showed a resistant reaction to races 32, 36, 37 and 44 with low severity, AUDPC and rate of disease progress (Tables 5.9, 5.10 and 5.11) (Figure 5.9F).

All inbred lines developed lesions and intermediate levels of severity after inoculation with race 46 (Table 5.9); however, differences were evident in disease reaction, severity and AUDPC (Tables 5.4, 5.9 and 5.10). Figure 5.10 shows the disease severity on B73, Mo17, Pr and H95 induced by race 46 which gave a susceptible, moderately susceptible, moderately resistant and resistant reaction, respectively, and the severity and AUDPC that they showed followed the same ranking.

Plants with susceptible-type lesions showed a higher severity; however, some line-race combinations produced few but susceptible-type lesions, resulting in a susceptible disease reaction with low severity. For example, Mo17 gave a moderately susceptible reaction with race 32, but it showed 7.25% of infected tissue (severity = 1.29) (Tables 5.4 and 5.9).

Discussion

Forty-nine isolates of *B. zeicola* were characterized by the disease reaction, severity and symptoms incited on 14 maize inbred lines. Eleven races of *B. zeicola* were distinguished based on their differential reaction on the 14 inbred lines. However, a minimum set of 6 inbred lines, i. e., Pr, B14, B37, B73, H95 and Mo17, differentiated the 11 races.

A system based on binary notation was used to designate the 11 races of *B. zeicola* because of the lack of knowledge about the genetic control of resistance and virulence for most inbred line-race interactions. This system was introduced by Habgood (1970) and applied to *Puccinia striiformis* on wheat by Johnson *et al.* (1972).

Pr and Pr1 and crosses with these lines to K61 have been used to distinguish the original races 1 and 2 (Ullstrup, 1944; Jennings and Ullstrup, 1957; Cantone and Dunkle, 1991) because they are near-isogenic lines differing in the *Hm* gene for resistance to race 1 (Ullstrup, 1944). Pr and Pr1 were useful to distinguish races that induce symptoms of HC-toxin production on Pr, i. e., races 1 and 17, from those that did not produced symptoms of HC-toxin on Pr. Additionally both inbred lines, Pr and Pr1, exhibited the same disease reaction with similar disease severity with the remaining 9 races, indicating that the *Hm* gene is not involved in the resistance to races that produce no HC-toxin. Resistance to races 1 and 17 was also expressed by other inbred lines in the minimum set of differentials, i. e., B14, B37, B73 and Mo17.

Resistance to isolates of *B. zeicola* that produce linear lesions is under polygenic control (Hamid *et al.*, 1982; Halseth *et al.*, 1991); however, resistance

to a race that causes oval lesions appeared to be controlled by one dominant gene (Chapter 4). Studies of inheritance of resistance to each race could identify major genes for resistance. Near-isogenic lines with major genes for resistance would be desirable to establish a set of differentials with a genetic basis and provide information on the virulence genes in the pathogen.

Races of *B. zeicola* have been originally described based on differential reactions to distinguish races 1 from 2 (Ullstrup, 1944) and the new pathotype (Dodd and Hooker, 1990), and on lesion shape to distinguish race 3 from the others (Nelson *et al.*, 1973). A set of differentials to distinguish races of *B. zeicola* previously identified has not been reported. Instead, races have been identified by the lesion shape incited in a single host genotype (Blanco *et al.*, 1974; Leonard, 1978; Lodge and Leonard, 1984; Leonard and Leath, 1990).

Our results suggest that the previous system has overlooked the variation of the population of *B. zeicola* since one host genotype can not reveal the diverse variability in virulence of the pathogen. Additionally, lesion shape is not a distinct character among the previously described races and may not allow for the differentiation of 11 races. The eleven races detected in this study would have been classified into 3 races by using the criteria of lesion shape on a single host or the Pr and Pr1 isolines with or without K61 systems.

Races 1 and 17 induced the typical symptoms of HC-toxin production on Pr, and agree with the original description of race 1 (Ullstrup, 1941). However, other inbred lines developed oval to irregular lesions which disagree with Ullstrup (1941) who reported flecks on resistant inbred lines 7 days after inoculation. Lesions on other inbred lines developed slowly and were mainly expressed 9 days or more after inoculation in the greenhouse at 24±3 C. The

difference in the time of evaluation could have accounted for the disagreement in lesion shape. Also race 17 gave a moderately susceptible reaction on H95 9 days after inoculation which would have been unnoticed by evaluating one week after inoculation. Segregation for the ability to produce HC-toxin in some crosses of race 1 × race 2 differs from the expected ratio for a single gene (Scheffer *et al.*, 1967). Some isolates carry additional genes for production of HC-toxin which may confer virulence to other hosts.

Twenty-seven isolates used in this research, represented 7 races that induced oval to irregular lesions in compatible interactions and were classified as race 2 by using either criterion, Pr and Pr1 as differentials or the lesion shape. These races induced chlorotic to necrotic flecks that developed into oval to irregular lesions which were evident between 5 and 9 days following inoculation depending on the race-inbred line combination. Ullstrup (1941) reported that race 2 produces elongated to irregular lesions on Pr, but later, Nelson and Ullstrup (1961) stated that race 2 produces only chlorotic to necrotic flecks on Pr. This discrepancy could be explained by differences in the time of evaluation, or by the use of different races in both experiments. Our results support either proposal. Additionally, Nelson and Ullstrup (1961) reported that lesions caused by race 2 are indistinguishable from resistant-type lesions caused by race 1. When our set of differentials was inoculated with most isolates that produced oval lesions, susceptible-type lesions developed 7 to 9 days after inoculation.

Some isolates of race 46 produced elongated lesions, wide and short, mixed with typical linear lesions. Also some resistant-type lesions of race 46 were small and oval. These symptoms agree with the original description of

race 3 by Nelson *et al.* (1973) who also reported some isolates of race 3 that predominantly caused oval lesions on certain inbred lines. Gregory *et al.* (1984) suggested that it would be difficult to distinguish isolates of race 3 that produce small lesions from isolates of race 2.

Two isolates of race 0 induced small oval to circular lesions on some inbred lines, and a third isolate of race 0 induced only resistant-type chlorotic to necrotic linear lesions. The criterion for race identification based on lesion shape would have placed, the former 2 isolates as race 2 and the latter as race 3 regardless of their virulence. In addition, the genetic mechanism for avirulence and lesion shape appeared to be independent from each other.

Differences in specificity were detected among isolates collected as the new pathotype. Two isolates induced symptoms that appear to produce HC-toxin on Pr and were classified as race 17. Nine isolates produced oval to irregular lesions but only 4 gave a compatible interaction with B73 as originally described (Dodd and Hooker, 1990). These isolates could not be distinguished based on symptoms from the other isolates that caused oval lesions and were classified as races 4, 11, 36, 37 and 38. However, Dodd and Hooker (1990) reported a zonate pattern in lesions caused by the new pathotype which was rarely observed in this experiment. Finally, another isolate of the new pathotype was avirulent on the 14 maize inbred lines and classified as race 0.

Lesion type incited by races 1 and 17 (HC-toxin producing) on Pr was susceptible-type at all times. However, the remaining races exhibited a mixture of susceptible- and resistant-type lesions at each rating time. Once infection took place, lesions developed immediately or stayed as chlorotic or necrotic flecks. *B. zeicola* remains viable in chlorotic flecks for at least 2 months after

inoculation (Jennings & Ullstrup, 1957). In compatible interactions, lesions evolved from resistant-type chlorotic or necrotic flecks to susceptible-type lesions with water soaked margins; however, in incompatible interactions, lesion development was restricted by the host. The age of the host tissue affected the development of lesions, more lesions enlarged in older leaves.

The time of evaluation is critical for race identification. The time of symptom expression differed from 2 to 12 days after inoculation for different races. Only races that induced symptoms of HC-toxin on Pr were distinguished from non-HC-toxin producing races 2 days following inoculation. However, most races developed differential reactions between 9 and 12 days after inoculation. Races of *B. zeicola* could have been overlooked because identification was done one week (Ullstrup, 1944; Leonard, 1978) or 10 days after inoculation (Blanco *et al.*, 1974). Disease reaction and severity indicated that the earliest time to differentiate all races except the HC-toxin isolates was 9 days after inoculation, although clearer differences were observed 12 days after inoculation; thereafter the host tissues started deteriorating, especially in more aggressive isolates, making the assessment more difficult and inaccurate.

Lesion types and disease severity were usually associated, the most susceptible inbred lines showed the highest severity; however, some race-inbred line combinations produced susceptible-type lesions and low severity which could have been overlooked when compared to susceptible reactions with high severity.

Races 1 and 17 induced high disease severity on Pr and low on all the other inbred lines. Races 46 and those that induced oval lesions but not HC-toxin, produced intermediate levels of severity; however, differential reactions

existed among the 14 maize inbred lines. These races were originally described as races 3 and 2, respectively, and both were indicated to be non-specific (Ullstrup, 1941, Blanco *et al.*, 1974, Castor *et al.*, 1976).

Most race-inbred line combinations developed lesions, but compatible interactions always gave a higher rate of disease progress and larger AUDPC than incompatible interactions.

The population of *B. zeicola* is highly variable in virulence, especially among isolates that produce oval lesions and symptoms described as non-HC-toxin isolates. This variability has been overlooked because races were distinguished (a) by shape of lesions instead of lesion type; (b) usually on one host genotype; and (c) between 7 and 10 after inoculation. The use of these criteria for race identification should be discouraged. A set of 6 differential inbred lines is proposed to distinguish races of *B. zeicola* based on the disease reaction 12 days after inoculation. This method should be more stable and permit detection of new variants in the pathogen population.

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Table 5.1: Isolate of *Bipolaris zeicola*, state of collection, original race designation, source and number of times tested in the greenhouse.

Isolate	State	Original race designation	Source	No. tests
M-2	IA	1	C. A. Martinson	2
84-1926	IL	1	D. R. Smith	2
85-2427	IN	1	D. R. Smith	5
Purdue	IN	1	H. L. Warren	2
84-1676	MN	1	D. R. Smith	2
Bz 23-55	NC	1	M. L. Carson	1
Bz 23-56	NC	1	M. L. Carson	1
84-3277	PA	1	D. R. Smith	2
Hc128=ATCC16185	VA	1	C. W. Roane	1
81-5173	CO	2	D. R. Smith	2
84-4842	GA	2	D. R. Smith	2
84-3033	IA	2	D. R. Smith	2
80-1910	IL	2	D. R. Smith	5
82-1026	MI	2	D. R. Smith	2
80-3666	MO	2	D. R. Smith	2
84-2533	NE	2	D. R. Smith	2
83-2462	OH	2	D. R. Smith	2
85-2600	PA	2	D. R. Smith	2
85-3326	TN	2	D. R. Smith	2
81-5032	CO	3	D. R. Smith	1
79-3521	IA	3	D. R. Smith	1
86-1074	MI	3	D. R. Smith	2
Bz 2-6	NC	3	M. L. Carson	4
Bz10-59	NC	3	M. L. Carson	2
81-1843	NY	3	D. R. Smith	2
85-1954	OH	3	D. R. Smith	3
ATCC26840	PA	3	R. R. Nelson	1
84-3306	PA	3	D. R. Smith	1
84-3335	PA	3	D. R. Smith	2
82-4427	VA	3	D. R. Smith	1
82-4439	VA	3	D. R. Smith	1
D-2	IA	4	J. Dodd	3
D-5	IA	4	J. Dodd	2
D-6	IA	4	J. Dodd	1
D-15	IA	4	J. Dodd	3
D-0	IL	4	J. Dodd	2
D-11	IL	4	J. Dodd	2
D-16	IL	4	J. Dodd	2
D-17	IL	4	J. Dodd	2
D-13	IN	4	J. Dodd	2
D-20	IN	4	J. Dodd	2
D-9	IN	4	J. Dodd	2
D-4	NE	4	J. Dodd	2
G3-4	VA		E. Traut	2
G47-29	VA		E. Traut	2
WT-1	VA		E. Traut	2
WT-2	VA		E. Traut	2
WT-3	VA		E. Traut	1
WT-4	VA		E. Traut	1

Table 5.2: Inbred lines used for inoculation with *Bipolaris zeicola* and their background.

Line	Developed from:
A632	(Mt42 × B14)$B14_3$
B14	Iowa Stiff Stalk Syn.
B37	Iowa Stiff Stalk Syn.
B73	Iowa Stiff Stalk Syn. recurrent sel. popul. C5
C103	Lancaster Surecrop
H84	(B37 × GE440)Ht
H95	Oh43 × CI90A
H111	Mayorbella × B37
Mo17	187-2 × C103
Oh43	Oh40B × W8
Pr	var. Pride of Saline
Pr1	((Pr x Hy) × $Pr)_6$
Va26	Oh43 × K155
W64A	$WF9_3$ × 187-2

Table 5.3: Classification of isolates of *Bipolaris zeicola* according to the lesion types determined on a set of 14 inbred lines.

Race	Isolate
0	84-2533
	D-6
	86-1074
1	Hc128 = ATCC16185
	WT-4
	M-2
4	D-20
11	D-0
	D-5
17	Purdue
	85-2427
	D-2
	D-15
	84-1926
	84-1676
	84-3277
32	80-3666
	81-5173
	84-3033
	84-3335
36	79-3521
	82-4439
	83-2462
	84-4842
	D-13
	WT-2
	WT-3
37	D-11
	G-3-4
38	D-9
	D-17
44	80-1910
	82-1026
	85-2600
	85-3326
46	82-4427
	84-3306
	85-1954
	Bz2-6
	Bz10-59
	G-47-29
	81-1843
	WT-1
	ATCC26840
Unclassified isolates	23-55
	23-56
	81-5032
	D-4
	D-16

Table 5.4: Disease reaction 12 days after inoculation with classified isolates of *Bipolaris zeicola*.

Inbred	Race										
line	0	1	4	11	17	32	36	37	38	44	46
A632	R	R	R	S	R	R	R	MR	MS	MR	S
B14	R	R	R	S	R	R	R	MR	S	MR	S
B37	R	R	MS	MR	R	MR	MS	MS	MS	MS	S
B73	R	R	R	MS	R	R	R	MR	MR	S	S
C103	R	R	R	R	R	MR	MS	MR	MR	MR	MS
H84	R	R	MR	MR	R	MR	MR	MR	MR	MR	S
H95	R	R	R	R	MS	R	R	R	R	R	R
H111	R	R	MR	MR	R	R	R	MR	MR	MR	MR
Mo17	R	R	MR	MR	R	MS	S	S	S	MS	MS
Oh43	R	R	MR	R	R	MR	MR	MR	MR	MR	MR
Pr	R	S	R	MS	S	MR	MR	S	MR	MR	MR
Pr1	R	R	R	MS	MR	MR	MR	S	MR	MR	MR
Va26	R	R	MR	R	R	R	MR	MR	MR	MR	S
W64A	R	R	MR	MR	R	MS	S	S	S	S	S

Table 5.5: Disease reaction 12 days after inoculation with unclassified isolates of *Bipolaris zeicola*.

Inbred line	Isolate D-16	D-4	23-55	23-56	81-5032
A632	S	S	MS	S	R
B14	S	S	MS	S	R
B37	MS	S	MR	S	R
B73	S	S	MR	S	R
C103	R	R	MR	MR	R
H84	R	R	MR	S	R
H95	R	R	MR	MS	R
H111	R	R	MR	MS	R
Mo17	MR	S	MS	MS	S
Oh43	R	R	MR	S	R
Pr	S	S	MS	S	S
Pr1	S	S	MS	S	S
Va26	R	R	MS	S	MR
W64A	R	MR	S	S	MS

Table 5.6: Correlation coefficients for the disease reaction between each pair of the 14 corn inbred lines evaluated 12 days after inoculation with *Bipolaris zeicola*.

Inbred line	B14	B37	B73	C103	H84	H95	H111	Mo17	Oh43	Pr	Pr1	Va26	W64A
A632	0.98	0.65	0.87	0.13	0.44	0.10	0.48	0.23	0.22	0.28	0.56	0.37	0.20
B14		0.65	0.83	0.15	0.43	0.08	0.50	0.27	0.23	0.24	0.52	0.36	0.24
B37			0.73	0.51	0.64	-0.04	0.53	0.54	0.58	-0.05	0.34	0.53	0.52
B73				0.17	0.44	0.05	0.45	0.22	0.27	0.24	0.51	0.36	0.23
C103					0.70	-0.02	0.35	0.54	0.60	-0.30	-0.05	0.66	0.83
H84						0.26	0.79	0.28	0.82	-0.20	0.07	0.87	0.67
H95							0.33	-0.23	0.43	0.38	0.18	0.33	0.02
H111								0.21	0.78	-0.08	0.18	0.74	0.60
Mo17									0.35	0.08	0.53	0.38	0.78
Oh43										-0.12	0.13	0.77	0.70
Pr											0.71	-0.07	-0.16
Pr1												0.18	0.24
Va26													0.73

Table 5.7: Frequency of disease reaction among the 11 races and 5 unclassified isolates of *Bipolaris zeicola*.

Inbred line	Frequency of reaction type			
	R	MR	MS	S
A632	0.44	0.13	0.13	0.31
B14	0.44	0.13	0.06	0.38
B37	0.25	0.19	0.38	0.19
B73	0.44	0.19	0.06	0.31
C103	0.50	0.38	0.13	0.00
H111	0.50	0.44	0.06	0.00
H84	0.38	0.50	0.13	0.02
H95	0.81	0.06	0.13	0.00
Mo17	0.19	0.19	0.31	0.31
Oh43	0.44	0.50	0.00	0.06
Pr	0.13	0.31	0.13	0.44
Pr1	0.19	0.38	0.13	0.31
Va26	0.44	0.38	0.06	0.13
W64A	0.25	0.19	0.13	0.44

Table 5.8: Designation of races of *Bipolaris zeicola* in binary and decimal codes and its correspondence with the old race designation.

Binary code						New race	Old race
Mo17	H95	B73	B37	B14	Pr	designation	designation
0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	1
0	0	0	1	0	0	4	NP
0	0	1	0	1	1	11	NP
0	1	0	0	0	1	17	1
1	0	0	0	0	0	32	2
1	0	0	1	0	0	36	2, NP
1	0	0	1	0	1	37	2, NP
1	0	0	1	1	0	38	NP
1	0	1	1	0	0	44	2
1	0	1	1	1	0	46	3

Table 5.9: Lesion type index 12 days after inoculation with different races of *Bipolaris zeicola*[a].

Inbred	Race										
line	0	1[b, c]	4[b]	11[b]	17[b, c]	32	36[c]	37[b]	38[b, c]	44	46[b, c]
A632	1.00 b	1.04	1.33 abc	3.89 a	1.47	1.22 c	1.54	1.00 e	1.72	1.39 ef	3.09
B14	1.06 b	1.07	1.33 abc	3.33 ab	1.28	1.31 c	1.35	2.32 bc	3.11	1.35 ef	2.99
B37	1.11 ab	1.37	2.22 a	2.00 de	1.58	1.50 c	2.02	1.99 bcd	1.78	2.00 bc	2.80
B73	1.00 b	1.15	1.11 bc	1.56 ef	1.11	1.20 c	1.24	1.33 de	1.17	1.88 bcd	2.90
C103	1.06 b	1.19	1.17 bc	1.50 ef	1.21	1.33 c	1.61	1.99 bcd	1.42	1.45 def	2.65
H84	1.00 b	1.06	1.78 abc	2.22 cde	1.26	1.47 c	2.01	1.99 bcd	1.78	1.66 bcdef	2.37
H95	1.00 b	1.26	1.00 c	1.44 ef	1.36	1.13 c	1.41	1.00 e	1.28	1.27 f	1.94
H111	1.00 b	1.00	1.56 abc	1.39 ef	1.00	1.33 c	1.58	2.21 bc	1.28	1.29 ef	2.21
Mo17	1.11 ab	1.04	1.83 abc	2.67 bcd	1.12	2.02 a	2.30	2.32 bc	2.39	2.08 b	2.51
Oh43	1.11 ab	1.30	2.00 ab	1.00 f	1.79	1.34 c	1.82	1.55 cd	1.72	1.51 def	2.47
Pr	1.00 b	4.00	1.11 bc	3.11 abc	4.00	1.57 abc	1.57	2.76 ab	1.83	1.62 cdef	2.16
Pr1	1.00 b	1.04	1.11 bc	2.67 bcd	1.36	1.35 c	1.46	1.88 cd	1.44	1.35 ef	2.14
Va26	1.06 b	1.19	1.67 abc	1.00 f	1.68	1.52 bc	1.73	1.88 cd	1.44	1.74 bcde	2.34
W64A	1.44 a	1.30	1.56 abc	2.78 bcd	1.61	1.98 ab	2.44	3.53 a	3.67	3.01 a	2.88

[a] Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

[b] Means of three replications and one repetition.

[c] Significant isolate × inbred interaction did not allow mean separation.

Table 5.10: Disease severity 12 days after inoculation with *Bipolaris zeicola*[a].

Inbred line	Race										
	0	1[b, c]	4	11	17[b]	32	36	37	38	44	46
A632	1.00	1.00	1.67 bcd	3.44 a	1.00	1.23 bc	1.86 bc	2.62 bcde	3.33 ab	1.19 ef	3.32 ab
B14	1.00	1.00	1.67 bcd	3.59 a	1.03	1.18 bc	1.74 bc	2.52 cde	4.25 a	1.17 ef	3.10 abc
B37	1.00	1.00	2.83 a	1.93 bc	1.10	1.22 bc	2.19 b	3.47 b	2.85 bc	1.56 cde	3.05 abc
B73	1.00	1.00	1.36 cd	2.11 bc	1.00	1.20 bc	1.50 bc	1.91 ef	2.50 bc	2.25 b	3.14 abc
C103	1.00	1.03	1.29 cd	1.28 c	1.06	1.23 bc	1.73 bc	2.22 de	2.51 bc	1.15 ef	3.06 abc
H84	1.00	1.00	1.92 abcd	1.31 c	1.03	1.20 bc	1.65 bc	2.14 de	2.42 bc	1.24 def	2.94 abc
H95	1.00	1.00	1.24 d	1.13 c	1.09	1.01 c	1.09 c	1.09 f	1.03 d	1.12 f	1.91 e
H111	1.00	1.00	1.75 bcd	1.78 bc	1.00	1.22 bc	1.67 bc	2.15 de	1.95 cd	1.21 ef	2.59 cd
Mo17	1.00	1.00	1.79 bcd	2.11 bc	1.00	1.29 bc	2.17 b	2.88 bcd	3.41 ab	1.66 cd	2.85 abcd
Oh43	1.00	1.00	2.26 abc	1.17 c	1.03	1.07 bc	1.78 bc	2.02 de	2.29 bc	1.17 ef	2.80 bcd
Pr	1.00	4.96	1.50 cd	3.20 a	5.00	1.40 b	1.87 bc	3.15 bc	2.65 bc	1.92 bc	2.30 de
Pr1	1.00	1.00	1.50 cd	2.59 ab	1.03	1.12 bc	1.81 bc	3.24 bc	2.40 bc	1.16 ef	2.15 e
Va26	1.00	1.00	2.14 abcd	1.15 c	1.05	1.10 bc	1.76 bc	2.08 de	2.60 bc	1.25 def	2.83 abcd
W64A	1.00	1.13	2.53 ab	2.13 abc	1.07	1.81 a	3.18 a	4.41 a	4.40 a	3.52 a	3.40 a

[a] Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

[b] Means of three replications and one repetition.

[c] Significant isolate × inbred interaction did not allow mean separation.

Table 5.11: Area under the disease progress curve (AUDPC) between 5 and 12 days after inoculation with different races of *Bipolaris zeicola*[a].

Inbred	Race										
line	0	1[b, c]	4	11	17[c]	32	36	37	38	44	46
A632	0.0	0.0	60.4 bcd	264.1 a	0.0	8.3 bc	70.5 bc	193.1 bc	214.4 bc	9.4 de	291.6 a
B14	0.0	0.0	67.4 bcd	264.4 a	1.7	6.9 c	63.7 bc	163.7 cd	340.5 a	8.1 de	270.3 a
B37	0.0	0.0	182.6 a	84.3 c	10.5	9.5 bc	88.3 bc	244.9 b	199.5 bcd	29.9 cde	281.4 a
B73	0.0	0.0	33.7 cd	99.8 c	0.0	22.1 bc	34.1 bc	72.0 e	130.4 bcd	103.0 b	256.5 a
C103	0.0	1.0	17.2 cd	40.3 c	4.7	16.7 bc	82.7 bc	106.3 de	134.7 bcd	7.6 e	244.6 ab
H84	0.0	0.0	67.7 bcd	41.4 c	1.9	9.2 bc	60.0 bc	86.5 e	162.2 bcd	8.9 de	242.5 ab
H95	0.0	0.0	15.6 cd	18.1 c	7.5	0.3 c	4.9 c	4.9 f	1.9 e	11.1 de	116.4 d
H111	0.0	0.0	61.1 bcd	70.4 c	0.0	9.1 bc	61.8 bc	89.6 e	95.0 de	8.2 de	217.7 abc
Mo17	0.0	0.0	74.7 bcd	111.8 bc	0.0	15.5 bc	104.8 b	186.6 c	236.1 ab	51.0 cd	237.1 abc
Oh43	0.0	0.0	141.3 ab	21.1 c	3.0	2.6 c	71.4 bc	84.5 e	113.0 cde	9.4 de	216.9 abc
Pr	0.0	658.6	31.3 cd	249.3 ab	690.4	48.5 b	79.9 bc	207.9 bc	154.3 bcd	72.5 bc	165.2 bcd
Pr1	0.0	1.9	31.3 cd	160.2 abc	1.0	5.6 c	62.8 bc	194.0 bc	125.0 bcd	5.5 e	151.7 cd
Va26	0.0	0.0	109.7 abc	19.0 c	4.2	5.0 c	75.6 bc	78.4 e	139.2 bcd	15.1 de	229.9 abc
W64A	0.0	8.6	173.6 a	138.7 abc	3.8	91.7 a	244.4 a	399.3 a	336.7 a	268.9 a	301.3 a

[a] Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

[b] Means of three replications and one repetition.

[c] Significant isolate × inbred interaction did not allow mean separation.

Table 5.12: Rate of disease progress after inoculation with different races of *Bipolaris zeicola*[a].

Inbred line	Race										
	0	1[b, c]	4	11	17[c]	32	36	37	38	44	46[c]
A632	0.000	0.001	0.021 bc	0.144 a	0.000	0.008 b	0.039 b	0.050 cdef	0.115 cd	0.007 c	0.160
B14	0.000	0.000	0.022 bc	0.143 a	0.002	0.008 b	0.029 b	0.061 bcdef	0.226 b	0.008 c	0.136
B37	0.000	0.003	0.073 a	0.028 bc	0.004	0.009 b	0.054 b	0.110 b	0.070 cde	0.021 c	0.085
B73	0.000	0.000	0.014 c	0.045 bc	0.000	0.005 b	0.019 b	0.038 ef	0.075 cde	0.120 b	0.125
C103	0.000	0.002	0.009 c	0.004 c	0.002	0.008 b	0.020 b	0.038 ef	0.073 cde	0.006 c	0.092
H84	0.000	0.000	0.033 bc	0.007 c	0.001	0.007 b	0.022 b	0.037 ef	0.054 cde	0.010 c	0.082
H95	0.000	0.000	0.008 c	0.003 c	0.003	0.001 b	0.003 b	0.003 f	0.001 e	0.003 c	0.032
H111	0.000	0.000	0.024 bc	0.025 bc	0.000	0.008 b	0.023 b	0.037 ef	0.030 de	0.008 c	0.057
Mo17	0.000	0.001	0.023 bc	0.038 bc	0.000	0.012 b	0.047 b	0.072 bcde	0.148 bc	0.021 c	0.078
Oh43	0.000	0.001	0.043 abc	0.005 c	0.002	0.004 b	0.034 b	0.037 ef	0.056 cde	0.006 c	0.092
Pr	0.000	0.345	0.017 c	0.087 ab	0.353	0.012 b	0.035 b	0.097 bcd	0.070 cde	0.044 c	0.053
Pr1	0.000	0.001	0.017 c	0.055 bc	0.001	0.005 b	0.035 b	0.106 bc	0.058 cde	0.007 c	0.046
Va26	0.000	0.001	0.036 abc	0.004 c	0.003	0.005 b	0.030 b	0.038 def	0.068 cde	0.012 c	0.080
W64A	0.000	0.010	0.057 ab	0.072 abc	0.002	0.039 a	0.183 a	0.254 a	0.378 a	0.231 a	0.162

[a] Means within a column followed by the same letter are not significantly different at P=0.05 by Tukey-Kramer HSD test.

[b] Means of three replications and one repetition.

[c] Significant isolate × inbred interaction did not allow mean separation.

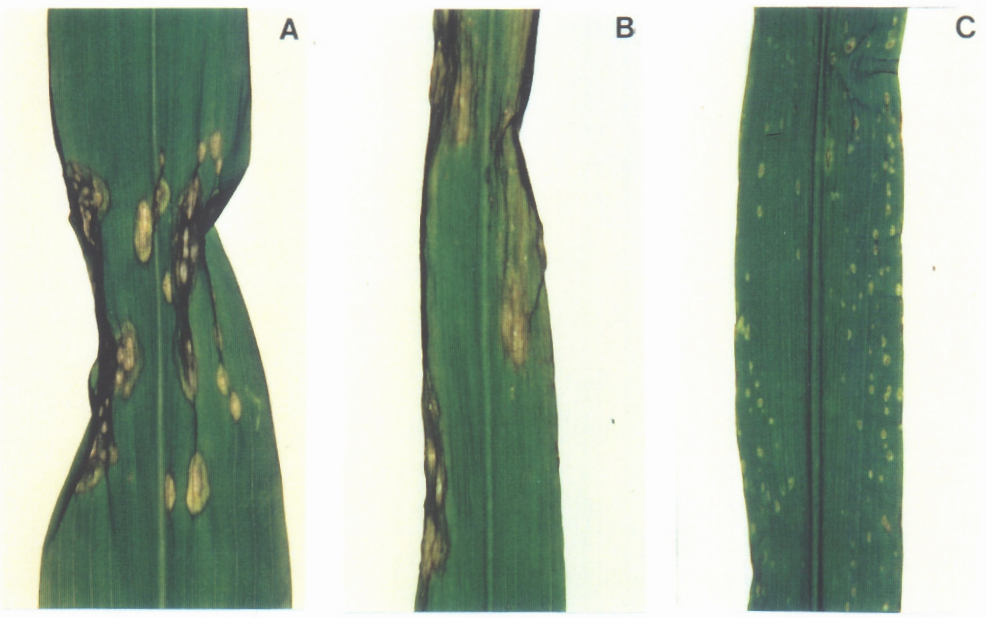

Figure 5.1: Disease reaction induced by *Bipolaris zeicola* race 17 on: (A) Pr 6 days after inoculation (susceptible); (B) H95 15 days after inoculation (moderately susceptible); and (C) B37 15 days after inoculation (resistant).

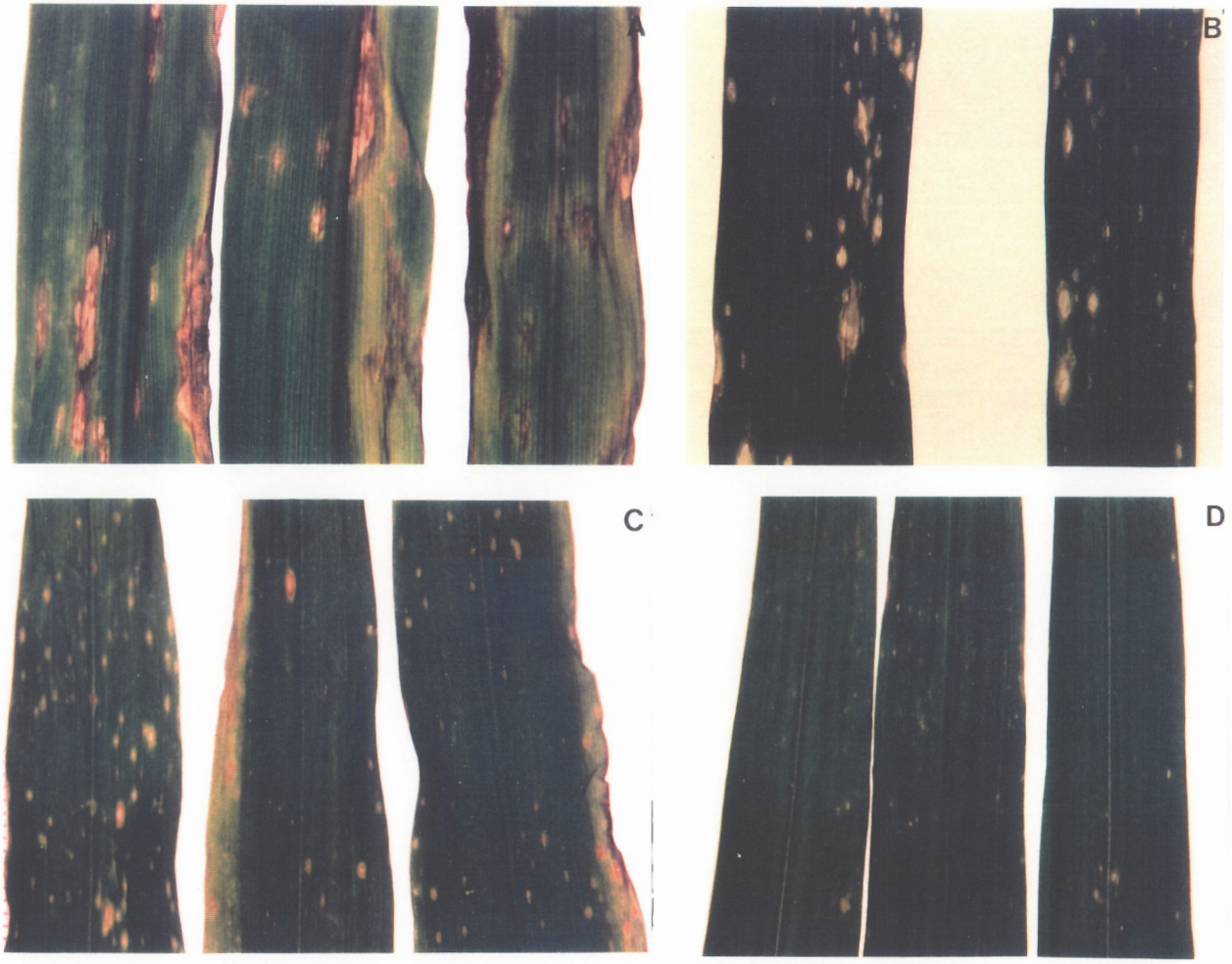

Figure 5.2: Disease reaction induced by *Bipolaris zeicola* race 44 15 days after inoculation on: (A) B73 (susceptible); (B) B37 (moderately susceptible); (C) Pr (moderately resistant); and (D) H95 (resistant).

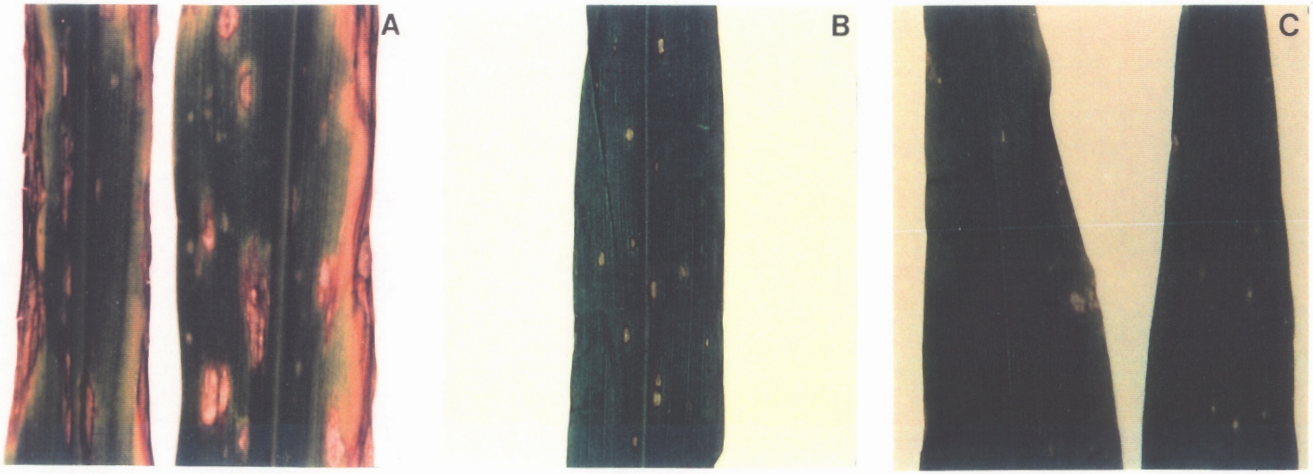

Figure 5.3: Disease reaction of W64A 15 days after inoculation with *Bipolaris zeicola* races: (A) 44 (susceptible); (B) 32 (moderately susceptible); and (C) 11 (resistant).

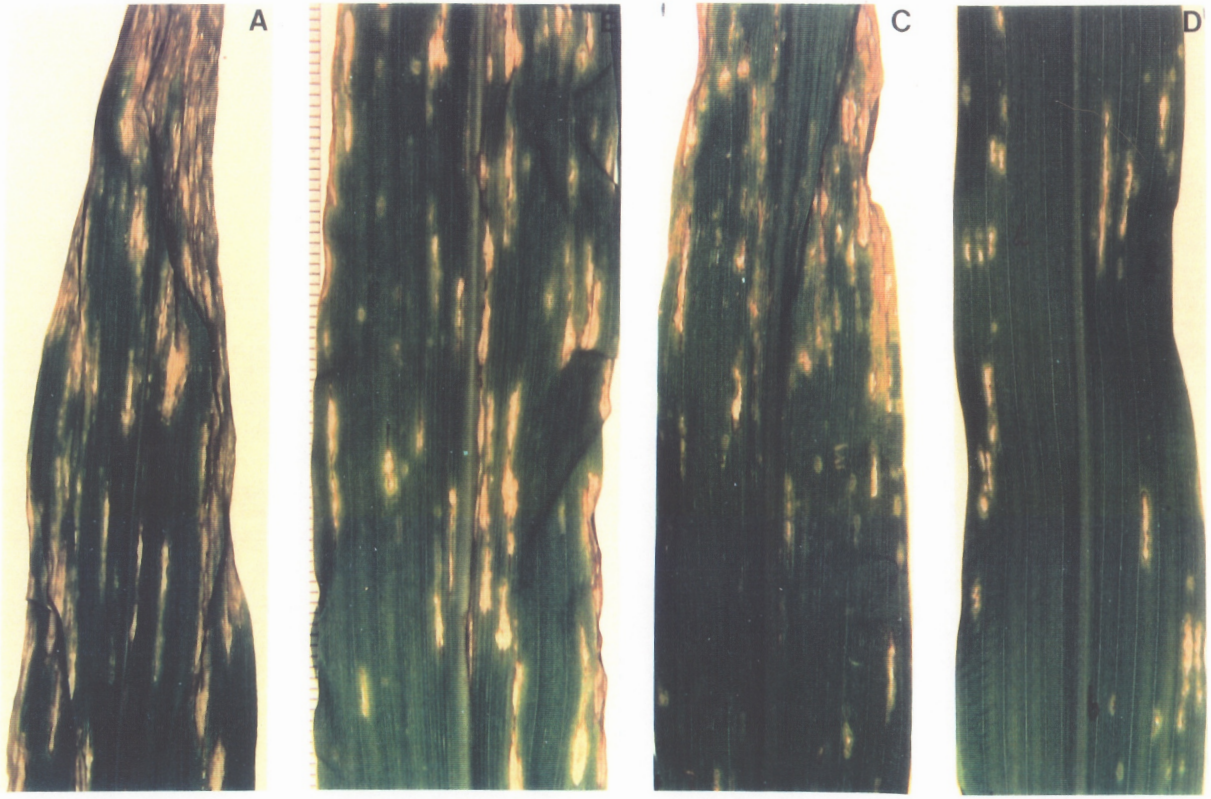

Figure 5.4: Disease reaction induced by *Bipolaris zeicola* race 46 15 days after inoculation on: (A) B73 (susceptible); (B) Mo17 (moderately susceptible); (C) Pr (moderately resistant); and (D) H95 (resistant).

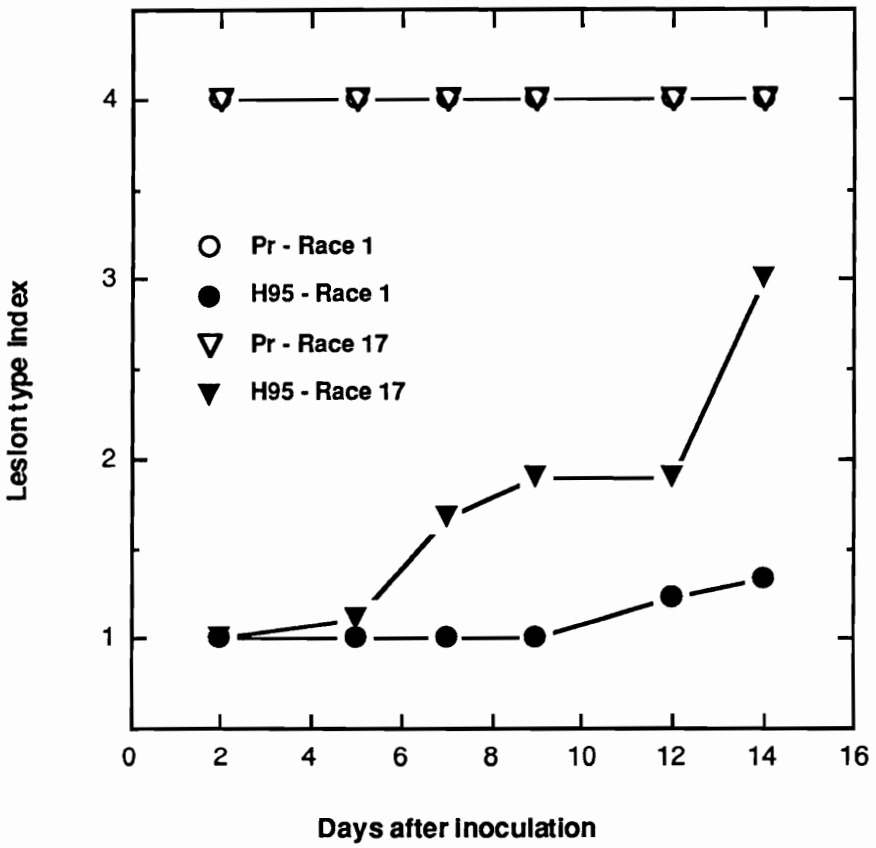

Figure 5.5: Progress of the lesion type index of *Bipolaris zeicola* races 1 and 17 on 2 maize inbred lines, Pr and H95.

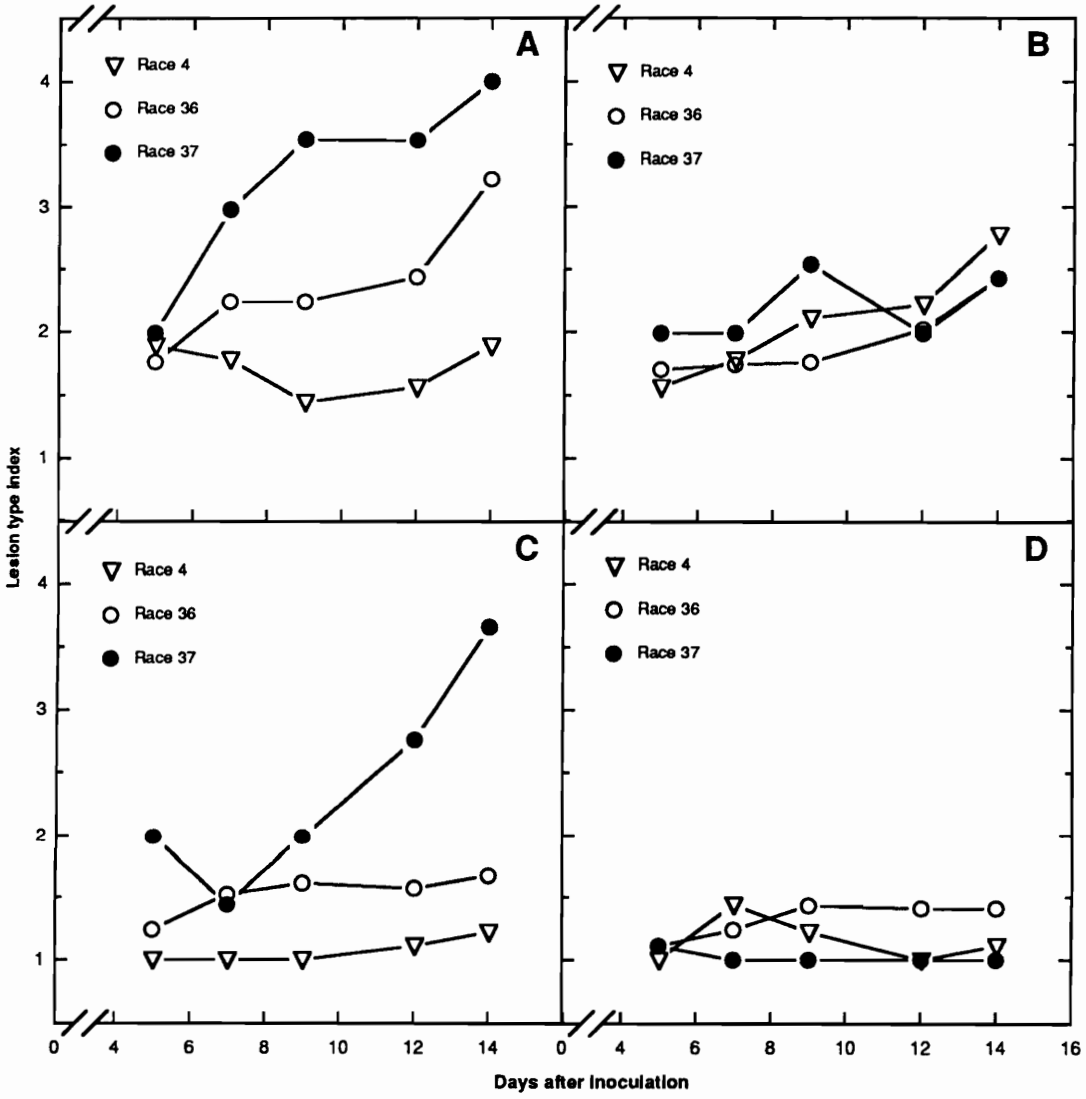

Figure 5.6: Progress of the lesion type index of *Bipolaris zeicola* races 4, 36 and 37 on inbred lines (A) W64A, (B) B37, (C) Pr and (D) H95.

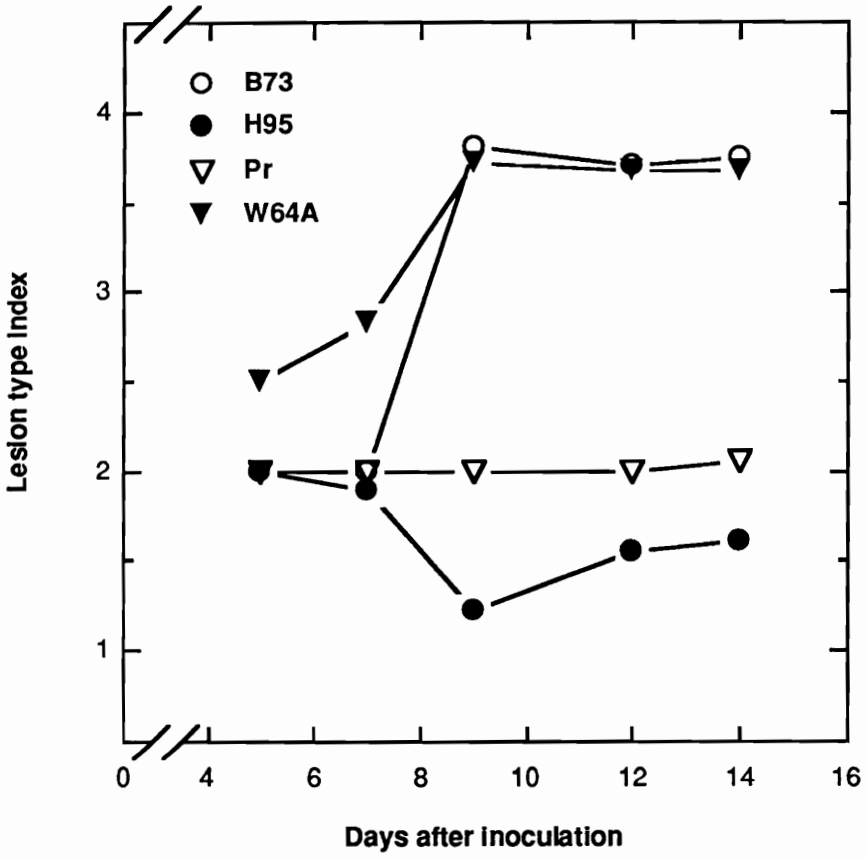

Figure 5.7: Progress of the lesion type index of *Bipolaris zeicola* race 46 (isolate 85-1954) on 4 maize inbred lines, B73, H95, Pr and W64A.

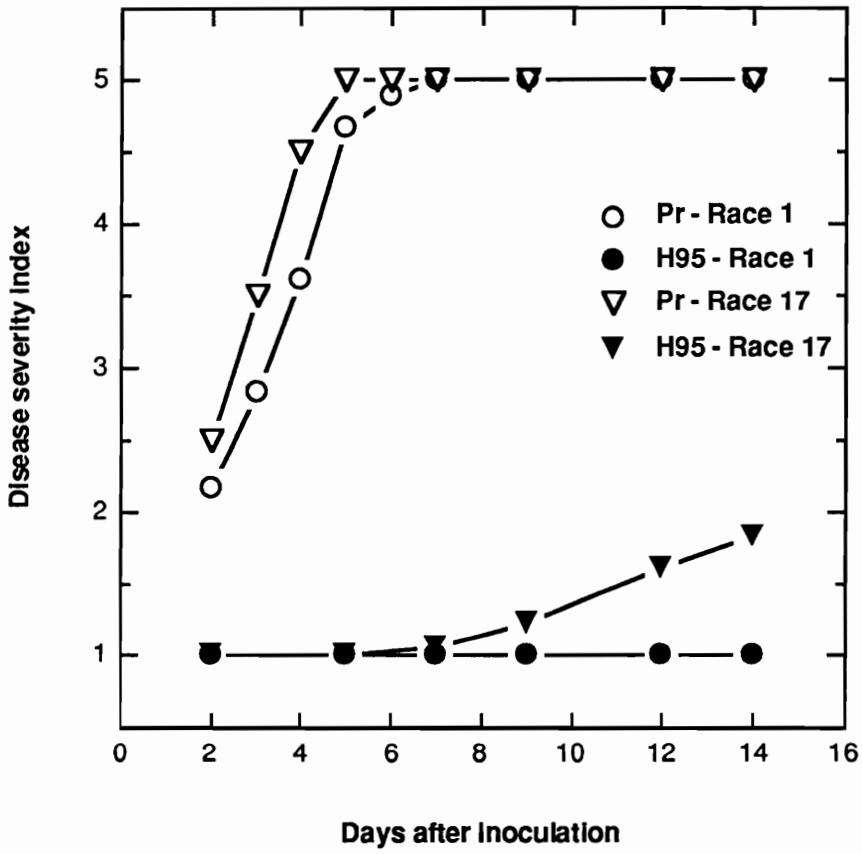

Figure 5.8: Disease severity induced by *Bipolaris zeicola* races 1 and 17 on 2 maize inbred lines.

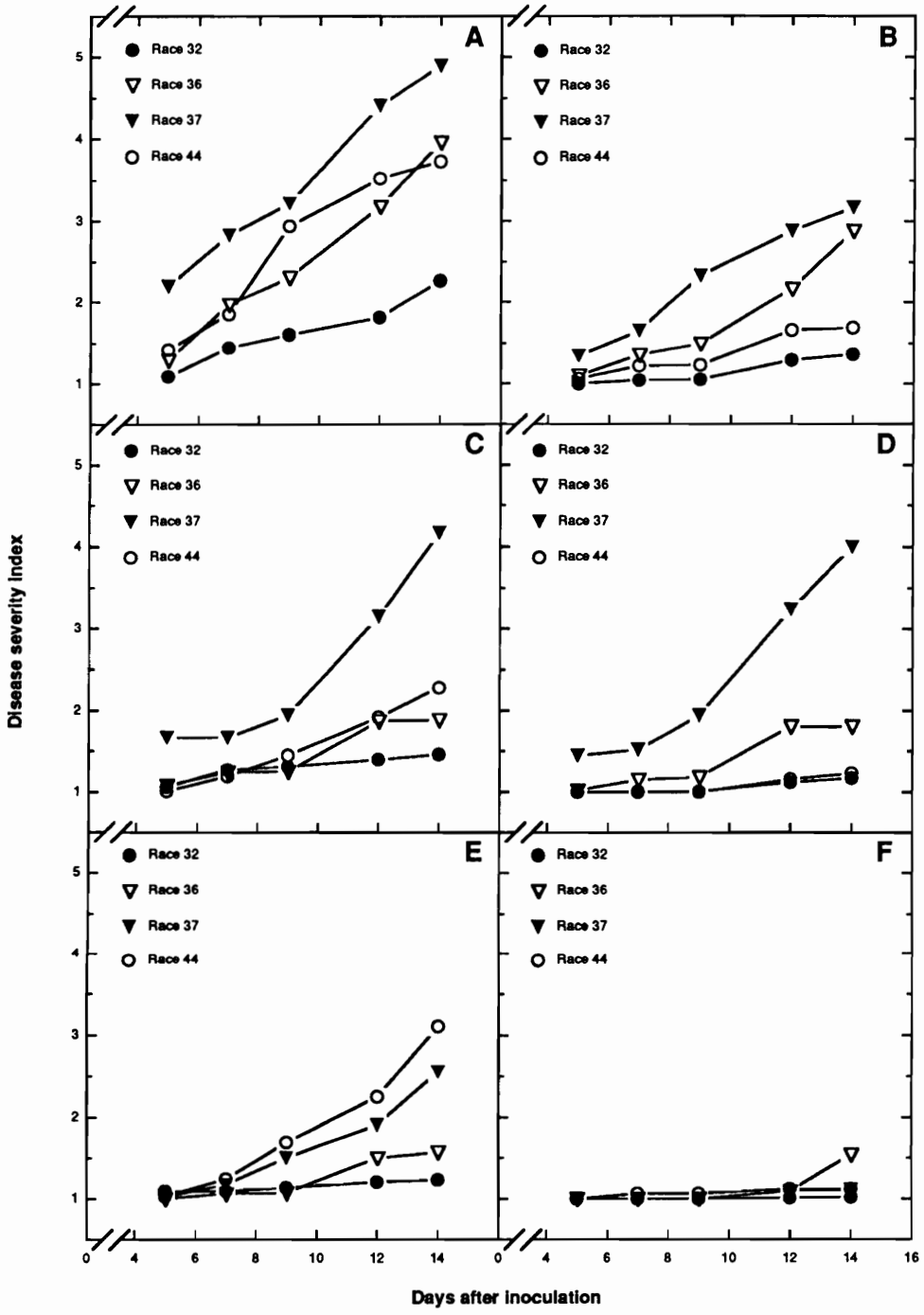

Figure 5.9: Disease severity induced by *Bipolaris zeicola* races 32, 36, 37 and 44 on maize inbred lines (A) W64A, (B) Mo17, (C) Pr, (D) Pr1, (E) B73 and (F) H95.

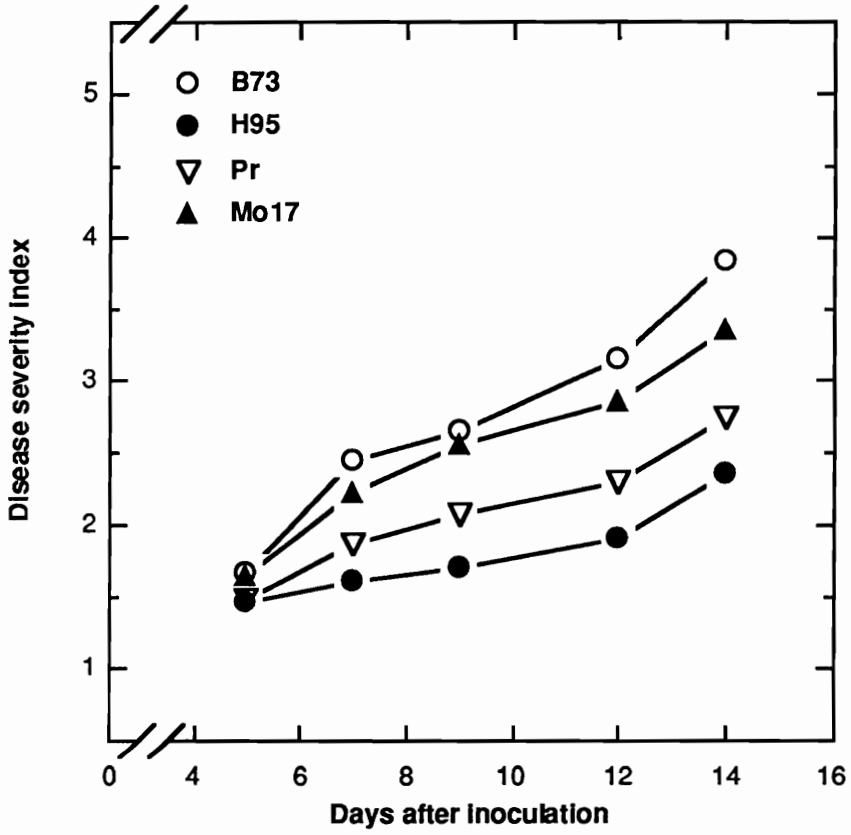

Figure 5.10: Disease severity induced by *Bipolaris zeicola* race 46 on 4 maize inbred lines, B73, H95, Pr and Mo17.

VITA

Eduardo Jorge Traut was born in Buenos Aires, Argentina, on March 22, 1955, to Adelia Skiba and Eduardo Lorenzo Traut.

He received his primary and secondary education in Buenos Aires, and was graduated from "Escuela Nacional de Comercio Juan XXIII" in 1972. In 1979, he received his professional degree in Agronomy, from Universidad de Buenos Aires, Argentina.

He worked as an intern in the Department of Plant Pathology, I.N.T.A. ("Instituto Nacional de Tecnología Agropecuaria") from 1978 to 1979, as a research assistant from 1980 to 1985 in the same Department, and as a research plant pathologist at Oliveros, Province of Santa Fe, Argentina in 1986.

He earned his Master of Science degree in plant pathology from Purdue University, Indiana in 1989. He enrolled at Virginia Polytechnic Institute and State University, Department of Plant Pathology, Physiology and Weed Science in August 1989 as a Ph. D. student in Plant Pathology.

He was married to Olga del Rosario Crespo on February 2, 1985. They are the parents of two boys, Nicolás and Maximilian.