DIALLEL ANALYSIS OF DIPLODIA EAR ROT RESISTANCE IN MAIZE AND AN ASSESSMENT OF THE GENETIC VARIABILITY OF *STENOCARPELLA MAYDIS* THROUGH ISOZYME ANALYSIS

by

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(ABSTRACT)

Diplodia ear rot (DER) of maize (*Zea mays* L.) caused by the fungus, *Stenocarpella maydis* (Berk.) Sutton has increased in incidence in localized fields over the past decade. My research focused on screening for resistance by examining the development of DER following inoculations prior to flowering, analyzing a diallel cross for DER resistance, and examining the genetic variability of the fungus from isolates collected from the U.S. and the Republic of South Africa. DER developed in maize following inoculations with a spore suspension prior to flowering in both greenhouse and field evaluations. A spore suspension gave a better differentiation of resistance responses than dried preparations of colonized millet, colonized ground popcorn, or kernels from a diseased maize ear, all applied in the whorl 10 to 15 days prior to flowering (V12 for inbreds), and natural occurrence of disease. General combining ability was significant for both 1994 and 1995 growing seasons in an analysis of the F_1 of the diallel cross, indicating that additive gene action may be responsible for resistance and could be introduced into commercial cultivars. Specific combining ability was significant in 1995 and indicates that

dominant gene action or epistasis may play role in DER resistance. There were minimal numbers of isozyme polymorphisms found in my *S. maydis* collection. Two isolates were polymorphic for esterase, two isolates were polymorphic for hexokinase and malate dehydrogenase and one isolate was polymorphic for hexose kinase. Fungi that have limited isozyme polymorphisms often are biotrophs or fungi with formae speciales which are usually limited to one host. These groups of fungi usually have races and this may indicate that a gene-for-gene interaction exists. These findings suggest that i) the whorl inoculation separates genotypes into resistant, intermediate, and susceptible groupings; ii) additive gene action is predominant form of inheritance, and iii) there are few isozyme polymorphisms in the population of *S. maydis* sampled.

To Tom, Hannah and Forrest

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Diplodia ear rot (DER) caused by the fungus, *Stenocarpella maydis* (Berk.) Sutton [=*Diplodia maydis* (Berk.) Sacc., = *D. zeae* (Schw.) Lev.], is an important disease of maize (*Zea mays* L.). Significant losses have been reported from many maize growing regions of the world. DER has periodically reached epidemic levels in the United States since it was first described as a maize pathogen by Heald in 1906. This was the major ear rot pathogen in the U.S. prior to 1960, after which time reports of DER were rare (Hooker and White, 1976). However, recent reports within the United States have included high levels of DER from isolated locales in Indiana (D. Scott, personal communication, 1995, Department of Botany and Plant Pathology, Purdue Unversity), Iowa (Munkvold and Yang, 1995), Kentucky (Vincelli, 1992, 1994), and Virginia (M. A. Hanson, 1992, VPI & SU Deptartment of Plant Pathology, Physiology and Weed Science, Plant Clinic Record 2102 and 2027). This pathogen may cause reduced germination of diseased or colonized seed, seedling blight, crown rot, and stalk rot (Christensen and Wilcoxson, 1966). Researchers in South Africa have associated a mycotoxicosis of poultry, lambs, and ruminants with feeding on *S. maydis* colonized grain (Kellerman et al., 1985; Kellerman et al., 1991; Rabie et al., 1985, 1987).

Several questions have been raised by the occurrence of high incidence of DER in isolated fields. Are the methods for screening germplasm effective in identifying

resistant genotypes? Was the germplasm resistant? Is the germplasm breaking down under high disease pressure resulting from changes in cultural practices (Latterell and Rossi, 1983)? Are there new biotypes or races of the fungus. This study addresses several of these questions.

Very few studies have been conducted in which the genetic factors that control resistance to DER were examined, in contrast to the number of studies on resistance factors for diplodia stalk rot. Where the genetic factors of resistance to DER have been examined in maize lines, different conclusions were reached. Koehler and Holbert (1930) found that resistance is dominant. Wiser et al. (1960) determined that resistance to DER is multigenic and they suggested that susceptibility may be dominant. Villena (1969) used nine corn populations to determine that additive effects played a major role in governing resistance. Most recently, Das et al. (1984) found highly significant specific combining effects where dominance played a major role in resistance.

There are at least two explanations for the variability in these four studies: 1) genetic composition in maize inbreds and hybrids as well as the fungus tested were different and 2) different methods were used to challenge the maize plants with the pathogen. Three different methods were used in these studies for obtaining disease: natural infection, conidial suspensions of *S. maydis* sprayed over the outside of the maize ear, and a conidial suspension or colonized toothpicks placed directly in the developing ear. Traditionally the best method for evaluating plant lines for resistance to a pathogen mimics the natural colonization process of the pathogen. To this day, etiology of DER

caused by *S. maydis* in maize is still not understood. The first report on mode of infection was by Smith and Hedges (1909) which indicated that maize was systemically colonized from diseased seed through the stalk to new ears. Several researchers disputed this hypothesis and showed that development of DER was a result of localized infections with subsequent colonization of the ears (Clayton, 1927; Durrell, 1923; Raleigh, 1930). A third possibility may be the genetic composition of the fungus as Young et al. (1959) demonstrated. They reported variability of disease reaction to stalk rot on a set of inbred lines from isolates collected from three different geographic regions.

In this study, I evaluated the gene action of DER resistance using a diallel cross and assessed the genetic variability of the fungus based on cultural characteristics and isozyme analyis. The objectives for this study were:

1) To evaluate a method of inoculation of maize for DER under controlled environmental conditions with a marked isolate to verify timing.
2) To determine gene action for resistance in maize lines.
3) To assess the genetic variability of isolates of *S. maydis* from the United States and South Africa based on cultural characteristics and isozyme analysis.

LITERATURE REVIEW

Inoculation methods to evaluate for DER resistance. There are many techniques available for challenging plants with plant pathogens for evaluation of host resistance.

Assaying inbred lines or hybrids for resistance to DER is no exception. Two methods most utilized involved spraying the developing ear from silks to shanks with an inoculum suspension (Klapproth and Hawk, 1991; Koehler, 1959; Ullstrup, 1949); or inserting a colonized toothpick into the developing ear (Chambers, 1988; DeVay et al., 1957; Villena, 1969; Young, 1943). These methods tend to separate differences in DER reactions to *S. maydis* among inbred lines and hybrids as resistant or susceptible interactions. However, neither of these methods resembles the natural mode of infection or the progression of the disease in maize. Considerable variability exists in the conclusions that have been drawn from studies to assess the genetic factors governing resistance. Variability, in part, may be explained by the use of a variety of inoculation techniques. There is a need then to determine precisely how *S. maydis* infects and colonizes the ear.

Christensen and Wilcoxson (1966) reviewed the literature concerning mode of infection. Smith and Hedges (1909) first suggested that *S. maydis* was a systemic pathogen moving from diseased or colonized seed to the ear. Several researchers questioned Smith and Hedges (1909) hypothesis. Most notable, Durrell (1923) proposed that *S. maydis* enters maize through the bases of leaf sheaths during the process of leaves "loosening" after the maize plant ceases vertical growth. Conidia, pollen, and moisture are trapped in the sheath at which point the fungus invades the plant. Durrell also examined numerous *S. maydis* infected maize plants and noted locations of infections within the plant. *S. maydis* could be found in lower internodes and ears but not in the middle

internodes. He reported very few plants where *S. maydis* could be continuously isolated from the crown to the ear. In several earlier studies, plantings of diseased and apparently healthy seed were compared (Durrell 1923). Clayton (1927) reported no significant increase in the amount of DER detected from fields planted with *S. maydis* diseased seed. The main result was a reduction in the initial plant stand.

Several researchers have followed the movement of *S. maydis* into maize. Craig and Hooker (1961) studied the movement of the fungus from infested soil through roots and into stalks. McNew (1937) described crown invasion by *S. maydis*, and reported that the fungus did not move up into the plant. Raleigh (1930) found that *S. maydis* can colonize the embryo in seed or the mesocotyl after the seed is planted. Raleigh (1930) also reported on the movement of *S. maydis* within the ear until checked by low moisture.

Several methods to challenge maize germplasm for resistance to DER have been reported (Klapproth and Hawk, 1991; Ullstrup, 1949, Villena, 1969; Warren and Onken, 1981). Ullstrup (1949) reported that spraying ears following silking resulted in a high incidence of disease and clear differences could be seen among inbreds. Villena (1969) examined nine different methods including; spore suspensions sprayed from silk to base of the ear, spore suspensions applied with a dropper at tip of the ear, spore suspension placed in the middle, tip or butt of the ear with a syringe, an agar suspension applied over the husk of the ear, toothpicks inserted in the middle of ear or between husks at the butt. Among these methods, syringe inoculation resulted in consistent and high

levels of disease severity. Warren and Onken (1981) reported from a field study that a spore suspension placed in the whorl of the plant two weeks prior to anthesis resulted in DER development in susceptible inbred lines. Klapproth and Hawk (1991) compared four methods of inoculation for DER: 1) dropping infested whole maize kernels into the whorl, 2) spraying silks with a spore suspension (silk spray), 3) placing spore suspension in the sheath cavity, and 4) injecting a spore suspension directly into the ear. They concluded that the silk spray technique gave the most reliable results based on environmental constraints and could differentiate disease response among inbred lines. They also concluded that infested whole kernels placed in the whorl resulted in a lower incidence of disease but could separate inbreds into resistant and susceptible groupings. Ullstrup (1949) stated that *S. maydis* is not a wound pathogen and that in the majority of diseased ears, ear colonization takes place through the shank and butt and not through the silks. Neither the injection or the silk spray methods utilized to differentiate germplasm for ear rot resistance resemble the natural infection process for development of DER as described by Ullstrup (1949). Villena (1969) compared spray vs. toothpick inoculations, and observed that 20 days were required for germination of conidia and penetratration of the cob. When *S. maydis* grew into the ear, the rate of fungal growth was equal to direct inoculation using a colonized toothpick.

DER resistance in maize. Resistance in maize to diseases caused by *S. maydis* has been examined for diplodia seedling blight (Hooker, 1956), diplodia root necrosis (Hooker,

1956), diplodia stalk rots (Craig and Hooker, 1961; Donahue et al., 1989; Hooker, 1956; Kappelman and Thompson, 1966), DER (Das et al., 1984; Koehler, 1953, 1959; Thompson et al., 1971; Ullstrup, 1949; Villena, 1969; Wiser et al., 1960), and relations with other maize pathogens (White, 1977), and insects (Jarvis et al., 1982; Nyhus et al., 1988,1989). Resistance to different diseases caused by *S. maydis*, *i.e.*, seedling blight, stalk rot, and DER is inherited independently (Hooker, 1956, Thompson et al., 1971).

Hooker (1956) reported no correlation in resistance between two ear rot fungi; *S. maydis* and *Gibberella zeae* (Schwein.) Petch. or with other disease reactions, seedling blights, stalk rots, or root necrosis with 25 dent maize inbred lines. He found a high degree of correlation for reaction to stalk rot on maize plants inoculated with *S. maydis* and *G. zeae*. He suggested that breeding programs need to test lines independently for resistance to stalk rot, seedling blight, DER, and gibberella ear rot. White (1977) reported a lack of correlation for stalk rot in maize inbred lines in reactions to *S. maydis* and *Colletotrichum graminicola* (Ces.) Wils. but in another study, White (1978) tested 102 maize inbred lines and found close correlations with *S. maydis* and *G. zeae* stalk rot. Donahue et al. (1989) reported no significant differences in stalk rot among eleven hybrids that were analysed for interactions of *S. maydis* and *Fusarium moniliforme* Sheldon.

Inheritance to stalk rot and factors affecting its development have been studied extensively and reviewed by Christensen and Wilcoxson (1966). Craig and Hooker (1961) determined that maize inbred lines and hybrids resistant to diplodia stalk rot and resist lodging, have a greater rind thickness, and a higher pith density. They theorized that stalk

rot disease occurs mainly as the plant begins to senesce and sugars are translocated to the developing grain. This results in a reduced amount of sugar available for vegetative activities. A senescing pith is suitable habitat for stalk-rotting organisms. Dodd (1980) proposed a similar hypothesis, "photosynthetic stress-translocation balance or PS-TB concept," which suggests plants that develop stalk rot are adversely affected by their environment. He stated that the lower part of the maize plant is colonized by a multitude of microorganisms, and after anthesis plants have little defense system due to movement of sugars from the stalk to the grain. After anthesis, the physiology of the plant is directed to fill the developing grain; therefore, fungi and bacteria that are already present in plant tissues as well as invading microbes, easily colonize and degrade the stalk and root, causing deterioration of stalk tissue and subsequent lodging.

Researchers have reported in a few studies definitive answers to the nature of inheritance of resistance to ear rots. Koehler and Holbert (1930) mention results of testing crosses between resistant and susceptible parents for ear rot. They reported that offspring tend to be resistant. In single crosses with ten inbred lines Ullstrup (1949) found that the most susceptible parent contributed susceptibility to the F_1 while three resistant inbred lines contributed resistance in crosses. Koehler (1953) examined yellow maize inbreds for ear rot resistance to four ear rot pathogens including *S. maydis*. He reported a significant correlation to the physical character of good husk coverage and reduced ear rot development for both *S. maydis* and *F. moniliforme*. He suggested that genes governing resistance may be additive.

Koehler (1959) reported on three characteristics of maize inbred lines that correlate with a reduced incidence in DER, inherent resistance, tight husk coverage, and upright ear position. Farrar and Davis (1991), found a similar relationship with fusarium ear rot and numbers of western flower thrips (*Frankliniella occidentalis* Perg.) and tightness of husk at brown silk stage.

Wiser et al. (1960) determined that resistance to DER was multigenic. They evaluated six maize lines, in the F_1, F_2, and backcross generations for resistance to DER. The values for percent cob infection in F_1's were closer to the susceptible parent. Crosses with two susceptible parents yielded progeny that were more susceptible than either parent. From their results with these inbreds the authors suggested susceptibility is dominant whereas Koehler and Holbert's (1930) suggestion was that resistance is dominant.

Villena (1969) reported that dominance factors were either small or not significant and that additive gene effects were more important. Villena's studies involved two series, nine maize populations consisting of six generations (P_1, P_2, F_1, F_2, B_1 and B_2) and a series of crosses between 'Indian Chief' and 'Jarvis' varieties. DER was evaluated on a scale of one to seven based on the percent of the ear colonized; a rating of one had zero percent colonization and a rating of seven was 100% colonization with the ear completely rotted by *S. maydis*. The inoculation technique consisted of a conidial suspension placed directly into the ear via an injection with a hypodermic syringe, 20 days after flowering. Ear rot ratings were taken 15 days after inoculation. Both studies were analyzed by

Hayman's Generation Mean Analysis, and in both sets additive effects were larger than dominant gene effects. In the nine populations, six had significant additive effects and dominance was significant in one.

Das et al. (1984) demonstrated highly significant specific combining effects for resistance to DER with open pollinated maize lines in a diallel cross. They inoculated plants with a conidial suspension sprayed over the ear from shank to silk. Evaluation of DER was based on the number of ears infected. Their results suggested that dominance factors played a major role in resistance to DER due to the highly significant specific gene combining effects.

Flett and McLaren (1994) evaluated germplasm from South Africa for DER over localities and seasons. Hybrids tested did not rank consistently over localities or seasons. They concluded that environmental conditions highly favorable for DER development and high disease potential may overcome resistant genotypes (Flett and McLaren, 1994). Their studies demonstrate that the maximum disease potential (defined as the mean DER incidence over all hybrids associated with a specific season, locality or inoculation treatment) for comparing resistant and susceptible genotypes was 50.6%.

The maternal effects in ear rot studies have been taken into account for fusarium ear rot. Headrick and Pataky (1989) reported the maternal influence of specific maize characters; *i.e.*, conditions of silk, pericarp and closing layer that have a significant impact on resistance to kernel infection by *F. moniliforme*. Cytoplasm did not appear to be the source of resistance in these studies, nor was there significant difference between

kernels produced on reciprocal F_1 and F_2 plants or on kernels of backcross generations when the F_1 was used as the ear parent. They concluded that efforts to reduce losses from fusarium ear rot should include selection of inbred parents for delayed silk senescence and use of identified sources of resistance as the ear parent. Scott and King (1984) demonstrated that the pericarp maybe the site of resistance to *F. moniliforme* in starchy inbred lines. Previously, Lunsford et al. (1975) demonstrated that in a diallel cross and its reciprocals, some maternal and additive genetic effects influenced resistance to seedling blight caused by *F. moniliforme*.

Diallel analyses have been used to study the resistance gene action to several maize diseases including; anthracnose stalk rot caused by *Colletotrichum graminicola* (Ces.) Wils. (Callaway et al., 1992), gibberella ear rot caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch] (Reid et al., 1992), and gray leaf spot caused by *Cercospora zeae-maydis* Tehon & E.Y. Daniels (Donahue et al. 1991, Gevers et al., 1994). The disease reaction is compared in a series of F_1 hybrids resulting from all possible combinations of crosses of parents. The parents have a range of disease reactions (resistant to susceptible) to the specific disease. General and specific combining abilities (GCA and SCA) as described by Sprague and Tatum (1942) are measures of the gene action that control the resistance or susceptibility. Based on the results of the diallel analysis, high GCA effects infer that resistance is influenced by additive gene effects and high SCA effects infers dominant or epistatic gene effects. Epistasis is defined as the influence one gene has on the phenotype of another.

Diallel analyses are used with phenotypes which are controlled by several genes or quantitatively inherited traits. Molecular markers have been utilized with diallel analyses. Lee et al. (1989) reported from an eight parent diallel that grain yield, and specific combining ability were significantly correlated with modified Roger's distance. Saghai Maroof et al. (1994) reported 27 loci on six of seven barley chromosomes were found to contribute to resistance to resistance to powdery mildew caused by *Eryisphe graminis* f. sp. *hordei*.

Genetic variability of *S. maydis*. Christensen and Wilcoxson's (1966) monograph on stalk rots addresses and reviews some of the fungal variability that has been found with *S. maydis*. Hoppe (1936) discovered aversions and inhibition of growth at the edge of cultures in a series of studies with different isolates on culture media and in host tissue. From host tissues he was able to re-isolate only one isolate even when ears were inoculated with several isolates.

Young et. al. (1959) examined isolates from Minnesota, Oklahoma, and Missouri on a set of F_1 entries for maize stalk rot in each of the three states and found differences in pathogenicity among the three isolates on the maize entries. They also reported differences in temperature growth requirements. Isolates from Minnesota grew better at lower temperatures than Oklahoma or Missouri isolates. At warmer temperatures, the isolates from Oklahoma and Missouri grew better. The same reactions occurred in the field. One field season, where Minnesota field temperatures were above

normal, the Oklahoma and Missouri isolates had higher stalk rot ratings than the Minnesota isolate. Environmental temperatures were thought to play a role in the amount of stalk rot that developed.

Kappelman et al. (1965) addressed the issue of variability further. They examined twenty isolates from different maize producing regions in the U.S. on nine single crosses. They conducted a field study to assess the development of the relative amount of diplodia stalk rot on single crosses and found significant differences among isolates. The selected inbreds that were used in the field study gave different rankings in virulence for some of the isolates. The authors discussed the possibility that while virulence of the fungus (virulence used as the ability to cause disease on a specific inbred line) is the reason for the differences in stalk rot ratings; genes in the host for resistance may also contribute to the order of ranking of the isolates. They concluded that highly virulent isolates specific to that geographical area should be used in a breeding program for resistance.

Ullstrup (1949) compared virulence of five different isolates from "widely separate geographic regions" and found no differences in the percent of diplodia rotted ears. Maxwell and Thompson (1974) reported significant differences in stalk rot incidence among combinations of maize lines and testers when challenged with highly virulent isolates and weakly virulent isolates of *S. maydis*.

In studies with *S. maydis*, several researchers have adapted their studies to account for some of these differences. Loesch et al. (1962) used five isolates which produced high numbers of pycnidia, pycnidiospores, and high levels of aggressiveness

(relative amount of stalk rot) in studies of diplodia stalk rot. Villena (1969) studied the inheritance of resistance to DER using three different isolates which demonstrated differences in virulence (possibly the amount of the ear colonized or severity). Chambers (1988) used an isolate of *S. maydis* that was known to be aggressive (possibly greater amount of tissue colonized) on maize.

Methods or Approaches to Study Genetic Variation. Genetic variation among isolates of fungi has been examined through a variety of techniques including: histochemistry, ultrastructural observation, anamorph connections, anamorph morphology, vegetative compatibility, isozyme analysis, DNA restriction fragment length polymorphisms (RFLP), polymerase chain reaction (PCR) with specific primers, restriction analysis of PCR products, direct sequencing, and random amplified polymorphic DNA analysis (Kohn, 1992; McDonald and McDermott, 1993; Meijr et al., 1994; Micales et al., 1986). Bonde et al. (1993) reviewed the uses of isozyme analysis by geneticists and plant pathologists. Briefly, isozyme analysis is a biochemical technique that can measure changes in gene coding and gene processing. Isozymes are different molecular forms of the same enzyme, which differ from each other in their amino acid sequence or protein modification. These differences are found during electrophoresis, where enzymes with different net charges or molecular weight migrate different distances on gels. This is a conservative estimate of genetic variability due to genetic changes can take place that will not affect the amino acid sequence or change the net charge of the protein molecule. Isozymes can be formed in

three manners; i) different alleles at the same locus (called allozymes) ii) multiple loci coding for a single enzyme, and iii) postranslational processing and formation of secondary isozymes. Isozymes are relatively inexpensive, fast and there is a large database from other fungi, plant and animal systems to compare results. A drawback of isozyme analysis is that there are few loci to look for genetic change.

Isozyme Analysis of Plant Pathogenic Fungi. Previous research has reported isozyme polymorphisms in facultative parasites *Rhynchosporium secalis* (Oud.)Davis on barley (*Hordeum vulgare* L.) (McDermott et al., 1989; Newman, 1985) and *Alternaria solani* Sorauer and *A. alternata* (Fr.) Keissl. (Petrunak and Christ, 1992). Pectolytic isozyme profiles have been used to study *Rhizoctonia* DC. (McNish and Sweetingham, 1993) and *Heterobasidion annosum* (Fr.:Fr.) Bref. (Karlsson and Stenlid, 1991). Rhizoctonia-like fungi could be separated into eleven zymogram groups based solely on their pectolytic profiles (Sweetingham et al., 1986). This was followed by a study providing evidence for the stability of these groupings (McNish and Sweetingham, 1993). Karlsson and Stenlid (1991) reported that six distinct zymogram groups may be used as markers for three intersterility groups in *H. annosum*.

Intraspecific differences have been detected using isozyme analysis and used for identification or confirmed classification of formae speciales. For example: i) Bosland and Williams (1987) could divide *Fusarium oxysporum* Schlechtend.:Fr. that caused yellows or wilt disease on Cruciferae into three groups that corresponded to their natural

host species, ii) Nygaard et al. (1989) divided isolates of *Phytophthora megasperma* Drechs. into six groups that corresponded to host range and iii) Julian and Lucas (1990) could separate wheat (*Triticum* L.) and barley (*Hordeum* L.) isolates of *Pseudocercosporella herpotrichoides* (Fron) Deighton.

Few or no isozyme variation has been found in several obligate parasites or biotrophs: i) *Puccinia recondita* f. sp. *tritici* Roberge ex Desmaz. and *P. graminis* f. sp. *tritici* Pers.:Pers. (Burdon et al., 1983), ii) *P. striiformis* Westend. (Newton et al., 1985), iii) two zymodemes were reported for *Peridermium harknessii* J.P. Moore (Vogler et al., 1991), and iv) *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal (Clark et al., 1989). The *Puccinia* spp. and *P. harknessii* in these studies reproduces primarily asexually. It was proposed in both of these studies that this may play a role in the uniformity of isozyme phenotypes. Clark et al. (1989) suggest as a possible explanation for isozyme uniformity in *E. graminis* f.sp. *hordei*, that barley as a host may impose particularly stringent biochemical requirement on mildew and exert strong selection pressure against any variation. Recombination does occur with this sexually reproductive mildew. If the recombinant progeny are not viable, sexually reproducing populations would show no greater isozyme variation than would clonal populations.

Different results have been found when isozyme phenotypes were linked with aggressiveness or virulence phenotypes (all possible definitions). Leung and Williams (1986) found that a majority of *Magnaporthe grisea* R. Krause & R.E. Webster isolates from rice fell into two groups despite variability in virulence among these isolates. Similar

results were reported by Linde et al. (1990) and Lu and Groth (1987) for *Uromyces appendiculatus* (Pers.:Pers.) Unger despite the presence of a wide array of virulence phenotypes.

Meijr et al. (1994) compared three techniques for detecting genetic variability in isolates of *Phomopsis subordinaria* (Desm.) Trav., a pathogen of *Plantago lanceolata* L.. They found uniform isozymes for 19 enzymes and reported variability among the isolates for vegetative compatibility and RAPD analysis.

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CHAPTER 2

Stenocarpella maydis: Maize Whorl Inoculations and Development of Diplodia Ear Rot

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ABSTRACT

Greenhouse inoculation studies were conducted to determine if whorl inoculation at the final vegetative (V12-V15) growth stage with a benomyl resistant *Stenocarpella maydis* strain, G3 could develop diplodia ear rot (DER). Three maize lines, B73 and H99 (susceptible) and Va26 (intermediate), were inoculated when the final leaf (V12) had emerged, and the tassel was formed but had not elongated. Five ml of a spore suspension containing 5 x 10^3 conidia/ml, was placed in the whorl and the plants were maintained for 4 to 6 wk past flowering. DER developed in 94% of inoculated plants. *S. maydis* strain G3 was recovered from 81% of the diseased plants. To compare inoculation effectiveness of greenhouse with field inoculations, four maize inbred lines [B37, H95 (resistant), Va26 and B73], were inoculated in the field at the final vegetative growth stage (V12) using four inoculum preparations: spore suspension, maize seed collected from diseased ears,

ground colonized popcorn, or colonized millet kernels. The spore suspension inoculum gave the clearest separation of susceptible, intermediate, and resistant responses. These results indicated that inoculation of germplasm to evaluate resistance to DER should begin one to two weeks prior to anthesis or at the final vegetative growth stages, V12-V15.

INTRODUCTION

Diplodia ear rot (DER) of maize (*Zea mays* L.) caused by the fungus *Stenocarpella maydis* (Berk.) Sutton can cause serious losses in isolated locales. Recent outbreaks have occurred in isolated areas in Indiana (D.H. Scott, personal communication, Department of Botany and Plant Pathology, Purdue University), Iowa (11), Kentucky (16, 17), and Virginia (M. A. Hanson, 1992, VPI & SU Deptartment of Plant Pathology, Physiology and Weed Science, Plant Clinic Record 2102 and 2027) over the past three growing seasons. Diplodiosis, a mycotoxin disease that affects animals, has been described from studies in South Africa (7). Management of the plant disease has focused on identifying resistant germplasm and incorporating the resistance into commercial hybrids. Several studies examined the mode of inheritance for resistance characteristics. These studies have resulted in conflicting conclusions: resistance may be dominant (10), resistance may be multigenic (19), susceptibility may be dominant (19), additive effects are most important (15), dominance played a major role in resistance (3) and environmental conditions may play a primary role in plant response (5). Different germplasm, isolates, and possibly more importantly, different inoculation methods to were used in these studies

to evaluate the germplasm. Two methods used in these studies and others to identify resistance to DER included applying a spore suspension from silk to base 10 days following silking (2,14,19) or insertion of inoculum into the ear with a colonized toothpick or a spore suspension with a syringe (15).

In three studies inoculation techniques for DER reactions were evaluated (8,15,18). Villena (15) examined nine techniques including: spore suspensions sprayed from silk to base of the ear, applied with a dropper at tip of the ear, placed in the middle, tip or butt of the ear with a syringe, an agar suspension applied over outside of ear, and toothpicks inserted in middle of ear or between husks at the butt. Among these tests, syringe inoculation resulted in consistent and high levels of disease. Warren and Onken (18) reported that a spore suspension placed in the whorl of the plant two weeks prior to anthesis resulted in DER developing in susceptible inbred lines. Klapproth and Hawk (8) compared four methods of inoculation for DER: 1) dropping infested whole maize kernels into the whorl, 2) spraying silks with a spore suspension (silk spray), 3) placing a spore suspension in the sheath cavity, and 4) injecting a spore suspension directly into the ear. They concluded that the silk spray technique gave the most reliable results based on environmental constraints and identified differences in disease response among inbreds. They also concluded that infested whole kernels placed in the whorl resulted in a lower incidence of disease but could also separate inbred lines into resistant and susceptible groupings. Ullstrup (14) stated that *S. maydis* is not a wound pathogen and that in the majority of diseased ears, ear colonization takes place through the shank and butt but not

through the silks. Neither injection or silk spray method utilized to differentiate germplasm for ear rot resistance (3,10,15,19) resemble the natural infection process for development of DER as described by Ullstrup (14).

A better understanding of how ears are infected and the timing of infection is necessary to determine which inoculation technique will accurately measure resistance to the development of DER (8). This study was undertaken to determine if DER develops after placing inoculum in maize whorls using a marked isolate under controlled environmental conditions in the greenhouse. A follow-up study to examine inoculation preparations was conducted in the field.

MATERIALS AND METHODS

Benomyl resistant strain. A single spore isolate from a diseased ear originating from the Virginia Polytechnic Institute and State University Kentland Agricultural Research Farm, Blacksburg , VA was used in the greenhouse study. Selection medium was prepared by dissolving benomyl (99.18% a.i.; E.I. DuPont De Nemours & Company, Wilmington, DE) in dimethyl sulfoxide, then adding this suspension to cooled potato dextrose agar for a final concentration of 1 μg/ml, (benomyl agar). One ml spore suspensions (6.6×10^6 spores) were plated onto each of ten plates of benomyl agar. After six day, benomyl resistant strains were isolated. One isolate, G3, was used in all the subsequent greenhouse inoculation studies.

Greenhouse experiments. Three separate experiments were conducted. In the first experiment, nine plants of susceptible inbred line B73 were inoculated with an aqueous spore suspension of *S. maydis* strain G3 and three plants were "mock"- inoculated with water. In the second experiment, *S. maydis* strain G3 was used to inoculate four B73 and four susceptible H99 maize plants and two plants were inoculated with water. In the third experiment, *S. maydis* strain G3 was inoculated into six H99 and eight intermediate Va26 maize inbred lines with two water controls. A total of 31 plants were inoculated with *S. maydis* strain G3 and seven were inoculated with water as controls.

Greenhouse experiments general procedures. Inbred lines B73, H99, and Va26 were planted in small pots (10 cm) in the greenhouse. Seedlings of each inbred line were transplanted into larger pots (11 L) after three wk. Soil medium consisted of two parts pasteurized sand and one part pasteurized field soil (Wheeling silt loam) amended with slow release fertilizer (14N-14P-14K, Osmocote®, Grace Sierra Horticultural Products Co., Milpitas, CA) at 250 ml per 36 L soil medium. Supplemental light (high intensity, 1000 W Cermalux C1000S52, Westinghouse and 120V Duratest, Dura-test Corp. N. Bergen, NJ) was provided for a 14 hr light and 10 hr dark period.

Whorl inoculation description. Plants were inoculated with 5 ml of a 5 x 10^3/ml spore suspension into the whorl using a sterile pipet. The plants were inoculated at the final

vegetative growth stage (V12 for inbreds) where the last leaf was fully emerged and the tassel had formed but had not elongated (12).

Recovery of *S. maydis* G3 isolate. Plants were harvested 4 to 6 wk following inoculation. Symptomatic tissue was plated onto benomyl amended agar plates as previously described and grown at room temperature (25-28 C) under continuous cool white fluorescent lamps (F40CW/RS/EW-II 34 W, Philips Co.) for a 24 h light period. Plates were examined for pycnidiospore production after two weeks.

Field treatments: Four different inoculum preparations were evaluated on the following inbred lines: B37 (R), H95 (R), Va26 (I), and B73 (S). These inbred lines were selected based on days to flowering and different resistance responses. Five ml of a 5 x 10^3/ml pycnidiospore suspension, maize seed collected from DER diseased ears from previous season, 5 ml of ground colonized popcorn kernels, or 5 ml of colonized millet seed was placed in maize whorls at V12 growth stage. Noninoculated plants served as the control. Each inbred was paired with each treatment and planted in two-row plots (3.3 m long and 0.75m wide) at the Kentland Agricultural Research Farm on Purdy silt loam soil with a 0-2% slope in a randomized complete block with three replications. The field was fertilized with 168 kg N, 45 kg P, and 67 kg K per ha prior to planting. Preplant herbicides applied were, atrazine (1.7 kg ai per ha), simazine (1.1 kg a.i. per ha) and postemergent herbicide

treatments included bentazone (1.12 kg ai per ha) and crop oil concentrate. The insecticide applied was tefluthrin (18.3 kg per ha) in the furrow at planting.

Field inoculum preparation: A single spore *S. maydis* isolate strain J211 from Kentland Agricultural Research Farm was used for the field study. The pycnidiospore suspension was prepared as described above. *S. maydis*-colonized millet and popcorn were prepared by soaking 250 ml of untreated seed in a mason jar with distilled water for 24 h. Water was drained off and the jars were autoclaved at 121 C for one hr on two consecutive days. Two 5 mm plugs of agar with mycelial growth from a 6 day old culture of *S. maydis* were placed in both popcorn and millet jars and incubated at room temperature (28-30 C) for two weeks. The popcorn and millet were removed from jars and allowed to dry for three days at 32 C. Popcorn was ground in a blender and millet was separated into individual seeds. Inoculum for the whole kernel maize treatment was a combined mixture of five diseased ears collected from the Kentland Agricultural Research Farm in 1994.

Field inoculations: Plants were inoculated at the V12 growth stage. A pipet was used to apply the spore suspension. A 5 ml spoon was used to apply colonized ground popcorn kernels and millet seed. Five whole maize seeds were placed into the whorls. All inbred lines matured at approximately the same time, therefore only one inoculation was conducted on July 16, 1994.

Field evaluation: Plants in each plot were rated for presence or absence of ear rot approximately 2 mo following inoculation. Percentage plants with DER was used for data analyses. Analysis of variance (ANOVA) was conducted and least squares means were calculated and comparisons made among inbred lines and methods at $P \leq 0.01$ (13).

RESULTS

Recovery of benomyl resistant isolate. Five benomyl-resistant isolates were recovered from benomyl agar plates where wild-type *S. maydis* pycnidiospores were added. One benomyl-resistant *S. maydis* isolate, G3, was recovered from the benomyl agar plates and showed wild-type growth habits. Comparisons were conducted with wild-type and G3 for several phenotypes; growth habit over four temperatures, pycnidiospore production, and plant colonization ability. The *S. maydis* strain G3 grew at a slower rate across a PDA plate when measured after 48 hr. The growth was significantly different from the wild type at one temperature 27 C (Table 1). The benomyl resistant mutant was colonized ear tissue as well as the wild-type in a greenhouse assay (Table 1). Plating of recovered *S. maydis* strain G3 spores onto benomyl agar revealed that some pycnidiospores were susceptible to benomyl. This finding indicates that not all of the nuclei carry resistance to benomyl. This fungus is multinucleate, to prevent those nuclei which were not susceptible to benomyl from increasing, all culturing of *S. maydis* strain G3 took place in the presence of benomyl.

Table 1. Comparison of benomyl resistant mutant, *S. maydis* strain G3 with wild type for growth on acidified potato dextrose agar (APDA) at 23, 27 and 33 C and ability to colonize maize ear tissue in a greenhouse

Character	Wild type[a]	Benomyl resistant
Growth on APDA at 31 C	51.3±5.9 a[b]	44.3±5.9 a
Growth on APDA at 27 C	47.7±0.5 a	37.0±0.5 b
Growth on APDA at 23 C	25.3±1.9 a	14.7±1.9 a
Plant colonization ability[c]	+	+

[a] Values represent average growth of mycelium (mm) and standard error from 3 plates, measured from the edge of a colonized agar plug, after 48 hr.

[b] A general linear model, ANOVA was conducted, values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

[c] + = maize ear was colonized 14 day following inoculation with *S. maydis* strain G3 or wild type. H99 was inoculated in the ear when silks were 4-5 cm with core borer, 5 mm colonized plug, and wrapped with parafilm. B73 a spore suspension was placed in the whorl.

Greenhouse inoculations. A total of 31 plants were inoculated with benomyl resistant isolate G3 and seven controls. Twenty-nine of 31 plants inoculated with *S. maydis* developed DER (Table 2). From these plants exhibiting symptoms of DER, benomyl resistant *S. maydis* was recovered from 81%. *Fusarium moniliforme* Sheldon was also recovered from all plants including controls. Figure 1 shows ear rot symptoms that developed in the greenhouse after six wk when inoculated by the whorl method. White mycelial growth developed in leaf axils, on ear shanks, and in ears. Some of the ears were heavily colonizied while others exhibited localized colonization. DER did not develop in plants mock-inoculated with distilled water.

Field inoculations. Table 3 summarizes the ANOVA for comparison of the four inbred lines to the inoculum preparations. There were highly significant differences among the inbred lines, methods, and inbred line by method interaction. Table 4 summarizes the field results. Whole maize kernels placed in the whorl did not result in significantly different DER levels compared to controls or natural infection and colonization. *S. maydis* colonized millet and popcorn had very high levels of DER for all inbreds. The spore suspension placed in the whorl gave the best separation of resistant levels among inbreds.

Table 2. Number of plants that developed diplodia ear rot (DER) following inoculations with benomyl-resistant *Stenocarpella maydis* strain G3 on three maize inbred lines over three greenhouse experiments

	S. maydis			Control	
Inbred	Inoculated[a]	DER[b]	Recovery of G3[c]	Water inoculated[a]	DER[b]
Experiment 1.					
B73	9[d]	7	7	3	0
Experiment 2.					
B73	4	4	2	1	0
H99	4	4	2	1	0
Experiment 3.					
H99	6	6	6	1	0
Va26	8	8	8	1	0
Total	31	29	25	7	0

[a] A spore suspension of 5 x 10^3 pycnidiospores/ml was placed in the whorl with a pipet at the V12 growth stage. Controls were mock-inoculated with distilled water.

[b] Number of plants that fourteen days after inoculations, had any colonization of the ear.

[c] Symptomatic tissue was plated onto benomyl agar (1μg/ml benomyl and potato dextrose agar). Plates were examined after 10 to 14 day for pycnidia and pycnidiospores.

[d] Number of plants

A

B

Fig. 1. Maize inbred B73, (A) inoculated with 5 ml of a benomyl resistant *S. maydis* pycnidiospore suspension ($5x10^3$ spores/ml), thick white mycelium is present at the base of the ear and (B) inoculated with 5 ml of distilled water. Inoculations took place in the greenhouse at the V12 growth stage, approximately 12 d prior to flowering. Pictures taken 4 wk post inoculation.

Table 3. Analysis of variance of a comparison of inbred lines reaction to different inoculum preparations of *Stenocarpella maydis* for development of diplodia ear rot

Source	df	MS	F Value[a]
Inbreds	3	3591.9	19.62 **
Methods	4	13950.8	76.22 **
Inbred x methods	12	685.2	3.74 **
Replication	2	778.6	4.25 *
Error	38	183.0	

[a] Asterisks indicate ** significance at $P \leq 0.01$ and * significance at $P \leq 0.05$.

Table 4. Least square mean values for maize with diplodia ear rot caused by *Stenocarpella maydis* from a field experiment where four inoculum preparations were applied in the whorl at the V12 growth stage on four maize inbred lines; B37 (R: resistant) , H95 (R: resistant), Va26 (I:intermediate), and B73 (S: susceptible)

Inbred	Inoculum preparation				
	Spore suspension[a]	Corn kernels[b]	Ground popcorn[c]	Millet[d]	Noninoculated
H95 (R)	4.2[e] a A[f]	15.4 a A	82.8 b A	64.3 b AB	0.0 a A
B73 (S)	82.5 a B	27.0 b A	82.6 a A	91.1 a AC	11.7 b A
Va26 (I)	49.3 a B	38.8 a A	100.0 b A	88.9 b ABC	4.6 a A
B37 (R)	5.8 a A	5.0 a A	73.5 b A	54.0 b AB	5.8 a A

[a] Five ml of 5 x 10^3 spores per ml, *S. maydis* single spore strain J211, isolated from diseased ear from Kentland Agricultural Research Farm.

[b] Five whole kernels collected from diseased ears at Kentland Agricultural Research Farm.

[c] Five ml colonized ground popcorn, *S. maydis* single spore strain J211.

[d] Five ml colonized millet, *S. maydis* single spore strain J211.

[e] Values represent percent mean incidence of DER in two row plots.

[f] Values followed by the same lowercase letter within a row are not significantly different at $P \leq 0.01$. values followed by the same uppercase letter in a column are not significantly different a $P \leq 0.01$.

DISCUSSION

An abstract of this research was presented earlier (4). Ullstrup (14) listed five criteria to evaluate usefulness of a method for inoculating maize to determine resistance reactions: 1) a clear differential between resistant and susceptible host materials, 2) disease reaction of inbred lines and hybrids comparable to those experienced under natural epidemics, 3) reproducibility of results from year to year with no great effect of environment on the relative disease reactions of plants, 4) simplicity of execution and freedom from complicated laborious procedures, and 5) simulation of those phenomena attributed to the natural mode of infection by the parasite. Several researchers have examined numerous methods for evaluating maize germplasm for resistance to DER. The results of these various studies have produced conflicting and variable results and have not met all of Ullstrup's criteria. Timing of inoculations in these studies varied from mid-silk to 20 days after silking (1,2,10,11,15,19) which may or may not simulate timing in natural infections. Our findings from both greenhouse and field evaluations demonstrate that DER can develop as a result of inoculations one to two weeks prior to anthesis or the final vegetative growth stage (V12-V15). At this growth stage the ear and the tassel are enclosed in the top leaves prior to elongation and flowering (12).

The method by which inoculum is applied is as important as the timing of the inoculations. Warren and Onken (18) utilized a spore suspension of *S. maydis* placed in the whorls, late in the evening and Flett (5) used dried colonized ground grain. Both studies were judged successful in evaluating germplasm. Klapproth and Hawk (8) tested

the whorl inoculation technique with five kernels of dried grain. DER did developed in these plants but at reduced incidence when compared to a spore suspension applied over the outside of the ear. When whole kernels were placed in the whorl in our 1995 field study, the inoculum did not directly reach the developing ear and we observed that as the whorl expanded some kernels were forced out of the whorl. Our field observations agree with the findings of Klapproth and Hawk (8) that whole kernels placed in the whorl do not differ significantly from natural infection of noninoculated control. In addition, kernels placed in the whorl failed to separate genotypes into resistant and susceptible groupings. However, a spore suspension, ground colonized popcorn, and colonized millet resulted in increased amounts of DER compared to natural infection levels. The colonized ground popcorn and colonized millet resulted in very high disease levels potentially overwhelming all defense responses. It should be noted that weather conditions during this season were very dry, with little rainfall, but with levels of high relative humidity. The inoculation technique of applying a spore suspension gave the best separation of genotypes into resistant, intermediate and susceptible disease reactions. This method, however, does require that inoculum be placed in the whorl late in the evening or on cool overcast days to ensure survival of the conidia. Further evaluation is needed on the millet and popcorn inoculum treatments. These could include reduced dosage levels, finer particles or a survey of another substrate that will not serve as a good nutrient source for the fungus.

Physical characters of the host plant which may block pathogen colonization include thickened cuticle, thick epidermal layer, and tightly wrapped leaves or husks.

Utilizing inoculation methods which breech host physical resistance mechanisms may confer susceptibility to otherwise "field resistance" genotypes. The same is true for high inoculum densities that may overwhelm the host defense responses. From our field study, there was a significant inbred line by method interaction which resulted from the different amounts of disease generated based on how the inoculum was applied. Specifically from our data, dried colonized millet and dried ground popcorn treatments resulted in very high levels of disease on all genotypes (54-100% DER). Quantitative resistance (resistance controlled by several genes), is often influenced by environmental factors, therefore finding a level of inoculum and timing of application where the genotypes are not overwhelmed but allow for expression of resistance can be difficult to attain under field conditions. We should focus on inoculation procedures which give a separation of genotypes but at reduced levels of severity and incidence, similar to an LD_{50} value used for pesticide evaluations. Environmental conditions highly favorable for DER development and high disease potential may overcome field resistant genotypes (5). Flett and McLaren (5) reported from South African studies that the maximum disease potential for comparing resistant and susceptible genotypes is 50.6%.

Hooker (6) reported that resistance to seedling blight, stalk rot and DER caused by *S. maydis* were all inherited independently in maize. It is possible to have a line resistant to stalk rot but susceptible to DER. Stalk rot is evaluated by placing inoculum directly into the stalk. Another difference between our findings and those of other workers is that different types of resistance responses are measured by different inoculation

procedures. For instance, direct inoculation into plant tissues (syringe, toothpick, etc.) measures the limitation of the pathogen colonization ability within host tissues; which includes only part of the process of pathogenesis--ramification and symptom development. The spore suspension inoculation method measures resistance to the whole process of pathogenesis (germination, penetration, establishment, colonization, and symptom development). Specific sites where resistance in maize to *S. maydis* may be genetically controlled is another question. Because resistance in the host may be separated by different locations (seedling, stalk, or ear) and resistance reactions may change with different host growth phenologies. H111 may be a key inbred line to test this hypothesis. Klapproth and Hawk (8) reported that H111 had a susceptible rating following inoculations with a spore suspension introduced into the ear with a syringe. In field studies (H.L. Warren, unpublished data) with whorl inoculations prior to flowering H111 was resistant to DER. The ear may serve as a perfect incubation chamber for *S. maydis* development. Results of direct inoculation into the ear may provide little protection or this inbred's resistance to DER may change with phenology? Determining if the resistance mechanisms are located at the outer surface would be crucial to our understanding the mechanics of resistance to DER.

In this study we have shown that a spore suspension applied into the whorl of the maize inbred lines prior to flowering results in the development of DER and a separation of genotypes into resistant, susceptible, and intermediate groupings. It is suggested that germplasm evaluated for resistance to DER should take into account these growth stages

for inoculation. Further studies to evaluate inoculation procedures may focus on environmental parameters that influence DER development and reduction of dosage of dried inoculum preparations.

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CHAPTER 3

Diallel Analysis of Diplodia Ear Rot Resistance in Maize

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ABSTRACT

Diplodia ear rot (DER) of maize has increased in occurrence in the US in the past few years. In this study, F_1 generations from eight parents in 1994 and nine parents in 1995 were analyzed for their reaction to DER. Genotypes were planted in a randomized complete block with four replications in 1994 and three replications in 1995. Plants were inoculated with a spore suspension ($5x10^3$ spores/ml) placed in the whorl at the V14-V15 growth stage. Plants were evaluated for presence or absence of DER at harvest. The percentage of plants with DER was used for analysis. In both years general combining ability (GCA) effects were significant indicating additive gene action is important. In 1995, specific combining ability effects were also significant, indicating that dominance or epistatic gene action were important. There was no significant interaction between year and genotypes indicating that crosses acted the same in both years. The following inbred lines had negative GCA effects contributing towards resistance in both years; B37, H111, B68, and MS.

INTRODUCTION

Diplodia ear rot (DER) caused by the fungus, *Stenocarpella maydis* (Berk.) Sutton, is an important disease in many maize (*Zea mays* L.) growing regions of the world and has caused significant losses in isolated locales within the United States (M. A. Hanson, 1992, VPI & SU Department of Plant Pathology, Physiology and Weed Science, Plant Clinic Record 2102 and 2027; Munkvold and Yang, 1994; D.H. Scott, 1995, personal communication, Department. of Botany and Plant Pathology, Purdue University; Vincelli, 1992, 1994). Recent studies in South Africa have associated a mycotoxicosis of poultry, lambs, and ruminants with feeding of *S. maydis* colonized grain (Kellerman et al., 1985; & 1991, Rabie et al., 1987). DER develops as a result of infection and subsequent colonization of the maize ear. Ears are usually colonized from the shank up into the ear and losses are due to reduced seed weight. Typically this does not occur as a wide spread phenomenon but in isolated fields. However, disease incidence of 1-2% has been recorded from field surveys (Scott et al., 1992).

Previous reports concerning DER resistance are conflicting. Koehler and Holbert (1930) reported resistance is dominant from studies with natural infection. Wiser et al. (1960) reported that susceptibility may be dominant from examining parents, F_1s and backcrosses. Villena (1969) found additive effects played a major role in resistance to DER. Das et al. (1984) reported specific combining ability (SCA) was most important. Resistance to DER is inherited independently from resistance to other ear rot pathogens

(Hooker, 1956) and other diseases caused by *S. maydis* (Hooker, 1956; Thompson et al, 1971). Flett and McLaren (1994) reported that resistance to DER was ineffective under high levels of inoculum potential and environmental conditions favorable for disease development.

Our research focused on how resistance is inherited from a set of inbred lines which vary in their resistance to DER through an analysis of a diallel cross. The inbred lines include germplasm that are used in maize line improvements, or related derivatives which have been used in the past, and two nonreleased inbred lines of tropical origin. One inbred line, B73 or its derrivatives, is highly susceptible to DER and is one of the most common female parents utilized in US maize seed production.

MATERIALS AND METHODS

Seven inbred lines and one synthetic (Table 1) were crossed in all possible combinations in Virginia during the 1992 and 1993 growing seasons. There was an initial evaluation during 1993, but due to poor growing conditions and poor stand count, these results are not included. An additional inbred parent was added to the diallel during the 1995 growing season. Parents (Table 1) were initially identified for disease reaction based on screenings conducted on inbred lines (H.L. Warren, unpublished data).

Germplasm response to DER inoculation was examined at the Virginia Polytechnic Institute and State University, Kentland Agricultural Research Farm, Blacksburg, VA. The genotypes were treated as treatments and planted in a complete randomized block

Table 1. Pedigree and origin for lines used in the diallel cross to determine inheritance of resistance for diplodia ear rot reaction

Inbred line	Reaction to DER[a]	Pedigree[b]	Origin	Date released	Year progeny tested
H95	R	OH43 X C.I.90A	Indiana	1968	1995
B37	R	Stiff Stalk Syn.	Iowa	1958	1994-95
H111	R	B37 X PI 209135 (Mayorbela)	Indiana	1978	1994-95
MS	MR	Mayorbela Selections	Tropical	Not released	1994-95
B68	I	(41.2504B X B14[3])selection	Iowa	1968	1994-95
Va26	I	OH43 x K155	Virginia	1967	1994-95
B73	S	Stiff Stalk Syn.	Iowa	1972	1994-95
H99	S	Il. Syn. 60 C	Indiana	1974	1994-95
Mb271[c]	S	[B73 x (B73 x Mb 118-2)]S_2Bx[1]	Tropical	Not released	1994-95

[a]Ratings for ear rot from 1993 and 1994 tests, R=resistant, MR=moderately resistant, I=intermediate and S=susceptible.

[b]References: Henderson (1980) and Warren (1982).

[c]This inbred has been readmitted into the breeding program due to drought sensitivity.

with single row plots and four replications in 1994 and two row plots with three replications in 1995. Seeds were planted by hand in 1994 and with a two-row seed planter Almaco Planter (Almaco Corp., Johnson City, IA) in 1995. The soil type was a Purdy silt loam with a 0-2% slope, row length was 3.3 m with 0.75 m spacing. Plots were separated by 1.52-m-wide nonplanted alleys. Lime was applied at the rate of 4,480 kg per ha in April 1994. Fertilizer was disk incorporated at the rate of 168 kg N, 45 kg P, 67 kg K per ha prior to planting. Preplant herbicides applied included, atrazine (1.7 kg a.i. per ha), simazine (1.1 kg a.i. per ha) and postemergent herbicide treatments included bentazone (1.12 kg a.i. per ha; BASF AG, Research Triangle Park, NC) and dicamba (0.14 kg a.i. ha; Sandoz Agro, Inc., DesPlaines, IL) and crop oil concentrate (1.0% v/v). In 1995, only bentazone (1.12 kg a.i. per acre) was applied as a postemergent herbicide. Nicosulfuron (0.02 kg a.i. per ha; DuPont Agricultural Products, Wilmington, DE) was used to spot treat some grasses. Tefluthrin insecticide (18.3 kg a.i. per ha; Zeneca Ag Products, Wilmington DE) was applied in the furrow at planting. In 1994, the plots were irrigated at 2.54 cm on June 22 and 23, and July 7 to assure plant survival during prolonged drought periods.

Inoculum was produced in the following manner: maize kernels were placed in glass Petri dishes and autoclaved (121 C) on two consecutive days for one h each (autoclaved kernels). Fifteen autoclaved kernels were then placed on acidified potato dextrose agar (APDA, pH 5.5). Plates were seeded with pycnidiospores of an isolate from a diseased ear originating from the Kentland Agricultural Research Farm. The same

diseased ear was used in each year of the study. *S. maydis* was grown at room temperature or in an incubator at 25-27 C under 24 h light (24 W cool white, General Electric, Cleveland, Ohio). After 3 wk, kernels were placed in a flask with distilled water and shaken for 10-20 min, and filtered through four layers of cheesecloth. Spore counts were made using a hemocytometer. The spore concentrate was diluted to a final concentration of 5×10^3 spores/ml in the field prior to inoculations. Inoculation of germplasm was conducted as described by Warren and Onken (1981) where, approximately 5 ml of the spore suspension was placed in the whorl of the maize plants at the V14-V15 growth stage (Ritchie and Hanway, 1992).

Germplasm was rated for disease incidence of DER per row at harvest time in both 1994 and 1995. The ear of each plant was husked and examined for the presence of *S. maydis* mycelium. When there was a question, ears were broken and examined for the presence of pycnidia. Disease severity, percent of ear colonized was noted, but was not used in the analysis. Percent DER incidence was analyzed as follows; in 1994, the full diallel was first analyzed with a nonorthogonal ANOVA due to the low stand count and missing crosses caused by the environmental conditions (Huber et al., 1992). Reciprocal crosses were combined and the subsequent data set was then analyzed using a software program from Linda (1991) for Model 1, Method 4 of Griffing (1956). Due to a large error term, the possibility of a cross by replicate interaction was also analyzed using Tukey's test for non-additivity (Tukey, 1949) with the following modified model:

$$y_{ijk} = \mu + g_i + g_j + s_{ij} + r_k + \theta c_{ij} r_k + e_{ijk}$$

where the g_i and s_{ij} represent the GCA and SCA response of the genotypes, r_k represent the replication effect and $\theta\ c_{ij}\ r_k$ represents a particular form of cross by replicate interaction. In 1995, the full diallel was analyzed again, reciprocal crosses were combined and the same analysis was conducted but without cross by replicate interaction. The genotypes that were common for both 1994 and 1995 were analyzed for general combining ability (GCA), specific combining ability (SCA), year effects, and cross x year interactions with the general least squares analysis of diallel experiment program (Schaffer and Usanis, 1995).

RESULTS

Environmental conditions during both growing seasons were marked by drought periods that occurred at different times during the season. In 1994 the drought period occurred during early growth and development stages and in 1995, drought occurred during anthesis. In 1994 and 1995, the number of *S. maydis* inoculated plants that developed DER out of the total stand count or percent disease incidence for the plot was 5.11% and 6.77% respectively. Tables 2 and 3 list the ANOVA for the crosses in the diallel analysis for GCA and SCA effects. In 1994 the genotypes from eight parents had GCA effects were significant and SCA effects were not significant. These results indicate

Table 2. Analysis of variance of diplodia ear rot ratings for a half diallel cross of eight parents[a], 1994

Source of variation	df	Sum of squares	Mean square	F value
Crosses	27	2,908.56	107.72	2.58 **
GCA[b]	7		277.80	6.65 **
SCA[c]	20		48.20	1.15
Replications	3	93.74	31.25	0.75
Cross x rep[d]	1	870.36	870.36	20.84 **
Error	80	3,341.90	41.77	
Total	111	7,214.56		

[a] Asterisks indicate that the effect is significant at $P \leq 0.01$

[b] GCA=General combining ability

[c] SCA=Specific combining ability

[d] Tukey's test $P \leq 0.01$

Table 3. Analysis of variance of diplodia ear rot ratings for a half diallel cross of nine parents[a], 1995

Source of variation	df	Sum of squares	Mean square	F value
Crosses	35	8,447.12	241.35	8.83 **
GCA[b]	8		103.33	11.34 **
SCA[c]	27		73.67	8.08 **
Replications	2	56.02	28.01	1.02
Error	70	1,913.84	27.34	
Total	107	10,416.97		

[a] Asterisks indicate that the effect is significant at $P \leq 0.01$

[b] GCA=General combining ability

[c] SCA=Specific combining ability

that in 1994, a cross by replicate interaction occurred as a result of the genotypes did not act the same across all replicates. In 1995, the crosses were highly significant with a small error term so the cross by replicate interaction was not tested. Both GCA and SCA effects were significant for the progeny of the nine parents in 1995. Reciprocal effects were not significant in this study for both years. This indicates that maternal effects, factors attributed to cytoplasmic genetic factors, were not active and breeders may use either line as the ear parent.

In 1995 there was a higher disease incidence across a majority of the genotypes. Percent mean disease incidence, general combining abilities and specific combining abilities are outlined in Table 4 (1994) and Table 5 (1995). Negative values for combining abilities indicate a contribution towards resistance to DER. In 1994, B37, H111, B68, MS, and H99 had GCA values for resistance. In 1995, B37, H111, B68, MS and H95 had GCA values for resistance. The parent H99, contributed towards resistance in 1994 while contributing toward susceptibility in 1995. The SCA was not significant in 1994, but it was highly significant in 1995. Parents B73, MB271, and VA26 contributed toward susceptibility in both years. The mean percentage DER ranged from 0.0% to 21.25% in 1994 and 0.0% to 52.07% in 1995. Table 6 lists the ranking and significance of the genotypes and parents. Many of the F_1s had similar or greater disease incidence levels in 1995 when compared to 1994 while few had less. More of the genotypes had some level of disease incidence in 1995 compared to 1994. Genotypes with H99 combinations had higher disease incidence. Table 7 lists the ANOVA for genotypes tested in both 1994 and 1995 to look for a year effect. There was no significant difference for cross by year

Table 4. F_1 cross means (above the diagonal), general combining ability effects (on the diagonal, underlined) and specific combining ability effects (below the diagonal) for diplodia ear rot reactions for a set of diallel crosses among maize inbred lines evaluated in 1994, Kentland Agricultural Research Farm, Blacksburg, VA

Parents	B37	H111	B68	VA26	MS	B73	H99	MB271
B37	-1.51	2.93	0.00	6.43	3.00	7.43	3.78	3.23
H111	2.51	-3.20	0.00	2.00	2.08	3.85	2.38	3.42
B68	0.66	2.35	-4.27	3.32	3.5	1.40	0.00	2.00
VA26	-1.16	-3.9	-1.5	3.98	9.25	16.13	5.20	17.38
MS	0.06	0.83	3.32	0.82	-0.68	7.30	2.78	3.88
B73	-0.27	-2.16	-3.54	2.94	-1.24	4.09	3.04	21.25
H99	2.21	2.50	1.19	-1.85	0.38	-4.13	-2.04	6.40
MB271	-4.00	-2.11	-2.47	4.65	-4.19	8.40	-0.29	3.62

Table 5. F_1 cross means (above the diagonal), general combining ability effects (on the diagonal, underlined) and specific combining ability effects (below the diagonal) for diplodia ear rot reactions for a set of diallel crosses among maize inbred lines evaluated in 1995, Kentland Agricultural Research Farm, Blacksburg, VA

Parents	B37	H111	B68	VA26	MS	B73	H99	MB271	H95
B37	-3.49	3.30	1.40	1.31	0.00	0.53	16.9	4.61	1.61
H111	0.12	-0.10	3.77	7.81	2.19	4.51	12.44	4.77	14.64
B68	2.42	1.41	-4.30	2.66	4.76	1.39	1.39	2.71	5.93
VA26	-2.22	0.88	-0.06	0.26	10.98	10.01	5.61	15.24	2.29
MS	0.04	-1.15	5.61	7.28	-3.31	4.16	0.74	6.96	1.11
B73	-7.47	-6.89	-5.81	-1.75	-4.02	4.73	10.86	52.07	3.71
H99	12.28	4.41	-2.44	-2.78	-4.08	-2.01	1.37	10.77	4.98
MB271	-5.57	-8.81	-6.67	1.31	-3.40	33.66	-4.28	6.92	5.39
H95	0.40	10.04	5.53	-2.67	-0.27	-5.72	-1.09	-6.23	-2.07

Table 6. Mean[a] percent diplodia ear rot caused by *Stenocarpella maydis* from a diallel cross in 1994 and 1995, Kentland Agricultural Research Farm, Blacksburg, Va.

1994		1995	
Cross or parent	Mean	Cross	Mean
B37	3.60	B37	5.80
H111	11.10	H111	NT
B68	25.00	B68	NT
VA26	24.40	VA26	49.30
MS	5.00	MS	NT
B73	32.80	B73	82.50
H99	26.90	H99	NT
MB271	27.70	MB271	NT
H95	NT [b]	H95	4.20
B73 X MB271	21.25 a	B73 X MB271	52.07 a
VA26 X MB271	17.38 ab	B37 X H99	16.91 b
VA26 X B73	16.13 abc	VA26 X MB271	15.24 bc
VA26 X MS	9.25 a-d	H111 x H95	14.64 b-d
B37 X B73	7.43 b-d	H111 X H99	12.44 b-e
MS X B73	7.30 b-d	VA26 X MS	10.98 b-f
B37 X VA26	6.43 b-d	B73 X H99	10.86 b-f
H99 X MB271	6.40 b-d	H99 X MB271	10.77 b-f
VA26 X H99	5.20 b-d	VA26 X B73	10.01 b-g
MS X MB271	3.88 cd	H111 X VA26	7.81 b-g
H111 X B73	3.85 cd	MS X MB271	6.96 b-g
B37 X H99	3.78 cd	B68 x H95	5.93 c-g
B68 X MS	3.50 d	VA26 X H99	5.61 c-g
H111X MB271	3.42 d	MB271 X H95	5.39 c-g
B68 X VA26	3.32 d	H99 X H95	4.97 d-g
B37 X MB271	3.23 d	H111 X MB271	4.77 d-g
B73 X H99	3.04 d	B68 X MS	4.76 d-g
B37 X MS	3.00 d	B37 X MB271	4.61 d-g
B37 X H111	2.93 d	H111 X B73	4.51 d-g
MS X H99	2.78 d	MS X B73	4.16 e-g
H111 X H99	2.38 d	H111 X B68	3.77 e-g
H111X MS	2.08 d	B73 x H95	3.71 e-g
H111 X VA26	2.00 d	B37 X H111	3.30 e-g
B68 X MB271	2.00 d	B68 X MB271	2.71 e-g
B68 X B73	1.40 d	B68 X VA26	2.66 e-g
B37 X B68	0.00 d	VA26 x H95	2.29 e-g
H111 X B68	0.00 d	H111X MS	2.20 e-g
B68 X H99	0.00 d	B37 x H95	1.61 fg
H95 X B37	NT	B37 X B68	1.40 fg
H95 X H111	NT	B68 X B73	1.39 fg
H95 X B68	NT	B68 X H99	1.39 fg
H95 X VA26	NT	B37 X VA26	1.31 fg
H95 X MS	NT	MS x H95	1.11 fg
H95 X B73	NT	MS X H99	0.74 fg
H95 X H99	NT	B37 X B73	0.53 fg
H95 X MB271	NT	B37 X MS	0.00 g

[a] Means represent the number of plants in a plot with DER over 4 replications (one row plots) in 1994 and 3 replications (two row plots) in 1995. Means followed by the same letter do not differ significantly at $P \leq 0.05$, Duncan's Multiple Range Test.

[b] NT=not tested

Table 7. ANOVA for comparison of general combining (GCA) and specific combining abilities (SCA) in years for a diallel analysis for resistance to diplodia ear rot in 1994 and 1995

Source	df	MS	F[a]
Mean	1	7066.20	
Year	1	239.62	5.72 **
Replicate	5	17.40	0.41
GCA	7	505.26	12.06 **
SCA	21	298.94	7.14 **
Cross x Year	7	42.34	1.01
Error	154	41.88	
Total	196		

[a] Numbers followed by asterisks significantly different at $P \leq 0.01$.

interaction but there was a significant difference for the year effect. This indicates that a majority of the genotypes acted the same in both years but there was a higher disease level in 1995 when compared to 1994. Table 8 summarizes the GCA of the parents tested in both 1994 and 1995. Inbred line B68, H111, MS, and B37 all had similar negative GCA indicating they had genes that contributed to resistance. MB271, VA26, and B73 had positive GCA effects that contributed toward susceptibility to DER. H99 had negative GCA effects in 1994 and positive GCA effects in 1995.

DISCUSSION

An abstract of this study was reported earlier (Dorrance et al., 1995). Four maize inbred lines B37, H111, B68, and MS contributed to negative GCA effects at about the same level in both 1994 and 1995. Combinations with these parents should allow for increased resistance to DER even in years with greater disease pressure. H99 was not consistent for GCA, but this parent is susceptible to DER and the low levels of DER in 1994 may have given a false reading for this genotype. MB271 in hybrid combination with B73 had very high levels of DER in both years. B73 contributed to susceptibility to DER in both years and it or its derivatives are common parents in U.S. maize production. Significant GCA in 1994 and 1995 indicate that additive gene action is predominant. Genes that contribute to GCA for resistance to DER indicates that genes for resistance can be easily transferred to progeny by crossing resistant inbred lines with the highest

Table 8. Comparison of general combining abilities (GCA) of parents tested in 1994 and 1995

1994		1995	
Parent	GCA[a]	Parent	GCA
B68	-4.27 a	B68	-5.48 a
H111	-3.20 a	B37	-3.82 a b
H99	-2.04 a b	MS	-3.52 a b
B37	-1.51 a b	H111	-2.03 a b
MS	-0.68 a b	VA26	0.44 b c
MB271	3.62 b	H99	1.29 b c
VA26	3.98 b	B73	5.42 c
B73	4.09 b	MB271	7.69 c

[a]Numbers followed by the same letter do not differ significantly at $P \leq 0.001$.

GCA effects. These results agree with Villena's (1969) earlier report where additive gene effects predominate. He also reported SCA were significant in one maize population; SCA indicate which combinations are better or worse compared to the group as a whole, these effects are indicative of dominant gene action or epistasis. However, the F_1 with the highest SCA effects, B73 x MB271, is a selection from the B73 by Mayorbela collection. Therefore, this cross may be homozygous for many genes and responds more like an inbred or B73 parent. Selfing in naturally cross-fertilized crops can result in yield and height reductions as well as reveal unfavorable plant characteristics (Stoskopf, 1993). The drought period in 1995 occurred during flowering through grain filling stages. This hybrid, B73 x MB271 showed severe drought stress and may have also influenced the disease response. The magnitude of SCA effects in 1995 indicate that susceptibility may be controlled by dominant gene action. This agrees with Wiser et al. (1960) who reported that susceptibility may be dominant.

From the F_1s that were tested in both years, only H99 was inconsistent in its GCA effects. As a parent, H99 is susceptible and flowers early (68d). The negative GCA effects of F_1s with H99 as a parent in 1994 may be due to the overall lower disease incidence level. The incidence of DER ranged from 2.38 to 6.40% in 1994 and 5.61 to 16.91% in intermediate and susceptible crosses. It is important in analyzing germplasm reaction to have sufficient number of plants for evaluation. Total stand counts for the analysis were 1994 and 3942 in 1994 and 1995 respectively. Changing the plot size,

reducing the number of replications and the occurrence of more favorable environmental conditions during the early growing season resulted in an increased stand count in 1995.

The cross by replicate interaction that was significant in 1994 may have been influenced by the severe environmental conditions in that season. The field was irrigated prior to and following inoculations. The responses of genotypes to irrigation during high temperature and uneven distribution of water may provide an explanation for the genotype by replication interaction in crosses. Some hybrids showed signs of drought stress, particularly B73 x MB271. Flett and McLaren (1994) concluded that environmental conditions highly favorable for DER development and high disease potential may overcome resistance. Environmental factors can affect the phenotype of additive gene action, and this may account for this response. There are few reports on the environmental effects that influence DER development in maize. With the increase in incidence of this maize pathogen and the predominance of additive gene action, research in this area may help answer several of these questions.

Maize hybrids with higher levels of resistance may be able to withstand higher levels of disease pressure. The environmental parameters that influence germplasm response to infection and colonization need to be further explored.

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CHAPTER 4

Comparison of *Stenocarpella maydis* Isolates for Isozymes, Aggressiveness, and Cultural Characteristics

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ABSTRACT

A collection of isolates of *Stenocarpella maydis* from seed companies and plant disease clinics in the U.S. and the Republic of South Africa was assembled and assayed for isozyme polymorphisms, aggressiveness, and cultural variability. A low level of polymorphisms was detected with isozymes in the collection of isolates. Isozyme polymorphisms were detected for α-esterase, hexose kinase and malate dehydrogenase in two isolates for each enzyme out of 47 isolates assayed. Fungi that have few or no isozyme polymorphisms often have host specialization such as biotrophs or fungi with formae speciales designations. Isolate aggressiveness was measured by the relative amount of a maize ear that was colonized and the amount of seedling blight and crown rot in two different greenhouse assays. The first measured the amount of ear colonization ability where only one isolate out of 24 tested had limited growth within plant tissues of

maize inbred line, B73. The second assay consisted of two separate greenhouse experiments which measured the ability of 25 isolates to cause diplodia seedling blight, crown rot or root rot . There were significant differences among isolates for total weight and top weight as a result of planting maize inbred line seed in infested soil. Cultural characteristics were measured for optimum temperature, colony color, and pycnidiospore production. All of the isolates had an optimum temperature of 28-31 C as measured from colony growth on acidified potato dextrose agar plates at four separate temperatures. Colony color and pycnidiospore production proved to be variable over the course of several experiments. These results indicate that *S. maydis* is variable in culture and these phenotypes would not make good genetic markers.

INTRODUCTION

Plant pathogenic fungi are examined for genetic variation for a variety of purposes. Population geneticists may use plant pathogenic fungi as a model to predict genetic change in fungal populations (McDermott et al, 1989; McDonald and McDermott, 1993), classification of fungi in fungal systematics (Kohn, 1992; Micales et al., 1986) and genetic structure of pathogen population can assist plant pathologists in disease management decisions (Vogler et al., 1991). Very little is known about the population genetics of, *Stenocarpella maydis* (Berk.) Sutton [= *Diplodia maydis* (Berk.)

Sacc.], the fungus that causes diplodia ear rot (DER) of maize (*Zea mays* L.). This fungus was the predominant ear rot pathogen in the early part of this century however, Hooker and White (1976) reported a decline in prevalence. Latterell and Rossi (1983) reported an increase in occurrence of this pathogen and in the past three years significant losses have been reported from DER in the U.S. (Hanson, M.A., 1992, VPI & SU Department of Plant Pathology, Physiology and Weed Science, Plant Clinic Record 2102; Munkvold and Yang, 1995, D.H. Scott, 1995, personal communication, Department. of Botany and Plant Pathology, Purdue University, Vincelli, 1992, 1994). The increase in incidences of DER may result from a decline in host resistance, changes in cultural practices resulting in an increase in inoculum (Latterell and Rossi, 1983), or changes in the population genetics of the fungus.

There are several previous reports of variability in *S. maydis.* Hoppe (1936) discovered aversions and inhibition of growth at the edge of cultures among isolates on culture media and in host tissue. Young et al. (1959) examined isolates from several states on a set of F_1 entries for maize stalk rot, and found differences in pathogenicity, and temperature growth requirements. Kappelman et al. (1965) reported significant differences among isolates for stalk rot severity. Ullstrup (1949) found no difference in "virulence" among five different isolates on crosses as measured by the incidence of ear rot from "widely separate geographic regions". Maxwell and Thompson (1974) reported significant differences in stalk rot severity among combinations of maize lines and testers challenged with "highly or weakly virulent" isolates; measured by the relative amount of

stalk rot. Several researchers have adapted their studies to account for some of these differences by using several isolates with varying degrees of "aggressiveness" (measured by the relative amounts of stalk rot or ear rot) or production of high numbers of pycnidiospores (Chambers, 1988; Loesch et al., 1962; and Villena, 1969).

Genetic variation among isolates of a fungus have been examined through a variety of techniques: histochemistry, ultrastructural features, anamorph connections, anamorph morphology, vegetative compatibility, isozyme analysis, DNA restriction fragment length polymorphisms with specific probes, polymerase chain reaction (PCR) length polymorphisms, restriction analysis, direct sequencing, and random amplified polymorphic DNA (see reviews by Kohn, 1992; McDonald and McDermott, 1993; Meijer et al., 1994; Micales et al., 1986). Isozyme analysis has proven to be a useful tool at the species level for several plant pathogenic fungi. Because variation in *S. maydis* has been reported, we chose to analyze a set of isolates from a broad geographic region of the U.S. and the Republic of South Africa for isozyme polymorphisms. Isozyme analyses was conducted on intracellular enzymes. Production of cellulolytic and pectolytic enzymes of this fungus have been reported previously (BeMiller et al., 1969), therefore the isozyme profiles of these two enzymes were also investigated.

The purpose of this study was to more completely characterize a set of *S. maydis* isolates for isozyme phenotypes, aggressiveness (measure of the amount of a maize ear that is colonized and decrease in seedling weight due to root and crown infection and colonization), cultural characteristics, and electrophoretic profile of total soluble proteins.

Additionally, a discussion of these results contribution to DER disease management decisions is presented.

MATERIALS AND METHODS

Fungal strains. Forty-seven single-spore isolates of *S. maydis* were started from diseased seeds collected from maize seed companies and plant disease clinics in the U.S. and Republic of South Africa (Table 1). Kernels from symptomatic DER maize ears were placed in 10% sodium hypochlorite solution for 1 min, rinsed twice in sterile distilled water and placed on acidified potato dextrose agar (APDA). Single pycnidiospores were placed on APDA with twelve autoclaved maize kernels (kernels placed in glass Petri dish, autoclaved at 121 C for 1 hr on two consecutive day). After two to three wk colonized kernels were shaken in tubes of sterile distilled water and the resulting spore suspension brought to 10 % glycerol (v/v) solution, and stored at -80 C until used. Colonized kernels were also stored in sterile glass tubes at 4 C. A few isolates were grown on sterile washed toothpicks (21 day), lyophilized, and stored at room temperature (Young, 1943). *Diplodia nataliensis* was used as an outgroup for isozyme analysis.

Sample preparation. To obtain mycelium extracts for intracellular enzymes, two 5mm colonized plugs of mycelium from 6-7 day culture were transferred to 75 ml of potato-dextrose broth in 125 ml Erlenmeyer flasks and incubated at 25 C for 5-6 day under 24 h light (24W cool white, General Electric, Cleveland, OH). Mycelial mats were collected

Table 1. Origin of *Stenocarpella maydis* isolates examined for phenotypes

Lab key[a]	Isolate number[b]	Location[c]	Source	Date
CB10	3869	AL	M. Miles, Ciba Seeds	1984
90-135-8	90-135	IL	J.G. Kinsey, Dekalb Plant Genetics	1990
87-109-1	87-109	IL	J.G. Kinsey, Dekalb Plant Genetics	1987
74-189	74-189	IL	J.G. Kinsey, Dekalb Plant Genetics	1974
74-206	74-206	IL	J.G. Kinsey, Dekalb Plant Genetics	1974
CB6	5318	IL	M. Miles, Ciba Seeds	....
CB12	6725	IL	M. Miles, Ciba Seeds	1992
CB13	6844	IL	M. Miles, Ciba Seeds	1993
CB18	6698	IL	M. Miles, Ciba Seeds	1992
CB19	6846	IL	M. Miles, Ciba Seeds	1993
CB21	5629	IL	M. Miles, Ciba Seeds	1992
J408	J400	IN	H.L. Warren, VPI & SU	1982
93-148-3	93-148	IN	J.G. Kinsey, Dekalb Plant Genetics	1993
74-193	74-193	IN	J.G. Kinsey, Dekalb Plant Genetics	1974
CB1	6773	IN	M. Miles, Ciba Seeds	1992
CB15	4552	IN	M. Miles, Ciba Seeds	1986
74-191	74-191	IO	J.G. Kinsey, Dekalb Plant Genetics	1974
A102	A100	KY	Vincelli, P., Univ. of Kentucky	1992
KY94-1	KY94-1	KY	Vincelli, P., Univ. of Kentucky	1994
74181-2	74-181	KY	J.G. Kinsey, Dekalb Plant Genetics	1974
CB2	5108	KY	M. Miles, Ciba Seeds	1987
74-194-1	74-194	MN	J.G. Kinsey, Dekalb Plant Genetics	1974
87-145	87-145	MO	J.G. Kinsey, Dekalb Plant Genetics	1987
87-124	87-124	MO	J.G. Kinsey, Dekalb Plant Genetics	1987
85-91	85-91	MO	J.G. Kinsey, Dekalb Plant Genetics	1985
86-137	86-137	MO	J.G. Kinsey, Dekalb Plant Genetics	1986
CB20	4796	MO	M. Miles, Ciba Seeds	1986
91-1-1	91-1	OH	J.G. Kinsey, Dekalb Plant Genetics	1991
87-153	87-153	OH	J.G. Kinsey, Dekalb Plant Genetics	1987
81-87-1	81-87	PA	J.G. Kinsey, Dekalb Plant Genetics	1981

Table 1. Continued

Lab Key[a]	Isolate Number[b]	Location[c]	Source	Date
85-2601	85-2601	PA	J.G. Kinsey, Dekalb Plant Genetics	1985
CB8	3419	PA	M. Miles, Ciba Seeds	1986
CB9	4554	PA	M. Miles, Ciba Seeds	1986
M111	M100	TN	H.L. Warren, VPI & SU	1985
84-84	84-84	TN	J.G. Kinsey, Dekalb Plant Genetics	1984
86-3326	86-3326	TN	J.G. Kinsey, Dekalb Plant Genetics	1986
CB3/CB16	5439	TN	M. Miles, Ciba Seeds	1989
A205	A200	VA	Henry Co., VPI & SU Plant Disease Clinic	1992
J211	J200	VA	H.L. Warren, VPI & SU	1991
J312	J300	VA	H.L. Warren, VPI & SU	1991
J602	J600	VA	Patrick Co., VPI & SU Plant Disease Clinic	1992
CB7	6845	....	M. Miles, Ciba Seeds	1973
Ced-4	....	Cedaro	B.C. Flett, Grain Crop Inst., Rep. S. Africa	1993
SA-2	....	Viljoenskroon	B.C. Flett, Grain Crop Inst., Rep. S. Africa	1993
SA-4	....	Ermelo	B.C. Flett, Grain Crop Inst., Rep. S. Africa	1993
SA-5	....	Winterton	B.C. Flett, Grain Crop Inst., Rep. S. Africa	1991

[a] Lab key designates those numbers used in collection of A.E. Dorrance.

[b] Isolate numbers are those used by original collector, . . . designates that there was no number associated with isolate.

[c] Locations are abbreviations for states in the U.S., . . . designates that no information on where the isolate was collected was received.

onto filter paper with vacuum filtration, rinsed with sterile distilled water, frozen with liquid nitrogen and ground to a fine powder using a mortar and pestle. Extraction buffer (approximately 2 ml of 0.05M Tris pH 7.5, 5% glycerol) was added to the powdered mycelium, and samples were centrifuged twice (Compac10F/Compac 10V centrifuge, Fisher Scientific, Pittsburgh, Penn) at 5500 x g for 10 min at 4 C. The supernatant was frozen at -80 C until used.

Electrophoresis. Intracellular enzymes were subjected to vertical polyacrylamide gel electrophoresis (PAGE). Native polyacrylamide electrophoresis was conducted using a BioRad Mini-PROTEAN II Apparatus (BioRad Laboratories, Hercules, Ca). The procedures, were followed as outlined in the BioRad Mini Protean II manual (Anonymous). All intracellular enzymes were run on 7.5 % acylamide except malate dehydrogenase and α-esterase which were separated on 10.0 % acrylamide. The gel buffer was 0.375 M Tris-HCl, pH 8.8; running buffer was Tris-Glycine (pH 8.3) and the stacking gel was 4.0 % acrylamide, 0.125 M Tris-HCl, pH 6.8. Approximately 10-15µl of protein extract was placed in wells with sample buffer (0.5 M Tris-HCl pH 6.8, 1 % glycerol and 0.05 % bromophenol blue). Electrophoresis was conducted for 45 min at 200V or until the dye front reached the bottom of the gel. One standard isolate was used in each electrophoretic assay. Table 2 lists the isozymes analyzed for polymorphisms and enzyme staining protocols. Electrophoretic mobility of the various enzymes was represented as R_f values, described by Bosland and Williams (1987), allowing the

Table 2. Enzymes examined for polymorphisms in a collection of *Stenocarpella maydis* isolates from the United States and Republic of South Africa

ENZYME	Abbrev	EC NO.	Reference
Aspartate aminotransferase	AAT	2.6.1.1	Wendel and Weeden (1989)
α Esterase (EST)	EST	3.1.1.2	Brewbaker, et al.(1968)
β-glucosidase	GLU	3.2.1.21	Wendel and Weeden (1989)
Glutamate dehydrogenase	GDH	1.4.1.4	Micales et al. (1986)
Hexokinase	HEX	2.7.1.1	Wendel and Weeden (1989)
Malate dehydrogenase	MDH	1.1.1.37	Wendel and Weeden (1989)
Phosphoglucomutase	PGM	5.4.2.5	Micales et al. (1986)
Superoxide dismutase	SOD	1.15.1.1	Wendel and Weeden (1989)
Xanthine dehydrogenase	XDH	1.1.1.204	Vallejos (1983)
Pectate lyase	PL	4.2.2.2	Reid and Collmer (1985)
Cellulase	C_x	3.2.1.4	Andro (1984)

bromophenol blue boundry front to migrate to the bottom of the gel from the origin then measuring the band distance from the origin and dividing by the length of the gel.

Forty *S. maydis* isolates were examined for pectolytic enzyme production. Isolates of *S. maydis* were grown on 25 ml liquid medium previously reported by Morant et al. (1993). Carbon source substitution was made with 5% acid washed polygalacturonic acid (PGA). Two 5 mm colonized plugs from 5-6 day cultures were placed in 100ml bottles, placed in a modified plastic recycling bin, under 12 h light (23 mm below light source, GE Rapid Start FC8T9-WW warm white, General Electric, Cleveland, Ohio) at room temperature (25-27 C). Following 10 to 14 day, 2 ml of culture liquid was placed in a 10 MW concentrator (Centricon®, Amicon, Inc., Beverly, MA). These were placed in a centrifuge for 90 min at 4000 x g. The concentrated protein was then immediately used for isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE).

Ultrathin acrylamide was cast on acrylamide support film (FMC, Rockland, ME). The acrylamide gels were composed of 16.6 % acrylamide, 5.0 % glycerol and ampholytes were 2 % 3-10 pH (Sigma, St. Louis, MO) and 4.3 % 3-5 pH (Serva Feinbiochemica GmbH & Co., Heidelberg, Germany). The gels were prefocused at 100V constant voltage for 20 to 30 min prior to sample application. Anode buffer used was Anode fluid 3 (Bucher Instruments, Inc., Saddlebrook, NJ) and the cathode buffer 0.1M NaOH. The samples (9µl) were focused at a constant power of 15W for 40 to 45 min on flatbed (Hoefer Scientific Instruments, CA). Marker proteins were from Serva Test Mix 9 (Serva Feinbiochemica GmbH &Co., Heidelberg, Germany). Pectate lyase isozymes were

detected following adaptations of the methods of Reid and Collmer (1985). Ultrathin overlays were cast on agarose gel support film (FMC, Rockland, ME). The PGA agarose overlays contain, 0.5 % acid washed PGA, 50mM EDTA, 0.1M potassium acetate (pH 5.5) and 1 % agarose. Following electrophoresis, overlays were incubated at 28 C for 2 to 3 h. Isozymes were detected by staining agarose overlays for 20 min with 0.05 % ruthenium red. Overlays were preserved by air drying.

Cellulase isozymes were assayed in 33 of the isolates using IEF-PAGE. Methods were the same as those used for pectate lyase enzyme detection with the following exceptions; isolates were grown on medium reported by Morant et al. (1993) but with 10 % carboxymethylcellulose (CMC) for the carbon source. Extracellular cellulase was focused on acrylamide gels (described above) containing; 2 % 3-10 pH and 4.3 % 4-6 pH (Bio-lyte, Bio-Rad Laboratories, Richmond, CA). The cathode buffer was cathode fluid 7 (Ephortec, Haake Bucher Instruments, Saddlebrook, NJ). Cellulase activity was detected following adaptations of the methods of Andro et al.(1984). CMC agarose overlays contained 0.5 % CMC, and 1 % Noble agar. Overlays were prepared with two different buffers, potassium acetate (pH 5.5) and Tris (pH 8.3). A separate IEF-PAGE was conducted for each overlay. Overlays were incubated for 3 h for pH 5.5 and 2 h for pH 8.3. Cellulase activity was detected with freshly prepared 0.1% Congo red in pH 5.5 potassium acetate for pH 5.5 overlays and distilled water for pH 8.3 for 20 min, followed by rinsing with 1M $NaCl_2$ (Andro et al., 1984).

Data analysis. Simple matching coefficients (SMC) were calculated as described by Nygaard et al. (1989). Isolates were placed into four groups based on their zymograms for intracellular enzymes. Representative isolates were then analyzed with the following formula:

$$SMC = (a + b)/n,$$

where a = the number of pairs of isozyme bands common to a pair of isolates, b = the number of pairs of isozme bands absent in both of these isolates but present in at least one isolate of the set and n = number of different isozyme bands detected in the set of isolates.

Protein electrophoresis. Thawed protein extract was mixed with SDS reducing buffer [20 % glycerol (vol/vol), 10 % SDS (wt/vol), 5 % 2-6-mercaptoethanol (vol/vol), and 0.05 % (wt/vol) bromophenol blue] and boiled for 3 min. Electrophoresis was conducted as described by Laemmili (1970) with 7.5 and 12 % acrylamide/bis in separating gel. Gels were focused for 4 h or until dye front reached the bottom. Gels were stained in 0.1 % Coomassie blue R-250 in 40 % methanol and 10% glacial acetic acid overnight and destained with 40 % methanol, 10 % glacial acetic acid. Sigma molecular weight markers of 14,000-70,000 were used in 12 % gels (MW SDS-70L, Sigma, St. Louis, MO).

Pycnidiospore production. Thirty-two isolates were analysed for pycnidiospore procuction in eight separate experiments. A 5 mm diameter plug of agar from a 5-6 day culture of *S. maydis* was placed at the center of an APDA plate with twelve autoclaved

maize kernels (described above). Three replicates were made for each isolate and plates were placed in an incubator 24cm from the light source, under 24 h light (24W GE Soft Light, General Electric, Cleveland, Ohio) at 25 C. After 3 wk, colonized kernels from one plate were placed in a blender with 100ml water and ground for 60 sec to dislodge pycnidiospores. The suspension was filtered through four layers of cheesecloth and the filtrate volume brought to 200 or 400 ml dependent on spore concentration. Two pycnidiospore counts were made from each flask for the total number of spores per plate with a hemocytometer for each flask. Each pycnidiospore determination was made with a standard isolate strain, J211. An ANOVA was conducted on the spore counts for the standard strain from each test for significance ($P \leq 0.05$) using the general linear model (GLM) procedure of SAS (SAS Institute, 1989, Cary, NC).

Mycelium color. Pycnidiospores of 41 *S. maydis* isolates were streaked on to separate APDA plates and placed in an incubator at 25 C for 12 day. A color guide was used to determine colors (Kornerup and Wanscher, 1966) This study was conducted three times with each isolate compared one to three times. A second study with 17 isolates was conducted in a growth chamber under full spectrum lights (15 amp F96T12/CWX/HO and bank of 10 F48T12/CW/VHO, Philips Lighting, Bloomhead, N.J. and 12, 25W incandescent bulbs for balanced illumination General Electric, Cleveland, Ohio) placed 113 cm below light source at 25 C to compare light effects on mycelial color development.

Optimum growth temperature. A *S. maydis* colonized 5 mm plug of agar from a 5-7 day culture was placed at the edge of an APDA plate. Three replicates for each isolate and temperature were made. Plates were placed in incubators under 24 h light (GE 24W cool white, General Electric, Cleveland, Ohio) at 18, 23, 28, and 33 C. Measurements of growth were taken daily with a mm ruler for four day from the edge of the plug to the edge of the mycelium. Optimum temperature was determined from the growth curves.

Aggressiveness assay on seedlings. As a measure of aggressiveness the incidence of seedling blight, and amount of root or crown rot caused by infesting soil with *S. maydis* was determined on three maize inbred lines in two greenhouse experiments. The inoculum was produced on nontreated millet seed (250 ml) that had been placed in a Mason jar with approx 400 ml distilled water overnight. Water was drained off and the jars covered with foil and autoclaved for 1 h on two consecutive days. Two *S. maydis* colonized 5 mm plugs of agar from a 5-7 day culture, were added to each jar and placed on a bench top at room temperature (25-27 C) for 3 wk for the first set of isolates and 6 wk for the second set. The colonized millet was then thoroughly mixed with a steamed soil mixture consisting of one part Wheeling silt loam and two parts sand and slow release fertilizer 14N-14P-14K (Osmocote®, Grace Sierra Horticultural Products Co., Milpitas, CA) was added at the rate of 250 ml per 15 L soil mix. The soil and colonized millet mix was placed in nine, 15-cm pots and planted with 10 seeds per pot of maize inbred lines, B37, H99, or Va26. Treatments were arranged in a randomized complete block design

arranged by three replicates and inbreds. After 6 wk, the plants were removed from pots, and the soil was gently washed off the roots, and stand counts, total fresh weight, root weight, and top weight were taken. Roots and tops of plants were placed in a drying oven at 80 C. After drying, dry top and root weight were taken. ANOVA was conducted on the tests as the whole plot, inbreds as split plot, and *S. maydis* isolates as the treatements; using the mixed model analysis procedure for fixed and random effects (Proc MIXED) of SAS version 6.0 (SAS Institute, Cary, NC).

Aggressiveness assay on maize ears. As a measure of aggressiveness the ability of 24 *S. maydis* isolates to colonize maize ears was measured. Maize inbred B73 seed was planted in the soil mixture (descibed above) in 10 cm pots (one seed/pot). Seedlings were transplanted to 30 cm pots (4 L soil) at the three leaf stage (one seedling/pot). Approximately 5 ml of fertilizer was applied every 3 wk. Inoculum was produced on toothpicks as described by Young (1943). Wooden applicators (Fisher Scientific, Pittsburgh, PA), approximately 5 mm in length, were boiled in distilled water for 1 h to eliminate any phenolics that might inhibit fungal growth. Wooden applicators were placed in vials, autoclaved for 1 h, then soaked overnight in sterile potato dextrose broth. Excess broth was decanted and vials with wooden applicators autoclaved for 1 h. Small 2 mm blocks of colonized agar were placed on the wooden applicators and incubated at room temperature (25-27 C) under 24 h light (24W soft light, General Electric, Cleveland, Ohio). The plants in the greenhouse were tagged when the silks emerged. Five day

following silk emergence, a colonized toothpick was placed in the middle of the ear by first making a hole with a dissecting probe then with forceps placing the wooden applicator in the ear until it was covered by the outside husk. Each isolate was inoculated into ears of three separate plants. The amount of the ear colonized by *S. maydis* was recorded.

RESULTS

Isozymes. There was a considerable amount of isozyme uniformity in the 47 *S. maydis* isolates assayed for enzyme polymorphisms. For intracellular isozyme analysis a total of 23 enzymes were assayed for staining activity, however only eleven showed sufficient reliable activity to be scored. The U.S. and South African isolates were monomorphic or produced the same isozyme pattern for ten of the eleven enzymes assayed. Two U.S. isolates were polymorphic for one of four bands of α-esterase. One U.S. isolate and one South African isolate were polymorphic for hexose kinase and malate dehydrogenase and one South African was polymorphic for hexose kinase. Figures 1, 2, and 3 illustrate the isozyme phenotypes for *S. maydis*. A single allele was detected in the enzyme systems, aminoaspartate transferase (AAT), β-glucosidase (GLU), glutamate dehydrogenase (GDH), hexose kinase (HEX), phosphoglucomutase (PGM), and xanthine dehydrogenase (XDH). Multiple bands were produced by α-esterase (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD), and pectate lyase. Cellulase produces multiple isoforms on CMC agarose overlays of pH5.5. The cellulase isoforms could not be

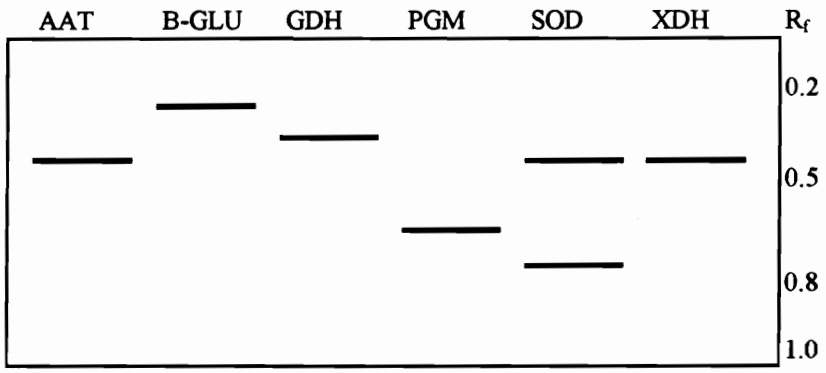

Figure 1. Isozyme banding patterns from intracellular protein extracts of *Stenocarpella maydis* determined on 7.5% vertical polyacrylamide gels. Protein extracts are from mycelium from 5 to 7 day old cultures grown on liquid potato dextrose broth. AAT=aminoaspartate transferase, β-GLU= β-glucosidase, GDH=glutamate dehydrogenase, PGM = phosphoglucomutase, SOD=superoxide dismutase and XDH=xanthine dehydrogenase. Staining protocol were conducted according to Wendel and Weeden (1989). Rf values are relative mobility from the enzyme front.

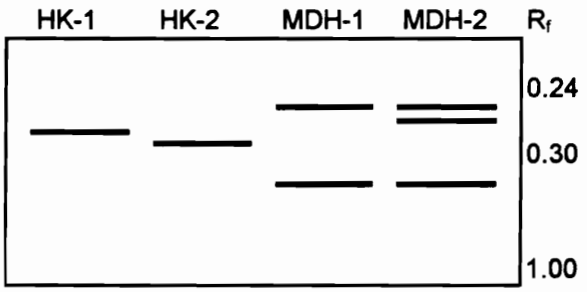

Figure 2. Polymorphic iszoyme banding patterns for two isozymes hexokinase (HK-1, HK-2) and malate dehydrogenase (MDH-1, MDH-2), from protein extracts of *Stenocarpella maydis* patterns were determined by electrophoresis on 7.5% acrylamide for HK and 10% acrylamide for MDH. HK-2 was recorded for three isolates and MDH-2 for two isolates out of the 47 isolates assayed. R_f values are relative mobility from the enzyme front.

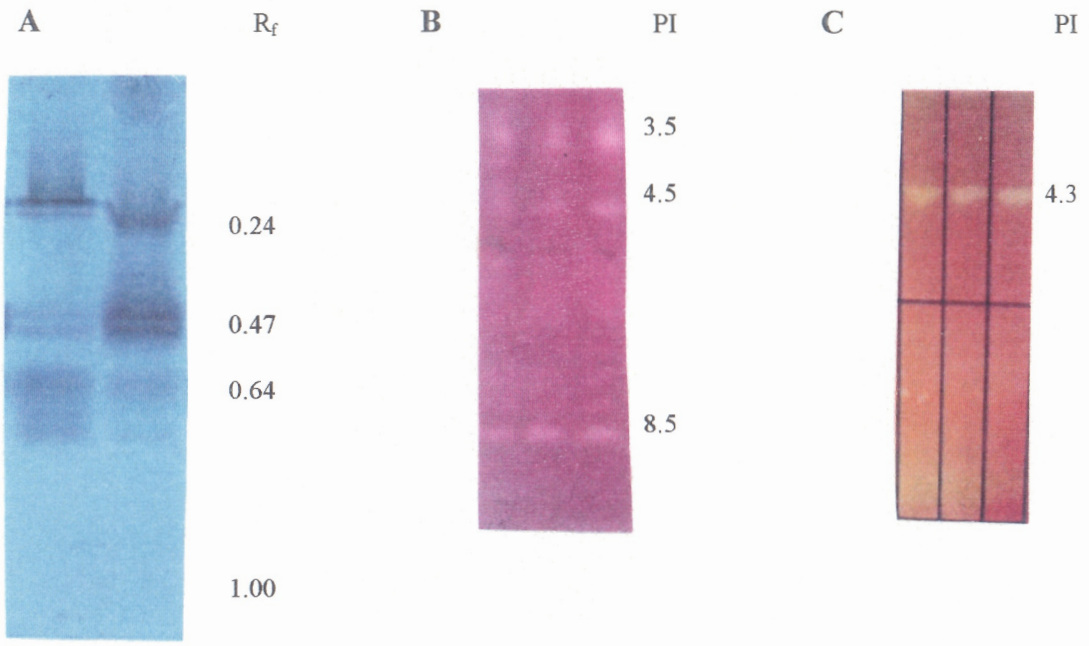

Figure 3. *Stenocarpella maydis* enzyme banding patterns for (A) Esterase from mycelial extracts in 10% polyacrylamide gels. Staining protocol according to Brewbaker et al. (1968). (B) Pectate lyase- isoelectric focusing gel (IEF) Agarose overlay, pH 5.5. Protein is from concentrated extracellular culture fluid where polygalacturonic acid served as the carbon source. (C) Cellulase IEF-Agarose overlay, pH 8.3. Protein is from concentrated extracellular culture where carboxymethlycellulose served as carbon source. Rf is mobility relative to enzyme front and PI markers are Protein test mix 9, Serva, Feinbiochemica.

differentiated clearly, therefore, only the pH 8.3 single band is reported here. Isozymes that formed faint bands or those that were inconsistent between analyses were not counted, similar to what other studies have reported (Clark et al., 1989, Zambino and Harrington, 1992). *D. nataliensis* had the same banding pattern for two of the enzymes, glutamate dehydrogenase and phosphoglucomutase. SMC are outlined in Table 3, even with the few differences reported, the values ranged from 0.91 to 0.97.

Protein electrophoresis. Interpretation of total denatured proteins was to difficult due to the large number of bands. Latorre et al., (1995) reported similar findings in comparisons of *Phytophthora cryptogea*. They reported a better analysis of nondenatured proteins.

Pycnidiospore production. There was a significant difference between the different tests for spore counts of the standard isolate *S. maydis* strain J211. The pycnidiospore production ranged from 4.63×10^7 to 1.37×10^8. There was considerable variation for isolates within tests. In some cases a plate would have no pycnidia while another plate had numerous develop for the same isolate in the same experiment. Fungi Imperfecti, such as *S. maydis*, can lose the ability to produce conidia as a result of repeated fungal transfers (Tuite, 1969). Because this group of fungi, including *S. maydis*, can lose this trait and these were from mycelial transfers, plates which did not produce pycnidia were not included in the final means.

Table 3. Simple matching coefficients (SMC) for 46 isolates of *Stenocarpella maydis* using ten enzymes

Group	I[a]	II	III	IV
I	. . .	34/35[b]	33/35	34/35
II	0.97[c]	. . .	32/35	33/35
III	0.94	0.91	. . .	34/35
IV	0.97	0.94	0.97	. . .

[a] Isozyme banding groups I = Most common isozyme group (40 isolates), II = α-esterase (two isolates), and III = hexokinase and malate dehyrogenase (two isolates) and IV = hexose kinase (one isolate)

[b] Above the diagonal: ratio of number of common pairs of isozyme bands present or absent to the number of different isozyme bands detected in this set of isolates

[c] Below the diagonal: simple matching coefficients

Mycelial color. The color of the mycelium changed for specific isolates between tests and the results were not repeatable. For example, strain 85-2601 was white to grayish red (p7B5) in test 2 but the results in test 3 were white to orange gray (p5B2). For a majority of the isolates, the color of *S. maydis* mycelium, was white with black pycnidia surrounded by areas of brownish orange (p7C4) or Sahara (6C5). Other mycelium color phenotypes recorded were; white to reddish gray (p7B2), grayish brown mycelium (6D3), brownish orange (p6C4), brownish red (8C4), and pinkish white (p7A2).

Optimum temperature. Growth curves were generated from the measurements of mycelial growth across a plate. The optimum temperature was estimated from these curves and the majority of isolates fell within the range 29 to 32 $\pm$ 2 C. One isolate, SA4-1 had an optimum of 27, but when retested it was 29 $\pm$ 2 C. The standard isolate was fairly consistent in its optimum temperature, 30-31 C, but in one assay its optimum was 32. This particular assay was started from an old culture.

Aggressiveness assay-seedlings. As a measure of aggressiveness symptoms of diplodia crown rot and diplodia seedling blight were evident as a result of soil infestation with *S. maydis* colonized millet seed. Table 4 summarizes the ANOVA results of the two assays for the different characters measured; stand count per pot, and average per plant of total fresh weight, top fresh weight, root fresh weight, top dry weight, and root dry weight. The analysis found no significant differences between tests and isolate within test by inbred

Table 4. Analysis of variance for greenhouse seedling assays[a] for development of seedling blight and crown rot caused by *Stenocarpella maydis* on maize inbred lines, B37, Va26 and H99. Effects measured include stand count, total fresh, top fresh, root fresh, root dry[b], and top dry weight

Variable	Source of variation	df	F	P
Stand count	Test	1	2.75	0.17
	Isolate (Test)	30	1.48	0.10
	Inbred line	2	7.21	0.00
	Test x inbred line	2	0.18	0.84
	Isolate x Inbred(Test)	60	1.27	0.13
Total fresh wt	Test	1	0.03	0.88
	Isolate (Test)	30	2.12	0.01
	Inbred line	2	7.15	0.00
	Test x inbred line	2	2.23	0.11
	Isolate x Inbred(Test)	60	0.70	0.94
Top fresh wt	Test	1	0.58	0.49
	Isolate (Test)	30	3.44	0.00
	Inbred line	2	6.09	0.00
	Test x inbred line	2	5.39	0.01
	Isolate x Inbred(Test)	60	0.82	0.81
Root fresh wt	Test	1	0.49	0.52
	Isolate (Test)	30	1.39	0.14
	Inbred line	2	12.50	0.00
	Test x inbred line	2	0.10	0.91
	Isolate x Inbred(Test)	60	0.65	0.97
Top dry wt	Test	1	0.01	0.94
	Isolate (Test)	30	1.71	0.04
	Inbred line	2	7.88	0.00
	Test x inbred line	2	0.83	0.44
	Isolate x Inbred(Test)	60	0.65	0.97
Root dry wt	Test	1	0.25	0.65
	Isolate (Test)	30	1.12	0.35
	Inbred line	2	3.10	0.05
	Test x inbred line	2	0.66	0.52
	Isolate x Inbred(Test)	60	0.72	0.92

[a] Greenhouse seedling assay consisted of infesting 5 L of soil with 250 ml of *S. maydis* colonized millet, separated into nine pots, each pot was planted with ten seeds of H99, Va26, or B37. After six wk stand counts were taken, plants were removed from pots and soil washed from roots.

[b] Plant tops and roots were separated and weighed, then placed in separate bags in a 80 C oven for 48 to 72 hr.

interaction was not significant. The means represented are combined for all three inbred lines. There were significant difference for the inbreds but because there was no isolate by inbred interaction, therefore, the combined inbred least squares means values are reported. There were no significant differences for stand count, root fresh weight, top dry weight, and root dry weight in the combined analysis. The standard error increased when comparisons were made between isolates and tests nullifying any significance, this is not unusual when comparing LS Mean values.

Aggressiveness assay-maize ears. Of the 24 *S. maydis* isolates screened for their ability to colonize ears only one isolate, 86-3326, failed to colonize the maize ear. Of the three plants that was inoculated with 86-3326, only one was less than 5% colonized, the other two ears were not colonized. The other 23 *S. maydis* isolates colonized ears 100%, many pycnidia had formed and the leaf sheath around the ear was dead. We did not have permission to use the South African isolates for greenhouse plant inoculations according to the limitations of the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine permit to import plant pests (No. 932847). Many plants aborted ear development in the greenhouse so there were not enough plants to assay all of the U.S. isolates.

DISCUSSION

Abstracts of this research were presented earlier; (Dorrance et al., 1995 and Dorrance et al., 1994). Our collection of *S. maydis* isolates from the U.S. and Republic of South Africa had minimal isozyme variation for the enzymes assayed. We fully expected to detect isozyme variation, based on previous reports of variability of this fungus (Chambers, 1988; Kappelman et al., 1965; Maxwell and Thompson, 1974; Villena, 1969; Young et al., 1959). However this fungus has only two reported hosts, maize and *Arundinaria* L. (Sutton, 1980). Several researchers have reported similar results with fungi that have a host specialization, as a formae speciales or biotroph where the fungus is an obligate parasite.

Studies which could not detect differences in isozyme patterns for formae speciales or obligate parasites include: *Ustilago hordei* (Hellman and Christ, 1991), *Erysiphe graminis* f.sp. *hordei* (Clark et al.1989), *Phytophthora megasperma* (Nygaard et al., 1989), *Fusarium oxysporum* (Bosland and Williams, 1987), and *Puccinia striiformis* (Newton et al., 1985). Wheat and barley isolates of *Pseudocercosporella herpotrichoides* could be separated by isozyme analysis (Julian and Lucas, 1990). In contrast, a great deal of isozyme variation for specific enzymes has been reported for *Rhynchosporium secalis* (Newman, 1985), and *Alternaria solani* and *A. alternata* (Petrunak and Christ, 1992).

Several hypotheses have been proposed for this lack of isozyme polymorphisms found in formae speciales and biotrophs. Hellmann and Christ (1991) proposed inbreeding, common origin and lack of necessity for enzyme variation as possible explantations for the few electrophoretic types in *U. hordei*. Burdon et al. (1983)

proposed that a founder effect may be responsible for the uniformity of isoymes detected among isolates of *P. graminis* f.sp. *tritici* and *P. recondita* f.sp. *tritici*. Hellmann and Christ (1991) also proposed that the isozymes may be subject to different selection pressures. The selection pressure on these enzymes may be so small that there is little advantage to be gained by enzyme variation.

In contrast others have suggested that it is the selection pressure placed on the pathogen by the specific host that results in minimal enzyme variability. Kurzeja and Garber (1973) reported little variation in amylase patterns for the haploid fungus, *Aspergillus nidulans*. They hypothesized that this is a result of immediate selection pressure placed on new mutation. Clark et al. (1989) suggest as a possible explanation that barley as a host may impose particularly stringent biochemical requirement on mildew and exert strong selection against any variation. Newton et al. (1985) hypothesized that highly specialized obligate parasites, with no sexual reproduction are likely to be under higher selection pressure and to exhibit less variation than necrotrophic fungi such as *R. secalis*. They hypothesized that obligate parasites seem to exhibit lower levels of isozyme variability because they encounter a more uniform substrate and environment than facultative parasites which often have broader host range and colonize different substrates in different environments.

From our study we can not determine if the lack of isozyme polymorphism is due to a lack of selection pressure or the result of a stringent biochemical requirement placed on the fungus by the host. What is significant about this finding is that it provides some

evidence of the possibility of races of *Stenocarpella maydis*, similar to other fungi which have uniform isozyme polymorphisms and a host specifity. Fungi which have host specificity often have races or gene-for-gene interactions with their hosts. Limited isozyme polymorphism may be due to the haploid state of this fungus so if there was a mutation, there would be an immediate selection pressure placed on it. A sexual stage has not been reported for this fungus, therefore recombination as a result of mating is not known. Due to the wide geographic regions from which the isolates were collected and the array of enzymes analyzed it is likely that we should have detected more variation. Therefore, this places this fungus in with other groups of obligate parasites which have specialized for their host. There does exist the possibility that these results are biased based on the enzymes that were tested, but many of these have shown variability with other pathogens. Further isozyme analysis with different enzyme stains and a collection of isolates from other geographic regions may eventually provide some level of variability. An analysis of these isolates on a set of maize differentials will provide evidence if there are races of this pathogen.

For a closer examination of genetic variability of *S. maydis*, vegetative compbatibility tests and RAPDs have provided information for several plant pathogens (McDonald and McDermott, 1993; Meijr, et al., 1994). Prior to this a thorough examination of the germplasm should be conducted to assess if there are gene-for-gene interactions. This will not be easy based on information that environmental influences may compound some of the phenotypes (Flett and McLaren, 1994) and it is unlikely that such

study could be carried out in the greenhouse. If races of *S. maydis* do exist this would compound management decisions for which hybrid to plant in any given area.

In the greenhouse evaluation of aggressiveness one strain failed to colonize maize ears. One of the three plants inoculated with this strain was colonized to a limited extent. These results confirm previous studies that there are differences in the ability of different strains to colonize host tissue. All of the isolates collected in this study were originally from diseased maize ears from seed companies or from locations where there was a high incidence of disease. These isolates were probably collected from ears that were completely dead. These factors may have biased our results to have a collection of highly aggressive forms of *S. maydis* for ability to colonize maize ears.

The three inbred lines examined in the greenhouse to determine aggressiveness on seedlings had similar effects from the treatment of soil with *S. maydis* colonized millet. This is evident from the ANOVA where there was no significance in the inbred by isolates within tests. This agrees with Hooker's (1956) report that different diseases caused by *S. maydis* are inherited independently. These three inbred lines have different interaction with the ear rot caused by this pathogen; H99 is susceptible, Va26 is intermediate, and B37 is resistant. Total fresh weight and top fresh weight were the only parameters measured that were significantly different as a result of root and crown infections and colonization of maize seedlings. The roots appeared brown and few in number, the seedlings appeared dry and pycnidia could be seen on those seedlings with crown rot. This assay took a total of nine to twelve weeks to complete and required a considerable

amount of manipulation. In each of the two assays the conditions in the greenhouse were different based on temperature and a final setup of this assay failed due to contamination. The controls had low stand counts most likely due to the contamination brought in by fungus gnats probably feeding on the uncolonized millet seed. The isolate that failed to colonize maize ears was included in the greenhouse assay that failed so a comparison of these two methods cannot be completed. A comparison is still necessary to determine if the ranking in the greenhouse evaluation would yield similar results in the field. There may be better, more applicable methods which take less time to measure specifically the aggressiveness or ability to infect and colonize maize by *S. maydis* isolates.

The cultural characteristics for *S. maydis* proved to be quite variable and unstable. There was a significant difference in the production of pycnidiospores by the standard isolate and the color phenotype could not be repeated consistently. Both of these traits are influenced by genetic and environmental cues. When pycnidiospores were transferred to two plates and placed under two different light systems there was some phenotype difference in nine out of 18 strains. It is unlikely that this would have been a mutation due to the number of pycnidiospores transferred. The same color phenotype could not be reproduced in successive runs of the experiment in the same incubator. The color ranges were white with numerous black pycnidia to white with areas of Sahara (p.5A2) to mostly grayish red. These two traits would not be good features on which to base a genetic study due to variability. This confirms previous reports that there is variability for pycnidiospore production; however, these studies suggest that it is not a stable phenotype.

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APPENDIX A

Field Evaluation of F_1 of Diallel Cross of nine maize inbred lines

In 1992, nine parents were crossed in all possible combinations, the parents listed in Chapter 2, Table 1 and an additional parent H84 (B37 X GE440)Ht

In 1993, a half diallel was planted at the Kentland Agricultural Research Farm at Blacksburg, VA in a Wheeling silt loam soil. The F_1's were the treatments and planted in a randomized complete block in three replicates with an Almaco two-row planter (Almaco, Johnson City, IA).

Hybrids were inoculated with approximately 5 ml of a spore suspension of 5,000 conidia/ml in the whorl at the V13-V14 growth stage (Ritchie and Hanway, 1992). Hybrids were self pollinated to ensure pollination. Evaluations for percent ear rot were taken at harvest.

This field had a hard pan under this test. In the early part of the growing season (V4-V5), heavy rains fell, what seed did germinate, many of the plants died as a result of standing water. All of the plants were combined across the whole plot to come come up with percent data (Table A1). The total number of plants was to few to conduct any type of genetic evaluation.

Table A1. Percent means of diplodia ear rot in a diallel evaluated at Kentland Agricultural Research Farm, Blacksburg, Va, 1993

Inbreds	H111	B68	VA26	MS	B73	H99	H84	MB271
B37	0.00	25.00	13.30	18.75	26.30	7.69	NS[a]	NS[a]
H111		18.18	NS[a]	26.67	0.00	16.00	NS[a]	NS[a]
B68			NS[a]	27.30	NS[a]	NS[a]	0.00	NS[a]
VA26				33.30	33.30	8.30	NS[a]	NS[a]
MS					NS[a]	NS[a]	NS[a]	8.70
B73						0.00	NS[a]	41.70
H99							18.80	NS[a]
H84								NS[a]
MB271								

[a] NS=No stand or not enough plants to calculate.

Table A2. Full diallel analysis for resistance to diplodia ear rot caused by *Stenocarpella maydis*, 1994

	Rep 1			Rep 2			Rep 3			Rep 4		
Cross	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent
B37XH111	0	10	0.0	0	13	0.0	0	12	0.0	0	11	0.0
B37XB68	0	10	0.0	0	15	0.0	0	8	0.0	0	13	0.0
B37XVA26	1	12	8.3	0	10	0.0	3	8	37.5	0	10	0.0
B37XMS	0	11	0.0	3	11	27.2	0	11	0.0	0	15	0.0
B37XB73	1	11	9.1	2	15	13.3	1	11	9.1	0	12	0.0
B37XMB	2	10	20.0	0	12	0.0	0	8	0.0	1	15	6.7
H111XB37	1	10	10.0	0	14	0.0	0	8	0.0	1	4	
H111XB68	0	8	0.0	0	11	0.0	0	10	0.0	0	14	0.0
H111XVA26	0	11	0.0	0	13	0.0	0	9	0.0	1	9	11.1
H111XMS	0	10	0.0	0	11	0.0	0	12	0.0	0	11	0.0
H111XB73	2	9	22.2	0	12	0.0	1	10	10.0	0	5	
H111XH99	0	10	0.0	0	8	0.0	0	12	0.0	1	13	7.7
H111XMB	1	11	9.1	1	14	7.1	1	9	11.1	0	14	0.0
B68XH111	0	10	0.0	0	13	0.0	0	7	0.0	0	14	0.0
B68XVA26	1	11	9.1	0	14	0.0	2	12	16.7	0	16	0.0
B68XMS	0	10	0.0	0	18	0.0	0	9	0.0	0	12	0.0
B68XB73	1	10	10.0	0	16	0.0	0	9	0.0	0	16	0.0
B68XH99	0	10	0.0	0	14	0.0	0	10	0.0	0	13	0.0
B68XMB	0	10	0.0	0	17	0.0	2	16	12.5	0	10	0.0
VA26XB37	1	10	10.0	0	15	0.0	1	16	6.3	0	13	0.0
VA26XH111	0	10	0.0	1	18	5.6	0	12	0.0	0	12	0.0
VA26XB68	0	9	0.0	0	11	0.0	0	12	0.0	0	9	0.0
VA26XMS	0	10	0.0	0	11	0.0	0	12	0.0	0	9	0.0
VA26XB73	0	10	0.0	2	12	16.7	1	10	10.0	2	13	15.4
VA26XMB	0	10	0.0	0	9	0.0	2	10	20.0	3	7	43.0
B73XB37	1	10	10.0	1	13	7.7	0	8	0.0	1	12	8.3
B73XB68	0	8	0.0	0	16	0.0	0	17	0.0	0	15	0.0
B73XVA26	3	10	30.0	3	15	20.0	2	12	16.7	2	10	20.0
B73XMS	0	10	0.0	0	9	0.0	0	10	0.0	2	13	15.4
B73XH99	0	10	0.0	0	11	0.0	0	9	0.0	0	14	0.0

Table A2. Continued.

Cross	Rep 1			Rep 2			Rep 3			Rep 4		
	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent
B73XMB	0	10	0.0	3	10	30.0	5	7	71.4	2	8	25.0
H99XB37	1	14	7.1	1	17	5.9	0	11	0.0	0	14	0.0
H99XH111	0	12	0.0	0	14	0.0	0	9	0.0	1	8	12.5
H99XB68	0	11	0.0	0	17	0.0	0	12	0.0	0	17	0.0
H99XVA26	0	15	0.0	0	16	0.0	1	8	12.5	1	16	6.3
H99XMS	0	8	0.0	0	15	0.0	0	7	0.0	0	10	0.0
H99XMB	2	13	15.3	0	14	0.0	0	15	0.0	0	9	0.0
MBXB37	0	12	0.0	0	18	0.0	0	12	0.0	0	11	0.0
MBXH111	0	10	0.0	0	14	0.0	0	10	0.0	0	15	0.0
MBXB68	0	10	0.0	0	12	0.0	0	9	0.0	0	9	0.0
MBXVA26	3	13	23.0	0	14	0.0	3	10	30.0	3	12	25.0
MBXMS	0	10	0.0	0	16	0.0	2	9	22.2	0	11	0.0
MBXH99	0	10	0.0	3	16	18.8	2	14	14.3	0	15	0.0

Table A3. Full diallel analysis for resistance to diplodia ear rot (DER) caused by *Stenocarpella maydis*, 1995 at Kentland Agricultural Research Farm for stand counts in plots and percentage of plants with DER

	REP 1			REP 2			REP 3		
Cross	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent
B37XH111	0	26	0.00	1	30	3.33	1	25	4.00
B37XB68	0	35	0.00	1	32	3.13	0	22	0.00
B37XVA26	0	34	0.00	1	26	3.85	0	29	0.00
B37XMS	0	21	0.00	0	9	0.00	0	34	0.00
B37XB73	1	34	2.94	0	17	0.00	0	33	0.00
B37XH99	2	24	8.33	3	13	23.08	7	35	20.00
B37XMB271	1	34	2.94	0	30	0.00	1	28	3.57
B37XH95	0	26	0.00	1	29	3.45	1	16	6.25
H111XB37	1	23	4.35	0	11	0.00	1	12	8.33
H111XB68	0	17	0.00	3	21	14.29	0	14	0.00
H111XVA26	1	30	3.33	0	24	0.00	3	20	15.00
H111XMS	0	23	0.00	0	28	0.00	0	11	0.00
H111XB73	3	31	9.68	1	23	4.35	1	20	5.00
H111XH99	3	24	12.50	3	27	11.11	1	21	4.76
H111XMB271	1	29	3.45	3	23	13.04	1	19	5.26
H111XH95	1	25	4.00	1	8	12.50	1	21	4.76
B68XB37	0	26	0.00	0	20	0.00	1	22	4.55
B68XH111	0	27	0.00	3	32	9.38	0	29	0.00
B68XVA26	0	27	0.00	0	20	0.00	1	31	3.23
B68XMS	0	8	0.00	0	25	0.00	0	21	0.00
B68XB73	0	19	0.00	0	28	0.00	2	32	6.25
B68XH99	0	17	0.00	0	14	0.00	0	12	0.00
B68XMB271	1	26	3.85	1	33	3.03	1	27	3.70
B68XH95	0	29	0.00	4	35	11.43	0	25	0.00
VA26XB37	1	19	5.26	0	23	0.00	0	32	0.00
VA26XH111	4	23	17.39	3	27	11.11	0	17	0.00
VA26XB68	0	24	0.00	3	33	9.09	0	12	0.00
VA26XMS	5	18	27.78	2	25	8.00	3	24	12.50
VA26XB73	3	16	18.75	3	23	13.04	0	33	0.00
VA26XH99	2	26	7.69	4	26	15.38	0	12	0.00
VA26XMB27	7	27	25.93	4	18	22.22	2	13	15.38
VA26XH95	0	25	0.00	0	12	0.00	1	21	4.76
MSXB37	0	35	0.00	0	40	0.00	0	8	0.00
MSXH111	0	23	0.00	1	27	3.70	2	31	6.45
MSXB68	6	34	17.65	0	32	0.00	0	20	0.00
MSXVA26	4	26	15.38	1	23	4.35	0	24	0.00
MSXB73	0	31	0.00	1	31	3.23	2	25	8.00
MSXH99	1	22	4.55	0	35	0.00	0	33	0.00
MSXMB271	1	13	7.69	0	24	0.00	0	10	0.00
MSXH95	missing								

Table A3. Continued.

	REP 1			REP 2			REP 3		
Cross	DER	Stand	Percent	DER	Stand	Percent	DER	Stand	Percent
B73XB37	0	29	0.00	0	25	0.00	0	34	0.00
B73XH111	2	28	7.14	0	29	0.00	0	12	0.00
B73XB68	0	31	0.00	0	31	0.00	0	16	0.00
B73XVA26	5	26	19.23	1	28	3.57	2	31	6.45
B73XMS	1	26	3.85	1	31	3.23	1	15	6.67
B73XH99	2	15	3.80	2	9	22.22	1	29	3.45
B73XMB271	8	22	36.36	14	26	53.85	12	20	60.00
B73XH95	1	27	3.70	0	14	0.00	1	24	4.17
H99XB37	3	22	13.64	4	19	21.05	2	15	13.33
H99XH111	0	13	0.00	1	23	4.35	6	12	50.00
H99XB68	0	10	0.00	0	10	0.00	1	12	8.33
H99XVA26	1	18	5.56	1	24	4.17	0	21	0.00
H99XMS	0	23	0.00	0	31	0.00	0	19	0.00
H99XB73	3	29	10.34	2	12	16.67	0	17	0.00
H99XMB271	2	28	7.14	3	39	7.69	2	33	6.06
H99XH95	0	27	0.00	1	28	3.57	0	22	0.00
MB271XB37	0	24	0.00	6	35	17.14	0	13	0.00
MB271XH111	0	29	0.00	1	22	4.55	0	8	0.00
MB271XB68	0	15	0.00	0	29	0.00	1	22	4.55
MB271XVA2	1	16	6.25	3	18	16.67	1	26	3.85
MB271XMS	4	24	16.67	2	17	11.76	1	30	3.33
MB271XB73	11	25	44.00	15	26	57.69	3	5	60.00
MB271XH99	1	18	5.56	3	21	14.29	10	43	23.26
MB271XH95	1	25	4.00	2	20	10.00	0	21	0.00
H95XB37	0	24	0.00	0	22	0.00	0	19	0.00
H95XH111	6	32	18.75	10	29	34.48	0	31	0.00
H95XB68	3	26	11.54	0	15	0.00	2	21	9.52
H95XVA26	0	25	0.00	2	31	6.45	0	24	0.00
H95XMS	0	27	0.00	1	30	3.33	0	26	0.00
H95XB73	3	29	10.34	0	30	0.00	1	26	3.85
H95XH99	5	27	18.52	2	25	8.00	0	25	0.00
H95XMB271	1	29	3.45	0	36	0.00	4	24	16.67

APPENDIX B

Table B1. Enzymes analysed for activity from protein extracts of *Stenocarpella maydis* from the United States and South Africa

ENZYME	EC NO.
Aconitase	4.2.1.3
Alcohol dehydrogenase	1.1.1.1
α-amylase	3.2.1.1
Aspartate aminotransferase	2.6.1.1
α-Esterase	3.1.1.2
β–galactosidase	3.2.1.23
β-glucosidase	3.2.1.21
Fumarase	4.2.1.2
Glucose-6-phosphate dehydrogenase	1.1.1.49
Glucose phosphate isomerase	5.3.1.9
Glutamate dehydrogenase	1.4.1.4
Hexose kinase	2.7.1.1
Isocitrate dehydrogenase	1.1.1.42
Lactate dehydrogenase	1.1.1.27
Malate dehydrogenase	1.1.1.37
Malic enzyme	1.1.1.4
NADH dehydrogenase	1.6.99.2
Phosphoglucomutase	5.4.2.5
6-phosphogluconate dehydrogenase	1.1.1.44
Succinate dehydrogenase	1.3.99.1
Superoxide dismutase	1.15.1.1
Xanthine dehydrogenase	1.1.1.204
Pectate lyase	4.2.2.2
α-Amylase	3.2.1.1
Cellulase	3.2.1.4

Table B2. Mean pycndiospore counts averaged from three plates of *Stenocarpella maydis*[a]

Isolate	Test[b]	Number pycnidiospores	Isolate	Test[b]	Number pycnidiospores
74-189-4	3	4.80	CB12-2	4	4.76
74-193-2	4	3.37	CB13-2	7	5.82
74-206-2	2	5.39	CB13-4	6	5.94
81-87-1	3	5.60	CB18-5	7	6.50
84-84	8	6.40	CB19-2	7	4.70
85-2601-3	5	4.73	CB21-1	6	2.69
85-91-4	4	3.46	CB21-3	5	7.62
86-137-2	3	9.14	J211	1	6.98
87-109-1	2	5.48	J211	2	8.05
87-153	8	11.30	J211	3	11.40
90-135-8	5	1.72	J211	4	8.52
91-1-7	3	5.70	J211	5	5.55
93-148-3	2	6.99	J211	6	8.97
A102	1	3.15	J211	7	4.63
A205	1	3.23	J211	8	13.60
A205	7	7.77	J312	3	0.70
CB 1-4	4	7.48	J408	1	4.00
CB 2-2	1	8.64	M111	4	1.90
CB3-2	6	5.70	SA2-3	8	11.30
CB7-2	8	10.30	SA4-3	8	6.50
CB8-3	6	4.15	SA5-4	2	5.17
CB9-4	5	7.92			

[a] Pycnidiospores were produced on APDA plates with twelve autoclaved maize kernels at 25 C, for three weeks. Kernels were placed in a blendor to remove conidia, total volume brought to 200 or 400 ml depending on concentration and counted with a hemocytometer.

[b] Strains were tested in groups of six or seven with J211 as the standard.

[c] Number of spores x 10^7.

Table B3. Summary of colony descriptions and color block[a] designations for single spore isolates of *Stenocarpella maydis*.

Isolate	Colony description[b]	Color	Test
74-181-2	Raised mycelial mat (RMM), white with pycnidia surrounded by areas of grayish red (25 pycnidia)	A1, 7B4	2
74-189-4	RMM, white with black pycnidia (400+)	A1	2
74-193-2	RMM+C48, white in color approx. 50 pycnidia, surrounded by areas of Sahara	A1, 6C5	2
74-194-2	RMM, white in color (100 pycnidia), pycnidia surrounded by areas of Sahara	A1, 6C5	2
74-206-2	RMM, color changes from reddish blonde to light brown, 2 pycnidia	5C3,5D6	2
81-87-1	Flocculant mycelium, light brown in color with white tufts, few pycnidia	6D3	3
81-87-1	RMM, white with areas of Sahara clumps of pycnidia (100)	A1, 7C5	2
84-135-8	RMM, numerous pycnidia, mycelium white to orange white	A1, 5A2	2
84-84-8	RMM, brownish orange in color	6C3	3
85-2601	RMM, some areas appressed, white to orange gray	A1, 5B2	3
85-2601-3	RMM, white to grayish red raised clumps of pycnidia (50)	A1, 7B5	2
85-91-4	RMM, white with areas of brownish orange, few pycnidia	7C4	3
85-91-4	RMM, white with pycnidia surrounded by areas of Sahara (200 pycnidia)	A1, 6C5	2
86-137-2	RMM, white to areas of grayish red	A1, 7B3	2
86-3326-2	RMM, white, pycnidia surrounded by areas of grayish red	7B3	3
86-3326-2	RMM, white with areas of Sahara, (40 pycnidia)	A1, 6C5	2
87-109-1	RMM white to orange gray	A1, 6B2	3
87-109-1	RMM, white with pycnidia surrounded by areas of Sahara	A1, 6C5	2

Table B3 continued.

Isolate	Colony description[b]	Color	Test
87-124-6	RMM, brownish orange in color	6C3	3
87-124-6	RMM, white with pycnidia surrounded by areas of Sahara (7 clumps)	A1, 6C5	2
87-153-2	RMM white, brownish orange areas surrounding pycnidia, numerous pycnidia	7C5	3
87-153-2	RMM, white with areas of brownish-orange (400 pycnidia)	A1, 7C5	2
90-135-8	RMM, numerous small pycnidia , mycelium white to orange white	A1, 5A2	2
91-1-7	RMM, white to areas of brownish orange, few pycnidia	7C3	3
91-1-7	RMM, white to brownish orange (200 pycnidia)	A1, 7C5	2
93-148-3	RMM, white, numerous submerged pycnidia	A1	2
A102	RMM, with areas of grayish brown pigment	6D3,51A	1
A103	RMM brown mycelium with brownish orange	6C3	3
A205	RMM, mostly white with areas of reddish gray surrounding pycnidia	9B2, 51A	1
CB 1-4	RMM, white with some cottony areas, numerous black pycnidia (400+)	5A1	1
CB 2-2	RMM, white with numerous pycnidia	A1	3
CB 2-2	RMM, white to pinkish white mycelium with few (15) pycnidia, edges of plate grayish white	7A2, 7B3	1
CB 3-2	RMM, brown mycelium some small raised cottony areas, no pycnidia formed	5(E)4	1
CB 3-2	RMM, white to grayish red surrounding pycnidia, raised clumps of pycnidia (10)	A1, 7B5	2
CB 3-2	RMM, white with pycnidia surrounded by areas of grayish red	8C5	3

Table B3 Continued.

Isolate	Colony description[b]	Color	Test
CB 6-1	RMM, brownish gray mycelium with white cottony areas, no pycnidia	6C2	1
CB 7-2	RMM, white to brownish-red mycelium (100 pycnidia)	A1,8C4	1
CB 7-2	RMM, white mycelium with pycnidia surrounded by areas of brownish orange (100 pycnidia)	A1	2
CB 7-2	RMM, white with areas of grayish red	7B3	3
CB 9-4	RMM, white mycelium, pycnidia surrounded by areas of brownish orange	7C4	1
CB 9-4	RMM, mycelium white to reddish gray, numerous small pycnidia	A1, 7B2	2
CB10-2	RMM, grayish brown mycelium with small areas of white cottony mycelium (4 pycnidia)	6D3	1
CB 12-2	RMM, pycnidia formed on center of plate surrounded by - grayish brown then rest of mycelium grayish orange	5D4,5B3	1
CB 13-2	RMM, brownish orange mycelium with areas of white to orange-gray cottony growth, no pycnidia	6C4,6B2	1
CB 13-2	RMM, white to large areas of orange-white, center is grayish-brown	A1, 5A2, 5E3	2
CB 15-3	RMM, orange gray mycelium with numerous (200) pycnidia	5B2	1
CB 16-3	RMM, white to gray pigment with pycnidia surrounded by areas of reddish gray (75 pycnidia)	8B2, 8C3	1
CB 19-2	RMM, white mycelium with pycnidia surrounded by areas of reddish gray (30 pycnidia)	8A1,8B2	1
CB 19-2	RMM, white to areas of brownish orange (200 pycnidia)	A1, 6C4	2
CB 19-2	RMM, white, pycnidia surrounded by areas of dull red	8C3	3

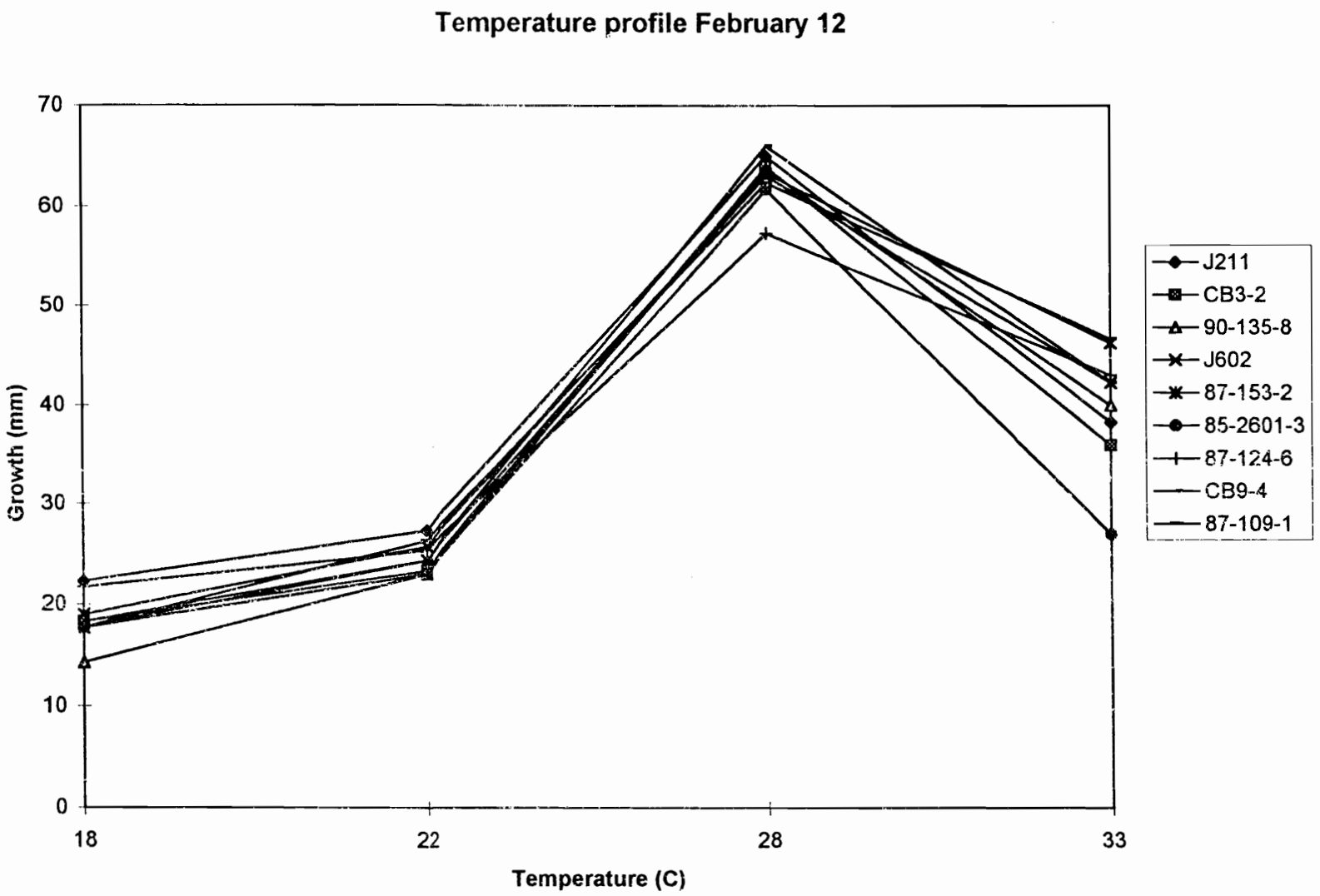
Table B3 Continued

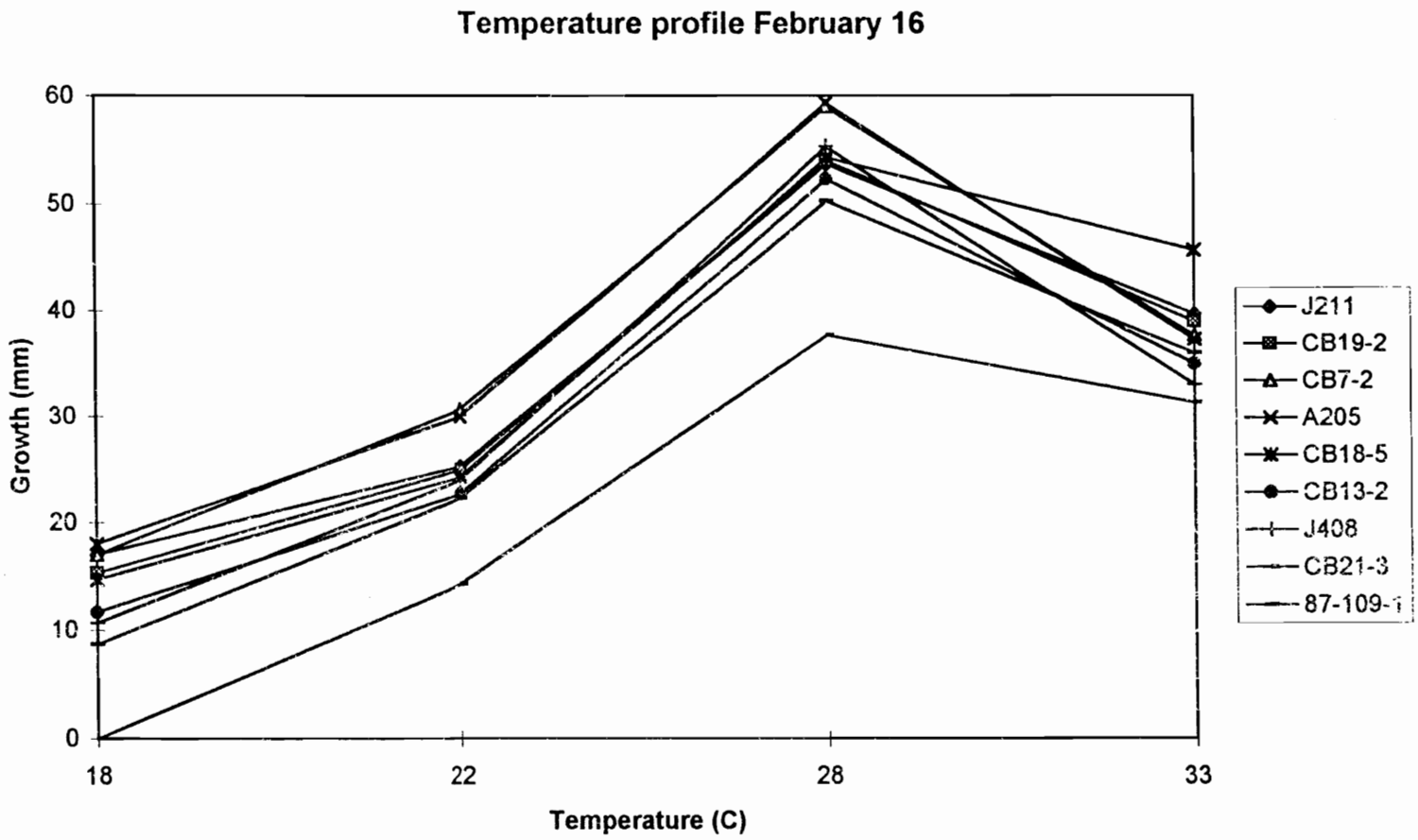
Isolate	Colony description[b]	Color	Test
CB 20-4	RMM, brownish orange-mycelium with areas of white, no pycnidia	5C4	1
CB 21-3	RMM, white to brownish orange mycelium	5A1,5C4	1
J211	RMM, numerous pycnidia (300+), white with brownish-gray white center	6C2	1
J211	RMM, orange gray in color, (100 pycnidia)	6B2	2
J312	RMM, brown mycelium with some small areas of white	5(E)4	1
J408	RMM, white center with brownish orange around edge (12 pycnidia)	5C4	1
J602	RMM, pycnidia formed on center of plate surrounded by - grayish brown then rest of mycelium grayish orange	5D4, 5E3	1
M111	RMM white, pycnidia surrounded by areas of grayish red	8C5	3
M111	RMM, no pycnidiospores, grayish brown mat	5(E)3	1
SA2-3	RMM, white with pycnidia surrounded by areas of brownish orange	5C4	3
SA2-3	RMM, white with small areas of brownish orange, clumps of pycnidia (100)	A1, 5C3	2
SA4-3	RMM, white with areas of brownish orange	6C3	3
SA4-3	RMM, white to brownish orange (200 pycnidia)	A1, 7C5	2
SA5-4	RMM, white, areas of brownish orange and pycnidia surrounded by areas of brownish orange	A1, 6C3	3
SA5-4	RMM white but mostly Sahara (100 pycnidia)	A1, 6C5	2

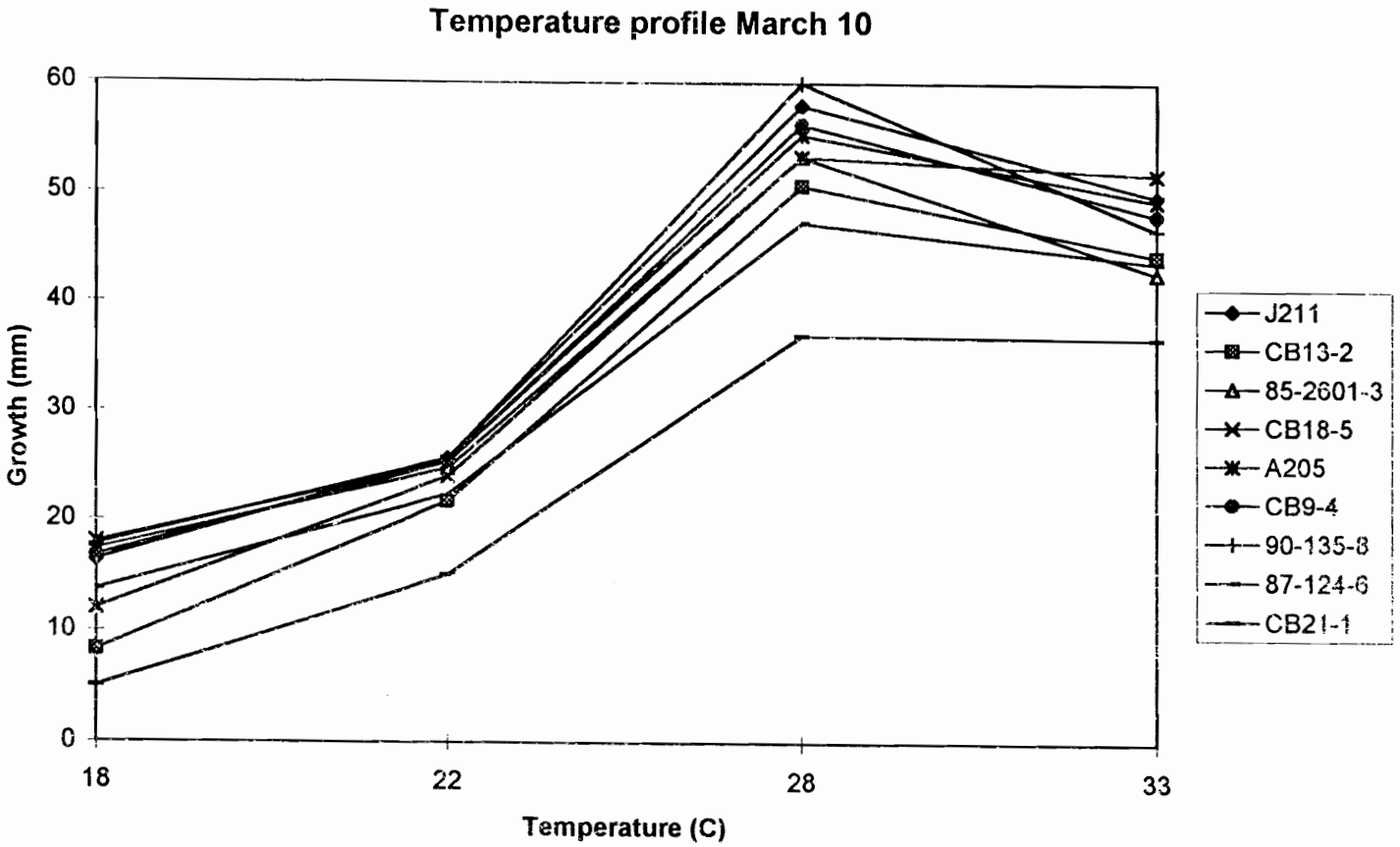
[a] color block from Kornerup and Wanscher (1966). First number is the page, followed by column and then row. A1=white in all pages.

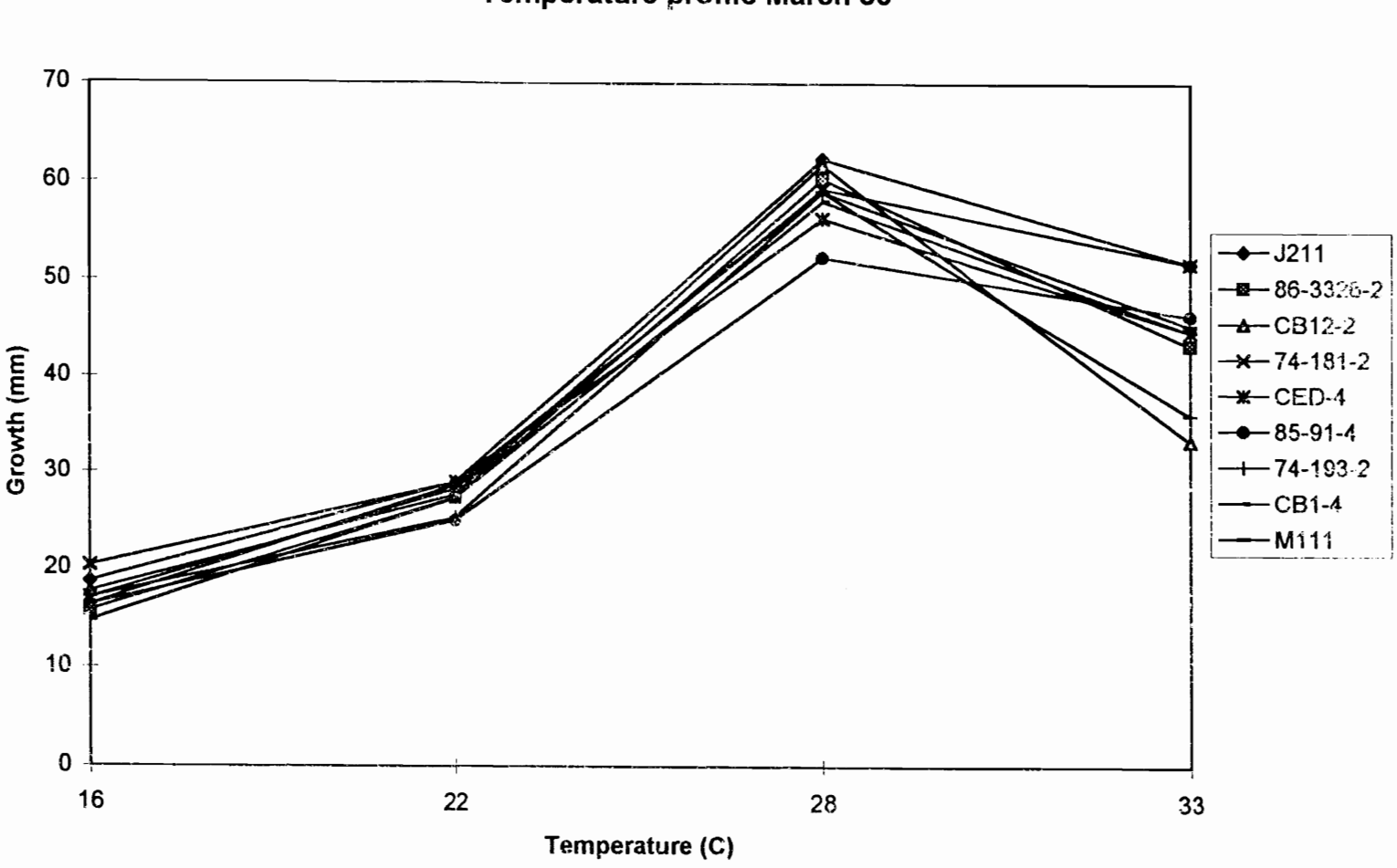
[b] Colony descriptions taken after 12 d at 25 C under 24 hr light (24W GE Soft light, General Electric, Cleveland, Ohio).

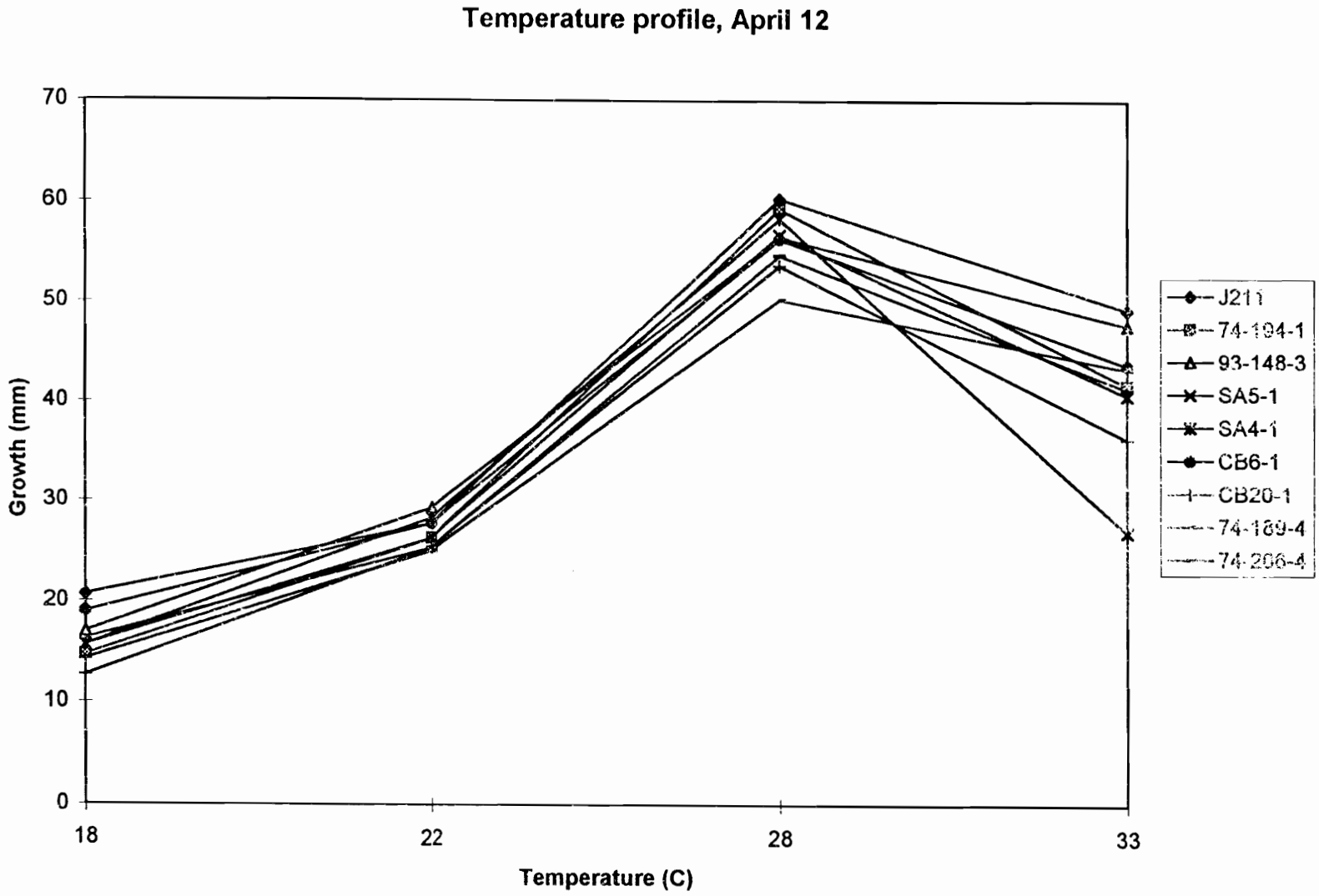
Figures B1-B7. Temperature growth profiles for *Stenocarpella maydis* isolates. A 5 mm colonized agar plug was placed at the edge of an acidified potato dextrose agar plate. Three plates were assembled for each temperature, 19, 23, 28, and 33 C. Measurements (mm) were taken from the edge of the agar plug to the edge of the mycelium daily. Graphs represent the mean of the three plates. Optimum temperatures were then estimated from these curves.


Temperature profile February 12
Growth (mm)
70
60
50
40
30
20
10
0
18
22
28
33
Temperature (C)
J211
CB3-2
90-135-8
J602
87-153-2
85-2601-3
87-124-6
CB9-4
87-109-1

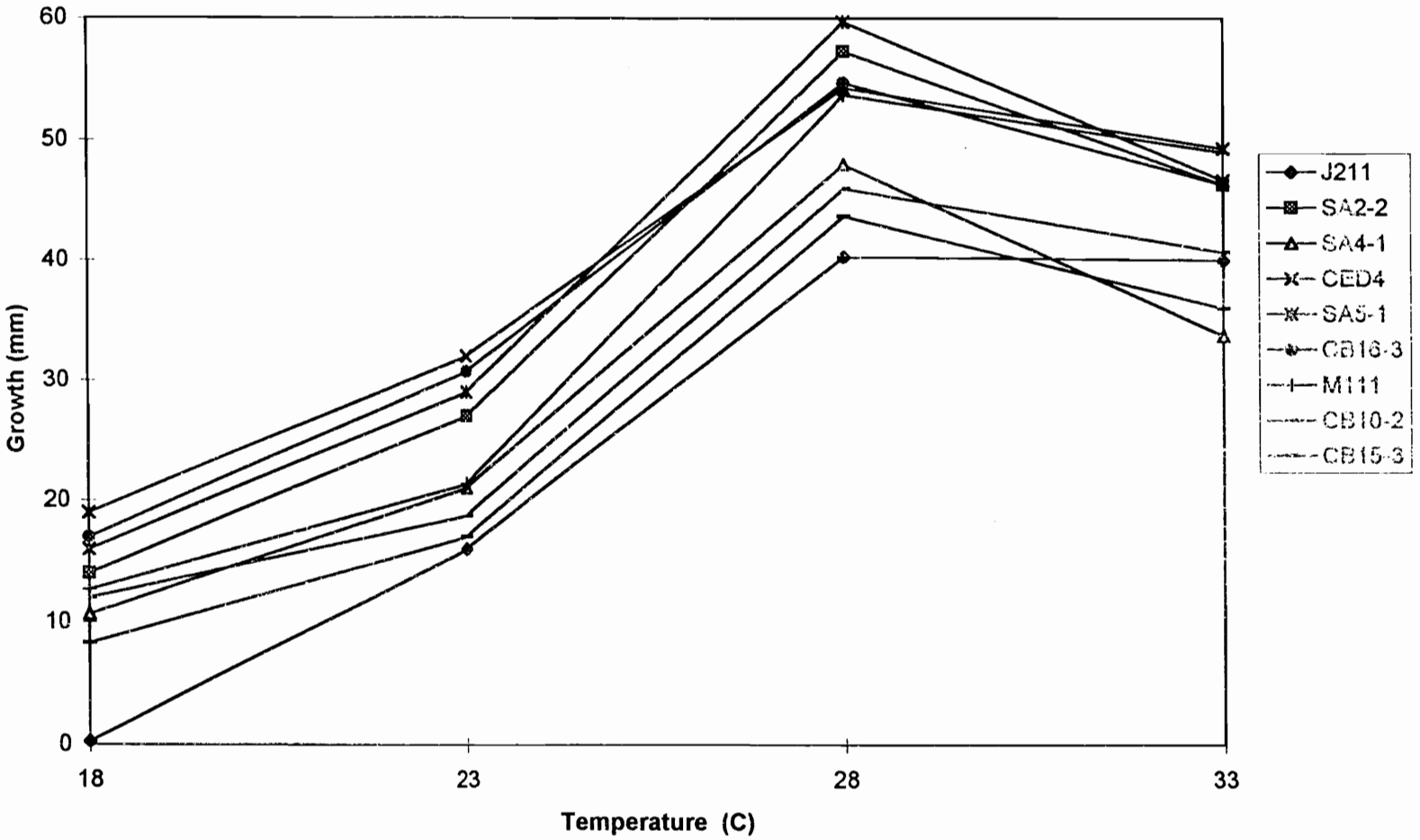

Temperature profile February 16
Growth (mm)
60
50
40
30
20
10
0
18
22
28
33
Temperature (C)
J211
CB19-2
CB7-2
A205
CB18-5
CB13-2
J408
CB21-3
87-109-1

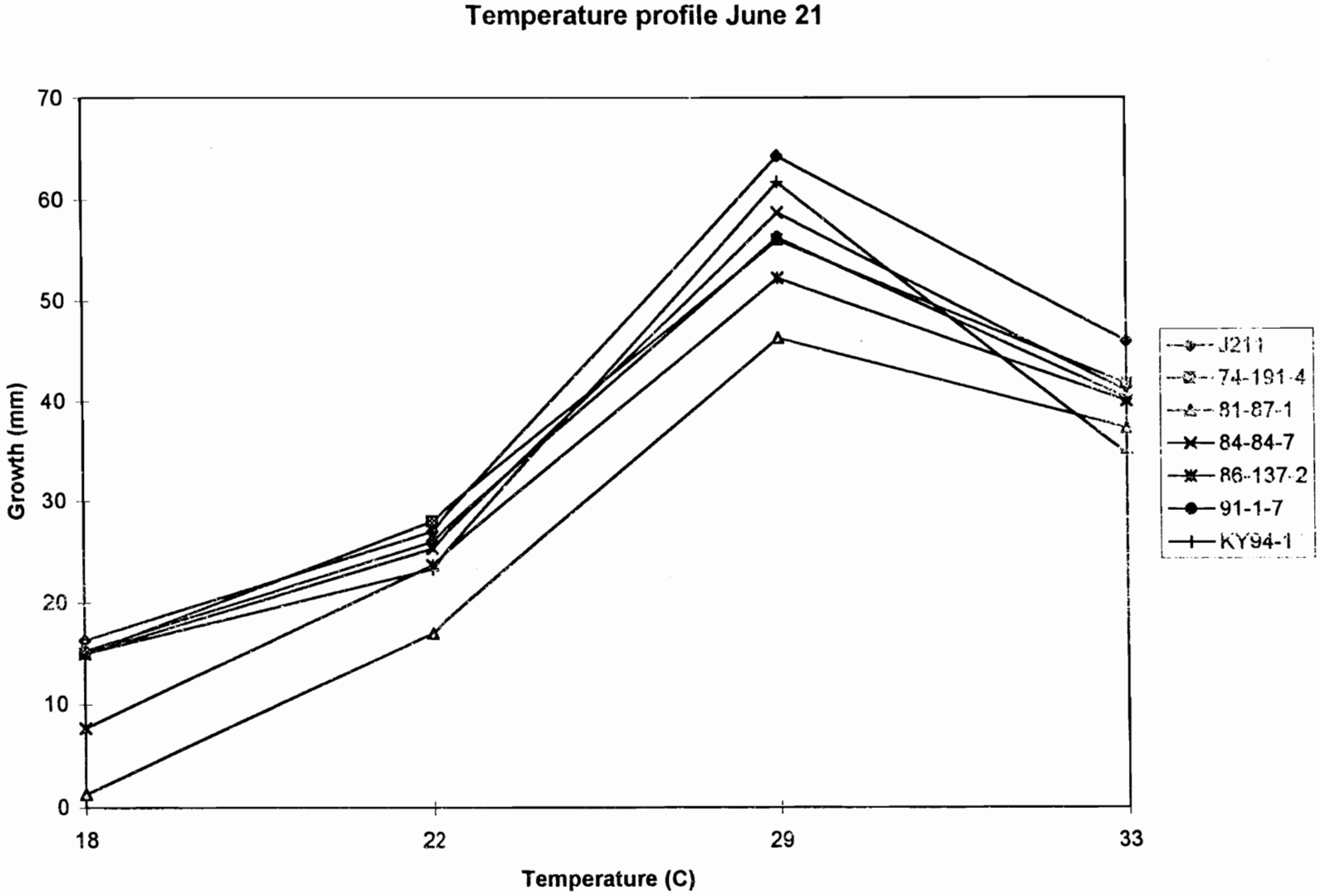

Temperature profile March 10
Growth (mm)
60
50
40
30
20
10
0
18
22
28
33
Temperature (C)
J211
CB13-2
85-2601-3
CB18-5
A205
CB9-4
90-135-8
87-124-6
CB21-1


Temperature profile March 30
70
60
50
40
30
20
10
0
Growth (mm)
16
22
28
33
Temperature (C)
J211
86-3326-2
CB12-2
74-181-2
CED-4
85-91-4
74-193-2
CB1-4
M111


Temperature profile, April 12
70
60
50
40
30
20
10
0
Growth (mm)
18
22
28
33
Temperature (C)
J211
74-194-1
93-148-3
SA5-1
SA4-1
CB6-1
CB20-1
74-189-4
74-206-4

Temperature Profile May 28




Temperature profile June 21
70
60
50
40
30
20
10
0
Growth (mm)
18
22
29
33
Temperature (C)
J211
74-191-4
81-87-1
84-84-7
86-137-2
91-1-7
KY94-1

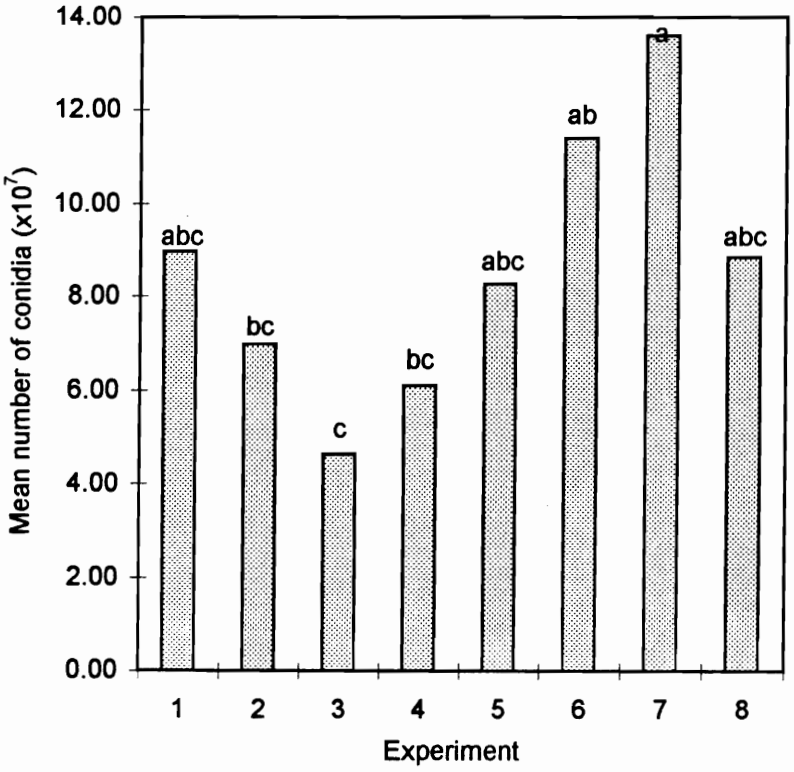


Figure B8. Mean pycnidiospore counts for *Stenocarpella maydis* strain J211. The isolate was grown on acidified potato dextrose agar with twelve autoclaved maize seeds (121 C, 1 h on two consecutive days) for 3 wk at 25 C under 24 h light (GE 24W cool white fluorescent bulbs, General Electric) Kernels were placed in a blendor to dislodge pycnidiospores, volume of water was brought to 400 ml, the spore suspension was then counted with a hemocytometer. Two counts per plate were conducted and the bar represents the mean of three plates. Data was analysed with general linear model of SAS and columns with the same letter are not significantly different at $P \leq 0.05$.

Table B4. Summary of two greenhouse seedling aggressiveness assays with 27 isolates of *Stenocarpella maydis* for greenhouse treatment number, greenhouse test, mean stand count per pot, plant averages for total fresh, root fresh, top fresh, root dry and top dry weight (g)

			Mean	Means per plant				
Isolate	Treatment number[a]	Test	Stand count	Total weight	Root weight	Dry root weight	Top weight	Dry top weight
Control	1	1	7.6	11.21	4.77	1.14	6.21	0.81
J211	2	1	8.4	10.35	3.40	0.81	6.81	0.89
74-193-2	3	1	8.2	13.35	3.41	0.79	9.80	1.19
74-189-4	4	1	8.5	14.83	5.69	2.60	9.00	1.12
87-109-1	5	1	8.5	12.55	3.26	0.67	10.30	1.18
CB 10-2	6	1	7.8	15.59	5.34	1.42	11.50	1.28
93-148-3	7	1	8.4	18.99	6.52	1.77	12.10	1.38
CB 6-1	8	1	8.3	17.31	6.19	2.66	11.00	1.46
74-206-4	9	1	8.4	17.00	6.01	1.54	10.80	1.34
74-181-1	10	1	8.9	14.37	4.33	1.05	9.88	1.13
87-124-6	11	1	8.2	15.49	3.99	0.77	11.41	1.27
74-194-1	12	1	9.4	10.84	3.76	0.87	6.77	1.01
CB 20-4	13	1	6.7	13.52	3.95	1.00	8.78	1.18
Control	14	2	7.2	12.75	4.61	1.18	7.82	0.97
74-181-2	15	2	8.7	11.39	4.03	0.86	7.20	1.01
J211	16	2	7.9	17.11	7.18	1.70	9.79	1.68
87-109-1	17	2	6.7	17.43	6.56	1.73	10.70	1.42
CB 21-3	18	2	7.8	10.57	3.89	0.80	6.45	0.87
A102	19	2	6.8	17.95	7.28	2.00	10.44	1.45
J312	20	2	5.9	15.32	5.80	1.25	9.33	1.40
CB 9-4	21	2	9.0	13.66	5.43	1.35	8.02	1.16
87-124-6	22	2	7.7	17.49	6.88	1.96	10.52	1.60
87-153-2	23	2	7.1	13.41	4.58	1.12	8.68	1.19
74-193-2	24	2	7.9	12.04	4.26	0.83	7.48	0.98
M111	25	2	8.2	13.69	4.94	1.11	8.60	1.28

Table B4 Continued.

			Mean	Means per plant				
Isolate	Treatment number[a]	Test	Stand count	Total weight	Root weight	Dry root weight	Top weight	Dry top weight
93-148-3	26	2	7.0	9.07	3.13	0.65	5.76	0.70
A205	27	2	7.8	14.25	5.35	1.13	8.65	1.22
85-2601-3	28	2	7.4	15.37	5.33	1.21	9.84	1.26
CB 13-2	29	2	7.9	13.94	4.27	1.01	9.53	1.19
CB 18-5	30	2	8.0	12.01	4.41	0.84	7.45	1.08
90.135-8	31	2	7.7	13.44	5.56	1.26	7.67	1.13
85-91-4	32	2	7.6	13.89	5.23	1.10	8.46	1.21

[a] Five L soil was mixed with 250 ml colonized millet, planted with 10 seeds of inbred lines, VA26, H99 or B37.

[b] After plant weights were recorded, tops and roots were placed in separate paper bags and placed in a drying oven at 80 C for 48 to 72 h.

Table B5. Analysis of variance for first greenhouse seedling assay[a] for development of seedling blight and corwn rot caused by *Stenocarpella maydis* on maize inbred lines, B37, Va26 and H99. Effects measured include stand count, total fresh, top fresh, root fresh, root dry, and top dry weight

Variable	Source	DF	MS	F	P
Stand count	Isolate	12	4.01	1.44	0.16
	Inbred	2	9.19	3.31	0.04
	Isolate x Inbred	24	2.67	0.96	0.52
	Error	78	2.78		
Total fresh weight	Isolate	12	62.5	2.25	0.02
	Inbred	2	21.13	0.76	0.47
	Isolate x Inbred	24	18.39	0.66	0.87
	Error	78	27.75		
Top fresh weight	Isolate	12	33.91	4.12	0.00
	Inbred	2	8.95	1.09	0.34
	Isolate x Inbred	26	4.83	0.59	0.94
	Error	78	8.22		
Top dry weight	Isolate	12	0.37	1.81	0.06
	Inbred	2	1.24	7.08	0.00
	Isolate x Inbred	24	0.13	0.72	0.81
	Error	78	0.18		
Root fresh weight	Isolate	12	12.12	1.76	0.07
	Inbred	2	47.02	6.83	0.00
	Isolate x Inbred	24	5.95	0.86	0.65
	Error	78	6.89		
Root dry weight	Isolate	12	4.03	1.11	0.37
	Inbred	2	3.5	0.96	0.39
	Isolate x Inbred	24	2.91	0.80	0.73
	Error	78	3.64		

[a] Greenhouse seedling assay consisted of infesting 5 L of soil with 250 ml of *S. maydis* colonized millet, separated into nine pots, each pto was planted with ten seeds of H99, Va26, or B37

Table B6. Analysis of variance for second greenhouse seedling assay[a] for development of seedling blight and crown rot caused by *Stenocarpella maydis* on maize inbred lines, B37, Va26 and H99. Effects measured include stand count, total plant, top fresh, root fresh, root dry, and top dry weight

Variable	Source	DF	MS	F	P
Stand count	Isolate	18	4.60	1.35	0.17
	Inbred	2	13.35	3.90	0.02
	Isolate x Inbred	36	4.50	1.32	0.14
	Error	114	3.42		
Total fresh weight	Isolate	18	52.95	1.55	0.09
	Inbred	2	274.33	8.03	0.00
	Isolate x Inbred	36	18.74	0.55	0.98
	Error	114	34.16		
Top fresh weight	Isolate	18	17.39	1.69	0.05
	Inbred	2	84.33	8.19	0.00
	Isolate x Inbred	36	6.49	0.63	0.94
	Error	114	10.29		
Top dry weight	Isolate	18	0.54	0.83	0.66
	Inbred	2	1.00	1.54	0.21
	Isolate x Inbred	36	0.20	0.31	1.00
	Error	114	0.65		
Root fresh weight	Isolate	18	12.00	1.19	0.28
	Inbred	2	54.06	5.36	0.01
	Isolate x Inbred	36	4.60	0.46	0.99
	Error	114	10.07		
Root dry weight	Isolate	18	1.35	1.21	0.27
	Inbred	2	4.64	4.15	0.02
	Isolate x Inbred	36	0.59	0.53	0.98
	Error	114	1.12		

[a] Greenhouse seedling assay consisted of infesting 5 L of soil with 250 ml of *S. maydis* colonized millet, separated into nine pots, each pot was planted with ten seeds of H99, Va26 or B37

Table B7. Ranking of means of isolates for total fresh and top fresh weight (g) from first greenhouse assay examine the effects of soil infestation with different isolates of *Stenocarpella maydis*

	Total fresh weight			Top fresh weight	
Isolate	Treatmen	Mean[a]	Isolate	Treatment	Mean
93-148-3	7	18.99 a	93-148-3	7	12.07 a
CB 6-1	8	17.31 ab	CB 10-2	6	11.48 ab
74-206-4	9	17.00 abc	87-124-6	11	11.41 ab
CB 10-2	6	15.59 abcd	CB 6-1	8	10.99 ab
87-124-6	11	15.49 abcd	74-206-4	9	10.83 ab
74-189-4	4	14.83 abcd	87-109-1	5	10.31 ab
74-181-1	10	14.37 abcd	74-181-1	10	9.88 ab
CB 20-4	13	13.52 abcd	74-193-2	3	9.8 ab
74-193-2	3	13.35 abcd	74-189-4	4	8.99 bc
87-109-1	5	12.55 bcd	CB 20-4	13	8.78 bcd
Control	1	11.21 cd	J211	2	6.81 cd
74-194-1	12	10.84 d	74-194-1	12	6.77 cd
J211	2	10.35 d	Control	1	6.22 d

[a] Average of nine pots planted with maize inbred lines, B37, H99 or Va26, means followed by the same letter do not differ significantly at P=≤ 0.05.

Table B8. Means from nine pots second greenhouse seedling assay for total plant and top fresh weight (g) from soil infested with *Stenocarpella maydis* and planted with inbred lines, B37, Va26 or H99

	Total fresh weight			Top fresh weight	
Isolate	Treatmen	Mean	Isolate	Treatment	Mean
A102	19	18.0 a	87-109-1	17	10.7 a
87-124-6	22	17.5 a	87-124-6	22	10.5 ab
87-109-1	17	17.4 a	A102	19	10.4 ab
J211	16	17.1 ab	85-2601-3	28	9.8 abc
85-2601-3	28	15.4 ab	J211	16	9.8 abc
J312	20	15.3 ab	CB 13-2	29	9.5 abc
A205	27	14.2 ab	J312	20	9.3 abc
CB 13-2	29	13.9 ab	87-153-2	23	8.7 abc
85-91-4	32	13.9 ab	A205	27	8.7 abc
M111	25	13.7 ab	M111	25	8.6 abc
CB 9-4	21	13.7 ab	85-91-4	32	8.5 abc
90-135-8	31	13.4 ab	CB 9-4	21	8.0 abc
87-153-2	23	13.4 ab	Control	14	7.8 abc
Control	14	12.7 ab	90-135-8	31	7.7 abc
74-193-2	24	12.0 ab	74-193-2	24	7.5 abc
CB 18-5	30	12.0 ab	CB 18-5	30	7.5 abc
74-181-2	15	11.4 ab	74-181-2	15	7.2 abc
CB 21-3	18	10.6 ab	CB 21-3	18	6.5 bc
93-148-3	26	9.1 b	93-148-3	26	5.8 c

APPENDIX C

Media:

1. Acidified Potato Dextrose Agar

Autoclave (121 C) for 10-15 min
44 g of dehydrated potatoes in 1 L distilled water
30 g of agar in 1 L distilled water

Add 20 g dextrose to flask with melted agar
Drain potatoes and bring volume up to 1 L with distilled water
Mix thoroughly, dispense in 500ml bottles

Autoclave (121 C) for 20 min

Cool to approximately 60 C and add 1 drop of 1 N Lactic Acid/100 ml

2. Basal Salts Mineral Medium (Morant et al. 1993)

5.6 g K_2HPO_4
2.3 g KH_2PO_4
1.0 g $MgSO_4.7H_2O$
2.64 g $(NH_4)_2SO_4$
1 L distilled water

3. Pridham Gottlieb Trace Salts (Tuite, 1969)

0.64 g $CuSO_4 \cdot 5H_2O$
0.11 g $FeSO_4 \cdot 7H_2O$
0.79 g $MnCl_2 \cdot 4H_2O$
0.15 g $ZnSO_4 \cdot 7H_2O$

100 ml distilled water

To 25 ml of Basal Salts Mineral Medium add 25 µl of Pridham Gottlieb Trace Salts

Staining recipes:

1. Aspartate amino transferase (AAT)

Stock solution, AAT substrate solution:

Distilled water	200 ml
α-ketoglutaric acid	73 mg
L-Aspartic acid	270mg
PVP-40	1 g
EDTA, Na_2salt	100 mg
Sodium phospate, dibasic	2.84 g

AAT substrate solution	30ml
Fast Blue BB salt	30mg

Incubate gel at room temperature in dark on stir plate.
Reference: Wendel and Weeden (1989)

2. Aryl Esterase (EST)

Na Phosphate 0.1M pH 6.5	50ml
α-napthl butyrate 1%	15ul in 1.5ml acetone
Fast blue RR salt	50mg

Reference: Brewbaker et al. (1968)

3. Fumarate hydratase (FUM) [=fumarase]

50mM Tris-HCl, pH8.0	30ml
NAD	600ul (10mg/ml stock)
Fumaric acid, sodium salt	120mg
Malate dehydrogenase	120 units
MTT	600ul (5mg/ml stock)
PMS	240ul (2mg/0.4ml stock)

Reference: Wendel and Weeden (1989)

4. ß-Glucosidase (GLU)

50mM Na-phosphate buffer, pH 6.5	30ml
6-Bromo-2-napthyl-ß -D-glucopyranoside	30mg
Fast Blue RR salt	30mg

Reference: Wendel and Weeden (1989)

Na-phosphate buffer:

Solution A = 27.6g NaH_2PO_4 (0.2M) in 1 L distilled water
Solution B = 53.65 g $Na_2HPO_4 \cdot 7H2O$

68.5 ml Solution A + 31.5 ml Solution B + 100 ml distilled water, pH 6.5

5. Glutamate dehydrogenase (GDH)

50mM Tris-HCl, pH 8.0	30 ml
NADP	600ul (10mg/ml)
$CaCl_2$	600ul (50mg/ml)
L-Glutamate, sodium salt	120mg
MTT	600ul (10mg/ml)
PMS	240 (2mg/0.4ml)

Reference: Wendel and Weeden (1989)

6. Hexokinase (HEX)

50mM Tris-HCl, pH 8.0	30ml
$MgCl_2$	600ul (50mg/ml)
NAD	600ul (10mg/ml)
Glucose	120mg
Adenosine triphosphate	75mg
Glucose-6-phosphate dehydrogenase	24 units
MTT	600ul (10mg/ml)
PMS	240ul (2mg/0.4ml)

Reference: Wendel and Weeden (1989)

7. Malate dehydrogenase (MDH)

50 mM Tris-HCl, pH 8.5	30 ml
NAD	600ul (10mg/ml)
Malic acid	90 mg
MTT	600ul (5mg/ml)
PMS	240ul (2mg/0.4ml)

Reference: Wendel and Weeden (1989)

8. Phosphoglucomutase (PGM)

50mM Tris-HCl, pH 8.5	30ml
$MgCl_2$	600ul (50mg/ml)
Glucose-1-phosphate, Na_2-salt	100mg
NADP	600ul (10mg/ml)
Glucose-6-phosphate dehydrogenase (NAD)	12 units
MTT	600ul (10mg/ml)
PMS	240ul (2mg/0.4ml)

Reference: Wendel and Weeden (1989)

9. Superoxide dismutase (SOD)

50mM Tris-HCl, pH 8.0	30ml
Riboflavin	1.2mg
EDTA	600ul (mg/ml)
NBT	6mg

Reference: Wendel and Weeden (1989)

10. Xanthine dehydrogenase (XDH)

Step 1. Heat to dissolve, 20mg Hypoxthanine in 30ml, 50mM Tris-HCl, pH 8.0

Let cool to room temperature.

Step 2.

NAD	600ul (10mg/ml)
MTT	600ul (5mg/ml)
PMS	240ul (2mg/ml)

Reference: Vallejos (1983)

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VITAE

Anne Elizabeth Dorrance was born in Philmont, New York on August 16, 1958. She moved shortly thereafter to Cazenovia, NY where she played extensively on her grandfathers dairy farm. Her family moved to Herkimer, NY where she graduated from Herkimer High School in 1976 and Herkimer County Community College in 1978. She transferred to the College of Environmental Sciences and Forestry at Syracuse, NY where she graduated with a B.S. in Forest Biology. Her first job was with the USDA, APHIS, PPQ working on a joint project with NYS Ag and Markets for Scleroderris scout, gypsy moth and golden nematode surveys. Anne enjoyed a winter in Vermont as a lift operator, then worked for NYS Agricultural Experiment Station on diseases of apple, beet, cabbage and lettuce. She attended the University of Massachusetts and received a M.S. in 1985 in Plant Pathology. She then worked at the State Plant Pathologists for the Vermont Dept. of Agriculture. Prior to beginning her studies at VPI & SU, she worked as a research biologist at the Illinois Natural History Survey and adjunct faculty member at Concord College, WV. Anne is married to Dr. Thomas F. Tierney and they have two children, Hannah and Forrest. Her next endeavor is a post doctoral position at WSU Mount Vernon Experiment Station.

Anne E. Dorrance