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ROLE OF EXTRACELLULAR PEROXIDASE IN MAIZE, *ZEA MAYS (L.)* RESISTANCE TO THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS (HÜBNER)*

by

© Susan MacIsaac

A thesis submitted to the

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ABSTRACT

A cell wall peroxidase (E.C. 1.11.17) mediates the formation of diferulic acid, (E,E)-4,4'-dihydroxy-5,5'-dimethoxy-3'-bicinnamic acid, through the cross-linking of hemicellulose bound ferulic acid, 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid, which increases the mechanical strength of the cell wall and its resistance to insects. It was hypothesized that the level of the cell wall bound peroxidase activity was positively correlated across genotypes with the concentration of cell wall bound diferulic acid concentration and resistance to the European corn borer (ECB), *Ostrinia nubilalis* Hübner, in field grown maize, *Zea mays* (L.).

Fourteen or twelve genotypes of maize of different sensitivity to the ECB from Agriculture and Agri-Food Canada and CIMMYT, Mexico, were planted in mid May, 1995 and 1996, respectively. Plants were harvested in July at the mid whorl stage. The yellow-green section of the whorl leaf was used in three separate analyses. First, leaf feeding bioassays of fresh tissue were performed on all genotypes (20 replicates per genotype) in 1995 and (40 replicates per genotype) in 1996. In every genotype tested, consumption rate varied widely and there were several replicates in each genotype in which no leaf tissue was consumed. This high variability was likely due to larval feeding characteristics. Secondly, wall bound ferulic and diferulic acid concentrations from freeze dried leaves were determined by HPLC. Resistant genotypes had higher ferulic and diferulic acid concentrations in their cell walls than susceptible genotypes. A strong positive correlation between the quantities of ferulic acid and diferulic acid was evident in both years ($r=0.80$, $p<0.001$, $n=14$ in 1995; $r=0.87$, $p<0.001$, $n=12$ in 1996). Thirdly, cell wall bound peroxidase was extracted and the activity determined spectrophotometrically using ferulic

acid as substrate. Activity of the peroxidase was highest at the base of the whorl leaf and rapidly declined with tissue age. Among genotypes, the activity varied widely with no apparent relationship with either ferulic acid, diferulic acid or consumption rate. These results suggest that while diferulic acid may be an indicator of resistance, cell wall bound peroxidase does not play a regulatory role in its formation. Subsequently, peroxidase cannot be a bioindicator of resistance. The strong relationship between ferulic acid and diferulic acid suggests that feruloylation is the rate limiting step in the formation of diferulic acid.

RÉSUMÉ

Une peroxydase de la paroi cellulaire (E.C. 1.11.17) est responsable de la formation de l'acide diférulique, acide (E, E)-4,4'-dihydroxy-5,5'-dimethoxy-3'-dicinnamique, à partir de la formation de liens croisés d'acide férulique, acide 3-(4-hydroxy-3-methoxyphenyl)-2-propenöique, lié à l'hémicellulose, qui augmente la force mécanique de la paroi cellulaire ainsi que sa résistance aux insectes. Nous avions postulé que, parmi les génotypes, l'activité de la peroxydase liée à la paroi cellulaire soit positivement corrélée avec la concentration d'acide diférulique lié à la paroi cellulaire et avec la résistance à la pyrale, *Ostrinia nubilalis* (Hübner), dans une étude au champ du maïs, *Zea mays* (L.).

Quatorze ou douze génotypes de maïs de sensibilité différente à la pyrale, d'Agriculture et agro-alimentaire Canada et de CIMMYT, Mexique, ont été semés à la mi-mai 1995 et 1996, respectivement. Les plants ont été récoltés en juillet au mi-stade du verticille. La portion foliaire de teinte jaune-verte du verticille a été utilisée et analysée selon trois composantes. Premièrement, les bio-essais de consommation ont été réalisés avec du tissu frais sur tous les génotypes (20 réplicats par génotype) de 1995 et (40 réplicats par génotype) de 1996. Pour chacun des génotypes étudiés, le taux de consommation variait grandement ou encore il n'y avait aucune consommation foliaire pour plusieurs réplicats de chaque génotype. Cette grande variabilité peut résulter des caractéristiques de consommation des larves. Deuxièmement, les concentrations d'acide férulique et d'acide diférulique liés à la paroi cellulaire à partir de feuilles lyophilisées ont été déterminées par HPLC. Les génotypes résistants avaient, dans leurs parois cellulaires, des concentrations d'acides férulique et diférulique plus élevées que les génotypes

plus sensibles. Une forte corrélation positive a été mise en évidence entre les quantités d'acides férulique et diférulique pour chaque année ($r=0.80$, $p<0.001$, $n=14$, en 1995 ; $r=0.87$, $p<0.001$, $n=12$, en 1996). Troisièmement, la peroxydase liée à la paroi cellulaire a été extraite et son activité mesurée par spectrophotométrie, utilisant l'acide férulique comme substrat. L'activité de la peroxydase était la plus élevée à la base du verticille foliaire et diminuait rapidement avec l'âge du tissu. Parmi les génotypes, l'activité variait grandement sans relation apparente avec les concentrations d'acide férulique ou d'acide diférulique ou les taux de consommation. Ces résultats suggèrent qu'alors que l'acide diférulique s'avère un indice de résistance, la peroxydase liée à la paroi cellulaire ne semble pas jouer un rôle de régulation dans sa formation. La peroxydase ne peut donc être considérée comme un bio-indicateur de résistance. La forte relation existant entre l'acide férulique et l'acide diférulique suggère que la "féruloylation" est l'étape limitante dans la formation de l'acide diférulique.

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LIST OF ABBREVIATIONS

AAC	Agriculture and Agri-Food Canada
CHU	corn heat units
CIMMYT	International Maize and Wheat Improvement Center
CML	CIMMYT maize line
DFA	diferulic acid
df	degrees of freedom
DTT	dithiothreitol
DW	dry weight
ECB	European corn borer
F	Fischer's statistic
FA	ferulic acid
FAX	feruloylarabinoxylan
MBR	multiple borer resistant
MS	mean square
n.s.	non significant
p	probability statistic
PAL	phenylalanine ammonium lyase
PVP	polyvinylpyrrolidine
r	correlation coefficient
RGR	relative growth rate
SCB	sugarcane borer

SWCB	southwestern corn borer
SS	sum of squares

1. INTRODUCTION

1.1 INTEGRATED PEST MANAGEMENT OF MAIZE

Maize along with rice and wheat are the three main crops that feed the world. With the human population increasing at 90 million a year (CIMMYT, 1996), increased production as well as increased yields have never been more important. Policies directed toward the development of sustainable agricultural systems have been initiated throughout the developed and developing countries. Most of these programs involve the implementation of integrated pest management (i.p.m.) practices. Within i.p.m., newer strategies attempt to reduce or eliminate the need for pesticides, with their associated dangers of environmental contamination, pest resistance and non-target effects. The use of cultural and biological control of pests is still important, but host plant resistance has become the cornerstone of pest management and new varieties of maize with built in resistance to pests are constantly being sought. Targeting resistance to a particular pest as well as developing cultivars tolerant to drought or nutrient deficient soils is possible through on-going conventional selection or new biotechnological advances including transformation with exotic genes.

Cultivars developed for resistance to the European corn borer (ECB), *Ostrinia nubilalis*, include the CML lines from Mexico, the BS9 synthetic series from Iowa and the inbred B86, also from Iowa. All employ to some extent a newly identified phenolic acid based defence (Bergvinson, Arnason and Towers, unpublished data) which results in tissue strong enough to reduce ECB feeding rather than toxic hydroxamic acid defences known in conventional maize. Susceptible varieties like the Argentinean landraces and the Mexican inbred, Hi34, express lower levels of this particular defence. Targeting the enzyme that regulates the concentration of the

phenolic acid defences can lead to the development of new technologies for enhanced resistance to the ECB via tissue strength.

1.2 MAIZE

The mature maize plant consists of roots, stalk, leaves and reproductive organs. The root system consists of the radicle, lateral seminal roots, nodal roots and brace roots (Ritchie and Hanway, 1984). The stalks are strong and erect and are divided by conspicuous nodes at which point a single leaf emerges (Bockholt, 1979). Many plants develop anywhere from 15 to 25 or more leaves. They are long and narrow with parallel venation and are spaced alternately on the stem (Bockholt, 1979; Hartman et al., 1981). Each plant contains both staminate and pistillate flowers located at the top of the stem and in the ears respectively (Bockholt, 1979; Hartman et al., 1981).

Germination of the seed entails emergence of the radicle and coleoptile. The root system immediately establishes the lateral seminal root which, together with the radicle, is functional throughout most of the plant life, although the nodal roots formed by the sixth leaf stage become the major supplier of nutrients and water thereafter. Brace roots develop from nodes above the surface just before the onset of the reproductive stage. They function in supporting the plant and supplying water and nutrients from the upper soil layers.

The coleoptile containing the embryonic plant emerges from the soil at which point the embryonic leaves begin to develop rapidly. Development can now be divided into two stages, vegetative and reproductive. Vegetative stages begin with the emergence of the coleoptile and end with tasseling. The growth and maturation of the leaves occur during this time. The

reproductive stages are silking, blister, milk, dough, dent and physiological maturity. The silks on the ears of the plant leading to the cob and the tassel containing pollen are now fully developed. Pollination occurs when pollen is released from the tassels and comes into contact with the silks of the ears. Fertilization takes place when the pollen grain grows down the silks and reaches the ovule which forms a kernel. The remaining stages are concerned with the maturity of the kernel (Ritchie and Hanway, 1984).

The growing season depends on the climate and can vary anywhere from 70 days to year round (Hartman et al., 1981). Each cultivar is genetically programmed to reach maturity in a particular time frame which varies greatly from about 60 days to 11 months (Bockholt, 1979). Environmental conditions play a key role in this developmental time. Long days increase the duration of the vegetative stage (Bockholt, 1979), whereas moisture deficiencies may lengthen the time between vegetative stages and shorten the time between reproductive stages (Ritchie and Hanway, 1984). Optimum conditions in which corn grows best include moist, warm, fertile sandy loams with slightly acidic pHs that have the capacity to retain water while not becoming waterlogged (Bockholt, 1979). Germination will only occur at temperatures above 9 ^{0}C and maximal growth is attained by daytime temperatures between 20 to 40 ^{0}C and night time temperatures above 13 ^{0}C (Hughes and Metcalfe, 1972).

1.3 PLANT DEFENCES

Integrated pest management (IPM) provides an ecological approach to pest control by utilizing a combination of biological, cultural and chemical controls. Host plant resistance is the basic component (Panda and Khush, 1995). All plants have a degree of resistance to insect pests

which involves an array of physical and chemical characteristics which render the plant toxic, repellent or otherwise unsuitable (Beck and Schoonhoven, 1980). The utilization of favorable plant physical characteristics to increase plant resistance to insects which increases plant productivity is an example of host plant resistance. The use of pesticides is no longer favored as a means of insect control and plowing down of stalks, burning of crop residues and crop rotation (Hudon and LeRoux, 1986a) do not provide adequate measures of insect control. Host plant resistance has arisen as a key element in IPM. (Bockholt, 1979).

It was not until the mid 1960's that host plant resistance was developed as a full scale breeding strategy for maize, beginning with the pioneer work of Guthrie (Guthrie et al., 1960). Much progress in breeding for resistance to insects had been made throughout the world. The use of tolerant plants has controlled insect pests below crop damaging levels, reduced the need for insecticides, improved yields and prevented catastrophic economic losses.

Each plant species has a number of defence traits that can alter herbivore behaviour or physiology. Tissue toughness and pubescence provide structural barriers while feeding suppressants, repellents and arrestants proved to be phytochemical barriers.

1.3.1 Phytochemical Factors

The quality and quantity of phytochemical constituents vary with plant age and tissue. Often these chemicals evoke a particular response in insects. Volatile compounds may repel the insect and prevent landing to feed while other higher molecular weight substances may suppress feeding, stop it altogether or reduce insect growth and development. The latter compounds are known as feeding deterrents, antifeedents or growth suppressants. One such compound present in immature maize whorl leaves which acts as a feeding deterrent to ECB larvae is the secondary

metabolite DIMBOA (2,4-dihydroxy-7-methoxy-1,4- benzoxazin-3-one). It increases insect developmental time and decreases fecundity. Its effects are dose dependent (Panda and Khush, 1995). The highest levels are found within the yellow whorl tissue which is most preferred by the ECB (Bergvinson et al., 1995).

1.3.2 Tissue Toughness

Thickening of tissues, solidity of stems, accumulation of surface waxes, incorporation of silica, anatomical adaptations and pubescence all constitute physical defence mechanisms (Maxwell and Jennings, 1980). These contribute to leaf toughness which in turn has been correlated with decreased foliage consumption by the mustard beetle, *Phaedon cochleariae* (Tanton, 1962), penetration of peapods by the cowpea curculio, *Chalcodermus aeneus* (Cuthbert and Davis, 1972), rice to the rice stem borer, *Chilo suppressalis* (Patanakamjorn and Pathak, 1967) and sorghum to the sorghum shoot fly, *Atherigona variasoccata* (Blum, 1968). Decreased consumption may result from the ingestion of indigestible cellulose and lignin or other materials not suitable for digestion, absorption, growth and development or perhaps critical nutrients are less available because of hydrogen bonding with lignins (Swain, 1979). Tougher leaves also generally contain less nutrients and water.

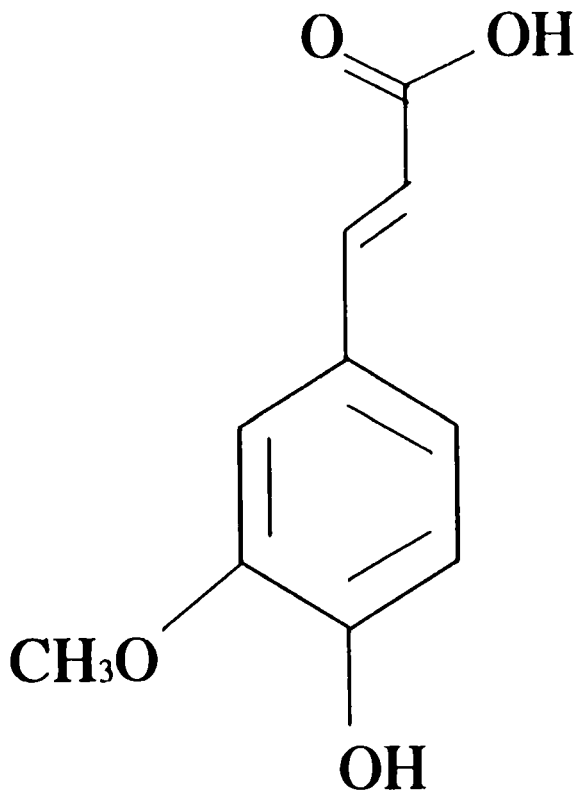
Increased mechanical toughness of corn via thickening of cell walls is a result of cellulose, lignin and silica deposits in the epidermal cell walls (Maxwell and Jennings, 1980). More recent findings suggest that phenolic fortification via diferulic acid crosslinks also contributes to mechanical resistance by decreasing the solubility of the wall and increasing intermolecular associations (Markwalder and Neukom, 1976; Fry, 1979; Shibuya, 1984).

1.3.3 Phenolic compounds

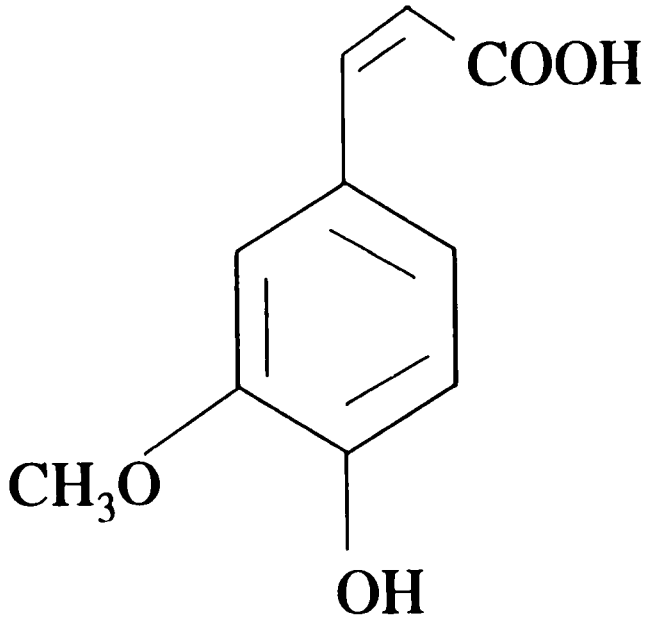
Phenolic compounds in maize as in all land plants are derived from the shikimate pathway (Swain, 1977) in which the amino acid phenylalanine is deaminated to form cinnamic acid. Cinnamic acid gives rise to two of the most abundant phenolic acids in maize tissue, *p*-coumaric acid and ferulic acid. Ferulic acid is of particular interest as the bound form dimerizes to diferulic acid, the mechanical strengthening factor.

The primary cell walls of monocotyledons contain large amounts of ferulic acid esterified to the hemicellulosic matrix (Harris and Hartley, 1976; Smith and O'Brien, 1979; Shibuya, 1984; Kamisaka et al., 1990). The *trans* (E) form dominates (Fig. 1). It is ester bound by the carboxylic acid group (Hartley, 1973) to matrix polysaccharides (Hartley, 1973; Whitmore, 1974; Fry, 1983; Kamisaka et al., 1990), specifically the *O*-5 position of the arabinofuranosyl residue of arabinoxylan (Ishii and Hiroi, 1990). The resulting molecule (Fig. 2) is called feruloylarabinoxylan (FAX), *O*-(5-*O*-feruloyl-alpha-L-arabinofuranosyl)-(1-3)-*O*beta-D xylopyranosyl-(1-4)-D-xylopyranose. The esterification of ferulic acid to the cell wall is thought to occur intracellularly, prior to its deposition in the cell wall (Myton and Fry, 1995). Not only are ferulic acid residues conjugated with monosaccharides and disaccharides but they are also associated with cell wall polysaccharides, glycoproteins, lignin, betacyanins and other insoluble cell wall biopolymers (Zimmerlin et al., 1994). Ferulic acid, being the major cinnamic acid derivative in the cell wall of a number of monocotyledons (Harris and Hartley, 1980), particularly the Poaceae (Smith and Hartley, 1983), is thought to play a significant role in modifying the mechanical properties of cell walls and digestibility of grasses by acting as a

Figure 1. *Trans* (E) and *cis* (Z) isomers of ferulic acid.

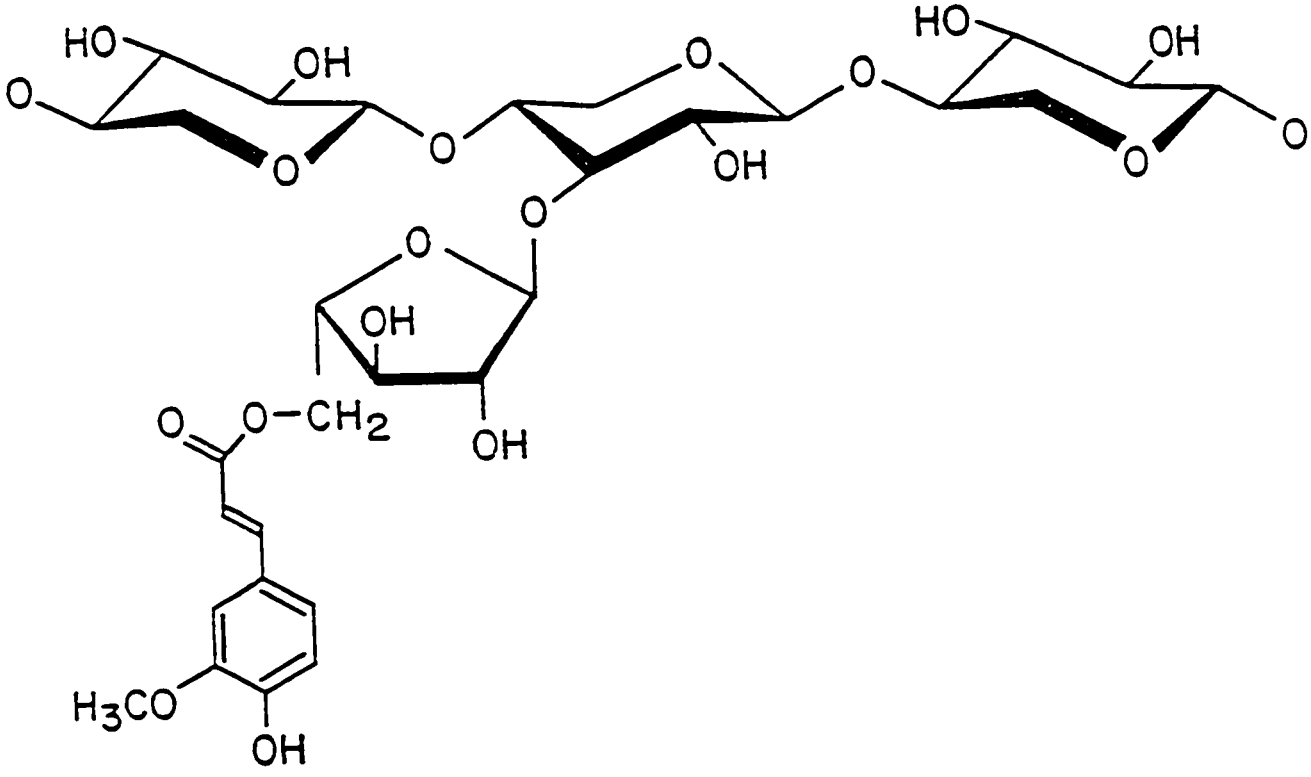


E-ferulic acid



Z-ferulic acid

Figure 2. Feruloylarabinoxylan (FAX).


HO
OH
O
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O
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O–CH2
OH
H3CO
OH

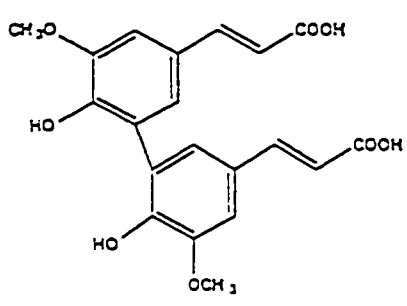
cross-linking agent between polysaccharides and lignins (Besle et al., 1994). In the free state, ferulic acid can act as an inhibitor of seed germination or plant growth in surrounding soil or act as a host plant recognition factor and feeding stimulant (Bergvinson et al., 1993). The roles of the esterified form involve the cross-linking of vicinal pentosan chains, arabinoxylans and hemicelluloses in cell walls (Zimmerlin et al., 1994). These linkages exist between the lignin core and structural carbohydrates (Hartley, 1973) and are responsible, in part, for the well established decrease in the digestibility of the structural carbohydrates of cell walls which occur with an increase in the lignin content (Van Soest, 1964). Later studies confirmed a good correlation between ferulic acid content and the mechanical properties of the cell wall (Miyamoto et al., 1994).

Bound ferulic acid (FAX) had an inhibitory effect on elongation caused by auxin in rice lamina joints (Ishii and Saka, 1992) and the elongation caused by gibberellin in dwarf rice (Ishii and Nishijima, 1995). It has been shown that the quantity of FAX during the time of maximum coleoptile elongation in the maize cell wall represents only a small portion of the total arabinoxylan but that more and more FAX domains are added during the course of development (Nishitani and Nevins, 1990). High phenolic acid content has been correlated with reduced feeding by grasshoppers and planthoppers in sorghum (Woodhead, 1983) and by ECB in maize. It is also correlated with increased leaf toughness in maize (Bergvinson et al., 1993) and decreases the amount of forage digested by ruminants of straw (Mueller-Harvey and Hartley, 1986). Consumption rate of adult grain weevils (*Sitophilus zeamais*), progeny produced and number of eggs laid are negatively correlated with FAX content in maize grain (Classen et al., 1990).

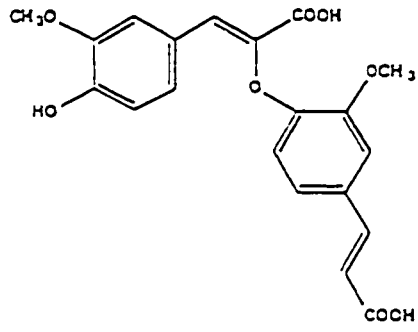
FAX has been isolated from maize shoots (Kato and Nevins, 1985), coastal Bermuda grass shoots (Borneman et al., 1990), sugar cane bagasse (Azuma et al., 1990), and barley straw and endosperm (Ahluwalia and Fry, 1986; Mueller-Harvey and Hartley, 1986). The highest levels of FAX appear to be in yellow tissue that will emerge in two days (Bergvinson et al., 1995) and ferulic acid concentration is higher in resistant varieties (Bergvinson et al., unpublished data). Biotic and abiotic conditions influence the amount of ferulic acid produced. Light tends to increase the amount of ferulic acid ester linked to the cell wall (Tan et al., 1992a; Miyamoto et al., 1994) as does aging (Kamisaka et al., 1990; Tan et al., 1991), temperature, and the presence of fungal elicitors (Bolwell et al., 1985). FAX undergoes a coupling reaction by peroxidase to produce diferulic acid (Kamisaka et al., 1990) thereby cross-linking this part of the wall matrix (Saulnier et al., 1995). The cross-linking of FAX to form diferulic acid occurs early in maize tissue development with the frequency of diferulic acid to arabinoxylan approximately 1:2600 (Shibuya, 1984).

Cross-links in cell walls dictate the extensibility, digestibility and adherence of the tissue (Fry, 1986). One of these cross-links is diferulic acid. They are formed *in situ* in the growing plant from ferulic acid units by oxidative coupling involving peroxidase (Hartley and Jones, 1976). A number of isomers have been recently identified in grass stems; 5-5, 8-O-4, 8-5, and 8-8 as seen in Fig. 3 (Ralph et al., 1994). These isomers are bound to the carbohydrates of the cell walls through their carboxyl groups (Hartley and Jones, 1976) insolubilizing them (Markwalder and Neukom, 1976). Diferulic acid has been isolated from wheat germ (Markwalder and Neukom, 1976) and from *Lolium multiflorum* (Hartley and Jones, 1976) as well

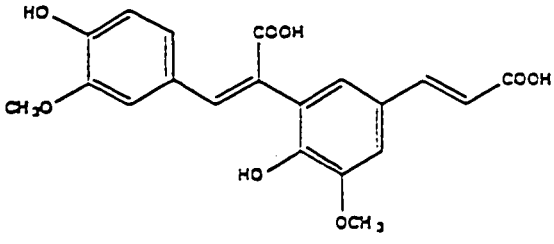
Figure 3. Isomers of dehydrodiferulic acid. The 5-5 and 8-O-4 each exists in one form while the 8-5 and 8-8 are found in two forms.



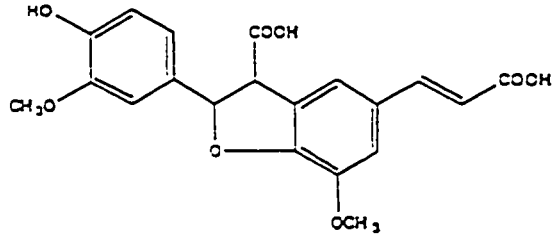
(1) 5,5'-Diferulic acid



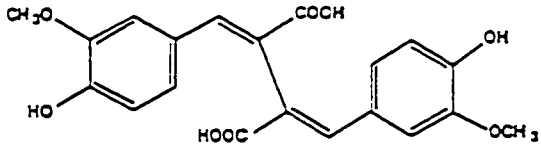
(2) 8-O-4'-Diferulic acid



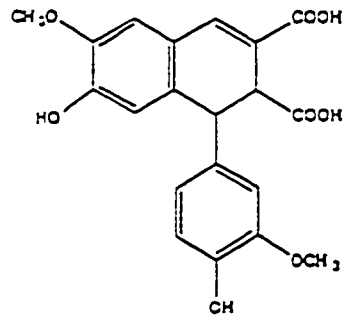
(3) 8,5'-Diferulic acid



(4) 8,5'-Diferulic acid benzofuran form



(5) 8,8'-Diferulic acid



(6) 8,8'-Diferulic acid aryltetralin form

Structures

as a number of other plants. Elevated levels of diferulic acid in the matrix polysaccharides result in a decrease in cell wall extensibility (Fry, 1979; Kamisaka et al., 1990; Tan et al., 1991), solubility (Markwalder and Neukom, 1976; Shibuya, 1984), leaf consumption rate in maize by ECB (Bergvinson et al., 1995), digestibility (Alibes et al., 1984; Hartley and Ford, 1989), and an increase in minimum stress relaxation time (Kamisaka et al., 1990), and tissue toughness (Fincher and Stone, 1986; Classen et al., 1990; Bergvinson et al., 1993). Like ferulic acid, diferulic acid increases in the presence of light (Tan et al., 1992b; Miyamoto et al., 1994) and with tissue age.

The fortification that these cross-links bring to immature tissue provides varying resistance to insect pests depending on the degree of cross-linking. This is important at this early stage as lignin does not yet play a role in the primary cell walls and there is flexibility for growth. Diferulic acid content is strongly related to a reduced tissue digestibility and an increase in tissue toughness (Hartley and Ford, 1989). In corn, diferulic acid content is negatively correlated with leaf feeding damage and field leaf ratings and positively correlated with tissue toughness to the ECB (Bergvinson et al., 1993). It is not only in the leaf but the kernel in which cell wall bound phenolic acids confer toughness as was shown by kernel resistance to the storage pest *Sitophilus zeamais*. In fact, the most consistent factor accounting for resistance in immature whorl tissue is diferulic acid (Bergvinson et al., 1993).

The formation of diferulic acid is controlled at several levels. The rate limiting step can be feruloylation, peroxidase activity, oxidant formation or hydrogen peroxidase availability. Because the ratio of ferulic acid to diferulic acid is often constant irrespective of tissue age, zone or growth conditions, some authors believe that feruloylation is the rate limiting step. This was

found in *Avena* (Kamisaka et al., 1990) and *Oryza* (Tan et al., 1991) coleoptiles. It is suggested that feruloylation itself may control wall extensibility by interfering with the enzymatic degradation of matrix polysaccharides (Miyamoto et al., 1994) as it is known that feruloylated matrix polysaccharides are hydrolase resistant (Fry, 1984) and that a turnover of these matrix polysaccharides are indispensable for an increase in the ability of cell walls to extend (Sakurai et al., 1979; Hoson and Nevins, 1989; Hoson, 1990). There are, however, studies that indicate peroxidase activity may be the regulator. Diferulic acid residues in the second leaf sheath of rice seedlings treated with FAX did not increase, suggesting peroxidase is the limiting factor (Ishii and Nishijima, 1995). Siegel (1953) and Grabber et al. (1995) suggested that diferulic acid formation is limited by the availability of hydrogen peroxide. While peroxidase activity and resistance do not seem to be directly related, peroxidase activity may well affect the extent of the resistance expressed (Van Loon, 1986).

1.4 IMPORTANCE OF MECHANICAL RESISTANCE FACTORS

Plants have a vast array of chemicals which are stored in various plant structures and can be used for defence (Beck and Schoonhoven, 1980). Maintaining these chemicals can be expensive to the plant in terms of energy expenditure yet their presence is invaluable in conferring resistance to certain pests and as insurance for future tolerance.

Many insect pests including the ECB are polyphagous, that is, they feed on a wide range of host plants. The ECB however does have a preferred host, corn, which it will utilize above all others in choice tests (Beck and Schoonhoven, 1980). The advantage of a wide host range is that the insect has developed extensive metabolic capabilities and is able to degrade a large array of

plant chemicals (Feeny, 1976). In the event that a mutant plant derives a novel deleterious compound, it is likely, in polyphagous insects, that the metabolic breakdown machinery is already present and functional for that particular compound. If it is not, the insect will likely develop resistance over time through coevolution. In the meantime, polyphagous insect populations will not suffer as there are a number of other hosts which can be utilized at this time. As plants evolvee and gain resistance to certain insect varieties, their success is often short lived because insects, as predicted in the coevolutionary theory, undergo mutations to overcome the plants novel compound.

Qualitative factors (plant toxic compounds such as DIMBOA) do provide resistance for a time at low energy cost. Quantitative factors such as mechanical strength may provide prolonged resistance at a higher energy expenditure. They provide resistance to both specialists and generalists. Qualitative factore eliminate the risk of the pests acquiring and utilizing plant chemicals for use against their natural enemies (Feeny, 1976).

Diferulic acid increases the mechanical strength of leaf tissue thereby decreasing the availability of food to neonate larvae of the ECB. Their small mandibles are not able to grind the tissue rendering the necessary nutrients for survival and growth unattainable. The result is death or reduced viability and growth which can impair reproduction. The more diferulic acid bridges present, the stronger the tissue and the more resistant to the ECB larvae.

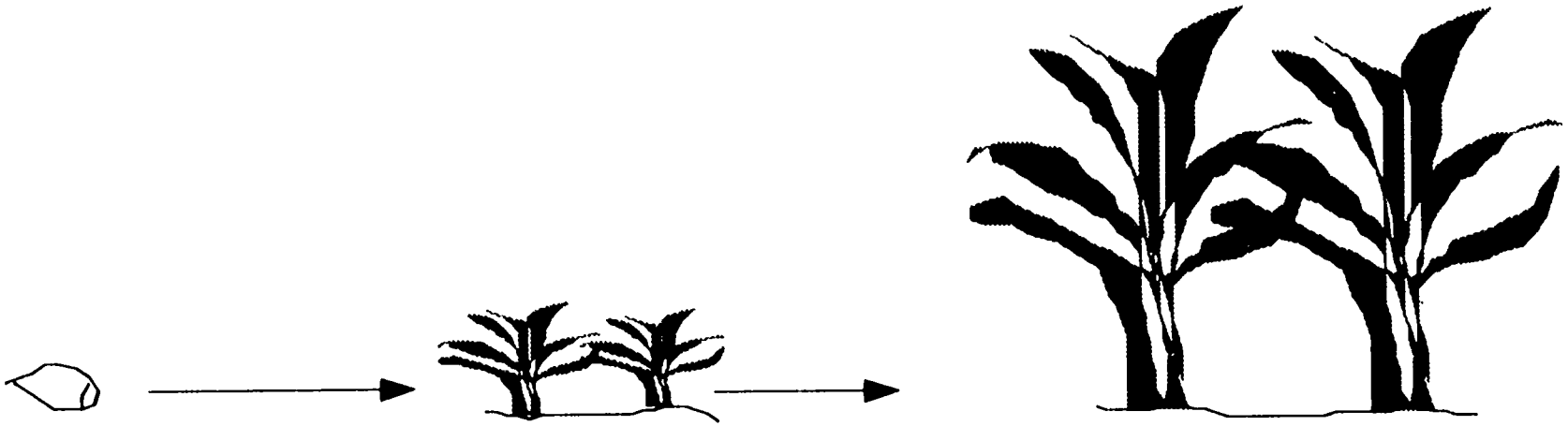
1.5 EUROPEAN CORN BORER

The ECB, first described in 1796, is a world wide pest of economic importance. After introduction into the United States in the 19th century, it was discovered in Ontario and Quebec in

1910 (Smith, 1920; Keenan 1927) and 1926 (Keenan, 1927), respectively. A member of the subfamily Pyraustinae, the ECB is a polyphagous insect utilizing over 200 plant species although maize is its preferred host (Hudon and LeRoux, 1986a). Three biotypes exist; the univoltine, bivoltine and multivoltine. The univoltine strain dominates in Ontario and Quebec although the bivoltine strain is not uncommon. Adult moths mate in spring and lay their eggs on the undersurface of whorl leaves of corn plants. The eggs hatch in five to seven days (Hudon and LeRoux, 1986a) and immediately begin feeding on the leaves of the plant. More than 80% of larvae move toward the center of the whorl during daylight hours to avoid the elevated temperatures on the surface of the leaves exposed to the sun, preventing their desiccation (Bergvinson et al., 1995). Not only is there protection from the elements but greater moisture and softer tissue for grinding. Often, however, larval mortality reaches 80% within the first 48 hours (Ross and Ostlie, 1990). The larvae move through five instars near the end of which they burrow into the stalk of the plant and spin a web around themselves and remain as pupae until they emerge as adults. The life cycle is depicted in fig. 4. For the bivoltine strain, the first generation pass through all stages of development in one season. When the pupae reach maturity they mate and lay eggs, starting the cycle over again. The second generation pupae, unlike the univoltine strain, are forced to overwinter in the stalks of corn as fifth instar larvae. It is not until next spring that they thaw, reach maturity, and continue their cycle.

Only a 1.3% ECB survival rate is needed to maintain levels of injury sufficient to decrease yields (Hudon and LeRoux, 1986b). First generation larval feeding destroys the leaf surface and breaks midribs thereby decreasing the leaf capability for producing photosynthates for the plant.

Figure 4. First generation ECB development demonstrating synchrony with maize development.



new crop

seedling

2-3 weeks

whorl stage

8-9 weeks

pupate → adult emergence → egg deposition on leaf lower surface → first, second, and third, instars (feed on leaves) → fourth and fifth instars (bore into stalk)

Stalk tunnelling by fourth and fifth instar larvae destroys food channels, weakens the plant and may decrease yield. Stalk tunnelling also allows easy access to stalk rot organisms which can further damage the plant (Salisbury and Ross, 1988). Second generation ECB larvae, however, are more injurious as they feed on pollen, decreasing fertilization and on ear husks and kernels causing poor ear development and possibly ear droppage (Hudon and LeRoux, 1986a).

ECB shows the highest consumption on immature yellow whorl tissue whereas the lowest is the point where the leaf emerges from the whorl (Bergvinson et al., 1995). In second generation ECB resistance, crude fiber, lignin and biogenic silica of leaf sheaths have been related to plant tolerance (Buendgen et al., 1990). Leaf feeding resistance of corn to the southwestern corn borer has also been attributed to crude fiber as well as decreased protein and increased hemicellulose in the whorls of resistant genotypes (Hedin et al., 1993). Rumen digestion studies of leaf tissue showed reduced breakdown of cell wall material with elevated levels of ferulic acid (Akin et al., 1990; Jung and Casler, 1990).

Control of ECB populations includes crop rotation, burning of crop residues and plowing down of stalks (Lagloire, 1936). A significant factor was the introduction of hybrid corn which had an increased tolerance compared to the open-pollinated cultivars previously used (Wressell, 1958). This and the development of host plant resistance are providing ever increasing resistance to the ECB.

1.6 PEROXIDASES

Peroxidases are hemoprotein enzymes which contain ferriprotoporphyrin prosthetic groups (Saunders et al., 1964; Scandalios, 1969). These monomers usually have a molecular weight

around 40,000 (Liu, 1975) and are synthesized, glycosylated and transported in the protoplast and then secreted into the extracytoplasmic space (Schlob et al., 1987). Hence, most of the peroxidases are found in the cell wall and vacuole (Mader et al., 1976) but others have been noted in the endoplasmic reticulum, Golgi apparatus, vesicles and chloroplasts (Gaspar et al., 1982). Within the extracellular space, peroxidases are mainly localized in the middle lamella and in the corner junctions between cells (Goldberg et al., 1987). The richest known sources of peroxidases are the root of horseradish and the sap of a fig tree (Sung et al., 1993).

Although peroxidase activity had been studied for a number of years, it was not until 1942 that Theorell noted that the activity consisted of more than one electrophoretically distinct species (Liu, 1975). Twelve years later, Jermyn recognized that the activity existed in multiple forms (Liu, 1975). Since then, it has been discovered that there are several independent genes coding for different peroxidases, there are temporal and spatial changes in peroxidase activity, and different peroxidases have different substrate specificity (Khavkin and Zabrodina, 1995). In maize, there are nine loci coding for peroxidase (Brewbaker et al., 1985) and 24 peroxidase isoenzymes in total (Hamill and Brewbaker, 1969).

Most peroxidases are unspecific for hydrogen donors (Ievinsh, 1992), have a pH optimum around 5.5 (Valero et al., 1991) and are inhibited at low pH (Fry, 1986). The ionically wall bound fraction of peroxidase is generally accepted to represent the cell wall activity (MacAdam et al., 1992). In poplar and corn, the wall bound activities represent 15% and 20%, of the total activity, respectively (Birecka and Catalfamo, 1975; Goldberg et al., 1993). Of the corn leaf cell wall bound peroxidases, there are six isoenzymes, four cathodic and two anodic (Birecka and Catalfamo, 1975).

Peroxidase catalyzes the oxidation of cellular components by H_2O_2 or other organic hydroperoxides. There are two groups of peroxidases, the first generally known as guaiacol peroxidases. The oxidation products of the electron donors have physiological roles and may play a role in wound healing or as fungicides or bactericides. The second group is mainly used to scavenge H_2O_2 or organic hydroperoxides. They are divided into heme, flavin and seleno proteins with respect to their prosthetic group and into ascorbate, NADH, glutathione and cytochrome c enzymes with respect to their electron donor. Plants mainly use ascorbate peroxidase as the enzyme scavenging H_2O_2. These enzymes, as all enzymes whose function lies in scavenging H_2O_2, lose their activity in the absence of an electron donor while the guaiacol peroxidases do not (Asada, 1992).

Peroxidase activity is one of the fastest mechanisms for metabolic regulation in plants (Gaspar et al., 1982). Peroxidases are particularly prevalent in defence related responses such as lignification, the cross-linking of glycoproteins and phenolics including diferulic acid and isodityrosine (Fry, 1982; McNeil et al., 1984) and suberization and phytoalexin production (Harkin and Obst, 1973; Whitmore, 1976, 1978a, 1978b; Fry, 1979; Mohan and Kolattukudy, 1990; Scott-Craig et al., 1995). These enzymes also participate in plant growth and differentiation (Khavkin and Zabrodina, 1995) by oxidizing and therefore inactivating the growth hormone IAA (Pilet and Lavanchy, 1969; Gebhardt, 1982). The synthesis and secretion of peroxidases are controlled by a number of biotic and abiotic factors including light, drought, salinity, temperature and plant growth regulators (McNeil et al., 1984).

In general, anionic peroxidases are known to be involved in the lignification process as they can rapidly oxidize phenolic acids such as ferulic and caffeic acids (Pickering et al., 1973). They

may also be involved in wound healing as they can bind ferulic and coumaric acids to suberin and cutin (Riley and Kolattukudy, 1975). Cationic peroxidases are associated more with H_2O_2 generation (Campa, 1991) and in IAA regulation (Gaspar et al., 1982).

It has been repeatedly documented that resistant plants have higher peroxidase levels as demonstrated in wheat (Arora and Wagle, 1985), tomato (Brenneman and Black, 1979), and lupin (Malolepsza et al., 1989). Elevated levels of peroxidase were correlated to high levels of certain phenolics such as ferulic acid, as demonstrated in rye (Rybka et al., 1993). An increase in cell wall peroxidase activity has been noted following cessation of elongation (Gardiner and Cleland, 1974; MacAdam et al., 1992). Resistant maize infected with corn leaf blight, *Helminthosphorium*, exhibited higher peroxidase activity in leaves than susceptible cultivars of maize (Jennings et al., 1969). This also occurred after elicitor treatment of bean suspension cultures (Malolepsza and Urbanek, 1994). During pathogenesis the peroxidase may participate in the formation of a physical barrier that may effectively localize the pathogen (Hrubcova et al., 1992).

A novel peroxidase that has been purified from *Bupleurum salicifolium* catalyzes the dimerization of ferulic and caffeic acids via oxidative coupling and formation of beta-beta linkages to the lignin type compounds 8,8-bisferulic acid and 8,8-biscaffeic acid. This peroxidase has a MW of 38,000, is soluble and more specific to caffeic acid than ferulic acid, hence the name caffeate peroxidase was coined (Frias et al., 1991). A similar peroxidase has been isolated from French bean suspension cultures but this one is more specific to ferulic acid, is cationic and has a MW of 46,000 (Zimmerlin et al., 1994). The initial content of this

peroxidase is only 0.3% of the total protein and has a pH optimum of 4.4 (Zimmerlin and Bolwell, 1993).

1.7 HYPOTHESIS AND OBJECTIVES

The hypothesis of this study is that the level of extracellular ferulic acid peroxidase activity is positively correlated across genotypes with cell wall bound diferulic acid concentration and resistance to ECB in field grown maize. This peroxidase is the rate limiting step in the formation of diferulic acid and can be used as a biochemical marker for resistance across genotypes as it is known that diferulic acid correlates positively with maize resistance to the ECB.

The objectives of this study were to:

1. Develop a suitable method to determine the activity of the peroxidase catalyzing the dimerization of ferulic acid to diferulic acid in maize.
2. Compare the activity of the peroxidase among the 14 genotypes of maize varying in resistance to the ECB.
3. Determine whether peroxidase activity correlates with cell wall bound diferulic acid concentration and resistance levels of the genotypes grown in the field.
4. Determine the resistance of the 14 genotypes of maize to the ECB in leaf feeding bioassays.

2. MATERIALS AND METHODS

2.1 GERMPLASM

Fourteen and twelve genotypes (1995 and 1996 respectively) of temperate and tropical maize varieties with differing insect resistance to the ECB were used in this study. Dr. R.I. Hamilton, Agriculture and Agri-Food Canada, supplied the synthetic Iowa Stiff Stalk BS9 selection series (C0, C2, C4, C5) which were derived from the recombination of ten inbred lines; Argentinean landraces, MB10 and MC3, formed from a cross between Cateto lines from Cargill International, Minneapolis, Minnesota and CM7; and the North American inbreds, CG16 from Guelph and B86 from Iowa. Dr. J. Bergvinson and Dr. G. Srinivasan, International Maize and Wheat Improvement Center (CIMMYT), Mexico, provided MEX 208 and the multiple borer resistant inbreds CML67, CML121, CML139, Ki3 and Hi34 (Table 1). These are adapted tropical materials that have undergone recurrent selection for insect resistance. As their name suggests, they are resistant to three borers; sugar cane borer (SCB), *Diatraea saccharalis*, southwestern corn borer (SWCB), *D. grandiosella*, and the corn earworm, *Heliothus zeae*.

2.2 FIELD PLOT

Plants in both the 1995 and 1996 field seasons were planted in early May in a 18.2 m by 10.7 m plot at the Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Ontario (Lat. 45° 24'N, Long. 75° 43'W). A four seeder planter was used to insert seeds 15 cm into the sandy loam soil. The rows were spaced 0.76 m apart with each row being 3.8 m long. Fifteen plants were grown per row, uniformly spaced at 0.25 m. The planting design was a completely randomized block with four replicates. One genotype was planted in each row (times

Table 1: Maize genotypes used in the study and their sources.

BS9 Synthetic Series (AAC, Ontario)	Argentinean Landraces (AAC, Ontario)	North American Inbreds (AAC, Ontario)	Multiple Borer Resistant Cultivars (CIMMYT, Mexico)
BS9 C0	MB10	B86	CML 67
BS9 C2	MC3	CG16	CML 121
BS9 C4			CML 139
BS9 C5			Hi34
			Ki3
			MEX 208

AAC = Agriculture and Agri-Food Canada, CIMMYT = International Maize and Wheat Improvement Center.

four rows or replicates) so that in the first season 48 rows were planted and in the second season, 56 rows were planted.

2.3 FIELD CONDITIONS

The soil was non-irrigated but received abundant rainfall between planting and harvest: 312.6 mm the first season and 248.0 mm the second season. Fertilizer (NH_4NO_3 at 100 kg N/ha) was applied just prior to planting. Herbicides were applied twice: Prime Exterior Light at 7 L/ha shortly after planting and atrazine at 2 L/ha later in the season.

2.4 PLANT SAMPLES

Within each row, eight plants were randomly sampled with the exception of the two on the ends. Plants reaching the mid-whorl stage, defined here as the ten leaf stage, were used for harvest. Leaf 12 in the whorl was the leaf of interest as larvae feed primarily on the leaves of the whorl. For harvest, this leaf plus all the younger leaves were pulled out of the whorl of the plant and the 12th leaf was unwrapped from the rest. Once all the leaves of a particular genotype had been harvested, they were divided into four sections. Section one and two comprised the mature green tissue, section three the yellow green tissue and section four the immature yellow tissue. The samples were stored in separate plastic bags and placed in a cooler containing dry ice. When all the samples had been collected for the day, they were brought into the lab where subsamples of section three were put aside for use in leaf feeding bioassays and the rest stored at -20 ^{0}C until freeze dried. Samples were then milled on a UD cyclone mill (UD Corp., Boulder, CO) with a 1.0 mm screen and stored at -20 ^{0}C until phytochemical analysis.

2.5 BIOGENIC DATA

To determine specific plant growth characteristics a number of plant parameters were measured throughout the 1996 field season. Measurements taken included early/lateness of maturity, height at maturity, number of ears and corn heat units (CHU).

Early/Lateness: Time from planting to silking.

Height: Measured from the base of the stalk to the top of the open tassel.

Number of ears (prolificness): The number of visible and mature ears developing silks.

CHU: Accumulation of CHU by Julian date as a measure of the thermal environment of corn (Brown, 1969) was provided by Agriculture and Agri-Food Canada for May, June and July in 1995 and 1996.

2.6 INSECT LEAF FEEDING BIOASSAY

Harvested leaves were placed in coolers on dry ice and immediately brought into the lab for use in the insect bioassays. Fresh yellow green tissue from section three of the leaves were roughly cut into 2.0 cm^2 sections and placed into a modified Ascher et al. (1981) apparatus. The bottom of a petri dish was filled with damp cotton and overlaid with filter paper. The top of the associated petri dish had a 1.0 cm^2 opening on the upper surface. Leaf tissue was placed on top of the filter paper with the undersurface facing upwards. This was covered with the surface petri dish plate with the 1.0 cm^2 hole. One third instar ECB larvae was placed in each petri dish where it was exposed to the leaf for feeding. The larvae and a small plastic cover for shade was enclosed in this space by adding a third petri cover over top and securing with an elastic. The petri dishes were placed in the incubator in which the ECB had been reared. The assay ran for 24

hours with 20 replicates per genotype in 1995 and 40 replicates per genotype in 1996. Area consumed was determined by placing tissue over 1.0 mm^2 grid paper and counting the number of consumed or damaged squares.

A continuous culture of larvae and adults were maintained on an artificial diet in incubators (Conviron Model E7) under controlled conditions. A diurnal 16:8 day/night photoperiod, 85% relative humidity and a 25 °C/ 19 °C day/night temperature had been set.

2.7 PHYTOCHEMICAL STUDIES

2.7.1 Extraction of Hydroxycinnamic Acids: Soluble metabolites were extracted before cell wall bound hydroxycinnamic acids were determined. Five hundred mg of freeze dried leaf tissue was weighed and placed on top of filter paper (Whatman #1) in a Buchner funnel and washed with 30 ml of water, 30 ml of 50% methanol, 30 ml of 100% methanol and 30 ml of ethyl acetate under vacuum. The liquid was discarded and the samples were dried in a dessicator for four days. From the remaining tissue, 250 mg was weighed and used for further analysis.

The phenolic acids, wall bound ferulic and diferulic acids, were extracted by base hydrolysis. Samples were shaken for four hours in darkness in 20 ml 4N NaOH under N_2 after which time the pH was lowered to pH 2.0 by the addition of HCl. Centrifugation for ten min at 1000 rpm eliminated particulate matter. The supernatant was poured into a separatory funnel and the pellet washed twice with deionized water, each time adding the supernatant into the separatory funnel. The phenolic acids were then extracted three times with 50 ml ethyl acetate. The ethyl acetate fractions were pooled, rotary evaporated and stored at -20 °C until HPLC analysis (Bergvinson et al., 1993).

2.7.2 High Performance Liquid Chromatography: For HPLC analysis, the samples were dissolved in 1.0 ml methanol and diluted ten fold. They were filtered into autosampler vials, capped and placed in specific order into the autosampler for analysis. A Beckman 168 System Gold HPLC with a 168 detector, 126 solvent module and a 502 autosampler was used for analysis. A new Ultratechsp 5ODS reverse phase column, 250 mm by 4.6 mm, 5 um particle size was used for the separations. The autosampler was programmed to inject 20 uL aliquots of extract into the column at a time. A solvent system of methanol (A) and 10 mM H_3PO_4, pH 2.4 (B) was used: flow rate of 1.0 ml/min; gradient of 15 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 15% A in 2 min and 15% A for 3 min. Absorbance was detected at 227 nm and 325 nm. Identification of ferulic acid and diferulic acid in samples was determined by comparison of retention times and UV spectra of samples with pure standards. The retention times were approximately 16.8 min for ferulic acid and 19.04, 19.25 and 19.52 min for the diferulic acid isomers. All had peak absorbances at 325 nm. Diferulic acid was prepared synthetically by Dr. J. Atkinson (Department of Chemistry)..

2.7.3 Cell Wall Bound Ferulic Acid Peroxidase Extraction: Three hundred mg of freeze dried tissue was homogenized on ice with mortar and pestle in 10 mL 50 mM Tris-HCl, pH 7.2, ontaining 0.25 M sucrose and 1.0 mM DTT (Zimmerlin and Bolwell, 1993). The solution was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant containing soluble proteins was separated from the pellet and stored at 4 °C for several hours until protein content could be determined. The pellet was resuspended in 5 mL 20 mM Tris-HCl, pH 7.2, containing 0.05g PVP and 1.0 M $CaCl_2$. It was stored at 4 ° C for one hour with periodical shaking on a vortex mixer. The solution was again centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant

contained ionically wall bound enzymes and was partially purified by a Concanavalin A Sepharose column by adding 2.5 ml of protein extract followed by five aliquots of 3.5 ml of elution buffer; 20 mM Tris-HCl, pH 7.4, 0.5 M NaCl and 0.5 M methylmannopyranoside (Zimmerlin and Bolwell, 1991). Five fractions were collected into separate test tubes and the activity determined in each. The column was equilibrated between each sample with 3.5 mL of 20 mM Tris-HCl, pH 7.4 containing 1.0 mM $CaCl_2$, 1.0 mM $MnCl_2$, 1.0 mM $MgCl_2$ and 0.5 M NaCl.

2.7.4 Cell Wall Bound Ferulic Acid Peroxidase Analysis: A Beckman DU 62 Spectrophotometer with a kinetics program was used to determine the ferulic acid peroxidase activity. The assay buffer consisted of 0.1 M Na acetate, pH 4.4, 0.1 mM ferulic acid (Sigma) and 0.1 mM H_2O_2 . Purified extract (0.1 ml) was added to the assay buffer (1.0 ml) at time zero, rapidly mixed and the reaction followed for 2.5 min at a wavelength of 310 nm. At the end of the run, change in absorbance over the first 15 seconds (the highest and most linear rate of reaction) was calculated, divided by 15 to obtain change in absorbance per second (rate), and multiplied by a factor of 1000 to obtain the peroxidase activity in units/sec. (Valero et al., 1991). The activity was determined for each of the five purified fractions and the total activity determined by combining the data. Each genotype was replicated four times.

The protein concentration of both the soluble and bound fractions were determined according to Bradford (1976). Extract (0.1 ml) was added to 5.0 ml of diluted BioRad reagent in a test tube and after standing at 20 °C for 15 min, the absorbance was measured at 595 nm. Protein concentrations were found by corresponding absorbance to the standard curve using bovine serum albumin.

2.7.5 Elemental Analyses: Nitrogen and carbon percentages were estimated in 10 mg dry samples, two replicates per year, using a Elemental (CHNS) Analyzer (Perkin Elmer Series 2 Analyzer 2400, USA).

2.8 STATISTICS

All statistical analyses were performed on Systat 5.2, statistical software for IBM computers. CHU were transformed by x^2 to satisfy the assumptions of the general linear model. The rest of the data remained untransformed and were analyzed by ANOVA followed by a mean Fischer's LSD comparison test. Multiple linear regression was performed on means with Statistix 4.0.

3. RESULTS

3.1 PHENOLIC ANALYSIS BY HPLC

The portion of the chromatogram corresponding to retention times between 15 min and 20 min was the region in which the two major groups of phenolic acids were eluted; ferulic acid (E and Z isomers) and diferulic acid (3 structural isomers). A typical analytic result for separation of phenolics from hydrolysed cell wall extracts is shown in Fig. 5.

E-ferulic acid is the major ferulic acid isomer. Z-ferulic acid was often not detectable or found in quantities low enough to be negligible. Retention time for ferulic acid averaged 16.8 min. Significant effects of year, genotype and a significant interaction between year and genotype (Table 2) were found in a two-way ANOVA. Hence, the years were analyzed separately. In the 1995 field season, one-way ANOVA showed significant differences in ferulic acid levels ($F=5.91$, $p<0.001$, $n=14$) among genotypes (Table 3). Figure 6 shows CML 139 had high levels of ferulic acid (6.09 mg/g DW) as did two other tropical MBR lines CML 67 (5.77), and CML 121 (5.46). The remaining two MBR lines, Ki3 and Hi34, were among the lowest in ferulic acid concentrations with 3.93 mg/g DW and 3.70, respectively. The landraces MC3 and MB10 were both low and MB10 was the lowest of all genotypes with 3.25 mg/g. The BS9 selection series had intermediate amounts of ferulic acid with no trend along the four cycles of selection. The North American inbreds, CG16 and B86, also showed intermediate amounts. Table 3 shows ferulic acid concentrations in 1996 varied significantly ($F=3.86$, $p=0.001$, $n=12$) among cultivars. Figure 7 shows CML 121 and CML 139 had the highest levels with 19.95 mg/g DW and 16.13 respectively, while CML 67 had a lower amount, 11.9. The landraces MB10 (8.81 mg/g DW) and MC3 (8.32) contained the lowest quantities of ferulic acid. The

Figure 5. Ferulic and diferulic acids resolved by HPLC from sample extracts. Retention times and absorbance spectra are indicated. The first diferulic acid peak has the absorbance spectrum shown in the top right inset, the middle peak has the absorbance spectrum shown in the middle right inset, while the last peak has the absorbance spectrum shown in the bottom right inset.

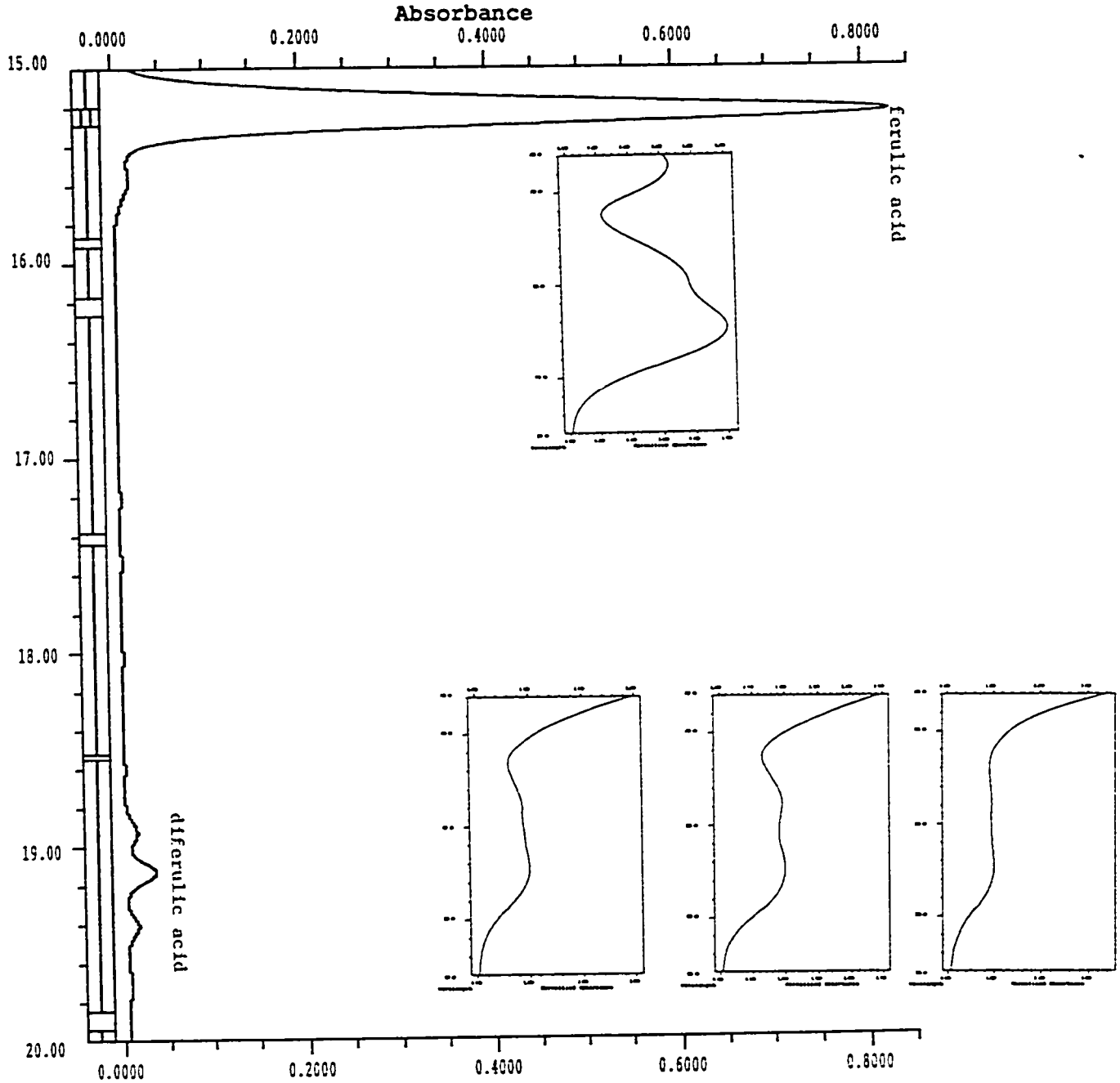

Absorbance
0.0000
0.2000
0.4000
0.6000
0.8000
15.00
16.00
17.00
18.00
19.00
20.00
Time (min)
ferulic acid
diferulic acid

Table 2: Two-way ANOVA on variation of ferulic acid with genotype and year.

Source of variation	df	SS	MS	F	p
Year	1	11.39	11.39	10.38	=0.002
Genotype	11	269.12	24.46	22.29	<0.001
Year x genotype	11	172.82	15.71	14.31	<0.001

df = degrees of freedom, SS = sum of squares, MS = mean square, F = F distribution, p = probability.

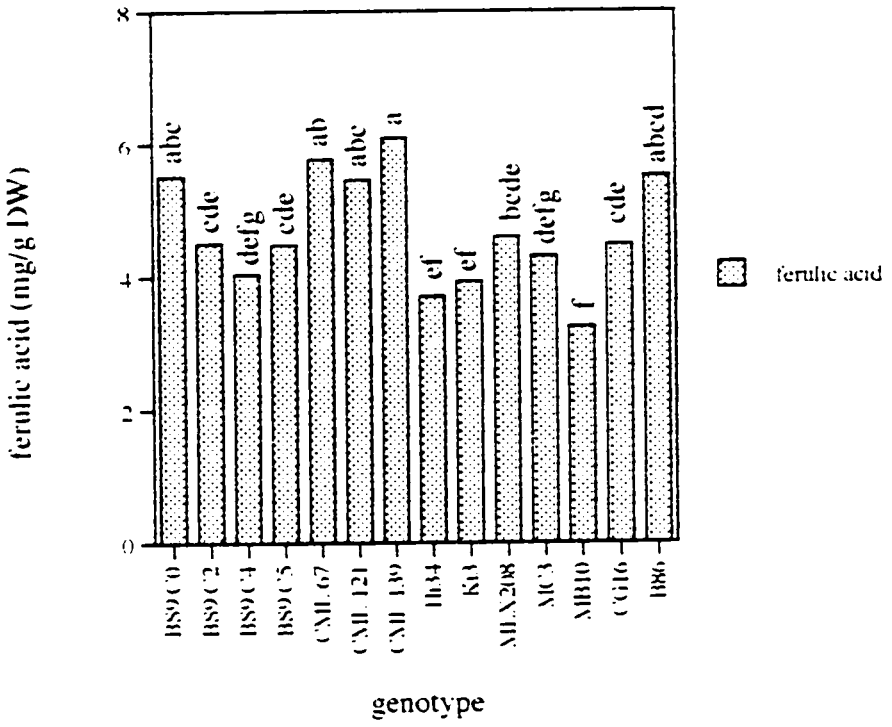
Table 3: One-way ANOVA on genotype variation for the biochemical parameters, diferulic acid and ferulic acid, measured in 1995 and 1996.

	1995	df	SS	MS	F	p
DFA		13	1.62	0.13	2.64	=0.01
FA		13	56.24	4.33	5.91	<0.001
	1996					
DFA		11	13.86	1.26	1.91	=0.07
FA		11	446.74	40.61	3.86	<0.001

DFA = diferulic acid in mg/g DW, FA = ferulic acid in mg/g DW. Other symbols as in Table 2.

Figure 6. Concentrations of ferulic acid and diferulic acid in the yellow-green tissue of leaf 12 of 14 maize varieties of temperate and tropical corn grown in the field in 1995. Bars with different letters indicated significant differences at $p < 0.05$.

Ferulic Acid (1995)



Diferulic Acid (1995)

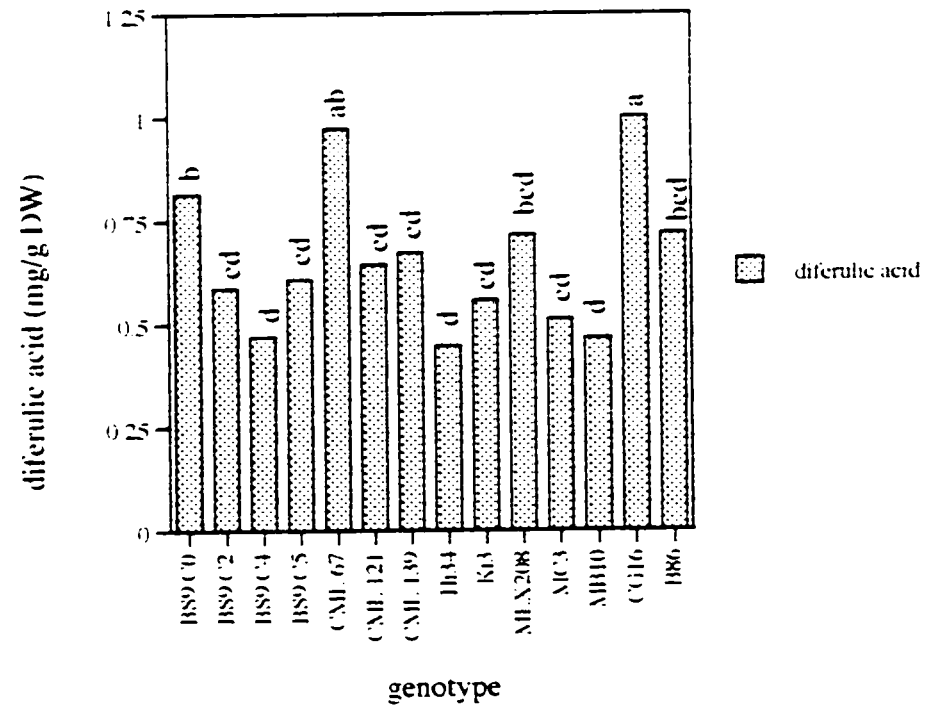
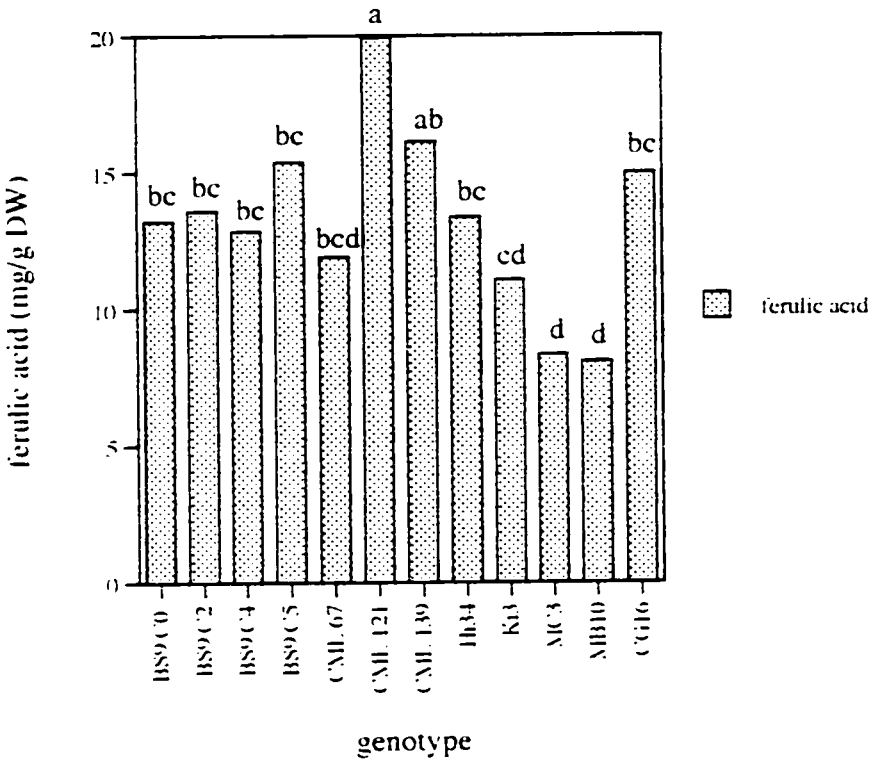
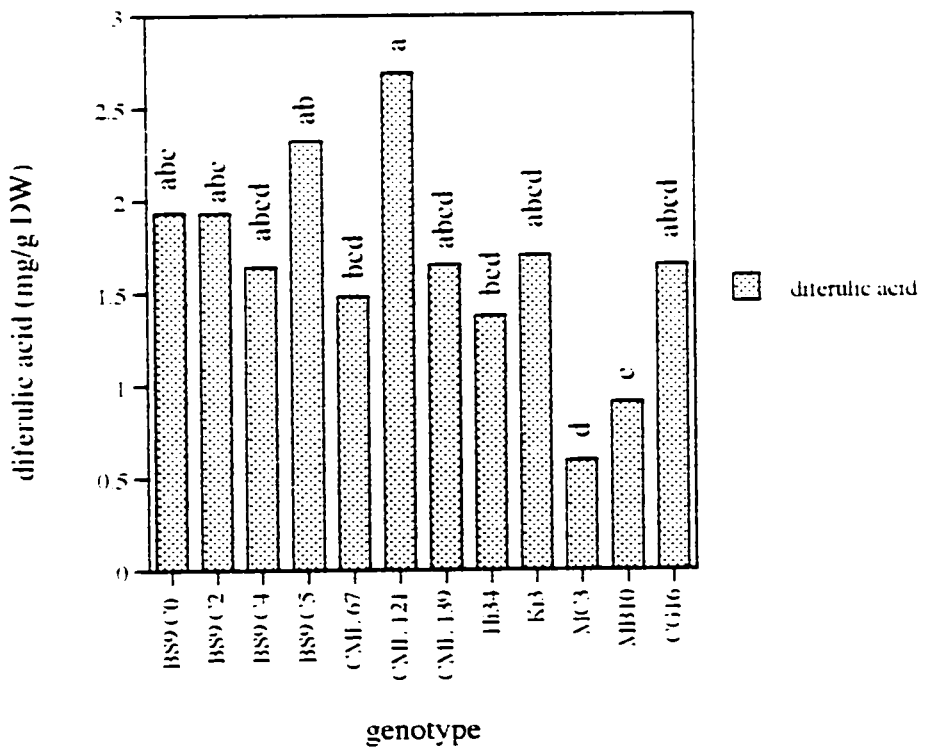


Figure 7. Concentrations of ferulic acid and diferulic acid in the yellow-green tissue of leaf 12 of 12 maize varieties of temperate and tropical corn grown in the field in 1996. Bars with different letters indicated significant differences at $p < 0.05$.

Ferulic Acid (1996)



Diferulic Acid (1996)



North American inbred and the BS9 selection series were all intermediate. No trends were evident in the BS9 selection series.

The 1996 results were significantly higher than the previous year showing genotypic differences in ferulic acid levels between years, $F=10.38$, $p=0.002$, $n=2$ (Table 2). Significant effects of year ($F=150.54$, $p<0.001$, $n=12$), and Julian date ($F=24843.37$, $p<0.001$, $n=2$), and a significant interaction between year and Julian date ($F=20.40$, $P<0.001$, $N=2$) were found in a two-way ANCOVA (Table 4). CHUs were higher and accumulation was more rapid in the first year (Fig. 8).

Multiple linear regression using ferulic acid as the dependent variable and absolute peroxidase activity, specific peroxidase activity, protein content, carbon, nitrogen, and carbon-nitrogen ratio as predictors yielded no significant models (data not shown). None of these predictors could explain the variance among ferulic acid quantities.

Diferulic acid exists as a number of isomers, each represented by a different peak at different elution times and absorbance spectra. Figure 5 shows diferulic acid isomers as three small, closely eluted, peaks. All have maximal peak absorbance at 325 nm and average retention times of 19.04, 19.25 and 19.52 minutes. The middle peak is the largest and the two smaller peaks have smaller, similar concentrations. The diferulic acid standard had the same absorbance spectrum as the middle peak and identical retention time. Each was quantified separately using the diferulic acid standard curve and the results summed (total diferulic acid). Significant effects of year and genotype and a significant interaction between year and genotype (Table 5) were found in a two-way ANOVA. Hence, the years were analyzed separately. Significant differences in total diferulic acid ($F=2.64$, $p=0.009$, $n=14$) were found among cultivars in 1995

Table 4: ANCOVA on corn heat unit accumulation for May to July, 1995 and 1996.

Source of variation	df	SS	MS	F	p
Year	1	52903.26	52903.26	150.54	<0.001
Jdate	1	8730628.07	8730628.07	24843.37	<0.001
Year x Jdate	1	7170.17	7170.17	20.41	<0.001

Jdate = Julian date. Other symbols as in Table 2.

Figure 8. Accumulation of corn heat units (CHU) by Julian date in 1995 and 1996.

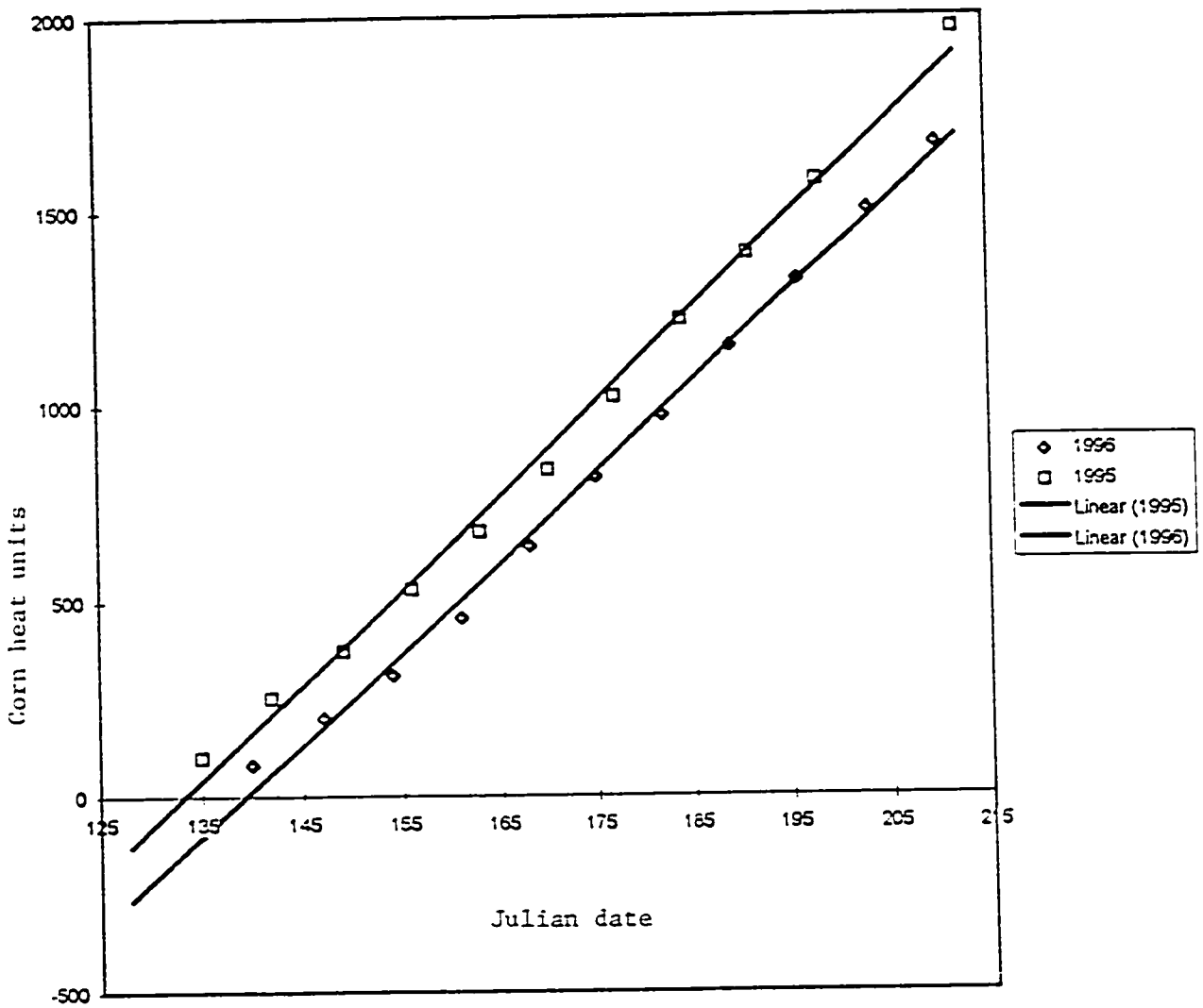

2000
1500
1000
500
0
-500
Corn heat units
125
135
145
155
165
175
185
195
205
215
Julian date
1996
1995
Linear (1995)
Linear (1996)

Table 5: Two-way ANOVA on variation of diferulic acid with genotype and year.

Source of variation	df	SS	MS	F	p
Year	1	0.29	0.29	5.29	=0.02
Genotype	11	6.74	0.61	11.03	<0.001
Year x genotype	11	4.22	0.38	6.91	<0.001

Symbols as in Table 2.

(Table 3). A high quantity was found in the North American inbred CG16 with 1.0 mg/g DW 0.178 (Fig. 6). CML 67 was also high (0.97 mg/g DW). Most were intermediate and there were no exceptional values. The differences among cultivars in 1996 were almost significant as determined in a one-way ANOVA (Table 3), $F=1.91$, $p=0.07$, $n=12$. CML 121 was high with 2.69 mg/g DW (Fig. 7) and different from the landraces MB10 (0.91) and MC3 (0.59). With the exception of BS9 C4 the rest of the BS9 selection series was different from MC3. In 1996 the diferulic acid concentrations were significantly higher than those of the previous year (Table 5), $F=5.29$, $p=0.02$, $n=2$.

In 1995 and 1996, ferulic acid was highly correlated with diferulic acid, $r=0.80$ and $p< 0.001$ (Table 6) and $r=0.87$ and $p<0.001$ (Table 7). Multiple linear regression with diferulic acid as the dependent variable was performed (Table 8) with ferulic acid, absolute peroxidase activity and specific peroxidase activity (peroxidase activity as a measure of total protein content) as independent variables. In both years, one significant regression model was found and included ferulic acid as the only variable. The line describing the relationship between ferulic acid and diferulic acid is illustrated in fig. 9 with 95% confidence limits. The equations describing the relationship are described below. The slope of the lines, 0.14 and 0.15, are similar.

DFA = 0.14(FA) - 0.03 for 1995 (1)

DFA = 0.15(FA) - 0.38 for 1996 (2)

Table 6: Pearson's correlation table showing the relationships among corn leaf biochemical parameters for 1995.

		CR	DFA	FA	POX	SPEC POX	PRO	N	C
DFA	r	n.s.							
	p								
FA	r	n.s.	**0.80**						
	p		**<0.001**						
POX	r	n.s.	n.s.	n.s.					
	p								
SPEC POX	r	n.s.	n.s.	n.s.	**0.96**				
	p				**<0.001**				
PRO	r	n.s.	n.s.	n.s.	n.s.	-0.51			
	p					0.08			
N	r	n.s.	n.s.	n.s.	**0.57**	n.s.	n.s.		
	p				**0.04**				
C	r	0.66	n.s.	n.s.	n.s.	n.s.	**-0.61**	0.51	
	p	0.07					**0.03**	0.08	
C/N	r	n.s.	n.s.	n.s.	-0.52	n.s.	n.s.	**-0.95**	n.s.
	p				0.07			**<0.001**	

CR = consumption rate in mm^2, DFA = diferulic acid in mg/g DW, FA = ferulic acid in mg/g DW, POX = absolute peroxidase activity in units/g DW, SPECPOX = specific peroxidase activity in mg/g protein, PRO = protein in mg/g DW, C = % carbon, N = % nitrogen, C/N = carbon-nitrogen ratio, n.s. = non significance, r = correlation coefficient, p = p-statistic. Boldface type indicates significance.

Table 7: Pearson's correlation table showing the relationships among corn leaf biochemical parameters for 1996.

		CR	DFA	FA	POX	SPEC POX	PRO	C	N
DFA	r	n.s.							
	p								
FA	r	n.s.	**0.87**						
	p		**<0.001**						
POX	r	n.s.	n.s.	n.s.					
	p								
SPEC	r	n.s.	0.53	**0.58**	**0.76**				
POX	p		0.08	**0.05**	**0.004**				
PRO	r	n.s.	n.s.	n.s.	n.s.	n.s.			
	p								
C	r	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
	p								
N	r	0.51	-0.50	n.s.	n.s.	n.s.	**0.59**	n.s.	
	p	0.09	0.10				**0.05**		
C/N	r	-0.24	0.30	0.29	n.s.	n.s.	**-0.49**	n.s.	**-0.99**
	p	0.10	0.08	0.08			**0.003**		**<0.001**

Symbols as in Table 6.

Table 8: Simple and multiple regression with diferulic acid as the dependent variable and ferulic acid, absolute peroxidase activity and specific peroxidase activity as the predictors.

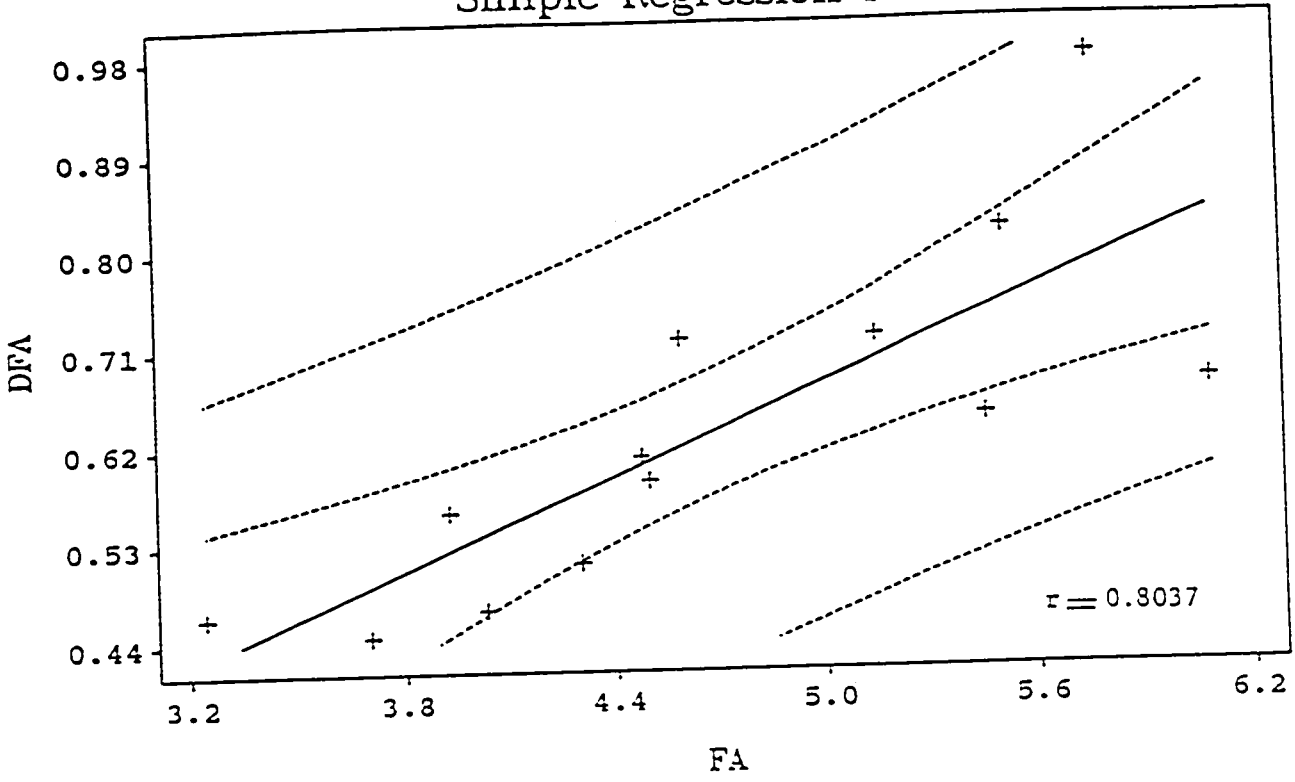
1995

Equation	r^2	p	n
DFA = -0.034 + 0.142(FA)	0.66	<0.001	14
DFA = -0.065 + 0.140(FA) + 6.19E-05(POX)	0.67	=0.004	14
DFA = 0.576 + 0.000(FA)(POX)	0.09	n.s.	14
DFA = -0.0571 + 0.140(FA) + 0.004(SPECPOX)	0.67	=0.004	14
DFA = 0.570 + 0.0029(FA)(SPECPOX)	0.09	n.s.	14
DFA = 0.575 + 8.39E-05(POX)	0.01	n.s.	14
DFA = 0.570 + 6.18E-03(SPECPOX)	0.01	n.s.	14

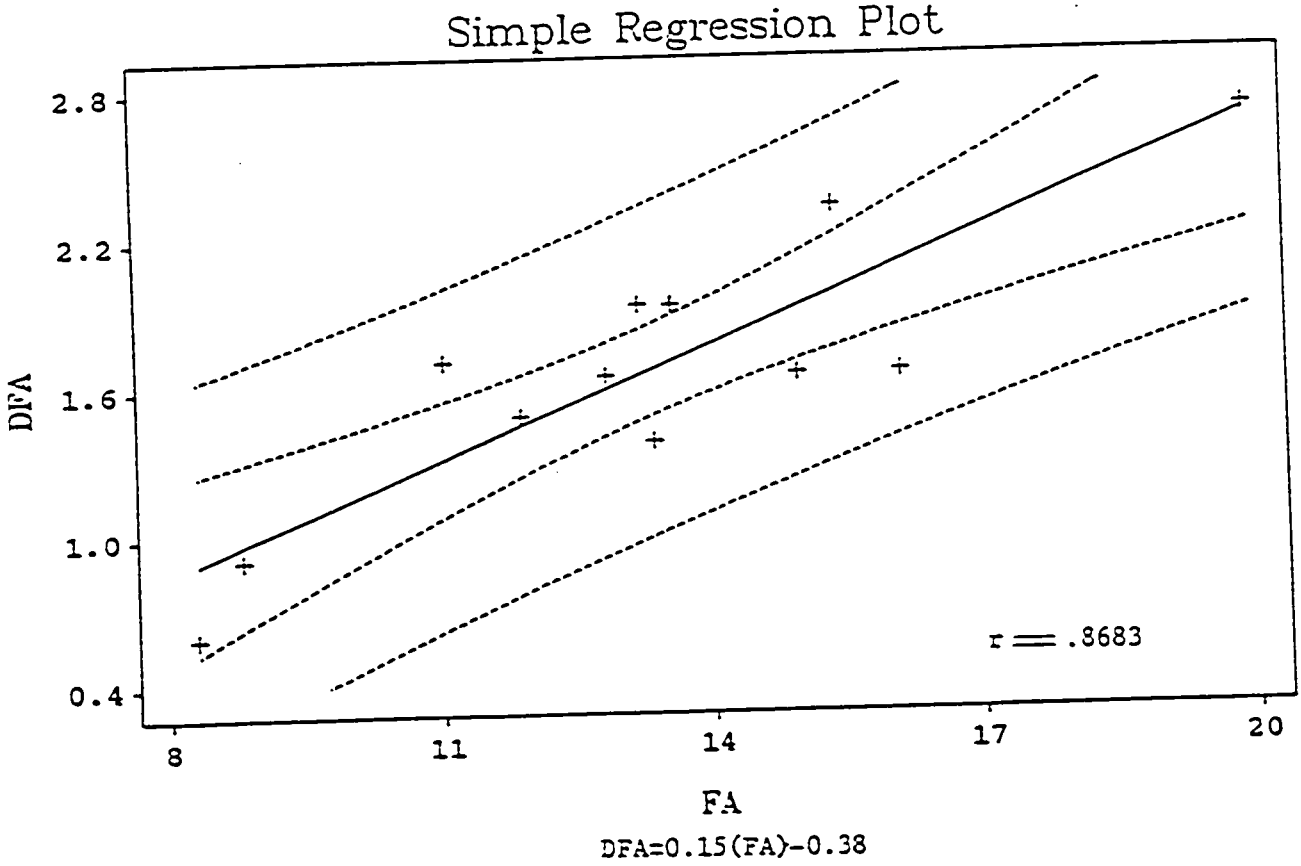
1996

Equation	r^2	p	n
DFA = -0.377 + 0.153(FA)	0.65	<0.001	12
DFA = -0.237 + 0.158 (FA) + -1.022E-04(POX)	0.77	=0.001	12
DFA = 1.113 + 0.000(FA)(POX)	0.21	n.s.	12
DFA = -0.435 + 0.152(FA) + 0.002(SPECPOX)	0.76	=0.002	12
DFA = 0.842 + 0.002(FA)(SPECPOX)	0.59	=0.004	12
DFA = 1.573 + 4.15E-05(POX)	0	n.s.	12
DFA = 1.485 + 0.005(SPECPOX)	0.28	=0.08	12

Figure 9. Simple linear regression plots illustrating the relationship between diferulic acid (DFA) and ferulic acid (FA).



DFA = 0.14(FA)-0.03



DFA=0.15(FA)-0.38

3.2 EXTRACELLULAR PEROXIDASE

Extracellular peroxidase activity was measured along the length of leaf 12 in BS9 C4 (Fig. 10). The activity was the highest in section one, the section at the base of the leaf. Section 2 had moderate peroxidase activity. The remaining sections (3-7) showed no activity. This indicates that activity was the highest in the youngest tissue and rapidly declined with age. Section 2 was used during subsequent investigations in the study as this was the yellow green tissue, the same tissue from which phenolic acids were quantified and leaf tissue samples were used in ECB leaf feeding bioassays.

Specificity of this peroxidase for various phenolic compounds revealed that it was two to eight times more specific for ferulic acid than any other compound tested (Table 9). This suggests that this extracellular peroxidase has the primary function of cross-linking ferulic acid monomers in the cell wall.

Significant effects of year and genotype and a significant interaction between year and genotype (Table 10) were shown in a two-way ANOVA. Hence, the years were analyzed separately. An ANOVA of absolute peroxidase activity (Table 11) showed significant genotypic variation in both years, $F=7525.04$, $p<0.001$, $n=14$ in 1995 and $F=1541.78$, $p<0.001$, $n=12$ in 1996. Figure 11 shows the peroxidase activity of the 14 genotypes tested in 1995. CML 67 had the highest peroxidase activity of all cultivars, 1364 units/g DW. The MBR lines had the highest overall activity of any type (synthetics, inbreds or landraces). BS9 C2 (1248 units/g DW) was also very high but the rest of the BS9 selection series was low. The landraces and inbreds had intermediate activity. In 1996, the inbred CG16 had 3890 units/g DW, significantly more

Figure 10. Extracellular peroxidase activity (units/g DW) along the length of leaf 12 from the whorl of BS9 C4.

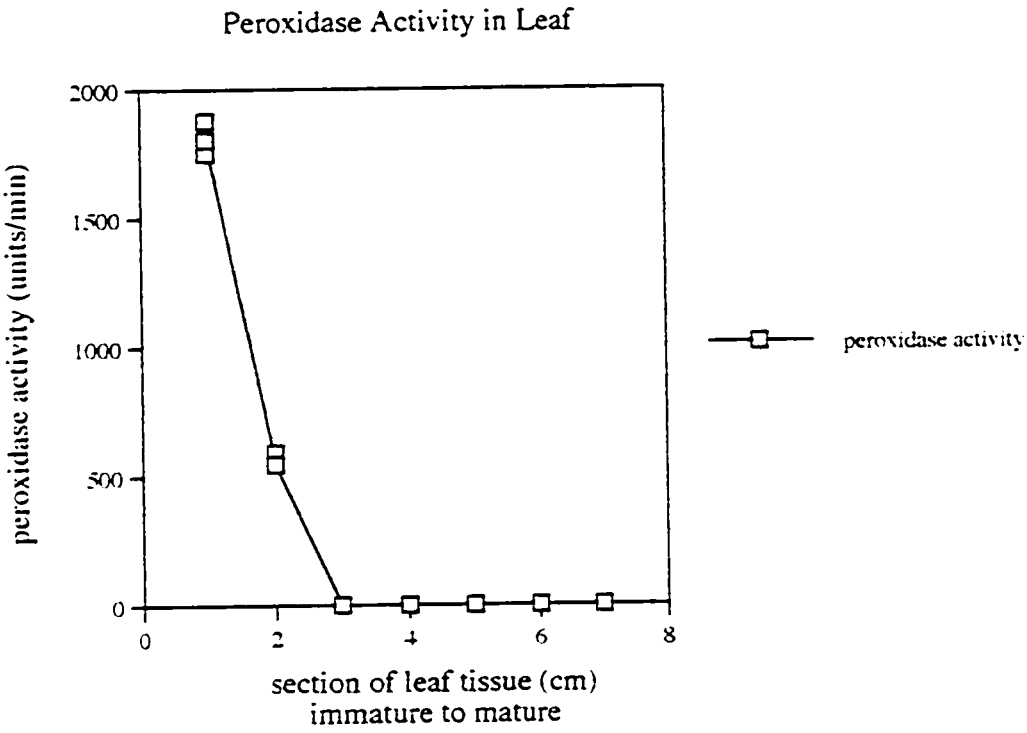

Peroxidase Activity in Leaf
peroxidase activity (units/min)
2000
1500
1000
500
0
0
2
4
6
8
section of leaf tissue (cm)
immature to mature
peroxidase activity

Table 9: Specificity of extracellular peroxidase for various phenolic compounds. The specificity is expressed as a rate based on units of activity.

Phenolic Acid	Rate (units of activity/min)	Specificity
ferulic acid	675.15	7.78
folic acid	314.08	3.62
catechol	217.43	2.5
ellagic acid	170.33	1.96
p-coumaric acid	168.93	1.95
diferulic acid	153.42	1.77
vanillin	138.37	1.59
coumarin	86.8	1

Table 10: Two-way ANOVA on variation of extracellular peroxidase activity with genotype and year.

source of variation	df	SS	MS	F	p
year	1	581259.84	581259.84	3118.26	<0.001
genotype	11	7542769.72	685706.34	368576	<0.001
year x genotype	11	.15E+08	1319141.66	7076.74	<0.001

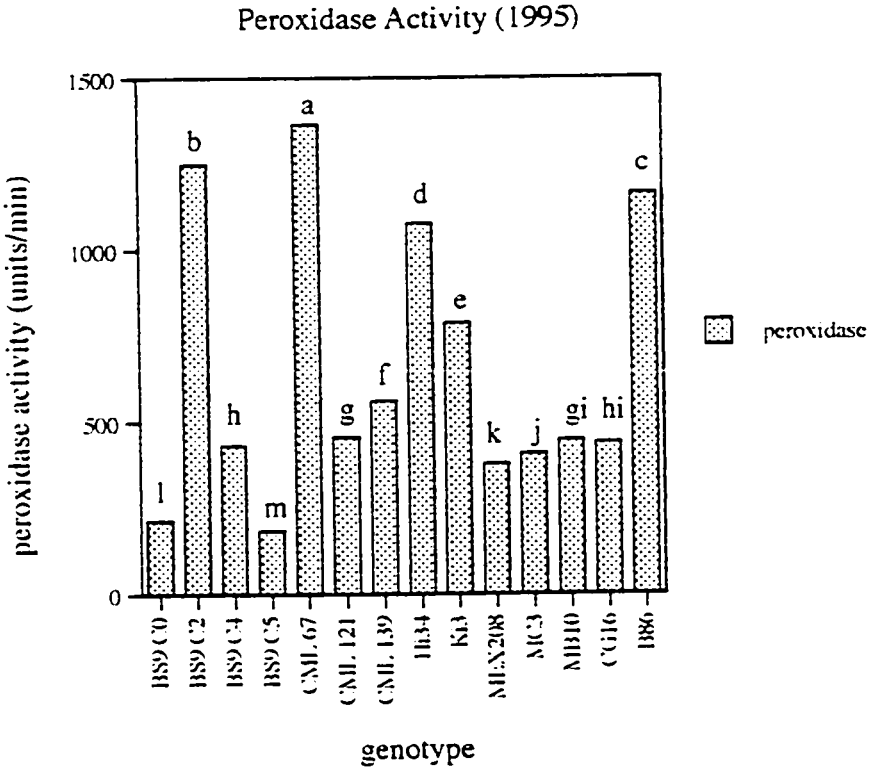
Symbols as in Table 2.

Table 11: One-way ANOVA on genotype variation for the biochemical parameters, peroxidase activity, specific peroxidase activity and protein content, measured in 1995 and 1996.

		df	SS	MS	F	p
	1995					
POX		13	.12E+08	896425.67	7525.04	<0.001
SPECPOX		13	1350.33	103.87	386.81	<0.001
PRO		13	0.01	0	35.43	<0.001
	1996					
POX		11	.24E+08	2147929.53	1541.78	<0.001
SPECPOX		11	7017.21	637.93	303.66	<0.001
PRO		11	0.02	0	360.65	<0.001

POX = absolute peroxidase activity in units/g DW, SPECPOX = specific peroxidase activity in mg/g protein, PRO = protein content in mg/g DW. Remaining symbols as in Table 2.

Figure 11. Extracellular peroxidase activity (units/g DW) in the yellow-green tissue of leaf 12 of 14 genotypes of field grown maize in 1995. Bars with different letters indicated significant differences at $p < 0.05$.


Peroxidase Activity (1995)
peroxidase activity (units/min)
1500
1000
500
0
a
b
c
d
e
f
g
h
i
j
k
l
m
gi
hi
peroxidase
genotype

activity than any other genotype (Fig. 12). BS9 C4 (1247 units/g DW) and MB10 (1051) had the lowest values and other genotypes were intermediate.

Peroxidase was significantly correlated with nitrogen in 1995, $r=0.57$, $p=0.04$ (Table 6). Simple linear regression was attempted to determine if the extracellular peroxidase activity is influenced by an increased allocation of resources to growth (Table 12). Prolificness, height and early/lateness did not appear to contribute to the level of peroxidase activity.

Turning our attention to the phytochemicals involved and assuming a product precursor relationship, multiple regression was performed (Table 8) using diferulic acid as the dependent variable and ferulic acid, absolute peroxidase activity and specific peroxidase activity as predictors of diferulic acid variability among genotypes. Only one significant multiple regression model was derived for both years. In this model diferulic acid is predicted using ferulic acid alone. Neither peroxidase nor specific peroxidase activity entered the model significantly.

3.3 PROTEINS

In 1995 and 1996 leaf tissue samples showed wide genotypic variation in protein content. Significant effects of year and genotype, and a significant interaction between year and genotype (Table 13) were found. Hence the years were analyzed separately. In 1995, the protein concentrations among genotypes were significantly different, $F=35.43$, $p< 0.001$, $n=12$ (Table 11). The MBR lines showed the highest overall protein (Fig. 13) with Ki3 having the highest (0.11 mg/g DW). The inbreds and landraces had the lowest protein concentration, B86 being the

Figure 12. Extracellular peroxidase activity (units/g DW) in the yellow-green tissue of leaf 12 of 12 genotypes of field grown maize in 1996. Bars with different letters indicated significant differences at $p < 0.05$.

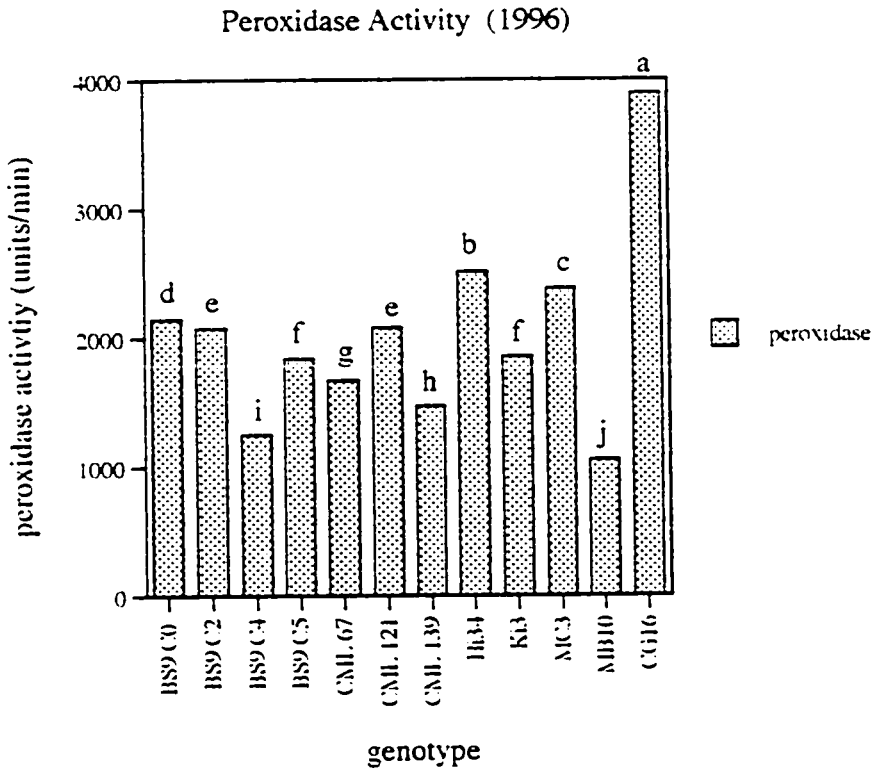

Peroxidase Activity (1996)
peroxidase activity (units/min)
4000
3000
2000
1000
0
a
b
c
d
e
e
f
f
g
h
i
j
BS9C0
BS9C2
BS9C4
BS9C5
CML67
CML121
CML139
B34
K3
MC3
MB10
CG16
genotype
peroxidase

Table 12: Simple linear regression in corn leaves with extracellular peroxidase activity as the dependent variable and biogenic data as the independent variables. Data collected in 1996.

Equation	r^2	p	n
POX = 2520.615 - 21.091(early/lateness)	0.17	n.s.	12
POX = 2290.714 - 1.455(height)	0.01	n.s.	12
POX = 2842.302 - 354.8(ears)	0.11	n.s.	12

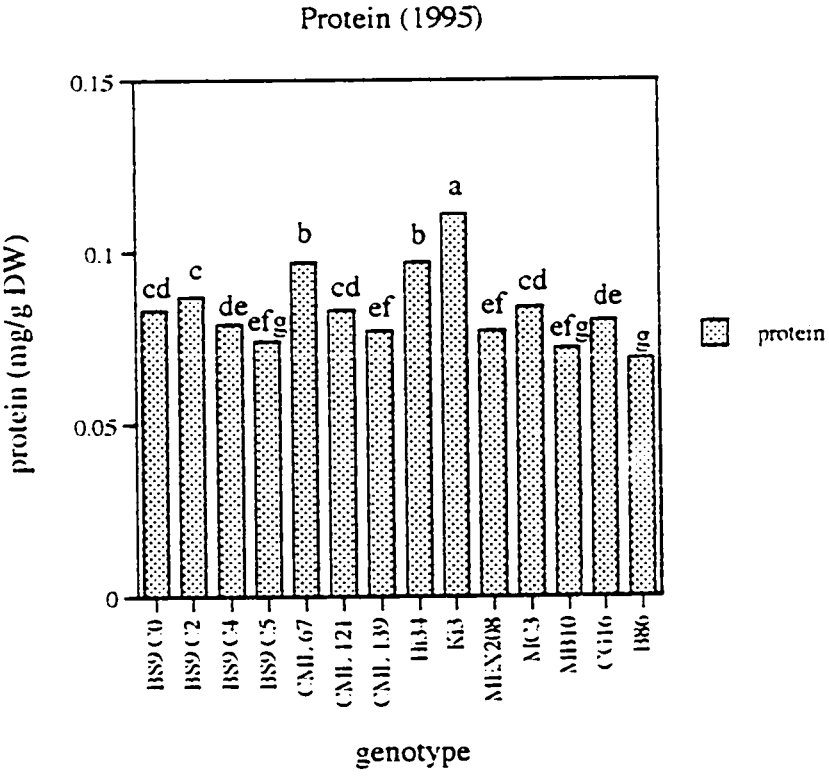
POX = absolute peroxidase activity in units/g DW, early/lateness = time from planting to emergence of silks (days), height = average height of plant at time of silking (cm), ears = number of developed ears at time of maturity.

Table 13: Two-way ANOVA on variation of leaf protein concentration with genotype and year.

source of variation	df	SS	MS	F	p
year	1	0.01	0.01	875.68	<0.001
genotype	11	0.01	0	90.67	<0.001
year x genotype	11	0.01	0	98.25	<0.001

Symbols as in Table 2.

Figure 13. Protein concentrations (mg/g DW) measured in the yellow-green tissue of whorl leaf 12 in 14 genotypes of field grown corn in 1995 Bars with different letters indicated significant differences.


Protein (1995)
protein (mg/g DW)
0.15
0.1
0.05
0
cd
c
de
efg
b
cd
ef
b
a
ef
cd
efg
de
g
protein
genotype

lowest (0.07 mg/g DW). The BS9 selection series was intermediate. BS9 C2 had the highest in the series and BS9 C5 had the lowest. In 1996, the protein concentrations among genotypes were significantly different, $F=360.65$, $p<0.001$, $n=12$ (Table 12) The MBR lines had intermediate concentrations except for CML 121 which had the lowest value (0.04 mg/g DW) along with BS9 C5 having the same concentration (Fig.14). The BS9 selection series had the lowest overall group concentrations. The inbred CG16 was intermediate.

Protein concentration was significantly correlated ($r=-0.61$, $p=0.03$) with carbon content in 1995 (Table 6). In 1996, protein level was significantly correlated with nitrogen content and the carbon-nitrogen ratio, $r=0.59$, $p=0.04$ and $r=-0.49$, $p=0.003$ respectively. Pearsons correlation did not reveal significant relationships with any other biochemical parameter measured.

3.4 CONSUMPTION RATE

In all genotypes tested, consumption rate by ECB larvae varied widely between replicates and there were several replicates in each genotype in which no leaf tissue was consumed by the insects. A significant effect of genotype, $F=8.61$, $p<0.001$, $n=12$, and a significant interaction between genotype and year, $F=3.33$, $p<0.001$, $n=12$, were shown (Table 14) in a two-way ANOVA.

In 1995, CG16 had the highest consumption rate (33.55 mm^2/day). BS9 C0 was also very high (28.30 mm) but the rest of the BS9 selection series was low. All other values were intermediate (Fig. 15). In 1996, Hi34 had the highest consumption rate (37.93 mm). Most values were intermediate. BS9 C5 (6.28 mm) and CML 139 (4.18) were the lowest (Fig. 16). It

Figure 14. Protein concentrations (mg/g DW) measured in the yellow-green tissue of whorl leaf 12 in 12 genotypes of field grown corn in 1996Bars with different letters indicated significant differences.

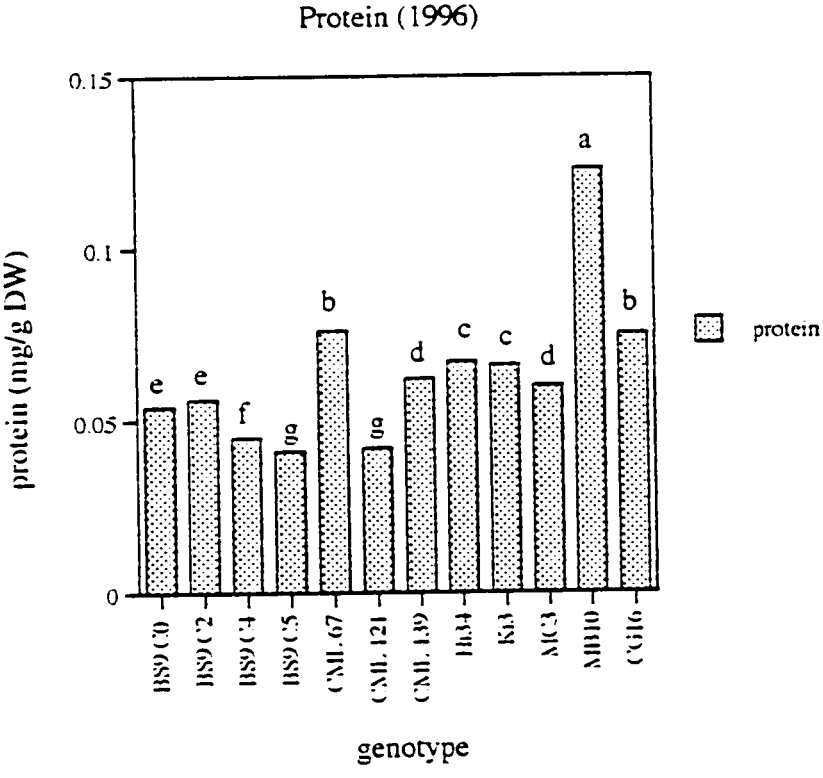

Protein (1996)
protein (mg/g DW)
0.15
0.1
0.05
0
e
e
f
g
b
g
d
c
c
d
a
b
protein
genotype

Table 14: Two-way ANOVA on genotype and year variation for consumption rate.

source of variation	df	SS	MS	F	p
year	1	137.07	137.07	0.52	n.s.
genotype	11	24960.47	2269.13	8.61	<0.001
year x genotype	11	9661.94	878.36	3.33	<0.001

Symbols as in Table 2.

Figure 15. Consumption rate (mm^2/day) by third instar ECB larvae over a 24 hour period as measured in the yellow-green tissue of whorl leaf 12 in 14 genotypes of field grown corn in 1995. Bars with different letters indicated significant differences.

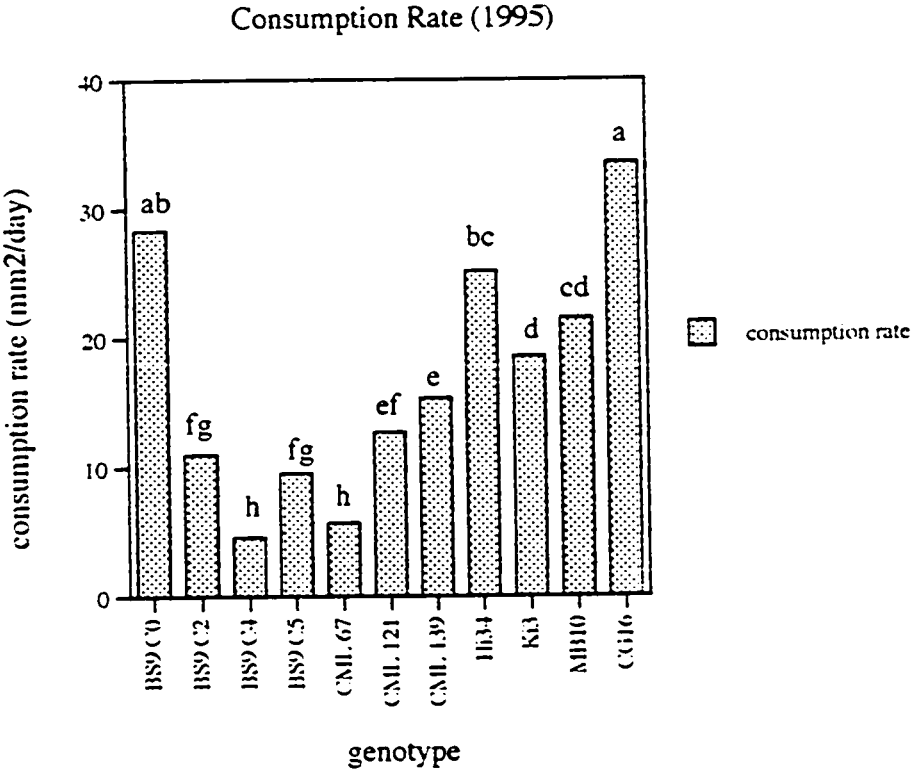
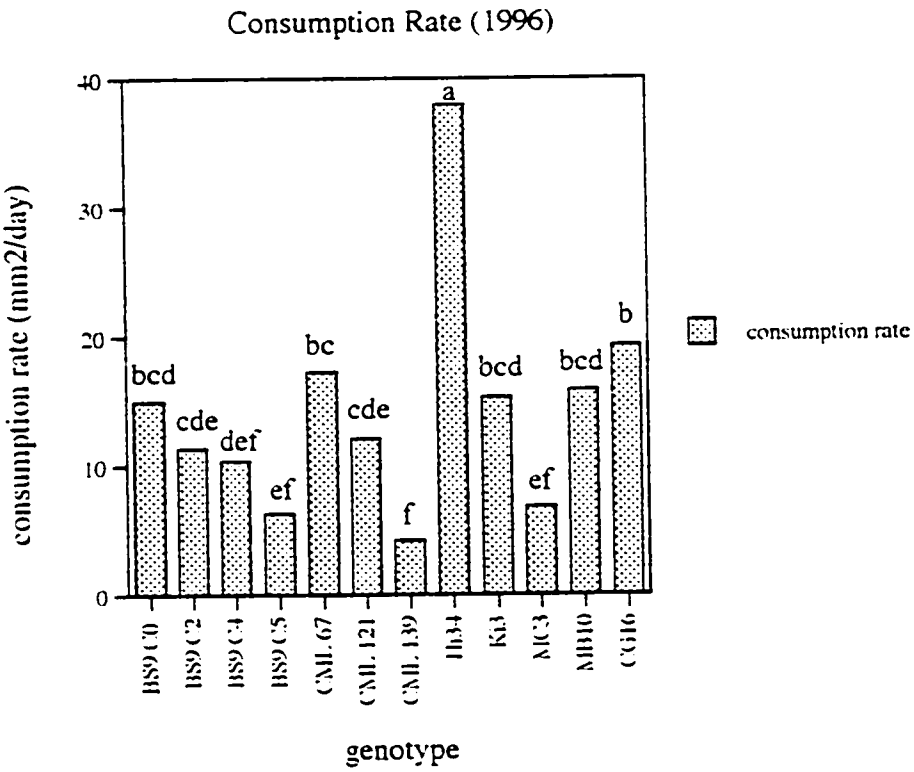

Consumption Rate (1995)
consumption rate (mm2/day)
40
30
20
10
0
ab
fg
h
fg
h
ef
e
bc
d
cd
a
BSDC0
BSDC2
BSDC4
BSDC5
CML67
CML121
CML139
H34
K63
MBI10
CG16
genotype
consumption rate

Figure 16. Consumption rate (mm^2/day) by third instar ECB larvae over a 24 hour period as measured in the yellow-green tissue of whorl leaf 12 in 12 genotypes of field grown corn in 1996. Bars with different letters indicated significant differences at $p < 0.05$.


Consumption Rate (1996)
consumption rate (mm2/day)
40
30
20
10
0
bcd
cde
def
ef
bc
cde
f
a
bcd
ef
bcd
b
genotype
consumption rate

is interesting to note that the BS9 selection series had a decreasing consumption rate from BS9 CO to BS9 C5.Simple linear regression showed that consumption rate was dependent on carbon content ($p=0.08$) in 1995 (Table 15) and on nitrogen content ($p=0.09$) in 1996 (Table 16).

3.5 ELEMENTAL DATA

Nitrogen level indicated significant differences with genotype and between year and genotype in Table 17, $F=8.95$, $p<0.001$, $n=12$. Carbon level and the carbon-nitrogen ratio indicated significant differences of year and genotype and a significant interaction between year and genotype. The years were then analyzed separately in a one-way ANOVA (Table 18). Carbon levels among genotypes were significantly different in both years, $F=6.23$, $p<0.001$, $n=14$ in 1995 and $F=2.71$, $p=0.012$, $n=12$ in 1996. The carbon-nitrogen ratios among genotypes were also significantly different in both years, $F=121.12$, $p<0.001$, $n=14$ in 1995 and $F=21.35$, $p<0.001$, $n=12$ in 1996.

Carbon: Hi34 had the highest percentage carbon with 48.50 (Fig. 17) in 1995. The lowest (41.40%) was found in BS9 C5. The rest had intermediate values. There was no trend in the BS9 selection series. In 1996 (Fig. 18), CG16 had the highest percentage carbon (43.80). The lowest belonged to CML 67 (41.60%). Most were intermediate.

Nitrogen: In 1995, Hi34 had the highest nitrogen with 3.77% (Fig. 17). MB10 had the lowest (2.42%). The rest of the genotypes were intermediate and no trends were evident in the BS9 selection series. Ki3 had the highest nitrogen content in 1996 with 3.47%. The landraces MC3 and MB10 were high, the BS9 selection series was low and the rest were intermediate (Fig. 18).

C/N Ratio: The carbon-nitrogen ratio in 1995 was highest in MB10, lowest in Hi34 (Fig. 19). No group stood out as having all high, low or intermediate values. There was much variability

Table 15: Simple linear regression with consumption rate as the dependent variable and various biochemical parameters as the independent variables for 1995.

Equation	r^2	p	n
CR = 9.353 - 11.436(DFA)	0.06	n.s.	11
CR = 26.159 - 1.997(FA)	0.04	n.s.	11
CR = 20.901 - 0.006(POX)	0.07	n.s.	11
CR = 21.749 - 0.524(SPECPOX)	0.08	n.s.	11
CR = 16.522 + 4.146(PRO)	0	n.s.	11
CR = -125.532 + 3.096 (C)	0.43	=0.08	11
CR = 3.924 + 3.419(N)	0.04	n.s.	11
CR = 13.745 + 0.036(CN)	0	n.s.	11

CR = consumption rate in mm^2, DFA = diferulic acid in mg/g DW, FA = ferulic acid in mg/g DW, POX = absolute peroxidase activity in units/g DW, SPECPOX = specific peroxidase activity in units/g protein, PRO = protein in mg/g DW, C = % carbon, N = % nitrogen, C/N = carbon-nitrogen ratio.

Table 16: Simple linear regression with consumption rate as the dependent variable and various biochemical parameters as the independent variables for 1996.

Equation	r^2	p	n
CR = 18.351 - 2.457(DFA)	0.03	n.s.	12
CR = 17.314 - 0.228(FA)	0.01	n.s.	12
CR = 5.661 + 0.004(POX)	0.13	n.s.	12
CR = 13.188 + 0.032(SPECPOX)	0	n.s.	12
CR = 6.705 + 118.529(PRO)	0.09	n.s.	12
CR = 8.965 + 0.128(C)	0	n.s.	12
CR = -20.584 + 11.910(N)	0.26	=0.09	12
CR = 50.647 - 2.445(CN)	0.27	=0.09	12

CR = consumption rate in mm^2, DFA = diferulic acid in mg/g DW, FA = ferulic acid in mg/g DW, POX = absolute peroxidase activity in units/g DW, SPECPOX = specific peroxidase activity in units/g protein, PRO = protein in mg/g DW, C = % carbon, N = % nitrogen, C/N = carbon-nitrogen ratio.

Table 17: Two-way ANOVA on variation of elemental data (carbon, nitrogen, carbon-nitrogen (C/N) ratio) with genotype and year.

	source of variation	df	SS	MS	F	p
carbon	year	1	95.04	95.04	72.92	<0.001
	genotype	11	90.06	8.19	6.28	<0.001
	year x genotype	11	60.27	5.48	4.21	<0.001
nitrogen	year	1	0.03	0.03	1.09	n.s.
	genotype	11	11.79	1.07	36.94	<0.001
	year x genotype	11	2.86	0.26	8.95	<0.001
C/N ratio	year	1	6.29	6.29	14.28	<0.001
	genotype	11	252.74	22.98	52.13	<0.001
	year x genotype	11	86.99	7.91	17.94	<0.001

Symbols as in Table 2.

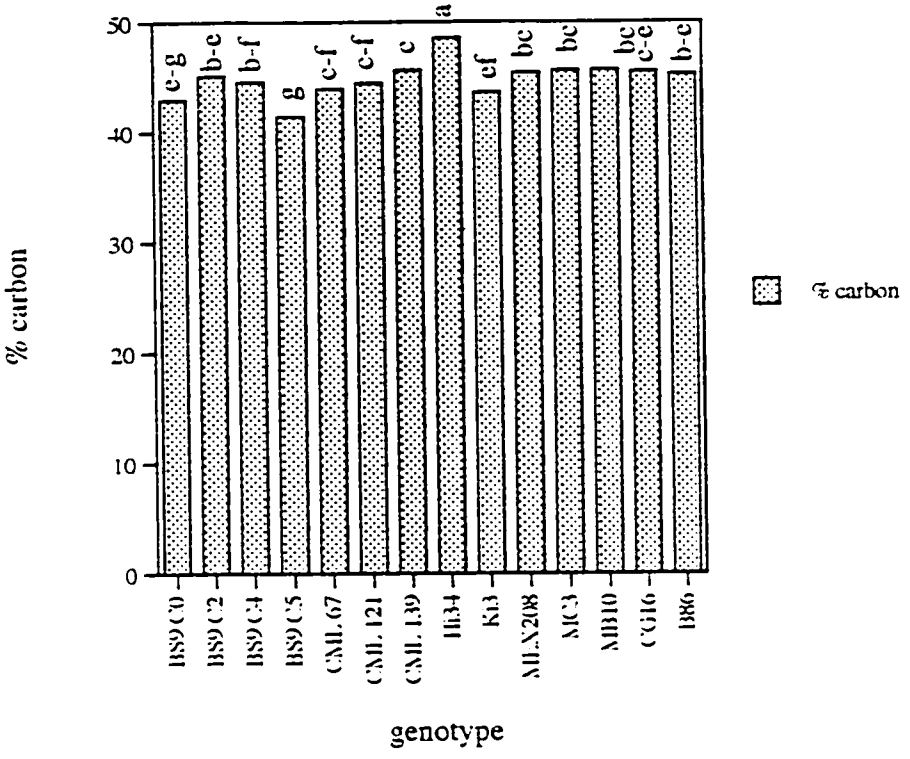
Table 18: One-way ANOVA on genotype variation for the biochemical parameters carbon, nitrogen and the carbon/nitrogen ratio, measured in 1995 and 1996.

	df	SS	MS	F	p
1995					
C	13	133.71	10.29	6.23	<0.001
C/N	13	183.69	14.13	121.12	<0.001
1996					
C	11	20.19	1.85	2.71	0.012
C/N	11	172.91	15.72	21.35	<0.001

C = % carbon, N = % nitrogen, C/N = carbon-nitrogen ratio. Remaining symbols as in Table 2.

Figure 17. Carbon and nitrogen percentages measured in the yellow-green tissue of whorl leaf 12 in 14 genotypes of field grown corn in 1995 Bars with different letters indicated significant differences.

Carbon in Leaves (1995)



Nitrogen in Leaves (1995)

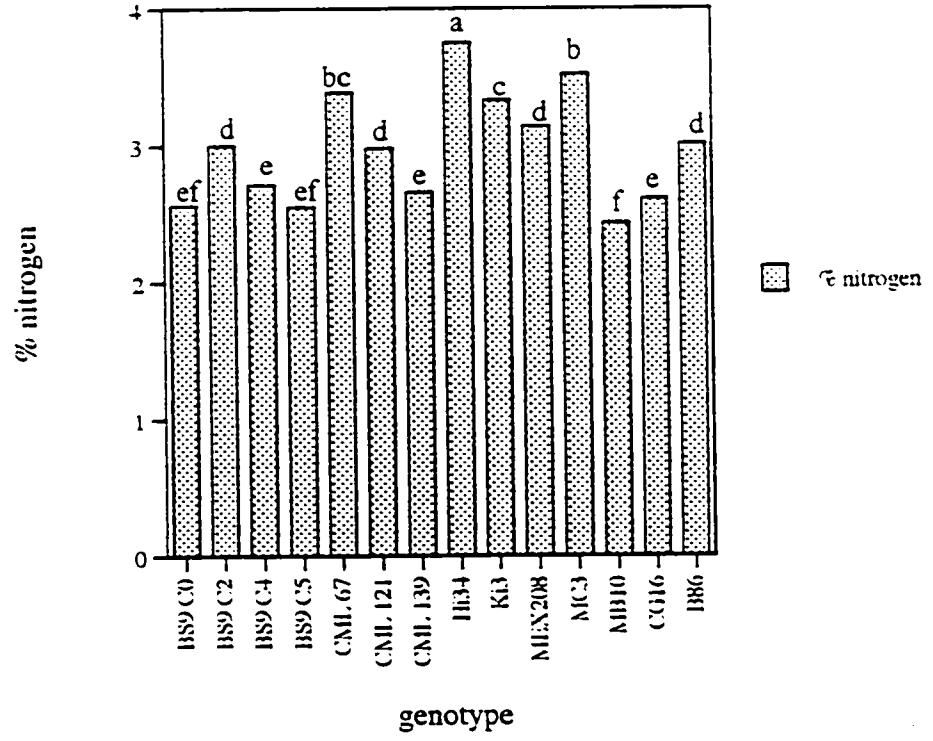
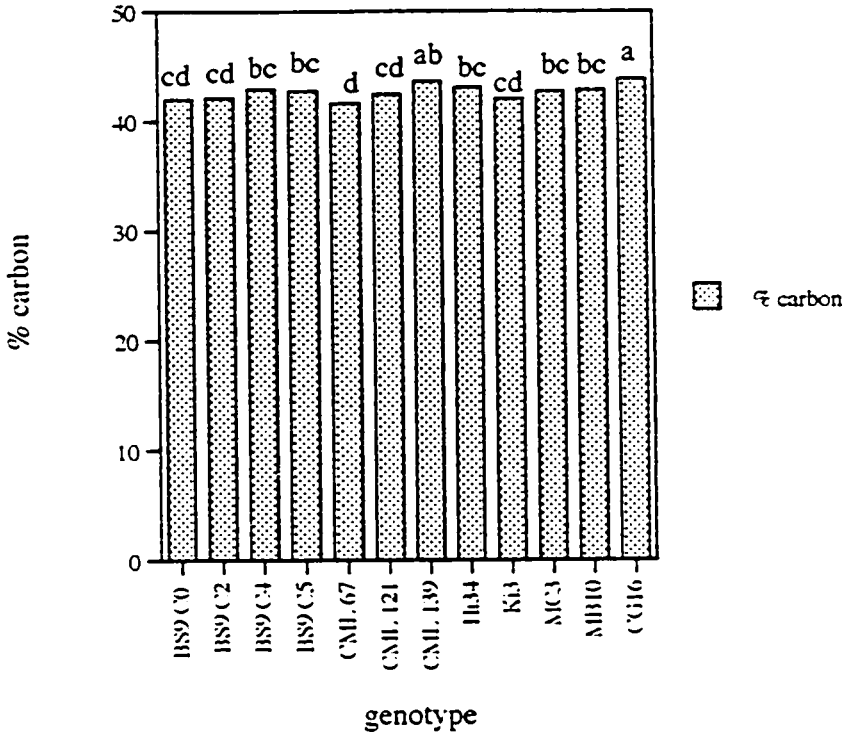


Figure 18. Carbon and nitrogen percentages measured in the yellow-green tissue of whorl leaf 12 in 12 genotypes of field grown corn in 1996. Bars with different letters indicated significant differences.

Carbon in Leaves (1996)



Nitrogen in Leaves (1996)

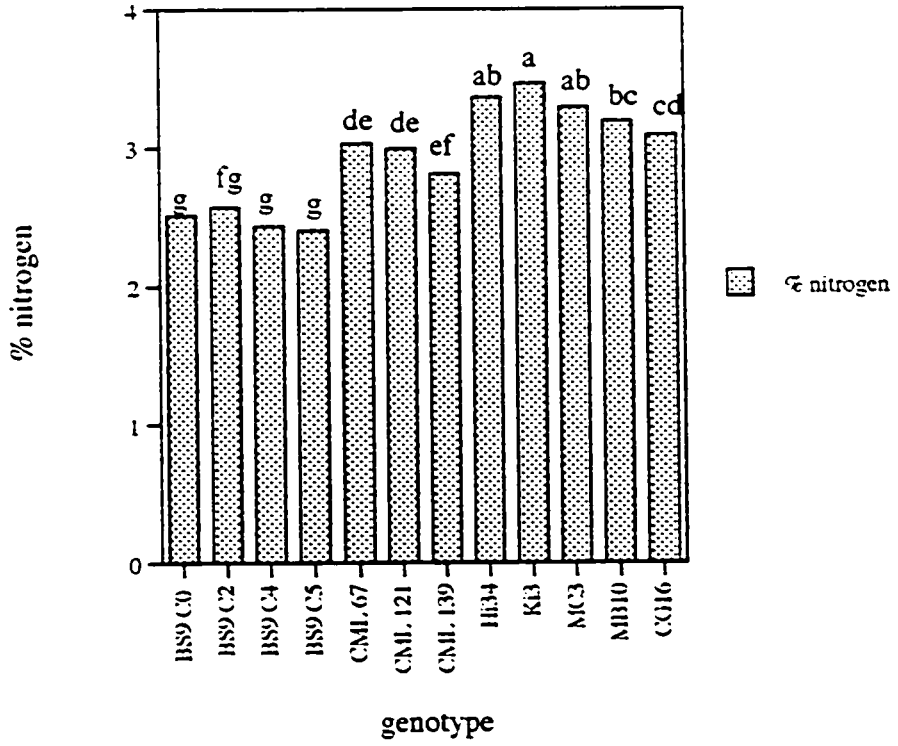
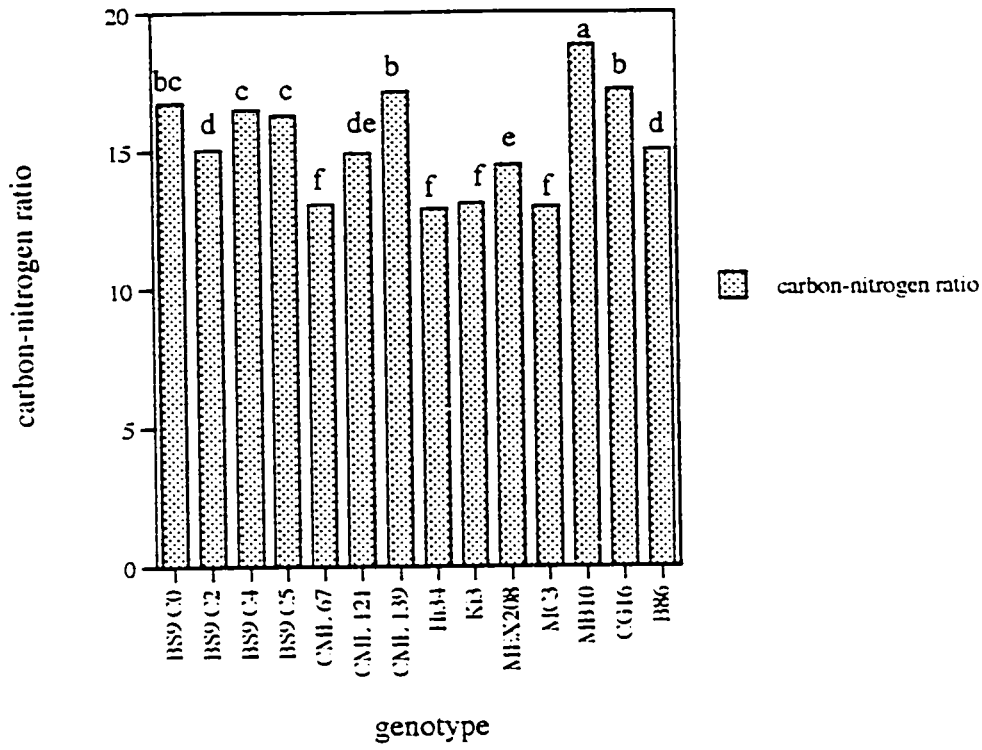
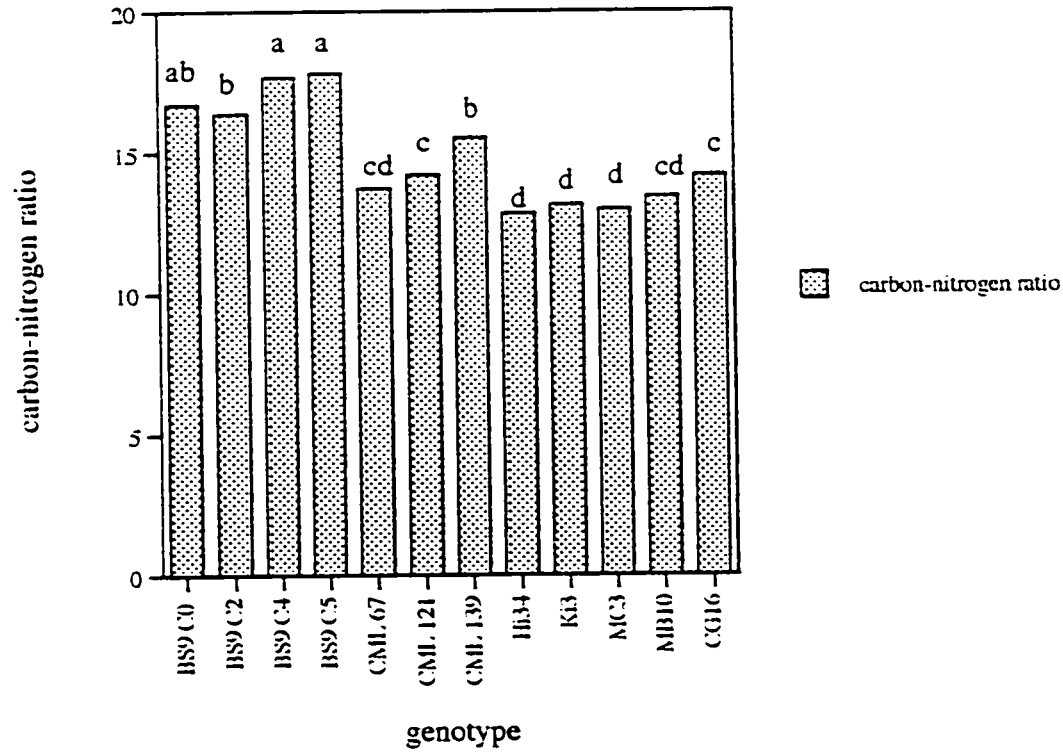


Figure 19. Carbon-nitrogen ratios measured in the yellow-green tissue of whorl leaf 12 in field grown corn in 1995 and 1996. Bars with different letters indicated significant differences.

Carbon-Nitrogen Ratio (1995)



Carbon-Nitrogen Ratio (1996)



within the BS9 selection series, MBR's, landraces and inbreds. In 1996, the BS9 selection series had the highest overall group ratios, the landraces the lowest and the rest intermediate.

A number of relationships between carbon and nitrogen and various biochemical parameters were evident from Pearson's correlation (Tables 6 and 7). In 1995, carbon was correlated with consumption rate, protein content and nitrogen content. Nitrogen content was correlated with peroxidase activity. In 1996, nitrogen was correlated with consumption rate, diferulic acid content and protein content.

3.6 BIOGENIC DATA

Early/Lateness: Maturity, measured as time to silking, showed significant genotypic variation in Table 19, ($F=.17E+17$, $p<0.001$, $n=12$), spanning 43 days among the 12 cultivars (Fig. 20). CG16 matured first, a full two weeks before any of the others. The BS9 selection series and landraces matured next. The MBR lines were late maturing.

Prolificness: The BS9 C4 had four ears per plant and BS9 C2 and BS9 C5 each had three ears. All other genotypes had two ears per plant. There was significant genotypic variation ($F=12.36$, $p<0.001$, $n=12$) with BS9 C4 being significantly different from all the cultivars (Fig. 21). While BS9 C2 and BS9 C5 were not different from each other, they were significantly different from the rest of the cultivars.

Height: The height of plants measured at maturity showed significant genotypic variation ($F=35.76$, $p<0.001$, $n=12$). Hi34 was the tallest averaging about 280 cm, MC3 the shortest, aroung 98 cm. The BS9 selection series was the tallest group overall (Fig. 22).

Table 19: One-way ANOVA on genotype variation for agronomic traits; plant height, prolificness and early/lateness for 1996.

Trait	df	SS	MS	F	p
Height	11	79226.97	7202.43	35.76	<0.001
Prolificness	11	22.67	2.06	12.36	<0.001
Early/Late	11	9264	812.18	.17E+17	=0.001

Height = average height in cm, prolificness = number of ears per plant, early/late = number of days from planting to silking. Remaining symbols as in Table 2.

Figure 20. Early/lateness of maturity measured as number of days from planting to silking in 12 genotypes of field grown corn in 1996. Bars with different letters indicated significant differences.

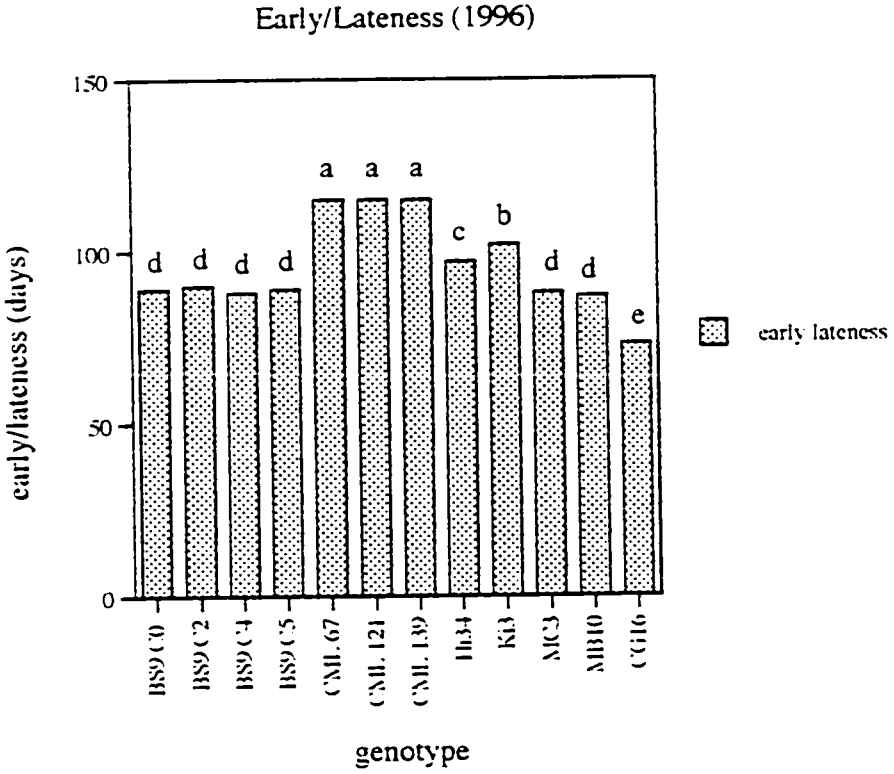

Early/Lateness (1996)
150
100
50
0
early/lateness (days)
d d d d a a a c b d d e
early lateness
genotype

Figure 21. Number of ears produced per plant at maturity in 12 genotypes of field grown corn in 1996. Bars with different letters indicated significant differences.

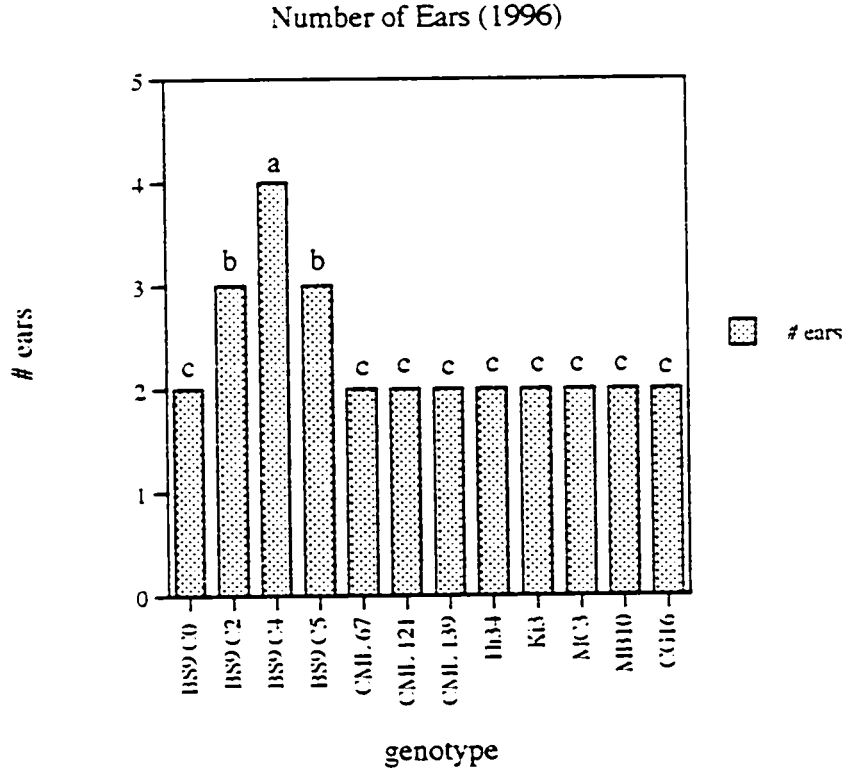
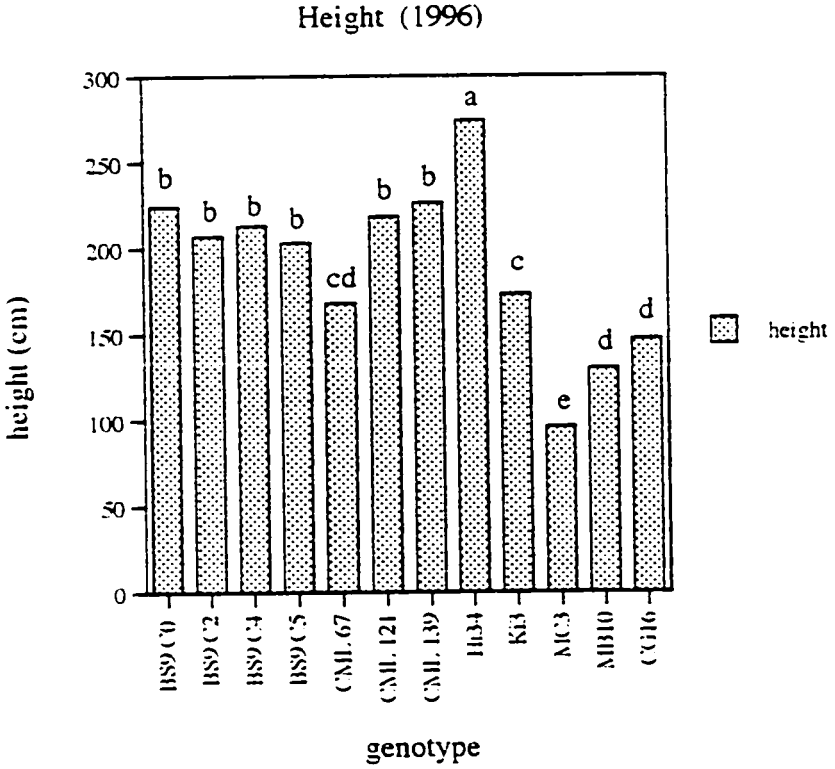

Number of Ears (1996)
ears
5
4
3
2
1
0
a
b
b
c
c
c
c
c
c
c
c
c
ears
genotype

Figure 22. Height of plant at maturity in 12 genotypes of field grown corn in 1996. Bars with different letters indicated significant differences.


Height (1996)
height (cm)
300
250
200
150
100
50
0
b
b
b
b
cd
b
b
a
c
e
d
d
height
genotype

4. DISCUSSION

4.1 PHENOLIC ACIDS

In the present study, the concentration of bound ferulic acid, measured as the amount of ferulic acid released by hydrolysis from arabinoxylan residues of the cell walls of maize whorl leaves, displayed consistent trends among all genotypes for both years. However, in the second season, (1996), the maize crop had concentrations significantly higher than the previous year (1995). The accumulation of CHU was significantly higher in the first season in comparison to the second. Biotic and abiotic conditions have been known to influence the amount of ferulic acid produced. A more rapid accumulation of CHU in 1995 than 1996 was associated with lower ferulic acid levels. Light (Tan et al., 1992a; Miyamoto et al., 1994), aging (Kamisaka et al., 1990; Tan et al., 1991), and the presence of fungal elicitors tend to increase the amount of ferulic acid ester linked to the cell walls (Bolwell et al., 1985). Temperature has a similar effect (Bolwell et al., 1985). However, this relationship was not found in this study. Perhaps differences in the concentrations of ferulic acid between the two years can be attributed to sampling variation along the length of the leaf (ie. more yellow or green tissue may have been included in analysis in one year as opposed to the next). The highest levels of ferulic acid in the whorl leaves appeared to be in the yellow-green tissue (Bergvinson et al., 1995). This tissue has reduced levels of DIMBOA and is not yet highly lignified. It was speculated that the primary means of insect resistance in this yellow-green tissue was due to the presence of elevated levels of phenolic acids (Bergvinson et al., 1993).

The Mexican inbred CML lines employ a non-conventional resistance mechanism as they contain low levels of DIMBOA (Benson, 1986), once thought to be the only mechanism of

resistance in whorl leaves. These plants have resistance to lepidopteran leaf feeders by a physical defence mechanism, since leaf feeding was shown to be significantly correlated with leaf toughness (Bergvinson et al., 1995). Leaf toughness may be mediated by lignin, detergent fiber content (Buendgen et al., 1990), and wall bound phenolic compounds (Eraso and Hartley, 1990). These create physical barriers to feeding and limit nutrient availability. Differences in amounts of ferulic acid among the genotypes investigated may indicate that plants employ this type of resistance to varying degrees. The CML lines originating from CIMMYT in Mexico had the highest levels of ferulic acid bound to the cell wall. Ferulic acid concentrations were higher in the leaves (Bergvinson et al., unpublished data) and grain (Classen et al., 1990) of insect resistant maize varieties. The CML lines have been specifically developed for resistance against three borers (Benson, 1986), and the high ferulic acid levels found in these cultivars may contribute to this enhanced resistance. Conversely, Hi34 and Ki3, the MBR intermediate and susceptible lines, had lower ferulic acid content.

In a previous study using these cultivars (Kumar and Mihm, 1996), non-preference by larvae in a no-choice feeding test, contributed to the overall resistance of the plants to SWCB and SCB. Larvae surviving on resistant inbreds remained smaller and gained less weight. Slower weight gain increased larval developmental time and exposure to parasites, predators and other adverse conditions. Antibiosis in these MBR inbreds, of which ferulic acid may have been involved, contributes to resistance as does the high ferulic acid content by imparting mechanical strength.

The temperate BS9 selection series from Iowa contained moderate to moderate-high amounts of bound ferulic acid in the cell walls in both years. Russell and Guthrie (1982) rated this series from moderate to good for first generation ECB resistance. Despite an increase in ECB

resistance throughout the series from C0, C2, C4 to C5, there was no evident trend in wall bound ferulic acid content. This suggests that ECB resistance in this series is not based solely on ferulic acid as a mechanical defence. The other temperate group, the North American inbreds, CG16 and B86, contained high and moderate (Russell and Guthrie, 1984) amounts of ferulic acid, respectively. There was a significant correlation between leaf toughness and leaf rating (Bergvinson et al., 1993) for these North American inbreds suggesting that ferulic acid content was the major defence against ECB in the yellow-green tissue. The Argentinean landraces, MB10 and MC3, had the lowest concentrations of ferulic acid in both years. These plants were developed for drought and heat tolerance, not for lepidopteran leaf feeding resistance. Their susceptibility was associated with low mechanical resistance due to low ferulic acid concentrations.

Maize sample extracts for 1995 and 1996 eluted three diferulic acid peaks by HPLC. Total diferulic acid was measured as the combined concentration of these three diferulic acid isomers (Fig. 5) which may underestimate the total diferulic acid concentration. Diferulic acid has recently been found to exist as six isomers in maize suspension culture corn cell walls (Ralph et al., 1994). The most abundant are the 8-5 linkages constituting 45% of all diferulic acid dimers followed by 8-8, 8-O-4 and 5-5 each comprising 10-25% of the total (Ralph et al., 1994; Grabber et al., 1995). Other plants possess these dimer isomers in varying amounts. In pine, the 8-8 isomer is the highest and the 5-5 isomer is not detected at all (Sanchez et al., 1996). In this study, the largest peak corresponds to the 8-0-4 diferulic acid isomer, identified by its absorbance spectrum (Waldron et al., 1996). The two smaller peaks appear to belong to 8-5 benzofuran diferulic acid and 8-5 diferulic acid, respectively. The 5-5 and 8-8 isomers were not detected in

my study. This may have been due to the absence of this isomer at the yellow-green stage of leaf development. It is possible that they may be more readily ether linked to lignin resulting in a bond not solubilized by base hydrolysis at 4N NaOH at 25 °C. Such a procedure only cleaves ester linked diferulic acid isomers (Argillier et al., 1996).

The Mexican MBR inbred CML lines contained medium-high to high amounts of diferulic acid in their cell walls. Diferulic acid content was strongly related to a reduced tissue digestibility and an increase in tissue toughness (Hartley and Ford, 1989) and a decrease in growth capacity (Sanchez et al., 1996). The high levels of diferulic acid in these lines then, can explain the resistance to the first generation ECB. The MBR inbreds, Hi34 and Ki3, had lower diferulic acid concentrations, explaining, in part, their reduced tolerance.

The temperate CG16 and B86 cultivars were moderate to high in diferulic acid concentration with corresponding moderate to high resistance to the ECB. The BS9 synthetic series increased in resistance through selection from C0 to C5 (Russell and Guthrie, 1984) but there was no corresponding increase in diferulic acid concentration. There did not appear to be any trend in diferulic acid in the present data set for BS9. Phenolic fortification is not the only factor increasing tissue resistance in these cultivars. Fiber, lignin, cellulose, hemicellulose, nitrogen and ash composition in BS9 whorl leaves did not change throughout the selection series either, indicating that other factors are acting (Buendgen et al., 1990). One of these is the qualitative toxin, DIMBOA, which is known to be almost 50% greater in C5 plants than in C0 plants (Buendgen et al., 1990).

The Argentinean landraces had the lowest diferulic acid concentration. This is consistent with their susceptibility to ECB leaf feeding. The diminished phenolic fortification may decrease

tissue toughness rendering nutrients more accessible and digestible. In corn, diferulic acid content was negatively correlated with leaf feeding damage and field leaf rating and positively correlated with tissue toughness to the ECB (Bergvinson et al., 1993). Field leaf rating is a system established by Guthrie whereby leaf damage due to insect feeding is qualified on a scale from 1-9. One indicates little damage while 9 indicates severe damage.

In this study, the correlation between ferulic acid and diferulic acid concentrations was significant among all cultivars in both years. Simple linear regression indicated that the variability in diferulic acid concentration is dependent on the amount of esterified ferulic acid present in the cell wall. Ferulic acid was the only significant factor in this study correlated with diferulic acid concentration. This suggests that the rate limiting step in the formation of diferulic acid is feruloylation of arabinoxylans. Because ferulic acid controls diferulic acid concentration and diferulic acid is an indicator of tissue toughness to insects, ferulic acid as well as diferulic acid might be used as bioindicators for ECB resistance. Screening maize germplasm for resistance can be done by quantifying ferulic acid concentration rather than diferulic acid concentration as ferulic acid exists in two easily extracted and detected forms, trans and cis. Diferulic acid, on the other hand, exists in four known forms which are more difficult to extract and identify. It had been previously suggested that feruloylation may be the rate limiting step in the formation of diferulic acid rather than peroxidase activity or oxidant formation. The ratio of ferulic acid to diferulic acid is almost always constant irrespective of age, zone or growth conditions as found in *Avena* (Kamisaka et al., 1990) and *Oryza* coleoptiles (Tan et al., 1991). It was suggested that feruloylation itself may control wall extensibility by interfering with enzymatic degradation of matrix polysaccharides (Miyamoto et al., 1994). It is known that

feruloylated matrix polysaccharides are hydrolase resistant (Fry, 1984) and a turnover of these matrix polysaccharides is indispensible for an increase in the ability of cell walls to extend (Sakurai et al., 1979; Hoson and Nevins, 1989; Hoson, 1990).

Because diferulic acid content increases the mechanical strength of tissue and may decrease digestibility in insects, concern about a decrease in digestibility of corn tissue in sheep, cows and bulls has been expressed. Argillier et al. (1996) found no obvious effect on these animals due to esterified ferulic acid. There was a decrease in degradibility but this was due to lignin content and ferulic ethers.

4.1.1 SUMMARY

1. The CML lines demonstrated good resistance to the ECB which can be attributed to the high levels of wall bound ferulic and diferulic acids in their whorl leaves. Ferulic acid concentration is known to be higher in the leaves of resistant than sensitive maize varieties (Bergvinson et al., unpublished data).

2. The BS9 selection series were developed for first and second generation ECB resistance. They had high levels of wall bound ferulic and diferulic acid in their whorl leaves. There was an increase in resistance from C0 to C5 but phenolic acid levels did not show a trend. Fiber, lignin, hemicellulose, ash and nitrogen content did not show a trend either (Hedin et al., unpublished data). DIMBOA, on the other hand, does increase by 50% from C0 to C5 suggesting that this toxin may play an important role in resistance in these cultivars.

3. The North American inbreds, B86 and CG16 have moderate and moderately good resistance to the ECB. They contained moderate and high amounts of wall bound ferulic acid and diferulic acid, respectively. There appears to be a good correlation between these compounds and tissue

resistance. In fact, Bergvinson et al. (1993) found a significant correlation between leaf toughness and leaf rating (for ECB feeding) for these two cultivars.

4. The Argentinean landraces, MB10 and MC3 were developed for heat and drought tolerance, not for ECB resistance. They showed the lowest concentrations of wall bound ferulic and diferulic acids, which may account for their susceptibility from reduced cell wall fortification.

5. A significant relationship between ferulic and diferulic acids based on regression models was indicated in both years.

6. Ferulic acid is the sole predictor of diferulic acid content in maize leaf tissue and can be used as a bioindicator for plant tissue resistance to the ECB.

4.2 PEROXIDASE

The leaf profile of extracellular peroxidase in the maize line BS9 C4 indicated that the isoenzyme dimerizing ferulic acid is highest in immature yellow tissue and rapidly declined with age to a level not detectable in mature tissue. Esterified ferulic acid undergoes a coupling reaction *in situ* by this peroxidase to produce dehydrodiferulic acid (Hartley and Jones, 1976; Kamisaka et al., 1990). The formation of dehydrodiferulic acid occurs early in maize tissue development and reaches its highest levels in the yellow-green leaf tissue (Bergvinson et al., 1995) which can be explained by the peroxidase profile.

In the corn leaf cell, there are six wall bound peroxidase isoenzymes, four cationic and two anionic (Birecka and Catalfamo, 1975). The ionically wall bound fraction of peroxidase is generally accepted to represent the cell wall activity (MacAdam et al., 1992). In corn, this represents 20% of the total peroxidase activity (Birecka and Catalfamo, 1975; Baier and Webster,

1992; Goldberg et al., 1993). It was shown that only these cell wall bound peroxidases participate in the oxidation of lignin monomers and cross-linking of phenolic constituents (Mader et al., 1976; Pang et al., 1989; Goldberg et al., 1993). Anionic peroxidases rapidly oxidize phenolic acids including ferulic and caffeic acids (Pickering et al., 1973) indicating that the peroxidase mediating the oxidation of ferulic acid is likely one of the two anionic isoenzymes. Isolation of the anionic isoenzyme in maize leaf tissue that rapidly decreases with age is likely the peroxidase isoenzyme involved in phenolic fortification.

All the cultivars of maize in this study significantly varied in peroxidase activity in the yellow-green leaf tissue. There was no correlation between this peroxidase activity and maize resistance to insects. This suggests that the peroxidase activity does not determine the degree of tissue toughness dictated by wall fortification despite its participation in phenolic cross-linking, lignin precursor bonding and the joining of the hemicellulosic matrix and cellulosic components in the cell wall, all of which do play a role in tissue toughness. Van loon (1986) recognized that peroxidase activity and resistance may not be directly related and that high peroxidase activity may be an unspecific response to stress. This has also been noted by Birecka and Catafamo (1975) who observed enhanced maize levels of peroxidase infected by *Helminthosphorium maydis*. Several studies showed, however, that peroxidase activity is a marker for resistance in plants. Oat leaves inoculated with *Puccinia coronata* showed an increase in ionically wall bound peroxidase activity and a corresponding increase in diferulic acid (Ikegawa et al., 1996). Ferulic acid content remained constant before and after inoculation (Ikegawa et al., 1996). It has been repeatedly documented that resistant plants of a species have higher peroxidase levels in the resting state compared to susceptible plants as demonstrated in wheat (Arora and Wagle, 1985).

and tomato (Brenneman and Black, 1979). Resistant cultivars of lupin have been shown to have higher levels of peroxidase than susceptible cultivars (Malolepsza et al., 1989). These authors have found higher levels of an ionic wall bound isoenzyme. This ionic wall bound peroxidase increased in activity after the lupin roots and calli were subjected to *Fusarium culmorum* indicating this peroxidase reacts to biotic stresses. The levels of peroxidase increased much sooner, more rapidly and to a greater extent in resistant cultivars than susceptible ones. Spanu and Bonfante-Fasolo (1988) also found the cell wall portion of peroxidase increased in *Allium porrum* roots when inoculated with vesicular arbuscular mycorrhizal fungi.

It has generally been found that peroxidase isoenzymes vary quantitatively among cultivars and hence confer varying resistance. In a number of studies, however, infection with pathogens had induced the production of previously undetected peroxidase isoenzymes. This was noted in the onion with *Botrytis alli* (Magro, 1984), in maize with *Helminthosphorium maydis* (Cadena-Gomez and Nicholson, 1987), in bean with southern bean mosaic virus (Farkas and Stahmann, 1966), and in alfalfa with alfalfa mosaic virus or TMV (Solymosy et al., 1967). Higher levels of these novel peroxidases were found in resistant cultivars of a species. Other enzymes have also been shown to be higher in resistant cell lines, such as PAL in chili peppers (Ochoa and Salgado, 1992) and polyphenol oxidases in tomato (Constabel et al., 1996).

Peroxidase levels as determinants of plant tolerance are a measure of overall peroxidase activity, cytoplasmic and wall bound. It is possible that only one or several of these have high activity in resistant cultivars which give the plant an overall elevated peroxidase status and the general classification as a resistant cultivar. Resistant plants can be identified by elevated peroxidase levels in the ionic wall bound fraction or by the presence of high levels of novel

isoenzymes during invasion. Resistant maize plants cannot be identified by the wall bound peroxidase isoenzyme activity specific to ferulic acid.

The accumulation of CHU showed a significant interaction between genotype and year. Lower peroxidase activity in the 1995 season, is associated with a more rapid accumulation of CHU in this study indicating that high internal temperatures may increase the rate of enzyme turnover. The synthesis and secretion of many peroxidase isoenzymes are controlled by a number of abiotic factors. These include light, drought, salinity, temperature (McNeil et al., 1984), and plant growth regulators such as ethylene (Campa, 1991). Ethylene increased peroxidase activity (Abeles, 1973) in the cytoplasmic and ionically wall bound spaces but not in the covalent fractions (Ridge and Osborne, 1970) as discovered in *Pisum sativum*. The plant growth regulator IAA, on the other hand, appeared to be controlled by peroxidase as this enzyme has the ability to inactivate it through oxidation (Pilet and Lavanchy, 1969; Gebhardt, 1982).

The linear regression model using diferulic acid as the dependent variable and peroxidase as the predictor was non-significant. Only 4.4% of diferulic acid variability among cultivars in the first year and 0.25% in the second year could be explained by the peroxidase activity. Our results suggest that this peroxidase cannot be used as a bioindicator for resistance in maize plants to first generation ECB. Screening cultivars for resistance should be done through phenolic acid quantification.

Despite peroxidase not being involved in plant tissue resistance through cell wall fortification, there does seem to be a positive correlation with nitrogen levels. In the first season, peroxidase activity was strongly dependent on nitrogen levels. The whorl leaves are in the midst of rapid growth and function as a sink for nitrogen. High nitrogen content is necessary for this growth

and is utilized in the production of proteins. Therefore, the young leaves must contain relatively high levels of nitrogen in comparison to other plant organs. Young leaves also tend to have high defence allocation (Bazzaz et al., 1987). In certain genotypes of maize this defence is found in carbon based phenolic acids.

4.2.1 SUMMARY

1. The cell wall bound extracellular peroxidase leaf profile indicated that the isoenzyme involved in dimerizing ferulic acid to diferulic acid was the highest in immature yellow tissue and rapidly declined with age to activity not detectable in mature tissue. The formation of diferulic acid occurred early in maize tissue development and levels of both ferulic and diferulic acids were highest in the yellow green leaf tissue.

2. Peroxidase activities within and between cultivars (i.e. BS9 synthetic series, MBR inbreds. North American inbreds and Argentinean landraces) were not consistent.

3. No relationship between extracellular peroxidase activity and plant resistance to insects was evident.

4. Regression models having extracellular peroxidase activity as the independent variable and diferulic acid as the dependent variable yielded non-significant results. Peroxidase, as measured in this study, is not an indicator of diferulic acid content.

4.3 PROTEIN

Plant protein is essential for insect growth and development. If protein is inaccessible or quantities insufficient, larvae suffer a delayed developmental time, impaired reproductive potential or mortality. Consequently, tissue consumption is highest in the yellow-green tissue as

larvae must consume more tissue to get the necessary protein for growth and survival (Scriber and Slansky, 1981). The maize yellow-green tissue contained the lowest amount of protein in the leaf, comprising only 17% of maximum levels (Bergvinson et al., 1995). Some resistant cultivars use a strategy whereby they contain lower protein content than susceptible cultivars (Bergvinson et al., 1993). These plants may not provide enough nutrition for development, encouraging the ECB to find more protein rich cultivars (Bergvinson et al., 1993). Low protein content correlated with decreased feeding by larvae. No correlation between plant resistance via larval feeding and protein content was observed for this study. The BS9 synthetic series have moderate to high resistance and low to intermediate protein content in contrast to the MBR lines which had moderate-high resistance and moderate-high protein content. Other studies demonstrated a positive correlation between leaf tissue resistance and protein content (Classen et al., 1990; Arnason et al., 1994).

The accumulation of CHU within genotypes between years was significantly different. A more rapid accumulation of CHUs was associated with higher protein content in this study. Higher internal temperatures increased the production of proteins which are essential for growth.

Protein content was positively correlated with nitrogen content and negatively correlated with the carbon/nitrogen ratio in 1996. This may indicate that protein measurement for this year was in agreement with the practice of estimating protein concentration from nitrogen content using a conversion factor (McKenzie and Wallace, 1954). In the previous year, protein content was not correlated with nitrogen content perhaps indicating a variance in sampling in the yellow-green tissue section (i.e. more yellow or more green tissue may have been used for either protein or

nitrogen estimation). This may also explain why carbon content was negatively correlated with protein content in the first year but not in the second year.

4.4 CONSUMPTION RATE

In other work, consumption rate was negatively correlated with leaf tissue resistance and was highest in the immature yellow whorl tissue (Bergvinson et al., 1995). There are three main parameters that can account for leaf feeding by ECB in maize; epidermal cell wall absorbance, toughness and fiber content (Bergvinson et al., 1995). Feeding resistance to the SWCB has been attributed to a decreased protein content, increased crude fiber and increased hemicellulose (Hedin et al., 1993). Ferulic and diferulic acids act in concert with fiber in increasing the mechanical resistance of tissue. There was no correlation found in my study between consumption rate and these phenolic acids which would indicate that fiber was not playing a significant role in tissue resistance.

The high variability in consumption rate among and within genotypes over the two years in the present study is likely due to larval feeding characteristics. When larvae approach the next developmental stage, they cease to feed. Within all genotypes, many of the samples showed an absence of feeding resulting in very high variability within genotypes. Relationships with plant parameters became less evident.

Over the two years, consumption rate showed a relationship between carbon, nitrogen and the carbon/nitrogen ratio. The positive correlation with nitrogen and the negative correlation with the carbon/nitrogen ratio is in agreement with Bergvinson (1993). The negative correlation with carbon is likely due to elevated concentrations of phenolics and other carbon based compounds which aid in increasing tissue resistance.

4.5 ELEMENTAL AND BIOGENIC DATA

Plants having abundant carbon usually allow reproductive processes to dominate earlier in the season (Janick, 1986). Carbon quantities are related to plant biomass and higher carbon content is usually indicative of higher plant biomass. A biomass threshold must be reached before reproduction begins (Werner, 1975). When this biomass is obtained,vegetative growth ceases in annual plants. Large supplies of nutrients are directed toward flower and seed production. It seems likely that cultivars with higher carbon content would reach their critical biomass sooner than cultivars with lower carbon content. CG16 had moderate-high to high amounts of carbon in both years and was the earliest maturing cultivar. CML 139, however, had similar levels of carbon as CG16 but it matured at a much later date. Perhaps using relative growth rate (RGR), measured as time to maturity, can better be used as a function of resource allocation. CG16 had a fast RGR, the BS9 series and Argentinean landraces, moderate RGRs, the MBR inbreds Hi34 and Ki3, moderate-slow RGRs and the CML lines had slow RGRs. According to Coley et al. (1985), cultivars with fast RGRs have low defence allocation and cultivars with slow RGRs have high defence allocation. This did not hold true for the cultivars of the present study as CG16 had moderately good resistance and the BS9 series had good to high resistance. On the other hand, Bazzaz et al. (1987) suggested that high RGR increases allocation to defence if the defence is carbon based. This hypothesis also does not hold true for the cultivars in this study as the slow growing CML lines had high carbon based defences (phenolic acids).

Prolific cultivars absorb and accumulate more nitrogen than nonprolific cultivars (Baligar and Duncan, 1990) and are able to partition more nitrogen to grain production. The BS9 synthetic series had low nitrogen content and were the most prolific. Perhaps these cultivars had good

photosynthetic productivity, good storage and efficient mineral utilization. High nitrogen encourages vegetative growth and can delay reproduction (Janick, 1986). Hi34 had high levels of nitrogen in both years and invested the most in vegetative growth (as height) among all cultivars. Hi34 is a susceptible cultivar to ECB leaf feeding, investing less in defence. Other susceptible cultivars, MB10 and MC3, had high nitrogen content in both years (except MB10 in 1996) but they invested less in vegetative growth as compared to the tallest cultivar as they were the two shortest cultivars.

Differences in genotypic variation in plant nutrient efficiency may be due to a number of factors. Root features including greater spread of roots, more root hairs, greater efficiency in ion uptake per unit root length, presence of mycorrhizae or longevity of roots may increase plant nutrient efficiency or perhaps nutrient movement across roots and delivery to the xylem or nutrient distribution within plants play a significant role (Gerloff and Gabelman, 1983).

4.6 RESOURCE DISTRIBUTION

Three processes competing for limited resources in plants are growth, reproduction and defence (Bazzaz et al., 1987). Within species, variation among cultivars grown under the same environmental conditions is likely due to genetic differences. Resource allocation pattern is correlated to resource availability, habitat, plant age and the internal resource balance (Bazzaz et al., 1987). Because all these factors except internal resource balance were constant among the cultivars, the differences in allocation can be a direct measure of this balance.

Corn planting is done in such a way as to maximize productivity by reducing competition with other corn plants (by optimal spacing) and other species of plants (by weeding and herbicide

application). Limited resources due to competition are then reduced. The plant is no longer driven to utilize most of its obtainable nutrients to maximize growth. This allows an increase in allocation of resources to defence as hypothesized by Herms and Mattson (1992). As a species then, corn plants allocate more resources to defence and less to growth in comparison to many other plant species.

Corn is an apparent plant as it is found in monocultures in the same geographic regions, year after year. According to Feeny (1976), such plants employ quantitative defences including wall bound phenolic acids or lignins. The plant can maintain these compounds at a relatively low cost. Low maintenance defences are necessary when herbivory is a constant stress. The presence of high concentrations of the wall bound phenolic compounds, ferulic and diferulic acids, is the quantitative defence used as a measure of resistance in this study. The resistant CML lines and BS9 synthetics had intermediate to high phenolic concentrations, the moderately resistant cultivars B86 and CG16 had intermediate phenolic concentrations and the susceptible Argentinean landraces as well as the MBR lines, Hi34 and Ki3, contained low phenolic concentrations. I conclude that resistant cultivars are high in wall bound phenolics and use these compounds in quantitative defence. This is further supported by the high carbon-nitrogen ratio of these cultivars. The BS9 synthetics had the highest carbon-nitrogen ratio followed closely by the CML lines. The inbreds, CG16 and B86, had moderate ratios and the Argentinean landraces and the MBR lines, Hi34 and Ki3, had low carbon-nitrogen ratios. I suggest that resistant cultivars have high carbon-nitrogen ratios and susceptible cultivars have low carbon-nitrogen ratios. Cultivars with higher carbon-nitrogen ratios such as the BS9 synthetic series and CML lines would allocate more resources to quantitative defences while the cultivars with the lower

ratios (Argentinean landraces and MBR lines, Hi34 and Ki3) would allocate more resources to growth.

Nutrition is also thought to play a role in defence. Mohan and Hamilton (1980) hypothesized that high or low plant nutrition to herbivores can be adaptative depending on the initiation and maintenance costs of defences to the plant and the insect mortality profile. In the case of corn and the ECB, low nutrition in the leaves is an adaptative trait designed for defence. About 80% of ECB larvae perish in the first 48 hours after hatching (Bergvinson et al., 1993). Low nutrition increases this number while high nutrition may significantly decrease it. According to this theory, resistant cultivars of corn will have low leaf nutrition (via protein content). The cultivars in this study did not show this relationship. The resistant CML cultivars showed moderate to high protein concentrations as did the susceptible MBR lines, Hi34 and Ki3. Using the same parameters (corn and ECB), Bergvinson's (1993) results agree with Moran and Hamilton's hypothesis.

A number of factors such as plant nutrition to herbivores, the carbon-nitrogen ratio, competition versus herbivory, and plant apparency have been implicated in defence. The two plant fundamentals, growth and defence, are at odds with one another. Coley et al. (1985) suggested defence is negatively correlated with plant growth rate and Pimentel (1976) suggested that pest resistant varieties often have lower yield than susceptible varieties in the absence of herbivores. When height is used as an indicator of growth, the tallest plants should allocate less to defence and be susceptible. The Hi34 cultivar was the tallest plant but MC3 and MB10 were the shortest cultivars. The CML lines and BS9 synthetics were also tall. Resistant cultivars are not shorter, susceptible cultivars are not taller. The BS9 synthetic series were also the most

prolific, having three or four ears per plant as opposed to two as in the rest of the cultivars. Pimentel's hypothesis is not supported.

4.7 CONCLUSION

Screening varieties of maize for first generation ECB resistance might continue by way of phenolic acid quantification. As shown in this study, diferulic acid levels may be used as a bioindicator of ECB resistance. A better and more accurate measure of resistance, however, would be to quantify wall bound ferulic acid. Ferulic acid is easily extracted and identified, existing in only two isomers, cis and trans. Diferulic acid, on the other hand, exists as four isomers which are harder to extract and identify.

4.7.1 Future Work

1. It may be relevant to characterize the enzyme that is involved in binding ferulic acid to arabinoxylose in the cell wall of maize. Perhaps activity of this enzyme is a determinant of esterifed ferulic acid concentration. If so, then this enzyme can be a measure of tissue resistance and screening can proceed via enzyme activity.

2. Elevating leaf tissue resistance by increasing the production of ferulic and diferulic acids could possibly be achieved by manipulating the expression of the peroxidase participating in feruloylation but these results suggest this strategy may not be successful.

5. REFERENCES

Abeles, F.B. 1973. *Ethylene in plant biology*. Academic Press, N.Y.

Ahluwalia, B. and Fry, S.C. 1986. Barley endosperm cell walls contain feruloylated and non-feruloylated arabinoxylan *Journal of Cereal Science 11*:287-295.

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

Alibes, X., Munoz, F. and Faci, R. 1984. Anhydrous ammonia treated cereal straw for animal feeding. Some results from the mediterranean area. *Animal Feed Science and Technology 10*:239-246.

Argillier, O., Barriere, Y., Lila, M., Jeanneteau, F., Gelinet, K. and Menanteau, V. 1996. Genotypic variation in phenolic components of cell walls in relation to the digestibility of maize stalks. *Agronomie 16*:123-130.

Arnason, J.T., Baum, B., Gale, J., Lambert, J.D.H., Bergvinson, D., Philogene, B.J.R., Serratos, J.A., Mihm, J. and Jewell, D.C. 1994. Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. *Euphytica 74*:227-236.

Arora, Y.K. and Wagle, D.S. 1985. Interrelationship between peroxidase, polyphenol oxidase activities and phenolic content of wheat for resistance to loose smut. *Biochemical and Physiological Pflanzen 180*:75-78.

Asada, K. 1992. Ascorbate peroxidase - a hydrogen peroxide-scavenging enzyme in plants. *Physiologia Plantarum 85*:235-241.

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

Azuma, J., Kato, A., Koshijima, T., and Okamura, K. 1990. Arabinosylxylotriose mixedly esterified with acetic and ferulic acids from sugar cane bagasse. *Agricultural Biology and Chemistry. 54*:2181-2182.

Baier, A.H. and Webster, B.D. 1992. Control of acanthoscelides-obtectus say coleoptera bruchida phaseolus-vulgaris l. seed stored on small farms 2. evaluation of damage. *Journal of Stored Products Research 28*:289-293.

Baligar, V.C. and Duncan, R.R. 1990. *Crops as enhancers of nutrient use*. Academic Press, N.Y.

Bazzaz, F.A., Chiariello, N.R., Coley, P.D. and Pitelka, L.F. 1987. Allocating resources to reproduction and defence. *Bioscience 37*:58-67.

Beck, S.D. and Schoonhoven, L.M. 1980. Insect behaviour and plant resistance. In: *Breeding Plants Resistant to Insects*. F.G. Maxwell and P.R. Jennings (Editors). John Wiley and Sons. N.Y.

Benson, P.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.

Bergvinson, D.J., Arnason, J.T., Hamilton, R.I. and Mihm, J.A. 1993. Putative role of dehydrodiferulic acids in maize resistance to the european corn borer. In: *Plant Peroxidases: Biochemistry and Physiology*. K.G. Welinder, S.K. Rasmussen, C. Penel and H. Greppin (Editors). University of Geneva. pp. 383-386.

Bergvinson, D.J., Hamilton, R.I. and Arnason, J.T. 1995. Leaf profile of maize resistance factors to european corn borer, *Ostrinia nubilalis*. *Journal of Chemical Ecology*. *21*:343-353.

Besle, J.M., Cornu, A. and Jouany, J.P. 1994. Roles of structural phenylpropanoids in forage cell wall digestion. *Journal of the Science of Food and Agriculture 64*:171-190.

Birecka, H. and Catalfamo, J.L. 1975. Cell wall and protoplast isoperoxidases of corn leaves in relation to cut injury and infection with *Helminthosporium maydis*. *Plant Physiology 55*:607-610.

Blum, A. 1968. Anatomical phenomena in seedlings of sorghum varieties resistant to the sorghum shoot fly (Atherigona varia soccata). *Crop Science 8*:388-390.

Bockholt, A.J. 1979. Biology and breeding of corn. *In: Biology and Breeding for Resistance to Arthropods and Pathogens in Agricultural Plants*. M.K. Harris (Editor). Texas A & M Univ., College Station, Texas.

Bolwell, G.P., Robbins, M.P. and Dixon, R. A. 1985. Metabolic changes in elicitor treated bean cells: Enzymatic responses associated with rapid changes in cell wall components. *European Journal of Biochemistry 148*:571-578.

Borneman, W.S., Hartley, R.D., Himmelsbach, D.S. and Ljungdahl, L.G. 1990. Assay for *trans-p*-coumaroyl esterase using a specific substrate from plant cell walls. *Analytical Biochemistry 190*:129-133.

Bradford, M.M. 1976. A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72*:248-254.

Brenneman, J.A. and Black, L.L. 1979. Respiratory and terminal oxidases in tomato leaves infected by *Phytophthora infestans*. *Physiological Plant Pathology 14*:281-290.

Brewbaker, J.L., Nagai, C. and Liu, E.H. 1985. Genetic polymorphisms of 13 maize peroxidases. *Journal of Heredity 76*:159-167.

Brown, D.M. 1969. *Heat units for corn in southern Ontario. Ontario Ministry of Agriculture and Food fact sheet*. Guelph, Ontario. Agdex 111.

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

Cadena-Gomez, G. and Nicholson, R.L. 1987. Papilla formation and associated peroxidase activity: a non-specific response to attempted fungal penetration of maize. *Physiological and Molecular Plant Pathology 31*:51-67.

Campa, A. 1991. Biological roles of plant peroxidases: known and potential function. *In: Peroxidase in Chemistry and Biology, Vol 3*. J. Everse, K.E. Everse and M.B. Grisham (Editors). CRC Press, Boca Raton, Florida.

CIMMYT, 1996. Canada and CIMMYT. Report, 1pp.

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

Coley, P.D., Byrant, J.P. and Chapin, F.S. 1985. Resource availability and plant antiherbivore defence. *Science 230*:895-899.

Constabel, C.P., Bergey, D.R., and Ryan, C.A. 1996. Polyphenol oxidase as a component of the inducible defence response in tomato against herbivores. *In: Phytochemical Diversity and Redundancy in Ecological Interactions*. J.T. Romeo, J.A. Saunders and P. Barbosa (Editors). Plenum Press. N.Y.

Cuthbert, F.P. and Davis, B.W. 1972. Factors contributing to cowpea curculio resistance in southern peas. *Journal of Economic Entomology 65*:778-781.

Eraso, F. and Hartley, R.D. 1990. Monomeric and dimeric phenolic constituents of plant cell walls - possible factors influencing wall biodegradability. *Journal of the Science of Food and Agriculture 51*:163-170.

Farkas, G.L. and Stahmann, M.A. 1966. On the nature of changes in peroxidase isoenzymes in bean leaves infected by southern bean mosaic virus. *Phytopathology 56*:669-677.

Feeny, P. 1976. Plant apparency and chemical defence. *Recent Advances in Phytochemistry 10*:1-40.

Fincher, G.B. and Stone, B.H. 1986. Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology* 8:207-295.

Frias, I., Siverio, J.M., Gonzalez, C., Trujillo, J.M. and Perez, J.A. 1991. Purification of a new peroxidase catalysing the formation of lignan-type compounds. *Biochemical Journal 273:*109-113.

Fry, S. 1979. Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth. *Planta 146:*343-351.

Fry, S. 1982. Isodityrosine, a new cross-linking amino acid from plant cell wall glycoprotein. *Biochemical Journal 204:*449-455.

Fry, S. 1983. Feruloylated pectins from the primary cell wall: their structure and possible functions. *Planta 157:*111-123.

Fry, S. 1984. Incorporation of (^{14}C) cinnamate into hydrolase-resistant components of the primary cell wall of spinach. *Phytochemistry 23:*59-64.

Fry, S. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review of Plant Physiology 37:*165-186.

Gardiner, M.G. and Cleland, R. 1974. Peroxidase changes during cessation of elongation in *Pisum sativum* stems. *Phytochemistry 13:*1095-1098.

Gaspar, T., Penel, C., Thorpe, T. and Greppin, N. 1982. *Peroxidases 1970-1980.* University of Geneva, Switzerland.

Gebhardt, K. 1982. Activation of indole-3-acetic acid oxidase from horseradish and Prunus by phenols and H_2O_2. *Plant Growth Regulation 1:*73-84.

Gerloff, G.C. and Gabelman, W.H. 1983. The search for an interpretation of genetic controls that enhance plant growth under deficiency levels of a macronutrient. *Plant Soil 73:*325-350.

Goldberg, R., Chabanet, A. and Catesson, A.M. 1993. Cell wall peroxidase and oxidase activities. In: *Plant Peroxidases: Biochemistry and Physiology*. K.G. Welinder, S.K. Rasmussen, C. Penel and H. Greppin (Editors). University of Geneva.

Goldberg, R., Liberman, M., Mathieu, C., Pierron, M. and Cattesson, A.M. 1987. Development of epidermal cell wall peroxidases along the mung bean hypocotyl: possible involvement in the cell wall stiffening process. *Journal of Experimental Botany 39:*1378-1390.

Grabber, J.H., Hatfield, R.D., Ralph, J., Zon, J. and Amrhein, N. 1995. Ferulate cross-linking in cell walls isolated from maize cell suspensions. *Phytochemistry 40:*1077-1082.

Guthrie, W.D., Dicke, F.F. and Neiswander, C.R. 1960. Leaf and sheath leaf feeding resistance to the European corn borer in eight inbred lines of dent corn. *Ohio Agricultural Experimental Station Bulletin,* 860.

Hamill, D.E. and Brewbaker, J.L. 1969. Isoenzyme polymorphism in flowering plants. IV. The peroxidase isoenzymes of maize (*Zea mays*). *Physiologia Plantarum 22:*945-958.

Harkin, J.M. and Obst, J.R. 1973. Lignification in trees: indication of exclusive peroxidase participation. *Science 180:*296.

Harris, P.J. and Hartley R.D. 1976. Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy. *Nature 259:*508-510.

Harris, P.J. and Hartley, R.D. 1980. Phenolic constituents of the cell walls of monocotyledons. *Biochemical Systematicss and Ecology 8:*153-160.

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

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

Hartley, R.D. and Ford, C.W. 1989. Phenolic constituents of plant cell walls and wall biodegradability. In: *Biogenesis and Biodegradation.* N.G. Lewis and M.G. Paice (Editors). ASC Sym. Ser. 399.

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

Hedin, P.A., Williams, W.P., Buckley, P.M. and Davis, F.M. 1993. Arrestant responses of southwestern corn borer larvae to free amino acids: structure-activity relationships. *Journal of Chemical Ecology 19:*301-311.

Herms, D.A. and Mattson, W.J. 1992. The dilemma of plants: to grow or to defend. *Quarterly Review of Biology* 283-335.

Hoson, T. 1990. Effect of auxin on autolysis of cell walls in azuki bean epicotyls. *Plant Cell Physiology 31:*281-287.

Hoson, T. and Nevins, D.J. 1989. Effect of anti-wall protein antibodies on auxin induced elongation, cell wall loosening, and beta-D-glucan degradation in maize coleoptile segments. *Physiologia Plantarum 77:*208-215.

Hrubcova, M., Cvikrova, M., Meravy, L., Eder, J. and Binarova, P. 1992. Phenolic accumulation and peroxidase activity in *in vitro* selected alfalfa callus cultures resistant to filtrate of *fusarium* spp.. *Biologia Plantarum 34:*203-211.

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 Quebec. I. Sytematics, 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 Quebec. III. Population dynamics and spatial distribution. *Phytoprotection 67:*93-115.

Hughes, H.D. and Metcalfe, D.S. 1972. *Crop Production.* MacMillan, N.Y.

Ievinsh, G. 1992. Characterization of the peroxidase system in winter rye seedlings: compartmentation and dependence on leaf development and hydrogen donors used. *Journal of Plant Physiology 140:*257-263.

Ikegawa, T., Mayama, S., Nakayashiki, H., and Kato, H. 1996. Accumulation of diferulic acid during the hypersensitive response of oat leaves to *Puccinia coronata* f.sp. *avena* and its role in the resistance of oat tissues to cell wall degrading enzymes. *Physiological Molecular Plant Pathology 48:*245-255.

Ishii, T. and Hiroi, T. 1990. Isolation and characterization of feruloylated arabinoxylan oligosaccharides from bamboo shoot cell walls. *Carbohydrate Research 196:* 175-183.

Ishii, T. and Nishijima, T. 1995. Inhibition of gibberellin induced elongation growth of rice by feruloyl oligosaccharides. *Plant Cell Physiology 36:*1447-1451.

Ishii, T. and Saka, H. 1992. Inhibition of auxin stimulated elongation of cell in rice lamina joints by a feruloylated arabinoxylan trisaccharide. *Plant Cell Physiol. 33:*321-324.

Janick, J. 1986. *Horticultural Science.* W.H. Freeman and Company. N.Y.

Jennings, P.H., Brannaman, B.L. and Zscheile, Jr., F.P. 1969. Peroxidase and polyphenol oxidase activity associated with *Helminthosporium* leaf spot of maize. *Phytopathology 59:*963-967.

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

Kamisaka, S., Takeda, S., Takahashi, K. and Shibata, K. 1990. Diferulic and ferulic acid in the cell walls of *Avena* coleoptiles - their relationship to mechanical properties of the cell wall. *Physiologia Plantarum 78:*1-7.

Kato, Y. and Nevins, D.J. 1985. Isolation and identification of o-(5-o-feruloyl-alpha -L-arabinofuranosyl)-(1-3)-o-beta-D-xylopyranoxyl-(1-4)-D-xylopyranose as a component of *Zea* shoot cell walls. *Carbohydrate Research 137:*139-150.

Keenan, W.N. 1927. The spread and degree of infestation of the european corn borer in Canada. *Entomological Society of Ontario Annual Report 57:*24-27.

Khavkin, E.E. and Zabrodina, M.V. 1995. Tissue-specific peroxidase patterns in maize. *Russian Journal of Plant Physiology 42:*249-257.

Kumar, H. and Mihm, J.A. 1996. Resistance in maize hybrids and inbreds to first generation southwestern corn borer, *Diatraea grandiosella* (Dyar) and sugarcane borer, *Diatraea saccharalis* Fabricus. *Crop Protection 15:*311-317.

Lagloire, P. 1936. *La menace de la pyrale du mäis. Le guide de collectionneur d'insectes.* Tremblay et Dion (Editors). Québec. pp.16.

Liu, E.H. 1975. Substrate specificities of plant peroxidase isozymes. In: *Isoenzymes 2: Physiological Function.* C.L Markert (Editor). Academic Press, N.Y.

MacAdam, J.W., Nelson, C.J. and Sharp, R.E. 1992. 1.Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. *Plant Physiology 99:*872-878.

Mader, M., Meyer, Y. and Bopp, M. 1976. Cell wall and peroxidase isoenzyme synthesis in isolated protoplasts of *Nicotiana tabacum* L. *Planta 129:*33-38.

Malolepsza, U. and Urbanek, H. 1994. Changes in peroxidase activity in bean suspension cultures after *B. cinerea* and elicitor treatment. *Journal of Phytopathology 141:*314-322.

Malolepsza, U., Urbanek, H. and Patykowski, J. 1989. Changes in peroxidase activity in lupin roots and callus tissues after infection by *Fusarium culmorum. Bulletin of the Polish Academy of Science 37:65-74.*

Markwalder, H.U. and Neukom, H. 1976. Diferulic acid as a possible crosslink in hemicelluloses from wheat germ. *Phytochemistry 15:*836-837.

Maxwell, F.G. and Jennings, P.R. 1980. *Breeding Plants Resistant to Insects.* Wiley and Sons. N.Y.

McKenzie, H.A. and Wallace, H.S. 1954. The Kjehldahl determination of nitrogen: a critical study of digestion condition-temperature, catalyst, and oxidizing agents. *Australian Journal of Chemistry 7*:55-70.

McNeil, M., Darvill, A.G., Fry, S.C. and Albersheim, P. 1984. Structure and function of the primary cell wall in plants. *Annual Review of Plant Physiology 53*:525.

Miyamoto, K., Ueda, J., Takeda, S., Ida, K., Hoson, T., Masuda, Y. and Kamisaka, S. 1994. Light induced increase in the contents of ferulic and diferulic acids in cell walls of *Avena* coleoptiles: its relationship to growth inhibition by light. *Physiologia Plantarum 92*:350-355.

Mohan, R. and Kolattukudy, P.E. 1990. Differential activation of expression of a suberization-associated peroxidase gene in near-isogenic resistant and susceptible tomato elicitors of verticillium-albo-atrum. *Plant Physiology 92*:276-280.

Moran, N. and Hamilton, W.D. 1980. Low nutritive quality as defence against herbivores. *Journal of Theoretical Biology 86*:247-254.

Mueller-Harvey, I. and Hartley, R.D. 1986. Linkage of p-coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw. *Carbohydrate Research 148*:71-85.

Myton K.E. and Fry, S.C. 1995. Dithiothreitol and cobalt effects on membrane-associated peroxidases oxidizing feruloyl-coA. *Phytochemistry.38*:573-577.

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

Ochoa, A.N. and Salgado, G.R. 1992. Phenylalanine ammonia-lyase activity and capsaicin-precursor c-p flurorphenylalanine-resistant and sensitive variant cells of pepper capsicum-annuum. *Physiol. Plant. 82(2)*:173-179.

Panda, N. and Khush, G.S. 1995. *Host Plant Resistance to Insects*.Wallingford, Oxon. UK.

Pang, A., Catesson, A.M., Francesch, C., Rolando, C. and Goldberg, R. 1989. On substrate specificity of peroxidases involved in the lignification process. *Journal of Plant Physiology 135*:325-329.

Patanakamjorn, S. and Pathak, M.D. 1967. Varietal resistance of the asiatic rice borer, *Chilo suppressalis* (Lepidoptera: Crambidae) and its association with various plant characteristics. *Annual Entomological Society of America 60*:287-292.

Pickering, J.W., Powell, B.L., Wender, S.H. and Smith, E.C. 1973. Ferulic acid: a substrate for two isoperoxidase *Nicotiana tabacum* tissue cultures *Phytochemistry 12*:2639-2643.

Pilet, P.E. and Lavanchy, P. 1969. Purification d'extraits peroxydasiques (racine de Lens) à activité < <auxin esoxydasiques> >. *Physiologie Végétale 7:*19-29.

Pimentel, D. 1976. World food crises: energy and pests. *Bulletin of the Entomological Society of America 22:*20-26.

Ralph, J., Quideau, S., Grabber, J.H. and Hatfield, R.D. 1994. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. *Journal of the Chemical Society of Perkin Transcripts 1:*3485-3498.

Ridge, I. and Osborne, D.J. 1970. Hydroxyproline and peroxidases in cell walls of *Pisum sativum. Journal of Experimental Botany 21:*843-856.

Riley, R.G. and Kolattukudy, P.E. 1975. Evidence for covalently attached p-coumaric acid and ferulic acid in cutins and suberins. *Plant Physiology 56:*650-654.

Ritchie, S.W. and Hanway, J.J. 1984. *How a corn plant develops.* J. Clayton Herman (Editor). Iowa State Univ., Ames, Iowa.

Ross, S.E. and Ostlie, K.R. 1990. Dispersal and survival of early instars of european corn borer (Lepidoptera: Pyralidae) in field corn. *Journal of Economic Entomology 83:*831-836.

Russell, W.A. and Guthrie, W.D. 1982. Registration for BS9(CB)C4 maize germplasm. *Crop Science 22:*694.

Russell, W.A. and Guthrie, W.D. 1984. Registration of B85 and B86 germplasm lines of maize. *Registration of Germplasms.*

Rybka, K., Sitarski, J. and Raczynska-Bojanowska. 1993. Ferulic acid in rye and wheat grain and grain dietary fiber. *Cereal Chemistry 70:*55-59.

Sakurai, N., Nishitani, K. and Masuda, Y. 1979. Auxin induced changes in the molecular weight of hemicellulosic polysaccharides of the *Avena* coleoptile cell wall. *Plant Cell Physiology 20:*1349-1357.

Sanchez, M., Pena, M.J., Revilla, G. and Zarra, I. 1996. Changes in dehydrodiferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pinus pinaster* hypocotyls. *Plant Physiology 111:*941-946.

Saulnier, L., Vigouroux, J. and Thibault, J.F. 1995. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res. 272:* 241-253.

Saunders, B.C., Holmes-Siedle, A.G. and Stark, B.P. 1964. *Peroxidase--The properties and uses of a versatile enzyme and some related catalysts.* Butterworth, London.

Scandalios, J.G. 1969. Genetic control of multiple molecular forms of enzymes in plants: a review. *Biochemical Genetics 3:*37-79.

Schlob, P., Walter, C. and Mader, M. 1987. Basic peroxidases in isolated vacuoles of *Nicotiana tobacum* L. *Planta 170:*225-229.

Scott-Craig, J.S., Kerby, K.B., Stein, B.D. and Somerville, S.C. 1995. Expression of an extracellular peroxidase that is induced in barley (Hordeum vulgare) by the powdery mildew pathogen (*Erysiphe graminis* f. sp. hordei). *Physiological Molecular Plant Pathology 47:*407-418.

Scriber, J.M. and Slansky Jr., F. 1981. The nutritional ecology of immature arthropods. *Annual Review of Entomology 26:*183-211.

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

Siegel, S.M. 1953. On the biosynthesis of lignins. *Physiologia Plantarum 6:*134-139.

Smith, H.E. 1920. Broom corn, the probable host in which *Pyrausta nubilalis* Hubn. reached America. *Journal of Economic Entomology 13:*425-430.

Smith, M.M. and Hartley, R.D. 1983. Occurrence and nature of ferulic acid substitution of cell wall polysaccharides in graminaceous plants. *Carbohydrate Research 118:*655-680.

Smith, M.M. and O'Brien, T.P. 1979. Distribution of autofluorescence and esterase and peroxidase activities in the epidermis of wheat roots. *Australian Journal of Plant Physiology 6:*201-219.

Spanu, P. and Bonfante-Fasolo, P. 1988. Cell wall bound peroxidase activity in roots of mycorrhizal *Allium porrum. New Phytologist 109*: 119-124.

Sung, H.S., Yu, R.H. and Chang, C.T. 1993. Purification and some properties of peroxidase isozymes from pineapple stem. *Biochemical and Molecular Biology International 29:*185-195.

Swain, T. 1977. Secondary compounds as protective agents. *Annual Review of Plant Physiology 6:*201-219.

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

Tan, K.S., Hoson, T., Masuda, Y. and Kamisaka, S. 1991. Correlation between cell wall extensibility and the content of diferulic and ferulic acids in cell walls of *Oryza sativa* coleoptiles grown under water and in air. *Physiologia Plantarum 83:*397-403.

Tan, K.S., Hoson, T., Masuda, Y. and Kamisaka, S. 1992a. Effect of ferulic and p-coumaric acids on *Oryza* coleoptile growth and the mechanical properties of cell walls. *Journal of Plant Physiology 140:*460-465.

Tan, K.S., Hoson, T., Masuda, Y. and Kamisaka, S. 1992b. Involvement of cell wall bound diferulic acid in light induced decrease in growth rate and cell wall extensibility of *Oryza* coleoptiles. *Plant Cell Physiology 33:*103-108.

Tanton, M.T. 1962. The effect of leaf 'toughness' on the feeding of larvae of the mustard beetle *Pheadon cochleariae* Fab. *Entomologia Experimentalis et Applicata 5:*74-78.

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

Van Loon, L.C. 1986. *Molecular and physiological aspects of plant peroxidases*. H. Greppin C. Penel and T. Gaspar (Editors). University of Geneva.

Van Soest, P.J. 1964. Symposium on nutrition and forage and pastures. New chemical procedures for evaluating forages. *Journal of Animal Science 23:*838.

Waldron, K., Parr, A.J. and Ng, A. 1996. Diode array HPLC analysis of dimeric cell wall phenolics. In: *Polyphenols Communications 96, Bordeaux, France.*

Werner, P.A. 1975. Prediction of fate from rosette size in teasel (*Dipsacus fullonum* L.). *Oceologia 20:*197-201.

Whitmore, F.W. 1974. Phenolic acids in wheat coleoptile cell walls. *Plant Physiology 53:*728-731.

Whitmore, F.W. 1976. Binding of ferulic acid to cell walls by peroxidases of *Pinus elliotti. Phytochemistry 15:*375-378.

Whitmore, F.W. 1978a. Lignin-protein complex catalysed by peroxidase. *Plant Science Letters 13:*241-245.

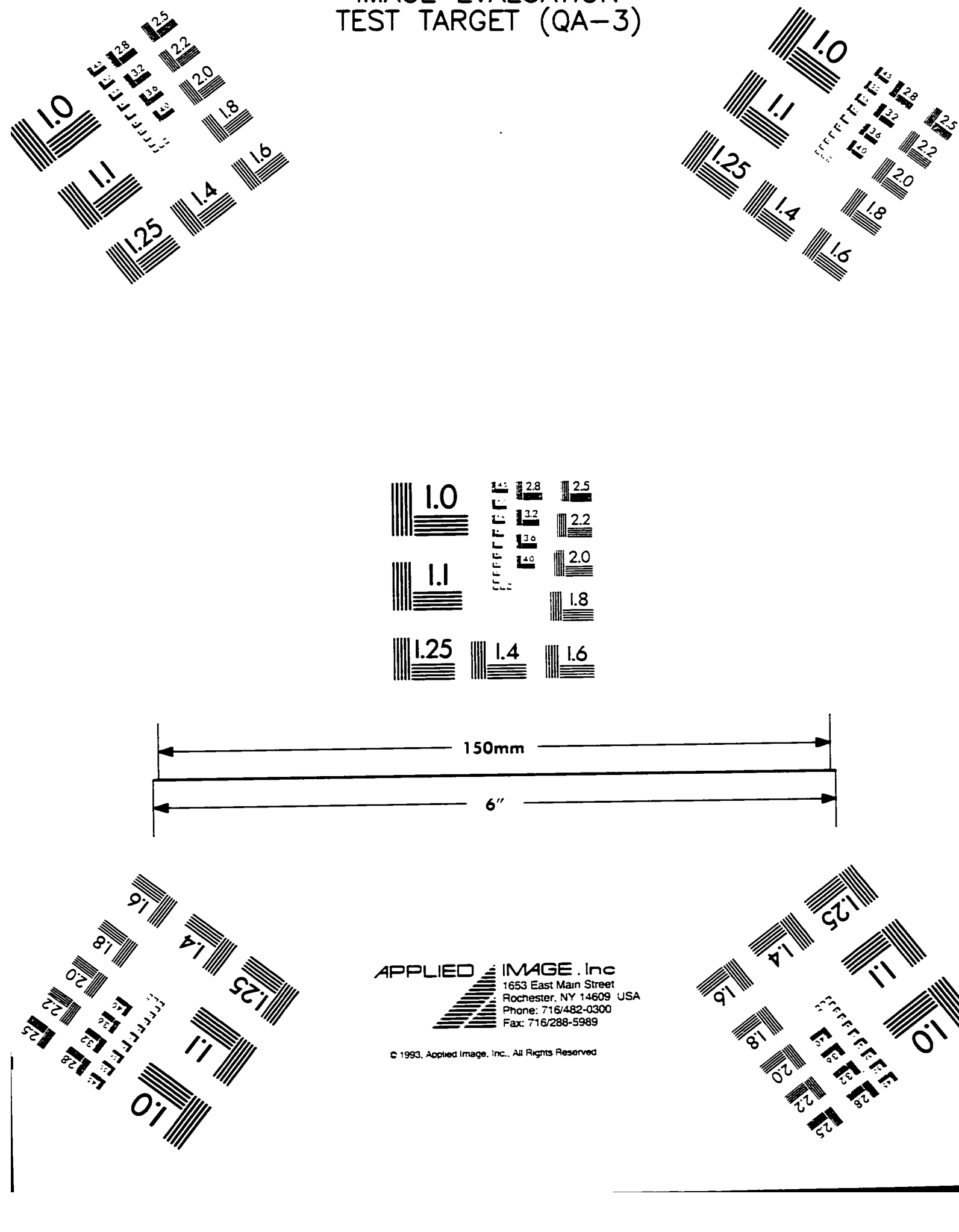
Whitmore, F.W. 1978b. Lignin-carbohydrate complex formed in isolated cell walls of callus. *Phytochemistry 17:*421-424.

Woodhead, S. 1983. Surface chemistry of *Sorghum bicolor* and its importance in feeding by *Locusta migratoria. Physiological Entomology 8:*345-352.

Wressel, H.B. 1958. The history of the european corn borer *Pyrausta nubilalis* (Hbn.) (Lepidoptera: Pyralidae) in Canada. *International Congress of Entomological Proceedings 10:*389-394.

Zimmerlin, A. and Bolwell, G.P. 1993. Characterization of a cell wall peroxidase from french bean which systhesizes diferulate *in vitro*. In: *Plant Peroxidases: Biochemistry and Physiology*. K.G. Welinder, S.K. Rasmussen, C. Penel and H. Grepin (Editors). University of Geneva.

Zimmerlin, A., Wojtaszek, P. and Bolwell, G.P. 1994. Synthesis of dehydrogenation polymers of ferulic acid with high specificity by a purified cell wall peroxidase from french bean (*Phaseolus vulgaris* L.). *Biochemistry 299*:747-753.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