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RESISTANCE OF WORLD GERMPLASM RESOURCES OF MAIZE, *ZEA MAYS*, TO THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS*

by

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A thesis submitted to the
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UNIVERSITY OF OTTAWA

To my parents,

ABSTRACT

The resistance of world germplasm resources of maize, *Zea mays*, to the European corn borer, *Ostrinia nubilalis*, was investigated using two major groups of germplasm: a latitudinal series of thirty-seven inbred lines and an almost complete set of the indigenous landraces of Mexico. In addition, three highly resistant control lines, three CIMMYT maize pools, three Canadian synthetic lines, a multi-borer resistant line, and ten Argentine landraces were studied.

A rapid extraction procedure for the analysis of hydroxamate concentrations in one-week-old etiolated maize seedlings was developed and used to screen all the above germplasm. All were found to contain three major hydroxamates: DIMBOA, HMBOA, and MBOA, a breakdown product of DIMBOA. Significant differences in concentrations of DIMBOA characterized the different germplasm groups.

Resistance of the germplasm to first-brood European corn borers was determined in laboratory and field evaluations. A laboratory method for evaluating leaf-feeding resistance was developed and results were found to correlate with both seedling DIMBOA concentrations and field evaluations of leaf-feeding resistance.

Two seasons of field studies were used to evaluate germplasm resistance to first-brood borer infestation and stalk rot, *Gibberella zeae*, in relation to planting density. Many correlations were found among the phytochemical and resistance variables. Suites of characters associated with high leaf-feeding resistance of the more modern lines, such as the latitudinal series of inbred lines, were mirror images of that for the indigenous races of Mexico which were associated with high borer tunneling resistance.

Analysis of the phytochemical and resistance data revealed global trends in European corn borer resistance. Resistance and DIMBOA concentrations were inversely correlated to both latitude and altitude..

The relationship of resistance of maize germplasm to the taxonomy of the indigenous landraces of Mexico was determined. Significant differences in resistance among the major taxonomic groupings were reflected in the existing taxonomy of maize developed by Wellhausen et al (1952).

The potential use of all the germplasm types, particularly the Mexican landraces, in Canadian maize breeding programs aimed at resistance to the European corn borer is discussed.

RÉSUMÉ

La résistance des ressources mondiales du plasma germinatif du maïs, *Zea mays*, à la pyrale du maïs, *Ostrinia nubilalis*, a été étudiée en utilisant deux groupes importants de plasma: une série latitudinale contenant trente-sept lignées endogames et un ensemble presque complet de populations naturelles indigènes du Mexique. En plus trois lignées contrôles hautement résistantes, trois lignées provénants du CIMMYT, trois lignées canadiennes de cultivars synthétiques, une lignée résistante aux insectes térébrants et dix lignées indigènes d'Argentine ont été étudiées.

Une méthode d'extraction rapide pour l'analyse de la concentration d'hydroxamate contenue dans les plantules de maïs étiolé agées d'une semaine, a été développée et utilisée pour sélectionner les plasmas germinatifs. Tous contiennent trois hydroxamates principaux: DIMBOA, HMBOA, et MBOA, un sous-produit du DIMBOA. Des différences significatives dans la concentration du DIMBOA caractérisent les différents groupes.

La résistance du plasma germinatif a l'attaque de la première génération de la pyrale du maïs a été étudiée en laboratoire et sur le terrain. On a développé une méthode en laboratoire pour évaluer la résistance à la foliophage et on a obtenu des résultats pour établir une corrélation avec la concentration du DIMBOA dans les plantules et les évaluations faites sur le terrain.

Deux saisons d'étude sur le terrain ont été nécessaires pour déterminer la résistance du maïs à une infestation par la première génération de la pyrale suivie de la pourriture fusarienne, *G. zeae*, en relation avec la densité de la plantation. On a trouvé des corrélations entre les variables phytochimiques et la résistance. Plusiers caractères associés à une résistance élevée à la foliophagie des lignées plus récentes, telles la série latitudinale des lignées endogames, sont des images inversées de ce qu

on trouve dans les races indigènes du Mexique associées à une résistance élevée au minage de la tige.

L'analyse des donnés phytochimiques et de la résistance révèle certaines tendances globales dans la résistance à la pyrale. La résistance et la concentration du DIMBOA sont inversement proportionnelles à la latitude et l'altitude.

On a déterminé la relation entre la résistance du plasma germinatif et la taxonomie des populations naturelles indigènes du Mexique. Il y avait des différences significatives dans la résistance parmi les groupes taxonomiques principaux tels que définis par Wellhausen et al (1952).

On discute finalement de l'utilisation potentielle de tous les types de plasma germinatif, particulièrement des populations naturelles mexicaines, dans les programmes de sélection du maïs canadien visant à développer la résistante à la pyrale.

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CHAPTER I

INTRODUCTION

1.1 THE IMPORTANCE OF GERMPLASM STUDIES

Until recently the major food crops of the world were produced from many highly genetically diverse populations of crop plants. Since the beginnings of agriculture variability has been developed and maintained in crops by the processes of hybridization, mutation, selection, and adaptation aided by many groups of traditional farmers working in different environments (Harlan, 1979). These processes resulted in thousands of known varieties or populations in major crops such as wheat, potatoes, rice, and maize. Such variability is an important safety factor since if one variety of a given crop were to become extremely susceptible to a given pest then new, more resistant varieties could be bred from the diverse germplasm available (Chang, 1984; Gale and Lawrence, 1984).

In recent years the demand for varieties of crops with dependable high yields has increased. Unfortunately this has resulted in the decrease of the genetic base of crops as breeding selections were made to produce more genetically uniform hybrids with superior agronomic traits (Feldman and Sears, 1981). Once such a variety is developed there is a tendency to culture it over a large geographical area in genetically uniform monocultures (Harlan, 1979). This erosion of natural diversity has a great potential for large scale destruction by an adapting pest. For example, the genetic base of most North American crops is very narrow; but the genetic base of its pests is virtually unrestricted and is therefore free to come into evolutionary balance in such an agricultural system (Harlan and Starks, 1980).

The genetic vulnerability of our modern crops was made evident in 1970 when approximately 18% of the U.S. corn crop was destroyed by an epidemic of Southern

corn leaf blight, *Helminthosporium maydis* (Hooker, 1982; Tatum, 1971). The vulnerability of corn was a result of the widespread use of Texas male sterile (T) cytoplasm in hybrid seed production. Incorporation of male sterility eliminates pollen shed and thus the need for highly labor-intensive and costly detasseling procedures. By 1969-1970 most of the U.S. and Canadian maize crop had a uniform cytoplasmic background. Unfortunately the Texas T gene also conferred susceptibility to the toxin produced by *H. maydis* and in 1970 the fungus proceeded to spread and destroy field after field of the genetically uniform hybrids. As a result U.S. farmers lost over 1 billion dollars for that year (Tatum, 1971).

Obviously man has little control over pest evolution or over the environment but we can control the evolution of our crops. This control is achieved by preserving the germplasm and thus the genetic diversity of the crops used in breeding and agriculture. More diverse hybrids can be developed that would decrease the risk presented by the use of a few elite varieties. This genetic diversity of crops does not assure disease and insect resistance, but it does give some protection against the unexpected (Harlan and Starks, 1980; Williams, 1984).

The realization of the need to maintain genetic resources led to the exploration, collection, preservation, and distribution of wild and ancestral crop germplasm. There has been a substantial increase in the number of germplasm "banks" throughout the world. Today, there are a total of thirteen International Agricultural Research Centers (IRACs) throughout the world. All have been founded to provide agricultural training and research assistance, especially to developing countries. One of the major IRAC organizations concerned with germplasm resources maintenance is the International Board for Plant Genetics Resources (IBPGR) located in Rome, Italy. Founded in 1974, its primary goal is to create an international network of genetic resource centers that would in turn increase the collection, conservation, evaluation, documentation, and use of plant germplasm (Williams, 1984; CIMMYT, 1984). Germplasm banks are living collections of genetic material consisting largely of the primitive landraces that went

into the evolution of today's modern cultivars. These ancient and ancestral types are preserved in case of emergencies or when agriculture and consumer needs require new breeding material (Galinat, 1982).

Even though we have these large stores of genetically diverse material the range of resistance and agronomic characters within them has been poorly examined and documented (Goodman, 1984). This fact makes it extremely difficult for the breeder to find the exact source of resistance or other characters required. Many studies have been carried out but they were on fragments of the large collections and cannot be compared with one another (Harlan and Starks, 1980). Therefore, there is a great need to screen the world collections systematically under controlled conditions and thus to accumulate data that would generalize the genetic sources of resistance and other agronomic traits. To find resistance material, screening must be carried out first on the major food crops and their most economically important pests.

1.2 LITERATURE REVIEW

1.2.1 MAIZE: ONE OF THREE CROPS THAT FEED THE WORLD

1.2.1.1 PLANT CHARACTERISTICS

Maize, *Zea mays* (L.), is a member of the grass family, Gramineae (Poaceae), and shares several features in common with other members of this family: conspicuous nodes on the stem; a single leaf at each node; parallel veined leaves; and, leaves composed of a sheath surrounding the stem and a blade connected to the sheath by a blade joint. Most of the maize plant body is composed of long narrow leaves spaced alternately in two rows on strong erect stalks. Unlike most of its relatives, maize is an annual monoecious plant with the staminate flowers in tassels at the top of the stem and the pistillate flowers in ears in the axils of leaves. Tillers originate from subterranean in-

ternodes and develop into structures identical to the main stem although somewhat smaller. The presence of tillers varies with different maize varieties.

Many divergent types of maize are grown over a wide range of climatic conditions. Various soil conditions are tolerated, but the best is a deep, well drained, fertile loam soil with a slightly acidic pH of approximately 5 (Bockholt, 1979). Maize is a warm weather crop requiring mean summer day temperatures higher than 19°C and night temperatures higher than 13°C (Hartman et al, 1981). It is classified as a short day plant; longer days tend to increase the duration of the vegetative stage, and the size and number of leaves of the plant (Bockholt, 1979).

1.2.1.2 CROP VALUE

Maize is the world's third largest cereal crop after rice and wheat. It can be grown throughout temperate, subtropical, and tropical zones wherever rainfall or irrigation is adequate. The major producer is the U.S. followed by China, Brazil, the U.S.S.R., Romania, Yugoslavia, Mexico, South Africa, Argentina, and India (Janick, 1981). More than 100 million hectares of land are planted each year to produce more than 250 million metric tons (Shurtleff, 1984). Maize is the major food crop for more than 100 million people in the world (Chiang, 1978). It is used after being parched, boiled, baked, ground, treated with lye, and/or mixed with other ingredients (Thruston, 1977; Hartmann et al, 1981).

In North America, mainly sweet corn is grown for human consumption; its production exceeded 11 tons/ha for the US and 8.5 tons/ha for Canada in 1983 alone (Hudon and LeRoux, 1986a). Corn is now the number one feed grain in the world (CIMMYT Maize Facts and Trends Report Two, 1984). Ninety percent of the corn grown in North America is grain corn (Chiang, 1978). Maize is also an important source of industrial products. It is used as raw material to produce sugar, starch, oil, and as a carbohydrate source for alcoholic beverages. More than 300 commercial items

are derived from the grain alone (Hartman, 1981). Diseases and insects are the major limiting factors of increasing maize yields (Jugenhiemer, 1976).

1.2.1.3 THE ORIGIN AND TAXONOMY OF MAIZE

There are a number of theories on the origin of maize. One theory suggests that maize was derived from its closest living relative teosinte, *Zea mexicana*. This theory is best supported by Iltis (1983) in his "Catastrophic Sexual Transmutation Theory" (CSTT) which proposes that the female maize ear was derived from the terminal central spike of the male teosinte tassel. Development was aided by selection by humans (Galinat, 1984). Mangelsdorf et al (1964) proposed a more widely accepted theory, at complete odds to that of Iltis and called it the tripartite theory. They proposed that: one, cultivated corn originated from a primitive pod corn; two, teosinte is a derivative of a hybrid between corn and *Tripsacum*, another wild grass; and three, most modern corn cultivars are the product of a mixture with teosinte or *Tripsacum* or both. This theory was mostly based on the archeological findings of MacNeish (Mangelsdorf et al, 1964) who found remains of corn plants in the Tehuacan Valley of Mexico and dated them back to approximately 5000 B.C. Since the earliest remains of teosinte found date only to 1500 B.C. (Wilkes, 1977), Mangelsdorf suggested that corn predated teosinte and therefore could not have been derived from it. This theory has recently lost some credibility since it was shown that teosinte is not of hybrid origin (Wilkes, 1982) although the other parts of the theory still stand. An alternative theory is that corn, teosinte, and *Tripsacum* descended along independent lines directly from a common ancestor (Randolf, 1976). However it is now almost universally accepted that modern maize is the product of the introgression of primitive maize with teosinte and possibly *Tripsacum* although the sequence of events remains unclear.

Today, the most controversial element of the origin of maize is the question of whether the ancestor was wild maize or ancestral wild teosinte, perhaps the perennial teosinte, *Zea diploperennis*. Much more archaeological evidence is needed to resolve

this question, although many aspects that surround the origin of maize are commonly agreed upon. The domestication of maize appears to have been in Southern Mexico about 10 to 14 thousand years ago in a highland site above 1500 m with limestone derived soils (Wilkes, 1977; Sprague, 1976; Beadle, 1980; Mangelsdorf, 1974).

With the help of many sponsored programs maize has been the most systematically collected crop of all (Harlan and Starks, 1980). It is believed that the greatest variation of maize occurs in Mexico. One of the major reasons for this is the large range in the geography of Mexico which resulted in several kinds of isolating factors (latitude, longitude, altitude) conducive to rapid differentiation of a given species (Wellhausen et al, 1952). Because of this diversity in the races it was necessary to create a classification that would represent an inventory of the morphological and physiological characteristics of each race for future reference, especially for that of maize breeders. This has lead to a number of taxonomic studies centering around the indigenous landraces of Mexico (McK. Bird, 1977, 1982a, 1982b; Camussi, 1979; Cervantes et al, 1978; Goodman, 1978; Goodman and Mck. Bird, 1977).

The most comprehensive and still most widely accepted classification of the indigenous landraces of Mexico was carried out by Wellhausen, Roberts, and Hernandez (1952). Others have repeated this work and expanded upon it slightly, but only minor changes have been made. Wellhausen et al grouped the many varieties found into twenty-five distinct races and then further placed these races into five major groups (TABLE 1). This classification was based upon external morphological characteristics, internal cytological features, and physiological characteristics such as earliness, yield, and resistance to disease. Their study is the only one that takes into account resistance to diseases. Wellhausen et al's first major group is classified as Ancient Indigenous Races. These are believed to have arisen in Mexico from the primitive pod corn. This group consists of four races: Palomero Toluqueno, Arrocillo Amarillo, Chapalote, and Nal-Tel. The second major group, the Pre-Columbian Exotic Races, is believed to have been introduced into Mexico from Central or South America in prehis-

TABLE 1

CLASSIFICATION OF MEXICAN LANDRACES (WELLHAUSEN ET AL, 1952)

A. ANCIENT INDIGENOUS RACES
1. Palomero Toluqueno
2. Arrocillo Amarillo
3 . Chapalote
4. Nal-Tel

B. PRE-COLUMBIAN EXOTIC RACES
5. Cacahuacintle
6. Harinoso de Ocho
7. Oloton
8. Maiz Dulce

C. PREHISTORIC MESTIZOS
9. Conico
10. Reventador
11. Tabloncillo
12. Tehua
13. Tepecintle
14. Comiteco
15. Jala
16. Zapalote Chico
17. Zapalote Grande
18. Pepitilla
19. Olotillo
20. Tuxpeno
21. Vandeno

D. MODERN INCIPIENT RACES
22. Chalqùeno
23. Celaya
24. Conico Norteno
25. Bolita

E. "POORLY DEFINED RACES"
26. Conejo
27. Complejo Serrano de Jalisco
28. Maiz Blando
29. Onaveno
30. Dulcillo del Noroeste
31. Bofo
32. Tabilla de Ocho
33. Zamorano
34. Gordo
35. Apachita
36. Azul

toric times and consists of four recognized races: Cacahuacintle, Harinoso de Ocho, Oloton, and Maiz Dulce. Although there is no historical evidence for its origin, the third group ,the Prehistoric Mestizos, is believed to have arisen through the hybridization of the Ancient Indigenous Races with the Pre-Columbian Exotic Races and also through the hybridization of both of the above with teosinte. There are thirteen recognized races in the Pre-Columbian Exotic group: Conico, Reventador, Tabloncillo, Tehua, Tepicintle, Comiteco, Jala, Zapalote Chico, Zapalote Grande, Pepitilla, Olotillo, Tuxpeno, and Vandeno. The fourth group is the Modern Incipient Races presently consisting of four recognized races: Chalqueno, Celaya, Conico Norteno, and Bolita. Wellhausen et al (1952) also lists some races which have been grouped together as the "poorly defined races" since more data are required to classify them. They consist of eleven races: Conjo, Comlejo Serrano de Jalisco, Zamorano Amarillo, Maiz Blando de Sonora, Onaveno, and Dulcillo del Noroeste, Bofo, Tabilla de Ocho, Gordo, Apachita, and Azul.

1.2.1.4 MAIZE PLANT DEVELOPMENT

Beard (1943) has broken deveopment of the maize plant into twelve stages. The first five are based on leaf characteristics: the seedling stage characterized by the primary leaf blade; the pre-whorl stage characterized by one to five leaf blades; the early-whorl stage when six to seven leaves are arranged in a distinct whorl; the mid-whorl stage when eight to nine leaves compose the whorl and the rudimentary tassel is completely enclosed by the whorl; and, the late-whorl stage when the tassel just becomes visible along with one or two small ear shoots. The next three stages are based on tassel characteristics: the early green tassel stage when the tip of the tassel shows above the whorl and two to four ear shoots are visible; the mid-green tassel stage when the tassel consists of a clump of adhering branches; and, the late green tassel stage when the tassel has unfurled, pollen sacs have swelled, and three to five ear shoots are visible. The final four stages are based on silk characteristics: the early-silk stage when the top ear shoots are showing fresh silk and the anthers of the tassel are dehis-

ing; the mid-silk stage when the top ear silk is fully extended, the true ears are distinct from the rudimentary ones, and there is maximum pollen shedding; the late-silk stage when the silk is now dried at the tips, the anthers are gone; and the final stage, the roasting ear when the cobs are ripe for harvest.

1.2.2 THE EUROPEAN CORN BORER: A MAJOR INSECT PEST OF MAIZE

There are a number of insect pests that limit maize production. The major ones are armyworms, earworms, rootworms, insect vectors of various pathogens, and the borers of which the major one in North America is the European corn borer, *Ostrinia nubilalis* (Hubner) of the Pyralidae family.

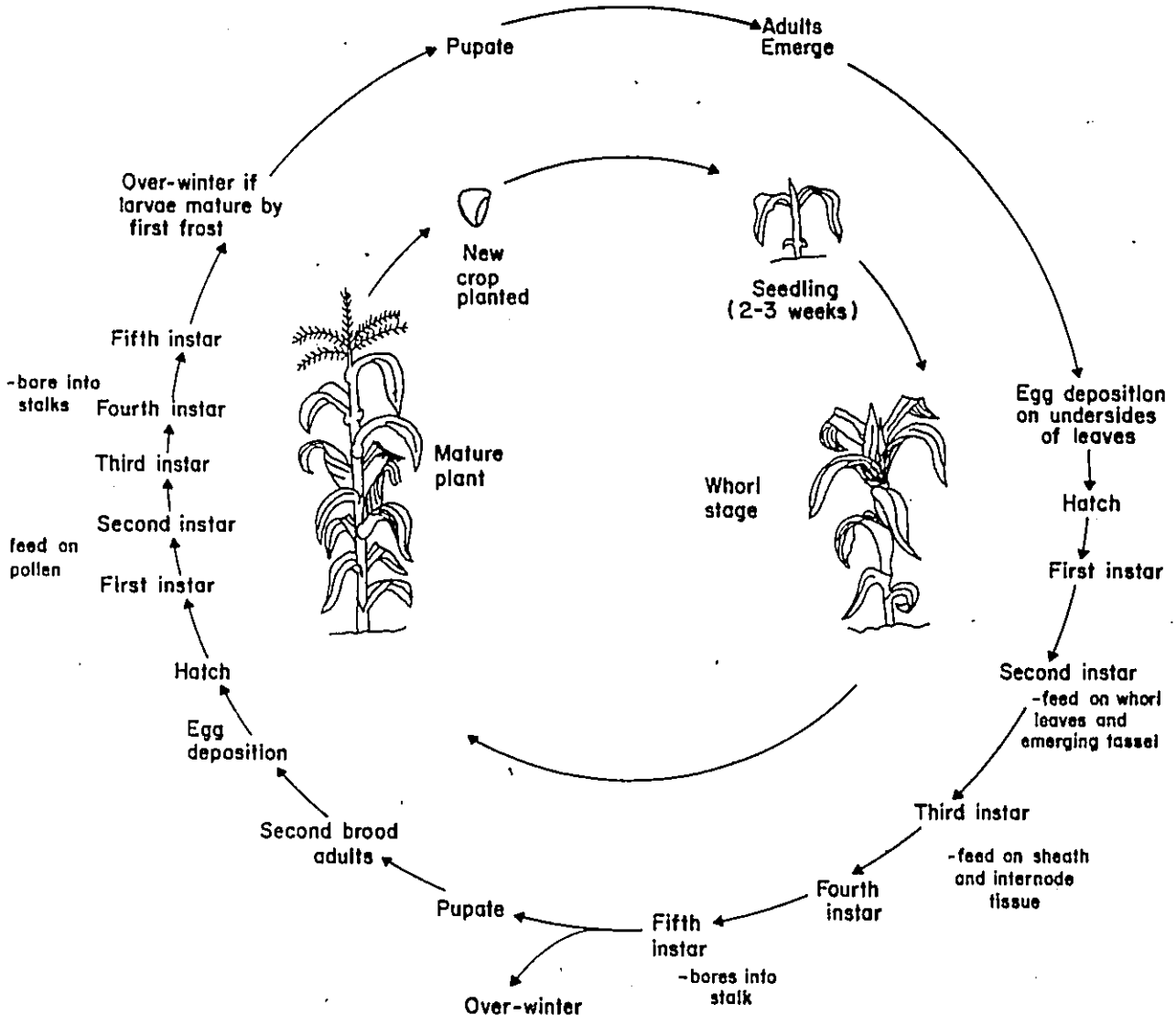
1.2.2.1 THE LIFE CYCLE OF THE EUROPEAN CORN BORER

The European corn borer is a polyphagous insect that feeds on green peppers, tomatoes, potatoes, beans, beets, oats, soybeans, many wild plants and its major host, maize. The life cycle of this insect is closely related to the developmental stages of maize (FIGURE 1). In most corn producing areas the insect is bivoltine, i.e. it has two generations or broods per season. In the period of egg deposition by the first brood adults, which overwintered as mature larvae, most of the corn is in the whorl stage. White eggs are laid in masses of 15-35 on the undersides of lower leaves. Each adult female can lay more than 500 eggs during her life span (Davidson, 1979). The eggs hatch in approximately seven days. The highest degree of damage is done by these first brood larvae. The first and second instar larvae feed primarily on the spirally rolled leaves in the whorl destroying the surface and breaking midribs. As the corn grows out of the whorl stage, the third and fourth instars feed primarily on sheath and collar tissue. The fifth instar tunnels all parts of the stalk and ear resulting in cavities in the stalk, broken stalks and tassels, poor ear development and dropped ears. When the corn has tasseled and is in the process of completing the pollen shedding stage egg deposition by second brood adults occurs. The second brood first and second instars

FIGURE 1

THE LIFE CYCLE OF THE EUROPEAN CORN BORER
(*Ostrinia nubilalis*)

This diagram represents the relationship between the maize plant and a bivoltine strain of the European corn borer.


Pupate
Adults Emerge
Over-winter if larvae mature by first frost
New crop planted
Seedling (2-3 weeks)
Fifth instar
-bore into stalks
Fourth instar
Third instar
Second instar
feed on pollen
First instar
Mature plant
Whorl stage
Egg deposition on undersides of leaves
Hatch
First instar
Second instar
-feed on whorl leaves and emerging tassel
Third instar
-feed on sheath and internode tissue
Fourth instar
Fifth instar
-bores into stalk
Pupate
Over-winter
Second brood adults
Egg deposition
Hatch
SECOND BROOD
FIRST BROOD

feed primarily on pollen accumulated at the axils of the leaves and on tissues of the sheath, collar, ear shoots, husk, and silk. The first to fourth instars can develop satisfactorily on a pollen diet while feeding extensively on sheath and collar tissue (Guthrie, 1981; Guthrie and Dicke, 1972). The fifth and final instar will over-winter in the stalks and the cycle will be repeated the following growing season.

European corn borer eggs are flat, white and less than 1 mm in diameter. They take five to seven days to develop to what is called the black head stage which is just before hatching. A fully grown adult larva is approximately 25 mm long and 3 mm wide. The head is dark brown to black in colour while the upper body surface ranges from light to dark brown. Each body segment has on its dorsal surface a row of small dark brown spots with several narrow dark brown lines extending lengthwise. The under surface has no distinctive markings and is usually a uniform flesh colour (Davidson, 1979). The pupa is cigar-shaped and light to dark brown in colour. The female pupa is approximately 15 mm long while the male pupa is slightly smaller at 14 mm. Fully grown female adults are yellowish-brown, 2 cm long, with a wing span of approximately 3 cm. The outer third of the females forewings are marked by two darker serrate lines running across the wings. The adult males are smaller than the females, reddish-brown, and have darker wings with correspondingly darker markings on the forewings (Davidson 1979).

1.2.2.2 HISTORY AND STATUS OF *O. NUBILALIS*

The European corn borer is not native to North America. It was accidently introduced in shipments of broom corn, *Sorghum vulgare* var *technica* (Koern.) in eastern U.S. and Canada from Austria, Hungary, and Italy (Brindley and Dicke, 1963). The presence of the insect was first reported near Boston in 1917 (Vinal, 1917). The first infestations were around broom factories and it is believed that the refuse from these factories was the main vehicle of distribution of the insect (Robinson, 1978). The insect has now spread to practically all of the major corn-producing areas of the

U.S. and Canada with a range extending from the Maritimes to the Prairies and in the southern U.S. as far as Georgia, Mississippi, and northern Florida. The number of generations per year or voltinism began to vary with geographical location until three different biotypes were recognized in North America: a northern univoltine strain in most of Canada and Minnesota; a more central bivoltine strain in the more southern corn areas of Canada and in Iowa, Nebraska, and Ohio; and a southern multivoltine strain in Alabama, Georgia, and Missouri (Hudon and LeRoux, 1986a).

Successful establishment of neonate larvae on the corn plant is accomplished mainly because of the large number of eggs laid by the female moths. Early studies revealed that fewer than 25% of the larvae survive the first 48 hours and an additional 10-15% fail to reach maturity (Painter and Fight, 1925; Caesar, 1926; Huber, 1936). This high mortality rate is due to a number of factors such as: mechanical crushing of the plant parts during cultivation; drowning in water accumulation in the whorl; dislodging; desiccation; predation; exposure to UV light; and chemical factors. Despite this the insect still manages to reach pest population levels because of a number of agro-bio-ecological factors such as its variable life cycle, a relatively low level of parasite activity, the low level of over-wintering larval mortality, the high host-plant density , and the relatively flat topography of Central North America. The latter three factors are the most important of all. The annual mean over-wintering larval mortality is only 1.6% of total fifth instar larvae (Hudon and LeRoux, 1986a). These authors have also shown that borer larvae can withstand supercooling to approximately -21°C by virtue of the fact that by January 9% of their body weight is glycerol. The most important factor contributing to the rise of borer populations is the increasing hectarage of grain and forage corn grown in large monocultures. Based upon Feeny's (1976) theories of plant apparency and the co-evolution of plants and insects, these monocultures have taken "unapparent" plants and made them apparent. Since most crops have not evolved defences appropriate for survival as an apparent plant, other control measures must be used to increase their defenses. Therefore, even though total larval mortality can be as high as 98%, with the large numbers of borers produced a

survival rate of only 2% or less is sufficient to maintain economic levels of infestations in Quebec alone (Hudon and LeRoux, 1986b).

Yield reductions are a result of a reduction in the amount of grain produced (physiological) and the amount of grain left in the field after mechanical harvest (Klenke et al, 1986). Heavy infestations have been known to decrease yields up to 25% (Davidson,1979), although this varies with time of infestation, stage of plant development, and geographical location (Everett et al, 1958; Kwolek and Brindley, 1959; Patch, 1942). First generation borers are more injurious to grain corn since tunneling results in lodging at harvest (Bailey and Pedigo, 1986). For sweet corn the second brood borers cause more problems because of boring into the ears. The borers are difficult to remove from harvested ears. Total annual losses in the U.S. alone now exceed 200 million dollars (Burburtis et al, 1984). In Canada, the economic threshold accepted for sweet corn is 5% and 10% infested ears for fresh market and processing (canning) respectively (Hudon and LeRoux, 1986a).

1.2.2.3 INSECT PATHOGEN ASSOCIATIONS: STALK ROT AND CORN SMUT

Losses and damage from insect feeding are usually compounded by many common insect-pathogen associations. The European corn borer is associated with corn smut and corn stalk rot. Common corn smut, *Ustilago maydis*, occurs worldwide and is most severe in young plants. Fungal galls are formed on any above-ground parts of the plant and can result in yield losses exceeding 10% in susceptible maize lines; although resistant lines have been developed that have less than 2% loss (Shurtleff, 1984). Control of this pathogen can be accomplished, along with the use of resistant hybrids and lines, by avoiding mechanical injury that would leave the plants more susceptible to infection, and by maintaining a well-balanced soil fertility to avoid stressing the plants unnecessarily (Shurtleff, 1984).

Stalk rots are devasting diseases of maize. The major rot associated with the European corn borer is *Gibberella zeae*. Yield losses of up to 20% in susceptible North American lines and 33% in other countries have been reported (Shurtleff, 1984). *G. zeae* usually attacks plants approaching maturity. Diseased stalk tissue has an internal reddish-brown colour followed by a shredding of pith tissue. Losses are due directly to poor ear development or indirectly through harvest loss because of stalk breakage. Development of the fungus is encouraged by a stressful late environment such as borer tunneling and crowding. Control can be achieved by the use of resistant hybrids and lines, balanced soil fertility, and lower plant densities (Shurtleff, 1984).

1.2.3 CONTROLLING THE EUROPEAN CORN BORER

A number of control measures have been and are still being used in an attempt to control the European corn borer. Cultural practices include deep plowing of stalks containing over-wintering larvae and crop rotation. The release of parasites, the use of light traps and trap crops to attract early egg laying moths have proved moderately successful (Hudon and LeRoux, 1986a,b; Barber and Witkowski,1984; Franklin and Holdaway, 1966; Legg and Chiang, 1984a,b). Planting time is an important factor since moths lay more eggs on taller early planted than shorter later planted lines (Patch, 1946; Andrews and Carlson, 1976). Unfortunately this method does not always coincide with the time of the crop's highest market value (Davidson, 1979). Insecticides are used but are considered to be uneconomical because of the relatively low net profit of corn, especially that of grain corn. In addition, insecticides are not always useful since larvae inhabit relatively protected areas of the plant; therefore, to be effective the insecticide must be applied directly, be systemic, be applied frequently, or have a long residual activity.

Maize, because it is a cereal crop, is lower priced than many other crops and hence there is a need to maximize economic control measures. The most effort put into control of the European corn borer has been in the selection and development of resis-

tant lines of maize. This approach has led to the discovery of natural resistance factors in the maize plant.

1.2.3.1 HOST-PLANT RESISTANCE

Resistant plants as described by Hedin et al, (1974, 1977) are "... plants that are inherently less severely damaged or less infested by a phytophagous pest under comparable environments in the field...". In other words, resistance is a property that enables a plant to avoid, tolerate, or recover from the injurious effects of insect feeding. There are three basic mechanisms of resistance: preference or nonpreference, the latter being characters of the plant making it less suitable for food, oviposition, or shelter; antibiosis, when the presence (or rarely absence) of various chemical compounds affects the development and survival of the insect; and tolerance, the ability of the plant to survive insect attack by repairing pest damage or by not being as severely affected by the damage as a more susceptible plant would be (Horber, 1980; Adkisson and Dyck, 1980; Harris, 1979; Hedin, 1977, 1983).

Resistant plants are often used in combination with some of the control measures stated above such as good cultural practice and insecticides (Waiss et al, 1977). This is because these resistant lines are compatible with both chemical and other biological control measures and are therefore very useful in Integrated Pest Management programs. The end result is a more effective, convenient, economical, and environmentally acceptable method of insect control. This approach has been carried out successfully for the corn earworm, *Heliothis zea*, on sweet corn (Wiseman et al, 1973).

Resistance to the European corn borer occurs at three stages of maize development. The first is the seedling stage, at which a large number of current cultivars are resistant. The second occurs at the whorl stage. Resistance at this stage is called first-brood borer resistance or leaf-feeding resistance because it is the first brood borer

population that is attacking the leaves at this time. The third stage is in the pollen-shedding stage and is called second-brood borer resistance. A variety resistant at one stage may or may not be resistant at another. Selections in breeding programs have emphasized resistance in the whorl stage when the larvae feed mainly on leaf tissue and cause great losses in grain corn (Russel, 1975; Pesho et al, 1965). This is because the largest amount of corn grown is grain corn and damage and crop loss is most severe following first-brood infestations.

Resistance is seldom due to the effect of only one factor but instead results from the combination of a number of factors of which a large proportion are chemical. Since host-plant resistance is heritable, knowledge of its chemical basis would play an important role in plant breeding.

1.2.3.2 PHYTOCHEMICAL BASIS OF RESISTANCE IN MAIZE

Researchers have long been interested in finding causative agents in the maize plant that are responsible for resistance to the European corn borer. Not only would this knowledge give a better understanding of the host-pest relationship, but it may be useful as a selection tool in a breeding program. Recently a great deal of attention has focussed on the role of secondary metabolites in insect resistance. These compounds are termed "secondary" because they play no known role in the essential metabolic processes of the plant. They may act as defensive substances which tend to render the plant repellant, toxic, or otherwise chemically unsuitable for utilization by herbivores and micro-organisms (Beck, 1976, 1980).

Two groups of secondary metabolites that have been implicated in insect resistance in maize are phenolic acids and hydroxamic acids. Phenolic acids are widespread throughout the plant kingdom. Many studies have been carried out on their role in desease and insect resistance. Reese et al (1982) and Waiss et al (1979) have both found that the flavone glycoside, maysin, from corn silks is inhibitory to *Heliothis zea*

larvae. Also, p-coumaric, caffeic, and ferulic acids have been shown to affect the viability of, or act as feeding deterrents, to a wide variety of insects (Swain, 1977, 1979), including the maize weevil (Serratos et al, 1987).

1.2.3.2.1 HYDROXAMIC ACIDS

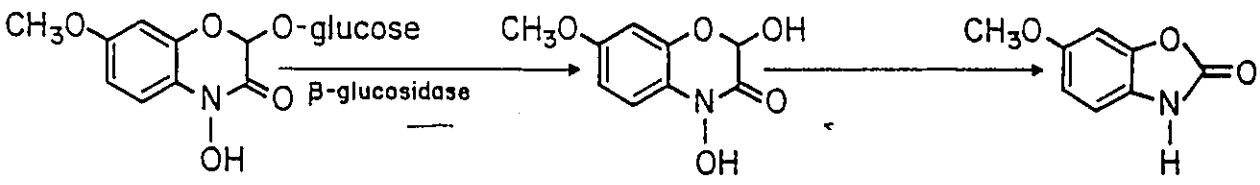
Many studies of maize resistance to the European corn borer have centred around the role of hydroxamic acids. These compounds occur in maize, wheat, and other members of the Graminae family. Hydroxamates contain one or more oxidized peptide (amide) bonds, -CON(OH)- (Emery, 1971). This bond is the oustanding chemical feature of these molecules and can therefore be expected to play an important role in their biological action (Neilands, 1967). Natural hydroxamates are classified according to the number of amide groups they contain. In maize and related Graminae, approximately fifteen hydroxamates have been identified, (Woodward et al, 1979a).

Hydroxamates occur as glucosides in intact tissue, but undergo enzymatic hydrolysis to the aglucone when plant tissues are crushed, i.e. by insect feeding. The major aglucone found in maize is 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one or DIMBOA. In hot aqueous solutions this compound decomposes to its benzoxazinone, 6-methoxy-benzoxazoline or MBOA, with the liberation of formic acid , FIGURE 2 (Honakanen and Virtanen, 1960). Various intermediates occur during this reaction and have been studied by many researchers (Niemeyer et al, 1982); Brendenberg et al, 1962; Bravo and Niemeyer, 1986; Woodward et al, 1978a, b; Copaja et al, 1982; Grambow et al, 1986). MBOA does not occur in vivo (Hofman and Hofmanova, 1971). The presence of MBOA was first detected in extracts of root tissue from the medicinal plant *Coix lachryma-joba* (Koyama et al, 1955). DIMBOA was first reported in maize and wheat in 1959 by Wahlroos and Virtanen. The lactam of DIMBOA, 2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one or HMBOA (FIGURE 3) is also found in maize (Woodward et al, 1979b). The biosynthetic relationship between this compound and DIMBOA is not known.

FIGURE 2

THE MAJOR HYDROXAMIC ACID FOUND IN MAIZE LEAF TISSUE

The major hydroxamic acid found in maize is 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one or DIMBOA. This secondary metabolite occurs as a glucoside in intact tissue, but undergoes enzymatic hydrolysis to a physiologically active aglucone when plant tissues are damaged by insect feeding. DIMBOA decomposes to 6-methoxy-benzoxazoline (MBOA) in aqueous solutions.



DIMBOA GLUCOSIDE

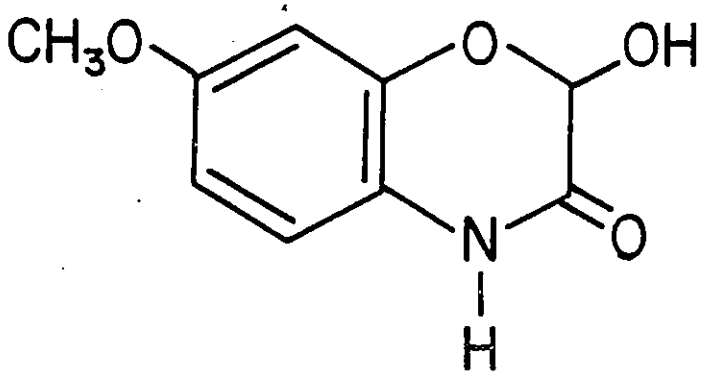
DIMBOA

MBOA

FIGURE 3

THE LACTAM OF DIMBOA

The lactam of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) is 2-hydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one or HMBOA.



HMBOA

DIMBOA is synthesized and accumulated throughout the growth of the plant. The biosynthetic pathway is not yet totally elucidated but it has been shown that radio-labelled (^{14}C) quinic acid, methionine, ribose, glycine, glycerate, and anthranilic acid are incorporated into DIMBOA (Reimann and Byerrum, 1964; Tipton et al, 1973). This suggests that synthesis is via the shikimic acid pathway.

Concentrations of DIMBOA are generally highest in the root and then in decreasing order of concentration in the stalk, whorl, and leaf (Klun and Robinson, 1968; Argandona et al, 1981; Guthrie er al, 1986a, b). If high concentrations are found in the whorl stage then they are also found to be high in the silking stage (Long et al,1975). In the leaves the highest concentrations are around the vascular bundles (Argandona and Corcuera, 1985). The total amount of DIMBOA and its glucoside may be more than 1% of the dry weight of the plant (Woodward et al, 1978).

DIMBOA has been directly implicated as a biochemical factor in the resistance of maize to the European corn borer, especially to the first brood larvae (Beck and Smissman, 1960). When DIMBOA was added to artificial diets and fed to larvae it was found to increase larval mortality, inhibit larval development, and decrease larval and pupal weights (Klun et al, 1967; Robinson et al, 1982; Argandona et al, 1983; Klun and Brindley, 1966). This result indicates that DIMBOA has toxic properties, but many more studies are needed to elucidate the mode of action. There is now much more evidence to indicate that DIMBOA controls borers by acting as an antifeedant. This term is applied to substances that prevent or interrupt feeding activity when they come into contact with an insect's buccal parts or any other organ provided with sensory cells (Schoonhoven, 1982). In maize lines with high concentrations of DIMBOA, borer larvae, when given the chance will move off the plant (Robinson et al, 1978,1982). This behavior has been observed for concentrations as low as 1 mM (Argandona et al, 1983). Such movement off the host-plant would increase larval mortality because migrating larvae would be more susceptible to environmental hazards such as predators and desiccation (Robinson et al, 1978). The result is higher larval feeding

rates on low DIMBOA lines (Manuwoto and Scriber, 1985a, b; Klun and Brindley, 1966). MBOA and HMBOA have as yet not been shown to have any biological activity towards borer larvae, but it is hypothesized that a combination of these compounds with DIMBOA may be more active than either compound alone (Klun et al, 1967). Therefore, resistance depends upon a higher concentration of glucosides present in the plant and their ability to split quickly into effective aglucones.

DIMBOA has also been shown to be a biochemical factor associated with resistance of maize to various pathogens. DIMBOA extracts inhibit growth of the bacterial corn stalk rot caused by various *Erwinia* species (Corcuera et al, 1978; Woodward et al, 1978a; Lacy, 1979). It also inhibits the growth of, and confers resistance to, two corn stalk rots, *Diplodia maydis* and *Gibberella zeae* (Whitney and Mortimore, 1959; BeMiller and Pappelis, 1965), along with Northern corn leaf blight, *Helminthosporium turcicum* (Long et al, 1975, Couture et al, 1971).

Besides conferring resistance to the European corn borer, DIMBOA has been found to have a diverse effect on the survival and reproductive rates of aphids in both wild and cultivated plants (Argandona et al, 1980; Zuniga et al, 1983; Corcuera et al, 1982, 1985). DIMBOA has also been shown to be mutagenic to *Salmonella typhimurium* (Hashimoto et al, 1979), allelopathic (Wolf et al, 1985), a chemical cue affecting reproductive cycles in meadow voles, *Microtus montanus* (Sanders et al, 1981; Berger et al, 1981), important in plant iron metabolism and mineral nutrition due to its chelating properties (Tipton and Buell, 1970; Dabed et al, 1982), and important in increasing the tolerance of maize to large doses of pesticides by catalyzing the hydrolysis of diazinon and the dechlorination of others such as atrazine (Hamilton, 1963, 1964; Malan et al, 1984; Ioannou et al, 1980). More recently, DIMBOA has been found to inhibit cyclic and noncyclic photophosphorylation in spinach chloroplasts and mitochondria (Niemeyer et al, 1982; Queirolo et al, 1981). It has also been found to react strongly with the sulfhydryl (thiol) groups of compounds such as cysteine (Ar-

gandona et al, 1982). This reaction may be the basis for its inhibitory action on some enzymes and its toxicity (Niemeyer et al, 1982).

The separation of hydroxamates in plant extracts has been accomplished by a number of methods. Paper, thin-layer, and column chromatography have been used with detection by a ferric chloride spray reagent and/or UV spectroscopy (Woodward et al, 1979a). These methods are time consuming and do not differentiate readily among the compounds of interest and other compounds which have similar physical properties. Other methods used have been isotopic dilution (Klun and Brindley, 1966), infrared spectrophotometry (Scism et al, 1974), and fluorometry (Bowman et al, 1967). More recently gas-chromatography and mass spectroscopy have been used to separate, detect and identify either free (Tang et al, 1975) or trimethylsilyl derivatives (Woodward et al, 1979a, b). The advantages of such a method are 100 ng sensitivity, reduced time of analysis, and increased amount of information obtained about structural properties of the compounds. High performance liquid chromatography (HPLC) is also used and has proven very useful in quantitation (Gutierrez et al, 1982; Clark and Brown, 1980).

Many of the above methods presume that MBOA is stoichiometrically related to DIMBOA, i.e. there is a 100% conversion of DIMBOA to MBOA. It was believed that if one could detect the amount of MBOA this would be equal to that of DIMBOA (Robinson et al, 1978). It has since been found that the amount of MBOA formed varies with temperature, pH, and solutes and that under a number of conditions less than 75% yield of MBOA was obtained from DIMBOA (Woodward et al, 1978a, 1979a). Therefore, the assumption of total yield of MBOA from DIMBOA may lead to erroneous estimates of DIMBOA concentrations in plant extracts. Today, most researchers quantitate DIMBOA concentrations directly.

Recently, factors other than DIMBOA have been found to confer resistance to the European corn borer. This is not a surprise since no one factor can be expected to account for all of the resistance in a given plant species. It was found that in maize,

silica and lignin, along with DIMBOA, account for a major percentage of the factors conferring resistance to first-brood borers (Rojanaridpiched et al, 1984).

1.2.3.3 BREEDING MAIZE FOR RESISTANCE TO THE EUROPEAN CORN BORER

Once it was found that certain lines of maize were more resistant than others and part of the cause of this resistance was elucidated, inheritance of resistance studies were carried out. The goal of these studies was to estimate the number of genes controlling resistance and to determine the best breeding system to use to increase resistance levels.

Reciprocal translocation studies have shown that at least twelve of the possible twenty chromosome arms in maize contribute a minimum of twelve genes that are involved in resistance to the Eurpopean corn borer (Onukogu et al, 1978; Scott et al, 1966). A partial dominant, primarily additive type of gene action is involved since crosses between a susceptible line and a resistant line result in a line intermediate in resistance (Russell et al, 1975; Scott et al, 1966; Guthrie et al, 1985b). Both monogenic and polygenic control, depending upon the maize line in question, have been found for the accumulation of hydroxamates (Dunn et al, 1981). Further translocation studies revealed that resistance is conditioned by separate genetic mechanisms, one for first-brood resistance and one for second-brood resistance (Klenke et al, 1986). This is because only two or three of the twelve chromosome arms are in common for genes resistant to both broods. Because of this it is very rare to find a line that is resistant to both borer broods.

Once the genetic control over resistance became more clear it was possible to select the type of breeding program that was best to increase resistance. Because of the large number of genes involved, a backcross breeding procedure could not be used to transfer resistance to susceptible genotypes. The most success has been obtained

using recurrent selection techniques. This breeding method concentrates genes for certain superior traits in the gene pool and enhances the possibilities for genetic recombination by maintaining a broad genetic base (Niles, 1979; Guhtrie, 1979). Recurrent selection has been found to increase DIMBOA levels in leaf tissue and to increase resistance to both first and second brood larvae (Barry et al, 1983; Tseng et al, 1984; Klenke et al, 1986). Resistance to both broods in the same line can also be obtained by combining a number of inbred lines to produce a synthetic line (Guthrie, 1979).

In breeding programs it is essential to study more than a single pest in order to explore the potential for multiple-pest resistance and to avoid the possibility of increased susceptibility. In maize, for example, selection for resistance to Northern corn leaf blight can be based on DIMBOA concentrations, but one cannot select for resistance to the borer and in turn get resistance to the leaf blight as well (Long et al, 1974; Guthrie et al, 1985).

The basic challenge in insect resistance breeding programs is that of identifying sources of resistance, and more importantly, having a broad genetic base of sources from which to choose.

1.2.3.4 GERMPLASM RESOURCES

When searching for a source of genetic resistance a breeder can look at several sources of genetic variability such as contemporary types, re-introduced types, polypoids, induced mutants, obsolete cultivated types, and exotic species (Niles, 1979). The latter consists of germplasm that is not fully acclimatized and would have to undergo physiological adjustments before use with adapted germplasm. These are often a very useful source of genes and gene combinations (Robinson, 1978). The search for resistance should be carried out in a logical sequence by first examining adapted cultivars, followed by plant introductions and exotic germplasm, and finally the near relatives of the cultivar (Ortman and Peters, 1980). In order to carry this out all of the

sources of resistance must be preserved and available to the breeder through germplasm banks.

There are a number of maize germplasm banks such as the International Rice Research Institute (IRRI) in the Philippines, the East-West Food Institute in Hawaii, the Food and Agriculture Organization (FAO) of the United Nations, and one the largest, the International Maize and Wheat Improvement Center (CIMMYT) located in El Batan, Mexico (Chiang, 1978). CIMMYT was founded in 1966 with the support of many private and national agencies, the major one being the Consultative Group on International Agricultural Research (CGIAR). Research at CIMMYT centers on improving yields of maize, wheat, triticale, and barley in order to improve food production in developing countries. The main emphasis is on high yield with wide adaptations, disease and insect resistance, higher quality protein, shorter growing period, and shorter plants that could be grown at a higher density without lodging (CIMMYT Report on Maize Production, 1980-1981). Very large germplasm collections from many countries are kept at CIMMYT and classified according to agroclimatic characteristics, grain types, and length of growing season. CIMMYT acts as the focal point of several international networks for germplasm exchange and testing involving more than 125 coountries (This Is CIMMYT pamphlet, 1984).

Despite these large germplasm collections only a small fraction of the total genetic diversity in maize has been used in breeding programs. There is a need to survey these resources systematically so that the breeder will know what is available (Williams, 1984).

Many researchers believe that the introgression of exotic maize germplasm should be intensified (Chiang, 1978; Robinson,1978). This would include all of the indigenous landraces of maize. Before this introgression is possible this germplasm must first be examined for resistance and organized in such a way as to allow a systematic evaluation and selection of resistant germplasm. In addition a logical clas-

sification of resistance as related to the geographic origin of maize germplasm needs to be created (Williams, 1984).

1.2.3 CONCLUSIONS

This review of the literature has revealed the following main points:

(1) Maize is a very important crop with substantial economic value. Diseases and insects are the major limiting factors in increasing yields of the crop throughout the world.

(2) One of the major insect pests of maize is the European corn borer. Its life cycle is closely tied to that of maize such that in some geographic areas the crop is hit by two or more broods of the insect per year. The major factor involved in elevating the borer to pest levels has been the increasing use of large monocultures of maize in North America.

(3) Insect-pathogen associations occur between the European corn borer, corn smut, and corn stalk rot. This compounds the loss of crop yield due to borer infestations.

(4) The most widely studied and currently used method of borer control involves the use of resistant lines of maize.

(5) Resistance to the European corn borer has a phytochemical basis. Secondary metabolites known as hydroxamic acids, occur as glucosides in intact maize tissue but undergo hydrolysis to a physiologically active aglucone when the tissue is damaged. Studies have revealed that these compounds act as antifeedants by reducing the amount of borer feeding. These compounds have also been shown to inhibit the growth of some pathogenic fungi.

(6) Breeding maize for resistance has best been accomplished through recurrent selection techniques. But, this requires a broad genetic base of resistant germplasm sources.

(7) Although large genetic resources have been accumulated in germplasm banks, there is a lack of studies that systematically and logically document the sources of resistance available. Two major areas that require more elucidation are the relationships between resistance and geographical origin and taxonomy of maize germplasm.

1.3 THE RESEARCH PROJECT AND ITS OBJECTIVES

The review of the literature has revealed that large sources of maize germplasm types exist, many of which have never been examined for resistance to the European corn borer. These germplasm types need to be systematically examined in a logical manner for sources of resistance. The main of goal of this research project was to further examine the resistance of maize to first-brood European corn borer and in particular to determine if any global trends exist between the resistance of world germplasm resources and the geographic origin and taxonomy of maize germplasm. These two areas, geographic origin and taxonomy, represent two of the more logical places to begin when approaching large amounts of germplasm.

The research was divided into four major parts:

(1) Knowing that resistance to the first-brood European corn borer is due to the presence of hydroxamic acids, all of the germplasm was screened for the presence of and concentrations of these compounds after developing a suitable rapid extraction method.

(2) A suitable laboratory method to evaluate leaf-feeding resistance of whorl tissue was developed followed by the screening of the germplasm using this

method. Resistance of the germplasm to three major agricultural stresses was determined by exposing the germplasm to European corn borer infestation, stalk rot infection, and high density planting in the field followed by ratings and measurements of resistance. The degree of correlation between these resistance results and the phytochemical results was determined, along with the potential use of each germplasm group in a Canadian breeding program.

(3) All the phytochemical and resistance data was combined and analyzed to determine the relationship between the resistance of maize germplasm and the latitudinal and altitudinal origin of maize.

(4) Again all of the data was combined and various taxonomic analyses were carried out to determine the relationship between resistance of maize germplasm and the taxonomy of the indigenous landraces of Mexico.

CHAPTER II

MATERIALS AND METHODS

2.1 GERMPLASM

Two major germplasm groups were used in this study: a latitudinal series of thirty-seven inbred lines and an almost complete set of the indigenous races of maize of Mexico. In addition to these, three highly resistant control lines, three resistant Canadian synthetic lines, three CIMMYT maize pools, and ten Argentine landraces were studied.

2.1.1 LATITUDINAL SERIES OF INBRED LINES

These consist of four broad based gene pools developed by CIMMYT for use in different latitudinal regions (FIGURE 4):

1. Gene pool for the Southern Temperate Region (STR).

2. Gene pool for the Intermediate Temperate Region (ITR).

3. Gene pool for the Northern Temperate Region (NTR-1).

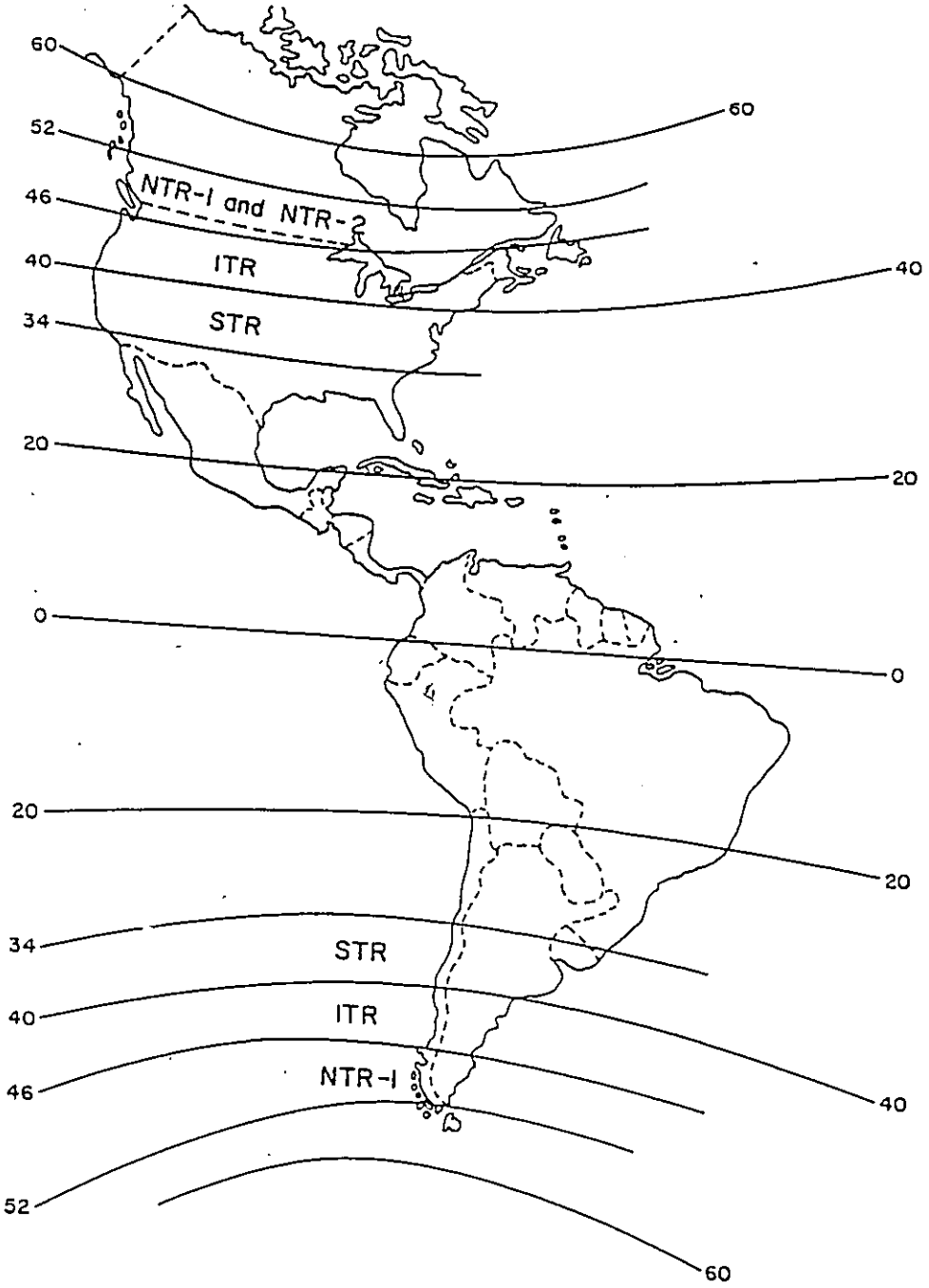
4. CIMMYT German Maize Gene Pool (NTR-2).

These pools are constantly being improved through selection by CIMMYT. Advances are being made in yield, height reduction, early maturity, and resistance to diseases and insects. They are being used as new sources of variation for temperate areas and as a mechanism for the transfer of genes from tropical to temperate areas and vice-versa.

FIGURE 4

MAP OF LATITUDINAL GROUPS OF INBRED LINES

STR = Southern Temperate Region
ITR = Intermediate Temperate Region
NTR-1 = Northern Temperate Region
NTR-2 = CIMMYT German Maize Gene Pool


60
52
46
40
34
20
0
20
34
40
46
52
60
60
40
20
0
20
40
60
NTR-1 and NTR-2
ITR
STR
STR
ITR
NTR-1

The STR gene pool is based on germplasm from the US corn belt and tropical lowlands and highlands. It has been developed for use in the winter maize growing areas of the tropics and subtropics and for low latitude temperate areas 34-40° north and south of the equator. In this study six lines of this gene pool were used. and are referred to by the numbers, STR: 3790, 3794, 3802, 3805, 3815, and 3823 (TABLE 2).

The gene pool for the ITR group consists primarily of European maize material. It is designed for use in latitudes 40-46° N-S of the equator and also the winter maize growing areas of the tropics and subtropics. A total of seven lines were used from this gene pool, ITR: 3853, 3857, 3862, 3865, 3872, 3877, and 3878 (TABLE 2).

The NTR-1 gene pool consists of materials from the US corn belt and is designed to serve maize growing areas 46-52° N-S of the equator. Six lines were used from this gene pool, NTR-1: 3945, 3946, 3947, 3962, 3971, and 3983 (TABLE 2).

Finally, the NTR-2 or CIMMYT German Maize gene pool consists of germplasm from Mexico, Peru, Bolivia, Pakistan, China, Hungary, and the USA. It has been designed to introduce tropical germplasm into temperate areas 46-52° north of the equator. Eighteen lines were used in this study. They are subdivided into five major groups: Holland 4018, 4019, 4020, 4021, and 4022; Switzerland 4034, 4035, and 4036; Germany 4042, 4046, and 4050; Poland 4064, 4065, and 4066; and Canada 4071, 4072, 4077, and 4081 (TABLE 2). The five countries refer to the places where selection for adaption to northern latitudes took place. All of the countries were co-operaters with CIMMYT where the lines over-wintered.

2.1.2 INDIGENOUS LANDRACES OF MEXICO

Sixty-three populations derived from thirty-seven distinct indigenous races of maize of Mexico were studied. The different populations are segregated on the basis of their geographical collection sites; for example, Oaxaca-179 was collected in the Mexican state of Oaxaca (FIGURE 5). A given race may have populations from one or

TABLE 2

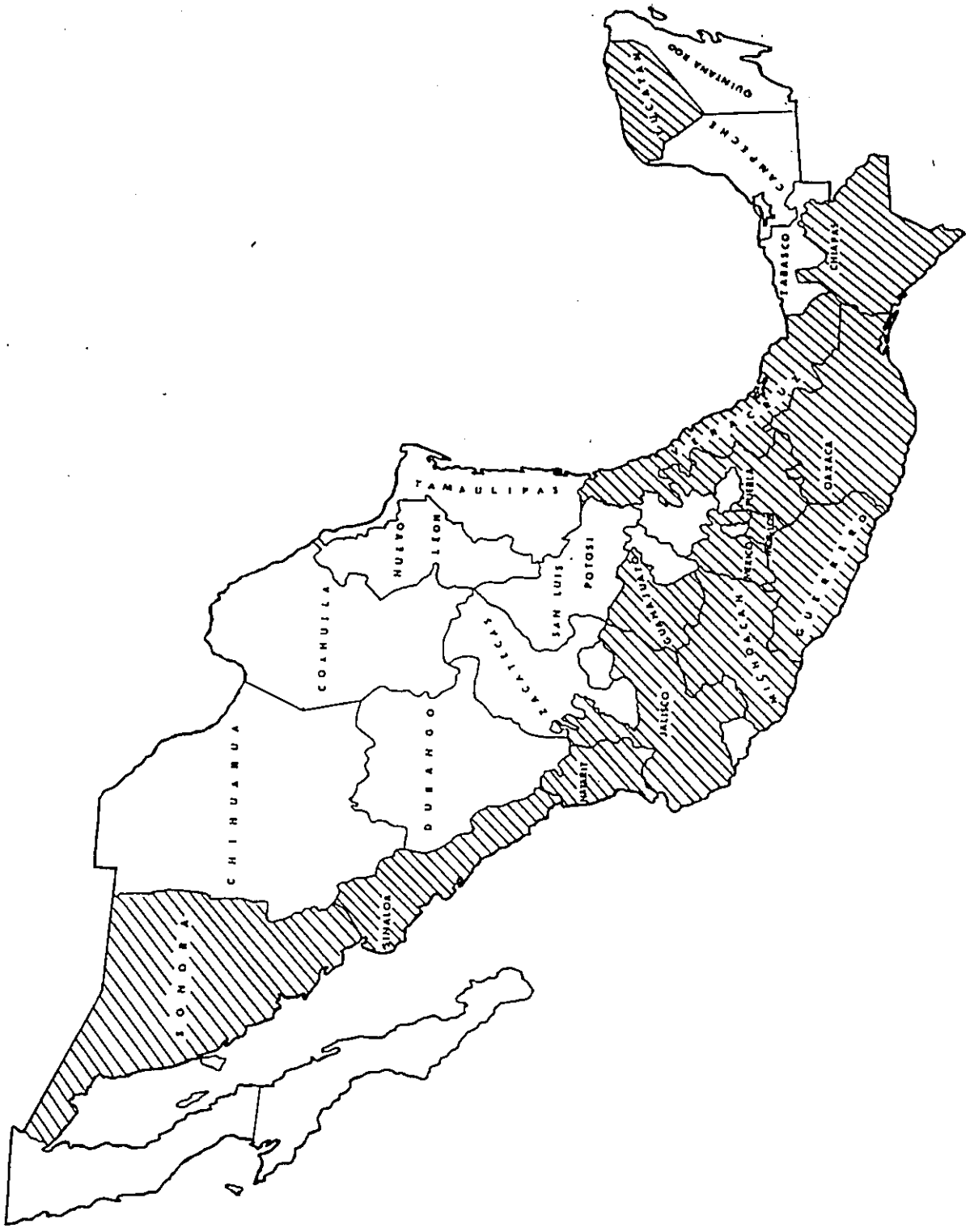
LATITUDINAL SERIES OF INBRED LINES

LATITUDINAL GROUPING		LINES
Southern Temperate Range (STR) 34°-40° N-S		3790 3794 3802 3805 3815 3823
Intermediate Temperate Range (ITR) 40°-46° N-S		3853 3857 3862 3865 3872 3877 3878
Northern Temperate Range (NTR-1) 46°-52° N-S		3945 3946 3947 3962 3971 3983
Northern Temperate Range (NTR-2) 46°-52° N	Holland	4018 4019 4020 4021 4022
	Switzerland	4034 4035 4036
	Germany	4042 4046 4050
	Poland	4064 4065 4066
	Canada	4071 4072 4077 4081

FIGURE 5

THE STATES OF MEXICO

States with cross-hatching are those from which landrace populations were collected for this study.


QUINTANA ROO
CAMPECHE
TABASCO
CHIAPAS
OAXACA
TAMAULIPAS
NUEVO LEON
COAHUILA
SAN LUIS POTOSI
ZACATECAS
DURANGO
CHIHUAHUA
SONORA
SINALOA
NAYARIT
JALISCO
COLIMA
MICHOACAN
GUERRERO

more Mexican states. The races and their corresponding populations are listed in TABLE 3.

2.1.3 ARGENTINE LANDRACES

In addition to the Mexican landraces, two groups of landraces from Argentina were studied: Cateto C and Cateto E (TABLE 4). These races of flint corn are thought to be derived from Mexican dent germplasm (Goodman and McK. Bird, 1977).

2.1.4 CIMMYT MAIZE POOLS

Three CIMMYT maize pools and three crosses between them were studied (TABLE 5). Pool 27 consists of subtropical-temperate early white flint corn primarily from Pakistan, the USA, and Europe. It has been found to have good yield potential and is currently under selection for tolerance to high density planting and resistance to ear, stalk, and leaf diseases. Pool 28 is more variable than Pool 27 and is a dent rather than flint corn, while Pool 30 is similar to Pool 28 but is a yellow dent rather than a white dent corn.

2.1.5 SYNTHETICS A, B, and C

Synthetics A, B, and C (TABLE 5) are all cultivars resulting from combining separately developed lines: Synthetic A is composed of nine inbred lines; Synthetic B consists of fourteen inbred lines seven of which are from Central Europe and seven from the USA and Canada; and Synthetic C is composed of seventeen inbred lines, thirteen of which are Canadian. All three synthetics have been selected for earliness and resistance to *O. nublilalis* (Henderson, 1984).

TABLE 3

MEXICAN LANDRACES AND THEIR RESPECTIVE POPULATIONS

LANDRACE	POPULATION(S)[a]
Arrocillo Amarillo	Puebla-463, Puebla-537
Apachito	Chiapas-166
Azul	Chiapas-133
Bofo	Nayarit-222
Bolita(BOLI)	Oaxaca-130, Oaxaca-40
Cacahuancintle (CATL)	Mexico-212
Celaya (CYLA)	Guanajuato-101, Guanajuato-71
Chalqueno (CHAL)	Mexico-46, Mexico-208
Chapalote (CHAP)	Sinaloa-2, Sinaloa-35
Comiteco (COMI)	Chiapas-235, Chiapas-46
Complejo Serrano de Jalisco	Jalisco-GP-12
Conejo	Guerrero-168
Conico (CONI)	Mexico-3, Mexico-182, Mexico-461
Conico Norteno (CONN)	Guanajuato-102, Guanajuato-22
Dulcillo del Noroeste	Sonora-159
Gordo	Chiapas-140
Harinoso de Ocho (HARO)	Nayarit-24, Sinaloa-66
Jala	Nayarit-72, Nayarit-59
(K-65-1)	Teosinte
Maiz Blando	Sonora-32
Maiz Dulce (MADU)	Guanajuato-93A, Jalisco-78
Nal-Tel (NALT)	Yucatan-7, Yucatan-16
Olotillo (OLTI)	Chiapas-237, Chiapas-239, Chiapas-218
Oloton (OLOT)	Chiapas-124
Onaveno	Sonora-139
Palomero Tolqueno (PALT)	Mexico-55, Mexico-5, Mexico-6
Pepitalla (PEPT)	Morelos-52, Morelos-17
Reventador (REVE)	Nayarit-39, Nayarit-15
Tablilla de Odso	Nayarit-185
Tabloncillo (TABL)	Jalisco-222, Jalisco-43
Tehua (THUA)	Chiapas-78
Tepecintle (TETL)	Guanajuato-207, Chiapas-26
Tuxpeno (TUXP)	V-520-C, Veracruz-39
Vandeno (VAND)	Oaxaca-4, Guerrero-130
Zamorano	Michoacan-GP-13
Zapalote Chico (ZAPC)	Oaxaca-179, Oaxaca-48
Zapalote Grande (ZAPG)	Chiapas-236, Chiapas-224
?	Oaxaca-139

a-refer to different states of Mexico from which populations were collected

TABLE 4

ARGENTINE LANDRACES

Major Grouping by CIMMYT	Number
CATETO E	2044 2045 2047 2048 2051
CATETO C	2025 2026 2027 2030 2032

TABLE 5

CIMMYT MAIZE POOLS, SYNTHETIC LINES, AND CONTROL LINES

CIMMYT Maize Pools	
POOL 27:	4106
POOL 28:	4107
POOL 30:	4108
POOL 27 X 28:	4094
POOL 27 X 30:	4095
POOL 28 X 30:	4098

Canadian Synthetic Lines	
SYNTHETIC	A
SYNTHETIC	B
SYNTHETIC	C

Resistant Control Lines

A619
OH43
B73

MBR
(Multi-borer Resistant Line)

2.1.6 CONTROL LINES

Three resistant control lines were used: A619, OH43, and B73 (TABLE 5). All of these are commonly used in studies for first-brood resistance to *O. nublilalis* and have always been found to be resistant.

The last line listed in TABLE 5 is the multi-borer resistant line (MBR). This is a CIMMYT line that has undergone recurrent selection from adapted tropical materials. It is resistant to three main borers: the sugar cane borer, *Diatraea saccharalis*; the Southwestern corn borer, *D. grandiosella*; and, the corn earworm, *Heliothus zeae*.

Seeds for the latitudinal inbreds, Argentine landraces, CIMMYT Pools, and controls were obtained from Dr. R. Hamilton of the Plant Research Center of Agriculture Canada, Ottawa, Ont. Synthetic A, B, and C lines were obtained from M. Hudon of Agriculture Canada Research Station, St. Jean sur Richelieu, Quebec. The Mexican landraces were obtained by Dr. J.T. Arnason through collaboration with Drs. J. Mihm, D. Jewell, and S. Taba of the CIMMYT maize program. Seed selected from bank entries at CIMMYT were multiplied at CIMMYT before shipment to Canada.

2.1.7 HYBRIDIZATION EXPERIMENTS

In the 1986 field season crosses were made between various lines and races, TABLE 6 (selection of lines and races was based upon concentrations of hydroxamic acids in the etiolated seedlings, see Section 2.2). Paper bags were placed on tassels to collect pollen and also on young ears to exclude pollen. Pollination was carried out by placing a tassel bag containing pollen on the appropriate ear for the cross. The pollen bag was fastened on the ear and shaken vigorously thus ensuring pollination and subsequent fertilization. At harvest the ears were collected and dried at 25° C for four weeks. The kernels were then removed by hand.

TABLE 6

LINES AND RACES USED IN HYBRIDIZATION EXPERIMENTS

PARENTAL CROSS		
RESISTANT	X	RESISTANT[a]
NTR-1, 3945	X	Pool 28 x 30, 4098
Cateto C, 2032	X	Pool 28 x 30, 4098
NTR-1, 3945	X	ITR, 3962
Cateto C, 2032	X	Cateto C, 2030
Pool 28 x 30, 4098	X	Cateto C, 2030
Cateto C, 2030	X	NTR-1, 3947
Cateto C, 2032	X	NTR-1, 3945
ITR, 3862	X	NTR-1, 3947
Cateto C, 2030	X	ITR, 3862
NTR-2, Poland, 4065	X	NTR-1, 3945
Cateto C, 2030	X	Pool 28, 4107
Pool 28, 4107	X	Pool 28 x 30, 4098
Pool 28, 4107	X	NTR-1, 3947
NTR-1, 3947	X	Cateto C, 2032
Cateto C, 2032	X	NTR-2, Poland, 4065
NTR-1, 3947	X	Pool 28, 4107
Pool 28, 4107	X	Cateto C, 2032
Cateto C, 2032	X	ITR, 3862
Pool 28 x 30, 4098	X	NTR-1, 3947
NTR-1, 3945	X	Pool 28 x 30, 4098
SUSCEPTIBLE	X	SUSCEPTIBLE[a]
NTR-2, Germany, 4042	X	Cateto C, 2025
NTR-2, Germany, 4042	X	NTR-2, Canada, 4081
NTR-2, Canada, 4081	X	NTR-2, Canada, 4071
NTR-2, Canada, 4081	X	Cateto C, 2025
RESISTANT	X	SUSCEPTIBLE
Pool 28 x 30, 4098	X	Cateto C, 2025

a-resistance or susceptibility determined by hydroxamic acid concentrations in etiolated seedlings (Section 2.2)

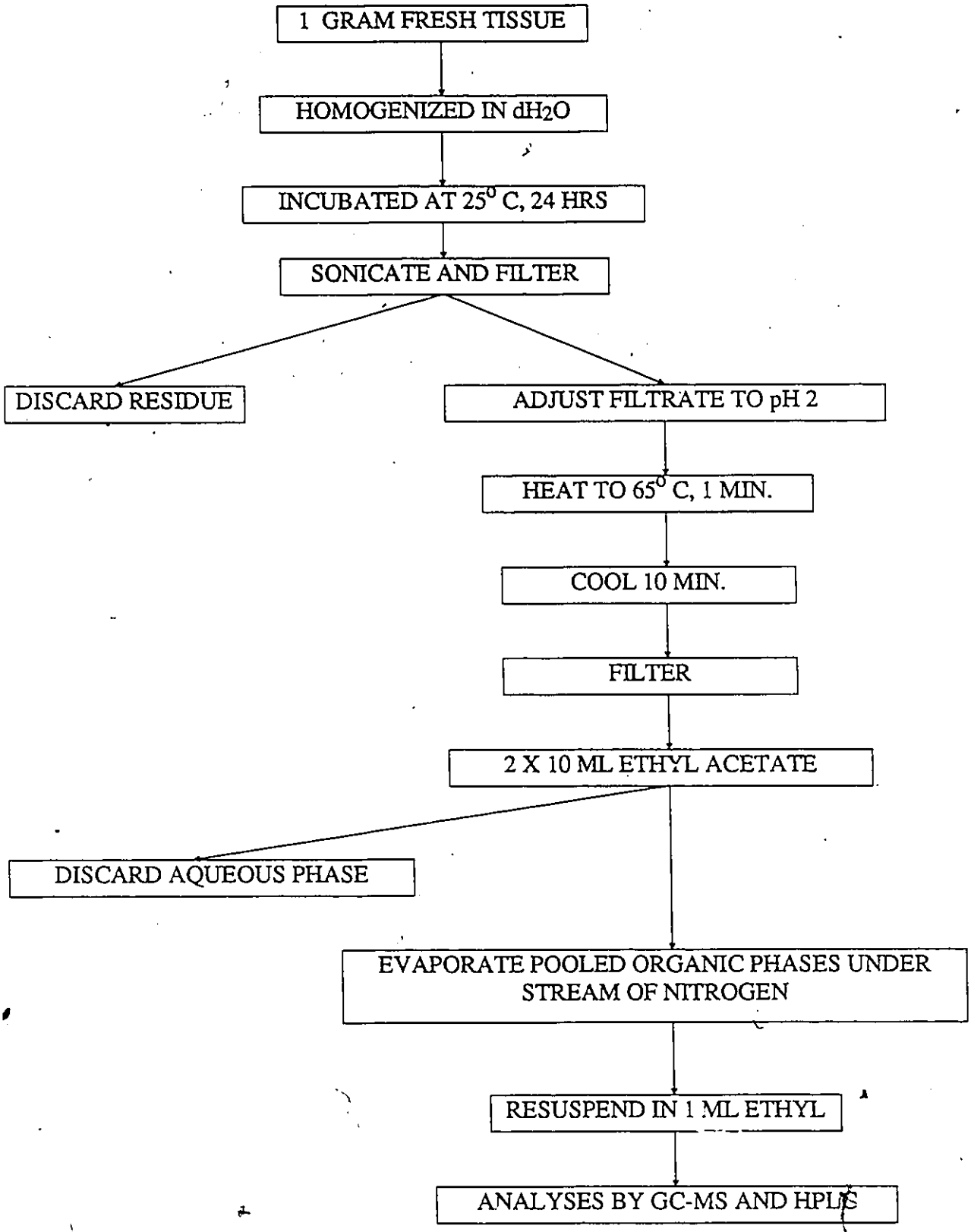
2.2 PHYTOCHEMICAL STUDIES - HYDROXAMIC ACIDS

2.2.1 EXTRACTION OF HYDROXAMIC ACIDS

A modified Gutierrez et al (1982) extraction was carried out to determine the levels of hydroxamic acids in the lines and races studied. Twenty kernels of each line or race were disinfected by soaking in a solution of 10% sodium hypochlorite (Javex) for 30 minutes, after which they were washed several times with distilled water. Each group of twenty kernels was then separated into two lots of ten kernels each and planted in plastic pots containing vermiculite. After being well watered with distilled water the pots were placed in sealed wooden boxes and the seeds allowed to germinate in the dark for seven days at 25° C. Shoots were then removed and cut into small (approx. 1 cm) pieces. One gram samples of this fresh tissue were taken in triplicate. Each one gram sample was suspended in 6 ml of distilled water and homogenized in a Sorval-Omni Mixer for 1 minute (FIGURE 6). The resulting slurry was removed from the mixer and placed in a 50 ml beaker. The apparatus was rinsed to get maximum yield. To facilitate enzymatic hydrolysis of the DIMBOA glucoside the slurry was incubated at 25° C for 24 hours. After incubation the homogenate was disrupted by sonic treatment in a cleaning bath for three minutes then filtered through four layers of cheesecloth into a 100 ml Erlenmeyer flask. The residue was rinsed with distilled water. The filtrate and rinsings (pH approximately 4.6) was acidified to pH 2 with 2N HCL. Next the filtrate was heated rapidly to 65° C in a water bath for 1 minute and then cooled in an ice bath for 10 minutes to coagulate the proteins. The proteins were removed by vacuum filtration through Whatman No. 42 filter paper in a Buchner funnel and discarded. The protein-free filtrate was extracted twice with 10 ml of HPLC grade ethyl acetate (B.D.H.). The aqueous phase was discarded and the pooled organic phases were evaporated down to almost complete dryness under vacuum at 40° C. Final evaporation was carried out under a stream of nitrogen to prevent oxidation and degradation of the samples. The residue was then resuspended in 1 ml of HPLC grade

FIGURE 6

FLOW CHART OF HYDROXAMIC ACID EXTRACTION PROCEDURE FOR ONE-WEEK-OLD ETIOLATED CORN SEEDLINGS


1 GRAM FRESH TISSUE
HOMOGENIZED IN dH2O
INCUBATED AT 25° C, 24 HRS
SONICATE AND FILTER
DISCARD RESIDUE
ADJUST FILTRATE TO pH 2
HEAT TO 65° C, 1 MIN.
COOL 10 MIN.
FILTER
2 X 10 ML ETHYL ACETATE
DISCARD AQUEOUS PHASE
EVAPORATE POOLED ORGANIC PHASES UNDER STREAM OF NITROGEN
RESUSPEND IN 1 ML ETHYL
ANALYSES BY GC-MS AND HPLC

ethyl acetate , filtered through a 0.5 micron nylon filter, and stored in a freezer for later analysis by HPLC and GC-MS. This procedure was carried out on all of the lines and races listed in TABLES 2 to 5 and also on the progeny resulting from the hybridization experiments (Section 2.1.7).

2.2.2 PREPARATION OF EXTRACT SAMPLES FOR ANALYSIS

All extracts were filtered through 0.5 micron nylon millipore filters with a 1.5 ml Luer lock syringe and a Millipore filter holder after resuspension and again before analysis. For GC-MS analysis 0.5 ml of the extract was filtered and evaporated down to approximately 15 ul under a stream of nitrogen. For quantitation by HPLC a one in ten dilution of each extract was prepared as follows: a 100 ul sample was taken from each extract, evaporated under nitrogen, resuspended in 1 ml of HPLC grade methanol, and refiltered.

2.2.3 IDENTIFICATION AND QUANTITATION OF EXTRACTS

2.2.3.1 Gas Chromatography - Mass Spectroscopy

Samples were sent to the Ottawa-Carleton Universities Mass Spectrometry Center for identification of compounds by Dr. Glement Kasikov. Samples were trimethyl silylated using tricyl-BSA then analyzed using a Varian 3300 Gas Chromatograph equipped with a J&W megabore DBS 30 meter column, column temperature (150° C) programmed for an increase of 10°/min. Mass spectra were obtained with a VG 7070 E medium resolution mass spectrometer and a DEC PDP8a data system. Resulting mass spectra and GC traces of hydroxamates found were then compared to that obtained by Woodward et al (1979a,b).

2.2.3.2 High Performance Liquid Chromatography

A Beckman HPLC equipped with a Model 165 Variable Wavelength detector, a Model 110A Solvent Metering Pump System, and a Model 420 System Controller Programmer was used to quantitate the hydroxamates found in the extracts. Twenty microliters of the diluted sample were injected into the system and processed under the following conditions: Ultrasphere ODS reverse-phase C18 column; isocratic elution with 40% methanol/58% water/ 2% acetic acid; flow rate of 1 ml/min; range=0.100; detection wavelength= 265 nm; and an online UV scan from 240-400 nm of eluted peaks.

Identification of DIMBOA, MBOA, and any other compounds was carried out by comparison of retention times, UV spectra and by peak enrichment of authentic standards. All standards were synthesized by Jeff Atkinson of the Department of Chemistry, University of Ottawa, Ottawa, Ont.

Standard curves were used to quantitate DIMBOA and MBOA concentrations in the extracts. Peak heights of standards were correlated to concentration of hydroxamates injected. Therefore, concentrations in extracts were determined by peak height and comparison to standard curves.

2.2.4 RECOVERY OF HYDROXAMIC ACIDS FROM EXTRACTS

Triplicates of a range of different quantities (0.1-1 mg) of DIMBOA and MBOA were added to beakers containing 1 g of homogenated corn tissue and water and then processed as before. The extraction procedure was also carried out in the absence of tissue, i.e. with solvent alone. The difference between DIMBOA concentrations for the two methods showed how much DIMBOA was lost by binding to the protein thiol groups of the tissue.

2.3 RESISTANCE STUDIES

2.3.1 LABORATORY LEAF-FEEDING TESTS

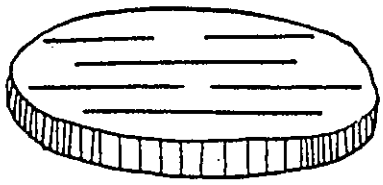
Preliminary feeding tests with corn tissue involved placing small (1-2 cm dia.) leaf disks of whorl tissue in a sealed vial with two third instar borer larvae for 24 hours. After this time the disks were examined and the percentage of leaf disk consumed by the larvae was recorded. This method was found to be unsatisfactory since the leaf disks often dried out very rapidly leaving the larvae with food that was inedible. In addition, by cutting the leaf disk out of the leaf tissue one was already exposing the insect to tissue that was excreting various compounds, including hydroxamic acids, from its cut edges. Feeding results with this method were not statistically significant; therefore, it was necessary to develop a more suitable method to evaluate resistance of a given maize line in the laboratory.

A modified Asher and Glotter (1981) method was used. The appararatus consisted of a system of petri plates (FIGURE 7). Humidity was maintained by placing a water saturated cotton wool square (B) into an inverted petri dish cover (A). A disk of filter paper (C) was then placed over the cotton to create a seal and a more even surface. An approximate 4 X 6 cm strip of corn whorl leaf tissue (D) ,midrib excluded, was then placed on the filter paper. Preliminary experiments showed that there was greater consumption on the lower surface of the leaves so the strip of leaf was placed with the lower surface exposed to the insect. A petri dish bottom with a 2.5 cm diameter circle cut out of it (E) was placed on the leaf strip. Two early third instar borer larvae were then placed on the exposed circular area of leaf. The apparatus was sealed with another petri dish cover (F) and secured with an elastic band. The entire apparatus was then placed in an incubator for 48 hours at 27° C and 16:8 hr. L:D.. After this time the total area consumed was determined by placing the leaf strip on a 1 mm^2 grid scale and counting the number of squares made visible by the larvae. This result was used

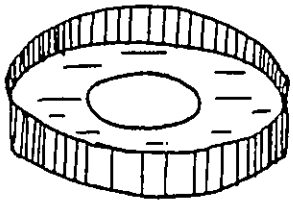
FIGURE 7

LABORATORY LEAF FEEDING TEST APPARATUS

A - inverted petri dish cover
B - cotton wool square
C - filter paper
D - whorl corn leaf section excluding midrib
E - petri dish bottom with 2.5 cm diameter central hole and two third instar European corn borer larvae
F - petri dish cover



F



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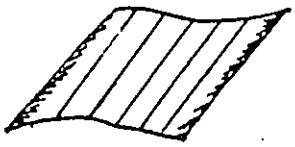

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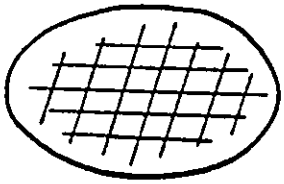

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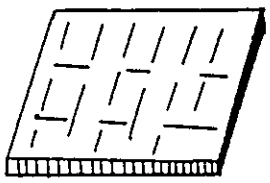
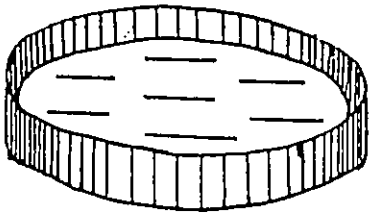
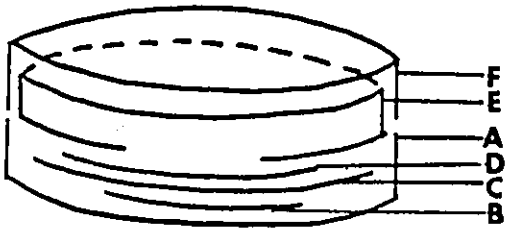


B



A



to calculate the percent consumption of the exposed circular leaf area by the larvae. Twenty replicates were carried out for each line or race studied.

European corn borer larvae for this study came from a laboratory colony that was initiated from eggs supplied by Dr. M. Hudon (Agriculture Canada, St. Jean, P.Q.). The colony was periodically boosted with eggs supplied by Dr. G. McLeod (Agriculture Canada, London. Ont.) and maintained on a meridic diet according to the method of Guthrie et al (1971). Larvae were reared in a controlled enviroment chamber (Conviron Model E7), with a photoperiod of 16/8 (L/D), at 25° C during the photophase, and 19° C during the scotophase. Relative humidity was kept constant at 85%. All leaf feeding tests were carried out in this chamber.

Plants for this part of the study were grown in a plastic outdoor greenhouse in the summers of 1986 and 1987 at the Plant Research Station, Agriculture Canada, Ottawa. Five seeds of each line or race were planted in separate 20 cm diameter peat pots. Normal cultural practices performed at this station were used. Leaf tissue sections for the consumption study were taken from each line as that line reached its mid-whorl stage of development (approx. 50 cm high, 8-10 leaves, and 10-12 days before tassel emergence). Time to reach this stage varied among lines and races.

Differences between means were analyzed by Duncan's multiple range test ($p=0.05$). Linear regression and Pearson's correlations were used to examine the relationship between consumption levels and DIMBOA concentrations in the etiolated seedlings.

2.3.2 FIELD STUDIES

2.3.2.1 PLANTING AND FIELD ARRANGEMENTS

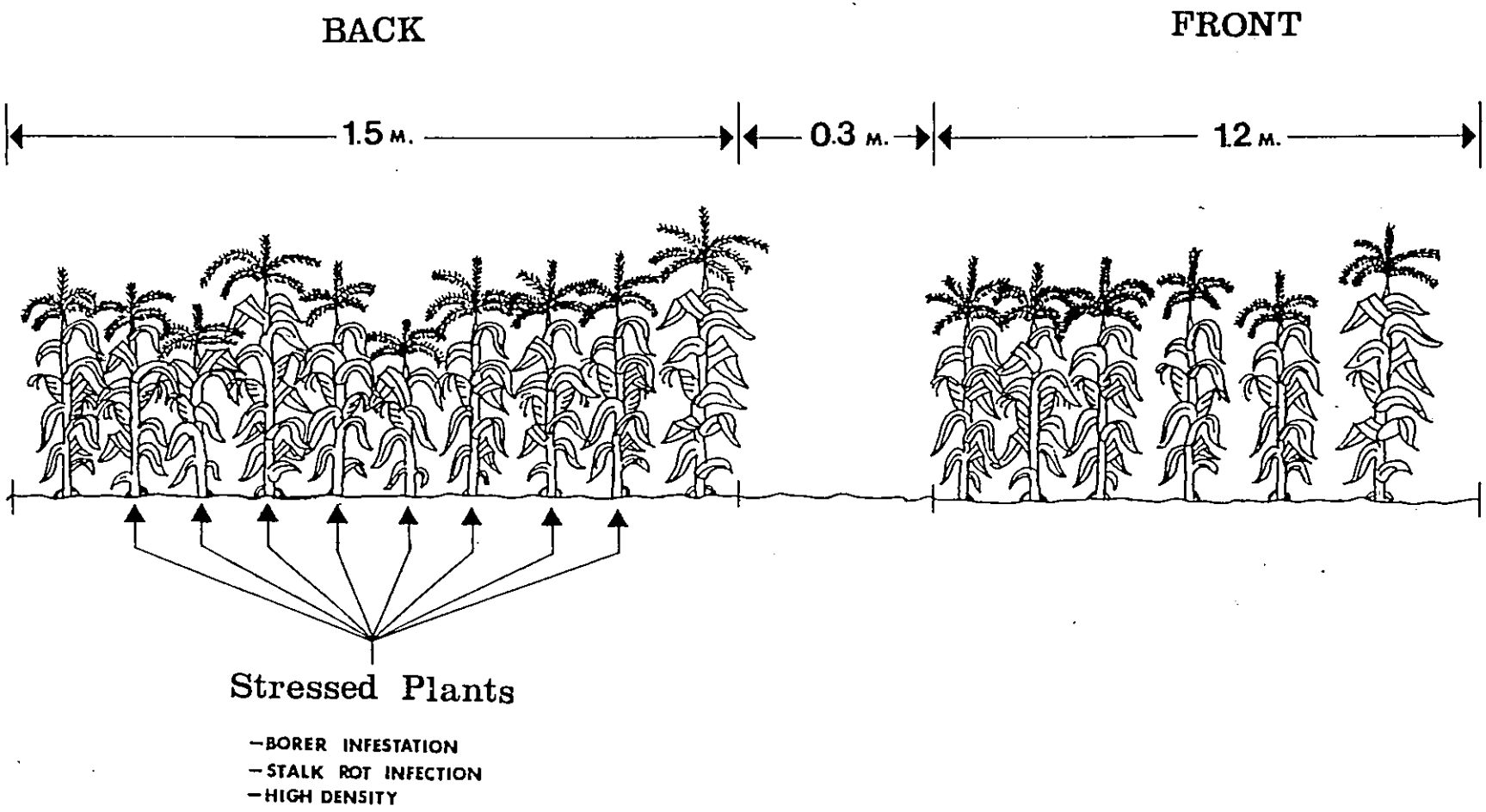
Three seasons of field studies (summers 1985, 1986 and 1987) were carried out at the Plant Research Station , Agriculture Canada, Ottawa, Ont. Data from the 1985 field season are not included in this study since this season was used as a preliminary technique-establishing season. All of the inbred lines, pools, controls, Argentine landraces, and ten of the Mexican landraces populations were studied in the 1986 and 1987 field seasons. Seed for the remaining 53 landrace populations was available only for the 1987 field season. The successive field seasons were used to evaluate germplasm showing resistance in the previous season to ensure that this resistance was real and not apparent as a result of low insect populations or climatic conditions. Resistant checks (control lines) commonly used at the Plant Research Center of Agriculture Canada were included to determine the level of borer damage and to act as a reference for rating and evaluation.

In 1986 the seeds were planted in 3 meter long rows with one row per line or race. The control line rows were replicated twice and randomly placed among the trial rows. In 1987 a randomized block design with six replications was used. Each of the six plots consisted of 104 rows with each row representing a different line or race. There was a space of 0.5 meters between each 3 meter long row. In mid-May 24 seeds of each line or race were planted in each of their six replicated rows. After three weeks all rows were thinned to 16 plants per row. This was done in such a manner that the front (East) 1.2 meters of the row contained 6 plants and the back (West) 1.5 meters contained 10 plants, leaving approx. 0.3 meters of space between the front and back parts of the row (FIGURE 8). The back 10 plants were then used to study the performance of each line or race when exposed to three main experimental stresses simultaneously:

FIGURE 8

ARRANGEMENT OF ROWS FOR THE 1987 FIELD SEASON

Each 3 meter row was subdivided into two 1.5 meter sections. The front (East) 1.5 meters consisted of 6 plants in the first 1.2 meters that were used for selfing and observing various morphological and agronomical traits. The back 1.5 (West) meters consisted of 10 plants of which the end two were left as border plants and the middle 8 plants were infested with European corn borer egg masses and inoculated with stalk rot, *Gibberella zeae*. Therefore, these 8 plants were exposed to three stresses: borer infestation, stalk rot infection, and high density. Level of overpopulation or high density was 6.7 plants/meter in the back part of the row compared to 5 plants/meter in the front of the row. The rows were spaced 0.5 meters apart.


BACK
FRONT
1.5 M.
0.3 M.
1.2 M.
Stressed Plants
—BORER INFESTATION
—STALK ROT INFECTION
—HIGH DENSITY

1. European corn borer infestation.

2. Stalk rot infection by *Gibberella zeae*.

3. High density planting (overpopulation).

Of these ten plants only the middle eight were infested and inoculated; the remaining two plants were used as border plants (FIGURE 8).

The 6 plants in the front 1.2 meters of each row were not experimentally stressed. These plants were selfed to produce more seed and were used for observing various agronomic and morphological characteristics such as silking date, leaf number, and plant height (under non-stressed conditions). Cultivation and fertilizer practices normal to the area were used throughout the study.

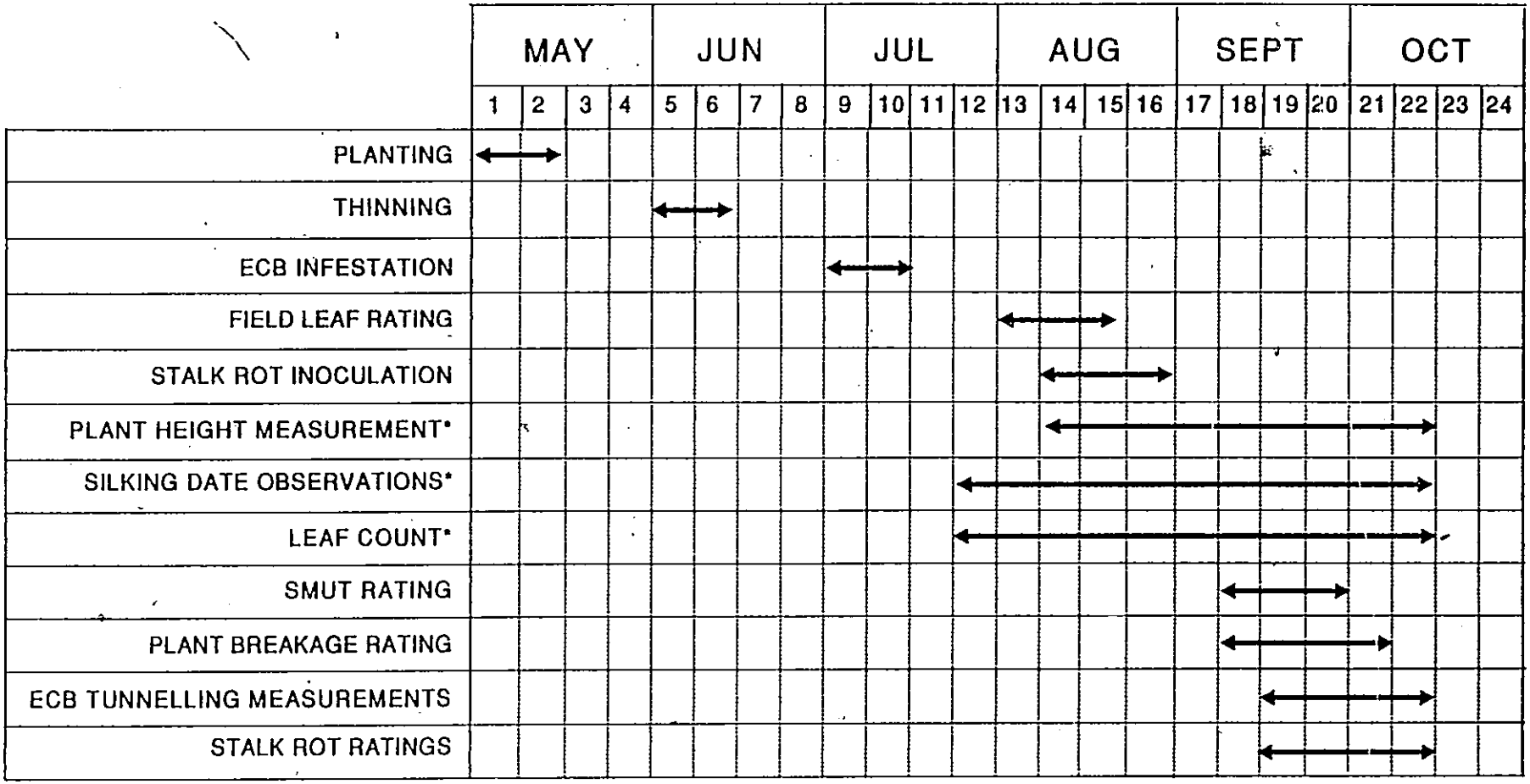
Plant damage was used to measure resistance rather than insect counts because many factors, including disease, parasitism, and predation can result in the absence of insects at the time of examination even though extensive plant damage can be observed (Pesho et al, 1965). The timing of infestations, inoculations, and measurements or ratings taken throughout the field season is listed in TABLE 7.

2.3.2.2 EUROPEAN CORN BORER INFESTATION

Plants were infested during the mid-whorl stage of development (approx. 50 cm high, 8-10 leaves, 10-12 days before tassel emergence). White coloured recently laid egg masses were supplied by Dr. M. Hudon of Agriculture Canada, St. Jean sur Richelieu, Quebec. Each egg mass was on a 1 cm dia. disk of waxed paper. Disks were spread out and sorted on a wire mesh then pinned on insect mounting needles, 20 disks per needle. Ten needles, each with 20 disks, were placed in glass petri dishes (2.5 cm deep) with a moistened filter paper at the bottom of the dish to retain humidity. The eggs were then incubated at 26° C until they reached the black-head stage in approx.

TABLE 7

TIMING OF FIELD INFESTATIONS, INOCULATIONS, MEASUREMENTS, AND RATINGS



*-DATES VARY ACCORDING TO TIME OF MATURITY

4 to 5 days and 1/2-1 days before hatching. Field infestation was carried out at this stage of development to prevent insect predation by coccinellids, syrphid, and chrysopid larvae which attack newly laid corn borer eggs. As a further preventive measure the egg masses were placed deep in the whorl of the plant by means of long forceps. Each plant was infested with a total of four egg masses (approx. 100 eggs per plant) in two applications of two egg masses each. The second application was carried out two days after the first.

In 1986 all the plants in a given row were infested. In 1987 only the middle 8 plants in the back 1.5 meters of each row were infested. The front part of each row was left uninfested to observe natural infestation damage (FIGURE 8).

2.3.2.3 EUROPEAN CORN BORER FIELD LEAF DAMAGE RATINGS

A nine-class rating scale developed by Guthrie et al (1960) was used to evaluate damage to whorl leaves resulting from borer feeding. Rating is based on size and number of leaf lesions produced by borer larval feeding. In this rating scale (TABLE 8) lines or races with little or no damage are given a rating of 1 or 2, while the heavily damaged lines are rated as an 8 or 9.

Ratings were taken three weeks after artificial infestation, when the plants were beginning to tassel and the borer larvae had ended their leaf feeding stage. Therefore, this leaf damage rating is a measure of the resistance of the plants to first-brood borer leaf-feeding damage. At the same time, ratings were also taken on the uninfested plants in the front 1.2 meters of each row to measure natural infestation damage.

2.3.2.4 STALK ROT INOCULATION

The same plants that were infested with borer egg masses were infected with *Gibberella zeae* during the mid to late tassel stage (early August). Plants were first

TABLE 8

FIELD LEAF DAMAGE RATING SCALE
(Guthrie et al, 1960)

RATING	DAMAGE
1	NO DAMAGE OR JUST A FEW PIN HOLES
2	SHOT-HOLE LESIONS/INJURY ON JUST A FEW LEAVES
3	SHOT-HOLE INJURY ON SEVERAL LEAVES
4	SHOT-HOLE AND ELONGATED INJURY ON SEVERAL LEAVES
5	ELONGATED LESIONS ON SEVERAL LEAVES
6	LARGER (2.5 cm) ELONGATED LESIONS ON SEVERAL LEAVES
7	LONG LESIONS ON HALF OF THE LEAVES
8	LONG LESIONS ON TWO-THIRDS OF THE LEAVES
9	ALMOST ALL LEAVES WITH LONG LESIONS

wounded by driving a small nail into the first internode of the stalk. Toothpicks inoculated with *G. zeae* spores were then inserted into the wound and pushed across the entire diameter of the stalk. Inoculated toothpicks were supplied by Dr. A. Bolton of the Plant Research Center, Agriculture Canada, Ottawa. Uninoculated toothpicks were used for the first six plants in the front 1.5 m of each row.

2.3.2.5 MEASUREMENT OF PLANT HEIGHT

Plant height (cm) was measured after each line or race had flowered and before the tassel had dried out. Measurements were taken from soil level to the tip of the extended tassel. Height measurements began in mid-August and continued until the end of the season (mid-October) as each line reached maturity (TABLE 7). Most of the landraces did not flower; however, height measurements for them were taken from soil level to the tip of the longest extended leaf.

In each row, measurements were taken on both the back middle eight infested plants and on the front six unstressed plants. The back eight height measurements were used for tunnelling/height ratios in further resistance ratings and for the measurement of height variation due to high density stress.

2.3.2.6 LEAF COUNTS AND DAYS TO SILKING

The front six plants of three replicates were used to obtain total leaf counts and silking dates of the lines, races, pools, and controls studied. To counter the difficulty created by the fact that early leaves tend to dry out and fall off, the fifth and tenth leaves were marked by clipping them with scizzors when the plants were at 5 and 8 weeks of development respectively. At maturity a final leaf count was made by counting from the tenth leaf upwards. For some races that did not flower, leaf counts were made at the end of the season. Days to silking were taken as the number of days from planting to the date when 50% of the plants had silk emerging from their ears.

2.3.2.7 SMUT RATINGS

After observing in the 1985 field season that many of the more tropical lines and especially the landraces were infected with corn smut, *Ustilago maydis*, all lines and races were examined for the absence or presence of smut and the number of plants infected was recorded. Plants that were infected with smut were given a presence rating of 1.0; those not infected were given an absence rating of 0.0.

2.3.2.8 EUROPEAN CORN BORER DAMAGE RATINGS AT HARVEST

Determination of plant injury was also made after plant maturity or at the latest possible date for non-maturing landraces. By this time (approx. 60 days after egg hatch) the larvae had had the opportunity to cause extensive damage to the stalk. Two measures of resistance were taken at this time: plant breakage and borer tunnelling.

Infested plants were evaluated to measure their tolerance to plant breakage until harvest. A rating scale of 1-10 developed by Guthrie et al (1960) was used (FIGURE 9). A rating of 1 is for a clean plant, 2 or 3 for a broken tassel, 4 or 5 for a stalk broken above the ear, and 6 to 10 for plants broken below the ear.

The degree of borer larval tunnelling was determined by taking each infested plant, removing the leaves, and splitting the stalk down the center. The cumulative lengths of borer tunnels were then measured in the split stalk. In addition to infested plants the uninfested front six plants were also dissected and rated.

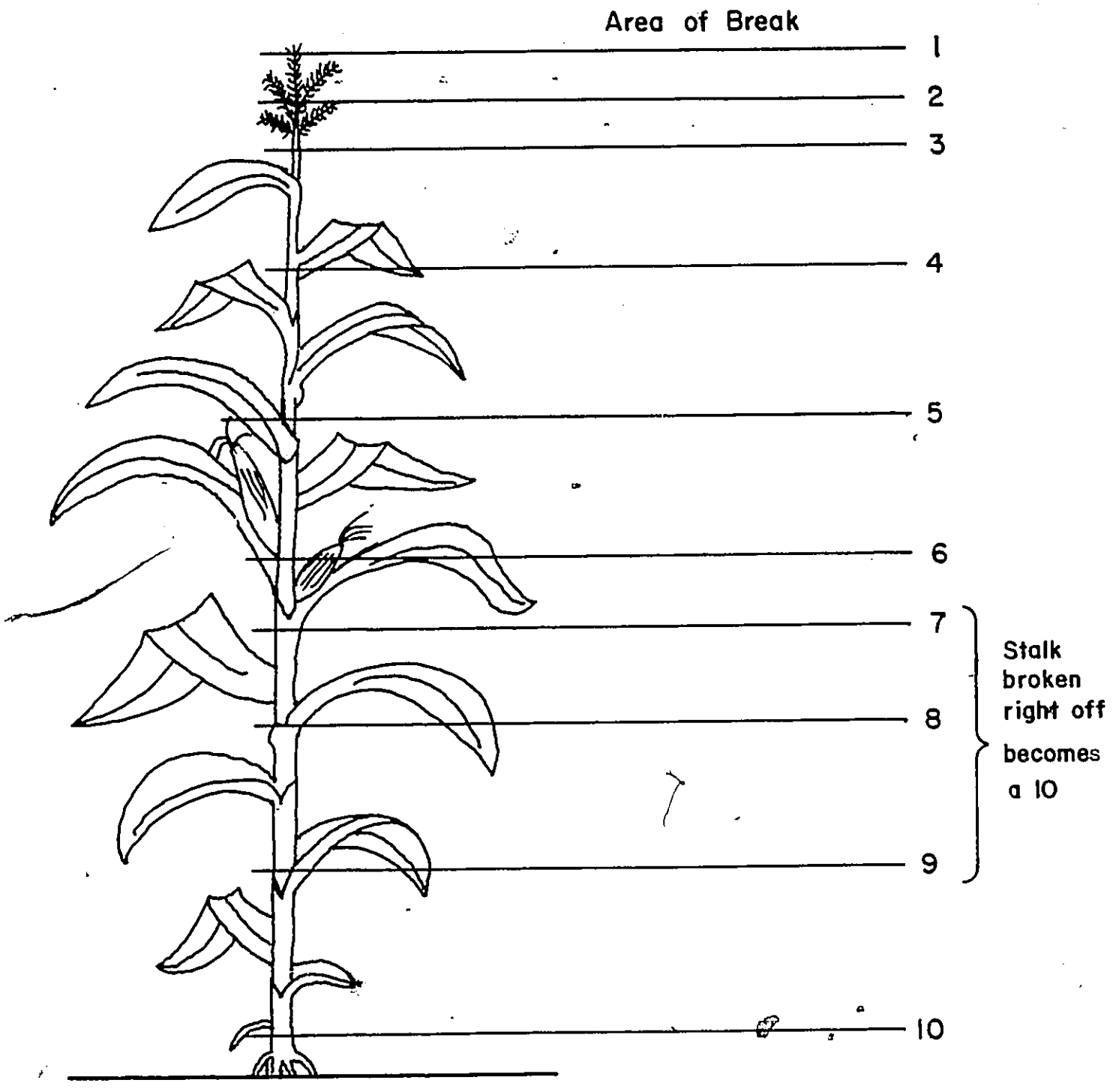
2.3.2.9 STALK ROT RATINGS

When the stalks were split to measure the degree of larval tunnelling the resistance of each line or race to stalk rot infection was also determined. A scale of 1-10 was used to evaluate the spread of the infection in the first internode of the plant

FIGURE 9

PLANT BREAKAGE RATING SCALE
(Guthrie et al, 1960)

Scale is based on the point of stalk breakage due to larval tunneling.


Area of Break
1
2
3
4
5
6
7
8
9
10
Stalk broken right off becomes a 10

(FIGURE 10). A rating of 1 means little spread of infection from the point of inoculation; a rating of 10 was given to infection that had spread to adjacent internodes.

Because of the different field planting arrangements and poor climatic conditions of 1986, analysis of the field data was kept separate for each year. For 1987, data from individual plots were averaged to obtain a plot mean for each line. Pearson's correlations and linear regressions were performed on all ratings and measurements (except leaf counts and silking dates). Laboratory leaf feeding ratings and DIMBOA concentrations were also included in this statistical analysis.

2.4 RESISTANCE OF MAIZE AS RELATED TO THE GEOGRAPHICAL ORIGIN OF MAIZE GERMPLASM

The relationship between the geographical origin of maize germplasm and resistance of maize to the European corn borer was examined. All of the phytochemical and resistance data obtained for the latitudinal series of inbred lines (TABLE 2) were combined to see what effect, if any, different geographical latitudes have on resistance. Similarily, all the data for the almost complete set of the indigenous races of maize of Mexico were used to examine the relationship, if any, of altitude and resistance. The races represent populations from various regions of Mexico; therefore, they are adapted to altitudes ranging from sea level to 2800 meters above sea level (TABLE 9).

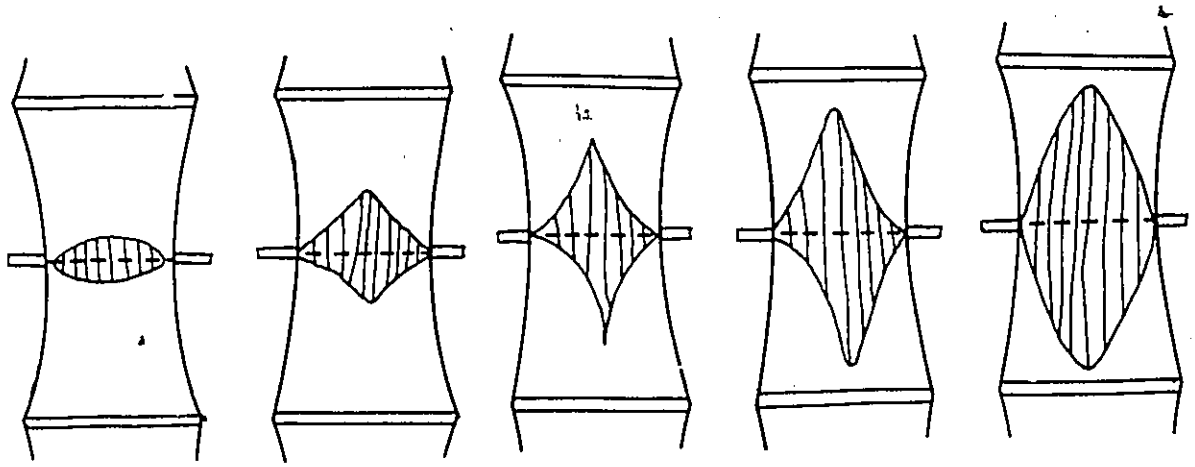
2.5 RESISTANCE OF MAIZE AS RELATED TO THE TAXONOMY OF MAIZE GERMPLASM

From the work in this study a large data set for the Mexican landraces was obtained, consisting mostly of phytochemical and resistance data. To examine the relationship between the taxonomy of maize germplasm and the resistance of maize to the European corn borer, the data were subjected to a number of numerical

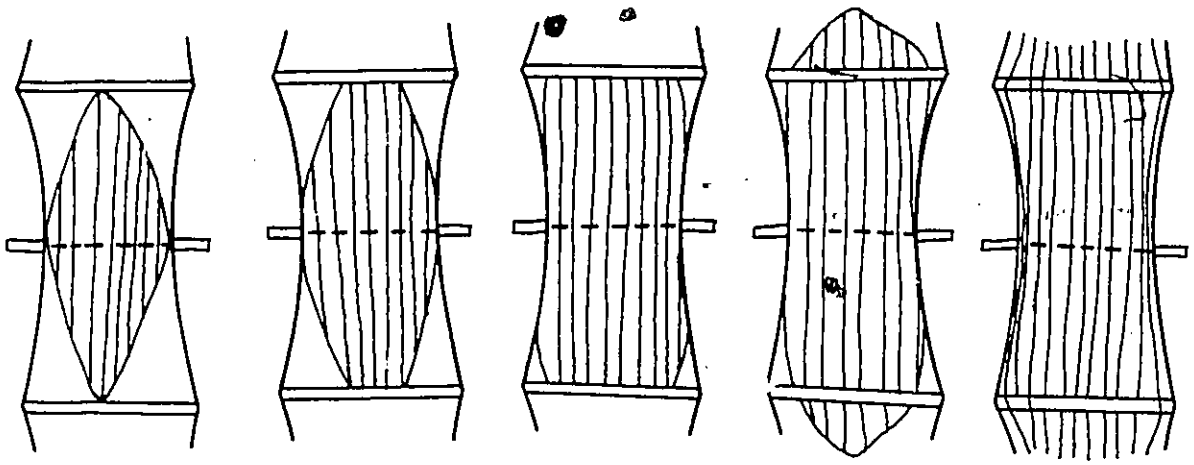
FIGURE 10

CORN STALK ROT, *Gibberella zeae*, RATING SCALE

Longitudinal sections through the first internode of a corn stalk where inoculated toothpicks were placed. Scale is based on the spread of infection from the point of inoculation.



RATING: 1 2 3 4 5



6 7 8 9 10

TABLE 9

ALTITUDE GROUPINGS OF THE INDIGENOUS RACES OF MAIZE OF MEXICO
(Wellhausen et al, 1952)

GROUP[a]	ALTITUDE[b] (meters)	LANDRACES
1	100	Nal-Tel; Harinoso de Ocho; Zapalote Chico
2	0-500	Tuxpeno; Vandeno
3	0-600	Tepecintle
4	100-600	Chapalote; Zapalote Grande
5	300-700	Olotillo
6	600-1000	Tehua
7	1000	Jala
8	0-1500	Reventador; Tabloncillo
9	900-1500	Bolita
10	1000-1500	Maiz Dulce
11	1100-1500	Comiteco
12	1000-1700	Pepitilla
13	1200-1800	Celaya
14	1600-2000	Arrocillo Amarillo
15	1600-2100	Conico Norteno
16	1800-2300	Chalqueno
17	2000-2400	Oloton
18	2200-2800	Palomero Tolqueno; Conico;Cacahuacintle

a-groupings determined by upper altitude limit of race
b-altitude at which the race is commonly found

taxonomic analyses to find whether they fit into Wellhausen's landrace groupings. To investigate this the following analytical steps were undertaken:

STEP 1

A data set for the Mexican landraces was created. It consisted of means for the following: field leaf damage ratings, plant breakage ratings, stalk rot ratings, plant height measurements, smut ratings, tunneling/height ratios, total DIMBOA concentrations and laboratory leaf feeding ratings. It was necessary to use means due to the inability of some of the taxonomic analyses to handle missing data and unequal replications. To this data set, one of Wellhausen's five major groupings of landraces was added as label to each observation (TABLE 1). Since there were more variables than races or populations representing group D, this group was removed from the data set for those analyses which required more individuals than characters.

STEP 2

Stepwise discriminant analysis (Klecka, 1980) was carried out to find a subset of variables that best revealed the differences among the Wellhausen groups. It was computed first with forward selection then with backward elimination. Forward selection discriminant analysis picks out those variables that would give the best separation of Wellhausen's groups. Actual selection is based on a series of sequential F tests. For each test the variable with the higher F value is selected in preference to the others with lower values. Backward elimination removes those variables that do not give a good separation of Wellhausen's groups, again by a series of sequential F tests.

STEP 3

Classificatory discriminant analysis (Rao, 1973) using a linear model was carried out using the variables selected in the previous step that give the best separation of Wellhausen's groups . The linear discriminant functions were generated from the pooled covariance matrix. In addition, this analysis was carried out using all of the

variables in the data set. A test of equality was carried out on the individual covariance matrices. If the test indicated unequality then step 4 was performed.

STEP 4

Step 3 was repeated but this time using a quadratic model of classificatory discriminant analysis. The results of the various analyses were compared in terms of percent total correct classifications. The one with the lowest misidentifications of races was selected as best.

STEP 5

The classification function coefficients obtained in the previous steps were used on the Mexican landraces and the Argentine landraces in an attempt to determine where these races fit into Wellhausen's classification based upon their resistance characteristics. The latitudinal series of inbred lines and CIMMYT maize pools were also analyzed in the same manner.

STEP 6

In order to give a better description of Wellhausen's groups and their resistance characteristics, a series of univariate summary statistics were computed and most were expressed in box plots. Variability profiles of each landrace group were then drawn from the coefficients of variation. The latter were computed for all variables in the data and for all the groups pooled and within each group.

STEP 7

To investigate the relationships among the groups ordination was carried out on the data by means of canonical discriminant analysis (Cooley and Lohnes, 1971).

CHAPTER III

RESULTS

3.1 PHYTOCHEMICAL STUDIES - HYDROXAMIC ACIDS

3.1.2 IDENTIFICATION AND QUANTITATION OF EXTRACTS

3.1.2.1 Gas Chromatography - Mass Spectroscopy

GC - MS was used to detect and identify hydroxamic acids in the etiolated corn seedling extracts (FIGURE 11). Separation of the silylated hydroxamates and other compounds revealed the presence of three major hydroxamic acid peaks with retention times of 7.17 min., 7.48 min., and 9.17 min. By comparing the spectra of the first (7.17 min.) and the last peak (9.17 min.) to those of standards, these peaks were identified as TMS-MBOA and TMS_2-DIMBOA respectively. The second peak (7.48 min.) was identified as TMS_2-HMBOA by comparison with mass spectra published by Woodward et al (1979b) since pure standards of this compound were unavailable at the time of this study. All extracts contained these three compounds, although in varying amounts.

3.1.2.2 High Performance Liquid Chromatography

HPLC was used to quantify the levels of DIMBOA and MBOA in the extracts. As with the GC-MS, three major hydroxamic acids were found in each of the extracts (FIGURE 12). The three peaks found were HMBOA, DIMBOA, and MBOA with retention times of 6.2 min., 7.6 min., and 10.2 min. respectively. These retention times correspond to that of standards for DIMBOA and MBOA. On line UV spectra (240-400 nm) obtained for all three peaks also corresponded to those of standards and litera-

FIGURE 11

TYPICAL GAS-CHROMATOGRAM AND MASS SPECTRA FOR A HYDROXAMIC ACID EXTRACT FROM ETIOLATED ONE-WEEK-OLD MAIZE SEEDLINGS

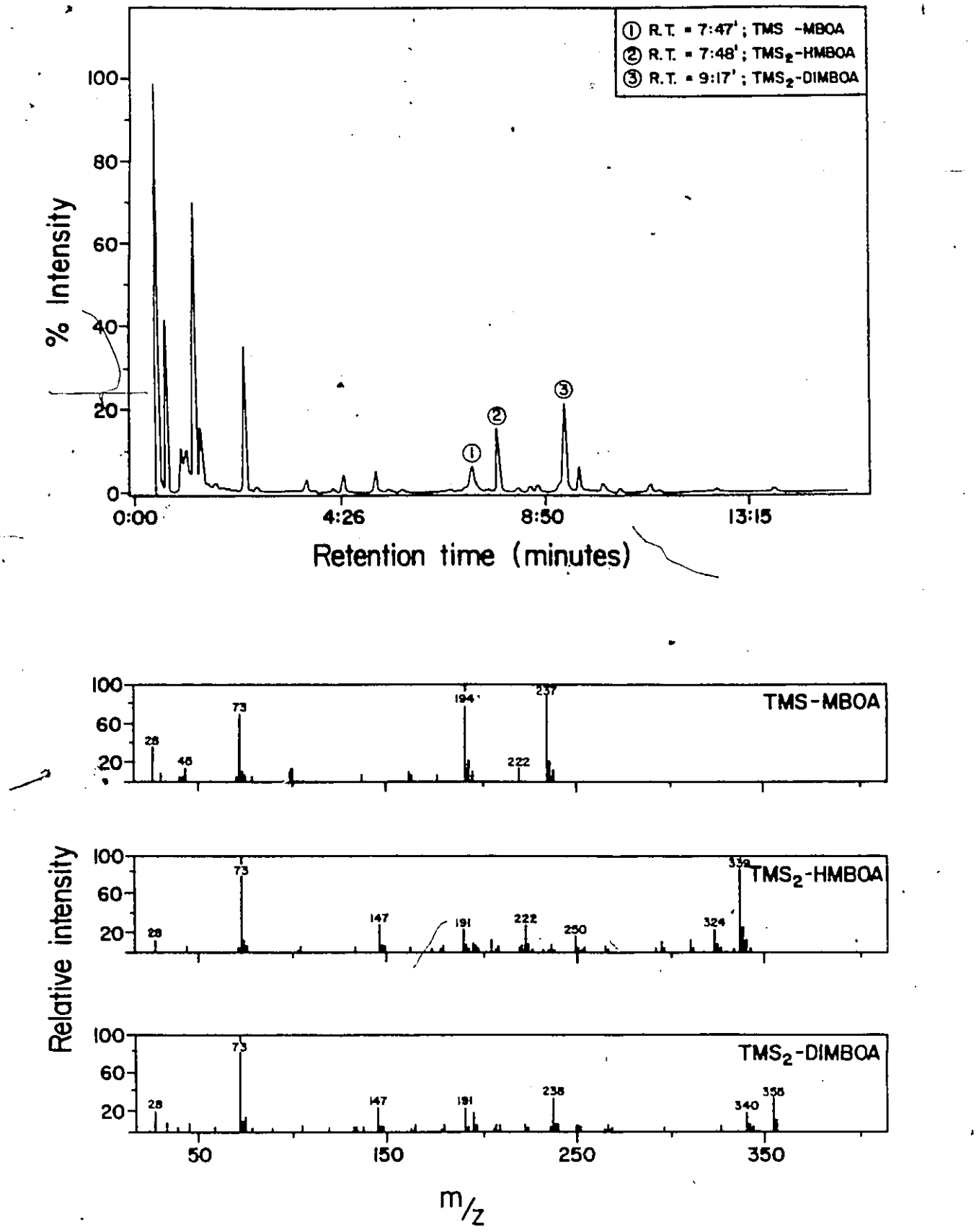
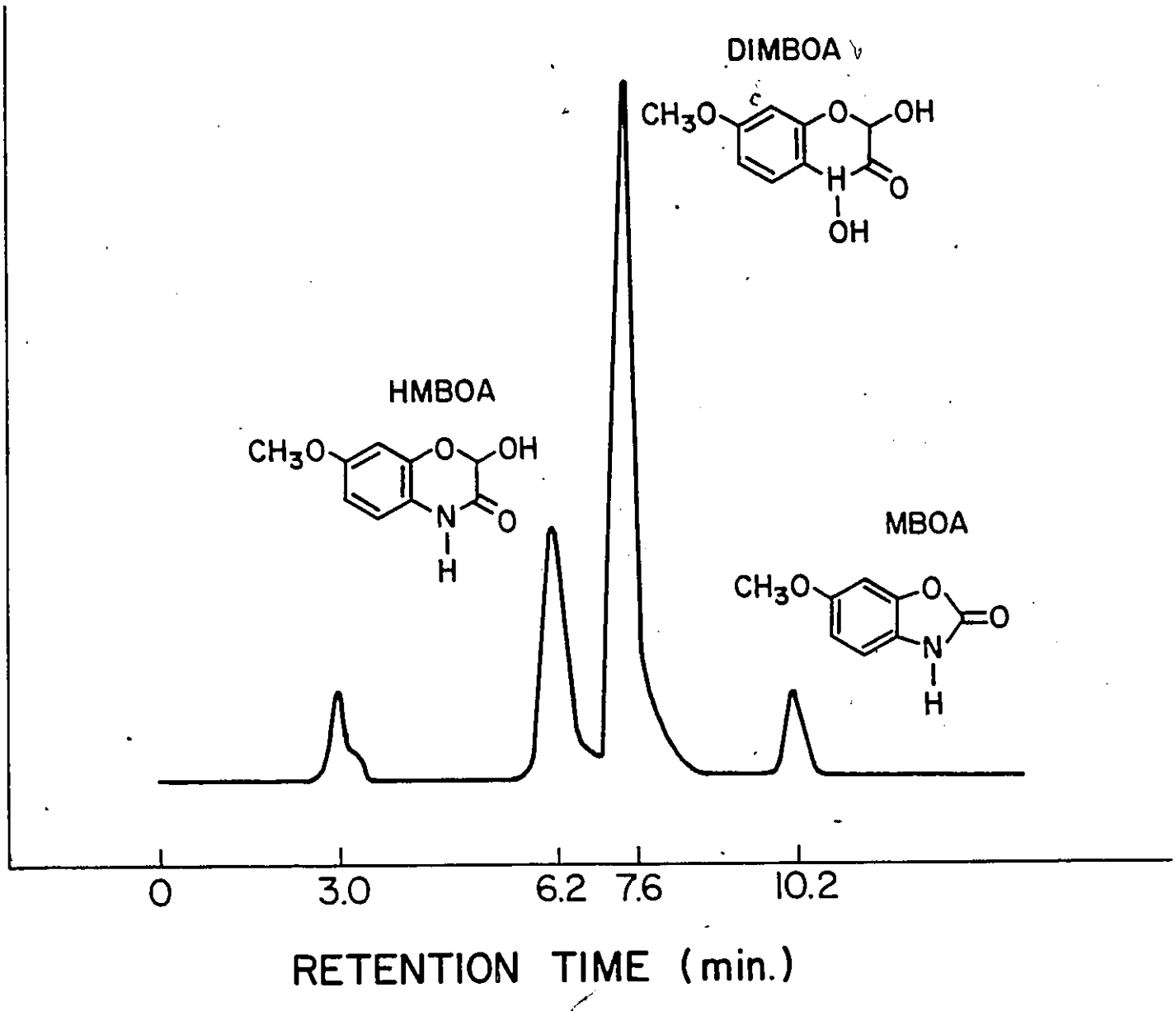

① R.T. = 7:47' ; TMS -MBOA
② R.T. = 7:48' ; TMS2-HMBOA
③ R.T. = 9:17' ; TMS2-DIMBOA
% Intensity
Retention time (minutes)
0:00
4:26
8:50
13:15
TMS-MBOA
TMS2-HMBOA
TMS2-DIMBOA
Relative intensity
m/z

FIGURE 12

TYPICAL HIGH PERFORMANCE LIQUID CHROMATOGRAM FOR A HYDROXAMIC ACID EXTRACT FROM ETIOLATED MAIZE SEEDLINGS

Ultrasphere ODS reverse-phase C18 column; isocratic elution with 40% methanol/58% water/2% acetic acid; flow rate of 1 ml/min; range = 0.100; and a detection wavelength of 265 nm.


DIMBOA
HMBOA
MBOA
0
3.0
6.2
7.6
10.2
RETENTION TIME (min.)

ture (Tipton et al, 1967). The order of elution was as expected for reverse-phase chromatography in that the most polar compound, HMBOA, eluted first followed by the less polar compounds DIMBOA and MBOA. The amounts of each compound varied between each extract; although, not within a given line or race. For all the extracts the consistently largest peak and largest concentration was always that of DIMBOA.

Standard curves created from a dilution series (0 ug - 50 ug) of DIMBOA and MBOA were linear (FIGURE 13). Absorbance units were measured as peak heights from base line to the top of the peak. Linearity of the curves indicated that the response of the detector to different concentrations of DIMBOA and MBOA was also linear. This justifies the use of peak heights and standard curves to calculate the concentrations of both compounds in the extracts.

3.1.3 RECOVERY OF HYDROXAMIC ACIDS FROM EXTRACTS

A 76 +/- 5.3% recovery of MBOA added to tissue is indicated by the slope of 0.764 +/- 0.053 shown in the plot of added vs. found MBOA (FIGURE 14 and APPENDIX 1). An approximate 15% increase in recovery, 90 +/- 7.2%, was found when no tissue was added. Reaction of these compounds to protein thiols is one of the unavoidable problems in quantitation and a major source of yield loss (Perez and Niemeyer,1985; Niemeyer et al, 1982b).

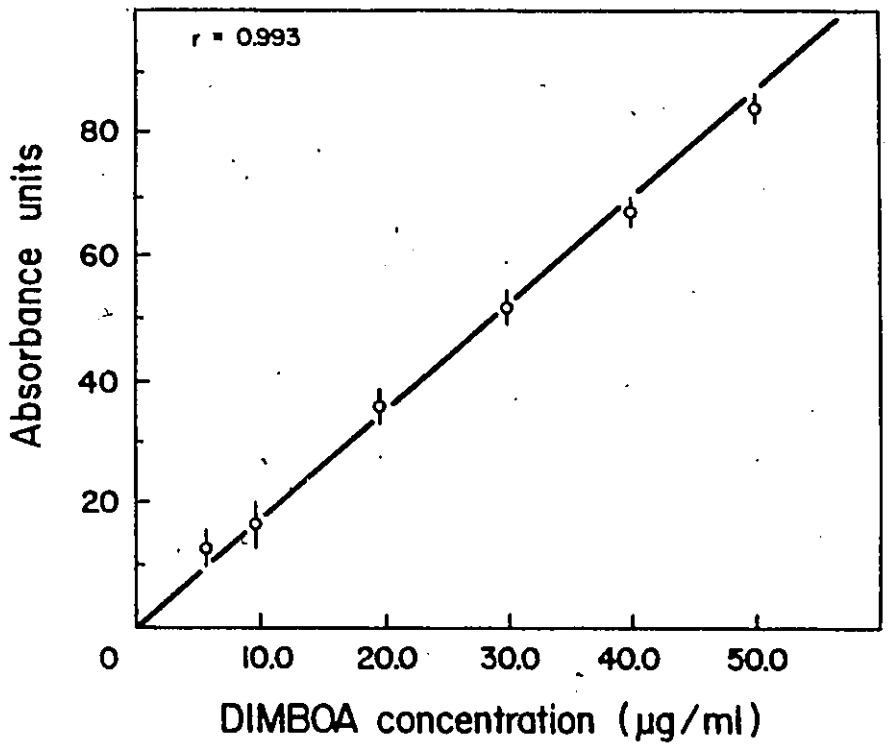
The recovery of DIMBOA when extracted with tissue was quite low, only 61 +/- 5.2% (FIGURE 14 and APPENDIX 1); but this decrease in recovery was accounted for by an increase in the concentration of MBOA. Attempts were made to decrease this degradation to a minimum by optimizing pH, reducing extraction times, and stabilizing temperatures, but some degradation was unavoidable. A substantial increase (28%) in recovery was found when extraction was carried out with solvent alone (recovery of 89 +/- 6.7%). Therefore, with the presence of tissue the degradation to

FIGURE 13

STANDARD CURVES OF DIMBOA AND MBOA

Dilution series (0 ug- 50 ug) of DIMBOA (A) and MBOA (B) were injected into the HPLC system. Resulting absorbance units for each concentration were measured from base line to top of peak (peak height). Vertical bars represent standard deviations.

A


r = 0.993
Absorbance units
0
20
40
60
80
10.0
20.0
30.0
40.0
50.0
DIMBOA concentration (µg/ml)

B

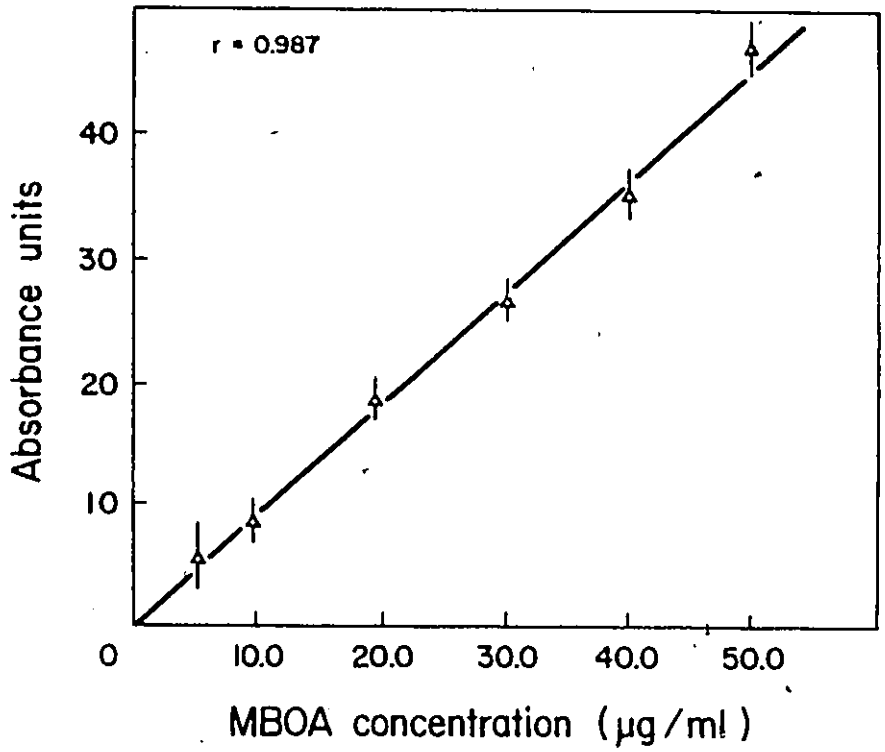

r = 0.987
Absorbance units
0
10
20
30
40
10.0
20.0
30.0
40.0
50.0
MBOA concentration (µg/ml)

FIGURE 14

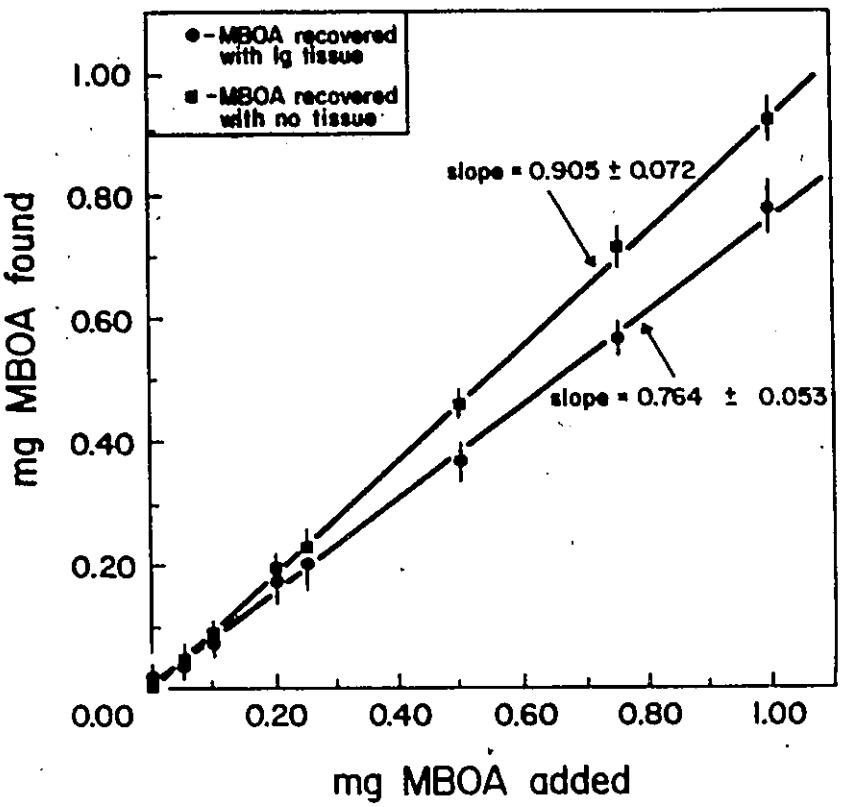
RECOVERY OF MBOA AND DIMBOA FROM EXTRACTS

To test the accuracy of the extraction method, aliquots of MBOA and DIMBOA were added to homogenated tissue samples and processed as with the other extracts in triplicate. Plots were made of added vs. found micrograms of MBOA or DIMBOA. In such plots intercept values with ordinate axes represent the original amounts of MBOA or DIMBOA present in the sample. Triplicated aliquots were also added to solvent alone and extracted without tissue.

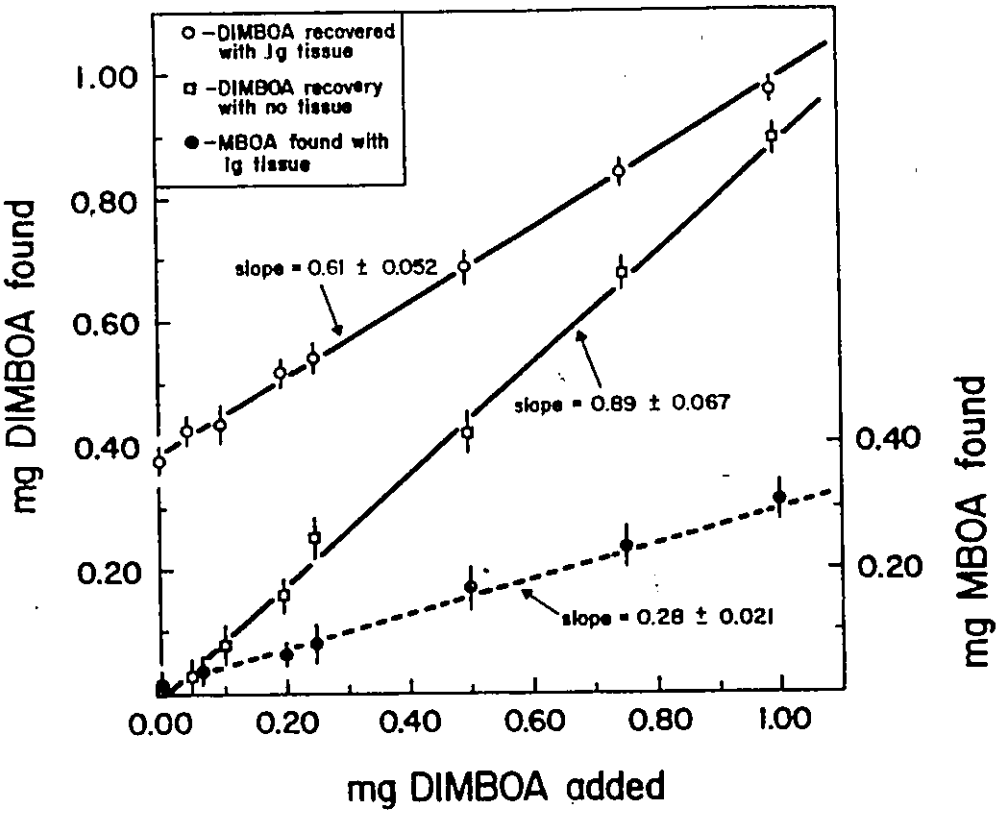
A - extraction of MBOA with tissue yielded a 76% recovery while extraction without tissue yielded a 90% recovery

B - extraction of DIMBOA with tissue yielded a 61% recovery of DIMBOA; remaining DIMBOA was accounted for in an increase in MBOA concentration. Extraction without tissue yielded an 89% recovery of DIMBOA with no breakdown to MBOA.

A


●-MBOA recovered with lg tissue
■-MBOA recovered with no tissue
slope = 0.905 ± 0.072
slope = 0.764 ± 0.053
mg MBOA found
mg MBOA added
0.00
0.20
0.40
0.60
0.80
1.00

B


○-DIMBOA recovered with lg tissue
□-DIMBOA recovery with no tissue
●-MBOA found with lg tissue
slope = 0.61 ± 0.052
slope = 0.89 ± 0.067
slope = 0.28 ± 0.021
mg DIMBOA found
mg MBOA found
mg DIMBOA added
0.00
0.20
0.40
0.60
0.80
1.00

MBOA is increased, since when extracted with solvent alone no degradation to MBOA was found.

3.1.4 TOTAL DIMBOA CONCENTRATIONS

Only DIMBOA and MBOA were quantified in the samples. The lactam, HMBOA, was not quantified for two reasons: one, a pure standard could not be obtained; and two, the role of this compound in resistance is unknown and further studies in this matter were beyond the scope of this study.

Since DIMBOA has been shown to be the major phytochemical in maize associated with resistance, and its degradation product, MBOA, has yet to be shown as a resistance factor, levels of total DIMBOA concentrations were calculated for each line or race based on a pre-determined conversion factor of DIMBOA to MBOA (see Appendix 2 for sample calculation). Mean concentrations of calculated total DIMBOA levels in ug/g fresh weight of etiolated tissue were determined for each line, race, pool, and control line studied (APPENDIX 3) and summarized in box plots (FIGURE 15).

3.1.4.1 LATITUDINAL SERIES OF INBRED LINES

In the latitudinal series of inbred lines DIMBOA levels ranged from 38.3 +/- 7.04 ug/g for Germany 4042, to 387.0 +/- 54.67 ug/g for NTR-1 3962 (APPENDIX 3). Large standard deviations of some lines may be due to the heterozygous nature of these lines which are still undergoing selection. DIMBOA concentration varied both within a given latitudinal grouping and between groupings. On average the lowest concentrations (FIGURE 15) were found in the NTR-2 groups, which is the latitudinal group adapted for use only in the Northern 46° - 52° latitudes. The relationship between latitude and DIMBOA concentrations is further discussed in Section 3.4.

FIGURE 15

'BOX PLOTS OF DIMBOA CONCENTRATIONS

Box plots of the DIMBOA concentrations (ug/g fresh weight of etiolated tissue) for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Concentrations were determined from HPLC analysis of one-week-old maize seedling extracts.

MBR
B73
A619
OH43
Syn. A, B, and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28 and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT Maize Pools
Argentine Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines
500.0
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
5.00
DIMBOA Concentration
(µg/g fr. wt. etiolated seedlings)

3.1.4.2 INDIGENOUS LANDRACES OF MEXICO

On average levels of DIMBOA were lower in the Mexican landraces than in the latitudinal inbred lines (FIGURE 15). Landrace levels ranged from as low as 8.4 +/- 2.89 ug/g for Puebla-537 of the landrace Arrocillo Amarillo (the lowest amount found in all the germplasm studied) to 227.1 +/- 18.28 ug/g for Chiapas-52 of the landrace Olotillo (APPENDIX 3). Concentrations were not consistent between populations for a given landrace, indicating that geographical collection sites rather than landrace groupings may have a greater bearing on DIMBOA levels. This aspect is further discussed in Sections 3.4 and 3.5.

3.1.4.3 ARGENTINE LANDRACES

The Argentine landraces had much higher levels than their Mexican counterparts (FIGURE 15) ranging from 31.4 +/- 10.75 ug/g for Cateto C: 2025 to as high as 460.9 +/- 69.88 ug/g for Cateto C: 2030 (APPENDIX 3). On average, higher levels were found in the Cateto C group.

3.1.4.4 CIMMYT MAIZE POOLS

All of the CIMMYT pools (27, 28, and 30) had relatively high levels of DIMBOA, greater than 170 ug/g (FIGURE 15 and APPENDIX 3). Crosses within these pools also had high levels indicating that perhaps levels of DIMBOA can be increased by crossing two lines with equivalent high levels.

3.1.4.5 SYNTHETIC LINES A, B, AND C

Synthetics A, B, and C had intermediate to high levels of DIMBOA (FIGURE 15 and APPENDIX 3). Large standard deviations may have resulted from an increase

in heterozygousity in the lines due to lack of selection. This is especially true of Synthetic C which is a result of the combination of seventeen inbred lines.

3.1.4.6 CONTROL LINES

The control lines had intermediate to high levels of DIMBOA (FIGURE 15). B73 with a concentration of 471.7 +/- 72.97 ug/g had the highest level of all the germplasm studied (APPENDIX 3). The multi-borer resistant line (MBR), which has been bred for resistance to three major borers had a low level of only 78.9 +/- 11.63 ug/g DIMBOA.

3.1.4.7 HYBRIDIZATION STUDIES

Total DIMBOA concentrations were determined for the progeny of crosses between lines with high DIMBOA levels and equivalent high levels or very low levels. High levels were taken to be greater than 200 ug of DIMBOA per gram fresh weight while low levels were 50 ug/g and less. In addition, high levels were assumed to indicate that a given line is resistant while low levels indicate susceptibility. Therefore, the crosses consisted of those between two resistant lines; a resistant and a susceptible line; and, two susceptible lines.

The majority of the progeny examined were from crosses between two resistant lines since crosses with susceptible lines failed to yield sufficient quantities of seed for study. In all crosses between resistant and resistant lines the resulting progeny had low to intermediate, 50-200 ug/g, levels of DIMBOA (TABLE 10). None of the progeny had DIMBOA levels greater than either of the parents.

In crosses between two susceptible lines all of the progeny had higher levels of DIMBOA then either of the susceptible parents. Only one cross between a resistant parent and a susceptible parent yielded sufficient progeny for study. Mean DIMBOA

TABLE 10

DIMBOA CONCENTRATIONS IN THE PROGENY OF THE HYBRIDIZATION STUDIES

RESISTANT X RESISTANT CROSS		
PARENTAL CROSS	DIMBOA CONCENTRATION (ug/g fr. wt.)[a]	
	CROSS	PROGENY
NTR-1, 3945 X Pool 28, 4107	267.1 +/- 32.59 228.1 +/- 56.52	102.3 +/- 33.16
Pool 28 X 30, 4098 X Cateto C, 2032	392.9 +/- 68.93 421.6 +/- 72.11	65.5 +/- 26.05
NTR-1, 3945 X ITR, 3862	267.1 +/- 32.59 380.6 +/- 44.95	52.5 +/- 44.75
Cateto C, 2032 X Cateto C, 2030	421.6 +/- 72.11 460.9 +/- 69.88	91.5 +/- 26.03
Pool 28 X 30, 4098 X Cateto C, 2030	392.9 +/- 68.93 460.9 +/- 69.88	45.2 +/- 33.51
Cateto C, 2030 X NTR-1, 3947	460.9 +/- 69.88 256.5 +/- 51.89	120.3 +/- 20.69
Cateto C, 2032 X NTR-1, 3945	421.6 +/- 72.11 267.1 +/- 32.59	108.6 +/- 33.83
ITR, 3862 X NTR-1, 3947	380.6 +/- 44.95 256.5 +/- 51.89	118.9 +/- 35.23

a- DIMBOA concentrations determined from one-week-old etiolated maize seedlings (Section 2.2.1)

(DIMBOA CONCENTRATIONS IN THE PROGENY OF THE HYBRIDIZATION STUDIES - CONT'D)

PARENTAL CROSS	DIMBOA CONCENTRATION (ug/g fr. wt.)[a]	
	CROSS	PROGENY
Cateto C, 2030 X ITR, 3862	460.9 +/- 69.88 380.6 +/- 44.95	92.3 +/- 28.83
NTR-2, Poland, 4065 X NTR-1, 3945	320.6 +/- 57.94 267.1 +/- 32.59	233.9 +/- 13.89
Cateto C, 2030 X Pool 28. 4107	460.9 +/- 69.88 228.4 +/- 56.28	128.6 +/- 60.26
Pool 28, 4107 X Pool 28 X 30, 4098	228.4 +/- 56.28 392.9 +/- 68.93	209.2 +/- 24.02
Pool 28, 4107 X NTR-1, 3947	228.4 +/- 56.28 256.5 +/- 51.89	97.5 +/- 73.93
NTR-1, 3947 X Cateto C, 2032	256.5 +/- 51.89 421.6 +/- 72.11	203.1 +/- 31.08
Cateto C, 2032 X NTR-2, Poland, 4065	421.6 +/- 72.11 320.6 +/- 57.94	67.9 +/- 29.35
NTR-1, 3947 X Pool 28, 4107	256.5 +/- 51.89 228.4 +/- 56.28	158.1 +/- 95.92
Pool 28, 4107 X Cateto C, 2032	228.4 +/- 56.28 421.6 +/- 72.11	97.6 +/- 63.64
Cateto C, 2032 X ITR 3862	421.6 +/- 72.11 380.6 +/- 44.95	122.3 +/- 48.10
Pool 28 X 30, 4098 X NTR-1, 3947	392.9 +/- 68.93 256.5 +/- 51.89	146.4 +/- 19.54

a- DIMBOA concentrations determined from one-week-old etiolated maize seedlings (Section 2.2.1)

(DIMBOA CONCENTRATIONS IN THE PROGENY OF THE HYBRIDIZATION STUDIES - CONT'D)

PARENTAL CROSS	DIMBOA CONCENTRATION (ug/g fr. wt.)[a]	
	CROSS	PROGENY
NTR-1, 3945 X Pool 28 X 30, 4098	267.1 +/- 32.59 392.9 +/- 68.93	45.5 +/- 15.11
Pool 28 X 30, 4098 X Cateto C, 2032	392.9 +/- 68.93 421.6 +/- 72.11	92.0 +/- 43.26
SUSCEPTIBLE X SUSCEPTIBLE CROSS		
NTR-2, Germany, 4042 X Cateto C, 2025	38.3 +/- 7.04 31.4 +/- 10.75	52.7 +/- 14.82
NTR-2, Germany, 4042 X NTR-2, Canada, 4081	38.3 +/- 7.04 40.9 +/- 5.97	69.1 +/- 14.81
NTR-2, Canada, 4081 X NTR-2, Canada, 4071	40.9 +/- 5.97 49.0 +/- 6.21	99.3 +/- 40.24
NTR-2, Canada, 4081 X Cateto C, 2025	40.9 +/- 5.97 31.4 +/- 10.75	44.7 +/- 17.08
RESISTANT X SUSCEPTIBLE CROSS		
Pool 28 X 30, 4098 X Cateto C, 2025	392.9 +/- 68.93 31.4 +/- 10.75	54.5 +/- 18.08

a- DIMBOA concentrations determined from one-week-old etiolated maize seedlings (Section 2.2.1)

levels for this progeny were higher than that of the susceptible parent yet much lower than that of the resistant parent.

In all crosses the standard deviation of the mean DIMBOA concentrations of the progeny was large. This may be due to the heterozygous nature of the progeny since they are a result of two widely different lines not all of which are entirely homozygous themselves.

3.2 RESISTANCE STUDIES

3.2.1 LABORATORY LEAF FEEDING TESTS

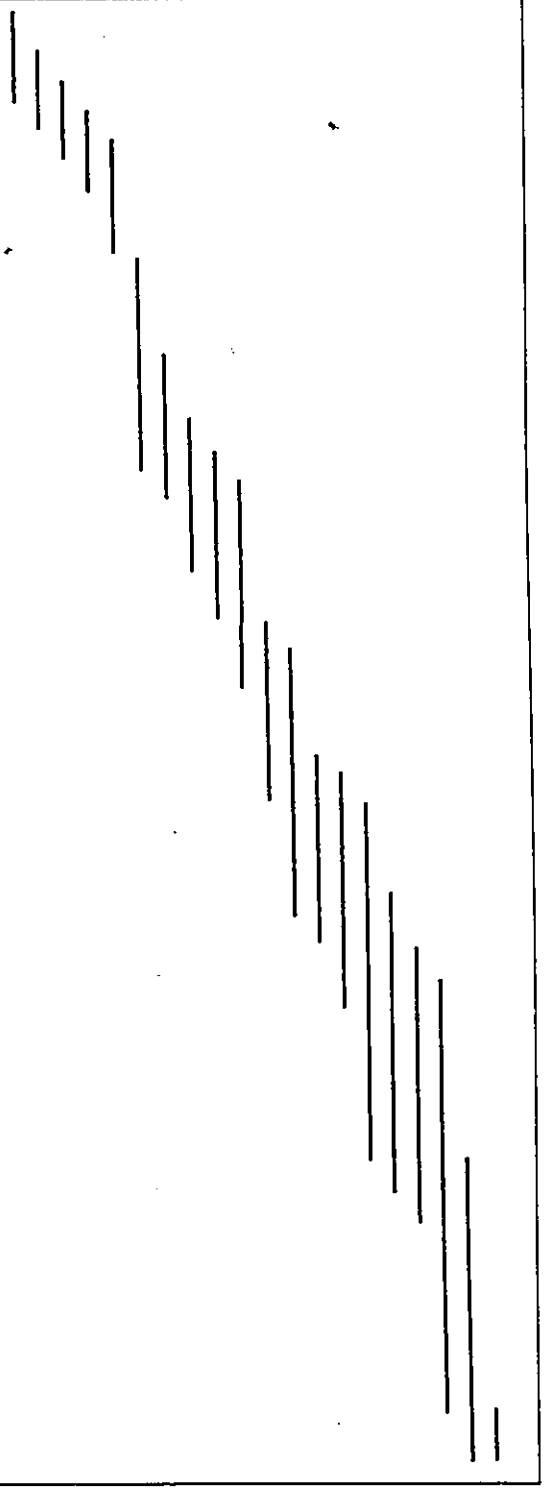
The laboratory leaf feeding test apparatus consisting of a system of petri plates was found to be quite satisfactory and yielded statistically significant results that differentiated between the various lines and races examined. A total of forty different lines and races were tested using greenhouse grown whorl tissue. TABLE 11 lists the mean percent leaf consumptions for each line or race. Percent consumptions ranged from 11.0 +/- 3.98% for NTR-2, Poland: 4065, to 90.5 +/- 2.90% for Nayarit-24 of the landrace Harosino de Ocho.

A high percent leaf consumption was taken to indicate a low level of leaf feeding resistance. Consumption values greater than 50% for the majority of the landraces indicates that they have a lower resistance to leaf feeding then many of the inbred lines which were consumed little, 11.0%. As expected the two control lines, A619 and OH43, had low consumption values of 29.5% and 21.5% respectively. The multi-borer resistant line (MBR) was also little consumed, rating 16,8%.

Linear regression and Pearson's correlations were used to examine the relationship between etiolated seedling DIMBOA concentrations and laboratory leaf feeding. As expected, there was a negative correlation (r= -0.572, p=0.0001) of the DIMBOA concentration with percent consumption, FIGURE 16. The landraces (represented by

TABLE 11
LABORATORY LEAF-FEEDING RATINGS ON GREENHOUSE GROWN WHORL TISSUE

LINE OR RACE	N	PERCENT LEAF CONSUMPTION[a] MEAN +/- STANDARD DEVIATION
NAYARIT- 24	20	90.5 +/- 2.90
MEXICO- 461	20	87.1 +/- 7.77
NAYARIT- 39	20	83.5 +/- 12.89
MEXICO- 208	40	81.6 +/- 5.12
NTR-2, SWITZ., 4034	40	77.3 +/- 2.33
MEXICO- 212	20	74.5 +/- 19.91
MEXICO-55	20	72.5 +/- 3.30
PUEBLA- 463	20	69.1 +/- 1.52
MORELOS- 52	20	59.7 +/- 5.24
V-520-C	20	59.3 +/- 5.63
SINALOA- 2	20	58.5 +/- 5.48
CHIAPAS- 236	20	56.0 +/- 8.61
GUANAJUATO- 102	20	55.6 +/- 7.64
CHIAPAS- 218	40	51.7 +/- 6.11
NTR-2, HOLL., 4022	20	50.3 +/- 17.41
NTR-2, GERM., 4042	20	47.7 +/- 3.17
GUANAJUATO- 207	20	46.2 +/- 4.82
OAXACA- 4	20	45.1 +/- 5.10
CATETO E, 2051	20	45.1 +/- 14.50
NTR-2, POL., 4064	20	42.1 +/- 9.51
CATETO C, 2025	20	38.9 +/- 8.95
GUANAJUATO- 101	20	38.3 +/- 3.90
STR, 3802	40	35.6 +/- 9.63
ITR, 3862	20	35.5 +/- 14.32
OAXACA- 130	20	34.9 +/- 8.95
GUANAJUATO- 93A	20	34.3 +/- 5.20
ITR, 3878	20	33.4 +/- 15.14
OAXACA- 179	20	31.2 +/- 2.01
JALISCO-222	20	30.0 +/- 3.49
A619	40	29.5 +/- 4.88
POOL 28 X 30, 4098	40	28.4 +/- 6.42
POOL 30, 4108	40	24.2 +/- 3.79
CATETO C, 2030	20	24.2 +/- 7.47
NTR-1, 3945	20	23.3 +/- 4.71
NAYARIT- 72	20	22.0 +/- 4.58
NTR-2, CAN., 4081	40	21.9 +/- 2.98
NTR-1, 3947	40	21.8 +/- 5.48
CATETO C, 2032	40	21.8 +/- 2.14
OH43	40	21.5 +/- 6.21
NTR-1, 3962	20	19.4 +/- 8.67
CHIAPAS- 237	20	17.6 +/- 3.62
YUCATAN- 16	20	17.2 +/- 3.51
STR, 3823	20	16.8 +/- 6.51
MBR	40	15.6 +/- 2.78
POOL 28, 4107	20	15.4 +/- 3.55
CHIAPAS- 235	20	15.1 +/- 4.80
NTR-2, CAN., 4071	40	14.2 +/- 2.48
NTR-2, POL., 4065	20	11.0 +/- 3.98

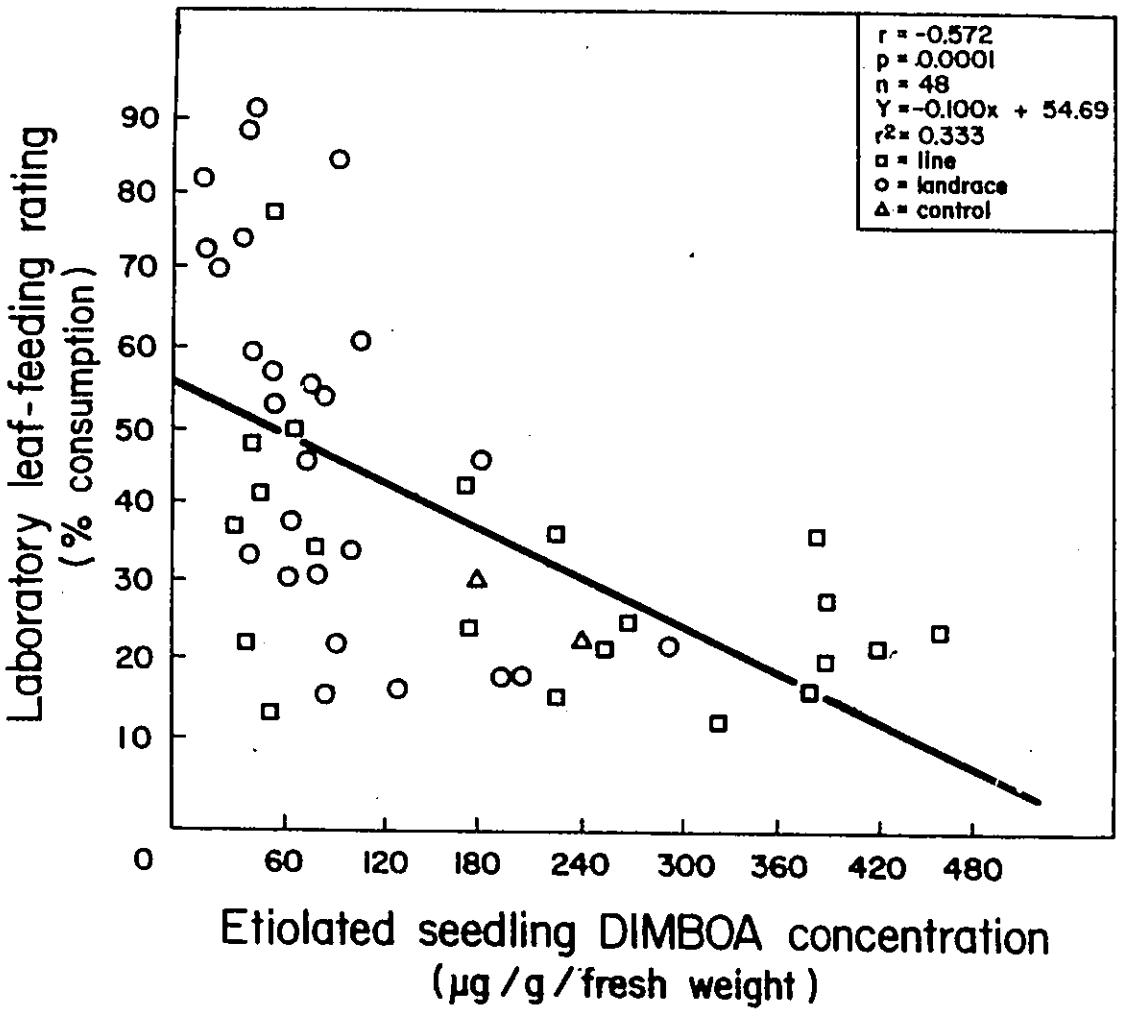


a= means opposite continuous lines not significantly different at 5% level of probability using Duncan's multiple range test

FIGURE 16

GRAPH OF LABORATORY LEAF-FEEDING RATING VS. ETIOLATED SEEDLING DIMBOA CONCENTRATIONS

Leaf-feeding ratings were taken as percent consumption of a 2.5 cm diameter circular area of whorl leaf tissue by two third instar European corn borer larvae in a period of 48 hours. DIMBOA concentrations were quantitated by HPLC analysis on extracts from 1 g of etiolated one-week-old maize seedlings.


r = -0.572
p = 0.0001
n = 48
Y = -0.100x + 54.69
r² = 0.333
□ = line
○ = landrace
△ = control
Laboratory leaf-feeding rating
(% consumption)
0
10
20
30
40
50
60
70
80
90
60
120
180
240
300
360
420
480
Etiolated seedling DIMBOA concentration
(µg / g / fresh weight)

circles in FIGURE 16) were the most consumed and had the lowest levels of DIMBOA. The inbred lines (represented by squares) varied in their DIMBOA concentrations, but all had consistently low consumption rates. This difference between the inbred lines and the landraces is further illustrated in FIGURE 17. When analysed separately from the inbred lines the landraces had a higher negative (m=-0.257) slope (A) than that (m=-0.055) of the inbreds (B) suggesting that DIMBOA concentration has a greater effect on percent consumption in the landraces than in the inbreds.

The control lines (indicated by triangles) all had intermediate to high levels of DIMBOA and low percent leaf consumptions.

3.2.2 FIELD STUDIES

3.2.2.1 EUROPEAN CORN BORER FIELD LEAF DAMAGE RATINGS

The resistance of each line or race to first-brood borer damage was determined by artificially infesting the plants then rating the amount of leaf feeding damage using the nine class rating scale of Guthrie et al (1960). Ratings were interpreted in the following manner: a line or race with a rating of 1 or 2 (little or no damage) was considered to be highly resistant; a rating of 3 or 4 was considered resistant; a rating of 5 and 6 indicated intermediate resistance; and, a rating of 7 to 9 was considered to be highly susceptible.

Box plots of the field leaf damage ratings for the major groups of lines and races are shown in FIGURE 18 (mean values for each line or race are tabulated in APPENDIX 4, and 5). The latitudinal series of inbred lines ranged from highly resistant to resistant; Mexican landraces were intermediate to susceptible in resistance; the Argentine landraces were highly resistant to resistant; the CIMMYT maize pools were all resistant; Synthetics A, B, and C were highly resistant to resistant; and, the control lines and multi-borer resistant line (MBR) were all resistant. The most significant

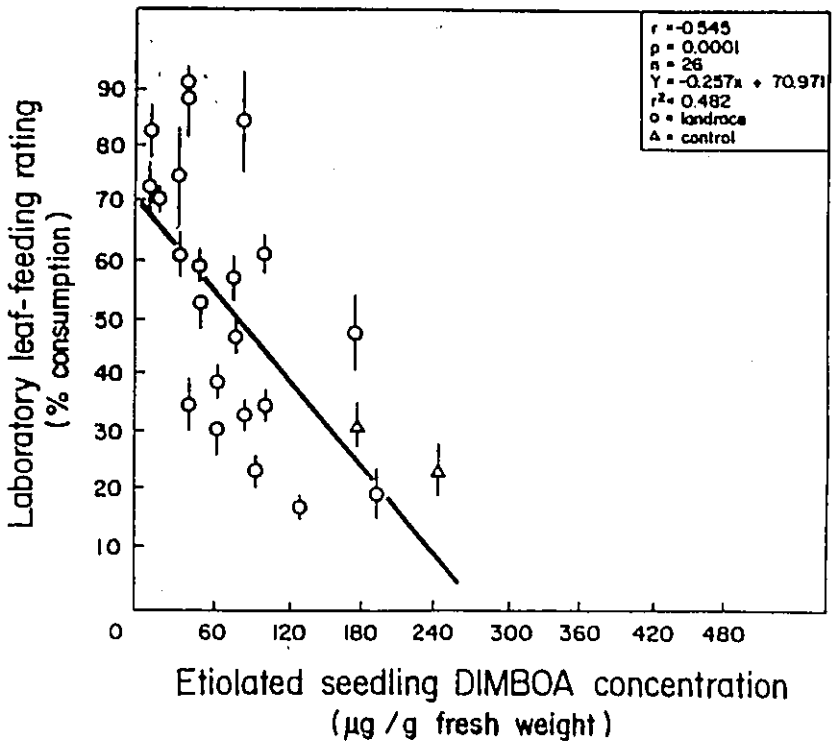
FIGURE 17

GRAPH OF LABORATORY LEAF-FEEDING RATING VS. ETIOLATED SEEDLING DIMBOA CONCENTRATION FOR THE INDIGENOUS LANDRACES OF MEXICO AND THE INBRED LINES

A - Indigenous Landraces of Mexico
B - Latitudinal Series of Inbred Lines

Leaf-feeding ratings were taken as percent consumption of a 2.5 cm diameter circular area of whorl leaf tissue by two third-instar European corn borer larvae in a period of 48 hours. DIMBOA concentrations were quantitated by HPLC analysis on extracts from 1 g of etiolated one-week-old maize seedlings.

A


r = -0.545
p = 0.0001
n = 26
Y = -0.257x + 70.971
r² = 0.482
o = landrace
Δ = control
Laboratory leaf-feeding rating
(% consumption)
0
10
20
30
40
50
60
70
80
90
60
120
180
240
300
360
420
480
Etiolated seedling DIMBOA concentration
(µg / g fresh weight)

B

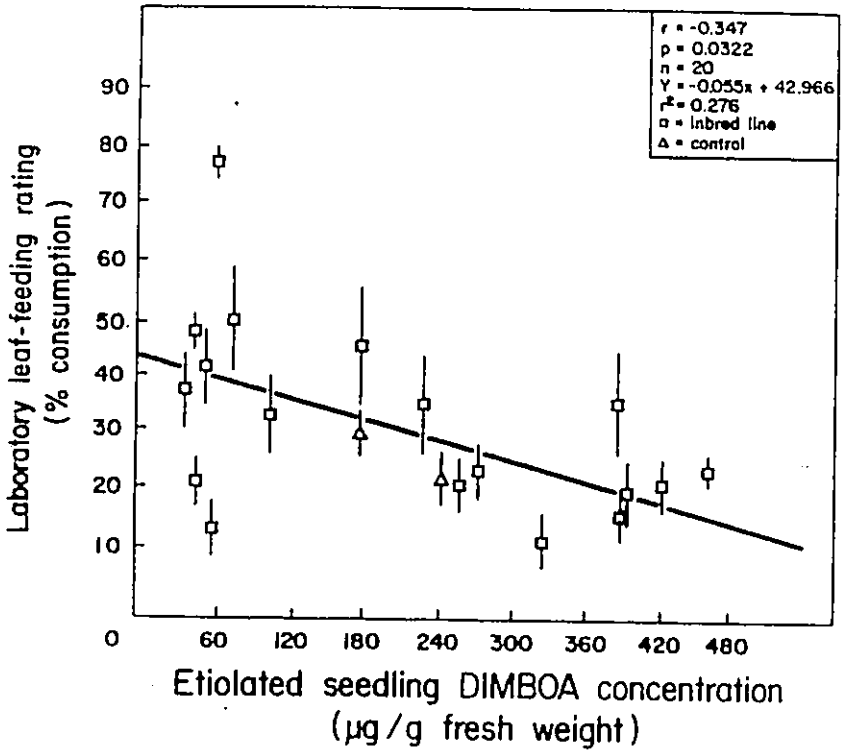
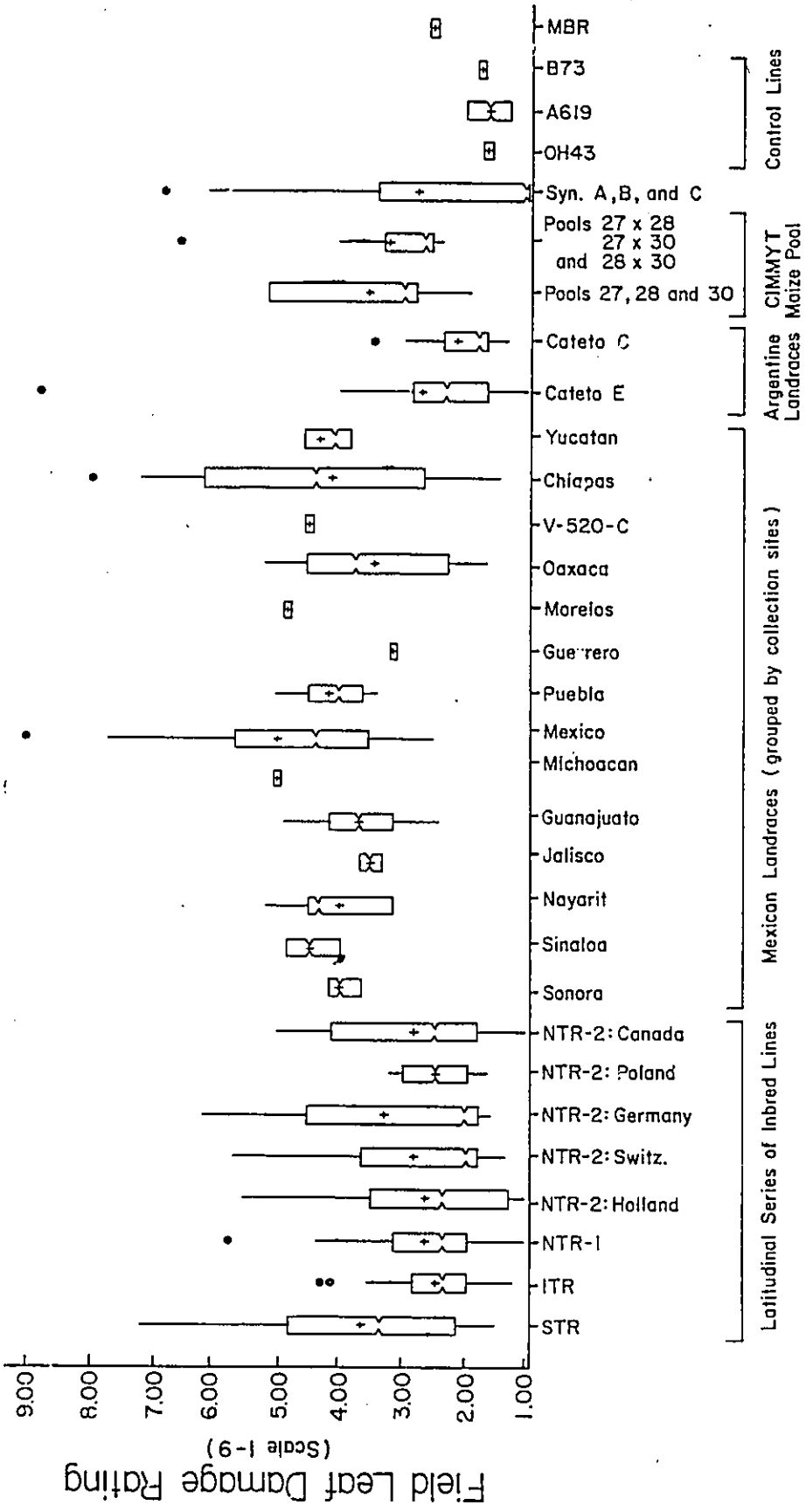

r = -0.347
p = 0.0322
n = 20
Y = -0.055x + 42.966
r² = 0.276
□ = inbred line
Δ = control
Laboratory leaf-feeding rating
(% consumption)
0
10
20
30
40
50
60
70
80
90
60
120
180
240
300
360
420
480
Etiolated seedling DIMBOA concentration
(µg / g fresh weight)

FIGURE 18

BOX PLOTS OF MEAN FIELD LEAF DAMAGE RATINGS

Box plots of the mean field leaf damage rating for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Ratings were made using the 1-9 rating scale developed by Guthrie et al (1960). Larger ratings indicate increased damage and susceptibility.


MBR
B73
A619
OH43
Syn. A, B, and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28 and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT Maize Pool
Argentine Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
(Scale 1-9)
Field Leaf Damage Rating

results from this study is the fact that the Mexican landraces as a group differed from the rest of the lines and races and were the most susceptible overall.

The uninfested six plants in the front 1.2 m of each row were rated to see what the level of natural infestation damage was. All of these plants rated 1 to 2 (APPENDIX 6) on the scale of leaf damage and were therefore rated as highly resistant, due to lack of infestation.

3.2.2.2 PLANT HEIGHT MEASUREMENTS

TABLE 12 lists the mean plant heights for stressed (high density) and non-stressed plants of each major group of lines and races studied. In all cases the standard deviations of the mean heights for stressed plants are much higher than those of the non-stressed plants. Therefore, a larger range in plant height was found when the plants were stressed. The mean heights were not significantly different between stressed and non-stressed plants.

FIGURE 19 is a box plot of mean plant heights of the major groups of lines and races studied (actual values are tabulated in APPENDIX 4 and 5). The tallest groups were the Mexican landraces and the CIMMYT maize pools (2.0 to 3.0 m). All other lines and races were of normal height, 1.5 - 2 meters.

3.2.2.3 LEAF COUNTS AND DAYS TO SILKING

The latitudinal series of inbred lines, Argentine landraces, CIMMYT maize pools, synthetic lines, and control lines all had 15 to 18 leaves per plant (TABLE 13); mean values for each line and race are tabulated in APPENDIX 7. The Mexican landraces had on average 16 to 22 leaves. It was noted that not only do these races have more leaves, but they were much taller plants with generally thicker densely pubescent stalks (3-5 cm dia.), and often have more than three large tillers. The multi-borer resistant line had a mean number of 23 leaves.

TABLE 12
PLANT HEIGHTS FOR STRESSED AND NON-STRESSED PLANTS

LINE OR RACE GROUPING	MEAN PLANT HEIGHT +/- S.D. NON-STRESSED[a]	STRESSED[b]
LATITUDINAL INBREDS		
STR	148.4 +/- 9.63	152.8 +/- 38.73
ITR	153.2 +/- 2.18	153.3 +/- 34.60
NTR-1	150.5 +/- 8.53	146.9 +/- 31.73
NTR-2		
HOLLAND	147.1 +/- 9.13	154.3 +/- 34.67
SWITZERLAND	155.1 +/- 8.97	159.1 +/- 23.41
GERMANY	134.7 +/- 8.86	144.7 +/- 26.55
POLAND	144.7 +/- 2.68	147.7 +/- 25.65
CANADA	135.4 +/- 6.59	135.7 +/- 28.67
MEXICAN LANDRACES[c]		
SONORA	215.4 +/- 1.41	208.1 +/- 20.90
SINALOA	213.6 +/- 3.96	195.5 +/- 12.39
NAYARIT	231.4 +/- 8.91	222.9 +/- 15.24
JALISCO	194.3 +/- 8.42	205.2 +/- 10.29
GUANAJUATO	207.7 +/- 5.01	221.7 +/- 17.13
MICHOACAN	278.0 +/- 6.41	240.0 +/- 8.15
MEXICO	201.8 +/- 7.67	207.1 +/- 17.44
PUEBLA	199.1 +/- 4.28	218.1 +/- 17.55
GUERRERO	199.0 +/- 8.65	170.2 +/- 18.65
MORELOS	200.0 +/- 6.85	255.4 +/- 19.56
OAXACA	216.4 +/- 4.83	211.5 +/- 23.21
V-520-C	207.6 +/- 8.66	261.9 +/- 10.85
CHIAPAS	200.8 +/- 5.48	237.3 +/- 28.78
YUCATAN	200.0 +/- 1.41	193.3 +/- 18.21
ARGENTINE LANDRACES		
CATETO C	149.4 +/- 5.58	147.3 +/- 23.04
CATETO E	157.6 +/- 9.26	159.4 +/- 25.75
CIMMYT MAIZE POOLS		
POOLS 27,28,30	148.3 +/- 9.87	170.8 +/- 28.43
POOL CROSSES	203.7 +/- 4.94	185.3 +/- 23.40
SYNTHETIC LINES	132.1 +/- 5.54	144.3 +/- 33.07
CONTROL LINES		
OH43	138.4 +/- 6.27	138.2 +/- 21.49
A619	152.8 +/- 3.63	132.3 +/- 28.21
B73	146.6 +/- 2.81	170.2 +/- 36.73
MBR LINE	153.0 +/- 8.74	171.0 +/- 45.10

a= front six plants grown in the front (East) 1.5 meters of each row (4 plants/meter)
b= back ten plants grown in the back 1.5 meters of each row and exposed to high density planting (6.7 plants/meter), borer infestion and stalk rot infection
c= Mexican landraces grouped by collection sites

FIGURE 19

BOX PLOTS OF MEAN PLANT HEIGHTS

Box plots of the mean plant heights (cm) for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Plant height was measured from soil level to the tip of the extended tassel or the tip of the longest extended leaf in those lines or races that did not flower. These measurements were used in calculating the tunneling/height ratios as a measure of resistance to borer tunneling.

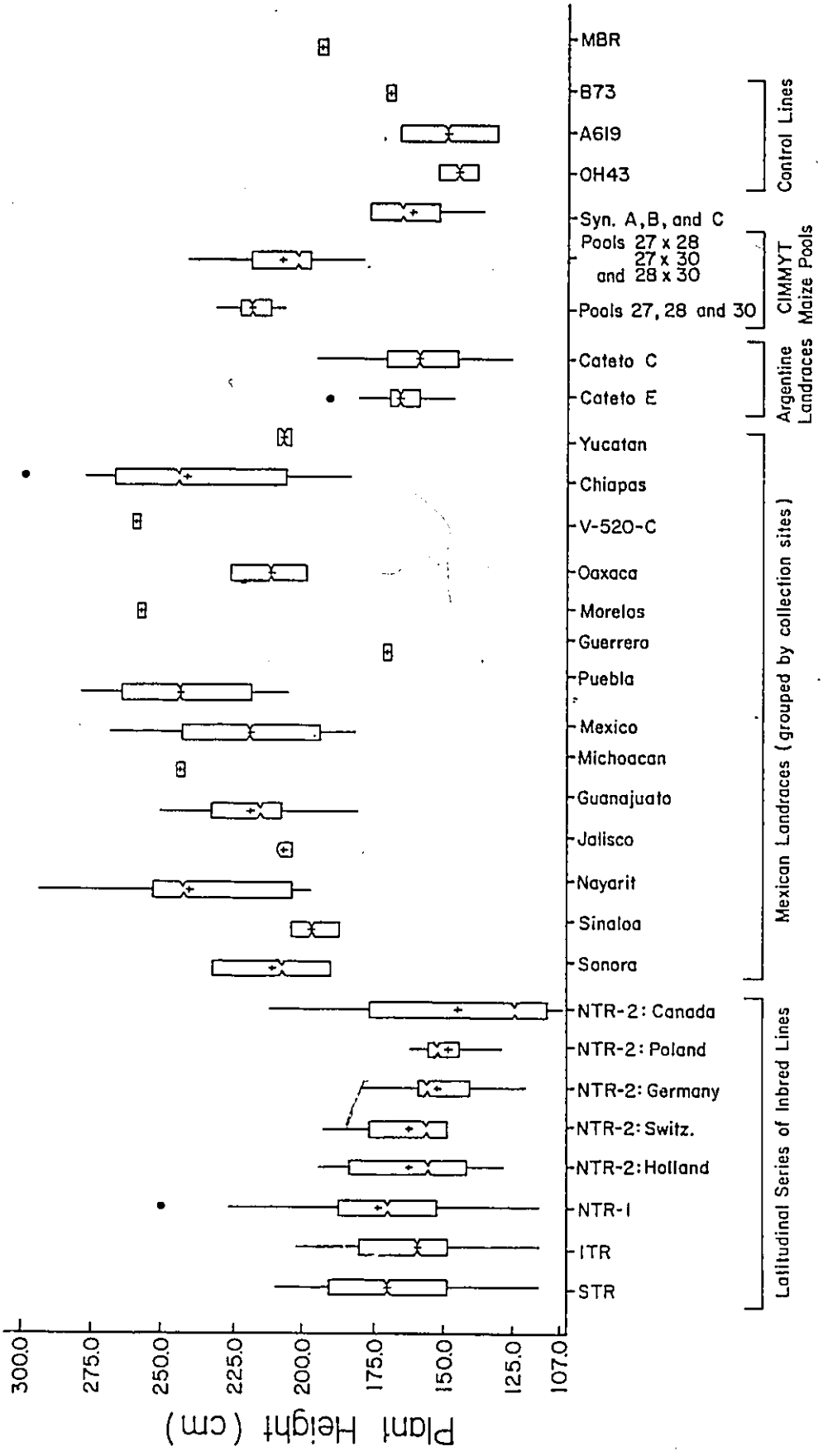

MBR
B73
A619
OH43
Syn. A, B, and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28 and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT Maize Pools
Argentine Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines
300.0
275.0
250.0
225.0
200.0
175.0
150.0
125.0
107.0
Plant Height (cm)

TABLE 13
MEAN LEAF COUNTS AND DAYS TO SILKING FOR THE MAJOR GROUPS OF LINES AND RACES STUDIED

LINE OR RACE GROUPING	MEAN NUMBER OF LEAVES PER PLANT	DAYS TO SILKING[a]
LATITUDINAL INBREDS		
STR	17.8 +/- 0.79	94
ITR	15.8 +/- 1.38	81
NTR-1	16.6 +/- 0.83	93
NTR-2		
HOLLAND	15.8 +/- 0.85	84
SWITZERLAND	15.5 +/- 1.22	85
GERMANY	16.2 +/- 1.39	84
POLAND	15.6 +/- 1.11	83
CANADA	14.5 +/- 1.73	76
MEXICAN LANDRACES[b]		
SONORA	20.9 +/- 3.06	---
SINALOA	21.1 +/- 4.38	---
NAYARIT	21.9 +/- 4.50	---
JALISCO	22.3 +/- 2.40	---
GUANAJUATO	19.9 +/- 1.35	---
MICHOACAN	20.8 +/- 0.84	---
MEXICO	16.4 +/- 1.18	98 to ---
PUEBLA	16.9 +/- 1.84	---
GUERRERO	15.4 +/- 0.89	---
MORELOS	20.6 +/- 1.52	---
OAXACA	20.6 +/- 1.22	90 to ---
V-520-C	22.0 +/- 1.00	---
CHIAPAS	19.1 +/- 4.32	83 to ---
YUCATAN	19.8 +/- 2.83	---
ARGENTINE LANDRACES		
CATETO C	15.0 +/- 0.63	80
CATETO E	13.4 +/- 2.51	83
CIMMYT MAIZE POOLS		
POOLS 27,28,30	18.1 +/- 2.27	92
POOL CROSSES	16.7 +/- 1.03	83
SYNTHETIC LINES	14.3 +/- 1.63	78
CONTROL LINES		
OH43	17.2 +/- 0.84	95
A619	18.0 +/- 2.71	92
B73	19.8 +/- 0.45	95
MBR LINE	23.0 +/- 1.00	83

a= days to silking when 50% of the plants were showing silk
b= Mexican landraces grouped by collection sites
---= no silking date available since plants did not flower before harvest, 149 days

In the latitudinal series of inbred lines the earliest group to silk was NTR-2, Canada (76 days), while the latest was the STR group (94 days),(TABLE 13 and APPENDIX 7). Days to silking were not obtained for the Mexican landraces except for populations Oaxaca-179, Mexico-55, Chiapas-140, Mexico-212, Mexico-208, Mexico-5, and Chiapas-133. The Argentine landraces showed silk at 80 days for the Cateto C group and 83 days for Cateto E. The CIMMYT maize pools showed silk at 92 days and their F1 hybrids at 83 days. The synthetic lines showed silk at 76 days. All of the control lines, OH43, A619, and B73, showed silk at three of the latest dates obtained, 95, 92 and 95 respectively. Finally, the multi-borer resistant line showed silk at 83 days.

3.2.2.4 SMUT RATINGS

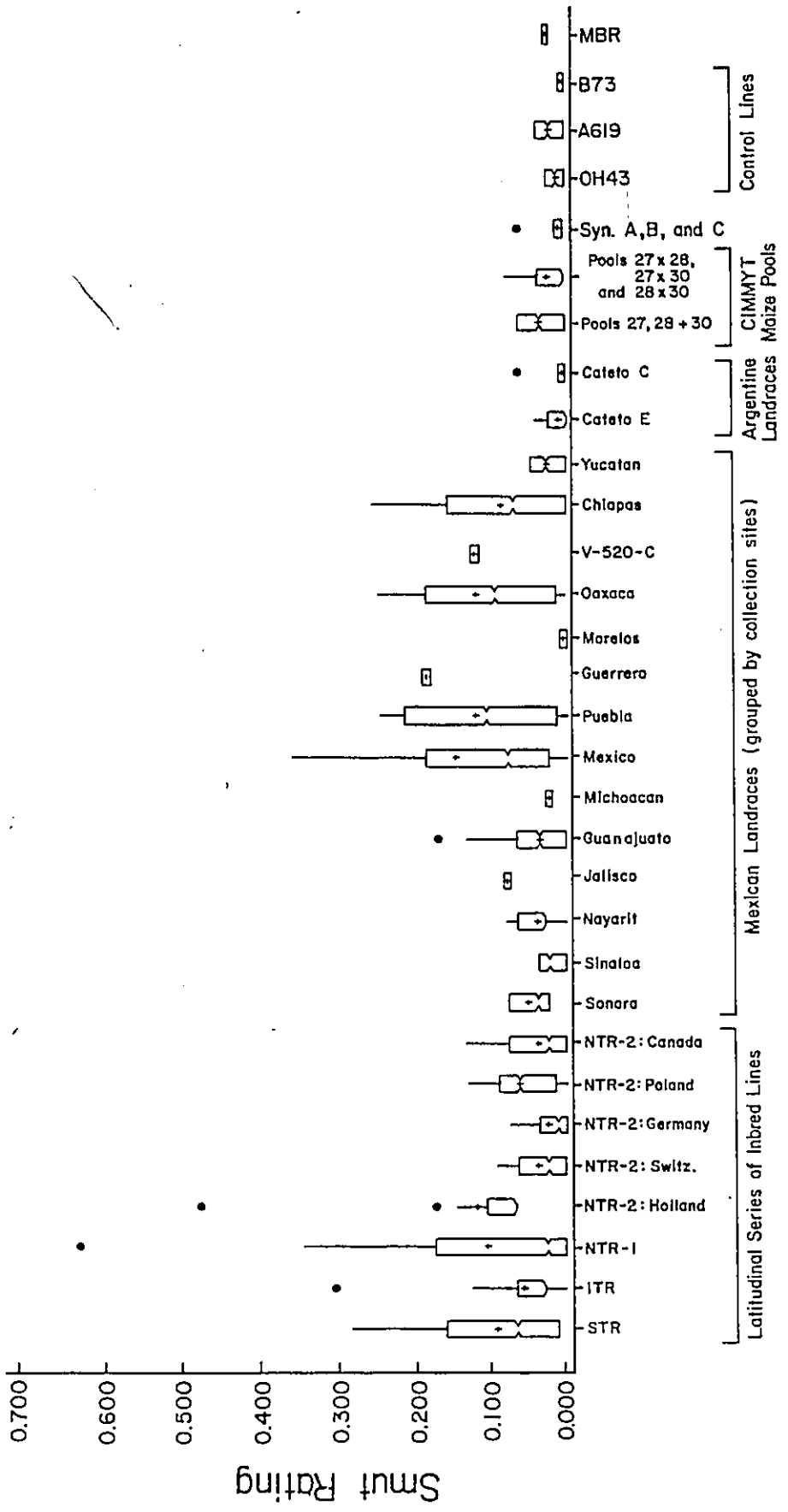
Means of the number of plants infected with smut (rating of 1.0) were calculated for each line or race (APPENDIX 4 and 5) then averaged for each major group of lines or races studied. A box plot of the latter (FIGURE 20) shows that the majority of the lines and races studied had smut ratings below 0.300; therefore, out of the eight plants examined in the back 1.5 m of each row three or less were infected with smut (approximately 38% infected).

A broad range of smut ratings was found for the latitudinal series of inbred lines and for some of the Mexican landrace populations (Mexico, Puebla, Oaxaca, and Chiapas). Both of these groups had lines and races with little or no smut infection while others had a 20-30% infection. The Argentine landraces had almost 0% infection along with the control lines, the multi-borer resistant line, and most of the CIMMYT maize pools. The six uninfested and low density plants in the front 1.2 m of each row had 0% infection for almost every line or race studied (APPENDIX 6).

FIGURE 20

BOX PLOTS OF MEAN SMUT RATINGS

Box plots of the mean smut ratings for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Plant were rated on absence or presence of smut, then total counts were averaged for each line.


MBR
B73
A619
OH43
Control Lines
Syn. A,B, and C
Pools 27x28, 27x30 and 28x30
Pools 27, 28 + 30
CIMMYT Maize Pools
Cateto C
Cateto E
Argentine Landraces
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
Mexican Landraces (grouped by collection sites)
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Latitudinal Series of Inbred Lines
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Smut Rating

3.2.2.5 EUROPEAN CORN BORER DAMAGE RATINGS AT HARVEST

Two measures of resistance to European corn borer stalk damage were taken: plant breakage and borer tunneling/height ratio. The tunneling of borer larvae often results in stalk or plant breakage thereby rendering a plant unharvestable. FIGURE 21 is a box plot of the mean plant breakage ratings obtained for the major groups of lines and races studied (actual values are tabulated in APPENDIX 4 and 5). Overall, the Mexican landraces exhibited the greatest degree of resistance with mean ratings of 2.0 and less with the exception of those from the state of Mexico. The latitudinal series of inbred lines were less resistant (mean ratings of 3.0 to 4.0) and had wider ranges of ratings within a given latitudinal group. The Argentine landraces with ratings similar to those of the latitudinal series were less resistant than their Mexican counterparts. The CIMMYT maize pools were highly resistant with mean ratings of 2.0. Synthetics A, B, and C with ratings between 3.5 and 5.0 were less resistant, while the control lines varied in their resistance (1.3 to 4.0) with B73 being the most resistant of the three (1.3). The multi-borer resistant line was rated as resistant with a rating of 2.3.

Measurements taken on the uninfested six plants in the front 1.2 m of each row showed that almost all plants had breakage ratings of 1.0 to 1.5 as a result of little or no damage (APPENDIX 6).

A box plot of mean tunneling/height ratios for each major group of lines and races (FIGURE 22) shows that the most resistant group, indicated by the lower ratios, was the Mexican landraces with mean ratios no higher than 0.075 (APPENDIX 4 and 5). The Argentine landraces were much more susceptible with ratings 0.125 to 0.150, almost two times higher than the Mexican races. The latitudinal series of inbred lines also had high ratios, but were more variable, ranging from mean ratios of 0.80 to 0.15. The CIMMYT maize pools were resistant (0.050). The synthetic lines (0.075) and the control (0.060 to 0.100) lines were less resistant than the Mexican landraces. The

FIGURE 21

BOX PLOTS OF MEAN PLANT BREAKAGE RATINGS

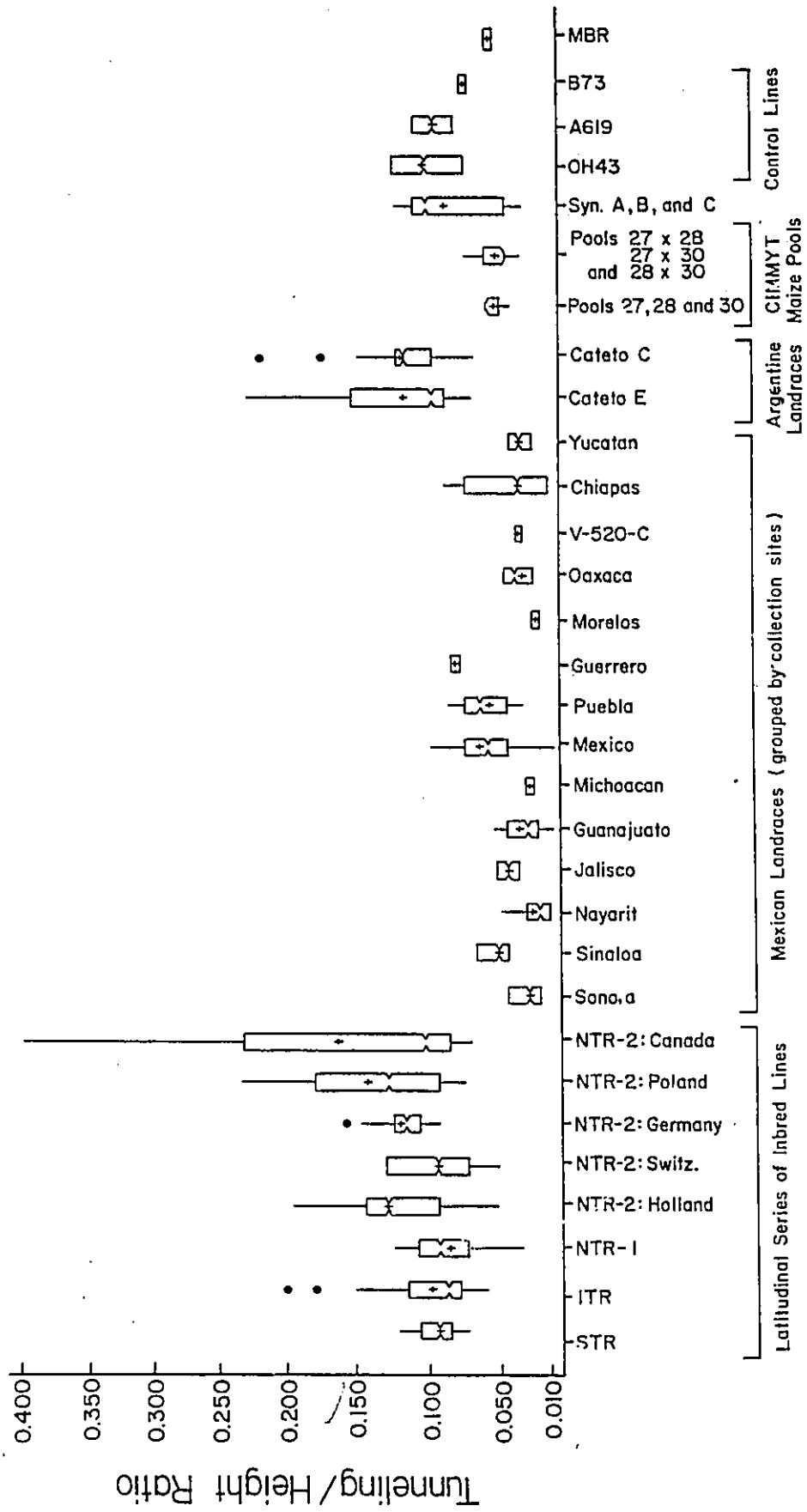
Box plots of the mean plant breakage ratings for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Plants were rated on a scale of 1-10 developed by Guthrie et al (1960). Larger ratings indicate greater breakage and increased susceptibility.

Plant Breakage Rating
(scale 1-10)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
MBR
B73
A619
OH43
Syn. A,B and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28
and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT
Maize Pools
Argentine
Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines

FIGURE 22

BOX PLOTS OF MEAN TUNNELING/HEIGHT RATIOS

Box plots of the mean tunneling/height ratios for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Cumulative lengths of tunnels (cm) were measured from stalks split down the center. Larger ratios indicate increased susceptibility.


MBR
B73
A619
OH43
Syn. A, B, and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28 and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT Maize Pools
Argentine Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.010
Tunneling / Height Ratio

multi-borer resistant line was resistant (0.060). Uninfested plants in the front of each row all had ratings of less than 0.05 for every line and race studied (APPENDIX 6).

3.2.2.6 STALK ROT RATINGS

A box plot of the mean stalk rot ratings for each major group of lines and races (FIGURE 23) shows that for almost all the groups a wide range in resistance was found. Overall, the most resistant groups were the Mexican landraces and the CIMMYT maize pools with mean ratings ranging from 1.75 to 3.50 (APPENDIX 4 and 5). The latitudinal series of inbred lines had large ranges of resistance, 4.00 to 6.50, as did the Argentine landraces, Synthetics A, B, and C, and the control line A619. The multi-borer resistant line had a mean rating of 3.8. Ratings taken on the front six plants of each row that were wounded but not infected, were all 1.0 to 2.0 for every line and race studied (APPENDIX 6).

3.2.3 CORRELATIONS AMONG PHYTOCHEMICAL AND RESISTANCE VARIABLES

To examine whether or not any of the phytochemical or resistance variables studied are interdependent or covary, data obtained on each were combined and subjected to tests of correlations using Pearson's product moment correlation. No distinction was made between independent and dependent variables, only the degree to which any of the variables vary together was estimated.

For the 1986 field data the following variable pairs were correlated with each other (TABLE 14): field leaf damage rating and plant height; field leaf damage rating and tunneling/height ratio; field leaf damage rating and DIMBOA concentration in etiolated seedlings; field leaf damage rating and lab leaf-feeding rating; plant breakage and stalk rot; plant breakage and plant height; plant breakage and tunneling/height ratio; stalk rot and plant height; stalk rot and tunneling/height ratio; plant height and lab leaf rating; and as discussed in Section 3.2.1, DIMBOA concentration in etiolated

FIGURE 23

BOX PLOTS OF MEAN STALK ROT RATINGS

Box plots of the mean stalk rot ratings for the major groups of lines and races studied: latitudinal series of inbred lines; Mexican landraces grouped by population collection sites; Argentine landraces; CIMMYT maize pools; Synthetics A, B, and C; control lines; and the multi-borer resistant line (MBR). Symbols for each box: +, median; notch mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. Ratings were based on the spread of infection from the point of inoculation. Larger ratings indicate greater infection spread from the point of inoculation and increased susceptibility.

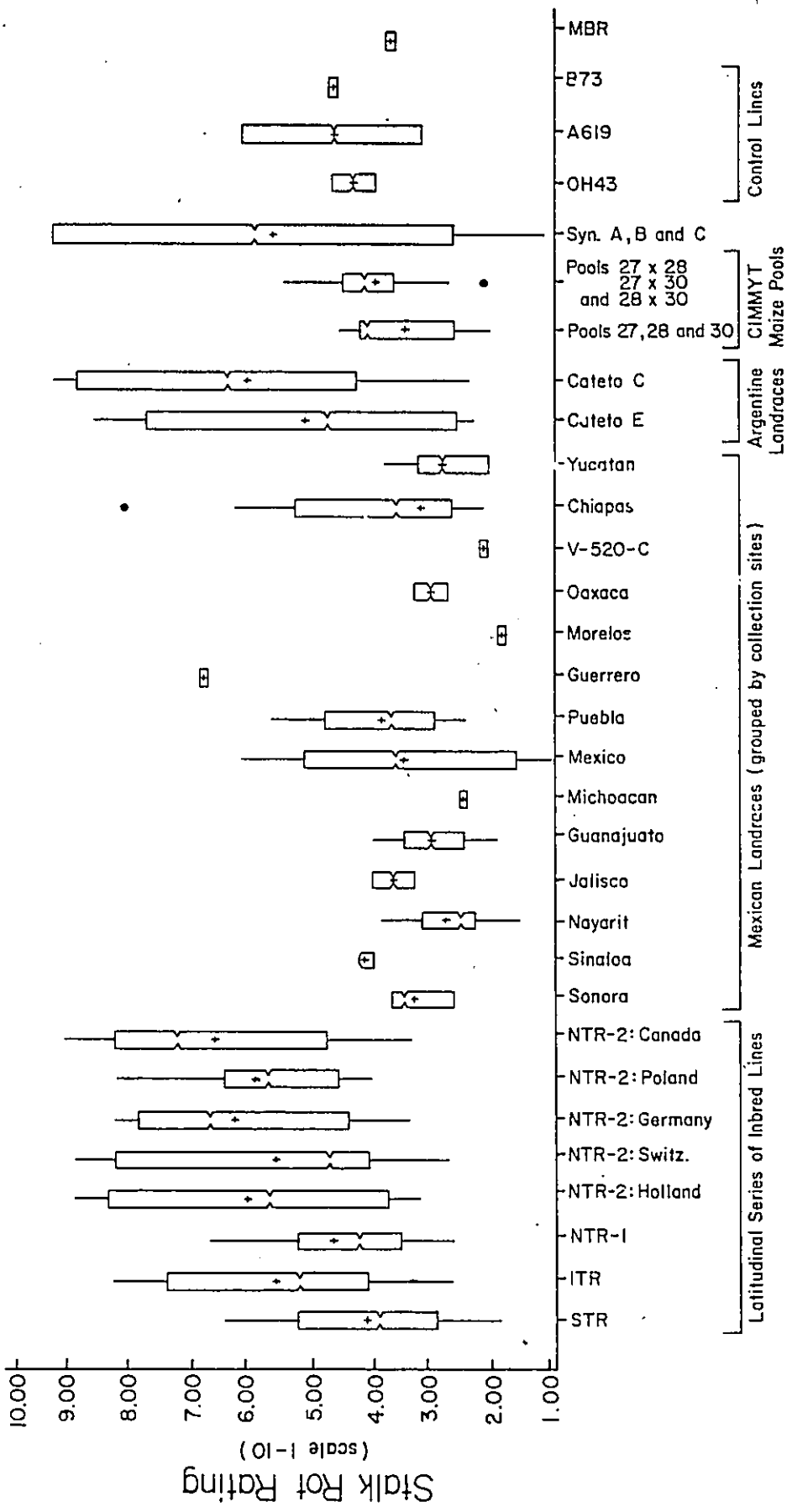

MBR
B73
A619
OH43
Syn. A, B and C
Pools 27 x 28
27 x 30
and 28 x 30
Pools 27, 28 and 30
Cateto C
Cateto E
Yucatan
Chiapas
V-520-C
Oaxaca
Morelos
Guerrero
Puebla
Mexico
Michoacan
Guanajuato
Jalisco
Nayarit
Sinaloa
Sonora
NTR-2: Canada
NTR-2: Poland
NTR-2: Germany
NTR-2: Switz.
NTR-2: Holland
NTR-1
ITR
STR
Control Lines
CIMMYT Maize Pools
Argentine Landraces
Mexican Landraces (grouped by collection sites)
Latitudinal Series of Inbred Lines
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
(scale 1-10)
Stalk Rot Rating

TABLE 14

PEARSON CORRELATIONS FOR 1986 FIELD DATA

	Plant Breakage	Stalk Rot	Plant Height	Smut Rating	Tunneling /Height	DIMBOA (ug/g)	Lab Leaf Rating
Field Leaf Rating	-0.101	-0.232	0.473[b]	0.146	-0.294[a]	-0.235[a]	0.581[a] n=30
Plant Breakage		0.497[b]	-0.269[a]	0.173	0.388[a]	-0.172	0.021 n=30
Stalk Rot			-0.346[a]	-0.157	0.481[b]	-0.098	-0.330 n=30
Plant Height				0.139	----	-0.184	0.410[a] n=30
Smut Rating					-0.004	-0.296	0.355 n=30
Tunneling /Height					-0.153	-0.102	n=30
DIMBOA (ug/g)							-0.641[b] n=30

a= r values significant at $p < 0.05$
b= r values significant at $p < 0.0001$
N.B.: n=68 unless otherwise stated
-higher r values indicate greater correlation

seedlings and lab leaf ratings. More correlations were found in the 1987 field data (TABLE 15) as highly significant at p.0001. The additional correlations for 1987 were: field leaf rating and stalk breakage; field leaf rating and stalk rot; plant height and DIMBOA concentration in etiolated seedlings; and tunneling/height ratio and DIMBOA concentration in etiolated seedlings. Part of this difference in field seasons is due to the smaller number of lines used in 1986 (n=68) as opposed to 1987 (n=102).

In many of the correlated pairs of variables it can be assumed that not only do the two variables correlate, but one is dependent upon the other. Therefore, for each of the pairs of correlated variables linear regression was carried out. FIGURES 24 to 37 are regression plots for these variables (only 1987 plots are shown for purposes of simplicity).

Relationships were apparent between the three major groups of data: DIMBOA concentrations; laboratory leaf feeding ratings; and field data. Results of DIMBOA concentration and lab leaf rating correlations are presented in Section 3.2.1. Three correlations were found between DIMBOA concentration and field data; DIMBOA concentration correlated with field leaf damage ratings, plant height, and the tunneling/height ratio (FIGURES 24, 25, and 26). The first two are negative correlations while the third is a positive correlation. In all three regression plots the landraces (circles) and the inbred lines (squares) are separated into two groups due to both variables, not just DIMBOA concentrations. Therefore, DIMBOA is not the only variable differentiating the two major germplasm groups.

Two correlations were found between lab leaf feeding ratings and field data. Lab leaf feeding was positively correlated to both field leaf damage ratings (FIGURE 27) and plant height (FIGURE 28). Again, the response of the landraces and inbred lines are distinct from one another.

The remaining nine 1987 correlations were between the field variables themselves. When field leaf damage rating was taken to be the independent variable, nega-

TABLE 15

PEARSON CORRELATIONS FOR 1987 FIELD DATA

	Plant Breakage	Stalk Rot	Plant Height	Smut Rating	Tunneling /Height	DIMBOA (ug/g)	Lab Leaf Rating
Field Leaf Rating	-0.300[a]	-0.467[b]	0.680[b]	-0.048	-0.564[b]	-0.463[b]	0.386[a] n=48
Plant Breakage		0.792[b]	-0.505[b]	0.020	0.575[b]	0.1016	0.023 n=48
Stalk Rot			-0.735[b]	-0.043	0.756[b]	0.176	-0.872 n=48
Plant Height				0.034	----	-0.387[b]	0.293[a] n=48
Smut Rating					0.029	-0.128	0.120 n=48
Tunneling /Height						0.319[a]	-0.170 n=48
DIMBOA (ug/g)							-0.572[b] n=48

a= r values significant at $p < 0.05$
b= r values significant at $p < 0.0001$
N.B.: n=102 unless otherwise stated
-higher r values indicate greater correlation

FIGURE 24

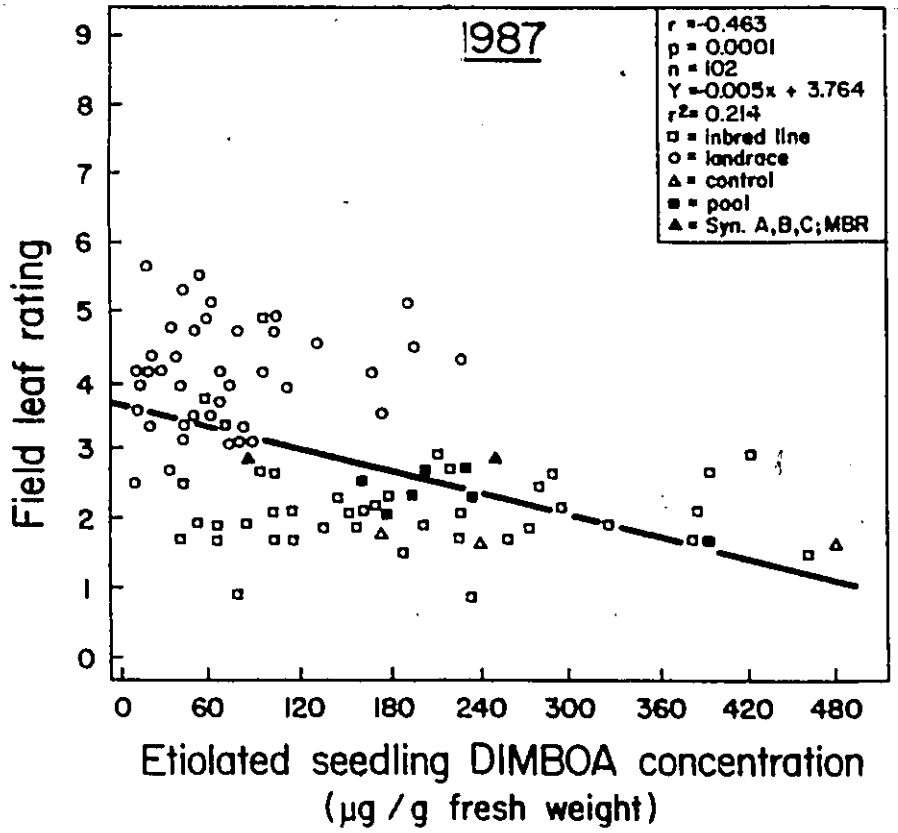
GRAPH OF DIMBOA CONCENTRATION AND FIELD LEAF DAMAGE RATING

(see FIGURE 15 and FIGURE 18)

FIGURE 25

GRAPH OF DIMBOA CONCENTRATION AND PLANT HEIGHT

(see FIGURE 15 and FIGURE 19)


1987
r = -0.463
p = 0.0001
n = 102
Y = -0.005x + 3.764
r² = 0.214
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C;MBR
Field leaf rating
0
1
2
3
4
5
6
7
8
9
0
60
120
180
240
300
360
420
480
Etiolated seedling DIMBOA concentration
(µg / g fresh weight)

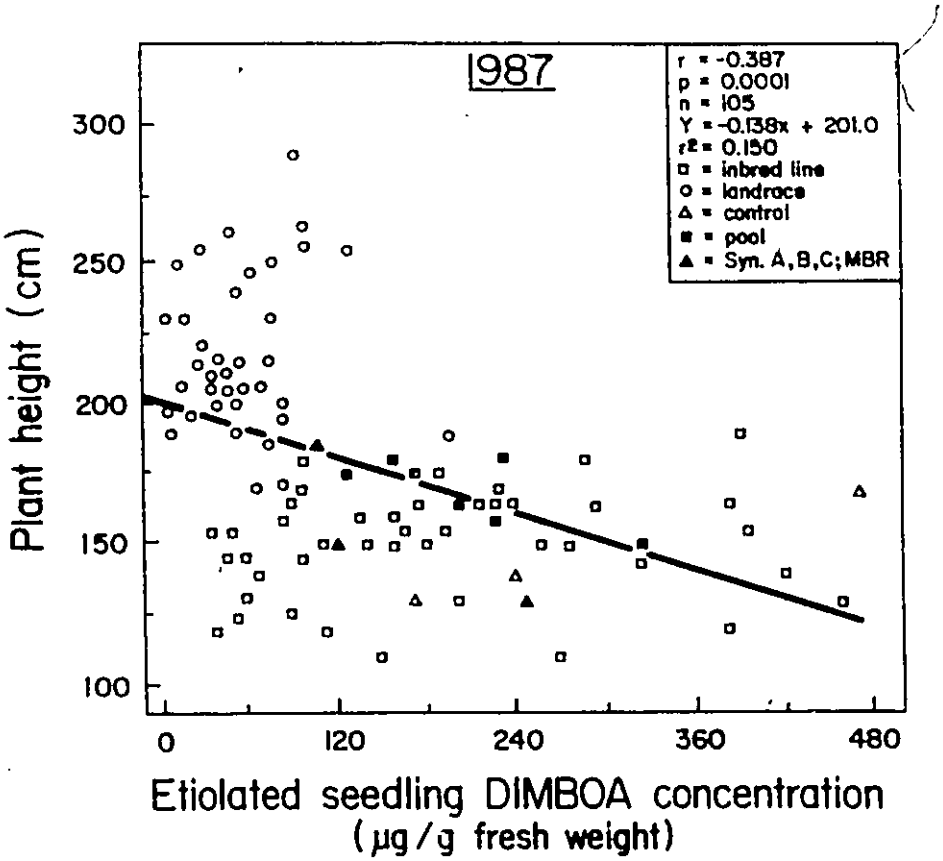

1987
r = -0.387
p = 0.0001
n = 105
Y = -0.138x + 201.0
r² = 0.150
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C;MBR
Plant height (cm)
100
150
200
250
300
0
120
240
360
480
Etiolated seedling DIMBOA concentration
(µg / g fresh weight)

FIGURE 26

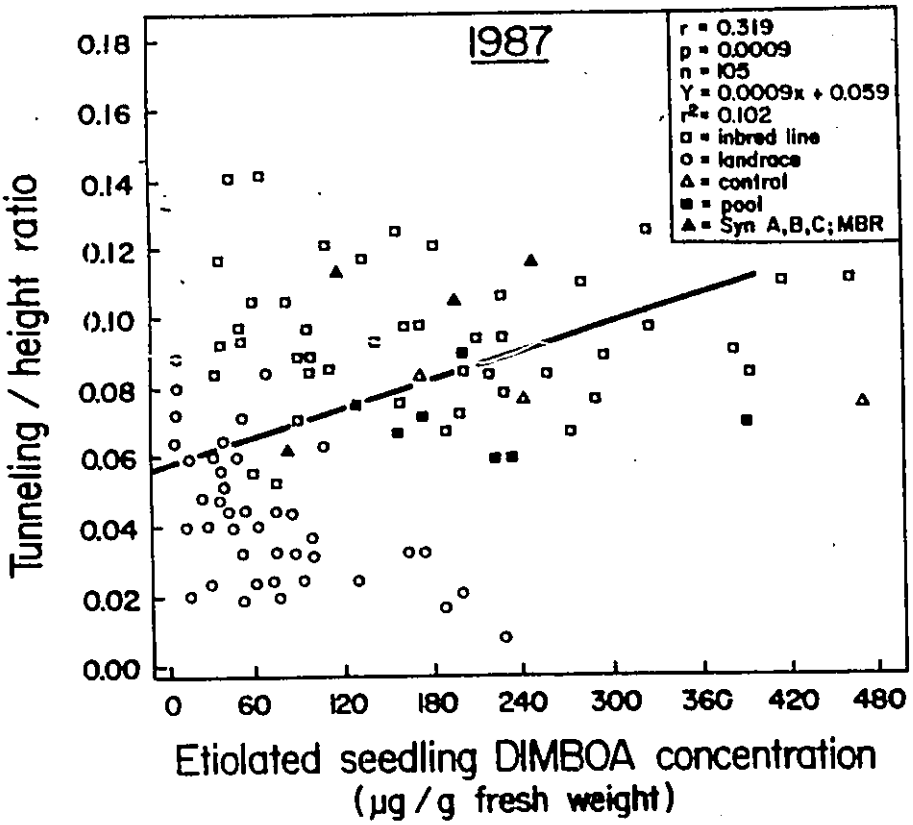
GRAPH OF DIMBOA CONCENTRATION AND THE TUNNELING/HEIGHT RATIO

(see FIGURE 15 and FIGURE 22)

FIGURE 27

GRAPH OF LABORATORY LEAF FEEDING RATING AND FIELD LEAF DAMAGE RATING

(see FIGURE 16 and FIGURE 18)


1987
r = 0.319
p = 0.0009
n = 105
Y = 0.0009x + 0.059
r² = 0.102
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn A,B,C;MBR
Tunneling / height ratio
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0
60
120
180
240
300
360
420
480
Etiolated seedling DIMBOA concentration
(µg / g fresh weight)

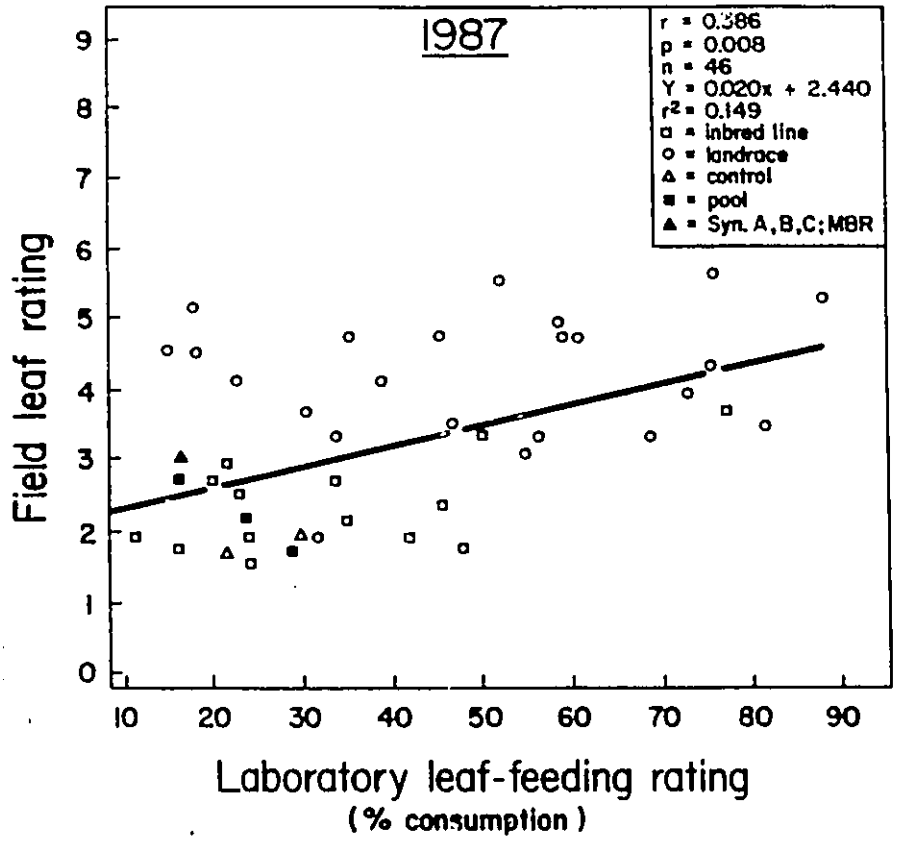

1987
r = 0.386
p = 0.008
n = 46
Y = 0.020x + 2.440
r² = 0.149
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C;MBR
Field leaf rating
0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
Laboratory leaf-feeding rating
(% consumption)

FIGURE 28

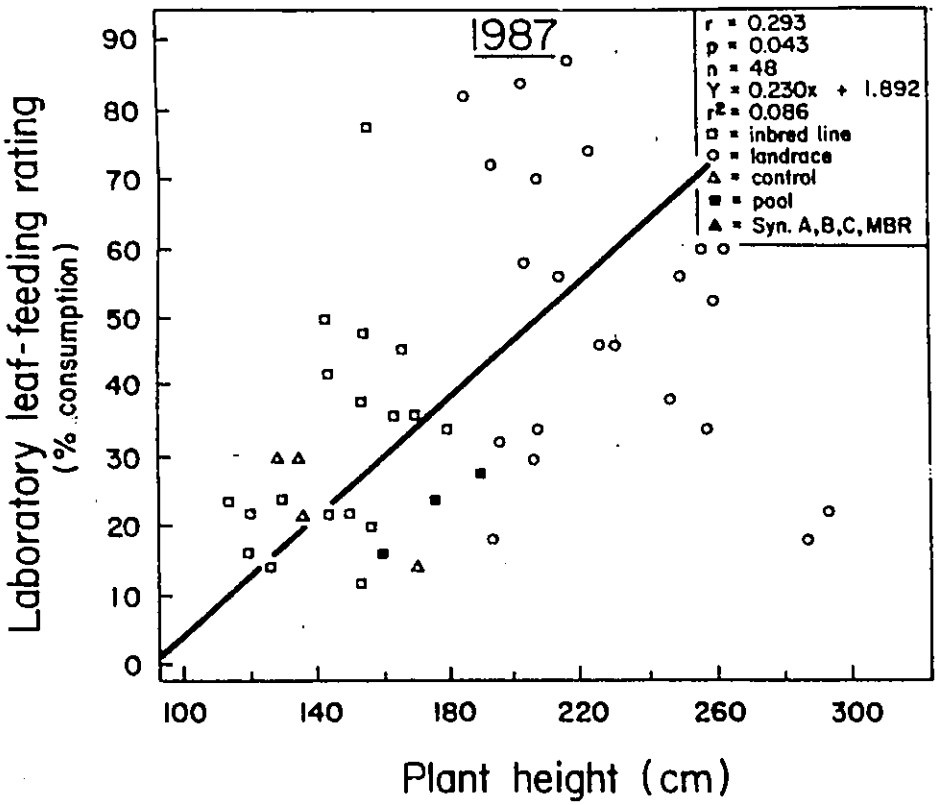
GRAPH OF PLANT HEIGHT AND LABORATORY LEAF FEEDING RATING

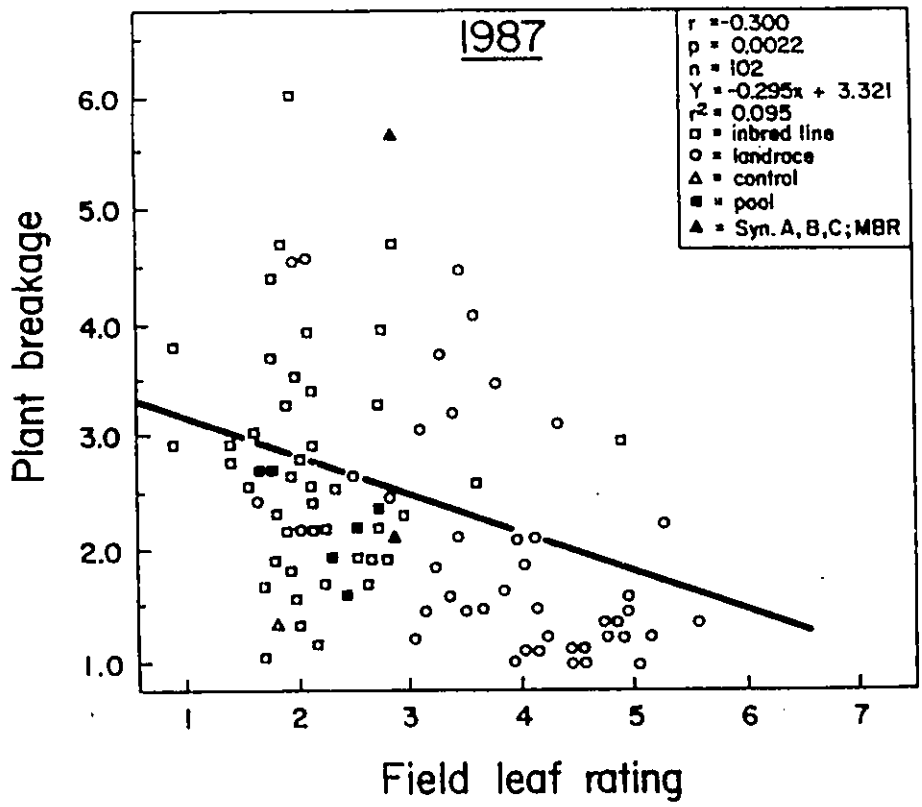
(see FIGURE 19 and FIGURE 16)

FIGURE 29

GRAPH OF FIELD LEAF DAMAGE RATING AND PLANT BREAKAGE RATING

(see FIGURE 18 and FIGURE 21)


1987
r = 0.293
p = 0.043
n = 48
Y = 0.230x + 1.892
r² = 0.086
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C, MBR
Laboratory leaf-feeding rating
(% consumption)
Plant height (cm)


1987
r = -0.300
p = 0.0022
n = 102
Y = -0.295x + 3.321
r² = 0.095
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Plant breakage
Field leaf rating

tive correlations were found with plant breakage, stalk rot, and the tunneling/height ratio (FIGURES 29, 30, and 31). The latter two were highly significant with p=0.0001. The tunneling/height ratio, when taken to be the independent variable, was found to have highly significant (p=0.0001) positive correlations to plant breakage (FIGURE 32) and to stalk rot (FIGURE 33). Stalk rot itself accounted for some plant breakage with a positive correlation (FIGURE 34). When plant height was taken as the independent variable it was found to have a positive correlation with field leaf damage rating (FIGURE 35) and two highly significant (p=0.0001) negative correlations with stalk rot and plant breakage (FIGURES 36 and 37).

3.3 RESISTANCE OF MAIZE AS RELATED TO THE GEOGRAPHICAL ORIGIN OF MAIZE GERMPLASM

3.3.1 LATITUDINAL SERIES OF INBRED LINES

The latitudinal series of inbred lines was used to examine the relationship between the latitudinal origin of maize germplasm and resistance to the European corn borer. Means of each variable (DIMBOA concentration, laboratory leaf feeding rating, field leaf damage rating, plant height, smut rating, plant breakage, tunneling/height ratio, and stalk rot) for each latitudinal group were summarized in bar graphs (FIGURES 38 to 45).

The latitudinal groups differed in their DIMBOA concentrations (FIGURE 38). The greatest difference for DIMBOA levels was between the first three groups (STR, ITR, and NTR-1) and the five groups making up the NTR-2 group. The former all had levels above 200 ug of DIMBOA per gram fresh weight of tissue, while the NTR-2 levels were all below 100 ug/g. There was no significant difference between the levels of DIMBOA for the STR, ITR, and NTR-1 groups.

FIGURE 30

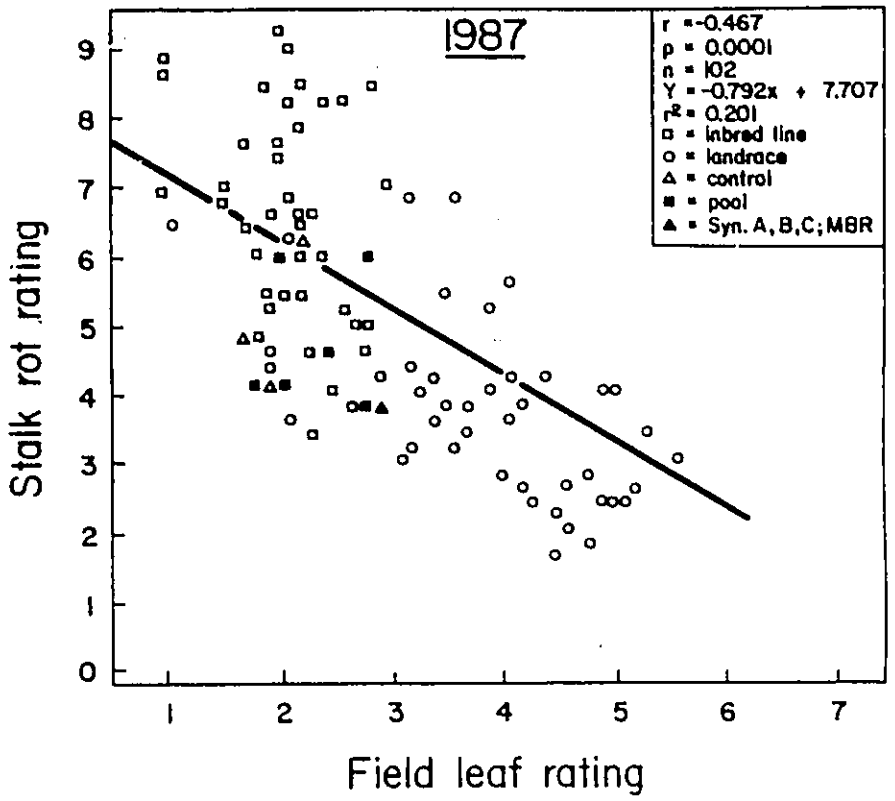
GRAPH OF FIELD LEAF DAMAGE RATING AND STALK ROT RATING

(see FIGURE 18 and FIGURE 23)

FIGURE 31

GRAPH OF FIELD LEAF DAMAGE RATING AND THE TUNNELING/HEIGHT RATIO

(see FIGURE 18 and FIGURE 22)


1987
r = -0.467
p = 0.0001
n = 102
Y = -0.792x + 7.707
r² = 0.201
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Stalk rot rating
Field leaf rating
0
1
2
3
4
5
6
7
8
9

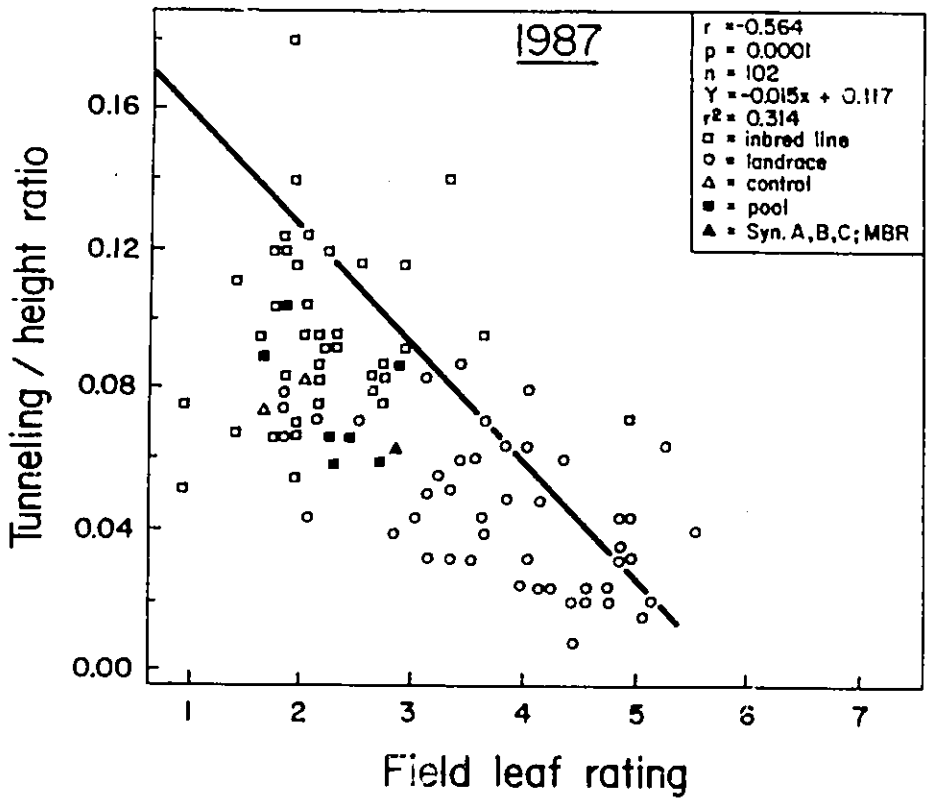

1987
r = -0.564
p = 0.0001
n = 102
Y = -0.015x + 0.117
r² = 0.314
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Tunneling / height ratio
Field leaf rating
0.00
0.04
0.08
0.12
0.16
1
2
3
4
5
6
7

FIGURE 32

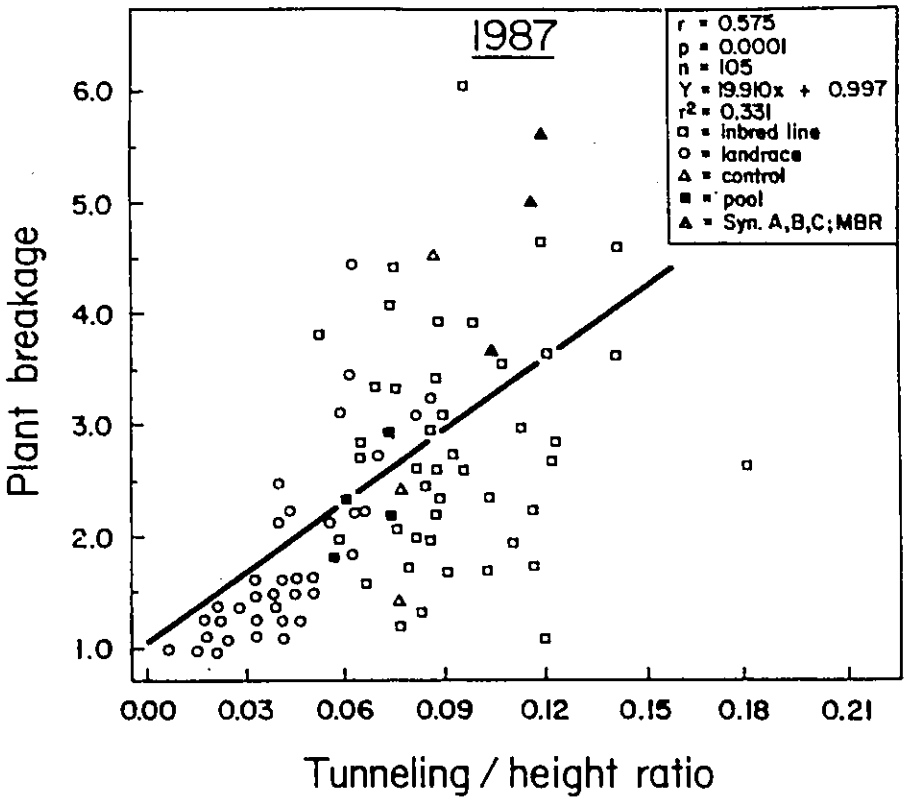
GRAPH OF THE TUNNELING/HEIGHT RATIO AND PLANT BREAKAGE RATING

(see FIGURE 22 and FIGURE 21)

FIGURE 33

GRAPH OF THE TUNNELING/HEIGHT RATIO AND STALK ROT RATING

(see FIGURE 22 and FIGURE 23)


1987
r = 0.575
p = 0.0001
n = 105
Y = 19.910x + 0.997
r² = 0.331
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Plant breakage
6.0
5.0
4.0
3.0
2.0
1.0
0.00
0.03
0.06
0.09
0.12
0.15
0.18
0.21
Tunneling / height ratio

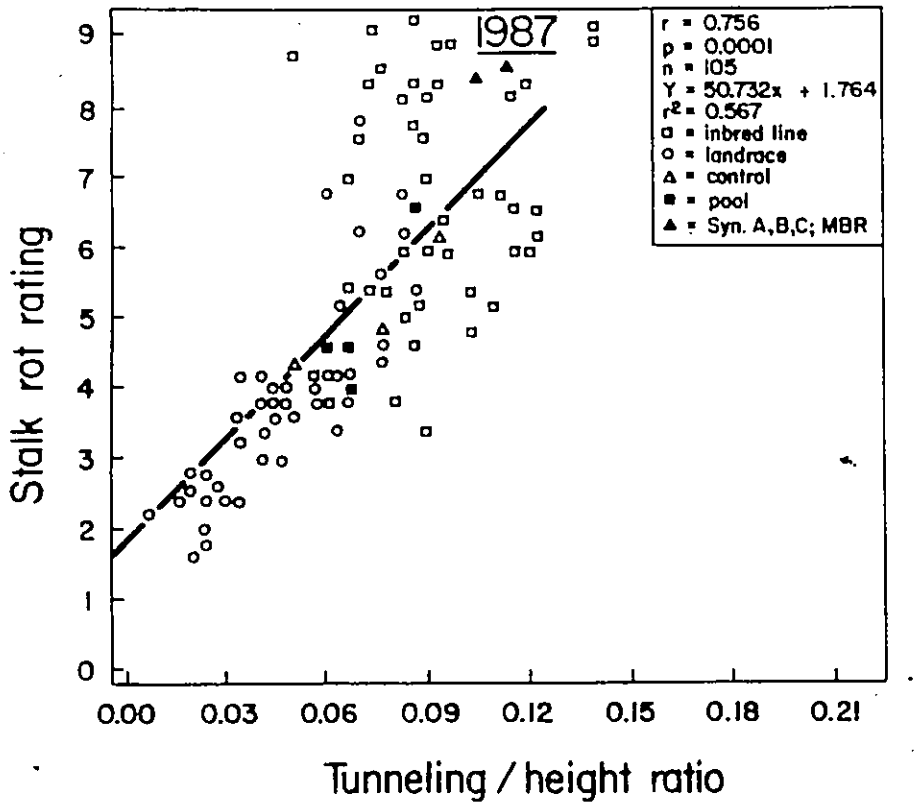

1987
r = 0.756
p = 0.0001
n = 105
Y = 50.732x + 1.764
r² = 0.567
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Stalk rot rating
9
8
7
6
5
4
3
2
1
0
0.00
0.03
0.06
0.09
0.12
0.15
0.18
0.21
Tunneling / height ratio

FIGURE 34

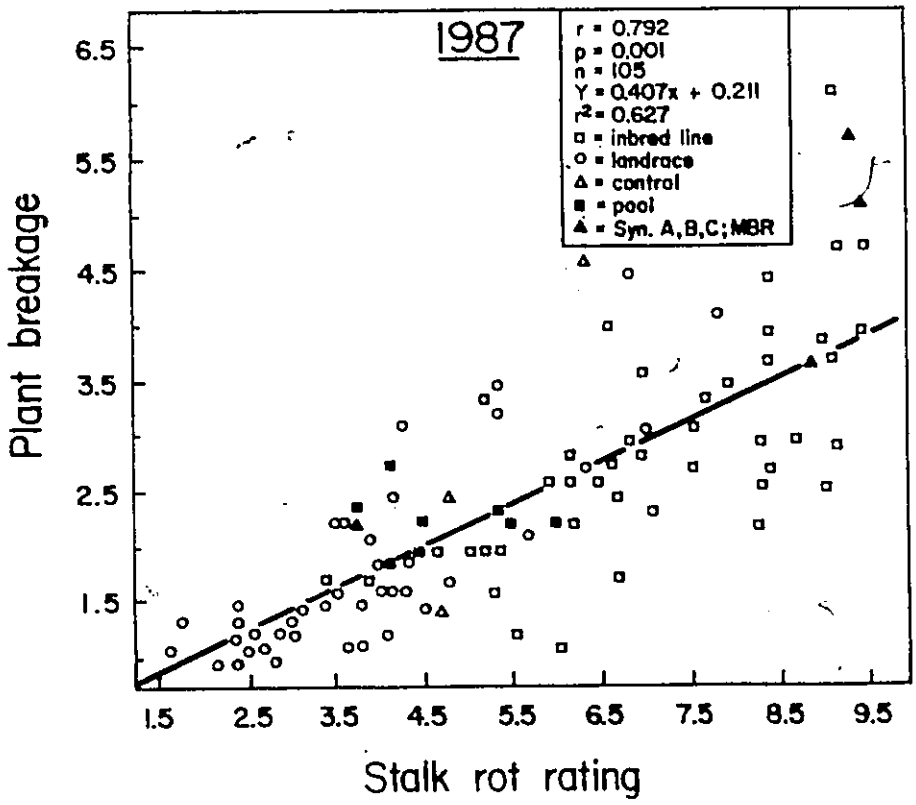
GRAPH OF STALK ROT RATING AND PLANT BREAKAGE RATING

(see FIGURE 23 and FIGURE 21)

FIGURE 35

GRAPH OF PLANT HEIGHT AND FIELD LEAF DAMAGE RATING

(see FIGURE 19 and FIGURE 18)


1987
r = 0.792
p = 0.001
n = 105
Y = 0.407x + 0.211
r² = 0.627
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C;MBR
Plant breakage
6.5
5.5
4.5
3.5
2.5
1.5
1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5 9.5
Stalk rot rating

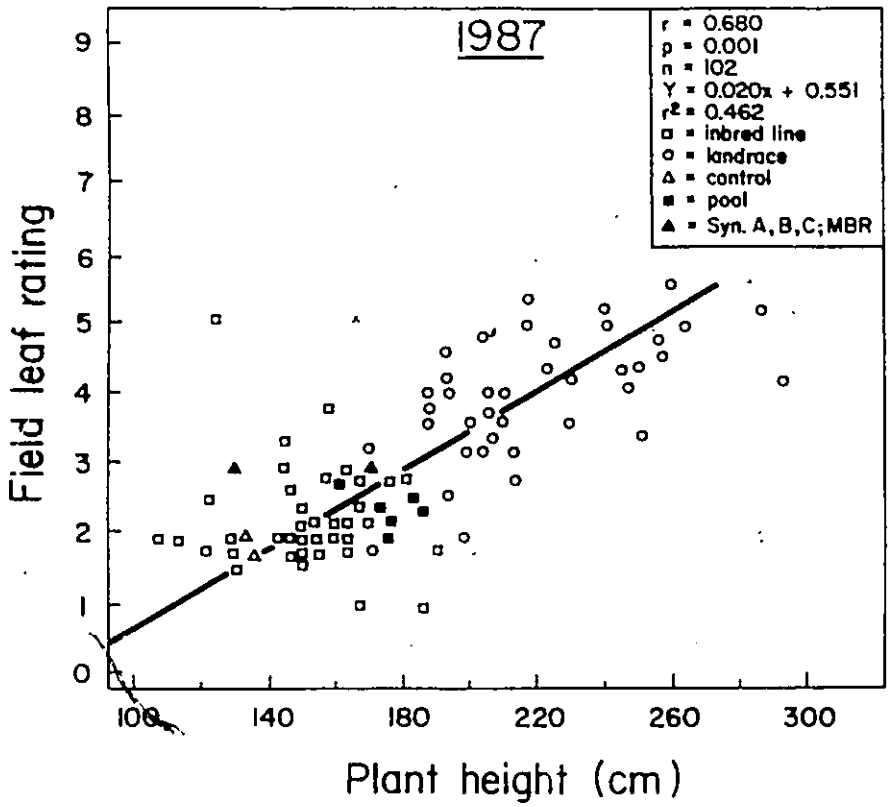

1987
r = 0.680
p = 0.001
n = 102
Y = 0.020x + 0.551
r² = 0.462
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C;MBR
Field leaf rating
9
8
7
6
5
4
3
2
1
0
100 140 180 220 260 300
Plant height (cm)

FIGURE 36

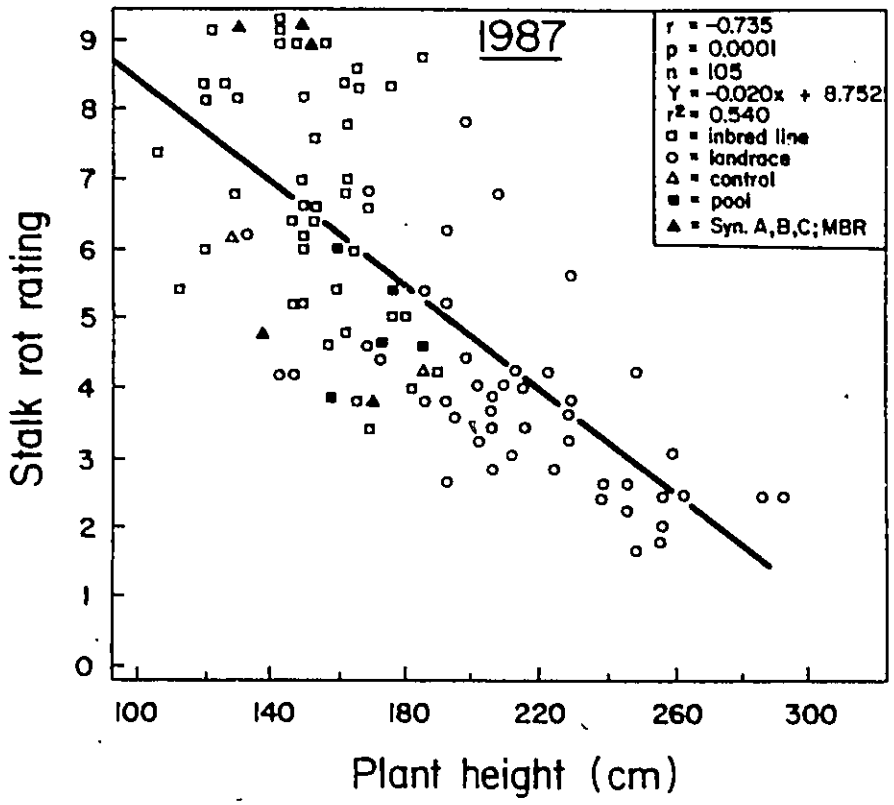
GRAPH OF PLANT HEIGHT AND STALK ROT RATING

(see FIGURE 19 and FIGURE 23)

FIGURE 37

GRAPH OF PLANT HEIGHT AND PLANT BREAKAGE RATING

(see FIGURE 19 and FIGURE 21)


1987
r = -0.735
p = 0.0001
n = 105
Y = -0.020x + 8.752
r² = 0.540
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Stalk rot rating
Plant height (cm)
0
1
2
3
4
5
6
7
8
9
100
140
180
220
260
300

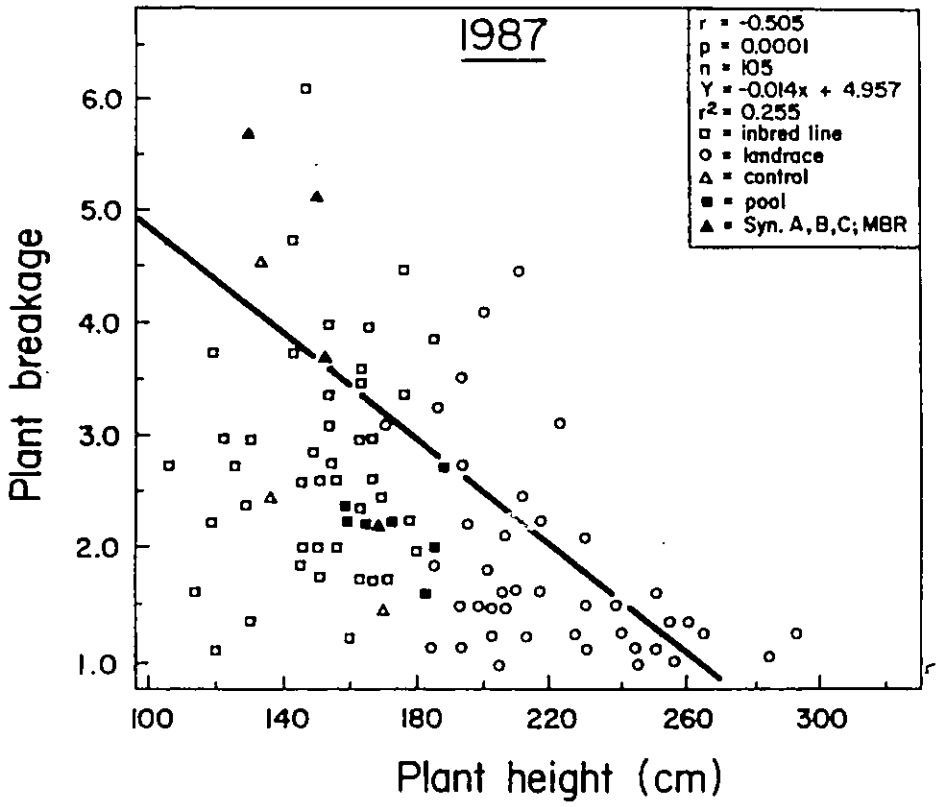

1987
r = -0.505
p = 0.0001
n = 105
Y = -0.014x + 4.957
r² = 0.255
□ = inbred line
○ = landrace
△ = control
■ = pool
▲ = Syn. A,B,C; MBR
Plant breakage
Plant height (cm)
1.0
2.0
3.0
4.0
5.0
6.0
100
140
180
220
260
300

FIGURE 38

BAR GRAPH OF DIMBOA CONCENTRATION FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 15)

Latitudinal Groupings:

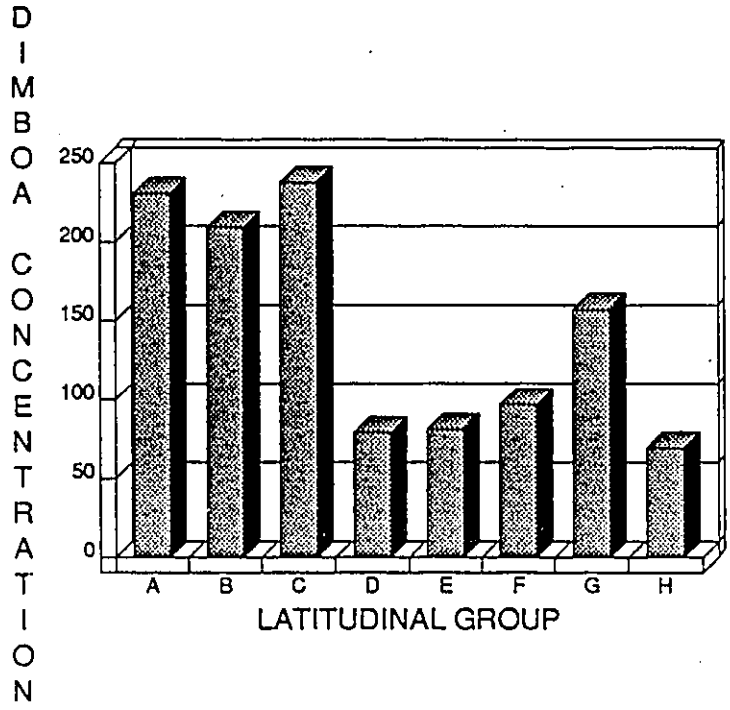
A= Southern Temperate Region (STR), 34-40° N-S
B= Intermediate Temperate Region (ITR), 40-46° N-S
C= Northern Temperate Region-1 (NTR-1), 46-52° N-S

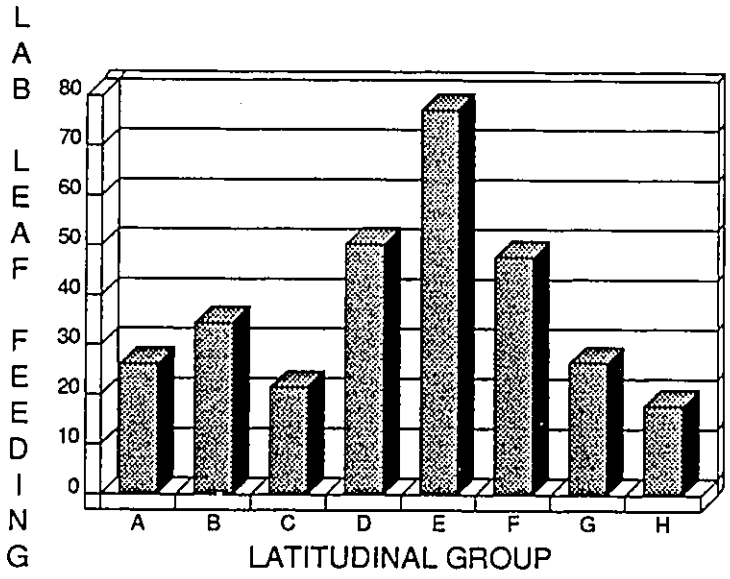
Northern Temperate Region (NTR-2), 46-52° N
D= Holland
E= Switzerland
F= Germany
G= Poland
H= Canada

FIGURE 39

BAR GRAPH OF LABORATORY LEAF-FEEDING RATINGS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 16)


DIMBOA CONCENTRATION
250
200
150
100
50
0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP


LAB LEAF FEEDING
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

There was no trend in laboratory leaf feeding ratings and latitudinal groupings (FIGURE 39). NTR-2, Switzerland (E) was the most consumed and therefore least resistant, followed by Holland, Germany, ITR, Poland, STR, NTR-1, and Canada. Similarily, no trend was found with field leaf damage ratings (FIGURE 40). All groups were classified as either highly resistant or resistant.

All groups were of almost equal height ranging from 1.3 meters to 1.6 meters (FIGURE 41). Smut varied tremendously between the groups (FIGURE 42). The most infected group was NTR-1 followed by STR, ITR, Holland, Poland, Canada, Germany, and the least infected, Switzerland. Therefore, the NTR-2 group had the lowest smut ratings overall.

Susceptibility to plant breakage, borer tunneling, and stalk rot increased with latitude (FIGURES 43, 44, and 45). In all cases the most heavily damaged by harvest and therefore most susceptible groups were those of the NTR-2 group, the most Northern of all groups. The most resistant group in all cases was STR, the most Southern of all groups. In summary, with increasing latitude there was a decrease in DIMBOA concentrations and an increased susceptibility to damage by borer tunneling and stalk rot infection.

3.3.2 INDIGENOUS LANDRACES OF MEXICO

There was no correlation of the geographical state of origin of landrace germplasm with resistance. Therefore, since altitude is another major factor segregrating the landraces of Mexico and Wellhausen et al (1952) used altitude one of their taxonomic characters, the relationship between altitude and resistance to the European corn borer was examined.

Altitude ranges for each landrace were taken from Wellhausen et al (1952) and ranked according to the maximum altitude in which a given landrace is commonly found, i.e. the upper altitude limit of each landrace (TABLE 9, Section 2.4.1). Pearson's

FIGURE 40

BAR GRAPH OF FIELD LEAF DAMAGE RATINGS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see Figure 18)

Latitudinal Groupings:

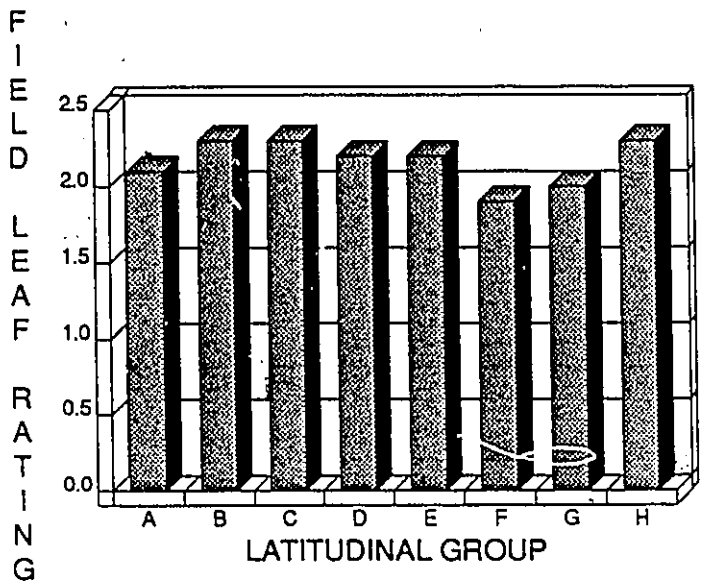
A= Southern Temperate Region (STR), 34-40° N-S
B= Intermediate Temperate Region (ITR), 40-46° N-S
C= Northern Temperate Region-1 (NTR-1), 46-52° N-S

Northern Temperate Region (NTR-2), 46-52° N
D= Holland
E= Switzerland
F= Germany
G= Poland
H= Canada

FIGURE 41

BAR GRAPH OF PLANT HEIGHT MEASUREMENTS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 19)


FIELD LEAF RATING
2.5
2.0
1.5
1.0
0.5
0.0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

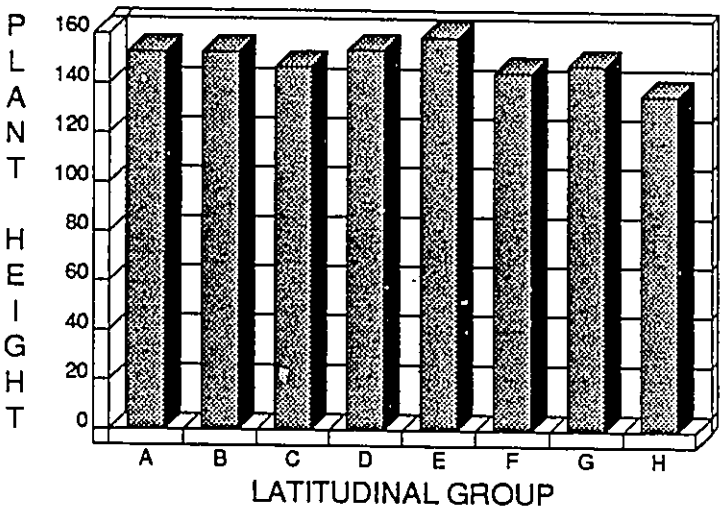

PLANT HEIGHT
160
140
120
100
80
60
40
20
0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

FIGURE 42

BAR GRAPH OF SMUT RATINGS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 20)

Latitudinal Groupings:

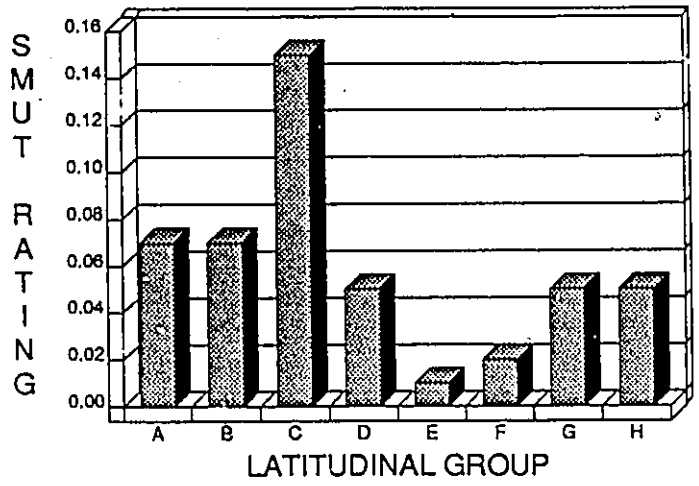
A= Southern Temperate Region (STR), 34-40° N-S
B= Intermediate Temperate Region (ITR), 40-46° N-S
C= Northern Temperate Region-1 (NTR-1), 46-52° N-S

Northern Temperate Region (NTR-2), 46-52° N
D= Holland
E= Switzerland
F= Germany
G= Poland
H= Canada

FIGURE 43

BAR GRAPH OF PLANT BREAKAGE RATINGS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 21)


SMUT RATING
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

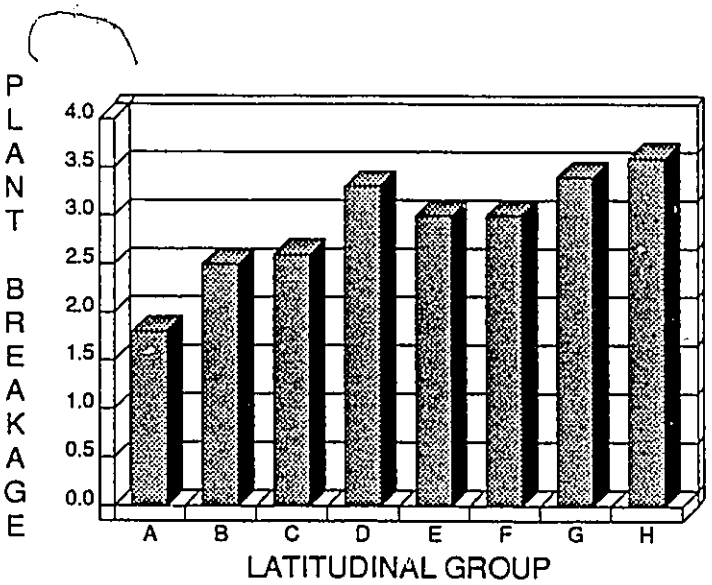

PLANT BREAKAGE
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

FIGURE 44

BAR GRAPH OF THE TUNNELING/HEIGHT RATIOS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 22)

Latitudinal Groupings:

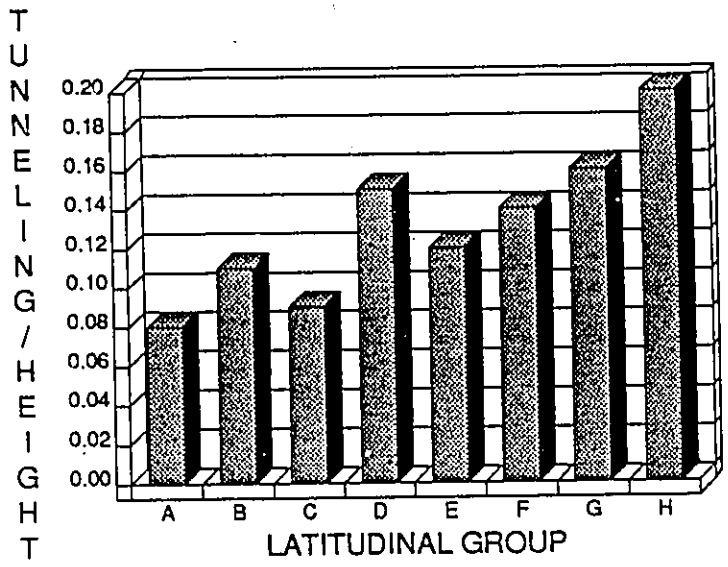
A= Southern Temperate Region (STR), 34-40° N-S
B= Intermediate Temperate Region (ITR), 40-46° N-S
C= Northern Temperate Region-1 (NTR-1), 46-52° N-S

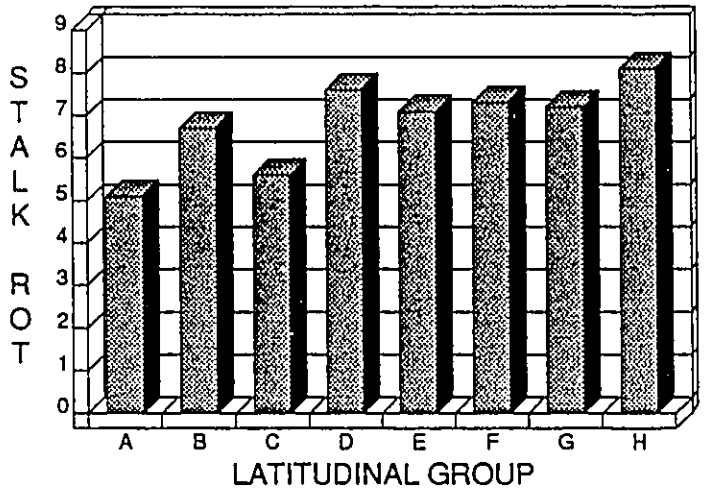
Northern Temperate Region (NTR-2), 46-52° N
D= Holland
E= Switzerland
F= Germany
G= Poland
H= Canada

FIGURE 45

BAR GRAPH OF STALK ROT RATINGS FOR THE LATITUDINAL SERIES OF INBRED LINES

(see FIGURE 23)


TUNNELING/HEIGHT
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A
B
C
D
E
F
G
H
LATITUDINAL GROUP


STALK ROT
9
8
7
6
5
4
3
2
1
0
A
B
C
D
E
F
G
H
LATITUDINAL GROUP

product moment correlation was used to see if these altitude rankings covaried with any of the phytochemical and resistance data on the landraces. Correlations were found with the following variables: DIMBOA concentration in the etiolated seedlings (r= -0.601); laboratory leaf feeding rating (r= 0.464); field leaf damage rating (r= 0.230); plant breakage (r= 0.525); and, tunneling/height ratio (r= 0.377).

Taking altitude to be the independent variable, regression plots were made for each correlating variable. The correlation with DIMBOA concentration was highly significant, p=0.0001 (FIGURE 46). The negative slope of the line indicates that DIMBOA concentration decreases with increasing altitude. The correlation with laboratory leaf feeding rating (FIGURE 47) was not as significant as that for DIMBOA concentrations; but, as expected, increasing altitude led to increased consumption of the whorl leaves and therefore less resistance. The correlation to field leaf damage ratings (FIGURE 48) was less significant although a positive correlation like that of the laboratory rating was obtained. A strong correlation was found for plant breakage ratings and altitude (FIGURE 49). With increasing altitude more of the plants were susceptible to stalk breakage. This is also reflected in the correlation with the tunneling/height ratio (FIGURE 50) where susceptibility again increased with altitude. To summarize, with increasing altitude DIMBOA concentrations decreased and susceptibility to borer damage by both leaf feeding and tunneling increased.

3.4 RESISTANCE OF MAIZE AS RELATED TO THE TAXONOMY OF MAIZE GERMPLASM

To examine the relationship between resistance of maize to the European corn borer and the taxonomy of maize, phytochemical and resistance data were subjected to various numerical taxonomic analyses to see how they fit into the existing classification of maize landraces by Wellhausen et al (1952).

FIGURE 46

GRAPH OF LANDRACE ALTITUDE GROUPINGS AND DIMBOA CONCENTRATIONS

(see FIGURE 15)

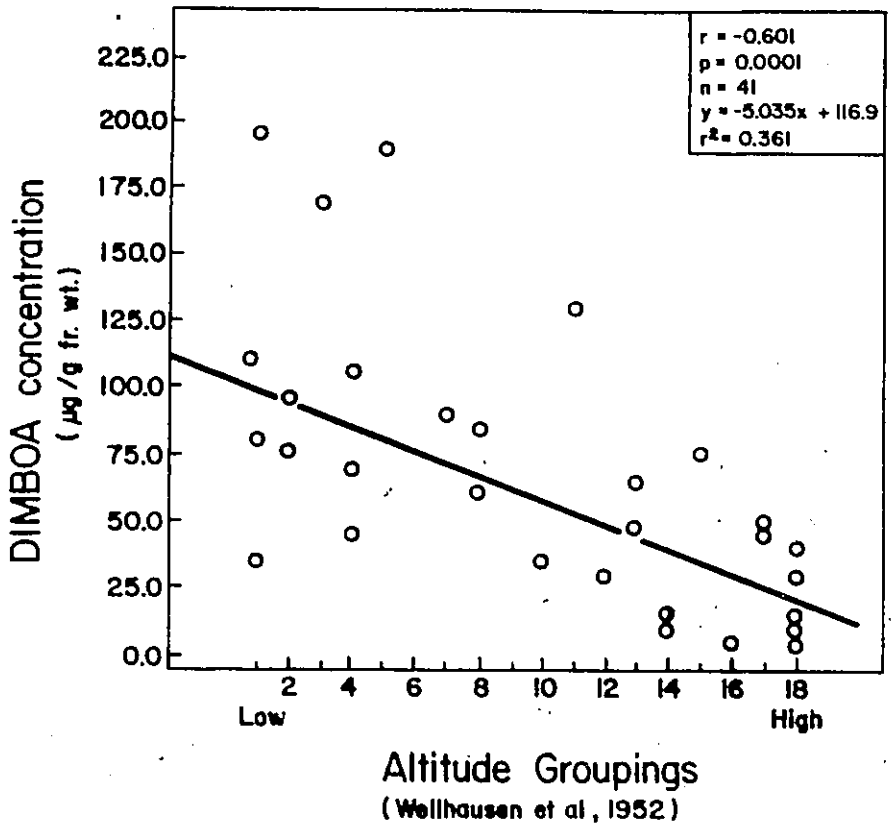
Altitude Groupings:

1= 100 meters
2= 0-500 meters
3= 0-600 meters
4= 100-600 meters
5= 300-700 meters
6= 600-1000 meters
7= 1000 meters
8= 0-1500 meters
9= 900-1500 meters
10= 1000-1500 meters
11= 1100-1500 meters
12= 1000-1700 meters
13= 1200-1800 meters
14= 1600-2000 meters
15= 1600-2100 meters
16= 1800-2300 meters
17= 2000-2400 meters
18= 2200-2800 meters

FIGURE 47

GRAPH OF LANDRACE ALTITUDE GROUPINGS AND LABORATORY LEAF FEEDING RATINGS

(see FIGURE 16)


r = -0.601
p = 0.0001
n = 41
y = -5.035x + 116.9
r² = 0.361
DIMBOA concentration
(µg/g fr. wt.)
225.0
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
2 4 6 8 10 12 14 16 18
Low
High
Altitude Groupings
(Wellhausen et al, 1952)

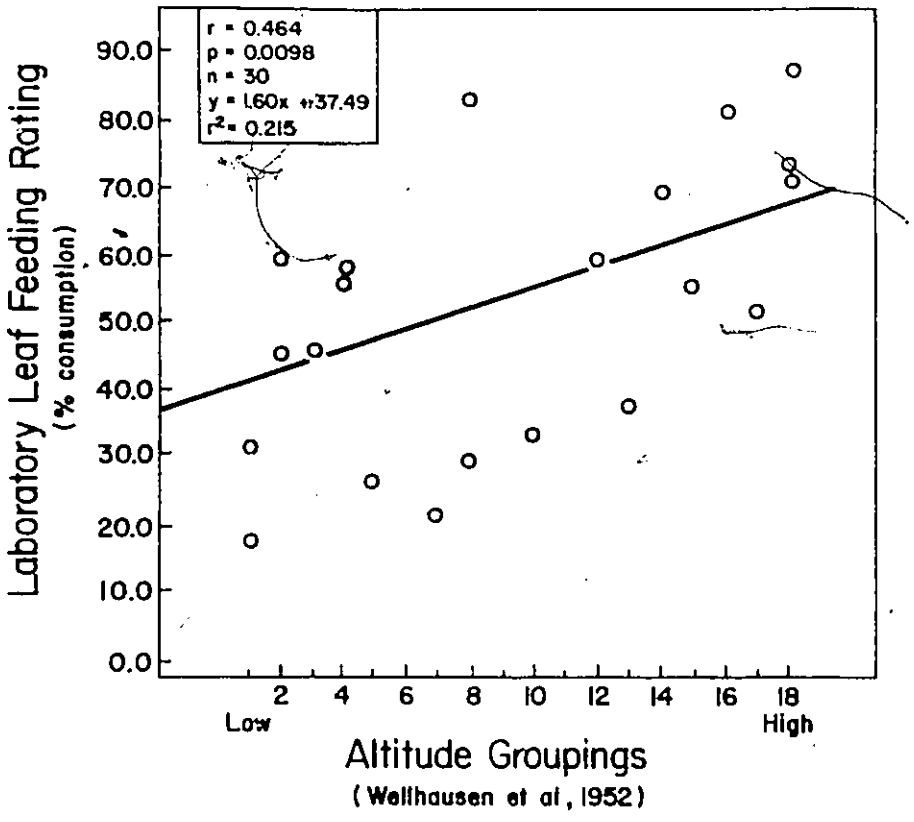

r = 0.464
p = 0.0098
n = 30
y = 1.60x +37.49
r² = 0.215
Laboratory Leaf Feeding Rating
(% consumption)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
2 4 6 8 10 12 14 16 18
Low
High
Altitude Groupings
(Wellhausen et al, 1952)

FIGURE 48

GRAPH OF LANDRACE ALTITUDE GROUPINGS AND FIELD LEAF DAMAGE RATING

(see FIGURE 18)

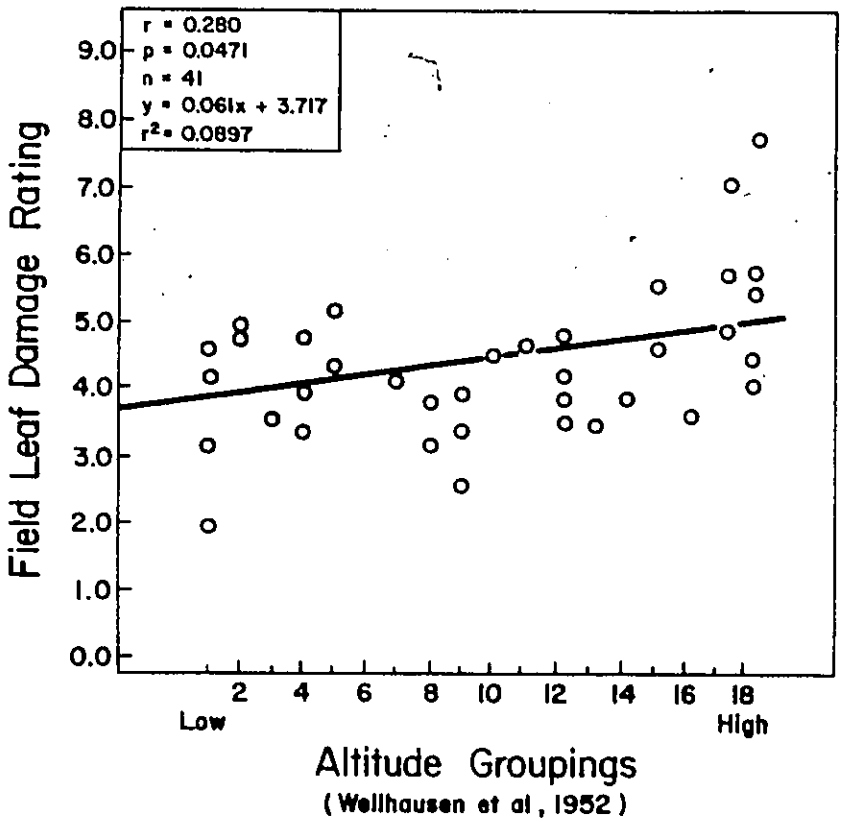
Altitude Groupings:

1= 100 meters
2= 0-500 meters
3= 0-600 meters
4= 100-600 meters
5= 300-700 meters
6= 600-1000 meters
7= 1000 meters
8= 0-1500 meters
9= 900-1500 meters
10= 1000-1500 meters
11= 1100-1500 meters
12= 1000-1700 meters
13= 1200-1800 meters
14= 1600-2000 meters
15= 1600-2100 meters
16= 1800-2300 meters
17= 2000-2400 meters
18= 2200-2800 meters

FIGURE 49

GRAPH OF LANDRACE ALTITUDE GROUPINGS AND PLANT BREAKAGE RATINGS

(see FIGURE 21)


r = 0.280
p = 0.0471
n = 41
y = 0.061x + 3.717
r² = 0.0897
Field Leaf Damage Rating
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2 4 6 8 10 12 14 16 18
Low
High
Altitude Groupings
(Wellhausen et al, 1952)

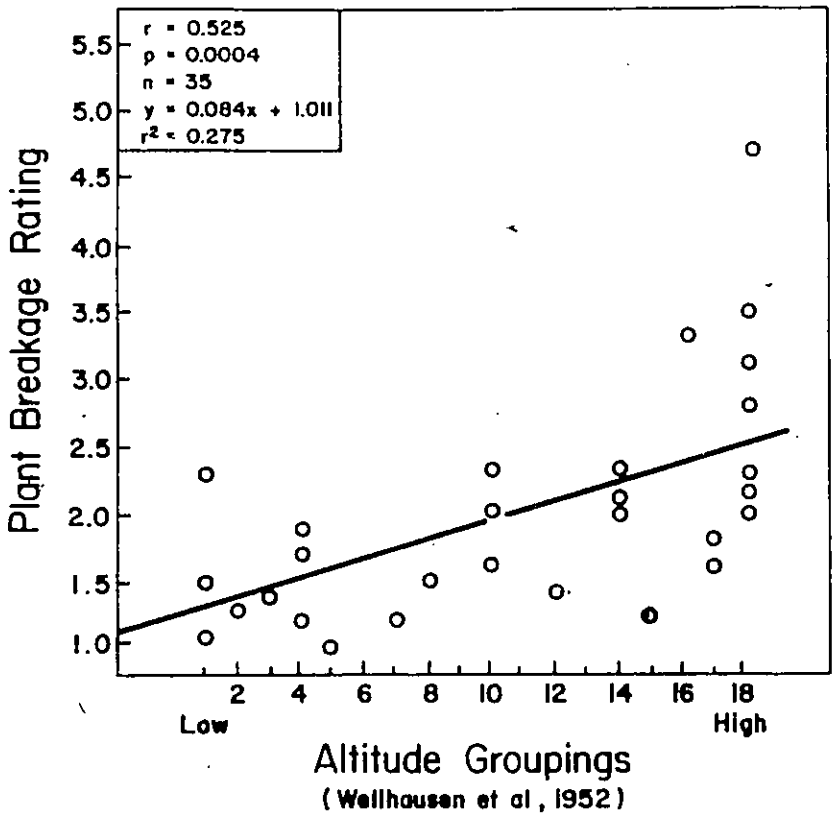

r = 0.525
p = 0.0004
n = 35
y = 0.084x + 1.011
r² = 0.275
Plant Breakage Rating
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
2 4 6 8 10 12 14 16 18
Low
High
Altitude Groupings
(Wellhausen et al, 1952)

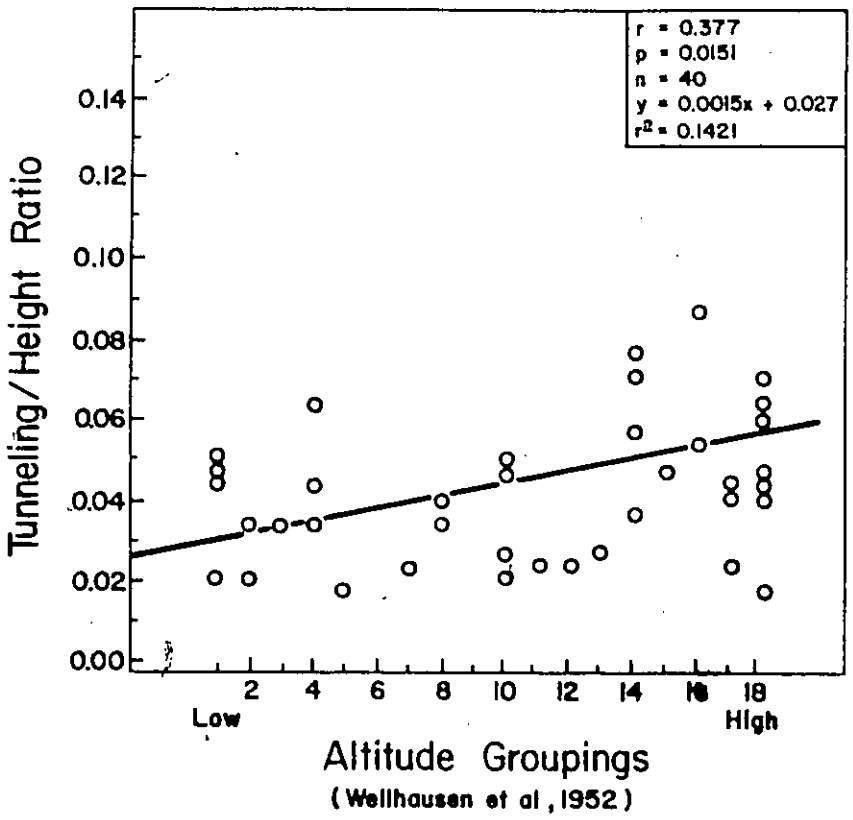
FIGURE 50

GRAPH OF LANDRACE ALTITUDE GROUPINGS AND THE TUNNELING/HEIGHT RATIO

(see FIGURE 22)

Altitude Groupings:

1= 100 meters
2= 0-500 meters
3= 0-600 meters
4= 100-600 meters
5= 300-700 meters
6= 600-1000 meters
7= 1000 meters
8= 0-1500 meters
9= 900-1500 meters
10= 1000-1500 meters
11= 1100-1500 meters
12= 1000-1700 meters
13= 1200-1800 meters
14= 1600-2000 meters
15= 1600-2100 meters
16= 1800-2300 meters
17= 2000-2400 meters
18= 2200-2800 meters


r = 0.377
p = 0.0151
n = 40
y = 0.0015x + 0.027
r² = 0.1421
Tunneling/Height Ratio
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
2
4
6
8
10
12
14
16
18
Low
High
Altitude Groupings
(Wellhausen et al, 1952)

3.4.1 STEPWISE DISCRIMINANT ANALYSIS

Of all the variables only one was selected as important in the forward selection, the tunneling/height ratio. In backward elimination the variables smut rating, plant breakage, and stalk rot were removed since they had the lowest sequential F values. Unlike the forward selection, backward elimination selected field leaf damage, DIM-BOA concentration, and plant height in addition to the tunneling/height ratio as those variables that give the best separation of Wellhausen's groups.

3.4.2 CLASSIFICATORY DISCRIMINANT ANALYSIS

This analysis was used to see which landraces would be classified into their "proper" Wellhausen group based upon their resistance characteristics. Two models were used: a linear model and a quadratic model.

3.4.2.1 Linear Model

The linear discriminant functions were generated from the pooled covariance matrix. Analysis was first carried out using only the four variables selected by the backward elimination stepwise discriminant analysis. Only 50% of the landraces were reclassified into Wellhausen's groups. The remaining 50% of misclassified races were classified into different groups then those that they were originally placed in by Wellhausen et al.

Due to this low level of reclassification, the analysis was repeated using all of the variables in the data set. Some improvement was found since there was a 60.4% reclassification. Moreover, the test of equality of the covariances revealed that they were unequal. This indicates that the data is more suited to classificatory analysis using the quadratic discrimant functions rather than the linear functions.

3.4.2.2 Quadratic Model

This model, unlike the linear model, assumes that the covariances are not equal. The quadratic discriminant functions are computed from the individual covariance matrices.

By using only the variables selected by the backward elimination an even lower percent reclassification (44%) then the linear model was found. But, when all the variables in the data set were used a 78.6% reclassification was found. Of a total of nine landraces originally classed by Wellhausen et al into group A, eight (77.7%) were again classified into group A based upon analysis of the resistance data (TABLE 16). Fifty percent of the landraces first classified into group B were again classified into this group. Of a total of sixteen group C landraces, thirteen or 81.2% were again classified into C. Ten (90.9%) of the eleven group E landraces were grouped into E once more. By adding together the total number of races that were reclassified into their original groups and dividing by the total number of landraces examined, the percent reclassification was calculated as 78.6% (33/42). This leaves a total of 21.4% or 9 landraces that were misclassified, i.e. the same races that were initially identified as A, C, or E were assigned to other groups based on the analysis of the resistance data. TABLE 17 summarizes these landraces, the groups they were reclassified into, and the probability of that reclassification.

3.4.3 CLASSIFICATION OF THE ARGENTINE LANDRACES, LATITUDINAL SERIES OF INBRED LINES, AND CIMMYT MAIZE POOLS

Now that it has been shown that Wellhausen's groups are justified with respect to resistance to the European corn borer, the next step was to classify the remaining races and lines used in this study into Wellhausen's groups. TABLE 18 summarizes the classification of the Argentine landraces, the latitudinal series of inbred lines, and

TABLE 16

SUMMARY OF CLASSIFICATORY DISCRIMINANT ANALYSIS USING THE QUADRATIC MODEL

WELLHAUSEN'S GROUP	NUMBER OF OBSERVATIONS AND PERCENTS CLASSIFIED INTO WELLHAUSEN'S GROUPS				
	A	B	C	E	Total
A	7 77.7	0 0.0	0 0.0	2 22.2	9 100.0
B	0 0.0	3 50.0	0 0.0	3 50.0	6 100.0
C	1 6.2	0 0.0	13 81.2	2 12.5	16 100.0
E	0 0.0	0 0.0	1 9.1	10 90.9	11 100.0
Total Percent	8 19.1	3 7.1	14 33.3	17 40.5	42 100.0

TABLE 17

SUMMARY OF MISCLASSIFIED LANDRACES

LANDRACE	CLASSIFICATION		PROBABILITY OF MEMBERSHIP IN RECLASSIFIED GROUPING			
	WELL.[a]	RESIST.[b]	A	B	C	E
Mexico-55, Palomero	A	E	0.302	0.000	0.257	0.440
Puebla-463, Arrocillo	A	E	0.303	0.000	0.276	0.421
Mexico-182, Conico	C	A	0.546	0.211	0.244	0.000
Guanajuato-93a(red), Maize Dulce	B	E	0.364	0.034	0.158	0.444
Chiapas-237, Olotillo	C	E	0.009	0.005	0.422	0.564
Chiapas-124, Oloton	B	E	0.018	0.000	0.084	0.899
Nayarit-24, Harinoso de Ocho	B	E	0.027	0.000	0.029	0.944
Chiapas-236, Zapalote Grande	C	E	0.004	0.000	0.290	0.710
Sonora-139, Onaveno	E	C	0.038	0.024	0.514	0.424

a= classification according to Wellhausen et al (1952)
b= classification according to the analysis of the resistance data

TABLE 18

CLASSIFICATION OF ARGENTINE LANDRACES, LATITUDINAL SERIES OF INBRED LINES, AND CIMMYT MAIZE POOLS INTO WELLHAUSEN'S GROUPINGS BASED ON RESISTANCE DATA

LINES/RACES	CLASSIFICATION	PROBABILITY
ARGENTINE LANDRACES		
Cateto C		
2026, 2030, 2032 and 2025	A	100.0
2027	E	67.2
Cateto E		
2044, 2045, 2047 and 2051	A	100.0
2048	E	81.3
LATITUDINAL SERIES		
STR		
3794, 3802, 3805, 3815 and 3823	A	100.0
3790	E	99.7
ITR		
3853, 3857, 3872 and 3878	A	100.0
3862, 3865, 3877	E	100.0
NTR-1		
all lines	A	100.0
NTR-2		
Holland		
4018	A	91.6
4019, 4022	A	100.0
4020	E	96.4
4021	C	94.3
Switzerland		
4034, 4035	A	100.0
4036	C	100.0
Germany		
all lines	A	100.0
Poland		
4064, 4065	A	100.0
4066	A	96.8
Canada		
4071	A	100.0
4072	A	88.4
4077	E	100.0
4081	A	88.6
CIMMYT MAIZE POOLS		
Pool 27	A	87.8
Pool 28	A	99.7
Pool 30	A	100.0
Pool 27 X 28	A	78.4
Pool 27 X 30	C	95.2
Pool 28 X 30	A	100.0

the CIMMYT maize pools. Of all the lines and races, 84.6% were classified into group A; 0% in group B; 7.7% in group C; and the remaining 7.7% in group E.

3.4.4 SUMMARY STATISTICS

Statistics showing the range of variation of each character for each Wellhausen group are summarized in box plots (FIGURE 51). Group C had the largest mean concentrations of DIMBOA in the etiolated one-week-old seedlings, followed by groups E, B, D, and A. For laboratory leaf feeding ratings group C was the most resistant followed by groups B, D, and A (no data was available for group E). Almost all groups had equal mean field leaf damage ratings ranging only from 4.0 to 5.0, and were therefore all intermediate in resistance. Plant height varied between groups with group C being the tallest followed by groups D, B, E, and A. Smut ratings were low (below 0.200) and almost equal for all groups; although, group D had the smallest range of ratings of all five groups. Group A was the most susceptible to plant breakage due to borer tunneling followed by groups B, E, C, and D in order of increasing resistance. Subsequently, group A was also the most susceptible to borer tunneling having the largest tunneling/height ratio of all the groups, followed by groups D, B, E, and the most resistant group, C. Group A was the most susceptible to the spread of stalk rot infection followed by groups E, B, D, and C in order of increasing resistance.

To further illustrate the differences between the groups, variability profiles were plotted from the coefficients of variation (FIGURE 52). The characters smut rating and DIMBOA concentration are the most notably different between the five groups. This is especially true for the DIMBOA character and group B, which has the lowest coefficient of variation of the all the groups. Of all five groups no two variability profiles are close to each other with respect to all characters.

FIGURE 51

SUMMARY STATISTICS OF WELLHAUSEN'S GROUPS

Statistics on the phytochemical and resistance data obtained for the five Wellhausen groups is summarized is box plots. The groupings include: A, Ancient Indigenous Races; B, Pre-Columbian Exotic Races; C, Prehistoric Mestizos; D, Modern Incipient Races; and E, "Poorly Defined Races". Symbols for each box: +, median; notch, mean; top of box, 75th percentile; bottom, 25th percentile; whiskers (stemming up and down of each box) extend to maximum of 1.5 of the range between the 25th and 75th sample percentiles; dots represent data that go beyond the former and represent the tail end of the distribution. See FIGURES 15 to 23 for variable descriptions.

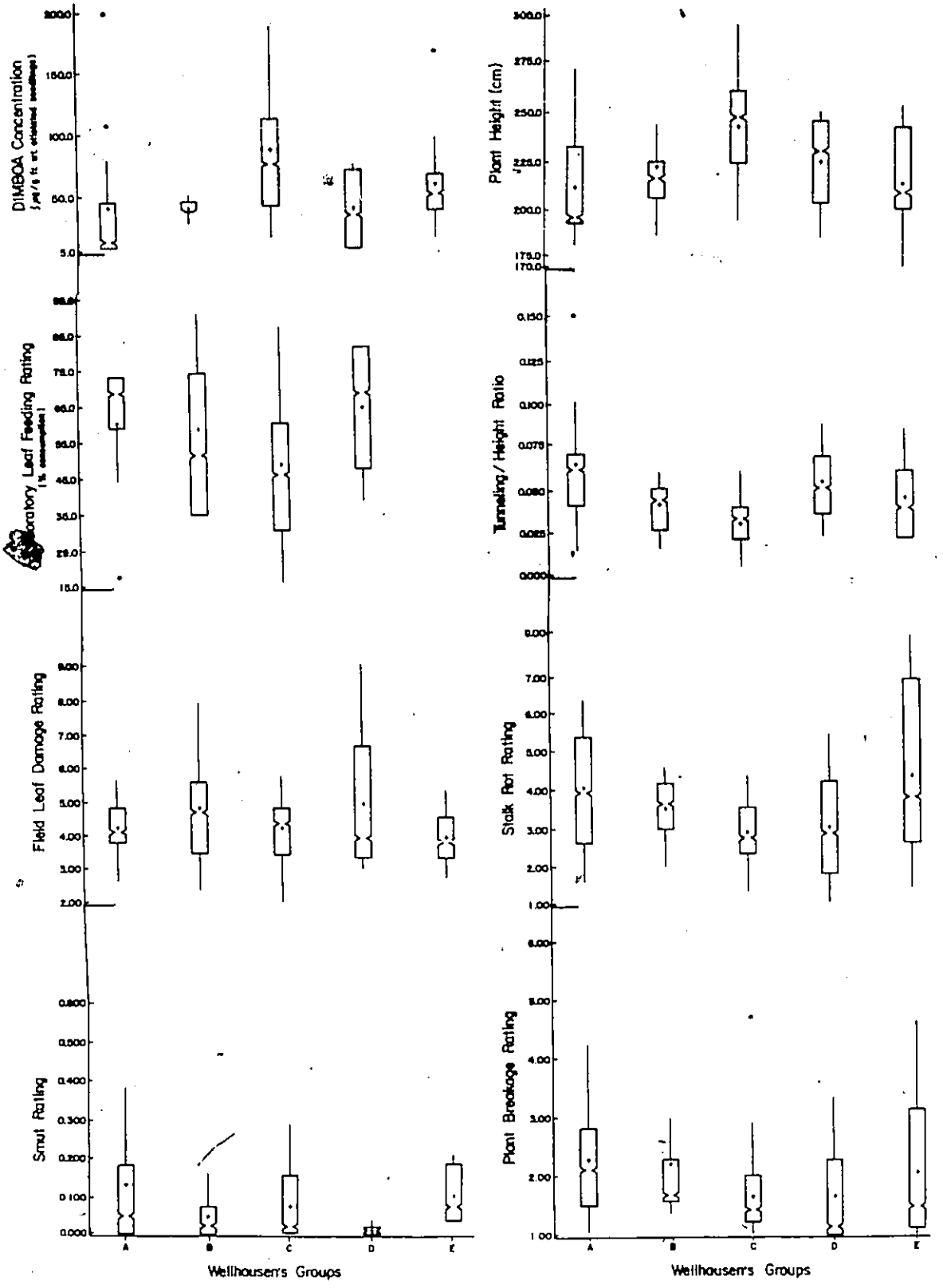
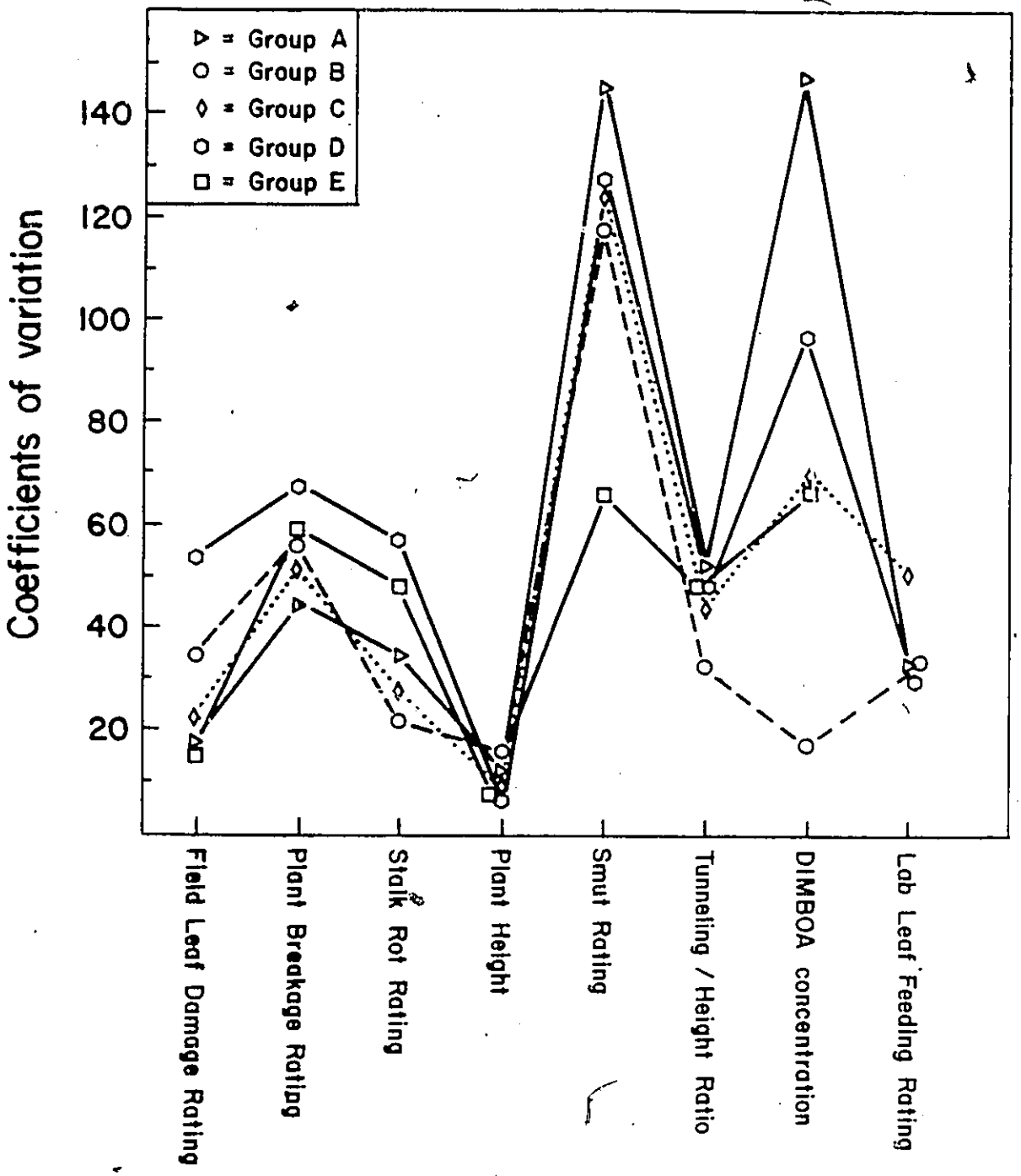

DIMBOA Concentration
Plant Height (cm)
Laboratory Leaf Feeding Rating
Tunneling / Height Ratio
Field Leaf Damage Rating
Stalk Rot Rating
Smut Rating
Plant Breakage Rating
A
B
C
D
E
Wellhousen's Groups
Wellhousen's Groups

FIGURE 52

VARIABILITY PROFILES OF THE FIVE WELLHAUSEN GROUPS

Profiles are based on the eight phytochemical and resistance characters measured (horizontal axis), and their coefficients of variation expressed in percent (vertical axis).


Δ = Group A
○ = Group B
◇ = Group C
○ = Group D
□ = Group E
Coefficients of variation
140
120
100
80
60
40
20
Field Leaf Damage Rating
Plant Breakage Rating
Stalk Rot Rating
Plant Height
Smut Rating
Tunneling / Height Ratio
DIMBOA concentration
Lab Leaf Feeding Rating

3.4.5 CANONICAL DISCRIMINANT ANALYSIS

Canonical discriminant analysis was carried out to investigate the relationships between the five Wellhausen groups formed from the phytochemical and resistance data. Mahalanobis distances were computed between the means of each group (TABLE 19). Larger values indicate a greater distance between the groups. Groups D and E are the furthest apart followed by groups C and D, A and C, C and E, B and C, A and D, A and B, B and D, B and E, and A and E. Therefore, groups A and E are closer to each other than to any other group on the basis of Mahalanobis distances.

A three dimensional ordination graph was plotted to visualize the relationships between the groups (FIGURE 53). Because this ordination is carried out on the pooled covariance matrix it is therefore somewhat distorted as the group covariances are unequal. The mean of each group is plotted in FIGURE 54. From both plots it can be seen that group A (flags) is fairly broad and also high on axis-3. Group B (circles) is characterized by short needles and is therefore much lower in the three dimensional stratum than group A. Group C (diamonds) is at the opposite end of the plot to group A and is also high on axis-3. The mean of group D (hexagons) is the highest of all groups on axis-3; although, this is the group consisting of only a few individuals. Finally, group E (squares) somewhat bridges the overlap between groups A and C.

The total canonical structure obtained determines the relative magnitude of contribution of the variables to the three axis on the ordination plot (TABLE 20). Those variables with the highest values for each axis have the greatest influence on that particular axis. For axis-1 the tunneling/height ratio is the strongest character and has the greatest influence on this axis followed by plant height, DIMBOA concentration, stalk rot, plant breakage, smut rating, and the weakest character, field leaf rating. For axis-2 stalk rot contributes the most followed by field leaf rating, smut rating, plant height, DIMBOA concentration, plant breakage, and the tunneling/height ratio. Finally, for axis-3 the strongest and most influencing character is the same as that for

TABLE 19

MAHALANOBIS DISTANCES BETWEEN THE MEANS OF THE GROUPS FORMED FROM THE ANALYSIS OF THE RESISTANCE DATA

	A	B	C	D	E
A	---	1.519	1.941	1.536	1.278
B		---	1.647	1.371	1.358
C			---	2.105	1.687
D				---	2.210
E					---

FIGURE 53

THREE-DIMENSIONAL ORDINATION PLOT OF WELLHAUSEN'S GROUPINGS FORMED FROM THE ANALYSIS OF THE PHYTOCHEMICAL AND RESISTANCE DATA

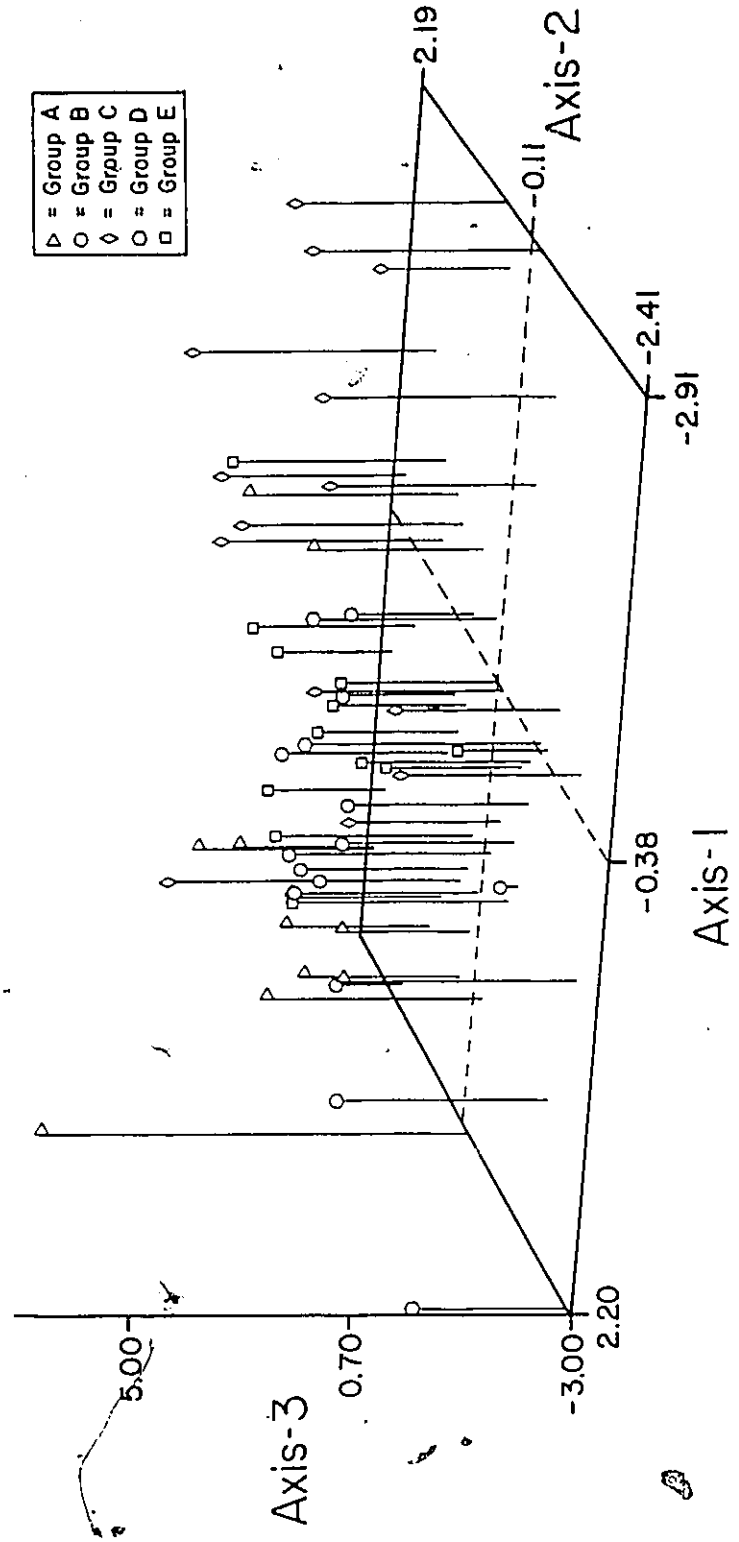

△ = Group A
○ = Group B
◇ = Group C
○ = Group D
□ = Group E
2.19
-0.11
-2.41
-2.91
-0.38
2.20
-3.00
0.70
5.00
Axis-2
Axis-1
Axis-3

FIGURE 54

THREE-DIMENSIONAL ORDINATION PLOT OF THE MEANS OF WELLHAUSEN'S GROUPINGS FORMED FROM THE ANALYSIS OF THE PHYTOCHEMICAL AND RESISTANCE DATA

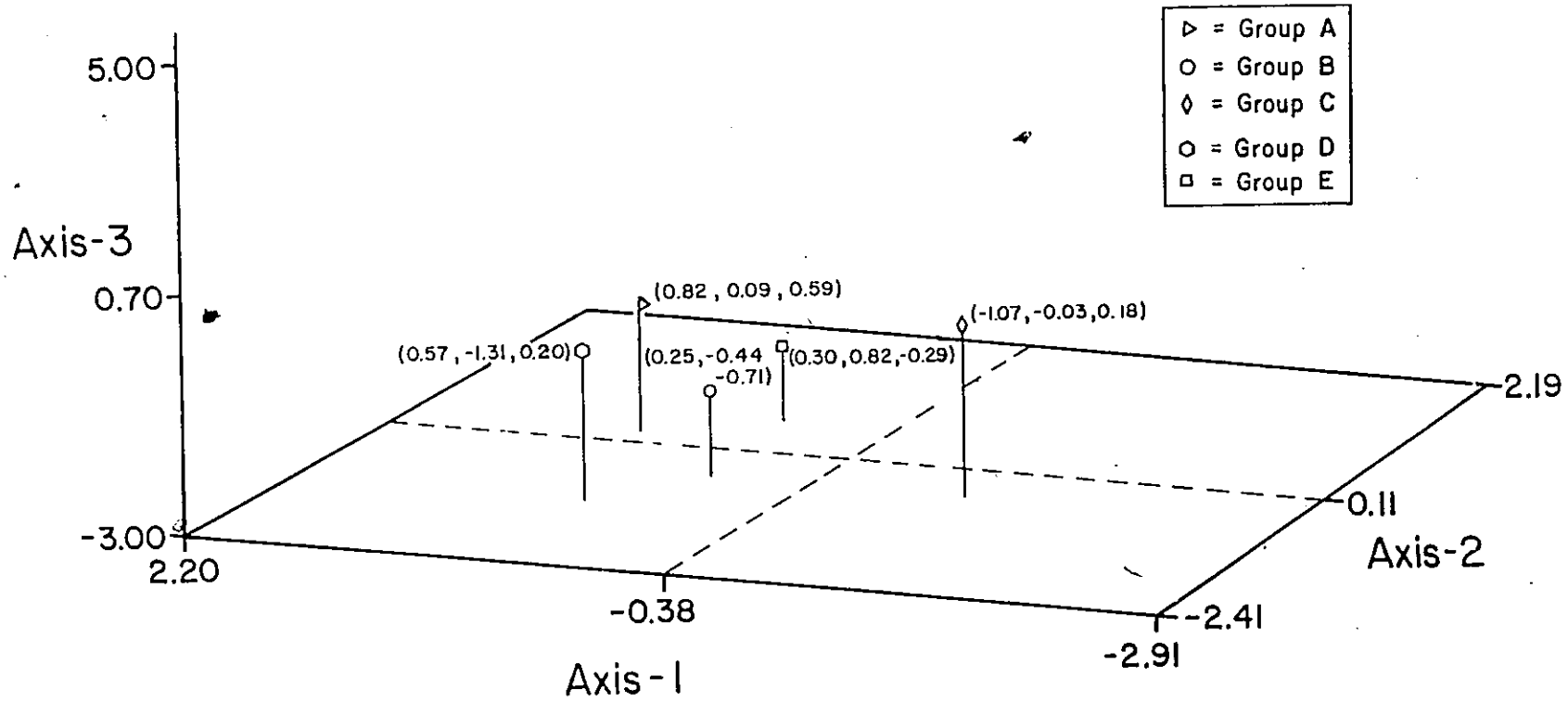

▷ = Group A
○ = Group B
◊ = Group C
○ = Group D
□ = Group E
5.00
Axis-3
0.70
-3.00
(0.82 , 0.09 , 0.59)
(-1.07, -0.03, 0.18)
(0.57 , -1.31 , 0.20)
(0.25, -0.44 , -0.71)
(0.30, 0.82, -0.29)
2.19
0.11
Axis-2
2.20
-0.38
-2.41
-2.91
Axis - 1

TABLE 20

TOTAL CANONICAL STRUCTURE

VARIABLE	AXIS-1	AXIS-2	AXIS-3
field leaf damage rating	0.056	-0.447	-0.206
plant breakage	-0.237	0.126	-0.124
stalk rot	0.444	0.458	-0.125
plant height	-0.562	-0.231	-0.032
smut rating	0.120	0.392	0.399
tunneling/ height ratio	0.664	-0.019	0.532
DIMBOA concentration	-0.557	0.201	0.191

axis-1, the tunneling/height ratio, followed by smut rating, field leaf damage rating, DIMBOA concentration, stalk rot, plant breakage, and plant height.

CHAPTER IV

DISCUSSION

4.1 PHYTOCHEMICAL STUDIES - HYDROXAMIC ACIDS

4.1.1 ADVANTAGES OF EXTRACTION METHOD

The DIMBOA extraction procedure used in this study was found to be rapid, sensitive enough to detect microgram quantities and to distinguish between the various hydroxamates, and suitable for screening a number of germplasm types at once. The most useful feature of this method is that seedlings can be used. This not only reduces the time required for growth of the plants but allows for easier extraction with less tissue to handle. The use of etiolated tissue avoids the presence of chlorophyll and therefore the steps necessary to remove it before hydroxamates can be assayed and also produces plants of nearly uniform size despite the diversity of germplasm used in the study. Long et al (1977) have demonstrated that DIMBOA levels in seedlings are significantly correlated to that of mature plants. Therefore measurements of seedling DIMBOA concentrations can be expected to give a reasonable estimate of the relative hydroxamate concentration in the more mature tissues where insect attack occurs.

4.1.2 DIFFERENCES BETWEEN GERMPLASM GROUPS

The wide difference between the Mexican landraces and today's modern cultivars of maize has led some researchers to question how closely related these two germplasm types are (Wilkes, 1982; Wellhausen et al, 1952; Randolf, 1976). It was found in this study that all of the germplasm contained three major hydroxamates:

HMBOA, DIMBOA, and MBOA. The only difference that existed among the inbred lines and the landraces was in the concentrations of the compounds. The range of concentrations are comparable to those found by other researchers in various lines of maize (Gutierrez et al, 1982; Argandona and Corcuera, 1985; Sullivan et al, 1974; Rojanaridpiched et al, 1984). These findings indicate that, at least with regards to hydroxamic acids, the landraces and the modern inbreds share a similar genetic background since no new or different compounds were found. This situation reflects the generally held view that the landraces and the modern cultivars all belong to one species, *Zea mays*, with significant gene flow between distinct populations. However, in different species of the Gramineae, such as wheat and rye, there are low levels of DIMBOA and high levels of the additional hydroxamate 2,4-dihydroxy-1,4- benzoxazin-3-one (DIBOA), which is rarely found in maize (Argandona, 1980, 1981).

The findings of this study indicate that there exists significant differences in the total DIMBOA concentrations among the germplasm types. Most significant was that the Mexican landraces had the lowest concentrations of all the groups studied. This is the same result that Scriber et al (1975) found when working with tropical lines. Sullivan et al.(1974) also found lower levels in exotic maize lines as compared to the higher levels in modern cultivars.

Unlike their Mexican counterparts, the Argentine landraces had very high DIMBOA concentrations. If these races are indeed derived from the Mexican ones (Goodman and Mck. Bird, 1977), then they may be further removed than first thought. Perhaps they have undergone more selection for resistance and are much younger than the Mexican races.

The latitudinal series of inbred lines had DIMBOA levels higher and more variable than those of the Mexican landraces. These higher levels may be the unintentional result of repeated selection for resistance to several pests carried out both at CIMMYT and the countries or latitudes of origin of each germplasm group. Many of today's resistant cultivars may well have been created by such a mechanism since high

levels of DIMBOA are found in the first-brood borer resistant control lines A619, OH43, and B73 and in the Canadian synthetic lines A, B, and C. An exception is the low level of DIMBOA in the multi-borer resistant line which has been bred for resistance against three species of tropical stem borers. The low levels may be explained as a result of selection for resistance to stalk boring insects rather than to leaf-feeding ones, since DIMBOA has been shown to confer resistance only to leaf feeding.

The CIMMYT maize pools, all intended for sub-tropical to temperate areas, had relatively high levels of DIMBOA. Again, these lines have already undergone a great deal of selection especially for resistance to leaf diseases. The crosses, which were carried out at CIMMYT, between these pools yielded progeny with even higher levels of DIMBOA. This result suggests that the inheritance of DIMBOA is primarily additive.

Inheritance of DIMBOA was further examined in the hybridization studies. The results indicate that inheritance is partially dominant since crosses between lines with high and low levels result in progeny with intermediate levels. These results are the same as that of Long et al (1977) who suggested that resistance is inherited multigenically. Many more crosses including reciprocal, backcross, and others will be required to further elucidate the mode of inheritance.

4.2 RESISTANCE STUDIES

4.2.1 LABORATORY LEAF FEEDING TESTS

The method used, consisting of a newly developed system using petri plates, was found to be quite satisfactory and gave results that showed significant differences among the germplasm types. There were three major advantages of this method: one, it tests the actual site of insect attack without exposing the insect to excised or dessicated tissue; two, field trials and seasonal restraints are avoided; and three, it permits a number of lines to be screened at one time.

The Mexican landraces were shown to be the most susceptible to leaf feeding as expected from their low content of DIMBOA. Unexpectedly, the multi-borer resistant line also with low DIMBOA levels was not consumed. This line is an example of non-conventional resistance. No lines or races with high DIMBOA levels were susceptible to leaf feeding, yet the multi-borer resistant line and a few of the inbred lines have low DIMBOA yet high resistance. This result indicates that factors other than hydroxamic acids play a role in the resistance of these lines. Perhaps these factors are of a morphological nature or perhaps other phytochemicals such as phenolics may be involved. Analysis of silica and lignin contents of these lines may prove useful (Rojanaridpiched et al, 1984). The other germplasm groups, all with higher DIMBOA levels, were much less consumed and therefore more resistant as expected.

4.2.2 FIELD STUDIES

Field studies carried out over two seasons revealed differences among the germplasm in their resistance to European corn borer infestations, stalk rot infection, and high density planting.

4.2.2.1 SUCCESS OF THE ARTIFICIAL INFESTATION OF EUROPEAN CORN BORERS

The six uninfested maize plants at the front (East) of each row in the field were all rated highly resistant, i.e. largely undamaged, to leaf feeding, plant breakage, and borer tunneling. This indicates that there was little natural borer infestation. This could also suggest that the lines that rated highly resistant after an artificial infestation may have done so because the artificial infestation was not successful. Evidence that such was not the case is provided by the consistent results with infested plants over two field seasons and the fact that borers were found to inflict heavy harvest damage on the plants. This means that lines rated resistant to borer damage/feeding were indeed resistant and that artificial infestation was successful.

4.2.2.2 INDIGENOUS LANDRACES OF MEXICO - IMPLICATIONS OF THEIR DIFFERENT MORPHOLOGY

The Mexican landraces grew the tallest of all the germplasm. Under the long photoperiod of an Ottawa summer their flowering was probably reduced with concomitant increased vegetative growth. A different response to a different climate is not unusual (Hudon and Chiang, 1985; Hartman et al, 1981) and was even somewhat expected (Bockholt, 1979). Nevertheless, it is necessary to evaluate all germplasm intended for a Canadian breeding program under Canadian conditions. Tall plants are not necessarily a desirable characteristic with regard to European corn borer resistance since it has been shown that the adult moths prefer taller plants for oviposition (Patch, 1946; Andrew and Carlson, 1976).

Leaf counts were used to examine the relative vegetative growth of the different lines and races. Wide variations in leaf number and number of tillers was found between the Mexican landraces and the inbred lines. There are no studies indicating any correlation of the number of leaves and resistance to insect feeding, although it can be expected that the number of leaves could play a role in the shape of plants and host-selection by the insects (Feeny, 1976). The large amount of vegetative growth in the landraces is not beneficial since it often results in a decrease in grain production.

The resistance of the Mexican landraces to plant breakage, borer tunneling, and stalk rot may be related to their thick, solid stalks, often as much as four times thicker than stalks of the average inbred line. A thick stalk has been found to be a source of resistance in wheat to the wheat stem fly, *Cephus cinctus,* where stem solidness results in damaged, desiccated eggs and impaired larval movements and also in sugarcane where the hardness of rind and fiber content of stalks increases resistance to *Diatrea saccharalis* larval boring (Norris and Kogan, 1980). The thicker stalks and numerous leaves of the Mexican races were densely pubescent by harvest. It has been shown that the presence of plant hairs interferes with insect oviposition, attachment

to the plant, feeding, and ingestion (Norris and Kogan, 1980). Mutant lines of soybeans differing only in pubescence have been shown to have leafhopper, *Empoasca fabae*, infestations directly related to hair density (Singh et al, 1971).

4.2.2.3 HIGH DENSITY STRESS AND RESISTANCE

Competition for resources, especially light, was severe among plants stressed by high density planting as shown by their widely varying heights. Seeds slow to germinate failed to reach maturity in most cases. Overcrowding often resulted in lodging particularily if there was a strong wind or rain. Shorter plants that can withstand high density planting yet still give high yields are one of the aims of the CIMMYT maize program.

The Mexican landraces were the most uniform in height whether stressed or not by high density planting. The greatest degree of variation in stressed plant heights was found with the inbred lines. This suggests that the inbred lines are not as tolerant of overcrowding since the natural height variability of the unstressed plants was quite low for the majority of the inbred lines. This result would indicate that between these lines and the Mexican races there is a difference in their response to high density planting with the landraces being able to tolerate it better. Many more studies carried out at differing densities need to be performed before any definitive conclusions can be made in this regard.

A high incidence of smut infection (up to 30%) was found in the stressed plants. There are two possible reasons for this: one, very few of the germplasm types have been selected for resistance to this pathogen; and two, high density stressed plants are particularily susceptible to fungal infection. The latter seems most probable since nearly all of the unstressed plants were uninfected. This clearly indicates the disadvantage of stressful environments especially high density planting. However, removal of this stress is not always possible due to the need to maximize yields, so another al-

ternative is to develop lines of maize either resistant to the fungus or to increase resistance by having lines better able to tolerate high density stress. Similarily, with resistance to stalk rot infection all unstressed plants were rated as resistant to stalk rot. This result indicates that the level of fungal inoculum in the field was low and those plants rated resistant after artificial infection were indeed resistant. It would be useful to repeat this study but to remove the other two stresses to see what the effect of borer infestation and high density planting have on a plant's resistance. In the long run, for a successful crop, the plants must be able to withstand all three stresses.

4.3 THE MAJOR GERMPLASM GROUPS AND THEIR POTENTIAL FOR USE IN CANADIAN MAIZE BREEDING PROGRAMS AIMED AT RESISTANCE TO THE EUROPEAN CORN BORER

One of the most important factors determining the potential use of the germplasm in a Canadian breeding program is the maturity or days to silking. Lines that take more than 96 days to silk are considered too late under Quebec growing conditions (Hudon and Chiang, 1983). All of the germplasm studied, with the exception of most of the Mexican landraces, had silked by 95 days and thus can be integrated into a Canadian breeding program. Some, such as the Argentine landraces and synthetic lines which silked by 85 days may have even more potential since it has been shown that European corn borer moths lay more eggs on later maturing lines (Andrew and Carlson, 1976). Later maturing lines have a higher proportion of plants at the preferred whorl stage at the time eggs are laid.

FIGURES 55 to 61 are suite of character graphs summarizing the phytochemical and resistance data obtained for each major germplasm group in this study. The latitudinal series of inbred lines (FIGURE 55) had intermediate to high levels of DIMBOA; was resistant to borer leaf feeding and smut infection; but, was susceptible to borer tunneling and stalk rot. This result indicates that these inbreds are potential-

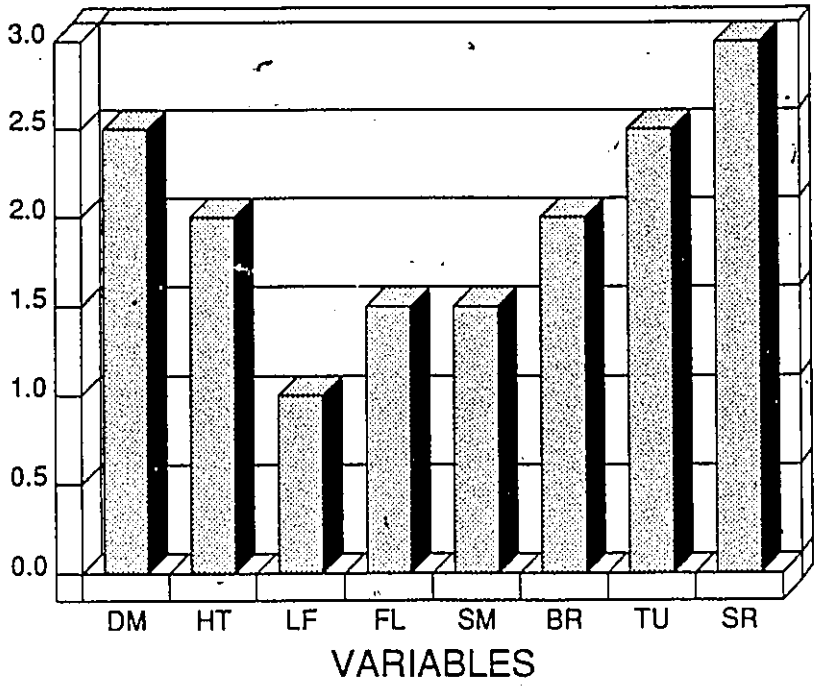
FIGURE 55

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE LATITUDINAL SERIES OF INBRED LINES

DM= relative concentration of DIMBOA in one-week-old etiolated maize seedlings; 1= low, 2= intermediate, 3= high
HT= relative plant height; 1= short, 2= average, 3= tall
LF= relative laboratory leaf-feeding rating;
1=resistant, 2= intermediate resistance, 3=susceptible
FL= relative field leaf damage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
SM= relative smut rating;
1=resistant, 2= intermediate resistance,3=susceptible
BR= relative plant breakage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
TU= relative tunneling/height ratio;
1=resistant, 2= intermediate resistance, 3=susceptible
SR= relative stalk rot rating;
1=resistant, 2= intermediate resistance, 3=susceptible

FIGURE 56

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE INDIGENOUS LANDRACES OF MEXICO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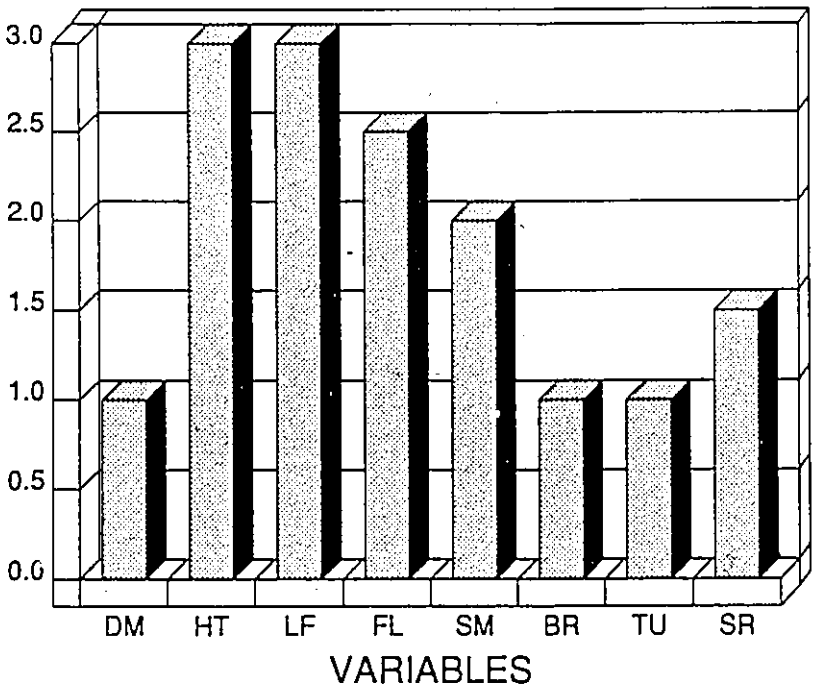
VARIABLES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ly good sources of first-brood resistance and, combined with their early silking dates, are good candidates for further study and integration into Canadian germplasm.

The indigenous landraces of Mexico (FIGURE 56) with low concentrations of DIMBOA and susceptibility to leaf-feeding borers do not have much potential in a breeding program for leaf-feeding resistance. But, this does not mean that they should be discarded especially since they are highly resistant to borer tunneling and stalk rot. It is important to note that FIGURES 55 and 56, the inbred lines and the Mexican races, are mirror images of each other. This indicates that two different selection pressures were acting on these two germplasm groups. The inbred lines were selected for leaf-feeding resistance while the races were selected for borer tunneling resistance. Unfortunately, failure to silk of the Mexican landraces puts a severe limitation on their potential use in a Canadian breeding program. These races were also most susceptible to smut. This is not surprising since these races are growing in a completely different climate than what they have been adapted to or bred for. They may not have developed any form of resistance to Northern strains of smut. A line with a too high incidence of smut is not worth the time and effort for a Canadian breeder to work with (Dr. R. Hamilton, Agriculture Canada, Personal communication). Their use will be limited until a great deal of breeding is carried out unless the genes that condition their borer tunneling resistance can be located and utilized. Such a prospect is limited until further advances in genetic engineering are made.

The Argentine landraces had some of the highest levels of DIMBOA (FIGURE 57). They were resistant to leaf-feeding, intermediate in resistance to borer tunneling, but susceptible to stalk rot. Since they were average in height and had early silking dates they show promise of being good germplasm for a Canadian breeding program. Their only drawback is that being more of an "exotic" variety the integration of their germplasm into commercial lines may take some time.

The Canadian synthetic lines A, B, and C (FIGURE 58) have previously been bred for resistance to the European corn borer. This was evidenced in their inter-

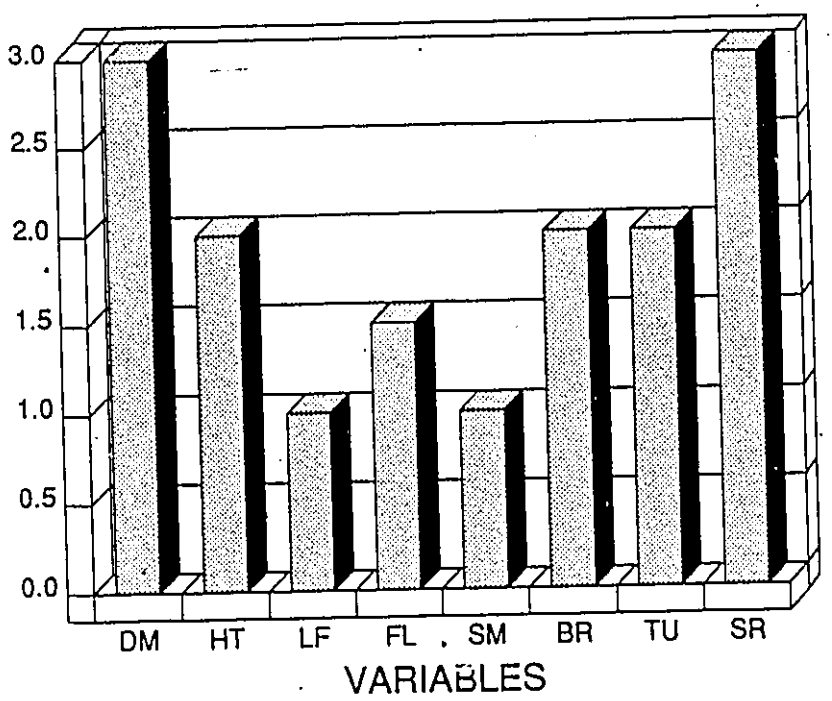
FIGURE 57

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE ARGENTINE LANDRACES

DM= relative concentration of DIMBOA in one-week-old etiolated maize seedlings; 1= low, 2= intermediate, 3= high
HT= relative plant height; 1= short, 2= average, 3= tall
LF= relative laboratory leaf-feeding rating;
1=resistant, 2= intermediate resistance, 3=susceptible
FL= relative field leaf damage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
SM= relative smut rating;
1=resistant, 2= intermediate resistance,3=susceptible
BR= relative plant breakage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
TU= relative tunneling/height ratio;
1=resistant, 2= intermediate resistance, 3=susceptible
SR= relative stalk rot rating;
1=resistant, 2=intermediate resistance, 3=susceptible

FIGURE 58

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE CANADIAN SYNTHETIC LINES A, B, AND C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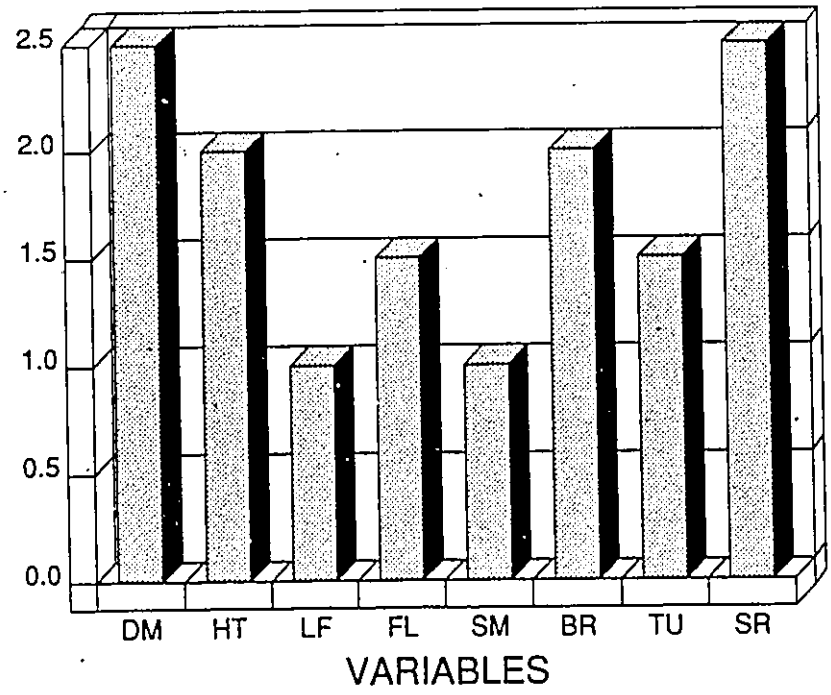
VARIABLES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VARIABLES

mediate to high resistance to both leaf feeding and tunneling borers. Coupled with their early silking dates, average heights, and intermediate to high DIMBOA levels, further studies and integration of these lines should be encouraged, especially with respect to improving their stalk rot resistance. An important point to emphasize with these lines is that their increased borer tunneling resistance may be a result of their high leaf-feeding resistance. This means that control of the borer by eliminating the first-brood leaf feeders may be quite feasible.

As expected the resistant control lines A619, OH43, and B73 were all resistant to the first-brood borers (FIGURE 59) and agrees with results obtained by Guthrie et al (1985). This was also reflected in their high levels of DIMBOA. Resistance of these lines to borer tunneling and stalk rot needs to be improved as evidenced by their intermediate to susceptible ratings.

The CIMMYT maize pools and the multi-borer resistant line (FIGURES 60 and 61) have both proven to be good sources of first-brood resistance along with intermediate resistance to stalk rot. Both are also early maturing and resistant to corn smut. Use of these germplasm in Canadian breeding programs should be encouraged. In addition, further studies on the multi-borer resistant line should be carried out to discern what other factors condition its first-brood resistance since DIMBOA levels are extremely low. Once elucidated, breeding programs to incorporate these resistance factors into more commercially desirable varieties of maize should be implemented.

4.4 CORRELATIONS AMONG PHYTOCHEMICAL AND RESISTANCE VARIABLES

The use of etiolated seedling DIMBOA concentrations to predict resistance to leaf-feeding was validated by the highly significant negative correlations of DIMBOA levels with both laboratory and field leaf-feeding ratings. Seedling extractions avoid difficult, time consuming extractions of whorl tissue, although it does not eliminate

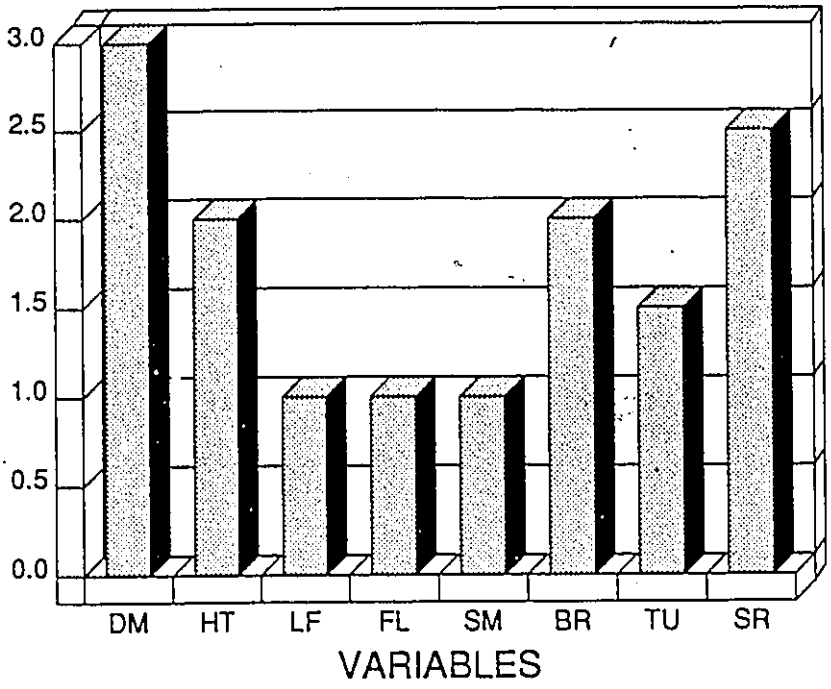
FIGURE 59

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE RESISTANT CONTROL LINES A619, OH43, AND B73

DM= relative concentration of DIMBOA in one-week-old etiolated maize seedlings; 1= low, 2= intermediate, 3= high
HT= relative plant height; 1= short, 2= average, 3= tall
LF= relative laboratory leaf-feeding rating;
1=resistant, 2= intermediate resistance, 3=susceptible
FL= relative field leaf damage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
SM= relative smut rating;
1=resistant, 2= intermediate resistance,3=susceptible
BR= relative plant breakage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
TU= relative tunneling/height ratio;
1=resistant, 2= intermediate resistance, 3=susceptible
SR= relative stalk rot rating;
1=resistant, 2= intermediate resistance, 3=susceptible

FIGURE 60

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE CIMMYT MAIZE POOLS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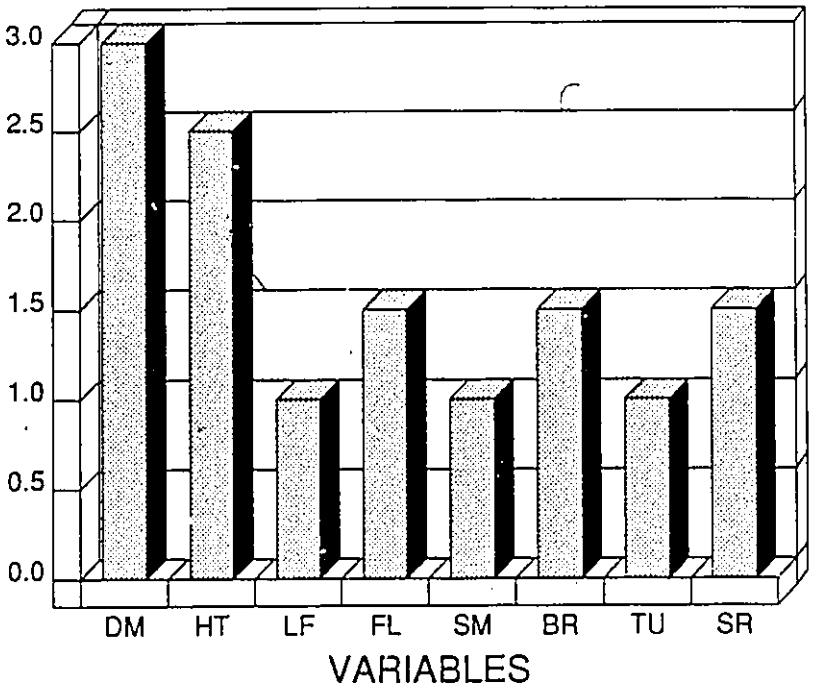
VARIABLES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2.0
1.5
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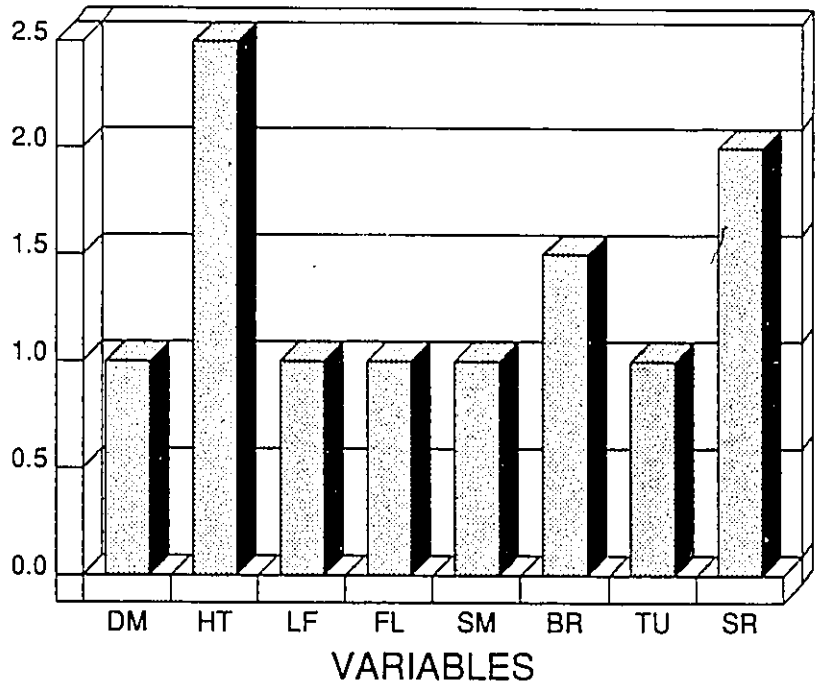
0.5
0.0
DM
HT
LF
FL
SM
BR
TU
SR
VARIABLES

FIGURE 61

THE MEAN PHYTOCHEMICAL AND MEAN RESISTANCE CHARACTERISTICS OF THE MULTI-BORER RESISTANT LINE

DM= relative concentration of DIMBOA in one-week-old etiolated maize seedlings; 1= low, 2= intermediate, 3= high
HT= relative plant height; 1= short, 2= average, 3= tall
LF= relative laboratory leaf-feeding rating;
1=resistant, 2= intermediate resistance, 3=susceptible
FL= relative field leaf damage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
SM= relative smut rating;
1=resistant, 2= intermediate resistance,3=susceptible
BR= relative plant breakage rating;
1=resistant, 2= intermediate resistance, 3=susceptible
TU= relative tunneling/height ratio;
1=resistant, 2= intermediate resistance, 3=susceptible
SR= relative stalk rot rating;
1=resistant, 2= intermediate resistance, 3=susceptible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1.5
1.0
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DM
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TU
SR
VARIABLES

the need to perform resistance tests as evidenced by the unexpected results with the multi-borer resistant line. The positive correlation of laboratory and field leaf-feeding ratings indicates that evaluation of resistance need not be in the field. This means that seedling DIMBOA concentrations along with laboratory leaf-feeding tests will give reliable predictions of the performance of a given maize line in the field with regards to first-brood leaf feeding resistance. One important point to emphasize is that the Mexican landraces had a larger negative correlation and slope for DIMBOA concentration and leaf-feeding correlations than the inbred lines. This result indicates that DIMBOA plays a larger role than other possible resistance factors for these races. The significance of these results is that the DIMBOA/leaf-feeding correlation, long observed in North American germplasm, obviously extends to a much broader tropical and subtropical collection.

The negative correlation of DIMBOA concentration with plant height is a result of dilution since biosynthesis of DIMBOA remains constant with plant age even though tissue mass increases (Reimann and Byerrum, 1964; Tipton et al, 1973). The dilution of DIMBOA is greatest in the Mexican races due to their extensive vegetative growth. This taken together with their low initial DIMBOA content may account for their increased susceptibility to leaf-feeding larvae. Positive correlations of both laboratory leaf-feeding ratings and field leaf damage ratings with plant height may also reflect DIMBOA dilution. If we had relied on natural infestation the same correlation might have been found but this time it may have been due to the oviposition preference of borer moths for taller plants. This preference for taller plants may be because the insects instinctively know that the taller plants are more edible because of DIMBOA dilution or it is just a preference for the morphology and apparency of taller plants.

One would expect that heavy field leaf damage by the borers would result in a large population of larvae that would tunnel into the stalks and result in greater plant breakage. However, the negative correlations of field leaf damage with plant breakage

and the tunneling/height ratio suggests that some other resistance mechanism(s) must come into play to make the plants resistant to borer tunneling (Klenke et al, 1986).

A negative correlation of field leaf damage with stalk rot resistance was also obtained. Again one would expect susceptibility to first brood larvae to lead to an increased insect population and subsequently more wounds for pathogens to enter into the plant. As with borer tunneling the results indicate that this is not the case and suggests that resistance to leaf feeding, borer tunneling, and stalk rot must involve a number of genes which operate independently.

The question of whether or not plant breakage is a result of borer tunneling or stalk rot was examined by observing the positive correlations found among these three variables. These correlations suggest that plant breakage may be due to both borer tunneling and stalk rot, with a major role attributed to borer tunneling because of the more significant correlation. Plants susceptible to borer tunneling were also more susceptible to the spread of stalk rot infection. This result indicates that the probability of existance of similar mechanisms of resistance to borer tunneling and stalk rot is greater than the probability of similar mechanisms between field leaf feeding and borer tunneling or stalk rot. All of these results emphasize the need to select for resistance to both insects and pathogens. However, further studies will have to be carried out to further test the relationship between borer resistance and stalk rot resistance. It has been shown that with regards to first-brood borer resistance and Northern corn leaf blight, selection cannot be made for one and expect to have resistance to the other (Guthrie et al, 1985).

Plant height was found to be negatively correlated with both plant breakage and stalk rot such that an increase in height led to a decrease in both plant breakage and stalk rot infection. The decrease in plant breakage is most likely due to the thicker, stronger stalks found on the taller plants, especially the Mexican landraces. Increased plant height would also result in longer internodes which, combined with thicker stems, would tend to suggest a morphological resistance to stalk rot infection.

Again, if this is so, it is not necessarily a desirable mechanism of resistance for a grain crop plant since too much of the plant's energy would be put into vegetative growth rather than grain production.

Comparison of many of the correlations found to those already reported by other researchers was not possible due to the wide range of methods used to evaluate resistance and the different parts of the maize plant that were used for DIMBOA analysis. In addition, many of the correlations have just been assumed to exist and have not been really tested or published as of yet.

4.5 RESISTANCE OF MAIZE AS RELATED TO THE GEOGRAPHIC ORIGIN OF MAIZE GERMPLASM

The purpose of this part of the study was to take the results obtained with the two major germplasm groups, the latitudinal series of inbred lines and the almost complete set of the indigenous landraces of Mexico, and to examine the relationship between geographical origin of germplasm and resistance to the European corn borer. Special emphasis was made on latitudinal and altitudinal origins. Such studies of sources of resistance are few (Wellhausen et al, 1952), even though such an approach is a logical one in the face of the extensive sources of germplasm in the world today. This study is the first to examine world germplasm resources of maize and has revealed definite global trends in the resistance of maize to the European corn borer.

The latitudinal series of inbred lines developed by CIMMYT for use in specific latitudinal ranges was idealy suited for this study. All of these lines have been selected and bred for optimal agronomic characters such as yield. The results of this study indicate that there are definite trends between the latitudinal origin and DIMBOA concentrations, plant breakage ratings, tunneling/height ratios, and stalk rot ratings.

None of the latitudinal groups has been selected for either borer resistance or DIMBOA concentrations. Thus the trend to increased DIMBOA concentrations ob-

served in the lines from more Southern latitudes is significant and expected. Studies on plants other than maize have revealed ecological and geographical patterns in the toxicity of plant alkaloids, another major group of secondary metabolites. Levin and York (1978) have shown that tropical plants have higher concentrations of, and more toxic, alkaloids than temperate plants. These authors hypothesize that this difference is due to more intense herbivory in the tropics and the superior ability of tropical pests to develop resistance.

Although latitudinal trends in DIMBOA concentrations were noted, no similar trends were found for resistance to leaf-feeding borers. This is unexpected since DIMBOA levels and leaf feeding resistance have already been shown to be closely correlated in the present study. Part of the reason for the lack of correlation may be that, at least for field leaf damage, the lines are almost equal in their resistance. This result indicates that although there is a decrease in DIMBOA concentrations in the more Northern latitudes, these lines compensate for lower DIMBOA levels by having other factors to bring their levels of resistance up to that of their Southern counterparts.

The most pronounced results were found for borer tunneling resistance and stalk rot resistance with increased susceptibility to both in higher latitudes. Previous results indicate that morphological resistance may be the key factor. Southern and tropical plants are exposed to many more insects, and since an adult plant is the chronologically largest portion of the corn plant's life cycle, those lines requiring a longer growing season will be exposed to more insects at all stages of their lives. In addition it is known that the number of generations of borers per year increases with decreasing latitude (Hudon and LeRoux, 1986). Thus the mature plants would be exposed to possibly three or more major attacks by a multi-voltine strain of borer, a circumstance that could lead to the development of morphological resistance features such as thicker, more pubescent stalks.

The more humid, hot climates of the tropics would promote greater fungal growth. This would mean that Southern plants would have to develop greater resis-

tance. It is interesting at this point to emphasize that the Mexican landraces are also the most resistant to borer tunneling and stalk rot.

Since the majority of indigenous races of maize are found in Central America and since this is believed to be the center of origin of maize, it stands to reason that practically all natural genetic variability for adaptations to all latitudes and altitudes, in addition to germplasm for special and general purposes, can be obtained in this area (Hallauer, 1978). Thus the Mexican landraces were examined for any correlations between their resistance and geographic origin. The lack of correlation of resistance and latitude is not surprising since in Mexico neither latitude nor longitude has much influence on the environment. Instead, altitude has a major influence and is predominantly associated with changes in precipitation, length of the growing season, and temperature. The result is hot, moist low altitudes and cooler, drier high altitudes with a shorter growing season (Doebley et al, 1985).

The highly significant negative correlation found between total DIMBOA concentrations and altitude means that the landraces adapted for growth in the lower altitudes have higher concentrations of DIMBOA. There are a number of possible reasons for increased levels in lower altitudes, the major one being that the longer growing season at lower altitudes would expose the plants to longer periods of attack; and possibly more generations of insects per year. Two positive correlations of altitude with laboratory leaf-feeding ratings and field leaf damage ratings show that higher altitude races were much more heavily damaged. Similar positive correlations were found between altitude and harvest damage. Therefore, the higher altitude landraces were less resistant to attack by both leaf feeding and tunneling borers. This suggests that if breeders are to choose sources of resistance from the Mexican landraces they should start with those adapted to conditions prevailing at lower altitdes.

One other reason could account for the increased resistance of the lower altitude races. All of these races are well adapted for use in primitive, traditional agriculture. Although low yielding, they are tough, as evidenced by their morphological charac-

teristics. It stands to reason that those races adapted for growth in lower altitudes where most farming occurs would be preferred over the others for farming. This would mean that once selected in farming the races would undergo continuous selection by the farmers for resistance along with other desirable agronomic characters. Selection would in time increase their resistance levels and perhaps their DIMBOA levels.

No study other than the present one has been carried out on the Mexican landraces with regard to their resistance characteristics and geographic origin. However, a few studies have been made of isozyme variation and geographic origin in an attempt to further classify these races. The most intensive isozyme study was carried out by Doebley et al (1985) using isozymes to determine the variation among thirty-four Mexican races. They found that the races originating from Northern Mexico were much more variable than those from the South. Even stronger correlations were found between isozyme alleles and altitudinal origin.

4.6 RESISTANCE OF MAIZE AS RELATED TO THE TAXONOMY OF MAIZE GERMPLASM

It has already been shown in this study that the indigenous landraces of Mexico exhibit some promise as potential sources of resistance to the tunneling larval stage of the European corn borer. Since there are a number of major and sub-races of maize from Mexico it is a formidable task to decide which races to select for intensive studies. Most of the races have already been classified into major groups by Wellhausen et al (1952) and their's is still the most widely accepted classification. For this reason it seems reasonable to use the existing taxonomy of this large group of germplasm to attempt to produce a more systematic outlook on the resistance of maize to the European corn borer.

4.6.1 STEPWISE DISCRIMINANT ANALYSIS

Of all the phytochemical and resistance variables only four (DIMBOA concentrations, field leaf damage ratings, plant height, and the tunneling/height ratio) were selected by stepwise discriminant analysis as the most useful in separating out Wellhausen et al's existing groups of landraces. This selection was expected since all these variables, except field leaf damage ratings, were the most variable among the races studied.

4.6.2 CLASSIFICATORY DISCRIMINANT ANALYSIS

Best results of classificatory discriminant analysis were obtained with the quadratic model. This is because the covariances were not equal and therefore more suited to the quadratic function. Using this model it was found that 38 out of a total of 48 (79%) landraces examined were reclassified into their original labeled races following Wellhausen et al. Therefore, based upon the resistance data alone and from this analysis, it can be assumed that Wellhausen et al's groups are justified. Given their classification and the observations made on resistance there is a good indication that Wellhausen et al's taxonomy predicts resistance. It is therefore justifiable to use this taxonomy when studying resistance to the European corn borer, although it must be remembered that even with a 79 % reclassification the "goodness" of fit has yet to be verified, ie. by simulation studies and the collection of more data.

With Wellhausen et al's classification justified with respect to resistance, it was of interest to see how some more modern lines fit into their scheme. Almost 85% of all the remaining germplasm in this study (latitudinal series of inbred lines, CIMMYT maize pools, and Argentine landraces) were classified into Wellhausen's group A. Group A, the Ancient Indigenous races, is believed to have arisen from primitive pod corn. This theory of origin is significant since many theories propose that our modern cultivars are descended from such pod corn along with some introgression with teosinte

(Mangelsdorf et al, 1964). It is not surprising that the diverse amount of germplasm studied falls into this group since the major factor differentiating the races of this group is their independent development in different localities and environments (Wellhausen et al, 1952). In addition these landraces are the earliest maturing.

4.6.3 SUMMARY STATISTICS

Summary statistic results allowed for two major conclusions about the various races. The races are best distinguished by their DIMBOA levels, plant height, and resistance to plant breakage and borer tunneling. This agrees with the results of the stepwise discriminant analysis. The second major conclusion is that group A, the group that 85% of the latitudinal series of inbred lines, Argentine landraces, and CIMMYT maize pools were grouped into, was the most susceptible of all to both leaf feeding and tunneling. This susceptibility indicates that although continued selection and breeding may have increased the levels of DIMBOA and first-brood resistance, it failed to increase levels of borer tunneling and stalk rot resistance. Further selection for resistance to both leaf feeding and tunneling should be made from germplasm material from the much more resistant group C, the Prehistoric Mestizos, rather than group A.

The races of group C are believed to be a result of hybridization between group A and group B, the Pre-Columbian Exotic races, and through the hybridization of these two with teosinte (Wellhausen et al, 1952). Groups A and B both lack high levels of resistance yet they have given rise to a highly resistant group when hybridized with teosinte. This indicates that teosinte may have imparted some degree of resistance to these groups. The high levels of DIMBOA found in group C most likely did not come from teosinte, which has low DIMBOA levels, but most likely resulted from continued selection for resistance. Field analysis of teosinte was not available due to insufficient seed material for analysis.

4.6.4 CANONICAL DISCRIMINANT ANALYSIS

Canonical discriminant analysis revealed further differences among the five Wellhausen et al groupings based on the analysis of phytochemical and resistance data. Mahalanobis distances between the means of each group indicated that group D, the Modern Incipient races, is the most distinguishable from group E, the "Poorly Defined" races, while group A, the Ancient Indigenous races, is much less distinguishable from group E. This indicates that many of the races that Wellhausen et al had difficulty in classifying, i.e. group E, due to a lack of data are most probably part of group A and least likely part of group D based on the analysis of resistance data.

The relationship among the groups was further illustrated with three-dimensional ordination plots. These results indicate there is overlap among the groups and that phytochemical and resistance data alone can not completely separate them although some degree of distinction was obtained. It is crucial at this point to emphasize that this ordination was carried out using the pooled covariance matrix. Since best results were obtained using the quadratic model of classificatory discriminant analysis, and it was shown that the covariances are not equal, this ordination plot is somewhat distorted since it assumes that the covariances are equal. Better results would have been obtained if this assumption could have been avoided.

The total canonical structure, also based on the linear model, indicated that for the ordination plots the borer harvest resistance variables, especially the tunneling/height ratio, and the stalk rot variable had the greatest influence on the separation of the groups. This agrees with previous results that also indicated that these were the most important variables in the analysis of the Mexican landraces.

CHAPTER V

CONCLUSIONS

5.1 MAIN CONCLUSIONS

(1) Using a rapid extraction method developed for the analysis of total DIMBOA concentrations in etiolated maize seedlings significant differences in DIMBOA levels exist among the maize germplasm types.

(2) Seedling DIMBOA concentrations, when combined with laboratory evaluation of resistance, give a reliable prediction of the performance of a given maize line in the field with respect to first-brood borer leaf-feeding resistance, thus the need to perform time consuming field trials when screening large sources of germplasm for resistance is eliminated.

(3) The Mexican landraces, when grown in the field, differ markedly in morphology and resistance characteristics from the other germplasm groups. These races are the most susceptible to leaf-feeding borers yet are the most resistant to borer tunneling and stalk rot. Morphological factors rather than phytochemical factors are believed to be responsible for this resistance.

(4) High density planting in the field increases the plant height variability and the susceptibility to corn smut and stalk rot.

(5) For Canadian breeding programs aimed at resistance to the European corn borer, all of the inbred lines and control lines are good sources of germplasm for first-brood leaf-feeding resistance. The Mexican races are potential sources of borer tunneling resistance although their failure to flower under Canadian conditions creates a barrier to their integration into a breeding program. The Argentine races and the Canadian synthetic lines all have high

resistance to leaf feeding and intermediate resistance to borer tunneling. The lines with the greatest potential are those of the CIMMYT maize pools and the multi-borer resistant line. These lines are the most resistant to both leaf-feeding and tunneling borers.

(6) Of the many correlations existing among the phytochemical and resistance variables, the most significant is a negative correlation of leaf feeding resistance and borer tunneling resistance. This means that very few lines are resistant to both. DIMBOA concentrations are not correlated to borer tunneling resistance or resistance to stalk rot and corn smut.

(7) There are global trends in European corn borer resistance. Inbred lines from the more Southern latitudes have higher DIMBOA levels and increased resistance to borer tunneling and stalk rot. Mexican landraces adapted to lower altitudes have higher DIMBOA levels and increased resistance to both leaf-feeding and tunneling borers.

(8) Taxonomic analyses reveals that the resistance of the Mexican landraces to the European corn borer is reflected in the existing taxonomy of maize developed by Wellhausen et al (1952). Almost all of the inbred lines used in this study are classified into the Ancient Indigenous group which is one of the most susceptible groups of all.

5.2 FUTURE WORK

The presence of HMBOA in all of the extracts indicates that the role of this compound, if any, in resistance should be elucidated. Further studies on the inheritance of DIMBOA need to be carried out.

The implications of planting density and its relationship to European corn borer, stalk rot, and corn smut resistance needs to be further elucidated.

Lines of maize exhibiting non-conventional resistance, such as the multi-borer resistant line, should be further studied to determine what other factors besides DIMBOA condition this resistance to the European corn borer. The levels of phenolic acids in the leaves of these plants should be quantified and the interaction between these factors should be determined. The basis of the proposed morphological resistance of the Mexican races needs to be elucidated by carrying out analyses such as content of silica and lignin.

All lines exhibiting resistance should be further studied with respect to integrating these resistant sources of germplasm into Canadian breeding programs. Particular emphasis should be placed on those lines originating from the more Southern latitudes and those adapted for growth at lower altitudes.

The integration into breeding programs of indigenous maize germplasm exhibiting high resistance to borer tunneling should be expanded with the goal of increasing the genetic base of modern cultivars in mind. All of the germplasm should be examined for resistance to other major pests of maize such as the corn rootworm, *Diabrotica* spps., and factors conditioning such resistance should be elucidated.

CHAPTER VI

REFERENCES

Adkisson, P.L., and Dyck, V.A. 1980. Resistant varieties in pest management systems. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell and P.R. Jennings (Editors). Wiley, N.Y. pp. 234-251.

Andrew, H.A., and Carlson, J.R., Jr. 1976. Preference differences of egg laying European corn borer adults among maize genotypes. *Hort. Science 11(2)*: 143.

Argandona, V.H., Corcuera, L.J., Niemeyer, H.M., and Campbell, B.C. 1983. Toxicity and feeding deterrency of hydroxamic acids from Gramineae in synthetic diets against the greenbug, *Schizaphis graminum. Ent. Exp. and Appl. 34:* 134-138.

Argandona, V.H., and Corcuera, L.J. 1985. Distribution of hydroxamic acids in *Zea mays* tissues. *Phytochem. 24(1):* 177-178.

Argandona, V.H., Luza, J.G., Niemeyer, H.M., and Corcuera, L.J. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochem. 19*: 1665-1668.

Argandona, V.H., Niemeyer, H.M., and Corcuera, L.J. 1981. Effect of content of hydroxamic acids in wheat on infestation by the aphid *Schizaphis graminum. Phytochem. 20(4)*: 673-676.

Argandona, V.H., Pena, G.F., Niemeyer, H.M., and Corcuera, L.J. 1982. Effect of cysteine on stability and toxicity to aphids of a cyclic hydroxamic acid from Gramineae. *Phytochem. 21(7)*: 1573-1574.

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

Bailey, W.C., and Pedigo, L.P. 1986. Damage and yield loss induced by stalk borer (Lepidoptera: Noctuidae) in field corn. *J. Econ. Entomol. 79:* 233-237.

Barber, D.T., and Witkowski, J.F. 1984. Concepts for combining suppression of European corn borer (Lepidoptera: Pyralidae) adults and larvae in center-pivot-irrigated corn. *J. Econ. Entomol. 77*: 1004-1007.

Barry, D., Zuber, M.S., Antonio, A.Q., and Darrah, L.L. 1983. Selection for resistance to the second generation of the European corn borer (Lepidoptera: Pyralidae) in maize. *J. Econ. Entomol. 76:* 392-394.

Beadle, G.W. 1980. The ancestry of corn. *Scientific Amer. 242(1)*: 112-119.

Beard, R.L. 1943. Investigations on the control of the European corn borer. Part I: Biological aspects. *Conn. Agric. Exp. Stn. Bull. 462:* 551-566.

Beck, S.D., and Rcese, J.C. 1976. Insect-plant interactions: nutrition and metabolism. *Recent Advances in Phytochem. 10*: 41-93.

Beck, S.D., and Schoovnhoven, L.M. 1980. Insect behavior and plant resistance. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell, and P.R. Jennings (Editors). Wiley. N.Y. pp. 116-133.

Beck, S.D., and Smissman. E.E. 1960. The European corn borer, *Pyrausta nubilalis*, and its principal host plant. VIII. Laboratory evaluation of host resistance to larval growth and survival. *Ann. Entomol. Soc. Amer. 53*: 755-762.

BeMiller,J.N.,and Pappelis, A.J. 1965. 2,4-Dihydroxy-7- methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, 1-butanol-soluble glycoside fraction content of pith cores and stalk rot resistance. *Phytopath. 55:* 1237.

Berger, P.J., Neges, N.C., Sanders, E.H., and Gardner, P.D. 1981. Chemical triggering of reproduction in *Microtus montanus. Science 214:* 69-70.

Bockholt, A.J. 1979. Biology and breeding of corn. In: *Biology and Breeding for Resistance to Arthopods and Pathogens in Agricultural Plants.* M.K. Harris (Editor). Texas A & M Univ., College Station, Texas. pp. 276-282.

Bowman, M.C., Beroza, M., and Klun, J.A. 1967. Spectrophoto- fluorometric determination of 6-methoxy-2-benzoxazolinone, an indicator of resistance to the European corn borer in *Zea mays. J. Econ. Entomol. 61(1):* 120-124.

Bravo,H.R., and Niemeyer, H.M. 1986. A new product from the decomposition of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a hydroxamic acid from cereals. *Heterocycles 24(2):* 335-337.

Bredenberg, J.B., Honkanen, E., and Virtanen, A.I. 1962. The kinetics and mechanism of the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one. *Acta. Chem. Scand. 16:* 135-141.

Caesar, L. 1926. Mortality of the European corn borer (*Pyraustra nubilalis*) adults and larvae. *Rep. Ent. Soc. Ont. 56:* 72-75.

Camussi, A. 1979. Numerical taxonomy of Italian populations of maize based on quantitative traits. *Maydica XXIV*: 161-174.

Cervantes, T., Goodman, M.M., Casas, E., and Rawlings, J.O. 1978. Use of genetic effects and genotype by enviromental interactions for the classification of Mexican races of maize. *Genetics 90:* 339-348.

Chang, T.T. 1984. Conservation of rice genetic resources: luxury or necessity? *Science 224*: 251-256.

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

Chiang, H.C., and Hudon, M. 1973. Inheritance of resistance to the European corn borer in grain corn. *Can. J. Plant Sci. 53:* 779-782.

Chiang, H.C., and Hudon, M. 1974. A good corn borer resistant line of grain corn, A619. *Phytoprotection 55*: 94.

Chiang, H.C., and Hudon, M. 1976. A study of maize inbred lines for their resistance to the European corn borer, *Ostrinia nubilalis (Hubner). Phytochem. 57(1)*: 36-40.

CIMMYT, 1984.

CIMMYT Maize Facts and Trends Report Two: An analysis of changes in Third World food and feed uses of maize, 1984.

CIMMYT Report on Maize Production. 1980-1981.

Clark, W.D., and Brown, G.K. 1980. Uses of high performance liquid chromatograpy in phytochemical studies. *Phytochem. Bull. 13(1)*: 2-23.

Cocley, N.W., and Lohnes, P.R. 1971. *Multivariate Data Analysis.* John Wiley and Sons. New York.

Copaja, S.V., Bravo, H.R., and Niemyer, H.M. 1982. Quantitation of N-(2-hydroxy-4-methoxy-phenyl)-glyoxylohydroxamic acid, a reactive intermediate in reactions of 2,4-dihydroxy-7- methoxy-1,4-benzoxazin-3-one. *J. Org. Chem. 19*: 1-16.

Corcuera, L.J., Argandona, V.H., Pena, G.F., Perez, F.J., and Niemeyer, H.M. 1982. Effect of a benzoxazinone from wheat on aphids. *Proc. 5th Int. Symp. Insect-Plant Relationships,* Wageningon. pp. 33-39.

Corcuera, L.J., Queirolo, C.B., and Argandona, V.H. 1985. Effects of 2-B-D-glucosyl-4-hydroxy-7-methoxy-1,4- benzoxazin-3-one on *Schizaphis graminum* (Rondani) (Insecta, Aphididae) feeding on artificial diets. *Experientia 41*: 514-516.

Corcuera, L.J., Woodward, M.D., Helgeson, J.P., Kelman, A., and Upper, C.D. 1978. 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin- 3-one, an inhibitor from *Zea mays* with differential activity against soft rotting *Erwinia* species. *Plant Physiol. 61*: 791-795.

Couture, R.M., Routley, D.G., and Dunn, G.M. 1971. Role of cyclic hydroxamic acids in mongenic resistance of maize to *Helminthosporium turricum. Physiol. Plant Pathol. 10*: 515-521.

Dabed, R.G.,Toral, M.I., Corcuera, L.J., and Niemeyer, H.M. 1982. Complexes of bivalent cations with a hydroxamic acid from maize extracts. *Polyhedron 2(2): 106-108.*

Davidson, R.H., and Lyon, W.F. 1979. *Insect Pests of Farm, Garden, and Orchard.* 7th Edition. Wiley and Sons. New York.

Doebley, J.F., Goodman, M.M., and Stuber, C.W. 1985. Isozyme variation in the races of maize from Mexico. *Amer. J. Bot 72(5):* 629-639.

Doebley, J.F., Goodman, M.M., and Stuber, C.W. 1986. Exceptional genetic divergence of Northern flint corn. *Amer. J. Bot. 73(1):* 64-69.

Dunn, G.M., Long, B.J., and Routley, D.G. 1981. Inheritance of cyclic hydroxamates in *Zea mays. Can. J. Plant Sci. 61*: 583-593.

Emery, T. 1971. Hydroxamic acids of natural origin. In: *Advances in Enzymology and Related Areas of Molecular Biology.* A. Meister (Editor). Wiley and Sons. New York. Vol. 35. pp. 135-185.

Everett, T.R., Chiang, H.C., and Hibbs, E.T. 1958. Some factors influencing populations of European corn borer *(Pyrausta nubilalis)* in the North Central States. *Minn. Agric. Exp. Stn. Tech. Bull.*: 63 pp.

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

Feldman, M., and Sears, E.R. 1981. The wild gene resources of wheat. *Scientific Amer. 224(1):* 102-112.

Franklin, R.T., and Holdaway, F.G. 1966. A relationship of the plant parasitism of European corn borer by the Tachinid parasite *Lydella grisescens. J. Econ. Entomol. 59*: 440-441.

Gale, J.S., and Lawrence, M.J. 1984. The decay of variability. In: *Crop Genetic Resources: Conservation and Evaluation.* Holden, J.H.W., and Williams, J.T. (Editors). Allen and Unwin. London, U.K.

Galinat, W.C. 1978. The inheritance of some traits essential to maize and teosinte. In: *Maize Breeding and Genetics.* International Maize Symposium, Sept. 1977. D.B. Walden (Editor). Wiley and Sons. New York. pp. 93-112.

Galinat, W.C. 1982. Maize breeding and its raw material. In: *Maize for Biological Research.* W.F. Sheridan (Editor). North Dakaota. pp. 331-334.

Galinat, W.C., Mangelsdorf, P.C., and Iltis, H.H. 1984. The origin of maize. *Science 225:* 1093-1096.

Goodman, M.M. 1978. A brief survey of the races of maize and current attempts to infer racial relationships. In: *Maize Breeding and Genetics.* International Maize Symposium. Sept. 1977. D.B. Walden (Editor). Wiley and Sons. N.Y. pp. 143-158.

Goodman, M.M. 1984. An evaluation and critique of current germplasm programs. In: *Conservation and Utilization of Exotic Germplasm to Improve Varieties.* Report of the 1983 Plant Breeding Research Forum. pp. 197-227. Des Moines, Iowa.

Goodman, M.M., and Mck. Bird, R. 1977. The races of maize IV: Tentative grouping of 219 Latin American races. *Econ. Bot 31:* 204-221.

Grambow, H.J., Luckge, J., Klausener, A., and Muller, E. 1986. Occurrence of 2-(2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin- 3-one)-B-D-glucopyranoside in *Triticum aestivum* leaves and its conversion into 6-methoxy-benoxazolinone. *Z. Naturforsch. 41c*: 684-690.

Guthrie, W.D. 1960. Techniques, accomplishments and future potential of breeding for resistance to European corn borers in corn. *Iowa State J. Sci. 35(1):* 214-253.

Guthrie, W.D. 1971. Resistance of maize to second-brood European corn borers. *26th Hybrid Corn Industry Research Conf.* pp. 165-179.

Guthrie, W.D. 1981. Maize whorl stage resistance to the first four instars of European corn borer larvae. *J. of the Kansas Ent. Soc. 54(4)*: 737-746.

Guthrie, W.D., Barry, B.D., Rossman, E.C., and Jarvis, J.L. 1985a. Correlation beween leaf-feeding resistance to European corn borer (Lepidoptera: Pyralidae) and resistance to Northern corn leaf blight. *J. Econ. Entomol. 78:* 811-814.

Guthrie, W.D., and Dicke, F.F. 1972. Resistance of inbred lines of dent corn to leaf feeding by first-brood European corn borer's. *Iowa State J. of Science 46(3):* 339-357.

Guthrie, W.D., Huggans, J.L., and Chatterji, S.M. 1969. Influence of corn pollen on the survival and development of second-brood larvae of the European corn borer. *Iowa State J. of Science 44(2):* 185-192.

Guthrie, W.D., Huggans, J.L., and Chatterji, S.M. 1970. Sheath and collar feeding resistance to the second-brood European corn borer in six inbred lines of dent corn. *Iowa State J. of Science 44(3):* 297-377.

Guthrie, W.D., Russell, W.A., Jarvis, J.L., and Robbins, J.C. 1985b. Performance of maize inbred line B86 in hybrid combinations: resistance to first- and second-generation European corn borers. *J. Econ. Entomol. 78:* 93-95.

Guthrie, W.D., Tseng, C.T., Russell, W.A., Coats, J.R., Robbins, J.C., and Tollefson, J.J. 1986a. DIMBOA content at seven stages of plant development in a maize synthetic cultivar. *J. of the Kansas Ent. Soc. 59(2):* 356-360.

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

Gutierrez, C., Guerrero, A., Castanera, P., and Torres, J.V. 1982. A high-performance liquid chromatographic method for quantitation of DIMBOA and MBOA in maize plant extracts. *J. Agric. Food Chem. 30(6)*: 1258-1260.

Hallauer, A.R. 1978. Potential of exotic germplasm for maize improvement. In: *Maize Breeding and Genetics.* International Maize Symposium, Sept. 1977. D.B. Walden (Editor). Wiley and Sons. N.Y. pp. 229-247.

Hallauer, A.R., and Miranda, J.B. 1981. *Quantitative Genetics in Maize Breeding.* Iowa State Univ. Press.

Hamilton, R.H. 1963. A corn mutant deficient in 2,4-dihydroxy-7- methoxy-1,4-benzoxazine-3-one with tolerance of atrazine. *Weeds 12:* 27-30.

Hamilton, R.H. 1964. Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *J. Agric. Food Chem. 12(1)*: 14-17.

Hamilton, R.H., Bandurski, R.S., and Reusch, W.H. 1962. Isolation and characterization of a cyclic hydroxamate from *Zea mays. Cereal Chem. 39:* 107-113.

Harlan, J.R. 1979. Origins of agriculture and crop evolution. In: *Biology and Breeding for Resistance to Arthropods and Pathogens in Agricultural Plants.* M.K. Harris (Editor). Texas A & M Univ. College Station, Texas. pp. 1-8.

Harlan, J.R., and Starks, K.J. 1980. Germplasm resources and needs. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell and P.R. Jennings (Editors). Wiley and Sons. N.Y. pp. 254-273.

Harris, M.K. (Editor). 1979. *Biology and Breeding for Resistance to Arthropods and Pathogens in Agricultural Plants.* Texas A & M Univ. College Station, Texas.

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

Hashimoto, Y., Shudo, K., Okamoto, T., Nagao, M., Takahasi, Y., and Sugimura, T. 1979. Mutagenicities of 4-hydroxy-1,4- benzoxazinones naturally occuring in maize plants and of related compounds. *Mutation Res. 66:* 191-194.

Hedin, P.A. 1977. Host plant resistance to pests. *ACS Symposium Series 62.* Amer. Chem. Soc. Washington, D.C.

Hedin, P.A. 1983. Plant resistance to insects. *ACS Symposium Series 208.* Amer. Chem. Soc. Washington, D.C.

Hedin, P.A., Jenkins, J.N., and Maxwell, F.G. 1977. Behavioral and developmental factors affecting host plant resistance to insects. In: *Host Plant Resistance to Pests.* P.A. Hedin (Editor). ACS Symposium #62.

Hedin, P.A., Maxwell, F.G., and Jenkins, J.N. 1974. Insect plant attractants, feeding stimulants, repellents, deterrents, and other related factors affecting insect behavior. *Proceedings of the Seventh Institute on Biological Control of Plant Insects and Diseases.* F.G. Maxwell and F.A. Harris (Editors). Univ. Press of Miss., Miss.

Henderson, C.B. 1984. Inbreds, breeding stocks, maize investigations, and academic research personnel. In: *Maize Research and Breeders Manual.* No. X. Illinois Foundation Seeds. Champaign, Ill., U.S.A.

Hofman, J., and Hofmanova, O. 1971. 1,4-Benzoxazine derivatives in plants: absence of 2,4-dihydroxy-7-methoxy-2H-1,4- benzoxazin-3-one from uninjured *Zea mays* plants. *Phytochem. 10:* 1444-1445.

Holden, J.H.W., and Williams, J.T. (Editors). 1984. *Crop Genetic Resources: Conservation and Evaluation.* Allen and Unwin. London. U.K.

Honkanen, E., and Virtanen, A.I. 1960. The synthesis of precursor II of benzoxazoline formed in rye plants, and the enzymic hydrolysis of precursor I, the glucoside. *Acta Chem. Scand. 14(2)*: 504-507.

Hooker, A.L. 1982. Genetic diversity of maize: disease resistance. In: *Maize for Biological Research*. W.F. Sheridan (Editor). North Dakota. pp. 361-366.

Horber, E. 1980. Types and classification of resistance. In: *Breeding Plants for Insect Resistance*. F.G. Maxwell and P.R. Jennings (Editors). Wiley. N.Y. pp. 15-20.

Huber, L.L. 1936. Mortality of first instar larvae of the European corn borer. *Bull. Ohio Agric. Exp. Sta. 561:* 44.

Hudon, M., Chiang, M.S., and Chez, D. 1979. Resistance and tolerance of maize inbred lines to the European corn borer *Ostrinia nubilalis* and their maturity in Quebec. *Phytoprotection 60(1)*: 1-22.

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

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

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

Iltis, H.H. 1983. From teosinte to maize: the catastrophic sexual transmutation. *Science 222*: 886-894.

Ioannou, Y.M., Dauterman, W.C., and Tucker, W.P. 1980. Degradation of diazinon by 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in maize. *Phytochem. 19:* 1607-1611.

Janick, J., Schery, R.W., Woods, F.W., and Ruttan, V.W. 1981. *Plant Science. An Introduction to World Crops.* Third Edition. Freeman and company. San Francisco.

Jugenheimer, R.W. 1976. *Corn: Improvement, Seed Production, and Uses.* Wiley and Sons. N.Y.

Kanour, W.W.Jr., and Burbutis, P.P. 1984. *Trihogramma nubilale* (Hymenoptera: Trichogramma-tidae) field releases in corn and a hypothetical model for control of European corn borer. *J. Econ. Entomol. 77:* 103-107.

Kehl, H. (Editor). 1982. *Chemistry and Biology of Hydroxamic Acids.* S. Karger. N.Y.

Kingston, D.G.I. 1979. High performance liquid chromatography of natural products. *J. Nat. Prod. 42:* 237-260.

Klecka, W.R. 1980. *Discriminant Analysis.* Sage Univ. Paper on Quantitative Applications in the Social Sciences, 07-019. Sage Publications, Beverly Hills and London.

Klenke, J.R., Russell, W.A., and Guthrie, W.D. 1986. Distributions for European corn borer (Lepidoptera: Pyralidae) resistance ratings of S1 line from "B59" corn. *J. Econ. Entomol. 79:* 1076-1081.

Klun, J.A., and Brindley, T.A. 1966. Role of 6-methoxybenzoxazolinone in inbred resistance of host plant (maize) to first-brood larvae of the European corn borer. *J. Econ Entomol. 59(3):* 711-718.

Klun, J.A., Guthrie, W.D., Hallaver, A.R., and Russell, W.A. 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. *Crop Science 10:* 87-90.

Klun, J.A., and Robinson, J.F. 1968. Concentrations of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. *J. Econ. Entomol. 62:* 214-220.

Klun, J.A., Tipton, C.L., Robinson, J.F., Ostrem, P.C., and Beroza, M. 1970. Isolation and identification of 6,7-dimethoxy-2-benzoxazolinone from dried tissues of *Zea mays* and evidence of its cyclic hydroxamic acid precursor. *J. Agric. Food Chem. 18(4):* 663-665.

Klun, J.A., Tipton, C.L., and Brindley, T.A. 1967. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in the resistance of maize to the European corn borer. *J. Econ. Entomol. 60(6):* 1529-1533.

Kobashi, K., Sakaguchi, K., Takebe, S., and Hosaka, K. 1985. A colorimetric method for the determination of hydroxamic acid by iodine oxidation. *Anal. Biochem. 146(1):* 7-12.

Kogan, M. 1986. Plant defense strategies and host-plant resistance. In: *Pest Management*. M. Kogan (Editor). Wiley and Sons. N.Y. pp. 83-134.

Koyama, T., Yamotota, M., and Kubota, K. 1955. Constituents of the root of *Coix lachryma. J. Pharm. Soc. Jpn. 76*: 1077.

Kwolek, W.F., and Brindley, T.A. 1959. The effects of the European corn borer, *Pyrausta nubilalis,* on corn yield. *Iowa State Coll. J. Sci. 33:* 293-323.

Lacy, G.H., Hirano, S.S., Victoria, J.I., Kelman, A., and Upper, C.D. 1979. Inhibition of soft-rotting *Erwinia* species strains by 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one in relation to their pathogenicity on *Zea mays. Phytopath 69(7):* 757-763.

Larson, K., Sjoberg, B.M., and Thelander, L. 1982. Hydroxamic acids and other hydroxylamine derivatives as inhibitors of DNA synthesis. In: *Chemistry and Biology of Hydroxamic Acids.* H. Kehl (Editor). N.Y. pp. 83-93.

Legg, D.E., and Chiang, H.C. 1984a. European corn borer (Lepidoptera: Pyralidae) infestations: Predicting second generation egg masses from blacklight trap captures and relating their abundance to several corn crop characters. *J.Econ. Entomol. 77:* 1432-1438.

Legg, D.E., and Chiang, H.C. 1984b. European corn borer (Lepidoptera: Pyralidae) infestations: Relating captures in pheromone and blacklight traps in Southern Minnesota cornfields. *J. Econ. Entomol. 77:* 1445-1448.

Levin, D.A., and York, B.M.Jr. 1978. The toxicity of plant alkaloids: an ecogeographic perspective. *Biochemical Systematics and Ecology 6*: 61-76.

Long, B.J., Dunn, G.M., Bowman, J.S., and Routley, D.G. 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. *Crop Science 17*: 55-58.

Long, B.J., Dunn, G.M., and Routley, D.G. 1974. Rapid procedure for estimating cyclic hydroxamate (DIMBOA) concentrations in maize. *Crop Science 14:* 601-603.

Long, B.J., Dunn, G.M., and Routley, D.G. 1975. Relationship of hydroxamic acid content in maize and resistance to Northern corn leaf blight. *Crop Science 15*: 333-335.

Long, B.J., Dunn, G.M., and Routley, D.G. 1975. Relationship of hydroxamic acid concentration in maize and field reaction to *Helminthosporium turcicum*. *Crop Science 18*: 573-574.

Lonnquist, J.H. 1982. Maize breeding and future goals. In: *Maize for Biological Research*. W.F. Sheridan (Editor). Univ. Press. North Dakota. pp. 327-330.

Lynch, R.E. 1980. European corn borer: yield losses in relation to hybrid and stage of corn development. *J. Econ. Entomol. 73(1):* 159-164.

Malan, C., Visser, J.H., and Van de Venter, H.A. 1984. Screening for DIMBOA (benzoxazinone) concentration among South African inbred maize lines and sorghum cultivars. *S. Afr. J. Plant Soil 1(4):* 99-102.

Manuwoto, S., and Scriber, F.M. 1982. Consumption and utilization of three maize genotypes by the Southern armyworm. *J. Econ. Entomol. 75:* 163-167.

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

Manuwoto, S., and Scriber, F.M. 1985b. Consumption and utilization of experimentally altered corn by Southern armyworm, iron, nitrogen, and cyclic hydroxamates. *J. Chemical Ecology 11(11):* 1469-1483.

Mangelsdorf, P.C. 1977. *Corn. Its Origin, Evolution, and Improvement.* Harvard Univ. Press. Cambridge.

Mangelsdorf, P.C., MacNeish, R.S., and Galinat, W.C. 1964. Domestication of corn. *Science 143*: 538-545.

Maxwell, F.G. 1985. Utilization of host plant resistance in pest management. *Insect. Sci. Applic. 6(3)*: 437-442.

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

Mck. Bird, R.M. 1982a. Systematics of *Zea* and the selection of experimental material. In: *Maize for Biological Research.* W.F. Sheridan (Editor). North Dakota. pp. 341-350.

Mck. Bird R.M. 1982b. Maize and teosinte germplasm banks. In: *Maize for Biological Research.* W.F. Sheridan (Editor). North Dakota. pp. 341-350.

Mck. Bird, R.M., and Goodman, M.M. 1977. The races of maize V: Grouping maize races on the basis of ear morphology. *Econ. Bot. 31*: 471-481.

Mihm, J.A. 1985. Breeding for host plant resistance to maize stem borers. *Insect Sci. Applic 6(3):* 369-377.

Munakata, K. 1970. Insect antifeedants in plants. In: *Control of Insect Behavior by Natural Products.* D.L. Wood, R.M. Silverstein, and M. Nakajima (Editors). Academic Press. N.Y. pp. 179-187.

Munson, J.W. 1982. Chemistry and biological activity of hydroxamic acids- an overview. In: *Chemistry and Biology of Hydroxamic Acids.* H. Kehl (Editor). N.Y. pp. 1-13.

Nagao, T., Otsuka, H., Kohda, H., Sato, T., and Yamasaki, K. 1985. Benzoxazinones from *Coix lachryma-joba* var. Ma-Yuen. *Phytochem. 24(12)*: 2959-2962.

Navon, A., and Melamed-Madjar, V. 1986. Screening of *Bacillus thuringiensis* preparations for microbial control of *Ostrinia nubilalis* in sweet corn. *Phytoparasitica 14(2):* 111-117.

Neilands, J.B. 1967. Hydroxamic acids in nature. *Science 156(3781):* 1443-1447.

Niemeyer, H.M., Bravo, H.R., Pena, G.F., and Corcuera, L.J. 1982. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one, a hydroxamic acid from Gramineae. In: *Chemistry and Biology of Hydroxamic Acids.* H. Kehl (Editor). N.Y. pp. 22-28.

Niemeyer, H.M., Corcuera, L.J., and Perez, F.J. 1982. Reaction of a cyclic hydroxamic acid from Gramineae with thiols. *Phytochem. 21(9):* 2287-2289.

Niles, G.A. 1979. The basics of plant breeding. In: *Biology and Breeding for Resistance to Arthopods and Pathogens in Agricultural Plants.* M.K. Harris (Editor). Texas A & M Univ. College Station, Texas. pp. 9-22.

Norris, D.M., and Kogan, M. 1980. Biochemical and morphological bases of resistance. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell and P.R. Jennings (Editors). Wiley. N.Y. pp. 24-40.

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

Oretaga, A., Vasal, S.K., Mihm, J., and Hersey, C. 1980. Breeding for insect resistance in maize. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell and P.R. Jennings (Editors). Wiley. N.Y. pp. 370-419.

Ortman, E.E., and Peters, D.C. 1980. Introduction. In: *Breeding Plants for Insect Resistance.* F.G. Maxwell and P.R. Jennings (Editors). Wiley. N.Y. pp. 3-13.

Painter, R.H., and Fight, G.A. 1925. A field study of the reduction of European corn borer larvae in standing crop. *Rep. Ent. Soc. Ont. 55:* 53-54.

Patch, L.H. 1942. Height of corn as a factor in egg laying by the European corn borer moth in the one-generation area. *J. Agric. Reseach 64(9):* 503-515.

Penny, L.H., Scott, G.E., and Guthrie, W.D. 1967. Recurrent selection for European corn borer resistance in maize. *Crop Science 7(5):* 407-409.

Perez, F.J., and Niemeyer, H.M. 1985. The reduction of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one by thiols. *Phytochem. 24(12):* 2963-2966.

Perez, F.J., and Niemeyer, H.M. 1986. Effect of borate on the decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one, a hydroxamic acid from Gramineae. *Heterocycles 24(1):* 13-16.

Pesho, G.R., Dicke, F.F., and Russell, W.A. 1965. Resistance of inbred lines of corn to the second brood of the European corn borer. *Iowa State J. of Science 40(1):* 85-98.

Philogene, B.J.R. 1981. Successes and future prospects for host plant resistance in integrated control systems. *International Symp. on "The Role of Biological Control in Pest Management Systems".* Santiago, Chile.

Poething, R.S. 1982. Maize: the plant and its parts. In: *Maize for Biological Research.* W.F. Sheridan (Editor). Univ. Press. North Dakota. pp. 9-18.

Queirolo, C.B., Andreo, C.S., Vallejos, R.H., Niemeyer, H.M., and Corcuera, L.J. 1981. Effects of hydroxamic acids isolated from Gramineae on adenosine 5'-triphosphate synthesis in chloroplasts. *Plant Physiol. 68*: 941-943.

Randolf, L.F. 1976. Contributions of wild relatives of maize to the evolutionary history of domesticated maize: a synthesis of divergent hypotheses. *Econ. Bot. 30*: 321-345.

Rao, C.R. 1973. *Linear Statistical Inference and its Applications.* John Wiley and Sons. N.Y.

Reese, J.C., Chan, B.G., and Waiss, A.C. 1982. Effects of cotton condensd tannin, maysin (corn) and pinotol (soybeans) on *Heliothis zea* growth and development. *J. Chem. Ecology 8(12)*: 1429-1436.

Reimann, J.E., and Byerrum, R.V. 1964. Studies on the biosynthesis of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one. *Biochem. 3(6)*: 847-851.

Ribereau-Gayon, P. 1972. *Plant Phenolics.* Oliver and Boyd. Edinburgh.

Robinson, H.F. 1978. Meeting the world's food problems. In: *Maize Breeding and Genetics.* D.B. Walden (Editor). Wiley and Sons. N.Y. pp. 1-10.

Robinson, H.F., Berry, E.C., Lewis, L.C., and Lynch, R.E. 1977. European corn borer: host-plant resistance and use of insecticides. *J. Econ. Entomol. 71(1)*: 109-110.

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

Robinson, H.F., Klun, J.A., Guthrie, W.D., and Brindley, T.A. 1982. European corn borer leaf feeding resistance: DIMBOA bioassays. *J. Kansas Ent. Soc. 55(2):* 357-364.

Rojanaridpiched, C., Gracen, V.E., Everett, T., Coors, G., Pugh, B.F.,and Bouthyetta, P. 1984. Multiple factor resistance in maize to European corn borer. *Maydica 29:* 305-315.

Russell, W.A., Guthrie, W.D., Klun, J.A., and Grindeland, R. 1975. Selection for resistance in maize to first-brood European corn borer by using leaf-feeding damage of the insect and chemical analysis for DIMBOA in the plant. *J Econ. Entomol. 68(1):* 31034.

Sanders, E.H., Gardner, P.D., Berger, P.J., and Neges, N.C. 1981. 6-Methoxybenzoxazolinone: a plant derivative that stimulates reproduction in *Microtus montanus. Science 214:* 67-69.

Sandlan, K.P., Jones, R.L., and Chiang, H.C. 1983. Influence of density of the European corn borer, *Ostrinia nubilalis,* on the parasitoid *Lydella thompsoni. Env. Entomol. 12:* 174-177.

Schoonhoven, L.M. 1982. Biological aspects of antifeedants. *Ent Exp. and Appl. 31:* 57-69.

Scism, A.J., BeMiller, J.N., and Caskey, A.L. 1974. Determination of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one glucosides in corn (*Zea mays*). *Anal. Biochem. 58*: 1-13.

Scott, G.E., Dicke, F.F., and Pesho, G.R. 1966. Location of genes conditioning resistance in corn to leaf feeding of the European corn borer. *Crop Science 6:* 444-446.

Scriber, M.J., Tingey, W.M., Gracen, V.E., and Sullivan, S.L. 1975. Leaf-feeding resistance to the European corn borer in genotypes of tropical (low-DIMBOA) and U.S. inbred (high-DIMBOA) maize. *J. Econ. Entomol. 68(6):* 823-826.

Serratos, A., Arnason, J.T., Nozzolillo, C., Lambert, J.D.H., Philogene, G., Fulcher, G., Davidson, K., Peacock, L., Atkinson, J., and Morand, P. 1987. Factors contributing to resistance of exotic maize populations to maize weevil, *Sitophilus zeamais. J. Chem. Ecol. 13(4):* 751-762.

Showers, W.B., Berry, E.C., and Von Kaster, L. 1980. Management of second-generation European corn borer by controlling moths outside to cornfield. *J. Econ. Entomol. 73:* 88-91.

Shurtleff, M.C. (Editor). 1984. *Compendium of Corn Diseases.* The American Phytopathological Society. St. Paul, Minnesota.

Simms, E.L., and Rausher, M.D. 1987. Costs and benefits of plant resistance to herbivory. *Amer. Nat. 130(4)*: 570-581.

Singh, B.B., Hadley, H.H., and Bernard, R.L. 1971. Morphology of pubescence in soybeans and its relation to plant vigour. *Crop Sci. 11:* 13-16.

Smissman, E.E., Corbett, M.D., Jenny, N.A., and Kristiansen, O. 1972. Mechanism of the transformation of 2,4-dihydroxy-1,4-benzoxazin-3-ones and 2-hydroxy-2-methyl-4-methoxy-1,4-benzoxazin-3-one to 2-benzoxazoline. *J. Org. Chem. 37(11):* 1700-1704.

Smith, S. 1985. Maize (*Zea mays* L.): Historical development, breeding, and utilization. *J. of Plant Foods 6:* 3-17.

Snelling, R.O. 1941. Resistance of plants to insect attack. *Bot Rev. 7:* 548-586.

Sokal, R.R., and Rohlf, F.J. 1969. *Biometry*. Freeman and Company. San Francisco.

Sprague, G.F. (Editor). 1976. *Corn: Its Origin, Evolution and Improvement.* Second Edition. Agronomy Soc. Amer. Madison, Wisconsin.

Sullivan, S.L., Gracen, V.E., and Ortega, A. 1974. Resistance of exotic maize varieties to the European corn borer, *Ostrinia nubilalis. Environ. Entomol. 3(4):* 718-720.

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

Swain, T. 1979. Phenolics in the enviroment. *Rec. Adv. in Phytochem. 12*: 617-641.

Tang, C.S., Chiang, S.H., Hoo, D., and Yanagihara, K.H. 1975. Gas chromatographic determination of 2(3)-benzoxazolinones from cereal plants. *Phytochem. 14:* 2077-2079.

Tatum, L.A. 1971. The Southern corn leaf blight epidemic. *Science 171*: 1113-1116.

Thurston, H.D. 1977. International crop development centers: a pathologist's perspective. *Ann. Rev. Phytopath. 15:* 223-247.

Tipton, C.L., and Buell, E.L. 1970. Ferric iron complexes of hydroxamic acids from maize. *Phytochem. 9: 1215-1217.*

Tipton, C.L., Klun, J.A., Husted, R.R., and Pierson, M.D. 1967. Cyclic hydroxamic acids and related compounds from maize. Isolation and characterization. *Biochem. 6(9):* 2866-2870.

Tipton, C.L., Wong, M.C., Tsao, F.H.C., Lin Tu, C.C., and Husted, R.R. 1973. Biosynthesis of 1,4-benzoxazin-3-ones in *Zea mays. Phytochem. 12:* 347-352.

Toler, R.W. 1979. Breeding for resistance to pathogens in corn. In: *Biology and Breeding for Resistance to Arthopods and Pathogens in Agricultural Plants.* M.K. Harris (Editor). Texas A & M Univ. College Station. Texas. pp. 283-289.

Tseng, C.T., Guthrie, W.D., Russell, W.A., Robbins, J.C., Coats, J.R., and Tollefson, J.J. 1984. Evaluation of two procedures to select for resistance to the European corn borer in a synthetic cultivar of maize. *Crop Sci. 24:* 1129-1133.

Vinal, S.C. 1917. The European corn borer, *Pyrausta nubilalis,* a recently established pest in Massachusetts. *Mass. Agric. Exp. Stn. Bull. 178*: 147-152.

Virtanen, A.I., and Hietala, P.K. 1960. Precursors of benzoxazolinone in rye plants. I. Precursor II, the aglucone. *Acta Chem. Scand. 14(2):* 499-502.

Wahlroos, O., and Virtanen, A.I. 1959. The precursors of 6-methoxy-benzoxazolinone in maize and wheat plants, their isolation and some of their properties. *Acta Chem. Scand. 13(9):* 1906-1908.

Waiss, A.C., Chan, B.G., and Elliger, C.A. 1977. Host plant resistance to insects. In: *Host Plant Resistance to Pests.* P.A. Hedin (Editor). ACS Symp. #62.

Waiss, A.C., Chan, B.G., Elliger, C.A., Wiseman, B.R., McMillian, W.W., Widstrom, N.W., Zuber, MS., and Keaster, A.J. 1979. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. *J. Econ. Entomol. 72:* 256-258.

Walden, D.B. 1978. *Maize Breeding and Genetics.* Wiley and Sons. New York.

Wellhausen, E.J. 1978. Recent developments in maize breeding in the tropics. In: *Maize Breeding and Genetics.* D.B. Walden (Editor). Wiley and Sons. N.Y.

Wellhausen, E.J., Roberts, L.M., Hernandez, E., and Mangelsdorf, P.C. 1952. *Races of Maize in Mexico: Their Origin, Characteristics and Distribution.* Bussey Institution of Harvard Univ.

Welsh, J.R. 1981. *Fundamentals of Plant Genetics and Breeding.* Wiley and Sons. N.Y.

Whitney, N.J., and Mortimore, C.G. 1959. An antifungal substance in the corn plant and its effect on growth of two stalk-rotting fungi. *Nature 183:* 341.

Wilkes, G. 1977. The origin of corn: studies of the last hundred years. In: *Crop Resources.* D.S. Seigler (Editor). Academic Press. N.Y. pp. 211-223.

Wilkes, G. 1982. Wild relatives of the maize gene pool. In: *Maize for Biological Research.* W.F. Sheridan (Editor). North Dakota. pp. 335-339.

Williams, J.T. 1984. A decade of crop genetic resources research. In: *Crop Genetic Resources: Conservation and Evaluation.* Holden, J.H.W., and Williams, J.T. (Editors). Allen and Unwin. London. U.K.

Wiseman, B.R., Harrell, E.A., and McMillian, W.W. 1973. Continuation of tests of resistant sweet corn hybrid plus insecticides to reduce losses from corn earworm. *Environ. Entomol. 2:* 919-920.

Witkowski, J.F., Barber, D.T., and Cirrier, D.R. 1986. Control of first-generation European corn borer larvae in Nebraska by applying insecticides by center-pivot irrigation systems. *J .Econ. Entomol. 79:* 1595-1598.

Wolf, R.B., Spencer, G.F., and Plattner, R.D. 1985. Benzoxazolinone, 2,4-dihydroxy-1,4-benzoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth. *J. Nat. Prod. 28(1):* 59-63.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P., Kelman, A., and Upper, C.D. 1978a. Factors that influence the activity of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one on *Erwinia* species in growth assays. *Plant Physiol. 61:* 803-805.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P., Kelman, A., and Upper, C.D. 1979a. Quantitation of 1,4-benzoxazin-3-ones in maize by gas-liquid chromatography. *Plant Physiol. 63*: 14-19.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P., and Upper, C.D 1978b. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in aqueous solutions. *Plant Physiol. 61*: 796-802.

Woodward, M.D., Corcuera, L.J., Schnoes, H.K., Helgeson, J.P., and Upper, C.D. 1979b. Identification of 1,4-benzoxazin-3-ones in maize extracts by gas-liquid chromatography and mass spectrometry. *Plant Physiol. 63:* 9-13.

Zuniga, G.E., Argandona, V.H., Niemeyer, H.M., and Corcuera, L.J. 1983. Hydroxamic acid content in wild and cultivated Graminae. *Phytochem. 22(12):* 2665-2668.

APPENDIX 1

RECOVERY OF MBOA FROM ETIOLATED CORN TISSUE EXTRACTIONS

MILLIGRAMS MBOA ADDED	RECOVERY	
	WITH TISSUE[a]	WITHOUT TISSUE[b]
0.00	0.021 +/- 0.0051	0.000
0.05	0.038 +/- 0.0091	0.039 +/- 0.0056
0.10	0.076 +/- 0.0106	0.089 +/- 0.0091
0.20	0.180 +/- 0.0046	0.183 +/- 0.0021
0.25	0.200 +/- 0.0298	0.238 +/- 0.0329
0.50	0.361 +/- 0.0461	0.463 +/- 0.0481
0.75	0.562 +/- 0.0682	0.718 +/- 0.0714
1.00	0.781 +/- 0.0786	0.932 +/- 0.0962

MILLIGRAMS DIMBOA ADDED	RECOVERY		
	WITH TISSUE[a]		WITHOUT TISSUE[b]
	mg DIMBOA	mg MBOA	mg DIMBOA
0.00	0.398 +/- 0.0276	0.021 +/- 0.0051	0.000
0.05	0.428 +/- 0.0323	0.027 +/- 0.0009	0.041 +/- 0.0076
0.10	0.449 +/- 0.0291	0.031 +/- 0.0011	0.087 +/- 0.0089
0.20	0.527 +/- 0.0415	0.063 +/- 0.0018	0.165 +/- 0.0146
0.25	0.548 +/- 0.0384	0.081 +/- 0.0076	0.273 +/- 0.0193
0.50	0.691 +/- 0.0581	0.177 +/- 0.0098	0.428 +/- 0.0583
0.75	0.848 +/- 0.0694	0.243 +/- 0.0198	0.677 +/- 0.0612
1.00	0.978 +/- 0.0561	0.031 +/- 0.0214	0.891 +/- 0.0261 N.B. no MBOA detected

a= extraction carried out with 1 gram of etiolated corn tissue
b= extraction carried out with solvent alone

APPENDIX 2

SAMPLE CALCULATION OF TOTAL DIMBOA CONCENTRATION

DIMBOA concentrations in one-week-old etiolated maize seedling extracts were quantified using HPLC. Twenty microliters of a 1/10 diluted sample were injected into the HPLC system and the peak height in absorbance units was measured.

eg. -a 20 ul injection of sample gives a peak height of 39.5 absorbance units

-a 20 ul injection of 0.1 mg/ml (2 ug) of DIMBOA standard gives a peak height of 90.0 absorbance units

$$2.0 \text{ ug}/90.0 = x \text{ ug}/39.5$$

$$x = 0.878 \text{ ug DIMBOA}$$

-therefore, 39.5 a.u. for the sample is equivalent to 0.878 ug of DIMBOA for the 20 ul injection of the sample

The sample was redissolved after final extraction in 1 ml of ethyl acetate;therefore, for the total sample there is an equivalent of 0.878 ug(1000 ul/20 ul= 43.89 ug in the 1/10 dilution or 438.9 ug of DIMBOA in the undiluted extract

Recovery experiments showed that during extraction, degradation of DIMBOA to MBOA was unavoidable; therefore total DIMBOA concentrations were determined by calculating MBOA concentrations in the same manner as that of DIMBOA, then using a conversion factor obtained from the recovery experiments to calulate the total DIMBOA concentration which included that degraded to MBOA.. Total DIMBOA concentration cannot be determined simply by adding DIMBOA and MBOA concentrations because the degradation of DIMBOA to MBOA is not stoichometric. Therefore, to determine total DIMBOA a conversion factor for MBOA may be calculated from the DIMBOA recovery in the absence of tissue when no degradation to MBOA takes place.

-eg. when 1 mg of DIMBOA was added to 1 g tissue and extracted, 0.610 mg of DIMBOA was recovered along with 0.284 mg of MBOA; when extracted without tissue, 0.890 mg of DIMBOA was recovered

$$0.610 \text{ mg DIMBOA} + a(0.284 \text{ mg MBOA}) = 0.890 \text{ mg DIMBOA}$$

where a= conversion factor of DIMBOA to MBOA
= 0.986

-therefore, total DIMBOA concentration = ug DIMBOA obtained + 0.986 (ug MBOA obtained)

APPENDIX 3

TOTAL DIMBOA CONCENTRATION IN ETIOLATED SEEDLINGS

LINE OR LANDRACE		DIMBOA CONCENTRATION (ug/g fresh weight) mean +/- S.D.
LATITUDINAL SERIES OF INBRED LINES		
	3790	177.7 +/- 51.89
	3794	220.7 +/- 42.88
STR	3802	225.1 +/- 39.93
	3805	225.2 +/- 43.73
	3815	159.1 +/- 35.77
	3823	379.1 +/- 75.77
	3853	282.7 +/- 32.61
	3857	197.0 +/- 58.11
	3862	380.6 +/- 44.95
ITR	3865	160.8 +/- 17.92
	3872	150.4 +/- 28.02
	3877	198.9 +/- 38.65
	3878	97.2 +/- 14.28
	3945	267.1 +/- 32.59
	3946	79.2 +/- 15.58
NTR-1	3947	256.5 +/- 51.89
	3962	387.0 +/- 54.67
	3971	274.4 +/- 28.64
	3983	163.4 +/- 28.83
NTR-2	4018	100.9 +/- 13.02
	4019	74.3 +/- 11.54
HOLLAND	4020	93.1 +/- 13.05
	4021	59.6 +/- 9.26
	4022	67.9 +/- 7.41
	4034	50.2 +/- 5.26
SWITZERLAND	4035	130.5 +/- 38.44
	4036	63.0 +/- 21.05
	4042	38.3 +/- 7.04
GERMANY	4046	114.6 +/- 17.46
	4050	137.5 +/- 20.09
	4064	42.5 +/- 4060
POLAND	4065	320.6 +/- 57.94
	4066	109.1 +/- 21.48
	4071	49.0 +/- 6.21
CANADA	4072	100.4 +/- 17.93
	4077	87.3 +/- 14.25
	4081	40.9 +/- 5.97

(TOTAL DIMBOA CONCENTRATION IN ETIOLATED SEEDLINGS CONTINUED...)

LINE OR LANDRACE		DIMBOA CONCENTRATION (ug/g fresh weight) mean +/- S.D.
MEXICAN LANDRACES[a]		
	26	223.7 +/- 49.48
	166	51.1 +/- 2.52
	133	31.1 +/- 6.47
	218	45.8 +/- 15.75
	235	131.2 +/- 27.02
	236	71.7 +/- 20.00
	237	191.1 +/- 46.55
CHIAPAS	239	226.8 +/- 36.19
	124	50.6 +/- 8.95
	236	71.7 +/- 20.00
	46	103.0 +/- 23.93
	140	45.2 +/- 8.32
	52	227.1 +/- 18.28
	78	150.5 +/- 36.13
	224	103.2 +/- 27.34
	159	53.2 +/- 2.10
SONORA	32	65.9 +/- 14.71
	139	166.2 +/- 40.67
	35	107.1 +/- 19.90
SINALOA	2	44.3 +/- 16.02
	66	162.1 +/- 18.69
	15	111.8 +/- 23.17
	72	92.4 +/- 18.31
	39	83.6 +/- 16.26
NAYARIT	24	36.5 +/- 3.82
	222	15.7 +/- 5.47
	185	53.1 +/- 7.44
	59	126.0 +/- 24.61
	43	146.5 +/- 40.43
JALISCO	GP-12	36.8 +/- 5.07
	222	62.0 +/- 12.53
	78	102.7 +/- 26.49
	22	25.2 +/- 5.31
	93A	36.0 +/- 6.50
GUANAJUATO	102	75.5 +/- 13.87
	207	169.8 +/- 33.78
	101	63.6 +/- 13.94
MICHOACAN	GP-13	49.5 +/- 13.50

a= Mexican landraces grouped by collection sites

(TOTAL DIMBOA CONCENTRATION IN ETIOLATED SEEDLINGS CONTINUED...)

LINE OR LANDRACE		DIMBOA CONCENTRATION (ug/g fresh weight) mean +/- S.D.
MEXICAN LANDRACES[a] (CONT)		
	46	4.7 +/- 3.76
	5	5.3 +/- 4.42
	212	27.8 +/- 3.94
MEXICO	55	10.8 +/- 2.10
	208	6.1 +/- 5.21
	182	16.1 +/- 2.10
	461	40.2 +/- 9.39
	6	143.1 +/- 38.37
PUEBLA	463	14.3 +/- 2.52
	537	8.4 +/- 2.89
GUERRERO	168	70.7 +/- 20.98
	130	28.3 +/- 10.48
MORELOS	52	31.0 +/- 11.57
	17	113.4 +/- 34.17
	40	25.8 +/- 5.79
	179	80.0 +/- 25.63
OAXACA	130	99.1 +/- 25.83
	4	73.1 +/- 2.10
	48	155.5 +/- 13.82
	139	43.2 +/- 2.10
V-520-C		94.3 +/- 21.92
VERACRUZ	39	83.3 +/- 13.26
YUCATAN	16	196.4 +/- 43.93
	7	20.5 +/- 2.10
Teosinte		37.2 +/- 10.00

a= Mexican landraces grouped by collection sites

(TOTAL DIMBOA CONCENTRATION IN ETIOLATED SEEDLINGS CONTINUED...)

LINE OR LANDRACE		DIMBOA CONCENTRATION (ug/g fresh weight) mean +/- S.D.
ARGENTINE LANDRACES		
CATETO: E	2044	230.2 +/- 77.34
	2045	295.4 +/- 57.97
	2047	187.2 +/- 38.18
	2048	139.1 +/- 25.68
	2051	175.1 +/- 41.35
CATETO: C	2025	31.4 +/- 10.75
	2026	324.8 +/- 65.21
	2027	213.4 +/- 62.18
	2030	460.9 +/- 69.88
	2032	421.6 +/- 72.11
CIMMYT MAIZE POOLS		
POOL 27	4106	188.4 +/- 38.72
POOL 28	4107	228.4 +/- 56.52
POOL 30	4108	172.4 +/- 40.03
POOL 27 X 28	4094	229.5 +/- 54.97
POOL 27 X 30	4095	153.9 +/- 32.87
POOL 28 X 30	4098	392.9 +/- 68.93
SYNTHETIC	A	247.7 +/- 55.37
SYNTHETIC	B	120.0 +/- 28.42
SYNTHETIC	C	194.0 +/- 10.68
CONTROL LINES		
	A619	170.4 +/- 31.14
	OH43	243.0 +/- 54.89
	B73	471.7 +/- 72.97
MULTI-BORER RESISTANT LINE		78.9 +/- 11.63

LIST OF LINES AND RACES USED IN APPENDICES 4, 5, AND 6

LINE		LINE	
0055	Canadian Synthetic Line A	0104	Morelos-52 (PEPT)
0056	Canadian Synthetic Line B	0105	Nayarit-39 (REVE)
0057	Canadian Synthetic Line C	0106	Jalisco-222 (TABL)
0058	Mexico-5 (PALT)	0107	Nayarit-24 (HARO)
0059	Mexico-212 (CATL)	0108	Guanajuato-207 (TETL)
0060	Mexico-55 (PALT)	0109	Guanajuato-101 (CYLA)
0061	Puebla-463 (ARRO)	0110	V-520-C (TUXP)
0062	Mexico-208 (CHAL)	0111	Oaxaca-4 (VAND)
0063	Mexico-182 (CONI)	0112	Oaxaca-179 (ZAPC)
0064	Puebla-537 (ARRO)	0113	Chiapas-236 (ZAPG)
0065	Mexico-461 (CONI)	0114	Guerrero-168 (CONEJO)
0066	Chiapas-218 (OLOT)	0115	Jalisco-GP-12 (CSERJ)
0067	Gaunajuato-93Ar (MADU)	0116	Sonora-32 (MABL)
0068	Gaunajuato-93Ay (MADU)	0117	Sonora-139 (ONAV)
0069	Mexico-6 (PALT)	0118	Sonora-159 (DULN)
0070	Sinaloa-2 (CHAP)	0119	Nayarit-222 (BOFO)
0071	Yucatan-7 (NALT)	0120	Nayarit-185 (TABO)
0072	Sinaloa-66 (HARO)	0121	Michoacan-13 (ZAMO)
0073	Jalisco-78 (MADU)	0122	Chiapas-140 (GORD)
0074	Mexico-3 (CONI)	0123	Chiapas-166 (APAC)
0075	Nayarit-15 (REVE)	0124	Chiapas-133 (AZUL)
0076	Jalisco-43 (TABL)	0125	Resist. cont. line, B73
0077	Chiapas-78 (THUA)	2025	Cateto C, 2025
0078	Chiapás-26 (TETL)	2026	Cateto C, 2026
0079	Chiapas-46 (COMI)	2027	Cateto C, 2027
0080	Nayarit-59 (JALA)	2030	Cateto C, 2030
0081	Oaxaca-48 (ZAPC)	2032	Cateto C, 2032
0082	Chiapas-224 (ZAPG)	2044	Cateto E, 2044
0083	Morelos-17 (PEPT)	2045	Cateto E, 2045
0084	Chiapas-52 (OLTI)	2047	Cateto E, 2047
0085	Veracruz-39 (TUXP)	2048	Cateto E, 2048
0086	Guerrero-130 (VAND)	2051	Cateto E, 2051
0087	Mexico-46 (CHAL)	3790	STR, 3790
0088	Guanajuato-71 (CYLA)	3794	STR, 3790
0089	Guanajuato-22 (CONN)	3802	STR, 3802
0090	Oaxaca-40 (BOLI)	3805	STR, 3805
0091	Teosinte	3815	STR, 3815

(LIST OF LINES AND RACES CONTINUED...)

LINE		LINE	
0092	Resistant control line, A619	3823	STR, 3823
0093	Resistant control line, OH43	3853	ITR, 3853
0094	Multi-borer resistant line	3857	ITR, 3857
0095	Oaxaca-130 (BOLI)	3862	ITR, 3862
0096	Sinaloa-35 (CHAP)	3865	ITR, 3865
0097	Chiapas-235 (COMI)	3872	ITR, 3872
0098	Guanajuato-102 (CONN)	3877	ITR, 3877
0099	Nayarit-72 (JALA)	3878	ITR, 3878
0100	Yucatan-16 (NALT)	3945	NTR-1, 3945
0101	Chiapas-237 (OLTI)	3946	NTR-1, 3946
0102	Chiapas-239 (OLTI)	3947	NTR-1, 3947
0103	Chiapas-124 (OLOT)	3962	NTR-1, 3962
3971	NTR-1, 3971	4064	NTR-2, Poland, 4064
3983	NTR-1, 3983	4065	NTR-2, Poland, 4065
4018	NTR-2, Holland, 4018	4066	NTR-2, Poland, 4066
4019	NTR-2, Holland, 4019	4071	NTR-2, Canada, 4071
4020	NTR-2, Holland, 4020	4072	NTR-2, Canada, 4072
4021	NTR-2, Holland, 4021	4077	NTR-2, Canada, 4077
4022	NTR-2, Holland, 4022	4081	NTR-2, Canada, 4081
4034	NTR-2, Switz., 4034	4094	Pool 27 X 28
4035	NTR-2, Switz., 4035	4095	Pool 27 X 30
4036	NTR-2, Switz., 4036	4098	Pool 28 X 30
4042	NTR-2, Germany, 4042	4106	Pool 27
4046	NTR-2, Germany, 4046	4107	Pool 28
4050	NTR-2, Germany, 4050	4108	Pool 30

VARIABLE ABBREVIATIONS USED IN APPENDICES 4, 5, AND 6

FLLF= mean field leaf damage ratings

BR= mean plant breakage ratings

ROT= mean stalk rot ratings

HT= mean plant heights (cm)

SMUT= mean smut ratings

TUHT= mean tunneling/height ratios

APPENDIX 4
1986 MEAN FIELD RESULTS

Line	FLLF	BR	ROT	HT	SMUT	TUHT
2025	1.500	2.917	6.083	163.417	0.000	0.212
2026	1.533	3.667	2.533	178.800	0.000	0.113
2027	1.333	2.833	4.333	193.727	0.000	0.118
2030	1.714	3.786	3.000	169.929	0.000	0.073
2032	1.700	3.100	4.800	143.800	0.000	0.171
2044	1.667	3.867	2.533	187.800	0.000	0.153
2045	1.200	2.600	3.600	167.800	0.000	0.128
2047	7.125	4.000	2.500	164.500	0.000	0.096
2048	3.200	2.500	3.312	171.000	0.000	0.170
2051	1.938	3.562	2.688	158.000	0.000	0.218
3790	7.182	3.364	2.454	171.091	0.091	0.095
3794	4.818	3.818	2.000	190.400	0.273	0.087
3802	4.812	2.688	4.125	191.733	0.062	0.091
3805	6.143	2.286	1.929	205.285	0.000	0.072
3815	3.875	3.062	4.000	191.438	0.188	0.071
3823	4.583	1.750	4.250	147.917	0.000	0.097
3853	4.143	3.438	3.938	155.286	0.062	0.120
3857	2.500	2.688	3.812	148.467	0.062	0.096
3862	2.769	3.077	4.154	185.000	0.000	0.113
3865	2.688	2.333	2.867	165.800	0.125	0.068
3872	1.400	3.267	4.267	125.533	0.000	0.200
3877	1.500	2.125	5.562	146.875	0.000	0.099
3878	4.267	2.133	6.733	198.600	0.067	0.094
3945	1.062	2.750	3.125	172.438	0.000	0.102
3946	1.400	3.267	4.133	190.467	0.000	0.121
3947	2.438	1.812	3.562	184.500	0.000	0.059
3962	3.533	2.200	2.800	245.933	0.000	0.041
3971	4.500	3.000	3.846	177.077	0.143	0.119
3983	5.867	2.500	4.000	193.714	0.200	0.088
4018	4.667	4.833	4.667	183.000	0.167	0.140
4019	1.312	3.688	3.812	190.562	0.062	0.117
4020	2.818	6.286	8.143	174.750	0.083	0.204
4021	1.231	3.364	3.364	141.273	0.077	0.145
4022	6.417	2.923	3.615	140.538	0.461	0.188
4034	1.500	4.077	5.077	157.154	0.071	0.129
4035	5.778	6.364	4.454	188.091	0.091	0.133
4036	1.786	2.214	2.857	147.429	0.000	0.100
4042	6.201	3.947	4.526	155.421	0.000	0.157
4046	.	9.999	8.000	141.000	0.000	0.126
4050	4.583	2.333	3.667	179.454	0.083	0.110
4064	3.182	6.000	5.667	131.667	0.091	0.231
4065	3.000	3.750	4.250	162.875	0.125	0.096
4066	2.714	1.714	4.714	156.286	0.000	0.176
4071	1.000	7.500	8.000	181.000	0.000	0.218
4072	4.250	2.875	4.250	208.188	0.125	0.085
4077	1.875	4.500	3.500	107.875	0.000	0.382

4081	2.500	2.800	5.400	109.800	0.000	0.242
0059	7.875	5.600	3.600	216.167	0.167	0.046
0060	5.500	4.667	1.667	183.333	0.667	0.156
0061	5.000	2.000	3.571	250.571	0.000	0.071
0062	9.000	1.000	1.000	240.000	0.000	0.053
0063	3.333	4.667	1.333	243.333	0.333	0.016
0064	3.800	2.250	2.500	275.000	0.250	0.038
0065	5.667	2.000	2.333	268.000	0.000	0.043
0066	7.182	1.750	2.917	305.000	0.083	0.023
0067	4.857	2.286	2.000	223.571	0.000	0.019
0068	2.500	2.000	3.000	182.500	0.000	0.027
0055	1.000	3.000	2.667	139.333	0.000	0.055
0056	1.000	3.500	1.000	159.000	0.000	0.048
0057	1.000	3.667	3.167	159.667	0.000	0.120
0092	1.333	2.950	3.200	163.850	0.000	0.107
0093	1.688	2.906	3.938	153.094	0.000	0.124
4094	4.250	1.750	2.250	191.000	0.000	0.070
4098	4.250	2.750	2.750	199.000	0.000	0.027
4102	2.714	3.143	2.571	205.429	0.000	0.041
4106	2.375	3.000	4.125	174.875	0.000	0.051
4107	2.250	1.500	2.250	190.375	0.000	0.047
4108	6.143	2.000	4.143	207.429	0.000	0.053

APPENDIX 5
1987 MEAN FIELD RESULTS

Line	FLLF	BR	ROT	HT	SMUT	TUHT
2025	.	4.062	9.391	154.979	0.062	0.08
2026	2.071	6.104	9.021	146.104	0.000	0.09
2027	3.000	2.354	7.042	164.625	0.000	0.09
2030	1.522	2.958	6.809	128.708	0.000	0.11
2032	3.000	4.792	9.354	142.271	0.000	0.11
2044	1.000	2.938	8.681	166.771	0.042	0.07
2045	2.200	3.521	7.848	163.270	0.021	0.08
2047	1.500	2.925	6.923	149.225	0.021	0.06
2048	2.429	2.604	8.250	150.104	0.000	0.09
2051	2.381	2.583	5.932	166.188	0.000	0.09
3790	2.312	1.723	6.575	150.106	0.188	0.11
3794	2.729	1.771	3.864	167.083	0.042	0.08
3802	2.255	1.688	3.432	168.583	0.000	0.09
3805	1.792	1.708	4.745	162.596	0.064	0.10
3815	2.083	2.833	6.125	148.375	0.125	0.12
3823	1.750	1.114	6.047	118.630	0.021	0.11
3853	2.762	3.396	5.087	178.000	0.042	0.07
3857	2.000	3.417	7.646	153.521	0.021	0.07
3862	2.193	3.000	8.302	164.833	0.021	0.08
3865	2.182	1.312	5.457	159.688	0.104	0.07
3872	1.958	2.708	7.479	108.021	0.021	0.18
3877	2.068	1.417	8.261	128.375	0.021	0.08
3878	2.729	2.062	4.938	181.000	0.292	0.08
3945	2.000	1.604	5.312	112.167	0.000	0.06
3946	2.089	3.625	6.830	161.854	0.042	0.10
3947	1.896	2.062	5.200	149.812	0.042	0.08
3962	2.787	2.000	4.565	155.404	0.021	0.08
3971	2.583	2.062	5.167	147.787	0.625	0.10
3983	2.230	4.000	6.489	154.583	0.188	0.09
4018	1.732	2.604	6.370	145.562	0.021	0.09
4019	1.000	3.896	8.851	187.062	0.104	0.05
4020	2.880	4.042	8.422	165.146	0.021	0.08
4021	1.850	2.417	5.422	131.500	0.062	0.10
4022	3.435	3.688	8.958	142.191	0.062	0.13
4034	3.714	2.667	8.958	155.542	0.021	0.09
4035	1.930	4.438	8.362	175.146	0.000	0.07
4036	2.000	1.896	4.104	146.542	0.021	0.05
4042	1.738	3.083	7.532	154.917	0.000	0.09
4046	1.875	3.761	8.395	120.319	0.042	0.12
4050	2.047	2.229	6.021	158.375	0.021	0.11
4064	2.032	4.812	9.125	143.104	0.042	0.13
4065	1.917	2.708	6.511	151.688	0.021	0.12
4066	2.206	2.681	6.064	148.425	0.085	0.08
4071	.	2.750	8.341	126.771	0.083	0.09
4072	2.219	2.458	6.644	171.250	0.021	0.08
4077	5.000	3.042	9.128	123.771	0.062	0.07

4081	2.555	2.271	8.250	120.702	0.021	0.11
0058	2.571	2.812	6.234	192.872	0.188	0.07
0059	4.354	3.083	4.227	221.851	0.083	0.06
0060	3.917	3.458	5.222	193.894	0.083	0.06
0061	3.480	2.083	3.867	205.717	0.167	0.05
0062	3.521	3.312	5.302	188.277	0.021	0.08
0063	4.188	2.104	3.891	228.886	0.021	0.04
0064	4.104	2.083	5.681	230.542	0.028	0.07
0065	5.312	2.250	3.488	217.021	0.042	0.06
0066	5.596	1.396	3.022	259.819	0.000	0.04
0067	3.438	1.604	3.521	205.354	0.062	0.05
0068	3.917	1.604	3.978	210.085	0.042	0.04
0070	4.854	1.208	4.021	204.333	0.042	0.04
0071	4.225	1.500	3.743	194.575	0.050	0.04
0096	4.083	1.875	4.227	186.783	0.000	0.06
0097	4.583	1.000	2.098	256.512	0.000	0.02
0098	3.125	1.188	2.979	214.812	0.000	0.04
0099	4.271	1.188	2.319	291.667	0.021	0.02
0100	4.625	1.075	2.538	192.000	0.000	0.01
0101	5.125	1.000	2.417	285.000	0.000	0.01
0102	4.458	1.000	2.167	246.667	0.021	0.00
0103	5.000	1.614	4.068	215.727	0.023	0.04
0104	4.812	1.354	1.787	255.425	0.000	0.02
0105	3.229	1.500	3.104	201.833	0.021	0.03
0106	3.729	1.500	3.409	206.167	0.083	0.03
0107	3.229	1.521	4.489	200.864	0.000	0.05
0108	3.646	1.438	3.128	231.438	0.167	0.03
0109	4.229	1.083	2.583	246.792	0.042	0.02
0110	4.925	1.275	2.487	261.900	0.150	0.03
0111	4.771	1.250	2.848	227.957	0.021	0.02
0112	2.064	2.292	3.630	195.130	0.188	0.04
0113	3.350	1.650	4.210	249.436	0.150	0.03
0114	3.229	3.104	6.875	170.250	0.188	0.08
0115	3.292	1.917	4.044	204.340	0.083	0.05
0116	3.979	1.000	2.729	206.292	0.042	0.02
0117	4.125	1.175	3.564	229.850	0.083	0.03
0118	3.739	1.146	3.745	188.167	0.021	0.04
0119	4.458	1.146	1.681	249.542	0.083	0.02
0120	5.229	1.229	2.562	239.375	0.062	0.02
0121	5.000	1.521	2.422	240.000	0.021	0.03
0122	3.555	4.550	6.769	208.400	0.200	0.06
0123	3.667	4.104	7.783	200.125	0.125	0.07
0124	2.884	2.438	4.133	213.646	0.186	0.04
0055	3.000	5.812	9.304	129.617	0.062	0.11
0056	7.000	5.083	9.375	148.667	0.000	0.11
0057	.	3.708	8.914	154.583	0.000	0.10
0092	2.062	4.583	6.239	132.324	0.044	0.08
0093	1.723	2.562	4.711	138.222	0.021	0.07
0094	2.900	2.250	3.743	171.000	0.042	0.06
0125	1.854	1.562	4.630	170.188	0.000	0.07
4094	2.372	1.979	4.523	185.362	0.042	0.06
4095	2.535	1.625	4.089	181.896	0.062	0.06
4098	1.787	2.792	4.130	188.729	0.062	0.06
4102	2.765	2.229	6.085	167.271	0.000	0.08

4106	2.341	2.188	4.542	174.583	0.083	0.06
4107	2.792	2.333	3.721	161.104	0.042	0.06
4108	2.182	2.208	5.458	176.646	0.000	0.07

APPENDIX 6
1987 MEAN FIELD RESULTS ON NON-STRESSED CONROL DATA

Line	FLLF	BR	ROT	HT	SMUT	THUT
0055	.	4.600	2.126	120.400	0.000	0.024
0056	.	6.600	1.113	135.200	0.000	0.007
0057	.	2.400	1.812	140.800	0.000	0.040
0058	.	1.000	1.000	206.250	0.250	0.006
0059	1.400	2.000	1.000	201.200	0.200	0.036
0060	1.800	3.000	1.000	201.200	0.200	0.047
0061	2.000	1.000	1.000	229.000	0.200	0.031
0062	1.000	1.600	1.000	170.600	0.000	0.049
0063	1.200	1.000	1.000	225.250	0.000	0.005
0064	1.000	3.200	1.000	169.200	0.000	0.028
0065	1.200	1.000	1.000	206.000	0.000	0.038
0066	1.400	1.000	1.000	210.000	0.200	0.009
0067	1.000	1.000	1.000	170.000	0.000	0.000
0068	1.000	1.200	1.000	194.400	0.000	0.044
0070	1.200	1.000	1.000	216.400	0.000	0.012
0071	1.000	4.000	1.000	201.000	0.600	0.013
0085	.	1.000	1.000	204.000	0.000	0.012
0092	1.000	1.000	1.268	152.800	0.000	0.022
0093	1.000	1.000	1.489	138.400	0.000	0.018
0094	1.400	1.000	1.899	153.000	0.000	0.024
0095	1.000	1.000	1.000	266.500	0.000	0.000
0096	1.600	1.000	1.000	210.800	0.000	0.018
0097	1.000	1.000	1.000	200.000	0.000	0.000
0098	2.000	1.000	1.000	213.400	0.000	0.012
0099	1.000	1.000	1.000	300.000	0.000	0.003
0100	1.500	1.000	1.000	199.000	0.000	0.019
0101	1.800	1.000	1.000	200.000	0.000	0.018
0102	1.000	1.000	1.000	200.000	0.000	0.000
0103	1.400	1.000	1.000	200.000	0.000	0.000
0104	1.000	1.000	1.000	200.000	0.000	0.003
0105	1.000	1.000	1.000	168.200	0.000	0.014
0106	1.000	1.000	1.000	214.400	0.200	0.000
0107	1.200	1.200	1.000	208.600	0.000	0.000
0108	1.000	1.000	1.000	231.600	0.200	0.003
0109	1.400	1.000	1.000	229.200	0.000	0.007
0110	1.625	1.000	1.000	207.625	0.125	0.001
0111	1.000	1.000	1.000	180.000	0.000	0.006
0112	1.000	1.000	1.000	202.800	0.000	0.004
0113	1.000	1.800	1.000	184.600	0.200	0.008
0114	1.000	1.600	1.000	199.000	0.000	0.013
0115	1.200	1.400	1.000	174.200	0.000	0.029
0116	1.200	1.000	1.000	246.600	0.000	0.000
0117	1.000	1.000	1.000	211.400	0.000	0.005
0118	1.000	1.000	1.000	188.200	0.000	0.008
0119	1.000	1.000	1.000	250.000	0.000	0.002
0120	1.400	1.000	1.000	230.000	0.000	0.004

0121	1.600	1.000	1.000	278.000	0.200	0.009
0122	.	1.200	1.000	225.600	0.000	0.006
0123	.	2.000	1.000	173.000	0.000	0.006
0124	1.200	1.200	1.000	214.200	0.000	0.013
0125	1.000	1.000	1.000	146.600	0.000	0.017
2025	1.000	4.400	1.112	127.800	0.000	0.000
2026	1.800	2.800	1.456	136.400	0.000	0.000
2027	.	1.200	1.000	191.000	0.000	0.027
2030	1.000	1.200	1.000	135.200	0.000	0.008
2032	.	2.800	1.000	156.400	0.200	0.029
2044	.	1.000	2.380	163.600	0.000	0.000
2045	.	1.000	1.000	170.000	0.000	0.031
2047	1.000	1.800	1.000	124.000	0.000	0.000
2048	.	1.200	2.310	170.200	0.000	0.024
2051	1.000	1.400	2.000	160.000	0.000	0.007
3790	1.400	1.000	1.000	134.400	0.000	0.028
3794	1.000	1.400	1.000	118.000	0.000	0.078
3802	1.200	1.000	1.000	165.600	0.000	0.013
3805	1.000	1.000	1.000	167.600	0.000	0.086
3815	1.200	2.400	1.000	159.800	0.000	0.013
3823	1.000	2.400	1.000	145.000	0.000	0.038
3853	1.000	2.400	1.000	168.800	0.000	0.040
3857	.	4.000	1.000	160.200	0.000	0.028
3862	1.000	1.200	1.000	158.800	0.000	0.073
3865	1.000	1.000	1.000	159.200	0.000	0.000
3872	1.000	1.000	1.000	107.600	0.000	0.004
3877	1.000	1.000	1.000	144.200	0.000	0.007
3878	1.400	1.400	1.113	174.000	0.000	0.000
3945	1.000	1.000	1.456	133.600	0.000	0.008
3946	1.000	1.400	1.000	165.600	0.000	0.076
3947	1.000	1.400	1.118	130.600	0.000	0.076
3962	1.200	1.000	1.000	176.400	0.000	0.059
3971	1.000	1.600	1.000	156.400	0.800	0.053
3983	1.000	1.200	1.000	140.400	0.400	0.019
4018	1.800	1.400	1.000	121.800	0.000	0.007
4019	.	1.200	1.000	187.400	0.200	0.020
4020	1.600	1.200	2.566	148.400	0.000	0.100
4021	1.000	1.200	2.000	116.600	0.200	0.026
4022	.	2.600	2.100	161.200	0.000	0.018
4034	.	1.600	1.000	148.800	0.000	0.023
4035	1.000	1.800	1.000	165.400	0.200	0.010
4036	.	1.000	1.000	151.200	0.000	0.020
4042	2.000	1.800	1.000	148.600	0.000	0.028
4046	1.000	1.600	1.000	113.200	0.200	0.047
4050	1.200	1.600	1.000	142.200	0.000	0.034
4064	2.000	2.000	1.000	125.200	0.000	0.040
4065	1.000	1.000	1.000	166.400	0.000	0.024
4066	1.600	1.400	1.000	142.600	0.000	0.060
4071	.	1.200	1.000	112.400	0.000	0.054
4072	1.000	1.000	1.000	189.600	0.000	0.020
4077	.	1.400	1.000	114.000	0.000	0.017
4081	1.000	1.000	1.000	125.800	0.000	0.000
4094	1.200	1.000	1.000	175.000	0.000	0.000
4095	2.000	1.000	1.000	219.800	0.000	0.018

4098	1.200	1.000	1.000	216.400	0.000	0.007
4102	1.400	1.000	1.000	212.400	0.000	0.000
4106	1.200	1.000	1.000	141.600	0.000	0.013
4107	1.400	2.200	1.000	141.200	0.000	0.056
4108	1.000	1.800	1.000	162.000	0.000	0.003

APPENDIX 7

MEAN LEAF COUNTS AND DAYS TO SILKING FOR ALL LINES AND RACES

LINE OR RACE		MEAN NUMBER OF LEAVES PER PLANT	DAYS TO SILKING[a]
LATITUDINAL INBREDS			
STR	3790	18.2 +/- 0.84	94
	3794	18.0 +/- 0.00	94
	3802	16.2 +/- 1.30	95
	3805	18.0 +/- 0.71	94
	3815	18.2 +/- 0.84	95
	3823	18.2 +/- 0.45	95
ITR	3853	17.2 +/- 1.64	81
	3857	15.2 +/- 1.10	77
	3862	14.7 +/- 0.58	77
	3865	15.0 +/- 0.00	83
	3872	14.0 +/- 0.71	83
	3877	17.4 +/- 0.89	81
	3878	17.0 +/- 1.22	85
NTR-1	3945	18.0 +/- 1.00	94
	3946	15.8 +/- 0.84	94
	3947	16.2 +/- 1.10	92
	3962	16.6 +/- 0.55	88
	3971	17.2 +/- 3.03	94
	3983	16.0 +/- 0.71	98
NTR-2	4018	15.4 +/- 1.82	85
Holland	4019	15.0 +/- 1.00	74
	4020	15.4 +/- 1.67	85
	4021	17.2 +/- 1.30	92
	4022	15.8 +/- 1.64	83
Switz.	4034	14.2 +/- 1.10	83
	4035	15.8 +/- 0.45	88
	4036	16.6 +/- 1.14	83
Germany	4042	17.8 +/- 0.45	84
	4046	15.4 +/- 0.89	86
	4050	15.4 +/- 0.89	83
Poland	4064	14.4 +/- 1.14	83
	4065	16.6 +/- 1.52	84
	4066	15.8 +/- 1.30	83
Canada	4071	13.8 +/- 1.64	76
	4072	16.6 +/- 1.67	76
	4077	12.6 +/- 0.55	76
	4081	15.2 +/- 1.30	77

a= days to silking when 50% of the plants were showing silk

(MEAN LEAF COUNTS AND DAYS TO SILKING CONTINUED...)

LINE OR RACE		MEAN NUMBER OF LEAVES PER PLANT	DAYS TO SILKING[a]
MEXICAN LANDRACES[b]			
SONORA	32	24.4 +/- 0.89	---
	139	19.2 +/- 2.49	---
	159	19.0 +/- 1.00	---
SINALOA	35	18.0 +/- 0.71	---
	2	24.2 +/- 0.45	---
NAYARIT	72	26.6 +/- 0.89	---
	39	18.4 +/- 1.14	---
	24	18.4 +/- 1.67	---
	222	19.0 +/- 0.71	---
	185	27.0 +/- 1.41	---
JALISCO	GP-12	20.6 +/- 0.55	---
	222	24.0 +/- 0.71	---
GUANAJUATO	93A	20.6 +/- 1.02	---
	102	18.0 +/- 0.71	---
	207	21.4 +/- 0.89	---
	101	19.2 +/- 0.84	---
MICHOACAN	GP-13	20.8 +/- 0.84	---
MEXICO	5	14.8 +/- 1.30	94
	212	18.4 +/- 1.14	92
	55	16.0 +/- 0.00	92
	208	16.2 +/- 0.96	98
	182	16.8 +/- 1.10	94
	461	16.2 +/- 0.45	---
PUEBLA	463	15.6 +/- 0.89	---
	537	18.2 +/- 1.30	---
GUERRERO	168	15.4 +/- 0.89	---
MORELOS	52	20.6 +/- 1.52	---
OAXACA	4	21.4 +/- 0.55	---
	179	19.2 +/- 2.05	90
	130	21.2 +/- 1.30	---
V-520-C		22.0 +/- 1.00	---
YUCATAN	16	21.8 +/- 1.48	---
	7	17.8 +/- 0.84	---

a= days to silking when 50% of the plants were showing silk
b= Mexican landraces grouped by collection sites

(MEAN LEAF COUNTS AND DAYS TO SILKING CONTINUED...)

LINE OR RACE		MEAN NUMBER OF LEAVES PER PLANT	DAYS TO SILKING[a]
MEXICAN LANDRACES[b]			
CHIAPAS	166	14.2 +/- 1.30	---
	133	14.8 +/- 1.40	94
	218	22.4 +/- 1.52	---
	235	22.8 +/- 0.84	---
	237	23.6 +/- 1.14	---
	239	23.0 +/- 1.00	---
	124	13.6 +/- 0.55	---
	236	21.8 +/- 0.45	---
	140	16.0 +/- 1.00	83
ARGENTINE LANDRACES			
CATETO: E	2044	9.4 +/- 2.61	83
	2045	15.0 +/- 1.22	85
	2047	12.6 +/- 2.30	83
	2048	15.4 +/- 0.55	83
	2051	14.8 +/- 0.45	83
CATETO: C	2025	14.4 +/- 0.89	74
	2026	15.2 +/- 0.84	83
	2027	14.6 +/- 1.82	76
	2030	16.0 +/- 1.22	92
	2032	14.8 +/- 1.30	77
CIMMYT MAIZE POOLS			
POOL 27:	4106	17.0 +/- 1.41	85
POOL 28:	4107	17.6 +/- 0.55	83
POOL 30:	4108	15.6 +/- 1.95	83
POOL 27 X 28:	4094	20.6 +/- 0.89	92
POOL 27 X 30:	4095	16.2 +/- 1.10	92
POOL 28 X 30:	4098	17.4 +/- 1.95	92
SYNTHETIC	A	15.0 +/- 0.71	83
SYNTHETIC	B	15.4 +/- 0.89	76
SYNTHETIC	C	12.4 +/- 1.14	76
CONTROL LINES			
	A619	18.0 +/- 2.71	92
	OH43	17.2 +/- 0.84	95
	B73	19.8 +/- 0.45	95
MULTI-BORER RESISTANT LINE		23.0 +/- 1.00	83

a= days to silking when 50% of the plants were showing silk
b= Mexican landraces grouped by collection sites