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Resistance of indigenous races of maize to infestation by maize weevil *Sitophilus zeamais* Motsch.

by

J. Antonio Serratos H.

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in
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ABSTRACT

Factors contributing to resistance of maize to infestation by *Sitophilus zeamais* were investigated in 10 populations of indigenous races from Mexico, and 4 indigenous races from Belize. Resistance was related to the antifeedant properties of grain as well as hardness. Grain extracts of maize significantly reduced insect feeding on treated artificial diets when compared to control diets. Consumption of treated diets was negatively correlated with phenolic content of the grain extract. An analysis by GC-MS indicated that ferulic acid and p-coumaric acid were the principal phenolics present in the extracts, and insect feeding was strongly deterred when pure substances were added to insect diets. The relationships among a number of variables connected to susceptibility in grain (Index of susceptibility, stability parameter, weight loss of seed, percent mortality of insect population, emergence of insects, preference in choice test) and characteristics of the grain (hardness, content of phenolics at surface of grain, concentration of ferulic acid and moisture content of grain), were analyzed through canonical correlation analysis. The better resistance characteristics of some indigenous races were linked to the presence of ferulic acid as a chemical deterrent to oviposition and feeding. A taxonomic study was, in addition, performed to develop a comprehensive resistance map of maize germplasm which suggested that the ancient indigenous group was the most resistant.

RÉSUMÉ

Certains facteurs contribuant à la résistance du maïs à l'infestation par *Sitophilus zeamais* ont été analysés chez 10 populations indigènes du Mexique et 4 populations indigènes du Belize. La résistance a été associée à la fermeté et aux propriétés phagorépressives du grain. L'alimentation des insectes a été sensiblement réduite par des extraits de grain appliqués à la nourriture artificielle, par comparaison avec celle des insectes ayant reçu un régime normal. Une correlation négative entre la consommation des régimes et le contenu des produits phénoliques du grain a été observée. Comme l'a indiqué l'analyse GC-MS, l'acide férulique et l'acide p-coumarique étaient les principaux agents phénoliques des extraits. De plus, l'addition de ces substances à une diète artificielle a sensiblement diminué l'alimentation des insectes. Les relations entre certaines variables associées à la susceptibilité du grain et certaines caractéristiques du grain ont été etudiées grâce à la méthode d'analyse de correlation canonique. Les meilleurs traits de résistance chez quelques races indigènes de maïs ont été relies au contenu en acide férulique qui intervient comme agent phagorepresseur et comme antagoniste de l'oviposition des insectes. En outre, une étude taxonomique a été realisée pour développer une carte de résistance des races de maïs. Cette carte a suggéré que l'ancien groupe des maïs indigènes était le plus résistant.

CONTENTS

FIGURES

TABLES

INTRODUCTION

1.1 The Grain Storage Problem

Losses of grain due to infestation by insects is an ever present problem all over the world, even in countries having well equipped storage facilities (CIMMYT, 1970-1971; Dobie, 1977; Hardman, 1978; MacGregor, 1980). However, this problem is especially severe for less developed countries (LDC) where post-harvest losses of grain to insects and pathogens remain one of the most serious of all agricultural problems. The magnitude of losses of stored grain to insects is sometimes catastrophic frequently representing up to 30 % of the total harvest per year. A successful program to reduce the losses during storage may be expected to increase the food for human consumption by 10 to 20 per cent in less developed countries (Ramirez, 1981).

Within the realm of management, improvement and use of germplasm of maize by international organizations such as the International Center for Improvement of Maize and Wheat (Centro Internacional de Mejoramiento de Maiz y Trigo, CIMMYT), there are many research programs for the enhancement of productivity, protein quality, insect and disease resistance, etc. It is the role of international organizations such as CIMMYT to improve maize germplasm for use in the less developed countries.

There are two main breeding approaches currently in use for the management and utilization of maize germplasm. The developed countries have focused their main research effort on investigating and developing hybrid maize. On the other hand, CIMMYT has taken the initiative for the less developed countries by turning its attention to the improvement of populations and the development of open pollinated varieties (synthetics). This seems to be the best approach for less developed countries, since small farmers cannot afford all the mechanical and chemical requirements of large scale farming needed for growing high yielding hybrid cultivars of corn, and because the lack of resistance to insects of many of these cultivars limits their advantages (Fortier *et al.*, 1982; Salamini *et al.*, 1970).

The priority stressed in almost all the programs of improvement of maize is the selection of resistance to pathogens and pests of vegetative tissues growth (Ullstrup, 1978; Vasal *et al.*, 1983). There is a large amount of information on many pests of corn: *Ostrinia nubilalis, Spodoptera frugiperda, Heliothis zea, Diatrea grandiosella,* and *Diabrotica* spp which commonly attack the maize plant in the field (Ullstrup, 1978). Resistance of the vegetative growth to insects can with some species be related to secondary compound content of leaves. For example, DIMBOA (2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one) concentration correlates with resistance to the European corn borer, *Ostrinia nubilalis*.

However, the study of grain resistance to storage insects has lagged behind research on resistance of the vegetative growth to herbivores. Many aspects of the maize-corn weevil (*Sitophilus zeamais* Motsch. Coleoptera:Curculionidae) interaction remain obscure, especially with respect to the physiology and behaviour of the insect in the grain as well as the study of the chemical mechanisms of defense which the grain may possess (Baker and Mabie, 1973; Baker, 1982; Brown and Chippendale, 1975; Dobie, 1974 and 1977; MacGregor, 1980; Sinha and Watters, 1985).

Given the lack of information on many aspects of the grain storage problem, the present project was initiated in collaboration with CIMMYT to investigate the germplasm resources of Mexico from the point of view of the mechanisms of resistance in grain to attack by the major storage pest, *Sitophilus zeamais*.

1.2 *Races of maize(Zea mays ssp. mays)*

Maize is the fundamental element of culture for all the ancient civilizations of America. Twenty five hundred years ago corn constituted a primary source of food in Central and South America. Among others, the Nahuatls and Mayas-Quiches of Central America and the Incas-Quechuas of South America shared a common culture and agroeconomy which was based on maize growth and improvement. It can be said that the development of these sophisticated ancient cultures was made possible by the discovery, domestication and improvement of maize (Bird, 1980; Mangeldorf, 1959; Mangeldorf, 1974; Wellhausen *et al.*, 1952; Wilkes, 1977).

In addition to the world wide importance of corn as a food plant and as a principal source of food to millions of people, the study of maize has been a fascinating area of research covering almost every field of biology (Bird, 1980; Hernandez, 1973; Harrison *et al.*, 1977; Iltis, 1983; Kato, 1976; Mangeldorf, 1974; Marx, 1983; Strommer *et al.*, 1982).

The selection of experimental maize germplasm to study maize can be a difficult task since its genetic composition is intimately linked to related species like *Tripsacum* spp. and Teosinte (*Zea mexicana*) (Bird, 1980; Mastenbroek *et al.*, 1981; Smith *et al.*, 1982; Smith *et al.*, 1984). Thus, an understanding of the systematics of *Zea* would help to improve the testing, the reliability of techniques and the selection of the great diversity found in this species (Bird, 1980). The classification of maize has been quite informal to date (Bird and Goodman, 1977; Goodman and Bird, 1977), and an incomplete hierarchy based on land races has been used. There have been some attempts to formalize a system of classification and for most races of maize of Latin America this goal has been accomplished (Hernandez, 1973). However, the known array of ca. 300 races cannot yet be analyzed as a body since there is a critical lack of information for many of the races of Africa, Asia and Europe (Bird and Goodman, 1977).

Three types of approaches to the systematics of maize have, so far, been used. The analysis of ear morphological characteristics has become the classic approach for grouping races of maize (Bird and Goodman, 1977; Goodman and Bird, 1977; Smith *et al.*, 1981). The disadvantage of these characteristics is that they are highly influenced by environmental factors. The other two approaches, which seem quite promising, are:

1. The study of patterns of position, sizes and frequencies of chromosome knobs (Kato, 1976; Kato and Blumenschein, 1967; McClintock *et al.*, 1981; Smith and Goodman, 1981), and
2. The use of biochemical analysis of coleoptile isoenzymes, cytoplasmic DNA, and seed proteins (Doebley, *et al.*, 1985; Smith and Lester, 1980)

High yielding maize often does not have any advantage over poor yielding maize when resistance is brought into consideration (Betanzos, 1980a and 1980b; Dobie, 1977; Fortier *et al.*, 1982). Since traditionally used varieties of maize have been replaced by improved ones, in which resistance has been compromised for better yield and protein quality, an increase in the severity of storage problems has been observed (Fortier *et al.*, 1982).

Therefore, the importance of studying exotic indigenous maize is obvious since, on the one hand, these are the basis of maize germplasm from which all selections must be made and on the other hand the identification of resistant lines in the maize gene pool will permit an understanding of the mechanisms of such resistance and focus attention on stable heritable traits for breeding programs.

1.2.1 The infesting insect (Sitophilus zeamais (Motsch):Calandra oryzae–).

About 30 species of weevils (Curculionidae) have been recorded in stored products in various parts of the world. They are distinguishable by having the head in front of the eyes to form a well-defined snout, the antennae elbowed and clubbed and all tarsi four-segmented.

The larvae have no legs, and are stout and slightly curved; they are white with a pale brown or yellowish head; they live entirely within the kernel, and produce white powdery excreta which, in large quantities, makes the grain unpalatable for human consumption (Freeman, 1980).

Sitophilus zeamais (the corn weevil) has a length of 2.3 to 4.5 mm, short elytra and 8-segmented antennae. The elytra usually show four reddish spots and hindwings are always present (Figure 1).

The adults of the corn weevil have a long life span, living a average of about 30 weeks for males and about 25 weeks for females under optimum conditions (27 °C and 70 % R.H.). The female excavates a hole in the grain where she lays the eggs. The presence of eggs can be detected by the plug that is placed to block the hole containing the eggs. Eggs are quite resistant to chemically-induced mortality, the duration of this stage being about 6 days. It is known that eggs may be laid anywhere in the grain but it has been noted that few are laid in the embryo and, in the case of wheat, the majority are placed at the end farthest from the embryo (Richards, 1947 in Longstaff, 1981).

Another characteristic of the egg-laying process is that females tend to avoid laying more than one egg when the population is dense. Cannibalism is known in this species. The rate of growth is influenced by temperature, moisture content of the grain, oxygen concentration and population density. The stage most susceptible to mortality induced by environmental changes is the newly hatched larva (Hardman, 1978; Longstaff, 1981).

Figure 1. Adult stage of corn weevil

Nothing has been reported with respect to the effect of secondary plant substances on the growth of weevils, neither at the individual level nor at the population level, although some indirect evidence in this matter can be found in Dobie (1974), Fortier *et al.*, (1982), Ivbijaro (1983) and Russell (1962).

Ninety percent of the total mortality occurs during the first instar but if conditions are favorable for the survival of this stage then the survival of subsequent stages is virtually 100 %. The total larval stage lasts for about 20 days with the fourth instar being the one of longest duration. Pupae have a duration of approximately 6.5 days and the pre-adult stage lasts for about 5 days.

Although the biology of *S. zeamais* is well documented (Howe, 1952; Longstaff, 1981; Segrove, 1951), knowledge of the physiology and metabolism of the immature stages is somewhat lacking (Baker, 1982; Brown and Chippendale, 1975; Yetter *et al.*, 1979).

To be able to achieve an understanding of the dynamics of the grain-insect relationship more information is needed regarding the physiological and biochemical aspects of this interaction.

1.3 Resistance in Maize to Weevils

S. zeamais causes damage to stored maize grain, through its vital activities both by the adults and by the development of the larval stages within the kernel (Betanzos, 1980b; Longstaff, 1981). The damage to the grain is of such magnitude that *S. zeamais* has been considered as a major primary pest of stored grain (Hill, 1983). It is well known that *S. zeamais* is able to infest the grain even in the field (Brauer and Ramirez, 1960; Dobie, 1977; Fortier *et al.*, 1982; Ramirez, 1981) and a number of studies have been made in this area (Eden, 1952; Hill, 1983; Quintana *et al.*, 1960; Ramirez, 1960; Ramirez, 1980; Wilbur *et al.*, 1961).

Several methods have been described for evaluating maize weevil resistance in stored grain. The relative number of weevils in free-choice experiments (McCain *et al.*, 1964) oviposition and adult emergence rates (Russell and Rink, 1965) and no-choice and free-choice tests (VanderSchaaf *et al.*, 1969), are some of the methods currently and most widely used. An appraisal of the above mentioned methods has been made elsewhere (Betanzos, 1980a; Schoonhoven *et al.*, 1976; Widstrom *et al.*, 1972; Widstrom *et al.*, 1978).

Some observations have been made in relation to the effect of the maize grain characteristics on the preference of weevils and the susceptibility of the grain (Howe, 1952; Ibvijaro, 1981; Russell and Rink, 1965; Segrove, 1951; Schoonhoven *et al.*, 1976). Among these characteristics, hardness of the grain has received the most attention in attempts to explain the different susceptibilities to insect infestation in the different types of maize material. Certainly the hardness of the kernel is the characteristic which has most often been cited as a factor of resistance (Betanzos, 1980b; Dobie, 1977; Eden, 1952; Gomez *et al.*, 1983b; Russell, 1962; Schoonhoven *et al.*, 1976; Singh and McCain, 1963; VanDer-Schaaf *et al.*, 1969) but in many cases the results obtained have shown that some grain types have certain degrees of susceptibility even when they are hard (Dobie, 1977; Fortier *et al.*, 1982; Ivbijaro, 1981; Russell, 1962). The hypothesis that the chemical constituents of the maize kernel may be components of susceptibility of the grain has been primarily studied by determining the major nutritional characteristics of the kernel (Betanzos, 1980; Gomez *et al.*, 1983a; Singh and McCain, 1963). Indeed, Gomez *et al.* (1983a) found that the efficiency of food utilization was increased with high protein levels in populations of *S. oryzae* growing on selected corn genotypes. Schoonhoven *et al.* (1976), have proposed that the main factor driving the susceptibility of corn grain may be the presence of an assumed feeding stimulant in grain. Furthermore, Dobie (1977) has suggested the existence of an oviposition stimulant which would explain the preference of females to lay in certain types of grain.

1.4 *Plan of Study*

The resistance of the maize grain to infestation by *Sitophilus zeamais* has been described as a process where the interaction between insect and grain is driven by the characteristics of the grain as food and habitat and which correspondingly determine two basic behavioural responses of the insect, namely, feeding and oviposition.

The feeding behaviour of the insect on maize grain has been analyzed in the past, primarily as a response to the nutritional content of the seed, undoubtely the emphasis has been put on how attractive the grain is to the insect. Several authors (Dobie, 1977; Gomez *et al.*, 1983a; Schoonhoven, *et al.*, 1976; Singh and McCain, 1963), have suggested that a feeding or oviposition stimulant may be present in the seed which would explain the preference of the insect for certain maize materials or the suitability of the latter for the success of the former. Instead of addressing the question of how attractive the grain is to the weevil, in the present thesis the question of how unsuitable the grain is to the insect was addressed. In particular the possibility was investigated as to whether or not there may be chemical deterrent factors in the seed which could be involved in its mechanism of resistance. In order to answer that question the present study has examined the effect of phenolic acid compounds present in the maize grain to feeding activity of *Sitophilus zeamais*. Also, an attempt has been made to describe the relationship of these chemical factors to other grain characteristics such as hardness which may be involved in the mechanism of the interaction of the grain-insect system. Another aspect of the study was the investigation of rare germplasm. One reason for using this material was the previous discovery of exceptional resistance in land races from Belize (Fortier *et al.*, 1982). It was also felt that a study of rare germplasm resources would provide the variability which could assist in identifying resistance factors. Finally, the goal of establishing a taxonomic resistance map so that breeders might choose future lines more selectively was a part of the present work.

MATERIALS AND METHODS

2.1 *Maize material*

Seven indigenous races of maize (a total of 10 collections) were used in this study and were supplied from the Germplasm Bank at CIMMYT by Drs. J. Mihm and D. Jewell of the maize program. Geneology of the material is referred to in Table 1.

All races listed in Table 1 were grown at high altitude (El Batan, Mexico o.s.l. 2600 m); however, low altitude is also indicated for Maiz Dulce since this is where it is usually grown. They represent all 4 groups of indigenous races of maize found in Mexico.

Since one of the main purposes of this investigation was to initiate the establishment of a resistance map of the races of maize it seemed appropriate to start by analyzing the existing literature related to the classification of maize. The races of maize listed in Table 3 were originally established by Wellhausen *et al.* (1952) in their classic study of the origin and taxonomy of the races of maize in Mexico. Wellhausen *et al.* (1952) analyzed several key characters from the maize germplasm populations found in Mexico and gave a thorough description of the many indigenous maize populations and their geneological relationships. Data from that study including a set of several morphological characters related to the corn ear were re-analyzed using modern numerical techniques unavailable to Wellhausen *et al.* (1952). The aim of this analysis was to establish a modern taxonomic frame of reference for future results since the populations used by Wellhausen *et al.* (1952) were from the same pools as the ones used in the present study.

Table 1: Geneology of collections of Mexican maize

Races and collections of Mexican maize used in the present study. All races were grown in the experimental fields of CIMMYT at El Batan, Mexico.

Races (a)		Collections (b)	Altitude (m)
Palomero	(1)	Mexico 5(2233)	2652
		Mexico 55(13735)	2600
Arrocillo	(1)	Puebla 463(7672)	2200
		Puebla 537(7746)	2200
Oloton	(2)	Chiapas 218(1784)	2000
Cacahuacintle	(2)	Mexico 212(1371)	2657
Maiz dulce	(2)	Guanajuato 93A(543)	700-1800
Conico	(3)	Mexico 182(1414)	2800
		Mexico 461(8458)	2600
Chalqueno	(4)	Mexico 208(2235)	1832

a. The number in parentheses is the Wellhausen group to which the race belong (see Table 3).
b. The name indicates the state of Mexico in which the collection was made. Numbers in parentheses are CIMMYT accession numbers of the collections.

The characters used to carry out analysis of principal components (PCA) are described in Table 2. A total of 13 quantitative characters was analyzed and the scores were the mean values of those variables. There were 25 races of Mexican maize which gave a total of the corresponding operational taxonomic units (OTU) (Table 3) that were analyzed by clustering techniques.

Cluster analysis of all 25 races in Table 3 was carried out using the statistical analysis system available at the University of Ottawa's AMDAHL computer. Clustering was performed using a standard agglomerative hierarchical algorithm known as average linkage on squared Euclidean distances (Procedures CLUSTER and TREE of SAS package), PCA was also conducted since there were no missing data.

Ordinations were also carried out using the external and internal characteristics of the corn ear as described in Table 2. The races of maize selected for this study were represented by 10 populations which belong to the main 4 groups of indigenous races of maize found in Mexico.

Maize grain collected in Belize, C.A. as described by Fortier *et al.* 1982 was also used. Three of these populations were designated as Belize local white, Belize local yellow and Belize local black. The improved hybrid variety, Pioneer 230, grown in Belize was also included. Ordination and cluster analysis of 16 taxonomic characters, derived from measurements on the local populations indicated that all had affinities to the ancient indigenous group of maize races (Fortier *et al.*, 1982). A very close relationship between Belize local yellow and the land race Nal-Tel was evident. Belize local black was linked to land race Negro de Tierra Caliente and Belize local white linked with land race Dzit-Bacal. The hybrid had greatest affinity with upland races of the exotic group of races of maize.

Table 2: Morphological characters used in Multivariate analysis

Quantitative external and internal characters of the maize ears and kernels in Mexican races. The abbreviations used are for reference to the principal component analysis given in appendix 1.

EARDIAM	Internal diameter of the corn ear
COBDIAM	Internal diameter of the cob
RACDIAM	Internal diameter of the rachis
RACKER	Rachilla/Kernel index
EAREXTL	External length of the corn ear
EAREXTD	External diameter of the corn ear
ROWNO	Number of rows
SHADIAM	Shank diameter
KERWID	Width of corn kernel
KERSTRIA	Striations of the corn kernel
KERTHICK	Thickness of the kernel

Table 3: Description of the Mexican land races of maize

Mexican maize races and their abbreviations. The numbers indicate the group of maize to which the Mexican maize belong according to Wellhausen *et al.* (1952).

1. Ancient Indigenous Group
2. Pre-Columbian Exotic Group
3. Prehistoric Mestizos Group
4. Modern Incipient Group

1.	Palomero Toluqueno*	PALT	1
2.	Arrocillo Amarillo*	ARAM	1
3.	Chapalote	CHAP	1
4.	Nal-Tel	NALT	1
5.	Cacahuacintle*	CATL	2
6.	Harinoso de Ocho	HARO	2
7.	Oloton*	OLOT	2
8.	Maiz dulce*	MADU	2
9.	Conico*	CONI	3
10.	Reventador	REVE	3
11.	Tabloncillo	TABL	3
12.	Tehua	THUA	3
13.	Tepecintle	TETL	3
14.	Comiteco	COMI	3
15.	Jala	JALA	3
16.	Zapalote chico	ZAPC	3
17.	Zapalote grande	ZAPG	3
18.	Pepitilla	PEPT	3
19.	Olotillo	OLTI	3
20.	Tuxpeno	TUXP	3
21.	Vandeno	VAND	3
22.	Chalqueno*	CHAL	4
23.	Celaya	CLYA	4
24.	Conico Norteno	CONN	4
25.	Bolita	BOLI	4

2.2 *Insect Culture*

The weevil culture has been maintained in Dr. J. T. Arnason's laboratory at the University of Ottawa since 1982 from an original population of corn weevil obtained from Dr. J. Hollebone (Agriculture Canada, Ottawa, Ontario). Initially the weevils were maintained on a whole wheat diet (1 g/insect) (Fortier *et al.*, 1982), but later on they were changed to a corn diet. Corn weevils (*S. zeamais* Motsch.) were grown in screened 1 l Mason jars with locally obtained sweet corn (Ritchie Feed and Seed Limited, Ottawa, Ontario).

Environmental conditions of the growth chamber were set to simulate humid tropical conditions and also to achieve optimum development of the insect. Jars were placed in the chamber at 27 +/- 2 °C and 70 % relative humidity. Photoperiod was L/D : 16/8.

In order to have a sequential production of adult weevils the age of which were known, the following procedure was used. Unsexed adult weevils were placed in Mason jars halffilled with local maize grain. The adults were left for 1 week and then sieved and transferred to another jar with cleaned corn. The jar containing the maize grain recently infested was then labeled and periodic inspections for emergence of new adults were undertaken. When emergence was noted, another label was written to define the age of the cohort at the particular jar.

2.3 *Extraction Procedure*

2.3.1 Hydrolyzed extracts

The extraction procedure was performed following that described by Krieger *et al.* (1982) with some modifications. Five grams of each variety were ground. One hundred ml of NaOH solution (2 N) was added to the samples which were left for alkaline hydrolysis overnight. HCl 10 N was used to acidify the hydrolyzed extracts to pH 2. Acidified samples were centrifuged at 10,000 g for 15 minutes and the supernatant was retained. The supernatant was extracted with 60 ml of hexanes to remove fat then extracted twice with 25 ml of ether : ethyl-acetate (1 : 1). Sodium sulfate (anhydrous) was added to the pooled ether phase.

The ether phase obtained was dried in rotavapor (30 °C) to complete dryness and dissolved in 1 ml of ethanol (95 %). The concentrated extract was centrifuged at 5,000 g during 10 minutes and the supernatant retained for analysis and feeding studies.

2.3.2 Unhydrolyzed extracts

Acetone extractions for total soluble phenolic glycosides were performed as follows: 10 g of corn seeds were placed in liquid nitrogen, ground in a ball mill and put into 35 ml acetone. After centrifugation (15 min 1000x g) the supernatant was reduced to dryness *in vacuo*, dissolved in 10 ml of 95 % ethanol, then microfuged and the supernatant retained.

2.4 Chemical Analysis

Before analysis the extracts were filtered through a 5 μ nylon millipore filter and then analyzed by using a Beckman HPLC with a reverse phase 5 micron ODS column and isocratic elution with 1 : 1 methanol : 2 % HOAc in water (HPLC grade). A dilution series of ferulic acid was used to make the calibration curve.

Gas chromatography and mass spectroscopy (GC-MS) was performed on TMS- derivatized extracts using a VG7070-E spectrometer with a 3 % OV-1 column programmed at 150 °C and 10 °C/min .

Since both HPLC and GC - MS confirmed the presence of isomers of ferulic acid it was decided to evaluate the area of the curve from the HPLC chromatogram and not only the peak height as is customarily performed for quantification of sustances by means of HPLC. In the present study the isomers of ferulic acid were not separated by the elution program used in the HPLC. To achieve the quantification of the total ferulic acid concentration a dot area in mm2 procedure was used (Batschelet, 1975).

2.5 *Preparation of diets and antifeedant assay*

In this assay only maize grain from Belize (Fortier *et al.*, 1981), was used. Two populations, Local Yellow and Local White had exceptional resistance to corn weevil and one population, Local Black, was moderately susceptible. The improved double-cross hybrid variety, Pioneer 230 was highly susceptible to weevils.

A diet based on wheat flour was prepared as follows : one gram white wheat flour, 1 ml of extract from corn in ethanol (the equivalent of 1 g corn grain) and 5 mg sorbic acid were placed in a 5 g mortar. The ethanol was evaporated and 0.5 ml water added to make a paste. The paste was rolled to a sheet 2 mm thick and seven 1 cm discs were cut. Control diets were made with addition of only ethanol. Diets treated with pure substances were made in a similar manner from stock solutions of the compounds in ethanol. For the feeding assay, four weighed discs of each diet type were placed in a 9 cm petri dish with 5 unsexed corn weevil adults and kept at 27°C, 70 % R. H. and L/D:16/8 in an incubator. The discs were later weighed at 2 day intervals to day 10. Three discs were kept under similar conditions to monitor weight changes not associated with insect feeding. Calculations of consumption were made from weight changes corrected for non-feeding changes. Wheat flour was chosen as the artificial diet because of its exceptionally low phenolic content as compared to corn (Krygier *et al.*, 1982).

2.6 *Consumption Tests*

One hundred grams of corn grain were divided in 5 replicates of 20 g each. The number of grain present in each replicate was counted and 100 unsexed adult weevils, 3 weeks old, were placed on each replicate. After 2 weeks, weight loss of grain, weight of frass produced (removed with a brush), number of live insects, number of dead insects and percent mortality were determined.

To follow the temporal profile of consumption within the grain-insect system, 5 replicates were divided into two subsets to measure oviposition and a parameter known as parameter of stability. Although analysis of variance of consumption data from this study and that of other authors and its test of equality of means has allowed classification of seed materials according to their level of resistance, at least two inconveniences have been reported (Betanzos, 1980b) :

1. ANOVA of consumption data uses only the information of one inspection, which means a great amount of lost information.
2. Frequently the test of equality of means forms too many groups or only two which does not allow for a precise distinction among resistant, intermediate and susceptible varieties.

Due to these problems a technique of analysis known as stability parameters estimation (Betanzos, 1980b) was applied. The original technique (Eberhart and Russell, 1966) was applied to the results by dividing the loss of weight of grain by the number of days elapsed since the beginnig of the test. The mean over all varieties and repetitions per inspection of weight loss of grain was calculated. Then a linear regression of weight loss of grain per day of each variety of maize against mean weight loss of grain over all varieties per day was evaluated. By applying this procedure, then each variety has a mean, a regression coefficient (beta) and a sum of squared deviations which then are all analyzed.

2.7 *Estimated Growth Curves*

Another type of analysis was carried out in order to estimate two parameters related to growth of organisms, namely, maximum weight reached at the time of emergence and the rate of growth as weight gain on a daily basis.

An active consumption period was calculated from the values of the median development time of the insect. Since egg and pupa do not contribute to the consumption of grain, these two stages were eliminated from the analysis by substracting the period of time they last in the whole life cycle. Then it was considered that the pre-adult stage contributed but not to a great extent to the final weight at emergence, and that in any case the inspections of grain were constant for all the races of maize, thus, the experimental error would be loaded in the same form for all populations of weevils.

After calculating the active period of consumption of weevils, the rate of growth was estimated by dividing the final weight of insect at emergence by the number of days of actual consumption. Having these two parameters a model of growth was used.

In this case a differential equation was derived as follows. Weight was considered as a function of time, therefore change in weight would be proportional to the weight at any time and, in the present situation, to a certain maximum which is represented by the weight at emergence, thus there is a limit which indicates a change in the form of increasing weight due to change in metabolism from larva to adult stage.

In mathematical form :

$$dy/dt = \lambda y(W - y) \qquad (1)$$

solving this equation the familiar logistic equation is obtained :

$$y = W/[1 + \exp(-\lambda Wt)k] \qquad (2)$$

where,

y weight of insect (mg)

W	weight reached at emergence
t	time in days
λ	rate of growth
k	a constant of integration.

Since k is unknown, an initial condition at $t = 0$ was set as follows :

$$y(0) = Wo = W/(1 + k) \tag{3}$$

therefore,

$$k = 1 - (W/Wo) \tag{4}$$

where,

Wo	Initial weight.

Substituting equation 4 into equation 2, the working equation is established as :

$$y = W/\{1 - [1-(W/Wo)] \exp(-\lambda Wt)\} \tag{5}$$

2.8 Indices of Nutrition

Three nutritional indices were calculated as follows (adapted from Walbauer, 1968):

$$AD = [(WCi - WF)/WCi](100) \tag{6}$$

$$ECD = [WI/(WCi - WF)](100) \tag{7}$$

$$ECI = [WI/WCi](100) \tag{8}$$

where,

AD	Approximate digestibility
ECD	Efficiency of conversion by digestion
ECI	Efficiency of conversion by ingestion
WCi	Weight consumed per insect
WF	Weight of estimated frass production
WI	Weight of insect at emergence

2.9 Choice Test

To test the preference of weevil populations under a more complex environment where all 10 populations under study were offered to the insects, a feeding arena was set as shown on Figure 2. Two hundred unsexed, 3 week old adults from the stock culture were offered samples of all maize populations contained in 50 ml vials. First, a position within the feeding arena was assigned at random by using a table of random numbers, to each one of the seed populations of maize in a clockwise direction, then the weevils were released at the center of the feeding arena.

Due to the great variance of grain size among the different races of maize, neither number nor weight of grain would have been constant for all types of maize, therefore the volume of the vial containing the seed sample was used as constant. Nevertheless, number of grains and weight were measured for all races of maize in every replicate.

After 10 days of exposure, the number of live adults was counted and all were removed. The samples were split in two subsamples in order to determine emergence and oviposition sites by the berberine staining method.

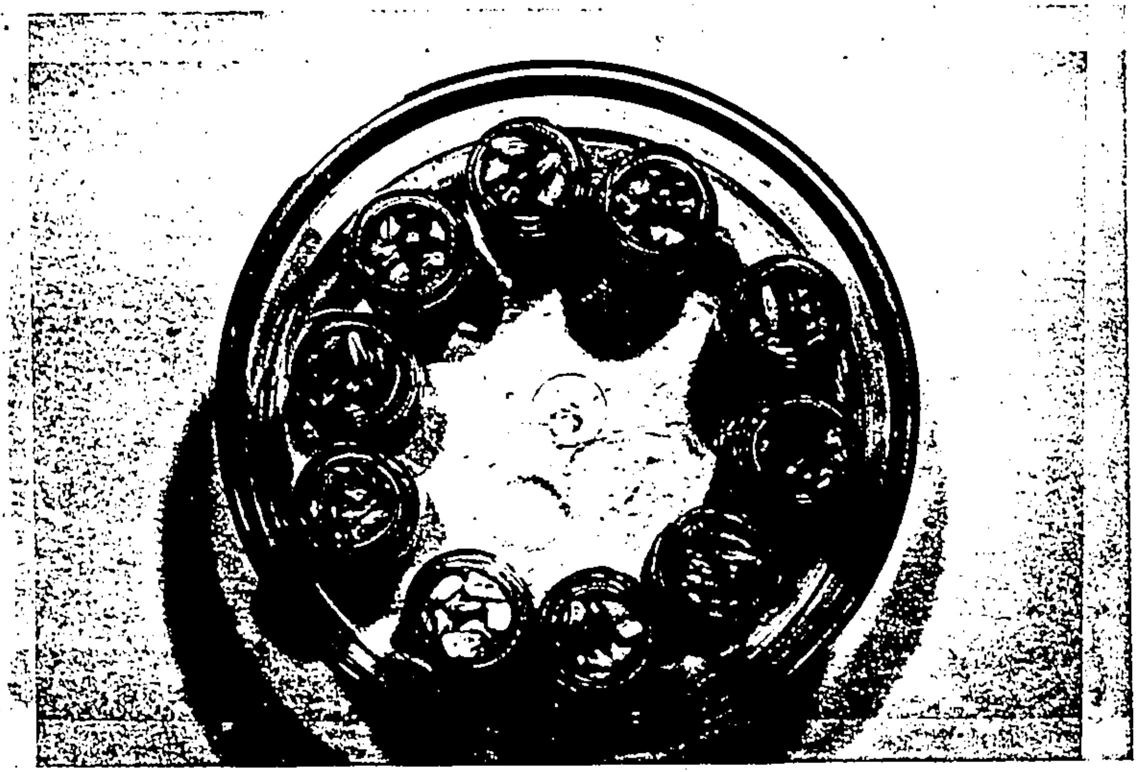


Figure 2: Feeding arena used in choice test.

2.10 *Emergence from Choice Tests*

New emergence was assessed up to the appearance of the next generation and statistics of median development and total emergence were calculated for the replicates. These estimations were used to obtain the index of susceptibility of Dobie (1977).

The Dobie index (I) relates progeny (N = total F1) and median development (D) in days using the following equation:

$$I = [(\ln N)/D]\ 100 \tag{9}$$

Although the method to obtain D and N is usually based on a no choice test (Dobie, 1977) no major difference was found when using either choice or no choice test for obtaining the estimates of D and N.

The plug left by the female weevil after laying the eggs was stained with a berberine solution that produces a bright yellow fluorescence under short wavelength ultraviolet (UV) light which is easily seen with the naked eye, although a stereoscope was used to make the observations more accurate, especially when dubious cases were present.

2.11 *Consumption and Insect Lyfe Cycle Characteristics*

To test the susceptibility of varieties of maize as related to performance through time of the adult cohort of weevils and the further performance through time of the progeny produced by these populations of weevils on the races of maize, a study was carried out where several indices of resistance of grain and some population characteristics of the insect were measured to account for a dynamic picture of the interaction of weevil on maize grain. This study entailed two sets of measurements: one, for the parental adult population and the other one for the progeny of new adults emerged.

The success of each population of weevils growing on the different types of maize was measured in terms of the number of emerging adults, weight of insects, consumption of grain by the parental population and efficiency of resource utilization expressed in production of progeny, capacity of adaptation to a new environment in terms of survival for the parental population.

One hundred grains of each maize population were placed in screened vials together with 50 unsexed 3 weeks old adult weevils. Every week the survivors were weighed, counted and transferred to 100 fresh grains of the same race of maize. The experiment was continued until there were enough survivors in each population of seeds which would allow comparisons among performances of the insect populations growing on each race of maize. Each of the exposed vials was kept in the growth chamber until total emergence of the new generation of adults. From this experiment, median development time in days, consumption of both the adult and immature stages of weevil, number of new emerged adults, mortality of adult stage and the Dobie's index were all calculated.

2.12 Hardness of Seed

Hardness of grain was measured by using a mill grinder (General Electric Co., Model 5X-B000B-D HP=1/4). The time elapsed until a known amount of whole grain was ground to dust was determined.

The relationship was :

$$H = t(sec)/w(g) \tag{10}$$

where,

H	Index of hardness
w	weight of grain in grams

t time in seconds

2.13 *Moisture Content*

The moisture content was measured by the method described by Fortier *et al.* (1982). Ten grains of each variety were accurately weighed to the nearest 0.1 mg in a prelabelled aluminum weighing dish using a Mettler AE-163 balance. The sample was dried for 12 hours in a preheated and calibrated oven (DESPATCH model). After the drying period, the sample was placed in a desiccator containing anhydrous calcium chloride until it reached room temperature, then it was reweighed. The moisture content was expressed as percentage of weight loss.

RESULTS

3.1 Races of Maize

3.1.1 Multivariate Analysis of Mexican Germplasm of Maize

A dendrogram (Figure 3) obtained by the TREE procedure of SAS package with a standard sorting technique along with the average linkage procedure shows the relationships among the various Mexican races of maize. A very close relationship between Palomero and Arrocillo (belonging to the Ancient Indigenous group of maize as defined by Wellhausen *et al.*, 1952) as well as Cacahuacintle and Oloton (Pre-columbian exotic group) is evident. Conico Populations of maize are quite isolated from the other races analyzed, although they cluster nicely within their group (Prehistoric mestizos).

The last two races analyzed in this study, Maiz dulce and Chalqueno do not fuse with any other member of their assumed groups. However, Chalqueno is immersed within its own group (Modern incipients) whereas for Maiz dulce (belonging to the Pre-columbian exotic group) seems to be lost in the Modern incipient group to which it does not belong as well as being closely related to Tuxpeno population of maize which is a member of the Prehistoric mestizos group.

Ordination by PCA reveals a good correspondence between the clusters obtained by the hierarchical procedure used in the dendrogram and the pattern observed by the PCA (Fig. 4). Projection onto the first two principal components shows a good separation between the Ancient group and the other 3 groups. The pre-columbian exotic group and the modern incipient group could be recognized in this projection although they seem to overlap. On the other hand, the Prehistoric mestizos group appears widely spread over the projection although forming two discernible sub-groups; namely group A = [TETL, ZAPG, VAND} and group B = [COMI, JALA, THUA}.

In order to identify the relationships among the races of maize represented by the populations of maize used in the present study, these representative races within the whole set of indigenous races were selected. By eliminating all races of maize but Palomero, Arrocillo, Cacahuacintle, Oloton, Maiz dulce, Conico and Chalqueno the same ordination and clustering techniques were applied to the ear characteristics taken from Wellhausen *et al.* (1952).

The dendrogram in Figure 5 shows the clusters obtained by this analysis. This time, only Palomero and Arrocillo grouped together separately from the others whereas for the other races Maiz dulce and Chalqueno fused and then linked sequentially to Conico and Oloton. Only the Ancient Indigenous group of maize represented by Palomero and Arrocillo clustered apart from the other groups.

The ordination performed on the same observations shows a great resemblance to the structure generated by means of the clustering technique (Figure 6).

A score based on the linear combination of the variables used to perform the PCA was calculated for the first principal component (eigenvalue = 8.6199 and proportion = 0.6631) in order to summarize the external morphological characteristics of the different races of maize.

Figure 3: Dendrogram of Mexican races of maize. Dendrogram showing relationships of Mexican races of maize. Races of maize analyzed in this study are marked with an *. Abbreviations are defined in Table 3.

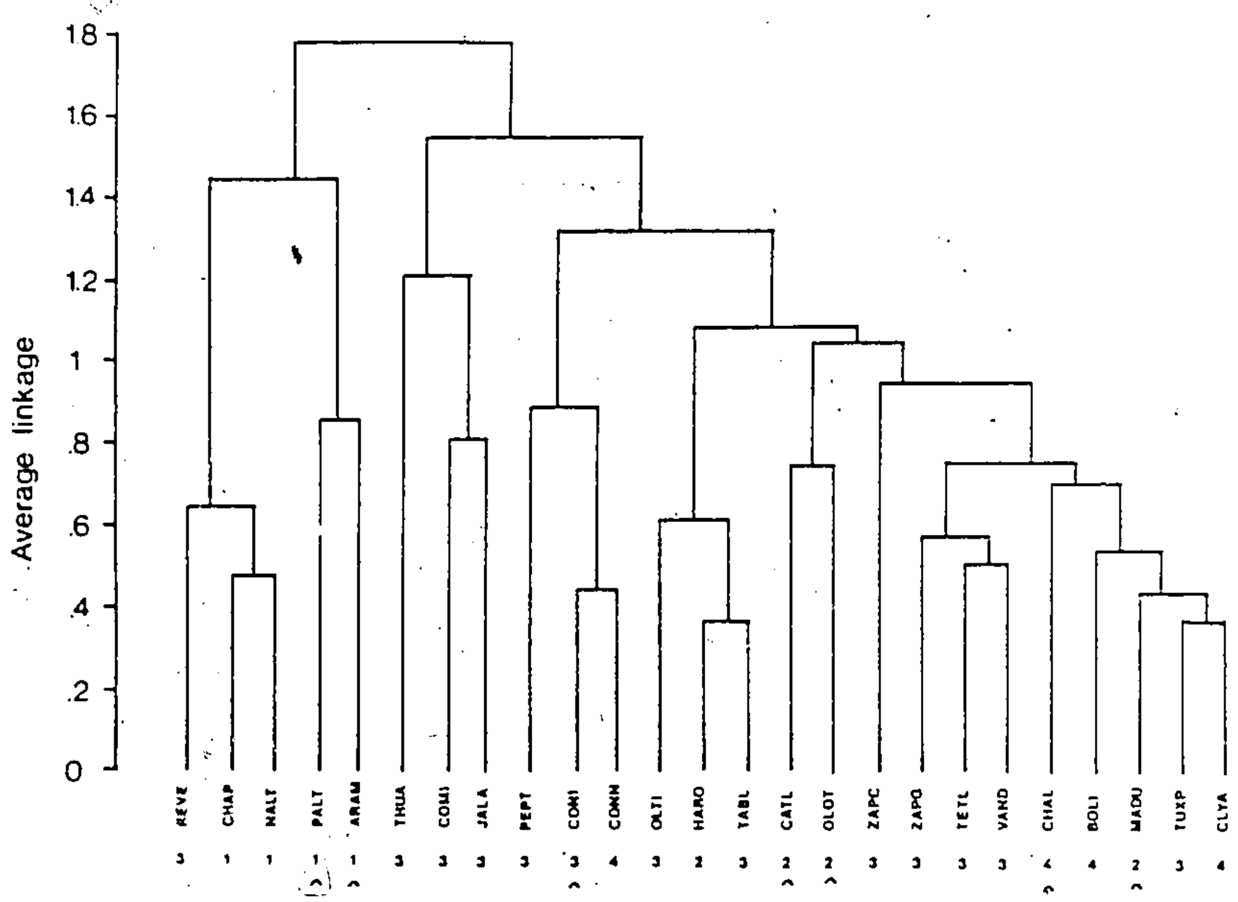
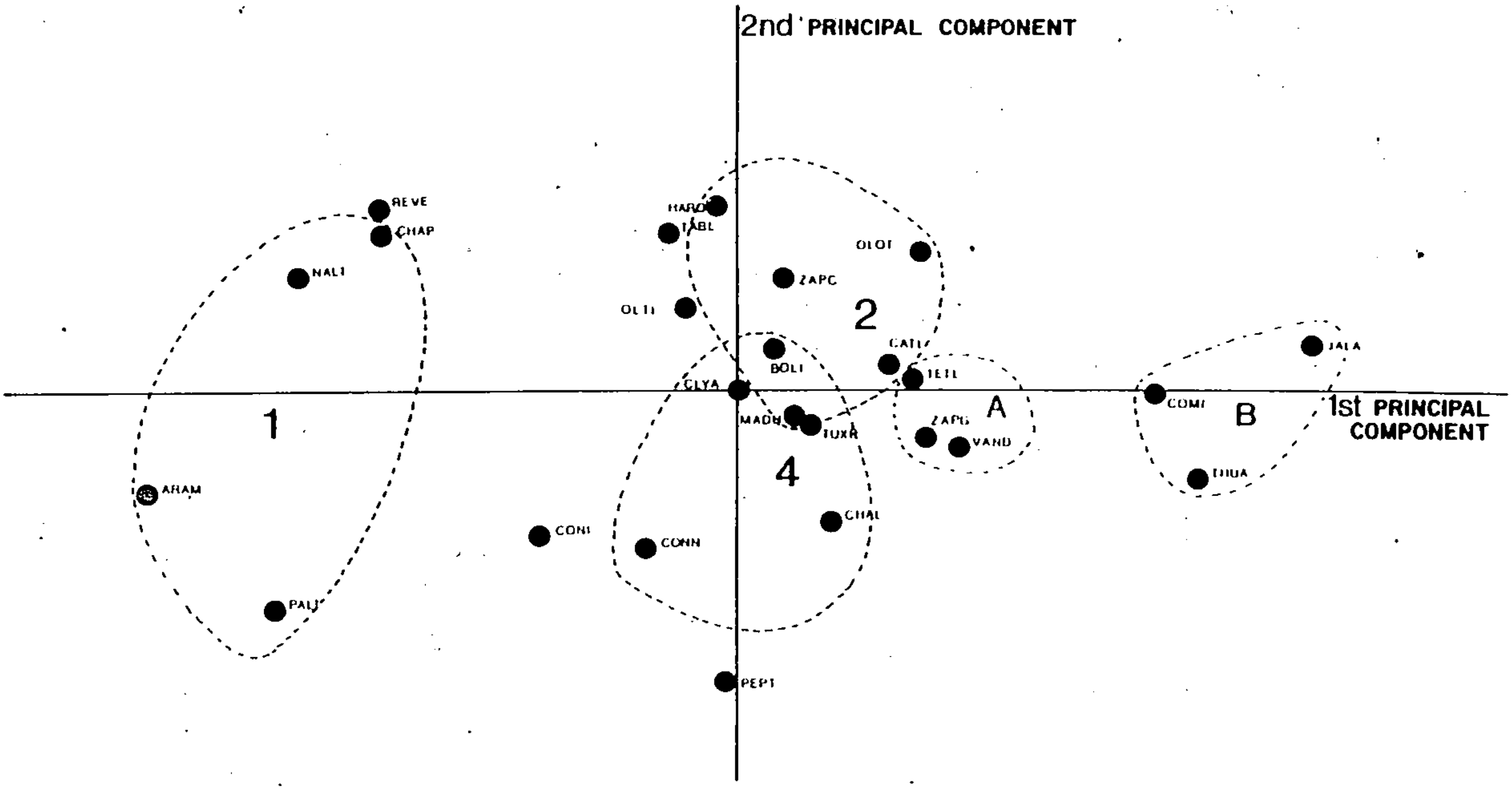


Figure 4: Ordination by principal components of Mexican races of maize. The first two principal components are plotted. Abbreviations are outlined in table 3. Numbers are the groups of maize defined in table 3. A and B are subsets of the Prehistoric Mestizos group.


2nd PRINCIPAL COMPONENT
1st PRINCIPAL COMPONENT
1
2
4
A
B
REVE
CHAP
NALI
ARAM
PALI
CONI
CONN
HARO
TABL
OLTI
ZAPC
OLOT
BOLI
CATI
TETL
CLYA
MADR
TUXR
CHAL
PEPT
ZAPG
VAND
COMI
JALA
CHUA

Dendrogram of selected Mexican races of maize.. Dendrogram showing relationships of selected Mexican races of maize analyzed in the present study. Abbreviations are as in Table 3.

1. PALT and ARAM belongs to Ancient Indigenous group
2. OLOT, CATL and MADU belong to Pre-Columbian Exotic group
3. CONI belongs to Prehistoric Mestizos group
4. CHAL belongs to Modern Incipient group

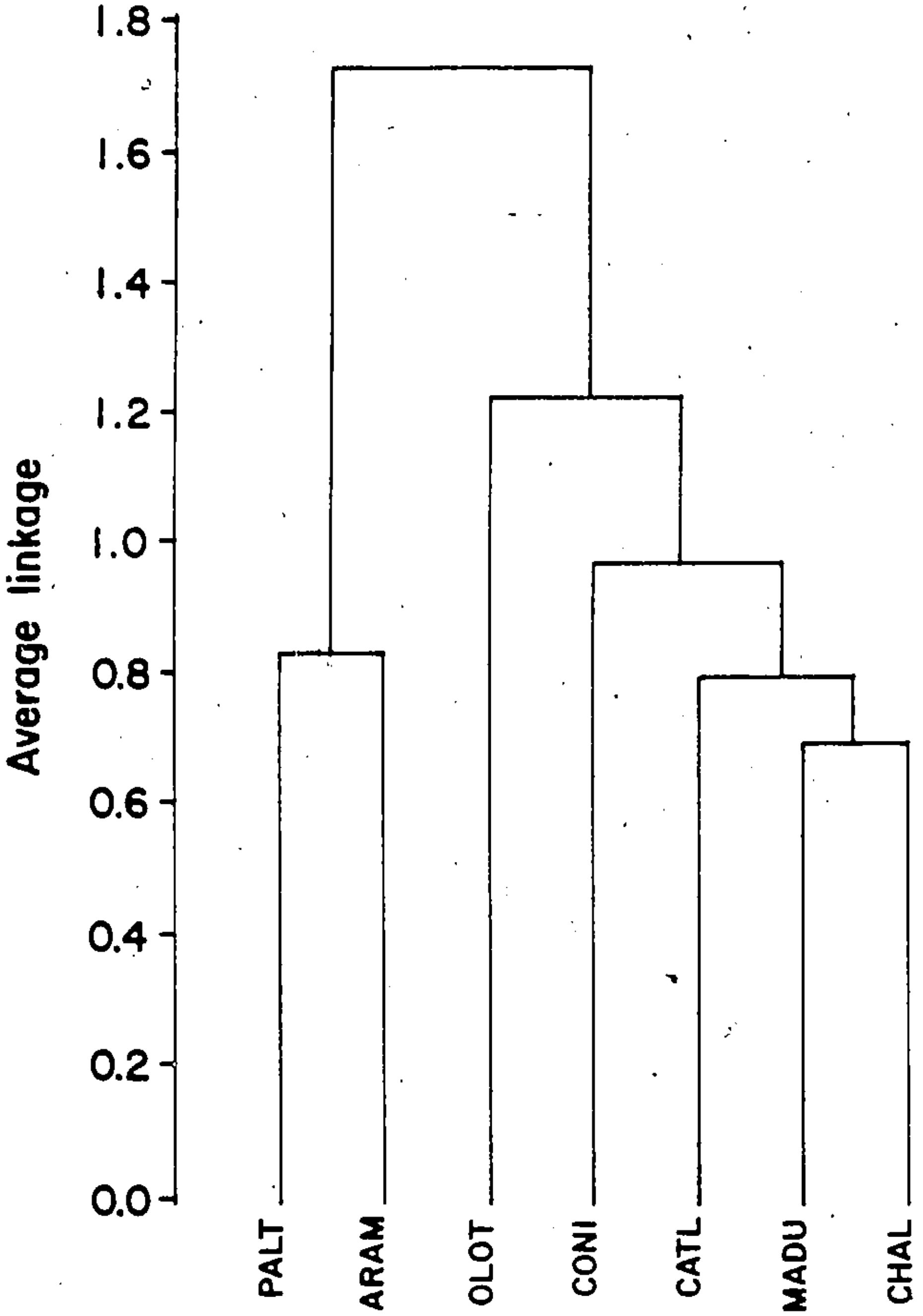
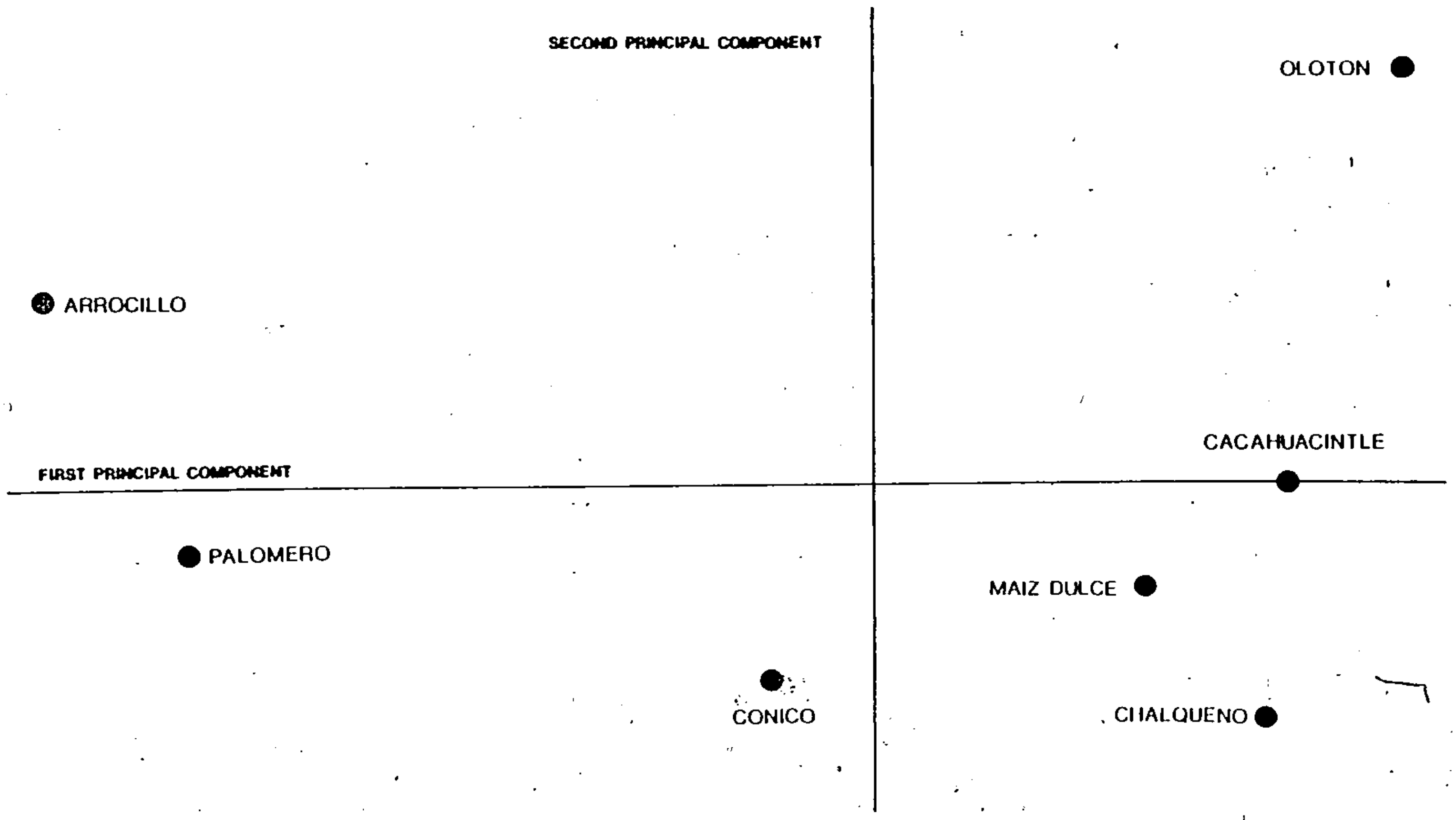


Figure 5: Dendrogram of selected Mexican races of maize.

Figure 6: Ordination by principal components of selected Mexican maize. Ordination by principal components analysis reflecting the major patterns of variation among 7 selected races of Mexican corn used in this study.

1. ARROCILLO = ARAM
2. PALOMERO = PALT
3. CONICO = CONI
4. MAIZ DULCE = MADU
5. CHALQUENO = CHAL
6. CACAHUACINTLE = CATL
7. OLOTON = OLOT


SECOND PRINCIPAL COMPONENT
FIRST PRINCIPAL COMPONENT
OLOTON
ARROCILLO
CACAHUACINTLE
PALOMERO
MAIZ DULCE
CONICO
CHALQUENO

To compare the affinities obtained by this analysis of morphological characteristics of the corn ear to the resistance characteristics of the collections of maize in the present study, the same type of clustering and ordination studies were carried out but using the interaction indices and the biological characteristics of the insect growing on the seed. The seed characteristics were not included in the analysis for further correlation analysis. The ordination and clustering performed by means of principal component analysis and average linkage procedure show that according to resistance factors an almost reverse image of affinities among collections of races is obtained (Figure 7 and 8). This time, the two collections of races in the Exotic Pre-Columbian Group fuse appart from the other collections of maize (Figure 7). These two collections of maize, are classified as very susceptible to insect attack. Then, according to morphological characteristics the two races of the Pre-Columbian Exotic Group are close to the Modern Incipient and Prehistoric Mestizos groups of maize (Figure 5) but according to the Resistance map the Ancient Indigenous group is linked to these later groups (Figure 7).

Figure 7: Dendrogram of selected collections of Mexican maize determined by resistance characteristics. Dendrogram for the collections used in the present study obtained by means of resistance characteristics (see Table 19).

1. P537 and P463 are Arrocillo Amarillo (ARAM) collections (group 1)
2. M182 and M461 are Conico (CONI) collections (group 3)
3. M208 is a Chalqueno (CHAL) collection (group 4)
4. M5 and M55 are Palomero Toluqueno (PALT) collections (group 1)
5. G93A is a Maiz Dulce (MADU) collection (group 2)
6. M212 belongs to Cacahuacintle (CATL) (group 2)
7. C218 belongs to Oloton race (OLOT) (group 2)

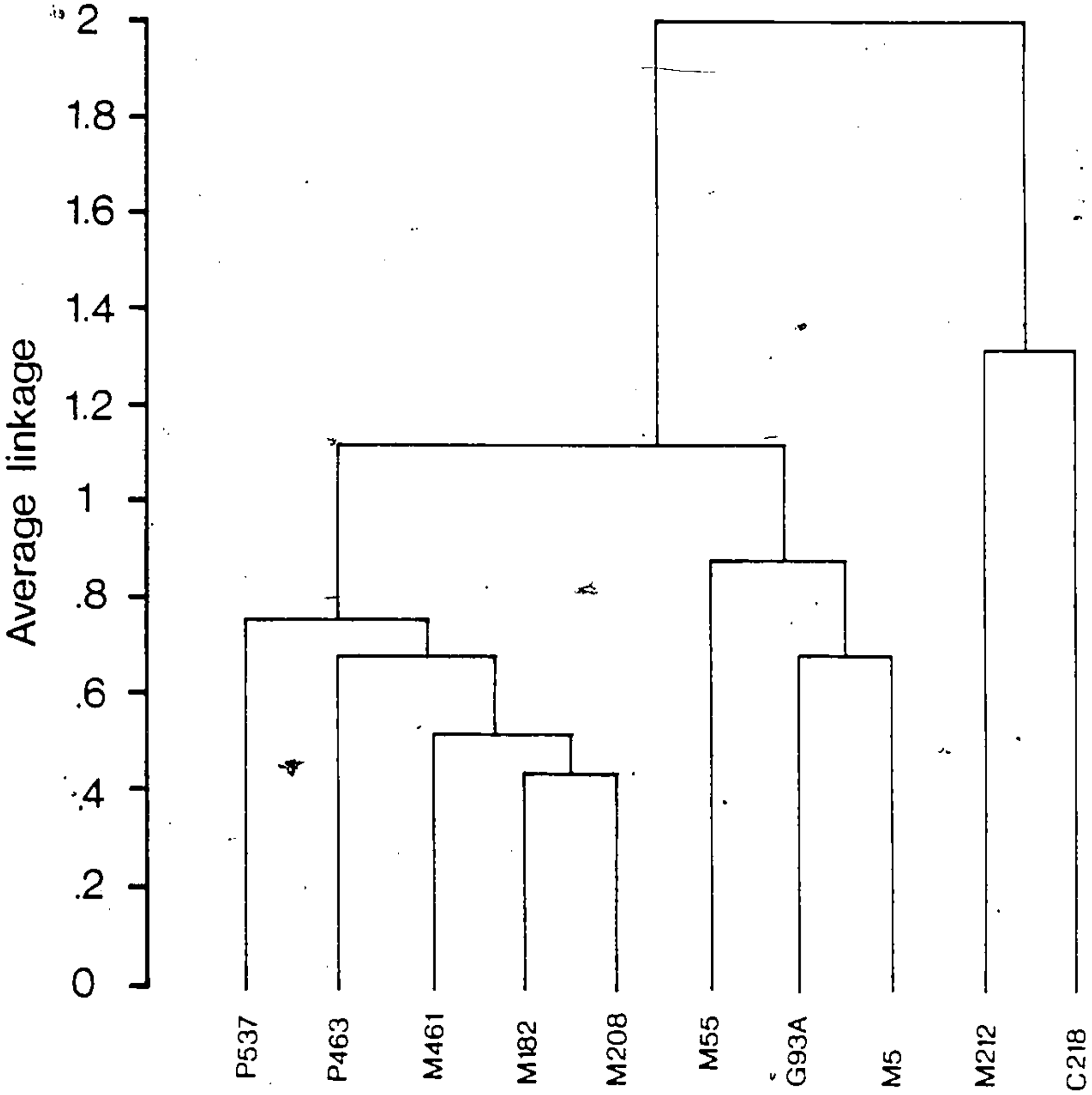
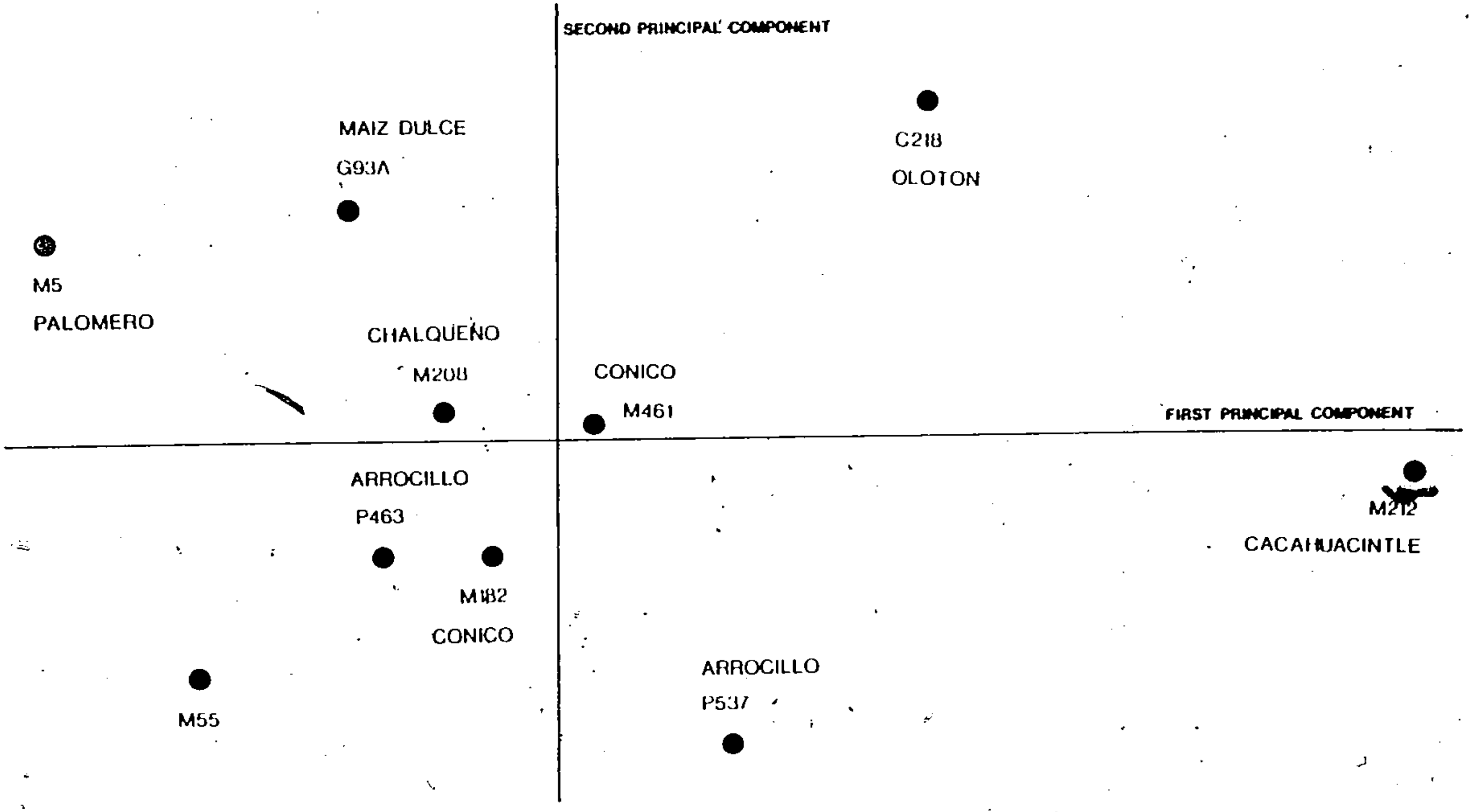

Average linkage
2
1.8
1.6
1.4
1.2
1
.8
.6
.4
.2
0
P537
P463
M461
M182
M208
M55
G93A
M5
M212
C218

Figure 8: Ordination of selected collections of Mexican maize. Ordination of the collections used in the present study obtained by means of resistance characteristics (see Table 19). See description in Figure 7.

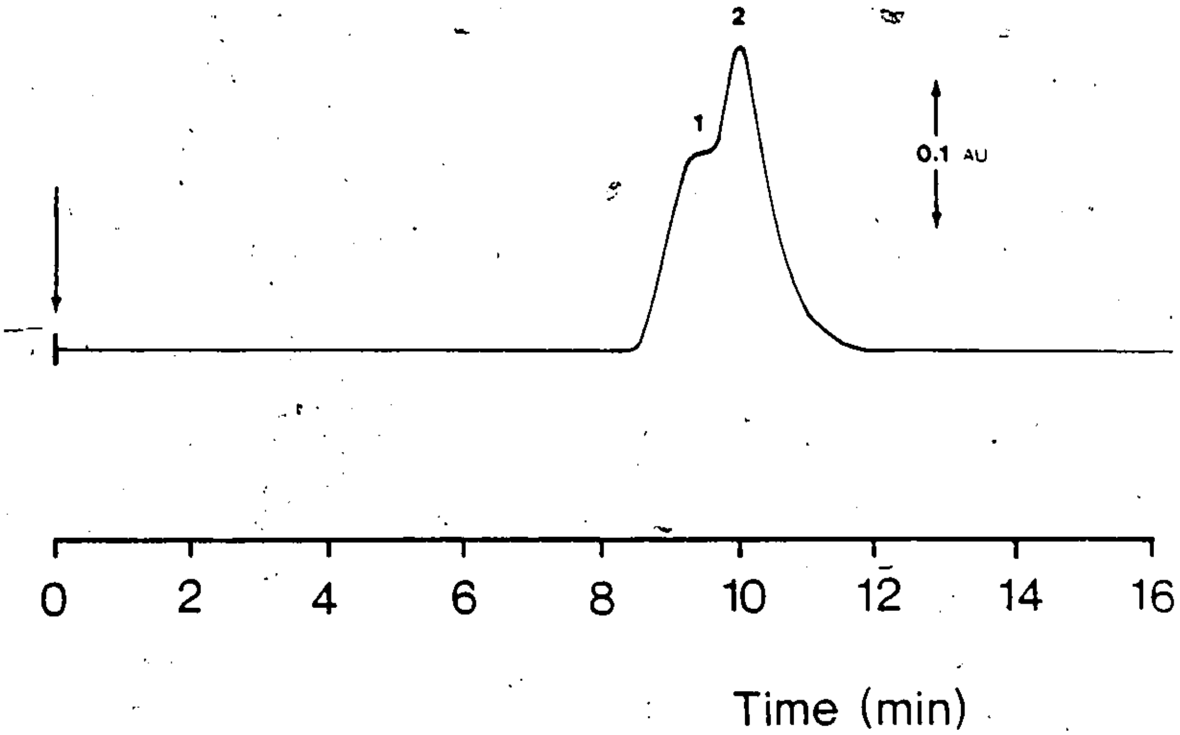

SECOND PRINCIPAL COMPONENT
FIRST PRINCIPAL COMPONENT
MAIZ DULCE
G93A
C218
OLOTON
M5
PALOMERO
CHALQUEÑO
M208
CONICO
M461
M212
CACAHUACINTLE
ARROCILLO
P463
M182
CONICO
M55
ARROCILLO
P537

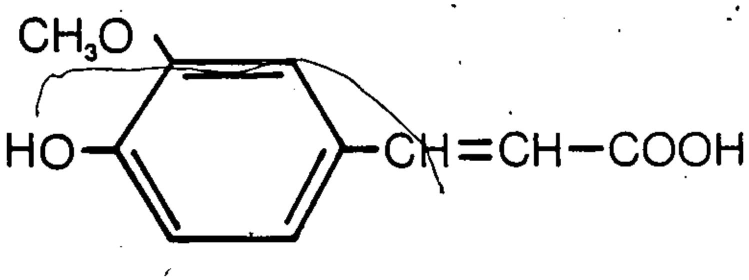
3.2 Chemical Analysis of Phenolics

The total phenolic extracts from 13 collections and 1 hybrid of maize listed in Table 4 were analyzed by using reverse phase liquid chromatography (HPLC) and combined Gas Chromatography and Mass Spectroscopy (GC - MS) for quantitative and qualitative analysis of the phenolic compounds present in the grain's crude extracts.

3.2.1 Qualitative Analysis

The HPLC chromatograms showing compounds absorbing at 254 nm in extracts of grain are depicted in Figure 9. Only one main peak was detected which displayed characteristic UV spectra of phenolics. This peak has comparable retention time on the chromatograms and UV spectrum to that of ferulic acid used as reference substance (Figure 9). Analysis of the mass spectra (Figure 10) and comparisons with the reference compound confirmed the presence of ferulic acid (Figure 9), with p-coumaric and other phenolic acids in much smaller proportions.





FERULIC ACID

Figure 9: Ferulic acid standard. Chromatogram of ferulic acid. (1) Z-form (2) E-form.

GC-MS analysis. GC chromatogram and normalized spectra of TMS derivatized total phenolic extracts from Local Yellow maize. (1) Z form of ferulic acid (2) p-coumaric (3) E form of ferulic acid.

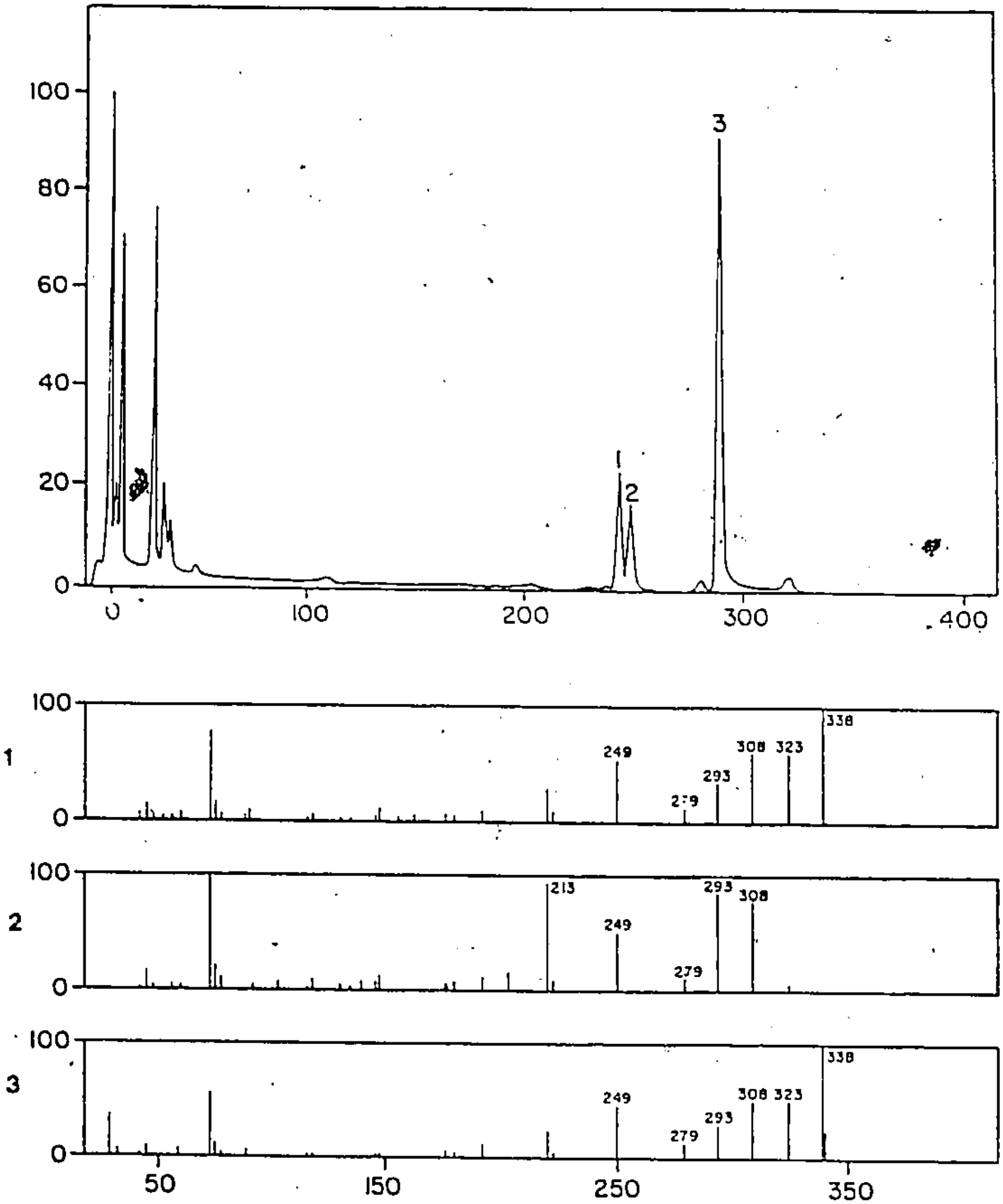


Figure 10: GC-MS analysis

3.2.2 Quantitative Analysis

A calibration curve (Fig. 11) was determined by means of a regression between area under the curves of the chromatogram and concentrations of the phenolic compound detected.

Quantitative work is indicated in table 4 where it is shown that the Belizean local yellow population of maize contained the highest concentration of phenolics whereas the lowest concentration was present in the Mexican Oloton (Chiapas 218) race of maize.

Isomer Z of phenolic compound is highly concentrated in Palomero (Mexico 005), again the lowest concentration is contained in the Oloton (Chiapas 218) race of maize. The proportion of isomer Z of phenolics as compared with the total phenolics amount is quite high in the Arrocillo (Puebla 463) maize and low in Oloton (Chiapas 218) race.

Calibration curve. Standard curve of ferulic acid as determined by using HPLC detector response recorded as the area of the curve from the chromatogram plotted against concentration of ferulic acid injected. Determination coefficient (r2) = 0.993 (n = 5).

Figure 11: Calibration curve

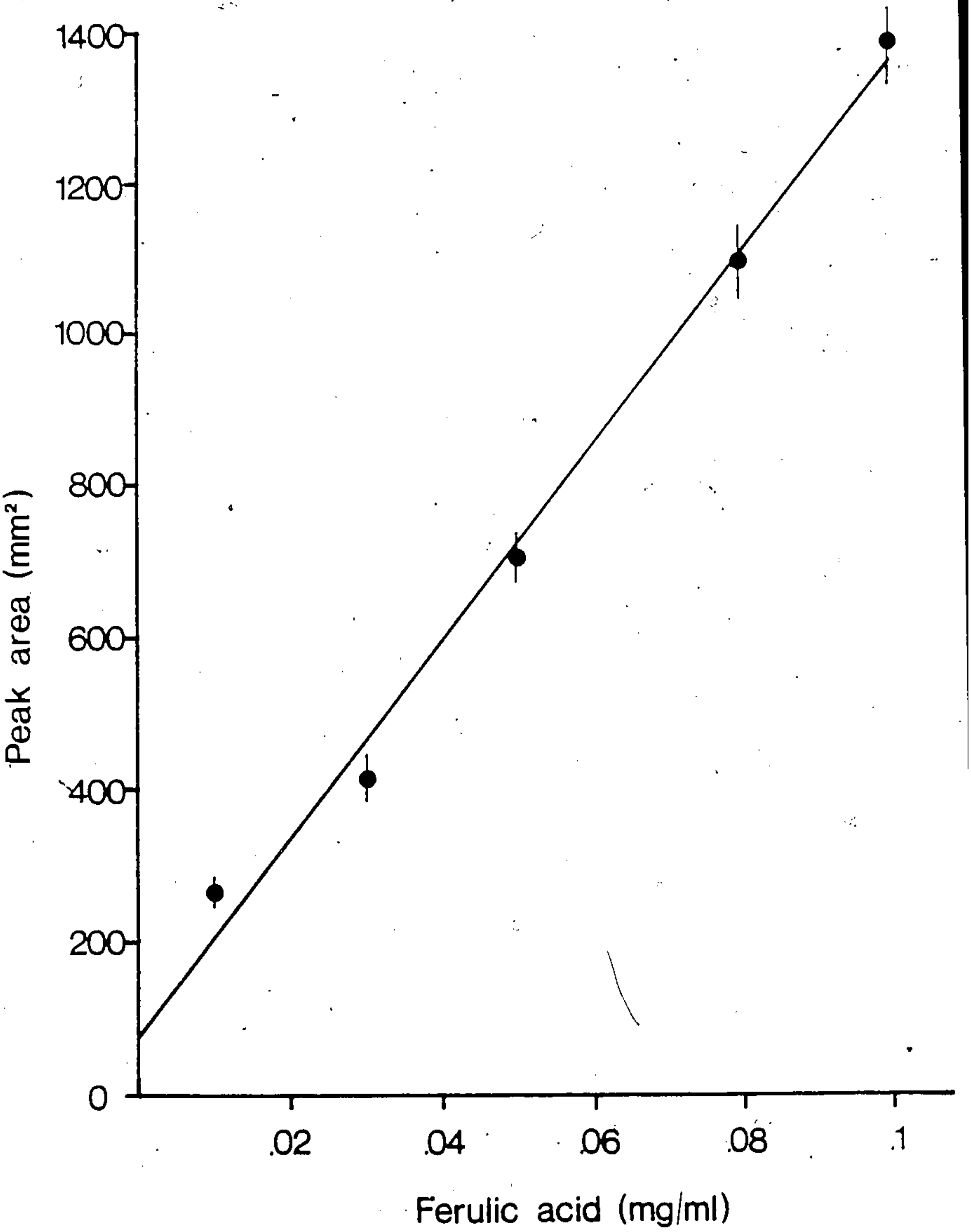


Table 4: Quantitation of phenolic compounds.

Hydrolyzed extracts from 10 Mexican maize collections, 3 Belizean collections and 1 hybrid.

	Phenolic concentration (mg/g Ferulic acid)			
Races and collections of maize	Total	E form	Z form	Z %
Arrocillo (Puebla 463)	.227	.168	.0593	35.32
Arrocillo (Puebla 537)	.352	.316	.0359	11.36
Cacahuacintle (Mexico 212)	.223	.204	.0185	9.05
Chalqueno (Mexico 208)	.258	.219	.0386	17.63
Conico (Mexico 182)	.504	.446	.0576	12.92
Conico (Mexico 461)	.282	.254	.0276	10.87
Local Black (*a)	.393	.348	.0451	12.96
Local White (*b)	.516	.462	.0540	11.69
Local Yellow (*c)	.554	.492	.0615	12.51
Maiz dulce (Guanajuato 93A)	.526	.476	.0498	10.47
Oloton (Chiapas 218)	.203	.188	.0150	8.00
Palomero (Mexico 005)	.491	.420	.0708	16.86
Palomero (Mexico 055)	.489	.426	.0634	14.89
Pioneer (d*)	.379	.348	.0305	8.75

*a : Affinity to land race Negro de Tierra Caliente
*b : Affinity to land race Dzit-Bacal (Belize)
*c : Affinity to land race Nal-Tel (Belize)
*d : Hybrid grown in Belize

3.3 Consumption of artificial diets treated with phenolic extracts

The presence of feeding deterrents in grain is demonstrated in Table 5. Treatment of artificial diets with hydrolyzed total phenolic extracts of Belizean maize populations of grain showed that consumption of diets containing extracts from grain was significantly lower when compared to consumption of untreated controls ($P<0.05$).

The feeding deterrent properties of the hydrolyzed extracts of grain as measured by consumption (Table 5) were negatively correlated ($r = -0.84$; $DF=4$, $P<0.05$) with the phenolic content of extracts (Figure 12).

When extracting at liquid nitrogen temperatures in order to extract the soluble glycosides, and adding these extracts to artificial diets, the consumption of diets treated in this way was also significantly different from controls ($P<0.05$). For the unhydrolyzed extracts the correlation between consumption and phenolic content was -0.96 ($DF=4$, $P=0.01$).

An assay was performed to check the effect of feeding response of weevils to pure phenolic and other substances. Again, Table 6 indicates that these substances have significant deterrent properties at a concentration of 0.3 mg/g which is close to the phenolic content in grain.

Table 5: Consumption of treated artificial diets

Antifeedant properties of maize extracts towards *Sitophilus zeamais* as evidenced by consumption of treated artificial diets. For ANOVA with total phenolic data F = 3.76 (P = 0.001, Df = 51). Means folowed by the same letter are not significantly different (P = 0.05) in Duncan's multiple range test.

Maize population from which extract was made	Consumption of treated diets	
	Total phenolic extract (mg/insect/day)	Acetone extract (mg/insect/day)
Control	4.88a	4.24a
Pioneer 230	4.48b	3.09b
Local Black	3.89c	3.43b
Local White	3.67c	3.57b
Local Yellow	3.66c	3.25b

Table 6: Antifeedant properties of pure phenolic substances.

Consumption of diets treated with pure phenolic substances by *Sitophilus zeamais.*

Treatment	Consumption (mg/insect/day)
Control	3.49a
BOA	2.70b
B-aminophenol	2.58b
6-MBOA	2.24c
ferulic acid	2.40c

Note: Concentration was 0.3 mg/g diet.
Mean followed by the same letter are not significantly different in Duncan's multiple range test (P - 0.05).
ANOVA values F - 15.48; P - 0.0001; df - 51.

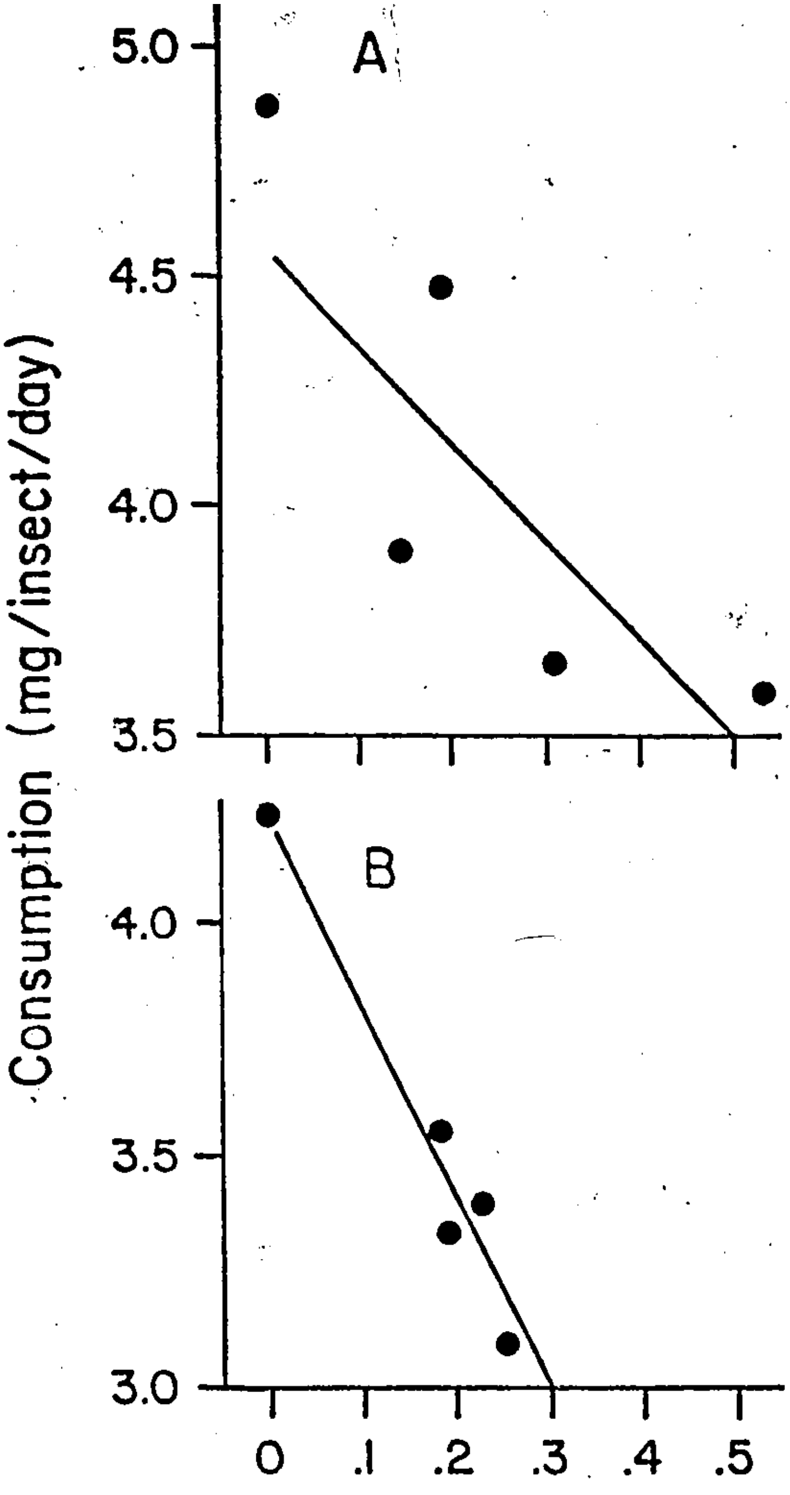


Figure 12: Plot of consumption versus concentration of phenolics in artificial diets.. Consumption by maize weevil of artificial diets treated with extracts from 4 Belizean populations of maize investigated plotted against the phenolic content of the extracts.. A. Total phenolic extracts (hydrolyzed). B. Acetone extracts (unhydrolyzed).

3.4 Resistance Tests using weevil populations

The resistance, in terms of consumption by weevil populations, of 10 populations of maize was compared by means of a no choice test (Table 7).

Weight loss of grain consumed by adult weevil populations after 2 weeks of exposure showed significant differences among races of maize subjected to infestation ($F = 7.63$, $P<0.0001$) and comparisons of means for consumption allowed the separation of 7 groups of corn according to their susceptibility to the insect population.

Another variable which was considered as an important index of resistance was the percent mortality of the weevil population after exposure to the new environment (i. e. test corn substrate). Again an analysis of variance was carried out and results are shown in Table 8. This time, a reversed image of consumption was obtained since the more grain consumed the less the mortality induced in the populations of weevils whereas the less consumed races had greater mortality of their infesting insect populations.

The data pertaining to the calculation of the parameter of stability (β) is summarized in Table 9. After obtaining the corresponding parameters of stability (β) which indicates the rate of consumption of grain by the insect population per day assuming a linear relationship, a qualitative index of susceptibility (R+ to S+) was assigned to each population of maize.

As shown in Table 9, the populations of maize; Puebla 463 (Arrocillo), Mexicó 005 (Palomero) and Mexico 182 (Conico) were classified as resistant. Four populations of maize were classified as intermediate since their values of β were closer to the mean consumption per day of all the varieties considered, these varieties were Mexico 055 (Palomero), Mexico 461 (Conico), Guanajuato 93A (Maiz dulce) and Mexico 208 (Chalqueno). At the top of the range were the susceptible populations of maize Chiapas 218 (Oloton) and Mexico 212 (Cacahuacintle).

As an illustration of the parameter evaluated Figure 13 shows the slopes or rates of consumption (β) compared against a dashed standard line which shows an assumed standard consumption per day, the lines above the standard line are the susceptible races of maize whereas the lines below it are the resistant varieties.

Table 7: Consumption of maize grain by weevil populations.

ANOVA values: F = 7.63; P = 0.0001;n = 5. Means followed by the same letter in a column are not significantly different (P = 0.05) in the Waller-Duncan multiple range test. Each replicate contains 20 g of grain and 100 +/- 10 adult insects.

Race (Accession No.)		Mean consumption by adults (g/week/100 insects)
Cacahuacintle	(Mexico 212)	0.843 a
Oloton	(Chiapas 218)	0.729 a,b
Arrocillo	(Puebla 537)	0.656 b
Conico	(Mexico 461)	0.634 b,c
Conico	(Mexico 182)	0.611 b,c,d
Chalqueno	(Mexico 208)	0.484 c,d,e
Arrocillo	(Puebla 463)	0.475 d,e
Palomero	(Mexico 055)	0.456 d,e,f
Maiz dulce	(Guanajuato 93A)	0.443 e,f
Palomero	(Mexico 005)	0.316 f

Table 8: Percent mortality of weevils

Weevils tested with 10 Mexican maize populations after 14 days. ANOVA values: F = 12.03; P = .0001; n = 5. Each replicate (vial) contains 20 g of seed and 100 insects. Treatment : maize seed. Variable of response : Percent mortality of adult weevils after 14 days.

Race (Collection)	Mean Mortality (%)
Palomero (Mexico 55)	53
Maiz dulce (Guanajuato 93A)	48
Palomero (Mexico 5)	40
Chalqueno (Mexico 208)	32
Conico (Mexico 461)	29
Conico (Mexico 182)	27
Oloton (Chiapas 218)	17
Arrocillo (Puebla 463)	16
Arrocillo (Puebla 537)	7
Cacahuacintle (Mexico 212)	7

Table 9: Calculation of Parameter of Stability Parameter

Consumption data used for calculation of Stability Parameter (β).

Note: Initial mean weight of maize samples at equilibrium moisture content was 60.25 g. There were 100 insects in the trial.

Inspection date	Mean Cconsumption all varieties (g)	Mean Consumption per day (g/day)
14 days	1.75	0.13
32 days	7.38	0.23
46 days	16.69	0.36

Values of consumption per day, at 3 inspections

Races of maize	(Coll.)	14 days (g)	32 days (g)	46 days (g)
Oloton	(C218)	0.16	0.25	0.46
Palomero	(M005)	0.08	0.10	0.22
Palomero	(M055)	0.10	0.21	0.31
Conico	(M182)	0.16	0.25	0.35
Chalqueno	(M208)	0.09	0.21	0.33
Cacahuacintle	(M212)	0.19	0.33	0.61
Conico	(M461)	0.14	0.26	0.36
Maiz dulce	(G93A)	0.11	0.24	0.33
Arrocillo	(P463)	0.09	0.16	0.22
Arrocillo	(P537)	0.14	0.31	0.42

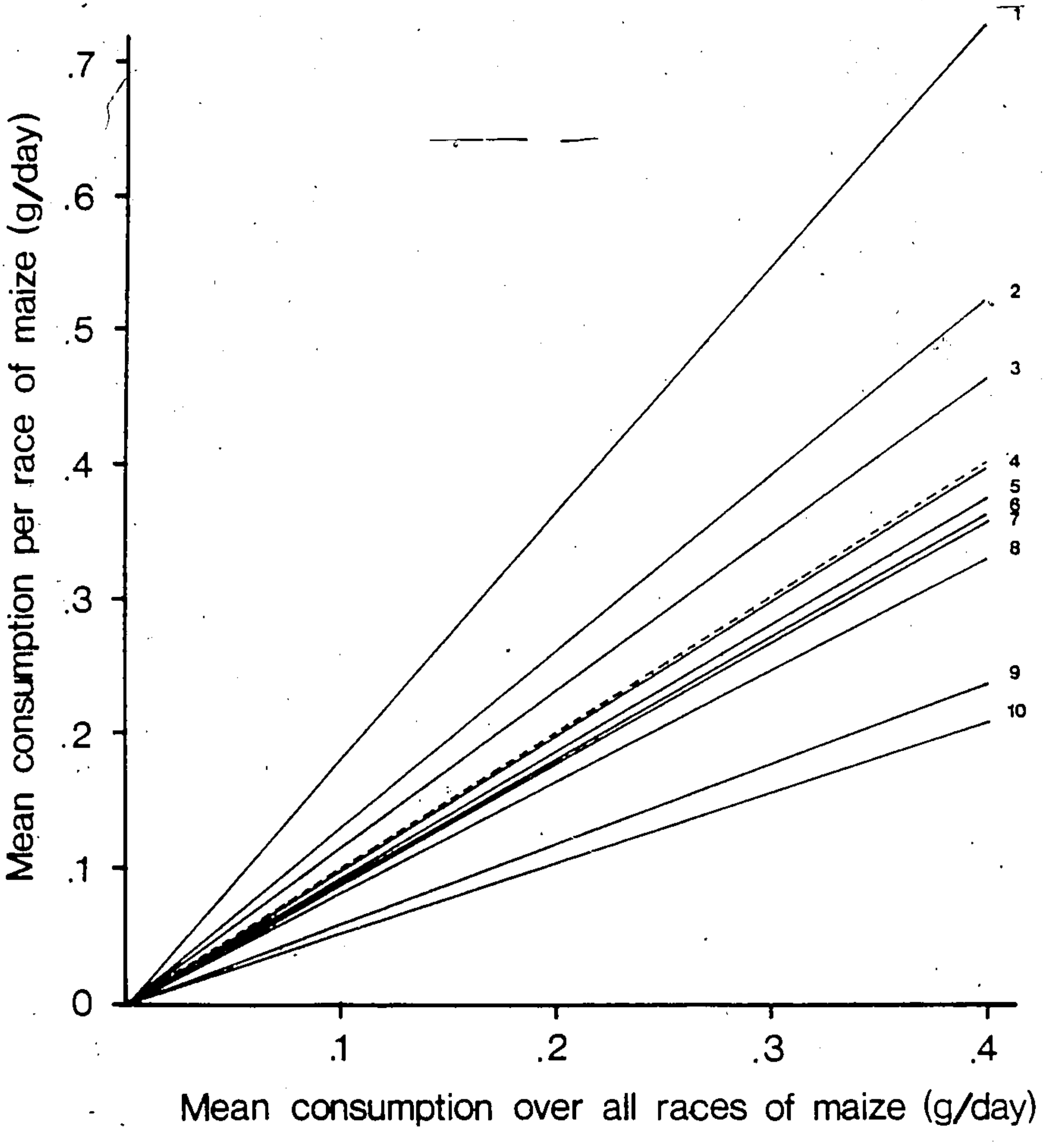
Land race (Collections)		rate of consumption (β)	Resistance score*
Arrocillo	(P463)	0.54	R+
Palomero	(M005)	0.61	R
Conico	(M182)	0.83	R-
Palomero	(M055)	0.90	I
Maiz dulce	(G93A)	0.92	I
Chalqueno	(M208)	0.94	I
Conico	(M461)	0.99	I
Arrocillo	(P537)	1.16	S-
Oloton	(C218)	1.30	S
Cacahuacintle	(M212)	1.81	S+

R - Resistant
S - Susceptible
I - Intermediate
* according to criteria of Betanzos (1980b).

Weevil populations' rates of consumption on different types of grain. Comparison of rates of consumption of populations of weevils, during 46 days, as determined by the parameter of stability (beta), assuming a linear feeding behaviour. Dashed line represents a standar consumption (mean of mean consumption by variety). The abbreviations are as in Table 3 and Figure 7. Numbers are:

1.	Mexico 212	(M212; CATL; 2)
2.	Chiapas 218	(C218; OLOT; 2)
3.	Puebla 537	(P537; ARAM; 1)
4.	Mexico 208	(M208; CHAL; 4)
5.	Guanajuato 93A	(G93A; MADU; 2)
6.	Mexico 461	(M461; CONI; 3)
7.	Mexico 55	(M55 ; PALT; 1)
8.	Mexico 182	(M182; CONI; 3)
9.	Mexico 5	(M5 ; PALT; 1)
10.	Puebla 463	(P463; ARAM; 1)

Figure 13:


Mean consumption per race of maize (g/day)
.7
.6
.5
.4
.3
.2
.1
0
.1
.2
.3
.4
Mean consumption over all races of maize (g/day)
1
2
3
4
5
6
7
8
9
10

3.5 Choice Tests

The number of adult insects found after 2 weeks of exposure in the different vials containing the seed are summarized in table 10. According to the results obtained by the analysis of variance the replicates do not contribute ($F = 0.05$, $P = 0.952$) to the variation of the number of insects present in the vials with grain implying that replicates are homogeneous, therefore the main effect is due to the different maize types or races of grain contained in the vials ($F = 8.82$, $P = .0001$).

Clearly there is a marked preference for two seed materials, namely Cacahuacintle (Mexico 212) and Oloton (Chiapas 218) which belong to the Pre-Columbian Exotic group of maize (Table 10) whereas the two populations of Palomero (Mexico 005 and Mexico 055, Ancient Indigenous group) were much less preferred by the infesting populations of weevils (Table 10).

Table 10: Preference of weevils in a Choice test.

Number of insects present in vials after 10 days of exposure to 10 races of maize seed. Two hundred unsexed adult weevils, 3 weeks old, were realesed per replicate.

Land race (collection)		------ Feeding Arena ------ Replicate A	B	C	Mean No. of insects (s.d.)
Palomero	(M055)	2	3	4	3.0 (1.0)
Palomero	(M005)	7	9	6	7.3 (1.5)
Conico	(M182)	10	3	13	8.7 (5.1)
Maiz dulce	(G93A)	14	6	7	9.0 (4.4)
Arrocillo	(P537)	12	6	16	11.3 (5.0)
Chalqueno	(M208)	18	5	11	11.3 (6.5)
Arrocillo	(P463)	10	14	11	11.7 (2.1)
Conico	(M461)	28	1	15	14.7 (13.5)
Oloton	(C218)	36	35	49	40.0 (7.8)
Cacahuacintle	(M212)	44	85	44	57.7 (23.7)

Mean infestation (blocks) = 17.5 insects per vial
Coeficient of variation = 57 %

3.6 Life Cycle Characteristics and Dobie Index of Susceptibility

The number of oviposition sites observed using the berberine stain method are presented in Table 11. The number of seeds showing oviposition sites was greater on the races Cacahuacintle (Mexico 212), Oloton (Chiapas 218) and one Conico (Mexico 461). On the contrary, races Palomero (both Mexico 005 and Mexico 055), Maiz Dulce (Guanajuato 93A) and one Conico (Mexico 182) showed a lesser degree of infestation when considering the presence or absence of oviposition sites.

An attempt was made to determine the expected number of oviposition sites assuming that the process was random. The problem was posed as follows : If all kernels are equal for the insect, then the distribution of the insects and their progeny would be random since the environment would be homogeneous and no preference for any sites, both among races of maize and within each population of kernels, would be noticeable.

On the other hand, when there exists a preference for some maize populations in the feeding arena the insects would have a greater probability of eventually mating since there would a greater number of insects in those more attractive maize populations, which in turn would be reflected in the number of oviposition sites.

Now, within the maize population the preference would be indicated by the frequency of oviposition sites both per grain (individual grain) and per maize population (grains in vials). This situation predicts that the data follow a Poisson distribution. Probabilities for a Poisson distribution are given by:

$$P(Y = k) = [\exp(-u)](u^{k}) / (k!) \tag{11}$$

where,

u = mean of distribution

k = (0,1,2,....)

Table 11: Oviposition sites detected by berberine staining method

Grain preference by weevils as determinated by the number of oviposition sites in diferent types of grain.

Land Race (collection)		Percentage of grain with oviposition sites	Total No. of oviposition sites	Mean No. of oviposition sites
PALT	(M055)	10.7	8	2.6
MADU	(G93A)	20.0	14	4.6
PALT	(M005)	7.5	15	5.0
CONI	(M182)	28.9	17	5.6
ARAM	(P537)	35.0	22	8.3
CHAL	(M208)	35.6	27	8.9
ARAM	(P463)	28.8	30	10.0
CONI	(M461)	46.7	46	15.3
OLOT	(C218)	80.0	74	24.6
CATL	(M212)	83.3	123	41.0

Maize samples were exposed for 2 weeks to 200 adult insects in a choice test before assessment.

P = Probability that the random variable Y

takes the value k

Y = Number of seeds with k number of oviposition sites

The substitution of the observed number of oviposition sites after calculating the probability u in equation 11 yields the expected number of grains with k number of oviposition sites. These expected values are compared to the observed values in table 12.

Evidently, there is a large departure from randomness (i. e. there are some sites, vials, which are more preferentially selected for oviposition) when considering the distribution of the number of eggs deposited in the grains of all populations (Figure 14). This strong preference for some maize populations is evidenced in Figure 15. The number of grains attacked and the number of oviposition sites per grain is clearly high for Cacahuacintle and Oloton maize. The populations of Palomero and Arrocillo grouped together as the least infested since the number of grains without oviposition sites is greatest and the number of oviposition sites per grain is smallest. Maiz Dulce (Guanajuato 93A), Conico (Mexico 182), Chalqueno (Mexico 208) and the second population of Conico (Mexico 461) are intermediate in terms of preference by the choice test.

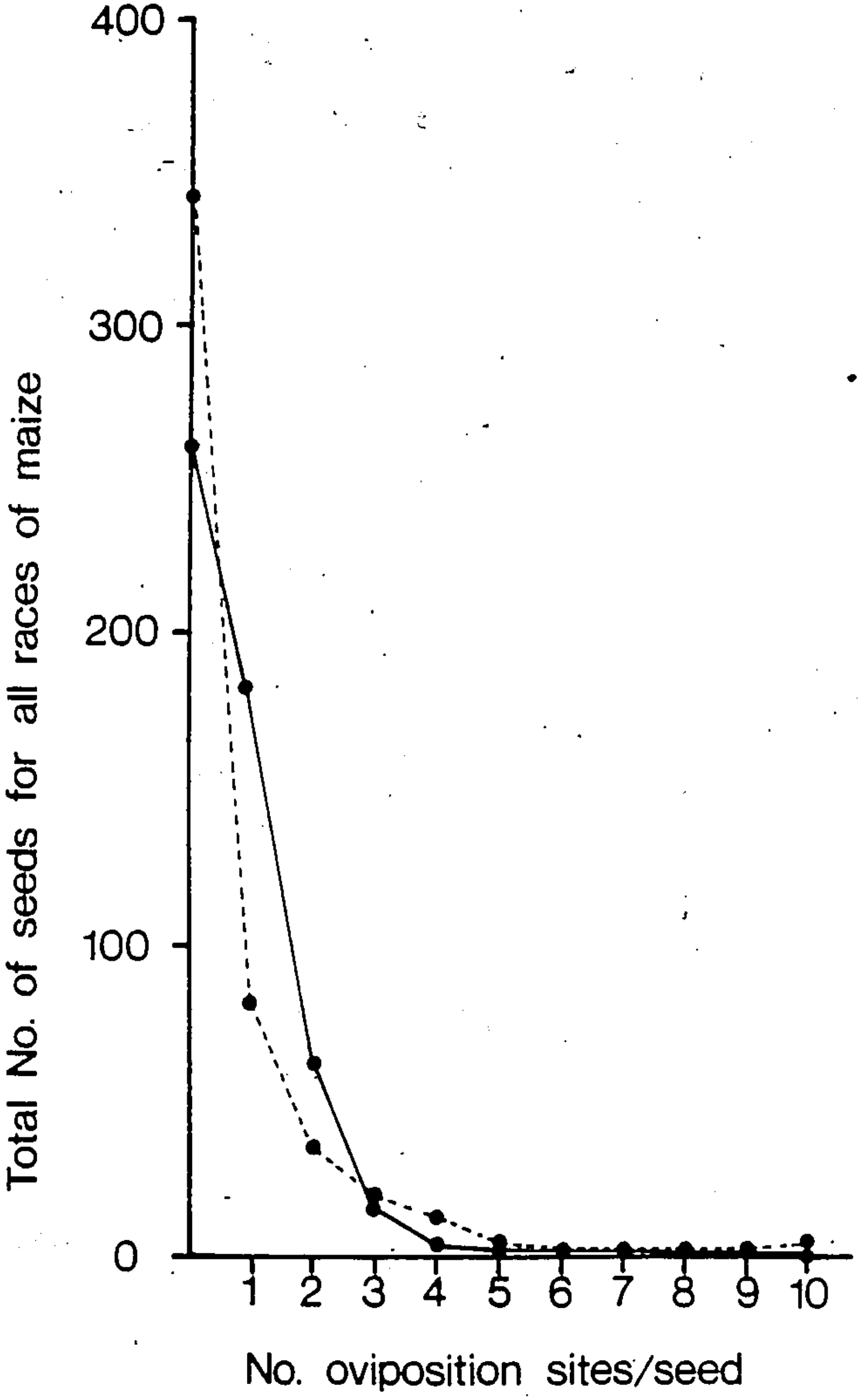
Table 12: Observed and expected values of oviposition sites (all races).

Table used to determine Poisson distribution.

Total No. of grains inspected	k oviposition sites per grain	Probability of k oviposition sites	No. of grains with k oviposition sites (expected)
524	0	0.497	260.2
	1	0.348	182.1
	2	0.122	63.8
	3	0.028	14.9
	4	0.005	2.6
	5	0.001	0.4
	6	0	0
	7	0	0
	8	0	0
	9	0	0
	10	0	0

Figure 14: Graphic representation of Poisson distribution. Graph of the values of expected and observed number of oviposition sites using combined data from all land races in feeding arena choice test.

Note : Dashed line, observed values; continous line, expected values. Chi square value of goodness of fit = 216 which rejects the null hypothesis of randomness (Poisson distribution).


400
300
200
100
0
Total No. of seeds for all races of maize
1
2
3
4
5
6
7
8
9
10
No. oviposition sites/seed

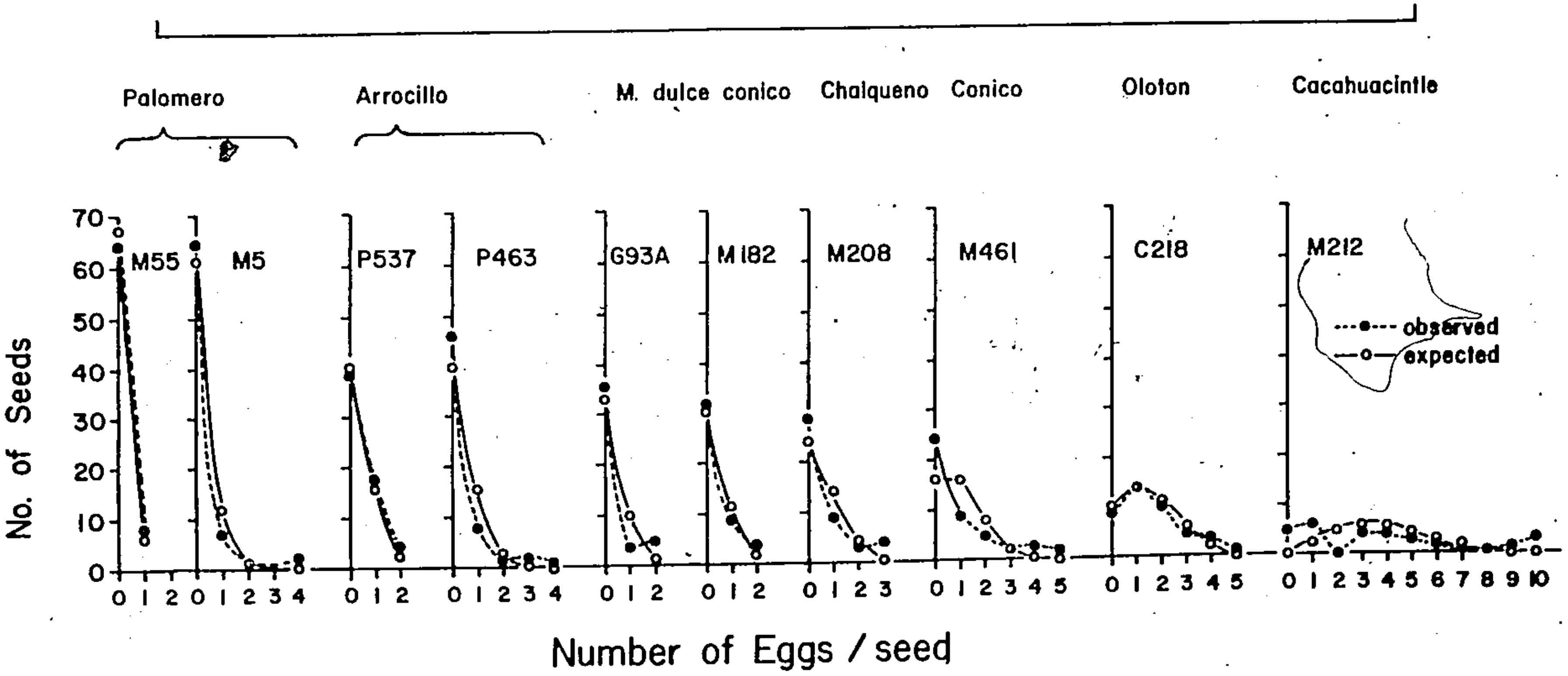


Figure 15: Poisson distribution and goodness of fit for individual populations of grain. Graph of observed and expected number of oviposition sites (punctures) in grain contained in vials. Analysis of each type of grain without considering the feeding arena.

3.7 Effect of Grain-Insect Interaction on Adult/Progeny Success

3.7.1 Performance of Parental Population of Insects through Time

Calculation of stability parameter gives information about the consumption behaviour of a population of weevils and their progeny in a restricted situation where there is a limited amount of resources. Also, the contribution of the progeny to the consumption of grain is undiscernible since it is confounded with the adult population feeding on the grain. The aim of this technique is to measure the consumption of a population of weevils (adults and progeny) after a period of time. In other words, the goal is to indicate the damage to the grain. However, other characteristics of the life cycle of the insect such as growth of individuals and populations related to consumption, number of individuals produced related to age, mortality in an environment free of density-dependent constraints, changes in weight of individuals, etc. are disregarded. To account for all these changes in a comprehensive form, the performance of adult and immature stages of weevil population were analyzed. Table 13 shows the results obtained after complete emegence of weevil progeny, as well as the characteristics of the parental population of weevils which produced that progeny.

Table 13: Biological characteristics of weevil progeny

Age of parental population: 3 weeks

Race of maize and collection (a)	Weight loss of grain. Feeding by immature stage (g)	No. of insects emerged	Median devel- opment period (days)	Total weight of insects emerged (mg)
CATL (M212)	1.839	44	49	165.2
ARAM (P463)	0.247	16	49	56.6
CONI (M461)	0.189	14	49	56.6
MADU (G93A)	0.070	13	49	47.0
OLOT (C218)	0.043	11	49	37.4
CONI (M182)	0.022	4	68	13.4
CHAL (M208)	0.002	1	54.5	3.2
PALT (M55)	0	0	0	0
PALT (M5)	0	0	0	0
ARAM (P537)	0	0	0	0

Age of parental population: 4 weeks

Race of maize and collection (a)	Weight loss of grain. Feeding by immature stage (g)	No. of insects emerged	Median devel- opment period (days)	Total weight of insects emerged (mg)
CATL (M212)	3.292	76	47	287.3
MADU (G93A)	0.662	24	49	92.0
OLOT (C218)	0.622	32	45	110.3
CONI (M461)	0.458	20	51	80.9
CONI (M182)	0.173	15	49	52.5
CHAL (M208)	0.081	13	59	47.8
ARAM (P537)	0.043	11	45	35.3
ARAM (P463)	0.023	4	55	11.6
PALT (M5)	*	1	*	3.2
PALT (M55)	0	0	0	0

Table 13: Biological characteristics of weevil progeny

Age of parental population: 5 weeks

Race of maize and collection (a)	Weight loss of grain. Feeding by immature stage (g)	No. of insects emerged	Median development period (days)	Total weight of insects emerged (mg)
CATL (M212)	2.077	44	50	164.7
CONI (M461)	0.679	24	50	93.1
OLOT (C218)	0.109	11	54	42.1
MADU (G93A)	0.070	6	52	25.6
ARAM (P537)	0.051	3	54	10.5
CONI (M182)	0.012	3	50	9.7
ARAM (P463)	0.010	2	48.5	7.3
CHAL (M208)	0.005	3	50	12.9
PALT (M55)	0	0	0	0
PALT (M5)	0	0	0	0

In these experiments adult weevils were provided with fresh substrate on a weekly basis, and the maize substrate from each week was conserved to observe progeny development.

As seen in Figure 16 and Table 14, there is a remarkable variation among the performances through time of parental populations, on different maize substrates both in survival and weight gain. Maize populations Cacahuacintle (Mexico 212), Conico (Mexico 461) and Oloton (Chiapas 218) supported the best growth of the infesting weevils.

A high rate of insect mortality is observed for both Palomero populations of maize (Mexico 005 and Mexico 055) as is seen in Figure 16, reaching 100 % of mortality for the infesting population of weevils by the begining of the 4th week. Arrocillo maize populations (Puebla 463 and Puebla 537) differed in their resistance in terms of the mortality of the weevil populations growing on them.

For Puebla 463 (Arrocillo) there was a low percentage of mortality at the end of the second week an a sudden increase in mortality from week two to week three while for Puebla 537 (Arrocillo) the rates of mortality did not vary that much (Figure 16). In the case of the two populations of Conico (Mexico 182 and 461) there was a great difference between these two as it is seen in Figure 16. Maiz dulce (Guanajuato 93A) and Chalqueno (Mexico 208) showed high mortality at the end of the third week however, while for Chalqueno the rate of mortality was constant, for Maiz dulce (Guanajuato 93A) a large change in mortality rates occured during the second and third week.

Table 14: Weight gain of parental weevil population.

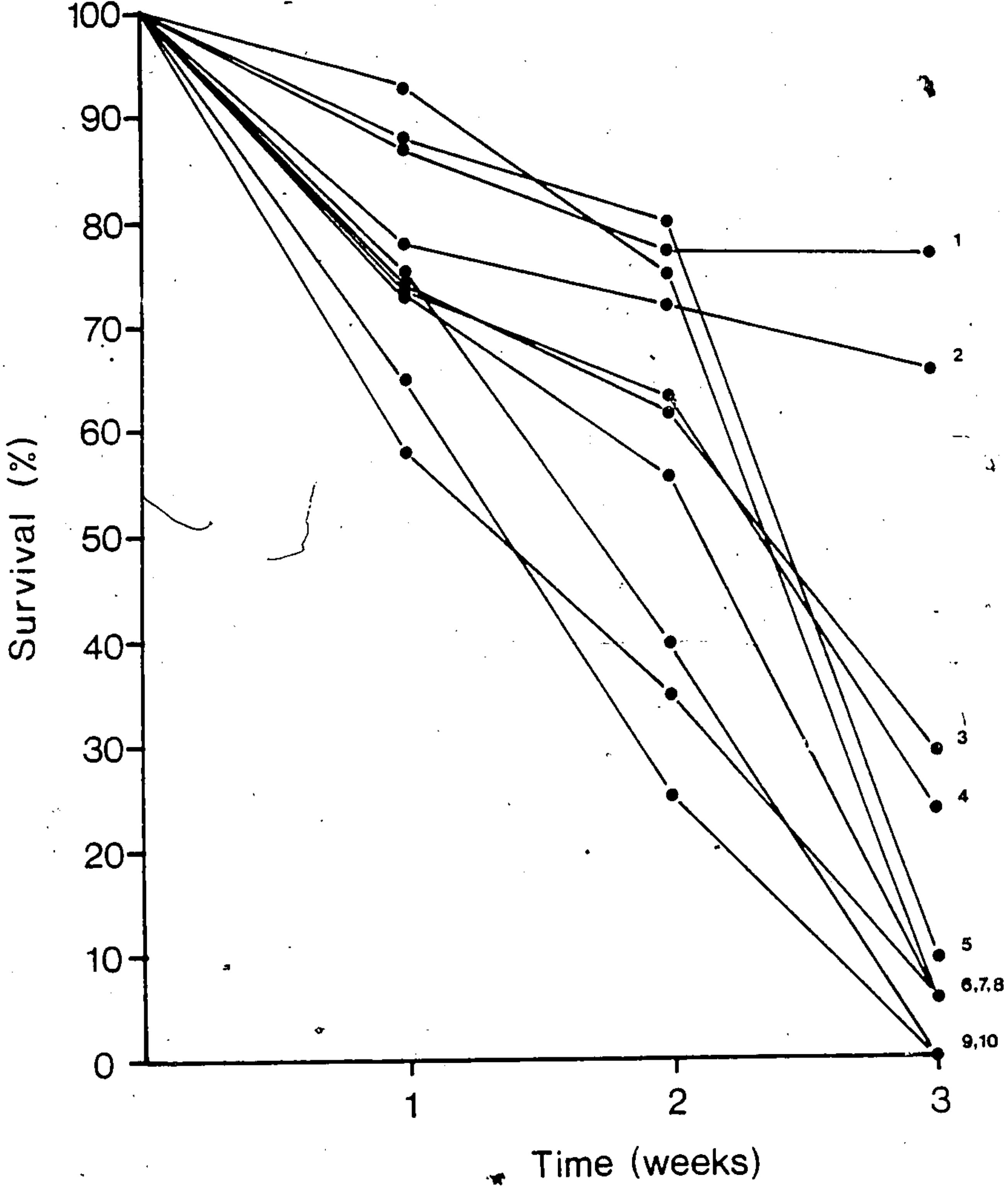
Kruskal-Wallis analysis of variance for adult weevil weight gain.

Race (Collection)		Mean change in weight of adult weevils during 3 week period (mg)
Palomero	(M055)	3.19 a
Arrocillo	(P463)	3.42 b
Chalqueno	(M208)	3.44 b,c
Palomero	(M005)	3.45 b,c
Conico	(M182)	3.47 b,c
Maiz dulce	(G93A)	3.62 c
Arrocillo	(P537)	3.69 c
Oloton	(C218)	3.70 c,d
Conico	(M461)	3.72 d
Cacahuacintle	(M212)	3.72 d

Chi square value : 16.06 (P = 0.067)
D.F. = 9

Figure 16: Survival profile of weevil populations. Parental weevil populations growing on different types of maize. Survival expressed as percentage. Time in weeks.

1. Mexico 212 (Cacahuacintle)
2. Mexico 461 (Conico)
3. Chiapas 218 (oloton)
4. Mexico 182 (Conico)
5. Guanajuato 93A (Maiz dulce)
6. Mexico 208 (Chalqueno)
7. Puebla 537 (Arrocillo)
8. Puebla 463 (Arrocillo)
9. Mexico 5 (Palomero)
10. Mexico 55 (Palomero)


Survival (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
Time (weeks)
1
2
3
4
5
6,7,8
9,10

3.7.2 Performance of Progeny of Parental Populations through Time

The number of progeny obtained from weekly changes of grain substrate are shown in Table 15. The number of progeny on the different races of maize grain range from a mean of 55 new adults emerged from Cacahuacintle to none emerged from Palomero. These results indicate that there exists a large gradient of success of adaptation to the different characteristics of each type of grain by the same species of weevil. In other words, the variation of adaptation of a single species to a new environment represented by different races of maize is very large both for the parental populations and for their progeny.

An analysis of covariance was performed in order to assist in interpreting the data with regard to the nature of treatment effects, in this case the effect of different types of grain and its relationship to the parental populations of insect weevils and their progeny. A number of derived variables were calculated from the original set of data indicated in Table 13. These new variables were analyzed by means of the covariance procedure since the information provided by them is related to the original variables but in a standardized format.

ANOVA and ANCOVA results are presented in the appendix 3, showing that weight loss of grain, development time of progeny, number of insects emerging, weight per individual and consumption of grain per individual per day could be analyzed as 3 replicates since no effect on the variable of response was observed due to the variation in time as well as in the number of parental insects and their respective weights.

On the other hand, analysis of variance and covariance indicated that most of the variation in the variables of response was due to the effect of the different types of grain (treatments) and their interaction with the weevil populations. Since the analysis of variance and covariance gave an indication of the effect of the treatments on the variables of response, a one way analysis of variance was performed to make comparisons among the different treatments or types of grain. The chosen variables were those related to consumption of grain per insect and weight of insect at the time of emergence because they were considered as a direct indication of growth and nutrition of insect within the grain.

Figure 17 and Table 16 indicate the differences in consumption and weight of the weevil progeny grown on the different classes of grain. Neither collection of maize Mexico 005 and Mexico 055 (Palomero) was successfully colonized by the infesting weevils and no emergence was observed from these races of maize, making it impossible to estimate any parameters for these two systems. At the other extreme it is observed that the consumption of grain per individual during development time of insects growing in the grain population of Cacahuacintle (Mexico 212) was twice than the mean consumption of grain within the other populations (Figure 17). Complete comparisons for other variables of response together with main statistics are shown in the appendix 2.

Table 15: Progeny obtained on maize grain

Number of insects emerged after 4 weeks produced by a single cohort of weevils on different types of maize.

Races and collections of maize	Number of insects emerged in a no-choice test
Cacahuacintle (Mexico 212)	55
Conico (Mexico 461)	19
Oloton (Chiapas 218)	18
Maiz Dulce (Guanajuato 93A)	14
Chalqueno (Mexico 208)	8
Arrocillo (Puebla 463)	7
Arrocillo (Puebla 537)	7
Conico (Mexico 182)	7
Palomero (Mexico 5)	1*
Palomero (Mexico 55)	0

* one insect in three replicates

Figure 17: Grain consumption by weevil during development time.

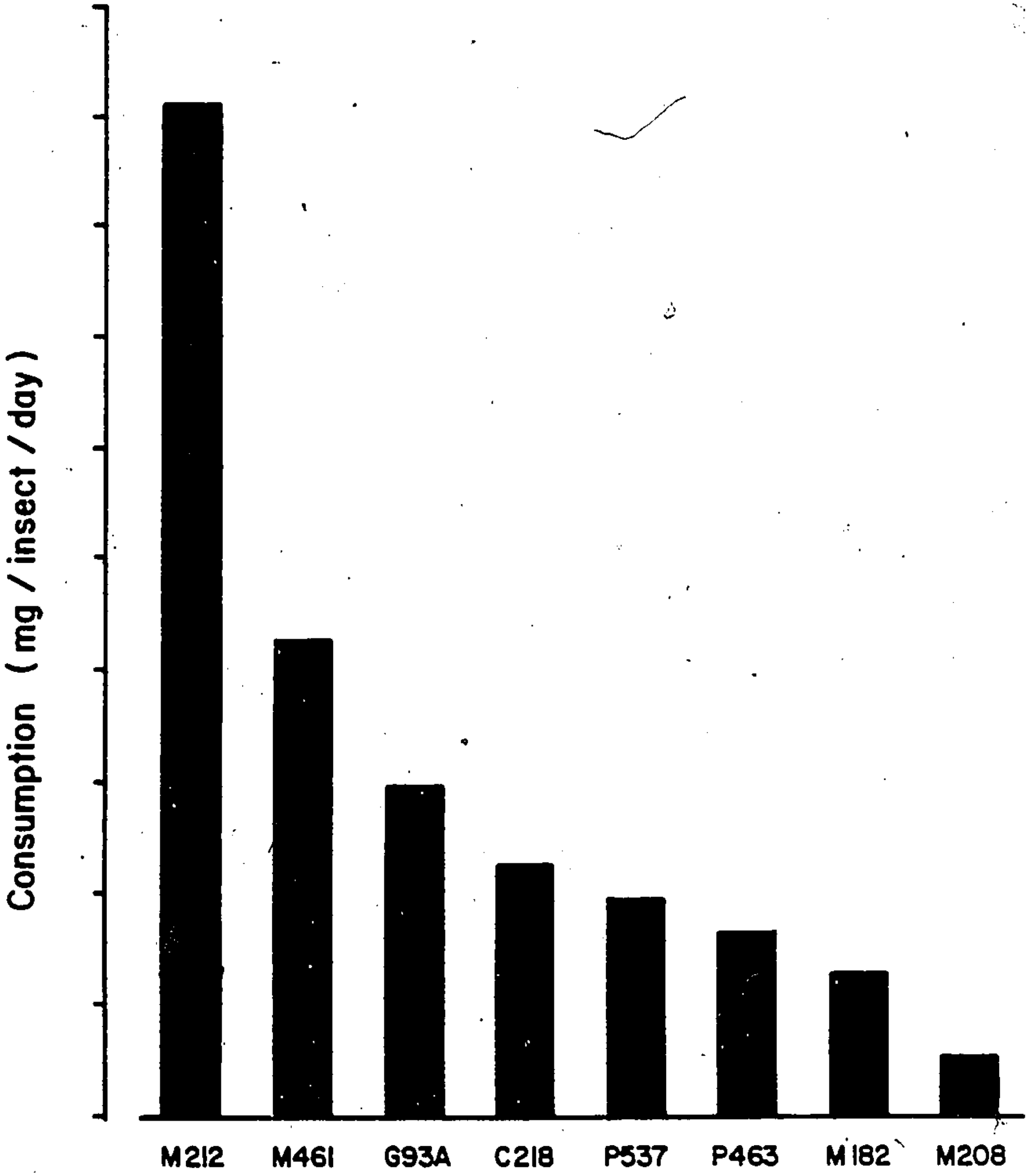


Table 16: Variables of weevil growth as related to consumption

Comparisons of several variables associated with the performance of weevil progeny.

Land Race		Mean Consumption	
		mg/insect/dev. time	mg/day
Cacahuacintle	(M212)	44.1 a	49.7 a
Conico	(M461)	21.5 b	8.8 b
Maiz dulce	(G93A)	14.9 b,c	5.4 b
Oloton	(C218)	11.1 b,c	5.6 b
Arrocillo	(P537)	10.3 b,c	0.9 b
Arrocillo	(P463)	8.8 c	1.9 b
Conico	(M182)	7.0 c	1.4 b
Chalqueno	(M208)	3.3 c	0.5 b

Land Race		Mean consumption mg/insect/day	Mean Weight insect (progeny) mg
Cacahuacintle	(M212)	0.91	3.76
Conico	(M461)	0.43	3.99
Maiz dulce	(G93A)	0.30	3.91
Oloton	(C218)	0.23	3.56
Arrocillo	(P537)	0.20	3.36
Arrocillo	(P463)	0.18	3.36
Conico	(M182)	0.13	3.36
Chalqueno	(M208)	0.06	3.73
Palomero	(M005)	----	3.20*

*Note: Only one insect in 3 replicates

3.8 *Nutritional indices of the immature stage of weevil*

Three indices of nutrition were estimated for each of the weevil populations emerged from the races of maize used as substrate. The small number of insects emerged from this experiment made it very difficult to measure the amount of frass accurately since these quantities were negligible in many cases. Therefore, the amount of frass was estimated by using the data obtained from a separate experiment, and assuming that the ratio of frass produced to grain consumed was constant for any one variety of grain.

The results of this evaluation are summarized in Table 17. Surprisingly, the most consumed grain (Mexico 212) was not the more nutritious, at least in terms of the indices used in this approach (AD, ECD, ECI). The ECI represents the efficiency of conversion of food taken into insect biomass. It varies by an order of magnitude from 10.7 for Cacahuacintle (M212) to 183.9 for Chalqueno (M208). The ECI has 2 components: the approximate digestibility (AD) and the efficiency of conversion of digested food (ECD). Evidently the variation in ECI stems from the considerable variation in conversion of digested food (ECD) not from variation in digestibility (AD).

Table 17: Nutritional indices of weevil on different maize material

Comparisons of the nutritional indices AD, ECD, and ECI obtained for weevil populations emerging from different races of maize. ANOVA values :

AD. F = 455.03; P = .0001; D.F.(Total) 22

ECD. F = 3.61; P = .0176; D.F.(Total) 22

ECI. F = 3.52; P = 0.0193; D.F.(Total) 22

Races (collections)		Mean AD %	 ECD %	 ECI %
Oloton	(C218)	92.1 a	51.6 b	47.6 b
Conico	(M182)	87.1 b	66.7 b	58.1 b
Conico	(M461)	86.7 b,c	23.6 b	20.5 b
Arrocillo	(P463)	86.4 c	55.7 b	48.1 b
Maiz dulce	(G93A)	85.9 d	45.5 b	39.1 b
Chalqueno	(M208)	85.6 d	183.9 a	157.6 a
Arrocillo	(P537)	83.8 e	62.1 b	52.0 b
Cacahuacintle	(M212)	79.8 f	10.7 b	8.5 b

Means followed by the same letter in a column are not significantly different in a Waller-Duncan test (P = .05).

3.8.1 Estimation of larval growth

Table 18 gives the constants used with equation 5 and Figure 18 shows the simulated results obtained. Figure 18 is a qualitative illustration of the growth of the weevil populations on different races of maize grain which allows one to visualize the type of growth which is related to consumption of grain.

All populations of weevil growing on the different races of maize showed no difference in growth during the first larval instar (I) but as soon as the second instar (II) was reached the differences in growth started to show up.

The function chosen is based on two parameters; one is the maximum weight reached by the particular weevil population and the second parameter is the rate of growth. Then, as seen in Figure 18, weevils on Conico (Mexico 461) together with Maiz Dulce (Guanajuato 93A) have the best performance in terms of weight gain through time. A second group includes Cacahuacintle (Mexico 212), Chalqueno (Mexico 208) and Oloton (Chiapas 218). A third group could be assigned to the two Arrocillo maize populations (Puebla 463 and Puebla 537) and the other Conico (Mexico 182).

One aspect to note is that if the standard time of development (bottom Figure 18) is compared against the curves depicted in Figure 18, one can observe a shift in the time of maturity of the larvae depending on the type of grain. Weevils growing on Mexico 461, as compared to the standard developmental time, would reach the pupae stage before any other weevil growing on any other grain.

Another aspect worthy of note is the great discrepancy between the two populations of Conico grain (Mexico 182 and Mexico 461) in terms of suitability for the growth of infesting insects.

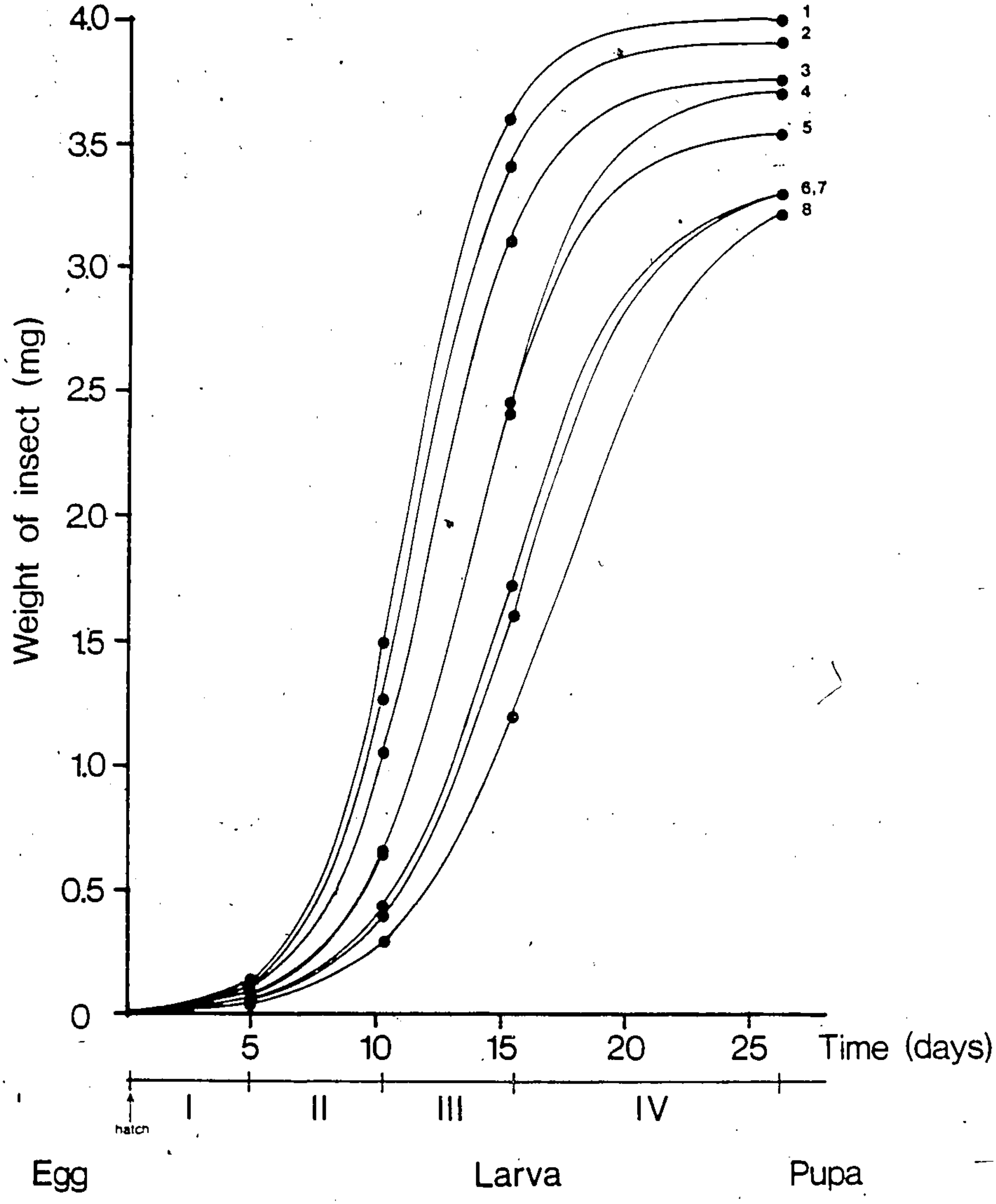
Table 18: Variables used in larval growth model

Land race (collection)		Mean Median development (days)	 Final Weight (insect) (mg) *	 Consumption mg/ins./day *	 Growth rate (mg/day)
Cacahuacintle	(M212)	48.7	3.76	0.91	0.129
Oloton	(C218)	49.3	3.56	0.23	0.120
Arrocillo	(P537)	49.5	3.36	0.20	0.113
Conico	(M461)	50.0	3.99	0.43	0.133
Maiz dulce	(G93A)	50.0	3.91	0.30	0.130
Arrocillo	(P463)	50.8	3.36	0.18	0.110
Chalqueno	(M208)	54.5	3.73	0.06	0.114
Conico	(M182)	55.8	3.36	0.13	0.100

* From table 16.

Figure 18: Simulation model of the weevil growth.. Numbers are:

1. Mexico 461 (M461; CONI; 3)
2. Guanajuato 93A (G93A; MADU; 2)
3. Mexico 212 (M212; CATL; 2)
4. Mexico 208 (M208; CHAL; 4)
5. Chiapas 218 (C218; OLOT; 2)
6. Puebla 537 (P537; ARAM; 1)
7. Puebla 463 (P463; ARAM; 1)
8. Mexico 182 (M182; CONI; 3)


Weight of insect (mg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
5
10
15
20
25
Time (days)
1
2
3
4
5
6,7
8
hatch
I
II
III
IV
Egg
Larva
Pupa

3.9 *Correlation Analysis*

The correlations between grain and insect characteristics, and three indices of interaction are shown in Table 19. The highest correlations were plotted and analyzed as type II linear regression where adequate. The scatter plots of these relationships are shown in figure 19 with the 95 % confidence interval.

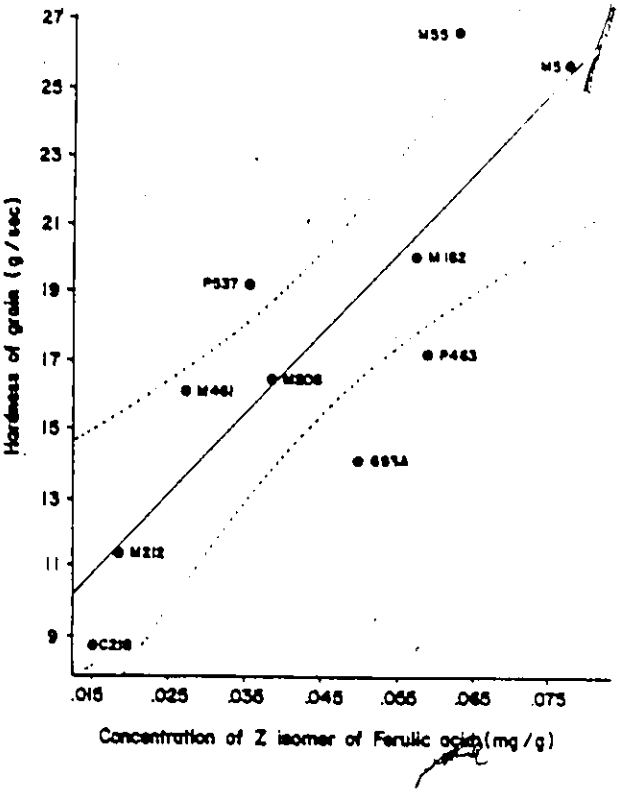
As indicated, principal component analysis was carried out without considering the grain characteristics in order to avoid spurious correlations. Figure 20 shows the correlations obtained for each one of the grain characteristics and the first principal component of the other variables analyzed. On the other hand, in order to examine the relationships between sets of components which characterize the grain populations and the characteristics of the insect population growing on them a canonical correlation analysis was carried out. The main goal was to summarize the multivariate system by finding those linear combinations of insect and grain variables which were most dependent, in a linear regression sense, on the values of the interaction indices. Three sets of variables were formed after analyzing the correlation matrix. The first set included the grain characteristics as follows: grain hardness (HARD), content of phenolics at surface of grain (SURFERUL), concentration of Z isomer of ferulic acid in grain (FERULZ) and moisture content of grain (MOIST). Number of grains (NSEEDS) and total ferulic acid content in grain (FERUL) were dropped since SURFERUL is an indirect index of these two characteristics and also because it was best correlated with other variables. The set of grain characteristics represents the independent set of variables in the interaction, since those are the factors affecting the insect behaviour. Percent mortality of insect populations (PCTMORT), frass produced by the insect (FRASS), number of insects emerging (EMERGEN) and number of insects found in the Choice test (CHOICE) were assigned to a second set called insect characteristics. The third set was formed with the Dobie index (INDEX), Parameter of stability (STABPAR) and weight loss of grain (WGTLOSS). This set represents the susceptibility of the grain as measured by the effect of insect activity in the system. The purpose of pursuing this canonical correlation analysis was to analyze in detail the more important relationships between sets of components and also to eliminate variables which were contributing little information to the description of the interaction. Although canonical correlation analysis and principal components analysis seem to be saying the same thing, in the sense these methods summarize data, they differ in that PCA is an attempt to uncover approximate linear dependencies among variables and canonical correlation analysis is to explain relationships between sets of variables by finding a small number of linear combinations from each set of variables that ~~have~~ the highest possible between-set correlations (Chatfield and Collins, 1980). In other words, these two methods will lead, hopefully, to a better understanding of the correlation structure and may generate hypotheses about the variables involved.

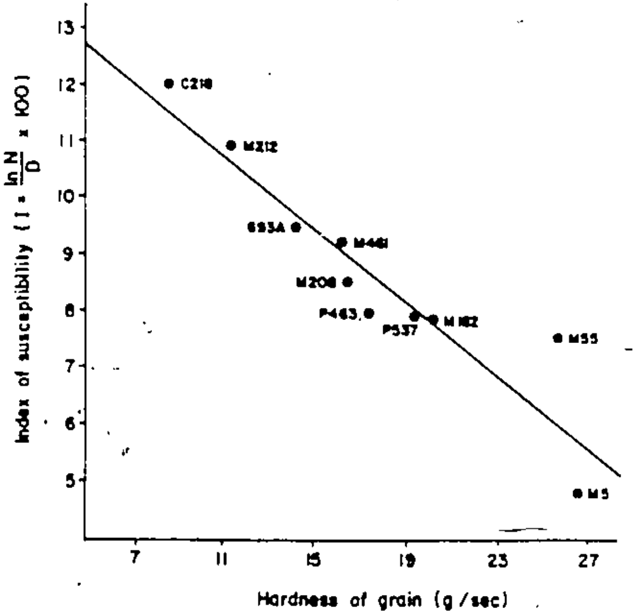
	FERULZ	SURFERUL	WGTLOSS	HARD	CHOICE	EMERGEN	INDEX	FERUL	STABPAR	PCTMORT	FRASS	NSEED	FERULE	MOIST
FERULZ	1													
SURFERUL	.80*	1												
WGTLOSS	-.87*	-.70*	1											
HARD	.85*	.80*	-.68*	1										
CHOICE	-.76*	-.64*	.81*	-.74*	1									
EMERGEN	-.81*	-.67*	.71*	-.86*	.88*	1								
INDEX	-.78*	-.72*	.64*	-.94*	.79*	.93*	1							
FERUL	.72*	.89*	NS	.67*	-.64*	-.66*	NS	1						
STABPAR	-.80*	NS	.[illegible]4*	NS	.86*	.70	NS	NS	1					
PCTMORT	.65*	.67*	-.82*	NS	NS	NS	NS	.69*	NS	1				
FRASS	-.63*	NS	.81*	NS	.69*	NS	NS	NS	.81*	-.75*	1			
NSEED	.74*	.88*	-.68*	.78*	NS	NS	NS	NS	NS	NS	NS	1		
FERULE	.64*	.86*	NS	NS	NS	NS	NS	.99*	NS	.67*	NS	NS	1	
MOIST	-.68*	NS	NS	NS	NS	NS	NS	NS	.70*	NS	.72*	NS	NS	1

Table 19. Correlation matrix (all variables studied)

Figure 19: Plots of the variables analyzed in the present study. Where indicated the regression line was calculated as a linear regression, type II. Broken line indicates the 95 % confidence interval for the regression line. Abbreviations are:

1.	Mexico 5	(M5 ; PALT; 1)
2.	Mexico 55	(M55 ; PALT; 1)
3.	Puebla 463	(P463; ARAM; 1)
4.	Puebla 537	(P537; ARAM; 1)
5.	Chiapas 218	(C218; OLOT; 2)
6.	Guanajuato 93A	(G93A; MADU; 2)
7.	Mexico 212	(M212; CATL; 2)
8.	Mexico 182	(M182; CONI; 3)
9.	Mexico 461	(M461; CONI; 3)
10.	Mexico 208	(M208; CHAL; 4)


27
25
23
21
19
17
15
13
11
9
Hardness of grain (g / sec)
M55
M5
M182
P537
P463
M461
M208
693A
M212
C218
.015
.025
.035
.045
.055
.065
.075
Concentration of Z isomer of Ferulic acid (mg / g)


13
12
11
10
9
8
7
6
5
Index of susceptibility (I = $\frac{\ln N}{D}$ x 100)
C218
M212
693A
M461
M208
P463
P537
M182
M55
M5
7
11
15
19
23
27
Hardness of grain (g /sec)

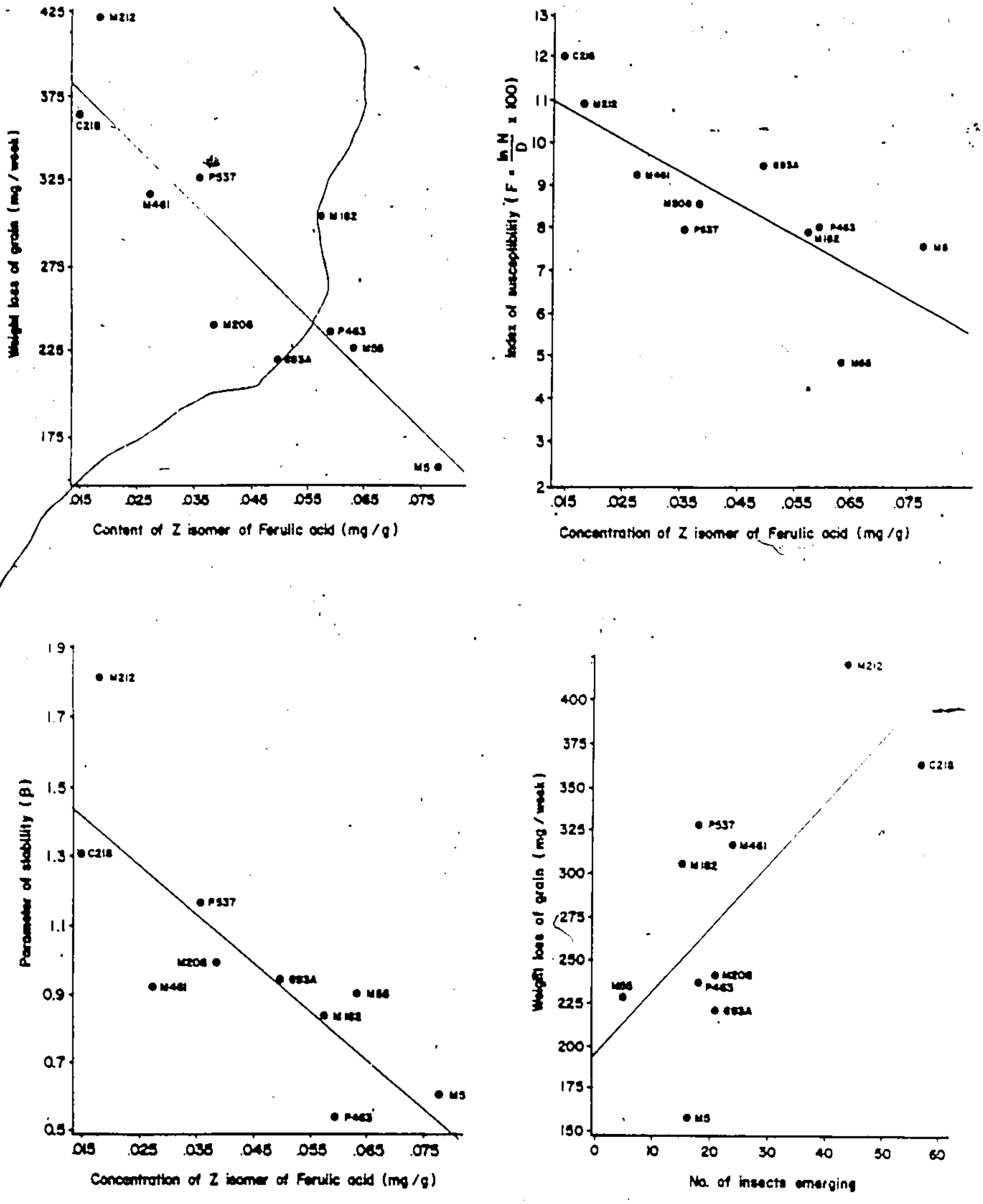
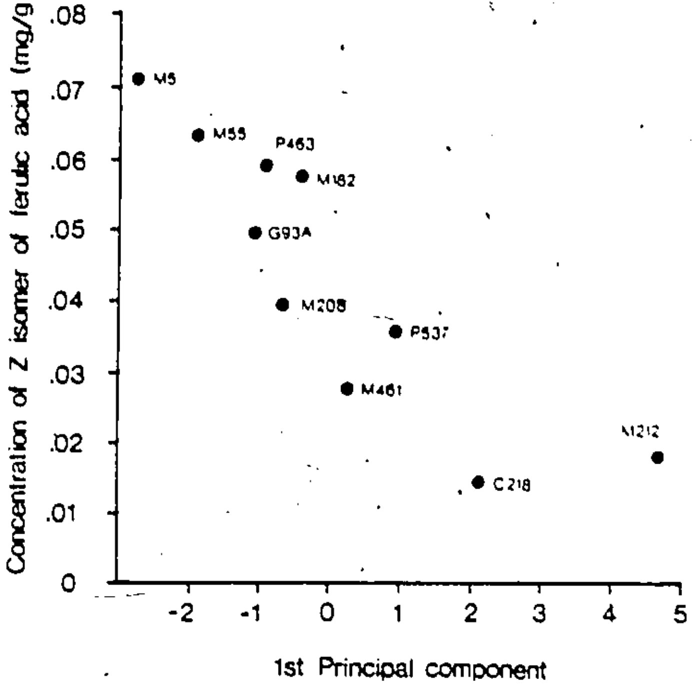
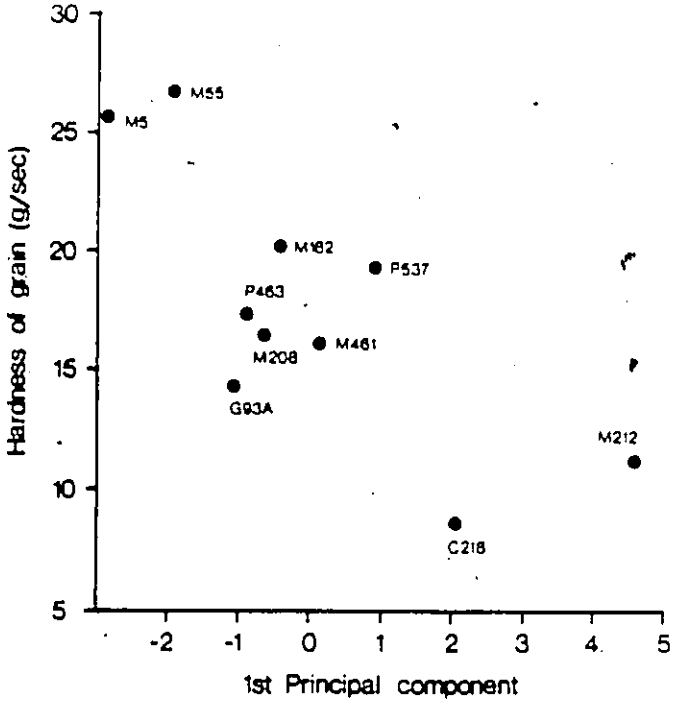


Figure 19: Plots of the variables analyzed in the present study

Figure 20: Multivariate correlations. First principal component from PCA as an score of resistance and its correlation with grain characteristics. characteristics. Abbreviations are:

1.	Chiapas 218	(C218; OLOT; 2)
2.	Guanajuato 93A	(G93A; MADU; 2)
3.	Mexico 5	(M5 ; PALT; 1)
4.	Mexico 55	(M55 ; PALT; 1)
5.	Mexico 182	(M182; CONI; 3)
6.	Mexico 208	(M208; CHAL; 4)
7.	Mexico 212	(M212; CATL; 2)
8.	Mexico 461	(M461; CONI; 3)
9.	Puebla 463	(P463; ARAM; 1)
10.	Puebla 537	(P537; ARAM; 1)


Concentration of Z isomer of ferulic acid (mg/g)
.08
.07
.06
.05
.04
.03
.02
.01
0
-2
-1
0
1
2
3
4
5
1st Principal component
M5
M55
P463
M182
G93A
M208
P537
M461
M212
C218


Hardness of grain (g/sec)
30
25
20
15
10
5
-2
-1
0
1
2
3
4
5
1st Principal component
M55
M5
M182
P537
P463
M461
M208
G93A
M212
C218

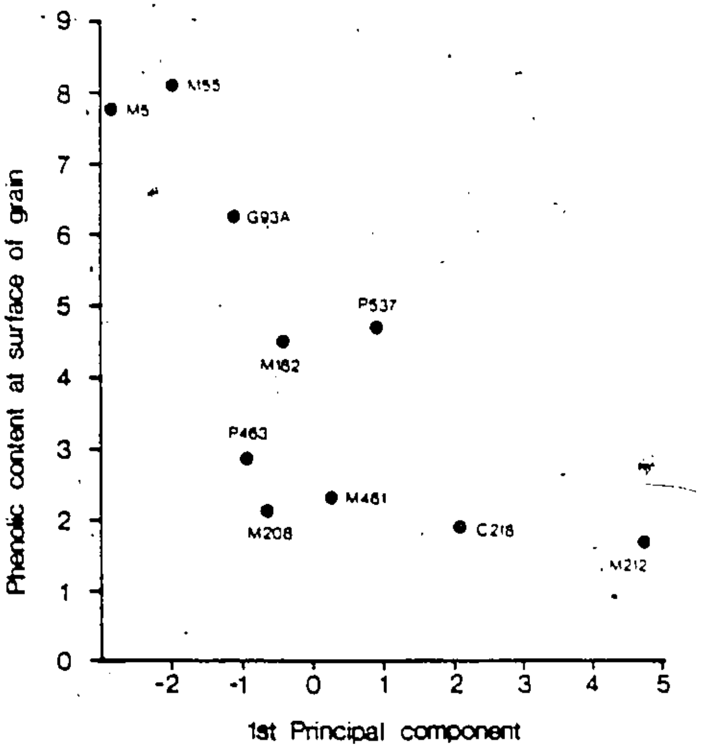


Figure 20: Multivariate correlations

3.9.1 Seed Characteristics and Indices of Interaction

The first canonical correlation is 0.9511 which appear to be closer to one of the between-set correlations (i. e. HARD and INDEX). The probability level for the null hypothesis, that all the canonical correlations are 0 in the population, is only 0.1264, which is not a high value but allows the interpretation of some tentative trends. For the remaining canonical correlations only the second canonical correlation has a moderate adjusted canonical correlation but its probability level is very poor therefore is not considered in the analysis. Since the variables have different units the standardized coefficients were analyzed. Also the correlations given in the canonical structure matrices were interpreted.

The first canonical variable for the grain variables is a weighted difference of HARD (-0.8740), MOIST (-0.1611) and FERULZ (-0.3347) from SURFERUL (0.0760), with the most weight on HARD. The correlations between HARD, MOIST and FERULZ and their first canonical variable are negative for both HARD (-0.9854) and FERULZ (-0.9046) whereas for MOIST it is positive (0.6338). After analyzing the correlation values among the grain variables it was found that MOIST is a suppressor variable, meaning that it is enhancing other variables.such as hardness and phenolic content of grain.

The first canonical variable for the interaction variables shows a weighted difference of INDEX (0.8149) and WGTLOSS (0.3243) from STABPAR (-0.0737), with the most emphasis on INDEX. Here STABPAR is a suppressor variable since its correlation has a positive value (0.6938). The correlations for INDEX and WLOSS, and their first canonical variable are 0.9779 and 0.7839 respectively.

The canonical redundancy analysis showed that the first pair of canonical variables are fairly good overall predictors of the opposite set of variables, the proportions of variance explained being 0.6458 (grain measurements) and 0.6188 (interaction indices). The second and third canonical variables add some information to the variance explained in the case of the grain variables, with a cumulative proportion of 0.7442 while for the interaction indices the cumulative proportion reaches 0.8012.

The squared multiple correlations indicated that the first canonical variable of the interaction indices has a high predictive value for HARD (0.8784) and FERULZ (0.7403), fairly good predictive value for SURFERUL (0.6011) and some for MOIST (0.3633).

The first canonical variable of the grain measurements shows a very good predictive power for INDEX (0.8650), fairly good for WLOSS (0.5559), and some for STABPAR (0.4354).

3.9.2 Insect Characteristics and Indices of Interaction

The first canonical correlation is 0.9986 with a value of F = 10.42 and a probabiliy level of 0.0011. The standardized canonical coefficients of the insect population variables showed that the highest weighted variable was EMERGEN (1.3396), on the other hand INDEX (0.6092) was again the highest weighted variable. The correlations between the four insect variables and their first canonical variable are : positive for CHOICE (0.9095), EMERGEN (0.9278) and FRASS (0.6475), and negative for PCTMORT (-0.6017) being CHOICE and PCTMORT suppressor variables.

The correlations of the indices and their first canonical variable are all positive: 0.9261 for INDEX, 0.8517 for WGTLOSS and 0.8511 for STABPAR; there is no suppressor variable in this set. The redundancy analysis gives an indication of a fairly good overall predictive power for both sets of variables being 0.6156 (variance explained by the opposite first canonical variable) for the insect characteristics, and 0.7670 for the interaction indices. The squared multiple correlations indicated that the first canonical variable of the interaction indices has a high predictive value for EMERGEN (0.8585) and CHOICE (0.8249), and some for FRASS (0.4181) and PCTMORT (0.3610). The first canonical variable of the insect measurements is a good predictor of INDEX (0.8553) and fairly good predictor of STABPAR (0.7224) and WLOSS (0.7234).

3.9.3 Grain Characteristics and Insect Characteristics

Although the correlations of insect and grain characteristics are negative and highly correlated (see Table 19), the first canonical correlation has a very poor probabilty level (P=0.2745). Nevertheless the correlation value of this first canonical correlation is very high (0.9808).

The squared multiple correlations of the grain characteristics and the first canonical variable of the insect characteristics show a high predictive value for HARD (0.8682) and FERULZ (0.8443). The only high predictive value between the first canonical variable of the grain characteristics and the insect characteristics was obtained for EMERGEN (0.8141). The variable HARD has the highest standardized canonical coefficient (0.7680) and the suppressor variables within its set are MOIST (positive to negative) and SURFERUL (negative to positive). For the insect characteristics the highest weighted variable was EMERGEN (-1.9577) as well, the suppressor variables were PCTMORT (negative to positive), CHOICE (positive to negative) and FRASS (positive to negative). The standardized variance of the grain and insect characteristics explained by the opposite first canonical variable were 0.6590 and 0.4716 respectively.

DISCUSSION

4.1 *Systematics of the Maize Seed Material*

Cluster and Ordination techniques (Figure 3 and 4), showing the relationship among the Mexican races of maize according to the external morphological characteristics of the corn ear, indicate a partial agreement with the groups described by Wellhausen *et al.* (1952). However, caution has to be used when trying to extrapolate these affinities to the collections of maize used here. Although the maize material analyzed in the present study represents the collections of maize analyzed by Wellhausen *et al.* (1952) there are some questions as to how representative these collections are of maize germplasm. In particular, the genetic variation of Mexican maize has been shown to be extremely high. Doebley *et al.* (1985) found that maize is rich in allelic diversity as compared to other plants. The variation within Mexican races as measured by isozyme variation shows that Chalqueno and Conico are the races of maize with the highest average number of alleles per collection and total number of alleles (Doebley *et al.*, 1985). The percentage of polymorphic loci per collection, the mean expected heterozygozity per collection, the panmictic heterozygosity per race and the relative genetic differentiation among collections within races confirm that, Chalqueno and Conico have a high level of isozyme variation whereas Arrocillo Amarillo, Cacahuacintle, Maiz Dulce, Palomero and Oloton are less variable in isozyme variation (Doebley *et al.*, 1985). The general trend, according to Doebley *et al*, (1985), appears to be that the northern and high elevation races of maize are the most variable. The fact that the indigenous germplasm has this wide genetic base complicates to some extent the interpretation of the results obtained.

The results shown in Figure 5 and in Figure 7 give an indication of the different origin of the Ancient Indigenous group and the Pre-Columbian Exotic group since these two groups do not link, at least,not in these two analysis.

Goodman and Bird (1977) in their attempt at ordering all races of maize from Latin America indicated that the Mexican races Cacahuacintle and Oloton are strongly linked to the South American races. This result, as well as the fact that the races of maize of Mexico and South America fall into mutually exclusive groups seems to point out the influence of geographical isolation and/or different selection techniques by the major pre-Columbian advanced civilizations of America in the domestication and improvement of maize. Thus, the results obtained here support the suggestion of Goodman and Bird (1977) since, according to resistance characteristics, the Pre-Columbian Exotic group forms an exclusive and characteristic group.

4.2 Feeding Deterrent Properties of Phenolic Acids from Grain

The results obtained demonstrate (Table 5) that treatment of artificial diets with hydrolyzed total phenolic extracts of grain leads to significantly lower consumption of diets by weevils as compared to consumption of untreated controls ($P<.05$). Although there exists a significant reduction of consumption of experimental diets it would be incorrect to describe these extracts as "absolute antifeedants" (Schoonhoven, 1982). In this case, the experiment did not prove that the compounds contained in the extracts cause death by starvation of adult weevils. However, the evidence shown in the present study allows for a preliminary definiton of the compounds as relative antifeedants or alternatively behavioural antifeedants.. According to Nakanishi (1982) although bioactive principles may be active as a crude mixture, no applications can be initiated until the compounds are isolated in pure form. The identification of phenolic acids as the materials in the hydrolyzed extracts and demonstration of their activity in pure form is the first step in this direction. One possible application, then, is the possibility of breeding resistant lines of maize.

The feeding deterrent properties of the total phenolic grain extracts as measured by consumption were negatively correlated (r = -0.84) with the phenolic content of the extracts (Figure 12). These results suggest that phenolics are the major substance contributing to reduced feeding. The effect of phenolics from sorghum on feeding of *Locusta* was tested by Woodhead and Cooper-Driver (1979) who arrived at similar conclusions.

Several pure phenolic substances were tested to examine the feeding response of *Sitophilus*. The results obtained indicated that these substances have significant feeding deterrent properties (Table 6). There are few publications concerned with the deterrent activity of secondary plant compounds against storage pests. The only other reference of this kind is the study of sesquiterpene lactones from Umbelliferae against storage pests (*S. granarius* included) by Nawrot *et al.* (1983), which also have feeding deterrent properties towards *Sitophilus*.

According to Dethier (1980) plants which are adequate sources of nutrition but which lack toxic principles are protected from attack if they elaborate deterrent compounds. Grain of maize fits the latter statement to a great extent. Although, in this case phenolics have to be classified as 'relative antifeedants' (Schoonhoven, 1982) since it was observed that there was some degree of habituation to the artificial diet if it is considered that the adult weevils did not stop feeding but rather they slowed their rate of consumption. Schoonhoven (1982) has pointed out that the information on long term adaptation or development of 'resistance' by the insect is still fragmentary and that more research is urgently needed. Ferulic acid is present in maize seed as a free acid in small quantities but soluble glycosides and polysaccharide-bound ferulic acid are probably released as the free acid during digestion in the insect's gut. Free ferulic acid release could make a substantial contribution to reduction of feeding by *Sitophilus* on grain. Indeed, the presence of these substances in the outer tissues of the grain could be correlated with their proposed defensive role (Fulcher *et al.*, 1972 and personal communication).

4.3 Grain characteristics and resistance to infestation by S. zeamais

Weevils have different feeding behaviour and performance depending on the type of grain they are feeding on. According to the tests performed in the present study (Choice and No-choice) along with several variables measured, there is a wide range of response of the weevil population related to the grain substrate. However, there is no single method for measuring corn kernel resistance. As seen in the tables and figures of the Results Section there are two races of maize which can be considered as opposite in the range of susceptibility to *Sitophilus* infestation. On one extreme, Palomero maize appears as the most resistant race (although, there is sometimes disagreement between the two collections), and on the other extreme of the range Cacahuacintle is clearly the more susceptible to weevil infestation. However, the differences within collections of the same race, besides the latter mentioned, are striking. Sometimes the collection can be classified as resistant in terms of consumption or slightly susceptible according to some other index. In other words, there are large inconsistencies both among and within collections. Therefore, it is evident that the real situation in the grain is complex and cannot be explained solely on the basis of one factor.

Since the only direct evidence of feeding deterrent activity of phenolics was obtained by means of artificial diets and because of the complexity of the real situation in grain, a second stage of evaluation of the role of phenolics in the mechanisms of defense, was achieved through a multivariate analysis. Since all multivariate techniques are in fact exploratory analysis of variables and especially techniques for reducing dimensionality, no attempt is made in trying to overemphasize any variable as a cause of a certain effect. This is not an analysis of causes, it is just a description of possible relationships between variables which could give indication of future experimentation and research.

The appraisal of methods for measuring resistance was done by Widstrom *et al.* (1972) as well as the advantages and disadvantages of such methods. Accordingly, it is assumed that each one of these methods, which were used here with some modifications, indicate some of the characteristics of the grain-weevil interaction system. Thus, the dynamics of the interactive process and some relationships of the races of corn to infestation by the corn weevil will be described for future modelling of the system.

The hardness of the grain, the content of phenolics both at its surface and the total amount, as well as the content of the Z isomer of phenolics are highly correlated to most of the biological variables relating to resistance (Table 19). This is an indication of the relevance of these characteristics as factors of resistance of the grain to weevil infestation. It has long been suspected that hardness is an important factor contributing resistance to maize grain (Betanzos, 1980a; Dobie, 1977; Schoonhoven, 1972; Schoonhoven *et al.*, 1976; VanDerSchaaf *et al.*, 1969). However, some striking discrepancies have been reported (Fortier *et al.*, 1982; Ibvijaro, 1981). In the present study it is clear that grain hardness is a characteristic which correlates to a certain extent with many of the variables which measure the performance of the weevil population, especially to Dobie's index. Furthermore, the grain hardness is correlated with phenolic content. This relationship indicates that there may exist a mechanism of resistance which involves these two factors. The PCA carried out for grouping the collections of maize races according to resistance indices was used to illustrate the relationship between these 'independent' factors of the grain to the first principal component obtained which is a summary of the weevil performance on the grain. In figures 20, it is shown that the best correlation was obtained for the content of Z isomer of phenolics and the first PC, followed by the hardness of the seed and the surface phenolics content. Therefore, a general trend of the interaction of these grain characteristics to the weevil performance and preference is established.

On the other hand, the canonical correlation analysis can be interpreted as follows: Two variables of the grain characteristics, hardness and content of Z isomer covaries closely with the first canonical correlation variable of the interaction indices. In a linear regression this means a high predictive power for these variables in both ways, from the grain characteristics to predict the indices of interaction and from the latter to predict the former. It can be said that the hardest grain tends to have more total phenolics (and consequently more Z isomer) and the insect population tends to consume it less, to avoid it and accordingly to perform poorly on these grains. Some inferences can be drawn regarding the immature stage, this is that since the Dobie index, an indirect measurement of the time of development of the insect, is highly correlated with the grain characteristics (moisture content, amount of Z isomer of phenolics, surface phenolic content and hardness of kernel), then an indication is given of the possible involvement of these grain characteristics in the performance of the weevil population within the grain. However, all the variables are confounded to some extent. The canonical correlation of the insect characteristics and the indices of interaction is highly redundant since the indices are largely based on the biological characteristics of the insect growing on the grain. However, the loss of weight of grain is well predicted by the linear compound of the insect characteristics indicating the tendency of the weevil population to 'prefer' grain with more 'favorable' characteristics. This suggestion is supported by the results of choice tests which clearly showed that the insects prefer the so called susceptible maize grain (Table 10 and Figure 14). Further indication of this type of selective process was found by Whitham (1983), who observed that there is a strong pattern of host preference, as related to phenol concentration, in the aphid-cottonwood tree system where the colonizing insects nearly always try to form their colonies in the leaves with the lowest phenol concentration. The canonical correlation analysis of the grain characteristics and the insect characteristics, although not significant (see canonical correlation section and appendix 4), shows a trend between only one of the insect characteristics, emergence of new adults, and the first canonical variable of the grain measurements. This may suggest that the emergence of new adults tends to be fewer for harder and high phenol grain. Because the moisture content and the surface phenolics were, according to the analysis, suppressor variables which therefore merely enhance the other two variables within the set, it can then be argued that at least to a certain degree the hardness of the kernel and the amount of phenolics in grain explain the variation in the number of weevils emerged. The low significance obtained might be due to the small sample used (10 races of maize).

These inferences derived from the correlation analysis seem to be in accordance with some earlier reports on the resistance of varieties of maize to weevil infestation. Ivbijaro (1981) found that adults of *Sitophilus zeamais* and *S. oryzae* were more numerous in soaked maize grain without pericarp than in unsoaked intact maize grain. Also, Schoonhoven *et al.* (1976) and Dobie (1974 and 1977) have suggested that resistance is mainly located in the pericarp which acts as a barrier against infestation. However, the interpretation of the above mentioned authors is largely based on stimulant factors which, they speculate, could be lacking in the resistant kernels (Schoonhoven *et al.*, 1976) or else, the possibility that the removal of the pericarp deprived the weevil larvae of the full complement of some nutritional factors. This conclusion was based on the observation that the mean development period of the new generation adults growing in soaked seeds was longer than in unsoaked seeds (Ivbijaro, 1981). Again, most of the research done in this area has concentrated on how attractive the grain is to the insect and not on how the balance between stimulant factors and deterrents influences the feeding behaviour of the insect. One exception is the earlier work of Russell (1962) who suggested that the high tannin content of one variety of sorghum is a deterrent to oviposition by *Sitophilus oryzae*.

4.4 Indices of Nutrition and Growth of the Immature Stage of Weevils

Deterrent chemicals play a large role in determining both the preference or initial choice of food and the amount consumed (Bernays and Simpson, 1982; Dethier, 1982; Schoonhoven, 1972). It has been observed, as well, that the effect of feeding deterrents are concentration-dependent and possibly they prevent feeding when concentrations are very high (Bernays and Simpson, 1982). In the present study, the estimated grain consumption per insect per day showed (Table 16) that the insect intake of food was markedly higher in the susceptible races of maize whereas for the resistant varieties the intake was lower with one exception presented by one of the intermediate races (Chalqueno-M208) which showed a remarkably low consumption. Relating this to the amount of phenolics present in grain, there is a general agreement with the proposed defensive role of these substances. However, another explanation, cannot be ruled out. The lack of some nutrients may be affecting, as well, the development of the weevil resulting in a reduced weight. Comparing the indices of nutrition and the rates of consumption (Tables 16-17) it is observed that the susceptible grain while more consumed did not always produce weevils of the highest weight. Thus, we could speculate that some of the susceptible grains (i. e. Cacahuacintle-M212) are more consumed not only because of its lack of deterrents but also its lack of nutritional value. The low nutritional indices (ECD and ECI) of the weevils feeding in the more susceptible grain (Cacahuacintle-M212) may then be explained from that perspective. Another explanation of the reduced weight of weevils on the most susceptible grain could be the effect of density-dependent factors like competition since susceptible races Oloton (C218) and Cacahuacintle (M212) had the highest number of oviposition sites per seed (Figure 15).

Dilution studies can be performed in which material lacking powerful feeding stimulants or deterrents or significant nutritional value are used to 'dilute' the food (e. g. cellulose). These studies indicate that there is an increase in intake of food containing cellulose which is correlated to its decreased digestibility (Browne, 1975). The present study perhaps illustrates a similar phenomenon. Furthermore, House (1965) examined the effect of food dilution on larvae of *Celerio euphorbidae* demonstrating that the diets containing lower nutrient value were consumed in greater quantities. Latheef and Harcourt (1972), using a more natural system, found that larvae of *Leptinotarsa decemlinata* feeding on potato and tomato leaves, showed higher indices of nutrition when feeding on potato than on tomato and that the consumption of tomato leaves was greater than potato foliage per day. Consequently, the final determinant of the suitability of a material as food seems to be the balance of phagostimulants and deterrents as well as the nutritional content of the food.

The estimated growth of the larval stages as depicted in Figure 18 shows that although differences in the rates of growth of immature weevil populations on different races are small (Table 18), the performance in terms of time of development and increase in weight are different for all weevil populations depending on the type of grain. An interpretation of these results is that the wide variation of grain quality presents a very strong determinant for weevils success. The change in the "degree of satisfaction" (Gadgil and Bossert, 1970), as an index of the extent to which a resource is limiting, seems to be affecting not only the survival of the population but also growth, maintenance and indirectly birth rate. This has strong implications to evolutionary-genetics. Lewontin (1965) in his theoretical essay about selection for colonizing ability, analyzes the effect of small changes in life cycle phenomena such as fecundity, longevity and length of developmental period on the intrinsic rate of increase. He concludes that the selection exerted on subtle variation of the life cycle components during colonization increases fitness although only by a small amount. The results obtained here regarding the differential performances of populations of a single insect species (weevil) on a number of plant races (maize) as related to biological processes such as reproduction, growth and maintenance and their influence on the life history strategy of the insect suggests that the grain-weevil system reacts according to Lewontin's model. Also, all the evidence shown in the present study seem to indicate a great potential for the use of this grain-insect system as a good model for analyzing genetic variation in resource use and has implications for coevolution. The relevance of understanding plant-herbivore systems is that it may permit prediction of evolutionary sequences of feeding habits (Mitter and Futuyma, 1983). On the other hand, the importance of these basic studies of genetic interactions between economically important crops and their pests could be enormous for breeders since they may be able to eventually develop and manage crop varieties which would maximize the stability of the crop's resistance to herbivores (Gould, 1983).

CONCLUSIONS

The presence of feeding deterrent compounds in maize grain has been shown. The importance of this factor within the defence system of the grain has been outlined, showing the relevance of this factor as an important part of the characteristics of grain involved in the mechanism of resistance of grain. Phenolic acids are characterized as relative antifeedants and their effect on oviposition and growth of the corn weevil seems to be concentration-dependent. Grain with higher concentrations of phenolics is more resistant to infestation and less suitable for growth of populations of *Sitophilus zeamais.* The presence of an isomer of ferulic acid (Z form) and its high correlation with some indices of resistance indicates that, at least tentatively, this compound could be considered as the major agent of defence for successful colonization of the grain by the weevils. Finally, the screening of land races of maize as a potential source of development and deployment of resistant varieties was initiated.

APPENDIX 1

Average linkage hierarchical cluster analysis (25 races)

Simple statistics

	Mean	std. dev.	skewness	kurtosis	bimodality
eardiam	43.3	9.0	-0.5	-0.7	0.5
cobdiam	25.7	5.8	0.8	1.2	0.4
racdiam	14.3	4.5	1.1	1.7	0.4
kerleni	12.1	2.9	0.6	2.1	0.3
raclen	2.4	1.0	-0.5	-0.1	0.4
racker	0.2	0.1	-0.7	0.4	0.4
earextl	15.6	5.4	1.2	1.9	0.5
earextd	4.3	0.9	-0.3	-0.3	0.3
rowno	13.5	3.2	0.8	1.9	0.3
shadiam	13.1	6.0	2.3	6.2	0.6
kerwid	8.7	1.8	-0.3	-0.3	0.4
kerstria	0.4	0.5	1.8	2.6	0.7
kerthick	3.9	0.7	0.9	3.0	0.3

eigenvalues of the correlation matrix:

eigenvalue	difference	proportion	cumulative
6.17	3.01	0.47	0.47
3.17	2.01	0.24	0.72
1.16	0.23	0.09	0.81
0.92	0.36	0.07	0.88
0.56	0.12	0.04	0.92
0.44	0.19	0.03	0.96
0.25	0.09	0.02	0.97
0.16	0.05	0.01	0.99
0.11	0.08	0.01	1.00
0.03	0.01	0.00	1.00
0.02	0.01	0.00	1.00
0.01	0.01	0.00	1.00
0.00	0.00	0.00	1.00

root-mean-square total-sample standard deviation = 1
root-mean-square distance between observations = 3.61

No. of clust	freq. of clust	rms std of new cluster	normalized average linkage	r-sq.	r-sq. expeted	cubic clust. criterion
10	2	0.58	0.82	0.87	0.87	0.096
9	3	0.53	0.87	0.85	0.85	0.273
8	9	0.51	0.94	0.83	0.82	0.275
7	11	0.58	1.00	0.78	0.79	-0.314
6	14	0.65	1.07	0.71	0.75	-1.573
5	3	0.76	1.19	0.67	0.71	-0.970
4	17	0.74	1.29	0.56	0.65	-2.213
3	5	0.83	1.42	0.48	0.56	-1.81
2	20	0.85	1.54	0.32	0.40	-1.297
1	25	1.00	1.77	0.00	0.00	0.000

APPENDIX 1

Average linkage hierarchical cluster analysis (7 races)

Simple statistics

	Mean	std. dev.	skewness	kurtosis	bimodality
eardiam	41.4	8.4	-0.9	0.3	0.3
cobdiam	23.4	5.2	-0.5	-1.9	0.4
racdiam	12.2	2.9	0.02	-2.0	0.3
kerleni	12.6	2.3	-0.4	-0.7	0.2
raclen	2.1	1.3	-0.5	-1.6	0.3
racker	0.2	0.1	-0.3	-1.9	0.3
earextl	13.7	3.0	0.03	-0.6	0.2
earextd	4.1	0.8	-1.2	0.5	0.4
rowno	16.1	3.4	1.4	3.5	0.3
shadiam	10.7	3.3	2.0	4.5	0.5
kerwid	7.4	2.0	-0.1	-1.6	0.3
kerstria	0.2	0.2	0.2	-2.4	0.3
kerthick	3.7	1.5	0.6	-0.9	0.3

eigenvalues of the correlation matrix:

eigenvalue	difference	proportion	cumulative
8.62	6.22	0.66	0.66
2.40	1.55	0.18	0.85
0.84	0.15	0.06	0.91
0.69	0.31	0.05	0.97
0.38	0.31	0.03	0.99
0.07	0.07	0.01	1.00
0.00	0.00	0.00	1.00
0.00	0.00	0.00	1.00
0.00	0.00	0.00	1.00
-.00	0.00	-.00	1.00
-.00	0.00	-.00	1.00
-.00	0.00	-.00	1.00

root-mean-square total-sample standard deviation = 1
root-mean-square distance between observations = 3.6

No. of clust	freq. of clust	rms std of new cluster	normalized average linkage	r-sq.	r-sq. expe-ted	cubic clust. crite-rion
6	2	0.48	0.68	0.96	0.99	-5.58
5	3	0.53	0.79	0.91	0.97	-3.06
4	2	0.59	0.83	0.85	0.92	-1.92
3	4	0.62	0.97	0.75	0.83	-1.29
2	5	0.73	1.25	0.58	0.65	-0.59
1	7	1.00	1.75	0.00	0.00	0.00

APPENDIX 1

```
Average linkage hierarchical cluster analysis
(10 collections)
                                  Simple statistics
                Mean      std.      skew-      kurt-      bimo-
                          dev.      ness       osis       dality

wgtloss        0.565     0.157     0.268     -0.374      0.270
pctmort        0.276     0.161     0.229     -1.076      0.321
stabpar        1.000     0.364     1.140      2.027      0.361
choice        17.500    17.424     1.881      2.807      0.635
emergen       23.900    15.220     1.417      1.777      0.492
index          8.639     1.952    -0.079      1.009      0.188
frass          0.077     0.039     1.592      3.790      0.435

eigenvalues of the correlation matrix:

eigenvalue  difference  proportion  cumulative

   5.78        4.71        0.72        0.72
   1.07        0.33        0.13        0.86
   0.74        0.58        0.09        0.95
   0.16        0.02        0.02        0.97
   0.14        0.05        0.02        0.99
   0.09        0.08        0.01        1.00
   0.01        0.01        0.00        1.00
   0.00        0.00        0.00        1.00

root-mean-square total-sample standard deviation = 1
root-mean-square distance between observations = 2.8

No.    freq.   rms std    normalized   r-sq.   r-sq.   cubic
of     of      of new     average              expe-   clust.
clust  clust   cluster    linkage              ted     crite-
                                                       rion
 9      2       0.31       0.43        0.99    1.00    -5.83
 8      3       0.35       0.52        0.97    0.99    -3.98
 7      4       0.42       0.69        0.94    0.98    -3.67
 6      2       0.49       0.69        0.91    0.95    -2.58
 5      5       0.47       0.76        0.87    0.92    -1.93
 4      3       0.58       0.88        0.83    0.87    -1.31
 3      8       0.67       1.11        0.66    0.79    -2.05
 2      2       0.93       1.31        0.56    0.63    -0.69
 1     10       1.00       1.99        0.00    0.00     0.00
```

APPENDIX 2

One way analysis of variance of variables related to parental/progeny weevil success

variable	DF	F	P	source of var.	DF	r2	CV	Mean
wgtloss	22	15.63	.0001	races	7	.88	74.4	0.47
consday	22	13.60	.0001	races	7	.86	80.4	9.64
meddev	22	0.76	.6317	races	7	.26	10.1	51.15
consind	22	10.54	.0001	races	7	.83	44.8	15.33
coninday	22	10.72	.0001	races	7	.83	45.7	0.31
numemerg	29	10.02	.0001	races	9	.82	66.7	13.17
wtotins	29	10.74	.0001	races	9	.83	65.8	48.87
parenpop	29	0.80	.6171	races	9	.27	28.8	39.60
windpapo	29	4.72	.0019	races	9	.68	4.4	3.59
index	21	3.30	.0273	races	7	.62	33.6	4.88
windins	23	2.03	.1133	races	8	.52	8.3	3.62

APPENDIX 3

APPENDIX 3

I. Responses by effect of races. Covariables: Weight and number of parental population.

Variable of Response	Model			Variable of Classification			Covariable			Covariable				
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	R^2	CV
WGTLOSS	9	11.04	.0001	7	14.11	.0001	1	0.27	.61	1	0.28	.61	.88	78
MEDDEV	9	1.37	.2930	7	0.94	.5070	1	0.94	.35	1	4.80	.05	.49	9
CONINDAY	9	9.20	.0002	7	11.41	.0001	1	1.33	.27	1	1.64	.22	.86	44
NUMEMERG	11	7.48	.0001	9	9.12	.0001	1	0.11	.75	1	0.09	.76	.82	70
WINDINS	10	3.75	.0144	8	3.28	.0281	1	9.65	.01	1	1.61	.23	.74	7

II. Responses by effect of blocks. Covariables: Weight and number of parental population.

Variable of Response	Model			Variable of Classification			Covariable			Covariable				
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	R^2	CV
WGTLOSS	4	0.45	0.77	2	0.32	0.73	1	0.35	0.56	1	0.81	0.38	.09	186
MEDDEV	4	0.30	0.88	2	0.44	0.65	1	0.31	0.58	1	0	0.95	.06	10
CONINDAY	4	1.12	0.38	2	0.30	0.75	1	0.93	0.35	1	2.96	0.10	.20	91
NUMEMERG	4	1.84	0.15	2	1.19	0.32	1	1.40	0.25	1	3.58	0.07	.23	123
WINDINS	4	1.14	0.38	2	1.63	0.22	1	1.03	0.32	1	0.26	0.62	.19	10

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