RELATIONSHIP OF RESISTANCE IN MAIZE (**Zea mays** L.) TO TWO RELATED SPECIES OF PYRALIDAE: **Diatraea saccharalis** (F.) AND **Zeadiatraea lineolata** (WLK.)

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RELATIONSHIP OF RESISTANCE IN MAIZE (*ZEA MAYS* L.) TO TWO RELATED SPECIES OF PYRALIDAE: *DIATRAEA SACCHARALIS* (F.) AND *ZEADIATRAEA LINEOLATA* (WLK.)

by

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Magor Department: Entomology and Nematology

Tests for resistance in 48 varieties of maize to *Diatraea saccharalis* (F.) and 86 varieties of maize to *Zeadiatraea lineolata* (Wlk.) were conducted respectively in Gainesville, Florida and Alajuela, Costa Rica. Data were taken on the mean number of eggs, egg masses, tunnels, and larvae plus pupae per variety for both insect species.

Mean number of eggs per variety and mean number of eggs per mass per variety were both good indicators of attractiveness to oviposition to the two insect species. The ratio of eggs per mass was independent of the density of egg masses per plant. The physiological or morphological basis of maize attractiveness to oviposition was not determined.

Different ovipositional sites on maize (upper leaf surface, lower leaf surface, etc.), for *D. saccharalis* differed in mean number of eggs, egg masses and eggs per mass.

The upper leaf surface along the midrib was the preferred site.

Mean tunnel and larval counts per variety were correlated for both species. Tunnel data were a more efficient index than larval counts. Variety Poey T-66 consistently was rated as most resistant to Z. lineolata. The physiochemical mechanism of this resistance was not determined.

Larval feeding of Z. lineolata resulted in an average of 21 cm of tunnel damage per plant. From 12 to 29% of the plants had tunnels in their ear shanks.

Varieties differed little in their content of 6MBOA and all varieties were quite low in comparison to published reports of varieties resistant to Ostrinia nubilalis (Hubn.).

INTRODUCTION

Corn or maize (Zea mays L.) is an important crop, both from an historical and current viewpoint, in the Western Hemisphere. Unfortunately, consumption of this crop exceeds production in several countries, e.g., Costa Rica. This disparity has caused concern and prompted the initiation of several programs to encourage greater production through better cultural practices and allocation of more land to this crop. The primary consideration for most farmers involves the economics of the crop. Unless high maize yields can be produced at a profit, such programs stand little chance of success.

Edaphic, climatic, and biotic factors are all related to the potential yield of maize for a given environment. The objectives of the agricultural investigator are to determine how these factors are related to each other and to crop yield. His studies are directed toward finding ways to manipulate these factors so higher yields can be obtained. It is important, however, that changes in the environment be biologically sound both for the present and the future.

The biotic environment involves the interaction of the agronomic crop with other biological organisms. This environment is more complex in the tropics than in temperate zones,

and cultural methods as practiced in the temperate zones have often proven unsatisfactory when applied indiscriminately in the tropics. Pesticides are widely used for the control of many insects in the tropics, and their continual use has led to the development of insecticidal resistance in the pests while diminishing populations of beneficial insects. Thus, many farmers have arrived at the untenable position of lacking both effective insecticides and biological controls to protect their crops. In addition, chemical controls, when improperly used, can cause acute effects in non-target organisms. Better methods of insect control are essential to obtain higher crop yields and reduction of the effects of ecologically unsound methods of insect control.

An integrated system, using a maximum of biotic controls with a minimum of chemicals, is a more desirable approach. One aspect of biotic control is breeding for host resistance. The basic flaw in chemical control stems from the use of a static entity, the insecticide, to control a dynamic and mobile group of organisms. When not all of a population is killed by pesticides, a selection pressure is applied to the genetically variable insect population. Selection pressure in this system favors certain insect genotypes, often resulting in the development of resistance. Biotic control, either by parasites, predators, or resistance in the host plant, differs from

chemical control in that selection operates against both organisms. This interaction often reaches an evolutionary equilibrium in which neither the host nor its parasite is eliminated.

Breeding plants for resistance to insects involves experimental evolution directed toward an increased advantage for the host plant. Any plant breeding program such as this necessitates a thorough understanding of the two organisms; their physiology, behavior, morphology, and genetics.

The adaptability of any plant species to any specific environment is partly a function of the individual plant's ability to develop and reproduce in spite of adverse environmental factors such as insect attacks. The interaction of phytophagous insects with their host plants often results in a selection pressure on the host plant for those physicochemical and morphological factors that will permit the plant to develop normally. The plant breeder may accelerate the process of selection by intensifying selection for those qualities he deems important for resistance.

Within a given randomly mating plant population, it is expected that variance will exist between plants in their response to insect attack. Plants may also change in their expression of resistance as they mature. The observed variance is composed of genetic and environmental variance and an interaction of the genetic and environmental components. In developing resistant varieties, we

are primarily interested in the inherited factors and the stage or stages of the plant's growth at which selection should be made.

Genetic advance for plant resistance to insects through selection may follow the equation $G_s = k\ \sigma_p\ h^2$ (Allard, 1960). This refers to selection for a single trait when G_s represents gain from selection, k represents selection intensity in standard units, σ_p represents the phenotypic standard deviation of the population, and h^2 represents heritability. Heritability, in the narrow sense, is the ratio of additive genetic variance to total variance. Genetic progress toward resistance is complicated by the great diversity of insect species and their various developmental stages that may interact with the host. In addition, there is the possibility of races developing that are able to attack resistant crops (Gallun, Deay, and Cartwright, 1961; Gallun, 1955).

Resistance in plants to a single species of insect may arise in any or all of three forms; antibiosis, tolerance, or non-preference. For phytophagous Lepidoptera, antibiosis and tolerance affect only the larval stage. Antibiosis is the characteristic of the plant to adversely affect the insect. Tolerance is a function of the plant's ability to survive despite insect damage. It does not adversely affect the insect. Plants may avoid insect damage by being unattractive for oviposition by the gravid

female moth. Non-preference may involve physicochemical or morpological characteristics which cause the normal sites for oviposition on the plant to be rejected or not identified by the female insect. Thus, for a particular species of insect, there are usually several factors contributing to the total resistance of the plant. Each factor may be genetically independent, having its own $\underline{k}$, $\sigma_{\underline{p}}$, and $\underline{h}^2$ values.

The selection intensity $\underline{v}$ for any single factor follows the formula $v = \sqrt[N]{X}$ when $\underline{N}$ equals the number of characters being selected and $\underline{X}$ equals the percent of the population left after selection (Allard, 1960). Assuming equal selection for two factors (A and B) with 5% of the population being saved, the selection intensity for each trait would be equivalent to saving 22% of the population. Therefore, the intensity of selection for any single genetic factor is reduced in proportion to the number of independent resistance factors involved.

A more complex situation occurs when a plant population is being selected for resistance to two or more insect species. In this case, we may consider several possible relationships between the resistance factors and the parasites. Plant resistance to two or more species may be positively correlated, negatively correlated, or not correlated. Lack of correlation occurs when the resistance is comprised of two distinct mechanisms, each of which

affects physiological systems that are peculiar to one of the species. Selection for resistance in the host plant to one of the species will not affect the host's relationship to the other species. Positively correlated resistance occurs when selection for resistance to one species has a similar effect on the host's relationship to the other species. Positive correlation may be partial or complete, depending on the degree that the physiological systems in the different species are affected the same by the resistant mechanisms of the plant. Negative correlation occurs when resistance developed to the selecting species renders the plant susceptible to other species.

Analysis for correlation of resistance to different insect pests of agronomic crops needs to be investigated for several reasons. Many closely related species are of economic importance in the same crop grown in widely separated geographical areas. Despite quarantine measures, new insect species are being introduced into agricultural areas. Knowledge of the type of resistance correlation in agronomic hosts of these insects would be of immense importance in designing breeding programs for resistance.

The objectives of this investigation were to determine some of the interactions between maize, Zea mays L., and two related species of Pyralidae; the sugarcane borer, Diatraea saccharalis (F.), in Florida and the neotropical cornstalk borer, Zeadiatraea lineolata (Wlk.),

in Costa Rica, Central America. The study involved analysis of resistance of the same lines of maize from Colombia, South America, to both species. In addition, investigations in Florida were carried out on single crosses from the Midwest which had previously been analyzed for resistance to the European corn borer, Ostrinia nubilalis (Hbn.). Zeadiatraea lineolata was also tested against local varieties from Costa Rica and a collection of lines from the Rockefeller Foundation in Mexico.

LITERATURE REVIEW

Cross resistance is the capacity of any single genetic factor in a plant species to contribute to the plant's resistance to more than one organism (i.e. different species of insects, fungi, nematodes, etc.) in the biosphere. The importance of cross resistance becomes evident upon consideration of the number and diversity of phytophagous organisms associated with a plant species in a specific locality. Among the more important phytophagous organisms are the insects. Painter (1955) catalogued over 180 species of insects associated with maize in Guatemala, Central America. Only about half of the species could be identified by specialists of the different insect groups. Although not all of these species were of significant economic importance, the list did include large populations of several economic pests; Diatraea saccharalis (Fabr.), Zeadiatraea lineolata (Wlk.), Euxesta major Wulp., Spodoptera frugiperda (Smith), and over 15 species of Diabrotica and related genera. In addition, intraspecific differences exist in many insect species in the form of races. Each stage of an insect species may also react differently to a resistant factor in a plant species. Bigger, Snelling, and Blanchard (1941), studying resistance in maize to the southern corn rootworm (Diabrotica

undecimpunctata Fabra.), found no correlation between resistance to larval feeding on roots and leaf injury by adults. Painter (1955) stressed that resistance is not developed to aphids, borers, or thrips as a group, but to individual species.

It is expected that the maximum resistance developed by a plant species would be toward the insect species most closely associated with it. To the degree that other insect species or biotypes differ in their behavior or physiology, we can expect a lack of correlation of resistance to these other species. Optimal plant resistance may result from the combination of several factors; one, a few, or all of which may be correlated to more than one species. Only when the factors causing the resistance are isolated can judgements be made concerning cross resistance. Failure to isolate these factors has led to contradictory results concerning cross resistance in insects. Huber and Stringfield (1940) found a correlation between resistance in maize to the corn leaf aphid and the European corn borer. Franklin (1964) found that susceptibility to the corn leaf aphid and the European corn borer is not consistent for all hybrids.

When an individual factor has been isolated, more meaningful data have been obtained concerning cross resistance. Resistance in maize to stalk rot, Diplodia zeae (Schw.), the European corn borer, and simazine may

all be correlated. Anderson (1964) found that five inbred lines with known resistance to stalk rot, and the European corn borer were much more resistant to the herbicides simazine and atrazine than were two susceptible lines. Resistance in the whorls of maize to the first instar larvae of the European corn borer has been correlated with a single factor; the concentration of 6-methoxybenzoxazolinone (6MBOA), a degradation product of 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) (Klun and Brindley, 1966; Klun and Robinson, 1969). BeMiller and Pappelis (1965a,b) found correlation of concentrations of glycosides, including DIMBOA, in maize, with stalk rot resistance. The fungistatic effects of 6MBOA and its analogues have been confirmed by several workers (Wahlroos and Virtanen, 1958; Honkanen and Virtanen, 1960; Loomis, Beck, and Stauffer, 1957). The aglucones, DIMBOA and DIBOA (2, 4-dihydroxy-1, 4-benzoxazin-3-one), and their glucosides were isolated from maize sap and found to detoxify simazine (Roth and Knusli, 1961; Hamilton and Moreland, 1962). Thus, a single factor, 6MBOA, has been shown to have a multifunctional effect, contributing to the plant's resistance not only to a species of insect, but also to a fungus and two herbicides.

Taxonomic Relationship of Several Corn Borers

The probability of a plant factor giving cross resistance in insects is related to the similarity of behavior and physiology of the insects involved. The species of the closely related phytophagous genera, Diatraea and Zeadiatraea (Lepidoptera, Pyralidae, Crambinae), are associated extensively with grasses (Graminae) in the Western Hemisphere. Their host plants include all of the major food and forage grasses; maize, Zea mays L.; sugarcane, Saccharum officinarum L.; rice, Oryza sativa L.; wheat, Triticum aestivum L.; sorghum, Sorghum bicolor L.; Johnson grass, Sorghum halepensis Pers.; Grama grass, Tripsacum latifolium Hitch.; Guatemala grass, Tripsacum laxum Nash.; bamboo, Bambusa vulgarus Schrad.; and Digitaria horizontalis Willd. (Box, 1935). Several species of Diatraea; D. saccharalis (F.) and D. zeacolella Dyar; and Zeadiatraea; Z. lineolata (Wlk.) and Z. grandiosella Dyar; are of economic importance on maize (Kevan 1943, 1944; Painter, 1955; Holloway, Haley, and Loftin, 1928; Metcalf, Flint, and Metcalf, 1962; Henderson, Bennett, and McQueen, 1966). Box (1955) erected the genus Zeadiatraea with D. lineolata Wlk. as the type specimen, but the two genera are closely related with no reliable characters apparent by which Zeadiatraea species can be separated as a group from those of Diatraea. More distantly related and separated

morphologically is the European corn borer, Ostrinia nubilalis (Hubn.) (Lepidoptera, Pyralidae, Pyraustinae). Like species of Zeadiatraea and Diatraea, the European corn borer is primarily a borer in graminaceous plants. It has become well adapted to feeding on maize in the USA and Europe.

Bionomics of Several Borers of Maize

Zeadiatraea lineolata:--Despite the economic importance of Z. lineolata, little has been published concerning its bionomics. The borer is almost completely restricted to maize; however, it has been reared on wheat, Guatemala grass, sorghum, and teosinte (Euchlaena mexicana Schrad.) (Box, 1951; Painter, 1955). It has occurred in sugarcane, but this infestation is thought to be "accidental"; most published reports of its occurrence in sugarcane are erroneous (Box, 1951). Kevan (1943) found that larvae, when introduced on the plants, bored into maize and teosinte but not adlay (Coix sp.). Myers (1935) concluded that the borer is the only well-studied borer of Diatraea or Zeadiatraea that is restricted to a cultivated host plant. The borer is unknown in the wild state as is its host plant maize.

Gravid females oviposit on the uppermost leaves or on the husks of young ears (Kevan, 1943). Maize plants are attractive for oviposition when they are between knee- and shoulder-height (Painter, 1955). Adults

appear to be most attracted to maize shortly before tasseling and scarcely at all after the ears are formed. Females may lay eggs not only on maize, but also on the sides of plastic containers (Painter, 1955). The number of eggs deposited per moth in Trinidad ranged from 187 to 448, with an average of 377.5. The average number of eggs per mass was 8.96 (Kevan, 1944). When two days old, the eggs develop bright red transverse bands. The average time from oviposition to eclosion is five days. Prior to hatching, the black head capsule is apparent through the chorion.

Newly hatched larvae feed externally on the epidermis of the leaves. Other leaf damage consists of transverse rows of tiny holes caused by the boring of the larvae in the whorl before the leaves unfold (Kevan, 1944). The larvae feed on the leaves up to the third instar, at which time they bore into the stalk. They begin tunneling upward and may bore into the shanks of ears. Burrows are usually continuous, but frequently the larvae will leave their tunnels and re-enter the stalk elsewhere (Kevan, 1944). Presence of tunnels is indicated exteriorly by frass holes in the stalks. There are from six to eight larval instars; the larval stage in the laboratory lasts from 22 to 48 days with an average of 31.2 days (Kevan, 1944). In dry stalks the larvae enter a resting stage and remain quiescent until

moisture becomes adequate. The duration of the pupal stage is from 6 to 13 days; the pupal case may or may not be left behind in the tunnel.

Adult females live from three to five days; males live approximately three days (Kevan, 1944). Adults are occasionally found at lights.

Damage to the host plant consists of disruption of the vascular system by the tunneling in the stem, loss of ears caused by tunneling in the pith of the cob and the shank of the ear, breakage of the tassel due to a weakened condition of the stalk, and reduction in overall yield.

Diatraea saccharalis:--Diatraea saccharalis is the most ubiquitous species of the genera Diatraea and Zeadiatraea. Its geographic distribution extends from Louisiana, Texas, and Florida in the United States to as far south as Buenos Aires Province in Argentina (Box, 1935). The species has been found infesting over 56 different species of grasses (Box, 1935). It is of economic importance on several major agronomic grasses; sugarcane, maize, rice, and sorghum (Box, 1935; Holloway et al., 1928; Painter, 1955; Kevan, 1943).

Oviposition begins at dusk and continues throughout the night. The eggs may be deposited on either side of the maize leaf, but usually are placed along the midrib (Painter, 1955). Holloway et al. (1928) found that

the number of eggs per mass varied from 2 to 50 or more. Kevan (1944) found the mean number of eggs per cluster in the laboratory to be 10.0.

The eggs are flattened and oval, about 1.16 mm long by 0.75 mm wide, and are deposited in clusters, overlapping one another like fish scales. The egg stage lasts from four to nine days, depending on temperature (Holloway et al., 1928). One to two days before eclosion, the black heads of the larvae are visible through the chorion. The eggs lack transverse red bands but are white in color when first laid and later take on an orange cast (Holloway et al., 1928).

Following eclosion the first instar larvae congregate in the whorl of the plant where they feed on patches of the leaf epidermis. They may burrow through the leaves of the whorl before the leaves unroll, leaving transverse rows of holes in the leaves. After the first molt the larvae may bore into the midrib of the leaves, feed in the leaf sheath, or bore into the stalk. Larvae usually do not bore into the stalk until the third instar. Upon entering the stalk the larvae generally tunnel upward, filling the tunnel behind them with frass. The shank of the ears may also be tunneled (Painter, 1955). The number of instars varies from 3 to 10, depending on the temperature. Under favorable conditions the larval period may be as short as 28 days, while for hibernating larvae

it can last up to 262 days (Holloway et al., 1928). In the USA, the larvae overwinter in the stalks of sugarcane and maize. The pupal period varies from 6 to 22 days when subjected to an average temperature of 82.9°F (Holloway et al., 1928).

Ostrinia nubilalis:--The European corn borer had its origin in Europe, being introduced in the United States in 1917. Its original hosts in Europe were probably graminaceous; but the insect is of economic importance on hops, Humulus lupulus L., and hemp, Cannabis sativa L., as well as maize. Since its introduction into the United States, the borer has become well adapted to maize and is a major pest of the crop.

The gravid female begins oviposition within five days after emergence, and the eggs are laid within two weeks. The opaque white eggs are deposited in masses with their edges overlapping. The number of eggs per mass ranges from a few to over 100. The moth, in selecting ovipositional sites, shows a preference for specific parts of maize plants of a certain height and stage of growth. The eggs are usually deposited next to the midrib on the underside of the leaves. Eggs may also be placed on the stem, on the upper surfaces of leaves, on leaf sheaths, and on ears (Everly, 1959).

The interval between oviposition and eclosion varies from 5 to 12 days and is related to temperature

(Hawkins and Devitt, 1953). Prior to eclosion the black head of the developing embryo is visible through the chorion of the egg.

The first instar larvae feed on the epidermis of leaves and bore into the leaf sheaths and the leaf whorl. Newly hatched larvae boring through the unfurled leaves cause pin-hole damage in the leaves. Tassel buds and stems are often bored by early instar larvae when the plant is more mature. In the second and third instars, the larvae tunnel in the stalks and may enter the base of ear shanks. The larvae may pass through as many as seven instars. They overwinter in stalks.

The pupal stage lasts approximately 14 days, and the adult stage approximately 6.0 days for males and 8.3 days for females (Hawkins and Devitt, 1953).

Maize Resistance to Ostrinia nubilalis

The economic importance of the European corn borer has engendered a vast amount of research. As early as 1925, a compilation of all known references pertaining to this species amounted to approximately 900 titles (Wade, 1925). Since then many workers have investigated different aspects of the insect's relationship to maize. They have shown that analysis for resistance is a complex process requiring the partitioning of the various factors; edaphic, climatic, genetic, and biotic; and determination of how these factors affect resistance. Many of the

concepts developed from these studies are applicable to studies of other borers.

Effect of plant height and maturity:--Under natural infestation, the uniformity of oviposition on the different varieties determines the type of experimental design and statistical analysis that can be used. Uniformity of egg deposition is in part due to several inherent factors in maize that affect the attractiveness of the plant to the female moth. These factors are dependent on plant height and stage of growth (Everly, 1959).

In a study of hybrids, Patch, Holbert, and Everly (1942) found that the half of the hybrids silking first were an average of 3.4 inches taller at the time of moth flight than the other half. The early silking varieties received more eggs and therefore tended to have more borers. In the early development of the plant, oviposition is correlated with plant height. During tasseling, the stage of the plant's development becomes the primary factor influencing oviposition (Everly, 1959). Jackson and Peters (1959), comparing brachytic and normal forms of the same hybrid, found that the stage of the plant's growth was more important than its height in determining infestation. Everly (1959) concluded that plant height and maturity are not in themselves the reason for attractiveness of the plant, but are related to conditions in the plant that

affect attractiveness. The influence of height and stage of growth on oviposition was found to be best expressed by a second-degree parabola (Everly, 1959). The population of borers in different strains has been predicted, and based on the multiple regression of borer population on strain height and silking date. The strains or varieties that consistently received fewer eggs or had fewer borers than predicted were classified as resistant (Patch et al., 1942).

Effect of soil fertility:--Various environmental factors (soil fertility, tilth, rainfall, and temperature) influence oviposition through their effect on factors related to the plant's height and stage of development (Everly, 1959). The effects of various plant nutrients on borer survival in maize have been investigated by several workers (Franklin, 1964; Taylor, Apple, and Berger, 1952 ; Cannon and Ortega, 1966). In field tests where plants were manually infested, corn borer survival was better on vigorous plants than on small, nutrient-deficient plants of the same age (Taylor et al., 1952; Franklin, 1964). Borer survival and crop yield were both higher under manure management than in the control plots lacking manure. Survival of the first generation larvae on the susceptible single-cross hybrid was 10-fold greater at 200ppm of nitrogen than at 10ppm, yet survival in the

resistant hybrid was low and not affected by nitrogen level (Cannon and Ortega, 1966). Few larvae survived on plants of either hybrid when they received 2.5ppm or less of phosphorous. Survival at 10ppm was triple that at 2.5ppm, but did not improve at concentrations from 20 to 80ppm. Quantitative measurements of feeding damage and tunnels were equally as effective in determining the effects of nitrogen and phosphorous on survival. The factors causing the differences in survival appeared to be operating when the larvae were in the first and second instars.

Effect of insect behavior:--Spatial distribution of the European corn borer population is due in part to factors which are independent of the host plant. The behavior of the insect in its different stages and the insects's interaction with parasites, predators, and diseases result in a heterogeneous distribution of the insect. Intraspecific interactions of a given stage of the insect may be density dependent. These interactions also affect the distribution of the borer (Beall, 1940; Bliss, 1953; McGuire, 1957; Cohen, 1960; Taylor, 1961; Katti and Gurland, 1962; Waters, 1959; Fisher, 1953).

Field populations of the European corn borer larvae are rarely, if ever, distributed at random, but rather show aggregation or grouping of individuals in a given spatial unit. Aggregation commonly results from the behavioral characteristic of the moth laying eggs

in masses. This aggregation parameter can be computed by either of two distribution formulae: k of the negative binomial, or b of Taylor's power law (Harcourt, 1965; Taylor, 1961). Based on the degree of aggregation and the goodness of fit required, the data may be transformed to make them amenable to analysis of variance.

Statistical analysis of biological data by analysis of variance is based on the assumed additivity of the data and the homogeneity of the variance. In addition, the variance should be independent of the mean. Frequently these assumptions are not fulfilled by the European corn borer data, but in many cases the analysis of variance is sufficiently robust to permit its use. When there is doubt concerning the data, the k or b values can be used in selecting the proper transformation technique for the data (Southwood, 1966).

Chemical basis of antibiosis:--Antibiosis in maize to the European corn borer is expressed by increased larval mortality, the inhibition of larval growth, and the reduction in larval feeding. Larval feeding by first instar larvae on resistant maize lines causes smaller and fewer leaf lesions than on susceptible lines (Beck, 1960).

Resistance to larval establishment is correlated with the concentration of 6-methoxybenzoxazolinone (6MBOA) (Beck, 1957; Klun and Brindley, 1966). Several techniques were developed for quantitating 6MBOA in maize tissue (Beck

and Stauffer, 1957; Klun and Brindley, 1966; Beck, Kaske, and Smissman, 1957; Bowman, Beroza, and Klun, 1968). The 6MBOA and related benzoxazolinones have been isolated from several graminaceous plants and also synthesized by several workers (Wahlroos and Virtanen, 1959; Honkanen and Virtanen, 1960; Tipton, Klun, Husted, and Pierson, 1967; Gahagan and Mumma, 1967; Smissman, LaPidus, and Beck, 1957 a,b; Hietala and Wahlroos, 1956).

Beck (1960) found that 6MBOA, when incorporated into an artificial diet, caused inhibition of larval growth and reduced feeding. Klun and Brindley (1966) found resistance in 11 inbred lines to be correlated with the concentration of 6MBOA. However, when 6MBOA was placed in diet media containing a vitamin supplement, it did not have a significant effect on borer development. In addition, it is doubtful that 6MBOA occurs free in plant tissue (Wahlroos and Virtanen, 1964). When the plant tissue is crushed 6MBOA is rapidly released from its precursors :2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) and its glucoside. DIMBOA is found free in appreciable concentrations in maize (Wahlroos and Virtanen, 1964).

The biosynthesis of DIMBOA was studied by application of isotopically labeled metabolites to mazie seedlings (Reimann and Byerrum, 1964). The aromatic ring is apparently derived from an intermediate in the shikimic acid

pathway. The O-methyl group is formed from compounds contributing to the one-carbon pool; such as methionine, glycine, and glyceric acid. The two heterocyclic ring carbons are derived from carbons 1 and 2 of ribose. The compound DIMBOA exists as a monoglucoside in the seedlings of maize and several other graminaceous plants, but the aglucone is released enzymatically when the tissue is crushed. Since there is a stoichiometric relation between 6MBOA and its precursors (Klun and Brindley, 1966), analysis for 6MBOA is an indirect measure of its precursors in plant tissue.

Klun and Brindley (1966) suggested that the active factor is DIMBOA and not 6MBOA. They subsequently found that DIMBOA inhibited larval development and caused 25% mortality when incorporated into a diet. The biological activity was not attenuated by alteration of the vitamin constituents.

The concentration of DIMBOA is not static throughout the development of the plant, nor is the concentration the same in different parts of the plant (Klun and Robinson, 1969). The levels of DIMBOA are initially very high in both resistant and susceptible lines, but decrease rapidly in susceptible lines and somewhat less in resistant lines. The average level for five inbred lines, 15-33 inches tall, was highest in the roots, followed by the stem, whorl, and leaf tissues. Analysis of the distribution in maize at pollen shedding

indicated that the concentration was highest in the developing ear and next highest in the stalk. Concentrations were lower in the leaf, sheath and collar, and tassel portions. The inbred line B49, which is moderately resistant to the first instar larvae of the second brood, had higher concentrations of DIMBOA than the other lines. The sheath and collar tissues are a primary site for the first instar larvae on more mature maize.

The two other aglucones 2, 4-dihydroxy-1, 4-2H-benzoxazin-3-one and 2-hydroxy-7methoxy-1, 4-2H-benzoxazin-3-one, have been identified from maize (Tipton et al., 1967; Gahagan and Mumma, 1967). The relationship of these two compounds and their glucosides to plant resistance has not been investigated.

METHODS AND MATERIALS

Biological Materials

Insect Species:--Determination of species of collected male moths was made by examination of genitalia. The abdomens were removed from the specimens and immersed in 10% KOH overnight or in boiling 10% KOH for 20 minutes. The tissue containing the genitalia was removed from the solution and placed in 70% alcohol. The genitalia were cleaned of any adhering tissue; the male genitalia were separated and mounted in Hoyer's Modified Berlese Medium on a slide. *Diatraea saccharalis* F. in Florida and *Zeadiatraea lineolata* (Wlk.) in Costa Rica were identified by reference to illustrations of *Diatraea* and *Zeadiatraea* sp. genitalia (Box 1931; Dyar and Heinrich, 1927).

Collecting:--Pupae and late-instar larvae of *D. saccharalis* collected from sugarcane fields east and south of Lake Okeechobee, Florida, served as the parent generation for a laboratory colony at Gainesville, Florida.

One hundred pupae of *Z. lineolata* were collected from a maize field at the Finca La Pacifica in Guanacaste, Costa Rica. Emerging adults were allowed to mate at

random and their egg masses collected for infesting maize in field experiments in Alajuela, Costa Rica.

Maise varieties, lines, and single crosses:--Inbred lines and varieties of maize were obtained from Dr. Dale Harpstead of the Rockefeller Foundation in Colombia and Dr. E.J. Wellhausen of the Rockefeller Foundation in Mexico. Several local Costa Rican varieties and a collection of Chirripo varieties from the mountainous region of Costa Rica were supplied by Ing. Carlos Salas F., of the University of Costa Rica. Mr. R.T. Everly of Purdue University, West Lafayette, Indiana, supplied several single-cross hybrids from the Midwest. The pedigree and source of the maize samples that were tested are shown in Table 1, together with a reference number for each entry.

Table 1. Pedigree and source of inbred lines, varieties, anc collections of maize tested in Costa Rica and Florida.

Pedigree	Source	Number
Variety Diacol V. 351	Colombia	1
Variety Blanco Comun	Colombia	2
Variety Diacol V. 153	Colombia	3
Variety Am. Theobromina-10	Colombia	4
Variety Am. Monteria-9	Colombia	5
Variety Cuba 362	Colombia	6
Variety USA 342	Colombia	7
Linea 114-9	Colombia	8
Linea Ath. 13B-2#-4-1-4#-1-D	Colombia	9
Linea Ath.-198c-1	Colombia	10
Variety Poey T-66	Costa Rica	11
Variety Eto Blanco	Costa Rica	12
Variety Eto Amarillo	Costa Rica	13
Variety Rocamex V-520-C	Costa Rica	14
Variety Tico H-1	Costa Rica	15
Variety Tico H-2	Costa Rica	16
Variety El Coyol	Costa Rica	17
Single Cross A x W23	Midwest	18
Single Cross A x Os426	Midwest	19
Single Cross Os420 x A	Midwest	20
Single Cross A x Oh02	Midwest	21
Single Cross Tr x A	Midwest	22
Single Cross L317 x A	Midwest	23
Single Cross WF9 x A	Midwest	24
Single Cross L317 x Os426	Midwest	25
Single Cross L317 x Oh02	Midwest	26
Single Cross L317 x W23	Midwest	27
Single Cross L317 x WF9	Midwest	28
Single Cross L317 x Tr	Midwest	29
Single Cross L317 x Os420	Midwest	30
Single Cross Oh02 x Os426	Midwest	31
Single Cross Oh02 x W23	Midwest	32
Single Cross Oh02 x WF9	Midwest	33
Single Cross Oh02 x Tr	Midwest	34
Single Cross Oh02 x Os420	Midwest	35

Table 1, continued

Pedigree	Source	Number
Single Cross Os420 x Os426	Midwest	36
Single Cross Os420 x W23	Midwest	37
Single Cross Os420 x WF9	Midwest	38
Single Cross Os420 x Tr	Midwest	39
Single Cross Os426 x W23	Midwest	40
Single Cross Os426 x WF9	Midwest	41
Single Cross Os426 x Tr	Midwest	42
Single Cross Tr x W23	Midwest	43
Single Cross Tr x WF9	Midwest	44
Single Cross W23 x WF9	Midwest	45
Collection 83	Costa Rica	46
Collection 151	Costa Rica	47
Collection 26	Costa Rica	48
Collection 20	Costa Rica	49
Collection 43	Costa Rica	50
Collection 88	Costa Rica	51
Collection 13	Costa Rica	52
Collection 50	Costa Rica	53
Collection 62	Costa Rica	54
Collection 65	Costa Rica	55
Collection 46	Costa Rica	56
Collection 11	Costa Rica	57
Collection 15	Costa Rica	58
Collection 52	Costa Rica	59
Colelction 64	Costa Rica	60
Mich. 166	Mexico	61
Ver. 181	Mexico	62
S.L.P. Gpo. 10	Mexico	63
S.L.P. Gpo. 12	Mexico	64
Ver. Gpo. 6	Mexico	65
Ver. Gpo. 7	Mexico	66
Ver. Gpo. 8	Mexico	67
Oax. Gpo. 5	Mexico	68
Ver. 133	Mexico	69
Ver. 143	Mexico	70
Ver. 141	Mexico	71
Ver. 165	Mexico	72
Ver. 179	Mexico	73
Ver. 208	Mexico	74
Ver. 215	Mexico	75
Ver. 228	Mexico	76

Table 1, continued

Pedigree	Source	Number
Ver. 225	Mexico	77
Ver. 43	Mexico	78
Ver. 14	Mexico	79
Ver. 8	Mexico	80
Ver. 39	Mexico	81
Ver. 187	Mexico	82
Ver. 168	Mexico	83
Ver. 213	Mexico	84
Cupurico	Mexico	85
Cuba Antibarsan	Mexico	86
Tuxpantigua	Mexico	87
Tuxp.-Sanvibag	Mexico	88
Tuxp. F.F. (Peru Crist.)	Mexico	89
Puerto Rico Gpo. 2	Mexico	90
Granada Gpo. 2	Mexico	91
J.S.Y.	Mexico	92
Saint Croix Gpo. 2	Mexico	93
Aztec-Tuxp.	Mexico	94
R. Dom. Gpo. 3	Mexico	95
R. Dom. Gpo. 8	Mexico	96
Pto. Rico Gpo. 6	Mexico	97
Trinidad Gpo. 1 & 2	Mexico	98
Sanvibag	Mexico	99
Antigua Gpo. 2	Mexico	100
Cuba Gpo. 1	Mexico	101
Cuba Gpo. 2	Mexico	102
Cuba Gpo. 4	Mexico	103
Cuba Gpo. 5	Mexico	104
Haiti Gpo. 1	Mexico	105
Pto. Rico Gpo. 1	Mexico	106
Pto. Rico Gpo. 3	Mexico	107
Pto. Rico Gpo. 6	Mexico	108
Saint Croix Gpo. 3	Mexico	109
San Vicente Gpo. 3	Mexico	110
Sta. Lucin Gpo. 1	Mexico	111
Tobago Gpo. 1	Mexico	112
Guad. Gpo. 1A	Mexico	113
Antigua Gpo. 1	Mexico	114
Barbados Gpo. 1	Mexico	115

Insect-Rearing Techniques

Zeadiatraea lineolata:--Emerging adults from field-collected pupae were placed in gallon jars lined with waxed paper. Crumpled waxed paper was placed in the jars and the tops were covered with waxed paper. Moistened cotton balls were placed in the jars to prevent dessication. The jars were kept at room temperature. Following oviposition, the areas of paper containing egg masses were cut out and kept in petri dishes lined with moist paper toweling until a short time before hatching. The eggs in each mass were counted and placed in the whorls of maize plants that were to be tested for resistance.

Diatraea saccharalis:--Ovipositional chambers were prepared from gallon pasteboard ice cream cartons. The bottom of each container was covered with moist vermiculite and the inside walls lined with waxed paper or green paper toweling. Folded waxed paper or toweling was placed in the chamber to provide additional surface for oviposition. The container was covered with a single layer of cheesecloth secured with rubber bands.

Newly emerged adults were introduced into the chamber and allowed to mate at random. The paper lining and the folded paper were removed periodically and replaced with fresh paper. Areas of paper containing egg masses were cut out and placed in petri dishes that had been lined with damp filter paper and their tops covered with

Alcoa Film (No. 5602). Pinholes were made in the covering to provide aeration and reduce condensation. Various sterilants were evaluated for surface sterilizing the egg masses. Immersion for 30 seconds in 70% ethanol proved the most effective.

Upon eclosion, one to five first instar larvae were placed on artificial media in one-ounce clear plastic cups. The cups were capped and the larvae left until pupation. Pupae were then removed from the cups, separated according to sex, and kept in half-pint ice cream containers until they emerged. Cartons for the pupae were prepared by covering the bottoms with moist vermiculite to prevent dessication of the pupae.

Several artificial diets were evaluated for rearing the sugarcane borer. The composition of three of these diets is given in Tables 2 and 3. The Shorey and Hale (1965) diet was used during the summer of 1967. Three generations of borers were reared in the laboratory and some of the progeny were used for field studies in Gainesville, Florida. The Shorey and Hale diet was modified by substituting soaked field corn for pinto beans. Two generations of borers were reared on this diet.

During 1967, Hensley and Hammond's diet (1968), which had been used extensively in Louisiana, was used to rear three generations.

Table 2. Composition of Shorey and Hale's artificial media and a modification of the diet.

Ingredient	Original Diet	Modified Diet
Soaked pinto beans	640 g	---
Soaked corn kernels	---	640 g
Brewer's yeast*	100 g	100 g
Ascorbic acid*	10 g	10 g
Methyl p-hydroxybenzoate	6 g	6 g
Sorbic acid*	3 g	3 g
Formaldehyde (40%)	6 ml	6 ml
Agar*	40 g	40 g
Water (Distilled)	1920 ml	1920 ml

*Nutritional Biochemicals Corp., Cleveland, Ohio.

Table 3. Composition of artificial medium used by Hensley and Hammond for rearing the sugarcane borer larvae.

Ingredient	Amount
Water	3116 ml
Wesson's salt*	36 g
Casein*	108 g
Sucrose	180 g
Wheat germ*	108 g
Choline chloride*	3.6 g
Vanderzant's vitamin mixture*	36 g
Ascorbic acid*	5 g
Formaldehyde (40%)	5 ml
Methyl p-hydroxybenzoate	5.4 g
Agar*	70 g

*Nutritional Biochemical Corp., Cleveland, Ohio.

All diets were prepared in basically the same manner. For the Shorey and Hale diet, either dry pinto beans or corn kernels were soaked overnight and blended in a Waring blender with half of the amount of water required by the diet. Formaldehyde solution and other dry ingredients, except agar, were added to the mixture while blending. For Hensley and Hammond's diet the formaldehyde and other dry ingredients, except the agar, were added to one half of the total amount of water and blended.

In all cases, the rest of the water was brought to a full boil and the agar added while stirring. The agar solution was allowed to cool to about 70°C before blending with the other ingredients.

The media were poured while still hot into one-ounce clear plastic cups (Premium Plastic, Inc., Chicago, Ill.). The cups were filled approximately half full, allowed to cool, and capped with plastic-lined cardboard lids (Smith-Lee Co., Inc., Oneida, N.Y.). The media were then kept refrigerated until needed.

Resistance in Maize to Diatraea saccharalis

A cage 40 feet long, 16 feet wide, and 11 feet high, was constructed of 20-mesh, 100% Saran insect screen (Chicopee Mills Ind., New York, N.Y.) with aluminum supports.

Diatraea saccharalis was used to study the relationship of its host plant maize under a restricted biotic environment. The bottom of the cage was covered with crushed rock. The screen was found to appreciably lower the wind velocity and reduce the light intensity in the cage.

Ovipositional Studies

Experiment 1:--Resistance in single-cross Midwestern varieties of maize to the sugarcane borer was studied under caged conditions in Gainesville. Seeds from source numbers 18-45 (Table 1) were planted in green plastic pots, 11 inches in diameter and 11 inches high. The pots were filled with a mixture of 2/3 fumigated Arredondo fine sand and 1/3 peat moss. The pots were placed 1-1/2 feet apart in the cage. A completely randomized design was used with each variety represented twice in each pot and each varietal pot replicated three times.

After the plants had reached an average height of 36 inches extended leaf measurement, 50 male and 50 virgin female adult sugarcane borers were placed in the cage in ten half-pint ice cream containers and allowed to emerge at dusk. The release stations were evenly distributed in the cage with the sexes in separate release cartons.

Data on the number of eggs, egg masses, and their location on the plants were taken every other day for a week following the release. Egg masses were circled with India ink to prevent recounting the same egg masses. Forty days after the initial infestation, the plants were dissected and data taken on the number of tunnels and larvae per plant. The length of each tunnel was also recorded.

Experiment 2:--Six Colombian varieties, references nos. 1-3 and 5-7 (Table 1), were analyzed for resistance to the sugarcane borer under caged conditions at Gainesville. The same soil mixture and pots as in the previous experiment were used. Two plants were grown per pot and each varietal pot was replicated six times. The pots were situated in the cage in a Latin Square design.

Thirty virgin females were placed in half-pint containers which were used as release sites. Six release sites containing five females per site were evenly placed throughout the experimental plot. Thirty males were placed in six separate release containers in the cage.

Data were taken for the week on the number of eggs and egg masses and their location on the plants. After 40 days, the plants were dissected and the number of tunnels, their length, and the number of surviving larvae for each plant recorded.

Antibiosis Studies

Experiment 3:--Seven Colombia varieties, reference nos. 1-7 (Table 1) were planted in plastic pots. The seven varieties, were grown with one plant per pot and each variety replicated seven times. A completely randomized design was used. When the plants averaged approximately 36 inches in height, each plant was infested with 10 newly hatched sugarcane borer larvae. After 30 days the plants were dissected and the number of tunnels and surviving larvae recorded.

Resistance in Maize to Zeadiatraea lineolata

Costa Rican Field Tests in 1967:--Two initial experiments were conducted in the summer, May through August, 1967, at the agronomy farm of the University of Costa Rica at Alajuela, Costa Rica. The purpose of these experiments was to survey maize sources for resistance to the neotropical cornborer, Zeadiatraea lineolata. Maize sources evaluated were reference nos. 1-16 (Table 1) in Experiment 4 and nos. 61-115 (Table 1) in Experiment 5. Borer infestation in the plots came from the natural population.

Experiment 4:--The 16 entries were planted five seeds per hill and later thinned to two plants per hill. The experimental design consisted of 16 blocks, each having 16 varietal hills of two plants. The hills within each

block were one meter apart and the blocks two meters apart. Cultural methods were under the supervision of Ing. Carlos Salas F.

During the ovipositional period of the borer, the number of eggs and egg masses on each plant was recorded. The height of the tallest extended leaf was measured for those plants receiving egg masses. At harvest, data were taken on the number of tunnels, larvae, and pupae per plant.

Experiment 5:--The 56 entries were planted in six randomized blocks. Five seeds were planted per hill and later thinned to two plants. The hills within blocks were one meter apart and the blocks were two meters apart. Measurements were recorded of the heights of plants receiving eggs and the number of eggs and egg masses per plant. At harvest, data were taken on the number of tunnels and larvae per plant.

Costa Rican field tests in 1968:--Three experiments were conducted during June through August, 1968, at the agronomy farm of the University of Costa Rica. The purpose of these experiments was to continue screening of maize varieties and lines from tests in 1967. In addition, a collection of Chirripo varieties from Costa Rica was surveyed for resistance under natural infestation.

Experiment 6:--Eight entries (reference nos. 1,3,6,

11, 15, and 61 in Table 1) were planted one hill per variety per block in 16 randomized blocks. Five seeds were planted per hill and thinned to two plants per hill when the average height of the plants was 36 inches. Distances between hills and blocks were the same as in Experiment 5. The plants were infested by placing 20 eggs in the black-head stage in the whorl of each plant. At maturity the plants were dissected and the number of larvae and tunnels per plant recorded.

Experiment 7:--Twelve entries (references nos. 17, 61, 67, 71, 81, 82, 88, 90, 98, and 109 in Table 1) were planted one hill per variety per replicate in eight randomized blocks. Distances between hills and blocks were the same as in Experiment 5. The plants were thinned to two per hill when their average height was 36 inches.

Twenty eggs in the black-head stage were placed in the whorl of each plant. When the plants had matured, they were dissected and the number of tunnels and larvae recorded.

Experiment 8:--Fifteen Chirripo varieties (reference nos. 46-60 in Table 1) were planted in rows one meter apart and hills within rows one meter apart. Hills were thinned to two plants per hill. Infestation came from the natural field population. Data were taken on the first 10 plants in each varietal row. The number of tunnels and larvae per plant were recorded.

Analysis for 2, 4-Dihydroxy-7-methoxy-1, 4-benzoxazin-3-one

Two cyclic hydroxamic acids; 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) and 2, 4-dihydroxy-1, 4-2H-benzoxazin-3-one (DIBOA), and their glucosides have been isolated from maize. No technique has been developed for directly quantitating these compounds, but several methods have been developed for quantitating the degradation products, 6-methoxybenzoxazolinone (6MBOA) and benzoxazolinone (BOA). The concentrations of 6MBOA and BOA are stoichiometrically related to the concentrations of their respective precursors in plant tissue, thus, the two cyclic hydroxamic acids may be indirectly quantitated.

Synthesis of Benzoxazolinones

Benzoxazolinone was commercially available from Distillation Products Industries, Rochester 3, N.Y. The 6-methoxy-benzoxazolinone was not available commercially and was synthesized by two different techniques.

Technique 1:--To 6 g of 5-methoxy-2-nitrosophenol (Distillation Products Ind.) in 150 ml water in a 250-mi Erlenmeyer flask, 18 g $Na_2S_2O_4$ (Fisher Sci. Co., Fiarlawn, New Jersey) were added slowly. The solution was then heated to 60-65°C for 15 minutes. The solution was subsequently cooled to room temperature and neutralized with solid Na_2CO_3. The neutralized solution was then extracted with three 50-ml aliquots of diethyl ether.

The ether fraction was extracted with three 15-ml aliquots of 4NHCl. The acid fractions, which turned a deep purple, were combined and evaporated under reduced pressure. The hydrochloride of 2-amino-5-methoxyphenol was added to an Erlenmeyer flask along with approximately three grams of urea. The flask was fitted with an air condenser, placed in an oil bath, and heated at 180°C for two hours. Following fusion, the residue was washed with dilute HCl and the acid fraction was extracted with diethyl ether. The ether fraction was evaporated and the residue taken up in hot water. Activated charcoal was added, the solution stirred, and the mixture filtered while still hot. Upon cooling, white needle-like crystals appeared. The charcoal was washed with diethyl ether, the ether evaporated, and the residue taken up in hot water. This was filtered and yielded crystals upon cooling. The two batches of needle-like crystals were combined and stored in a dessicator until needed.

Technique 2:--To 10 g of 5-methoxy-2-nitrosophenol in 200 ml water, 30 g $Na_2S_2O_4$ were added slowly with constant stirring. The solution was heated to 60-65°C for 15 minutes, cooled, neutralized with solid Na_2CO_3, and extracted with three 100-ml aliquots of diethyl ether. The ether extracts were combined and evaporated to dryness under reduced pressure. The crude residue (7.3 g) was taken up in 200-ml anhydrous ether. Dichloroacetyl

chloride (Distillation Products Ind.) was dissolved in anhydrous ether (35-ml of a 10% solution by volume) and added by drops with constant stirring.

The blue-purple precipitate of 2-amino-5-methoxyphenol hydrochloride (2.15 g) was filtered. The precipitate was added to an Erlenmeyer flask along with 0.75 g urea. The flask was fitted to an air condenser and the mixture was heated at 180°C for 2 hours. The residue was washed with dilute HCl and the remainder taken up in anhydrous ether. The HCl wash was extracted with diethyl ether and the ether fractions combined and evaporated. The residue was taken up in a small quantity of acetone, and hexane was added until the solution became turbid. The red crystals which appeared were placed in 50-ml boiling water, a small quantity of activitated charcoal added, and the solution filtered. Upon cooling, white needle-like crystals appeared. Ultraviolet spectra were taken using a Beckman DB spectrophotometer. Excitation and emission maxima for fluorescence were determined with an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Maryland). Spectra compared favorably with published spectra of 6MBOA isolated from maize seedings.

Standard Curves for 6MBOA and BOA

The 95% ethanol was redistilled before using. The synthesized 6MBOA and the BOA were weighed and stock

solutions made up in 95% ethanol. The stock solution of 6MBOA was diluted with 95% ethanol to give concentrations of 0.025, 0.05, 0.08, 0.1, 0.25, 0.50, 0.80, 1.0, 2.5, 5.0, 8.0, 10.0, 25.0, 50.0, 80,0, and 100.0 μg/ml. The stock solution of BOA was diluted with 95% ethanol to give concentrations of 0.1, 0.5, 1.0, 5.0, 8.0, 10.0, 50.0, 80.0, and 100 μg/ml.

Fluorescence was measured with an Aminco-Bowman spectrophotofluorometer equipped with a xenon lamp and 1P21 detector tube; a 1-cm^2 cell was used with a slit program of 3-2-3-3-2-3-3 mm. The relative intensity of 6MBOA was read with the excitation wavelength of 280 mμ and emission wavelength of 335 mμ. The relative intensity of BOA was read with excitation set at 264 mμ and emission at 327 mμ . The 95% redistilled ethanol was used as a blank.

Recovery Standards for 6MBOA

The efficiency of the extraction procedure was determined by fortifying plant samples with 6MBOA and subjecting the samples to the complete extraction process used for analysis of 6MBOA.

The Midwestern inbred line WF9 was grown in pots. When the plants had reached an extended leaf height of 36 inches, the plant whorls were dried and ground in a Wiley mill. One-gram samples were placed in 250-ml round-bottom flasks. The 6MBOA in 95% ethanol or only

the ethanol was added to the flasks to give plant samples at four different levels of fortification (0, 75, 750, and 1500 ppm). Boiling chips and 150-ml distilled water were added to each flask and the flasks fitted with water-cooled condensers. The flasks were placed on a hot plate and refluxed for two hours. After extraction, the liquor was cooled and filtered. The filtrate was brought up to 150-ml with distilled water and then kept refrigerated.

Twenty milliliters of the solution were placed in a 60-ml separatory funnel and partitioned with three 10-ml portions of diethyl ether. The ether fractions were combined and filtered through a plug of Na_2SO_4 and the ether was evaporated under reduced pressure.

Alumina columns were prepared for cleaning the samples. A glass column (10 mm ID) was fitted with a scintered glass disc and 5 g alumina (Fisher Scientific Corp., no. A-540) were added. The column was washed with 20-ml 95% ethanol and the washings were discarded.

The residue from the ether extraction was taken up in 10-ml 95% ethanol and added to the column. The receptacle that had contained the residue was rinsed with an additional 10-ml ethanol and the rinse added to the column. The material on the column was eluted with enough 95% ethanol to provide 100-ml effluent. The effluent was collected in 100-ml volumetric flasks.

The relative intensity of the ethanol solutions was determined by spectrophotofluorometry. The ppm of 6MBOA were determined by reference to the standard curve for 6MBOA. The calculated ppm of the unfortified samples were subtracted from the readings for the fortified samples to arrive at the level of added 6MBOA that was recovered.

Analysis of Varieties from Experiments 6 and 7

Field plots 6 and 7 in Costa Rica in 1968 were thinned to two plants per hill when the average height of the plants was 36 inches. The cut plants were placed in bundles according to variety and dried at 30-50°C for 3 days. The dried plants were individually ground in a Wiley mill and the plant material derived from each was weighed and placed in a plastic bag.

One-gram samples were placed in 250-ml round-bottom flasks. Several boiling chips and 150-ml distilled water were added. The flasks were fitted with water-cooled condensers and heated on a hot plate for two hours while the mixture refluxed. The contents were allowed to cool to room temperature, filtered through Whatman no. 1 filter paper on a Buchner funnel, and the filtrate was brought up to 150-ml with distilled water.

Twenty milliliters of the filtrate were added to a 60-ml separatory funnel and shaken with three 10-ml portions of diethyl ether. The ether fraction was

filtered through a prewashed plug of Na_2SO_4 in a glass column. Two boiling chips were added to the effluent, which was then evaporated under reduced pressure.

The residue was taken up in 10-ml 95% ethanol and added to an alumina column. The flask was rinsed with another 10-ml ethanol, which was added to the column. The column was eluted with ethanol to give 100-ml of effluent.

The 6MBOA content of the effluent was determined by spectrophotoflurometry, using the excitation and emission values already mentioned. The relative intensity (RI) was recorded and the amount of 6MBOA related to this RI was read from the standard curve for 6MBOA. The amount of 6MBOA in the original one-gram sample was determined by the following formula:

ppm for one-gram sample=6MBOA (g/ml) X dilution factor/ Ef.

Ef refers to the efficiency of the extracting procedure.

RESULTS

The expression of resistance in maize to Diatraea saccharalis and Zeadiatraea lineolata results from the interaction of plant factors with the insect's biology to reduce the adaptation of the insects to their host plants. Reduction in adaptation can result from antibiosis or failure of the moth to oviposit on the plant. Consequently, the analysis for resistance must be designed to represent the effect of both factors on the insect, unless from some prior knowledge it is known that one of them is not significant.

Ovipositional Studies:--The distribution of insects in an area determines the design and the efficiency of field tests for resistance. Uneven distribution increases variance within treatments and necessitates more replication. Unfortunately, for experimental purposes, insect distributions are rarely, if ever, evenly distributed.

There are three basic distribution patterns; regular, random, and aggregated. The regular pattern is an overdispersed distribution in which the individuals are not independent. Presence of an individual in a unit area decreases the probability of finding other individuals in that area. In the random pattern there is no interaction of individuals. Each unit area has equal

of having other individuals in that area regardless of how many are already there. The aggregated pattern is commonly found among insects. There is a positive interaction among individuals. The presence of an individual in a unit area increases the probability of finding other individuals in that area.

The analysis of variance is the common test used to determine if there are significant differences between means. This test is based on the assumption of normality, homogeneity of variances for the treatments, additivity of the data, and independence of the mean from the variance. Insect counts seldom describe a normal curve. Many insect counts are approximated by the Poisson curve. In this distribution, the mean is proportional to the variance. Where the insects are aggregated, the binomial distribution generally gives a better fit.

Diatraea saccharalis:--Replicated field experiments with maize in south Florida gave non-significant results due to the low infestations of the borer. To obtain a larger insect population, further investigations were conducted in the screen cage at Gainesville. Investigations under caged conditions have the advantages of reducing the effects of certain parasites and predators of D. saccharalis and restricting the moths' choice of host

plants to those being tested. The screen reduced wind velocity, evapotranspiration, and light intensity. These factors probably affected plant growth and perhaps the behavior of the insects. Whether the cage biased the effects of only certain plant varieties is not known.

The distribution of egg masses per plant site in Experiment 1 is given in Table 4. The fit of the observed distribution to the expected Poisson was very poor. The underestimation of zero values indicated that aggregation might be producing the poor fit. The expected negative binomial distribution reduced the chi-square value, but the fit was still above the rejection level.

There were several ovipositional sites on the plant with the possibility of each site exhibiting a different distribution. A number of egg masses were found at the bases of several plants in Experiment 1. By considering only those egg masses placed on the leaves, the fit of the distribution was improved to both the Poisson and negative binomial distributions. By excluding the egg masses found on the stalks, the category of 5 or more masses per plant was significantly reduced.

The locations of the egg masses on the plants were observed and compared (Table 5). Using the criterion of eggs per mass as an indicator of preferred ovipositional

Table 4. Distributions of egg masses of D. saccharalis per hill in Experiment 1 and their fit to the expected Poisson and negative binomial models.

No. Masses Per Hill	Total Masses				Masses only on Leaves			
	Observed		% Expected		Observed		% Expected	
	No.	%	Poisson	Negative Binomial	No.	%	Poisson	Negative Binomial
0	13	15.9	7.4	24.9	15	18.3	17.6	26.5
1	27	32.9	19.2	20.5	30	36.6	30.5	28.2
2	13	15.9	25.0	15.4	15	18.3	26.6	20.7
3	5	6.1	21.8	11.2	10	12.2	15.4	13.0
4	8	9.8	14.2	8.1	6	7.3	6.7	7.4
5+	16	19.4	12.5	19.9	6	7.3	3.2	4.2
Chi-Square			39.331	13.458			9.810	7.655[a]

[a]Chi-Square fit acceptable at 5% level.

Table 5. Comparisons between means of ovipositional sites of maize by D. saccharalis in Experiments 1 and 2.

Experiment No.	Comparisons Made	df	Mean No.	t-Value
1	Eggs per mass on stalk base vs.	70	27.5	1.67
	Eggs per mass on leaves	142	32.7	
1	Eggs per mass along midrib vs.	113	35.8	
	Eggs per mass on blade	28	20.4	3.95[a]
1	Eggs per mass on upper leaf surface vs.	100	35.1	
	Eggs per mass on lower leaf surface	41	27.0	1.95
1	Eggs per hill on base of stalk vs.	81	23.8	
	Eggs per hill on leaves	81	57.0	3.86[a]
1	Eggs per hill on upper leaf surface vs.	81	43.2	4.73[a]
	Eggs per hill on lower leaf surface	81	13.9	
1	Eggs per hill along midrib vs.	81	49.8	6.26[a]
	Eggs per hill on the blade	81	7.2	
1	Egg masses per hill on the stalk vs.	81	0.866	
	Egg masses per hill on the leaves	81	1.744	3.38[a]
1	Egg masses per hill on upper leaf surface	81	1.23	5.05[a]
	vs. Egg masses per hill on lower surface	81	0.51	

Table 5, Continued

Experiment No.	Comparisons Made	df	Mean No.	t-Value
1	Egg masses per hill along midrib vs.	81	1.35	9.42[a]
	Egg masses per hill on the blade.	81	0.39	
2	Eggs per mass on upper leaf midrib vs.	51	34.3	1.28
	Eggs per mass on lower leaf midrib.	30	28.7	
2	Eggs per mass on upper leaf blade vs.	82	9.3	
	Eggs per mass on lower leaf blade.	25	22.3	3.55[a]
2	Egg masses per hill on upper leaf midrib	35	1.44	1.57
	vs. Egg masses per hill on lower midrib.	35	0.86	

[a]Difference between means highly significant at the 1% level.

sites, the leaf midrib was preferred over the leaf blade. There was no difference between the upper leaf midrib and the lower leaf midrib. In Experiment 2, the lower leaf blade was preferred over the upper leaf blade.

Site preference may be exhibited as a difference in egg masses between sites. In Experiment 1, the leaf was preferred over the stem. The number of eggs per site is determined by the number of eggs per mass and the number of masses per site. Comparisons of total eggs per site indicated that leaves were preferred over stalks, upper leaf surfaces were preferred over lower, and the midrib was preferred over the blade.

A comparison of the different varieties in Experiment 2 was desired. Using the number of egg masses per variety as an indicator of attractiveness, the variance for each variety was determined. The variances were found to be heterogeneous. Transformation of the data (Table 6) removed the heterogeneity and permitted analysis of variance (Table 7). No significant differences were found between varieties.

Although no significant differences were found between varieties in the number of egg masses, the number of eggs per mass might have been different between varieties. No correlation was found between the number of eggs per mass and the number of egg masses per plant.

Analysis of variance for differences between varieties in the number of eggs per mass indicated significant differences between varieties (Table 7). Comparisons between varieties by Duncan's multiple range test indicated Diacol V-153 contained significantly more eggs per mass than USA 342, Blanco Comun, and Cuba 362 (Table 8).

In Experiment 1, the number of egg masses per plant was correlated with both the total number of eggs per plant and the number of egg masses on plants in the same pot (Table 9). The majority of the eggs (68.6%) were placed on leaves 5-7 (Table 10). Several egg masses were found on the pots.

Diatraea saccharalis in South Florida is adapted to sugarcane, with other hosts being strictly secondary. The ovipositional characteristics are adapted to the dense plant population of sugarcane, a characteristic that may not be adapted to the more open stands of maize. Dispersion away from the site of oviposition is thought to occur for several reasons. No zero values were found in the tunnel data from Experiments 1 and 2, as compared to more than 15% zero values in the egg data. There was no correlation between the numbers of eggs and tunnels per plant. Distribution of tunnel data in Experiment 1 was found to approximate a binomial distribution; the chi-square value was 7.12 with 7 degrees of freedom. More than half of the pots, each pot containing two plants,

Table 6. Analysis for homogeneity of variance of varieties.

Experiment No.	Data Analyzed	df	Chi-Square	Significance
	Zeadiatraea lineolata Data			
4	Larvae and pupae per variety	11	8.02	n.s.
4	Tunnels per variety	11	14.78	n.s.
4	Eggs per variety	11	22.68	Sig.
4	Eggs per variety after transformation[a]	11	9.66	n.s.
5	Eggs per variety	54	75.18	Sig.
5	Eggs per variety after transformation	54	43.49	n.s.
6	Tunnels per variety	7	6.68	n.s.
6	Larvae and pupae per variety	7	8.66	n.s.
8	Tunnels per variety	11	19.16	n.s.
	Diatraea saccharalis Data			
2	Eggs per variety	5	45.77	Sig.
2	Eggs per variety following transformation[b]	5	3.742	n.s.
2	Eggs per variety following transformation[a]	5	6.444	n.s.

[a]Transformation by the square root of individuals plus 1.
[b]Transformation by taking the square root of each individual value.

Table 7. Analysis of variance for eggs, larvae and pupae, and tunnels for D. saccharalis and Z. lineolata per maize variety.

Experiment No.	Data Analyzed	Source of Variance	df	Mean Square	F-test
Diatraea saccharalis					
2	Egg masses per variety	Varieties	11	0.079	1.21
		Error	60	0.065	
2	Eggs per mass per variety	Varieties	5	876.0	2.88[c]
		Error	183	304.0	
3	Tunnels per variety	Varieties	5	1.89	1.12
		Error	35	1.69	
3	Larvae per variety	Varieties	5	5.80	2.33
		Error	35	2.06	
Zeadiatraea lineolata					
4	Eggs per variety[a]	Varieties	11	0.51	
		Error	165	0.69	
4	Tunnels per variety[b]	Varieties	11	12.27	1.92[c]
		Error	156	6.38	
5	Eggs per variety[a]	Varieties	54	0.66	2.24[d]
		Error	270	0.30	
5	Tunnels per variety	Varieties	54	3.22	1.21
		Error	598	2.66	
6	Larvae + pupae per variety[b]	Varieties	7	5.14	2.14[c]
		Error	103	2.40	
6	Tunnels per variety[b]	Varieties	7	13.71	2.76[c]
		Error	103	4.96	
7	Tunnels per variety	Varieties	14	5.86	2.43[d]
		Error	135	2.41	
7	Larvae + pupae per variety	Varieties	14	2.42	3.17[d]
		Error	135	0.76	
8	Tunnels per variety	Varieties	11	6.04	
		Error	77	7.81	
8	Larvae + pupae variety	Varieties	11	4.64	
		Error	77	4.17	

[a]Data transformed.
[b]Data corrected for missing values.
[c]Difference significant of 5% level of probability.
[e]Difference significant at 1% level of probability.

Table 8. Comparisons between mean number of D. saccharalis eggs per mass per maize variety in Experiment 2.

Variety	Mean No. of Eggs Per Mass
Diacol V-153	30.4 a
Amarillo Theobromina	24.8 ab
Diacol V-351	22.9 ab
USA 342	18.4 b
Blanco Comun	17.5 b
Cuba 362	16.5 b

Means of varieties having same letter are not significantly different at 5% level.

Table 9. Analysis for correlation of eggs, larvae and pupae, and tunnels for *D. saccharalis* and *Z. lineolata*.

Experiment No.	Data Analyzed	df	Correlation Coefficient
Diatraea saccharalis			
3	Larvae per plant with tunnels per plant	41	.506[a]
1	Tunnels with larvae	81	.814[a]
1	Egg masses per plant with total eggs	163	.497[a]
1	Egg masses on plants in same pot	81	.866[a]
Zeadiatraea lineolata			
4	Tunnels per plant with sum of tunnel lengths	35	.846[a]
4	Tunnels per plant with average of tunnel length	35	.098
6	Larvae with tunnels	251	.764[a]
6	6MBOA per variety with tunnels per variety	7	.2414
6	6MBOA per variety with larvae per variety	7	.512

[a]Significant correlation at 1% level.

Table 10. Mean number of D. saccharalis eggs per mass and percent of masses laid on different leaves in Experiment 1

Leaf No.[a]	Mean Eggs Per Mass	Standard Deviation	% of Total Leaf Masses
1	0	0	0
2	20.0	3.6	2.8
3	32.4	17.2	13.3
4	35.9	27.0	7.0
5	29.8	13.3	18.9
6	29.8	19.9	23.1
7	35.3	25.4	26.6
8	50.4	27.2	5.6
9	23.3	9.6	2.8

[a]Leaves were numbered from base of plant to top.

received more than 50 eggs per pot, yet this apparently had little effect on the number of tunnels.

Zeadiatraea lineolata

The data concerning eggs and egg masses per plant in Experiments 4 and 5 were recorded on two dates during the peak of the ovipositional period. The distribution of eggs and egg masses from Experiment 5 (Tables 11 and 12) gave better fit to the expected Poisson and negative binomial distributions than did the D. saccharalis data (Table 4). The number of egg masses per hill showed a better fit to the negative binomial than the Poisson. The Poisson distribution consistently underestimated the number of zero values, thereby suggesting a degree of aggregation. The negative binomial gave excellent fit in all cases.

Varietal differences were examined in both experiments. Variances associated with varieties were sufficiently heterogeneous to require transformation before analysis of variance could be performed (Table 6). The transformations removed the heterogeneity so analysis of variance (Table 7) and Duncan's multiple range test (Table 13) were applied. Significant differences were found between varieties in Experiment 5, but not in Experiment 4.

Table 11. Distributions of Z. lineolata egg masses per hill in Experiment 5 and their fit to the expected Poisson and negative binomial models.

No. Masses Per Hill	Deposited on 18				Deposited on 27			
	Observed		% Expected		Observed		% Expected	
	No.	%	Poisson	Negative Binomial	No.	%	Poisson	Negative Binomial
0	151	48.8	43.2	49.7	104	31.5	28.9	34.2
1	103	30.9	36.2	29.0	99	30.0	35.7	31.6
2	43	13.0	15.2	12.8	68	20.6	22.1	18.5
3	11	3.3	4.2	5.1	33	10.0	9.1	8.8
4	6	1.8	1.2	3.5	21	6.4	2.8	3.7
5-7	7	2.1			5	1.5	1.4	3.2
Chi-Square			8.22[a]	0.824[a]			5.965[a]	3.569[a]

[a]Chi-Square test indicated good fit of expected to observed.

Table 12. Distributions of Z. lineolata eggs per hill in Experiment 5 and their fit to the Poisson and negative binomial models.

Eggs Per Hill	Deposited on 18 Observed		% Expected		Deposited on 27 Observed		% Expected	
	No.	%	Poisson	Negative Binomial	No.	%	Poisson	Negative Binomial
0	161	48.8	32.6	48.1	104	31.5	15.9	26.5
1	76	23.0	36.5	24.5	68	20.6	29.2	26.0
2	44	13.3	20.5	12.9	56	17.0	26.9	18.8
3	24	7.3	7.6	6.9	43	13.0	16.5	12.0
4	10	3.0	2.1	3.7	28	8.5	7.6	7.2
5	5	1.5	.6	4.1	21	6.4	4.0	4.1
6	5	1.5			5	1.5		5.4
7	5	1.5			5	1.5		
Chi Square			41.32	0.308[a]			29.621	4.911[a]

[a]Chi Square test indicated good fit of expected to observed.

In both experiments the number of egg masses per hill was not correlated with the number of eggs per mass (Table 14). In Experiment 5, the seven varieties rated as having the most eggs were grouped together, as were the eight varieties having the least number of eggs (Table 13). The ratio of number of eggs per plant to masses per plant averaged 1.56±.75 in the first group and 1.09±.27 in the second group. Differences between means were significant after analysis according to Snedecor (1956).

Assuming that no non-random errors were made in counting eggs, the number of eggs received by each plant should be correlated to the number of tunnels, larvae, and pupae for each plant of a variety. Analysis for correlation of eggs with tunnels and larvae plus pupae was found to be nonsignificant (Tables 15 and 16). This lack of correlation indicates that factors in addition to oviposition are important in determining infestation per plant.

Antibiosis

Diatraea saccharalis:--The efficiency of testing for antibiosis in maize is related to the evenness of insect dispersal at the time of egg eclosion and the degree of migration to surrounding plants. To insure that each plant was exposed to the same number of insects, each plant in Experiment 3 received the same number of first instar

Table 13. Comparisons between mean number of Z. lineolata eggs per maize variety in Experiment 5.

Variety	Mean No. of Transformed Data	Variety	Mean No. of Transformed Data
Granada Gpo.2	2.44a	Ver.181	1.88abcdefg
Tuxpantiqua	2.42ab	Antiqua Gpo.1	1.87abcdefg
Barbados Gpo.1	2.40abc	Cuba Gpo.2	1.85abcdefg
S.L.P. Gpo.10	2.25abcd	Ver.Gpo.6	1.77abcdefg
Ver. 187	2.25abcd	St.Vicente Gpo.3	1.73abcdefg
S.L.P. Gpo.12	2.24abcde	Ver.179	1.72abcdefg
Sta.Lucin Gpo.1	2.17abcdef	Trinidad Gpo.1&2	1.72abcdefg
Azteca-Tuxp.	2.17abcdef	Antigua.2	1.72abcdefg
St.Croix Gpo.1	2.15abcdef	Ver.228	1.70abcdefg
Ver. 133	2.12abcdef	R.Dom.Gpo.8	1.70abcdefg
Cuba Gpo.5	2.12abcdef	Pto.Rico Gpo.3	1.70abcdefg
Cupurico	2.10abcdef	Ver.225	1.69abcdefg
Sanribag	2.08abcdef	Ver.43	1.69abcdefg
St. Croix Gpo.3	2.08abcdef	J.S.Y.	1.67abcdefg
Cuba Antibarsan	2.06abcdef	R.Dom Gpo.3	1.65abcdefg
Pto.Rico Gpo.1	2.06abcdef	Ver.215	1.63abcdefg
Pto.Rico Gpo.6	2.04abcdef	Tobago Gpo.1	1.62abcdefg
Cuba Gpo.1	2.04abcdef	Ver.Gpo.8	1.62abcdefg
Oax. Gpo.5	2.02abcdef	Ver.14	1.58 bcdefg
Haiti Gpo.1	1.98abcdef	Ver.8	1.57 cdefg
Ver.Gpo.7	1.97abcdef	Ver.143	1.56 cdefg
Ver.168	1.97abcdef	Pto.Rico Gpo.6	1.55 defg
Tuxp.FF(Peri Crista)	1.97abcdef	Ver.165	1.48 defg
Ver.213	1.95abcdef	Ver.208	1.40 efg
Tuxp.-Sanribag	1.95abcdef	Mich.166	1.30 fg
Pto.Rico Gpo.2	1.95abcdef	Ver.141	1.05 g
Guad.Gpo.1A	1.94abcdef		
Ver.39	1.92abcdef		
Cuba Gpo.4	1.90abcdefg		

Means of varieties having same letter are not significantly different at 5% level.

Table 14. Correlation of Z. lineolata egg masses per hill with eggs per hill and with eggs per mass in Experiments 4 & 5.

Date of Data	Experiment	Degrees of Freedom	Correlation	Coefficients
			Egg Masses with Egg	Egg Masses with Eggs per Mass
July 18	1	103	.645 a	-.059
July 27	1	150	.781 a	+.040
July 18	2	169	.814 a	-.057
July 27	2	225	.847 a	-.007

a = Significant correlation of 1% level.

larvae. Analysis of variance and Duncan's multiple range test indicated no differences between larval and tunnel data from the different varieties (Table 7). Larval counts were significantly correlated with tunnel damage (Table 9).

Zeadiatraea lineolata:--Tunnel and larvae plus pupae data from all experiments were tested for homogeneity of variance to determine if transformation of the data was required (Table 6). All variances were homogeneous. Analysis of variance (Table 7) and Duncan's multiple range test (Tables 17, 18, 19, and 20) were applied to tunnel data. There were significant differences between varieties in Experiments 4, 5, 6, and 7, but not in Experiment 8. The number of tunnels per plant was correlated with the larvae plus pupae data for most varieties at the time of plant dissection (Tables 9 and 21).

Analysis of variance (Table 7) and Duncan's multiple range test (Tables 22 and 23) were applied to the larvae and pupae data. The number of tunnels per plant was correlated for plants in the same hill (Table 24). The number of tunnels per plant was correlated to the sum of tunnel lengths per plant, but not to the mean length of tunnels per plant (Table 9). Tunnels were located throughout the upper portion of the stalk, with 85% of the tunnels occurring in internodes 3 through 9 (Table 25).

Table 15. Correlation of Z. lineolata eggs per hill with tunnels in Experiment 4.

Variety	Degrees of Freedom	Correlation Coefficient
Diacol V-153	15	-0.277 a
Poey T-66	14	+0.522
Diacol V-351	14	+0.351 a
Eto Blanco	15	+0.078 a
Tico H-1	14	+0.358 a
Tico H-2	15	+0.415 a
Blanco Comun	15	+0.311 a
Eto Amarillo	13	-0.012 a
USA 342	13	-0.247 a
Cuba 362	13	-0.312 a
Amarillo Theobromina	15	-0.364 a
Rocamex V-520-C	15	-0.195 a

a = correlation coefficient not significantly different from zero at 5% level.

Table 16. Correlation of Z. lineolata larvae plus pupae with eggs per hill in Experiment 4.

Variety	Degrees of Freedom	Correlation Coefficient
Diacol V-153	15	-0.251 a
Poey T-66	14	+0.013 a
Diacol V-351	14	+0.457 a
Eto Blanco	15	-0.116 a
Tico H-1	14	+0.268 a
Tico H-2	15	+0.166 a
Blanco Comun	15	+0.386 a
Eto Amarillo	13	+0.405 a
USA 342	13	-0.271 a
Cuba 362	13	+0.129 a
Amarillo Theobromina	15	+0.036 a
Rocamex V-520-C	15	-0.120 a

a = correlation coefficient not significantly different from zero at 5% level.

Table 17. Comparisons between mean number of Z. lineolata tunnels per maize variety in Experiment 4.

Variety	Mean No. of Tunnels Per Hill*
Rocamex V-520-C	7.13 a
Amarillo Theobromina	6.94 a
Cuba 362	6.88 a
USA 342	6.75 a
Eto Amarillo	6.50 ab
Blanco Comun	6.31 ab
Tico H-2	5.94 abc
Tico H-1	5.94 abc
Eto Blanco	5.69 abc
Diacol V-351	5.13 abc
Poey T-66	4.50 bc
Diacol V-153	4.19 c

*Means of varieties having same letter are not significantly different at 5% level.

Table 18. Comparisons between mean number of Z.lineolata tunnels per maize variety in Experiment 6.

Variety	Mean No. of Tunnels Per Hill
Mich 166	6.38 a
Cuba 362	5.47 ab
Eto Blanco	5.34 ab
Diacol V-153	5.22 abc
Tico H-1	4.63 abc
Diacol V-351	4.09 abc
Eto Amarillo	3.91 bc
Poey T-66	3.56 c

Means of varieties having same letter are not significantly different at 5% level.

Table 19. Comparisons between mean number of Z. lineolata tunnels per maize variety in Experiment 7.

Variety	Mean No. of Tunnels Per Plant
Collection 50	3.7 a
Collection 52	3.2 ab
Collection 46	2.9 abc
Collection 26	2.9 abc
Collection 15	2.7 abc
Collection 43	2.6 abc
Collection 62	2.2 abc
Collection 88	2.0 abc
Collection 13	2.0 abc
Collection 20	1.9 bc
Collection 151	1.5 bc
Collection 64	1.5 bc
Collection 83	1.3 c
Collection 65	1.3 c
Collection 11	1.3 c

Means of varieties having same letter are not significantly different at 5% level.

Table 20. Comparisons between mean number of Z. lineolata tunnels of maize varieties in Experiment 5.

Variety	Mean No. of Tunnels per Plant	Variety	Mean No. of Tunnels per Plant
Granada	4.17a	Cuba Antibarsan	2.75abcde
Trinidad Gpo. 1&2	3.75ab	Taxp. FF(PeruCrist)	2.67abcde
Ver. 181	3.58abc	Ver. 215	2.67abcde
Ver. Gpo. 8	3.50abcd	Cuba Gpo. 4	2.58abcde
Ver. Gpo. 6	3.50abcd	Saint Croix Gpo.3	2.50 bcde
Ver. 208	3.50abcd	Saint Vicente Gpo.3	2.50 bcde
Ver. 8	3.50abcd	Pto Rico Gpo.1	2.50 bcde
Ver. Gpo. 7	3.33abcde	Ver. 141	2.50 bcde
Ver. 143	3.33abcde	Ver. 228	2.50 bcde
Ver. 187	3.33abcde	Ver. 39	2.50 bcde
J.S.Y.	3.33abcde	Ver. 168	2.42 bcde
Haita Gpo. 1	3.33abcde	Taxpantigua	2.42 bcde
Ver. 213	3.25abcde	S.L.P. Gpo.12	2.42 bcde
R. Dom. Gpo. 8	3.17abcde	St. Croix GPO.1	2.42 bcde
Pto Rico Gpo. 3	3.17abcde	Barbados Gpo.1	2.33 bcde
Azteca-Taxp.	3.08abcde	Guad. Gpo. 1A	2.33 bcde
Cuba Gpo. 1	3.08abcde	Ver. 43	2.33 bcde
Sta.Lacin Gpo. 1	3.00abcde	Antigua Gpo.2	2.33 bcde
Tobago Gpo. 1	3.00abcde	Cuba Gpo. 5	2.23 bcde
S.L.P. Gpo. 10	3.00abcde	Cuba Gpo. 2	2.23 bcde
Ver. 225	3.00abcde	R.Dom.Gpo. 3	2.17 bcde
Ver. 133	2.92abcde	Taxp.-Sanribag	2.17 bcde
Pto.Rico Gpo. 6	2.83abcde	Antigua Gpo. 1	2.08 bcde
Oax. Gpo. 5	2.83abcde	Pto.Rico Gpo. 1	2.00 cde
Ver. 14	2.83abcde	Sanribag	1.92 de
Ver. 165	2.75abcde	Mich. 166	1.83 e
Ver. 179	2.75abcde		
Cupurico	2.75abcde		

Means of varieties having same letter are not significantly different at 5% level.

Table 21. Correlation of Z. lineolata larvae and pupae with tunnels per hill in Experiment 4.

Variety	Degrees of Freedom	Correlation Coefficient
Diacol V-153	15	0.727 b
Poey T-66	14	0.727 b
Diacol V-351	14	0.732 b
Eto Blanco	15	0.487 c
Tico H-1	14	0.606 c
Tico H-2	15	0.400 a
Blanco Comun	15	0.707 b
Eto Amarillo	13	0.434 a
USA 342	13	0.457 a
Cuba 362	13	0.386 a
Amarillo Theobromina	15	0.600 c
Rocamex V-520-C	15	0.566 c

a = not significant at 5% level.
b = significant at 1% level.
c = correlation significantly different from zero at 5% level.

Table 22. Comparisons between mean number of Z. lineolata larvae and pupae per maize variety in Experiment 6.

Variety	Mean No. of Larvae and Pupae per Hill
Mich 166	3.75 a
Diacol V-153	3.63 a
Cuba 362	3.50 a
Tico H-1	3.13 ab
Eto Blanco	2.89 ab
Eto Amarillo	2.63 ab
Diacol V-351	2.50 ab
Poey T-66	2.19 b

Means of varieties having same letter are not significantly different at 5% level.

Table 23. Comparisons between mean number of Z. lineolata larvae and pupae per maize variety in Experiment 7.

Variety	Mean No. of Larvae and Pupae per Plant
Collection 26	1.8 a
Collection 50	1.5 ab
Collection 88	1.0 abc
Collection 20	0.9 abcd
Collection 62	0.9 abcd
Collection 15	0.9 abcd
Collection 13	0.8 bcd
Collection 52	0.7 bcd
Collection 43	0.5 cd
Collection 83	0.4 cd
Collection 65	0.4 cd
Collection 46	0.4 cd
Collection 151	0.3 cd
Collection 11	0.1 cd
Collection 64	0.0 d

Means of varieties having same letter are not significantly different at 5% level.

Table 24. Correlation of *Z. lineolata* tunnels in plants of the same hill for Experiment 5.

Block	Degrees of Freedom	Correlation Coefficient
A	53	0.333 a
B	52	0.766 b
C	50	0.427 b
D	53	0.566 b
E	54	0.588 b
F	54	0.608 b

a = Significant correlation at 5% level of probability.
b = Significant correlation at 1% level of probability.

Table 25. Location of Z. lineolata tunnels in maize stalks in Experiment 4.

Internode[a]	No. of Tunnels	% of Total Tunnels	Tunnel Length in CM
1	1	0.4	10.0
2	6	2.3	2.0
3	21	8.1	4.8
4	41	15.8	6.0
5	40	15.4	6.6
6	43	16.6	6.5
7	32	12.4	5.9
8	23	8.9	6.0
9	22	8.5	5.0
10	9	3.5	4.8
11	10	3.9	4.1
12+	11	4.3	5.0

[a]The higher the number, the higher the internode is on the stalk.

Effect of larvae on plants:--Larval damage to maize consisted of leaf damage caused by first instar larvae, rupture of the stems' vascular systems by tunneling larvae, broken stems caused by tunnel-weakened stalks, and fallen ears due to larvae tunneling in the shank of the ears.

The sum of all tunnel lengths per plant averaged 21 cm. A significant percentage of the ear shanks contained tunnels. In Experiment 4, 9.9% of 303 ears contained tunnels. Experiments 6, 7, and 8 had 19, 29, and 12%, respectively, of their plants with tunneled ears.

Analysis of 6MBOA:--Plant samples from Experiments 6 and 8 were assayed for their content of 6MBOA. Readings were compared with a standard curve (Fig. 1). A correction factor of 80% was computed (Table 26) and used for correcting plant sample readings. Results of the analysis are reported in Table 27.

The 6MBOA content was not correlated with either tunnel or larval data in Experiment 6 (Table 9). The analysis of 6MBOA was low for all varieties tested. Due to technical problems, the dried tissue was not assayed until a year after the plants were cut. The drying process or storage might have resulted in a loss of activity in the samples. Further testing is necessary to determine the validity of the procedure used.

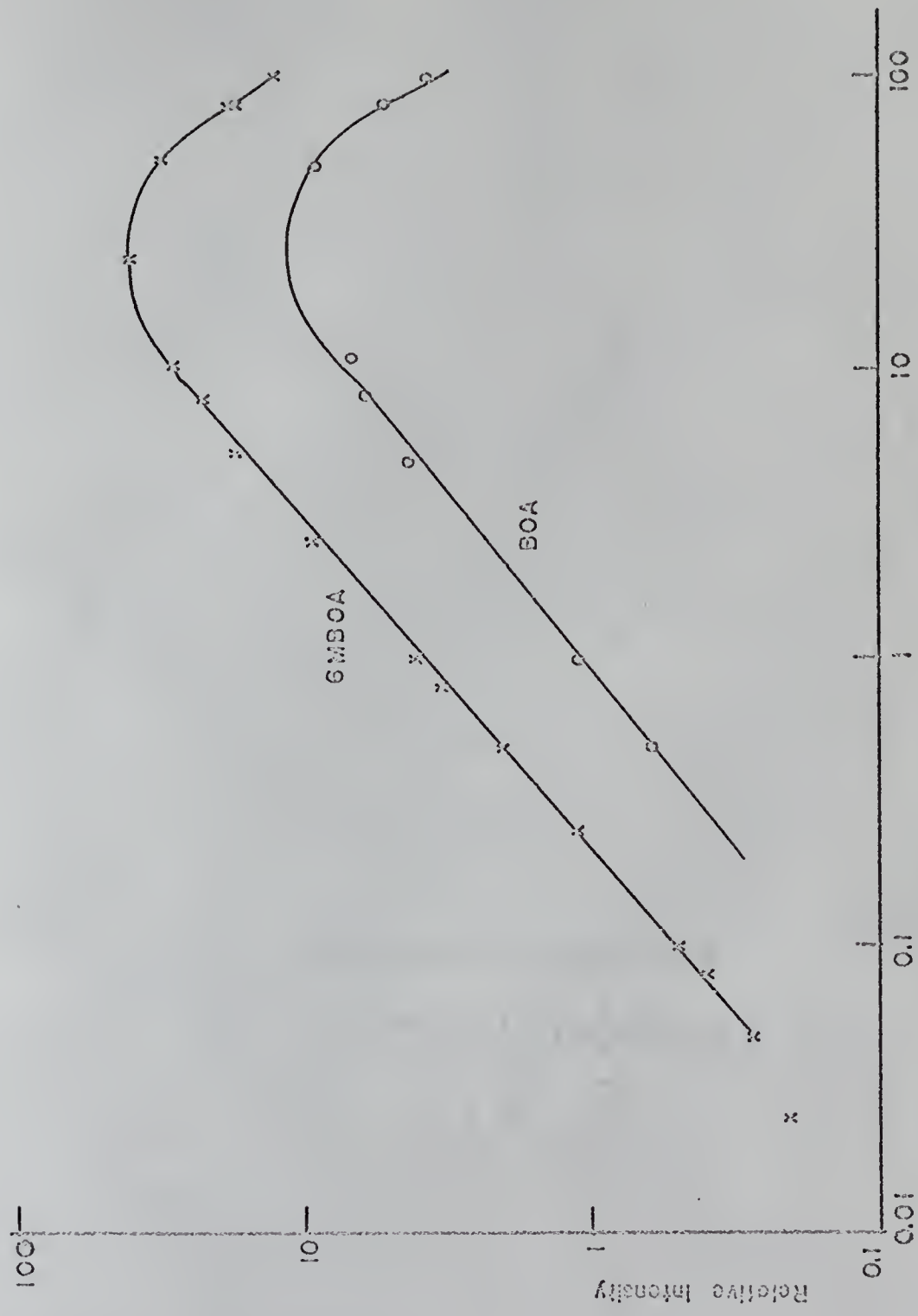

Fig. 1. Standard curves of 6MBOA and BOA in 95% ethanol.

Table 26. Recovery of 6MBOA from maize sample WF9 fortified and unfortified with 6MBOA

6MBOA Added (ppm)	6MBOA Found (ppm)	Added 6MBOA Recovered	% of added 6MBOA Recovered
0	270	0	0
0	270	0	0
75	330	60	80
750	900	630	84
750	825	555	74
1500	1500	1230	82

Table 27. Results of analyses for 6MBOA in varieties in Experiments 6 and 8.

Variety	No. Samples Assayed	Average ppm of MBOA
Mich 166	8	430
Cuba 362	7	570
Tico H-1	7	380
Eto Blanco	7	379
Eto Amarillo	7	420
Diacol V153	7	510
Diacol V351	7	420
El Coyol	7	330
J.S.Y.	7	600
Tuxp. - Sanvibag	1	330
Ver. 8	1	520
Ver. 187	1	340
R. Dom. Gpo. 3	1	750
Ver. 39	1	600
Trinidad Gpo 1 & 2	1	1030
Ver. 41	1	520
Pto. Rico Gpo. 2	1	390
St. Croix Gpo. 3	1	490

DISCUSSION

The proper testing procedures and the determination of indices of resistance are important considerations in the analysis for resistance. The interaction of maize with D. saccharalis and Z. lineolata may result in ovipositional resistance and resistance to larval survival.

The mean number of eggs per variety and the mean number of eggs per mass per variety were both good indices for varietal differences caused by ovipositional attraction. The eggs per mass ratio were not correlated with the number of masses per plant. The efficiency of the test could therefore be improved by considering each mass as a replicate and using the number of eggs per mass as an indicator of resistance. Dispersal of egg masses gave a skewed distribution and resulted in data requiring transformation prior to analysis of variance. By using the ratio index, non-normality and heterogeneity of variance associated with egg mass distribution were removed. Using this index, Diacol V-153 was found to be significantly more susceptible to oviposition by D. saccharalis than USA 342, Blanco comun, and Cuba 362 (Table 8).

The ratio index was tested in Costa Rica on varieties that had shown differences in mean number of Z.lineolata eggs per variety. The seven most susceptible varieties

composed Group 1, while the 8 most resistant composed Group 2. The difference between ratios of the two groups was significantly different.

The psysiological or morphological basis for attraction resistance to oviposition by the two species was not determined. Differences in oviposition sites for D. saccharalis were shown to result in significant differences between the mean number of eggs per site. Varietal differences relating to differences in site selection by the insect were not determined

The mean number of eggs per mass is a function of the moths' behavior. The response of the insect to the plant factors resulting in deposition of eggs probably results from an interaction of the genotype with the environment. The mean number of eggs per mass for Z. lineolata averaged less than two and is comparable to that of Z. grandiosella, which averages 3 eggs per mass (Rolston, 1955). The average number of eggs per mass found here is not consistent with the nine eggs per mass reported for the same species by Kevan (1944) in Trinidad. The heritability and environmental significance of this difference might prove academically interesting and economically significant.

Sites of oviposition of D.saccharalis were found to be comparable to published data concerning sites of oviposition of Z. grandiosella (Rolston, 1955). Both the

sugarcane borer and the southwestern corn borer showed a preference for the upper surfaces of the leaves along the midrib. Both species occasionally oviposit on the stalk. In contrast, Ostrinia nubilalis oviposited more than 80% of its eggs on the undersides of the leaves with only about 5% on the upper surfaces. Less than 2% of the eggs were placed on the stem and leaf sheaths (Everly, 1959). The importance of site differences in resistance was difficult to evaluate without knowledge of the mechanism or mechanisms of resistance to oviposition.

Larval counts and tunnel damage in the stalks of maize are common indices of resistance to any of the stalk borers. Tunnel counts were found to be correlated with the sum of tunnel lengths and larval counts per stalk for both D. saccharalis and Z. lineolata. The average length of each tunnel was not correlated with the number of tunnels per stalk.

No significant difference was found between mean number of tunnels per Colombian variety tested against the sugarcane borer. In Costa Rica, these same varieties were found to differ significantly between mean number of tunnels and larvae per variety for Z. lineolata.

Based on larval survival and tunnel data from experiments conducted under natural infestation of Z. lineolata and natural infestations supplemented with egg

masses, variety Poey T-66 consistently was found to have the greatest degree of resistance. Significant differences were also found between other varieties.

The location of tunnels caused by Z. lineolata occurred principally in internodes 3 - 9. Appreciable numbers of tunnels, more than 10%, were found in ear shanks. Unlike Z.grandiosella, Z.lineolata did not girdle the stalks. Z. grandiosella occurs mainly in the lower part of the stalk (Rolston, 1955). Ostrinia nubilalis is more frequently found in the upper portion of the stalk and frequently caused breakage of tassels (Hawkins and Devitt, 1953). In cage studies, D. saccharalis occurred throughout the stalk, causing both tassel damage and broken stalks.

Breeding programs for resistance to D. saccharalis and Z. lineolata will be more efficient when the physiological or morphological basis for resistance is recognized. Identification of the physicochemical resistant factors and their location in the plants, and the development of laboratory techniques for their identification and quantitation will permit more efficient selection procedures in breeding programs. Biological variation and the non-uniform nature of D. saccharalis and Z. lineolata distributions in the field experiments reduce the efficiency of field testing.

Varieties from Cost Rica were analyzed for their content of 6MBOA to determine if maize resistance to Z. lineolata was correlated with concentration of 6MBOA or its precursors. Varieties differed little in their content of 6MBOA and all were quite low in comparison with published reports of varieties resistant to the European corn borer. No correlation was found between field resistance as measured by tunnel or larval counts and average ppm of 6MBOA for 5 - 7 samples of each variety. Further analysis of these varieties is needed to verify the observations. The results obtained so far neither prove nor disprove any relationship between 6MBOA and resistance to D. saccharalis or Z. lineolata.

Ability to quantitate resistant factors in the laboratory would be of great use in the study of cross resistance of a plant variety to different insects in different environments.

SUMMARY

Tests for resistance in 48 varieties of maize to Diatraea saccharalis (F.) and 86 varieties of maize to Zeadiatraea lineolata (Wlk) were conducted respectively in Gainesville, Florida, and Alajuela, Costa Rica. Data were taken on the mean number of eggs, egg masses, tunnels, and larvae plus pupae per variety for both insect species.

Mean number of eggs and mean number of eggs per mass were both indicators of resistance in varieties to oviposition by the two insects. The number of eggs per mass was independent of density of masses per plant. Variety Diacol V-153 had significantly more D. saccharalis eggs per mass than Cuba 362, USA 342, and Blanco comun. The physiological or morphological basis of resistance to oviposition was not known.

Different ovipositional sites on maize for D. saccharalis differed in mean number of eggs, egg masses and eggs per mass. The upper leaf along the midrib was the preferred site.

Mean tunnel and larval counts per variety were correlated for both species. Tunnel data were a more efficient index in that dissection of the plant was not required. Variety Poey T-66 consistently was rated as

most resistant to Z. lineolata. The physicochemical mechanism of this resistance was not identified.

Larval feeding of Z. lineolata resulted in an average of 21 cm of tunnel damage per plant. From 12 to 29% of the plants had tunnels in their ear shanks.

Varieties differed little in their content of 6MBOA and all varieties were quite low in comparison with published reports of varieties resistant to Ostrinia nubilalis (Hubn.). Further analysis of these varieties is needed to verify the 6MBOA readings.

Ability to quantitate resistance in the laboratory would be of great use in the study of resistance of a plant variety to different species in different environments.

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BIOGRAPHICAL SKETCH

James Lynn Overman was born June 12, 1941, at New Castle, Indiana, the son of Mr. and Mrs. Jack Overman. He graduated from Carthage High School, Carthage, Indiana, in May, 1959. He completed his Bachelor of Science degree at Purdue University in January, 1964. While at Purdue, he was president of the Thomas Say Entomological Society.

He did graduate work at Rutgers, the State University of New Jersey, and the University of Wisconsin before coming to the University of Florida in 1966.

On August 7, 1965, James Overman was married to Mary Ann Newhouse. He is a member of the Newell Entomological Society, the Society of Sigma Xi, and the Florida Entomological Society.

The Center for Tropical Agriculture supported his doctoral research, part of which was conducted in Costa Rica. He has been awarded a certificate of Tropical Agriculture.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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