Maize Resistance to Infection by *Fusarium graminearum*: Mechanisms and Inheritance

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Doctor of Philosophy

Short Title:

Kernel Resistance to *Fusarium graminearum*

DEDICATION

To my mother, Lister Musonda and father, Aaron Chungu (Sr)

THESIS FORMAT DESCRIPTION

This thesis consists of papers submitted or to be submitted for journal publications. The format is consistent with all other requirements outlined in part B, section 2 of the thesis format. The following is a quotation of part of the text that applies to the guidelines for **Manuscripts and Authorship**:

*"The candidate has the option, **subject to approval of the Department** of including as part of the thesis text of paper(s) submitted for publication. The style of the manuscripts should however, conform to all other requirements outlined in the "Guidelines Concerning Thesis Preparations" approved by the Faculty of Graduate Studies and Research of McGill University. The thesis should not be a mere collection of manuscripts published or to be published. It must include: Table of contents, a general abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive literature review, final overall conclusion and/or summary and a thorough reference list. Additional material (procedural and design data as well as description of equipment) must be provided where appropriate and in sufficient detail (eg. Appendices) to allow clear and precise judgement to be made of the importance and originality of the reported research. Inclusion of manuscripts co-authored by candidate and others is acceptable, but the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent. The supervisor(s) must attest to the accuracy of such claims at the Ph.D Oral Defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the*

responsibilities of the authors of co-authored papers".

This thesis is a collection of papers and observation notes. It includes a general introduction, abstract, general literature review and references. Each chapter consists of a preface and names of contributing authors, an abstract, an introduction, materials and methods, results and discussion. In addition, the thesis includes a general discussion, conclusions and suggestions for future research. All references including those cited within each chapter are listed at the end.

GENERAL ABSTRACT

MAIZE RESISTANCE TO INFECTION BY *Fusarium graminearum*: MECHANISMS AND INHERITANCE

Ph.D **Chibwe Chungu** **Plant Science**

In evaluating ear rot resistance in maize, choice of inoculation method(s) should be considered since different methods may assess different resistance mechanisms. Comparison of six inoculation methods showed that the silk-channel injection and kernel-stab techniques were effective in detecting silk and kernel resistance, respectively. Inheritance of kernel resistance was assessed after ears were inoculated with the kernel-stab method. Resistance was predominantly due to additive gene effects, and appears to be controlled by several loci. The reaction of waxy and non-waxy endosperm maize was also evaluated with the silk and kernel methods. No significant differences were observed between these isogenic lines. Evaluation of deoxynivalenol (DON), a toxin produced by *F. graminearum*, indicated that the distribution of DON in individual maize kernels was very variable, with a few kernels containing high levels of DON. The presence of DON in kernels with no detectable fungal hyphae and low correlation between DON and fungal DNA suggested that translocation of DON does not always depend on fungal hyphae. Differences in ear rot symptom development were observed in several hybrids between 25 and 85 days after silking. Symptoms enlarged more slowly in one hybrid than others. These differences were attained before physiological maturity. The resistant hybrid had a thicker pericarp at all sampling times than other hybrids. Correlation between disease assessment methods, image analysis and visual rating scale was significant and high.

RÉSUMÉ

RÉSISTANCE DU MAIS A L'INFECTION PAR *Fusarium graminearum*: MÉCANISMES ET HÉRÉDITÉ

Ph.D **Chibwe Chungu** **Phytotechnie**

Lors de l'évaluation de la résistance du maïs à la fusariose de l'épi, le choix de la (des) méthode(s) d'inoculation doit être pris en compte, car différentes méthodes mettent en évidence différents mécanismes de résistance. La comparaison de six méthodes d'inoculation a montré que la technique d'injection des canaux des soies et la technique de piquage des grains étaient efficaces pour détecter la résistance des soies et détecter la résistance des grains respectivement. La transmission héréditaire de la résistance des grains a été évaluée après que les épis aient été inoculés avec la technique de piquage des grains. La résistance était principalement dûe à des effets génétiques additifs, et semblait être controlée par plusieurs loci. La réaction d'endospermes de *Zea mays ceratina* et *Zea mays indentata* a également été évaluée avec les techniques d'injection des canaux des soies et de piquage des grains. Aucune différence significative n'a été observée entre ces deux lignées isogéniques. La mise en évidence de déoxynivalénol (DON), une toxine produite par *F. graminearum*, a montré que sa distribution était très variable d'un grain de maïs à l'autre, avec quelques grains contenant de grandes quantités de DON. La présence de DON dans les grains en absence de détection d'ADN fongique, ainsi que la faible corrélation entre le DON et l'ADN fongique, suggèrent que la translocation du DON ne se fait pas toujours par l'intermédiaire des hyphes fongiques. Les différences de développement des symptômes de la fusariose de l'épi ont été observées chez plusieurs hybrides, entre 25 et 85 jours après l'apparition des soies. Les symptômes ont augmentés plus lentement chez l'un des hybrides que chez les autres. Les différences entre cet hybride résistant et les autres étaient atteintes avant le stade de la maturité physiologique. Lors de tous les prélèvements, l'hybride résistant avait un péricarpe plus épais que les autres hybrides. La corrélation entre les méthodes d'évaluation de la maladie, l'analyse d'image et l'échelle de classement visuelle était significative et élevée.

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Chapter 1

CONTRIBUTIONS OF CO-AUTHORS

All manuscripts are co-authored by C. Chungu and D.E. Mather, my supervisor. Dr. Mather provided guidance from the onset of this research, arranged technical assistance during field operations, offered valuable suggestions and reviewed all the manuscripts and presentations.

Dr. R.I. Hamilton developed and provided all the genetic materials needed for the entire research, and also reviewed the manuscripts.

Dr. L.M. Reid provided the inoculum and technical assistance required to inoculate the Ottawa experiments, provided advice pertaining to all inoculation methods used in this research, and offered valuable suggestions during the review of manuscripts.

Dr. T. Ouellet provided laboratory facilities and technical assistance, and taught and advised me on how to handle molecular biology analysis of maize kernels.

Chapter 2

GENERAL INTRODUCTION

Maize [*Zea mays* L.] is an important grain crop for human consumption, livestock, and industrial use in many parts of the world. Ear rot caused by *Fusarium graminearum* Schwabe [sexual state: *Gibberella zeae* (Schwein) Petch] is a disease of importance in areas where maize is cultivated. The disease is characterized by ear rot symptoms which usually originate from the tip of the ear but occasionally start from the butt of the ear. The characteristic symptom of infection caused by this pathogen is a pink to reddish mold on kernels and husks. Symptoms may appear dark if perithecia (fruiting bodies of *G. zeae*) are present.

Effective screening to identify resistant maize germplasm depends on reliable inoculation techniques. Several inoculation methods with various degrees of efficacy have been used to artificially inoculate maize genotypes with ear rotting pathogens (Boling and Grogan, 1965; Odiemah and Manninger, 1982; Ullstrup, 1970; Drepper and Renfro, 1990; Reid *et al.* 1992a, 1994). In most of these studies, either the silk or both kernel and cob tissues were wounded. Evidence of a resistance mechanism in the silk tissue has been reported by Reid *et al.* (1992a). This mechanism acts by slowing the rapid growth of the fungus down to the kernels. However, if the fungus bypasses the silk tissue, silk resistance may not be sufficient. In such situations, kernel resistance mechanisms may be useful, because these might inhibit or slow down the spread of infection from kernel to kernel. Direct evaluation of kernel resistance, requires a means of introducing inoculum to the kernels without wounding

other tissues.

Whether plants are naturally or artificially infected, the development of symptoms may differ among genotypes. In addition, symptoms may depend on weather patterns, fungal aggressivity, inoculum type, time of inoculation, time of harvest, and inoculation methods. Little is known about the early pattern of ear rot development on maize ears and possible kernel developmental stage when differences in resistance might first be observed. Evaluation of ear rot development requires a reliable assessment method. Often, visual rating scales are used, but these methods are not precise when symptoms are moderate.

Fusarium graminearum produces toxins that are highly toxic to both humans and livestock. Consumption of grain contaminated with these mycotoxins can lead to reproductive and digestive disorders. One toxin produced under field conditions by *F. graminearum* is deoxynivalenol (DON). The presence of DON reduces the market value of the grain. Several studies have reported that the amount of DON produced is correlated with symptom levels. However, information on the distribution of DON in individual kernels is lacking.

Chapter 3

3. LITERATURE REVIEW

3.1. The Maize (Corn) Plant

Maize (*Zea mays* L.), also known as corn, is an annual crop indigenous to the Western hemisphere. It belongs to the large and important family, Gramineae. The crop is grown on more than 120 million hectares (Russell and Hallauer, 1980) in the world, and is important in world trade.

3.1.2. Production and Consumption

The United States (U.S.) produces 50% of the world maize total. Brazil and China are the next largest maize producing countries. In Canada, three quarters of grain and forage maize is produced in the provinces of Québec and Ontario.

Maize is consumed directly or indirectly by millions of people worldwide. In North America and Europe, almost three quarters of the maize produced is fed to domesticated animals (Russell and Hallauer, 1980). In other parts of the world, such as Africa, Asia, Central America and parts of South America, maize is consumed as a primary food grain.

3.1.3. Agronomic Aspects

3.1.3.1. Climate: Maize requires considerable moisture and warmth from the time of planting to the flowering period. According to Metcalfe and Elkins (1980), maize germinates best at temperatures above 10° C, and mean summer temperatures

of 21-32° C are required for optimal growth. The level of precipitation required to produce a crop without irrigation is about 38-50 cm. Moisture availability is most critical a few weeks before to a few weeks after tasselling and pollination (Hughes and Henson, 1957; Rouanet, 1987). Maturity of the crop is likely to be delayed when temperatures are below normal and rainfall is above normal. Cool, cloudy weather results in heavy vegetative growth and low grain yields.

3.1.3.2. Soil: Maize has wide adaptation to soil conditions. It is grown on a wide variety of soils but performs best on well-drained, aerated, deep loams and silt loam, containing abundant organic matter with high water holding capacity to supply water during the critical periods (Wallace and Bressman, 1949). According to Martin *et al.* (1975) maize can be grown on soils with pH ranging between 5.5 and 8.

3.1.3.3. Yield: Improved yields have been attained through breeding and use of fertilizers. Single-cross hybrids made and continue to make major contributions to yield increase because the genetic potential of hybrids takes advantage of improvements in cultural practices. Improved resistance to root and stalk rots have made machine harvesting possible and have contributed to stable high yields. Insects such as corn borers [*Ostrinia nubilalis* (Hubner)] and leaf aphids (*Rhopalosiphum maidis)* can reduce grain yield (Dicke and Guthree, 1988). Leaf diseases such as bacterial leaf blight (*Pseudomonas alboprecipitans* Rosen), goss's bacterial wilt (*Corynebacterium nebraskense* Vidaver and Mandel), anthracnose (*Colletotrichum*

graminicola), northern corn leaf blight (*Helminthosporium turcicum* Pass.), reduce maize yield by reducing the photosynthetic area. Ear rot diseases, such as Diplodia ear rot may reduce yield in severe cases and can lead to poor grain quality thus reducing the market value of the crop.

3.1.4. Morphology of the Maize Plant

In temperate production systems, maize is normally 2 to 3 m tall. Some very early-maturing flint types are very short, with ears close to the ground. In tropical and subtropical regions, maize plants can reach heights of 6 to 7 m. Stems of maize normally possess between 8 to 21 internodes (Berger, 1973). The stem internodes are fairly thick and short at the base, and become longer and thinner higher up the stem, tapering into the male inflorescence (tassel) which terminates the axis. The maize plant bears its flowers in spikelets, the characteristic units of the inflorescence of all grasses.

3.1.5. Flowering and Pollination

The plant is monoecious with the female flowers on the ear shoots and the male flowers in the tassel. The tassel is a fairly compact, branched panicle. Its axis is erect and its branches are sometimes flexuous, producing a drooping panicle (Russell and Hallauer, 1980). The tassel bears spikelets, each with two florets. Each floret contains three stamens which produce pollen grains. According to Metcalfe and Elkins (1980), a single plant is capable of producing about 10 million pollen grains.

However, only one pollen grain is required to pollinate the silk of each kernel. The female inflorescence is known as the 'ear'. The ear consists of a modified lateral branch derived from an axillary bud of the main stem. The internodes of this lateral branch have become short such that the overlapping sheaths of the leaves cover the terminal inflorescence, forming the husk of the ear. The ovary is topped by a long style, the silk, which grows rapidly and emerges from the top of the husk. The silk is bifurcated at the tip, but is stigmatic and receptive along most of its length (Berger, 1973; Martin *et al.* 1975).

Maize is normally cross-pollinated, but pollen shedding and silk receptivity may overlap to permit some self-pollination. Pollination is mainly by wind and gravity, although to a limited extent it is also accomplished by insects. Pollen is produced in large quantities from the opening flowers of the tassel. Silks usually emerge at the top of the ear node 1 to 3 days after anther dehiscence has begun. Tassel development seems to control development of the ear shoot, and this dominance is strong in genotypes that produce only one ear per plant (Berger, 1973; Russell and Hallauer, 1980). In genotypes that are prolific, no dominance for the tassel exists.

3.1.6. Maize Plant Developmental Stages

The plant development employed by Ritchie *et al.*, (1986) is sub-divided into vegetative (V) and reproductive (R) stages. The sub-divisions of the vegetative stages are: emergence (VE), first leaf (V1), second leaf (V2), third leaf (V3) through the nth

leaf (Vn), where *n* represents the last leaf stage before the tasselling stage (VT). The six reproductive stages are defined according to the development of the kernel and its parts. The reproductive stage encompasses the silking stage (R1), blister stage (R2), milk stage (R3), dough stage (R4), dent stage (R5) and physiological maturity stage (R6). The last reproductive stage is reached when all the kernels on the ear have reached maximum dry matter accumulation. A good indicator of the R6 stage is the black layer formation from the basal kernels of the ear.

3.1.7. The Kernel: The mature maize kernel is made up of the pericarp, the endosperm, and the embryo.

3.1.7.1 Pericarp - This forms the outer covering of the kernel and protects the internal parts. According to Kiesselbach (1949) there is also a thin suberized nucellar membrane which serves as a continuous covering between the aleurone and the pericarp. The dry-weight of the pericarp is usually less than 2% of the total kernel weight (Benson and Pearce, 1987). The pericarp has the silk scar at the apical end of the kernel.

3.1.7.2 Endosperm - When the pericarp is removed, only the endosperm and the embryo remains. The endosperm constitutes 82 - 84% of the kernel dry weight and is 86 - 89% of starch by weight (Watson, 1987). Except for the surface, the endosperm consists of cells filled with grains of starch. The basal cells of the endosperm are modified as conducting cells which serve for conducting food from the mother plant to the growing endosperm and indirectly to the embryo (Berger, 1973).

The outer layer of the endosperm, the aleurone tissue, is a single layer of cells. The aleurone cells are granular in appearance and contain protein granules but no starch. The cells are also rich in minerals. The proteins and minerals, which are of high quality, are not available nutritionally to digestive enzymes unless the cells are opened by grinding.

3.1.7.3. Embryo - The young maize plant is embedded near one surface of the endosperm. It has a central axis with the primary root at the basal end and the plumule with stem and leaves at the apical end. The outer layer of the embryo is the scutellum. The scutellum functions as a food storage organ and is specialized in producing enzymes required to digest the starch in the endosperm during embryo and seedling growth (Aldrich, 1975; Kiesselbach, 1949).

3.1.8. Types of Maize

Many kinds of maize can be grouped effectively according to their kernel texture. Several groups of maize that are of economic importance include:

3.1.8.1. Dent Corn - According to Delorit et al. (1984) about 95 percent of all grown maize is of this type. Dent corn is characterized by a hard, horny starch at the sides and back of the kernels, with only the soft starch extending to the crown. Metcalfe and Elkins (1980) indicated that a dent forms at the crown of the kernel when the soft starch dries rapidly and shrinks. Shrinkage is mainly limited to the soft starch at the crown end of the grain since the starch in other parts is much harder. Dent corn varies in color and in size and shape of ear. Great variation is also

observed in size and shape of kernels, and in maturity among the different races and cultivars within this group.

3.1.8.2. Flint Corn - Northern flint corn has small amount of soft endosperm that is enclosed in a layer of horny endosperm (Metcalfe and Elkins, 1980). Most of the kernels are large and broad, and ears long and slender. Flint corn tends to be resistant to injury by maize weevils because the seed is hard. Plants of this type of maize are relatively short and tiller abundantly.

3.1.8.3. Sweet Corn - The endosperm of sugary corn is transparent in appearance. When dry, sugary kernels have a glassy, gumlike appearance, and a wrinkled, irregular form. Before drying, the mature endosperm is distended and cohesive. Some varieties within this group, grown for table use, have been selected for a number of quantitatively measurable traits, including tenderness of pericarp and resistance to pests.

3.1.8.4. Waxy Corn - The kernels of waxy corn are characterized by a dull, soft endosperm with a wax-like appearance when cut or broken. The *waxy* gene affects the synthesis of endosperm starches. As the result only branched chain starch (amylopectin) is formed (Delorit et al., 1984; Martin *et al.* 1987). This starch has pasting and viscosity properties similar to tapioca starch. The *waxy* corn is also useful for industrial purposes because its homogenous starch make separation into distinct components unnecessary.

3.1.8.5. Floury Corn (*Zea mays amylacea*) - The floury corn kernels are not dented, and have opaque endosperm. This type of maize has a large amount of soft

starch present, and hence can be ground into flour easily.

3.1.8.6. High Lysine Corn - This type of maize is high in the amino acid lysine. The protein of this type of maize has relatively well balanced amino acid content and has a nutritional value comparable to skimmed milk (Bressani, 1992). High-lysine corn varieties generally yield less (Glover, 1992), contain higher moisture early in the season, suffer greater kernel damage and are more susceptible to some diseases than normal dent corn. However, recent studies by Bockholt and Rooney (1992) have shown that some quality protein maize (QPM) hybrids exceeded the yield of some of their best yielding white food maize hybrids. They also found that some QPM hybrids had excellent standability, disease and insect resistance, and grain quality. Quality protein maize contains amino acids lysine and tryptophan in the endosperm. The amino acid tryptophan is important in the synthesis of B-vitamin, niacin.

3.2. Fusarium Ear Rot

Gibberella zeae (Schwein) Petch, the sexual stage of *Fusarium graminearum* Schwabe, can cause ear rot in maize. The disease is a common problem in Quebec, Ontario (Sutton, 1982) and many other parts of the world (Marasas *et al.* 1979a,b; Koehler, 1959). It is often associated with damage to ears caused by birds or insects such as earworm (*Heliothis spp).* The disease is characterized by ear rot that usually starts from the tip, but occasionally may originate at the butt of the ear (Burgess, *et al.,* 1981). The fungus causes a pronounced reddish discoloration of the rotted grain

and husk tissues and produces a pink to reddish mold on the surface of colonized grain. In severe situations, the husks adhere tightly to the kernels. In wet conditions large numbers of perithecia form on the outside of the husks.

3.3. *Fusarium graminearum*

The population of *Fusarium graminearum* in North America is subdivided into Group I and Group II. According to Burgess *et al.* (1981) members of Group I are mostly soil-borne and cause crown rot of cereals, and rarely produce perithecia in nature. Members of Group II cause a variety of diseases of aerial plant parts, i.e. ear rot of maize, head blight of wheat, barley and oats. This group forms perithecia readily on host material and sometimes in culture media (Cook, 1981).

Although both populations probably occur in most areas, their relative abundance varies according to the prevailing environmental conditions and/or the presence of a particular host (Francis and Burgess, 1977). Members of Group I are often found in drier areas, possibly because dry conditions favour the survival of this fungus in residues (Burgess and Griffin, 1968). According to Francis and Burgess (1977), Group II tends to be more common in areas where it is warm and humid for at least part of the year when suitable substrates are available. Warm humid conditions favour the formation of perithecia and ascospore discharge (Tschanz et al., 1976) and therefore infection and colonization of aerial plant parts. *Fusarium graminearum* attacks a wide range of plant species in addition to cereals. The pathogen causes seedling blight in red clover (*Trifolium spp.)*, alfalfa (*Medicago spp.)*

and wheat (*Triticum spp.)*, and root rot in soybeans. According to Cook (1981), *F. graminearum* is the main fungal species on cereals in warm regions.

3.4. Factors Influencing Fusarium Ear Rot Development

3.4.1. Moisture: Moisture conditions affect the development of kernel ear rot. In Illinois, Koehler (1959) compared monthly rainfall data with Gibberella ear rot development. He found significant correlation between ear rot prevalence and rainfall in August, September and October, but September rainfall had more pronounced effect on ear rot development. Work done in Indiana by Tuite *et al.*, (1974) also found that August and September rainfall favoured ear rot development. The apparent increase in ear rot development in September could be associated with the effect high moisture has on increasing the production of inoculum, coupled with washing the spores down among or between the husks and ears when the ear tip is exposed. In general, high humidity supports spore germination and infection. High moisture levels during the month of October may facilitate disease development because it slows the natural drying process of the ears, making the ears more prone to infection for extended periods.

3.4.2. Temperature: Temperatures of 15 to 35°C favour mold infection (Sutton, 1982). The optimum temperature for ear rot growth is usually between 20 and 30°C, but in general *F. graminearum* can grow at temperatures as low as 5°C. Grain moisture content greater than 14% allows ear rot to develop at temperatures greater

than 5° to 10°C. Tuite *et al.,* (1974) reported that incidence of ear rot seemed to be associated with cool weather during silking. However, Lovelace and Myathi (1977) have reported that severe epidemics occur in some warm countries such as Zambia.

3.4.3. Ear Declination and Husk Coverage: Koehler and Holbert (1930) reported that erect ears are more susceptible to seed infection than ears that are in a declined position. Similar results were obtained by Koehler (1959) who observed a significantly higher percentage of Fusarium rots in erect ears than in declined ears. Kyle (1918) reported that ears with low husk coverage had more rotten and discoloured kernels than those with high husk coverage. Lower husk coverage make it easy for insects or birds to invade and deposit inoculum of developing kernels. Johnson and Christensen (1935) found positive association between ear rots and smut [*Ustilago zeae* (Beckm) Ung.] infections in 1933. They made 52 comparisons with ear-smutted plants and found that 90 percent of the smutted ears also had ear rots. They attributed this association to smut infection on the ear affording an accessible avenue for the entrance of ear-rotting organism.

3.5. Life Cycle of *Fusarium Graminearum*

The principal inoculum source of *F. graminearum* is host debris such as old maize stalks, ears, and stubble, and debris of other small grain cereals. Minimum or no-tillage practices heighten the problems of this fungal disease, whereas ploughing of plant debris and crop residue accelerates decomposition of the moldy plant material.

According to Hunter and Sutton (1985); Seaman (1982); and Sutton (1982) crop residues are the principal sources of *F. graminearum*, however, seeds, soils, and various grasses are also sources of inoculum. Spores are usually dispersed in the air by insects or birds. Warren and Kommedahl (1973) observed that *F. graminearum* appears to survive longer in tissues that resist breakdown. Similar observations were reported by Burgess and Griffin (1968) that the fungus survived longer in nodal than internodal tissues of wheat stems.

3.5.1. Types of inoculum: Propagules that may constitute inoculum include ascospores, macroconidia, chlamydospores, and hyphal fragments. Ascospores are formed in fruiting structures called perithecia that arise from inconspicuous stromata on host tissue and host debris. Macroconidia are formed in sporodochia, are more variable in size and septation, and have a distinctive foot cell (Booth, 1971). Macroconidia can be transformed into thick-walled chlamydospores, and these have the capacity to infect wheat and maize. Chlamydospores can also arise from hyphae (Wearing and Burgess, 1977). However, macroconidia or ascospores are the most important source of inoculum under natural conditions.

3.6. Inheritance of Ear Rot Resistance

Increased genetic resistance to *F. graminearum* is a more feasible method of control than are fungicides. The nature of resistance to ear rots caused by various *Fusarium* species has been studied, but variation in experimental methods, inoculation

techniques, time of inoculation, and pathogen has possibly contributed to differences in information obtained. Koehler (1959) found that inherited resistance to any one ear rot is largely independent of inherited resistance to other rots.

Boling and Grogan (1965), studying the genetics of host reaction to ear rot in maize caused by *F. moniliforme*, found additive and dominance gene effects to be important in the inheritance of host resistance. The estimated number of genes controlling host resistance were approximately 1.5. These authors also found epistasis in addition to additive and dominance gene effects to be important in the inheritance of ear rot resistance. Based on these results, they speculated that reciprocal recurrent selection would be the best breeding approach in accumulating genes for resistance.

Warren (1978) compared normal and high lysine maize inbred lines for resistance to kernel rot caused by *Fusarium moniliforme*. Ear rot was more severe on susceptible *opaque-2* maize inbreds than on normal maize. The author also found susceptibility or resistance in *opaque-2* maize endosperm to be positively correlated with susceptibility or resistance in the normal maize endosperm. In general, *opaque-2* maize tends to be softer, which may account for easier penetration of fungi.

Genotypic differences in maize to kernel infection by *F. moniliforme* were evaluated by King and Scott (1981). They observed more kernel infection on inbreds than on crosses involving the inbreds. They postulated that greater susceptibility of inbreds to infection could be related to their low plant vigour relative to the hybrids. Cullen et al. (1983) investigated resistance of maize inbreds and hybrids to Gibberella ear rot. They found that severity to ear rot differed significantly among the material

used, with inbred lines consistently being more susceptible than hybrids.

Odiemah and Manninger (1982) found genetic variation for resistance to ear rot caused by *Gibberella zeae* in maize to be associated with significant general combining ability (GCA) effects. Use of Vr, Wr graphic analysis indicated that disease resistance was due to additive effects. Generation means analysis by the same authors revealed additive, dominance and digenic epistatic gene effects to be important in the inheritance of host resistance.

Hart *et al.* (1984) screened 58 inbred maize lines and selected 10 lines with varying degrees of susceptibility. A diallel cross among these 10 lines was assessed for resistance to *G. zeae*. They found significant differences among lines in their response to the pathogen. General combining ability (GCA) was significant and specific combining ability was not, indicating that the reaction of single cross hybrids to infection to *G. zeae* could be predicted on the basis of the parental inbred reaction to *G. zeae*.

Crosses involving various generations derived from two susceptible and two resistant inbred maize lines were evaluated for their resistance to *G. zeae* by Gendloff *et. al* (1986). The generation means analysis revealed that the major genetic effect in the susceptible and resistant crosses was due to the additive gene effects. They also found genetic differences between the reciprocal crosses, which indicated possible maternal effects in disease response in segregating generations. Similar results were obtained by Chiang *et al.* (1987) studying the inheritance of resistance to Gibberella ear rot in maize. Their data also showed high narrow-sense heritability value

(68.8%) with three or more groups of genes to be involved in inheritance of the disease.

Szel (1984) studied resistance to *F. graminearum* in nine inbred *opaque-2* maize lines and 36 F_1 hybrids produced from them in diallel crossing. The inbreds used were chosen on the basis of their resistance to *F. graminearum*. They found general combining ability to be of importance in controlling resistance.

A diallel analysis study on the resistance of maize to *F. graminearum* infection via the silk was conducted by Reid *et al.* (1992b). The results of this study showed general combining ability (GCA) and specific combining ability (SCA) effects for mean disease ratings and incidence, to be significant, with the most resistant parent having a more negative GCA value than the most susceptible parent. Reid *et al.* (1994) examined resistance to infection via the silk in six generations of crosses between a highly resistant inbred line and three susceptible inbred lines. There appeared to be one major dominant gene affecting resistance, but its expression seemed to be sensitive to environmental conditions.

3.7. Resistance Mechanisms

Understanding of resistance mechanisms conferring resistance can facilitate selection of cultivars with high resistance. Both physical factors (eg. pericarp thickness, moisture content, kernel hardness) and chemical factors (eg. phenolic compounds) can play an important role in resistance.

According to Ullstrup (1953), inbred lines differ in resistance to ear rot.

Susceptible inbred lines often have 'silk cuts' or 'popped kernels'. These lines transmit weak pericarps to their hybrids and tend to be susceptible to ear rot. Schroeder and Christensen (1963) found two major factors to be involved in scab resistance caused by *G. zeae* in wheat. These factors include establishment of initial infection and rapidity of hyphal invasion of the host tissue. These authors speculated that resistance to pathogen spread in the host is probably due to physiological factors. Some factors may prevent initial penetration of the pathogen whereas different physiological factors may control both phenomena, resulting in varieties possessing either 1 or 2 types of resistance.

Ullstrup (1970) found that incidence of ear infection by *F. graminearum* was high two weeks after full silking. Similar observations were made by Reid *et al.* (1992a) after evaluating genotypes inoculated at different times after silking. It is possible that the decreasing moisture content of silk and/or kernels may affect the germination of the macroconidia and subsequent invasion of other kernels by fungal hyphae. Reid *et al.* (1992d) also suggested that accumulation of phenolic compounds in silk could be a resistance mechanism against *F. graminearum*.

The relative thickness of the pericarp may be of importance in the resistance to ear rot. Hoenisch and Davis (1994) compared the thickness of pericarp and aleurone layer in 12 dent hybrids representing a range of resistance to *F. moniliforme*. Pericarps were significantly thicker in intermediate and resistant hybrids than in susceptible hybrids.

The chemical composition of maize have been reported to be associated with

resistance to insects and diseases. Arnason *et al.* (1992) assessed phenolic compounds in maize grain as a possible indicator of resistance to two stored insects *Prostephanus truncatus* and *Sitophilus zeamais* (Motsch). Maize inbreds tested represented a taxonomic and latitudinal series. They reported that the mean weight loss of genotypes and the level of major phenolics were significantly different among the cultivars. They found (E)-ferulic acid content to be highly correlated with mean weight loss of the grain.

The relationship between (E)-ferulic acid content and disease resistance caused by *F. graminearum* in maize was investigated by Assabgui *et al.* (1993). This study showed a significant linear correlation between (E)-ferulic acid content and disease severity. Reid *et al.* (1992d) observed significant differences in the total concentration of phenolic compounds in the silk tissue of inoculated and non-inoculated plants. They found that following inoculation, the amount of phenolic compounds in a resistant inbred increased more than in susceptible inbreds.

Bergvinson and Reid (1995) recently found morphological differences between the silk tissue of resistant (CO272) and susceptible (CO266 and CO265) inbreds. They reported that the resistant inbred had a visibly thicker wax coating along the entire length of the silk. Analysis of silk wax revealed that the resistant inbred had four major wax constituents at higher levels than in susceptible inbreds. These wax constituents were simple hydrocarbons (C25-C31).

3.8. Inoculation Techniques

Screening genotypes for resistance to disease is an integral part of most breeding programs. Natural infection is unreliable in screening for resistance to ear rot by *F. graminearum*. Artificial inoculation methods often yield higher and more uniform levels of infection and allow differentiation of genotypes. Many studies have attempted to identify the most effective and efficient inoculation techniques for screening resistant genotypes.

Ullstrup (1970) evaluated two inoculation methods: a) spraying silks with a macroconidial suspension 1-2 weeks after silking, followed by covering of inoculated ears with wet paper towel and water repellent cap to ensure establishment, and b) inserting a toothpick colonized with fungal mycelium into the silk channel of the ear approximately 1 week before full silk emergence. While the toothpick method increased the incidence of ear rot, it was not effective in distinguishing between resistant and susceptible genotypes. On the contrary, Sutton and Baliko (1980) found placement of a toothpick colonized by *F. graminearum* in the silk channel near the cob tip at mid silk to be the most effective method for inoculating maize ears and differentiating resistance and susceptible genotypes.

Gulya *et al.* (1980) used ear-puncture and silk-spray techniques and compared them for their efficacy in screening resistance to maize ear rot incited by *F. graminearum*. They reported that the ear-puncture method produced high ear rot severity and made it easy to identify resistant genotypes.

Bolton *et al.* (1990) compared three inoculation methods to evaluate hybrid

tolerance to *F. graminearum*. The three inoculation techniques used were: (1) culturing the fungus on the toothpicks and inserting these directly through the husk into the middle of the ear 5-10 days after silk emergence, (2) immersing pipecleaner segments in a spore suspension and inserting these into holes made directly through the husks into the mid-ear 5-10 days after silk emergence, and (3) delivering approximately 2 ml of spore suspension into the upper silk channel using a hypodermic needle 4-7 days after silk emergence. They found the silk channel methods to closely approximate natural infection and to give consistent results for hybrids across strains. On the other hand, the results of the pipecleaner and toothpick methods were not clear, because these methods both failed to satisfactory and consistently differentiate resistance between hybrids. Reid *et al.* (1992a) used silk-channel injection and silk-spray inoculation methods to evaluate resistance of maize genotypes to *F. graminearum*. The results indicated that both techniques were able to incite disease, but the spray inoculation technique tended to yield lower disease ratings relative to the silk-channel injection.

Inoculation methods have also been compared in studies on *F. moniliforme* sheld. Boling *et al.* (1963) used three methods of inoculation (applying several ml of spore suspension on the exposed ear tip, injecting several ml of spore suspension into the tip of the ear with a hypodermic needle and syringe, and shooting one spore-covered BB into the ear about 2 inches below the ear tip with a pistol) to evaluate resistance to infection caused by *F. moniliforme*. The BB-pellet method yielded higher mean infection scores than the other two methods. Different ear and stalk

inoculation methods were compared for their effectiveness in creating high disease incidence and severity to infection caused by *F. moniliforme* (Drepper and Renfro, 1990). Ear inoculations methods used in this study were: injecting 2 ml of the spore suspension into the silk channel, shooting an inoculum-covered BB pellet with a gas pistol into the midear, inserting an infested toothpick into the middle of the ear and punching the middle of the ear with the nail punch. They found that shooting a BB pellet and using a nail punch were the most effective methods.

3.9 Distribution and Accumulation of Mycotoxins

Biosynthesis of mycotoxins depends on many factors, including the strain of the pathogen, the substrate, the period of colonization of the substrate, temperature, moisture and competing organisms (Eugenio *et al.,* 1970, Mirocha and Christensen, 1974; Naik *et al.*, 1978).

Distribution of aflatoxin content in individual maize kernels from ears naturally infected with *Aspergillus flavus* was assessed by Lee *et al.* (1980). They reported aflatoxin in kernels with no visible fungal development. These authors found highest levels of toxins in the germ portion of the kernel. They suggested that fungal infection was in the damaged pericarp region of the kernel, and subsequent intrakernel growth spread the toxins to adjoining kernels with no apparent fungal infection. They also postulated that fungal infection came from the cob and moved through the hilar layer into the kernel and continued to grow only in the lower region of the kernel.

Smart *et al.* (1990) analyzed individual kernels for aflatoxin produced by *A.*

flavus using enzyme-linked immunosorbent assay. To determine if aflatoxin can be translocated through the ear in the absence of hyphae, they sampled 21 kernels around wound-inoculated sites on maize ears. They found high variability in toxin levels within the ear. Some kernels with high toxin levels were adjacent to kernels lacking detectable aflatoxin. Among the 413 kernels assayed, 80% were aflatoxin-positive and showed signs of the fungus, and only 14% had detectable toxins levels without fungal signs. Based on this they concluded that long distance transport of aflatoxin does not happen in the absence of the hyphae.

Deoxynivalenol (DON) belongs to a group of compounds known as 12, 13-epoxytrichothecenes. These compounds are potent inhibitors of protein synthesis in animals. Inhibition of protein synthesis by the pathogen prevents or delays the host defenses, hence facilitating infection by the pathogen. DON is also implicated in a veterinary problem known as maize toxicosis of swine, the symptoms of which include a refusal to eat, digestive disorders, and diarrhea, leading to death.

Hart *et al.* (1982, 1987) observed that DON was produced consistently by several isolates of *F. graminearum* in each of inoculated varieties. They found that increased concentration of DON was positively correlated with increased disease ratings. The production of DON in each variety and by each isolate suggested that DON was a common metabolite of *F. graminearum* even though the toxin is difficult to quantify from cultures grown in a defined medium.

Variability in the concentrations of mycotoxins in fusarium infected crops may be related more to the time and extent of an infection and the genetics of the isolate

than to general weather pattern (Hart *et al.,* 1982). Host plant enzymes also play a role in determining the final level(s) of mycotoxin metabolites in the grain crop. The spread of fungal infection is however, governed by factors such as host-pathogen relationships, nutritional status and stress on the plant (Hart *et al.,* 1982; Sutton, 1982).

Miller *et al.* (1983b) stated that, after infection, toxin production is related to the extent of fungal infection. Young and Miller (1985) observed DON, a water soluble compound in regions of stalk not invaded by the fungi, which made them rule out *in situ* production. To explain distribution of DON below the cob attachment, they postulated that DON was being loosely conjugated to a substance being transported to the roots.

The types and levels of *F. graminearum* mycotoxins in kernels were assessed from three visibly identifiable zones of rotted ears as well as underlying portions of the cob by Bennett *et al.* (1988). Highest DON levels were found in severely rotted zones. Most of the symptomless kernels contained less than the detectable amounts of DON.

Visconti *et al.* (1990) investigated if mycotoxins are produced in maize ears infected by *F. graminearum* and *F. crookwellense* in the field. Kernels from zones of ear with more than 50% moldy kernels as well as underlying portions of cobs were analyzed. DON was detected in samples with *F. graminearum.* Reid *et al* (in press) inoculated ears with *F. graminearum* to examine the relationship between toxin content and disease severity. The amount of DON relative to severity was assessed in

three ear tissue fractions: symptomless kernels, symptomatic kernels and the cob tissue. The authors found visual evaluations were positively correlated with DON levels, and reported higher concentration of DON in cob tissue.

Preface of Chapter 4

This experiment was carried out to compare kernel, silk and cob tissue inoculation methods to identify methods that would be suitable for assessing kernel and silk resistance. This manuscript has been accepted for publication in Plant Disease and was co-authored by C. Chungu, D.E. Mather, L.M. Reid and R.I. Hamilton. Additional supporting data are presented in Appendix A.

COMPARISON OF TECHNIQUES FOR INOCULATING MAIZE SILK, KERNEL AND COB TISSUES WITH *Fusarium graminearum*

Abstract

Six inoculation techniques differing in the method of application of a conidial suspension and in the part of the ear inoculated were evaluated for their effectiveness in assessing maize (*Zea mays* L.) resistance to ear rot caused by *Fusarium graminearum*. Silk channel injection and ear-tip flooding inoculation techniques were carried out 7 days after silk emergence. The other four techniques (wound-spray, kernel-stab, pipecleaner and cob-tip) were carried out 15 days after silk emergence. A 7-class rating scale was used to assess disease severity at harvest. Significant differences ($P < 0.05$) in incidence and severity of ear rot symptoms were detected among the inbred lines and inoculation techniques. There were significant inbred x inoculation technique interactions, but inoculation techniques intended to measure the same resistance mechanism ranked inbred lines similarly in three of the four environments. All inoculation techniques except the ear-tip flooding technique identified CO325 as the most resistant inbred. Among the techniques used, the silk channel and the kernel-stab techniques appeared to be the most effective in measuring silk and kernel resistance, respectively.

Introduction

Ear rot caused by *Fusarium graminearum* Schwabe [sexual state: *Gibberella zeae* (Schwein) Petch] is a destructive disease of maize [*Zea mays* (L.)] in many parts of the world, including maize-growing regions of eastern Canada (Sutton, 1982; Ullstrup, 1970). Disease symptoms usually start from the tip of the ear, but occasionally they originate at the butt of the ear (Burgess *et al.* 1981). Ear rot can also be associated with damage caused by birds or insects such as corn borers. The fungus causes a pronounced reddish discoloration of the rotted grain and husk tissues and produces a pinkish-white mold on the surface of colonized grain. The disease has economic implications in that infection may lead to contamination of grain with mycotoxins, including deoxynivalenol (Mirocha and Christensen, 1974). These mycotoxins affect the performance of different species of livestock, with swine being the most susceptible.

Development of resistant maize hybrids could help control this disease. Because of the sporadic nature of epidemics, selection of resistant genotypes requires artificial inoculation. Numerous methods have been used for artificially inducing epiphytotics of maize ear rots (Reid *et al.* 1992a; Sutton and Baliko, 1981; Ullstrup, 1970). Ullstrup (1970) studied two inoculation techniques; spraying silks with a macroconidial suspension 1 to 2 weeks after silking and inserting a toothpick colonized with mycelium into the silk channel of the ear approximately 1 week before full silk emergence. Both methods established infection at levels that allowed differentiation between genotypes. Sutton and Baliko (1981) used the toothpick

method, spraying of silks, and a silk-channel injection method. They reported that silk-channel injection was ineffective in differentiating between resistant and susceptible genotypes. However, Reid *et al.* (1992a) reported that injection of a conidial suspension into the silk channel gave consistent results and allowed for differentiation between resistant and susceptible genotypes.

In the present study, six inoculation techniques, differing in the method of applying a macroconidial suspension and in the part of the ear inoculated, were evaluated with respect to their effectiveness in assessing genotypic differences in resistance to infection via the silk and to spread of infection on developing kernels of maize.

Materials and Methods

Field Trials: Experiments were grown in two locations, Macdonald Campus (Ste-Anne-de-Bellevue, Quebec) and Central Experimental Farm (Ottawa, Ontario) in 1992 and 1993. The experimental design was a split-plot design with inbred lines as main-plot factors and inoculation methods as subplot factors. Treatment combinations were replicated four times in both years. Each main plot consisted of seven rows. Rows were 2.4 m long at Ste-Anne-de-Bellevue and 3.8 m long at Ottawa. The experiments were seeded on May 12 in 1992 and 1993 at Ste-Anne-de-Bellevue, and on May 17, 1992 and May 20, 1993 at Ottawa. After emergence, plants were thinned to 12 plants per row.

Maize Genotypes: Six inbred lines (A641, CO266, CO265, CO272, CO325 and F2)

were evaluated. These lines were chosen to represent a range of levels of resistance to *F. graminearum*, based on results reported by Reid *et al.* (1992b).

Inoculum Preparation: *Fusarium graminearum* DAOM194276, a highly aggressive isolate, originally obtained from infected maize in Ottawa, and registered in the National Agriculture and Agri-Food Canada Culture Collection, was maintained on Synthetic Nutrient Agar (SNA), a modified form of Bilay's medium (Tuite, 1969). A macroconidial suspension was prepared as described by Reid *et al.* (1992c).

Ear Inoculation Techniques: Primary ears of the 10 middle plants of each row were inoculated with a 5 x 10^5 conidia/ml suspension using the following techniques:

1) **Silk Channel Injection**: 2 ml of inoculum were injected into the silk channel using a hypodermic needle (Fig. 1A).

2) **Ear-Tip Inoculation**: 2 ml of inoculum were dispensed with a hypodermic needle to flood the kernels at the tip of the ear (Fig. 1B).

3) **Kernel-Stab Inoculation**: A probe consisting of four nails (1.5 cm) fixed to a cylindrical wooden handle was used to inoculate the ear. The nails were dipped into inoculum and then used to stab through the husks and wound only three to four kernels in the middle of the ear (Fig. 1C).

4) **Wound and Spray Inoculation**: A probe rinsed in sterile water was used to wound the kernels as described above, then approximately 2 ml of spore suspension was sprayed on the wounded area with an atomizer (Fig. 1D)

5) **Pipecleaner Inoculation:** A 2-cm tunnel was made directly through the kernel and cob in the midear area with a nail. A 2-cm piece of pipecleaner

saturated with spore suspension was then inserted into the tunnel (Fig. 1E)

6) **Cob-Tip Inoculation**: Husks at the apex of the ear were opened to expose the tip of the cob. A tunnel about 1-cm deep was made into the cob tip using a battery operated drill. A 1-cm piece of pipecleaner saturated with spore suspension was then inserted into this apical tunnel. The apical husks were then clasped and a rubber band was used to hold the husks in position (Fig. 1F).

Inoculation methods 1 and 2 were carried out 6-7 days after silk emergence (between July 29 and August 10). The other four inoculation techniques (3 to 6) were carried out 15 days after silk emergence (between August 7th and 17th).

The trials were harvested in mid-October. Disease severity was assessed by rating the percentage of rotted area using a 7-class rating scale where; 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the infected ear (Bolton *et al.* 1990). Disease incidence was calculated as a percentage of inoculated ears that had ratings of 2 or above.

Data Analysis: Analysis of variance was performed using SAS PROC GLM (SAS/STAT User's Guide, version 6, 1994) on subplot means for disease severity values and disease incidence after verifying the assumptions for normality of data and homogeneity of variances. The effects of the inbred lines, inoculation techniques, and interactions were evaluated, and mean separation was tested by Duncan's Multiple Range test (Steel and Torrie, 1980). Spearman rank correlation coefficients were

calculated using SAS PROC CORR among inoculation techniques intended to measure the same resistance mechanisms.

Results and Discussion

Data were analyzed separately for each location and year because of significant location by year interactions for disease incidence and disease severity.

There were significant differences ($P<0.05$) in disease incidence among inbreds and among inoculation methods in all four environments, and highly significant ($P<0.01$) interactions of inbreds with inoculation methods in three of the four environments (Table 1). In 1992, disease incidence ranged from 9 to 100% (Table 2). In 1993, disease incidence was higher in both environments, with incidence values ranging from 65 to 100% (Table 3). Inbred CO266 was consistently among those with the highest disease incidence in all four environments. With most inoculation methods, inbreds CO272 and CO325 exhibited low disease incidence in 1992 at Ste-Anne-de-Bellevue and Ottawa. These lines may have mechanisms that inhibit the initial onset of infection.

Variation in disease severity (Table 1) was highly significant ($P<0.01$) for inbreds, inoculation methods, and interactions between inbreds and inoculation methods, in all four environments. Symptoms were least severe on inbred CO325, while CO266 and CO265 were among the most severely infected lines over the four environments (Tables 4 and 5).

In both locations, disease severity was higher in 1993 than in 1992. This was

probably due to differences in weather conditions between the 2 years. Rainfall was above normal in both years. July and August were wetter and cooler in 1992 than in 1993. Precipitation in September and October was higher in 1993 than in 1992. September and October air temperatures were similar in the four environments except that it was relatively warm in Ottawa in September 1992. The high disease incidence and disease severity in 1993 relative to 1992 may be explained by warm moist conditions during and shortly after the inoculation period. Tuite *et al.* (1974) reported that mean temperatures of 21° C during August are very conducive to outbreaks of ear rot. The high rainfall during September and October 1993 may also have favoured disease development. Koehler (1959) has postulated that high moisture in these months may facilitate infection by increasing inoculum production and by washing spores between the husks and ears when the ear tip is exposed. He also suggested that high moisture during October slows the natural drying process, making ears more prone to colonization for longer periods.

For disease severity, significant correlations were obtained between the kernel-stab and wound-spray inoculation techniques, and between the kernel-stab and pipecleaner inoculation techniques in three of the four environments (Table 6). Among these inoculation techniques, the wound-spray and the kernel-stab consistently identified CO325 and F2 as the most resistant inbreds. However, the wound-spray technique was not consistent in identifying inbreds that were intermediate or susceptible in their reaction.

With silk-channel inoculation, inbreds differed in resistance in all four

environments. Inbred CO272 showed high resistance at Ottawa, but was susceptible at Ste-Anne-de-Bellevue in both years (Tables 4 and 5). Although the silk-channel technique was not consistent in ranking inbreds that were susceptible or intermediate in their reaction, it did identify inbreds CO325 and CO272 as the most resistant.

The ear-tip and wound-spray inoculation techniques were not consistent in how they ranked the inbreds in the four environments. These techniques do not seem to be effective in identifying differences in resistance to ear rot.

The kernel-stab and pipecleaner inoculation techniques produced relatively severe disease symptoms in all four environments (Table 4 and 5). These inoculation techniques produced highly localized infections, probably because inoculum was deposited at a single point. The size of lesions should depend only on kernel resistance, and not on passive movement of inoculum as in the ear-tip inoculation method. The high level of ear rots obtained with these two inoculation techniques may be due to the circumvention of a normal physical barrier that serves to reduce the amount of inoculum reaching the kernels. Both techniques were able to identify one inbred (CO325) possessing a high level of kernel resistance. The pipecleaner technique, however, incited very severe infection in most of the inbreds in all four environments. Better separation of resistant and susceptible inbreds was obtained with the kernel-stab technique, and this method may be the most efficient for identifying true differences in kernel resistance to ear rot.

The kernel-stab technique requires very small amounts of inoculum, is easy to implement, causes minimal damage to kernels and mimics damage caused by insects

or birds. The relative inefficiency of the pipecleaner technique may have been due to comparatively larger amounts of inoculum, and to severe damage caused by piercing the ear. The latter may have resulted in more intense wound healing reactions, leading to other possible adverse effects that may have affected disease development.

The cob-tip technique was used to attempt to evaluate resistance to infection in the cob tissue. It resulted in very low disease severity in both years. It failed to clearly differentiate among inbreds, even in 1993 when the weather conditions were very favorable for infection.

Using the silk channel technique, Reid *et al.* (1992a, 1992c) reported that the inbred CO272 had a high level of silk resistance. In the present study, CO272 appeared resistant at Ottawa but susceptible at Ste-Anne-de-Bellevue. This is consistent with the observation of Reid *et al.* (1994) that resistance in silk tissue can be environmentally sensitive. Although CO272 was susceptible at Ste-Anne-de-Bellevue, ears inoculated with the silk-channel injection technique had less disease than ears inoculated with other methods (Table 2 and 3). Resistance in the silk may have slowed infection sufficiently to limit the development of symptoms. The results of this study support previous reports that CO272 resists infection via the silk. However, this inbred does not appear to have any resistance mechanism to slow the spread of the fungus from kernel to kernel.

Inbred CO325 exhibited a high level of resistance when inoculated with the silk-channel injection in all four environments (Table 2 and 3). Reid *et al.* (1993) also reported that CO325 was more resistant than other inbreds when inoculated in the

silk channel. This inbred also had the least disease from wound-spray, kernel-stab, and pipecleaner inoculation (Table 4 and 5). The resistance mechanism(s) of CO325 may be located in the kernel and/or cob tissue (Reid *et al.* 1992d), and perhaps in the silk tissue. An inbred with both silk and kernel resistance would be very useful in maize breeding.

In conclusion, genetic differences for resistance to infection caused by *F. graminearum* in ears of maize were observed among maize inbreds. All inoculation techniques except the ear-tip flooding method identified CO325 as the most resistant inbred. This study also confirms the results of previous studies that CO272 possesses some silk resistance, and that this resistance seems to be environmentally sensitive. In this study the silk-channel method was effective in measuring silk resistance, while the kernel-stab technique was able to clearly and consistently differentiate between inbreds possessing kernel resistance. To reduce infection via the silk and silk-channel and/or keep disease from spreading on developing kernels, breeders may need to incorporate both silk and kernel resistance mechanisms into maize hybrids.

Figure legend

Figure. 4.1. Diagram of six inoculation techniques evaluated: A. Silk-channel injection; B. Ear-Tip flooding; C. Kernel-stab inoculation; D. Wound and Spray inoculation; E. Pipecleaner inoculation; F. Cob-tip inoculation.

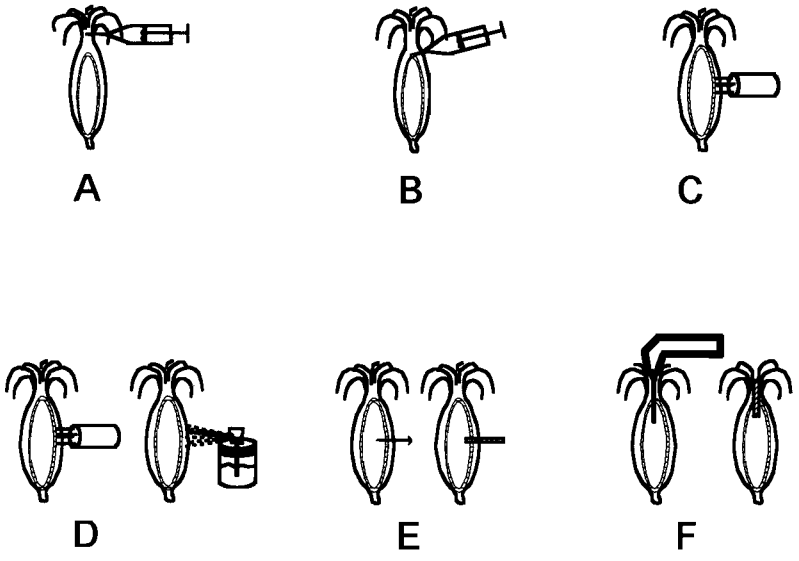

A
B
C
D
E
F

Table 4.1. Mean squares for disease severity and disease incidence in experiments conducted at Macdonald Campus (Ste-Anne-de-Bellevue) and Central Experimental Farm (Ottawa) in 1992 and 1993.

Source of variation	Df	1992		1993	
		Severity	Incidence	Severity	Incidence
Ste-Anne-de-Bellevue					
Block	3	0.12	281.07	0.48	159.56
Inbred	5	25.77**	10288.16**	14.48**	258.50*
Block × Inbred	15	1.18**	472.32	0.37	99.15
Inoculation (I)	5	17.13**	4146.68**	9.38**	179.31
Inbred × I	25	0.93**	591.23**	3.00**	97.16
Error	90	0.45	274.58	0.52	99.86
Ottawa					
Block	3	0.39	595.51	0.57	218.32
Inbred	5	26.14**	7880.17**	14.81*	513.61*
Block × Inbred	15	1.15**	408.95	0.73	118.77
Inoculation (I)	5	20.18**	7053.16**	16.10**	367.15**
Inbred × I	25	1.85**	1228.57**	2.39**	216.67*
Error	90	0.47	325.31	0.41	101.55

*,** significant at 0.05 and 0.01 probability level, respectively

Table 4.2. Mean disease incidence of six inbred lines of maize inoculated with *Fusarium graminearum* using six inoculation techniques at Macdonald Campus (Ste-Anne-de-Bellevue) and Central Experimental Farm (Ottawa) in 1992.

Inbred	Silk-Channel	Ear-Tip Flooding	Wound-Spray	Kernel-stab	Pipecleaner	Cob-Tip
Ste-Anne-de-Bellevue						
A641	72.5ab[z]	57.5b	72.5b	85.0ab	82.5ab	85.0a
CO265	80.0ab	45.0bc	76.9ab	87.2ab	85.0ab	77.5a
CO266	93.7a	86.1a	100.0a	100.0a	100.0a	100.0a
CO272	58.3bc	42.5bcd	57.5b	60.8c	72.2b	27.5b
CO325	31.9c	15.0d	68.9b	75.0abc	65.8b	26.9b
F2	58.3bc	20.6cd	32.2c	49.0c	77.3b	23.4b
Ottawa						
A641	89.7a	62.3ab	78.9ab	85.0a	100.0a	75.0a
CO265	97.2a	57.5b	35.0c	70.0a	100.0a	81.9a
CO266	97.2a	94.7a	95.0a	97.2a	97.2a	97.2a
CO272	35.3a	35.3bc	25.0c	95.0a	97.5a	30.0b
CO325	35.0b	9.2c	56.9bc	75.0a	97.5a	25.0b
F2	84.4a	35.0bc	57.5bc	81.7a	95.0a	83.9a

[z]Means within column and site followed by same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

Table 4.3. Mean disease incidence of six inbred lines of maize inoculated with *Fusarium graminearum* using six inoculation techniques at Macdonald Campus (Ste-Anne-de-Bellevue) and Central Experimental Farm (Ottawa) in 1993.

Inbred	Silk-Channel	Ear-Tip Flooding	Wound-Spray	Kernel-stab	Pipecleaner	Cob-Tip
Ste-Anne-de-Bellevue						
A641	95.0a[z]	100.0a	100.0a	100.0a	100.0a	100.0a
CO265	100.0a	80.6a	100.0a	100.0a	100.0a	94.4a
CO266	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
CO272	96.9a	100.0a	91.7a	100.0a	100.0a	100.0a
CO325	83.3a	81.1a	93.7a	100.0a	100.0a	87.3b
F2	92.3a	100.0a	90.0a	100.0a	100.0a	92.7ab
Ottawa						
A641	97.5a	96.9a	100.0a	100.0a	100.0a	100.0a
CO265	97.5a	100.0a	90.0ab	100.0a	100.0a	86.7a
CO266	95.8a	97.5a	100.0a	100.0a	100.0a	100.0a
CO272	86.7a	84.4b	95.0a	97.2a	100.0a	97.5a
CO325	65.0b	92.5ab	85.0ab	97.5a	97.5a	85.0a
F2	97.5a	95.0ab	68.9b	90.0a	100.0a	97.5a

[z]Means within column and site followed by same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

Table 4.4. Mean disease severity (and rankings) of six inbred lines of maize inoculated with *Fusarium graminearum* using six inoculation techniques at Macdonald Campus (Ste-Anne-de-Bellevue) and Central Experimental Farm (Ottawa) in 1992.

Inbred	Silk-Channel	Ear-Tip Flooding	Wound-Spray	Kernel-stab	Pipecleaner	Cob-Tip
Ste-Anne-de-Bellevue						
A641	3.7ab[z] (3)	2.9bc (4)	4.5ab (4)	4.2ab (3)	5.9ab (3)	3.8ab (5)
CO265	4.8a (6)	3.3b (5)	5.0a (5)	5.7a (6)	6.6a (4)	3.4b (4)
CO266	4.7a (5)	4.5a (6)	5.5a (6)	5.2a (5)	5.9ab (3)	4.6a (6)
CO272	4.3ab (4)	2.5bcd (3)	3.8bc (3)	5.0a (4)	5.0b (2)	2.4c (3)
CO325	2.3c (1)	1.6d (1)	3.0cd (2)	3.3bc (2)	3.6c (1)	1.8c (1)
F2	3.2bc (2)	2.1cd (2)	2.1d (1)	2.8c (1)	3.6c (1)	2.3c (2)
Ottawa						
A641	3.9bc (3)	2.9bc (4)	3.7b (5)	3.9b (3)	5.8a (3)	3.5bc (3)
CO265	5.0ab (5)	3.5b (5)	3.0a (4)	4.6a (4)	6.0a (5)	4.0b (5)
CO266	6.0a (6)	4.8a (6)	6.1a (6)	6.0a (6)	6.7a (6)	5.2a (6)
CO272	2.9cd (2)	2.8cd (3)	2.3b (1)	5.9a (5)	5.9a (4)	2.5cd (2)
CO325	2.7d (1)	1.6d (1)	2.7b (2)	3.1b (1)	3.8b (1)	2.0d (1)
F2	4.9b (4)	2.4cd (2)	2.8b (3)	3.2b (2)	4.3b (2)	3.4cb (4)

[z]Means within column and site followed by same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

Table 4.5. Mean disease severity (and rankings) of six inbred lines of maize inoculated with *Fusarium graminearum* using six inoculation techniques at Macdonald Campus (Ste-Anne-de-Bellevue) and Central Experimental Farm (Ottawa) in 1993.

Inbred	Silk-Channel	Ear-Tip Flooding	Wound-Spray	Kernel-stab	Pipecleaner	Cob-Tip
Ste-Anne-de-Bellevue						
A641	4.1ab[z] (2)	4.3b (2)	4.6b (3)	4.8b (2)	5.8a (4)	3.4b (3)
CO265	4.4ab (3)	4.3b (2)	5.4ab (5)	5.5ab (3)	5.7a (3)	3.7b (5)
CO266	4.5ab (4)	4.2b (1)	5.8a (6)	5.9ab (4)	6.4a (5)	5.9a (6)
CO272	4.8b (5)	6.0a (5)	5.2ab (4)	6.5a (5)	6.5a (6)	3.8b (4)
CO325	3.4b (1)	4.6ab (3)	3.4c (2)	3.6c (1)	4.3b (2)	2.4c (1)
F2	5.3a (6)	5.9a (4)	2.1d (1)	3.6c (1)	3.8b (1)	2.4c (2)
Ottawa						
A641	4.7ab (4)	4.5a (1)	4.8a (3)	5.3a (3)	6.2b (6)	3.5b (5)
CO265	4.2abc (3)	4.6a (2)	4.9a (4)	5.6a (4)	5.7cd (4)	2.0cd (2)
CO266	5.2a (6)	5.5a (5)	4.9a (4)	5.7a (5)	6.0a (5)	4.8a (6)
CO272	3.3c (2)	4.5a (1)	5.1a (5)	5.7a (5)	5.5c (3)	2.6c (4)
CO325	2.8c (1)	4.7a (3)	2.6b (2)	3.4b (1)	3.6b (1)	1.8d (1)
F2	5.3a (5)	5.0a (4)	2.3b (1)	3.6b (2)	4.0cd (2)	2.5cd (3)

[z]Means within column and site followed by same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

Table 4.6. Rank correlations of disease severity at Ste-Anne-de-Bellevue and Ottawa in 1992 and 1993.

	Year	Location	Wound-Spray	Kernel-Stab	Pipecleaner
Kernel-Stab	1992	Ste-Anne-de-Bellevue	0.89*		
		Ottawa	0.37		
	1993	Ste-Anne-de-Bellevue	0.83*		
		Ottawa	0.99**		
Pipecleaner	1992	Ste-Anne-de-Bellevue	0.89*	0.87*	
		Ottawa	0.54	0.94**	
	1993	Ste-Anne-de-Bellevue	0.71	0.94**	
		Ottawa	0.43	0.43	
Cob-tip	1992	Ste-Anne-de-Bellevue			0.83*
		Ottawa			0.83*
	1993	Ste-Anne-de-Bellevue			0.89*
		Ottawa			0.77

*,** significant at 0.05 and 0.01 probability levels, respectively

Preface of Chapter 5

In the experiment described in Chapter 4, the kernel-stab inoculation method was identified as an effective method for screening for kernel resistance. This study was designed to assess the inheritance of kernel resistance to *F. graminearum* using the kernel-stab inoculation method. The manuscript has been submitted to the Journal of Heredity and was co-authored by C. Chungu, D.E. Mather, L.M. Reid and R.I. Hamilton.

INHERITANCE OF KERNEL RESISTANCE TO *Fusarium graminearum* IN MAIZE [*Zea mays* (L.)].

Abstract

Inheritance of maize (*Zea mays* L.) kernel resistance to ear rot caused by *Fusarium graminearum* Schwabe was investigated in generations derived between resistant (CO325) and susceptible (CO265) maize inbred parents. Parents, F_1, F_2 and backcross generations were evaluated in two locations in eastern Canada in 1993 and 1994. Plants were inoculated with macroconidial suspension using a kernel-stab method, 15 days after silk emergence. Disease severity was assessed at harvest using a 7-class kernel rating scale. Significant differences were observed among the six generation means in all the environments. In general, the F_1 did not differ significantly from the resistant parent except at one location in 1993. The frequency distribution of the F_2 and backcross generations showed continuous variation. Generation means analysis indicated that resistance to *F. graminearum* was under genetic control with both simple (additive and dominance) and digenic (dominance x dominance) effects contributing to the total genetic variation among the generation means. Weighted least square regression indicated that more than 68% of the genetic variation could be explained by additive effects. Estimates of the number of effective factors affecting kernel resistance ranged from 4.6 to 13.7.

Introduction

Fusarium graminearum Schwabe [sexual state: *Gibberella zeae* (Schwein) Petch] may enter maize (*Zea mays* L.) ears via the silk or through wounds made by insects or birds. According to Koehler (1942) entry via the silk and/or silk channel is the most common mode of entrance by many pathogens. Plants may require different resistance mechanisms to defend themselves against these modes of fungal entry. Reid *et al* (1992a,c) have presented evidence for resistance in the silk tissue, which acts by preventing the fungus from growing rapidly down the silk to the kernels. However, genotypes possessing this type of resistance mechanism may not have any means of inhibiting the spread of the fungus from kernel to kernel should the fungus bypass the silk or succeed in overcoming the silk resistance. Resistance mechanisms in the kernel tissue might be useful as alternatives or complements to silk resistance.

There have been several reports on the inheritance of resistance to *F. graminearum* and other *Fusarium* ear rotting pathogens, all based on studies in which artificial inoculation techniques were used. Boling and Grogan (1965) evaluated six generations derived from two inbred lines for gene effects involved in the resistance to *F. moniliforme* Sheld. They inoculated plants 10 days after silking by shooting a spore-covered BB pellet into the ear about 5 cm below the tip. They reported that inheritance of resistance to *F. moniliforme* was due to additive, dominance and epistatic gene effects and estimated that it involved two gene pairs. Odiemah and Manninger (1982) assessed the inheritance of resistance to *F. graminearum* in six generations using two inoculation methods: that study involved injection of about 2 ml

of spore suspension between the silks and placement of a colonized toothpick between the silks. They concluded that additive, dominance and epistatic effects were important. Chiang *et al.* (1987) used diallel analysis to investigate resistance to *F. graminearum*. Plants were inoculated by placing a colonized toothpick at the tip of the ear within the base of the silks. Results of that study showed that additive gene action were important in resistance to ear rot. Reid *et al.* (1992b) also used diallel analysis to study resistance to *F. graminearum* infection. They inoculated plants by injecting 2 ml of spore suspension into the silk channel. They reported that inheritance of silk resistance involves dominance. In another study, Reid *et al.* (1994) examined resistance to infection via the silk in six generations of crosses between a highly resistant inbred line and three susceptible inbred lines. There appeared to be one major dominant gene affecting resistance, but its expression seemed to be sensitive to environmental conditions.

In most of the studies cited above inoculum was either applied to the silks, or was introduced to the kernels by methods that wounded both the kernel and the underlying cob tissue. Wounding of the cob tissue could allow the pathogen to enter the kernels from the cob tissue rather than simply spreading from kernel to kernel. To assess differences in kernel resistance, it may be better to introduce inoculum to the kernels without wounding of the cob tissue. Chungu *et al.* (1996) and Reid and Hamilton (In press) have described the use of a kernel-stab inoculation technique that introduces macroconidial suspension to developing kernels without wounding of the cob tissue. The use of a conidial suspension may be preferable to the introduction of

mycelial tissue (as in the 'toothpick method'). The use of mycelial inoculum can lead to intense disease symptoms even in resistant lines, making it difficult to discriminate effectively between lines (Ullstrup, 1970). The purpose of the current study was to use the kernel-stab inoculation method to examine the inheritance of kernel resistance to *F. graminearum* ear rot in six generations of a cross between susceptible and resistant maize inbreds.

Materials and Methods

The parental lines used in this study were CO325, a resistant inbred, and CO265, a susceptible inbred. These inbreds were selected on the basis of their kernel resistance to ear rot caused by *F. graminearum* from an experiment conducted by Chungu *et al.* (1996). To acquire genetic material required for the experiments, we made a cross between resistant [CO325 (P_1)] and susceptible [CO265 (P_2)] parents. The resistant parent was used as the female parent. The F_2 generation was obtained by selfing the F_1 plants, and backcross generations derived by crossing the F_1 plants with each parent, using the F_1 as the pollinator. The six populations (P_1, P_2, F_1, F_2, BCP_1 and BCP_2) were evaluated at Ste-Anne-de-Bellevue (Quebec) and Ottawa (Ontario) in 1993 and 1994. We planted the six generations in single rows of 14 plants in a randomized complete block design with four replicates. However, only primary ears of the 12 center plants, in each row were used for disease assessments. In all experiments, the inter-row and intra-row spacings were 0.75 m and 0.20 cm, respectively. The parental and F_1 generations were grown in 7-row plots and only the

5 centre rows were inoculated and harvested. The segregating generations were planted in 10-row plots with only the 8 centre rows used for evaluating resistance. We produced inoculum using modified Bilay's liquid medium consisting of 2 g KH_2PO_4, 2 g KNO_3, 1 g $MgSO_4$, 1 g KCl, 1 g dextrose, 0.2 g $MnSO_4$, $FeCl_3$, 2 g $ZnSO_4$ in 1 L of water. A 1 cm^2 plug of *F. graminearum* culture maintained on potato dextrose agar was suspended in 250 ml of autoclaved liquid medium in a 500-ml erlenmeyer flask. Prior to inoculation, inoculum was filtered through two layers of cheese cloth and diluted to a spore count of 5 x 10^5 spores/ml. Ears were inoculated with the kernel-stab technique, 15 days after silk emergence. In this inoculation technique, a probe consisting of four nails (1.5 cm) fixed to a cylindrical wooden handle was dipped into inoculum and then used to stab through the husk to wound three to four kernels in the middle of the ear (Chungu *et al.* (1996), and Reid and Hamilton (In press). Inoculated ears were harvested in mid-October and assessed for the severity of infection, using a 7-class kernel-rating scale where; 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the ear infected (Bolton *et al.*, 1990). Ears with severity rating of three or lower were considered to be resistant, whereas those with rating of four were moderately resistant and above five were susceptible.

Data Analyses

A separate analysis of variance for disease severity was calculated for each environment. The generation means were computed and compared using the

Duncan's multiple range test (Steel and Torrie, 1980). Within-plot variances were estimated and pooled across the four replicates. Each generation mean was weighted by the reciprocal of the variance of the mean for that generation and the method of Mather and Jinks (1982) was used to estimate additive, dominance and epistatic genetic effects. Parameters not significantly different from zero were eliminated from the model, and goodness-of-fit of each model was tested by a weighted χ^2. Tests of significance for the gene effects were obtained by dividing estimates by their corresponding standard errors and subsequently comparing them with the two-tailed Student's t-test at 0.01 and 0.05 probability levels.

The additive genetic variance (V_A) was estimated using the following formula: $V_A = 2(V_{F2}) - (V_{BC1} + V_{BC2})$ *where* V_{F2}, V_{BC1}, and V_{BC2} represent variances of F_2 and of backcrosses to P_1 and P_2, respectively. This method assumes that the non-heritable component of variation are comparable for the F_2 and backcross generations. Narrow-sense heritability was computed as follows: $h^2 = V_A / V_{F2}$. The number of effective factors (K) controlling kernel resistance was estimated using the equation: $R^2_T / 8V_A$ (Falconer, 1989), where R_T is the difference between parental generations.

Results and Discussion

The kernel inoculation technique used in this study incited sufficient infection in most of the inoculated ears in 1993 and 1994 to differentiate between resistant and susceptible genotypes at both locations.

Due to heterogeneity of error variances, we performed separate analyses of

variance for each environment. Significant differences ($P < 0.05$) in disease severity were observed among the six generation means in each environment (Table 1). Means for the F_1 generation were similar to those for the resistant parent (CO325). Disease severity values were higher in 1993 than in 1994 at Ste-Anne-de-Bellevue, but the reverse was true at Ottawa. September rainfall was higher in 1993 than in 1994 at Ste-Anne-de-Bellevue. However, at Ottawa, it rained more frequently in 1994 than in 1993 during the two weeks after inoculation, thus providing the moisture needed for fungal growth. The differences observed in disease severity among these environments may be due to differences in rainfall.

The F_2 and the backcross generations showed continuous variation (Fig. 1). The distributions of the backcrosses were skewed towards their recurrent parents. From these distributions, it appears that several loci control the inheritance of kernel resistance to the pathogen. Contrary to expectations, the nonsegregating generations were as variable as the segregating generations in most environments. For the parents, inbreeding might have caused sensitivity to environmental effects. However, it is difficult to account for the variability observed in F_1 generations.

The regression analysis indicated that additive gene effects were predominant, explaining more than 68% of the total genetic variation among the generations (Table 2). Odiemah and Manninger (1982), who used diallel analysis, also found significant additive effects for resistance to *Fusarium graminearum* ear rot, but these effects were relatively small compared to the dominance gene effects. In this study, the magnitudes of the additive gene effects in comparison with the mean effects were

higher than those of dominance and dominance × dominance genetic effects, suggesting that additive gene effects are important in the inheritance of kernel resistance in the cross studied.

Dominance gene effects for resistance were negative and significant at Ste-Anne-de-Bellevue in 1994, but not in the other three environments (Table 2). However, in those three environments there were small but significant dominance × dominance interactions. Perhaps the locus or loci that caused the dominance effects in one environment interacted with other loci (or with each other) in the other three environments. There could also be opposing dominance effects at different loci; generation means analysis can fail to detect dominance in such cases (Boling and Grogan, 1965; Hallauer and Miranda, 1988). Certainly, it appears that the dominance component of the genetic variation in this cross interacts with environmental factors; this is similar to what Reid *et al.* (1994) reported for a dominant gene conditioning resistance to *F. graminearum* infection via the silk. The negative signs associated with the dominance effects suggest that dominance was in the direction of greater resistance.

Because we collected data on an individual plant basis, it was possible to examine variances as well as means. The variances obtained from the segregating generations permitted estimation of the heritable component of variation and the number of effective factors controlling kernel resistance. More than 56% of the total variation was attributable to additive variation (Table 3), corresponding to the results of the generation means analysis. The estimates for number of genes affecting kernel

resistance were 5.8 and 13.6 at Ste-Anne-de-Bellevue, and 13.7 and 4.6 at Ottawa, in 1993 and 1994, respectively. These estimates support the conclusion of Chiang *et al.* (1987) that 3 or more genes could be involved in controlling resistance to ear rot.

The kernel-stab inoculation method was consistent at inciting infection in all four environments, a prerequisite for effective selection. Results presented here indicate that additive, dominance and dominance × dominance gene effects are important in the inheritance of kernel resistance to *F. graminearum*, with a predominance of additive gene effects. It may therefore be possible to identify plants with intermediate resistance from crosses derived between resistant and susceptible parents. Since only two inbred parents were used, caution should be exercised when making inferences on results obtained in this work. The limitation(s) involved in using two fixed parents, could be addressed by carrying out a more elaborate study with several inbred parents. However, the experiments described here provide some evidence that inheritance of kernel resistance can be due to several genes.

Table 5.1. Mean ear rot symptom severity ratings[a] of generations from a cross between maize inbreds CO325 and CO265 inoculated with *Fusarium graminearum* at two sites in two years.

	Ste-Anne-de-Bellevue		Ottawa	
Generation	1993	1994	1993	1994
CO325 (P1)	3.43c[z]	2.99e	2.93e	4.38b
BCP1	3.59c	3.22d	3.28d	4.39b
F_1	3.48c	3.15de	3.14d	4.16b
F_2	4.74b	3.67c	3.79c	4.42b
BCP2	5.41a	4.10b	4.46b	5.38a
CO265 (P2)	5.29a	5.10a	4.71a	5.63a

[z]means followed by the same letter within the columns are not significantly different.

[a]Based on a scale of 1-7, where 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the ear infected.

Figure Legend

Figure 5.1. Frequency distribution of disease severity of six generations at Ste-Anne-de-Bellevue in 1993 (A) and 1994 (B), and at Ottawa in 1993 (C) and 1994 (D).

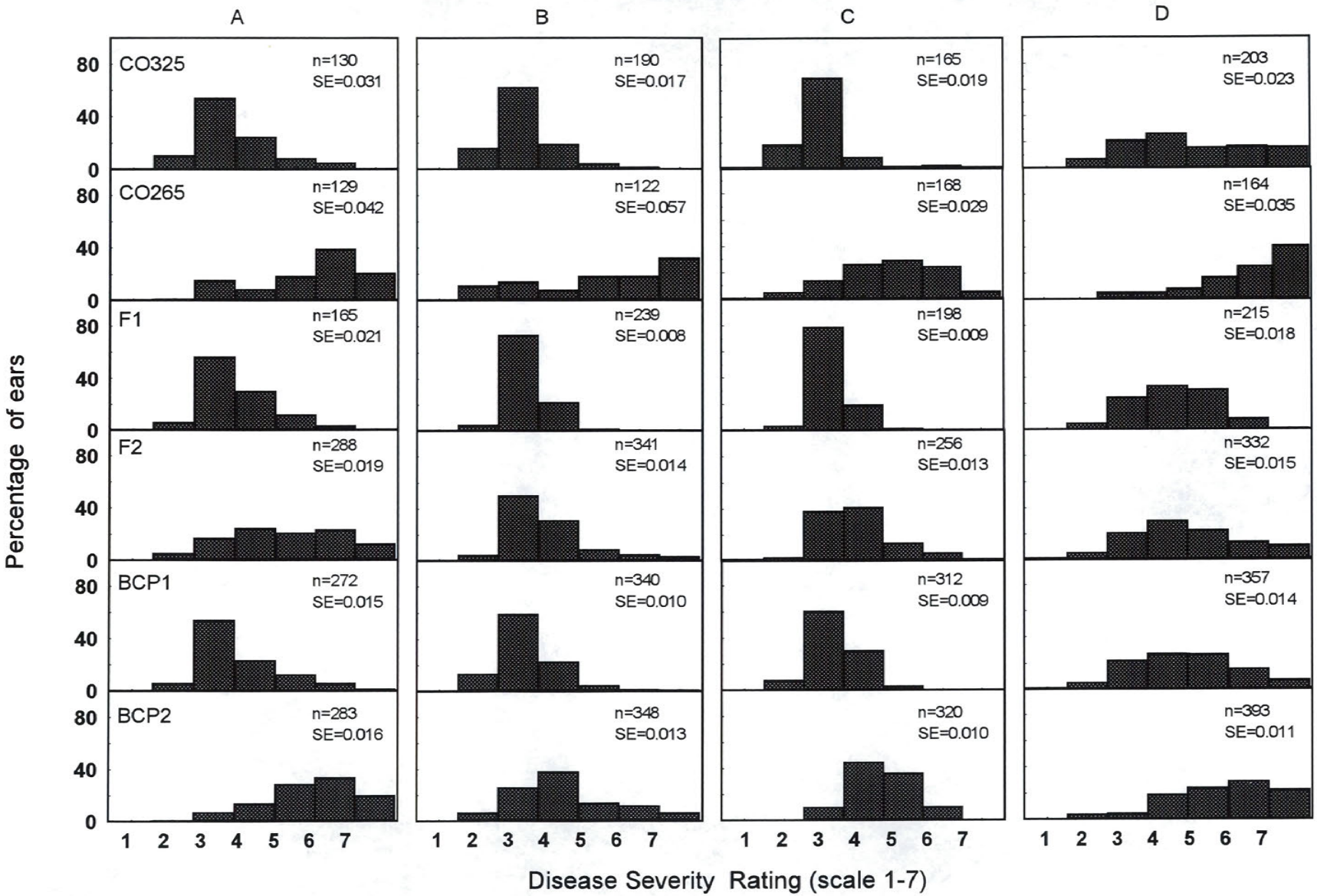

A
B
C
D
CO325
n=130
SE=0.031
n=190
SE=0.017
n=165
SE=0.019
n=203
SE=0.023
CO265
n=129
SE=0.042
n=122
SE=0.057
n=168
SE=0.029
n=164
SE=0.035
F1
n=165
SE=0.021
n=239
SE=0.008
n=198
SE=0.009
n=215
SE=0.018
F2
n=288
SE=0.019
n=341
SE=0.014
n=256
SE=0.013
n=332
SE=0.015
BCP1
n=272
SE=0.015
n=340
SE=0.010
n=312
SE=0.009
n=357
SE=0.014
BCP2
n=283
SE=0.016
n=348
SE=0.013
n=320
SE=0.010
n=393
SE=0.011
0
40
80
1 2 3 4 5 6 7
Percentage of ears
Disease Severity Rating (scale 1-7)

Table 5.2. Estimates of additive, dominance and dominance × dominance genetic effects on *Fusarium graminearum* ear rot symptom severity, and the relative contributions of these effects to the genotype sum of squares (% SS), in generations from a maize cross CO325 × CO265, at two sites and two years.

	Ste-Anne-de-Bellevue				Ottawa			
Estimate	1993	%SS	1994	%SS	1993	%SS	1994	%SS
m[a]	4.73**		4.73**		4.02**		5.01**	
[d]	-1.33**	98.8	-1.00**	68.5	-1.05**	95.9	-0.77**	83.9
[h]	-		-0.90*	31.5	-		-	
[i]	-		-		-		-	
[l]	-		-		-		-	
[j]	-1.17**	1.2	-		-0.87*	4.1	-0.86**	16.1

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

[a] m=mean, d=additive, h=dominance, i=additive x additive, l=additive x dominance and j=dominance x dominance.

Table 5.3. Estimates of additive genetic variance (V_A), F_2 variance, narrow-sense heritability (h^2) and number of effective factors (K) for kernel resistance to *Fusarium graminearum* ear rot in generations derived from a maize cross CO325 × CO265, in experiments grown at two sites in two years.

	Ste-Anne-de-Bellevue		Ottawa	
Generation	1993	1994	1993	1994
V_A	0.075	0.041	0.029	0.042
V_{F2}	1.104	0.067	0.043	0.075
h^2	0.720	0.610	0.670	0.570
K	5.760	13.570	13.660	4.650

Preface of Chapter 6

Waxy and non-*waxy* lines were compared for their resistance to infection via the silk and kernels after inoculation with the silk-channel injection and the kernel-stab methods. This work has been submitted as an observational note to Maize Genetics Cooperation Newsletter. The note is co-authored by C. Chungu and D.E. Mather.

REACTION OF WAXY AND NON-WAXY MAIZE INBREDS INFECTED WITH *Fusarium graminearum*

Abstract

The responses of *waxy* and non-*waxy* maize (*Zea mays* L.) inbreds to ear rot caused by *Fusarium graminearum* were compared in 1993. The experiment was carried out in a split-plot design with three replications. Inbreds were randomized as main-plot units and the two inoculation methods (silk channel and kernel-stab) as subplot units. Disease severity was significantly different among inbreds and inoculation methods. Although differences were observed among these inbreds, the reaction of *waxy* and non-*waxy* inbreds to infection by *F. graminearum* was similar.

Introduction

Fusarium graminearum Schwabe, the asexual state of *Gibberella zeae* (Schwein) Petch causes ear rot of maize in most maize growing areas in the world. The pathogen penetrates ears by the growth of the mycelia down the silks to the kernels or through wounds made by insects or birds. The characteristic symptom of the disease is a pink to reddish coloration on the surface of infected kernels and husks.

Warren (1978), observed that some *opaque-2* maize inbreds were more susceptible to *F. moniliforme* ear rot than their normal-endosperm counterparts. Similar observations were reported by Reid *et al.* (1992c) with *F. graminearum.* Kernels of *opaque-2* maize tend to be soft, which may allow pathogens to penetrate the kernels easily.

In normal dent maize, the endosperm starch is composed of 75% amylopectin and 25% amylose, whereas in *waxy* maize, the endosperm starch is 100% amylopectin. This difference is important to manufacturers of food and industrial products. According to Coe *et al.* (1988), the *waxy* kernel type displays uniform marble-like opacity and has kernel hardness similar to that of normal kernels. Little is known about the relative resistance of *waxy* inbreds and their non-*waxy* counterparts to ear rot caused by *F. graminearum.* The objective of this study was to compare the responses of *waxy* and non-*waxy* inbreds to *F. graminearum.*

Materials and Methods

An experiment was conducted at Ste-Anne-de-Bellevue (Quebec, Canada) in 1993. (The experiment was seeded again in 1994, but failed due to poor germination). Eleven *waxy* and non-*waxy* inbreds (seed provided by David Bauté and R.I. Hamilton) were planted in a split-plot design with four replications. Inbreds were randomized as main-plot units and two inoculation methods (silk channel injection and a kernel-stab technique) as subplots. Individual ears were inoculated by: (a) injecting 2 ml of the macroconidial suspension in the centre of the silk channel using the silk-channel injection method, seven days after silk emergence, and (b) by inoculating the ears using a kernel-stab technique, 15 days after silk emergence. In the latter technique, a probe consisting of four nails (1.5 cm) fixed to a cylindrical wooden handle was dipped into inoculum and then used to stab through the husk to wound three to four kernels in the middle of the ear. Primary ears of the *waxy* inbreds were bagged before silking to avoid contamination with pollen from their normal counterparts and were later hand-pollinated. Inoculated ears were harvested in mid-October and disease severity assessed by rating the percentage of rotted area using a 7-class kernel rating scale where; 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the infected ear. Disease incidence was calculated as the percentage of ears with severity rating of 2 or greater. Data were analyzed using general linear models, and mean comparisons were performed using Duncan's multiple range test.

Results and Discussion

Effects due to inbreds were statistically significant ($P<0.05$) for both disease incidence and severity (Table 1). Differences between the two inoculation methods were significant only for disease severity.

Disease incidence values were high for both *waxy* and non-*waxy* inbreds. Most inbreds exhibited high disease severity with both inoculation methods. Three inbreds (A641wx, LH82 and MO17wx) had only moderate disease severity after silk-channel injection. However, these inbreds were all susceptible with the kernel-stab method. One inbred (MO17wx) exhibited lower disease severity than its normal counterpart. It appears that most of the inbreds evaluated in this study do not have sufficient resistance in the silk and kernels to slow or inhibit the spread of ear rot.

To avoid pollen contamination, the ears of the *waxy* inbreds were bagged prior to silking and the bags remained on the ears four weeks postinoculation. The environmental conditions within the bags could have influenced the spread of ear rot on the *waxy* inbreds. Enerson and Hunter (1980), found increased colonization intensity in ears inoculated with a toothpick and bagged for 35 to 63 days. In contrast, Sutton and Baliko (1981) found that bagging after inoculation suppressed the growth of *F. graminearum*. In this study, it was not possible to determine the effect bagging had on disease development.

This study shows that the inbreds differed in their reaction to infection by *F. graminearum* when the silk channel method was used, however none of the *waxy* inbreds differed from their non-*waxy* counterparts. No significant difference was

observed among inbreds when inoculum was directly applied to the kernels. We did not find any evidence that the *waxy* endosperm trait confers ear rot resistance or susceptibility. However, our comparisons of *waxy* vs non-*waxy* lines were confounded by the fact that ears of the *waxy* lines were bagged to prevent pollen contamination.

Table 6.1. Mean values for disease severity and incidence *waxy* and non-*waxy* inbred lines within inoculation techniques at Ste-Anne-de-Bellevue in 1993.

	Silk-Channel		Kernel-stab	
Genotype	Severity	Incidence	Severity	Incidence
A632	5.4ab[†]	92[a]	5.6	96
A632Htwx	5.5ab	100	5.4	96
A641	5.1ab	100	5.7	100
A641Htwx	4.7ab	100	5.7	100
CM105	6.1a	100	5.1	100
CM105wx	5.8ab	100	6.1	100
LH74*LH146wx	5.8ab	100	6.2	100
LH82	4.5b	100	5.5	100
LH82wx	5.3ab	100	5.8	100
Mo17Ht	6.2a	100	6.1	100
Mo17wx	4.5b	100	4.9	100

[†]Means followed by the same letter within columns are not significantly different at 0.05 probability level.

[a]Not significantly different

Preface of Chapter 7

Zapalote Chico has been reported to be a possible germplasm source for resistance to ear rot caused by *F. graminearum*. This study was conducted to screen individual plants of Zapalote Chico and their selfed progenies for resistance to ear rot in an effort to determine if resistance in this population can be manipulated in a breeding program.

RESISTANCE OF ZAPALOTE CHICO TO EAR ROT CAUSED BY *Fusarium graminearum*

Abstract

In 1992, 200 Zapalote Chico plants were evaluated for resistance to *F. graminearum* in a preliminary test. Based on the results of this test, 20 selfed progenies (S1 lines) and a resistant inbred check (CO272) were evaluated in 1993. The experiment was carried out in a randomized complete block design with three replications. Individual ears were inoculated with a silk-channel injection method seven days after silk emergence. Disease severity was assessed using a 7-class rating scale in mid-October. Severity ratings among the individual plants and S1 lines ranged between 1 and 7. Plant pigmentation was also quite variable in Zapalote Chico, but was not associated with resistance. Several S1 lines had disease severity and incidence values that were significantly lower than the resistant check (CO272). Correlation between the parents and their progenies was relatively high and significant. The results suggest that the resistance exhibited in this population may be useful in a breeding program.

Introduction

Ear rot caused by *Fusarium graminearum* Schwabe [Teleomorph: *Gibberella zeae* (Schwein) Petch] is a disease of concern in many parts of the world (Sutton, 1982; Marassas et al. 1979). Few presently available inbreds and hybrids possess high and stable resistance to *F. graminearum.* Zapalote Chico is a Mexican maize landrace known for its resistance to insect damage (Wiseman and Widstrom, 1992; Wilson *et al.* 1991).

Reid *et al.* (1992e) observed that after inoculation of macroconidial suspension of *F. graminearum* into the silk channel, some plants within Zapalote Chico had disease ratings of 6 or 7, but most had disease ratings of 1 or 2. They speculated that there may be genetic segregation for resistance in this population.

Anthocyanins and related flavonoid pigments are found in different parts of the maize plant, including the tassel, cob, nodes, silk and pericarp. Plant pigments have been reported to aid in pollination because they attract insects and may also act as a feeding deterrent to some insects (Jayaram and Peterson, 1990). These authors also reported that these compounds have a negative effects on fungal growth.

Resistance to any pathogen is more useful if it can be transmitted to progenies. This study was conducted to evaluate the variation in resistance to infection by *F. graminearum* in Zapalote Chico, to assess whether that resistance is heritable and to examine if resistance is related to plant pigmentation.

Materials and Methods

In 1992, 200 Zapalote Chico plants were screened in a preliminary test for their resistance to ear rot caused by *F. graminearum*. Plants were labelled as ZAPC1 to ZAPC200 for identification purposes. Primary ears were bagged and self-pollinated. Secondary ears were inoculated by injecting 2 ml of the macroconidial suspension 7 days after silk emergence using the silk channel injection technique (Reid *et al.* 1992a). Plant pigmentation was assessed for each plant on a scale of 1 - 5, where 1 = no purple pigmentation, 2= very light purple, 3= light purple, 4= dark purple, and 5= very dark purple pigmentation. Inoculated ears were harvested in mid-October. Disease severity was assessed by rating the percentage of rotted area using a 7-class kernel rating scale where; 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the infected ear (Bolton *et al.*, 1990). Based on the results of the preliminary test, eight S1 lines from plants with severity ratings of four or less, and twelve S1 lines from plants with ratings greater than 4 were evaluated along with the inbred CO272 in a randomized complete block design with three replications. The centre plants of each row were inoculated with a macrocondial suspension using the silk channel injection technique. Disease severity was assessed in mid-October. Analyses of variance were conducted with General Linear Model and Duncan's multiple range test for mean comparisons (Steel and Torrie, 1980).

Results and Discussion

From our previous experiments with Zapalote Chico, we expected plants to be prolific (i.e. multiple ears per plant) but of the 200 plants screened in 1992, only 38 produced two ears. Sixteen of these exhibited severity ratings of 1 to 4, and 22 had severity ratings ranging from 5 to 7 (Table 1). Only 20 of the selfed ears produced sufficient seeds for replicated evaluation in the S1 generation.

Plant pigmentation among the inoculated plants ranged from no purple color to very dark purple, with both resistant and susceptible plants exhibiting pigments that ranged between these two colors. This observation suggests that resistance of individual plants within the population can not be predicted based on plant pigmentation. Plant pigmentation was also variable, among and within the S1 lines.

There were significant ($P<0.01$) difference among S1 lines for disease incidence and severity (Table 1). Disease incidence and severity levels of some lines were lower than the resistant check CO272 (Table 1). Three Zapalote Chico lines, ZapC154, ZapC18 and ZapC37 were highly resistant. Among these, ZapC154 had disease incidence of 13% and severity of 1.73. Some S1 lines (ZapC7, ZapC24, ZapC40, ZapC71, ZapC72, ZapC93 and ZapC97) had severity values greater than 5. Within each of these lines, individual plants exhibited disease severity ranging from 1 to 7. This observation suggests a wide variation in resistance to infection by *F. graminearum*, consistent with the observation made by Reid *et al.* (1992e).

Variation within five S1 lines (ZapC11, ZapC37, ZapC118, ZapC139, and ZapC154) was lower compared to other S1 lines. These five lines had a range of 3 to

4 compared to 6 for other lines. It appears that resistance in these lines could be fixed through selfing.

Disease severity of parents and their progenies are plotted in Fig. 1. The means of the resistant S1 lines were all clustered on one side and that of susceptible of the other. Significant differences ($P<0.05$) were observed between the overall mean of the S1 progeny of resistant and susceptibles plants. This observation indicates that improvement in resistance in this population can be achieved through breeding.

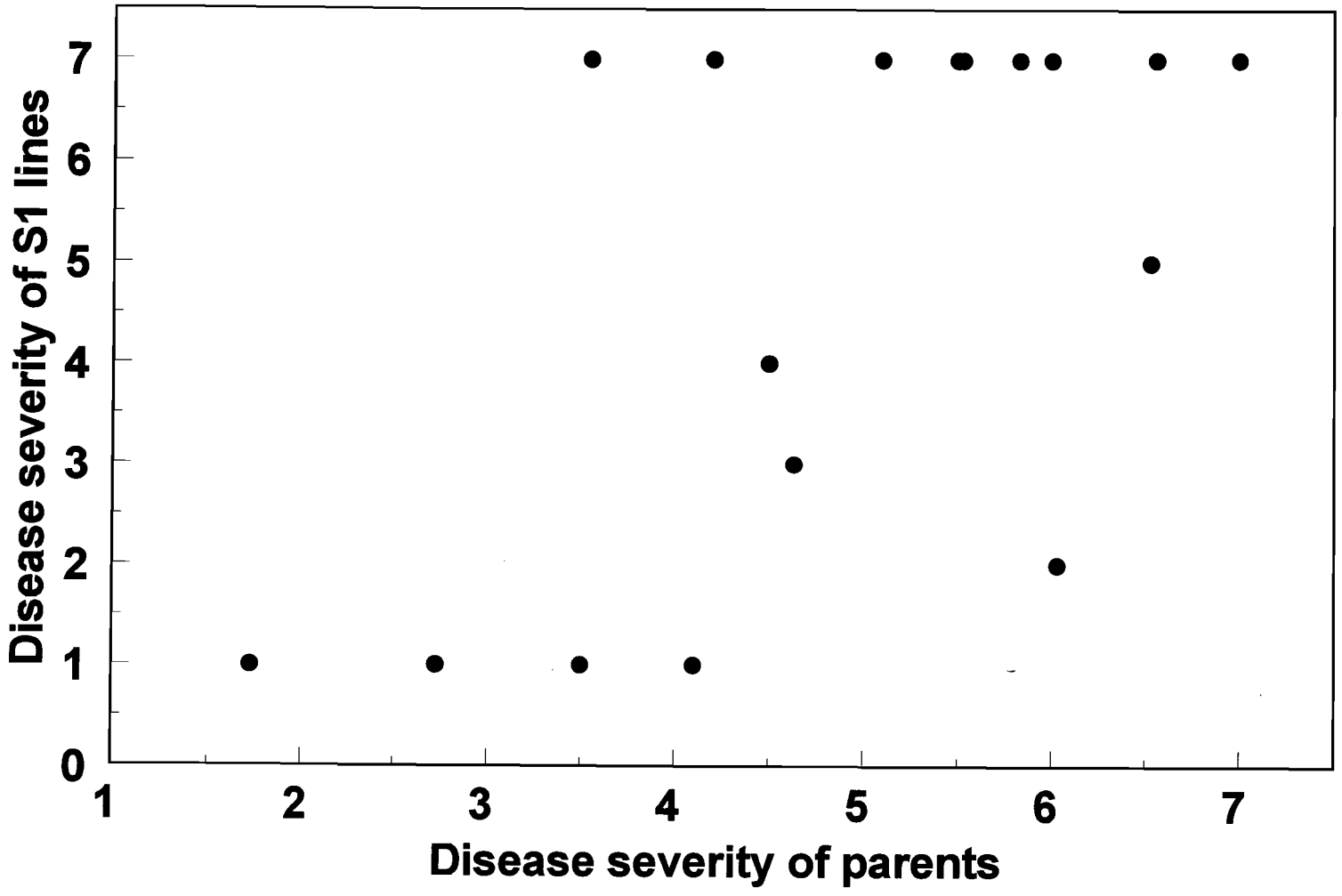
This study shows a wide variation in resistance to ear rot by *F. graminearum* in Zapalote Chico plants. Variation in plant pigmentation did not correspond to variation for disease resistance, indicating that resistance may not be predicted based on plant pigmentation. However, it seems that the silk resistance observed in Zapalote Chico is heritable and could be useful in breeding for resistance.

Table 7.1. Plant pigmentation, disease severity and incidence values of Zapalote Chico plants and S1 lines at Ste-Anne-de-Bellevue.

Genotype	Single Plants in 1992		S1 Lines in 1993	
	Pigmentation	Severity	Severity	Incidence
CO272	-	-	4.03cde[z]	100.00a
ZapC154	5	1.00	1.73f	13.33d
ZapC11	3	1.00	2.73ef	60.00abc
ZapC37	4	1.00	3.50def	48.33c
ZapC57	5	1.00	4.10cde	80.67abc
ZapC24	5	2.00	6.03abc	100.00a
ZapC39	5	3.00	4.63bcde	82.00abc
ZapC118	5	4.00	4.50bcde	83.33abc
ZapC139	4	4.00	4.50bcde	72.00abc
ZapC40	5	5.00	6.53ab	100.00a
ZapC18	5	7.00	3.55def	55.00bc
ZapC7	3	7.00	4.20cde	95.33ab
ZapC1	5	7.00	4.20cde	72.67abc
ZapC5	5	7.00	4.26cde	63.33abc
ZapC61	5	7.00	5.10bcde	77.67abc
ZapC104	5	7.00	5.50abcd	93.00ab
ZapC83	4	7.00	5.53abcd	83.33abc
ZapC71	4	7.00	5.83abc	80.33abc
ZapC72	5	7.00	6.00abc	94.33ab
ZapC97	5	7.00	6.56ab	100.00a
ZapC19	4	7.00	7.00a	100.00a

[z]means followed by the same letter within columns are not significantly different.

Figure 7.1. Disease severity of 20 Zapalote Chico S1 plants in 1992 and mean disease severity of their progeny in 1993, after inoculation with *Fusarium graminearum*.


Disease severity of S1 lines
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
Disease severity of parents

Preface of Chapter 8

Deoxynivalenol (DON) is a toxin produced by *F. graminearum* in infected ears. The distribution of DON in infected ears is poorly understood, therefore this study was undertaken to evaluate the distribution of DON in individual kernels from ears inoculated with *F. graminearum*. The manuscript is co-authored by C. Chungu, D.E. Mather and T. Ouellet and has been submitted to Plant Disease. Examples of the banding patterns of *F. graminearum* produced by PCR-based amplifications are shown in Appendix B.

DISTRIBUTION OF *Fusarium graminearum* AND DEOXYNIVALENOL IN INDIVIDUAL KERNELS FROM INOCULATED MAIZE EARS

Abstract

Deoxynivalenol (DON), a mycotoxin produced by *Fusarium graminearum*, and fungal DNA were assayed in individual kernels from inoculated maize ears. Series of adjacent kernels were sampled from four ears of a highly susceptible inbred and four ears of a moderately resistant inbred. PCR amplifications confirmed that *F. graminearum* was present in all ears. The concentration of DON was assessed by enzyme-linked immunosorbent assay (ELISA) and the relative amount of fungal tissue present was estimated by DNA hybridization. Toxin levels were high in ears with severe symptoms. Kernels from ears sampled from the moderately resistant inbred had higher levels of DON than those from the susceptible inbred. Some kernels with detectable DON had no detectable fungal DNA. DON concentration was highest near the inoculated point. Correlations between DON and fungal DNA were positive and generally low. Apparently the distribution of DON does not depend simply on the presence and quantity of the fungal hyphae.

Introduction

Deoxynivalenol (DON) is a toxin produced by several fungi including *Fusarium graminearum* Schwabe [sexual state: *Gibberella zeae* (Schwein) Petch], a causal organism of ear rot of maize (*Zea mays* L.). Deoxynivalenol can be produced in liquid cultures of *F. graminearum* (Miller *et al.* 1983a) or in maize ears infected with *F. graminearum* (Bennett *et al.* 1988; Neish *et al.* 1983). The toxic effects of DON include: vomiting, immuno-suppression and diarrhoea. Thus DON contamination of grain is of concern if the grain is to be used for human food or animal feed.

Visconti *et al.* (1990) assessed the production of mycotoxins in three maize ears naturally infected by *F. graminearum* and exhibiting more than 50% moldy kernels. Kernels as well as underlying portions of cob tissues from visibly moldy zones were analyzed. They found no consistent difference in the distribution of DON between kernels and cob tissues. They observed very high levels of DON (over 12 ppm) in heavily damaged kernels.

Bennett *et al.* (1988) reported on the distribution of mycotoxins in rotted ears of a commercial dent maize hybrid grown in a controlled environment. In their study, maize ears were inoculated with *F. graminearum* using colonized toothpicks. Kernels from ear fractions with severe rot, light to moderate rot and no infection were assayed for mycotoxins. The highest levels of DON were in the zones with severe rot with a dramatic drop in toxins further from these zones.

Reid *et al.* (Accepted) measured DON concentration in samples from

symptomless and symptomatic kernels and in cob tissue of ears inoculated with *F. graminearum* using a silk channel injection method. Toxin levels were positively correlated with ear rot severity, and high concentrations of DON were detected in the cob tissue.

In maize, Young and Miller (1985) detected DON in areas of the stalk not invaded by *F. graminearum*. This led them to speculate that DON could be loosely conjugated to a substance being transported to the root system.

Miller *et al.* (1985) evaluated DON and fusarium head blight resistance in spring cereals. Amounts of fungal biomass (estimated by measuring ergosterol) and of DON were compared in the kernels of resistant and susceptible cultivars of wheat, rye and triticale. They found high levels of fungal biomass in susceptible cultivars, confirming that these had little resistance to fungal invasion. In wheat and triticale, they found less DON in resistant cultivars than in susceptible cultivars.

Lee *et al.* (1980) evaluated aflatoxin, a toxin produced by *Aspergillus flavus* Link:Fr., in individual kernels from three naturally infected maize ears. They found highly variable levels of aflatoxin among kernels within a given ear. They observed highly contaminated kernels adjacent to kernels with no detectable aflatoxin. Smart *et al.* (1990) inoculated maize ears with colonized toothpicks, then analyzed individual kernels for aflatoxin and observed them for fungal signs with a stereomicroscope. Highly contaminated grains were rarely adjacent to each other and there was considerable variability in toxin levels within spikelet pairs. This led them to rule out translocation of aflatoxin through the vascular system. Furthermore, aflatoxin was

only rarely detected in kernels with no signs of the fungus, so it appears that long distance transport of aflatoxin depends on fungal hyphae.

Some of the studies cited above have examined DON concentrations in composite samples of maize kernels and(or) in samples of cob tissue, but none have done so in individual kernels. In the experiment reported here, we assessed the distribution of DON and *F. graminearum* DNA in individual kernels of inoculated ears from susceptible and resistant inbreds, to examine the contribution of each kernel to the overall toxin level, and to examine whether the presence of DON in each kernel depends on the presence of fungal hyphae.

Materials and Methods

Ears of susceptible (CO265) and resistant (CO325) maize inbreds were inoculated with a macroconidial suspension of *F. graminearum* using a kernel-stab inoculation technique (Chungu *et al.* accepted; Reid and Hamilton, accepted) in 1993 and 1994. Severity of the disease on ears was assessed visually. In each year, two ears of each inbred were selected for toxin analysis and DNA detection: one with a low level of symptoms (1-3% of the ear) and one with a moderate level of symptoms (11-25% of the ear). After harvesting, individual kernels were sampled from the same row of kernels as the inoculated point (Fig. 1). In addition, contiguous series of kernels to either side of the inoculated point (all the way around the ear) were sampled. Eight additional kernels were sampled around the inoculated point. Each kernel was visually assessed for disease symptoms, placed in a vial, and stored at

-20° C to prevent further fungal development. Prior to analysis, each kernel was sterilized in 10% NaOCl for 3 min and rinsed three times in sterile distilled water, then dried in sterile paper towels. Individual kernels were ground into fine powder using a Retsch Vibrating Mill (F. Kurt Retsch GmbH and Co., West Germany). Subsamples of ground kernels were used to characterize the fungus, and determine levels of deoxynivalenol (DON) and fungal DNA. Seven cob tissue samples underlying adjoining kernels on either side of the inoculated point were taken using the core-sampler. These samples were ground and later exposed to the same extraction and analyses as the kernels.

Fungal Characterization

Culture Maintenance: A subsample of the milled grain of each kernel was plated on fusarium-selective medium consisting of 15 g of peptone, 0.5 g $MgSO_4.7H_2O$, 1.0 g KH_2PO_4, 20 g agar, 1 g PCNB (added prior to autoclaving) and 0.1 g chloramphenicol and incubated at room temperature. Colonies were observed for pink to reddish coloration, characteristic of *F. graminearum* and related species. Single spore cultures of the fungus were isolated from several kernels and maintained on potato dextrose agar.

DNA Extraction From Mycelial Tissue: DNA was extracted from mycelial tissue of single spore cultures as described by Edwards *et al.* (1991). After a final resuspension, the DNA pellets were dissolved in TE (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA), DNA solutions were stored at 4 °C overnight, centrifuged for 5 minutes

to eliminate insoluble particles, and the supernatants were stored at -20 °C. The amount of DNA in each sample was visually quantified on 0.8% agarose gel containing ethidium bromide by comparisons with four known amounts of marker DNA (Lambda DNA, Promega Co., Madison WI).

DNA amplification: Reactions were performed and analyzed as described by Ouellet and Seifert (1993), using the Gene Amp kit (Perkin-Elmer Cetus, Norwalk, CT). Four polymerase chain reaction (PCR) based markers were used, three using individual 10-base primers (A11, B10 and B12) and the other using a pair of 19 and 18-nucleotide primers (A11a + A11b) specific to *F. graminearum*.

Deoxynivalenol (DON) Assay

DON was extracted from portions of ground kernels and quantified using direct competitive enzyme-linked immunosorbent assay (ELISA, Neogen Inc, Michigan). Absorbance readings were obtained using a microplate reader (model 450, Bio-Rad Laboratories, Hercules, CA) at 650 nm. Ground samples of 0.05 g were used for each kernel. Each sample was soaked in 2.5 ml of double distilled water further purified using a Milli-Q Plus Water System (Millipore, Canada Ltd) for 20 minutes and mixed. Particulates were filtered out through Whatman #1 filter paper. Samples with toxin levels higher than 5 ppm were diluted and re-analyzed. DON concentration was determined twice for each kernel and cob position. The minimum detectable level of DON was 0.1 ppm.

Fungal DNA Assay

DNA Extraction From Infected Maize Kernels: DNA was extracted from all subsamples of ground kernels as described by Möller *et al.* (1992).

DNA Layering: DNA samples, diluted in 5X SSC were immobilized on nylon membranes (Biotrans, INC Biomedicals) using a Manifold II Slot Blotter (Schleicher and Schuell Inc, Keene, N.H). The membranes were treated with denaturation solution (0.5M NaoH, 1.5M NaCl) then neutralizing solution (1M Tris pH 7.2, 1.5M NaCl) as described by the manufacturer. The immobilized DNA was fixed by exposing the membranes to a UV transilluminator for 3 min. The membranes were stored at -20° C until hybridization.

DNA Hybridization with a Radio-labelled DNA probe: Membranes were prehybridized overnight at 65° C in a buffer solution containing 5X SSC, 50 mM $NaPO_4$, 0.1% SDS, 5X Denhardt's solution and 250 ng/μl denatured herring sperm DNA. The prehybridization solution was poured off and replaced by the hybridization solution (1X SSC, 20 mM $NaPO_4$, 0.1% SDS, 1X Denhardt's solution, 5% dextran sulphate and 100 ng/ml denatured herring sperm DNA) containing a labelled probe (FG5E1H) and then incubated overnight at 65° C. The probe FG5E1H, a genomic DNA fragment from *F. graminearum*, was labelled with [alpha-^{32}P]-dCTP (Dupont) using a random priming kit (Promega). Final washes of the membranes were done in 0.1X SSC, 0.1% SDS at 65° C. Membranes were wrapped in plastic wrap and exposed to X-Omat AR film (Eastman Kodak, New York) at -70° C for three days. The amount of fungal DNA in each sample was estimated from

autoradiograms scanned and quantified with Gel Print Tools software (BioPhotonics Corporation, Michigan).

Results and Discussion

All eight maize ears showed pink to reddish symptoms as expected for *F. graminearum*. On plate cultures of ground kernels from these ears, most of the fungal colonies exhibited features expected of *F. graminearum*. About 20% of the kernels sampled from ears with low symptoms, and 65% of the kernels sampled from ears with moderate symptoms had visible symptoms in both years. All cob tissues from ears with moderate symptoms exhibited visible symptoms. Of the cob tissue samples obtained from ears with low symptoms, only tissues adjacent to the inoculated point showed visible symptoms.

Use of the PCR amplifications confirmed the presence of *F. graminearum* in the inoculated ears, but brought into question the identity of the isolate used in inoculations in one of the two years. We intended to use DAOM194276 in both years, and banding patterns from the 1994 samples agreed with expectations for that isolate. However, patterns from the 1993 samples differed from those expected, indicating that another isolate (possibly DAOM178148) may have been inadvertently used in 1993.

Quantities of fungal biomass (estimated by DNA hybridization) and mean deoxynivalenol (DON) concentrations of individual kernels are presented in Tables 1 and 2 for 1993 and 1994, respectively. Duplicate samples gave very similar results

when DON concentration was below 5 ppm, but occasionally differed from each other by as much as 3 to 4 ppm when DON concentration was above 10 ppm. Kernels from ears with moderate symptoms had higher DON concentration and more fungal DNA than those from ears with low symptoms. Most of the kernels with visible symptoms also had detectable DON and fungal DNA. Many of the kernels had very low levels of toxin but several kernels contained levels greater than 20 ppm. In composite samples, a few such kernels could be the major source of contamination.

Fungal DNA and toxin levels were generally higher in 1994 than in 1993. Warmer conditions in 1994 favored fungal growth, resulting in more fungal hyphae producing DON. Tuite *et al.* (1974) suggested that warm conditions favor ear rot development and Miller *et al* (1985) speculated that warm temperatures may be conducive to the production of DON. It is possible that the different isolates detected in the ears from 1993 and 1994 may have differed in aggressiveness and/or their ability to produce DON.

The distributions of DON and fungal DNA were quite similar in different ears. The cob tissues underlying the kernels to the left and right of the inoculated point frequently contained more fungal DNA and had higher DON concentrations than did the kernels. This difference between cob and kernel contamination is consistent with the results of Visconti *et al.* (1990) and Reid *et al.* (In press).

The concentration of DON was generally highest at the inoculated point with a decline further from this point in most of the ears. Higher DON levels were observed in kernels above the inoculated point, corresponding to the high levels of fungal

DNA. Some kernels with trace amounts of toxin levels were adjacent to highly contaminated kernels. Data presented in Table 4 allow for comparisons of toxin levels within pairs of laterally adjacent kernels (Fig. 1). DON concentrations of any two laterally adjacent kernels were rarely similar. If the toxin was moving mainly through the vascular system, laterally adjacent kernels should contain very similar DON concentrations (because of direct vascular connections between paired spikelets). The lack of a pairwise pattern of variation in toxin levels among kernels suggests that other mechanisms contributed to the differences of DON concentration in the kernels. There was more DON above than below the inoculated point, indicating that the movement of DON was in accordance with the generally upward movement of water and/or photosynthates through the cob. DON is water soluble and may have moved through the rachis tissue to the kernels independently of the hyphae. The presence of DON in kernels with no detectable fungal DNA supports this hypothesis. Young and Miller (1985) detected DON in the areas of the maize stalk not invaded by the pathogen.

Correlations between DON concentration and the amounts of fungal DNA in the kernels and cob tissues (Fig. 2) were positive but not high ($r < 0.46$ for kernels and $r < 0.28$ for cob tissues). Amount of fungal tissue in a kernel is not the only factor determining the concentration of DON in that kernel.

Similar amounts of fungal DNA were detected in the resistant (CO325) and susceptible (CO265) inbreds in 1993. In 1994, kernels from ears of the resistant inbred exhibited less fungal DNA than those from the susceptible inbred eventhough

ears exhibiting similar symptoms were compared. However, kernels from ears of the resistant inbred (CO325) had higher DON concentration than those of the susceptible inbred (CO265). One might speculate that the pathogen produced more toxin in ears from CO325 to overcome the resistance mechanisms in these ears. However, little is known about whether DON plays a role in fungal invasion of maize tissues, or if differences in resistance can be attributed to differences in sensitivity to DON. Hart *et al.* (1987) have investigated the inhibition of protein synthesis by DON, and found that a moderately resistant inbred was less sensitive to DON than susceptible inbreds. However, susceptibility to *F. graminearum* ear rot of maize inbred lines did not always correspond to their sensitivity to DON.

The results reported here show considerable kernel to kernel variation in fungal DNA and DON concentrations in ears of two inbreds differing in resistance. Some kernels exhibited DON levels that were far greater than 2 ppm, the level known to decrease feed intake and weight gain in pigs (Putnam and Binkerd, 1992; Trenholm *et al.* 1983). In 1994, ears from the resistant inbred exhibited more DON than ears from the susceptible inbred, whereas this difference was not apparent in 1993. Presence of DON but no fungal biomass in some kernels, varying levels of DON in neighboring kernels, and generally low correlations between DON and quantity of fungal DNA provide evidence that the movement of DON among kernels does not always depend on the hyphae.

Figure Legend

Figure 8.1. Positions of kernels removed from 8 ears of maize inbreds inoculated with *Fusarium graminearum*. IP indicates inoculated point. Kernels in the same row as the inoculated kernel are labelled from -10 to +10. Kernels to the left and right of the inoculated point are labelled from -7 to +7. Additional samples are labelled +a to +d, -a to -d.

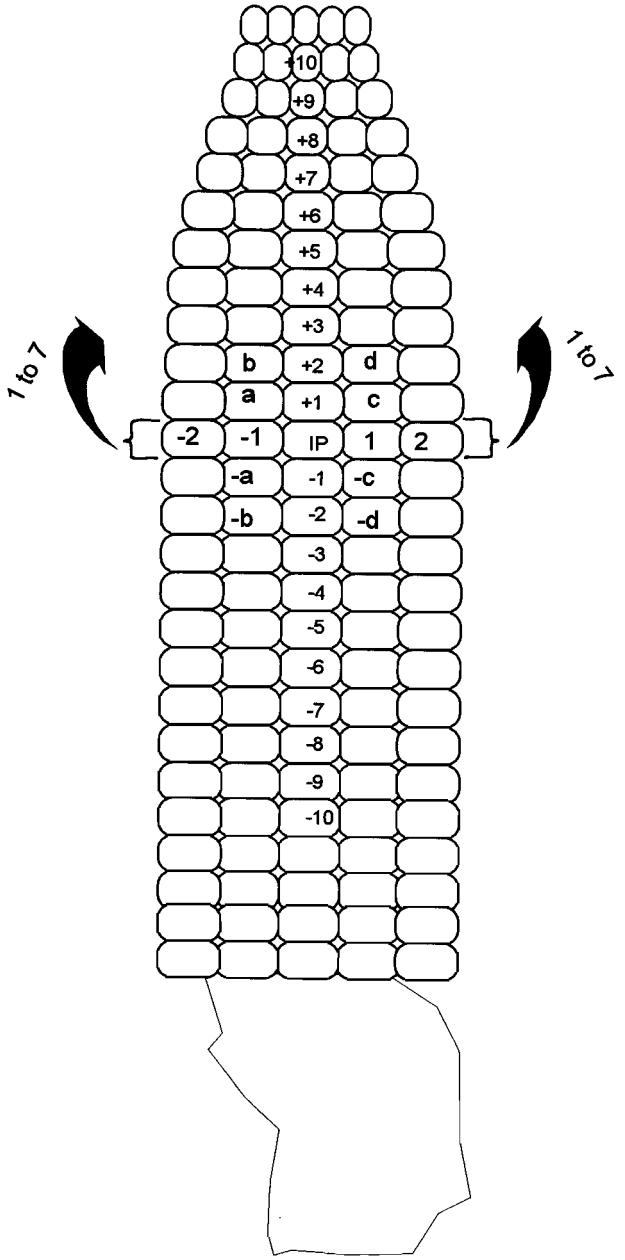

+10
+9
+8
+7
+6
+5
+4
+3
b
+2
d
a
+1
c
-2
-1
IP
1
2
-a
-1
-c
-b
-2
-d
-3
-4
-5
-6
-7
-8
-9
-10
1 to 7
1 to 7

Table 8.1. Deoxynivalenol (DON) concentrations and relative amounts of fungal DNA in individual kernels from ears of inbreds CO265 and CO325 with low and moderate symptoms in 1993.

Position of kernel relative to inoculated point		CO265				CO325			
		Low symptoms		Moderate symptoms		Low symptoms		Moderate symptoms	
		DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)
Above	11	0.38	2.0	0.16	-†	*‡	*	*	*
	10	0.06	-	0.13	-	*	*	*	*
	9	0.29	-	0.22	-	*	*	*	*
	8	0.11	-	0.22	-	0.93	-	0.40	-
	7	0.15	-	0.27	-	0.83	-	0.85	2.5
	6	0.21	-	-	-	0.64	-	0.41	7.4
	5	0.10	-	0.10	-	0.80	-	0.90	5.6
	4	0.15	-	0.10	2.8	0.67	2.8	0.75	6.5
	3	0.10	-	2.75	13.2	0.50	1.4	1.60	35.5
	2	1.45	-	0.40	4.1	3.25	6.4	47.00	59.6
	1	1.76	-	16.50	63.8	3.70	6.2	54.00	8.6
Inoculated Point		0.68	5.1	29.00	13.7	13.50	38.6	45.00	40.4
Below	-1	0.37	9.8	7.50	87.2	1.18	6.2	16.50	39.5
	-2	0.29	10.8	15.00	29.4	0.41	0.8	37.00	45.5
	-3	0.28	-	11.75	29.0	0.82	2.6	33.00	31.0
	-4	0.31	4.5	11.50	9.9	-	-	1.85	29.9
	-5	0.23	8.4	0.45	17.5	-	-	0.96	30.1
	-6	0.27	18.1	1.95	-	-	-	1.25	14.4

	-7	0.27	5.5	0.77	-	0.22	-	0.63	7.1
Left	-7	0.34	-	0.43	10.4	*	-	*	*
	-6	0.42	-	4.70	28.9	*	-	*	*
	-5	0.18	0.9	3.80	7.1	0.14	-	4.75	51.8
	-4	0.19	0.9	2.20	25.1	0.25	1.4	4.25	21.5
	-3	0.20	0.9	6.25	33.6	0.40	2.2	5.70	53.5
	-2	0.36	1.0	18.25	47.0	1.69	6.4	12.50	64.3
	-1	0.22	-	24.75	12.9	2.55	12.4	24.50	51.0
Inoculated	Point	0.68	5.1	29.00	13.7	13.50	38.6	45.00	40.4
Right	1	0.50	30.4	11.25	37.1	2.55	20.6	11.95	68.7
	2	0.32	17.3	3.03	11.4	0.52	8.3	3.75	40.7
	3	0.32	7.1	0.82	1.1	0.47	13.6	1.35	64.0
	4	0.48	3.5	0.54	12.7	0.10	2.3	4.60	17.8
	5	0.39	2.0	0.89	4.7	-	-	3.00	-
	6	0.24	-	3.50	15.7	*	*	*	*
	7	0.34	-	4.45	22.6	*	*	*	*

† no kernel because of ear length or width
‡ not detected

Table 8.2. Deoxynivalenol (DON) concentrations and relative amounts of fungal DNA in individual kernels from ears of inbreds CO265 and CO325 with low and moderate symptoms in 1994.

Position of kernel relative to inoculated point		CO265				CO325			
		Low symptoms		Moderate symptoms		Low symptoms		Moderate symptoms	
		DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)
Above	10	-	-	0.37	-	0.39	12.1	-	-‡
	9	-	-	0.29	-	0.27	4.2	-	1.6
	8	-	3.3	0.90	14.8	0.20	12.8	-	5.6
	7	-	17.1	0.46	6.4	0.13	8.4	0.19	10.7
	6	-	12.2	0.81	7.7	0.22	8.2	0.40	15.5
	5	0.26	75.8	0.89	17.0	0.23	13.9	6.00	44.6
	4	6.80	20.5	22.75	77.9	1.83	15.5	1.65	35.5
	3	1.20	90.7	20.75	98.6	1.35	10.3	3.70	46.0
	2	5.10	80.6	22.50	81.1	3.75	18.7	21.25	55.6
	1	4.20	67.3	8.13	109.6	2.40	2.1	22.50	59.5
Inoculated Point		2.20	+§	10.20	+	5.10	+	32.00	+
Below	-1	2.10	37.0	14.75	107.2	0.68	12.1	18.00	81.6
	-2	1.28	5.2	12.75	95.5	0.37	4.9	13.00	24.6
	-3	0.10	-	7.00	41.4	0.32	2.9	16.00	35.5
	-4	0.12	2.6	3.48	58.5	0.46	6.3	5.25	-
	-5	-	8.4	0.90	48.5	0.25	-	0.62	-
	-6	-	2.5	0.07	23.3	0.12	-	-	-
	-7	-	8.5	-	3.1	0.14	-	-	-

	-8	-	11.4	-	10.5	-	-	-	-
	-9	-	3.4	-	2.1	0.10	-	-	-
	-10	-	7.7	-	2.5	0.10	-	-	-
Left	-7	0.52	44.4	12.00	89.5	*†	*	*	*
	-6	0.35	19.1	12.00	109.2	*	*	*	*
	-5	0.89	38.9	22.50	95.4	2.65	22.6	37.50	83.5
	-4	0.38	10.1	18.00	92.5	0.62	10.9	38.50	95.3
	-3	0.52	70.0	11.75	17.3	0.84	-	43.50	74.2
	-2	0.72	47.3	15.00	92.0	3.30	29.0	31.50	75.2
	-1	2.95	47.5	15.00	129.5	4.70	-	25.50	23.7
Inoculated	Point	2.20	+	10.20	+	5.10	+	32.00	+
Right	1	6.60	71.7	24.50	55.3	0.24	19.7	17.50	73.1
	2	5.50	41.4	10.75	61.8	0.63	3.8	16.50	137.9
	3	3.70	47.9	23.00	59.6	0.11	3.9	29.50	46.5
	4	0.42	14.7	14.75	70.7	0.52	27.4	37.50	80.5
	5	1.45	75.1	23.00	93.4	0.44	61.9	42.00	68.3
	6	0.13	9.3	18.00	106.2	*	*	*	*
	7	0.10	9.1	15.00	88.4	*	*	*	*

† no kernel because of ear length;
‡ not detected
§ not determined due to insufficient sample

Table 8.3. Deoxynivalenol (DON) concentrations and relative amounts of fungal DNA in composite cob tissue from ears of inbreds CO265 and CO325 with low and moderate symptoms in 1993 and 1994.

Position of cob tissue relative to inoculated point		CO265				CO325			
		Low symptoms		Moderate symptoms		Low symptoms		Moderate symptoms	
		DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)	DON (ppm)	DNA (ng)
Left	-3	4.00	20.9	13.00	39.7	4.05	22.2	19.50	-‡
	-2	3.00	17.3	13.00	38.1	2.52	-	27.50	-
	-1	2.37	11.8	23.00	17.0	7.50	11.8	20.00	26.3
Inoculated Point		1.55	31.8	25.50	7.5	6.75	35.5	24.00	-
Right	1	3.02	34.2	20.50	89.3	7.90	19.5	25.50	31.4
	2	3.64	28.8	23.50	31.0	1.90	23.8	29.00	94.6
	3	2.27	10.7	18.50	40.9	-	-	-	-
1994									
Left	-3	4.55	11.3	69.00	68.9	1.43	24.1	26.00	12.6
	-2	3.75	32.9	42.50	107.5	1.85	27.0	13.00	20.4
	-1	4.30	24.0	40.25	46.1	3.95	61.4	29.00	3.9
Inoculated Point		3.85	27.9	60.75	56.1	4.50	30.5	20.00	10.7
Right	1	5.55	42.1	59.25	51.0	4.25	15.5	7.10	24.5
	2	4.50	45.1	46.50	75.9	0.95	22.6	9.10	13.2
	3	4.65	18.6	45.25	56.0	2.18	-	20.50	13.9

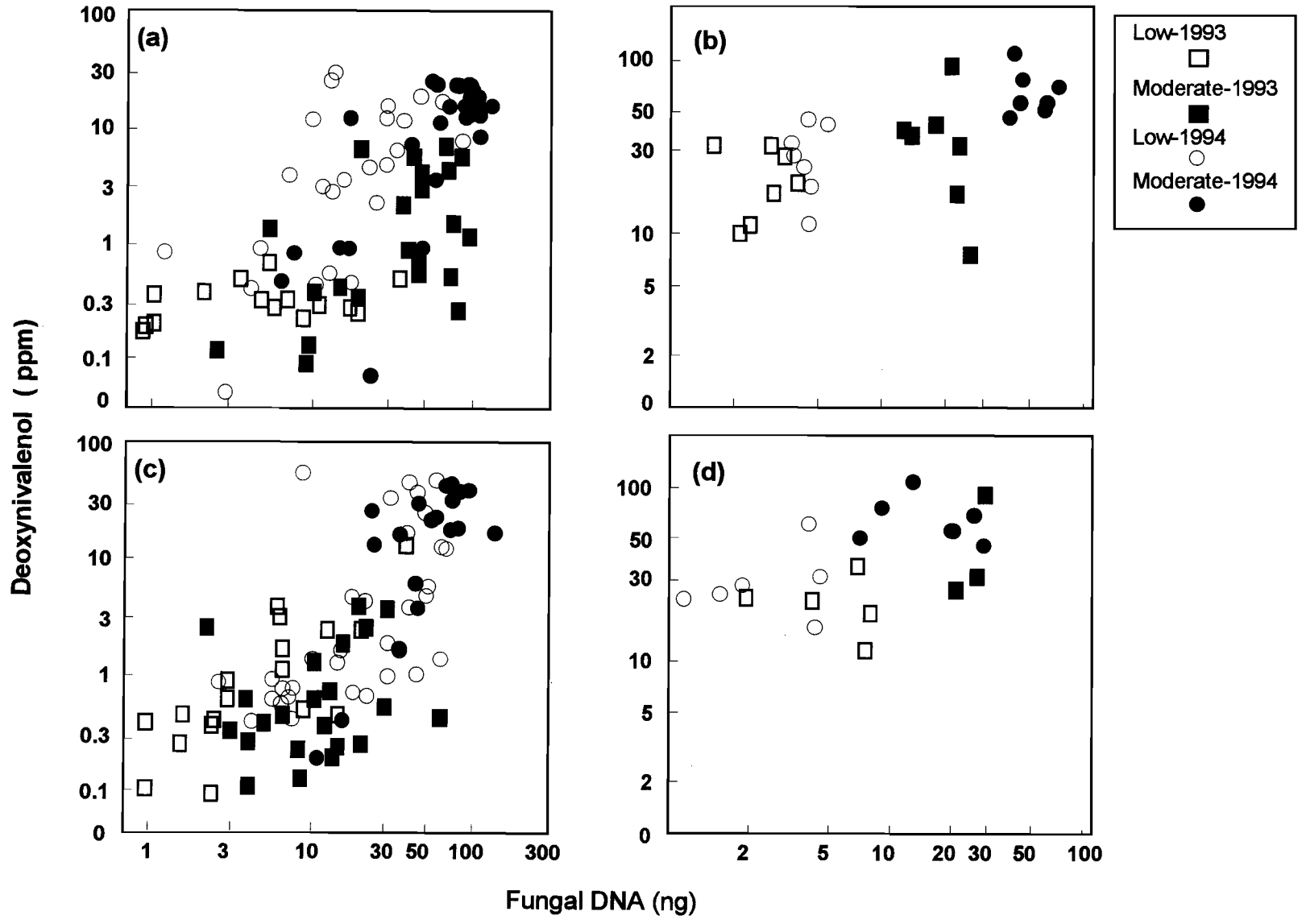
‡ not detected

Table 8.4. Deoxynivalenol (DON) concentrations (ppm) in each kernel harvested in a pattern around the inoculated point in inbred CO265 and CO325.

Position of kernel relative to inoculated point	CO265				CO325			
	Low symptoms		moderate symptoms		Low symptoms		moderate symptoms	
	1993	1994	1993	1994	1993	1994	1993	1994
a	-	-	7.80	2.70	0.60	0.68	3.20	2.10
b	0.29	-	10.00	11.60	0.39	0.27	7.40	10.50
c	-	-	11.20	2.10	0.16	0.89	3.80	1.60
d	-	-	11.20	2.20	0.33	2.50	14.00	1.92
2	1.45	5.10	0.40	22.50	3.25	3.75	47.00	21.25
1	1.76	4.20	16.50	8.13	3.70	2.40	54.00	22.50
Inoculated Point	0.68	2.20	29.00	10.20	13.50	5.10	45.00	32.00
-1	0.37	2.10	7.50	14.75	1.18	0.68	16.50	18.00
-2	0.29	1.28	15.00	12.75	0.41	0.37	37.00	13.00
-a	-	4.00	4.60	1.42	1.99	0.89	3.80	1.00
-b	-	0.11	2.30	1.16	0.68	0.51	7.80	1.00
-c	-	-	9.20	3.00	0.50	1.20	8.00	2.40
-d	-	-	-	4.20	0.33	0.51	0.78	3.40

‡ not detected

Figure 8.2. Deoxynivalenol concentrations and amounts of fungal DNA in individual kernels from (a) CO265, (b) CO325, and samples of cob tissue from (c) CO265 and (d) CO325 maize inbred ears inoculated with *Fusarium graminearum* in 1993 and 1994. Low= ear with low symptoms and Moderate= ears with moderate symptoms. Plotted on a logarithmic scale.


(a)
(b)
(c)
(d)
Low-1993
Moderate-1993
Low-1994
Moderate-1994
Deoxynivalenol (ppm)
Fungal DNA (ng)
100
30
10
3
1
0.3
0.1
0
50
5
2
1
3
10
30
50
100
300
20

Preface of Chapter 9

This study was designed to evaluate the pattern of ear rot symptom development in hybrids differing in resistance using image analysis and other disease assessment methods, and to monitor pericarp thickness as a possible mechanism affecting symptom development. Other mechanisms of resistance examined in this chapter are presented in Appendix C. The manuscript will be co-authored by C. Chungu, D.E. Mather, L.M. Reid and R.I. Hamilton and will be submitted to Phytopathology.

EAR ROT SYMPTOM DEVELOPMENT AND DISEASE ASSESSMENT ON MAIZE HYBRIDS INOCULATED WITH *Fusarium graminearum*

Abstract

Image analysis was used to examine the pattern of ear rot symptom development in six maize hybrids (A641 × CO251, A641Ht × CM105, Dekalb DK415, Mamouth, Pioneer 3902 and Pride K127) in 1993 and 1994. Ten ears were sampled from each hybrid at six developmental stages (milk, dough, early dent, late dent, physiological maturity and 30 days after physiological maturity). Lesion size differed significantly among the hybrids and sampling times. The most resistant hybrid, Pride K127, exhibited a slower rate of symptom development, lower disease severity and lower area under the disease progress curve (AUPDC) than other hybrids. Pericarp thickness was greater in Pride K127 than in susceptible hybrids. Significant differences in ear rot symptoms were apparent after the third sampling time (early dent). It may be possible to make selections before physiological maturity. Correlations between image analysis measurements and visual ratings of disease severity were high. The visual method can be applied easily in assessing ear rot symptoms.

Introduction

In maize or corn (*Zea mays* L.) breeding programs aimed at improving resistance to ear-rotting pathogens, selection of resistant plants or families is generally based on assessments of disease symptoms on mature ears. Repeated observations can not be made on same ears, since the husks must be removed from each ear so that symptoms can be observed on the kernels. This makes evaluation of disease progress on maize ears quite difficult. This is in contrast with foliar diseases such as northern leaf blight [*Exserohilum turcicum* (Pass.) K.J. Leonard & E. G. Suggs], where the same leaf or leaves can be observed repeatedly as symptoms progress (Perkins and Pedersen, 1987).

Miller *et al.* (1983b) evaluated number of *F. graminearum* colony forming units in ground samples of maize taken weekly between 14 and 63 days after inoculation. They found that the number of colony forming units increased slowly until 42 days after inoculation and then declined thereafter. Symptom development was however, not recorded in this study. The development of naturally occurring *F. moniliforme* Sheld maize ear rot symptoms has been studied between 56 and 98 days after silking (Ochor *et al.* 1987) and between 56 and 140 days (Kedera *et al.* 1994) after silking. In both cases symptom development differed significantly among maize hybrids and harvest times and the incidence of ear rot increased with time.

In most studies on ear rots, assessments of symptoms are done using ordinal rating scales. Enerson and Hunter (1980) and Hart *et al.* (1984) used a 5-class rating scale to assess ear rot symptoms caused by *F. graminearum* Schwabe. Bolton *et al.*

(1990) developed and used 7-class rating scales to assess *F. graminearum* ear rot symptoms. These have been used by Reid *et al.* (1992a,b,c, 1993, 1994) and by Chungu *et al.* (In press). These scales can be quickly and easily used in selecting plants or genotypes with high resistance.

For quantifying disease severity, ratio scales should be superior to ordinal scales. However, the required measurements (e.g. lesion size, proportion of the ear infected) could be too time consuming to integrate into breeding programs. For some foliar diseases, image analysis techniques have been used to facilitate measurement of symptom severity on ratio scales. For example, Lindow and Webb (1983) and Nutter *et al.* (1993) used image analysis to quantify disease severity caused by *Alternaria solani* (Ell. G. Martin) on tomato and *Ascochyta pteridium* Bres. on bracken fern. Application of image analysis to ear rot diseases is not straightforward. Firstly, the three-dimensional nature of the ear and the lesions makes it difficult to obtain measurements directly from the ears. Secondly, image analysis systems can only distinguish disease lesions that are consistently different in color from healthy tissue. Healthy maize kernels are consistently yellow (or white), but the space between them can appear quite dark. Ear rot symptoms can vary considerably in color, even within one ear. For ear rot caused by *F. graminearum* [sexual state: *Gibberella zeae* (Schwein) Petch] the symptoms can range from white to red, and even to black if perithecia (fruiting bodies of the sexual state) are present. Many image analysis systems use only a monochrome camera to capture images. These cameras are only able to distinguish disease lesions that are consistently darker or lighter than healthy

tissues.

The objectives of the research reported here were: (1) to develop a method of using image analysis to assess symptom severity of maize ear rot caused by *F. graminearum*; (2) to use that method to study the development of symptoms between 25 and 85 days after silking (10 and 70 days after inoculation); and (3) to evaluate kernel pericarp thickness at each sampling time as a possible mechanism of resistance. In previous research, Scott and King (1984) showed that resistance of ear rot caused by *F. moniliforme* Sheld was under maternal genetic control and suggested that the site of resistance could be the pericarp. Hoenisch and Davis (1994) found that hybrids resistant to *F. moniliforme* had thicker pericarps than susceptible hybrids.

Materials and Methods

Inoculum preparation and inoculation: *Fusarium graminearum* isolate DAOM194276, originally obtained from infected maize in Ottawa, and registered with the National Agriculture and Agri-Food Canada Culture Collection, was used. The isolate was maintained on a synthetic nutrient agar, a modified form of Bilay's medium (Tuite, 1969). Macroconidial suspensions were prepared by suspending a 1-cm^2 plug of the culture in 250 ml of autoclaved liquid medium in a 500-ml erlenmeyer flask. Inoculum was filtered through two layers of cheese cloth and diluted to a spore count of 5×10^5 conidia/ml, prior to inoculation. Primary ears were inoculated 15 days after silk emergence with a kernel-stab (Chungu *et al.* In press) inoculation method.

Symptom development: Six hybrids (A641 × CO251, A641Ht × CM105, Dekalb DK415, Mamouth, Pioneer 3902 and Pride K127) were evaluated for ear rot symptom development at Macdonald Campus (Ste-Anne-de-Bellevue, Quebec, Canada) in 1993 and 1994. Plants were grown in six-row plots, 2.4 m long, in a randomized complete block design in three blocks. Inoculated ears from the six hybrids were harvested at six kernel developmental stages (milk, dough, early dent, late dent, physiological maturity, and 30 days after physiological maturity), corresponding to 10, 15, 20, 30, 40 and 70 days after inoculation. In both years, ten randomly selected ears were harvested from each plot at each sampling time. Data collected included: diseased area (assessed by image analysis), severity of ear rot symptoms of inoculated ears visually assessed using a 7-class rating scale *where* 1= no symptoms present, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50, 6=51-75%, and 7=76-100% of the infected ear, lesion length, lesion width, and the number of diseased kernels.

Diseased Area (Image analysis): Diseased kernels showed white to reddish discoloration, while non-infected kernels were yellow. The image analyzer could not distinguish between healthy and symptomatic kernels directly from the ear. Therefore, sampled ears were photographed twice (from opposite sides of the ear), and 10 x 15-cm color prints were made. Outlines of the total and diseased area of each ear were traced onto clear acetates. The acetates were placed on a copy-stand light table with substage illumination. Images of these outlines were captured by a camera lens mounted onto the stand and connected to a Leco 2001 image analyzer (Leco Instrument Ltd, Mississauga, Ontario) providing a 482 x 482 pixel image

frame. The image was calibrated in millimeters, representing a spatial resolution of 0.2075 mm/pixel.

Area Under the Disease Progress Curve (AUDPC): Disease progress curves for diseased area were plotted for each hybrid. The AUDPC was calculated for each curve and standardized by dividing by the number of days in the epidemic.

Lesion Width and Length: These measurements were recorded in centimeters. Lesion width was measured around the ear where the lesion was the widest, whereas lesion length was taken along the kernel row with the longest lesion size.

Number of Diseased Kernels: The number of visibly diseased kernels was counted on each individual ear and averaged over ten ears.

Pericarp Thickness: Kernels were sampled from the tip and butt of the dried ears and bulked. Nine kernels were sampled at random from each treatment and block. Each kernel was cut transversely and mounted on the microscope stage using plasticine. The thickness of the pericarp was measured at magnification of 50X with an eyepiece micrometer on a dissecting microscope. Two readings were taken from the crown areas of each kernel and averaged.

Meteorological Data: Daily temperature and rainfall data were collected from a meteorological center (Dorval International Airport) located 20 km from Macdonald Campus. Mean temperature and total rainfall values between sampling times were calculated.

Statistical Analysis: Data sets were analyzed with PROC GLM procedure of SAS (SAS Institute, 1994). Due to heterogeneity of error variances, data were analyzed

separately for each year. Duncan's multiple range test was used for mean separation. Image analysis data was also correlated against disease severity visually collected using a 7-class ordinal rating scale and to number of visibly diseased kernels on the ear.

Results and Discussion

Symptom development: Due to heterogenity of variances, data is presented separately for each year. There were highly significant differences ($P<0.0001$) among hybrids and sampling times for all variables used to assess symptom development in both years (Table 1).

The development of the diseased area for each hybrid is shown in Fig 1. By the first sampling time, all hybrids exhibited similar symptoms. By the second sampling time, symptom development in Pride K127 was significantly lower than in the three most susceptible hybrids. After the fourth sampling time, symptoms in Pride K127 were lower than those of all the other hybrids. This difference between Pride K127 and other hybrids continued throughout the remaining period. The difference is reflected in the low final AUDPC value of Pride K127 (Table 2). The smaller lesion size of Pride K127 compared to the more susceptible hybrids appear to be due to slow symptom development and not to differences in disease establishment.

Lesion width (around the ear) was greater than lesion length (along the ear) (Fig. 2). Variations in lesion width accounted for much of the difference in lesion size between Pride K127 and the more susceptible hybrids by the fourth sampling

time. Pride K127 had significantly narrower lesions than Dekalb DK415, A641Ht × CM105 and A641 × CO251 at most sampling times. In contrast, the increase in lesion length was usually not very different among most hybrids, except that in 1994 it levelled off after the fourth sampling time in Pride K127. In 1993, symptoms continued to increase longitudinally in two susceptible hybrids, Dekalb DK415 and A641 × CM105. All the hybrids silked within three days of each other therefore differences in hybrid maturity would not have had much effect on the results.

Ear rot symptoms were more severe in 1993 than in 1994. Seasonal variations in rainfall and temperature could have influenced symptom development. In general, warm wet conditions (eg. above 23° C) are expected to favor ear rot development (Koehler, 1959; Tuite *et al.* 1974). In 1993, the first 20 days after inoculation were warmer than in 1994 (Fig. 1). Total rainfall during this period was less in 1993 than 1994. Nevertheless, it rained as frequently in 1993 as in 1994 during the first 20 days after inoculation. In September and October, rainfall was more frequent in 1993 than in 1994. The frequent rainfall in 1993 probably favored symptom development by providing a constant supply of moisture required for mycelial growth. In spite of the variations in weather, Pride K127 exhibited a similar pattern of symptom development in both years. The resistance mechanism(s) present in this hybrid slow(s) the spread of ear rot on kernels. This type of resistance could be useful in managing ear rot in conjunction with other control strategies.

Pericarp Thickness: Significant differences ($P < 0.05$) were observed among hybrids and sampling times for pericarp thickness (Fig. 3). The resistant hybrid had a thicker

pericarp than other hybrids at most harvest times. Perhaps kernel pericarp thickness is one resistance mechanism that influences symptom development in Pride K127, by inhibiting or slowing fungal penetration into kernels. Hoenisch and Davis (1994) found that hybrids resistant to *F. moniliforme* had greater pericarp thickness than susceptible hybrids.

Disease Assessment: Both image analysis (as used here) and visual ratings of disease symptoms involve subjective decisions by a researcher. With image analysis, the researcher only delineates tissue considered diseased, and quantification is automated and objective. With visual rating, the entire assessment process relies on subjective decisions of the researcher. Nevertheless, the visual disease severity ratings were highly correlated ($r=0.79$ $P<0.001$) with estimates of mean percent diseased area calculated using image analysis method (Fig. 4A). The relationship between the two sets of values appears linear, even though the rating scale was not designed to be linear. The open circles on Fig. 4A show the midpoints of each of the seven intervals on the scale. Most of the data points lie below the curve defined by these points, indicating a slight but consistent tendency to underestimate disease severity and also a tendency to apply the scale in a more linear manner than intended. In addition, points were more dispersed in the middle of the lines than on both ends of the line. This is usually expected with visual rating scales (Horsfall and Barratt, 1945) since differences in symptoms can not be precisely assessed when symptoms are moderate.

The relationship of diseased area assessed by image analysis with the number of diseased kernels is shown in Fig. 4B. Counting of the number of diseased kernels

is strictly quantitative and should be objective, but is very laborious. In addition, its usefulness as an indicator of lesion size would be limited by any variation in kernel size. This may explain the dispersion of points from the regression line. The points with the higher numbers of diseased kernels may represent ears on which the symptoms extended in length to spread over very small kernels at the tip of the ear. These aspects render this assessment method hard to use especially when lines differing in kernel size are being evaluated.

Since differences in symptom development between Pride K127 and more susceptible hybrids (Dekalb DK415, A641 × CO251 and A641HT × CM105) were attained as early as the fourth sampling time and continued throughout the rest of the growing season, it might be possible to select resistant lines as early as the dent stage. However, at this stage, a visual rating scale might not be sufficient because symptoms would only have reached moderate levels. Some means of measuring lesion size might be needed. Image analysis could be used, but based on our observations, measurement of lesion width might be adequate; it clearly differentiated Pride K127 from other hybrids at this stage.

The research reported here found that ear rot symptom development was slower and final symptoms were lower in Pride K127 than other hybrids. The thicker pericarp of the more resistant hybrid in this study may be a cause of its resistance. This characteristic might be useful in developing resistant genotypes. Differences in resistance to the spread of ear rot on kernels were apparent by the dent stage. It might, therefore be possible to select resistant genotypes before physiological

maturity. When two growing seasons per year are possible, early selection and identification of resistant genotypes could expedite the development of resistant cultivars. Researchers evaluating disease severity currently assess ear rot by visually estimating ear rot severity. Our study has shown that while image analysis can be used to quantify ear rot severity, it may not be necessary. The visual rating scale developed by Bolton *et al.* (1990) provided adequate estimates of symptom severity and was fast and easy to use. However, if symptoms are assessed early, it might be necessary to measure the size of lesions, either by image analysis, or simply by measuring lesion width.

Table 9.1. Analysis of variance for variables used to assess symptoms of ear rot caused by *Fusarium graminearum* on six maize hybrids in 1993 and 1994.

Source of variation	Df	Lesion Width	Lesion Length	Diseased[a] Area	Disease Severity	Diseased Kernels
1993						
Block	2	1.37	2.78	6309.40	0.37	1935.11
Hybrid	5	13.02**	18.38**	138972.90**	1.74**	13580.10**
Time(Hybrid)	30	64.66**	19.16**	124912.38**	3.29**	25698.20**
Error	70	1.31	1.37	4926.23	0.13	1018.54
1994						
Block	2	0.14	0.81	1472.96	0.028	3.54
Hybrid (H)	5	16.08**	12.08**	34800.92**	0.96**	4727.93**
Time(Hybrid)	30	34.73**	7.53**	15823.36**	1.29**	6581.44**
Error	70	1.39	0.56	1218.86	0.08	364.99

** significant at 0.0001 probability level

[a]infected diseased area estimated from acetate drawing using the image analyzer

Figure Legend

Figure 9.1. Development of diseased area of six maize hybrids inoculated with *Fusarium graminearum*, and mean temperature and cumulative rainfall in 1993 and 1994.

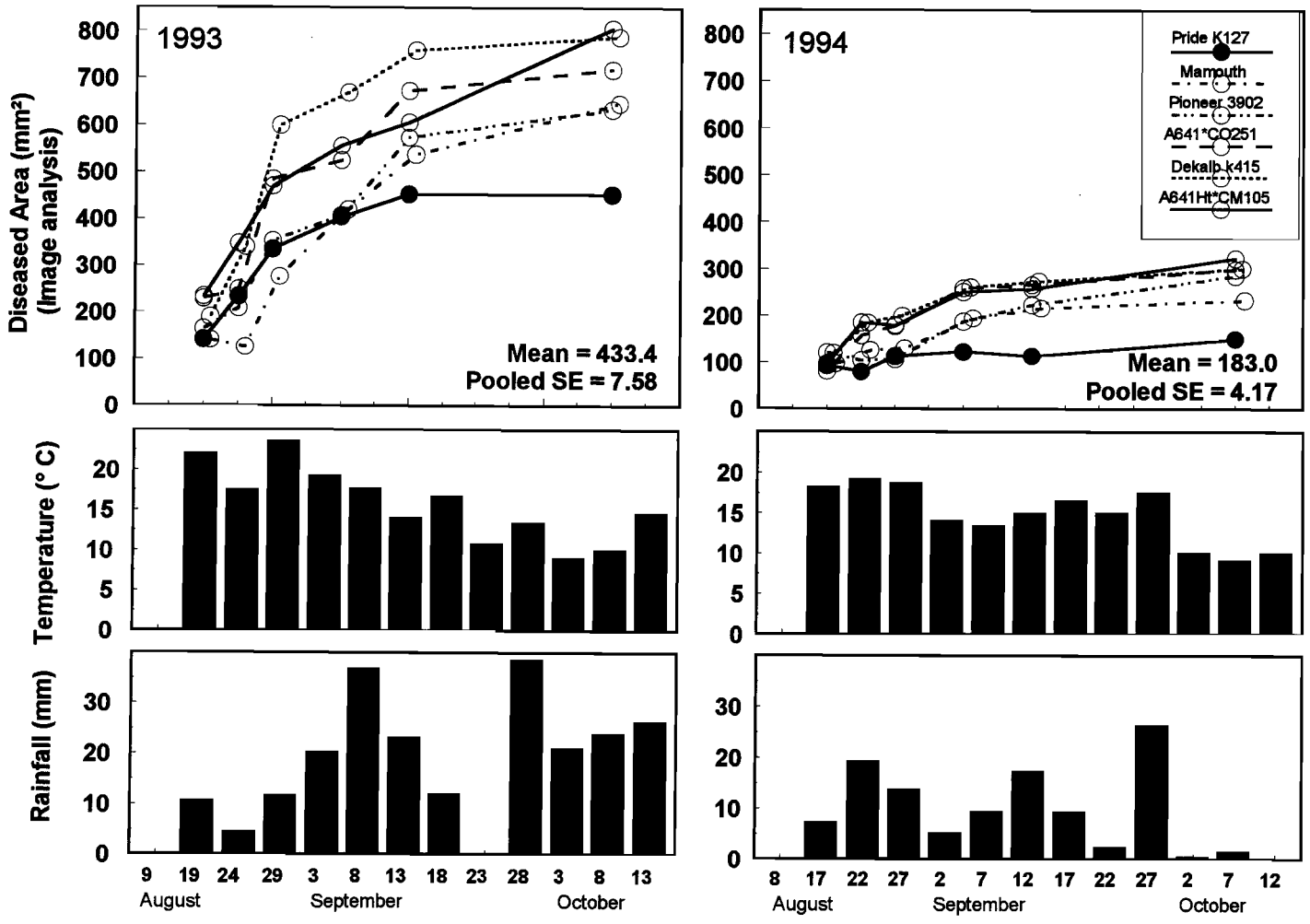

1993
1994
Diseased Area (mm²) (Image analysis)
Mean = 433.4
Pooled SE = 7.58
Mean = 183.0
Pooled SE = 4.17
Pride K127
Mamouth
Pioneer 3902
A641*CO251
Dekalb k415
A641Ht*CM105
Temperature (° C)
Rainfall (mm)
9 19 24 29 3 8 13 18 23 28 3 8 13
August September October
8 17 22 27 2 7 12 17 22 27 2 7 12
August September October

Table 9.2. Final area under the disease progress curve (AUDPC) of six maize hybrids inoculated with *Fusarium graminearum* in 1993 and 1994.

Hybrid	1993	1994
A641 × CO251	209.58ab	100.06ab
A641Ht × CM105	208.22ab	97.68ab
Dekalb DK415	225.42a	115.27a
Mamouth	177.56bc	82.14b
Pioneer 3902	202.56ab	86.43b
Pride K127	168.46c	49.76c

Means followed by the same letter within the columns are not significantly different.

[a]Standardized values of AUDPC =area divided by time span of disease assessments

Figure 9.2. Lesion length and width of ear rot on six maize hybrids inoculated with *Fusarium graminearum* at 1993 and 1994.

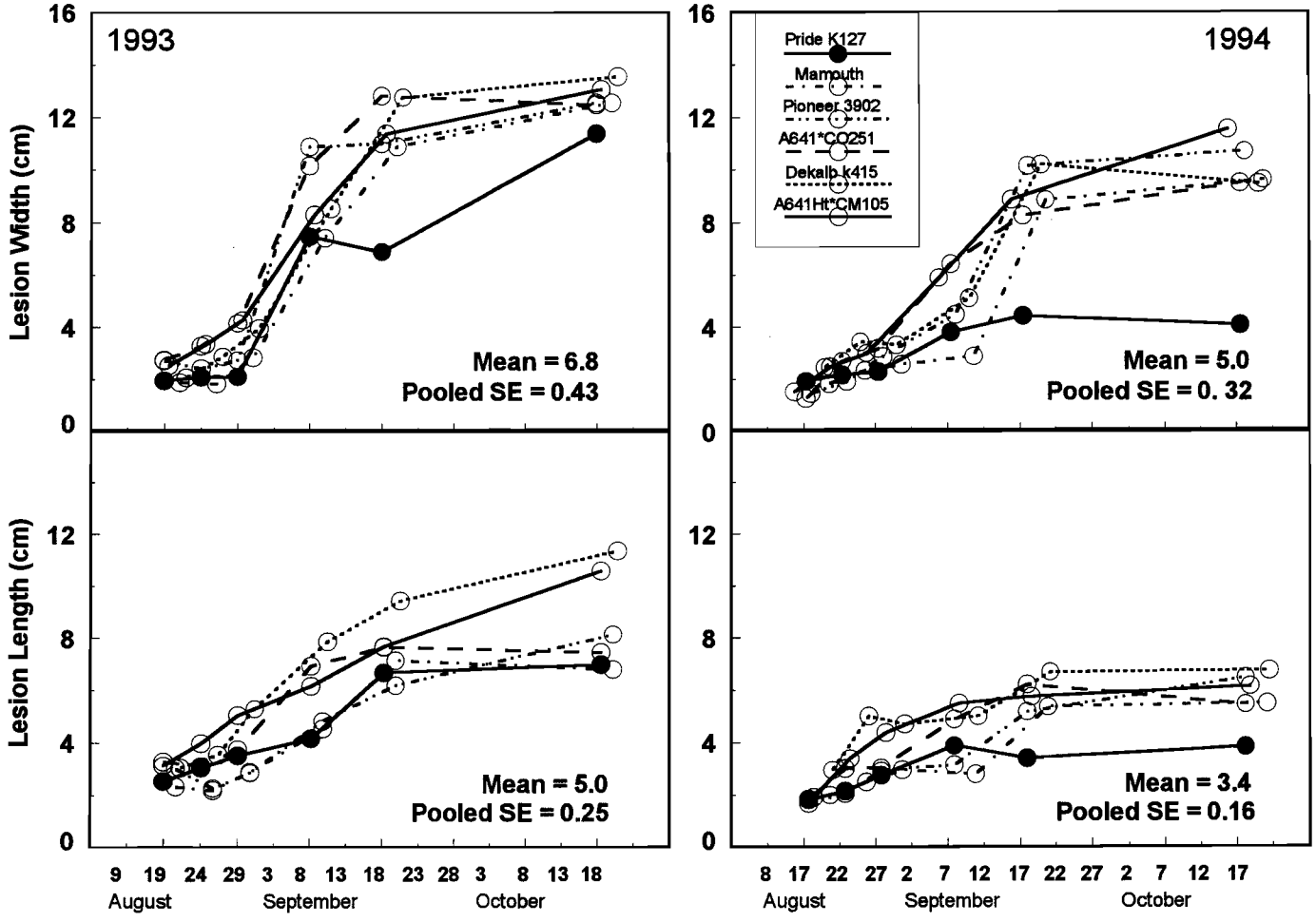

1993
1994
Pride K127
Mamouth
Pioneer 3902
A641*CO251
Dekalb k415
A641Ht*CM105
Lesion Width (cm)
Lesion Length (cm)
Mean = 6.8
Pooled SE = 0.43
Mean = 5.0
Pooled SE = 0. 32
Mean = 5.0
Pooled SE = 0.25
Mean = 3.4
Pooled SE = 0.16
0
4
8
12
16
9 19 24 29 3 8 13 18 23 28 3 8 13 18
August
September
October
8 17 22 27 2 7 12 17 22 27 2 7 12 17
August
September
October

Figure 9.3. Pericarp thickness in maize hybrids inoculated with *Fusarium graminearum* in 1993 and 1994.

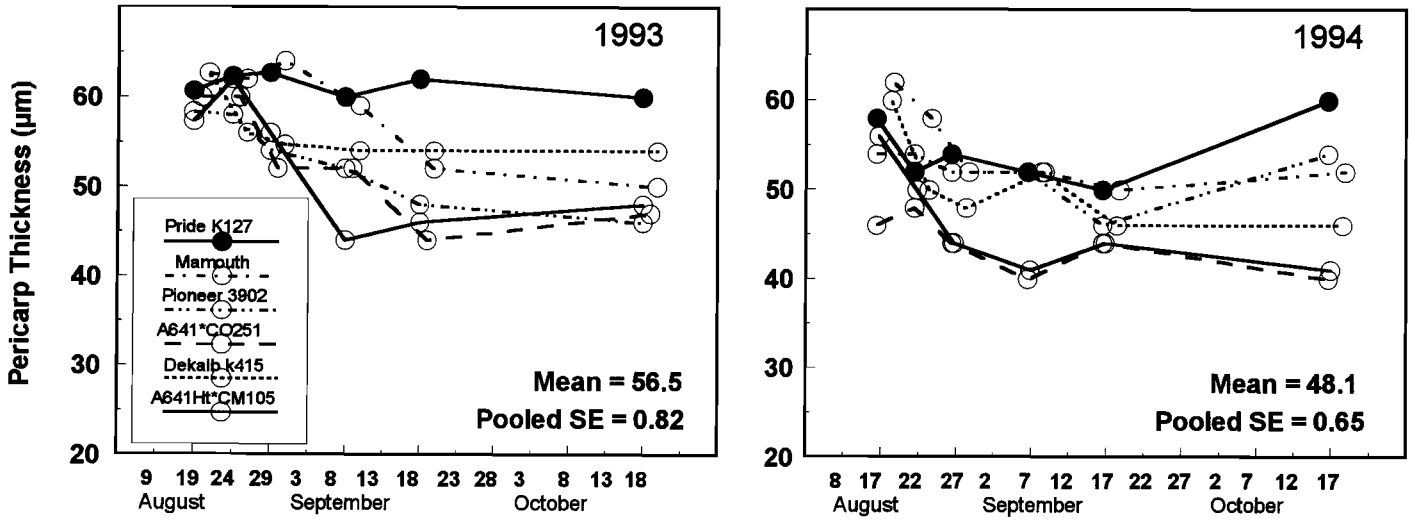
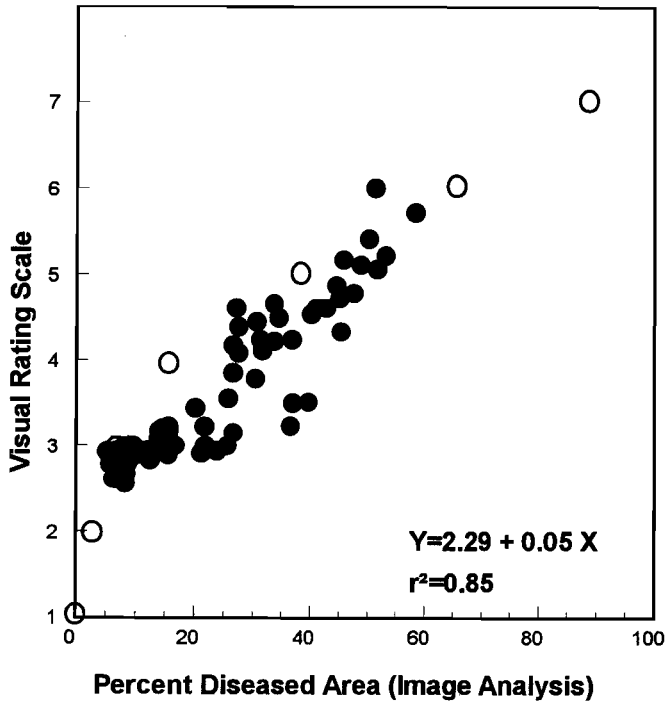
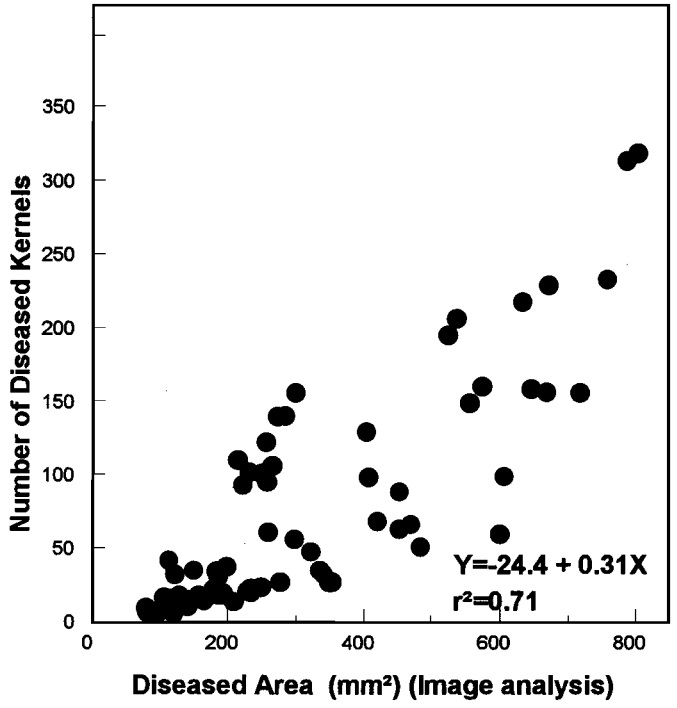

Pericarp Thickness (µm)
1993
1994
Pride K127
Mamouth
Pioneer 3902
A641*CO251
Dekalb k415
A641Ht*CM105
Mean = 56.5
Pooled SE = 0.82
Mean = 48.1
Pooled SE = 0.65
20
30
40
50
60
9 19 24 29 3 8 13 18 23 28 3 8 13 18
August
September
October
8 17 22 27 2 7 12 17 22 27 2 7 12 17
August
September
October

Figure 9.4. Correlation between mean disease severity values from (a) image analysis and a visual rating scale, (b) image analysis and number of diseased kernels, for ears inoculated with *Fusarium graminearum* in 1993 and 1994. Open circles represent midpoints of the interval on the visual rating scale.


Visual Rating Scale
Percent Diseased Area (Image Analysis)
Y=2.29 + 0.05 X
r²=0.85
1
2
3
4
5
6
7
0
20
40
60
80
100


Number of Diseased Kernels
Diseased Area (mm²) (Image analysis)
Y=-24.4 + 0.31X
r²=0.71
0
50
100
150
200
250
300
350
0
200
400
600
800

Chapter 10

GENERAL DISCUSSION

Types of Resistance: In previous research conducted by Reid *et al.* (1992a,b,c), a macroconidial suspension of *F. graminearum* was injected into the silk channel of maize ears. Among the inbreds evaluated, they identified CO272 as being more resistant than other inbreds. Later, results from phytochemical studies (Reid *et al.* 1992d) and an *in vitro* assay (Reid *et al.* 1992a) of detached silk tissue both pointed towards the silk as the tissue responsible for CO272's resistance. In genetic experiments, CO272's silk resistance appeared to be controlled by a single dominant gene (Reid *et al.* 1994). However, the expression of this gene was sensitive to environmental conditions. Recent studies by Bergvinson and Reid (1995) have demonstrated that CO272 has a thicker wax coating along the entire length of the silk. They identified the wax constituents to be simple hydrocarbons (C25-C31). All studies have so far shown that major resistance mechanism in CO272 are in the silk tissue. Zapalote Chico has also exhibited high level of resistance when a macroconidia suspension was injected in the silk channel in studies carried out by Reid *et al.* (1992) and in Chapter 6. The wide variation in resistance observed among individual plants or lines in this population indicated that Zapalote Chico is a potential source of silk and/or kernel resistance.

Silk resistance can only be effective if the mode of fungal entry into maize ears is via the silk. Infection that results from insect or bird wounds may require an alternative or complementary form of resistance that could slow or suppress the

spread of infection on kernels. Identification of such kernel resistance would be facilitated by inoculation methods that apply inoculum directly to kernel tissue.

Inoculation Methods: In Chapter 4, six inoculation methods (silk-channel injection, ear-tip flooding, wound-spray, kernel-stab, pipecleaner and cob-tip) differing in inoculum application were employed. The silk-channel injection and the cob-tip methods were used to assess silk and cob-tissue resistance, respectively. The ear-tip flooding, wound-spray and kernel-stab were used to assess kernel resistance. Although the pipecleaner method was intended to detect kernel resistance, it probably assessed a combination of kernel and cob-tissue resistance since inoculum was applied to both tissues. Among the methods used, the silk-channel injection and the kernel-stab methods caused minimal wounds and were effective in discriminating between resistant and susceptible inbreds. These methods mimicked natural infection through the silk and that incited by insects, respectively. It should, however, be pointed out that the silk-channel method should be able to detect either silk or kernel resistance because even if the silk tissue is breached, resistance mechanisms in the kernels could stop or minimize symptom development. The kernel-stab method, on the other hand, can only detect kernel resistance. Therefore, using both methods in a breeding program could allow differentiating between silk and kernel resistance mechanisms.

Inheritance: From the data presented in Chapter 4 and Appendix A, it was evident that inbred CO325 was more resistant than other inbreds (A641, CO265, CO266, CO272, and F2) with the kernel-stab method. This inbred appears to have high kernel resistance. It therefore represents a different form of resistance from that

reported in CO272. The inheritance of this resistance was investigated in the experiments reported in Chapter 5, in which CO325 was used as a source of resistance. Generation means analysis indicated additivity was the predominant type of genetic effect in all four environments. The magnitudes of the additive gene effects were also larger than those of dominance gene effects, in contrast to results reported by Boling and Grogan (1965) and Odiemah and Manninger (1982). Predominance of additivity in resistance to *F. graminearum* should make incorporation of resistance into agronomically useful maize lines practical since genes for a susceptible or resistance disease response would not be masked by other dominant or epistatic alleles. Other effects that were sometimes significant were dominance and dominance × dominance interactions. However, caution should be exercised when making conclusions on dominance effects because the generation means analysis may not detect opposing effects. For example, dominant genes for resistance and susceptibility could be present in the cross but not evident in the analysis. This problem could have been minimized if each inbred had been analyzed in more than one cross. The presence of significant dominance × dominance interactions signify that more than one gene is involved and that some or all the genes contributing to ear rot resistance interact with each other in producing their effects. The frequency distribution of the three segregating generations showed continuous variation, indicating that resistance may be under the influence of several genes, consistent with the estimates obtained in the analysis. The variation observed in the nonsegregating generations reflected the influence of the environment on the response

of these generations. The method employed to estimate the minimum number of effective factors (*n*) is dependent upon the assumptions that (1) all favorable alleles have been fixed at both limits, (2) all genes have equal effects and (3) no linkage. Failure of conditions (1) and (2) could have led to underestimation of *n*. Failure of condition (3) could lead to *n* being overestimated since the additive variance would be less than it would be with independent segregation. Due to the number of genes improvement in resistance to *F. graminearum* can be accomplished through the use of pedigree selection method. The method is more appropriate than recurrent selection since agronomically superior inbreds were used. However, if resistant inbreds were to be developed from populations, recurrent selection could be appropriate since this method would be the best in accumulating genes for resistance.

Environmental Effects: Genotype × environmental effects are important to a plant breeder because of the confounding effects they introduce in comparisons among genotypes tested in different environments. Although environmental effects are known to play a significant role in the establishment of ear rot caused by *F. graminearum* (Koehler, 1959; Tuite *et al.* 1974) little information is available concerning genotype × environmental interactions for resistance of maize to ear rot. The environment can be represented by important weather factors such as temperature and rainfall. The effect of these two factors on the development of *F. graminearum* was evident in Chapter 4, 5, 8 and 9. Although some genotypes were consistently resistant across environments, the effect of environment was reflected in the amount of symptoms that developed. In 1993 temperatures were warm and rainfall was high.

Rainfall in 1993 was more frequent and therefore provide a continuous surface wetness required by the fungus. On the other hand, 1994 was cooler than normal and infection was low in all hybrids. As long as environmental conditions allow for the expression of sufficient differences in disease among genotypes and do not cause important rank reversals in any genotypes, such effects are not so critical to a breeder. However, genotype × environment interaction that strongly affects the response of genotypes, as seen with inbred CO272 in Chapter 4 and in the manifestation of the dominance genetic effects in Chapter 5, is of concern to breeders. Such environmental effects could require breeding hybrids specifically for different environments, or preferably identifying hybrids with stable resistance. Among genotypes, inbred CO325 and hybrid Pride K127 were stable in resistance across all environments. The effect of the environment was also observed in Chapter 8 with respect to differences between years in the level of deoxynivalenol produced and amount of fungal tissue. Nonetheless, no major changes in the trend of toxin distribution were observed.

Components of Kernel Resistance: Characterization of resistance mechanisms is often helpful in determining the best screening method procedure to adopt. Furthermore, histological and biochemical studies of resistance may bring an understanding of the basis of resistance. Bergvinson and Reid (1995) reported that in maize kernels both physical (cell wall bound phenolic compounds) and toxin (soluble secondary metabolites) mechanisms are involved in resistance. Assabgui *et al.* (1993) demonstrated that resistant inbreds consists of higher kernel (E)-ferulic acid content

than susceptible inbreds. Total phenolic compounds of maize kernels for the six hybrids assessed in Chapter 9 are shown in Appendix C. Three susceptible hybrids (A641Ht × CM105, A641 × CO251 and Dekalb DK415) had higher phenolic compounds than Pride K127 at all sampling times. The method used (ultra fluorescence spectrophotometry) measured total phenolic compounds. Among these compounds, some probably play no role as plant resistance factors.

In the experiment described in Chapter 9, pericarp thickness was also identified as a possible component of kernel resistance. The pericarp of Pride K127 was thicker than other hybrids at most sampling times. If pericarp thickness is indeed a major mechanism of resistance in Pride K127, progeny of crosses involving sources of resistance could be screened microscopically before embarking on extensive field screenings.

Nutritional status of the ear could be a possible factor affecting ear rot development. Standen (1943) found that poorly matured cob tissues had higher percent nitrogen and fungal growth (*Nigrospora oryzae)* than well-matured cobs. Data presented in Appendix C show that percent nitrogen of Pride K127 was lower than other hybrids in 1993, but not in 1994. Other kernel factors that were observed include moisture content and kernel hardness (Appendix C). No clear differentiation between resistant and susceptible hybrids were observed for these factors. Previous work by Warren (1978) has demonstrated that endosperm type affects the response of maize genotypes to ear rot caused by *F. moniliforme*. The endosperm of *opaque-2* maize tended to be susceptible to fungal invasion. In the experiment described in

Chapter 6, the *waxy* endosperm type was assessed to determine if this endosperm type would affect resistance of maize inbreds to ear rot caused by *F. graminearum*. Waxy maize inbreds had comparable disease symptoms to their normal counterparts. Unlike *opaque-2* maize, *waxy* endosperm is similar in hardness to non-*waxy* endosperm. This could explain the similarity in reaction between these isogenic lines.

Symptom Development: For a given disease, variation in the disease progression may occur, but the form of disease progress may depend on environmental, genotypic and/or cultural practices. The spread of symptoms seem to have differed not only between genotypes but also across the years (Chapter 9). Differences in symptom development between hybrids could not be distinguished on the basis of diseased area before the second sampling time (dough stage), but after the fourth sampling time (dent stage) differences were observed readily. This is of practical significance because it allows a greater time-span for identifying resistant breeding germplasm than when selections are made only at the normal harvesting time. Observations made at various intervals can also help in selecting genotypes with stable resistance, since genotypes are observed over an extend time. In addition, this may also reveal the basis of resistance if biochemical and histological studies are undertaken.

Spatial Distribution of *F. graminearum* and Deoxynivalenol in ears: The presence of deoxynivalenol (DON, vomitoxin), in maize ears infected with *F. graminearum* is a cause of concern for grain growers, livestock producers and cereal processors. DON can cause a complete crop loss and consumption of contaminated grain is deleterious to the health of both humans and livestock. Understanding the distribution of fungal

tissue and particularly DON in infected ears is of practical significance in determining how infected ears could be used. Work done by Reid *et al.* (accepted) and Miller *et al.* (1985) have shown that levels of toxins tend to be higher in susceptible than resistant genotypes. In contrast, in the research reported in Chapter 8 it was found that a resistant inbred (CO325) exhibited higher toxin and lower fungal DNA than the susceptible inbred (CO265). Although only a few ears were evaluated in this study, the comparisons were made between ears with similar symptom levels. In contrast, Reid *et al.* (In press) and Miller *et al* (1985) compared genotypes with varying levels of ear rot symptoms. In all previously published reports, bulk samples were used, whereas in Chapter 8, individual kernels were evaluated. If resistant genotypes contained many of uncontaminated and/or uninfected kernels, it may explain why Reid *et al.* (accepted) and Miller *et al.* (1985) observed lower levels of toxins in resistant than susceptible genotypes. Consistent with results of Visconti *et al.* (1990), and Reid *et al.* (In press), fungal DNA and DON were high in kernels from ears with moderate symptoms. In addition, the cob tissues contained higher DON levels than the kernels. Use of cob tissue from infected ears as feedstuff for pigs should consider this aspect of spatial distribution of DON.

Disease Assessment Methods: In maize breeding programs, ear rot assessments are usually carried out using visual rating scales. These scales are easily integrated in breeding programs, but very subjective because of the irregularity of the lesions, coupled with the eyes ability to only accurately assess very low and very high disease. Appropriate disease assessment methods should provide accurate information that

satisfies the goals of the research. An image analysis method such as that used in Chapter 9 could minimize human bias in visual assessments, since it shifts the quantification of lesion size from the person making the outlines to a machine. As a result, image analysis provides a more accurate disease assessment than visual rating particularly when symptoms are moderate. If early selection could be embarked on as suggested in Chapter 9, use of visual rating scale(s) alone may not be sufficient since at this stage symptoms are moderate. Although image analysis is more accurate than the visual rating scale, it is quite laborious and may be quite costly. If selections are to be carried out before physiological maturity, use of a combination of visual rating and measurements of lesion width may be appropriate.

Chapter 11

CONCLUSIONS

1. The silk-channel injection and kernel-stab inoculation methods were effective in distinguishing between lines carrying silk and/or kernel resistance, respectively.

   Inbred CO325 exhibited high resistance with both silk-channel injection and kernel-stab inoculation methods.

   Correlations between inoculation methods intended to assess same resistance mechanisms were significant and high.

2. Inheritance of kernel resistance to *F. graminearum* was under genetic control with both simple (additive and dominance) and digenic (dominance × dominance) effects contributing to the total genetic variation among the generation means.

   More than 4 genes may be involved in controlling kernel resistance.

3. No differences in resistance were observed between the waxy inbred and their non-waxy counterparts.

4. Wide variation in disease severity was observed among Zapalote Chico plants and selfed progenies when the silk-channel injection was used.

   Plant pigmentation does not appear to be related to the resistance level of

individual plants extracted from Zapalote Chico.

Resistance within some S1 lines was less variable indicating that resistance can be improved through selfing.

5. Amount of fungal tissue and concentrations of deoxynivalenol were variable among individual maize kernels.

Some kernels with detectable DON were negative for *F. graminearum*, indicating that DON could have been translocated to these kernels independent of fungal hyphae.

Kernels from the resistant inbred exhibited higher levels of DON than the susceptible inbred.

The correlation between fungal DNA and DON of individual kernels was positive but low.

6. Symptoms developed more slowly in Pride K127 than in more susceptible hybrids.

Significant differences in symptom development between Pride K127 and other hybrids were observed before physiological maturity.

The pericarp thickness in Pride K127 was thicker than in other hybrids and may be one resistance mechanism that influenced ear rot development in this hybrid.

Image analysis can be used to measure maize ear rot symptoms.

Correlations between image analysis with visual rating scale and number of diseased kernels were significant and high.

Chapter 12

CONTRIBUTION TO KNOWLEDGE

Research on maize resistance to *F. graminearum* has been reported by several workers. However, this research was focused on the inheritance and mechanisms of kernel resistance. The following are the contributions to knowledge from the work presented in this thesis.

1. Inoculation of maize ears with six methods differing in the application of inoculum showed that some of these methods evaluated inbred lines differently. This study was the first to show that different resistance mechanisms to *F. graminearum* may be involved when inoculum is applied to the silk, kernel and cob tissue. In addition, this research was the first to reveal that inbred CO325 may possess both silk and kernel resistance mechanisms. This study also reports on the effectiveness of the kernel-stab inoculation method in evaluating kernel resistance.

2. Evaluation of the inheritance of kernel resistance revealed that both simple and digenic effects were important. Although previous studies have shown significant additive, dominance and digenic effects, this study was the first to reveal that additive gene effects were not only predominant but also larger than dominance effects. This research was also the first to show that more than 4 genes may be involved in controlling kernels resistance to *F. graminearum*.

3. No difference was observed in the response of *waxy* and non-waxy inbreds to *F. graminearum* after inoculation with the silk-channel injection and kernel-stab methods. This was the first study to compare the resistance levels of *waxy* and non-*waxy* isogenic lines.

4. S1 lines derived from individual plants of Zapalote Chico, segregated for both plant pigmentation and silk resistance. Some lines from resistant plants were still resistant in the S1 generation. This was the first observation to show that resistance in this population is heritable.

5. Amounts of fungal DNA and DON concentrations were found to be variable among individual maize kernels within an ear. This study was the first to report on the distribution of DON in individual kernels and show variations in DON and fungal tissue in kernels. It provided some evidence of possible DON translocation within an ear.

6. Assessment of ear rot symptom development showed that symptoms developed more slowly in the resistant than most susceptible hybrids. To my knowledge, this research was the first to use image analysis in assessing ear rot symptom development. It also showed that Bolton's visual rating scale gave similar results as image analysis. It was also the first study to monitor pericarp thickness at various kernel developmental stages and show that the resistant hybrid had thicker pericarp at

most growth stages. In addition, this was the first study to shown that differences in resistance to *F. graminearum* are attained before physiological maturity, an observation that could be useful to breeders in speeding up the development of resistant genotypes.

Chapter 13

SUGGESTIONS FOR FUTURE RESEARCH

1. Results of this research revealed that even breeding materials possessing silk or kernel resistance mechanisms may exhibit low symptoms in years conducive to disease. It seems therefore that breeding work could place emphasis in incorporating both silk and kernel resistance into agronomically useful inbred lines to develop hybrids that are effectively resistant to *F. graminearum.*

2. Screening for resistance requires methods that are effective and consistent in inciting infection. The silk-channel injection and the kernel-stab could be appropriate methods to use in evaluate lines carrying resistant genes to other ear-rotting pathogen. This could help determine if resistance to other fungal pathogens could be useful in screening for resistance to ear rot caused by *F. graminearum.*

3. Several lines differing in resistance and susceptibility to *F. graminearum* could be evaluated to have a broader picture of DON distribution in infected ears. An understanding of when DON accumulates in individual kernels could provide some information of the importance of DON to fungal invasion. Harvesting of ears inoculated with *F. graminearum* at various intervals could provide the necessary information.

4. To obtain a more definitive conclusion on whether the *waxy* endosperm trait affects susceptibility to ear rot, isogenic lines such as those used in Chapter 6 could be evaluated more rigorously, taking into account the possible confounding effects of bagging of ears. Treatments in such an experiment could include *waxy* inbreds with bagging to prevent pollen contamination and non-waxy inbreds with and without bagging.

5. Inheritance of silk resistance could be evaluated in more advanced selfed progenies of Zapalote Chico. Parents and offsprings could be used to obtain this information. Kernel resistance could also be investigated by inoculating the ears with the kernel-stab method.

Chapter 14

REFERENCES

Aldrich, S.R., W.O. Scott and E.R. Leng. 1975. Modern Corn Production. 2nd ed. A & L Publications.

Arnason, J.T., J. Gale, B. Conilh de Beyssac, A. Sen, S.S. Miller, B.J.R. Philogene, J.D.H. Lambert, R.G. Fulcher, A. Serratos and J. Mihm. 1992. Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). J. Stored Prod. Res. 28:119-126.

Assabgui, R.A., L.M. Reid, R.I. Hamilton and J.T. Arnason. 1993. Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. Phytopathol. 83:949-953.

Bennett, G.A., D.T. Wicklow, R.W. Caldwell and E.B. Smalley. 1988. Distribution of trichothecenes and zearalenone in *Fusarium graminearum*: Rotted corn ears grown in a controlled environment. J. Agric. Food Chem. 36:639-642.

Benson, G.O. and R.B. Pearce. 1987. Corn perspective and culture. Pages 34-50. *In*: Principles of Field Crop Production. 3rd ed. Macmillan Pub. Co., Inc. New York.

Berger, J. 1973. Maize Production and the Manuring of Maize. Conzett and Huber, Zurich.

Bergvinson, D.J. and L.M. Reid. 1995. Phytochemical mechanisms of resistance to ear rots. Maize Genetics Cooperation Newsletter 68:114-115.

Bockholt, A.J. and L. Rooney. 1992. QPM hybrids for the United States. Page 111-121. *In*: Quality Protein Maize. (ed. E.T. Mertz). The American Association of Cereal Chemists. St. Paul, Minnesota, U.S.A.

Boling, M.B. and C.O. Grogan. 1965. Gene action affecting host resistance to Fusarium ear rot in maize. Crop Sci. 5:305-307

Boling, M.B., C.O. Grogan and J.W. Broyles. 1963. A new method of artificially producing epiphototics of Fusarium ear rot of maize. Plant Dis. Rep. 47:315-317.

Bolton, A.T., R.I. Hamilton. and A.R. McElory. 1990. Measuring ear mold tolerance in corn. In: Proceedings 45th Annual North Eastern Corn Improvement Conference NEC. Newark, Delaware.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute. Kew. Surrey, England. 237 pp.

Bressani, R. 1992. Nutritional value of high-lysin maize in humans. Page 205-224. *In*: Quality Protein Maize. (ed. E.T. Mertz). The American Association of Cereal Chemists. St. Paul, Minnesota, U.S.A.

Burgess, L.W. and D.M. Griffin. 1968. The recovery of *Giberrella zeae* from wheat straws. Australian J. Exp. Agric. Anim. Husb. 8:364-370.

Burgess, L.W., R.L. Dodman, W. Pont and P. Mayers. 1981. *Fusarium* diseases of wheat, maize and grain sorghum in eastern Australia. Pages. 39-52. *In Fusarium*: Disease, Biology, and Taxonomy. P.E., Nelson, T.A. Toussoun, and R.J.Cook (eds). Pennsylvania State Univ. Press, Univ. Park, USA.

Chiang, M.S., M. Hudon, A. Devaux, and I. Ogilvie. 1987. Inheritance of resistance to *Gibberella zeae* ear rot in maize. Phytoprotection 68:29-33.

Chungu, C., D.E. Mather, L.M. Reid and R.I. Hamilton. 1996. Comparison of techniques for inoculating of maize silk, kernel and cob tissue with *Fusarium graminearum*. Plant Dis. 80:81-84.

Coe, E.H. Jr., M.G., Neuffer, and D.A., Hosington. 1988. The genetics of corn. Page 142. *In* Corn and Corn Improvement. American Society Agronomy Madison, Wisconsin, U.S.A.

Cook, R.J. 1981. *Fusarium* diseases of wheat and other small grains in North America. Pages 39-52. *In Fusarium*: Disease, Biology, and Taxonomy. P.E., Nelson, T.A. Toussoun, and R.J.Cook, (eds). Pennsylvania State Univ. Press, Univ. Park, USA.

Cullen, D., R.W. Caldwell, and E.B. Smalley. 1983. Susceptibility of maize to *Giberrella* ear rot: Relationship of host genotype, pathogen virulence, and zearelenone contamination. Plant Dis. 67:89:91.

Delorit, R.T., L.J. Greub, and H.L Ahlgren. 1984. Corn. Pages 69-130. *In* Crop Production. 5th ed. Prentice-Hall, INC., Englewood Cliffs, London.

Dicke, F.F. and W.D. Guthrie. 1988. The most important corn insects. Pages 767-869. *In* Corn and Corn Improvement. American Society Agronomy. Madison, Wisconsin, U.S.A.

Drepper, W.J., and B.L. Renfro. 1990. Comparison of methods for inoculation of ears and stalks of maize with *Fusarium moniliforme*. Plant Dis. 74:952-956.

Edwards, K., C. Johnstone, and C., Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Enerson, P.M. and R.B. Hunter. 1980. A technique for screening maize (*Zea mays* L.) for resistance to ear mold incited by *Gibberella zeae* (Schw.) Petch. Can. J. Plant Sci. 60:1123-1128.

Eugenio, C.P., C.M. Christensen, and C.J. Mirocha. 1970. Factors affecting production of the mycotoxin F-2 by *Fusarium roseum*. Phytopathol. 60:1055-1057.

Falconer, D.S. 1989. Introduction to Quantitative Genetics. 3rd ed. Longman Scientific & Technical, Essex, England.

Francis, R.G., and L.W. Burgess. 1977. Characteristics of two populations of *Fusarium roseum* 'Graminearum' in eastern Australia. Trans. Brit. Mycol. Soc. 68:421-427.

Gendloff, E. H., E.C. Rossman, W.L. Casale, G.G. Isleib and L.P. Hart. 1986. Components of resistance to Fusarium ear rot in field corn. Phytopathol. 76:684-688.

Glover, D.V. 1992. Corn Protein: Genetics, breeding and value in foods and feeds. Page 9-26. *In*: Quality Protein Maize. (ed. E.T. Mertz). The American Association of Cereal Chemists. St. Paul, Minnesota, U.S.A.

Gulya, T.J. Jr., C.A. Martinson and P.J. Loesch, Jr. 1980. Evaluation of inoculation techniques and rating dates for Fusarium ear rot of opaque-2 maize. Phytopathol. 70:1116-1118.

Hallauer A.R. and J.B. Miranda. 1988. Quantitative Genetics in Maize Breeding. 2nd ed. Iowa State Univ. Press, Ames, Iowa.

Hart, L.P., W.L. Casale, E. Gendloff, C. Rossman, and T. Isleib. 1987. Resistance of corn inbred lines to Fusarium and Fusarium toxins. 42nd Annual corn and sorghum research conf.

Hart, L.P., E. Gendloff, and C. Rossman. 1984. Effects of corn genotypes on ear rot infection by *Gibberella zeae*. Plant Dis. 68:296-298

Hart, L.P., E. Gendloff, and T.C. Stebbins. 1982. Production of zearalenone and deoxynivalenol in commercial sweet corn. Plant Dis. 66:1133-1135.

Hoenisch, R.W. and R.M. Davis. 1994. Relationship between kernel pericarp thickness and susceptibility to Fusarium ear rot in field corn. Plant Dis. 78:517-519.

Horsfall, J.G. and R.W., Barratt. 1945. An improved grading system for measuring plant diseases (Abstr.) Phytopathology 35:655.

Hughes, H.D. and E.W. Henson. 1957. The corn crop. Pages 189-239. *In* Crop Production: Principles and Practices. Macmillan Company, New York.

Hunter, R.B., and J.C. Sutton. 1985. Plant resistance and environmental conditions. Pages 62-67. *In* Mycotoxins: A Canadian perspective. Scott, P.M., Trenholm, H.L., Sutton, M.D., eds. National Research Council of Canada, Ottawa, Ontario. Publ. No. 22848.

Jayaram, Ch. and P.A. Peterson. 1990. Anthocynanin pigmentation and transposable elements in maize aleuron. Plant-Breeding 8:91-137.

Johnson, I.J. and J.J. Christensen. 1935. Relation between number, size and location of smut infection to reduction in yield of corn. Phytopathol. 25:223-244.

Kedera, C.J., T.E. Ochor, J.A.W. Ochieng and R.E. Kamidi. 1994. Incidence of maize ear rot in western Kenya. International J. Pest Management. 40:117-120.

Kiesselbach, T.A. 1949. The structure and reproduction of corn. Research Bulletin 161. Agric Exp. Station. Univ. of Nebraska.

King, S.B. and G.E. Scott. 1981. Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. Phytopathol. 71:1245-1247.

Koehler, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. J. Agric. Res. 64:421-442.

Koehler, B. 1959. Corn ear rots in Illinois. I11. Agric Exp. Stn. Bull. 639. 87 pp.

Koehler, B. and J.R. Holbert. 1930. Corn diseases in Illinois. I11. Agr. Exp. Bull. 354.

Kyle, C.H. 1918. Shuck protection for ear corn. U.S. Dept. Agr. Bull. 708c.

Lee, L.S., E.B. Lillehoj, and W.F. Kwolek. 1980. Aflatoxin distribution in individual corn kernels from intact ears. Cereal Chemistry 57:340-343.

Lindow, S.E., and R.R. Webb. 1983. Quantification of foliar plant disease symptoms by microcomputer-digitized video image analysis. Phytopathol. 73:520-524

Lovelace, C.E.A. and C.B. Myathi. 1977. Estimation of the fungal toxins, zearalenone and aflatoxin, contaminating opaque maize beer in Zambia. J. Sci. Food Agric. 28:228-292.

Marasas, W.F.O., N.P.J. Kriek, V.M. Wiggins, P.S. Steryn, D.K. Towers and T.J. Hastie. 1979a. Incidence, geographic distribution and toxigenicity of *Fusarium* species in Southern Africa corn. Phytopathol. 69:1181-1185.

Marasas, W.F.O., S.J., van Rensburg, and C.J. Mirocha. 1979b. Incidence of *Fusarium* species and mycotoxins, deoxynivalenol and zearalenone in corn produced in oesophageal cancer areas in Transkei. J. Agric. Food Chem. 27:1108-1112.

Martin, J.H., W.H. Leonard and D.L. Stamp. 1975. Indian corn or maize. Pages 323-382. *In* Principles of Field Crop Production. 3rd ed. Macmillan Pub. Co., Inc. New York.

Mather, K and J.L. Jinks. 1982. Biometrical Genetics: The study of continuous variation. 3rd ed. Chapman and Hall Ltd. London.

Metcalfe, D.S. and M.D. Elkins. 1980. Corn or maize. Pages 333-365. *In* Crop Production: Principle and Practices. 4th ed. MacMillan Publishing Co., Inc New York.

Miller, J.D., A. Taylor and R., Greenhalgh. 1983a. Production of deoxynivalenol and related compounds in liquid culture by *Fusarium graminearum*. Can. J. Microbiol. 29:1171-1178.

Miller, J.D., C.J. Young and D.R. Sampson. 1985. Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopath. Z. 113:359-367.

Miller J.D., C.J. Young, and H.L. Trenholm. 1983b. *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can. J. Bot. 61:3080-3087.

Mirocha, C.J. and C.M. Christensen. 1974. Fungus metabolites toxic to animals. Ann. Rev. Phytopathol. 12:303-330.

Möller, E.M., G. Bahnweg, H. Sandermann, and H.H. Geiger. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acid Res. 20:6115-6116.

Naik, D.M., L.V. Busch, and G.L. Barron. 1978. Influence of temperatures on the strain of *Fusarium graminearum* Schwabe in zearalenone production. Can. J. Plant Sci. 58:1095-1097.

Neish, G.A., E.R. Farnworth, R. Greenhalgh and J.C. Young. 1983. Observations on the occurrence of *Fusarium* species and their toxins in corn in eastern Ontario. Can. J. Plant Pathol. 5:11-16.

Nutter, F.W., Jr., M.L. Gleason, J.H., Jenco, and N.C., Christians. 1993. Assessing the accuracy, intra-rater repeatability and inter-rater reliability of disease assessment systems. Phytopathol. 83:806-812.

Ochor, T.E., L.E. Trevathan and S.B. King. 1987. Relationship of harvest date and host genotype to infection of maize by *Fusarium moniliforme*. Plant Dis. 71:311-313.

Odiemah, M. and I. Manninger. 1982. Inheritance of resistance to *Fusarium* ear rot in maize. Acta Phytopath. Acad. Sci. Hung. 17:91-99.

Ouellet, T. and K.A., Seifert. 1993. Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplifications. Phytopathol. 83:1003-1007.

Perkins, J.M. and W.L., Pedersen. 1987. Disease development and yield losses associated with Northern leaf blight on corn. Plant Dis. 71:940-943.

Putnam, M.L. and K.A., Binkerd. 1992. Comparison of a commercial ELISA kit and TLC for detection of deoxynivalenol in wheat. Plant Dis. 76:1078.

Reid, L.M., A.T. Bolton, R.I. Hamilton, T. Woldermariam and D.E. Mather. 1992a. Effect of silk age on resistance of maize to *Fusarium graminearum*. Can. J. Plant Pathol. 14:293-298.

Reid, L.M. and R.I. Hamilton. In press. Effect of inoculation position, timing, macroconidial concentration and irrigation on resistance of maize to *Fusarium graminearum* infection through kernels (Accepted in Can. J. Plant Pathol.).

Reid, L.M., D.E. Mather, J.T. Arnason and R.I. Hamilton. 1992d. Changes in phenolic constituents of maize silk infected with *Fusarium graminearum*. Can. J. Bot. 70-1697-1702.

Reid, L.M., D.E. Mather, A.T. Bolton and R.I. Hamilton. 1994. Evidence for a gene for silk resistance to *Fusarium graminearum* Schw. ear rot of maize. Heredity 85:118-121.

Reid, L.M., D.E. Mather, R.I. Hamilton and A.T. Bolton. 1992b. Diallel analysis of resistance in maize to *Fusarium graminearum* infection via the silk. Can. J. Plant Sci. 72:915-923.

Reid, L.M., D.E. Mather, R.I. Hamilton and A.T. Bolton. 1992c. Genotypic differences in the resistance of maize silk to *Fusarium graminearum*. Can. J. Plant Pathol. 14:211-214.

Reid, L.M., D.E. Mather and R.I. Hamilton. 1996. Distribution of deoxynivalenol in *Fusarium graminearum* infected maize ears. (Accepted in Phytopathology)

Reid, L.M., D.E. Mather, and R.I. Hamilton. 1992e. Observations of silk resistance in germplasm with resistance to *Aspergillus flavus* and *heliothis zeae*. Maize Genetics Cooperation Newsletter: 66:85-86.

Reid, L.M., D. Spaner, D.E. Mather, A.T. Bolton and R.I. Hamilton. 1993. Resistance of maize hybrids and inbreds following inoculation with three isolates of *Fusarium graminearum*. Can. J. Plant Sci. 72:915-923.

Ritchie, S.W., J.J. Hanway and G.O. Benson. 1986. How a Corn Plant Develops. Special report no. 48. Iowa State Univ. of Science and Technology Cooperative Extension Service. Ames, Iowa.

Rouanet, G. 1987. Maize. Pages 22-28. Macmillan Press Ltd. London and Basingstoke.

Russell, W.A., and A.R. Hallauer. 1980. Corn. Pages 299-312. *In* Hybridization of Crop Plants. W.A. Fehr and H.H. Hadley (eds). American Society of Agronomy, Madison, Wisconsin.

SAS Institute. 1994. SAS/STAT user's guide, version 6. 4th ed. SAS Institute Inc., Cary, North Carolina

Schroeder, H.W., and J.J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathol. 53:831-838.

Scott, G.E. and S.B. King. 1984. Site of action of factors for resistance to *Fusarium moniliform* in maize. Plant Dis. 68:804-806.

Seaman, W.L. 1982. Epidemiology and control of mycotoxigenic Fusaria on cereal grains. Can. J. Plant Pathol. 4:187-190.

Smart, M.G., O.L. Shortwell, and R.W. Caldwell. 1990. Pathogenesis in Aspergillus ear rot of maize: Aflatoxin B_1 levels in grain around wounded-inoculated sites. Phythopathol. 80:1283-1286.

Standen, J.H. 1944. Chemical and physical characteristics of maize cobs in relation to the growth of *Nigrospora oryzae*. Phytopathol. 34:315-323.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company.

Sutton, J.C. 1982. Epidemiology of wheat head blight. Can. J. Plant Pathol. 4:195-209.

Sutton, J.C. and W. Baliko 1981. Methods for quantifying partial resistance to *Gibberella zeae* in maize ears. Can. J. Plant Pathol. 3:26-32.

Szel, S. 1984. Study of resistance to *Fusarium graminearum* in *Opaque* maize lines and in hybrids produced from them by diallel crossing. Acta Agron. Acad. Sci. Hung. 33:444-448.

Tschanz, A. T., R. K. Horst, and P.E. Nelson. 1976. The effect of environment on sexual reproduction of *Gibberella zeae*. Mycologia 68:327-340.

Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Company. Minneapolis, MN.

Tuite, J., Shaner, G., Rambo, G., Foster, J., and Caldwell, R.W. 1974. The Gibberella ear rot epidemics of corn in Indiana in 1965 and 1972. Cereal Sci. Today 19:238-241.

Trenholm, H.L., D.B. Prelosky, J.C. Young and J.D. Miller. 1983. Reducing mycotoxins in animal feeds. Agric. Can. Publ. 1827/E.

Ullstrup, A. J. 1953. Several ear rots of corn. *In* Diseases of Corn, the Yearbook of Agric. U.S Dept Agric.

Ullstrup, A.J. 1970. Methods for inoculating corn ears with *Gibberella zeae* and *Diplodia maydis*. Plant Dis. Rep. 54:658-662.

Ullstrup, A. J. 1977. Diseases in corn. In G.F. Sprague (ed), Corn and Corn Improvement. pp. 391-500. American Society Agronomy, Madison, WI.

Visconti, A. J.Chelkowski, M. Solfrizzo and A. Bottalico. 1990. Mycotoxins in corn ears naturally infected with *Fusarium graminearum* and *F. crookwellense*. Can. J. Plant Pathol. 12:187:189.

Wallace, H. A. and E. N. Bressman. 1949. Corn and Corn Growing. 5th ed. John Wiley & Sons, Inc., New York. pp. 52-79.

Warren, H. L. 1978. Comparison of normal and high-lysine maize inbreds for resistance to kernel rot. Phytopathol. 68:1331-1335.

Warren, H. L., and T. Kommedahl. 1973. Prevalence and pathogenicity to corn Fusarium species from roots, rhizosphere residues and soil. Phytopathol. 63:1288-1290.

Watson, S. A. 1987. Structure and composition. Pages 53-82. *In* Corn: Chemistry and Technology. S.A. Watson and P.E. Ramstad, eds. American Association of Cereal Chemists. Inc., St. Paul, Minnesota.

Wearing, A. H. and L. W. Burgess. 1977. Distribution of *Fusarium roseum* 'Graminearum' Group I and its mode of survival in eastern Australia wheat belt soils. Trans. Br. Mycol. Soc. 69:429-442.

Wilson, R.L., B.R. Wiseman and G.L. Reed. 1991. Evaluation of J.C. Eldredge popcorn collection for resistance to corn earworm, fall armyworm *Lepidoptera: Noctuidae*), and European corn borer (*Lepidoptera: Pyralidae*). J. Econ. Entomol. 84:693-698.

Wiseman, B.R. and N.W. Widstrom. 1992. Resistance of corn populations to larvae of the corn earworm (*Lepidoptera*: *Noctuidae*). J. Econ. Entomol. 85:601-605.

Young, J.C and J.D., Miller. 1985. Appearance of fungus, ergosterol and *Fusarium* mycotoxins in the husks, axial stem and stalk after ear inoculation of field corn. Can. J. Plant Sci. 65:47-53.

APPENDICES

Appendix A:

Data presented in Appendix A is from experiments conducted at Ste-Anne-de-Bellevue and Ottawa in 1994. These experiments were carried out to supplement the 1992 and 1993 results reported in Chapter 4. Six inbreds and four hybrids were evaluated using three inoculation methods. Genotypes were evaluated in a split-plot design with genotypes as main plots and inoculation methods as subplots. The effects due to genotype, inoculation methods and interaction between genotype and inoculation methods were significant (Table A1). Ears inoculated with the pipecleaner and kernel-stab inoculation methods exhibited higher disease symptoms than those inoculated with the silk-channel injection method (Table A2), consistent with previous observations made in Chapter 4. Inbred CO325 and two hybrids Funks 4106 and Pride K127 were the most resistant.

Table A1. Mean squares for disease severity and incidence of maize inbreds and hybrids at Ste-Anne-de-Bellevue and Ottawa inoculated with *Fusarium graminearum* in 1994.

Source of Variation	Df	Ste-Anne-de-Bellevue		Ottawa	
		Severity	Incidence	Severity	Incidence
Block	3	0.44	130.94	0.59	1024.60**
Genotype	9	8.56**	57.61	8.60**	577.91**
Block × Genotype	27	0.56	115.16	0.52	54.18
Inoculation (I)	2	2.69**	325.73*	54.48**	7425.83**
Genotype × I	18	1.22**	64.51	3.93**	807.07**
Error	60	0.49	97.48	0.37	165.39

Table A2. Mean values for disease incidence and severity of inbred and Hybrids at Ste-Anne-de-Bellevue and Ottawa in 1994.

	Ste-Anne-de-Bellevue			Ottawa		
	Silk-Channel	Kernel-Stab	Pipecleaner	Silk-Channel	Kernel-Stab	Pipecleaner
Disease Incidence						
A641	100.00a	91.75a	100.00a	97.50a	100.00a	100.00a
CO265	100.00a	93.75a	100.00a	61.75cd	100.00a	100.00a
CO266	100.00a	100.00a	91.50a	100.00a	100.00a	100.00a
CO272	95.00a	87.50a	93.75a	42.75d	100.00a	100.00a
CO325	95.00a	86.25a	96.50a	70.50bc	100.00a	100.00a
F2	93.75a	89.25a	100.00a	90.00a	67.50b	100.00a
Funks 4106	90.25a	88.00a	100.00a	40.00d	100.00a	100.00a
Pioneer 3790	91.75a	97.50a	100.00a	87.50ab	100.00a	100.00a
Pioneer 3902	95.00a	95.75a	100.00a	80.00abc	97.50a	100.00a
Pride K127	90.00a	95.00a	100.00a	77.50abc	97.50a	100.00a
Disease Severity						
A641	3.57cd	3.44c	3.97bcd	4.22bc	5.40b	6.02ab
CO265	4.91ab	4.80ab	4.33bc	2.42de	6.05ab	5.00c
CO266	5.87a	5.65a	5.26a	5.67a	6.55a	6.65a
CO272	4.89ab	2.93c	4.53b	1.68e	6.49a	4.80c
CO325	2.86cd	2.60c	3.31de	1.92e	4.37cd	4.84c
F2	5.10ab	3.14c	3.63cde	4.31bc	3.07e	6.25ab
Funks 4106	3.18cd	2.59c	3.192e	1.62e	3.57de	4.72c
Pioneer 3790	2.87cd	3.33c	3.92bcde	3.27cd	4.45c	5.40bc
Pioneer 3902	3.95bc	3.69bc	3.85bcde	4.67ab	4.00cd	6.38ab
Pride K127	2.50d	2.93c	3.51de	2.42de	4.07cd	4.92c

means followed by the same letter within columns are not significantly different

Appendix B

Figures presented in Appendix B show the banding patterns of the *Fusarium graminearum* isolated from some individual maize kernels and characterized using PCR-based assays in Chapter 8. The fungus present on the ears was characterized by the random amplified polymorphic DNA (RAPD) and restriction analysis of amplified fragments fron the polymerrase chain reaction (PCR). The two RAPD primers produced different banding patterns. The strain of *Fusarium* could not be identified by RAPD amplifications, although single spore cultures were used. RAPD assays are not easily reproducible and interpreted, and are more sensitive to assay conditions than PCR amplifications (Ouellet and Seifert, 1993). The PCR amplifcations produced banding patterns that were consistent with *F. graminearum*. In 1994, the fungus identified agreed with the expectations based on the fungal isolate (DAOM194276) used in inoculations.

Figure B1. Example of patterns observed with random amplified polymorphic DNA (RAPD) assays. Primers A11, and B10 were used. Positive controls were DAOM180378 (A), DAOM178148 (B) and DAOM194276 (C) and a negative control (-) containing water instead of DNA. Molecular weight marker (M) was a mixture of HindIII- and TaqI-digested pBR322, with sizes indicated on the left in base pairs.

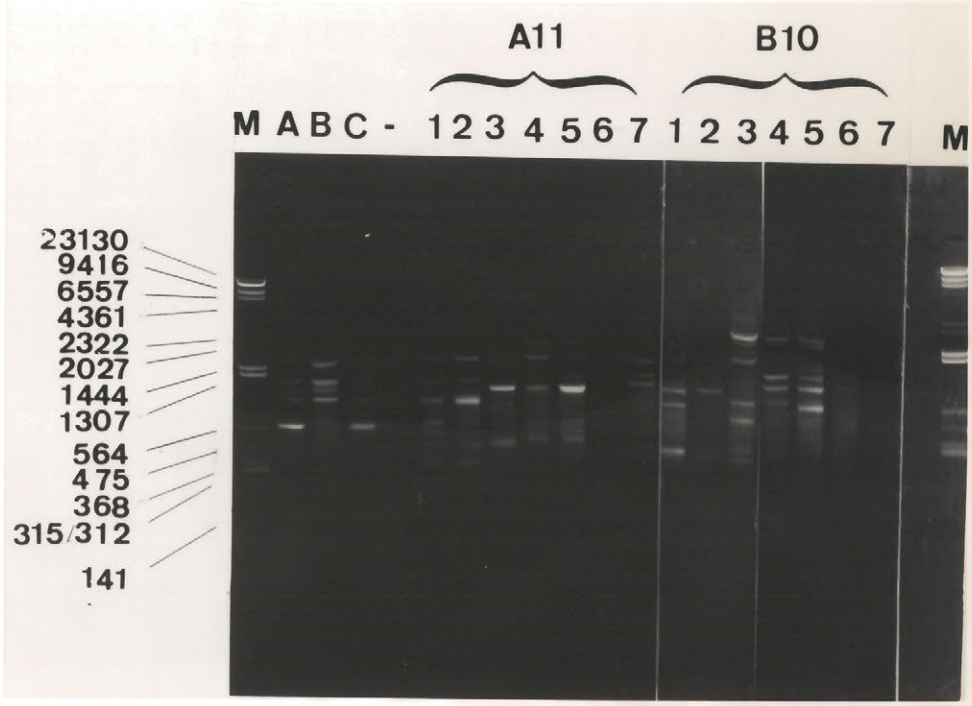
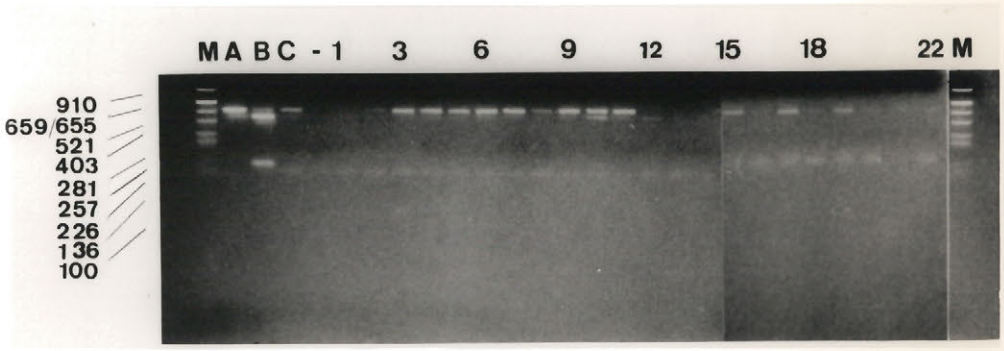

A11
B10
M A B C - 1 2 3 4 5 6 7 1 2 3 4 5 6 7 M
23130
9416
6557
4361
2322
2027
1444
1307
564
475
368
315/312
141

Figure B2. Example of patterns observed with the polymerase chain reaction (PCR) assays using primer pair A11a + A11b. Positive controls were DAOM180378 (A), DAOM178148 (B) and DAOM194276 (C) and a negative control (-) containing water instead of DNA. Molecular weight marker (M) was pBR322, with sizes indicated on the left in base pairs.


MA B C - 1 3 6 9 12 15 18 22 M
910
659/655
521
403
281
257
226
136
100

Appendix C

In the same experiments reported in Chapter 9, nineteen hybrids from Canada, France and United States were screened using the kernel-stab method. Additional mechanisms of resistance examined among the six hybrids reported in Chapter 9 were: percent nitrogen content, moisture content, kernel hardness and total phenolic compound (only in 1993).

Table C1. Mean disease severity of 25 maize hybrids from France, Ontario and Northern-U.S. inoculated with *Fusarium graminearum* in 1993 and 1994.

		Ste-Anne-de-Bellevue		Ottawa	
Source	Hybrid	1993	1994	1993	1994
France	Botan	5.0abcdefg	3.8cdefg	3.6bcd	4.7bcdef
France	Garant	5.0bcdefg	4.0bcde	4.5ab	5.3abcde
France	Koala	4.6bcdefgh	5.1a	3.1bcd	4.5cdef
France	Mamouth*	5.1abcdef	4.2bcd	3.8abcd	4.6cdef
France	Santos	4.2cdefgh	4.3bc	3.8abcd	5.7abc
U.S.A	A554Ht × W117Ht	5.4abc	4.1bcde	3.9abcd	5.4abcd
U.S.A	A619 × A632	3.9hg	3.8cdefg	4.1abcd	5.2abcde
U.S.A	A632Ht × W117Ht	4.1fgh	4.0bcdef	4.3abc	5.1abcde
U.S.A	A641 × CO251*	5.2abcde	4.3bc	3.2bcd	5.9ab
U.S.A	A641Ht × CM105*	5.7ab	4.1bcde	3.7bcd	5.5abcd
U.S.A	B73 × 78N9	3.9hg	3.0hij	3.5bcd	4.8abcdef
U.S.A	B73 × H99	4.2defgh	2.7j	4.3abc	6.0a
U.S.A	MO17Ht × 78N9	3.6h	2.9ij	4.4ab	5.1abcde
U.S.A	MO17Ht × F2	3.7h	3.7cdefgh	3.8abcd	3.9f
U.S.A	W64AHt × CM105	4.1bfgh	3.5efghi	4.0abcd	5.2abcde
Canada	Cargill 5157	3.7h	3.1ghij	4.0abcd	4.9abcdef
Canada	Delkab DK415*	6.0a	4.7ab	5.2a	5.5abc
Canada	FUNKS 4106	4.3cdefgh	3.0hij	3.5bcd	4.2ef
Canada	HL 2803	4.4cdefgh	2.9ij	3.9abcd	5.4abcd
Canada	NK N2001	4.6bcdefgh	3.5defghi	4.1abcd	4.5cdef
Canada	Pioneer 3902*	5.2abcdef	4.5ab	2.8cd	4.6cdef
Canada	Pioneer 3921	4.1fgh	3.6cdefghi	3.0cd	4.9abcdef
Canada	Pioneer 3953	5.3abcd	3.3fghij	3.6bcd	5.3abcde
Canada	Pioneer 3979	4.2cdefgh	3.4efghi	3.0cd	4.5cdef
Canada	Pride K127*	4.3cdefgh	3.2ghij	3.0cd	4.3def

means followed by the same letter(s) within columns are not significantly different

* Hybrids used for ear rot symptom development

Data were analyzed separately for each site-year due to heterogeneity of error variances. Most of the 25 hybrids had 100% disease incidence in all four environments. There was only one instance in which a hybrid had a disease incidence significantly ($P<0.05$) below 100% at Ste-Anne-de-Bellevue in 1994.

Significant differences ($P<0.05$) were observed for disease severity among the hybrids in all the four environments. Disease severity was lower in 1994 than 1993 at Ste-Anne-de-Bellevue, but the reverse was true for Ottawa (Table C1). The susceptible check, Dekalb DK415 had high disease severity in all environments. Two hybrids (Mamouth and Santos) from France exhibited lower disease severity in three of the four environments. Among the hybrids from Northern U.S., B73 × 78N9 and MO17Ht × F2 were the most resistant. Funk 4106, Cargill 5157 and Pride K127 exhibited low infection among the hybrids from eastern Canada. The response of these seven hybrids was consistent in most environments, suggesting that these hybrids could be potential sources of kernel resistance to ear rot.

Figure C1. Percent nitrogen content of six maize hybrids inoculated with *Fusarium graminearum* at 1993 and 1994.

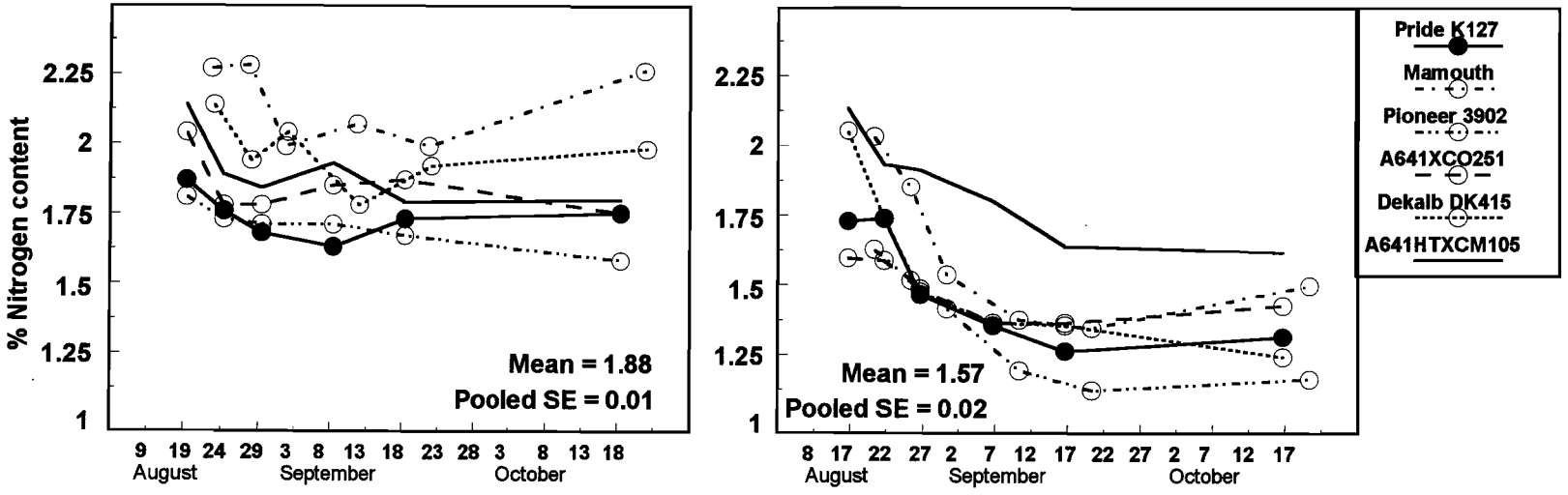

% Nitrogen content
2.25
2
1.75
1.5
1.25
1
Mean = 1.88
Pooled SE = 0.01
9 19 24 29 3 8 13 18 23 28 3 8 13 18
August
September
October
Mean = 1.57
Pooled SE = 0.02
8 17 22 27 2 7 12 17 22 27 2 7 12 17
August
September
October
Pride K127
Mamouth
Pioneer 3902
A641XCO251
Dekalb DK415
A641HTXCM105

Figure C2. Percent moisture content of six maize hybrids inoculated with *Fusarium graminearum* in 1993 and 1994.

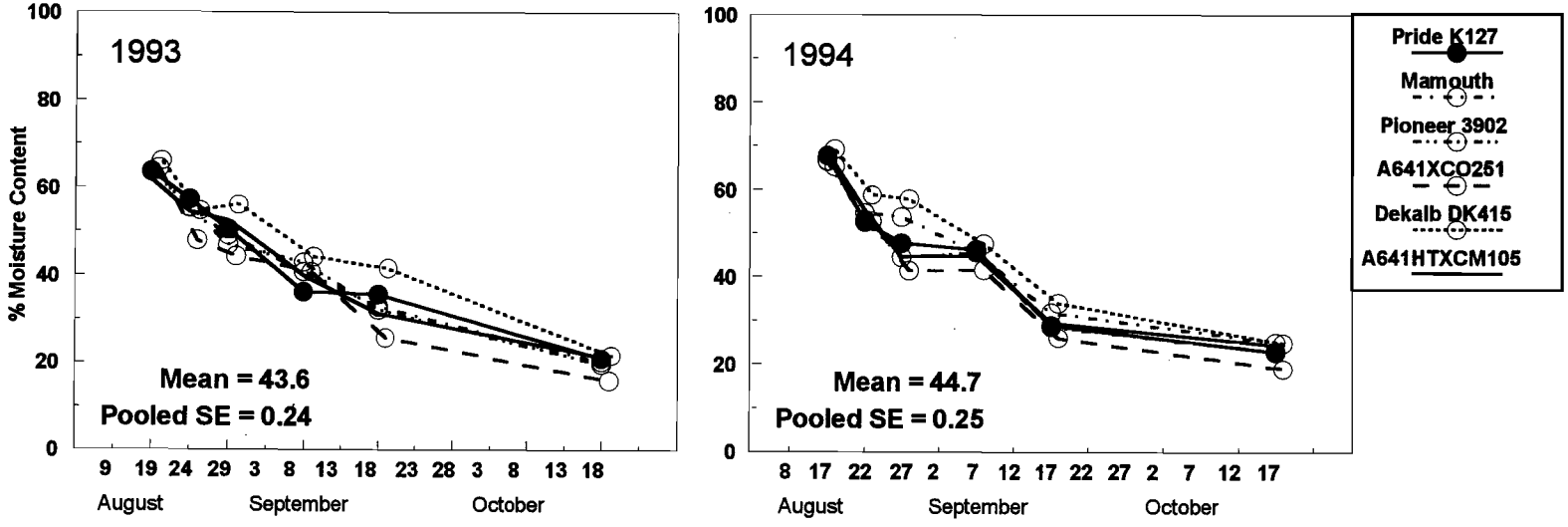

1993
1994
% Moisture Content
100
80
60
40
20
0
Mean = 43.6
Pooled SE = 0.24
Mean = 44.7
Pooled SE = 0.25
9 19 24 29 3 8 13 18 23 28 3 8 13 18
8 17 22 27 2 7 12 17 22 27 2 7 12 17
August
September
October
Pride K127
Mamouth
Pioneer 3902
A641XCO251
Dekalb DK415
A641HTXCM105

Figure C3. Kernel hardness for six maize hybrids inoculated with *Fusarium graminearum* at 1993 and 1994.

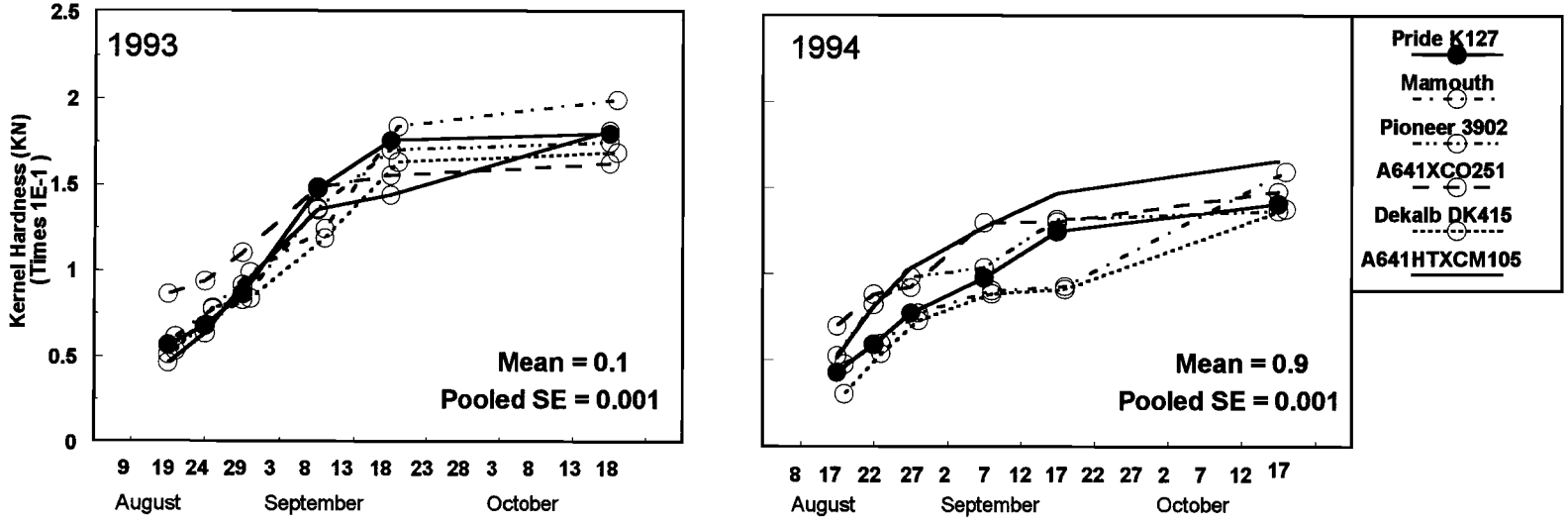
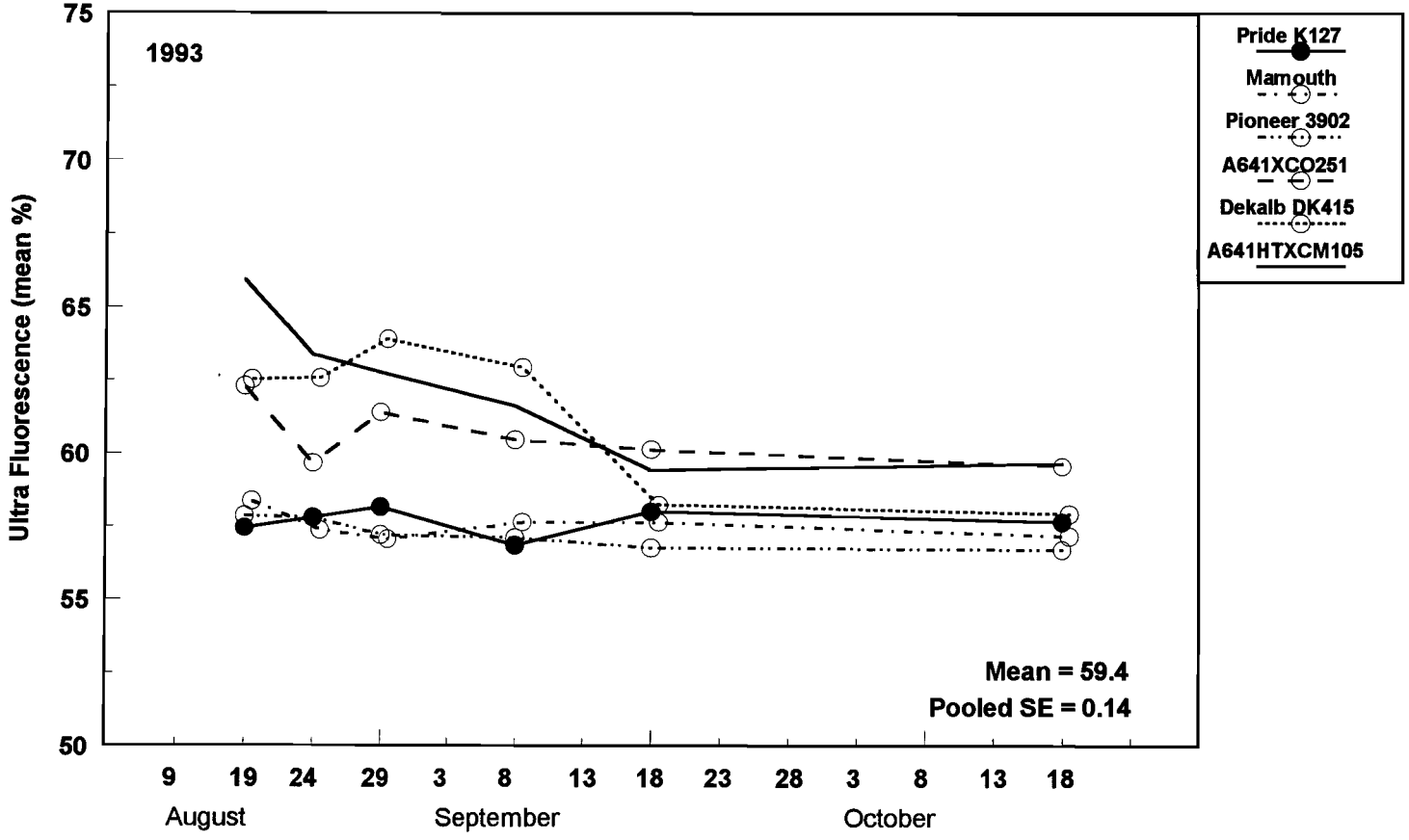
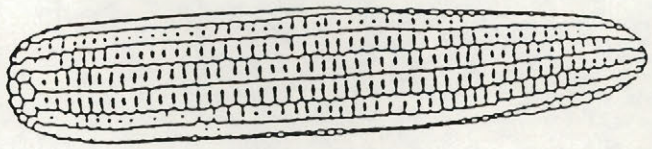
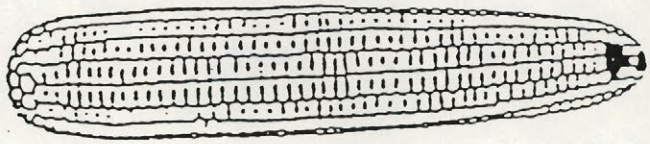
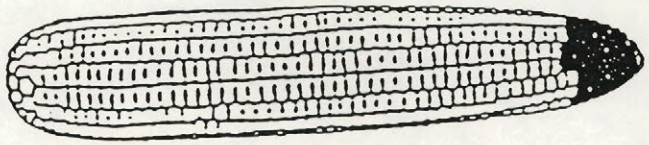
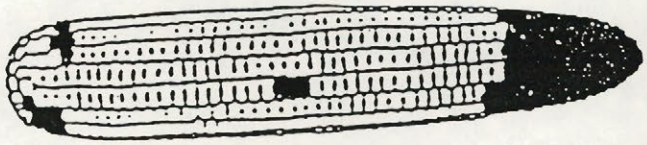
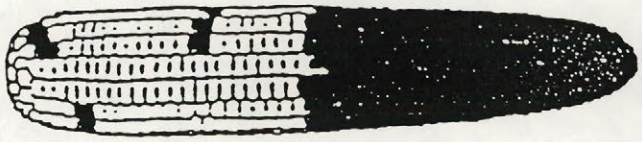

1993
Kernel Hardness (KN)
(Times 1E-1)
2.5
2
1.5
1
0.5
0
Mean = 0.1
Pooled SE = 0.001
9 19 24 29 3 8 13 18 23 28 3 8 13 18
August
September
October
1994
Mean = 0.9
Pooled SE = 0.001
8 17 22 27 2 7 12 17 22 27 2 7 12 17
August
September
October
Pride K127
Mamouth
Pioneer 3902
A641XCO251
Dekalb DK415
A641HTXCM105

Figure C4. Ultra fluorescence (total phenolic compounds) for six maize hybrids inoculated with *Fusarium graminearum* in 1993.


1993
Ultra Fluorescence (mean %)
75
70
65
60
55
50
9
19
24
29
3
8
13
18
23
28
3
8
13
18
August
September
October
Mean = 59.4
Pooled SE = 0.14
Pride K127
Mamouth
Pioneer 3902
A641XCO251
Dekalb DK415
A641HTXCM105

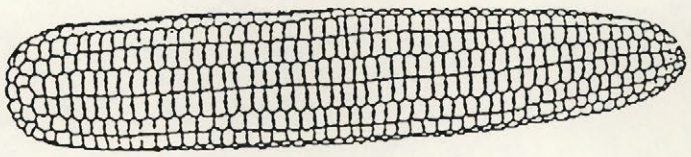
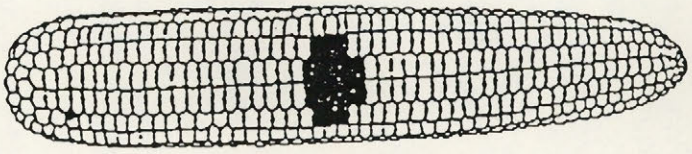
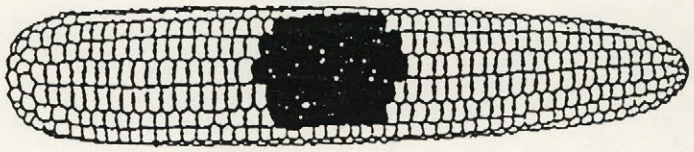
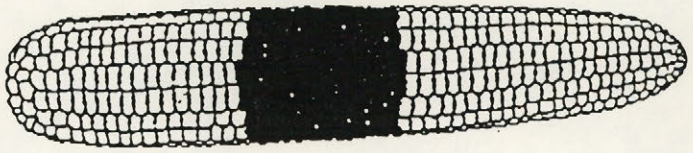
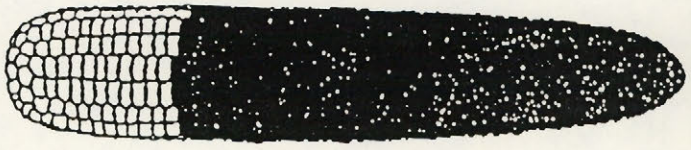
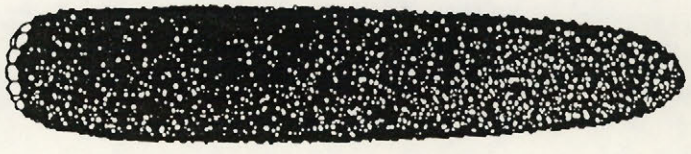
Appendix D

The following two visual rating scales were used in all experiments presented in the thesis.

Rating		Percentage of kernels infected
1		No Infection
2		1-3%
3		4-10%
4		11-25%
5		26-50%
6		51-75%
7		75-100%

Fusarium graminearum

Silk-channel inoculation rating scale

Rating		Percentage of kernels infected
1		No Infection
2		1-3%
3		4-10%
4		11-25%
5		26-50%
6		51-75%
7		75-100%

Fusarium graminearum

Kernel inoculation rating scale

Appendix E

Deoxynivalenol raw data of mean values presented in Chapter 8.

Table E1. Deoxynivalenol raw data for CO265 in 1993

Ear with low severity				Ear with moderate severity		
Kernel	Run1	Run2	Avg	Run1	Run2	Avg
Above inoculated Point (IP)						
11	0.36	0.39	0.38	0.17	0.14	0.16
10	0.07	0.05	0.06	0.11	0.14	0.13
9	0.27	0.30	0.29	0.24	0.20	0.22
8	0.11	0.11	0.11	0.20	0.23	0.22
7	0.14	0.16	0.15	0.24	0.30	0.27
6	0.19	0.23	0.21	0.00	0.00	0.00
5	0.11	0.09	0.10	0.12	0.08	0.10
4	0.11	0.18	0.15	0.10	0.10	0.10
3	0.09	0.11	0.10	2.40	3.10	2.75
2	1.32	1.58	1.45	0.42	0.38	0.40
1	1.65	1.87	1.76	17.50	15.50	16.50
Below Inoculated Point						
-7	0.25	0.29	0.27	7.50	7.50	7.50
-6	0.30	0.24	0.00	16.00	14.00	15.00
-5	0.25	0.20	0.23	13.00	10.50	11.75
-4	0.32	0.30	0.31	10.50	12.50	11.50
-3	0.25	0.31	0.28	0.42	0.48	0.45
-2	0.35	0.22	0.29	2.20	1.70	1.95
-1	0.40	0.34	0.37	0.84	0.70	0.77
Left						
-7	0.31	0.36	0.34	0.52	0.33	0.43
-6	0.39	0.44	0.42	4.90	4.50	4.70
-5	0.17	0.18	0.18	4.10	3.50	3.80
-4	0.21	0.15	0.18	2.00	2.40	2.20
-3	0.21	0.18	0.20	5.70	6.80	6.25
-2	0.40	0.31	0.36	16.00	20.50	18.25
-1	0.19	0.25	0.22	24.50	25.00	24.75
IP	5.50	6.00	5.75	30.00	27.00	29.00
1	0.53	0.46	0.50	11.00	11.50	11.25
2	0.37	0.27	0.32	3.50	2.60	3.05
3	0.13	0.17	0.15	0.94	0.70	0.82
4	0.50	0.46	0.48	0.57	0.50	0.54
5	0.40	0.37	0.39	1.00	0.77	0.89

6	0.27	0.20	0.24	3.80	3.20	3.50
7	0.31	0.36	0.34	4.70	4.20	4.45
cob tissue						
Left of IP						
-3	4.20	3.80	4.00	13.00	13.00	13.00
-2	3.50	2.50	3.00	12.00	15.00	13.50
-1	2.15	2.60	2.38	21.00	25.00	23.00
IP	1.70	1.40	1.55	23.00	28.00	25.50
Right of IP						
1	2.85	3.10	3.03	19.00	22.00	20.50
2	3.40	3.90	3.65	22.00	25.00	23.50
3	2.60	1.95	2.28	17.00	20.00	18.50

Table E2. Deoxynivalenol raw data for CO325 in 1993

Ear with low severity				Ear with moderate severity		
Kernel	Run1	Run2	Avg	Run1	Run	2Avg
Above inoculated point						
8	0.92	0.94	0.93	45.00	50.00	47.50
7	0.78	0.87	0.83	40.00	44.00	42.00
6	0.70	0.59	0.65	14.00	18.00	16.00
5	0.74	0.85	0.80	0.50	1.00	0.75
4	0.64	0.69	0.67	0.60	1.20	0.90
3	0.59	0.41	0.50	0.42	0.40	0.41
2	2.90	3.59	3.25	0.20	0.19	0.20
1	3.50	3.90	3.70	0.10	0.70	0.40
IP	16.00	11.00	13.50	43.00	47.00	45.00
Below inoculated point						
-1	1.10	1.25	1.18	21.00	12.00	16.50
-2	0.46	0.36	0.41	30.00	41.00	35.50
-3	0.40	0.49	0.45	22.00	41.00	31.50
-4	0.00	0.00	0.00	1.60	2.10	1.85
-5	0.00	0.00	0.00	1.10	0.82	0.96
-6	0.00	0.00	0.00	1.20	1.30	1.25
-7	0.00	0.00	0.00	0.76	0.50	0.63
-8	0.17	0.19	0.18	1.30	0.82	1.06
-9	0.50	0.60	0.55	0.90	0.22	0.56
-10	0.32	0.20	0.26	0.81	0.40	0.61
-11	0.28	0.31	0.30	0.68	0.70	0.69
left of IP						
-5	0.16	0.12	0.14	4.50	5.00	4.75
-4	0.29	0.20	0.25	4.75	3.75	4.25
-3	0.48	0.32	0.40	5.00	6.40	5.70
-2	1.60	1.78	1.69	13.00	12.00	12.50
-1	2.70	2.40	2.55	25.00	24.00	24.50
Right of IP						
1	2.30	3.10	2.70	4.40	9.00	11.70
2	0.54	0.50	0.52	3.20	8.80	11.00
3	0.54	0.40	0.47	6.80	8.00	7.40

4	0.09	0.11	0.10	5.00	4.20	4.60
5	0.00	0.00	0.00	3.00	3.00	3.00
Cob tissue						
left of IP						
-3	4.70	3.40	4.05	24.00	15.00	19.50
-2	2.60	2.45	2.53	29.00	26.00	27.50
-1	9.50	5.50	7.50	22.00	18.00	20.00
IP	7.00	6.50	6.75	26.00	23.00	24.50
Right of IP						
1	7.40	8.40	7.90	28.00	23.00	25.50
2	2.10	1.70	1.90	30.00	28.00	29.00
3	3.00	0.00	0.00	0.00	0.00	0.00

Table E3. Deoxynivalenol raw data for CO265 in 1994

Ear with low severity				Ear with moderate severity		
Kernel	Run1	Run2	Avg	Run1	Run	2Avg
above inoculated point						
1	4.40	4.00	4.20	7.25	9.00	8.13
2	5.20	5.00	5.10	25.00	20.00	22.50
3	1.30	1.10	1.20	20.50	21.00	20.75
4	7.00	6.60	6.80	24.50	21.00	22.75
5	0.24	0.28	0.26	1.05	0.83	0.94
6	0.00	0.00	0.00	0.80	0.81	0.81
7	0.00	0.00	0.00	0.51	0.40	0.46
8	0.00	0.00	0.00	0.79	1.00	0.90
9	0.00	0.00	0.00	0.31	0.26	0.29
10	0.00	0.00	0.00	0.34	0.39	0.37
IP	1.90	2.50	2.20	11.00	9.40	10.20
below inoculated point						
-1	2.00	2.20	2.10	15.50	14.00	14.75
-2	1.50	1.05	1.28	11.50	14.00	12.75
-3	0.10	0.10	0.10	7.50	6.50	7.00
-4	0.11	0.12	0.12	3.40	3.55	3.48
-5	0.00	0.00	0.00	0.98	0.81	0.90
-6	0.00	0.00	0.00	0.00	0.13	0.07
-7	0.00	0.00	0.00	0.00	0.00	0.00
-8	0.00	0.00	0.00	0.00	0.00	0.00
-9	0.00	0.00	0.00	0.00	0.00	0.00
-10	0.00	0.00	0.00	0.00	0.00	0.00
left of inoculated point						
-7	0.51	0.52	0.52	13.00	11.0	12.00
-6	0.37	0.33	0.35	13.00	12.0	12.50
-5	1.05	0.73	0.89	23.00	22.0	22.50
-4	0.33	0.45	0.39	20.00	16.0	18.00
-3	0.44	0.60	0.52	12.00	11.5	11.75
-2	0.69	0.74	0.72	14.00	16.0	15.00
-1	3.15	2.75	2.95	14.00	16.0	15.00

right of inoculated point						
1	7.20	6.00	6.60	24.00	25.00	24.50
2	5.60	5.40	5.50	10.00	11.50	10.75
3	3.80	3.60	3.70	21.00	25.00	23.00
4	0.42	0.42	0.42	14.00	15.50	14.75
5	1.50	1.40	1.45	25.00	21.00	23.00
6	0.11	0.14	0.13	18.00	18.00	18.00
7	0.19	0.00	0.10	14.00	16.00	15.00
cob tissue						
left of IP						
-3	4.10	5.00	4.55	71.00	67.00	69.00
-2	3.50	4.00	3.75	41.00	44.00	42.50
-1	4.10	4.50	4.30	40.50	40.00	40.25
IP	4.00	3.70	3.85	60.00	61.50	60.75
Right if IP						
1	6.00	5.10	5.55	58.50	60.00	59.25
2	5.00	4.00	4.50	45.00	48.00	46.50
3	4.30	5.00	4.65	43.50	47.00	45.25

Table E4. Deoxynivalenol raw data for CO325 in 1994

Ear with low severity				Ear with moderate severity		
Kernel	Run1	Run2	Avg	Run1	Run	2Avg
Above inoculated point (IP)						
Kernel	Run1	Run2	Avg	Run1	Run2	Avg
10	0.41	0.36	0.39	0.00	0.00	0.00
9	0.29	0.25	0.27	0.00	0.00	0.00
8	0.17	0.23	0.20	0.00	0.00	0.00
7	0.14	0.13	0.14	0.10	0.27	0.19
6	0.23	0.20	0.22	0.40	0.40	0.40
5	0.21	0.25	0.23	5.00	7.00	6.00
4	1.75	1.90	1.83	1.80	1.50	1.65
3	1.25	1.45	1.35	3.80	3.60	3.70
2	4.00	3.50	3.75	22.00	20.50	21.25
1	2.30	2.50	2.40	24.50	20.50	22.50
Below inoculated point						
-1	0.64	0.71	0.68	18.00	18.00	18.00
-2	0.41	0.32	0.37	12.50	13.50	13.00
-3	0.28	0.36	0.32	15.00	17.00	16.00
-4	0.40	0.51	0.46	5.50	5.00	5.25
-5	0.22	0.27	0.25	0.63	0.60	0.62
-6	0.13	0.11	0.12	0.00	0.00	0.00
-7	0.18	0.10	0.14	0.00	0.00	0.00
-8	0.00	0.00	0.00	0.00	0.00	0.00
-9	0.08	0.04	0.06	0.00	0.00	0.00
-10	0.10	0.00	0.05	0.00	0.00	0.00
left of inoculated point						
-5	3.10	2.20	2.65	35.00	40.00	37.50
-4	0.50	0.74	0.62	36.00	41.00	38.50
-3	0.82	0.86	0.84	41.00	46.00	43.50
-2	3.30	3.30	3.30	30.00	33.00	31.50
-1	4.80	4.60	4.70	26.00	25.00	25.50
Right of inoculated point						
1	0.28	0.19	0.24	17.00	18.00	17.50
2	0.54	0.71	0.63	17.00	16.00	16.50

3	0.09	0.12	0.11	27.00	32.00	29.50
4	0.52	0.52	0.52	36.00	39.00	37.50
5	0.35	0.52	0.44	40.00	44.00	42.00
IP	5.20	5.00	5.10	28.00	36.00	32.00
Cob tissue						
left of IP						
-3	1.6	1.25	1.43	27.00	25.0	26.00
-2	1.7	2.1	1.90	14.00	12.0	13.00
-1	4.0	3.9	3.95	27.00	31.0	29.00
IP	4.5	4.5	4.50	20.00	20.0	20.00
Right of IP						
1	4.0	4.5	4.25	8.00	6.2	7.10
2	1.0	0.9	0.95	22.00	19.00	20.50
3	2.5	1.85	2.18	10.00	8.2	9.10