ROLE OF PHENOLIC ACIDS IN MAIZE RESISTANCE TO THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS* (HÜBNER).

by

David Bergvinson

A thesis submitted to the
School of Graduate Studies and Research,
University of Ottawa,
in partial fulfilment of the requirements for the
Degree of Doctor of Philosophy
in Biology.

ISBN 0-315-93557-X

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

Host plant resistance of maize, *Zea mays* L., to the European corn borer (ECB), *Ostrinia nubilalis* Hübner, has been largely attributed to the hydroxamic acid DIMBOA. However, DIMBOA does not consistently account for observed resistance in both field and greenhouse environments. It was hypothesized that phenolic acids, a major secondary metabolite in plants, make an important contribution to resistance, through cell wall carbohydrate complexes.

Preliminary studies incorporating ferulic acid into the meridic diet of the ECB did not show any adverse effects until 10 mg/g, a dosage that is approximately 30 times that found in maize. Semi-preparative samples of sugar conjugates of ferulic and p-coumaric acids were tested using leaf-feeding bioassays that showed phagostimulant activity at ecologically relevant dosages. These observations suggested that soluble phenolics did not make a major contribution to host plant resistance but may be used for host plant recognition by the ECB.

Phenolic acids, namely *E*-ferulic and *E*-p-coumaric acids, occur largely in the cell wall as complexes bound by ester linkages to an arabinose moiety of the major hemicellulose of maize cell walls, arabino-xylose. Bound phenolics can form dimers with adjacent phenolic acids through the absorption of near ultraviolet light to form cyclobutane dimers or by the action of peroxidase to form dehydrodiphenolic acids. It was hypothesized that these dimers, cross-linked phenolic acid-carbohydrate complexes, could constitute a quantitative defence against the ECB. To assess quantitative defences an instron technique for leaf toughness was developed that showed significant correlations of leaf toughness and field resistance for five diverse germplasm sources. Microspectrophotometry and staining techniques for localizing and quantifying cell wall phenolics were also developed for maize tissue.

Developmental studies of a resistant maize synthetic using leaf bioassays and standard phytochemical procedures showed ECB larvae prefer to feed on immature tissue within the whorl and on plants between the 3- and 7-leaf stages of development. These preferred tissues had elevated levels of DIMBOA and lower levels of cell wall phenolics and lower levels of detergent fibre than more mature leaf tissue. Leaf toughness related inversely to leaf consumption. Microspectrophotometer readings of abaxial, epidermal cell walls best predicted insect preference for leaf tissues of different maturity. These studies showed that

leaf toughness, *vis a vis* phenolic acid-carbohydrate compiexes, is one of the major resistance factors which can account for larval feeding behavior.

Environmental factors also influence maize resistance, with light quality having a significant impact. Plastic with UV blocking agents was used to reduce the incidence of near ultraviolet light. Under reduced UV light conditions, maize resistance in both greenhouse and field environments was reduced as were the levels of cell wall bound cyclobutane dimers. Cyclobutane dimers appear to increase the mechanical strength of leaf tissue by cross-linking hemicelluloses.

Using the synthetic BS9 (cycle C0, C2, C4, C5), the changes in maize phytochemistry and nutritional quality that accompanied increased resistance were monitored for leaf (mature and immature), sheath, stalk, node and pith tissues. DIMBOA levels increased with selection as did fibre content and the cell wall phenolics p-coumaric acid and dehydrodiferulic acid. Microspectrophotometry results show that epidermal cell wall absorbance by phenolics provides the best prediction for the observed changes in resistance over cycles of selection.

Cultivars developed at the International Center for Maize and Wheat Improvement have resistance to several lepidopteran borer species but the mechanism of this resistance is unknown. Phytochemical screening of 13 cultivars was conducted for field grown plants during the mid-whorl stage. Correlations of field resistance were highest for fibre, dehydrodiferulic acid content and protein content. A nutritional model for host plant resistance in maize based on these three plant components was proposed that accounts for 78 to 87 percent of the variation in field damage ratings observed over two years.

According to Feeny's hypothesis of plant apparency, maize appears to rely heavily on quantitative defences. This is logical given the extensive areas planted in maize, with many areas employing no-till practices and continuous cropping which elevate the apparency of maize to insect pests. Phenolic acid - carbohydrate complexes have been demonstrated in this study to be a major component in this defence strategy.

RESUME

La résistance du maïs, *Zea mays* L., à la pyrale, *Ostrinia nubilalis* Hübner, a été attribuée à l'acide hydroxamique DIMBOA. Mais, le DIMBOA ne peut pas être considéré comme l'unique facteur de résistance en plein champ et en serre. Il est proposé que peut-être les acides phénoliques, produit secondaire de métabolisation des plantes, participeraient également à la résistance par l'entremise du complexe carbohydrate dans la paroi cellulaire.

Des études préliminaires ou l'acide ferulique a été incorporé à une diète artificielle pour la pyrale n'a produit aucun effet secondaire en-deça de 10 mg/g, c'est-à-dire une dose d'environ 30 fois supérieur à la concentrationobservée dans le maïs. Des échantillons préparés d'un conjugué de sucre comprenant l'acide ferulique et p-coumarique (concentrations égales à celle trouvées dans le milieu naturel) ont été étudiés, dans des bio-essais foliaires, de consommation et ont démontré une augmentation de l'activité phagostimulante. De ces observations on peut conclure que les solutés phénoliques ne participent pas signigicativement à la résistance chez la plante, mais peuvent être utilisés comme facteur de reconnaissance de la plante par la pyrale.

Les acides phénoliques *E*-ferulique et *E*-p-coumarique sont particulièrement présents, dans la paroi cellulaire comme complexe éther lié à l'arabinose avec un radical éther, particulièrement dans la paroi cellulaire, l'hémicellulose, et l'arabino-xylose.

Les liens phénoliques des composés adjacents peut être modifier par la lumière ultraviolette pour former de nouveaux dimères tel le cyclobutane ou par l'action de péroxydase pour former l'acide dehydro-phénolique. Il est proposé que ces dimères, un mélange d'acide phénolique et de carbohydrate, puissent constitués un moyen de défense quantitatif contre la pyrale. Pour évaluer quantitativement ce mécanisme de défense, un instrument pouvant mesurer la force requise pour percer la surface foliaire a été utilisé. Ainsi, la force requise pour percer la surface foliaire corrélate significativement avec la résistance chez 5 sources de lignées. Des techniques de micro-spectrophotométrie et de colorant ont également été développés pour le tissu foliaire afin de localiser et quantifier les phénols présents dans la paroi cellulaire.

L'amélioration d'un cultivar utilisant des bio-essais foliaires et des techniques standard d'étude phytochimique montre que la larve de la pyrale préfère s'alimenter de tissus en

croissance à l'intérieur du cornet ou de plants ayant 3 à 7 feuilles. Ces tissus préférés ont un niveau élevé de DIMBOA et faible en composé phénolique et fibre détergente dans leur paroi cellulaire comparativement aux tissus foliaires plus agés. Ainsi, la résistance foliaire est inversement reliée à la consommation foliaire. Les résultats micro-spectrophotométriques de la paroi cellulaire épidermale, non située près de l'axe, a prédit la préférence de l'insecte à divers degrés de maturité du tissu foliaire.

Ces résultats montrent que la dureté foliaire, reliée à la présence d'acide phénolique et du complexe carbohydrate, est l'un des principaux facteurs de résistance à l'alimentation larvaire.

Des facteurs naturels, comme la qualité de la lumière, agissent également sur les mécanismes de résistance. Un polythène possédant un agent réfractant aux rayons ultraviolets a été utilisé pour réduire l'intensité des rayons. Sous ce régime, la résistance du maïs en serre et dans un milieu naturel a été réduit tout comme le dimère cyclobutane dans la paroi cellulaire. Le dimère de cyclobutane agirait en augmentant la résistance mécanique du tissu foliaire par ces liens ave l'hémicellulose.

Des changement phytochimiques et la qualité nutritive du maïs reliés à l'augmentation du niveau de résistance dans la variété synthétique BS9 (cycle 0, 2, 4, et 5) ont été suivi sur du feuillage (jeune ou agée), sur l'enveloppe foliaire, la tige, les noeuds, et le tissu moelleux. Des cultivars ayant de plus en plus de DIMBOA, de fibre détergente, et d'acide phénolique (p-coumarique et dehydro-ferulique) dans la paroi cellulaire ont ainsi été sélectionnés. Les résultats micro-spectrophotométriques montrent que l'absorbance d'acides phénoliques par la paroi cellulaire épidermale aide à prédire le degré de résistance suivant les cycles de sélection.

Des variétés résistantes à plusieurs ravageurs développées par le Centre Internationale d'Amélioration du Maïs et du Blé, sont également résistantes à plusieurs espèces de lépidoptères ravageurs mais les mécanismes de résistance ne sont pas connus. La sélection phytochimique a été faite au stade mi-cornet sur 13 génotypes cultivés en plein champ. La corrélation de la résistance en plein champ était la plus élevée pour les fibres, l'acide dehydrodiferulique et le contenu en protéine. Un modèle nutritionel de la résistance du maïs aux dommages en plein champ basé sur ces 3 composés explique 78 à 87% de la variation observée au-cours des 2 années d'étude.

Selon l'hypothèse de Feeny sur les plantes apparentes, le maïs semble dépendre grandement de son mode de défense quantitatif. Ceci est logique puisque le maïs est cultivé sur de grandes surfaces et que dans plusieurs régions le maïs est cultivé de façon continue, avec un travail superficiel de la terre, ce qui le rend apparent aux insectes ravageurs. L'acide phénolique et les complexes carbohydrates ont démontré au-cours de cette étude, être des composantes majeures de ce mécanisme de défense.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my research supervisor, Dr. J.T. Arnason. From first suggesting the project and providing moral support to providing the resources and funding to see the project to completion, I acknowledge his substantial contribution.

Sincere thanks are extended to the members of my research advisory committee, Drs. C. Nozzolillo, R.I. Hamilton, B.J.R. Philogene and H. Damman, for their valuable suggestions.

Special thanks to Dr. R.I. Hamilton and his coworkers at the Plant Research Station of Agriculture Canada, Ottawa for providing land, increasing seed and providing *Ostrinia nubilalis* eggs. Their assistance made the field component of this study both feasible and enjoyable. I thank Drs. L.N. Pietrzak, J. Fregeau-Reid and S. Miller for access to the microspectrophotometer and the micro-Kjehldahl. I especially thank F. Meloché for his camaraderie as we together explored maize resistance and trophic level interactions.

Sincere thanks to Dr. J.A. Mihm and his collaborators at the International Center for Maize and Wheat Improvement for providing diverse sources of maize germplasm and for their insights on maize resistance to insect pests.

Thanks to Dr. J. Atkinson for the synthesis of hydroxamates and MBOA standards and to Drs. G.H.N. Towers and S. Tachibana for providing phenolic dimer standards.

Thanks to all the graduate students and technicians from Dr. Arnason's lab who provided assistance and companionship in the laboratory and field, with special thanks to J. Larsen and M. Labelle. I also thank Dr. D. Brown and B. Valintine for technical support in microscopy and plate preparation.

Finally, I would like to thank my parents for their moral support and for giving me the courage to make dreams a reality. Most of all, I thank my wife, Mary, for keeping me optimistic when all seemed lost and for the numerous hours of field assistance.

TABLE OF CONTENTS

LIST OF FIGURES

TABLES

CHAPTER I.

GENERAL INTRODUCTION

1.1 Economic importance of maize pests

Maize, *Zea mays* L., is the world's third largest cereal crop after rice and wheat, being grown throughout temperate, subtropical and tropical zones and constituting approximately 8.9 percent of the total world crop production (Ortega et al. 1980). In North America, three types of maize are grown: sweet, silage and grain (Hudon and Ogilvie 1984), with grain constituting 90% of the total tonnage (Chiang 1978). Diseases and insects are two of the major factors limiting further increases in maize yields . Annual losses to insect damage in both the United States and Canada exceed 50 million dollars (Anon. 1988, Isenhour et al. 1987). Moreover, chemical controls used to combat insect pests of maize cost over 30 million dollars a year in the United States and are a source of environmental contamination. To reduce losses and pesticide loading into the enviroment, host plant resistance in maize has been actively pursued over the last 60 years.

Maize is subject to attack by a complex of insects from the time it is planted until it is used as food or feed. Included in the North American complex are root feeders (corn rootworms, *Diabrotica* spp.), leaf feeders (armyworms, *Spodoptera* spp., corn leaf aphid, *Rhopalosiphum maidis*), stalk borers (European corn borer, *Ostrinia nubilalis*; southwestern corn borer, *Diatraea grandiosella*), earworms (corn earworm, *Heliothis zea*) and stored grain insects (maize weevil, *Sitophilus* spp., Angoumois grain moth, *Sitotroga cerealella*). A more complete list of 93 species of corn insects is given in Dicke and Guthrie (1988).

From this list, one of the most economically important and extensively studied pests is the European corn borer (ECB) (Dicke and Guthrie 1988). ECB is distributed in Europe, the Middle East, North Africa, and North America. After introduction to the United States (Everett, Mass.) in 1916, the ECB rapidly spread in Eastern North America. The ECB is the only major stem-boring insect of maize that can survive in temperate regions (Ortega et al. 1980, Mihm 1985). At present, the ECB is found in most states east of the Rocky Mountains and in several Canadian provinces (Showers 1979). Damage by this pest results not only from its feeding on all above ground portions of maize, but because it also vectors several *Fusarium* stalk-rot fungi which lead to further destruction of plant material. Stalk-rot fungi

known to be carried by the ECB include *Fusarium moniliforme* Sheld., *F. graminearum* Schwabe and *F. poae* (Peck). Common corn smut, *Ustilago zeae* (Beckm.) Unger, is also carried by ECB and is considered to be the most severe pathogen in young maize plants (Komedahl and Windles 1981).

ECB control methods vary depending on the maize crop. Insecticides are used in high value crops such as sweet corn for fresh markets and canning when ECB populations reach an economic threshold (Hudon and LeRoux 1986a). However, insecticide use is generally uneconomic for grain and silage crops (Hudon and Leroux 1986a). Since a higher economic threshold for ECB damage occurs in grain and silage corn, producers rely upon maize resistance as the centrepiece of an integrated pest management program.

1.2 History and host relationships of *Ostrinia nubilalis*

The European corn borer, *Ostrinia nubilalis* (Hübner), a pyralid moth of the subfamily Pyraustinae, was considered an economic pest in Europe by 1835, long before its introduction to North America in 1917 (Parks 1926). The pest was introduced in shipments of broomcorn (*Sorghum vulgare* var. *technica* (Koern.) Fiori) from Austria, Hungary and Italy. Broom factories facilitated ECB distribution in both Canada and the U.S.A. (Caffrey and Worthley 1927). Less than a decade was required for this pest to cause extensive crop loss in Ontario and Québec (Parks 1926).

Hübner was the first taxonomist to describe the ECB in 1796, calling the male *Pyralis nubilalis* but assigning the female to a different species *P. silacealis* (Hudon and LeRoux 1986a). In the late 1890's the genus changed from *Pyralis* to *Botys* Latreille and then to *Pyrausta* Schrank. Pierce and Metcalfe (1938) provided adequate characteristics to separate *P. nubilalis* from other species of the genus *Pyrausta*, but placed it incorrectly into *Anania* Hübner. This misclassification started a series of reclassifications which ended in 1956 with Capps verifying that *Ostrinia palustralis* larvae shared diagnostic and morphological characters with those of *nubilalis* in both Europe and America (Hudon and Leroux 1986a).

Wild hop (*Humulus lupulus* L.) and wild hemp (*Cannabis sativa* L.) growing in Hungary were two of the original native host plants of the ECB and continue to be infested (Nagy 1976). The ECB is polyphagous and can attack nearly every robust herbaceous wild

and cultivated plant that has a stem large enough for larval tunnelling and overwintering (Hudon and LeRoux 1986a). Artificial infestation studies of eight cultivated crops and eight weed species, showed a greater plant infestation and borer density on corn, hemp and hop, with corn being identified as the preferred host (Manojlovic 1984).

1.3 Life cycle of the European Corn Borer

The bionomic data for the ECB have been reviewed (Hudon and LeRoux 1986b, c). ECB populations in Canada have historically had one generation per season (the univoltine strain). Increasingly, corn regions of central Ontario are reporting two generations (bivoltine strain) each year. Differences in voltinism have generally been accounted for by the different genetic strains of ECB that exist across the Americas (Hudon and LeRoux 1986c). Molecular techniques detect no genetic differences between these two apparent strains (Sperling, unpublished data), which suggests that larvae can display univoltine or multivoltine development given the right environmental cues. Because of its increasing importance, the bivoltine lifecycle will be reviewed.

It is surprising to find the tight synchrony that exists between maize and ECB development given the polyphagous nature of the ECB. ECB overwinters as a late larval instar in the corn stalk and stubble (Figure 1.1). Adults emerge and are ready for oviposition when corn is in the mid-whorl stage. Each female can lay more than 500 eggs, depositing them in masses ranging from 2 to 74 on the undersurface of lower leaves. Fatty acid methyl esters extracted from ECB egg masses are oviposition deterrents to facilitate spatial distribution of egg masses (Thiéry and Le Quéré 1991). Eggs hatch in approximately 7 days. The greatest plant damage is caused by the first-generation larvae. Early instar larvae feed primarily on the succulent spirally rolled leaves in the whorl, producing "shot holes" in the leaf and breaking midribs. Most first-generation larval mortality on resistant maize varieties occurs during the first few days after hatch, with 95% mortality occurring within 5 days after hatch (Guthrie and Barry 1989). As the plant grows out of the whorl stage, third and fourth instar larvae feed primarily on sheath and collar tissues. The fifth instar larvae, the final stadium, tunnel all parts of the stalk and ear, resulting in cavities in stalks, broken stalks and tassels, poor ear development and dropped ears. When corn is in the pollen shedding stage,

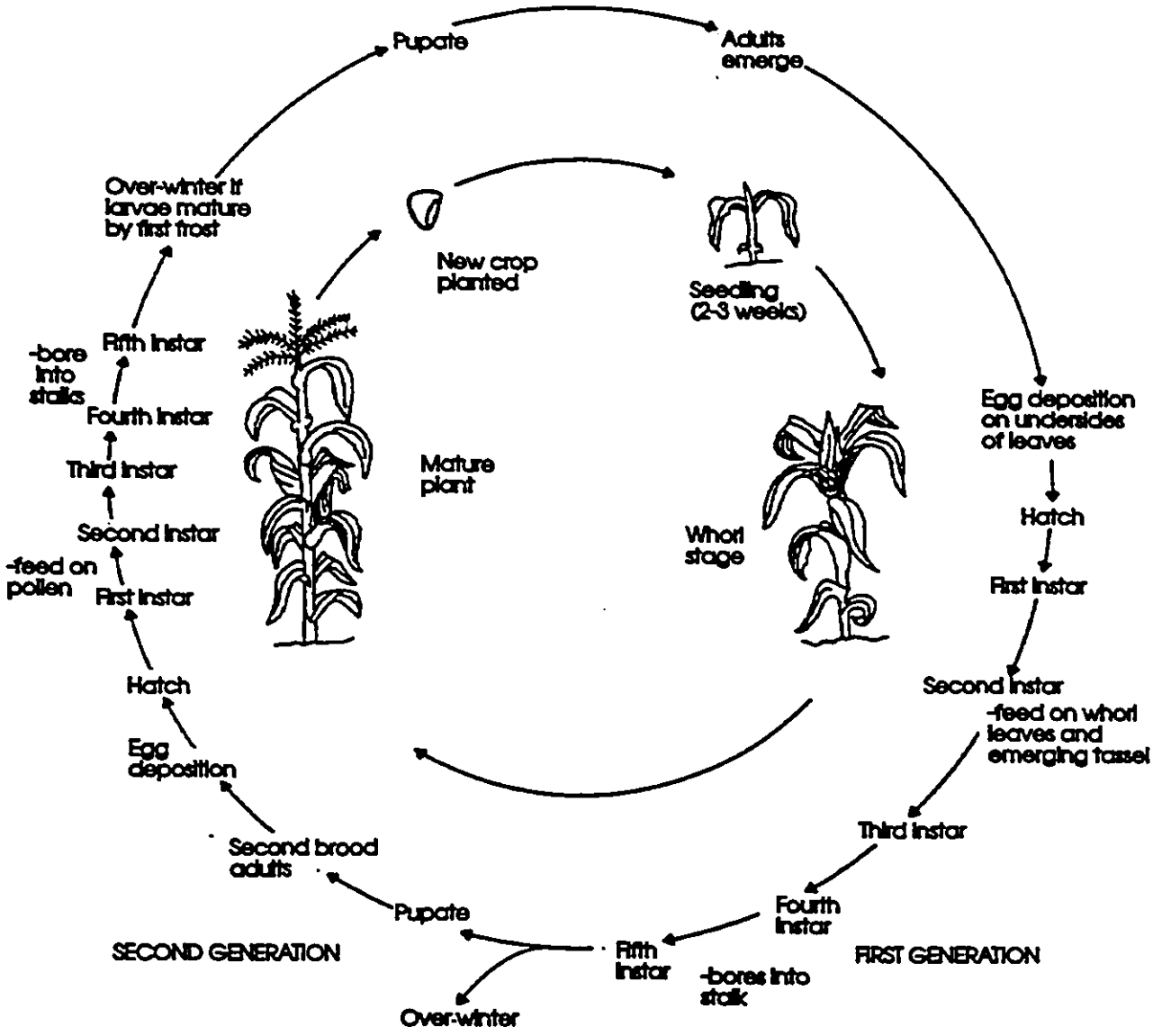


Fig. 1.1. The life cycle of the European corn borer (adapted from Reid 1989).

egg deposition of the second generation occurs. Early instars feed primarily on pollen deposits in the leave axils and also on sheath, collar, ear shoots, husk and silk tissue (Guthrie et al. 1969). The first four instars all feed on sheath-collar tissue (Guthrie et al. 1970). Fifth instar larvae bore into the stalk to overwinter.

1.4 Control Practices

Integrated pest management (IPM) has become the predominant philosophy of pest control, and involves the combined use of plant resistance, cultural controls, biological control agents and pesticides are used to provide economical and environmentally acceptable pest control (Metcalf and Luckmann 1982). Management of the ECB has led to development of resistant varieties, with different genotypes sought for the different generations of the ECB (Dicke and Guthrie 1978). Few genotypes have resistance to more than one generation. Painter (1951) proposed three general mechanisms to account for plant resistance to insects: 1) **non-preference** [now termed "antixenosis" by Kogan and Ortman (1978)], which is the insect response to unattractive or unsuitable plants to serve as hosts; 2) **antibiosis**, which adversely affects insect biology, causing mortality, reduced growth or reproduction, and prolong periods of development in immature stages; and 3) **tolerance**, which enables a host plant to withstand infestation or recover from damage caused by an insect population that would severely damage susceptible plants of the same species.

Given the polyphagous nature of the ECB, breeding for antixenosis has not been actively pursued. The traits that make the plant a desirable host (thick stalk, pollen production, high protein content in moist whorl tissue) are the very traits that define the plant's agronomic potential. Moreover, monocultures of maize often result in no alternative hosts being available to ECB larvae.

Breeding for first generation resistance is primarily concerned with antibiosis targeted at neonate larvae. First generation neonate larvae feed primarily on leaf tissue, with most larval mortality occurring during leaf feeding on resistant varieties (Guthrie et al. 1960). Young larvae of the second generation feed primarily on sheath-collar tissue, with 95% larval mortality occurring within three days after eggs hatch on some inbred resistant lines with sheath-collar feeding resistance (Guthrie et al. 1971).

The second direction taken by plant breeders is tolerance, and for maize this refers to resistance to lodging (stalk collapse) and ear drop (Dicke and Guthrie 1978). To address the problem of lodging, the synthetic BSSS (acronym for Iowa Stiff Stalk Synthetic) was developed by G.F. Sprague using 16 lines during the 1940's to produce a synthetic that had a tough stalk to reduce ECB stalk penetration and allow infested stalks to remain standing (Hallauer et al. 1983). Seven lines developed from this program (B49, B50, B52, B54, B55, B57, B68) have been combined with a line with good leaf feeding resistance (CI31A) a line with good yield and early maturity (Mo17) and a line with a good root system for root worm tolerance (SD10) (Russell and Guthrie 1982). This synthetic, BS9, was used in a recurrent selection program to provide a geneotypic resistance to insect pests during all stages of vegetative plant development and employing to varying degrees all three resistance mechanisms outlined by Painter (1951).

The chemical nature of antibiosis and tolerance has been investigated by numerous researchers, although the biological mechanisms through which these phytochemicals act has not been well defined (See section 1.6).

1.5 The maize plant

Maize is a member of the grass family, Gramineae (Poaceae). Maize is an annual crop characterized by a strong erect stalk with conspicuous nodes, long narrow leaves spaced alternately on the stem, a fibrous root system and separate male and female flowers on the same plant (monoecious). Maize is considered to be a warm-season plant, requiring temperatures higher than 20°C during the day and 14°C during the night with abundant sunlight for optimium yield (Hartman et al. 1981). Maize is classified as a short day plant, with longer days extending the duration of vegetative growth, which may preclude seed maturity before a killing frost (Hartman et al. 1981).

Corn probably originated from teosinte, *Zea mexicana* (Schrad.) Kuntze (Galinat 1988). Although some considered maize to have originated from an ancestral corn (Mangelsdorf 1986) the majority of evidence favors the teosinte ancestor (Galinat 1988). Isozyme and chloroplast DNA studies have furthered the argument that teosinte is ancestral to maize and in Doebley's new classification of maize and teosinte, one of the annual teosinte, *Z.*

mays subsp. *parviglumis*, was the most probable ancestral teosinte taxon (Doebley 1990). This work also suggested that maize originated in the Balsas River drainage southwest of Mexico City. The development of *Zea mays* began more than 8000 years ago, the corn cob developed from 1 to 8 rows between 7000 years before present (YBP) in Mexico to 3000 YBP in New Mexico. The evolution of larger kernels from eight-rowed maize (later called "Northern Flints") occurred 2500 YBP in Mexico. This corn was introduced to New Mexico 1200 YBP and to New England 600 YBP. At the same time, Palomero Toluqueno, a Mexican race of corn with long kernels was used in the development of cobs with extremely narrow and deep kernels, which formed the genetic base for Southern Dents. The corn belt dent appeared 100 YBP and is a hybrid between the Northern Flints and the Southern Dents, having kernel traits intermediate between the two parents (Galinat 1988).

1.6 Plant Defense Chemicals

1.6.1 *The Role of Hydroxamic Acids*

Hydroxamic acids (Fig. 1.2) are known to occur in maize, wheat, and other members of the Gramineae, and in bioassays can act as potent growth factors, antibiotic antagonists, tumour inhibitors, or cell-division factors (Niemeyer 1988, Neilands 1967). Klun & Brindley (1966) showed a positive correlation between the concentration of MBOA (6-methoxybenzoxazolinone) in dried whorl tissue and field resistance to ECB. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), which is chemically labile and decomposes to MBOA, was identified as the biochemical factor in leaf-feeding resistance (Klun and Robinson 1969). DIMBOA is present in uninjured corn tissue in the glucoside form and is present as the aglycone when damaged plant tissue releases the hydrolytic enzyme, glucosidase (Wahlroos and Virtanen 1959). Hydroxamic acid levels are highest in the leaves of embryonic maize plants and seedlings (Argandona and Corcuera 1985), with DIMBOA content typically decreasing with the age of the plant, thus providing little protection from second generation ECB (Guthrie et al. 1986a). During early stages of plant development, hydroxamic acid levels can be elevated by plant damage, suggesting a defence response against insect attack (Morse et al. 1991).

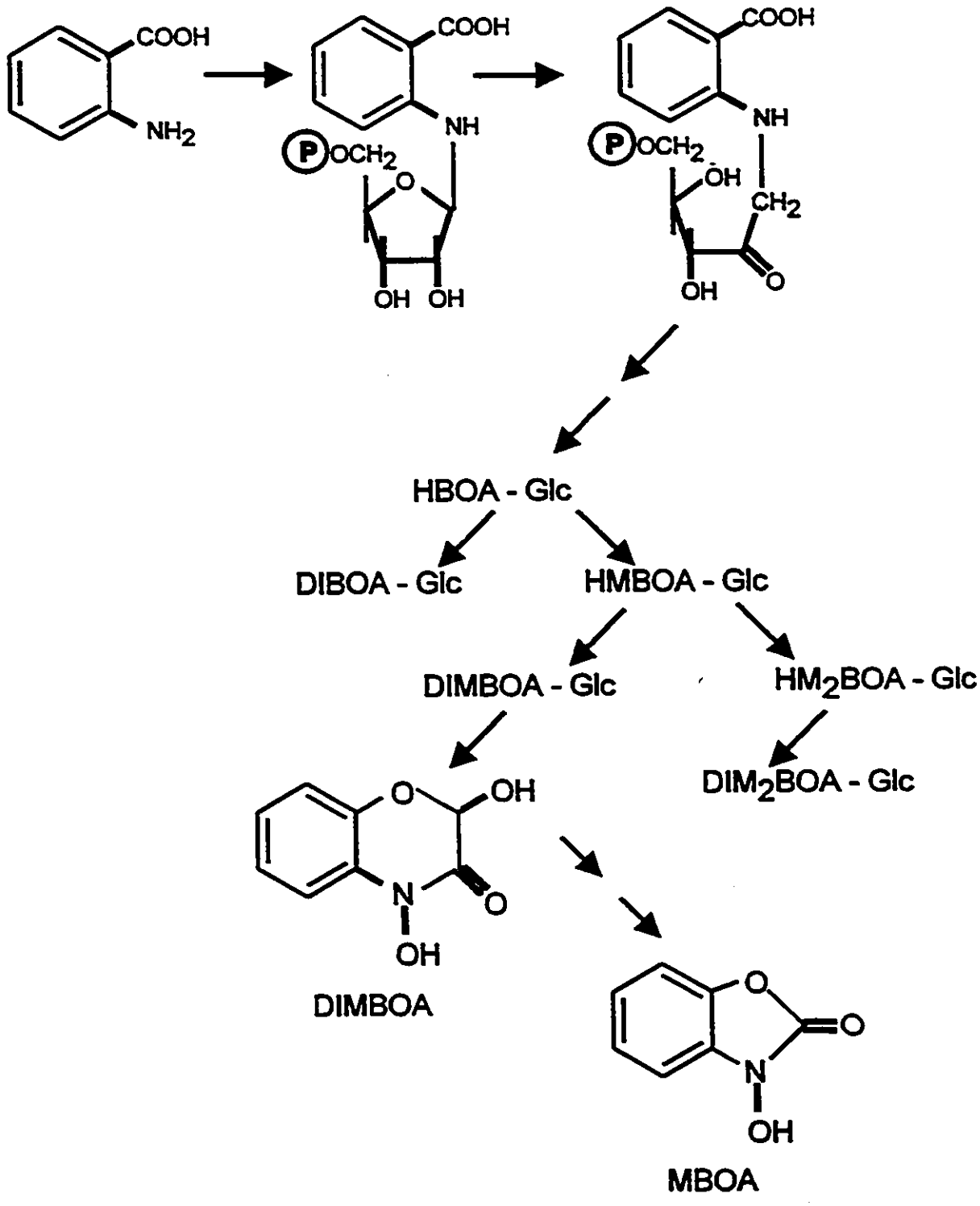


Fig. 1.2. Biosynthesis and degradation of hydroxamic acids (adapted from Niemeyer 1988).

The role of DIMBOA and MBOA in resistance to the European corn borer has been studied extensively. ECB larvae demonstrated higher larval mortality, slower larval development, lower larval and pupal weight, reduced egg mass size and female reproductive potential when DIMBOA was added to their artificial diets (Campos et al. 1989). Nutritional studies showed DIMBOA to decrease the approximate digestibility (AD); whereas MBOA resulted in a decrease in the efficiency of conversion of digested food (ECD) (Houseman et al. 1992). This study also showed that DIMBOA was a non-competitive inhibitor of the digestive protease, chymotrypsin.

Some studies suggested that DIMBOA does not account for the observed resistance of maize. Guthrie et al. (1986b) found maize grown in the greenhouse to be more susceptible to leaf feeding despite having higher levels of hydroxamic acids than field-grown plants. Manuwoto and Scriber (1985b) found plants grown under a reduced light regime had higher leaf consumption by the ECB and higher DIMBOA levels, proposing that the higher nitrogen levels found in the reduced light treatment may have accounted for the increased consumption rate. DIMBOA has little activity against the southwestern corn borer (*Diatraea grandiosella*) (Hedin et al. 1984) and environments that promote DIMBOA production in plants can even increase the growth rate of the southern armyworm (*Spodoptera eridania* Cram.) (Manuwoto and Scriber 1985a).

Although DIMBOA has provided an insight into first-generation resistance, a reduced level of DIMBOA does not necessarily imply susceptibility. Sullivan et al. (1974) and Scriber et al. (1975) showed that borer-resistant lines derived from Antigua and other exotic material had low leaf damage ratings despite having DIMBOA levels well below those of susceptible North American inbreds. Given these observations, there must be other resistance mechanisms operating that contribute to the defensive arsenal of maize. Additional components that have been proposed include both inorganic (eg. silica) and organic (eg. lignin) chemical components of maize tissue (Rojanaridpiched et al. 1984).

1.6.2 *Role of Silica*

Silica is the second most abundant element in the earth's crust and is present in soil solutions as a monosilic acid, $Si(OH)_4$. Monocots can absorb monosilic acid in large amounts,

which is then deposited in its opaline form, $SiO_2 \cdot nH_2O$, in several tissues (Coors 1988). Its role in insect resistance has been demonstrated in wheat, *Triticum aestivum* L. and rice, *Oryza sativa* L. (Wellso 1973, Djamin and Pathak 1967). The mechanism of silica resistance is unknown, though hypothesized mechanisms include stem tissue stiffness (Miller et al. 1960), digestibility reducer (Peterson et al. 1988), physical wearing on insect mandibles (Djamin and Pathak 1967) or silicated trichomes piercing the insect's gut wall (Wellso 1973). On sites where silica content is high, silica concentration in maize leaves was the most important single component determining leaf feeding resistance to ECB (Coors 1988). However, development of a high-silica-content variety, FISIHI, resulted in increased susceptibility to second-generation ECB (Coors 1988).

1.6.3 *Phenolic Acids*

Perhaps the most important phytochemical change that occurred in the evolution of land plants was the biosynthesis of the phenolic compounds, which are in all present day land plants (Swain 1979). They were thought to first evolve as UV light screens but were soon employed as anti-insect agents (Swain 1977). Phenolic acids are a class of phenolic compound with a monocyclic phenol and a carboxylic acid group, i.e. C6-Cx-COOH (Harborne 1980). Like most phenolics, phenolic acids are reactive substances that are susceptible to oxidation by phenolases, and may bond with sugars and peptides, including proteins and enzymes (Harborne 1980). Biosynthesis of phenolics, and phenolic acids in particular, occurs *via* the shikimic acid pathway, a metabolic pathway unique to plants, fungi and bacteria. The end product of the pathway is chorismate which in turn is the precursor of the aromatic amino acids, tyrosine, phenylalanine and tryptophan (Van Sumere 1989). As shown in Fig. 1.3, L-phenylalanine is of particular importance as it is deaminated by phenylalanine ammonia lyase (PAL) to *trans*-cinnamic acid, the precursor of most phenolic compounds.

Ferulic and p-coumaric acids are the most prevalent phenolic acids (phenylpropanoids) in corn grain (Sosulski et al. 1982), as well as occurring in leaf and stalk tissue (Hartley and Jones 1978). Phenolic acids such as these have been pursued as plant resistance factors. Corn-grain resistance to the maize weevil, *Sitophilus zeamais*, has been investigated, with

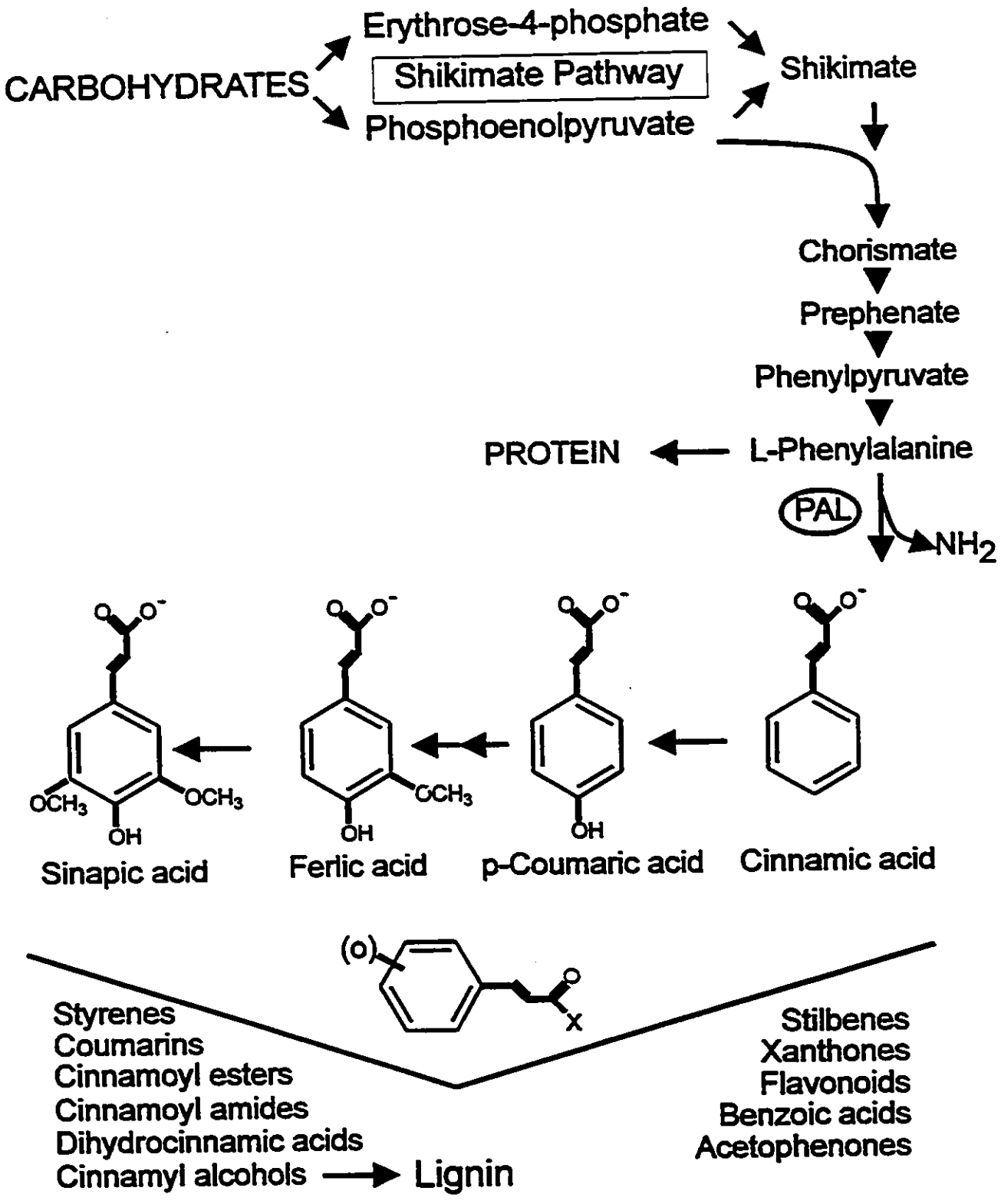


Fig. 1.3. Biosynthesis of phenolic acids and related compounds (adapted from Van Sumere 1989). PAL = phenylalanine: ammonia lyase.

ferulic acid being negatively correlated with parameters of insect development (Classen et al. 1990, Serratos et al. 1987).

Phenolic acids can be divided into two groups: soluble and cell wall bound. A review of each group will be given as their roles in resistance differ significantly.

1.6.3.1 Soluble Phenolic Acids

Free phenolic acid content is generally low in comparison to phenolic acids that are conjugated through ester linkages to sugars. Sosulski et al. (1982) determined the total phenolic composition in corn flour to consist of 5.3% free phenolic acids and 25.5% phenolic acid esters. In particular, hydroxycinnamic acids (ferulic and p-coumaric acids) occur naturally in a much wider range of conjugated forms than any other group of plant phenolics (Harborne 1980).

Most phenolics occurring in plant leaf, stem or flower tissue occur as water-soluble glycosides. Hydroxycinnamic acids are usually attached to sugars by the carboxylic acid group rather than to a phenolic residue (Harborne 1980). Ferulic acid is usually conjugated with glucose or gentiobiose while p-coumaric acid is often bound to glucose, fructose or rutinose (Harborne 1980). Feeding responses elicited by p-coumaric acid and ferulic acid differ with the insect species. Both compounds were phagostimulants for the spotted stem borer, *Chilo parellus* (Swinhoe) (Torto et al. 1991), while p-coumaric acid was toxic to the two-spotted spider mite (*Tetranychus urticae*) (Leszczynski et al. 1988). Other soluble phenolics such as gentisic or salicylic acids tend to be more toxic to insects (Fischer et al. 1990). Sap beetles, *Carpophilus hemipterus* (L.), are pests of maize, and larval feeding studies showed ferulic and p-coumaric acids to accelerate weight gain for this species (Dowd 1990). The above studies suggest that soluble ferulic and p-coumaric acid are not toxic to most insects and may serve as phagostimulants for insects. The studies did not consider plant-derived enzymes like phenoloxidase that oxidize foliar phenols which can reduce the efficiency of utilization of dietary amino acids and proteins in larvae (Felton et al. 1989). This could explain why pure phenolics in artificial diets did not dramatically affect insect feeding behavior or performance.

Phenolic acids conjugated to polyamines are relatively poorly studied. Their biosynthetic pathway is shown in Fig. 1.4. Besides being essential for cell growth and division (Heby 1981, Smith 1980, 1985), polyamines have been identified as radical scavengers (Bors et al. 1989) as well as possessing growth-regulator or hormonal-messenger activity (Davies 1987). Spermidine, putrescine and agmatine have been isolated as conjugates of caffeic, ferulic and p-coumaric acid (Evans and Malmberg 1989). It is of interest to note that one of the biosynthetic pathways of polyamines is mediated by arginine decarboxylase (Fig. 1.4), an enzyme known to be linked to stress responses of plants (Flores and Galston 1982). Within maize, both di-p-coumaryl putrescine and di-feruloyl putrescine have been isolated (Martin-Tanguay et al. 1978). Putrescine conjugated to ferulic acid has been localized in the aleurone layer of maize kernels and was found in high levels in kernels resistant to *S. zeamais* (Sen et al. 1993).

1.6.3.2 Plant cell wall phenolics

The evolution of phenolic acid carbohydrate complexes in plant cell walls is considered one of the most important determinants of early land plant success (Swain 1979). The cell wall of plants has been generally characterized as cellulose microfibrils embedded in a lignin-hemicellulosic macromolecule to which acetyl and phenolic acid groups are bound (Morrison 1979). During cell development, the primary cell wall is deposited initially, containing cellulose, hemicellulose and pectins (Bacon 1979). Arabinoxylans are the characteristic hemicellulose of cereals (Fry 1989). Pectins are a minor component in *Zea mays*, accounting for about 0.3% of the total cell wall polysaccharide (Kato and Nevins 1989). Lignin becomes part of the cell wall during establishment and thickening of the secondary cell wall (Theander and Aman 1984).

The phenolic constituents of the cell walls of graminaceous plants consist largely of p-coumaric and ferulic acids (Hartley and Jones 1978, El-Basyouni et al. 1964). Ferulic and p-coumaric acids are bound to cell wall polysaccharides through ester bonds (Fry 1986). Based on polysaccharide hydrolase digests of sugar cane and maize cell walls, ferulic acid is linked to arabinose, which in turn is linked to xylose (Kato and Nevins 1985, Kato et al. 1983). These compounds were identified as *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]

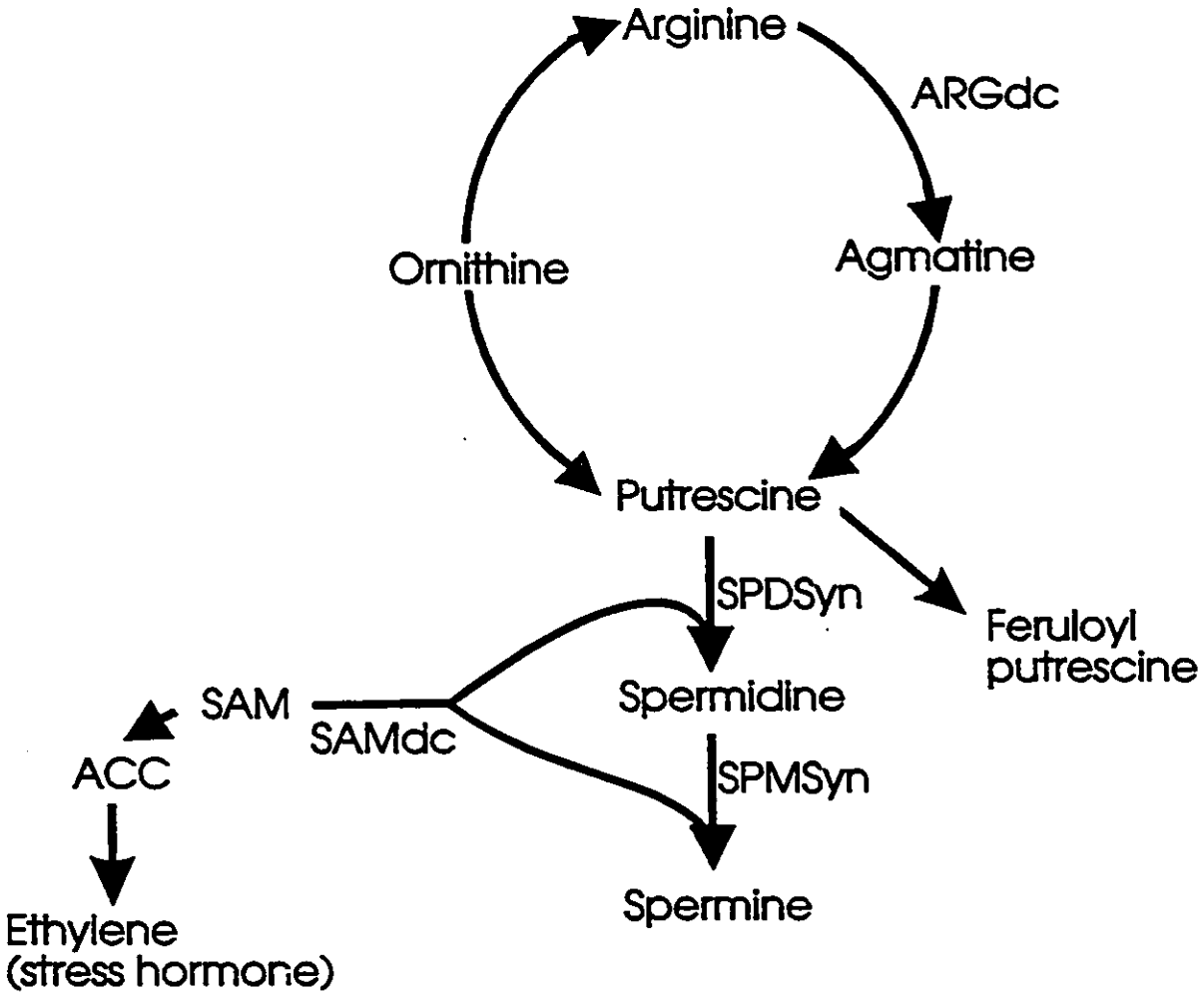


Fig. 1.4. Biosynthesis of phenolic amides (adapted from Evans and Malmberg 1989). ARGdc-arginine decarbosylase; ORNdc-ornithine decarboxylase; SPDSyn-spermidine synthase; SAM-S-adenosylmethionine; SAMdc-SAM decarboxylase; ACC-aminocyclopropane-1-carboxylic acid; SPMSyn-spermine synthase; PAO-polyamine oxidase; GABA-γ-aminobutyric acid.

(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX) and *O*-[5-*O*-(*trans*-p-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (PAXX) (Fig. 1.5) (Mueller-Harvey et al. 1986, Kato and Nevins 1985). Kinetic evidence suggests that the placement of these phenolic acids on polysaccharides is site-specific (Fry 1986). Levels of cell wall phenolics change with development, with ferulic acid predominating in primary cell walls (Nishitani and Nevins 1990), while p-coumaric acid is associated with secondary cell wall formation and is correlated with core lignin (Jung 1989). Not only do the levels of these cell wall phenolics differ among varieties, tissue type and maturity (Hanna et al. 1976), but environment also plays an important role in levels, with elevated temperatures (20 to 30°C) increasing ferulic and p-coumaric acid and lignin (Akin et al. 1987). These changes also reduced the digestibility of the tissue by rumen microorganisms. Incorporation of phenolic acids into the cell wall can also be induced by fungal invasion (Southerton and Deverall 1990). The importance of cell wall bound ferulic acid (FAXX) to maize resistance is illustrated in corn grain in which FAXX was the only significant factor explaining the variation in kernel damage by larvae and adult preference by *S. zeamais*, and has been suggested as a means of identifying resistance to grain storage pests although the mechanism of this resistance is not certain (Classen et al. 1990). P-coumaric acid and the ratio of p-coumaric acid to ferulic acid is highly correlated with lignin and reduced digestibilty of tissue by ruminants (Hartley et al. 1992, Akin and Chesson 1989, Cherney et al. 1988, Hartley 1972), although the causal agent for the reduced digestibility cannot be unequivically attributed to either p-coumaric acid or lignin.

Cell wall bound phenolic acids can also form dimers by two different mechanisms to facilitate cross-linking of hemicellulose. The significance of cross-linking is the increase in tissue toughness that could result (Hartley and Morrison III 1991, Hartley and Ford 1989). Increased digestibility of grasses after mild base hydrolysis which removes simple phenolics and their dimers is indirect support for this hypothesis (Hartley and Morrison III 1991). The first form of dimer to be discovered was the dehydrodiferulic acids (Hartley and Jones 1976, Markwalder and Neukom 1976). The dimer is produced by peroxidase in the presence of peroxide to form a ring linkage at the *meta* position (Fig. 1.6). The frequency of this cross-linking agent of arabinoxylans is one dehydrodiferulic acid /2600 sugar residues of

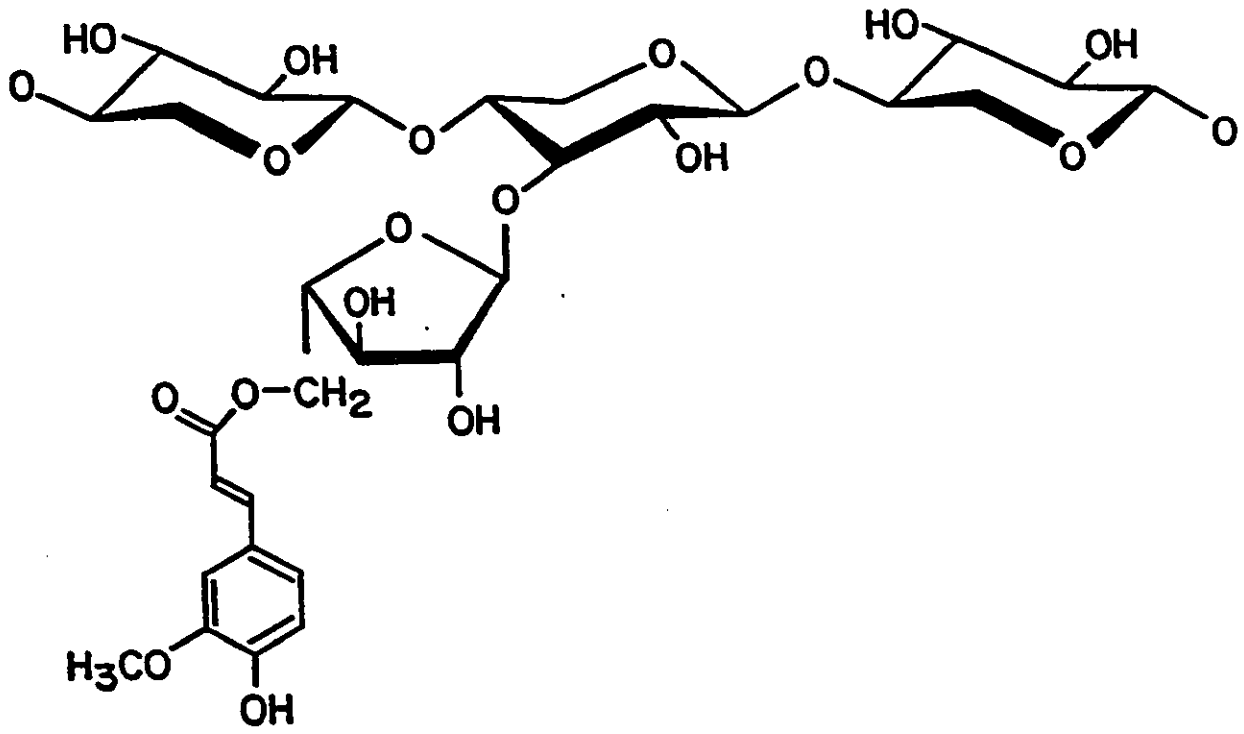


Fig. 1.5. Structure of phenolic acid - carbohydrate complexes. Complex shown is O-(5-O-feruloyl--α-L-arabinofuranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose (Kato and Nevins 1985).

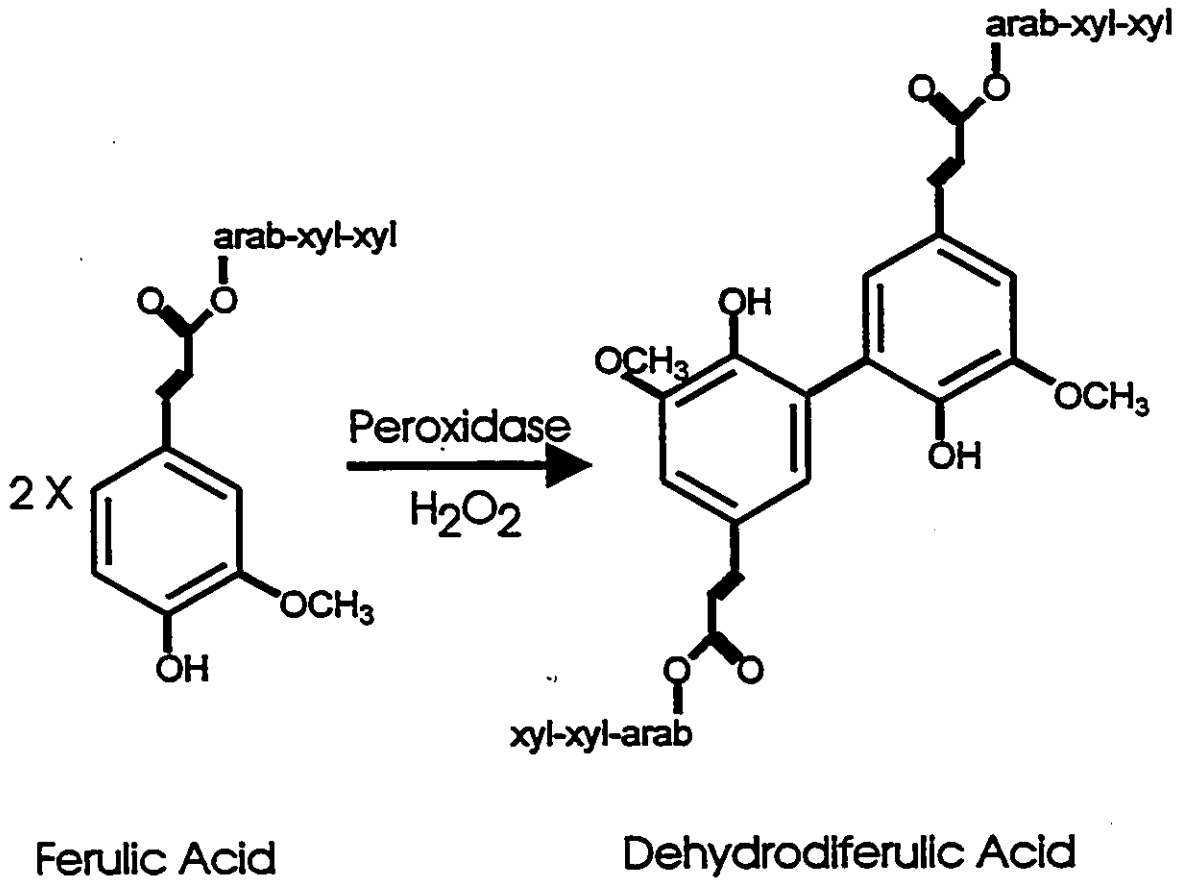


Fig. 1.6. Biosynthesis of dehydrodiferulic acid.

arabinoxylans based on 50% arabinoxylan content (Shibuya 1984). These dehydrodiferulic acid bridges appear to form early in maize tissue development (Nishitani and Nevins 1990). Irrespective of tissue age or the presence of light, the ratio of dehydrodiferulic acid to ferulic acid is constant, suggesting that the rate limiting step in the formation of these phenolic bridges is feruloylation of the cell wall (Tan et al. 1992).

The second mechanism of phenolic bridging involves the absorption of ultraviolet light. The energy of the photon is used to form a four-membered ring from the double bonds of the side chains in either a head-to-tail fashion to form truxillic acids or in a head-to-head fashion to form truxinic acids (Fig. 1.7) (Hartley et al. 1988). Again the concept of cross-linking applies, with both phenolics being attached to arabinose and xylose prior to the phytochemical reaction. Although the production of these dimers has been proposed to occur during milling (Tachibana et al. 1992), this would likely be minor in comparison to the light activated dimers (Morrison et al. 1992). Unlike the dehydrodiferulic acids that occur only as ferulic dimers, the cyclobutane dimers consist of both ferulic and p-coumaric acid. The levels of these cyclobutane dimers are highest in tissues that intercept sunlight, with leaf blades having the highest levels, followed by sheath and stem tissue (Eraso and Hartley 1990). Head-to-tail dimers are generally in the greatest abundance (Morrison et al. 1991). The formation of these dimers in full sunlight may explain why maize plants grown in greenhouses are more susceptible to insect feeding on leaves as reported by Guthrie et al. (1986b). In addition to phenolic-mediated cross-linking in the cell wall, many other forms of bonding occur within the cell wall to increase the mechanical strength of the cell wall (Fig. 1.8).

Lignin is the second most abundant organic component of cell walls and has been studied extensively to determine its biosynthesis, composition and function. Several reviews of lignin research are available (Lewis and Yamamoto 1990, Monties 1989, Janshekar and Fiechter 1983, Swain 1979). Lignins are high-molecular-weight polymers of which the main building units are reduced phenylpropanoids, namely p-coumaryl, coniferyl and sinapyl alcohols. Once the cinnamyl alcohol is formed, the hydroxyl group is oxidized by peroxidase or laccase, yielding a free radical species in mesomeric forms. These radicals then couple in a non-enzymatic and random fashion to form dilignols, oligomeric intermediates, and finally

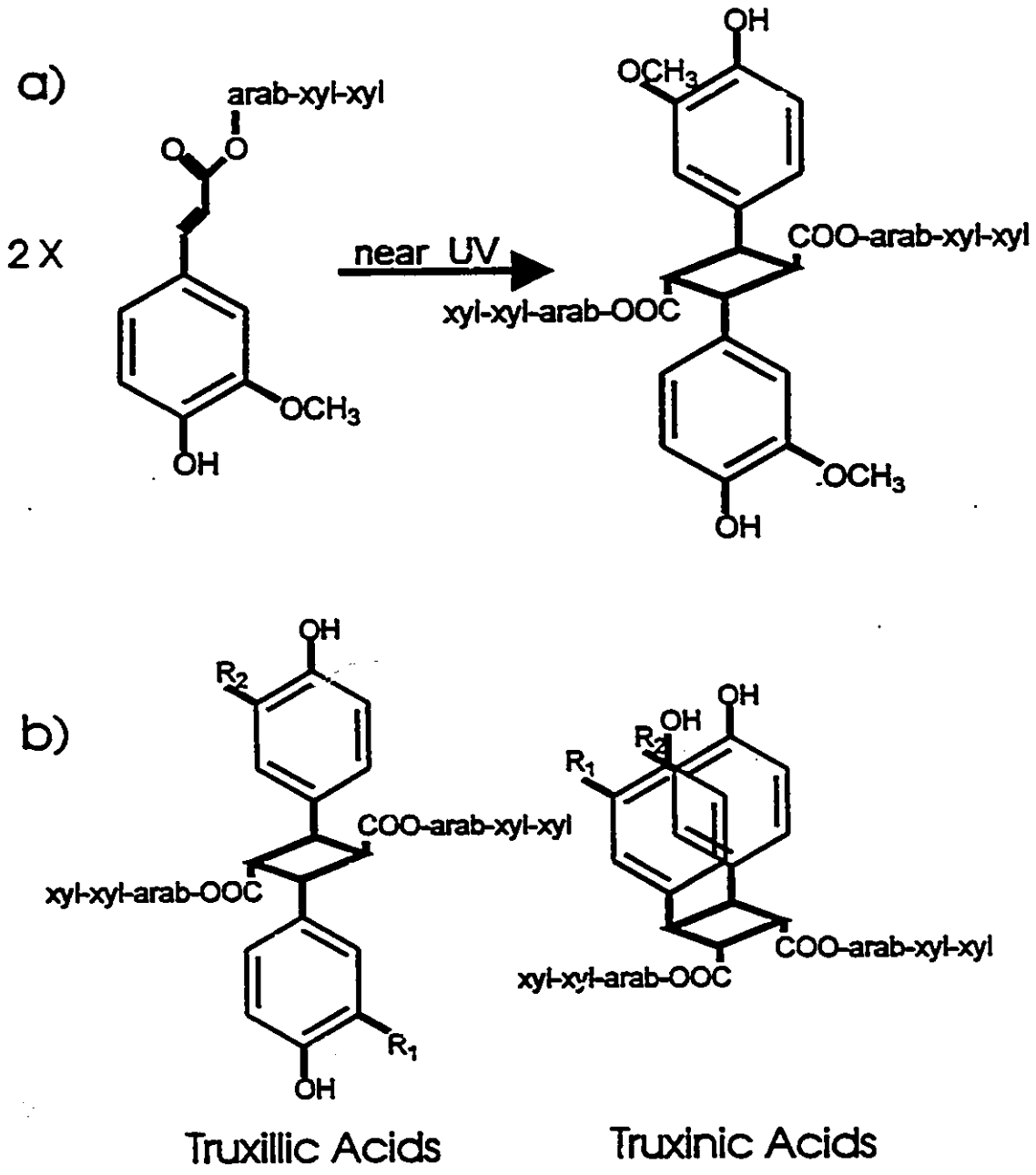


Fig. 1.7. Photosynthesis of cyclobutane dimers. a) ferulic-ferulic dimer formation. b) head-to-tail dimerization (truxillic acid) and head-to-head dimerization (truxinic acid). R=H (E-p-coumaric acid), R=OCH_3 (E-ferulic acid).

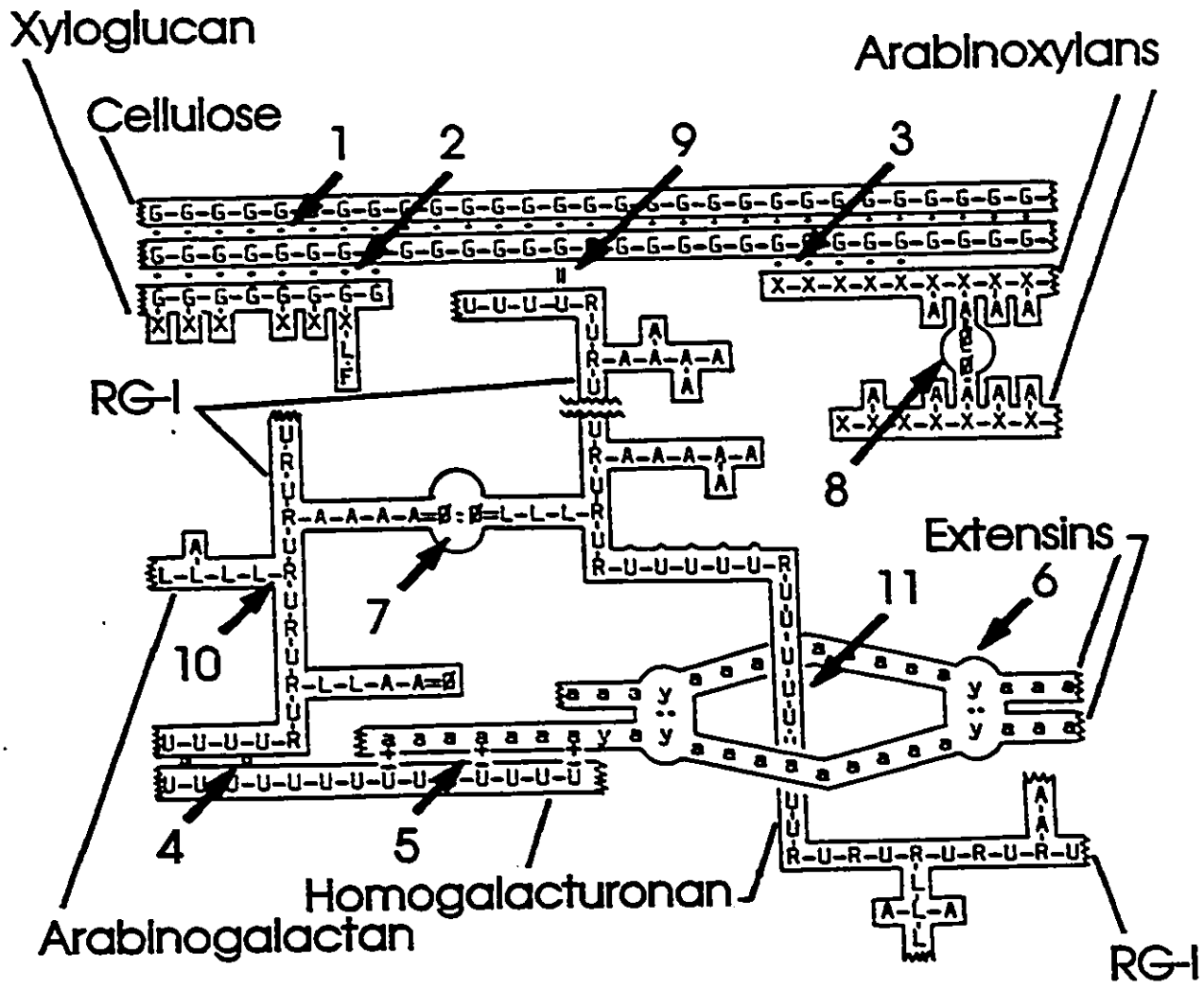


Fig. 1.8. Schematic of cell wall cross-linking (adapted from Fry 1986).
(o) Calcium bridges; (.) Hydrogen-bonds; (:) Coupled phenols;
(=) Ester bonds; (-) Glycosidic bonds; A=Arabinose; F=Fucose;
G=Glucose; L=Galactose; R=Rhamnose; U=Galacturonic acid;
U=Galacturonic acid methyl ester; a=Aminoacid other than tyr;
y=Tyrosine; y:y=Isodityrosine; ϕ =Ferulic acid;ϕ:ϕ=Diferulic acid.
1. cellulose-cellulose; 2. xyloglucan-cellulose; 3. xylan-cellulose;
4. homogalacturonan-homogalacturonan; 5. extensin-extensin;
6. extensin-extensin; 7. pectin-pectin; 8. arabinoxylan-arabinoxylan;
9. pectin-celiulose; 10. arabionogalactan-rhamnogalacturonan;
11. pectin-in-extensin.

lignin macromolecules. Given the random nature of free radical polymerization, the structure of lignin is not predictable although its composition is. Monocotyledonous angiosperms use p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, reduction products of p-coumaric, ferulic and sinapic acids. The biosynthesis of coniferyl, p-coumaryl and sinapyl alcohols takes place in the protoplast of the cell but their free radicals are produced only within the cell wall. Lignins may have arisen originally by cross-linking of adjacent feruloyl groups attached to the cell wall polysaccharide matrix (Swain 1978). The rest of the polymer would then be attached later. This is substantiated by the isolation of deoxydiferulic acid from cell wall polysaccharides (Hartley and Jones 1976). The role of lignins in plant defence may either stem from hardening of cell walls or from reduced digestibility of cell wall polysaccharides (Hartley 1973).

1.7 Hypothesis and Objectives

1.7.1 Central Hypothesis

For the purposes of the present study, it is proposed that maize was historically an unapparent plant as defined by Feeny (1976), in which its irregular occurrence made it difficult for specialized insects to find. The characteristic allelochemicals that unapparent plants used to deter polyphagous insects are toxins of which DIMBOA is an example. During the history of its cultivation, maize has become an increasingly apparent plant, making it vulnerable to the adaptive plasticity of insects, in particular the European corn borer. It is proposed that inadvertently, breeding programs are moving away from DIMBOA and other qualitative defence mechanisms and incorporating quantitative defences. Derived from Feeny's theory, quantitative defenses will be defined as phenolic-carbohydrate complexes for the puposes of this thesis which would encompass lignin, FAXX, PAXX, phenolic dimers, hemicellulose and detergent fibre content.

The biological effects and mechanisms of action of phenolics and phenolic acids in maize leaves and stalks on phytophagous insects are unknown. It is logical to hypothesize that phenolic acids, being ubiquitous in corn tissue, provide some form of resistance to corn varieties against such economic pests as the European corn borer. The project will investigate the biological effects and mechanisms of action of phenolics in plants to polyphagous insects, using the maize - European corn borer as a model system.

1.7.2 Research Objectives

The overall objective of this study is to elucidate the role phenolic acids and their cell wall - carbohydrate complexes play in maize resistance to the European corn borer. Preliminary studies (Appendix 1) showed that ferulic acid incorporated into a meridic diet did not cause significant larval mortality or reduce insect fecundity but only delayed insect development time at high levels (10 and 33 mg ferulic acid/g diet), levels much higher than observed in maize (0.3 mg/g fresh weight). Sugar conjugates of ferulic and p-coumaric acids extracted from resistant lines of maize and applied to maize leaves were found to be phagostimulants at moderately high dosages (Appendix 2). Higher dosages were phytotoxic causing tissue browning within 12 hours. Antifeedant activity at the higher dose may be due

to necrosis of substrate and not due to phenolic acid conjugates directly. From these observations, soluble phenolic acids do not appear to make a major contribution to maize resistance. Based on these preliminary studies the objectives for further work were based on phenolic acids contributing to a quantitative resistance mechanism in maize.

Objective 1: Determine if quantitative defenses play a role in host plant resistance in maize.

Objective 2: If phenolic acids are a biochemical basis of quantitative defences, where are they located in maize of different susceptibility?

Objective 3: Using a leaf profile to represent leaf development and plants at different stages in development, determine the role of toxic versus quantitative defences and nutritional factors in larval feeding preferences within the maize plant.

Objective 4: Determine the evolution of plant defence in the recurrent selection of maize for insect resistance.

Objective 5: Determine which resistance factors account for the most variation in field resistance of maize genotypes known for non-conventional resistance.

Objective 6: Determine the role of key environmental factors in quantitative phenolic acid defences.

Objective 7: Propose a composite resistance model for host plant resistance in maize.

CHAPTER II.

MONITORING QUANTITATIVE DEFENSES IN MAIZE AND THEIR RELATIONSHIP WITH FIELD RESISTANCE TO THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS*.

2.1 Introduction

Artificial infestation of maize genotypes has been the primary tool in screening for European corn borer resistance. Larval leaf feeding damage is rated on a nine point scale from resistant (1) to susceptible (9) (Guthrie et al.1960). This system of screening is essential for a resistance breeding program but it has some disadvantages which include the cost of mass rearing insects, variations in plant damage due to environment, personnel changes for infesting and rating plants, and changes in the insect population associated with artificial rearing (Klun et al. 1970). Monitoring the levels of the resistance toxic factor 2,4-dihydroxy-7-methoxy- 4,1-benzoxazin-3-one (DIMBOA), in whorl leaf tissue has facilitated resistance screening (Russell et al. 1975), but such a screening protocol is expensive and depends strongly on sampling date due to changes in DIMBOA levels over the maturity of the plant (Morse et al. 1991). Another screening technique tested was tissue pH but only low level correlations between pH and leaf feeding were observed (Byrne et al. 1990). Consequently it is evident that other objective and quantitative screening techniques are still needed for maize breeding. The first objective of this study was to determine if instron measurements in neutons (N) of leaf toughness correlated with field leaf feeding ratings.

Maize resistance based on the toxic hydroxamic acids does not explain many situations where host plant resistance is observed (Guthrie et al. 1986b, Scriber et al. 1975, Sullivan et al. 1974). Based on field observations and leaf feeding bioassays, ECB larvae prefer tender tissue within the whorl. In view of these observations and the predictions of the plant apparency hypothesis of Feeny (1976), it is hypothesised that maize may employ quantitative (structural) defences against the ECB in mature leaf tissue. The first objective of this study was to develop a quantitative technique for measuring leaf toughness. The second objective of this study was to identify how broadly structural defences are employed by studying diverse sets of maize germplasm.

2.2 Materials and Methods

Germplasm: Five genotype categories were investigated: multiple borer resistant (MBR) cultivars, MBR inbreds, Mexican landraces, BS9 selection cycles, and North American inbreds (Table 2.1). MBR lines and hybrids were developed at the International Maize and Wheat Improvement Center (CIMMYT). Mexican and Argentinean landraces were obtained from the CIMMYT maize germplasm seed bank. For 1991, five selections from a cross between propriety lines derived from the landrace Cateto from Cargill International, Minneapolis, Minnesota and CM7 were included in the landrace group: mk12, mb10, mk9, mc3, me3. Four cycles of selection from the BS9 population (provided by A.R. Hallauer) were also included.

Field plot: In 1991 and 1992 the same locations were planted in mid-May at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. The soil was a sandy loam. The experimental design was a randomized-complete block with four replicates for each year. The rows were spaced 0.9 m apart and plants spaced 0.15 m apart with each row consisting of 25 plants.

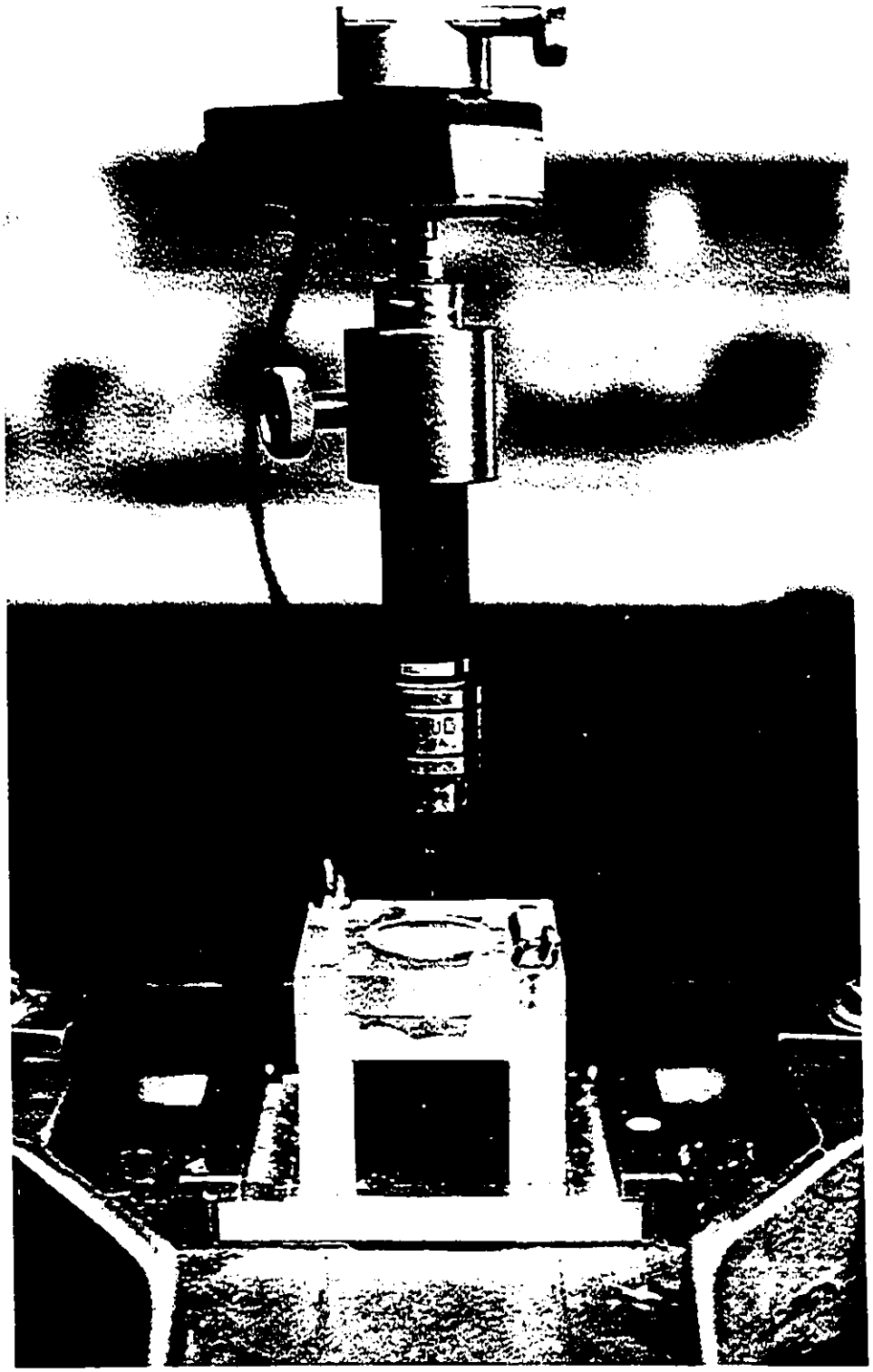
Artificial infestation: Infestations were made at the mid-whorl stage in early July with two applications (4 days apart) of 4 egg masses (ca. 200 eggs) per plant and 15 plants per row. Leaf-feeding damage was rated on an individual plant basis from 1 (no damage) to 9 (extensive damage) three weeks after infesting (Guthrie et al. 1960).

Leaf toughness measurements: In 1991 leaf toughness readings were taken at plant tasselling in late July and early August. In 1992 readings were taken at both the whorl stage in early July and at tasselling in early August. Leaf samples were taken from ten plants within the row. The second fully exposed leaf from the top of the whorl was sampled at mid-whorl and the ear leaf during tasselling with the sample consisting of a 10-cm length excluding the midrib from the middle of the leaf length. Samples were kept submerged in distilled water and stored at 4°C until analysed. Leaves were blotted dry and inserted between the stainless steel stage and a plexiglas plate with the lower surface of the leaf facing up (Fig. 2.1). Both the stage and the plexiglas plate had a 2.0 cm diameter hole to expose the leaf surface to the instron probe. The leaf was secured to the stage by screwing the plexiglas down while keeping the leaf surface taut. A standard instron (model TM-M, Intron Corp., Canton, Mass.) was equipped with a 5 lb. load cell (Lebow load cell, model 3108, Eaton Corp., Troy Mich.)

Table 2.1. Genotypes from five sources tested for instron study, 1991 and 1992.

North American Inbreds	Multiple Borer Resistant Inbreds	Multiple Borer Resistant Cultivars	Mexican and Argentinean Landraces	BS9 Synthetic Series
B73	CML67	Across 86590(IR)	Oaxaca 179	BS9 C0
A619	CML121	CML135XCML139	Oaxaca 4	BS9 C2
DE811	CML127	Ki3xTx601	Guanajuato 207	BS9 C4
ND241	CML135	Mbita 86590	Guanajuato 102	BS9 C5
CG16	CML131	Poza Rica 86590	Mexico 212	
CI31A	CML139	Across 86590-2	Mexico 208	
CO266	Hi34	Tlaltizapan85590(SWCB)	Nayarit 24	
CM7	Ki3	6796-13	Nayarit 72	
CK44		6796-49	Cateto:C 2030	
F7		**Checks:**	Cateto:C 2032	
B86		Fontanelle 6220	MK 12	
MS72		Pioneer 3184	MB10	
72E44		Dekalb 435	MK9	
OH43		Picseed 4533	MC3	
			ME3	

Fig. 2.1. Plate showing stage apparatus, probe and head of instron. Stainless steel stage has a 2 cm dia. hole through the plate's centre. Leaf is placed on stage and covered with Plexiglas plate with 2 cm dia. hole through its centre. Leaf is pulled taut and Plexiglas plate is tighly secured against stage by wing-nuts. Drill chuck is attached to a 5 lb. load cell.



and a 9 mm chuck to hold probes. The instron-computer interface was a strain gage conditioner/indicator (model 3278P, Daytronic Corp., Miamisburg, Ohio). Computer software used was the ESRC texture program 1987, developed by Agriculture Canada Engineering and Statistical Research Centre and Scientific System Development Micro-computer Centre. Various probe shapes and sizes were tested to determine the best probe. Different probes included a #3 insect pin, a 0.5 cm tapered cone, a 1 mm flat-bottomed cylinder and a 1 mm round bottomed cylinder (Fig. 2.2 and 2.3). A 1 mm dia. stainless steel cylinder probe with a rounded tip gave the best results and was used for all subsequent toughness readings. The cross-head speed was set to 1 cm per min. and was calibrated against 10 g . Peak force to penetrate the lower epidermis was the only reading used as the deflection to peak force and tensile force were not found to be useful in predicting field resistance. The probe was positioned between secondary veins in the middle of the leaf. The probe was lowered until the leaf was completely punctured and the force readings had returned to baseline (Fig. 2.3). All tests within a genotype group were measured the same day with samples being stored for no longer than 3 hours prior to measurement. Twenty readings were taken for each genotype.

Statistical analysis: All statistical analyses were performed on SAS v. 6.03 (SAS, 1988). Data was transformed by $\ln(x + 1)$ to satisfy the assumptions of the general linear model. Regressions were done on means for each variety tested.

2.3 Results and Discussion

Force profiles for different probe shapes are shown in Fig. 2.2. Pins were too narrow (even when dull) to generate sufficient force on the load cell to provide good measurements of varietal difference in leaf toughness. Cone probes were pointed and peak force readings were also undesirably low. An additional disadvantage of the cone was the continued force against the load cell after penetrating the leaf. Cylinders gave the best readings (Fig. 2.2 and 2.3). Flat cylinders (Fig. 2.2) tended to cut the leaf due to the sharp edge on the cylinder. This resulted in a reduction in peak force and inconsistent readings. Rounded cylinders (Fig. 2.3) gave the most consistent results with the puncturing of both the lower and upper epidermis layers being clearly recorded. The diameter of the cylinder used was 1 mm, larger cylinders

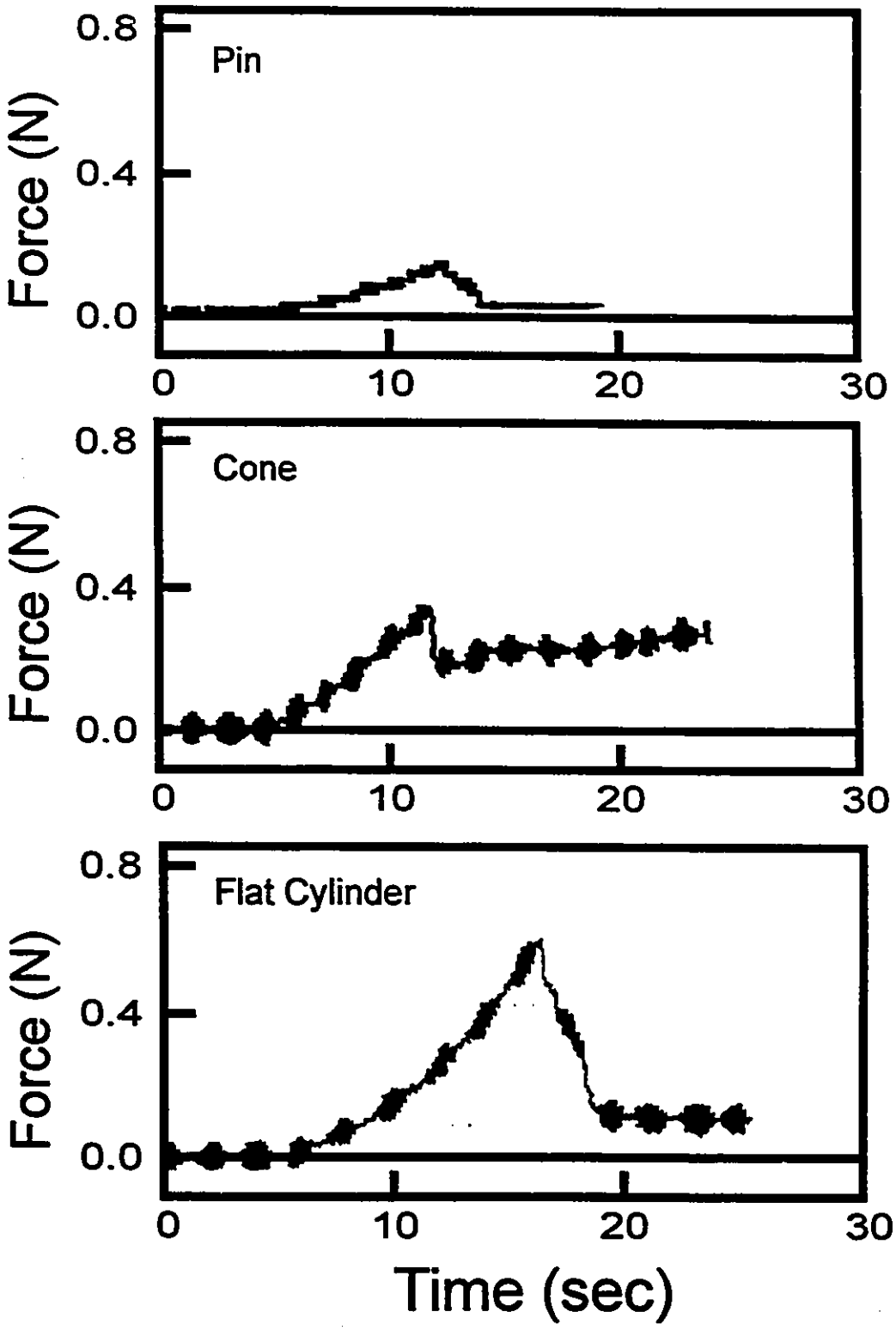


Fig. 2.2. Computer plots of force profiles for different shaped probes. Profiles of mature leaf tissue from B86. N=newtons.

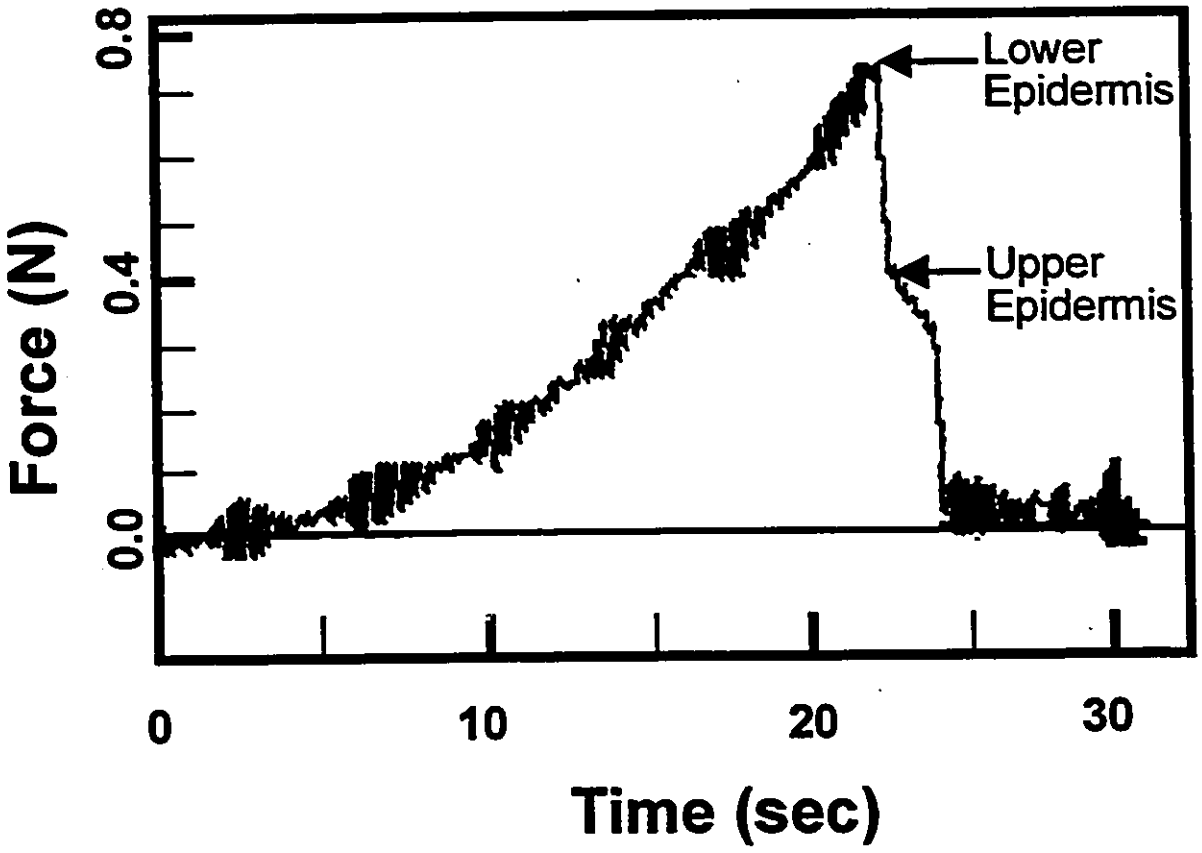


Fig. 2.3. Computer plot of force profile for B86 mature leaf tissue. N=newtons.

were rejected as they did not fit between the secondary veins of most genotypes. Peak force readings at penetration ranged from 0.4 N to 1.2 N depending on the genotype and leaf age.

Regression analysis for 1991 data (Table 2.2) demonstrates the significant inverse relationship between leaf toughness and leaf damage rating by borers. For all groups the tougher leaves tended to suffer less leaf feeding damage during the first three weeks after artificial infestation. Although the r^2 for BS9 selection cycles was high ($r^2 = 0.87$) the P value was low since only four cycles were available and the variation within a cycle was moderately high. For the other genotype groups the regressions were significant with MBR hybrids having the most significant regression. Figure 2.4 shows the regression of the untransformed data for MBR hybrids. MBR inbreds and hybrids are known to employ a non-conventional resistance mechanism as they contain low levels of DIMBOA (Benson 1986). It is likely that these genotypes rely on a physical defence mechanism to provide resistance to several lepidopteran foliage feeders. Mechanical mechanisms may include lignin and detergent fibre content (Buendgen et al. 1990) and monomeric and dimeric phenolic consistuents of the cell wall (Eraso and Hartley 1990, Chapter 8) which may act as nutritional or physical barriers to insect feeding. Leaf toughness has also been associated with plant resistance to species of Acrididae (Williams 1954) and Coleoptera (Shade and Wilson 1967, Tanton 1962), although no biochemical mechanisms for increased toughness was proposed. Instron measurements of stem strength for bermudagrass and alfalfa have shown lignified vascular ring structures to be the major factor affecting textural strength of stem residues (Akin et al. 1990c).

Cool wet weather in 1992 delayed the development of both the insect and the plant. Toughness readings taken in early July were highly variable especially for late maturing genotypes within the inbred and BS9 groups. Regression equations continued to be significant for all groups except inbreds and BS9 selection cycles (Table 2.3). Plant development within these two groups was delayed by the cool weather which may have delayed the establishment of resistance mechanisms associated with leaf toughness. BS9 regressions between toughness and leaf-damage rating actually changed from a negative relationship during mid-whorl to a positive relationship at tasselling (Tables 2.3 and 2.4). Again this is likely due to a small number of genotypes tested, 4, and small differences between the cycles in both leaf rating and toughness.

Table 2.2. Regression equations for leaf toughness and leaf rating during tasselling, 1991.

Genotype	Regression Equation[1]	r^2	F value	Prob > |T|	(n)
BS9 synthetic	Ln(LR) = 2.5(±0.4) - 2.7(±0.7) x Ln(LT)	0.87	13.14	0.07	4
Inbreds	Ln(LR) = 2.5(±0.4) - 2.1(±0.9) x Ln(LT)	0.31	5.09	0.05	14
Landraces	Ln(LR) = 2.8(±0.5) - 2.5(±1.1) x Ln(LT)	0.27	5.26	0.04	15
MBR inbreds	Ln(LR) = 5.8(±1.8) - 7.2(±2.8) x Ln(LT)	0.47	6.37	0.04	8
MBR hybrids	Ln(LR) = 4.1(±0.6) - 4.8(±1.0) x Ln(LT)	0.67	22.41	0.001	13

[1] LR = leaf rating (scale of 1-9), LT = leaf toughness in newtons; Data were transformed by ln(x +1).

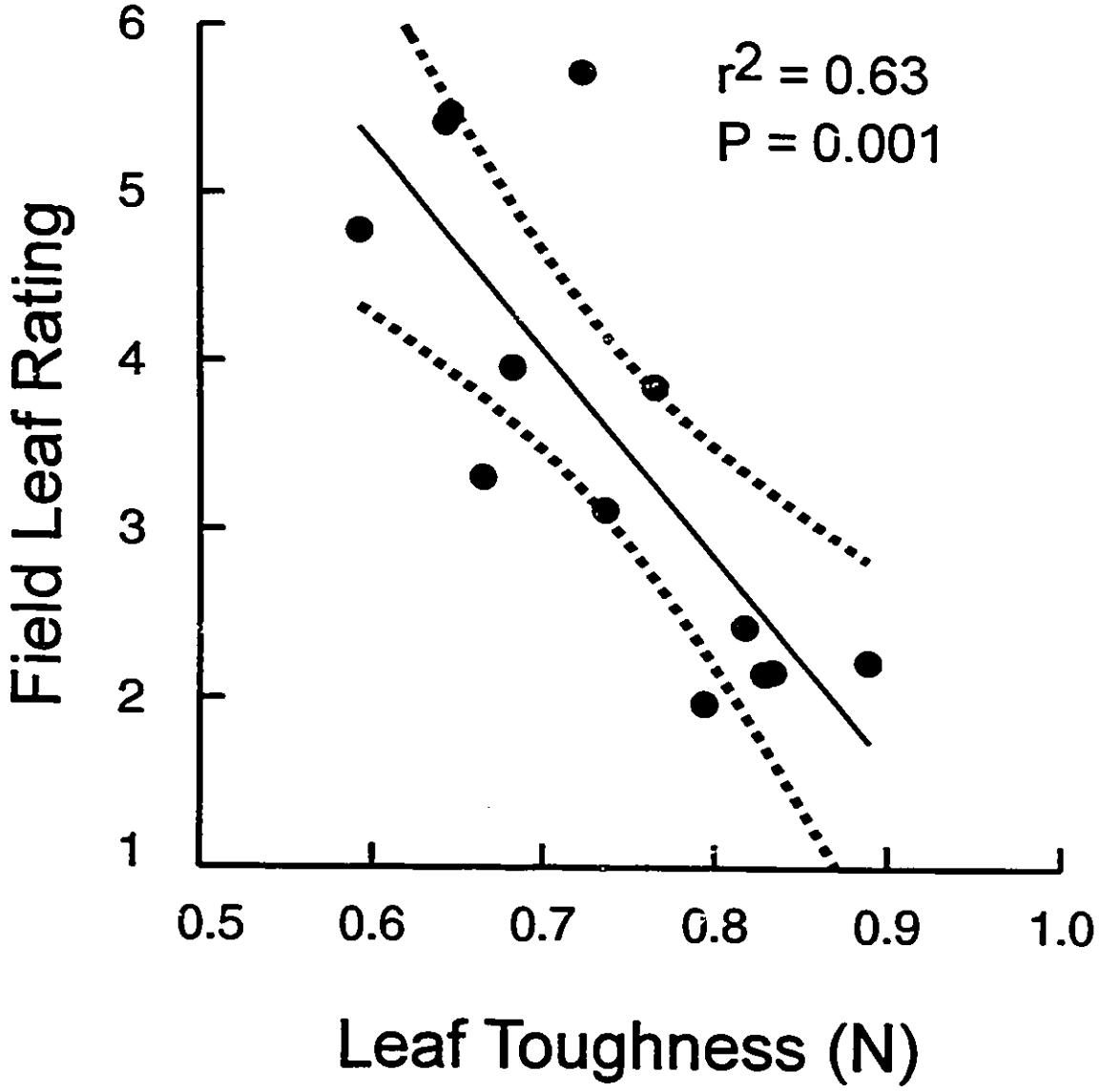


Fig. 2.4. Regression plot of leaf rating against leaf toughness for MBR cultivars.. N=newtons and field leaf rating according to Guthrie et al. (1960).

Table 2.3. Regression equations for leaf toughness and leaf rating during tasselling, 1992.

Genotype	Regression Equation[1]	r^2	F value	Prob > |T|	(n)
BS9 synthetic	Ln(LR) = -0.6(±0.7) + 4.2(±1.4) x Ln(LT)	0.81	9.03	0.1	4
Inbreds	Ln(LR) = 3.2(±0.8) - 3.8(±1.9) x Ln(LT)	0.31	4.23	0.07	14
Landraces	Ln(LR) = 2.6(±0.4) - 2.1(±0.7) x Ln(LT)	0.51	8.05	0.02	10
MBR inbreds	Ln(LR) = 3.6(±0.7) - 4.2(±1.3) x Ln(LT)	0.67	10.61	0.02	8
MBR hybrids	Ln(LR) = 4.1(±1.2) - 5.1(±2.2) x Ln(LT)	0.37	5.28	0.05	13

[1] LR = leaf rating (scale of 1-9), LT = leaf toughness in newtons; Data were transformed by ln(x +1).

Table 2.4. Regression equations for leaf toughness and leaf rating during mid-whorl, 1992.

Genotype	Regression Equation[1]	r^2	F value	Prob > |T|	(n)
BS9 synthetic	Ln(LR) = 3.3(±0.9) - 5.1(±2.3) x Ln(LT)	0.71	4.65	0.16	4
Inbreds	Ln(LR) = 2.9(±1.0) - 4.5(±3.5) x Ln(LT)	0.18	1.71	0.22	14
Landraces	Ln(LR) = 3.3(±0.5) - 4.4(±1.2) x Ln(LT)	0.63	13.37	0.01	10
MBR inbreds	Ln(LR) = 2.9(±0.6) - 3.8(±1.4) x Ln(LT)	0.53	6.86	0.04	8
MBR hybrids	Ln(LR) = 2.7(±0.6) - 3.0(±1.4) x Ln(LT)	0.33	4.46	0.06	13

[1] LR = leaf rating (scale of 1-9), LT = leaf toughness in newtons; Data were transformed by ln(x +1).

Inbreds incorporated into this study (except CML lines) have a DIMBOA based resistance mechanism. As a soluble secondary product, DIMBOA and other hydroxamic acids do not contribute to the structural strength of the leaf. Even so, leaf toughness accounts for approximately 31 percent of the variation in leaf feeding damage by the European corn borer, suggesting that leaf toughness is an important component in maize resistance to the European corn borer for all five genotype groups studied over two varied growing seasons.

Leaf toughness of immature tissue within the whorl of the plant did not correlate with leaf-feeding damage (data not shown) as the tender immature leaf tissue gave variable and low peak force readings (<0.2 N compared to 0.6-1.0 N for mature tissue). It is likely that DIMBOA is the principle mechanism of resistance for such tissue as DIMBOA levels are highest within the immature portions of the plant (Morse et al. 1991, Chapter 4). During the mid-whorl stage, the leaf readings were most consistent when readings were taken seven days after leaf emergence from the whorl. Although most leaf-feeding damage occurs within the whorl, emerged leaf tissue may be a good indicator of leaf toughness within the whorl where instron measurements were too variable to provide reliable quantitative information. Excluding BS9 for 1992, regressions between peak force readings taken at mid-whorl and tasselling were significant with the lowest r^2 of 0.49 observed for the North American inbreds ($F=7.92$, $P=0.02$). All regressions showed proportional increases in leaf toughness with maturity, and therefore, the best sampling time appears to be during tasselling in which the ear leaf is sampled. The advantages of this sampling time are: a higher and more consistent force reading, moderate to good correlations between leaf feeding damage and toughness, and more time available for measurements to be taken.

Applying this technique to a breeding program is feasible as each genotype can be analysed within 30 min. This time includes sample collection (5 min.), sample blotting, loading and 20 readings (25 min.). Travel time between the field plot and instron will vary but samples can be preserved in cool water for up to 4 hours without significantly affecting readings. However, if samples are left out of water for longer than 10 minutes, results will vary and if left out of water for one hour peak force readings drop significantly. During one day, 14 to 16 varieties can be measured accurately. With a sampling window of 15 to 20 days a maximum of 320 varieties could be sampled by one operator during the season. The training

time to become proficient is from one to three days, with the most critical component being the probe placement within the leaf (i.e., between secondary veins).

The power of this screening technique is the ability to further our understanding of European corn borer feeding behaviour by providing quantitative data on tissue toughness within the plant to see the role toughness plays in insect feeding behaviour. Having quantitative data on leaf toughness will also further our understanding of which leaf components contribute most to leaf toughness by genotype correlations and monitoring plant changes during maturation.

Correlations between leaf toughness and field-damage ratings for five diverse genotype groups suggests that quantitative defenses, namely leaf toughness, are important in maize resistance, and are employed to varying degrees within different germplasm groups. MBR cultivars appear to depend most on quantitative defenses which is consistent with previous observations of low DIMBOA levels and broad resistance to several borer species (Benson 1986). Quantitative resistance mechanisms are a plausible defence strategy for future resistance breeding-programs given the large expanses of corn planted in monoculture in present day agronomy practices which make corn an "apparent" plant according to Feeny's (1976) theory of plant defence. Apparent plants are predicted by Feeny to be most successful if they employ a quantitative defence strategy against herbivores.

CHAPTER III.

LOCALIZATION AND QUANTIFICATION BY MICROSPECTROPHOTOMETER OF HYDROXYCINNAMIC ACIDS IN CELL WALLS OF EUROPEAN CORN BORER RESISTANT AND SUSCEPTIBLE MAIZE INBREDS.

3.1 Introduction

In Chapter 2, the role of quantitative defences in leaf feeding resistance to the ECB was demonstrated. Structure and toughness of the leaf tissue is determined mainly by cell wall carbohydrates and their complexes with a variety of phenolics (Hartley and Ford 1989). It is hypothesized that cell wall phenolics are a major component of quantitative defences. This hypothesis is suggested by rumen digestion studies of leaf tissue which show reduced breakdown of cell wall material with elevated levels of the hydroxycinnamic acids, p-coumaric and ferulic acid (Akin et al. 1990a, Jung and Casler 1990).

Cell wall bound phenolic acids have not been studied as a line of defence against insect pests feeding on vegetative tissue. The purpose of this study was to localize and quantify cell wall bound phenolic acids within the different cell types of ECB susceptible and resistant lines of maize to critically evaluate a phenolic acid based defence mechanism. A second objective was to observe changes in cell wall phenolic composition between preferred (immature) leaf tissue and more resistant (mature) leaf tissue. To this end, colour staining, and autofluorescence were evaluated as techniques for micro-localization and relative quantification of cell wall phenolic acids. A microspectrophotometer technique developed for grasses (Akin et al. 1990b) was adapted for quantification of maize cell wall phenolics.

3.2 MATERIALS AND METHODS

Plant Samples: Insect resistant (MBR 6796-15, B86, CI31A) and susceptible (MS72) inbred lines of maize plants were grown in greenhouses during December through April at 22°C. Plants were harvested at 10 weeks with leaf sections from mature tissue coming from the middle of leaf 8 while immature tissue came from leaves 13 to 15 depending on the inbred. Immature leaf tissue consisted of leaf sections within the whorl that had not

been exposed to light. During 1991, field grown plants were harvested as above and used to make comparisons with greenhouse grown material. Leaf sections were prepared at -20°C to a thickness of 12 µm and mounted on glass for staining. All sections were used within four hours of sectioning.

Insect bioassay: The same tissues described above were incorporated into a modified Ascher et al. (1981) apparatus to measure the percent consumption of a 1.0 cm^2 disk by two third-instar ECB larvae in a 48 hr period on mature, and 24 hrs on immature, tissue. The bioassay apparatus consisted of three halves of plastic petri plates (5 cm dia.) with the bottom plate having a 1 cm dia. hole. The top plate in inverted and wet cotton is placed inside and covered with filter paper. Leaf tissue is placed with the top surface placed down onto the filter paper and the bottom plate with the hole is centered onto the leaf to expose the feeding surface. Plates were taped together and two early third instar larvae were placed into the apparatus. A small plastic covering was placed inside to shade the larvae and the third plate was taped over the top to seal the larvae into the apparatus. Larvae were reared and tested under a 16:8 (light:dark) photoperiod at 85% R.H. and a 25°C/19°C (day/night) temperature. Area consumed was measured using 1 mm^2 graph paper. Forty bioassays were performed for each tissue type and variety.

Nitrogen Determination: An automatic micro-Kjeldahl nitrogen analyzer (Tecator model 1030) was used to determine percent nitrogen and estimate protein content of 0.3 g dwt leaf samples using the conversion factor 6.25 for estimating protein content from nitrogen (McKenzie and Wallace 1954).

Phenolic Quantification: Hydroxycinnamic acids tested included *E*-p-coumaric acid and *E*-ferulic acid purchased from Sigma Chem. Co. Hydroxycinnamic acids were suspended in acetone and applied to Whatmann #1 filter paper at 5, 0.5, 0.05 and 0.005 mg of p-coumaric or ferulic acid per cm^2. Double-sided tape held spiked filter paper to a glass slide. Slides were incubated for the same time as tissue sections and were photographed while wet. Pictures were taken out of focus to minimize heterogeneity of colour due to cellulose fibres.

Staining of filter paper loaded with defined amounts of phenolic acids provided a concentration-based colour gradient for diazonium salt stains but not a pronounced colour

gradient for the other stains tested (Appendix 4). However, the coloration differed from stained leaf tissue and could not be used reliably to estimate phenolic acid levels.

Microscope: A Zeiss universal microscope fitted with a 75 W xenon lamp was used for staining and fluorescent micrographs. Only neutral density filters were used.

Preparation of Histochemical Stains: Lignin and simple phenolic acids were localized using the following procedures and stains: 1) Phloroglucinol - a saturated solution (60 g/L) phloroglucinol in water was added to 6 M HCl (4:1) as needed; 2) Azure B - azure B bromide crystals were added (0.25 g/L) to 0.1 M citrate buffer, pH 4.0 and mixed as needed; 3) Chlorine-sulfite - a sodium sulfite (20 g/L) solution was prepared and cooled to < 8°C before use; household bleach containing 6% sodium hypochlorite was acidified to pH 1 using 6N HCl with constant stirring. Both solutions were prepared fresh and used within 2 hours (Jensen 1962); 4) diazotized p-nitroaniline - 10 ml of a p-nitroaniline solution (1 g/L) in 2 N HCl were added to 10 mL of an aqueous $NaNO_2$ solution (2 g/L) and then mixed with 20 mL of an aqueous K_2CO_3 solution (100 g/L); 5) diazotized sulfanilic acid - 2.25 g sulfanilic acid was dissolved in 45 mL of 6 N HCl by warming and the solution was diluted to 500 mL with water. Ten mL of the sulfanilic acid solution were added to 10 mL of cold aqueous KNO_2 (45 g/L) followed by 20 mL of aqueous Na_2CO_3 (100 g/L) (Krebs et al. 1969). All reagent mixtures were combined just prior to staining.

Staining of Standards and Plant Cell Walls: For all staining, good results were obtained by gently submerging slides into stain reagent and gently rocking to ensure good stain penetration without dislodging sections. Incubation time was two minutes for all stains except diazotized sulfanilic acid which required only 15 seconds. A second staining technique for azure-B involved heating of slides soaking in azure-B at 50°C for two hours followed by washing once with distilled water and twice for two-hours each time in t-butanol (Akin et al. 1990a). All slides were drained of excess stain reagent and mounted with a drop of 80 % glycerol and covered with a cover slip to reduce oxidation and crystallization of stained sections.

Microspectrophotometry: A computer-controlled Zeiss UMSP-80 microspectrophotometer equipped with a high-pressure xenon lamp (XBO 75W) and a series-connecting grating monochromator (bandwidth 10 nm) was used to measure transmittance spectra at

wavelengths from 230 to 350 nm (5 nm steps). Sections for microspectrophotometry were cut 8 µm thick from mature and immature tissue. The microscope was fitted with a 100x ultrafluar Zeiss quartz objective and a 10x ultrafluar Zeiss quartz condenser lens. The measuring aperture placed over the middle of the cell wall was 0.32 mm, which provided a measuring field diameter of 2 µm. The microspectrophotometer was adjusted for 326 nm which gave a high signal to noise ratio for taking readings of sclerenchyma, parenchyma bundle sheath, xylem and epidermal cells walls.

Phytochemical Analysis:

DIMBOA was extracted from a 0.5 g sample of lyophilizes tissue. Samples were extracted 4 x 20 ml of 70% methanol and mixed with a polytron mixer. After centrifugation the supernatants were pooled and dried to near water on a rotary evaporator (35°C). The pH was then lowered to 2.0 using 1N HCl and the extract was extracted with 4 x 50 ml of ethyl acetate (BDH, Omni-Solv grade). Ethyl acetate fractions were pooled and dried by rotary evaporator and stored at -20°C until HPLC analysis.

The pellet that remained after extracting DIMBOA was washed in a Büchner funnel with 30 ml of water, 30 mL of methanol and 30 mL of ethyl acetate to remove residual chlorophyll and provide a crude cell wall preparation. Cell wall samples were dried in a desiccator for four days. Samples were shaken in 20 mL of 2N NaOH for 4-h under N_2 and the beakers were wrapped in foil, then the pH was lowered to 2 with 6N HCl. After centrifugation the supernatant was extracted with 3 x 50 mL ethyl acetate. The pellet was resuspended in water and centrifuged twice more with both supernatant fractions pooled and extracted with 3 x 50 mL ethyl acetate. Ethyl acetate fractions were pooled and dried by rotary evaporator under darkness and stored at -20°C until HPLC analysis.

HPLC analysis: All analyses were performed with a Perkin-Elmer system consisting of an LC 480 diode scan array detector and a Perkin-Elmer LC250 binary pump fitted with a 10 µL injection loop. Separations were achieved using a C18 ODS reverse phase column (Beckman), 250 x 4.6 mm, 5 µm particle size.

DIMBOA extracts were suspended in 4 mL of 50% methanol and centrifuged at 2000 g for 5 min. The supernatant was filtered and injected onto the column. The solvent system was comprised of methanol (A) and 10 mM H_2PO_4, pH 2.4 (B) at a flow rate of 1.5 mL/min

and the gradient was a follows: 25 to 55% A in 15 min, 55 to 80% A in 5 min, 80 to 100% A in 2 min, 100% A for 8 min, 100 to 25% A in 2 min and 25% A for 3 min. DIMBOA (Rt = 10.5 min) and MBOA (Rt = 13.6 min) standards were synthetically prepared by Atkinson et al. (1991). Peak identity was confirmed by on-line UV spectra and spiking of extracts with authentic standards. Recovery of DIMBOA equivalents (MBOA plus DIMBOA) exceeded 80%.

Cell wall bound phenolic acids were suspended in 1 mL of methanol, diluted ten fold in methanol, filtered and injected onto the column. The solvent system was the same as above except the starting mixture was 15% methanol. Standards of p-coumaric (Rt = 15.2 min) and ferulic acid (Rt = 15.6 min) were purchased from Sigma. Recovery of *E*-p-coumaric and *E*-ferulic acid exceeded 75%.

Statistical analysis: All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Data were transformed to satisfy the assumptions of the general linear model.

3.3 Results and Discussion

Leaf feeding by ECB larvae was greatest on immature leaf tissue, with the entire exposed leaf area being consumed in 48 hours. Reducing the exposure time to 24 hours enabled differences to be observed between the genotypes. Varietal differences for mature tissue were best observed in the 48 hour bioassays (Table 3.1). Rankings of the varieties by mature tissue bioassay is comparable to field damage ratings on a scale of 1 to 9 with 9 being susceptible. Field ratings are usually 2 for CI31A and MBR 6796-15, 4 for B86 and 8 for MS72. After the bioassay all that remained of incompletely consumed feeding areas was the secondary and primary venation and upper epidermis.

Comparisons between leaf consumption and levels of the conventional resistance factor DIMBOA (Table 3.2) suggest that other mechanisms of resistance are operating. For the immature tissue that was completely consumed in 48 hours, the levels of DIMBOA are highest, 3 to 4 times that of mature tissue which is more resistant. However, DIMBOA content likely to influence feeding on immature tissue as MBR 6796-15 with the lowest levels of DIMBOA suffered the highest consumption rate for immature tissue. Comparing the mature tissue there is a trend in the conventional genotypes (CI31A, B86 and MS72) for

Table 3.1. ECB bioassays for immature and mature leaf tissue from maize inbreds at mid-whorl.

	Leaf Area Consumed (mm^2)[1]	
Genotype	Immature Tissue[2]	Mature Tissue
CI31A	45.1 ± 10.2 a	10.5 ± 1.0 b
MBR 6796-15	71.9 ± 4.4 b	6.9 ± 0.7 a
B86	53.3 ± 5.2 ab	26.8 ± 2.6 c
MS72	67.1 ± 7.3 b	33.4 ± 1.9 d

[1] Data were transformed by ln(x +1) prior to statistical analysis. Means within the same column followed by the same letter are not significantly different, SNK test, $P<0.05$.
[2] Immature tissue bioassays ran 24 hrs., mature tissue bioassays ran 48 hrs.

Table 3.2. DIMBOA content of immature and mature leaf tissue from maize inbreds at mid-whorl.

Genotype	mg/g dry weight[1] Immature Tissue	Mature Tissue
CI31A	5.67 ± 0.76 a	1.51 ± 0.62 a
MBR 6796-15	1.30 ± 0.16 c	0.47 ± 0.05 b
B86	3.87 ± 0.41 b	1.03 ± 0.40 a
MS72	1.93 ± 0.23 c	0.60 ± 0.04 a

[1] Means within the same column followed by the same letter are not significantly different, SNK test, P<0.05.

feeding to be inversely correlated to DIMBOA content. The MBR line does not fit this trend since it possessed the highest resistance but the lowest DIMBOA level.

Plant nutrition plays a role in insect preference, especially for the C4 plants with low protein content (Bernays and Barbehenn 1987). In theory, insects feeding on tissue with low protein content would have to consume more to acquire the required amount of protein to complete development. This is especially true of lepidopteran larvae that do not feed as adults. Increased protein intake may be achieved by an increased rate of consumption or by delayed development. For the immature tissue it appears the insect may compensate for low protein content by increasing the consumption rate. For example, CI31A with the highest protein content had the lowest amount consumed (Table 3.3). However, this relationship does not apply to the mature tissue as the MBR genotype, with the lowest protein content, also had the least tissue consumption.

The role of cell wall phenolics in reducing ruminant digestibility of grasses has been well documented (Hartley and Ford 1989). For maize the most prominant cell wall phenolic acids are ferulic and p-coumaric (Table 3.4). Immature maize tissue has approximately 75% more p-coumaric acid and 90 to 125% more ferulic acid content by weight than mature tissue. This observation contradicts what might be expected based on phytochrome regulation and UV induction of the main regulating enzyme L-phenylalanine ammonia lyase (EC 5.4.99.5) (Luckner 1980). One possible explanation for elevated pools of phenolic acids in the immature cell wall is the relative absence of lignin and cell wall cross-linking due to cell wall extensibility requirements for leaf expansion. In immature tissue phenolic acids are present mainly as monomers esterified to cell wall hemicellulose, but may later form phenolic dimers or be linked to lignin (Hartley and Ford 1989, Iiyama et al. 1990). Mature tissue may divert phenolic acid production in the cell wall to lignin incorporation, building upon ferulic and p-coumaric acids linked to hemicellulose during early stages of leaf development. However, a trend was evident within both immature and mature tissue for lower leaf consumption with increasing levels of cell wall phenolic acids (Tables 3.1 and 3.4). While the statistical verification of this trend is weak, it will be verified in more detail in later chapters. Similar observations have been reported for maize kernels. Ferulic acid is known as a major

Table 3.3. Esitmated protein content of immature and mature leaf tissue from maize inbreds during mid-whorl.

	Percent Protein (dry wt.)[1]	
Genotype	Immature Tissue	Mature Tissue
CI31A	16.85 ± 0.52 a	15.49 ± 0.65 a
MBR 6796-15	12.04 ± 1.66 b	12.04 ± 0.91 b
B86	13.77 ± 0.73 ab	13.77 ± 0.58 ab
MS72	14.36 ± 0.59 ab	13.54 ± 0.86 ab

[1] Means within the same column followed by the same letter are not significantly different, SNK test, P<0.05.

Table 3.4. Levels of cell wall bound phenolic acids in immature and mature leaf tissue from maize inbreds at mid-whorl.

Genotype	mg/g dry weight[1]			
	Immature Tissue		Mature Tissue	
	p-Coumaric Acid	Ferulic Acid	p-Coumaric Acid	Ferulic Acid
CI31A	4.44 ± 0.29 a	5.04 ± 0.16 a	3.02 ± 0.29 a	2.63 ± 0.22 a
MBR 6796-15	3.81 ± 0.36 a	4.58 ± 0.38 a	2.75 ± 0.17 a	2.36 ± 0.18 ab
B86	3.68 ± 0.34 a	4.46 ± 0.38 a	2.17 ± 0.14 b	1.98 ± 0.10 b
MS72	3.68 ± 0.09 a	4.45 ± 0.09 a	2.09 ± 0.12 b	2.06 ± 0.14 b

[1] Means within the same column followed by the same letter are not significantly different, SNK test, $P<0.05$.

consistuent of maize pericarp in kernels. It has been correlated with storage pest resistance and mechanical strength of the pericarp (Classen et al. 1990, Serratos et al. 1987).

In order to localize phenolics in leaves a variety of staining techniques was used (Table 3.5, Appendix 4). Differences in tissue maturity were apparent for all varieties and stains. Phloroglucinol is a standard lignin stain which showed higher levels of lignin in the mature section of B86 (Fig. 3.1) with extensive lignification occurring in the epidermal cell wall. Autoflurescence of phenolic acids revealed the localization of phenolic acids throughout the cell walls of vascular and epidermal tissue (Fig. 3.2). Comparing this to the immature section (Figs. 3.3 and 3.4) the epidermal cell wall was not lignified but the autofluorescence of phenolic acids was located throughout the various cell wall tissues. Quenching which reduced the autofluorescence in micrographs was found to occur at a faster rate in immature tissue than mature tissue. As with phloroglucinol the same observations were made with Azure-B, sodium chlorite, and the diazonium salts of p-nitroaniline and sulfanilic acid. The best stain for comparing lignification was p-nitroaniline which showed the greatest contrast between mature and immature epidermal cell wall fortificaton (Figs. 3.5 and 3.6).

While maturity differences were very evident in stained tissues, varietal differences among the four genotypes could not be clearly distinguished with the exception of MS72 which was clearly different. As a result, staining for phenolics does not presently offer a means of differentiating between resistant and susceptible genotypes. However, staining does show the heterogeneity of cell wall phenolic complexes when combined with autofluorescence such as shown with chlorine sulfite which colormetrically distinguished the different cell wall components (Fig. 3.7). Chlorine-sulfite and UV illumination of sclerenchyma cells, which are structural cells resistant to microbial breakdown (Jung and Casler 1990), had a characteristic light green colour. Epidermal cells that possess a similar light green coloration may likewise resist microbial breakdown or penetration by insects. Differential staining of this nature may assist in better characterizing the cell wall composition in future work.

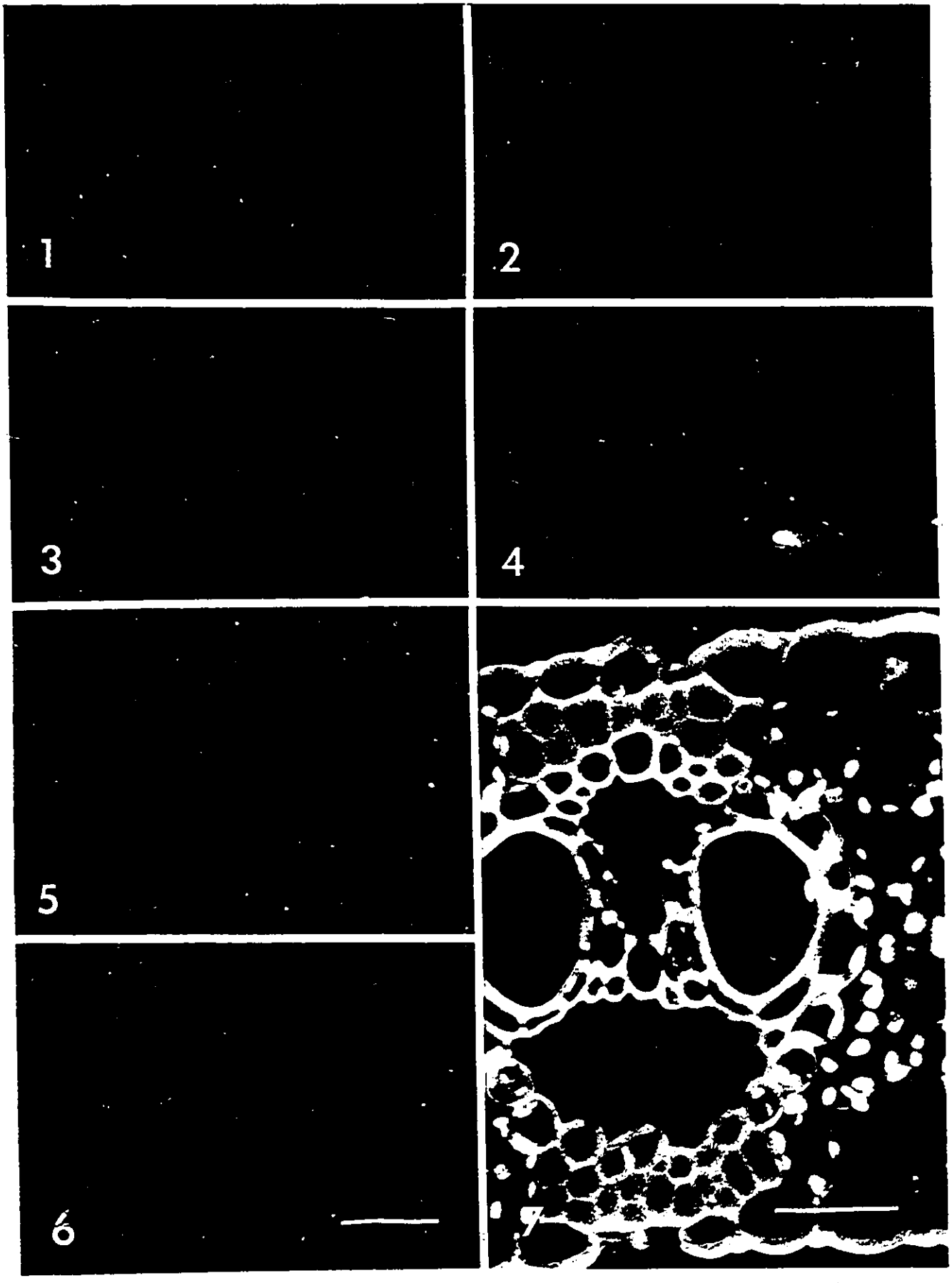
Although staining could not provide reliable quanitative data for characterizing resistant genotypes, consistent results were obtained using a microspectrophotometer (Fig. 3.8). Microspectrophotometry of bermudagrass, *Cynodon dactylon* (L.), has shown that cell walls with high absorption readings resisted digestion by rumen fluid (Akin et al. 1990b) and

Table 3.5. Colorimetric reactions of histochemical stains for phenolic acid standards and cell walls of maize leaf tissue.

		5.0 mg/cm²		
Stain	Control[1]	p-Coumaric Acid	Ferulic Acid	Leaf Section
Autofluorescence	dBl	lT	lBl	lBl to dT
Phloroglucinol	YB	dYB	B	lB to dB
Azure-B	Gr	dGr	dGr	lGr to dGr
Chlorine-sulfite	Y	Y	YR	YO to B
Diazotized p-nitroaniline	lB	GB	G	lO to dB
Diazotized sulfanilic acid	B	RO	R	lO to dR

[1] Control is the reaction with unspiked filter paper.
The following letters for colors are used: Bl = blue, T = turquoise, Y = yellow, B = brown, Gr = green, G = grey, O = orange, R = red. Intensity is indicated by: l = light, d = dark.

Fig. 3.1-7. Phloroglucinol staining of maize (B86) leaf-cross-sections of mature (1, 2) and immature (3, 4) tissue observed under white light (1, 3) and ultraviolet light (2, 4). Diazotized sulfanilic acid staining of mature (5) and immature (6) leaf tissue (bar = 100 μm). Mature leaf section stained with chlorine-sulfite and observed under ultraviolet light and white light with neutral density filters (7) (bar = 50 μm).


1
2
3
4
5
6

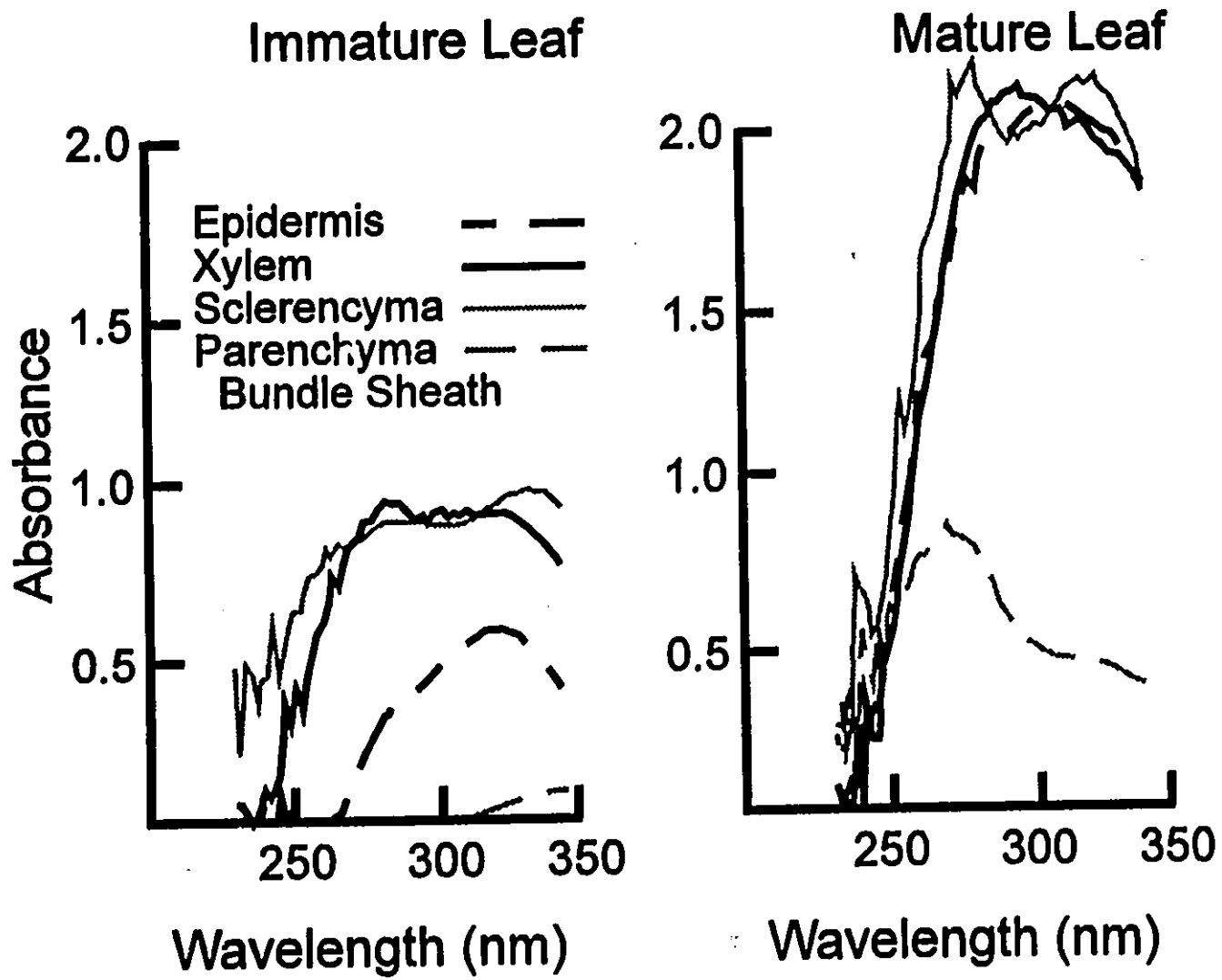


Fig. 3.8. Microspectrophotometer scans from 230 nm to 35 nm of immature and mature leaf tissue from inbred B86.

microspectrofluorimetry of pericarp phenolics predicts resistance to stored grain insects (Arnason et al. 1992). Comparing a typical series of tissue scans of genotype CI31A, the changes that occurred in cell wall absorption of ultraviolet light reflect changes largely in cell wall phenolics such as phenolic acids and lignin. The most dramatic change that occured was in adaxial epidermal cell wall tissue, which had an absorbance of less than 0.6 in immature tissue but attained an absorbance above 2.0 for some mature epidermal tissue. These results are consistent with staining observations but provide quantitative data for assessing resistance.

An important new result with the microspectrophotometer was that varietal differences could be found using the epidermal absorbance readings taken from the adaxial epidermis (Table 3.6). These results agree with not only the mature tissue ranking for consumption rate but also provide a suitable explanation for why immature tissues are completely consumed regardless of the elevated levels of secondary metabolites such as DIMBOA (Table 3.2). Lignification of mature tissue appears to be the most important factor accounting for differences in consumption rates between immature and mature tissue (Figs. 3.1-8). Upon maturity other factors such as simple phenolic acid levels in the cell wall may then contribute to differential resistance between genotypes (Table 3.4).

These observations are consistent with field observations that tissue toughness plays an important role in field resistance to the ECB (Chapter 2). Phenolic fortification of the epidermal cell wall would act as a physical barrier to penetration by neonate larvae thus depriving them of water and nutrients during the first 48 hours. Failure to ingest water during this time could result in desiccation during this stage of development when mortality can exceed 80% (Ross and Ostlie 1990). Tissue toughness is likely a major reason for post-eclosion migration from the undersurface of the leaf into the whorl to ingest tender tissue and avoid desiccation. Oviposition on the undersurface of the leaf is also advantagous to the neonate as the abaxial epidermis does not have as high a cell wall absorbance and is easier to penetrate using an instron (data not shown).

For mature tissue, the major factor for resistance appears to be leaf toughness which is manifested through cell wall fortification by phenolic acids largely in the form of phenolic-carbohydrate complexes. This form of mechanical resistance may explain the broad range of resistance reported for multiple borer resistant (MBR) varieties such as the genotype

Table 3.6. Microspectrophotometer readings of epidermal cell wall absorbance at 326 nm for resistant and susceptible inbreds.

	Absorbance at 326 nm
Genotype	Mature Tissue[1]
CI31A	2.00 ± 0.08 a
MBR 6796-15	2.11 ± 0.08 a
B86	1.77 ± 0.05 b
MS72	1.56 ± 0.08 c

[1] Means followed by the same letter are not significantly different, SNK test, $P<0.05$.

presently studied. Varietal differences in resistance can be quantified using a microspectrophotometer to record cell wall absorption at 326 nm light to estimate relative phenolic levels, with resistant genotypes tending to have higher epidermal absorption. Quantitative data derived from the microspectrophotometer can be used not only for screening purposes but also as a tool to better understand larval feeding behavior within the plant.

CHAPTER IV.

DEVELOPMENTAL CHANGES IN HOST-PLANT RESISTANCE: MAIZE-LEAF PROFILE OF FACTORS OF RESISTANCE TO THE EUROPEAN CORN BORER.

4.1 Introduction

Maize resistance to the European corn borer can be better understood using phenolic staining and microspectrophotometry procedures developed in Chapter 3 to relate intra-plant differences in cell wall fortification with insect feeding-preferences. From the perspective of insect feeding-behavior, the insect will feed on plant tissue that provides the most nutrition at the lowest possible cost including detoxification, mastication, digestion and absorption of nutrients. Larvae prefer the immature tissue of the whorl despite its high DIMBOA levels, because the absence of lignin renders the tissue tender and accessible to early instar larvae (Chapter 3).

Leaf resistance factors such as DIMBOA change over time (Morse et al. 1991) and among different tissues (Argandona et al. 1985). For hydroxamic acids these changes appear to be gradual in healthy plants (Morse et al. 1991). Given this continuum of hydroxamic acid levels in maize, it is reasonable to expect other defence compounds, namely quantitative defences, to also show a continuum of change with leaf tissue development which is represented by an extending whorl leaf. Such changes in leaf resistance factors during leaf development may influence insect feeding performance and behavior. The first objective of this study was to determine the changes in resistance and nutritional factors along the length of leaf from a resistant synthetic (BS9 (C4)). The second objective was to relate these changes to insect feeding performance to elucidate which resistance factor(s) most dictate insect feeding preference.

4.2 Materials and Methods

Plant Samples: The fourth cycle of selection from the maize population BS9 was used with the method of selection outlined by Russell and Guthrie (1982). Planting occurred in mid-May of 1992 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. The rows were spaced 0.9 m apart and plants spaced 0.15 m apart with 30 plants per

row. The soil was a sandy loam. Three blocks of four rows were planted with one row from each being infested with four egg masses (ca. 200 eggs) for field observations. At the mid-whorl stage the 13th leaf was harvested by pulling the 12th leaf and above whorl out of the plant and unwrapping the leaves to expose the 13th leaf which was green along 1/4, green-yellow along 1/2, and yellow along 1/4 of the leaf length. Ten leaves from each block were harvested for phytochemical analysis. The mid-ribs were removed and the leaves were cut into eight sections along the length. Four sections were predominantly green and four sections being predominantly yellow, with section 1 originating at the leaf tip and section 8 originating from the auricles. Tissue from 10 plants was pooled for each section within a block and frozen at -20° C. To release DIMBOA from its glucoside, each frozen sample was thawed for two hours and then refrozen and freeze-dried. Samples were milled on a UD cyclone mill (UD Corp., Bolder, CO) with a 1-mm screen. Milled samples were stored at -20° C until analyzed.

Bioassays: Thirty plants were dissected and sectioned as above. Bioassays were conducted as reported in Chapter 3.

Leaf Toughness: Using the method reported in Chapter 2, five force measurements were taken for each of the eight sections.

Microspectrophotometer: Five microscope sections from sections numbered 2, 4, 6 and 8 were collected and kept at -20° C. Sections were embedded in water containing glycerol (5 drops per 10 ml water) and 6 μm sections were prepared. Microspectrophotometry protocol is outlined in Chapter 3.

Protein Determinations: Protein content was estimated by the micro-Kjehldahl method on 0.3 g samples using the conversion factor 6.25 to estimate protein from N.

Phytochemical Analysis: Phenolic conjugates, hydroxamic acids and cell wall phenolics were determined using the method outlined in Chapter 3. Truxillic acid and diferulic acid were obtained from G.H.N. Towers (UBC).

Statistical analysis: All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Regressions were preformed on means. Microspectrophotometer data satisfied the assumptions of the general linear model and were not transformed.

4.3 Results and Discussion

Field observations of recently eclosed larvae showed that approximately 80 % of the larvae move towards the centre of the whorl during daylight hours. Similar observations can be found with other stem borers such as *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in which 95 to 100 % of live larvae are within the whorl (Ampofo and Kidiavai 1987). A possible explanation for this behavior is simply survival. The hot, dry micro-environment on the exposed whorl leaves causes desiccation of neonate larvae which have a high surface-to-volume ratio. This explanation is supported by high egg mortality at low relative humidities (Lee 1988) and a larval mortality that often exceeds 80% during the first 48 hrs after eclosion (Ross and Ostlie 1990).

A profile of leaf consumption by ECB on BS9 C4 is found in Fig. 4.1. ECB larvae show the highest consumption rate for immature, yellow, tissue (sections 6 to 8). The leaf section with the lowest consumption was at the point where the leaf emerges from the whorl (section 4). By conducting leaf-feeding bioassays in growth chambers the effects of relative humidity over the leaf length are controlled and there is an obvious reduction in feeding on mature, green, tissue even when relative humidity is not a restricting factor.

Other parameters commonly thought to be associated with feeding behavior are shown in Fig. 4.1. Protein was lower for the sections around the green-yellow interface, reaching a level as low as 17%. Based on protein alone, one would expect consumption to be higher at the interface so as to fulfill nutritional requirements for development (Scriber and Slansky 1981). This is not observed for the leaf profile for BS9 during the first 48 hours of feeding. Soluble metabolites washed from milled leaf tissue included sugars, soluble proteins, chlorophyll, phenolic conjugates such as flavonoids and hydroxycinnamic acids, and hydroxamic acids such as DIMBOA. The trend for soluble metabolites follows that of leaf consumption. Phenolic conjugates of maize act as phagostimulants (Appendix 2) and may account for higher consumption as the level of sugar conjugates of p-coumaric acid are higher for immature whorl tissue (Fig. 4.2). Other soluble secondary metabolites such as ferulic acid conjugates or HMBOA fluctuate and show no consistent trend.

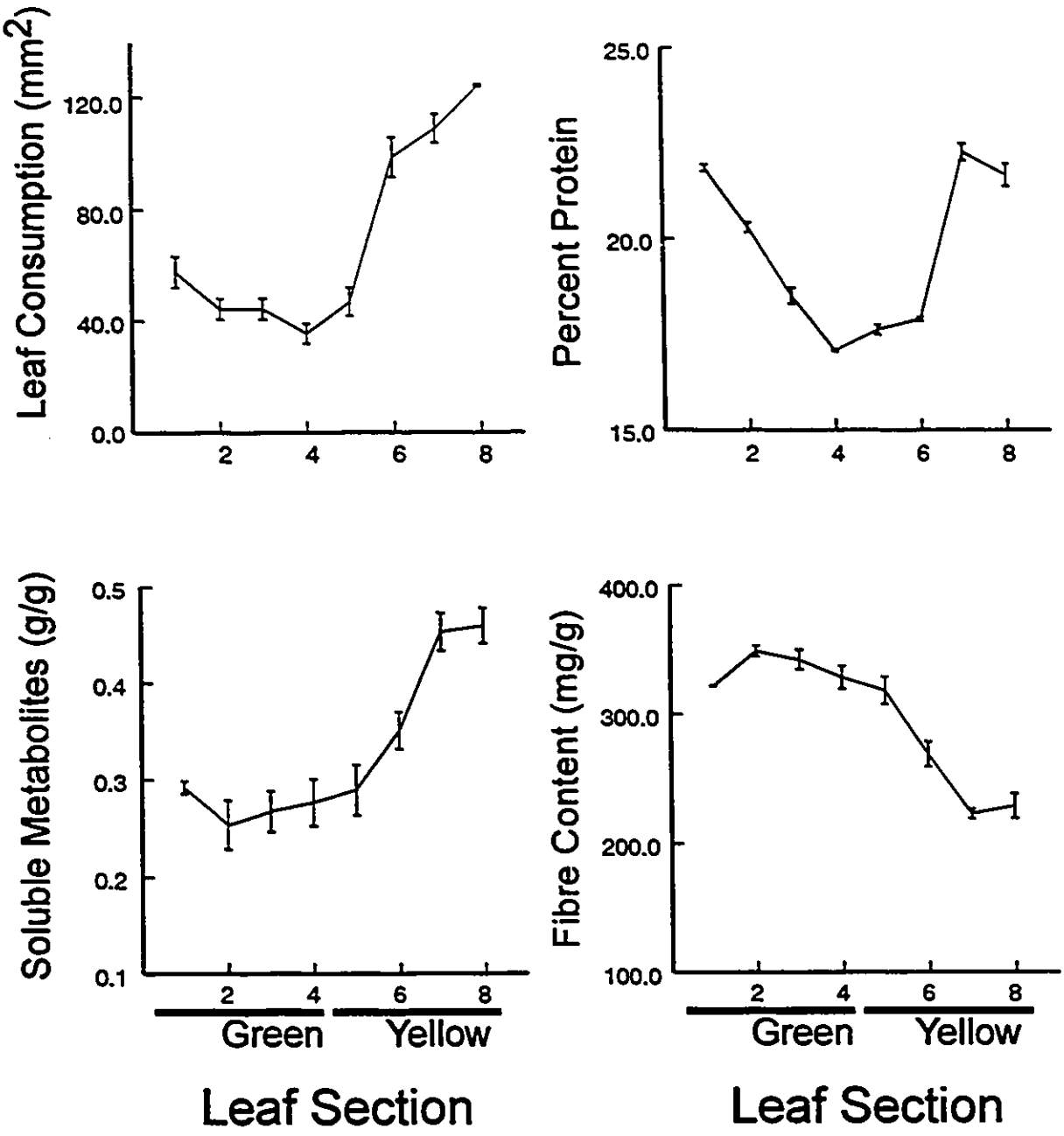


Fig. 4.1. Line graphs of BS9 leaf profile for leaf consumption, percent protein, soluble metabolites and fibre content. N = 4 for each section.

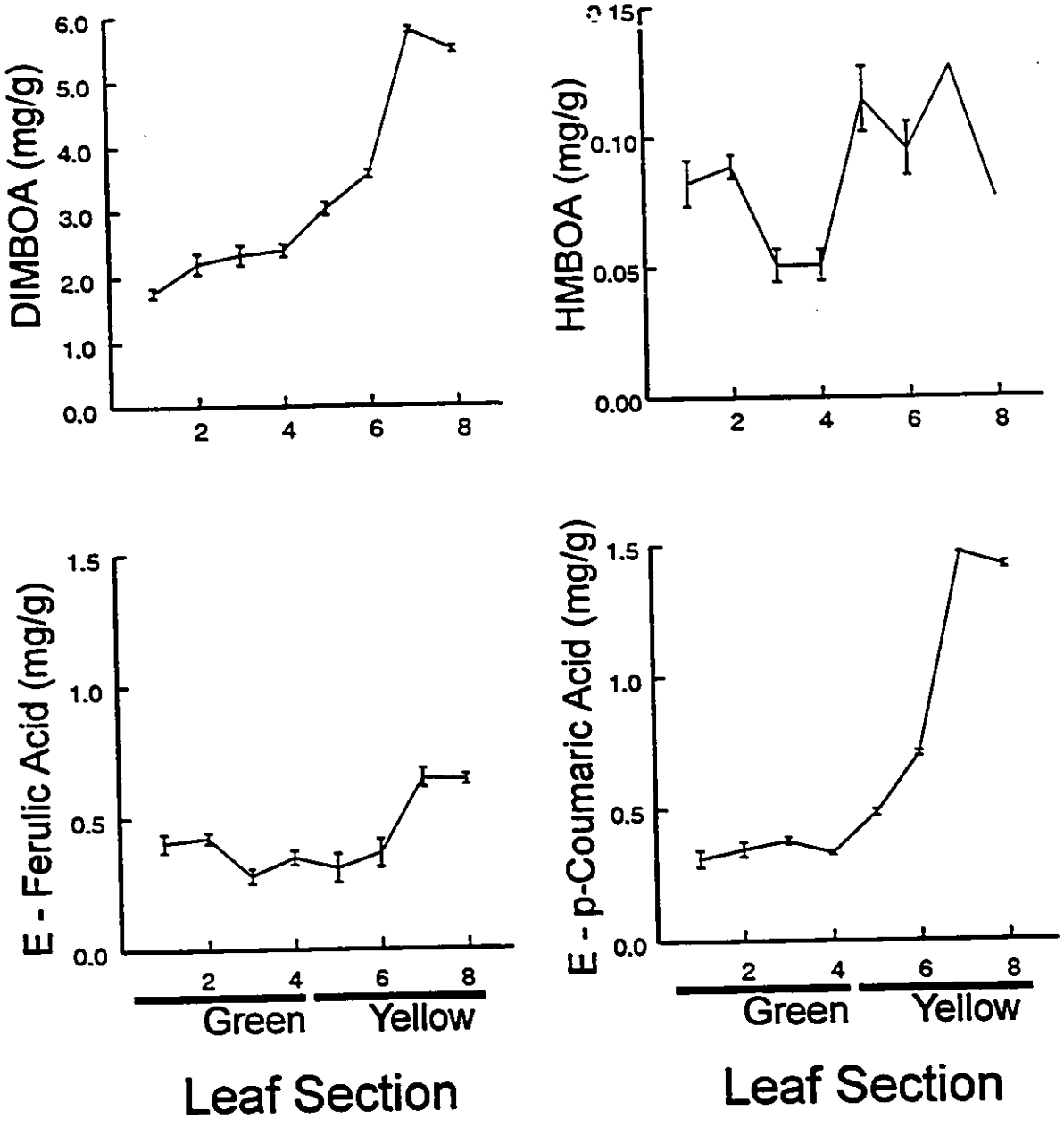


Fig. 4.2. Line graphs of BS9 leaf profile for soluble secondary compounds (DIMBOA, HMBOA ferulic and p-coumaric acids). N = 4 for each section.

DIMBOA reached highest levels within the leaf whorl (Fig. 4.2). Based on previous feeding preference studies the converse would be expected (Robinson et al. 1978). Nutritional studies have shown that DIMBOA incorporated into meridic diet increases ECB feeding while reducing the efficiency of nutrient assimilation and various fitness parameters (Houseman et al. 1992). Larvae that continue to feed in whorl tissue throughout their development may have a lower reproductive fitness due to reduced adult weight and delayed development.

The high feeding preference for tissue with elevated levels of DIMBOA can be rationalized by observing the relative absence of physical defense mechanisms. Fibre content in immature tissue is low (Fig. 4.1) and the relative absence of phenolic fortification in epidermal cell walls, as demonstrated by the low microspectrophotometer readings (Table 4.1), render nutrients within the cell walls more accessible and hence makes the tissue more desirable (Bernays and Barbehenn 1987, Scriber and Slansky 1981). The tissue toughness profile found in Fig. 4.3 best illustrates the absence of mechanical resistance factors *vis-a-vis* fibre and phenolic fortification of cell walls.

The toughness profile could account for field observations of neonate behavior. Immature tissue is more easily consumed by the small mandibles of neonates than tougher mature tissue. By migrating to the inner whorl, larvae would not only be in a higher humidity micro-environment but could also easily ingest water and nutrients to avoid desiccation and starvation during early stages of development. During the field study, another insect, the corn leaf aphid (*Rhopalosipum maidis*), was most prominent within the whorl of several maize varieties with high DIMBOA levels (unpublished data). DIMBOA has been identified as a feeding deterrent for aphids (Niemeyer 1988) as well as accounting for varietal differences in resistance to aphids (Beck et al. 1983). However, like the ECB there may be physical, environmental, or nutritional factors that are of greater importance to aphid performance that necessitate feeding on high DIMBOA tissue within the whorl.

The major cell wall bound phenolic acids are *E*-ferulic and *E*-p-coumaric acids which are attached to hemicellulose through pentose sugars (Kato and Nevins 1985). Both phenolic acids reach their highest levels in sections 5 and 6, the yellow tissue which will mature next.

Table 4.1. Microspectrophotometer readings of epidermal cell wall absorbance at 326 nm along the length of maturing leaf tissue from BS9(C4).

Leaf Section	Absorbance at 326 nm[1]
2	1.49 ± 0.11 a
4	1.46 ± 0.10 a
6	0.45 ± 0.06 b
8	0.04 ± 0.01 c

[1] Means followed by the same letter are not significantly different, SNK test, $P<0.05$.

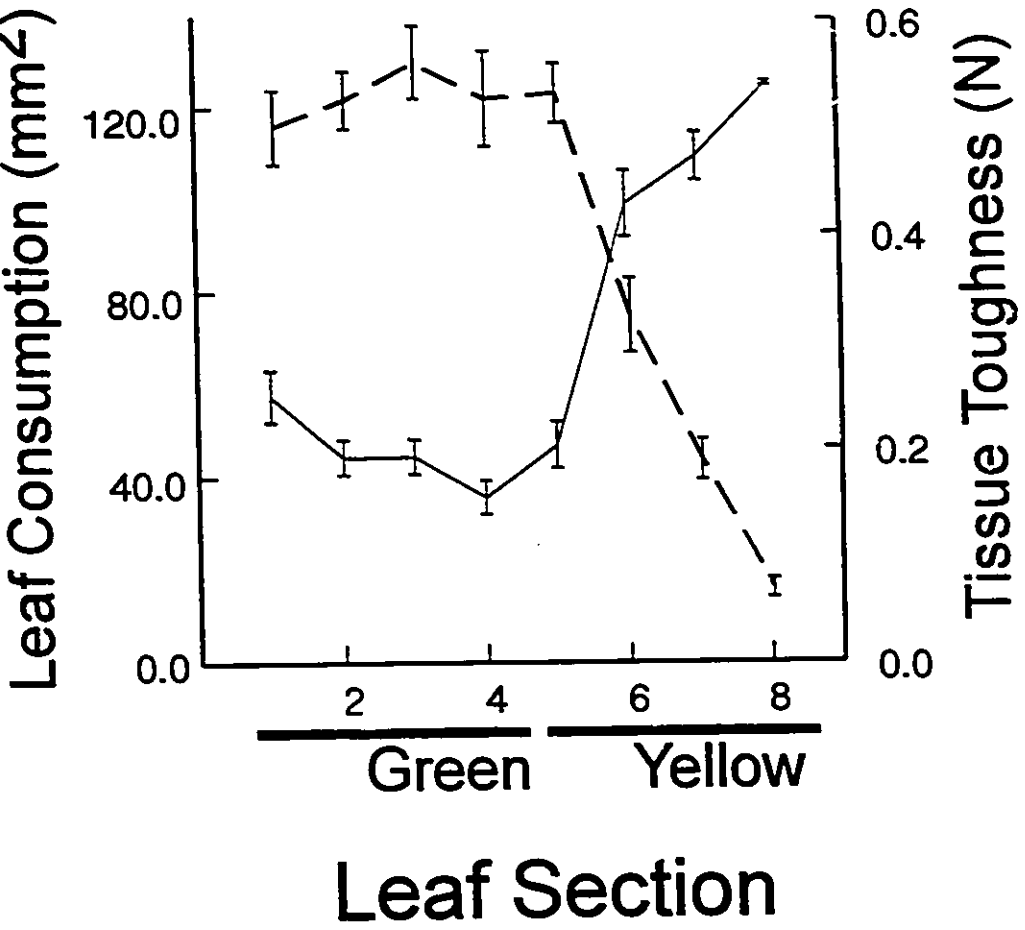


Fig. 4.3. Line graphs of BS9 leaf toughness profile (dashed line) in relation to leaf consumption (solid line). Force is measured in newtons (N). N = 4 for each section.

Cell wall bound ferulic and p-coumaric acids can form dimers either enzymatically through peroxidase to form diphenolic acids (Hartley and Jones 1976) or through photochemical reactions to form truxillic and truxinic acids (Hartley et al. 1988, Ford and Hartley 1989). Formation of such dimers may increase the mechanical strength of the cell wall by cross-linking hemicellulose (Ford and Hartley 1989). From the profiles in Fig. 4.4 no individual component provides a suitable explanation for leaf consumption or toughness. However, when taken together it appears that at the leaf tip that has been exposed to sunlight the longest, high levels of truxillic and truxinic acids could cross-link maturer tissue to provide structural resistance. For sections 4 through 6, elevated levels of cell wall bound p-coumaric, ferulic and diphenolic acids may sustain leaf toughness and thereby maintain a moderate leaf consumption rate. For sections 7 and 8 all cell wall phenolics are reduced with concurrent increases in leaf consumption (Fig. 4.1). It is of interest to note the dramatic reduction in cell-wall bound p-coumaric acid which has been shown to have a positive correlation with lignin in bromegrass, *Bromus inermis* Leyss. (Jung and Casler 1990). It may be that cell wall bound p-coumaric acid is an indicator for lignin by facilitating lignin attachment to the cell wall in maize. A similar role has been proposed for ferulic acid in wheat (Iiyama et al. 1990). Moreover, Akin et al. (1992) has shown increased digestibility of bermudagrass leaves with alkaline release of phenolic monomers and their photoactivated dimers. This increase in digestibility was noticed especially in the epidermis. These observations support the hypothesis that phenolic acid monomers and dimers contribute to the mechanical resistance of maturing tissue that are not yet highly lignified.

Simple linear regression of leaf consumption against each of the 12 parameters studied identified 4 parameters that could account for over 89% of the variation in leaf consumption (Table 4.2). The microspectrophotometer absorbance, toughness, weight of soluble metabolites and fibre content are all components or indicators of mechanical resistance. Elevated fibre content would not only increase the bulk density of the insect's diet to make nutrient and water requirements less attainable (Bernays 1986), but would also increase the area for phenolic cross-linking. Acting in concert, fibre and cell wall fortification in epidermal tissue could increase leaf toughness of "apparent" (*sensu* Feeny 1976) mature leaf tissue,

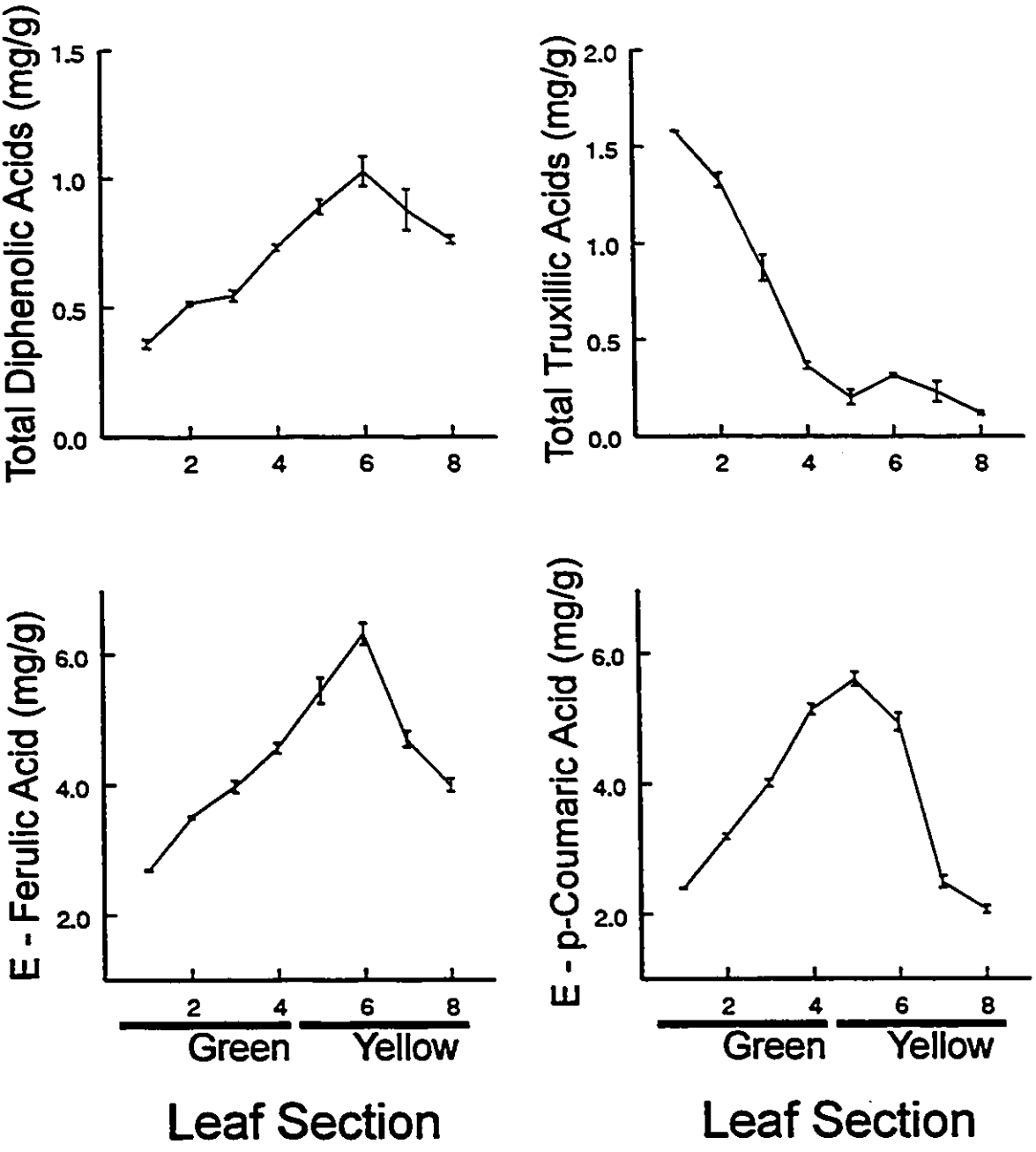


Fig. 4.4. Line graphs of BS9 leaf profile for cell wall bound phenolics. N = 4 for each section.

Table 4.2. Regression equations of bioassay leaf feeding against resistance factors for the maize synthetic BS9(C4) at mid-whorl.

Parameter	Regression Equation[1]	r^2	F value	Prob > |T|
Absorbance	LF = 126(±5) - 58(±4) x Abs	0.98	181.1	0.005
Toughness	LF = 142(±7) - 181(±17) x T	0.94	111.9	0.001
Fibre	LF = 540(±46) - 2688(±312) x Wt	0.93	74.2	0.001
Solubles	LF = -62(±18) + 403(±52) x Ratio	0.89	59.1	0.001
HMBOA	LF = 29(±10) + 58(±12) x HMBOA	0.76	23.1	0.03
DIMBOA	LF = -13(±25) + 198(±58) x DIMBOA	0.61	11.7	0.01

[1] LF = leaf feeding (mm^2/ 48 hrs); T = leaf toughness in newtons; Wt = weight in mg/g; Ratio = grams of soluble / total weight; DIMBOA = mg/g dry weight. (n=8).

making it inaccessible to neonate larvae which are forced to feed on softer whorl tissue which has higher leaf moisture but is defended by high levels of DIMBOA.

This study has shown that within a plant, resistance to leaf feeding varies considerably. Likewise, the nutrient and phytochemical composition of the maize leaf varies considerably and it is this variation which likely dictates the feeding behavior of early larval instars. Neonate larvae tend to migrate deep within the whorl to initiate feeding on tender immature leaf tissue which is not well fortified with fibre and cell wall phenolic acids. The young larvae may suffer from reduced weight gain associated with the high levels of DIMBOA in whorl tissue (Houseman et al. 1992) but would minimize physical dangers such as desiccation and predation. As the insect matures its mandibles may be better able to better cope with tougher mature tissue (Bernays 1986) which has lower levels of DIMBOA. Leaf toughness appears to be the predominate factor which dictates ECB feeding preference within maize during the mid-whorl stage of plant development.

CHAPTER V.

TEMPORAL CHANGES IN MAIZE SECONDARY METABOLITES AND RESISTANCE AGAINST THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS* (HUBNER).

5.1 Introduction

Studies on the temporal changes in maize hydroxamic acid content (Morse et al. 1991, Guthrie et al. 1986a) have found there is a decline in these defences with plant age, although these studies did not relate the changes in insect performance on tissues of varying ages. In addition to these investigations, plant-insect interaction studies using aphids and lepidopteran larvae have shown induction of hydroxamic acid production during insect feeding (Niemeyer et al. 1989, Gutiérrez et al. 1988). Hydroxamic acid levels are also influenced by the environment. Comparisons of greenhouse and field grown plants have shown higher levels of DIMBOA, with concurrent increases in plant damage by ECB larvae, in greenhouse grown plants (Guthrie et al. 1986b, Manuwoto and Scriber 1985b). I have presented new mechanisms of first generation resistance in earlier chapters that are based on phenolic acid - cell wall carbohydrate complexes that act by increasing the mechanical strength of the leaf (Chapters 2, 3 and 4). From these studies it appears that maize resistance-factors such as DIMBOA and cell wall phenolics change over time and are also influenced by the environment.

One objective of the present study was to relate changes in resistance and nutritional factors of a resistant synthetic (BS9 (C4)) over time and between growth environments to insect feeding preference. A second objective was to see if the maize plant utilizes different defense strategies at different stages in whorl development.

5.2 Materials and Methods.

Plant Samples: The maize synthetic BS9(CB)C4 was used with the method of selection outlined by Russell and Guthrie (1982). Plants were grown under three different environments. Greenhouse plants were grown at 22°C and 16:8 L:D. Plant density was 3 plants per 6" pot except for the three-leaf sampling in which plants were planted in flats of 40

to obtain sufficient tissue for phytochemical analysis. Plants grown under reduced UV light were covered by greenhouse plastic (CIL Durafilm 3, 6 mil) in such a way as to allow adequate ventilation. Plastic blocked 90-99% of the UV light which can be used for cyclobutane production (Fig. 5.1). Greenhouse plants grown with supplemental UV light were grown under black lamps that emitted light from 300 to 400 nm. Radiometric measurements were taken at pot height in both treatment areas and the daylight fluorescent lights and tungsten filament lights were adjusted to deliver 2.2×10^4 ergs/cm^2/sec for each treatment. Field grown plants were planted in mid-May of 1992 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. The rows were spaced 0.9 m and plants spaced 0.15 m apart with 30 plants per row. The soil was a sandy loam. Plants were harvested at full leaf extension for the 3, 5, 7, 9, and 10 leaf stages of development for all three treatments with a minimium of 10 g fresh weight of tissue being collected. The midribs were removed and tissue frozen at -20° C. The frozen sample was thawed for two hours and then refrozen and freeze-dried. Samples were milled on a Wiley® mill fitted with a 20 mesh screen. Milled samples were stored at -20° C until analyzed.

Bioassays: Thirty leaves were collected from each treatment and leaf age at the same time phytochemical samples were taken. Bioassay protocol is described in Chapter 3.

Leaf Toughness: Using the method reported in Chapter 2, fifteen force measurments were taken for each stage of plant development.

Microspectrophotometer: Three microscope sections from three replicates of greenhouse grown plants were collected during the aforementioned sampling and stored at -20°C until sectioned. Sectioning and microspectrophotometry procedures are outlined in Chapter 3.

Protein Determinations: Protein content was estimated by the micro-Kjehdahl method on 0.3 g samples using the conversion factor 6.25 to estimate protein from N.

Phytochemical Analysis: Soluble phenolic acid conjugates, hydroxamic acids and cell wall bound phenolic acid levels were determined using the protocol outlined in Chapter 3.

Lignin Determinations: Residue left after base hydrolysis was dried in a desiccator and weighed. The acetyl bromide procedure of Iiyama and Wallis (1990) was used on the residue to determine core lignin content.

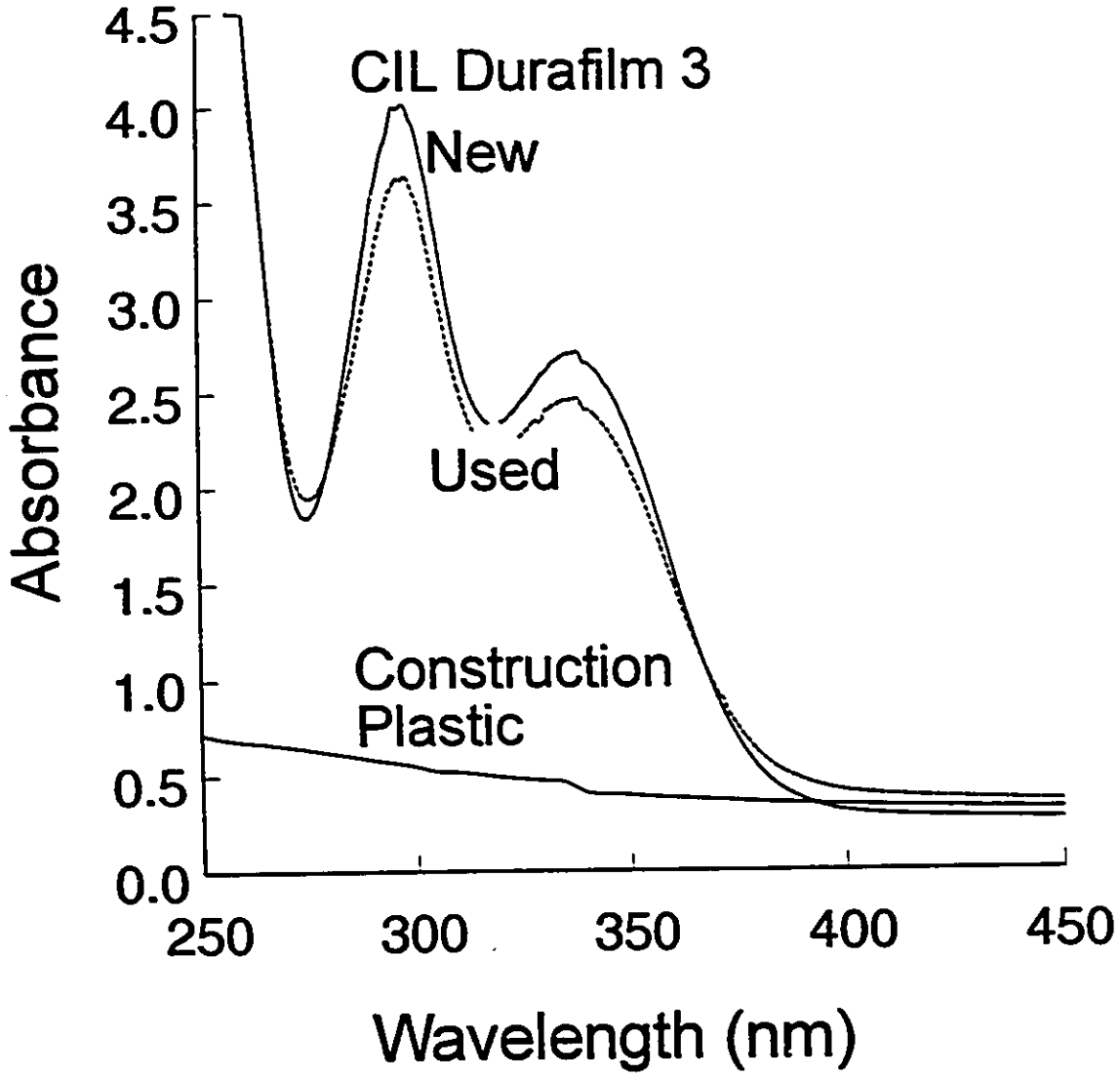


Fig. 5.1. Spectral absorbance between 250 and 450 nm for greenhouse and construction plastics. Used plastic was tested at the end of the field season.

Statistical analysis: All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Data were transformed to satisfy the assumptions of the general linear model. Student-Newman-Keuls test was used to compare means within a given treatment.

5.3 Results and Discussion.

Changes in feeding by ECB and secondary metabolites in maize leaves were monitored over the first 10 leaf stages of development under 3 conditions: 1) outside in full sunlight, 2) in a greenhouse under reduced UV irradiation and 3) in a greenhouse with supplemental UV irradiation. Bioassay results reveal a preference by ECB larvae for younger plants and for greenhouse grown plants grown under reduced UV light conditions (Fig. 5.2). Field-grown plants were least consumed at the three leaf stage, less than even 9 leaf plants grown under reduced UV light. These observations are consistent with those of Manuwoto and Scriber (1985b) who reported greater ECB feeding on plants grown under reduced light. In the present study it appears that UV light is the major component to account for this change as light intensity between the two greenhouse treatments was equilibrated. All treatments showed a significant reduction in leaf consumption with increasing leaf number, with the most dramatic reduction occuring in the reduced UV treatment between the 9 and 10 leaf stages of development.

DIMBOA dropped to significantly lower levels after the three leaf stage for all three environments with the 9 and 10 leaf stages having the lowest levels with the exception of the outside fifth leaf sample (Fig. 5.3). Since the older leaf stages had lower levels of DIMBOA than the younger leaf stages, it is evident that reduced consumption of older leaves (Fig. 5.2) cannot be explained by non-preference for DIMBOA. Similar observations have been reported in other greenhouse studies which have made comparisons to field-grown maize (Guthrie et al 1986b) or to plants grown under elevated artifical light conditions in the greenhouse (Manuwoto and Scriber 1985b). From both studies it appears that in addition to DIMBOA, another defence mechanism is operating that is light dependent.

Soluble phenolic acids conjugated to various sugars followed the same trends as DIMBOA with later leaves having lower levels than earlier leaves (Table 5.1). This trend in soluble phenolic levels has been observed for *Sorghum bicolor* (Woodhead 1981). However,

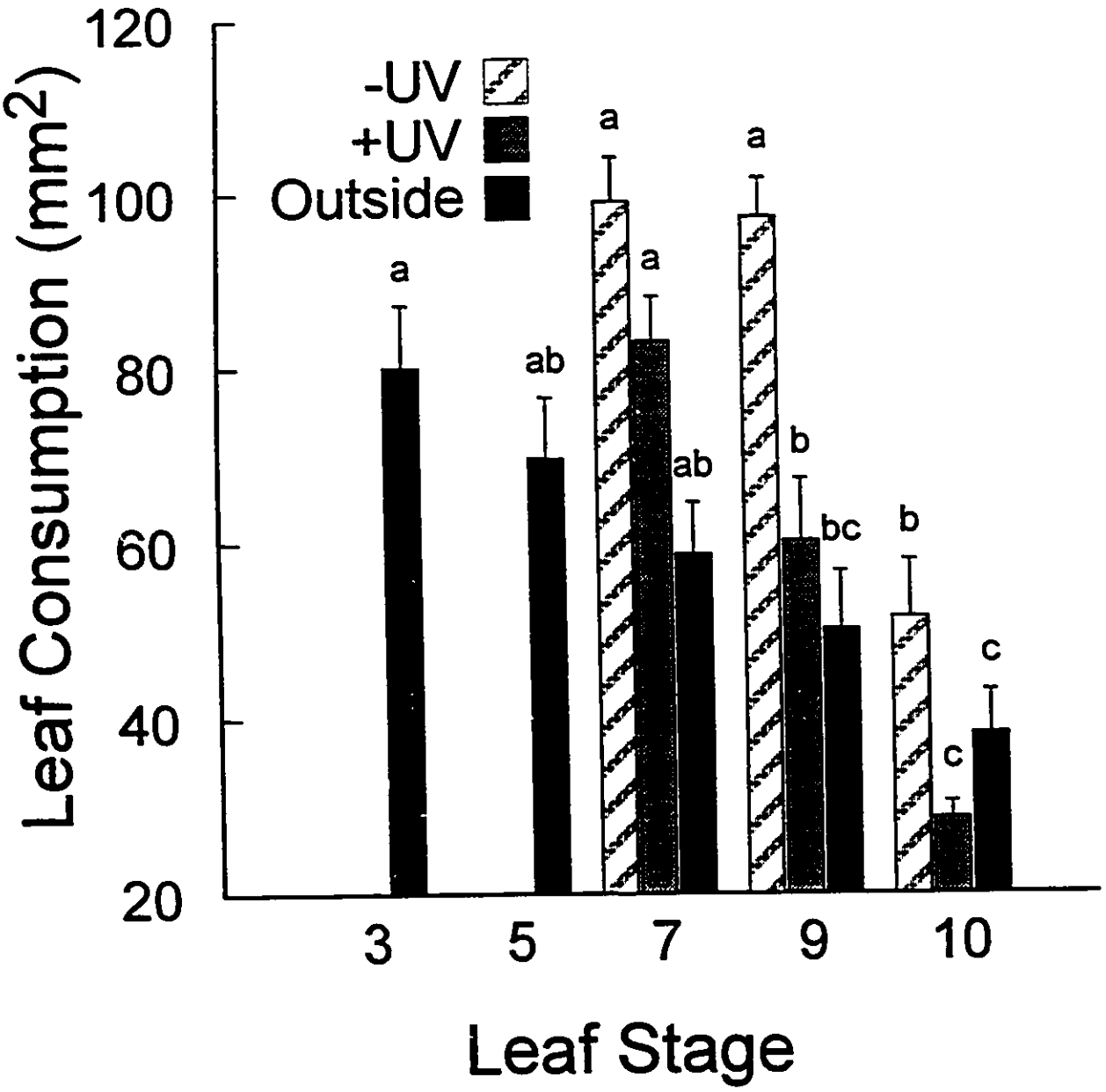


Fig. 5.2. Bar graph of leaf consumption for BS9 leaves at different stages in development and grown under different light conditions. N = 30 for each treatment and development stage. Bars topped with different letters within the same treatment are significantly different, SNK ($P < 0.05$).

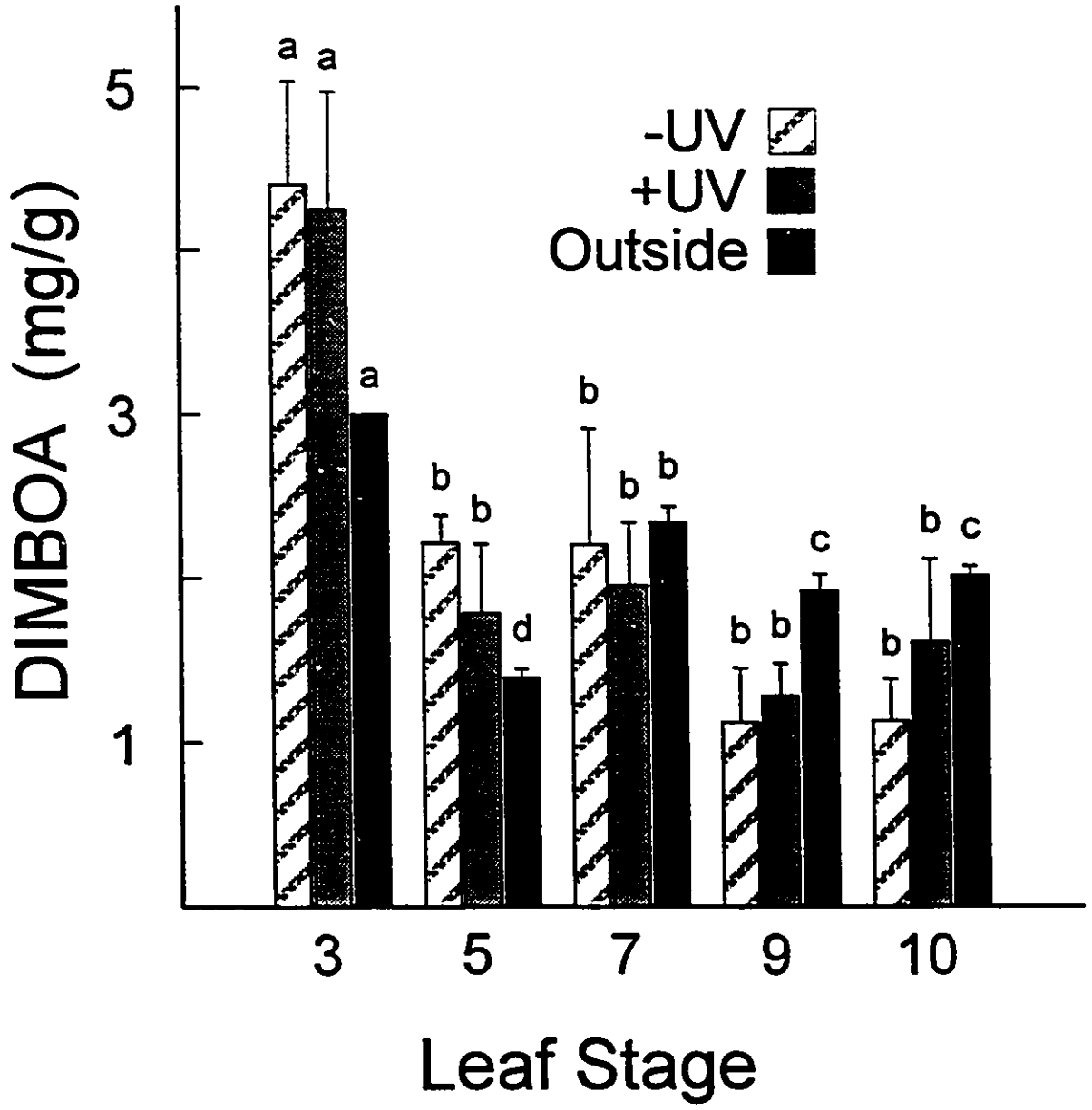


Fig. 5.3. Bar graph of DIMBOA content in BS9 leaves at different stages in development and grown under different light conditions. N = 4 for each treatment and development stage. Bars topped with different letters within the same treatment are significantly different, SNK ($P < 0.05$).

Table 5.1. Levels of soluble phenolic acid conjugates in BS9(C4) at different stages of development and grown under different light conditions.

		mg / g dry weight‡	
Treatment	Leaf	p-Coumaric Acid	Ferulic Acid
Greenhouse	3	1.95 ± 0.12 a	2.43 ± 0.13 a
Reduced UV	5	1.30 ± 0.21 ab	1.03 ± 0.07 b
	7	1.10 ± 0.23 b	0.84 ± 0.20 bc
	9	1.56 ± 0.18 ab	0.44 ± 0.09 c
	10	0.92 ± 0.16 b	0.75 ± 0.13 bc
Greenhouse	3	1.71 ± 0.10 a	2.19 ± 0.34 a
UV Supplement	5	1.37 ± 0.26 a	1.26 ± 0.24 b
	7	1.14 ± 0.10 ab	0.94 ± 0.15 b
	9	0.67 ± 0.14 b	0.80 ± 0.17 b
	10	0.78 ± 0.13 b	0.92 ± 0.09 b
Field Grown	3	1.09 ± 0.21 a	0.67 ± 0.10 a
	5	0.53 ± 0.02 b	0.73 ± 0.03 a
	7	1.06 ± 0.12 a	1.48 ± 0.15 b
	9	0.78 ± 0.03 b	1.55 ± 0.01 b
	10	0.89 ± 0.02 ab	0.92 ± 0.04 a

‡ Means within the same column and treatment followed by the same letter are not significantly different, SNK ($P<0.05$).

field-grown sorghum had higher levels of soluble phenolics than lab-grown plants which is the converse of the findings reported in the present study. *Locusta migratoria* nymphs are reported to take smaller meal sizes when feeding on seedling leaves of *Lolium perenne* which have higher levels of alkaloids than mature tissue. For maize and the ECB the opposite is true with higher feeding occurring on leaves with more soluble secondary metabolites. For some insects phenolic acids can act as feeding deterents (Dowd 1990) but based on ECB feeding bioassays (Appendix 2) it appears that soluble phenolic acids conjugated to sugars act as phagostimulants.

Protein content dropped significantly from the third to the fifth leaf and then gradually declined with subsequent leaves (Table 5.2). Nutritional requirements of insects often necessitate higher consumption of protein-poor tissue to complete development (Bernays and Barbehenn 1987, Scriber and Slansky 1981). However, leaves in the present study with the highest protein content were subjected to the most feeding, suggesting that low protein content is not the factor that dictates elevated feeding of younger leaf tissue by ECB larvae. Gravimetric determination of soluble metabolites provides a crude estimate of sugars, soluble secondary metabolites, proteins and chlorophyll (Table 5.3). The same trend as protein is observed suggesting that younger leaves provide more accessible nutrients and water to larvae than do older leaves. Fibre content has been hypothesized to increase the bulk density of the insect's diet to the point that insects cannot ingest enough nutrients (Peterson et al. 1988, Bernays 1986). Fibre content did not change significantly within the age range tested and would not likely account for the observed feeding preferences (Tables 5.1 and 5.3).

The major cell wall bound phenolic acids are *E*-ferulic and *E*-p-coumaric acid,s which are attached to hemicellulose through pentose sugars (Kato and Nevins 1985) in forms such as FAXX and PAXX. Cell wall phenolic acids have been studied as digestibility-reducing factors in ruminants (Hartley and Ford 1989) and have been correlated with maize resistance to storage pests (Classen et al. 1990) and to leaf feeding by the ECB (Chapter 8). Both phenolic acids reach their highest levels in the 9 and 10 leaf stages showing levels 2- to 5-fold higher than younger leaves (Table 5.4). The highest levels reported are for the older leaves of plants grown under reduced UV light. Within a given growth environment, phenolic acid levels

Table 5.2. Estimated percent protein of field grown BS9(C4) at different developmental stages.

Leaf stage	Percent Protein (dry wt)[‡]
3	29.2 ± 1.2 a
5	24.5 ± 0.5 b
7	23.7 ± 0.1 cb
9	23.5 ± 0.1 cb
10	21.5 ± 0.1 cd

[‡] Means followed by the same letter are not significantly different, SNK ($P < 0.05$).

Table 5.3. Gravimetric determinations of soluble metabolites and fibre content of field grown BS9(C4) leaf tissue at different stages in development.

Leaf stage	Soluble Metabolites‡ (g /g dry wt.)	Fibre Content‡ (mg / g dry wt.)
3	0.356 ± 0.013 a	414 ± 14 a
5	0.319 ± 0.009 a	384 ± 24 a
7	0.267 ± 0.036 a	438 ± 16 a
9	0.248 ± 0.043 a	356 ± 26 a
10	0.283 ± 0.041 a	336 ± 20 a

‡ Means within the same column followed by the same letter are not significantly different, SNK ($P<0.05$).

Table 5.4. Levels of cell wall bound phenolic acids in BS9(C4) at different stages of development and grown under different light conditions.

		µg / g dry weight[‡]	
Treatment	Leaf	p-Coumaric Acid	Ferulic Acid
Greenhouse	3	517 ± 88 a	1139 ± 102 a
Reduced UV	5	519 ± 28 a	1299 ± 89 a
	7	1591 ± 371 b	2257 ± 379 b
	9	3223 ± 206 c	3429 ± 168 c
	10	3942 ± 355 c	3491 ± 158 c
Greenhouse	3	385 ± 47 a	764 ± 111 a
UV Suppliment	5	585 ± 103 a	1297 ± 160 ab
	7	863 ± 176 a	1160 ± 153 ab
	9	3079 ± 259 c	3084 ± 405 c
	10	2024 ± 126 b	1722 ± 44 b
Field Grown	3	483 ± 14 a	981 ± 20 a
	5	590 ± 5 a	1229 ± 27 b
	7	615 ± 5 a	1490 ± 14 c
	9	1163 ± 47 b	2057 ± 82 d
	10	1557 ± 66 c	1986 ± 27 d

[‡] Means within the same column and treatment followed by the same letter are not significantly different, SNK ($P<0.05$).

reflect ECB feeding rates with higher consumption occurring on leaves with lower cell wall phenolics.

Cell wall bound ferulic and p-coumaric acids can form dimers either enzymatically through peroxidase to form dehydrodiphenolic acids through ring linkage (Hartley and Jones 1976) or through UV absorption to form cyclobutane dimers called truxillic and truxinic acids (Ford and Hartley 1989, Hartley et al. 1988). Formation of such dimers may increase the mechanical strength of the cell wall by cross-linking hemicellulose (Ford and Hartley 1989). Formation of such dimers may explain the anomaly reported in Table 5.4 with plants grown under reduced UV light having higher feeding but also higher levels of cell wall phenolics. In the absence of UV light phenolic dimers were not produced in significant quantities while field-grown plants had high levels of cyclobutane dimers through all leaf stages (Fig. 5.4). All treatments showed a sharp increase in cyclobutane dimers at the 9 leaf stage (10 leaf stage for the reduced UV treatment) which coincides with increased levels of phenolic acids and reduced insect feeding (Table 5.4, Fig. 5.2). Similar results are reported for sorghum, with cyclobutane dimer levels being highest at later stages in plant development (Goto et al. 1991). Dehydrodiferulic acid levels generally increased with leaf stage with the field grown leaves having the highest levels (Fig. 5.5). However, this family of phenolic dimers did not appear to reflect all the feeding preferences consistently because greenhouse grown plants under reduced UV light conditions tended to have higher levels than UV-exposed plants. A possible explanation for this is that cell wall phenolic monomers exposed to UV light formed dimers thereby reducing the probability of further dimerization reactions occurring enzymatically. Lignin content did not differ significantly (SNK, $P<0.05$) between different development stages within a treatment or between treatments (Appendix 5). Buendgen et al. (1990) showed that between different cycles of S_1 selection for BS9, lignin content of whorl tissue did not increase as insect resistance increased which is consistent with these results.

The overall impact cell wall phenolic acid - carbohydrate complexes have on leaf toughness is shown in Fig 5.6. Leaf stages with the lowest levels of cell wall phenolics have the lowest leaf toughness, with the 3-leaf stage being the softest of all stages. It is hypothesized that leaf toughness *vis-a-vis* phenolic fortification of cell wall tissue is the primary defence for mature tissue in maize against the ECB. Immature tissue and early leaf

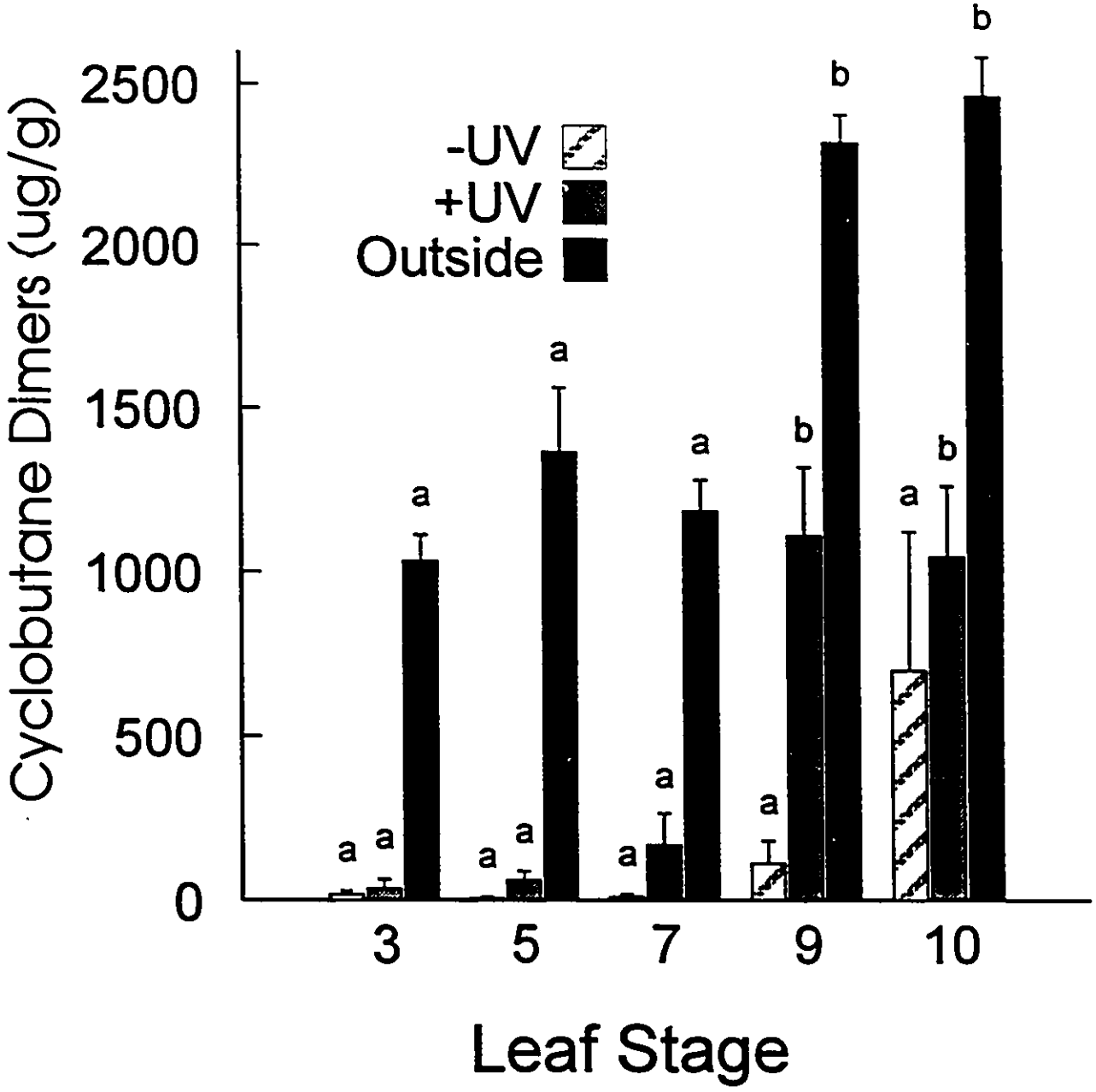


Fig. 5.4. Bar graph of total concentration of cyclobutane dimers in BS9 leaves at different stages in development and grown under different light conditions. N = 4 for each treatment and development stages.

Bars topped with different letters within the same treatment are significantly different, SNK ($P < 0.05$).

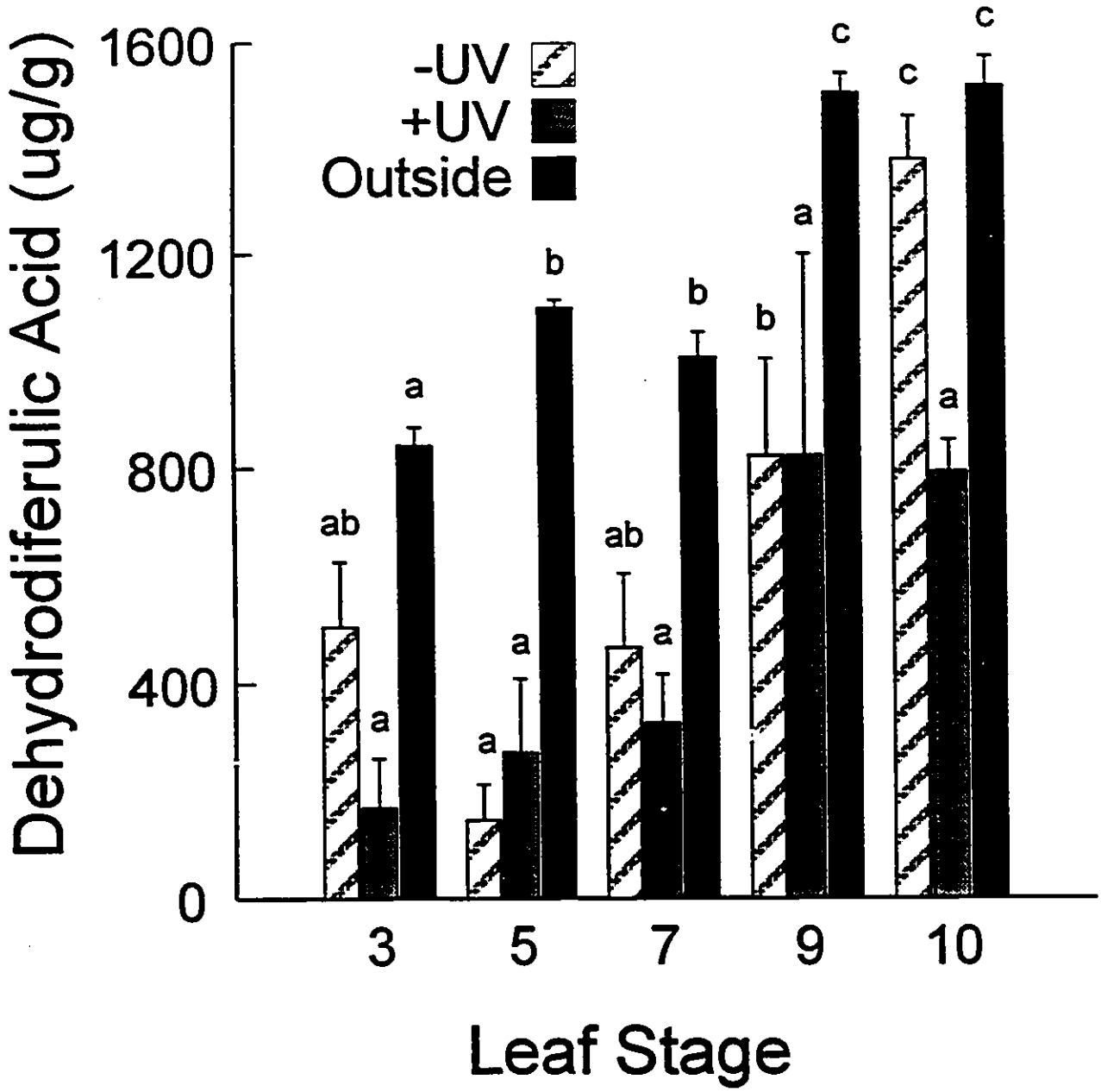


Fig. 5.5. Bar graph of dehydrodiferulic acid in BS9 leaves at different at different stages in development and grown under different light conditions. N = 4 for each treatment and development stages.

Bars topped with different letters within the same treatment are significantly different, SNK ($P < 0.05$).

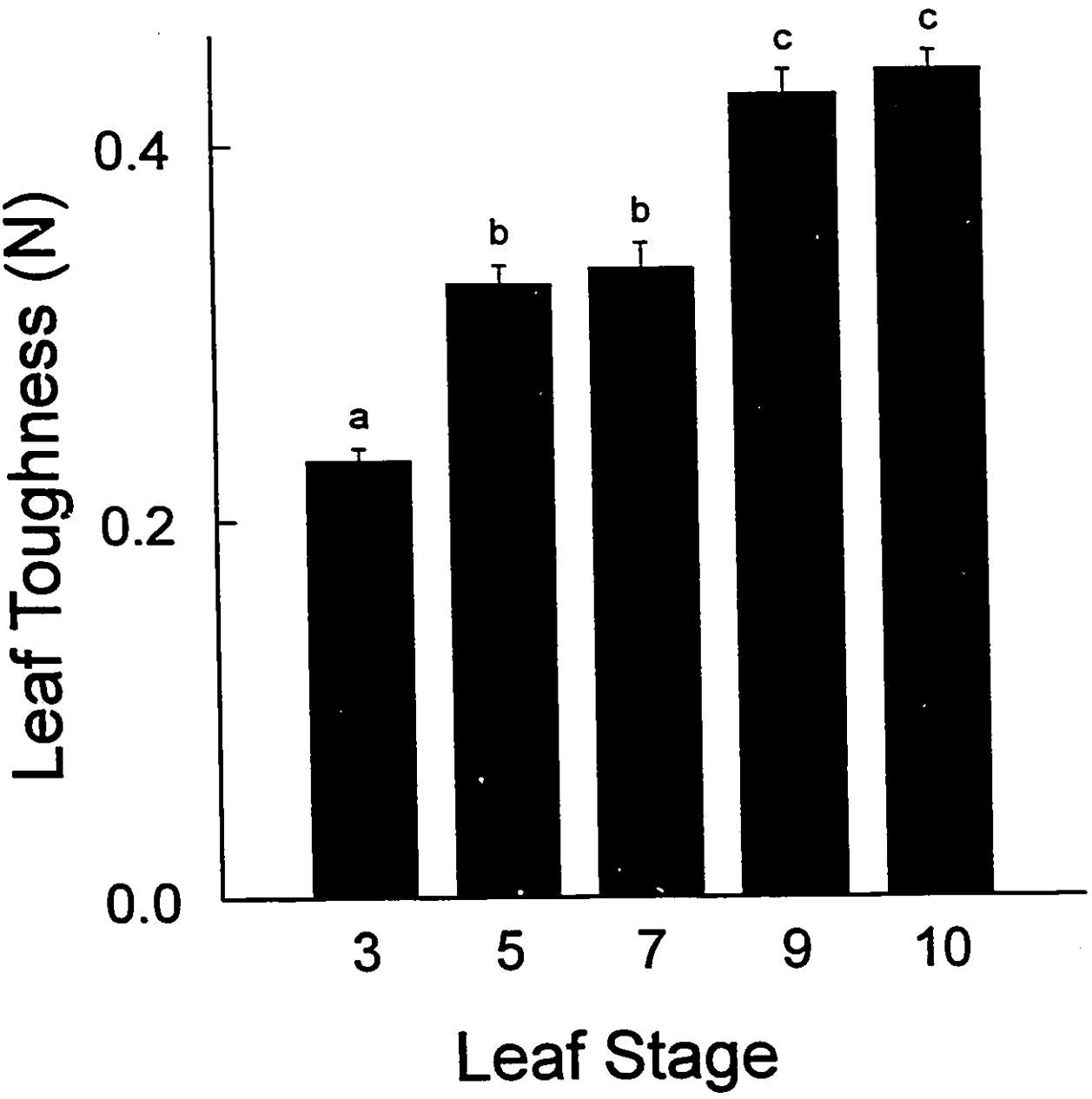


Fig. 5.6. Bar graph of leaf toughness of field grown BS9 leaves at different stages in development. N = 15 for each development stage.
Bars topped with different letters within the same development stage are significantly different, SNK ($P < 0.05$).

stages rely upon DIMBOA to protect tender tissue which is not well fortified. As the tissue matures the DIMBOA levels drop with insect consumption also dropping as the tougher tissue is less accessible and probably less palatable.

Changes in phenolic acids within the epidermal cell wall can be estimated using the microspectrophotometer (Chapter 3). Absorbance readings showed that the least fortified epidermal cell walls were found at the 3 leaf stage with a dramatic jump in absorbance occurring at the 9 leaf stage of development for both greenhouse treatments (Table 5.5). In spite of the high epidermal absorbance for 9 leaf plants grown under reduced UV light, leaf consumption continued to be high. A probable explanation is that cyclobutane dimers that cross-link the cell wall hemicellulose are at extremely low levels (Fig. 5.4) and are not detected by the microspectrophotometer due to changes in absorption spectra during dimerization. Field-grown plants tend to have epidermal cell wall absorbances that are 50 to 100% higher that those reported here for greenhouse-grown plants (Chapter 4).

From the above it appears that the preferred feeding substrate for ECB larvae is early plant tissue which is high in protein and water content and is accessible due to low phenolic fortification of cell walls. However, adult emergence and oviposition usually coincide with the mid-whorl with little evidence of ECB feeding damage to plants in the 3 to 7 leaf stages of development. This apparent discrepancy from field observations may be accounted for by considering the reduced growth rate that ECB larvae experience when reared on high-DIMBOA diets (Houseman et al. 1992) such as that found during the early leaf stages . Such a delay may reduce the reproductive potential for such individuals through asynchrony while exposing them to an extended period of predation within a small canopy plant that offers little physical protection from natural enemies (Lawton 1983) or from desiccation (Lee 1988). Moreover, during larval diapause and pupation the stalk may not be of sufficient diameter or may have excessively high levels of DIMBOA during earlier stages of stalk development (Gutiérrez et al. 1986).

This study demonstrates that maize resistance strategies to foliage feeders appears to switch from a qualitative defence during early stages of development to quantitative defenses after the 9 leaf stage. Elevated levels of DIMBOA during early plant development may act as an antifeedant to deter insect feeding but quantitative mechanisms involving cell wall

Table 5.5. Microspectrophotometer readings of epidermal cell wall from greenhouse grown BS9(C4) leaf tissue at different stages in development.

Treatment	Leaf stage	Absorbance at 326 nm[1]
Greenhouse	3	0.18 ± 0.04 a
Reduced UV	5	0.36 ± 0.08 a
	7	0.41 ± 0.08 b
	9	0.83 ± 0.07 b
	10	0.98 ± 0.11 b
Greenhouse	3	0.18 ± 0.02 a
UV Suppliment	5	0.29 ± 0.09 a
	7	0.30 ± 0.04 a
	9	1.12 ± 0.10 c
	10	0.82 ± 0.04 b

[1] Means within the same treatment followed by the same letter are not significantly different, SNK ($P < 0.05$).

fortification by phenolic acids appear to be a more prominent resistance factor during later stages of plant development as is evidenced by lower consumption rates by ECB larvae. Phenolic fortification within the epidermal cell wall would hinder penetration by insects, especially for early instar larvae with small, underdeveloped mandibles. In the presence of UV light, phenolic acids can dimerize to form cross-links between hemicelluloses, which may explain the observations from previous studies where greenhouse-grown maize with elevated levels of DIMBOA show more feeding damage than field-grown plants that would be exposed to high intensity UV light (Guthrie et al. 1986b, Manuwoto and Scriber 1985b). Given the extensive planting of maize and its high apparency according to Feeny's (1976) classification, it is reasonable to expect a quantitatively based resistance strategy to operate in maize as the plant matures.

CHAPTER VI.

ROLE OF PHOTODIMERIZED PHENOLIC ACIDS IN MAIZE RESISTANCE TO THE EUROPEAN CORN BORER.

6.1 Introduction

In chapter 5 the effect of environment on the levels of resistance factors and leaf consumption by ECB larvae was studied. Near-UV light regime manipulated in a greenhouse affected insect feeding performance and it was hypothesized that light activated phenolic dimers accounted for the reduced consumption observed for leaf tissue grown under UV light conditions. Guthrie et al. (1986b) found similar results for inbreds grown in greenhouses compared to those grown in the field. These observations suggest that resistance mechanisms that operate in first generation ECB resistance attenuate when maize grows in greenhouses. Major differences between these two growth environments include light quality, temperature, and soil composition. We hypothesized that reduced levels of near-UV light (300-400 nm) in greenhouse environments due to absorption by soft glass, would reduce the production of photoactivated dimers that appear to contribute to resistance (Chapter 5). To test this hypothesis, greenhouse and construction plastics were used to cover field grown plants to observe the effects of near UV light on field resistance.

The first objective was to determine if plants grown under reduced ultraviolet light (300-400 nm) in the field would be more susceptible to ECB feeding damage. The second objective was to observe if photodimers of the cell wall bound phenolic acids could account for differential feeding damage in five genotypes grown under three different light regimes in a field environment.

6.2 Materials and Methods

Germplasm: Four categories of inbred lines of dent maize were studied: high resistance and high DIMBOA levels (CI31A), moderate resistance and moderate DIMBOA levels (B86), low resistance and low DIMBOA levels (MS72), and high resistance and low DIMBOA levels (MBR 6796-24 in 1990 and MBR 6796-15 in 1991). MBR lines originated from CIMMYT and were adapted to northern temperate regions at Plant Research Centre, Ottawa, Ontario, Canada.

Field plot: In 1990 and 1991 the same location was planted in mid-May at the Plant Research Centre. The soil was a sandy loam. The experimental design was a randomized-complete block with four replicates. Row distance was 0.9 m and distance between plants 0.15 m. Infested plants had 15 plants per row and those intended for phytochemical analysis had 10 plants per row. Outside ends of each row were bordered by a resistant commercial hybrid (Dekalb 435).

Greenhouses: At the time of emergence two 12 m x 12 m x 2 m aluminum framed greenhouses were erected over two of the field plots. One frame was covered by 6 mil construction plastic which blocked less than 10% of the UV light (300-400 nm) (UV+ treatment) and the other was covered with greenhouse plastic (CIL Durafilm-3 6 mil) which blocked 90-99% of the UV light that can be absorbed by phenolic acids (UV- treatment) (See Fig. 5.1). Plastic hung over all sides of the frame by 1 m to reduce low level incident light at dawn and dusk but did not impede ventilation for cooling. The third treatment was left uncovered (Outside). The UV- and UV+ plastics reduced the incidence of 700 nm light by 15% and 12%, respectively. The spectral characteristics of the plastics did not change over the course of the summer (Fig. 5.1). To reduce stress on the plants and maintain adequate soil moisture status, soaker hoses were suspended from the frame to spray ca. 300 L water every other day. Outside plants were watered to maintain comparable soil moisture levels. In 1990 outside plants were transplanted after the green houses were constructed but planted with the other treatments in 1991. Afternoon temperatures within greenhouses were as much as 3°C higher than ambient temperature but were lowered by irrigation. Snow fencing was placed around the area encompassing the three plots to reduce damage from wind storms.

Artificial Infestation: Infestations were made at the mid-whorl stage in early July with two applications (4 days apart) of 4 egg masses (ca. 200 eggs) per plant. Leaf-feeding damage to each plant was rated from 1 (no damage) to 9 (extensive damage) (Guthrie et al. 1960). Laboratory bioassays were also conducted to account for any modifications in insect behaviour associated with reduced UV light (Thoms and Philogene 1979). One week after artificial infestation, twenty 10-cm leaf sections from the middle of the first fully extended leaf from each variety and treatment were incorporated into bioassays as outlined in Chapter 3.

Plants were dissected in early October to record the number of larvae, number and length of tunnels, percentage cross-sectional area excavated and height of tunnelling.

Sampling for Phytochemical Analysis: One week after infestation, uninfested plants were cut at ground level. Whorl tissue was pulled out and the three top most exposed leaves were pooled as a sample. Mid-ribs were removed and the middle portion of each leaf was cut up, placed in paper bags and frozen on dry ice, then stored at -20°C. Frozen tissue was freeze-dried and milled on a UD cyclone mill (UD Corp., Bolder, CO) with a 1-mm screen. Milled samples were stored at -20°C until analyzed.

Phytochemical Analyses: Procedure for phenolic acid and hydroxamic acid determinations is outlined in Chapter 3. 4,4'-dihydroxy--truxillic acid (FA-FA) was identified by standards prepared by S. Tachibana. HPLC peaks were confirmed by semi-preparative HPLC using the same gradient to collect peak fractions which were then extracted with 3 x 5 mL ethyl acetate, dried, and derivatized by mixing N,O-bis(trimethyl-silyl)trifluoracetamide with pyridine (1:1) and shaken for 5 min. at 23 °C. Aliquots (5 µL) were injected into a GC-MS fitted with a J & W Megabore 15 m x 0.15 um column packed with DB1. Temperature profile was 150°C for 2 min and then increased by 10°C/min to 350°C. Mass spectra showed the presence of only two parent ions m/z 338 and 308, the molecular ions for fully silyated ferulic and p-coumaric acid, respectively.

Statistical analysis: All statistical analyses were performed on SAS v. 6.03 (SAS, 1988). Data was transformed to satisfy the assumptions of the general linear model. Comparison of means within varieties and between treatments was carried out only if the treatment F-ratio was significant. Regressions were done on means for each variety and treatment.

6.3 Results and Discussion.

Under field conditions, a reduction in incident UV consistently and significantly increased the leaf feeding rating on the five genotypes tested in each of two years (Table 6.1). However, unlike the dramatic increase (up to 400%) in susceptibility demonstrated by Guthrie et al. (1986b) for greenhouse-grown maize, the increases observed for this study were smaller, ranging from 11% to 44% increase in leaf feeding by ECB larvae on plants grown under reduced UV light. CI31A and MBR lines continued to be highly resistant while B86 and

Table 6.1. Leaf feeding ratings and DIMBOA levels in mid-whorl leaf tissue from inbreds grown in the field under different light conditions.

		Leaf-feeding rating[1]		DIMBOA mg/g dry weight[1]	
Genotype	Treatment	1990	1991	1990	1991
CI31A	UV-	2.11 ± 0.11a	2.33 ± 0.12a	3.45± 0.24a	3.43 ± 0.26a
	UV+	1.52 ± 0.07b	2.05 ± 0.07b	2.88 ± 0.05b	2.98 ± 0.11b
	Outside	1.59 ± 0.12b	1.79 ± 0.07b	2.66 ± 0.23b	2.66 ± 0.16b
MBR	UV-	2.75 ± 0.14a	2.3 ± 0.1a	0.70 ± 0.30a	1.04 ± 0.35a
6796-24	UV+	2.04 ± 0.08b	2.08 ± 0.09a	0.25 ± 0.06a	0.41 ± 0.22a
6796-15	Outside	2.85 ± 0.21a	2.09 ± 0.08a	0.35 ± 0.17a	0.74 ± 0.32a
B86	UV-	4.07 ± 0.18a	4.69 ± 0.14a	2.13 ± 0.08a	2.21 ± 0.08a
	UV+	2.80 ± 0.13b	3.29 ± 0.14b	1.99 ± 0.08a	2.14 ± 0.10a
	Outside	2.44 ± 0.19b	3.30 ± 0.11b	2.05 ± 0.08a	2.10 ± 0.09a
MS72	UV-	7.15 ± 0.09a	6.72 ± 0.12a	1.65 ± 0.21a	1.83 ± 0.25a
	UV+	6.52 ± 0.16b	5.52 ± 0.15b	1.36 ± 0.14a	1.65 ± 0.25a
	Outside	3.93 ± 0.36c	5.98 ± 0.09c	1.31 ± 0.12a	1.46 ± 0.14a

[1] Means within the same genotype and year followed by the same letter are not significantly different, SNK test, $P<0.05$. Leaf rating data were transformed by ln(x+1) prior to statistical analysis.

MS72 showed the greatest increase in susceptibility. These changes could not be accounted for by changes in DIMBOA since DIMBOA also increased for maize grown under reduced UV light (Table 6.1). This result is consistent with earlier observations by Guthrie et al. (1986b). Based on DIMBOA levels alone the leaf feeding ratings should have increased (Klun and Robinson 1969) which suggests that other resistance mechanisms are operating in this altered growth environment.

Nutritional quality of grass foliage can dramatically affect the growth and behaviour of foliage-chewing insects such as the ECB (Bernays and Barbehenn 1987). For C4 plants such as maize, the levels of available nitrogen (N) are generally lower than for C3 plants (Boutton et al. 1978) and may tend to amplify insect responses to changes in N quality of the host plant (Caswell et al 1973). Based on micro-Kjehldahl determinations, the estimated protein concentrations were slightly, but generally not significantly, lower for maize grown under reduced UV light (Table 6.2). Part of the observed difference in susceptibility could reflect the insects' attempt to compensate for foliage of reduced nutritional value by increasing consumption.

Protein levels for 1991 were higher than for 1990 because the latter were harvested for chemistry samples one leaf stage later (14 leaf stage). Protein content drops rapidly with plant maturity but is also influenced by soil fertility, drought, flooding, light levels and temperature (Butler and Bailey 1973). Although the growth environments between treatments differed in light quality and quantity (see Fig. 5.1), temperature and water regime, all treatments were within one leaf stage of each other at the time of infestation and protein levels within a given genotype and year were similar.

The relationships of lignin and silica to maize resistance have been studied (Buendgen et al. 1990; Coors 1987; Rojanaridpiched et al. 1984), but the contribution made by cell wall hydroxycinnamic acids (HCA) to leaf resistance has not been reported. The UV- treatment resulted in higher levels of monomeric HCAs (Table 6.3). This observation would indicate that it is unlikely that cell wall bound phenolic acids played a direct role in ECB resistance. Slightly higher temperatures (up to 3°C) were associated with both plastic coverings. According to Akin et al. (1987), elevated temperatures increase the levels of cell wall

Table 6.2 Percent protein estimates in mid-whorl leaf tissue from inbreds grown in the field under different light conditions.

Genotype	Treatment	Percent protein (dry wt)[1] 1990	1991
CI31A	UV-	13.3 ± 0.42a	18.8 ± 0.70a
	UV+	14.3 ± 0.67a	21.2 ± 0.46b
	Outside	16.7 ± 0.54b	19.4 ± 0.36a
MBR	UV-	9.9 ± 0.77a	15.3 ± 0.72a
6796-24	UV+	10.3 ± 0.75a	15.9 ± 0.22ab
6796-15	Outside	13.8 ± 0.75b	17.4 ± 0.77b
B86	UV-	12.1 ± 0.08a	17.5 ± 0.38ab
	UV+	13.0 ± 0.93ab	16.1 ± 0.16a
	Outside	14.5 ± 0.56b	18.7 ± 0.83b
MS72	UV-	13.5 ± 0.35b	18.6 ± 0.66a
	UV+	11.7 ± 0.28a	18.8 ± 0.29a
	Outside	15.4 ± 0.51c	19.2 ± 0.36a

[1] Means within the same genotype and year followed by the same letter are not significantly different, SNK test, P<0.05.

Table 6.3. Levels of bound phenolic acids in mid-whorl leaf tissue from inbreds grown in the field under different light conditions, 1990.

		p-coumaric acid (mg/g)[1]		ferulic acid (mg/g)[1]	
Genotype	Treatment	*trans*	*cis*	*trans*	*cis*
CI31A	UV-	5.45 ± 0.15a	0.38 ± 0.01a	2.28 ± 0.05a	0.75 ± 0.02a
	UV+	3.99 ± 0.32b	0.41 ± 0.04a	2.22 ± 0.07a	0.74 ± 0.01a
	Outside	3.06 ± 0.28b	0.33 ± 0.01a	1.58 ± 0.16b	0.75 ± 0.05a
MBR	UV-	5.45 ± 0.15a	0.47 ± 0.05a	2.82 ± 0.14a	1.11 ± 0.05a
6796-24	UV+	4.16 ± 0.22b	0.46 ± 0.05a	2.08 ± 0.23a	1.24 ± 0.03a
	Outside	3.49 ± 0.29b	0.40 ± 0.02a	2.16 ± 0.22a	0.77 ± 0.05b
	UV-	3.51 ± 0.32a	0.32 ± 0.04a	3.96 ± 0.77a	1.32 ± 0.06a
6796-15	UV+	2.16 ± 0.14b	0.30 ± 0.01a	2.28 ± 0.16a	1.07 ± 0.09a
	Outside	2.05 ± 0.13b	0.33 ± 0.03a	2.30 ± 0.18a	0.88 ± 0.02b
B86	UV-	4.98 ± 0.03a	0.49 ± 0.02b	3.14 ± 0.05a	0.79 ± 0.02a
	UV+	3.09 ± 0.09b	0.41 ± 0.01a	1.79 ± 0.19b	0.78 ± 0.01a
	Outside	2.77 ± 0.03b	0.37 ± 0.01a	1.68 ± 0.08b	0.56 ± 0.05b
MS72	UV-	3.23 ± 0.17a	0.37 ± 0.01a	1.78 ± 0.20a	0.69 ± 0.04a
	UV+	3.16 ± 0.08a	0.37 ± 0.01a	1.84 ± 0.05a	0.74 ± 0.03a
	Outside	3.22 ± 0.51a	0.37 ± 0.05a	1.99 ± 0.37a	0.69 ± 0.08a

[1] Means within the same genotype and year followed by the same letter are not significantly different, SNK test, $P<0.05$.

hydroxycinnamic acids which could explain differences observed between the outside and the two plastic coverings.

With reduced ultraviolet light the production of cyclobutane dimers was reduced (Table 6.4). A reduction in the extent of cell wall cross-linking may render the leaf tissue a more accessible feeding substrate (Ford and Hartley 1990). The effect of reduced UV light on truxillic and truxinic acid production is best demonstrated when the probability of cross-linking is taken into account by taking the ratio of total cyclobutane dimers over total HCA monomers (Fig. 6.1). For both years the UV-treated plants had a significantly lower proportion of cell wall bound photodimers.

Given the behaviour-modifying effects of light (Thoms and Philogène 1979) and temperature (Slansky and Scriber 1985) on insect feeding, bioassays of field grown material from each treatment were tested under controlled environmental conditions (Table 6.5). The same trends observed in the leaf damage ratings (Table 6.1) were observed for the bioassays. The dramatic reduction in consumption rate for 1991 is likely attributable to the experimental growth chamber running 3°C cooler (22°C) during photophase. Correlations of the mean consumption rate within year or within treatment with DIMBOA level, protein content, total hydroxycinnamic acid content or total cyclobutane dimer content were not significant (r^2<0.10). There was a weak correlation (r^2 between 0.03 and 0.28, n=4) for the ratio of total cyclobutane dimers / total HCA. However, the best regressions were obtained when taking into account the background proportion of truxillic acids (UV- treatment) for each UV treatment (UV+ and Outside) (data not shown). The P-values for the slope of the regression lines were not significant, perhaps because only 4 genotypes were examined, but the same trend was evident for all four regression lines with increased levels of truxillic acids correlated with reduced feeding. This is the first reported study of a correlation of modified levels of cell wall photodimers of HCAs and leaf feeding resistance by a foliage-chewing insect. This proposed explanation of the mechanism of physical resistance would be largely under environmental control, namely UV light intensity. As a result, regressions over genotype would likely provide a weak association.

The effect of light quality on plant damage is shown in Table 6.6. Tunnelling was generally more extensive in plants grown under reduced UV light. Likewise, the mean

Table 6.4. Levels of cyclobutane dimers in mid-whorl leaf tissue from inbreds grown in the field under different light conditions.

Genotype	Treatment	4,4'dihydroxy-a-truxillic acid (ug/g)[1]		Total cyclobutane dimers (ug/g)[1]	
		1990	1991	1990	1991
CI31A	UV-	202 ± 90.4a	155 ± 91.1a	599 ± 84.9a	546 ± 154.4a
	UV+	306 ± 93.6b	172 ± 91.9a	744 ± 110.6a	685 ± 159.4a
	Outside	340 ± 87.2b	227 ± 94.1b	755 ± 98.7a	823 ± 144.8a
MBR	UV-	171 ± 104.7a	153 ± 85.5a	459 ± 127.1a	459 ± 128.2a
6796-24	UV+	430 ± 94.3b	254 ± 90.2b	838 ± 138.4b	884 ± 145.1b
6796-15	Outside	258 ± 152.0a	242 ± 90.9b	785 ± 160.7b	888 ± 170.2b
B86	UV-	256 ± 90.2a	171 ± 92.0a	580 ± 155.8a	642 ± 127.1a
	UV+	300 ± 130.2a	208 ± 87.0a	866 ± 150.0b	789 ± 144.0a
	Outside	298 ± 152.9a	196 ± 98.8a	902 ± 152.9b	824 ± 193.0a
MS72	UV-	121 ± 98.3a	153 ± 93.0a	623 ± 92.5a	540 ± 141.6a
	UV+	243 ± 177.8a	192 ± 88.5a	771 ± 291.1a	681 ± 138.1a
	Outside	298 ± 187.3a	182 ± 93.7a	814 ± 169.9a	710 ± 147.2a

[1] Means within the same genotype and year followed by the same letter are not significantly different, SNK test, $P<0.05$.

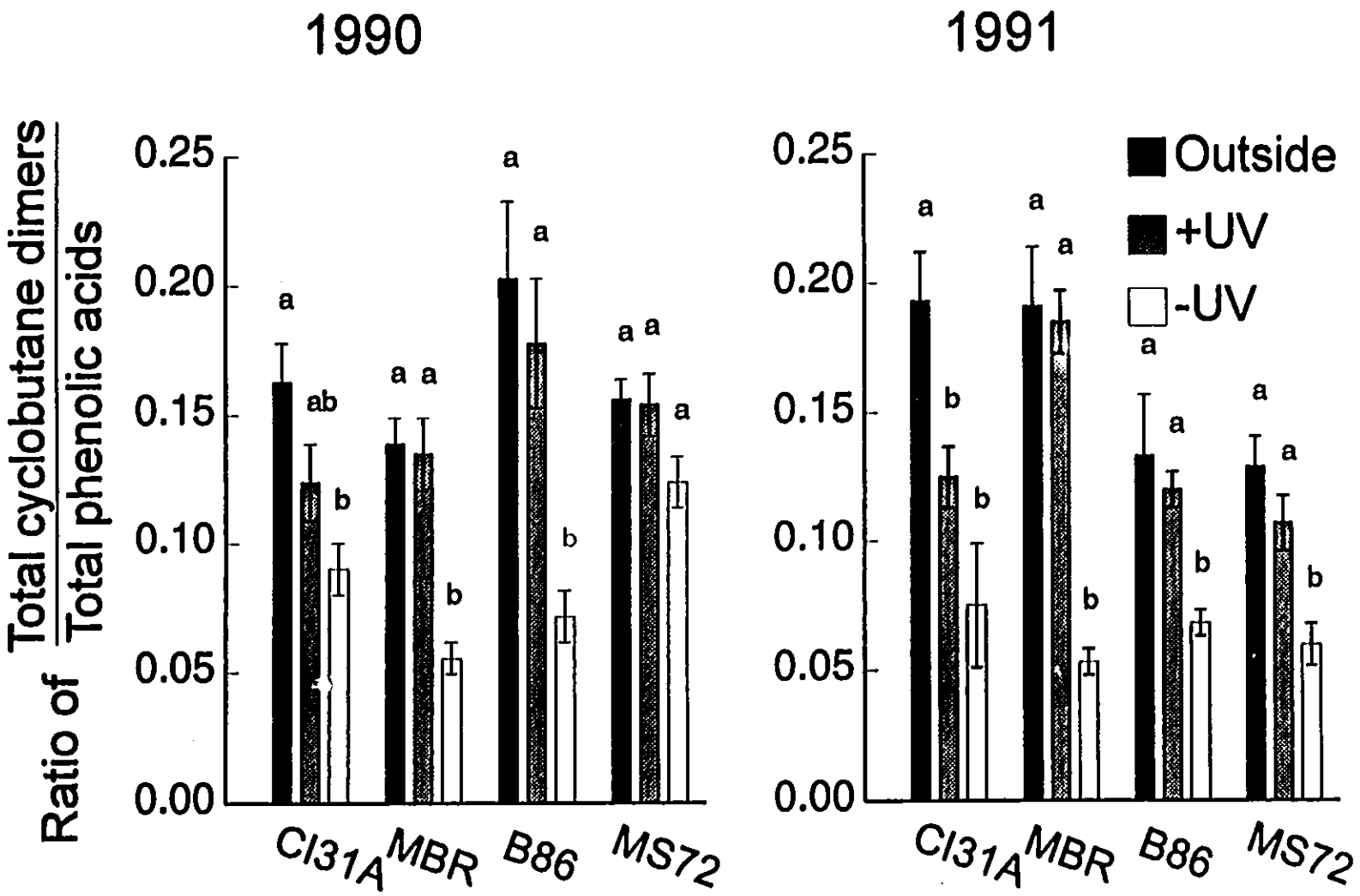


Fig. 6.1. Bar graph of ratios of total cyclobutane dimers over total hydroxycinnamic acids. Maize inbreds were grown in full sunlight (Outside), under construction plastic (+UV), and under greenhouse plastic in the field (-UV). Bars topped with different letters within a given genotype are significantly different, SNK ($P<0.05$).

Table 6.5. ECB feeding bioassays on mid-whorl leaf tissue from inbreds grown in the field under different light conditions.

Genotype	Treatment	Area consumed (mm^2)[1] 1990	1991
CI31A	UV-	44.1 ± 4.1a	12.2 ± 1.6a
	UV+	36.5 ± 3.0a	7.2 ± 0.8a
	Outside	23.5 ± 2.7b	13.5 ± 1.7a
MBR	UV-	22.8 ± 2.4a	13.7 ± 2.9a
6796-24	UV+	15.3 ± 2.6b	6.1 ± 0.9b
6796-15	Outside	25.5 ± 2.7a	6.1 ± 0.9b
B86	UV-	69.4 ± 6.9a	46.3 ± 4.1a
	UV+	54.9 ± 5.1a	21.6 ± 3.3b
	Outside	24.0 ± 3.7b	30.4 ± 3.8b
MS72	UV-	79.2 ± 6.1a	38.6 ± 2.5a
	UV+	70.4 ± 5.7a	30.4 ± 2.7a
	Outside	80.6 ± 7.0a	31.5 ± 2.8a

[1] Means within the same genotype and year followed by the same letter are not significantly different, SNK test, $P<0.05$. Bioassay data was transformed by ln(x+1) prior to statistical analysis.

Table 6.6. Plant damage by ECB for inbreds grown in the field under different light conditions.

Genotype	Treatment	Tunnel length (cm)[1]		Percent cross-section excavated[1]	
		1990	1991	1990	1991
CI31A	UV-	15.2 ± 2.6a	6.6 ± 1.2a	45.2 ± 3.6a	70.0 ± 4.0a
	UV+	8.6 ± 1.4b	5.5 ± 1.0a	43.4 ± 3.4a	56.0 ± 4.8b
	Outside	-	5.9 ± 0.8a	-	56.7 ± 3.1b
MBR	UV-	11.0 ± 2.2a	6.8 ± 1.3a	37.9 ± 3.2a	67.0 ± 5.0a
6796-24	UV+	5.0 ± 1.0b	3.8 ± 1.1a	37.9 ± 4.4a	57.1 ± 8.1a
6796-15	Outside	-	3.5 ± 1.6a	-	62.5 ± 12.5a
B86	UV-	10.1 ± 1.6a	2.9 ± 0.5a	52.0 ± 4.0a	67.1 ± 7.3a
	UV+	4.7 ± 0.7b	3.5 ± 0.8a	38.1 ± 3.4b	59.1 ± 4.7a
	Outside	-	3.4 ± 1.0a	-	47.6 ± 8.1a
MS72	UV-	37.9 ± 2.9a	12.7 ± 1.4a	62.3 ± 2.4a	70.1 ± 3.7a
	UV+	19.2 ± 1.8b	6.6 ± 0.9b	46.9 ± 2.3b	60.5 ± 3.4a
	Outside	-	8.9 ± 1.1b	-	67.1 ± 3.2a

[1] Means within the same genotype and year followed by the same letter are not significantly different, 1990 t-test, 1991 SNK test, $P<0.05$. Tunnel length data were transformed by ln(x+1) and percent excavation data transformed by arcsin ($\sqrt{x}$) prior to statistical analysis.

cross-sectional area excavated within the stalk tended to be higher for plants grown under reduced UV light. The number of larvae found within the dissected plants was not significantly different between treatments (P=0.43; df=1,4; N=168) so the increased tunnelling is probably not due to higher insect populations but larval canabalism may have occurred in extensively tunnelled stalks, thereby lowering the number of larval counts. Two probable explanations for increased tunnelling might be the reduced production of cyclobutane dimers and elevated temperature. Lower levels of cyclobutane dimers may have facilitated accelerated feeding rates by making nutrients more accessible to early leaf-feeding instars and subsequently accelerating development so that third-instar larvae, the first instar that feeds within the stalk, developed earlier and thus would feed within the stalk for an extended period of time. ECB development is influenced dramatically by temperature with an increase from 20 to 25°C resulting in a 55% increase in development rate (Ellsworth et al. 1989). Both plastic houses had comparable temperatures above ambient but the UV-blocking-plastic house reached temperatures 1-2 °C higher than the UV transmitting plastic house on hot, bright days.

Manipulation of cell wall composition by altering light quality in the field has enabled observation of the impact that UV light has on maize resistance and suggests a new resistance mechanism which operates at the structural level to reduce the accessibility of plant nutrients to foliage feeders such as the ECB. Maize plants grown under overcast conditions are often more susceptible to leaf feeding damage by a variety of leaf feeding Lepidoptera (J.A. Mihm, pers. comm.) which according to our results may be attributed to reduced levels of substituted cyclobutane dimers to cross-link cell wall macromolecules. Although mechanical strengthening of cell wall tissue by phenolic cross-linking may be compensated for by over-development of larval head capsules and increased mandibular power in later instars (Bernays 1986), early instars may not have time to adjust. In nature, approximately 80% larval mortality occurs within the first 48 h after emergence (Ross and Ostlie 1990). The physical barrier imposed by tougher leaf tissue *vis-a-vis* cell wall fortification may play a larger role in maize resistance than has been previously considered, by reducing lavral access to leaf nutrients and water.

CHAPTER VII.

EVOLUTION OF RESISTANCE FACTORS DURING RECURRENT SELECTION FOR MAIZE RESISTANCE TO THE EUROPEAN CORN BORER.

7.1 Introduction

Earlier chapters have provided a body of evidence to support the hypothesis of quantitative defenses in maize, with the level of quantitative defence being dependent on genotype, tissue maturity and environment. Early work done on exotic accessions demonstrated quantitative defence mechanisms in maize, namely lignin (Sullivan et al. 1974). Recently, quantitative defences such as fibre, lignin and silica have been assocated with second-generation ECB resistance (Buendgen et al. 1990, Coors 1987, Rojanaridpiched et al. 1984). Buendgen et al. (1990) investigated changes in cell wall composition over five cycles of selection in the BS9 synthetic and observed negative correlations between cell wall constituents and plant damage by the European corn borer. A comparable study looking at DIMBOA levels in this same synthetic population found DIMBOA levels to increase with resistance in advancing cycles of selection (Grombacher et al. 1989a).

The two studies described previously did not take into account protein, cell wall phenolic acids and cell wall dimers such as deoxydiferulic or cyclobutane dimers. The first objective of this study was to see if cell wall phenolics change over cycles of selection in maize using both standard phytochemical techniques and microspectrophotometer absorbance readings of epidermal cell walls. The second objective was to observe phytochemical and nutritional differences between four cycles of selection (C0, C2, C4, C5) and relate these differences to field resistance.

7.2 Materials and Methods.

Germplasm and Screening: Cycles 0, 2, 4 and 5 from the maize population BS9 were evaluated with the method of selection outlined by Russell and Guthrie (1982). Planting occurred in mid-May of 1991 and 1992 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. In 1991 a second location was planted in Prescott, Ontario, Canada (44°18'18"N, 78°31'50"W) where a high natural European corn borer population existed.

The rows were spaced 0.9 m apart and plants were spaced 0.15 m apart with 30 plants per row. The soil was a sandy loam at the Ottawa site and a sandy clay soil at the Prescott site. Two rows for each cycle within a block were planted for each of the four blocks. In 1991 the Ottawa site was infested using the Bazooka method (Guthrie and Barry 1989) on one row and egg masses on the adjacent row with ca. 80 larvae per plant being applied over two applications. The Prescott site was infested naturally. The 1992 Ottawa site was infested by Bazooka method only with 80 larvae per plant. Three weeks after infestation (July 20 to 27) plants were rated according to Guthrie et al. (1960) 9-point scale. Plants were dissected from late September through early October to count the number of larvae, number and length of tunnels, position of tunnelling and estimated cross-section of pith excavated by larval feeding.

Sample Collection: At the mid-whorl stage the 13th leaf was harvested by pulling the 10th leaf and above whorl out of the plant and unwrapping the leaves to expose the 13th leaf which was green along 1/2 and yellow along 1/2 of the leaf length. This leaf was used for microscopy studies. The green portions of leaves 11, 12 and 13 were cut into paper bags as was the immature tissue within the whorl and all were frozen on dry ice and held at -20°C. Three plants per row were pooled from each row. Frozen tissue was thawed for 2 hours and then refrozen for freeze drying. Samples were milled on a UD cyclone mill (UD Corp., Bolder, CO) with a 1-mm screen. Milled samples were stored at -20° C until analyzed. At early silking, three plants from each row were harvested for sheath, stalk, node and pith tissue. Stalk tissue was taken from the third internode above ground and was split in half. The pith was excavated using an apple corer and the node tissue was removed. Samples were processed as above.

Bioassays: Thirty plants were dissected and sectioned as above. Two leaf sections (3x7 cm) were taken from the middle of the green and yellow portions of the 13th leaf. Bioassays were conducted on both yellow (24 hour bioassay) and green (48 hour bioassay) tissue. Bioassay procedure is outlined in Chapter 3.

Leaf Toughness: Using the method reported in Chapter 2, seven force measurments were taken from each row for a total of 25 measurements.

Microspectrophotometer: Sectioning and microspectrophotometry techniques are reported in Chapter 3.

Protein Determinations: Protein content was estimated by the micro-Kjehldahl method on 0.3 g samples using the conversion factor 6.25 to estimate protein from N.

Phytochemical Analysis: Hydroxamic acids and cell wall phenolics were determined using the methods reported in Chapter 3.

Statistical analysis: All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Leaf rating data and number of larvae per plant were transformed by ln(x+1) to satisfy the assumptions of the general linear model. Microspectrophotometer data satisfied the assumptions of the general linear model and were not transformed.

7.3 Results and Discussion

Leaf-feeding damage was significantly greater on plants of cycles 0 and 2 than for those of cycles 4 and 5 for both forms of artifical infestation (bazooka and egg masses) and for the natural infestation screening (LDR, Table 7.1). The bazooka application method provided the greatest leaf feeding damage so this infestation method was continued for 1992. Despite rating only those plants within the natural infestation site that showed signs of insect feeding, this group had the lowest rating suggesting the insect population per plant was lower either by reduced oviposition or higher larval mortality.

Leaf bioassays under controlled conditions by third instar larvae was significantly greater from earlier cycles within both the mature and immature leaf tissues (Bioassay, Table 7.1). Estimated protein levels remained relatively constant at about 20% (Table 7.1). Nitrogen concentration of plant tissue is a major determinant in insect growth (Scriber and Slansky 1981), but this component does not seem to play a role in recurrent selection for ECB resistance as leaf protein was not correlated with field or bioassay ratings. DIMBOA levels in fully extended leaves have previously been reported to increase over cycles of selection for the BS9 population (Grombacher et al. 1989a), but this is the first report of changes in DIMBOA levels within the immature, yellow, whorl tissue for this maize population (Table 7.1). As with mature leaf tissue, the immature tissue shows an increase in DIMBOA levels with the largest increase occurring between C0 and C2. DIMBOA levels are 2 to 5 times higher for the immature tissue within the whorl than for mature leaf tissue, with DIMBOA content in immature tissue being significantly different over selection cycles and correlating with bioassay

Table 7.1. Nutritional and resistance factors in mature and immature leaf tissue from different cycles of BS9.

		1991					1992				
Tissue	Parameter[†]	C0	C2	C4	C5	LSD[‡]	C0	C2	C4	C5	LSD[‡]
Mature	Protein (%)	19.75	18.55	20.66	19.38	1.67	20.68	20.42	19.84	20.99	2.05
	DIMBOA	1.46	1.72	1.67	1.72	1.22	3.99	3.74	5.01	5.76	1.32
	Fibre	329	359	359	350	50	344	334	347	359	42
	FA	1.91	1.66	1.91	1.82	0.39	2.62	2.12	2.24	2.51	0.83
	pCA	1.99	1.78	2.11	2.26	0.52	2.29	2.06	2.2	2.94	0.81
	CBD	1.04	0.91	1.09	1.19	0.36	1.2	0.98	1.47	1.36	1.01
	DFA	0.52	0.27	0.62	0.51	0.24*	0.48	0.52	0.75	0.63	0.23
	Bioassay	26.6	28.3	22.2	25.2	1.2**	46.6	40.1	35.1	35.9	1.5*
Immature	Protein (%)	19.79	18.35	20.09	18.97	1.44	20.11	20.39	20.09	20.89	1.93
	DIMBOA	6.72	8.78	7.14	7.46	3.93	8.55	13.69	15.23	11.17	4.62
	Fibre	191	230	208	213	20*	203	239	240	255	40
	FA	3.08	2.81	2.68	2.68	0.54	3.43	3.85	4.66	3.58	1.42
	pCA	2.27	2.19	1.83	2.28	0.36	2.66	2.19	2.8	2.59	1.52
	DFA	0.35	0.27	0.69	0.38	0.09**	0.56	0.55	0.83	1.02	0.28**
	Bioassay	61.5	48.4	21.3	36.4	1.6**	41.4	31.4	27.1	26.2	1.8*
	LDR(B)	3.88	3.49	2.89	2.48	0.41**	3.56	3.22	2.73	2.87	0.37**
	LDR(EM)	3.11	2.71	2.19	1.76	0.39**	-	-	-	-	
	LDR(N)	1.63	1.46	1.29	1.32	0.18**	-	-	-	-	
	Toughness	0.71	0.76	0.81	0.79	0.06**	0.43	0.42	0.47	0.45	0.03*

[†] DIMBOA, fibre, cell wall bound ferulic acid (FA), p-coumaric acid (pCA), cyclobutane dimers (CBD), and dehydrodiferulic acid (DFA) are reported as mg/g dry weight. Leaf damage rating (LDR) for plants infested by bazooka (B), egg masses (EM), and natural infestations (N). Leaf toughness reported in neutons.

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

consumption (r=0.72*). Despite having higher DIMBOA levels, immature tissue was completely consumed within the normal 48-hour run time of the bioassays so immature tissue bioassays were run for 24 hours. Localization of DIMBOA within the tender whorl tissue is likely the main defence mechanism for this tissue. DIMBOA levels in stalk and sheath tissue were found to be very low during silking (Tables 7.2 and 7.3) so DIMBOA is not likely to contribute to second generation resistance.

Fibre content of the different tissues was estimated from gravimetric measurements of tissue following base hydrolysis. Such an extraction removes base lignins and ester linked phenolic acids attached to the cell wall as well as protein. Although significant differences could not be detected, a trend in leaf tissue fibre content for both immature and mature tissue is evident with C0 consistently having a lower fibre content (Table 7.1). Fibre content of immature leaf tissue was only 65% of that found in mature tissue. Elevated fibre content in the diet of insects can increase the bulk density so that larvae cannot eat enough to complete development (Bernays 1986).

Cell wall composition of BS9 whorl tissue showed increased levels of acid detergent fibre and ash but lignin content did not change from C0 through to C5 (Buendgen et al. 1990). Likewise, base-hydrolyzed lignin in the present study did not change significantly (Appendix 5). Other cell wall constituents that are more precisely quanitified include the simple phenolic acids (*E*-p-coumaric and *E*-ferulic acids) and their associated dimers dehydrodiferulic acid and substituted cyclobutane dimers. For leaf tissue the levels of simple phenolic acids in the cell wall varied considerably with ferulic acid showing no trend while p-coumaric acid in mature tissue increased over cycles of selection (Table 7.1). Substituted cyclobutane dimers are produced by the absorption of high energy light. Therefore immature whorl tissue had only trace amounts (data not shown) while C4 and C5 of mature tissue had higher levels than earlier cycles of mature leaf tissue. This may in part be accounted for by the slower growth rate and shorter leaf extension observed for cycles 4 and 5 which may permit greater light penetration into the lower canopy for photochemical reactions (see Chapter 4, leaf profile of cyclobutane). Dehydrodiferulic acid in immature whorl tissue differed significantly among selection cycles and correlated with bioassay consumption (r=-0.75*, n=4) with comparable increases being observed for mature tissue. Dehydrodiferulic acid may contribute to

Table 7.2. Nutritional and resistance factors in sheath tissue from different cycles of BS9, 1991.

Parameter†	C0	C2	C4	C5	LSD‡
Protein	3.79	3.55	3.51	4.01	0.65
DIMBOA	0.48	0.16	0.16	0.23	0.37
Fibre	303	376	376	390	39
FA	2.03	4.85	4.55	3.11	2.79
pCA	3.99	6.71	5.55	5.21	1.31*
CBD	0.49	0.51	0.74	0.58	0.35
DFA	0.99	1.06	1.25	1.35	0.17**

† Refer to Table 1 for abreviations.

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

Table 7.3. Nutritional and resistance factors in stalk, node and pith tissue from different cycles of BS9.

		1991					1992				
Tissue	Parameter[1]	C0	C2	C4	C5	LSD[1]	C0	C2	C4	C5	LSD[1]
Stalk	Protein	3.17	3.25	2.67	2.58	1.47	3.35	3.22	3.05	2.81	1.24
	DIMBOA	0.72	0.28	0.33	0.41	0.59	0.23	0.39	0.47	0.21	0.82
	Fibre	389	370	366	419	61	424	434	456	444	23
	FA	2.22	1.96	1.96	1.99	0.55	1.8	2.1	2.29	2.4	0.53
	pCA	8.42	8.58	8.36	8.15	0.76	7.75	8.36	8.62	8.29	1.04
	CBD	0.65	0.59	0.66	0.53	0.18	0.57	0.59	0.47	0.72	0.25
	DFA	0.75	0.89	0.75	0.82	0.12	0.74	0.88	0.87	0.85	0.12
Node	Protein	4.13	4.76	4.09	4.27	1.55	5.72	4.81	4.29	4.03	1.34
	DIMBOA	1.53	0.99	1.42	1.38	1.04	0.41	0.59	0.59	0.47	0.36
	Fibre	351	362	368	383	44	366	361	406	389	39
	FA	2.23	2.24	2.12	2.54	0.37	2.18	2.88	2.02	-	1.04
	pCA	7.58	7.71	7.95	8.42	0.84	7.77	8.11	7.8	-	1.01
	CBD	0.42	0.42	0.76	0.96	0.43*	0.8	0.63	0.52	-	0.32
	DFA	0.94	0.89	0.88	1.02	0.13	0.59	0.98	0.84	-	0.29*
Pith	Protein	4.52	5.11	4.75	4.32	2.37	5.36	4.62	3.97	4.49	1.31
	DIMBOA	0.19	0.29	0.21	0.09	0.23	0.17	0.24	0.28	0.49	0.21
	Fibre	185	190	174	173	38	182	174	191	192	19
	FA	1.2	2.25	1.09	1.28	0.49*	1.4	1.13	1.4	1.2	0.89
	pCA	3.69	4.88	3.59	4.21	0.98*	3.84	4.23	4.28	3.82	0.43
	CBD	0.06	0.23	0.05	0.04	0.15*	0.16	0.08	0	0.06	0.11*
	DFA	0.85	0.8	0.91	0.87	0.43	0.85	0.75	1.15	1.15	0.38
	N Larvae	0.53	0.36	0.17	0.22	0.23*	0.42	0.47	0.32	0.32	0.16
	N Tunnel	1.67	1.56	0.77	0.92	0.98	1.27	1.15	0.45	0.65	0.45**
	Tun.	6.67	6.33	5.77	8.33	2.77	8.44	7.16	6.84	4.18	2.71**
	Tunnel Ht.	1.83	1.71	2.01	1.87	0.51	1.81	1.43	0.86	0.98	0.69*
	X-section	52.5	40.8	40.3	45.1	9.1*	34.8	32.2	15.1	27.4	12.9*
	Toughness	4.1	4.7	5.12	5.98	0.5**	3.3	4.1	5.8	4.9	0.5**

[1] Refer to Table 1 for abreviations. N larvae and N tunnel are average number of larvae and tunnels per plant. Stalk toughness reported in kg. X-section is percent cross-sectional area of stalk excavated by ECB.

immature-tissue resistance by fortifying primary cell walls to provide modest levels of tissue toughness in the absence of lignin while maintaining tissue extensibility required for leaf growth.

Leaf-sheath tissue differs considerably in composition from leaf blade tissue in having lower protein and DIMBOA content while having higher fibre content and higher levels of simple phenolic acids and higher dehydrodiferulic acid content (Table 7.2). Buendgen et al. (1990) found that high concentrations of neutral detergent fibre, acid detergent fibre, cellulose, lignin and ash in a high silica population of maize did not render this population more resistant to second gerneration ECB than the BS9 population which had lower levels of these cell wall constituents. It may be that the high silica population reported had low levels of simple phenolics and dehydrodiferulic acid which appear to be selected for in the BS9 population. High fibre content alone may increase the bulk density of the tissue, but in the presence of phenolic-carbohydrate complexes, fibre strength may increase, providing a tougher physical barrier to restrict insect penetration into sheath tissue and render nutrients within the tissue less accessible.

Insect performance on the four different selection cycles indicates that cycles 4 and 5 are more resistant to plant damage (Table 7.3). Larval survivorship was significantly lower for the later two cycles while the number of tunnels per stalk and the total tunnel length was also reduced. The mean height of stalk tunneling was lower for 1992 with C4 and C5 having the lowest average tunnelling height which may in part be attributed to reduced plant height over selection cycles (Nyhus et al. 1989, Grombacher et al. 1989b). Excavation of pith tissue dropped significantly over the cycles of selection (X-section, Table 7.3) The importance of pith excavation is most prevalent in drought stressed environments in which tunneling causes a reduction in yield through reduced kernel size (Godfrey et al. 1991).

Stalk tissue (Table 7.3) was much like sheath tissue (Table 7.2) in having low protein content and DIMBOA levels while having higher levels of cell wall phenolic acids and fibre content. p-Coumaric acid levels were particularly high in stalk and node tissue. Ferulic acid remained relatively constant between different tissue types. Consistent levels of ferulic acid may in part be explained by little change in ferulic acid between different cell types (Grabber and Jung 1991). Pith tissue differed from node and stalk tissue in having marginally higher

protein and lower levels of phenolic acids and containing only trace amounts of cyclobutane dimers presumably due to the absence of UV light. For each of the three stalk tissues, p-coumaric acid and dehydrodiferulic acid levels correlated negatively with the number of larvae and tunnels, and cross-section excavation of stalk. Over selection cycles, protein content decreased within stalk tissues, which correlated with the above plant damage parameters. From these observations it appears that a nutritional mechanism of resistance is operating whereby stalk tissue is rendered tougher (Table 7.3) by the combined effects of elevated fibre and phenolic-carbohydrate complexes which may serve as cross-linking agents and by a lower nutritional value of plant tissue as indicated by reduced nitrogen content over cycles of selection. Cell wall bound phenolic acids have previously been correlated with maize-kernel toughness and maize resistance to the storage pest *Sitophilus zeamais* (Classen et al. 1990). ECB larvae develop and migrate to the protective tissue within the stalk where the low levels of protein and fibre of the pith (Tables 7.3) may facilitate higher consumption rates by larvae to sequester sufficient nitrogen for development within the pith. According to Scriber and Slansky (1981) insect consumption usually increases with reductions in diet nitrogen; this is observed for pith protein in which the average tunnel length per tunnel (calculated from Table 7.3) correlated negatively with pith protein ($r=-0.66$, $n=4$).

Localization of phenolic constituents within the cell wall of grasses by microspectrophotometry has provided a quantitative measure of total phenolics within specific cell types (Hartley et al. 1990, Akin et al. 1990b). For maize resistance to ECB, the abaxial epidermal absorbance of ultraviolet light by cell wall phenolics appears to be a good indicator of leaf-feeding resistance (Chapter 3). For the BS9 population, leaf, sheath and stalk epidermal cell-wall absorption generally increased over the cycles of selection for (Table 7.4). For ECB resistance, fortification of epidermal cell wall tissue in leaf, sheath and stalk is likely most important as a physical barrier to restrict tissue penetration rather than to reduce digestibility. Increased epidermal cell wall absorption of UV light (Table 7.4) is parrelled by increased leaf (Table 7.1) and stalk (Table 7.3) toughness. Previous chapters suggested that the importance of epidermal toughness is to restrict neonate larval access to water, thus leading to their desiccation resulting from exposure and large surface-area-to-volume ratio.

Table 7.4. Microspectrophotometer absorbance readings (327 nm) for epidermal cell wall of BS9 leaf, sheath and stalk tissue.

Tissue	C0	C2	C4	C5	LSD‡
Leaf	0.9	0.91	1.07	1.15	0.16**
Sheath	1.98	1.82	2.03	1.86	0.21
Stalk	1.58	1.84	2.06	1.94	0.21*

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

Given the Iowa stiff stalk (BSSS) genetic background of BS9, plant toughness probably represents an important component of the improved leaf-feeding resistance and reduced plant damage observed over the six cycles of selection represented in this study. Selection also resulted in elevated levels of DIMBOA in the immature leaf whorl tissue, which is not physically fortified. Larval feeding on mature leaf tissue is further reduced by elevated levels of cell wall phenolics. Based on the negative correlation of leaf epidermal absorbance and field leaf rating ($r=-0.97^*$, $n=4$), epidermal cell wall fortification to enhance leaf toughness is likely a major component of field resistance by restricting larval access to nutrients. Second generation larvae feeding on leaf sheath tissue are also faced with elevated tissue toughness through increased levels of phenolic acids and phenolic dimers such as dehydrodiferulic acid (Tables 7.2 and 7.3) in addition to higher fibre content (Buendgen et al. 1990). Plant damage by first generation ECB is considered more extensive than second generation ECB (Hudon et al. 1991); one explanation for this could be the increased toughness in plant tissue associated with maturity which would be more difficult for neonate larvae to penetrate and establish leaf feeding.

Selection for ECB resistance in the BS9 population has resulted in a synthetic that shows good resistance to both leaf feeding and stalk damage. This resistance is achieved by the combination of several resistance factors that operate within different tissues at different times in the plant's development. First generation ECB larvae prefer to feed within the immature whorl tissue where the more resistant C4 and C5 have considerably higher levels of DIMBOA than other cycles. In addition to DIMBOA, dehydrodiferulic acid within the immature leaf tissue increased with selection cycles and may serve to increase the toughness of this tender tissue. Feeding on mature leaf and stalk tissues appears to be affected by toughness factors, with p-coumaric acid, dehydrodiferulic acid and fibre probably making the major contributions to varietal differences in tissue toughness. The overall quantity of phenolics within the plant may not be as important as the positioning of the phenolic resources within the epidermal cell wall to restrict larval penetration. Over the course of selection epidermal cell wall fortification increased significantly for leaf and stalk tissue.

CHAPTER VIII.

PHYTOCHEMICAL BASIS FOR MULTIPLE BORER RESISTANT MAIZE.

8.1 Introduction

Lepidopteran stalk-boring larvae cause economic losses to maize production throughout the world (Dicke and Guthrie 1988, Ortega et al. 1980). Host plant resistance is an effective and environmentally safe means for control of these pests. A source population with multiple borer resistance (MBR) was developed by recombination and recurrent selection under infestation with southwestern corn borer (SWCB), sugarcane borer (SCB), European corn borer (ECB) and fall armyworm (FAW) (Smith et al. 1989, Benson 1986, Mihm 1985). Tropical maize resistant to both generations of ECB were found to have low levels of the the conventional resistance factor DIMBOA (Benson 1986, Scriber et al. 1975, Sullivan et al. 1974). Silica and lignin content appear to be important resistance factors in tropical maize (Rojanaridpiched et al. 1984). Caribbean germplasm, which also does not involve a DIMBOA-based resistance, showed an association between fibre content of the whorl and SWCB resistance (Hedin et al. 1984). Tropical maize resistance appears to be polygenically controlled and involves primarily additive variation (Hinderliter 1983, Onukogu et al. 1978, Scott et al. 1967).

The primary objective of this study was to conduct a phytochemical screening of MBR cultivars developed at CIMMYT and commercially available checks to elucidate the phytochemical components that best predict the observed field resistance, bioassay studies and leaf toughness. Although not exhaustive, the list of parameters measured included all resistance parameters studied to date as well as cell wall bound phenolics mentioned in previous chapters. The second objective was to develop a simple phytochemical model that would account for the field resistance of MBR material to the European corn borer.

8.2 Materials and Methods

Germplasm and Screening: MBR hybrids included Across 86590(IR), Mbita 86590 (Chilo), Poza Rica 86590 (SCB), Across 86590-2 (ECB), Tlaltizapan 85590 (SWCB), CML135xCML139, Ki-3 x Tx601. MBR adapted inbreds included 6796-13, -49 and -48

(1990 only). Four commercial checks included Fontanelle 6220, Pioneer 3184, Dekalb 435 and Picseed 4533. MBR lines and hybrids were developed at CIMMYT. Planting occurred in mid-May of 1990 and 1991 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. The rows were spaced 0.9 m apart and plants were spaced at 0.15 m with 30 plants per row. The soil was a sandy loam. Four replicates were planted in a complete randomized block design. In 1990 fifteen plants in each row were infested with two applications of three egg masses (ca. 120 eggs) and for 1991 the Bazooka infestation method (Guthrie and Barry 1989) was used to apply ca. 80 larvae per plant. Three weeks after infestation (July 20 to 27) plants were rated according to Guthrie et al. (1960) 9 point scale. Plants were dissected from late September through early October to count the number of larvae, number and length of tunnels, position of tunnelling and estimated cross-section of pith excavated by larval feeding.

Sample Collection: Leaf sampling procedure is outlined in Chapter 7.

Bioassays: Bioassay procedure is outlined in Chapter 3. Mean areas consumed were determined from 40 leaf disks per genotype and tissue type.

Leaf Toughness: Using the method reported in Chapter 2, five force measurements were taken from each row for a total of 20 measurements.

Protein Determinations: Protein content was estimated by the micro-Kjelhdahl method on 0.3 g samples using the conversion factor 6.25 to estimate protein from N.

Phytochemical Analysis: Phenolic conjugates, hydroxamic acids and cell wall bound phenolics were determined using the procedure outlined in Chapter 3.

Lignin Determinations: A modified acetyl bromide procedure outlined by Iiyama and Wallis (1990) was used. After base hydrolysis, the fibre pellet that remained was dried in a desiccator and 50 mg were used for lignin analysis. These determinations were carried out on 1990 tissue only.

Statistical analysis: All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Leaf rating data, bioassay consumption, leaf toughness and number of larvae per plant were transformed by ln(x+1) to satisfy the assumptions of the general linear model. Forward regressions were done using the forward option in PROC REG.

8.3 Results and Discussion

Leaf feeding damage and bioassay consumption of green tissue by ECB larvae were both negatively correlated (r=-0.58* and -0.81**, respectively (1990)) with leaf toughness (Table 8.1). Likewise, the number of larvae, number of stalk tunnels, length of tunnelling and cross-section of pith excavated were negatively correlated with leaf toughness (Tables 8.1 and 8.2). These correlations indicate a reduced capacity of insects to establish on genotypes that have tougher leaf tissue. Neonate mortality often exceeds 80% for the first two days post-eclosion (Ross and Ostlie 1990). One mortality factor is desiccation (Lee 1988), wereby larvae that cannot penetrate leaf tissue shortly after eclosion may desiccate. Although later instars have little difficulty penetrating mature leaf tissue as observed in leaf bioassays, neonates may not have the mandibular strength to penetrate tougher leaves. This incapacity may account for their migration into the whorl of the plant. This reasoning has been used to explain the feeding behavior of the southwestern corn borer, *Diatraea grandiosella* (Hedin et al. 1984).

Plant nitrogen is a major determinant of insect growth and can be a component of plant resistance (Scriber and Slansky 1981). Leaf protein content correlated positively with number of larvae (r=0.55* for 1991), length of tunnelling for both 1990 and 1991 (r=0.56* and 0.47, respectively) and with cross-sectional consumption of pith in 1990 (r=0.53*) (Tables 8.1 and 8.2). These observations suggest that more resistant genotypes with lower leaf-protein content may not provide sufficient accessible protein to facilitate larval development beyond early instars. This hypothesis is supported by field observations of differential size and biomass of SWCB larvae grown on resistant (MBR) and susceptible plants (Davis et al. 1988). Protein availability likely works in conjunction with elevated levels of cell wall components because protein content is inversely related to both leaf toughness (Tables 8.3 and 8.4) and cell wall weight for mature leaf tissue (data not shown), a relationship observed for many plant species (Hedin et al. 1984, Van Soest 1981). However, water content of maize leaf tissue does co-correlate with nitrogen content (Manuwoto and Scriber 1985a), and could provide an explanation for the observed correlations. Water content of leaf tissue was not determined in the present study.

Table 8.1. Correlations between plant damage parameters for multiple borer resistant cultivars, 1990[1].

	Bioassay	Leaf Toughness	Leaf Protein	N Larvae	Tunnel Length	X-section	Stalk Rot	Stalk Toughness
Leaf Rating	0.38	-0.58*	0.53	0.37	0.55*	0.51*	0.18	0.21
Bioassay		-0.81**	0.74**	0.26	0.84***	0.74**	0.70**	-0.13
Leaf Toughness			-0.65**	-0.48	-0.57*	-0.82***	0.73**	-0.11
Protein				0.15	0.56*	0.53*	0.33	-0.08
N Larvae					0.62**	0.64**	0.64**	-0.08
Tunnel Length						0.94***	0.88***	0.73**
X-section							0.83***	0.13
Stalk Rot								0.16

[1] Leaf rating, bioassay, leaf toughness, and number of larvae were transformed by ln(x+1) prior to statistical analysis (n=13).

Table 8.2. Correlations between plant damage parameters for multiple borer resistant cultivars, 1991[1].

	Bioassay	Leaf Toughness	Leaf Protein	N Larvae	N Tunnel	Tunnel Length	X-section	Tunnel Height
Leaf Rating	0.37	-0.81***	0.27	0.66**	0.65**	0.55*	0.65**	-0.49
Bioassay		-0.45*	0.83***	0.58*	0.63**	0.58*	-0.02	-0.32
Leaf Toughness			-0.36	-0.66**	-0.64**	-0.57*	-0.43	0.25
Protein				0.55*	0.55*	0.47	-0.17	-0.26
N Larvae					0.95***	0.84***	0.24	-0.29
N Tunnel						0.92***	0.25	-0.17
Tunnel Length							0.24	-0.17
X-section								-0.37

[1] Leaf rating, bioassay, leaf toughness, and number of larvae were transformed by ln(x+1) prior to statistical analysis (n=13).

Table 8.3. Correlations of biochemical parameters with plant damage parameters for multiple borer resistant cultivars, 1990.

Tissue Type	Independent Variable	Field Leaf Ratings[1]			Bioassay Feeding[1]			Leaf Toughness[1]		
		r	F	P > |T|	r	F	P > |T|	r	F	P > |T|
Green	Protein	0.82	23.9	<0001	0.67	9.88	0.009	-0.65	7.94	0.01
	Wt. Solubles	-0.13	0.22	0.64	0.08	0.09	0.77	0.06	0.05	0.82
	DIMBOA	0.24	0.74	0.41	0.27	0.94	0.35	-0.45	2.86	0.12
	pCA (sol.)	0.39	2.02	0.18	0.37	1.73	0.21	-0.51	3.58	0.09
	FA (sol.)	0.63	7.91	0.02	0.69	10.9	0.006	-0.64	7.81	0.02
	Flavonoids	0.16	0.33	0.57	0.31	1.23	0.28	-0.45	2.81	0.12
	Wt. Cell Wall	-0.14	0.26	0.62	-0.13	0.21	0.65	0.12	0.16	0.69
	pCA (CW)	-0.55	5.24	0.04	-0.65	8.91	0.01	0.69	10.2	0.009
	FA (CW)	-0.52	4.38	0.05	-0.44	2.82	0.11	0.51	3.84	0.07
	Tx	0	0.01	0.93	0.08	0.074	0.79	-0.12	0.17	0.68
	DFA	-0.76	16.9	0.001	-0.66	9.31	0.01	0.68	9.27	0.01
	Lignin (%)	0.06	0.01	0.94	-0.07	0.07	0.79	0.16	0.29	0.59
Yellow	Protein	0.32	1.37	0.26	-	-	-	-0.46	3.05	0.11
	Wt. Solubles	0.22	0.65	0.44	-	-	-	-0.54	4.43	0.05
	DIMBOA	0.11	0.13	0.73	-	-	-	-0.47	3.12	0.11
	pCA (sol.)	0.54	5.13	0.04	-	-	-	-0.19	0.41	0.54
	FA (sol.)	0.51	4.04	0.06	-	-	-	-0.14	0.24	0.64
	Flavonoids	0.31	1.31	0.27	-	-	-	-0.22	0.57	0.47
	Wt. Cell Wall	-0.36	1.85	0.2	-	-	-	0.83	24.3	<0.001
	pCA (CW)	-0.34	1.59	0.23	-	-	-	0.59	5.95	0.03
	FA (CW)	-0.25	0.79	0.38	-	-	-	0.42	2.35	0.15
	Tx	-0.14	0.25	0.62	-	-	-	0.05	0.02	0.88
	DFA	-0.42	2.56	0.13	-	-	-	0.66	8.29	0.01

[1] Field rating, bioassay feeding and leaf toughness were transformed by ln(x +1) prior to statistical analysis (n=13).
Tx=total truxillic and truxinic acids, DFA=dehydrodiferulic acid.

Table 8.4. Correlations of biochemical parameters with plant damage parameters for multiple borer resistant cultivars, 1991.

Tissue Type	Independent Variable	Field Leaf Ratings†			Bioassay Feeding†			Leaf Toughness†		
		r	F	P > |T|	r	F	P > |T|	r	F	P > |T|
Green	Protein	0.81	20.1	<0.001	0.65	8.48	0.01	-0.71	10.5	0.009
	Wt. Solubles	0.51	3.83	0.07	0.66	8.92	0.01	-0.63	6.54	0.03
	DIMBOA	0.53	4.28	0.06	0.51	3.98	0.07	-0.35	1.41	0.26
	pCA (sol.)	0.73	12.8	0.004	0.54	4.53	0.05	-0.51	3.52	0.09
	FA (sol.)	0.69	10.1	0.009	0.54	4.53	0.05	-0.5	3.27	0.1
	Flavonoids	0.11	0.12	0.73	0.44	2.64	0.13	-0.22	0.49	0.49
	Wt. Cell Wall	-0.74	13.2	0.004	-0.56	5.24	0.04	0.75	12.7	0.005
	pCA (CW)	-0.58	5.65	0.03	-0.69	10.4	0.008	0.37	1.65	0.22
	FA (CW)	-0.71	10.7	0.007	-0.74	13.5	0.004	0.47	2.78	0.12
	Tx	-0.64	7.81	0.02	-0.81	21.9	<0.001	0.39	1.77	0.23
	DFA	-0.89	46.1	<0.001	-0.56	5.23	0.04	0.73	11.37	0.007
Yellow	Protein	0.77	16.4	0.002	0.31	1.21	0.29	-0.62	6.32	0.03
	Wt. Solubles	-0.39	2.01	0.18	-0.35	1.55	0.24	0.69	9.28	0.01
	DIMBOA	0.32	1.24	0.28	0.46	2.96	0.11	-0.31	1.06	0.33
	pCA (sol.)	0.14	0.24	0.63	0.12	0.15	0.71	0.43	2.22	0.17
	FA (sol.)	0.59	6.16	0.03	0.29	1.06	0.32	-0.28	0.83	0.38
	Flavonoids	0.47	3.07	0.11	0.47	3.12	0.11	-0.31	1.06	0.33
	Wt. Cell Wall	-0.09	0.02	0.89	0.08	0.07	0.78	0.07	0.05	0.83
	pCA (CW)	0.43	2.56	0.13	0.24	0.74	0.41	-0.74	12.2	0.005
	FA (CW)	0.09	0.09	0.77	0.09	0.11	0.75	-0.26	0.85	0.38
	Tx	-0.26	0.81	0.39	-0.08	0.06	0.81	0.16	0.27	0.61
	DFA	-0.52	4.18	0.06	-0.32	1.25	0.28	0.62	6.23	0.03

† Field rating, bioassay feeding and leaf toughness were transformed by ln(x +1) prior to statistical analysis (n=13).

Soluble phytochemicals such as DIMBOA, ferulic and p-coumaric acid conjugates and flavonoids were correlated positively with leaf feeding and negatively with toughness, with the stronger correlations being observed for mature leaf tissue (Tables 8.3 and 8.4). Two possible explanations for this correlation are: 1) larvae are induced to feed in the presence of these phytochemicals which are host recognition factors or 2) elevated levels of soluble secondary metabolites may reflect poor efficiency of incorporation of phenolics into cell wall components that correlate with leaf toughness. Semipurified preparations of phenolic conjugates obtained from MBR genotypes were phagostimulants at ecologically relevant dosages (10 $\mu g/cm^2$) and became phytotoxic and antifeedants at 100 $\mu g/cm^2$ (Appendix 2). DIMBOA can increase consumption due to inhibition of digestive proteases in insects, thus requiring greater consumption to assimilate sufficient nitrogen for larval development (Houseman et al. 1992). It is probably a combination of all these factors that contributes to the higher consumption of genotypes with higher levels of soluble components.

Cell wall weight and cell wall bound phenolic acids in mature, green, tissue were negatively correlated with field leaf damage ratings and bioassay feeding and positively correlated with leaf toughness (Tables 8.3 and 8.4). The same trends were observed for the 1990 immature tissue. One reason why immature tissue from 1991 did not follow this trend was that the harvesting of plants occurred over a shorter time so that plants with higher heat unit requirements (ie. MBR cultivars) were less developed at the time of phytochemical sampling. This may have contributed to lower cell wall phenolic levels in these genotypes. Maize-grain resistance to storage pests has been previously correlated with cell wall ferulic acid levels and kernel toughness (Classen et al. 1990). Cell wall bound p-coumaric acid showed stronger correlations with the dependent variables than ferulic acid. This can be attributed to p-coumaric acid being more prevalent in secondary cell wall tissue due to its prominent role in lignin linkage to polysaccharides (Goto et al. 1991, Lam et al. 1990, Higuchi et al 1967). Such lignin linkages likely contribute to cell wall fortification and tissue toughness.

The most consistent relationship was observed between dehydrodiferulic acid and variables of insect resistance. Dehydrodiferulic acid, like the cyclobutane dimers, is presumed to facilitate cell wall cross-linking to increase mechanical strength (Ishii 1991, Fry 1986,

Hartley and Jones 1976, Markwalder and Neukom 1976). This is shown by the positive correlation of dehydrodiferulic acid (DFA) content and leaf toughness ($r=0.73^{**}$ to 0.62^{*}, Tables 8.2 and 8.3). Because cyclobutane dimer (Tx) production is largely under environmental control a poor correlation was observed for both leaf toughness and leaf damage rating for this photoactivated dimer. On the other hand, dehydrodiferulic acid is produced by a peroxidase that could be manipulated in the future to increase production of phenolic dimers and possibly cell wall toughness.

A peroxidase is also involved in lignin formation by oxidizing the hydroxyl group of cinnamyl alcohols to yield free radical species which then couple in random fashion (Lewis and Yamamoto 1990). With dehydrodiferulic acids occurring earlier in maturing tissue it may be an early mechanism of cell wall fortification in which the predominant primary cell wall phenolic, ferulic acid, is the major substrate for cell wall-associated peroxidases to act upon. If the same peroxidase isozymes catalyze both dehydrodiferulic acid and lignin production, then dehydrodiferulic acid levels may serve as a quantitative indicator for *in situ* peroxidase activity. Two cell wall peroxidases (Px1, Px2) have recently been isolated from epicotyls of *Cicer arietinum*, with Px2 showing increased specific activity with tissue age and Px1 showing no relationship (Valero et al. 1991). Based on acidic pH inhibition and association with cell wall stiffening, Px2 is likely involved in lignin production but dehydrodiferulic acid production could conceivably be produced by Px1 which shows age independent activity which is consistent with dehydrodiferulic acid production in immature tissue (Appendix 7). The cell wall peroxidases in maize have not been characterized to date but would be useful in elucidating the enzymatic origin of these two phenolic groups.

Forward regressions between biochemical parameters as independent variables and plant resistance parameters as dependent variables are shown in Table 8.5. With the exception of immature leaf bioassays for 1991, all regression models exceeded an r^2 value of 0.7. The most common independent variables within these models were protein content (PRO), fibre content (CW) and dehydrodiferulic acid (DFA) content. A fourth variable that occurred often was p-coumaric acid either in soluble form or cell wall bound form with the former being a positive predictor in the regression model for leaf rating and the latter being a positive predictor of leaf toughness (Table 8.5). Incorporating protein, fibre and dehydrodiferulic acid

Table 8.5. Forward multiple regressions of biochemical parameters with plant resistance parameters for multiple borer resistant cultivars, 1990 and 1991.

Tissue Type	Year	Dependent Variable	Regression Equation[1]	r^2	F	P > |T|
Green	1990	Leaf Rating	LLR = -0.621 + 0.129(PRO) - 0.541(DFA) + 0.0015(pCA sol.)	0.78	10.6	0.003
		Bioassay	LBA = 0.605 + 0.223(PRO) - 0.458(FA) + 0.0043(FA sol.)	0.79	11.1	0.002
		Leaf Toughness	LLT = 0.408 + 0.124(DFA) + 0.061(pCA) - 0.0004(FA sol.)	0.78	9.45	0.005
	1991	Leaf Rating	LLR = 2.114 - 2.61(DFA) + 0.354(pCA sol.) - 0.156(FLAV)	0.91	29.8	<0.001
		Bioassay	LBA = 1.859 + 0.089(PRO) - 0.804(TX) + 0.445(FLAV)	0.81	12.7	0.001
		Leaf Toughness	LLT = 0.058 + 0.0017(CW) + 0.414(DFA) - 0.089(FA)	0.81	10.7	0.004
Yellow	1990	Leaf Rating	LLR = 2.236 - 0.0033(CW) - 0.850(DFA) + 0.0011(pCA sol.)	0.71	8.11	0.005
		Leaf Toughness	LLT = -0.167 + 0.0029(CW) + 0.0419(SOL) - 0.0001(pCA sol.)	0.89	24.8	<0.001
	1991	Leaf Rating	LLR = 0.472 + 0.127(PRO) - 1.661(DFA) - 0.828(TX)	0.84	15.8	<0.001
		Bioassay	LBA = 0.729 + 0.215(FA) 0.436(D) + 0.436(FLAV)	0.16	0.29	0.59
		Leaf Toughness	LLT = 0.766 - 0.013(PRO) + 0.0006(CW) - 0.037(pCA)	0.71	6.55	0.01

[1] Leaf rating, bioassay and leaf toughness were transformed by ln(x+1) prior to statistical analysis (n=13 for both years).

content into a fixed regression model for each year and tissue type accounts for a reasonable amount of the variation, with the more consistent and more significant models being observed for mature leaf tissue (Table 8.6). Although not providing direct evidence for the mechanism of MBR resistance, it is apparent that much of the variablity of field leaf ratings and leaf toughness can be accounted for by these three parameters. These regressions support the hypothesis that MBR genotypes employ a nutritional resistance mechanism whereby lower protein content acts in concert with increased cell wall mechanical strength, manifested through higher fibre content and higher levels of cell wall phenolics, to reduce nutrient availability to early instar larvae. This model is consistent with early reports of fall armyworm showing reduced digestion of Bermuda grass with high cell wall content (Quisenberry and Wilson 1985) and may explain the reduced growth rate of southwestern corn borer larvae feeding on MBR cultivars compared to those feeding on susceptible cultivars (Davis et al. 1988).

Inheritance of multiple borer resistance appears to be polygenically controlled, and involves primarily additive variation (Smith et al. 1989, Hinderliter 1983). This observation is consistent with our proposed mechanism of resistance which involves three polygenic components, namely protein, fibre and cell wall phenolic acid content. A fourth component of this resistance model is the peroxidase-mediated production of dehydrodiferulic acid which may account for the nominal dominant inheritance observed for MBR germplasm (Smith et al. 1989). The main advantages of polygenic resistance are the reduced likelihood of development of resistance by pest populations and an effectiveness over a broader spectrum of pest organisms. To date, resistance break down of MBR material has not been reported and some MBR germplasm is effective against several borers belonging to different genera (Mihm 1985).

Table 8.6. Multiple regressions of protein, fibre and dehydrodiferulic acid levels with plant resistance parameters for multiple borer resistant cultivars, 1990 and 1991.

Tissue Type	Year	Dependent Variable	Regression Equation[†]	r^2	F	P > |T|
Green	1990	Leaf Rating	LLR = 0.108 +0.136(PRO) - 0.0023(CW) - 0.683(DFA)	0.78	12	0.001
		Bioassay	LBA = 1.794 + 0.188(PRO) - 0.0032(CW) - 1.30(DFA)	0.58	4.51	0.03
		Leaf Toughness	LLT = 0.664 - 0.015 + 0.0003(CW) + 0.146(DFA)	0.54	3.47	0.06
	1991	Leaf Rating	LLR = 4.175 - 0.032(PRO) - 0.0034(CW) -3.746(DFA)	0.87	19.8	<0.001
		Bioassay	LBA = 1.390 + 0.150(PRO) - 0.0035(CW) +0.785(DFA)	0.46	2.58	0.11
		Leaf Toughness	LLT = 0.165 + 0.00069(PRO) + 0.00088(CW) + 0.329(DFA)	0.68	5.44	0.02
Yellow	1990	Leaf Rating	LLR = 1.869 + 0.0075(PRO) - 0.0018(CW) - 0.512(DFA)	0.22	0.92	0.46
		Leaf Toughness	LLT = 0.221 - 0.00038(PRO) + 0.00179(CW) - 0.016(DFA)	0.69	6.61	0.01
	1991	Leaf Rating	LLR = 0.625 +0.138(PRO) - 0.0024(CW) - 1.332(DFA)	0.76	9.71	0.004
		Bioassay	LLB = 2.144 + 0.069(PRO) + 0.0025(CW) -1.878(DFA)	0.18	0.64	0.61
		Leaf Toughness	LLT = 0.651 - 0.016(PRO) - 0.00013(CW) + 0.364(DFA)	0.65	4.98	0.03

[†] Leaf rating, bioassay and leaf toughness were transformed by ln(x+1) prior to statistical analysis (n=13 for both years).

CHAPTER IX.
GENERAL CONCLUSIONS AND DISCUSSION

9.1 Main Conclusions

Although maize resistance to the European corn borer has been the most extensively studied interaction of all the lepidopteran pests of maize, some questions remain that this work has attempted to address. These questions included: 1) Why do larvae prefer feeding within the whorl despite the elevated levels of DIMBOA, the conventional resistance factor? 2) At what stage is the leaf tissue most susceptible to larval feeding? 3) Why are greenhouse-grown plants more susceptible despite having higher levels of DIMBOA? 4) What traits are being selected for in recurrent selection of resistant maize synthetics? 5) What is the phytochemical basis of multiple borer resistant genotypes developed at CIMMYT? To gain further insights into resistance and to better address these questions two new tools to monitor plant resistance were developed. A microspectrophotometer technique was developed to determine cell wall absorbance which provided an estimate of phenolic content and density of cell walls between different cell types, tissues and genotypes (Chapter 3). The second tool consisted of quantitative leaf toughness measurements using an instron. Leaf toughness was found to correlate well with field leaf ratings for all five different germplasm groups tested which points to a physical resistance component common to several maize groups (Chapter 2).

To determine why larvae prefer to feed on immature tissue within the whorl, the 13th leaf which contained both mature and immature leaf tissue of the resistant synthetic BS9 was partitioned into eight sections along the entire leaf length (Chapter 4). Bioassay results showed that third instar larvae consume immature tissue at a higher rate than mature tissue despite the presence in this tissue of over twice the level of hydroxamic acids of mature tissue. Protein content did not account for the feeding preference. Leaf consumption increased with increases in soluble metabolites, including phenolic glycosides, which appear to be phagostimulants at lower dosages (Appendix 2). Leaf consumption was inversely related to leaf toughness which in turn was related to fibre and cell wall phenolics. Water content is generally inversely related to toughness and could also contribute to the observed insect

feeding preference for moist immature tissue. Although feeding behaviour is also dictated by the abiotic environment and avoidance of natural enemies, it appears that larvae also migrate into the whorl to feed on tender tissue which is lower in fibre and cell wall phenolics. The apparent preference for immature tissue within the whorl with high DIMBOA content can be rationalized by considering leaf toughness to be the major component in resistance. Leaf toughness cannot be employed in the immature tissue because of cell wall extensibility requirements, so DIMBOA is utilized. Larval consumption may have been even higher if no DIMBOA were present.

The stage at which maize leaves are most aggressively consumed by ECB larvae is in the early stages of plant development (Chapter 5). Leaf consumption of plants in the 5 leaf stage was significantly higher than that observed for 7, 9 and 10 leaf stages. Leaf toughness again provided an explanation for this preference as DIMBOA was highest in the earlier stages of plant development. Cell wall phenolics increased markedly from the 7 to 9 leaf stage which corresponded to a drop in leaf consumption. Leaf consumption and phenolic complexes were also influenced by the growth environment with greenhouse-grown plants having lower levels of cyclobutane dimers which are capable of crosslinking cell wall hemicellulose to increase leaf toughness. This may provide an explanation for increased feeding damage observed for greenhouse-grown plants (Guthrie et al. 1986b). To observe just the effect of near UV light on field resistance, greenhouse plastic was used to block ultraviolet (UV) light during plant development (Chapter 6). Plants grown under reduced UV light had higher leaf-damage ratings and bioassay consumption rates than plants grown under UV-transparent plastic or those grown in open sunlight. The major phytochemical difference was the higher ratio of cyclobutane dimers to cell wall ferulic and p-coumaric acids. This observation may also account for the increased damage rating observed in CIMMYT screening trials during cloudy summers or during the winter when sunlight intensity is lower (J.A. Mihm, pers. comm.).

Recurrent selection has become a popular method of obtaining resistant genotypes but the mechanism(s) of this resistance are not understood although they are known to be polygenic. Based on the Iowa stiff stalk synthetic, BS9 has been taken through several cycles of selection with the most improvement occurring over the first 5 cycles (Klenke et al. 1986).

These genotypes not only have good leaf-feeding resistance but also show resistance to stalk boring and tunnelling by later instars. Recent work has shown fibre, cellulose and lignin in the stalk and sheath to increase but no changes were reported for whorl composition (Buendgen et al. 1990). Based on instron and penetrometer readings, both the leaf and stalk tissues increased in toughness over the first five cycles of selection (Chapter 8). This is the first study to report phenolic acid content for this selection series with p-coumaric and dehydrodiferulic acid increasing over selection cycles in leaf (both mature and immature tissue), sheath, stalk and pith tissue. Fibre content also increased. These observations fortify the theory that cell wall phenolics contribute to maize resistance by enhancing tissue toughness.

Multiple borer resistant material developed at CIMMYT has received little phytochemical attention (Benson 1986, Hedin et al. 1984). Like the BS9 synthetic, it employs polygenic resistance factors that involve additive variation (Smith et al. 1989). Leaf toughness is related inversely to leaf damage ratings for this germplasm, with fibre and dehydrodiferluic acid content being the best regression variables to account for toughness and leaf feeding in both the field and laboratory (Chapter 8). Protein content is also a good regression variable which points to a nutritionally based resistance in which lower protein levels are acting in concert with elevated fibre and cell wall phenolic dimers to reduce the accessibility of nitrogen to preclude larval development. Such a hypothesis would account for the slower larval development rates and reduced tunnelling reported for MBR genotypes (Davis et al. 1988).

9.2 Evolution of maize and the European corn borer

A stepwise progression in coevolution was proposed by Ehrlich and Raven (1964) who outlined five steps of the coevolution process in which plants produce novel secondary phytochemicals to reduce their suitablity to insects and in turn the insect undergoes adaptive radiation, evolving mechansims to overcome these phytochemical defences. Even if one considers the more sophisticated modern models such as diffuse co-evolution, for the European corn borer and maize there is no evidence of coevolution given the geographic origin of both species. European corn borer was introduced from Europe in 1917 and by 1925 was a major economic pest (Dicke and Guthrie 1988). However, maize was exposed

for several millenia to tropical and subtropical lepidopteran folivores, namely the southwestern corn borer, *Diatraea grandiosella*, the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), and the fall armyworm, *Spodoptera frugiperda* (Smith). As a result of this intense selection pressure by these multivoltine pests, tropical and subtropical germplasm has provided good sources of resistance for breeding programs to develop multiple borer resistant germplasm (Smith et al. 1989). Temperate germplasm not exposed to this selection pressure undoubtedly lost much of the resistance traits, given the relative absence of insect attack and the practice of open pollinated cultivars for seed production that focussed on agronomic traits. Within 17 years of its discovery the ECB had invaded a total of 18 states and Ontario and Québec (Hudon and LeRoux 1986a). A host range of over 200 plant species in 131 genera of 40 families indicates the polyphagous nature of the ECB.

"Qualitative defences" (Feeny 1976) or "toxins" (Rhoades and Cates 1976) are the typical defence mechanisms employed against polyphagous herbivores. For maize that qualitative defence has been identified as the hydroxamic acid DIMBOA which acts as a digestive protease inhibitor to reduce nitrogen assimilation efficiency (Houseman et al. 1992). However, other lepidopteran folivores are not affected by hydroxamic acids (Hedin et al. 1984), suggesting the evolution of insect resistance to this phytochemical family.

Tropical and subtropical maize resistance to borers has likely been selected for during the development of Meso-American shifting agriculture (Milpa agriculture) which employed landraces. Resistance to insect folivores was likely one of the major traits required for successful cropping. In an open pollinated system resistant traits were probably maintained due to severe insect selection pressure. Since Milpa agriculture was conducted in a spatially heterogeneous environment, resistance likely constituted both qualitative and quantitative mechanisms with a gradual emphasis on quantitative mechansims as the agriculture systems became more intensified (Fig. 9.1). Given this pressure, "quantitative defences" would likely emphasize defences which restrict the availability of essential nutrients to the herbivore (Feeny 1976). For maize it is proposed that these defences consist of elevated levels of fibre and cell wall carbohydrate-phenolic complexes to fortify the mechanical barrier of the epidermis to restrict access of neonate larvae to nutrients. Reduced levels of accessible nitrogen would act in concert with this toughness factor to provide a common defence strategy against all

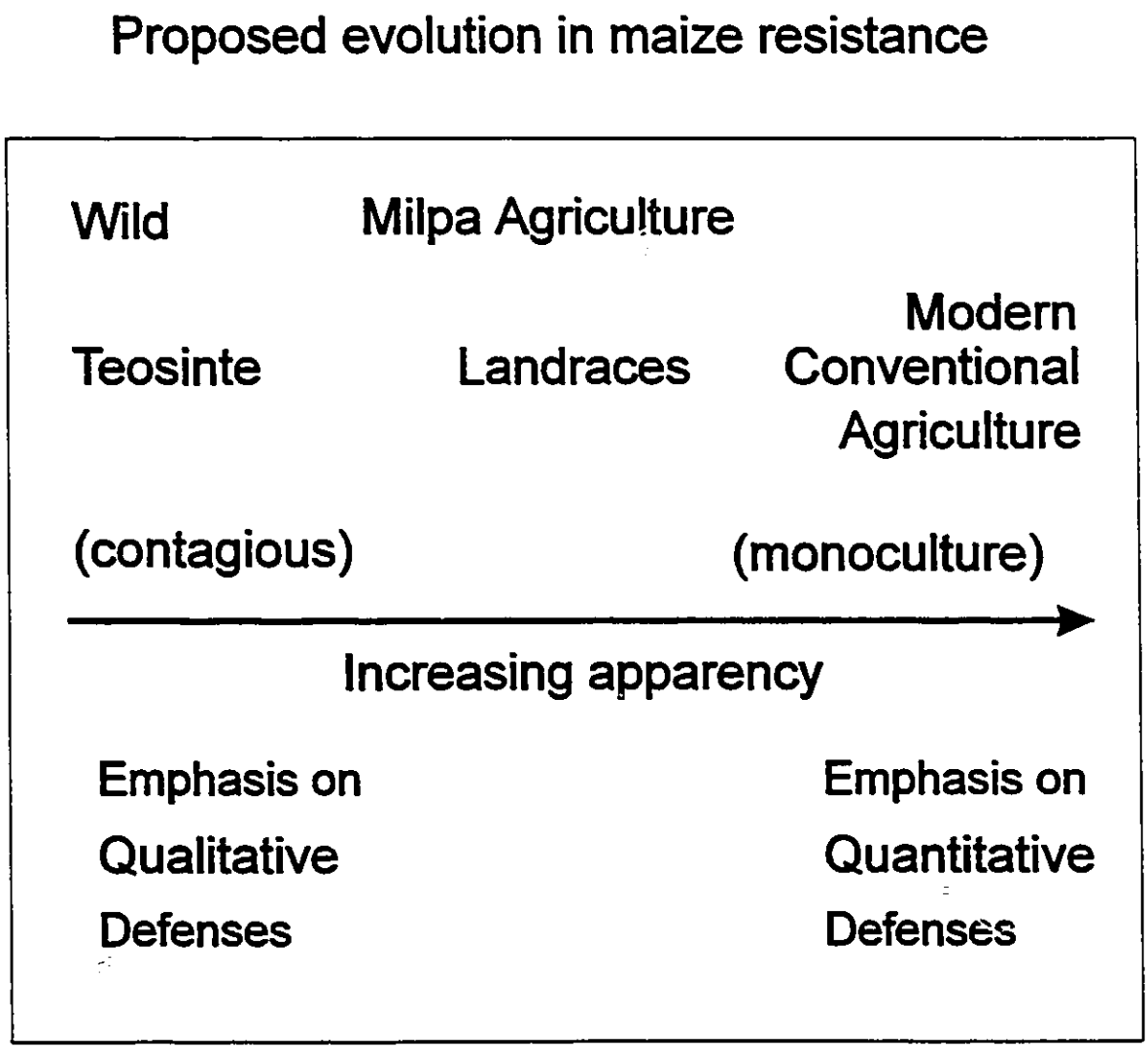


Fig. 9.1. Diagram of proposed evolution of host plant resistance fro maize.

folivores. However, given that lepidoptera larvae have the highest relative consumption rates for folivores (Slansky and Rodriguez 1987), leaf toughness would be the most important component of this nutritionally based resistance.

Feeny's theory is best suited to late-successional, temperate systems, but corn has now become a crop facing many of the same defence paradigms. Modern conventional cropping has resulted in extensive tracts of land being planted to one crop of similar genetic background, constituting an "apparent" (Feeny 1976) or "predictable" (Rhoades and Cates 1976) resource. Late successional systems are largely undisturbed year after year but with the no-till practices now being employed by Canadian and American farmers, the agroecosystem is less disturbed, with maize crops often grown over several consecutive years, again resulting in a predictable resource. As a result of this new direction in agricultural practices and the continued ability of insects such as the ECB to extend infestation boundaries, maize resistance will continue to develop quantitative defences as described previously. The advantages of this form of resistance are: 1) reduced probability of break-down given the polygenic regulation involved in fibre, protein and phenolic acid production; 2) broad range resistance to both specialists and generalist folivores alike; and 3) enhanced natural enemy populations by delaying development and not providing herbivores with toxic chemicals for defence against natural enemies (Price et al. 1980, Feeny 1976).

9.3 Implications of the present study on European corn borer management

Integrated pest management of ECB populations now utilizes all suitable techniques to maintain insect populations below an established economic injury thresholds (Lewis 1975). However, biological control agents in North America have not provided consistent control of ECB populations. This failure necessitates the use of insecticides for high value maize crops such as sweet corn, popcorn and seed corn (Hudon et al. 1986a). New soil conservation strategies also restrict cultural control practices, which also tend to be labour or resource intensive. This leaves plant resistance to be the centerpiece of present day integrated control programs. The demand for plant resistance to multipest complexes is high, with plant protection from seedling stage through to grain storage being the ultimate goal. Given the numerous defence strategies that would be operating simultaneously in different tissues (root,

leaf, stalk, silk, ear) the need for phytochemical screening techniques is becoming increasingly important in order to monitor progress in pulling together resistance genes into an elite germplasm.

Techniques that have been developed during the course of this research that will assist in meeting this germplasm screening mandate include both physical and phytochemical parameters. The instron can provide reliable leaf toughness measurements in less than 20 minutes per genotype. These measurements can be standardized using assigned checks that are adapted for different environments (North Temperate, South Temperate, Sub-tropical and Tropical). The advantage of this technique is its simplicity and ability to quantify tissue toughness for specific tissue sites on the plant. The second screening tool is the microspectrophotometer which measures another physical parameter. It determines cell wall fortification for different cell types and tissues although epidermal cell walls are presently considered to be the most important in insect resistance. The disadvantages of this method are the capital expense of equipment, labour-intensive determinations and extensive training to obtain consistent results.

Phytochemical parameters that can be determined in a two-extraction protocol and two HPLC runs include soluble secondary metabolites (including DIMBOA), cell wall phenolics (including phenolic dimers such as dehydrodiferulic acid), estimated levels of base hydrolyzed lignin, weight of soluble metabolites and estimated detergent fibre content. This procedure has the same drawbacks as the microspectrophotometer, in addition to requiring 5 g of fresh tissue to be freeze dried and milled. However, the procedure can quantify all phytochemical resistance factors presently considered in ECB resistance and will serve as a tool by which more rapid phytochemical screening techniques will be compared.

9.4 Future work

With the exception of the greenhouse studies to attenuate cyclobutane production in cell walls, the present study has relied on genotypic correlations or changes in recurrently selected material to make inferences on the role cell wall phenolics play in maize resistance. To this end, future work should use the screening techniques described previously to direct breeding strategies and to establish the extent of genetic-phytochemical variation present and

its utilization in breeding strategies for satisfactory levels of resistance/tolerance (biological equilibrium plus economic yields). This implies study of elite homozygous lines developed from the best present sources of resistance. Future work would entail the following:

1) To purify the peroxidase isozyme responsible for the production of dehydrodiferulic acid and to elucidate if the same isozyme is responsible for lignin production. If different isozymes are involved then characterizing an oligopeptide sequence would facilitate gene location and future manipulation of expression once molecular techniques for modified regulation are available. Altered levels of dehydrodiferulic acid would enable the testing of dimer cross-linking for heritable traits.

2) Using resistant genetic lines/synthetics with major gene complexes of resistance will allow classical phytochemical-genetic studies to identify, locate and manipulate resistance factors. Implications for other insect pests should also be investigated through the use of broadly adapted elite germplasm presently being developed in the PRC-CIMMYT-South American plant improvement program. Broad horizontal resistance strategies could then be obtained for a sustainable biological equilibrium.

3) Insect feeding studies are needed to characterize the mechanism by which leaf toughness operates. Is toughness just a physical barrier to restrict all feeding access or does it merely hamper digestion of ingested food? Microspectrophotometer studies of ingested food breakdown for different larval instars may provide further insights into this question.

4) Rapid screening techniques for breeding purposes need to be developed to facilitate the development of superior phytochemical based resistance. Such techniques may employ solid state NMR or Fourier transform-infrared reflectance spectroscopy (FT-IR). Near infrared reflectance spectroscopy (NIRS) is presently used in screening forage crops for *in vitro* dry matter digestibility by ruminants (Villalobos et al. 1991).

CHAPTER X.
REFERENCES

Akin, D.E., R.D. Hartley, L.L. Rigsby and W.H. Morrison III. 1992. Phenolic acids released from bermudagrass (*Cynodon dactylon*) by sequential sodium hydroxide treatment in relation to biodegradation of cell types. J. Sci. Food Agric. 58: 207-214.

Akin, D.E., R.D. Hartley, W.H. Morrison III, and D.S. Himmelsback. 1990a. Diazonium compounds localize grass cell wall phenolics: relation to wall digestibility. Crop Sci. 30: 985-989.

Akin, D.E., N. Ames-Gottfred, R.D. Hartley, R.G. Fulcher, and L.L. Rigsby. 1990b. Microspectrophotometry of phenolic compounds in bermudagrass cell walls in relation to rumen microbial digestion. Crop Sci. 30: 396-401.

Akin, D.E., L.L. Rigsby, C.E. Lyon and W.R. Windham. 1990c. Relationship of tissue digestion to textural strength in bermudagrass and alfalfa stems. Crop Sci. 30: 990-993.

Akin, D.E. and A. Chesson. 1989. Lignification as the major factor limiting forage feeding value especially in warm conditions. XVIth Int. Grassld. Congr. 16: 1753-1760.

Akin, D.E. S.L. Fales, L.L. Rigsby and M.E. Snook. 1987. Temperature effects on leaf anatomy, phenolic acids, and tissue digestibility in tall fescue. Agron. J. 79: 271-275.

Ampofo, J.K.O. and E.L. Kidiavai. 1987. *Chilo partellus* (Swinhoe) (Lepid., Pyralidae) larval movement and growth on maize plants in relation to plant age and resistance or susceptibility. J. Appl. Ent. 103: 483-488.

Anonymous. 1988. The economics of insect control on wheat, corn and canola in Canada, 1980-1985. Bulletin Entomol. Soc. Canada 20 (suppl. 2) 22 pp.

Argandona, V.H. and L.J. Corcuera. 1985. Distribution of hydroxamic acids in *Zea mays* tissues. Phytochemistry 24: 177-178.

Arnason, J.T., J. Gale, B. Conilh de Beyssac, A. Sen, S.S. Miller, B.J.R. Philogène, 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.

Ascher, K.R.S, H. Schmutterer, E. Glotter, and I. Kirson. 1981. Withanolides and related ergostane-type steroids as antifeedants for larvae of *Epilachna varivestis* (Coleoptera: Chrysomelidae). Phytoparasitica 9: 197-205.

Atkinson, J., P. Morand, J.T. Arnason, H.M. Niemeyer and H.R. Bravo. 1991. Analogues of the cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one: Decomposition to benzoxazolinones and reaction with ß-mercaptoethanol. J. Org. Chem. 56: 1788-1800.

Bacon, J.S.D. 1979. Plant cell wall digestibility and chemical structure. Rep. Rowett Inst. 35: 99-108.

Beck, D.L., G.M. Dunn, D.G. Routley and J.S. Bowman. 1983. Biochemical basis of resistance in corn to the corn leaf aphid. Crop Sci. 23: 995-998.

Benson, D.L. 1986. Evaluation of stalk borer resistance mechanisms and the development of a population for multiple stalk borer resistance in maize, *Zea mays* L. Ph.D. Thesis, Cornell University. pp. 179.

Bernays, E.A. 1986. Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. Sci. 231: 495-497.

Bernays, E.A. and R. Barbehenn. 1987. Nutritional ecology of grass foliage-chewing insects. pp. 147-175. In: Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. F. Slansky Jr. and J.G. Rodriguez (eds.) John Wiley & Sons, N.Y.

Bors, W., C. Langebartels, C. Michel and H. Sandermann Jr. 1989. Polyamines as radical scavengers and protectants against ozone damage. Phytochemistry 28: 1589-1595.

Boutton, T.W., B.N. Cameron and B.N. Smith. 1978. Insect herbivory on C3 and C4 grasses. Oecologia 36: 21-32.

Buendgen, M.R. J.G. Coors, A.W. Grombacher, and W.A. Russell. 1990. European corn borer resistance and cell wall composition of three maize populations. Crop Sci. 30: 505-510.

Butler, G.W. and R.W. Bailey (eds.). 1973. Chemistry and Biochemistry of Herbage. Vol. 1. Academic Press, London.

Byrne, P.F., L.L. Darrah, K.B. Simpson, A.J. Keaster, B.D. Barry and M.S. Zuber. 1990. Relationship of maize tissue pH and resistance to whorl leaf feeding and stalk

tunneling by the European corn borer (Lepidoptera: Pyralidae). Environ. Entomol. 19: 1091-1096.

Caffrey, D.J. and L.H. Worthley. 1927. A progress report on the investigations of the European corn borer. USDA Bull. 1476. U.S. Gov. Print. Office, Wash., D.C.

Campos, F., J. Atkinson, J.T. Arnason, B.J.R. Philogene, P. Morand, N.H. Werstiuk and G. Timmins. 1989. Toxicokinetics of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in the European corn borer, *Ostrinia nubilalis* (Hübner). J. Chem. Ecol. 15: 1989-2001.

Caswell, H., F. Reed, S.N. Stephenson, and P. Werner. 1973. Photosynthetic pathways and selective herbivory: a hypothesis. Am. Nat. 107: 465-480.

Cherney, J.H., J.D. Axtell, M.M. Hassen and K.S. Anliker. 1988. Forage quality characterization of a chemically induced brown-midrib mutant in pearl millet. Crop Sci. 28: 783-787.

Chiang, H.C. 1978. Pest management in corn. Ann. Rev. Entomol. 23: 101-123.

Classen, D., J.T. Arnason, J.A. Serratos, J.D.H. Lambert, C. Nozzolillo, B.J.R. Philogene. 1990. Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil in CIMMYT's collections. J. Chem. Ecol. 16: 301-315.

Coors, J.G. 1988. Nutritional factors related to European corn borer resitance in maize. In: Report of Forty-second annual corn and sorghum research conference, American Seed Trade Assoc., Chicaga, Ill.

Coors, J.R. 1987. Resistance to the European corn borer, *Ostrinia nubilalis* (Hübner), in maize, *Zea mays* L., as affected by soil silica, plant silica, structural carbohydrates, and lignin. pp. 445-456. In: W.H. Gabelman and B.C. Loughman (eds.) Genetic aspects of plant mineral nutrition. Nijhoff Publ., The Hague, the Netherlands.

Davies, P.J. 1987. The plant hormones: their nature, occurrence, and functions. In: Plant Hormones and Their Role in Plant Growth and Development. P.J. Davies (ed.). Ch. Al. Boston: Martinus Nijhoff.

Davis, F.M., W.P. Williams, J.A. Mihm, B.D. Barry, J.L. Overman, B.R. Wiseman, T.J. Riley. 1988. Resistance to multiple lepidopterous species in tropical derived corn

germplasm. Tech. Bulletin, Mississippi Agricultural and Forestry Exp. Station. 157: 1-6.

Dicke, F.F. and W.D. Guthrie. 1988. The most important corn insects. In: Corn and Corn Improvement. G.F. Sprague and J.W. Dudley (eds.). Amer. Soc. Agron., Inc., Madison, WIS. pp. 767-867.

Dicke, F.F. and W.D. Guthrie. 1978. Genetics of insect resistance in maize. pp. 299-317. In: Maize Breding and Genetics. D.B. Walden (ed.). John Wiley & Sons, Toronto.

Djamin, A. and M.D. Pathak. 1967. Role of silica in resistance to Asiatic rice borer, *Chilo suppresalis* (Walker), in rice varieties. J. Econ. Entomol. 60: 347-351.

Doebley, J. 1990. Molecular evidence and the evolution of maize. Econ. Botany 44(suppl.): 6-27.

Dowd, P.F. 1990. Responses of *Carpophilus hemipterus* larvae and adults to selected secondary metabolites of maize. Entomol. exp. appl. 54: 29-36.

Ehrlich, P.R. and P.H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586-608.

Ellsworth, P.C., R.P. Patterson, J.R. Bradley, Jr., G.G. Kennedy and R.E. Stinner. 1989. Developmental consequences of water and temperature in the European corn borer - maize interaction. Entomol. exp. appl. 53: 287-296.

El-Basyouni, S. Z., A.C. Neish and G.H.N. Towers. 1964. The phenolic acids in wheat--III. Insoluble derivatives of phenolic cinnamic acids as natural intermediates in lignin biosynthesis. Phytochemistry 3: 627-639.

Eraso, F. and R.D. Hartley. 1990. Monomeric and dimeric phenolic constituents of plant cell walls - possible factors influencing wall biodegradability. J. Sci. Food Agric. 51: 163-170.

Evans, P. T. and R.L. Malmberg. 1989. Do polyamines have roles in plant development? Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 235-269.

Feeny, P. 1976. Plant apparency and chemical defense. Rec. Adv. in Phytochem. 10: 1-40.

Felton, G.W., K. Donato, R.J. Del Vecchio and S.S. Duffey. 1989. Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J. Chem Ecol. 15: 2667-2694.

Fischer, D.C., M. Kogan and J. Paxton. 1990. Deterrency of Mexican bean beetle (Coleoptera: Coccinellidae) feeding by free phenolic acids. J. Entomol. Sci. 25: 230-238.

Flores, H.E. and A.W. Galston. 1982. Polyamines and plant stress: activation of putrescine biosynthesis by osmotic shock. Science 217: 1259-61.

Ford, C.W. and R.D. Hartley. 1990. Cyclodimers of p-coumaric and ferulic acids in the cell walls of tropical grasses. J. Sci. Food Agric. 50: 29-43.

Ford, C.W. and Hartley, R.D. 1989. GC-MS characterization of cyclodimers from p-coumaric and ferulic acids by photodimerization - a possible factor influencing cell wall biodegradability. J. Sci. Food Agric. 46: 301-310.

Fry, S.C. 1989. Analysis of cross-links in the growing cell walls of higher plants. pp. 12-36. In: Plant Fibers. H.F. Linskens and J.F. Jackson (eds.). Springer-Verlag, New York.

Fry, S. C. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. Ann. Rev. Plant Physiol. 37: 165-186.

Galinat, W.C. 1988. The origin of corn. In: Corn and Corn Improvement. 3rd Ed. G.F. Spague and J.W. Dudley (eds.). Amer. Soc. Agron., Inc. Madison, WIS. pp. 1-31.

Godfrey, L.D., T.O. Holtzer, S.M. Spomer and J.M. Norman. 1991. European corn borer (Lepidoptera: Pyralidae) tunneling and drought stress: effects on corn yield. J. Econ. Entomol. 84: 1850-1860.

Goto, M., A.H. Gordon, and A. Chesson. 1991. Changes in cell-wall composition and degradability of sorghum during growth and maturation. J. Sci. Food Agric. 54: 47-60.

Grabber, J.H. and G.A. Jung. 1991. *In-vitro* disappearance of carbohydrates, phenolic acids, and lignin from parenchyma and sclerenchyma cell walls isolated from cocksfoot. J. Sci. Food Agric. 57: 315-323.

Grombacher, A.W., W.A. Russell and W.D. Guthrie. 1989a. Resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. J. Kansas Entomol. Soc. 62: 103-107.

Grombacher, A.W., W.A. Russell and W.D. Guthrie. 1989b. Effects of recurrent selection in two maize synthetics on agronomic traits of S_1 lines. Maydica 34: 343-352.

Guthrie, W.D. and B.D. Barry. 1989. Methodologies used for screening and determining resistance in maize to the European corn borer. pp. 122-129. In: Toward Insect Resistant Maize for the Third World. Proc. Int. Symp. Methodologies for Developing Host Plant Resistance to Maize Insects, El Batán, Mexico. 9-14 Mar. 1987. CIMMYT, El Batán, Mexico.

Guthrie, W.D., C.T. Tseng, W.A. Russell, J.R. Coats, J.C. Robbins, J.J. Tollefson. 1986a. 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one content at seven stages of plant development in a maize (Zea mays) synthetic cultivar. J. Kansas Entomol. Soc. 59: 356-360.

Guthrie, W.D., R.L. Wilson, J.R. Coats, J.C. Robbins, C.T. Tseng, J.L. Jarvis and W.A. Russell. 1986b. European corn borer (Lepidoptera: Pyralidae) leaf-feeding resistance and DIMBOA content in inbred lines of dent maize grown under field versus greenhouse conditions. J. Econ. Entomol. 79: 1492-1496.

Guthrie, W.D., W.A. Russell and C.W. Jennings. 1971. Resistance of maize to second-brood European corn borers. pp. 165-179. In: Proc. Ann. Corn-Sorgum Res. Conf. Amer. Seed Trade Assoc., Inc. Wash., D.C.

Guthrie, W.D., J.L. Huggans and S.M. Chatterji. 1970. Sheath and collar feading resistance to the second-brood European corn borer in six inbred lines of dent corn. Iowa State J. Sci. 44: 297-311.

Guthrie, W.D., J.L. Huggans and S.M. Chatterji. 1969. Influence of corn pollen on the survival and development of second-brood larvae of the European corn borer. Iowa State J. Sci. 44: 185-192.

Guthrie, W.D., F.F. Dicke, and C.R. Neiswander. 1960. Leaf and sheath feeding resistance to the European corn borer in eight inbred lines of dent corn. Ohio Agric. Exp. Stn. Bull. 860.

Gutiérrez, C., P. Castanera and V. Torres. 1988. Wound-induced changes in DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4 benzoxazin-3 (4H)-one) concentration in maize plants caused by *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Annals of Appl. Biol. 113: 447-454.

Hallauer, A.R., W.A. Russell and O.S. Smith. 1983. Quantitative analysis of Iowa Stiff Stalk Synthetic. Stadler Symp. 15: 83-104.

Hanna, W.W., W.G. Monson and G.W. Burton. 1976. Histological and *in vitro* digestion study of 1- and 4-week stems and leaves from high and low quality bermudagrass genotypes. J. Agron. 68: 219-222.

Harborne, J. B. 1980. Plant phenolics. pp. 330-402. In: Secondary Plant Products. E.A. Bell and B.V. Charlwood (eds.). Springer-Verlag, New York.

Hartley, R.D. 1973. Carbohydrate esters of ferulic acid as components of cell-walls of *Lolium multiflorum*. Phytochem. 12: 661-665.

Hartley, R.D. 1972. p-Coumaric acid and ferulic acid components of cell walls of ryegrass and their relationships with lignin and digestibility. J. Sci. Food Agric. 23: 1347-1354.

Hartley, R.D. D.E. Akin and D.S. Himmelsbach. 1990. Microspectrophotometry of bermudagrass (*Cynodon dactylon*) cell walls in relation to lignification and wall biodegradability. J. Sci. Food Agric. 50: 179-189.

Hartley, R.D., W.H. Morrison III, W.S. Borneman, L.L. Rigsby, M. O'Neill, W.W. Hanna and D.E. Akin. 1992. Phenolic constituents of cell wall types of normal and brown midrib mutants of pearl millet (*Pennisetum glaucum* (L) R Br) in relation to wall biodegradability. J. Sci. Food Agric. 59: 211-216.

Hartley, R.D. and W.H. Morrison, III. 1991. Monomeric and dimeric phenolic acids released from cell walls of grasses by sequencial treatment with sodium hydroxide. J. Sci. Food Agric. 55: 365-375.

Hartley, R.D. and C.W. Ford. 1989. Phenolic constituents of plant cell walls and wall biodegradability. pp. 137-145. In. Biogenesis and Biodegradation. N.G. Lewis and M.G. Paice (eds.). ACS Sym. Ser. 399.

Hartley, R.D., F.R. Whatley and P.J. Harris. 1988. 4,4'-Dihydroxytruxillic acid as a component of cell walls of *Lolium multiflorum*. Phytochemistry 27: 349-351.

Hartley, R.D. and E.C. Jones. 1978. Phenolic components and degradability of the cell walls of the brown midrib mutant, bm3, of *Zea mays*. J. Sci. Food Agric. 29: 777-789.

Hartley, R. D. and E.C. Jones. 1976. Diferulic acid as a component of cell walls of *Lolium multiflorum*. Phytochemistry 15: 1157-1160.

Hartman, H.T., W.J. Flocker and A.M. Kofranek. 1981. Plant Science: Growth, Development, and Utilization of Cultivated Plants. Prentice-Hall, N.J.

Heby, O. 1981. Role of polyamines in the control of cell proliferation and differentiation. Differentiation 19: 1-20.

Hedin, P.A., F.M. Davis, W.P. Williams, and M.L. Salin. 1984. Possible factors of leaf-feeding resistance in corn to the southwestern corn borer. J. Agric. Food Chem. 32: 262-267.

Higuchi. T., Y. Ito and I. Kawamura. 1967. p-Hydroxyphenylpropane content of grass lignin and role of tyrosine ammonia-lyase in its formation. Phytochemistry 6: 875-881.

Hinderliter, D G. 1983. Host plant resistance in two tropical maize, *Zea mays* L., populations to the southwestern corn borer, *Diatraea grandiosella* Dyar, and the sugarcane borer, *D. saccharalis* F. Ph.D. thesis, University of Wisconsin, Madison, Wisconsin, USA.

Houseman, J.G., F. Campos, N.M.R. Thie, B.J.R. Philogene, J.Atkinson, P. Morand, and J.T. Arnason. 1992. Effect of the maize-derived compounds DIMBOA and MBOA on growth and digestive processes of European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 85: 669-674.

Hudon, M. R.E. Pitblado, R.I. Hamilton, G. Bourgeois, S. Kuzir and R. Girgis. 1991. Response of maize inbred lines to two European corn borer (*Ostrinia nubilalis*) strains in Canada. Phytoprotection 72: 69-76.

Hudon, M. and LeRoux E. J. 1986a. Biology and population dynamics of the European corn borer (*Ostrinia nubilalis*) with special reference to sweet corn in Québec. I. Systematics, morphology, geographical distribution, host range, economic importance. Phytoprotection 67: 39-54.

Hudon, M. and LeRoux E. J. 1986b. Biology and population dynamics of the European corn borer (*Ostrinia nubilalis*) with special reference to sweet corn in Québec. II. Bionomics. Phytoprotection 67: 81-92.

Hudon, M. and LeRoux E. J. 1986c. Biology and population dynamics of the European corn borer (*Ostrinia nubilalis*) with special reference to sweet corn in Québec. II. Population dynamics and spatial distribution. Phytoprotection 67: 93-115.

Hudon, M. and I. Ogilvie. 1984. Corn pest management. In: The Role of Biological Control in Pest Management. G. Allen (ed.). University of Ottawa Press.

Isenhour, D.J., R.D. Hudson and J.N. All. 1988. Field corn insects. p. 5. In: Summary of Losses From Insect Damage and Costs of Control in Georgia, 1987. G.K. Douce and R.M. McPherson (eds.). The Georgia Agricultural Experimental Stations College of Agriculture, the University of Georgia. Publ. 54.

Iiyama, D., T.B.T. Lam and B.A. Stone. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry 29:733-737.

Iiyama, D., and A.F.A. Wallis. 1990. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. J. Sci. Food Agric. 51: 145-161.

Ishii, T. 1991. Isolation and characterization of a diferuloyl arabinoxylan hexasaccharide from bamboo shoot cell-walls. Carbohydrate Res. 219: 15-22.

Janshekar, H. and A. Fiechter. 1983. Lignin: biosynthesis, application and biodegradation. pp. 119-178. In:Advances in Biochemical Engineering/Biotechnology. Vol 27. A. Fiechter (ed.). Springer-Verlag, New York.

Jensen, W.A. 1962. Botanical Histochemistry. W.H. Freeman and Co., London. 408 pp.

Jung, H.G. 1989. Forage lignins and their effects on fiber digestibility. Agron J. 81: 33-38.

Jung, H.G. and M.D. Casler. 1990. Lignin concentration and composition of divergent smooth bromegrass genotypes. Crop Sci. 30: 980-985.

Kato, Y. and D.J. Nevins. 1989. Structure of a pectic polysaccaride fraction from *Zea* shoots. Plant Physiol. 89: 792-797.

Kato, Y. and D.J. Nevins. 1985. Isolation and identification of *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-β-D- xylopyranoxyl-(1→4)-D-xylopyranose as a component of *Zea* shoot cell-walls. Carbohydr. Res. 137: 139-150.

Kato, A., J. Azuma and T. Koshijima. 1983. A new feruloylated trisaccharide from bagasse. Chem. Lett. 137-140.

Klenke, J.R., W.A. Russell and W.D. Guthrie. 1986. Distributions for European corn borer resistance ratings of S_1 lines from 'BS9' corn. J. Econ. Entomol. 79: 1076-1081.

Klun, J.A. W.D. Guthrie, A.R. Hallauer and W.A. Russell. 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin3(4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. Crop Sci. 10:87-90.

Klun, J.A. and T.A. Robinson. 1969. Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of plant and its relation to resistance of host plant to the European corn borer. J. Econ. Entomol. 62: 214-220.

Klun, J.A. and T.A. Brindley. 1966. Role of 6-methoxybenzoxazolinone in inbred resistance of host plant (maize) to first-brood larvae of European corn borer. J. Econ. Entomol. 59: 711-718.

Kogan, M. and E.E. Ortman. 1978. Antixenosis--a new term proposed to replace Painter's "Nonpreference" modality of resistance

Komedahl, T. and C.E. Windles. 1981. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the U.S.A. pp 94-103. In: *Fusarium*, disease, biology and taxonomy. P.E. Nelson T.A. Toussoun and R.J. Cook (eds.) Pennsylvania University Press.

Krebs, K.G., D. Heusser, and H. Wimmer. 1969. Spray reagents. pp. 854-909. In: E. Stahl (ed.). Thin-layer chromatography. Springer-Verlag, N.Y.

Lam, T.B.T., K. Iiyama, and B.A. Stone. 1990. Lignin in wheat internodes. Part 2: Alkaline nitrobenzene oxidation by wheat straw lignin and its fractions. J. Sci. Food Agric. 51: 494-506.

Lawton, J.H. 1983. Plant architecture and the diversity of phytophagous insects. Ann. Rev. Entomol. 28: 23-39.

Lee, D.A. 1988. Factors affecting mortality of the European corn borer, *Ostrinia nubilalis* (Hübner), in Alberta. Can. Ent. 120: 841-853.

Lewis, L.C. 1975. Natural regulation of crop pests in their indigenous ecosystems and in Iowa agroecosystems: bioregulation of economic insect pests. Iowa State J. Research 49: 435-445.

Lewis, N.G. and E. Yamamoto. 1990. Lignin: occurrence, biogenesis and biodegradation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 455-496.

Leszczynski, B., L.C. Wright, W.W. Cone and S.T. Kenny. 1988. Hop leaf phenolics and resistance to the two spotted spider mite. J. Agric. Entomol. 5: 257-266.

Luckner, M. 1980. Expression and control of secondary metabolism. pp. 23-63. In: Secondary Plant Products. E.A. Bell and B.V. Charlwood (eds.). Springer-Verlag, N.Y.

Mangelsdorf, P.C. 1986. The origin of corn. Sci. Am. (August): 72-78.

Manojlovic', B. 1984. [Some characteristics of development of European corn borer (*Ostrinia nubilalis* Hbn., Lepidoptera: Pyralidae) on different host plants.] (in Serbo-Croatian). Zast. bilja 35: 23-35.

Manuwoto, S. and J.M. Scriber. 1985a. Consumption and utilization of experimentally altered corn by southern armyworm: iron, nitrogen and cyclic hydroxamates. J. Chem. Ecol. 11: 1469-1483.

Manuwoto, S. and J.M. Scriber. 1985b. Neonate larval survival of European corn borers, *Ostrinia nubilalis*, on high and low DIMBOA genotypes of maize: effects of light intensity and degree of insect inbreeding. Agric. Ecosystems Envir. 14:221-236.

Markwalder, H.U. and H. Neukom. 1976. Diferulic acid as a possible crosslink in hemicelluloses from wheat germ. Phytochemistry 15: 835-837.

Martin-Tanguy, J., Cabanne F., Perdrizet E. and Martin C. 1978. The distribution of hydroxycinnamic acid amides in flowering plants. Phytochemistry 17: 1927-1928.

Metcalf, R.L. and W.H. Luckmann. 1982. Introduction to Insect Pest Management. 2nd Ed. John Wiley & Sons Inc., Toronto. 577 pp.

McKenzie, H.A. and H.S. Wallace. 1954. The Kjehldahl determination of nitrogen: a critical study of digestion condition-termperature, catalyst, and oxidizing agents. Aust. J. Chem. 7: 55-70.

Mihm. J.A. 1985. Breeding for host plant resistance to maize stem borers. Insect Sci. Appl. 6: 369-377.

Miller, B.S., R.J. Robinson, J.A. Johnson, E.T. Jones and B.W.X. Ponnaiya. 1960. Studies on the relation between silica in wheat plants and resistance to Hessian fly attack. J. Econ. Entomol. 53: 995-999.

Monties, B. 1989. Lignins. pp. 113-157. In: Methods in Plant Biochemistry vol. 1 Plant Phenolics. P.M. Dey and J.B. Harborne (eds.). Academic Press, London.

Morse, S., S.D. Wratten, P.J. Edwards and H.M. Niemeyer. 1991. Changes in the hydroxamic acid content of maize leaves with time and after artificial damage; implications for insect attack. Ann. appl. Biol. 119: 239-249.

Morrison, I.M. 1979. Carbohydrate chemistry and rumen digestion. Proc. Nutr. Soc. 38: 259-274.

Morrison, W.H. III, R.D. Hartley and D.S. Himmelsbach. 1992. Synthesis of substituted truxillic acids from p-coumaric and ferulic acid: simulation of photodimerization in plant cell walls. J. Agric. Food Chem. 40: 768-771.

Morrison, I.M., G.W. Robertson, D. Stewart and F. Wightman. 1991. Determination and characterization of naturally occurring phenolic acids. Phytochemistry 30: 2007-2011.

Mueller-Harvey, I.M., R.D. Hartley, P.J. Harris and E.H. Curzon. 1986. Linkage of p-coumaryl and feruloyl groups to cell wall polysaccharides of barley straw. Carbohydr. Res. 148: 71-85.

Nagy, B. 1976. Host selection of the European corn borer (*Ostrinia nubilalis*) populations in Hungary. Symp. Biol. Hung. 16: 191-195.

Neilands, J.B. 1967. Hydroxamic acids in nature. Science 156: 1443-1447.

Niemeyer, H.M. 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27: 3349-3358.

Niemeyer, H.M., E. Pesel, S.V. Copaja, H.R. Bravo, S. Franke and W. Francke. 1989. Changes in hydroxamic acid levels in wheat plants induced by aphid feeding. Phytochemistry 28: 2307-2310.

Nishitani, K. and D.J. Nevins. 1990. Enzymic analysis of feruloylated arabinoxylans (feraxan) derived from *Zea mays* cell walls. Plant Physiol. 93: 396-402.

Nyhus, K.A., W.A. Russell and W.D. Guthrie. 1989. Distributions among S_1 lines for European corn borer (Lepidoptera: Pyralidae) and stalk rot resistance ratings in two maize synthetics improved by recurrent selection. J. Econ. Entomol. 82: 239-245.

Onukogu, F.A., W.D. Guthrie, W.A. Russell, G.L. Reed and J.C. Robbins. 1978. Locations of genes that condition resistance in maize to sheath-collar feeding by second-generation European corn borers. J. Econ. Entomol. 71: 1-4.

Ortega, A., K. Vasal, J. Mihm and C. Hershey. 1980. Breeding for insect resistance in maize. pp 372-419. In: Breeding Plants Resistant to Insects. F.G. Maxwell and P.R. Jennings (eds). John Wiley & Sons, Toronto.

Painter, R.H. 1951. Insect Resistance in Crop Plants. Macmillan Co., N.Y., 520 pp.

Parks, T.H. 1926. The European corn borer in the old world and Canada. European Corn Borer 3: 1-4.

Peterson, S.S., J.M. Scriber, and J.G. Coors. 1988. Silica, cellulose and their interactive effects on the feeding performance of the Southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). J. Kansas Ent. Soc. 61: 169-177.

Pierce, F.N. and J.W. Metcalfe. 1938. The genitalia of the British pyrales with the deltoids and plumes. E.W. Classey Limited, Farrington, England.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.N. Thompson and A.E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Ann. Rev. Ecol. Syst. 11: 41-65.

Quisenberry, S.S. and H.K. Wilson. 1985. Consumption and utilization of bermuda grass by fall armyworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 78: 820-824.

Reid, L.M. 1989. Resistance of world germplasm resources of maize, *Zea mays*, to the European corn borer, *Ostrinia nubilalis*. M.Sc. thesis. University of Ottawa.

Rhoades, D.F. and R.G. Cates. 1976. A general theory of plant antiherbivore chemistry. Rec. Adv. Phytochem. 10: 168-213.

Robinson, J.F., J.A. Klun and T.A. Brindley. 1978. European corn borer: a non preference mechanism of leaf feeding resistance and its relationship to 1,4-benzoxazin-3-one concentration in dent corn tissue. J. Econ. Entomol. 71: 461-465.

Rojanaridpiched, C., V.E. Gracen, H.L. Everett, J.G. Coors, B.F. Pugh and P. Bouthyette. 1984. Multiple factor resistance in maize to European corn borer. Maydica 14: 305-315.

Ross, S.E. and K.R. Ostlie. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. J. Econ. Entomol. 83: 831-836.

Russell, W.A. and W.D. Guthrie. 1982. Registration for BS9(CB)C4 maize germplasm (Reg. No. 97). Crop Sci. 22: 694.

Russell, W.A., W.D. Guthrie, J.A. Klun and R.Grindeland. 1975. Selection for resistance in maize to first-brood European corn borer by using leaf-feeding damage of the insect and chemical analysis for DIMBOA in the plant. J. Econ. Entomol. 68: 31-34.

SAS Institute Inc. 1988. SAS/STAT User's Guide. Version 6.03. SAS Institute Inc., Cary, NC. 1028 pp.

Scriber, J.M. and F. Slansky Jr. 1981. The nutritional ecology of immature arthropods. Annu. Rev. Entomol. 26: 183-211.

Scriber, J.M., W.M. Tingey, V.E. Gracen and S.L. Sullivan. 1975. Leaf feeding resistance to the European corn borer in genotypes of tropical (low-DIMBOA) and U.S. inbred (high-DIMBOA) maize. J. Econ. Entomol. 68: 823-826.

Scott, G.E., W.D. Guthrie and G.R. Pesho. 1967. Effect of second-brood European corn borer infestation on 45 single-cross hybrids. Crop Sci. 7: 229-230.

Sen, A., D.J. Bergvinson, S.S. Miller, J.T. Arnason and R.G. Fulcher. 1993. Distribution and microchemical detection of phenolic acids, flavonoids and phenolic acid amides in maize kernels. J. Chem. Ecol. (submitted).

Serratos, A., J.T. Arnason, C. Nozzolillo, J.D.H. Lambert, B.J.R. Philogene, G. Fulcher, K. Davidson, L. Peacock, J. Atkinson and P. Morand. 1987. Factors contributing to resistance of exotic maize populations to maize weevil, *Sitophilus zeamais*. J. Chem. Ecol. 13: 751-762.

Shade, R.E. and M.C. Wilson. 1967. Leaf-vein spacing as a factor affecting larval feeding behavior of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chyrsomelidae). Ann. Ent. Soc. Amer. 60: 493-496.

Shibuya, N. 1984. Phenolic acids and their carbohydrate esters in rice endosperm cell walls. Phytochemistry 23: 2233-2237.

Showers, W.B. 1979. Effect of diapause on the migration of the European corn borer into the southeastern United States. pp. 420-429. In: Movement of Higly Mobile Insects: Concepts and Methodology in Research. R.L. Robb and G.C. Kennedy (eds.). North Carolina State University Press, Raleigh, NC.

Slansky, F. Jr. and J.G. Rodriguez. 1987. Nutritional ecology of insects, mites, spiders, and related invertebrates: an overview. pp. 1-69. In: Nutritional Ecology of Insect, Mites, Spiders, and Related Invertibrates. F. Slansky Jr. and J.G. Rodriquez (eds.). John Wiley, New York.

Slansky, F. Jr. and J.M. Scriber. 1985. Food consumption and utilization. pp. 87-163. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 4. G.A. Kerkut and L.I. Gilbert (eds.). Pergamon, Oxford.

Smith, M.E., J.A. Mihm, and D.C. Jewell. 1989. Breeding for multiple resistance to temperate, subtropical, and tropical maize insect pests at CIMMYT. pp. 222-234. In: Toward Insect Resistant Maize for the Third World. Proc. Int. Symp. Methodologies for Developing Host Plant Resistance to Maize Insects, El Batán, Mexico. 9-14 Mar. 1987. CIMMYT, El Batán, Mexico.

Smith, T.A. 1985. Polyamines. Ann. Rev. Plant Physiol. 36: 117-143.

Smith, T.A. 1980. Plant Amines. pp. 433-460. In: Secondary Plant Products. E.A. Bell and B.V. Charlwood (eds.). Springer-Verlap, Berlin.

Sosulski, R., K. Krygier and L. Hogge. 1982. Esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic in cereal and potato flours. J. Agric. Food Chem. 30: 337-340.

Southerton, S.G. and B.J. Deverall. 1990. Changes in phenolic acid levels in wheat leaves expressing resistance to *Puccinia recondita* f. sp. *tritici*. Physiol. Mol. Plant Path. 37: 437-450.

Sullivan, S.L., V.E. Gracen and A. Ortega. 1974. Resistance of exotic maize varieties to the European corn borer, *Ostrinia nubilalis* (Hübner). Envir. Entomol. 3: 718-720.

Swain, T. 1979. Tannins and lignins. pp. 657-682. In: G.A. Rosenthal and D.H. Janzen (eds.) Herbivores: Their interaction with secondary plant metabolites, Academic Press, N.Y.

Swain, T. 1978. Plant-animal coevolution; a synoptic view of the Paleozoic and Mesozoic pp. 1-19. In: Biochemical Aspects of Plant and Animal Co-evolution. J.B. Harborne (ed.). Academic Press, New York.

Swain, T. 1977. Secondary compounds as protective agents. Ann. Rev. Plant Physiol. 28: 479-501.

Tachibana, S. K. Ohkubo and G.H.N. Towers. 1992. 4,4'-dihydroxytruxillic acid as a component of the cell walls of the bamboo *Phyllostachys edulis*. Phytochemistry 31: 81-83.

Tan, K.S., T. Hoson, Y. Masuda and S. Kamisaka. 1992. Involvement of cell wall-bound diferulic acid in light-induced decrease in growth rate and cell wall extensibility of *Oryza* coleoptiles. Plant Cell Physiol. 33: 103-106.

Tanton, M.T. 1962. The effect of leaf "toughness" on the feeding of larvae of the mustard beetle *Phaedon cochleariae* Fab. Ent. exp. and appl. 5: 74-78.

Theander, O. and P. Aman. 1984. Anatomical and chemical characteristics. pp. 45-78. In: F. Sundstol and E. Owen (eds.). Straw and other fibrous by-products as feed. Elsevier, Amsterdam, Holland.

Thiéry, D. and J.L. Le Quéré. 1991. Identification of an oviposition-deterring pheromone in the eggs of the European corn borer. Naturwissenschaften 78: 132-133.

Thoms, D.A. and B.J.R. Philogène. 1979. Quality of light effects on immature stages and adults of *Pieris rapae* (L.). Rev. Can. Biol. 38: 157-165.

Torto, B., A. Hassanalli, K.N. Saxena and S. Nokoe. 1991. Feeding responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) larvae to sorghum plant phenolics and their analogs. J. Chem. Ecol. 17: 67-78.

Valero, P., G. Nicolás and E. Labrador. 1991. Variations of cell wall peroxidases in epicotyls of *Cicer arietinum* during growth. Plant Sci. 74: 171-178.

Van Soest, P.J. 1981. Limiting factors in plant residues of low biodegradability. Agric. Environ. 6: 135-143.

Van Sumere, C.F. 1989. Phenols and phenolic acids. pp 29-73. In: Plant Phenolics. J.B. Harborne (ed.). Vol. 1 in Methods in Plant Biochemistry. Academic Press, N.Y.

Villalobos, J.L., J.C. Burns, D.S. Fisher and K.R. Pond. 1991. *In vitro* dry matter disappearance and cell-wall concentrations of flaccidgrass masticates predicted by near infrared reflectance spectroscopy. Crop Sci. 31: 1571-1574.

Wahlroos, O. and A.I. Virtanen. 1959. The precursors of 6-MBOA in maize and wheat plants, their isolation and some of their properties. Acta Chem. Scand. 13: 1906-1908.

Williams, L.H. 1954. The feeding habits and food preferences of Acrididae and the factors which determine them. Trans. R. Ent. Soc. Lond. 105: 423-454.

Wellso, S.G. 1973. Cereal leaf beetle: larval feeding, orientation, development, and survival of four small grain cultivars in the laboratory. Ann. Entomol. Soc. Am. 66: 1201-1208.

Woodhead, S. 1981. Environmental and biotic factors affecting the phenolic content of different cultivars of *Sorghum bicolor*. J. Chem. Ecol. 7: 1035-1047.

APPENDIX 1
FERULIC ACID FEEDING STUDY

Male larval performance on meridic diet spiked with *E*-ferulic acid.†

Treatment	N	Larval weight (mg)	Days to Pupation‡	Pupal weight (mg)	Days to Adult‡	Adult weight (mg)‡
Control	16	59.6 ± 5.7	22.9 ± 1.0	70.2 ± 3.1	39.0 ± 1.0	31.0 ± 1.4
0.1 mg/g	19	65.4 ± 5.3	22.2 ± 0.5	72.2 ± 1.5	38.1 ± 0.6	32.5 ± 1.3
0.3 mg/g	13	75.3 ± 4.8	22.5 ± 0.7	73.5 ± 1.5	37.4 ± 0.7	31.7 ± 1.9
1.0 mg/g	8	76.0 ± 6.8	21.7 ± 0.5	72.6 ± 2.0	36.7 ± 0.5	30.8 ± 1.6
3.3 mg/g	13	74.2 ± 5.8	23.7 ± 0.6	67.9 ± 1.9	39.0 ± 0.7	30.8 ± 0.9
10.0 mg/g	14	75.8 ± 2.8	24.8 ± 0.4	67.8 ± 1.5	40.1 ± 0.6	31.7 ± 0.9
33.3 mg/g	8	74.0 ± 5.5	32.6 ± 1.5*	64.7 ± 4.0	49.9 ± 2.3*	25.8 ± 2.8*

† Diet preparation and ECB life study protocol found in Campos *et al.* 1989.
‡ Means significantly different from other means within a column, SNK ($P<0.05$).

Female larval performance on meridic diet spiked with *E*-ferulic acid.†

Treatment	N	Larval weight (mg)	Days to Pupation‡	Pupal weight (mg)	Days to Adult ‡	Adult weight (mg)
Control	12	101.4 ± 9.9	23.6 ± 0.7	95.1 ± 2.8	37.6 ± 0.6	57.8 ± 2.9
0.1 mg/g	10	102.4 ± 8.8	24.5 ± 0.6	89.4 ± 4.1	39.2 ± 0.7	53.7 ± 2.4
0.3mg/g	16	110.2 ± 6.5	24.1 ± 0.4	98.1 ± 2.9	38.7 ± 0.5	57.9 ± 2.2
1.0 mg/g	14	89.8 ± 6.4	23.7 ± 1.1	88.3 ± 6.5	38.2 ± 1.1	54.7 ± 1.7
3.3 mg/g	11	101.3 ± 7.3	26.2 ± 0.7	88.9 ± 3.1	41.1 ± 0.8	51.7 ± 2.5
10.0 mg/g	15	105.3 ± 5.5	26.9 ± 0.6	93.1 ± 2.8	40.6 ± 0.7	53.9 ± 1.8
33.3 mg/g	13	103.8 ± 7.7	34.7 ± 0.9*	92.8 ± 4.1	54.7 ± 1.2*	47.9 ± 3.1

† Diet preparation and ECB life study protocol found in Campos *et al.* 1989.
‡ Means significantly different from other means within a column, SNK ($P<0.05$).

(FERULIC ACID FEEDING STUDY CONTINUED...)

Effects of *E*-ferulic acid spiked diet on ECB fecundity[†].

Treatment	N	Eggs per egg mass	Egg masses per female	Eggs per female
Control	5	20.27 ± 1.27	6.9	139.8
0.1 mg/g	5	22.26 ± 1.63	7.2	160.2
0.3mg/g	5	20.84 ± 1.68	5.8	122.9
1.0 mg/g	6	16.05 ± 1.35	7.8	126.8
3.3 mg/g	6	20.00 ± 1.27	6.4	127.0
10.0 mg/g	6	16.57 ± 1.05	7.4	122.6
33.3 mg/g	2	19.29 ± 2.11	4.3	81.9

[†] Mating cages (20cm x 20 cm x 20 cm) contained two males and two females. Eggs were oviposited onto wax paper and counted after both females died. Cages were kept at 80% R.H., 16:8 (L:D), 25°C and supplied with sugar water.

APPENDIX 2.
FEEDING BIOASSAYS WITH PHENOLIC ACID CONJUGATES FROM MAIZE

Leaf feeding bioassay for phenolic conjugates extracted from MBR cultivar Mbita[†].

Dosage (ug/cm^2)	Ferulic Acid Glycoside	p-Coumaric Acid Glycoside	Control[‡]
100	97.0 ± 6.5	105.2 ± 5.4	115.3 ± 6.4
10	121.5 ± 6.8	124.5 ± 6.5	

[‡] Means were not significantly different from control (t-test, P=0.05).

[†] Assays ran 48h with 2 L^{III} ECB larvae at 16:8 (L:D), 25°C and 85% R.H.

Conjugates were prepared using 1mm guage paper chromatography with the solvent system n-butanol, acetic acid, water (4:1:5). Bands were identified by UV light and phenolic acid bands were eluted off with 50% and 80% methanol.

Silver Queen sweet corn leaves (leaf 7 on 8 leaf plants) were used with abaxial surface exposed in leaf feeding bioassay. Assay procedure is a method modified from Ascher et al. 1981. Area consumed was counted using 1 mm graph paper.

APPENDIX 3
MEAN INSTRON READINGS AND LEAF DAMAGE RATINGS FOR 1990 AND 1991 USED FOR REGRESSION EQUATIONS IN CHAPTER 2.

Mean leaf toughness readings and leaf ratings for cycles of recurrent selection of the maize synthetic BS9.

		1991‡		1992‡		
Cycle	Infestation	Rating	Force	Rating	Force (Mid-whorl)	Force (Tasselling)
0	1	3.1±0.2	0.78±0.02			
	2	3.9±0.2	0.70±0.02	3.6±0.2	0.43±0.01	0.66±0.01
	3	1.6±0.1	0.75±0.02			
2	1	2.7±0.2	0.83±0.02			
	2	3.5±0.1	0.76±0.02	3.2±0.2	0.42±0.01	0.63±0.01
	3	1.5±0.1	0.79±0.02			
4	1	2.2±0.1	0.87±0.02			
	2	2.9±0.1	0.81±0.02	2.7±0.1	0.46±0.01	0.61±0.02
	3	1.3±0.1	0.84±0.02			
5	1	1.8±0.1	0.81±0.02			
	2	2.5±0.1	0.79±0.02	2.9±0.1	0.45±0.01	0.58±0.01
	3	1.3±0.1	0.80±0.02			

‡ Force measurements were determined using the instron technique reported in Chapter 2. Force readings are in newtons. Leaf ratings are based on Guthrie et al. (1960) scale of 1 (resistant) to 9 (susceptible).

(MEAN INSTRON READINGS AND LEAF DAMAGE RATINGS FOR 1990 AND 1991 CONTINUED...)

Mean leaf toughness readings and leaf damage ratings for North American Inbreds.

Genotype	1991‡		1992‡		
	Rating	Force	Rating	Force (Mid-whorl)	Force (Tasselling)
CG16	6.6±0.2	0.55±0.02			
F7	5.5±0.2	0.63±0.02			
B73	4.5±0.2	0.55±0.02	5.8±0.1	0.31±0.01	0.47±0.01
MS72	4.6±0.2	0.62±0.01	7.0±0.1	0.37±0.01	0.49±0.01
CM7	3.8±0.2	0.62±0.02	5.8±0.2	0.36±0.01	0.52±0.02
CK44	3.8±0.2	0.54±0.03	3.9±0.1	0.30±0.01	0.46±0.01
72E44	3.6±0.1	0.54±0.01	3.7±0.2	0.33±0.01	0.50±0.01
CO266	3.7±0.2	0.53±0.02			
DE811	3.4±0.2	0.60±0.02			
OH43	3.2±0.2	0.63±0.02	2.9±0.1	0.42±0.01	0.66±0.02
A619	2.2±0.1	0.63±0.03	2.0±0.1	0.40±0.01	0.63±0.02
B86	2.1±0.1	0.69±0.03	3.8±0.2	0.31±0.01	0.61±0.02
CI31A	2.0±0.1	0.70±0.02	1.9±0.2	0.39±0.01	0.61±0.01

‡ Force measurements were determined using the instron technique reported in Chapter 2. Force readings are in newtons. Leaf ratings are based on Guthrie et al. (1960) scale of 1 (resistant) to 9 (susceptible).

(MEAN INSTRON READINGS AND LEAF DAMAGE RATINGS FOR 1990 AND 1991
CONTINUED...)

Mean leaf toughness readings and leaf damage ratings for multiple borer resistant cultivars developed at CIMMYT and commercial hybrids.

Genotype	1991‡		1992‡		
	Rating	Force	Rating	Force (Mid-whorl)	Force (Tasselling)
Across Bugs	2.2±0.1	0.83±0.04	1.8±0.2	0.53±0.01	0.77±0.02
Across ECB			2.8±0.2	0.62±0.01	0.65±0.01
Mbita-Chilo	2.4±0.1	0.82±0.03	2.8±0.2	0.51±0.02	0.75±0.02
SCB	2.2±0.1	0.83±0.04	2.5±0.2	0.59±0.01	0.70±0.02
SWCB	2.0±0.1	0.79±0.02	3.2±0.2	0.65±0.02	0.66±0.02
CML135x139i	2.2±0.1	0.89±0.03	2.9±0.2	0.65±0.01	0.71±0.02
Ki3xTx601	4.0±0.2	0.68±0.04	4.6±0.3	0.51±0.01	0.73±0.02
Pion 3184	3.9±0.2	0.77±0.02	3.7±0.3	0.43±0.01	0.61±0.01
Font 6220	5.7±0.1	0.72±0.03	5.8±0.3	0.42±0.01	0.64±0.02
6796-48	5.4±0.1	0.64±0.03			
6976-49	5.5±0.1	0.64±0.02			
6796-13	3.1±0.2	0.74±0.02	2.8±0.2	0.46±0.01	0.64±0.01
DK 435	3.3±0.1	0.67±0.02			
Pic 4533	4.8±0.1	0.59±0.03	6.2±0.2	0.44±0.01	0.60±0.02

Mean leaf toughness readings and leaf damage ratings for multiple borer resistant lines developed at CIMMYT

Genotype	1991‡			1992‡		
	Rating	Force (Whorl)	Force (Tasselling)	Rating	Force (Whorl)	Force (Tasselling)
CML67	1.9±0.1	0.56±0.03	0.88±0.03	1.7±0.1	0.59±0.02	0.58±0.03
CML135	4.7±0.3	0.48±0.03	0.84±0.02	4.3±0.2	0.44±0.01	0.56±0.02
CML121	2.0±0.1	0.58±0.04	0.98±0.03	2.1±0.1	0.54±0.01	0.76±0.03
CML139	2.5±0.2	0.58±0.03	1.00±0.03	2.2±0.1	0.61±0.08	0.72±0.09
CML131	5.9±0.2	0.47±0.04	0.86±0.03	4.6±0.1	0.46±0.05	0.65±0.02
Ki3	4.4±0.2	0.53±0.05	0.80±0.03	5.6±0.2	0.42±0.01	0.55±0.02
CML127	2.3±0.1	0.67±0.05	0.93±0.03	2.9±0.2	0.66±0.02	0.79±0.03
Hi34	2.3±0.1	0.59±0.04	0.85±0.04	3.1±0.2	0.42±0.01	0.63±0.03

‡ Force measurements were determined using the instron technique reported in Chapter 2. Force readings are in newtons. Leaf ratings are based on Guthrie et al. (1960) scale of 1 (resistant) to 9 (susceptible).

**(MEAN INSTRON READINGS AND LEAF DAMAGE RATINGS FOR 1990 AND 1991
CONTINUED...)**

Mean leaf toughness readings and leaf damage ratings for Mexican and Argentinian landraces.

Genotype	1991‡		1992‡		
	Rating	Force	Rating	Force (Whorl)	Force (Tasselling)
MK12	6.2±0.2	0.56±0.02			
MB10	4.3±0.3	0.59±0.03			
MK9	3.0±0.2	0.55±0.02			
MC3	2.1±0.2	0.63±0.02			
ME3	5.1±0.3	0.59±0.03			
Oaxaca 179	3.0±0.3	0.63±0.03	3.0±0.2	0.49±0.01	0.73±0.02
Oaxaca 4	5.4±0.3	0.58±0.02	4.8±0.2	0.48±0.01	0.55±0.02
Guanajuato 207	4.0±0.3	0.61±0.03	4.4±0.4	0.47±0.01	0.56±0.01
Guanajuato 102	3.8±0.3	0.65±0.03	3.4±0.3	0.53±0.01	0.74±0.02
Cateto:C 2030	4.2±0.3	0.61±0.04	3.4±0.2	0.52±0.02	0.56±0.01
Cateto:C 2032	4.3±0.2	0.63±0.04	3.4±0.3	0.47±0.02	0.63±0.02
Nayarit 72	5.4±0.2	0.72±0.02	4.6±0.3	0.51±0.01	0.71±0.02
Nayarit 24	4.1±0.3	0.67±0.03	3.0±0.3	0.36±0.01	0.62±0.02
Chiapis 218	4.2±0.3	0.69±0.03	3.3±0.2	0.54±0.02	0.77±0.02
Mexico 208	2.4±0.3	0.89±0.03	2.1±0.2	0.64±0.02	0.78±0.02
Mexico 212	2.3±0.3	0.82±0.04	2.4±0.2	0.61±0.02	0.90±0.02

‡ Force measurements were determined using the instron technique reported in Chapter 2. Force readings are in newtons. Leaf ratings are based on Guthrie et al. (1960) scale of 1 (resistant) to 9 (susceptible).

APPENDIX 4
PLATES FROM THE HISTOCHEMISTRY STUDY REPORTED IN CHAPTER 3

FIGURE 1. Colour gradients for hydroxycinnamic acids. Autofluorescence (a) of p-coumaric acid (top) and ferulic acid (bottom). Staining by phloroglucinol (b), diazotized p-nitroaniline (c) and diazotized sulfanilic acid (d). Columns from left to right are: control (no phenolics added to filter paper), 0.005, 0.05, 0.5 and 5.0 mg phenolic acid/cm^2. For each colourimetric test p-coumaric acid is above ferulic acid.

FIGURE 2. Unstained leaf sections of MS72, a line susceptible to insect feeding. Mature tissue (a) has thicker epidermal cell walls and more developed secondary vascular tissue than immature leaf tissue (b) (Bar = 100 μm). Immature vascular tissue of MS72 without staining (c), autofluorescence (d), and autofluorescence under white light conditions (e) (Bar = 50 μm).

FIGURE 3. Phloroglucinol staining of maize leaves observed under white light (left) and ultraviolet light (right). Leaf sections of mature (a, b) and immature (c, d) tissue of MS72. Sections of mature (e, f) and recently emerged (g, h) leaf tissue of B86 (Bar = 100 μm).

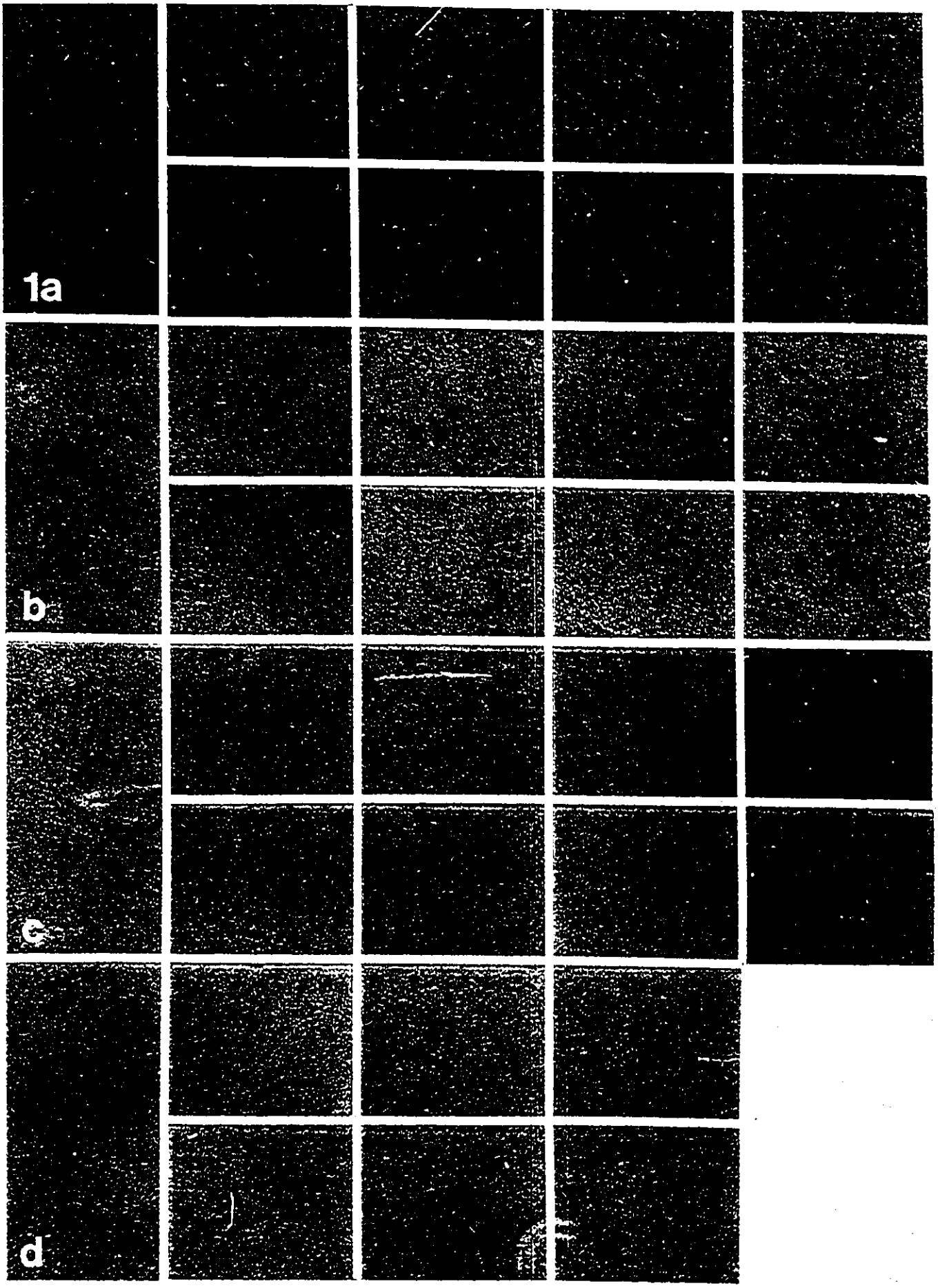
FIGURE 4. Azure B staining of leaf sections at room temperature (a-e) and after 2 hrs. of heating (f, g). Mature (a) and recently emerged (b) leaf sections of B86 (Bar = 100 μm). Vascular tissue of mature (c) and immature (d) leaf sections of MS72 (Bar = 50 μm). Trichome of mature leaf (e) (Bar = 10 μm). Mature (f) and immature (g) leaf sections of MS72 (Bar = 100 μm).

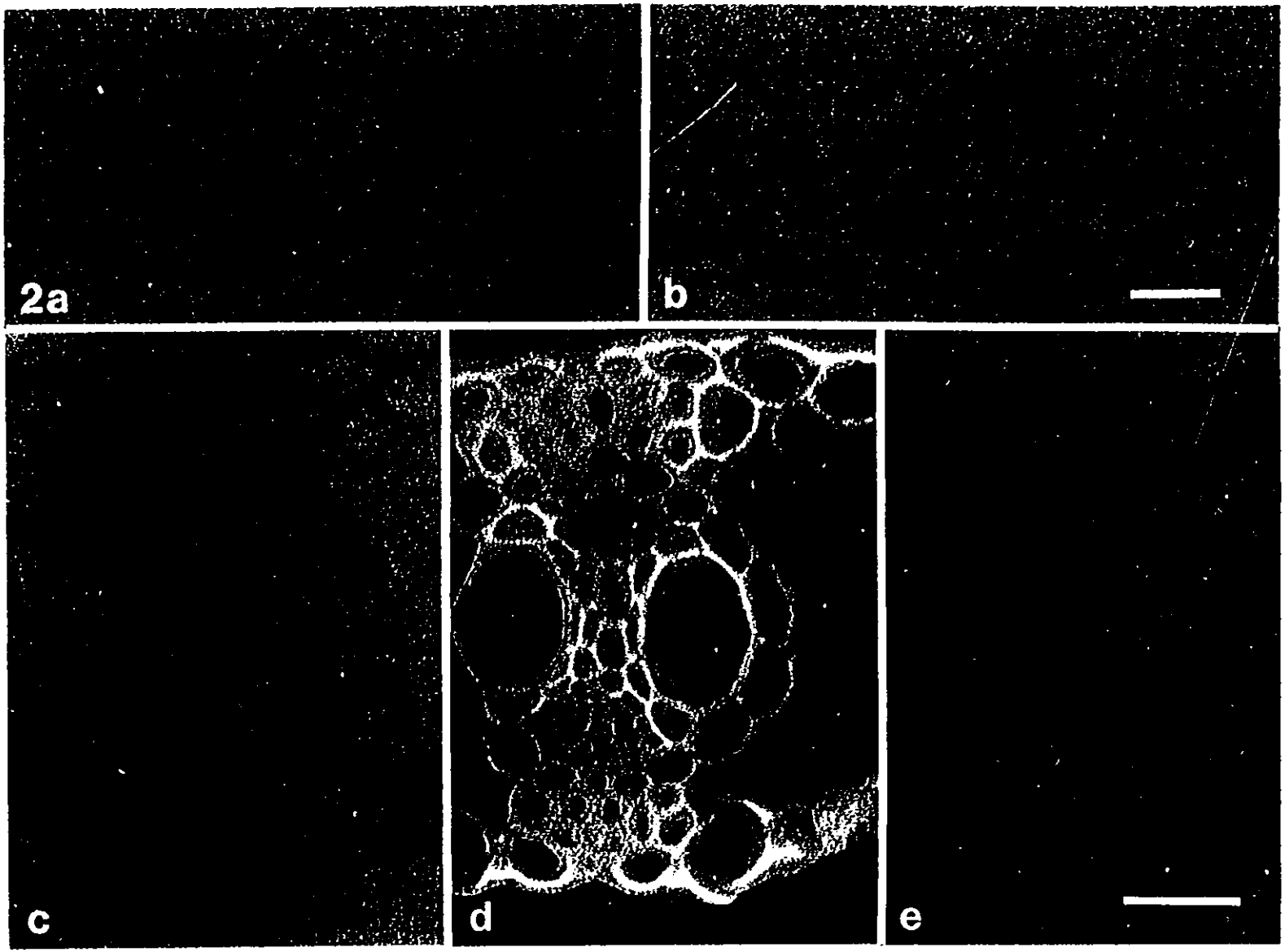
FIGURE 5. Chlorine-sulfite staining of leaf sections. Mature (a) and immature (b) leaf sections of MS72. Mature leaf sections of B86 vascular tissue under white light (c) and ultraviolet light (d). Vascular tissue of immature leaf from B86 (e) (Bar = 50 μm).

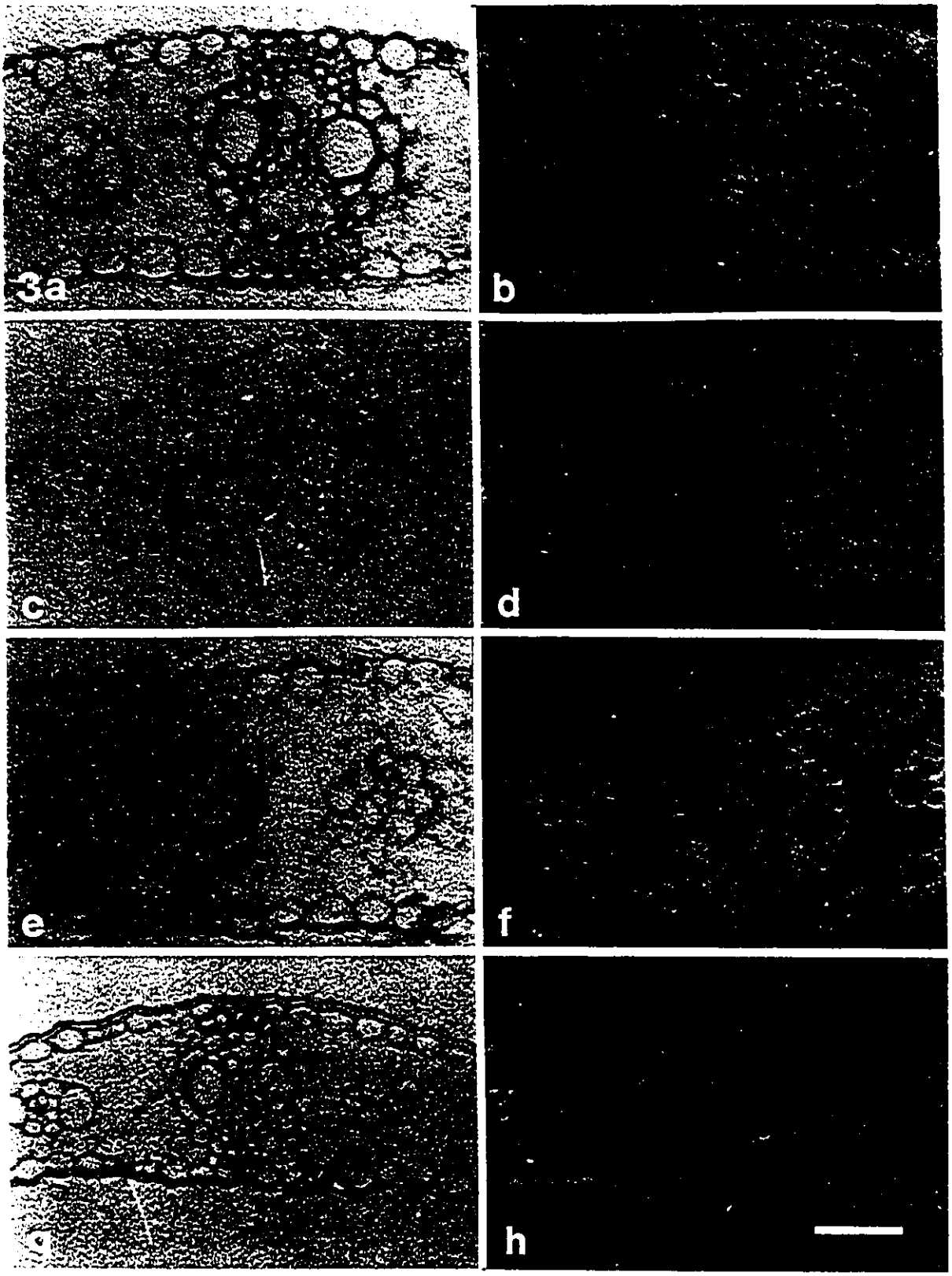
(PLATES FROM HISTOCHEMISTRY STUDY CONTINUE...)

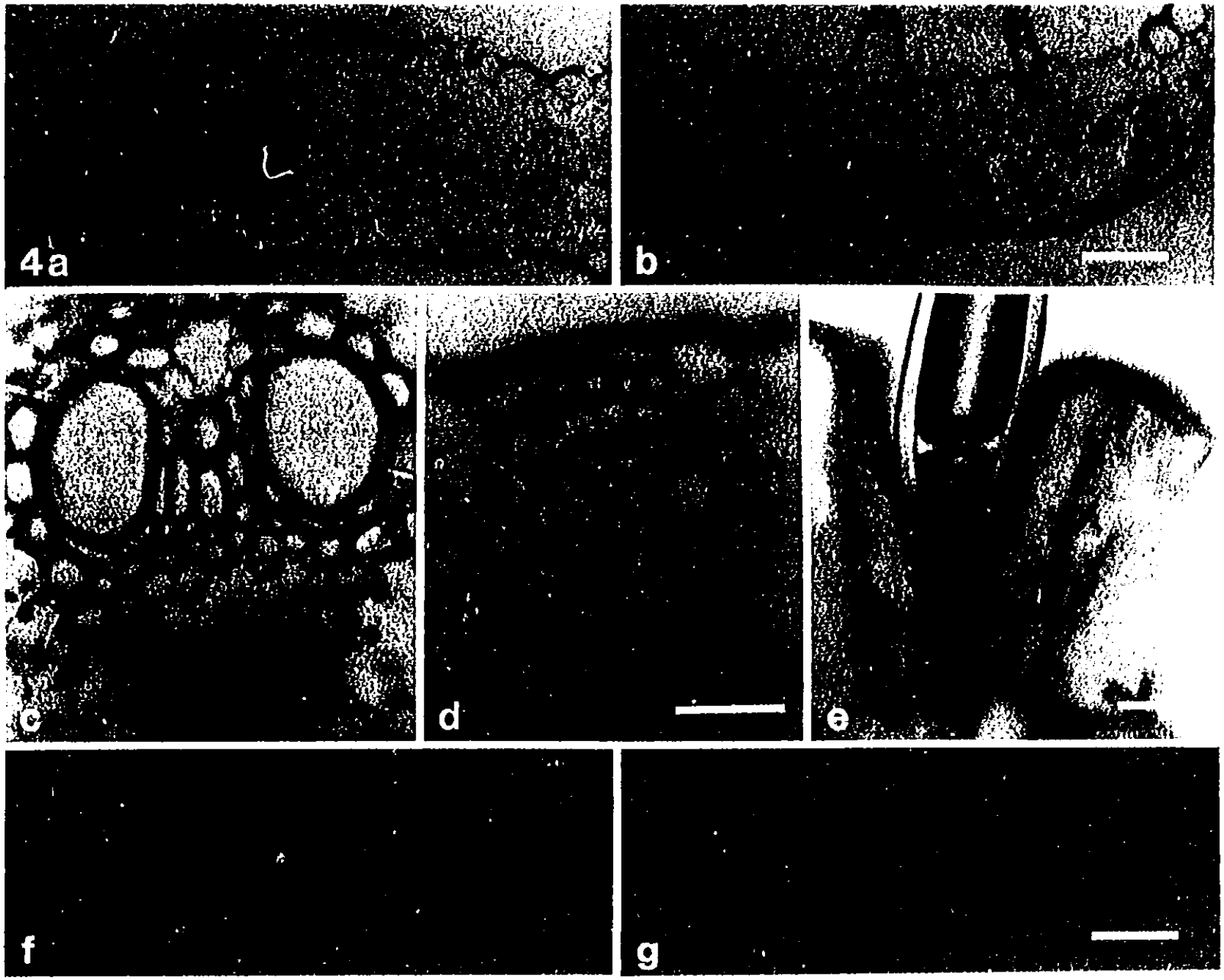
FIGURE 6. Diazotized 4-nitroaniline staining of maize leaf sections. Mature leaf sections of MS72 (a) and B86 (b). Immature leaf sections of MS72 (c) and recently emerged leaf of B86 (d) (Bar = 100 μm). Vascular tissue of immature (e) and mature (f) leaves from MS72 (Bar = 50 μm).

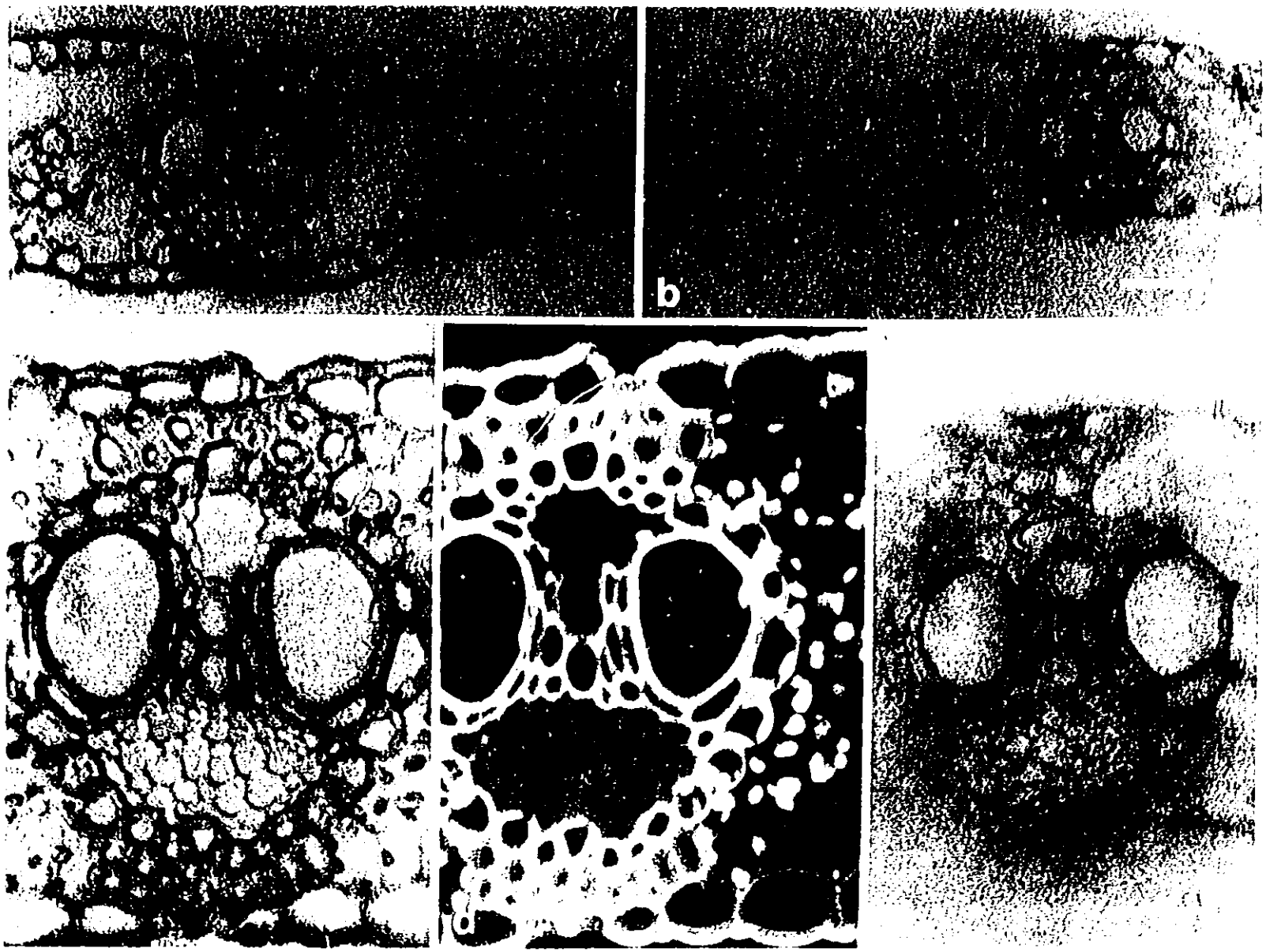
FIGURE 7. Diazotized sulfanilic acid staining of maize leaf sections. Mature (a) and immature (b) leaf sections of MS72. Mature (c) and recently emerged (d) leaf sections of B86 (Bar = 100 μm). Vascular tissue of mature (e) and immature (f) leaf sections of MS72. Autofluorescence of mature leaf section from B86 (Bar = 50 μm). Oxidation of stained section when not covered by glycerol solution (h) (Bar = 100 μm).

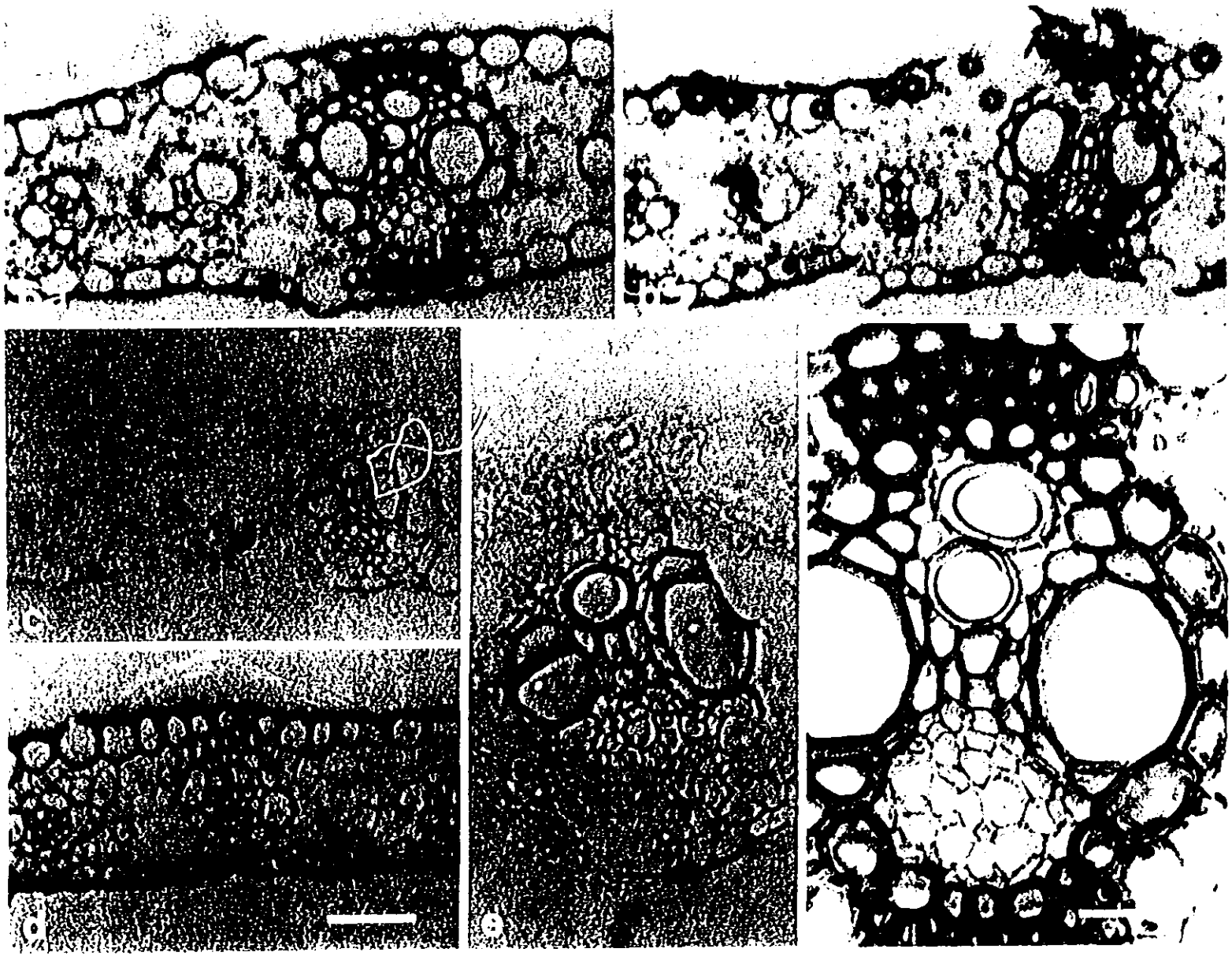

1a
b
c
d

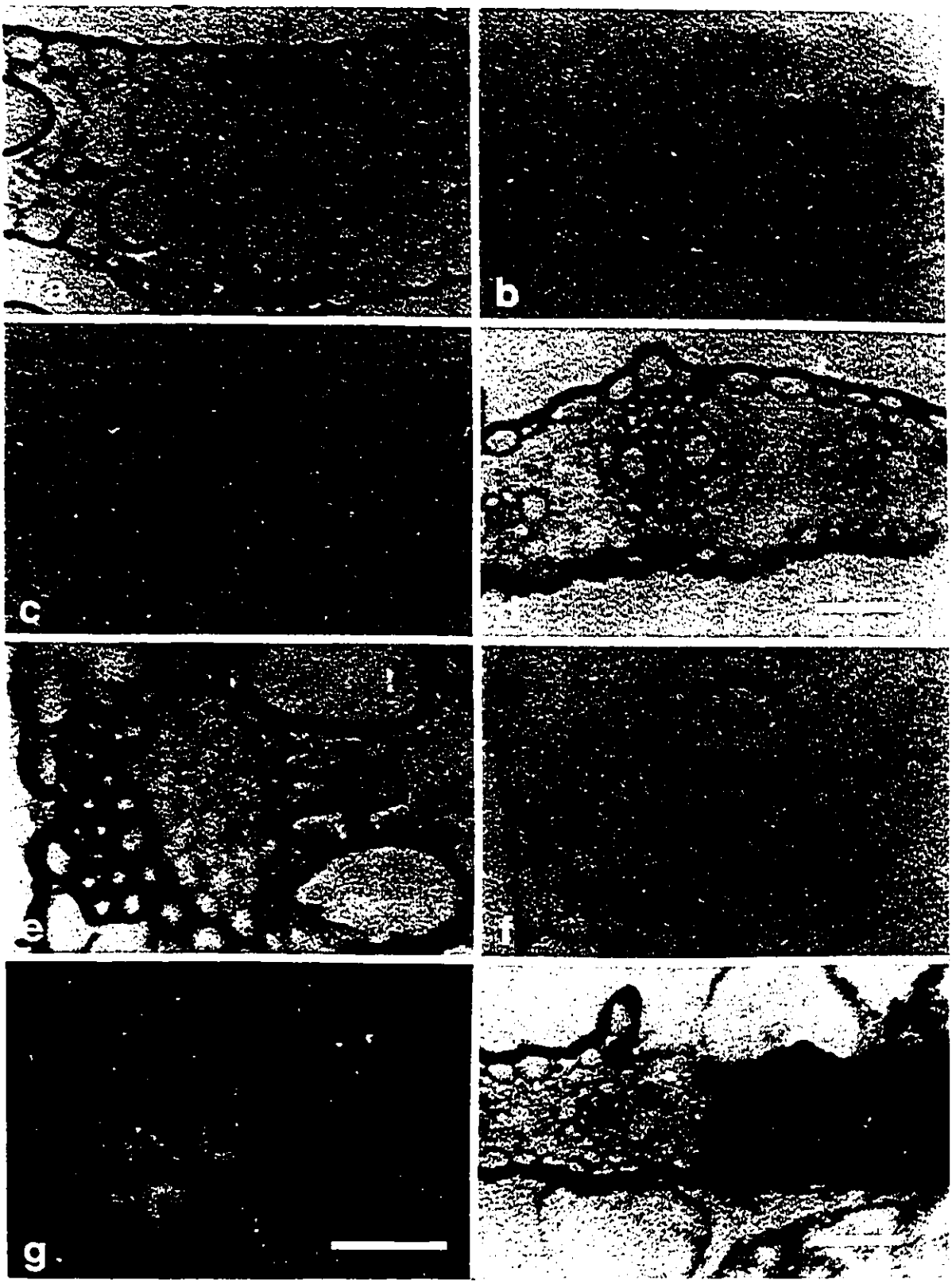

2a
b
c
d
e


3a
b
c
d
e
f
g
h


4a
b
c
d
e
f
g


b




b
c
e
g

APPENDIX 5

LIGNIN CONTENT OF BS9

Lignin determinations of different tissues from four selection cycles of BS9, field grown in 1991 and 1992.

		1991					1992				
Tissue	Parameter[†]	C0	C2	C4	C5	LSD[‡]	C0	C2	C4	C5	LSD[‡]
Stalk	Lignin 1	563	606	671	498		451	620	695	744	
	Lignin 2	8.95	9.71	13.5	9.07		8.91	10.37	12.32	11.65	
Node	Lignin 1	360	394	489	652	**	373	627	442	354	
	Lignin 2	6.21	8.03	10.1	19.2	**	14.37	13.66	6.33	-	
Pith	Lignin 1	354	346	277	355		352	164	398	252	
	Lignin 2	12.58	7.85	5.84	7.23		6.76	5.96	12.18	5.06	
Sheath	Lignin 1	370	380	520	602		-	-	-	-	
	Lignin 2	8.17	5.62	8.79	12.68		-	-	-	-	
Leaf (M)	Lignin 1	348	289	330	343		351	330	362	548	
	Lignin 2	2.75	3.01	3.32	2.91		2.11	2.31	1.61	2.78	
Leaf (I)	Lignin 1	101	292	286	291		247	146	250	322	
	Lignin 2	0.48	0.19	0.28	0.46		0.88	0.36	1.36	0.89	

[†] Lignin 1 determinations based on Iiyama and Wallis (1990), Lignin 2 are relative determinations from HPLC analysis of base hydrolyzed cell wall preparations from maize tissue (method reported in Chapter 3). Leaf tissue (M=mature, I=Immature) was harvested at mid-whorl and stalk, node, pith and sheath tissue were harvested during silking.

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

(LIGNIN CONTENT OF BS9

CONTINUED...)

Lignin determinations for BS9(C4) at different stages in development.

Treatment	Leaf	Percent Lignin[†]
Greenhouse	3	5.86 ± 0.55
Reduced UV	5	5.03 ± 0.77
	7	5.75 ± 0.58
	9	6.36 ± 0.75
	10	4.94 ± 0.98
Greenhouse	3	7.98 ± 0.48
UV Suppliment	5	5.55 ± 0.59
	7	4.82 ± 0.83
	9	5.77 ± 0.95
	10	6.43 ± 0.69

[†] Lignin determinations based on Iiyama and Wallis (1990) Leaf tissue was harvested two days after emergence from whorl.

[‡] * Significant at the 0.05 probability level.

APPENDIX 6
PHYSICAL TRAITS AND PLANT DAMAGE PARAMETERS FOR BS9

Measurements of physical parameters for field grown selection cycles of BS9, 1991.

	Parameter[†]	C0	C2	C4	C5	LSD[‡]
Stalk	Force (kg)	2.57	3.89	4.2	3.41	0.82
Height (m)	Ear	1.53	1.43	1.42	1.34	0.17
	Tassel	2.42	2.33	2.31	2.22	0.06
	Extended leaf	2.51	2.26	2.22	2.26	0.12

[†] Penetrometer readings taken at third internode during silking. Height measurements taken at maturity.
[‡] * Significant at the 0.05 probability level.

Measurements of physical and plant damage parameters for field grown selection cycles of BS9, 1991.

		Light Regime									
		UV-					UV+				
	Parameter[†]	C0	C2	C4	C5	LSD[‡]	C0	C2	C4	C5	LSD[‡]
Leaf	LDR	2.94	2.45	2.48	2.03	0.55	2.53	2.39	2.18	2.26	0.43
Stalk	Tough (kg)	2.5	3.88	3.95	2.85	1.43	2.65	3.91	4.48	3.96	1.75
Height (m)	Ear	1.57	1.42	1.48	1.31	0.16	1.49	1.43	1.36	1.36	0.19
	Tassel	2.43	2.29	2.37	2.21	0.21	2.41	2.35	2.25	2.22	0.23
	Leaf	2.49	2.33	2.22	2.24	0.06	2.51	2.19	2.22	2.29	0.31

[†] LDR Leaf damage rating (1-9), Guthrie et al. 1960. Physical measurements are same as above table.
[‡] * Significant at the 0.05 probability level.

(PHYSICAL TRAITS AND PLANT DAMAGE PARAMETERS FOR BS9 CONTINUED...)

Plant damage by natural ECB infestation, Prescott 1991.

Parameter[†]	C0	C2	C4	C5	LSD[‡]
N Larvae / plant	1.01	0.73	0.6	0.37	0.15
N Tunnels / plant	2.07	1.45	1.22	0.82	0.35
X-section excavated (%)	44.4	43.7	41.5	44.1	6.4
Tunnel length (cm)	7.02	6.67	4.85	4.56	1.93
Mean tunnel height (m)	1.4	1.31	1.03	1.19	0.33

[†] N=number of, X-section is percentage of pith cross section excavated by ECB.
[‡] * Significant at the 0.05 probability level.

APPENDIX 7
MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY

Means for biochemcial and physical resistance factors in mature leaf tissue of multiple borer resistant maize, 1990.

Genotype	Leaf type	Leaf rating	Bio-assay	Protein (%)	Leaf Force	PCA	FA	Tx	DFA	Soluble	Fibre	Hx	sPCA	sFA
						mg/g dry weight							µg/g d.w.	
Bugs	g	2.46	20	15.59	0.83	2.65	2.8	0.85	0.42	300	320	0.89	36	67
Mbita	g	2.56	22	16.98		1.81	2.46	0.97	0.35	240	360	1.13	25	17
SCB	g	2.14	29	15.89	0.82	2.4	2.52	0.73	0.58	340	300	1.45	36	47
ECB	g	2.12	19	16.07	0.83	2	2.81	1.04	0.6	300	280	1.17	41	52
SWCB	g	2.22	27	15.65	0.79	2.14	2.68	1.13	0.58	300	300	0.95	73	80
P47xKi3	g	2.22	14	16.11	0.89	2.53	3.16	1.37	0.4	320	300	0.39	30	37
Ki3xTx601	g	3.61	20	16.47	0.68	2.07	2.14	0.73	0.41	340	260	1.2	40	15
Pion 3184	g	3.9	27	15.39	0.77	2.5	2.62	1.01	0.4	280	300	1.48	115	64
Font 6220	g	5.71	40	18.64	0.72	1.31	1.25	0.51	0.2	320	280	0.23	136	100
6796-48	g	5.11	70	18.37	0.64	1.76	2.48	1.52	0.18	280	300	0.65	80	92
6796-49	g	5.36	78	19.17	0.65	1.73	2.41	0.89	0.23	300	300	1.06	31	122
6796-13	g	2.41	44	15.61	0.74	1.62	1.9	0.54	0.25	380	260	0.73	25	32
DK 435	g	3.47	41	16.28	0.67	1.78	1.65	1.2	0.28	320	300	1.29	190	139
Pic 4533	g	4.11	62	17.6	0.59	1.63	2.45	1.26	0.3	300	300	2.12	140	173

Leaf rating is Guthrie's (1960) 1-9 scale, bioassay is mm^2 tissue consumed, leaf toughness of mature ear leaf at tasselling, PCA=p-coumaric acid, FA=ferulic acid, Tx=total cyclobutane dimers, DFA=dehydrodiferulic acid, soluble is gravimetric determination of soluble metabolites on a dry weight basis, fibre is estimated detergent fibre, Hx=DIMBOA equivalents, sPCA and sFA are soluble conjugates of PCA and FA.

(MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY CONTINUED...)

Means for biochemcial and physical resistance factors in immature leaf tissue of multiple borer resistant maize, 1990.

Genotype	Leaf type	Leaf rating	Bio-assay	Protein (%)	Leaf Force	PCA	FA	Tx	DFA	Soluble	Fibre	Hx	PCA	FA
						mg/g dry weight							µg/g d.w.	
Bugs	y	2.46	20	18.54	0.83	4.07	4.44	0	0.49	440	200	1.06	53	61
Mbita	y	2.56	21	18.79		3.37	4.17	0.01	0.64	500	160	1.82	70	57
SCB	y	2.14	29	18.28	0.82	2.86	3.24	0.01	0.42	500	200	2.36	109	28
ECB	y	2.12	19	12.38	0.83	4.73	6.93	0.05	0.49	340	240	1.54	35	68
SWCB	y	2.22	27	12.84	0.79	5.76	6.97	0.08	0.57	340	200	1.66	60	35
P47xKi3	y	2.22	13	12.68	0.89	5.59	6.45	0.01	0.39	380	220	0.87	56	53
Ki3xTx601	y	3.61	20	14.01	0.68	3.43	4.27	0	0.28	560	160	0.88	53	20
Pion 3184	y	3.9	27	11.14	0.77	5.95	7.49	0.08	0.58	380	220	0.94	453	50
Font6220	y	5.71	39	15.27	0.72	3.82	4.24	0	0.51	400	220	1.65	653	623
6796-48	y	5.11	70	19.42	0.64	2.48	3.7	0.02	0.08	500	160	1.62	30	40
6796-49	y	5.36	78	21.72	0.65	2.42	3.58	0	0.2	480	180	1.75	31	46
6796-13	y	2.41	44	16.27	0.74	2.49	2.98	0	0.21	500	180	1.83	32	41
DK 435	y	3.47	41	15.46	0.67	3.1	3.76	0.13	0.35	460	180	4.9	79	117
Pic 4533	y	4.11	62	17.02	0.59	3.68	5.49	0.02	0.26	460	180	3.07	345	153

Leaf rating is Guthrie's (1960) 1-9 scale, bioassay is mm^2 tissue consumed, leaf toughness of mature ear leaf at tasselling, PCA=p-coumaric acid, FA=ferulic acid, Tx=total cyclobutane dimers, DFA=dehydrodiferulic acid, soluble is gravimetric determination of soluble metabolites on a dry weight basis, fibre is estimated detergent fibre, Hx=DIMBOA equivalents, sPCA and sFA are soluble conjugates of PCA and FA.

(MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY CONTINUED...)

Means for biochemcial and physical resistance factors in mature leaf tissue of multiple borer resistant maize, 1991.

Genotype	Leaf type	Leaf rating	Bio-assay	Protein (%)	Leaf Force	PCA	FA	Tx	DFA	Soluble	Fibre	Hx	PCA	FA
						mg/g dry weight							µg/g d.w.	
Bugs	g	2.15	16	14.41	0.83	2.7	2.6	1.15	0.32	300	348	0.79	58	81
Mbita	g	2.56	21	14.98		2.5	2.6	1.4	0.32	270	355	1.14	25	17
SCB	g	2.43	11	12.73	0.82	2.8	2.7	1.33	0.41	280	346	0.62	83	40
ECB	g	2.16	17	15.31	0.83	1.9	2.5	0.83	0.35	270	341	0.52	35	42
SWCB	g	1.97	4	13.32	0.79	3	3	1.65	0.36	220	348	0.24	23	23
P47xKi3	g	2.22	10	14.14	0.89	2.9	2.9	1.49	0.37	240	351	0.21	36	49
Ki3xTx601	g	3.97	15	15.01	0.68	2.9	2.3	1.01	0.31	370	288	1.45	89	68
Pion 3184	g	3.85	15	16.12	0.77	2.7	2.2	0.96	0.24	280	320	1.03	94	59
Font 6220	g	5.73	27	18.64	0.72	1.3	1.3	0.52	0.2	320	280	1.28	136	123
6796-48	g	5.42	11	14.09	0.64	3.7	3.41	1.43	0.29	200	352	0.87	75	86
6796-49	g	5.48	18	16.31	0.65	2.1	2.3	1.03	0.21	260	329	0.83	138	142
6796-13	g	3.12	19	15.41	0.74	2.4	2.5	1.08	0.31	300	336	0.82	68	46
DK 435	g	3.31	30	15.89	0.67	2.2	1.7	0.97	0.3	350	280	0.65	136	118
Pic 4533	g	4.78	21	19.51	0.59	2.2	2.5	1.09	0.21	340	295	0.47	50	56

Leaf rating is Guthrie's (1960) 1-9 scale, bioassay is mm^2 tissue consumed, leaf toughness of mature ear leaf at tasselling, PCA=p-coumaric acid, FA=ferulic acid, Tx=total cyclobutane dimers, DFA=dehydrodiferulic acid, soluble is gravimetric determination of soluble metabolites on a dry weight basis, fibre is estimated detergent fibre, Hx=DIMBOA equivalents, sPCA and sFA are soluble conjugates of PCA and FA.

(MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY CONTINUED...)

Means for biochemcial and physical resistance factors in immature leaf tissue of multiple borer resistant maize, 1991.

Genotype	Leaf type	Leaf rating	Bio-assay	Protein (%)	Leaf Force	PCA	FA	Tx	DFA	Soluble	Fibre	Hx	PCA	FA
						mg/g dry weight							µg/g d.w.	
Bugs	y	2.15	16	13.28	0.83	4.1	5	0.24	0.47	420	236	1.42	215	57
Mbita	y	2.56	21	14.13		4.6	5.9	0.08	0.52	420	236	1.82	70	57
SCB	y	2.43	11	15.38	0.82	3.9	4.9	0.34	0.52	460	226	0.75	46	43
ECB	y	2.16	17	12.6	0.83	2.9	5.4	0.16	0.58	450	215	0.59	97	99
SWCB	y	1.97	4	13.61	0.79	4.3	5.3	0	0.56	390	236	0.91	108	30
P47xKi3	y	2.22	10	12.8	0.89	4.5	5.7	0.12	0.44	460	217	0.54	77	67
Ki3xTx601	y	3.97	15	14.9	0.68	4.6	4.2	0.12	0.43	490	200	1.82	71	72
Pion 3184	y	3.85	15	13.2	0.77	3.8	4.6	0	0.41	480	213	2.22	68	43
Font6220	y	5.73	27	16.8	0.72	3.8	4.3	0	0.52	400	220	1.67	62	166
6796-48	y	5.42	11	15.4	0.64	4.5	5.8	0	0.42	390	226	1.05	165	19
6796-49	y	5.48	18	17.7	0.65	5.5	7.4	0.08	0.44	340	262	1.07	100	252
6796-13	y	3.12	19	13.3	0.74	4.3	5.6	0.07	0.49	360	237	1.15	101	53
DK 435	y	3.31	30	14.3	0.67	5.6	5.8	0	0.43	320	251	2.24	84	53
Pic 4533	y	4.78	21	15.2	0.59	5.9	5.8	0.24	0.3	270	204	0.71	364	50

Leaf rating is Guthrie's (1960) 1-9 scale, bioassay is mm^2 tissue consumed, leaf toughness of mature ear leaf at tasselling, PCA=p-coumaric acid, FA=ferulic acid, Tx=total cyclobutane dimers, DFA=dehydrodiferulic acid, soluble is gravimetric determination of soluble metabolites on a dry weight basis, fibre is estimated detergent fibre, Hx=DIMBOA equivalents, sPCA and sFA are soluble conjugates of PCA and FA.

(MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY CONTINUED...)

Means for physical resistance and plant damage factors for multiple borer resistant maize, 1990 field trial.[‡]

Genotype	# larvae	# larvae in ear	Tunnel length (cm)	Percent area	Stalk break (ft)	Length ear tunnel (cm)	Stalk rot (cm)	Stalk (lb/in^2)	Plant height (m)	Ear height (m)
Bugs	0.93	1.33	4.8	35.8	nb	0.36	1.8	7.75	3.35	1.95
Mbita	0.9	0	3.1	28	nb	0.03	1.3	5.4	3.41	1.99
SCB	1.2	0.13	8.3	39.6	nb	0.23	2	7.6	3.44	2.08
ECB	1.4	0.1	7.2	46.8	nb	0.36	2.4	4.91	3.44	2.12
SWCB	1.07	0.07	7.2	37.1	nb	0.1	2.4	6.65	3.29	2.09
P47	1.1	0.1	6	27.9	nb	0.03	1.6	6.95	3.51	2.12
Ki3xTx601	1.1	0.13	7.3	41	nb	0.3	2.7	6	3.26	1.99
Pion 3184	1.17	0.13	6.4	29.6	nb	1.1	1	8.85	3.77	1.44
Font 6220	1.93	0	16	52.4	0.93	0	3.1	9.95	3.17	1.55
6796-48	0.86	0	28	65.7	2.35	0.78	4.9	0.91	2.02	1.14
6796-49	0.12	0	18	51.7	2.05	1.76	6.7	4.76	2.21	1.31
6796-13	1.33	0.1	17	47.5	nb	0.4	7.9	2.3	2.67	1.59
DK 435	2.42	0.39	25	67.7	3.25	4.12	9.7	12.1	2.92	1.06
Pic 4533	3.3	0.63	30	67.6	2.03	6.66	8.9	12.2	2.67	1.06

[‡] # larvae=number of larvae in disected plant in October; #larvae in ear=number of larvae in ear (not included in plant count); percent area=percentage of pith cross-section excavated by ECB; stalk break=height of stalk breakage (nb=negligible breakage); Stalk=stalk toughness measured by penetrometer at the third internode during tasselling;.

(MEAN FIELD AND PHYTOCHEMICAL RESULTS FOR MULTIPLE BORER RESISTANT STUDY CONTINUED...)

Means for physical resistance and plant damage factors for multiple borer resistant maize, 1991 field trial.[1]

Genotype	# larvae	# Tunnels	Percent area	Tunnel length (cm)	Mean height (m)
Bugs	0.07	0.83	48.07	6.14	0.69
Mbita	0.07	0.62	42.59	3.64	0.54
SCB	0.15	0.45	38.41	4.18	0.47
ECB	0.1	0.45	41.05	4.73	0.68
SWCB	0.07	0.42	43.41	3.14	0.88
P47	0.15	0.62	43.26	5.22	0.54
Ki3xTx601	0.05	0.58	38.33	4.08	0.65
Pion 3184	0.12	0.52	48.04	4.22	0.49
Font 6220	0.51	2.12	47.51	8.65	0.54
6796-48	0.34	1.11	59.17	4.73	0.46
6796-49	0.18	0.91	58.21	7.46	0.48
6796-13	0.18	0.62	47.21	4.66	0.54
DK 435	0.42	1.48	41.12	7.52	0.56
Pic 4533	0.57	2.36	46.75	12.22	0.59

[1] # larvae=number of larvae in disected plant (stalk plus ear) in October; #Tunnels=number of tunnels per plant; percent area=percentage of pith cross-section excavated by ECB; Mean height=mean height of ECB tunnelling by October.